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QUELQUES RÉFLEXIONS SUR L'AVENIR DE LA CAFÉICULTURE *(L'IMPACT POSSIBLE DE LA RECHERCHE SUR LE SECTEUR PRODUCTIF)*

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Ce titre peut paraître bien ambitieux, pour ne pas dire prétentieux.

Aussi les thèmes qui seront abordés aujourd'hui sont-ils limités à quelques considérations sur l'impact possible de la recherche sur la caféiculture au cours des prochaines décennies, et sur ce qu'il paraît raisonnable de faire afin que cet impact soit à la hauteur des efforts des chercheurs.

Les acquis du passé

Il y a deux ans, lors du précédent Colloque de l'ASIC à Montpellier, on avait tenté de répondre à la question : "Quelles caféicultures pour demain ?"([1]). Une analyse de l'évolution des techniques et du matériel végétal mis en oeuvre au cours des cinquante ou soixante dernières années avait alors conduit à brosser un tableau des connaissances actuelles applicables dans la pratique courante.

Ces acquis du passé, aussi bien pour le robusta que pour l'arabica, constituent un paquet technique cohérent dont chaque élément interagit avec les autres, et qu'il convient d'utiliser dans sa globalité.

S'il existe des clones de robusta ou des variétés d'arabica à haute productivité, et, pour les arabica, des variétés naines pouvant être plantées aux très hautes densités qui sont un facteur déterminant d'augmentation du rendement, ce matériel végétal n'est rien s'il n'est accompagné :

> d'un des systèmes de taille qui ont fait leur preuve comme clés de la productivité dans des conditions économiques certaines, parmi lesquels en particulier le recépage cyclique après quatre à six cueillettes selon les régions ;

[1] R.A.MULLER, XVe Colloque ASIC, Montpellier, 1993

- d'une fumure minérale en général à dominante azotée mais adaptable à tous les cas grâce aux résultats du diagnostic-sol complétés par le diagnostic foliaire, et qui n'est valorisée qu'en culture de plein ensoleillement avec possibilité de production endogène d'azote et d'enrichissement du sol en matière organique par intégration dans la plantation, partout où elles poussent bien, des Erythrines, Légumineuses arborescentes, utilisées non comme plantes d'ombrage, mais comme plantes à mulch grâce à des émondages fréquents, en notant qu'elles sont compatibles avec l'emploi des hautes densités du caféier, puisque n'occupant que peu de place au sol ;

- des mesures de lutte phytosanitaire lorsque nécessaire, en les adaptant aux contraintes de chaque zone écoclimatique donnée, moyennant des observations épidémiologiques simples.

Utilisé d'une façon rationnelle et cohérente, c'est-à-dire dans son ensemble, ce paquet technique conduit à l'obtention de rendements à l'hectare pouvant atteindre jusqu'à 4-5 tonnes de café marchand pour l'arabica, au Costa Rica par exemple, et à plus de 3 tonnes pour le robusta dans les meilleurs cas.

On peut compléter les propositions à faire aux caféiculteurs, en recommandant :

- une valorisation du terrain et du travail d'entretien par la culture de vivriers intercalaires limitée aux deux années qui suivent la plantation ou le recépage, périodes où le caféier libère l'espace nécessaire ;

- un traitement post-récolte soigné suffisamment au point aujourd'hui pour répondre aux exigences du marché ;

- une valorisation des sous-produits, en particulier par récupération des déchets (pulpe, parche), résultant du traitement des cerises après récolte, sous forme de compost après culture de champignons comestibles.

Les espérances pour le futur proche

Les recherches orientées dans le sens de l'allégement des coûts de production et de la valorisation du travail humain, sont de nature à améliorer encore les performances de la caféiculture, et surtout sa rentabilité :

- la création de variétés résistantes aux grands aléas parasitaires (maladies, insectes, nématodes), et les résultats attendus des études sur la lutte biologique éviteront, ou au moins réduiront, l'emploi des pesticides polluants et coûteux à l'achat et en travail ;

- la création de variétés fournissant un meilleur produit permettra de vendre mieux, en particulier les robustas, grâce aux méthodes traditionnelles de la génétique (hybrides "arabusta" au sens large, sélection directe au sein du pool robusta qui recèle des éléments de qualité), et grâce aux biotechnologies qui devraient pouvoir fournir des caféiers moins riches en caféine et plus riches en arôme ;

- la réduction des quantités de fertilisants par la production endogène d'azote, grâce à la généralisation de l'emploi des Légumineuses arborescentes d'accompagnement adaptées à chaque zone écologique et améliorées dans le sens de leur aptitude à fixer l'azote atmosphérique, et par la récupération des sous-produits des traitements post-récolte comme engrais de complément, toutes pratiques enrichissant le sol en matière organique et lui permettant de mieux fixer les engrais, est de nature à diminuer le poids des intrants achetés sur les prix de revient ;

- l'utilisation d'un matériel végétal adapté à une formule de taille allégée telle que l'écimage afin de réduire le nombre des années creuses consécutives au recépage cyclique contribuera à réduire la masse de travail ;

- la création de variétés naines de robusta augmentera la productivité à l'hectare grâce à de hautes densités de plantation, et améliorera la productivité du travail de cueillette ;

- l'obtention d'une maturation groupée simplifiera la récolte en réduisant le nombre des passages de cueillette, et sera de plus, plus favorable à la mise en oeuvre de récolteuses mécaniques adaptées à chaque type de plantation ;

- l'amélioration des méthodes de récupération des sous-produits du traitement post-récolte évitera la pollution des rivières et la production, sous forme de gaz, d'une énergie non négligeable, rentabilisera l'opération.

Le possible et le réel

A l'énoncé du possible, et des espérances pour le futur, les chercheurs et techniciens de la caféiculture, conscients d'avoir fait évoluer les connaissances et les techniques, et sûrs d'aller dans la bonne voie, pourraient ressentir une légitime satisfaction.

Or c'est plutôt une certaine amertume qui les habite. Car les caféiculteurs qui mettent en pratique les connaissances disponibles, et obtiennent les rendements annoncés plus haut, soit entre 3 et 5 tonnes de café marchand à l'hectare, sont une exception.

D'après le tableau présenté ici, la moyenne de la production mondiale se situe en effet à environ 500 kg/ha, soit entre le sixième et le dixième du possible selon que l'on parle robusta ou arabica ; environ la moitié des surfaces plantées atteignent à peine cette moyenne ou se situent très en-dessous.

	SURFACES PLANTEES 1990-1991 (x 1000 ha)	**TONNAGES PRODUITS 90/91-91/92 (x 1000 t)**	**MOYENNES A L'HECTARE (x kg)**
AFRIQUE	3589	1089	303 (de 21 à 1522)
ASIE	1295	854	659 (de 116 à 1809)
AMERIQUE DU SUD	4721	2775	588 (de 244 à 951)
AMERIQUE CENTRALE	1752	1079	616 (de 175 à 1716)
OCEANIE	57	52	912 (-)
			MOYENNE GENERALE
TOTAL	**11414**	**5848**	**512**

Un fossé large et profond sépare donc ce qui pourrait être fait de ce qui se fait réellement. Si les caféiculteurs les plus performants mettent en pratique le paquet technique décrit plus haut, le plus grand nombre en est encore à une caféiculture rudimentaire : ou bien ils ignorent la taille, la fumure, la lutte phytosanitaire, l'entretien des champs étant réduit au minimum nécessaire pour pénétrer les plantations en vue de la récolte, et ils en sont restés à une activité proche de la cueillette, rognant sur le capital forestier dans l'exploitation de fronts pionniers, ou bien ils n'appliquent que l'un ou l'autre des éléments du paquet technique disponible, et le plus souvent imparfaitement, ce qui les conduit à n'en tirer qu'un profit dérisoire.

Il en résulte que, tandis que les caféiculteurs qui utilisent les meilleures variétés et mettent en pratique les acquis scientifiques et techniques, tirent de leurs activités des ressources leur assurant une vie décente et même un certain bien-être, ceux qui ignorent ou ne peuvent utiliser ces techniques se maintiennent au niveau de pauvreté qui, malheureusement, caractérise globalement la plupart des pays du Sud.

Le monde des producteurs de café donne ainsi une fidèle image du monde : d'un côté ceux qui ont accès aux acquis de la recherche et de la technique, et à un environnement leur permettant de les mettre en oeuvre, et qui en reçoivent les fruits, de l'autre côté ceux qui restent étrangers au progrès technique ou qui se trouvent placés dans un environnement ne leur permettant pas d'en bénéficier, et qui sont les laissés pour compte.

Un futur proche alarmant

Ce fossé entre une caféiculture productive permettant l'accès aux biens matériels, et une caféiculture rudimentaire, apanage de la pauvreté, ne fait que se creuser. La crise des années passées a accentué la cassure : les pays qui ont le plus diminué leur production par suite de la désaffection des paysans pour une culture peu rémunératrice, sont aussi les pays où l'utilisation du paquet technique disponible était la plus faible : Côte d'Ivoire qui passe de 4 799 000 sacs en 89-90 à 2 245 000 en 92-93 et 2 417 000 en 93-94 ; Cameroun qui passe de 1 928 000 sacs en 89-90 à 800 000 en 92-93 ; Zaïre qui passe de 1 799 000 sacs en 89-90 à 1 145 000 en 93-94, par exemple.

Ce sont surtout les robustas et particulièrement les robustas africains qui accusent cette baisse, les robustas brésiliens restant étrangers à ce mouvement, de même que les robustas indonésiens.

En revanche, les pays où les rendements sont les plus élevés n'ont apparemment pas été affectés : Costa Rica 2 300 000 sacs en 89-90, 2 620 000 en 92-93 et 2 458 000 en 93-94 ; El Salvador, 2 788 000 sacs en 89-90, 3 026 000 en 92-93 et 2 070 000 en 93-94 ; Honduras 1 881 000 sacs en 89-90, 1 919 000 en 92-93 et 1 851 000 en 93-94, par exemple.

Si la crise a entraîné une baisse de la production, ce fut donc principalement dans les pays à faible niveau technologique par abandon de surfaces plantées et par une baisse du niveau technologique déjà faible.

N'est-il pas inquiétant qu'un certain nombre de pays qui n'ont pourtant pas beaucoup d'autres ressources, s'éliminent d'un des secteurs de production qui leur assurait une part importante de leurs revenus et s'engagent davantage dans la voie de la pauvreté ?

Ne peut-on craindre que, dans ces pays, les habitudes n'ayant pas changé, la remontée de la production consécutive à la remontée des prix se fasse, à terme proche, par la simple remise en cueillette de surfaces abandonnées pendant la crise, et, à terme plus lointain, par une extension des surfaces plantées partout où ce sera possible, plutôt que par la mise en pratique des acquis scientifiques disponibles ?

Si donc on ne change pas les logiques paysannes, la caféiculture risque, dans cette hypothèse, de rester à la fois majoritairement faiblement technifiée et très au-dessous de ses potentialités, faute de savoir mettre en oeuvre les outils qui lui ont été donnés par les chercheurs et les techniciens, et fragile puisqu'il apparaît, contre l'opinion de beaucoup de prévisionnistes, que les pays les plus touchés par la crise ont été ceux où la caféiculture était la moins intensifiée.

Une obligation : mettre à la portée des paysans les acquis scientifiques et techniques

Il convient, à ce stade de réflexion, de citer les paroles du Ministre français de la Coopération de l'époque, Monsieur Michel ROUSSIN, qui, lors d'une visite au Centre CIRAD de Montpellier en septembre 1994, a dit, s'adressant aux chercheurs :

..."Je vous demande simplement de savoir traduire vos résultats en termes pratiques et de faciliter la diffusion des acquis au coeur du monde rural...
..."Si vous ne multipliez pas les passerelles avec le monde du développement, sur le terrain comme avec les responsables de l'aide au développement, vos travaux scientifiques, aussi excellents soient-ils, risquent de rester dans les tiroirs ou dans les éprouvettes. Vous devez communiquer et faire connaître vos travaux...
..."L'obligation, pour la recherche [....], c'est de voir ses résultats utilisés au service du développement."

On ne peut que faire siennes ces paroles qui sont exactement dans la ligne de ce qui avait été dit il y a deux ans ([1]) et que l'on peut, sans hésiter, répéter aujourd'hui.

Faire de la recherche utile, ce n'est pas seulement faire une recherche adaptée aux problématiques variées de la caféiculture -cela, c'est facile, et toutes les activités de recherche ont, jusqu'ici, globalement répondu à cet impératif-.

Il avait été fait, il y a deux ans ([1]), un inventaire des axes de recherches à privilégier pour alléger les tâches des caféiculteurs et valoriser leur travail. Cette liste toujours actuelle a été reprise plus haut. Mais si les variétés attendues de ces travaux, dotées d'un fort capital d'autodéfense contre les grands aléas parasitaires, ou de meilleures qualités à la tasse, sont par elles-mêmes porteuses de progrès, elles ne seront rien si elles ne sont pas accompagnées du paquet technique existant qui seul peut leur permettre d'exprimer toutes leurs potentialités. De même, si les méthodes de lutte biologique sont intrinsèquement un progrès par rapport à l'environnement, il faut qu'elles s'appliquent à une caféiculture payante car elles ne seront pas gratuites. Dans le cas contraire, les résultats de ces recherches, aboutissement d'un travail considérable, et remarquable par l'enrichissement des connaissances et les services potentiels dont ils sont porteurs, n'auront pas l'impact attendu des chercheurs, de la même façon que, par le passé, le matériel végétal sélectionné et distribué sous forme de boutures racinées en Côte d'Ivoire ou au Cameroun, aura à peu près servi à rien.

Faire de la recherche utile, c'est donc aussi et surtout assurer l'application des résultats. Il ne faut pas craindre de le répéter car le problème est toujours actuel : si l'on veut que les résultats de la recherche de demain soient utiles, il faut impérativement que les acquis du passé soient utilisés et maîtrisés par le plus grand nombre.

[1] R.A. MULLER. loc. cit.

Comment faire passer le message scientifique ?

Tout le problème est donc là : comment faire passer le message scientifique et technique chez les utilisateurs ? Tous les pays producteurs s'étaient dotés dans le passé de Sociétés d'Etat ou paraétatiques de développement qui ont oeuvré pendant des années selon des axes qu'elles croyaient efficaces. Ces efforts, pour coûteux qu'ils aient été, n'ont porté, globalement, que peu de fruits puisque l'on est conduit à constater le décalage énorme entre ce que l'on peut faire et ce qui se fait, entre les rendements possibles et les rendements réels.

Ce constat d'échec étant fait, il convient d'en tirer les conséquences, et de forger les méthodes de pénétration du monde paysan les mieux adaptées et les plus aptes à atteindre l'objectif fixé.

Si la recommandation du Ministre cité précédemment, d'établir des passerelles entre organismes de recherche et organismes chargés du développement, est bonne en soi, il semble qu'elle ne soit cependant pas suffisante pour que le message scientifique soit saisi et transmis, et adopté par les paysans. Ces passerelles, d'une façon ou d'une autre, ont toujours existé :

- les écrits des chercheurs ont toujours été largement diffusés ;

- certains organismes de recherche ont eu, au sein de certains organismes de développement, des représentants censés être porteurs du message scientifique de leur maison-mère.

Pourtant certaines recommandations de la recherche ne sont jamais passées dans la pratique (par exemple le nécessaire recépage, en Côte d'Ivoire), des dérives sont apparues, le message scientifique étant plus ou moins simplifié ou allégé pour en faciliter la transmission et l'adoption, oubliant qu'un message tronqué n'avait plus la cohérence lui assurant l'efficacité.

Plus que des passerelles entre recherche et développement, il faut rechercher une intégration effective des organismes de recherche aux opérations de transfert mises en place, leur donnant qualité à la fois pour définir les actions et les techniques, et pour intervenir, en cas de dérive, afin de redresser la barre.

Mieux encore, les organismes de recherche devraient eux-mêmes prendre une part directe à la transmission du message scientifique, en montant eux-mêmes le plus possible d'opérations de transfert de technologie en milieu rural : agissant alors sans intermédiaire, leur message ne serait pas dénaturé, ils pourraient définir les mesures les plus aptes à rendre applicables les innovations proposées, ils pourraient juger plus directement des travaux nécessaires pour améliorer les connaissances du moment, les adapter aux différents cas particuliers rencontrés, et les rendre plus fécondes.

S'ils sont les plus aptes à établir le message à diffuser en milieu paysan, les organismes de recherche sont aussi les plus aptes à élaborer en même temps, et à l'occasion de chacune des opérations mises en place, les méthodes de transfert les plus adaptées à chaque situation. Des études sont en effet à faire pour comprendre les milieux socio-économiques dans lesquels on veut agir, et que l'on prétend modifier par l'injection de technologies qui leur sont jusque-là étrangères, pour comprendre les contraintes et les motivations des agriculteurs, et pour identifier les modifications à apporter à leur environnement pour que ces technologies puissent être adoptées (accès au crédit par exemple, groupement des paysans en organismes coopératifs capables de gérer des structures communes destinées soit à l'acquisition des matériels et produits utiles pour le travail au champ, soit au traitement de la récolte en vue d'une qualité meilleure, soit à l'accession à un stade avancé de la commercialisation, etc.).

On voit bien par là qu'il y a encore une grande part de recherche dans les actions de transfert, et d'une recherche-clé puisqu'elle doit donner à tous les acteurs du développement les outils ou les orientations nécessaires à leur tâche.

Qu'il soit donc permis de faire ici une suggestion.

Au départ, les colloques de l'ASIC avaient pour seul champ de préoccupations, les problèmes relatifs à la technologie et à la chimie du produit, et à la physiologie de sa consommation.

Elargis au Colloque de Hambourg en 1975, à l'Agronomie au sens le plus large, ils se sont alors enrichis des travaux conduits sur le terrain et dans les laboratoires, dans les domaines de l'amélioration du matériel végétal, de la lutte contre les maladies, les insectes et les nématodes, des techniques agricoles allant de la pépinière à la récolte, en passant par la fumure minérale, la taille, et l'entretien des plantations, des biotechnologies et de la connaissance du génôme.

La santé a tenu et tient toujours une grande place dans les préoccupations de l'ASIC puisque le Colloque de Montpellier en 1993 était placé sous ce signe et que celui de 1995 est riche de communications sur le thème. On ne peut que se réjouir d'une telle tendance renforcée par la journée "Cafés et qualité" organisée conjointement avec le CIRAD à Montpellier en octobre 94, et qui montre que l'ASIC, forum scientifique, est bien engagée au service des hommes.

Ne serait-il pas bon que l'ASIC, dans le même esprit, fasse la place, en amont, aux problèmes posés par le transfert de technologie en milieu rural afin de contribuer à l'utilisation des données scientifiques par les petits caféiculteurs ?

Une demi-journée lors de chaque colloque, consacrée à des échanges sur les résultats d'expériences concrètes conduites en milieu rural serait de nature à compléter dans le bon sens sa panoplie de discussions et à donner à ses débats une encore plus grande dimension humaine.

Les difficultés du transfert de technologie en milieu paysan

Il est sans doute permis de rêver un peu. Nous voici donc quelques années plus avant, l'appel a été entendu, l'ASIC fait une grande place aux échanges d'expériences de diffusion du message technique et d'application concrète de ce message en milieu rural ; les acquis du passé, les résultats les plus récents sont saisis et transmis par les organismes de développement en liaison avec la recherche, celle-ci a affiné les méthodes les plus adéquates pour ce transfert, et elle multiplie ses propres opérations ; sommes-nous donc entrés dans une ère nouvelle avec la perspective d'une caféiculture radicalement transformée et performante ?

Certes il ne serait guère réaliste d'imaginer que, en quelques années, les 3 ou 4 millions d'hectares de caféiers actuellement au-dessous ou juste à la limite de la moyenne mondiale, aient atteint sinon les rendements exemplaires dont il est fait état au début de cet exposé, du moins des rendements très améliorés. Ce serait d'ailleurs désastreux, l'effarante surproduction qui en résulterait aurait l'effet contraire au but recherché.

Pour les Etats producteurs, pour les organismes nationaux s'occupant de l'industrie caféière, pour les organisations internationales, la modernisation de la caféiculture doit donc se faire avec un impératif : **ne pas produire trop**, s'entendre pour que l'offre ne dépasse pas la demande afin de maîtriser les cours.

Et pour ne pas produire trop, il faut que les caféiculteurs soient conscients que les propositions qui leur sont faites ne visent ni à augmenter les surfaces plantées, ni à technifier la totalité des surfaces déjà sous caféiers, mais, seulement, **à augmenter les rendements sur les plus petites surfaces possibles pour une production donnée.**

Le transfert des données de la recherche devra donc se faire avec l'idée que la caféiculture n'est qu'une partie de l'ensemble que constitue l'exploitation agricole, dans une approche globale des systèmes de production

permettant d'améliorer la productivité des caféiers et le rendement du travail qui leur est consacré, en même temps que se développeront et se perfectionneront les autres activités de la ferme selon les particularités de chaque région et les ouvertures des marchés intérieur et extérieur : au total en effet la modernisation de la caféière se traduira par un gain de surface, un gain de temps et une meilleure valorisation des intrants mieux utilisés.

Le premier mot d'ordre pour les paysans n'est donc pas de **produire plus** mais de **produire mieux sur des surfaces réduites**, et de diversifier en même temps leurs activités sur les surfaces libérées.

Un problème surgit alors : que produire d'autre sur les surfaces libérées, que faire du temps gagné ? C'est une question difficile, le café apparaissant en effet souvent comme la production la plus lucrative malgré les fluctuations des cours. Il convient de tenter d'y répondre en tenant compte de la conjoncture du moment, de la localité, des habitudes aussi, en considérant par exemple que le gain de temps obtenu dans la culture améliorée du café permettra d'élargir les activités rétribuées à l'extérieur.

Le second mot d'ordre devra être de **produire meilleur pour vendre mieux.**

Il importe en effet que les techniques bien connues de préparation du produit, qu'il s'agisse du séchage et du décorticage de la voie sèche, du dépulpage, de la fermentation, du séchage, du déparchage de la voie humide, ou du triage pour les deux méthodes, soient mises en pratique avec soin afin que, grâce à une qualité éprouvée, on conserve les clientèles actuelles et l'on puisse en convaincre de nouvelles. Le couronnement serait d'obtenir des labels de qualité faisant état des terroirs, mais aussi des savoir-faire, afin de toucher une clientèle particulière prête à payer plus cher un produit de classe : il y a là une ouverture pour un certain nombre de producteurs.

Mais cela suppose des efforts importants de formation. L'expérience conduite récemment au Mexique par le CIRAD ([1]) permet de dire que cette formation doit être technique, les responsables des unités paysannes de transformation devant apprendre à veiller avec soin à la qualité des cerises livrées par les paysans, en termes de maturité et de fraîcheur, et à toutes les étapes du procès (qualité de l'eau, séchage suffisamment poussé). Cette formation doit être également apportée en matière de gestion et de commercialisation. Des associations avec les torréfacteurs et distributeurs sont en outre nécessaires.

Produire mieux et produire meilleur, la voie est tracée, mais la tâche demeure immense.

Ceux qui auront pour mission de faire adopter les innovations proposées par la recherche, devront savoir convaincre le monde rural du bien-fondé de ces innovations car il n'est pas d'exemple qu'une innovation puisse être mise en application sans un effort en travail ou financier.

Des expériences nombreuses -avec autant d'échecs- permettent de dire que l'on ne convaincra pas en établissant des champs de démonstration conduits par des agents rémunérés de l'extérieur et par conséquent non intégrés dans l'économie de la pratique mise en oeuvre. Il faudra au contraire amener les paysans eux-mêmes à être les acteurs véritables des mutations envisagées en mettant en pratique plus ou moins progressivement suivant leurs capacités de travail et leurs moyens financiers, les innovations en question.

On va se heurter aussi à de vieilles habitudes, à des lourdeurs, à des carences.

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[1] Programme DIMAC : Développement intégré des marges de l'aire caféière Jalapa-Coatepec, Etat de Vera Cruz.

Quelles vieilles habitudes ? D'abord, **l'attrait de l'extensif.** On a bien vu dans le passé qu'étendre à l'infini les surfaces plantées sans se préoccuper de la qualité du matériel végétal mis en place, sans se soucier davantage de la conduite culturale, sans songer à renouveler les vieilles parcelles, avait séduit les populations paysannes de Côte d'Ivoire dont la préoccupation principale fut l'appropriation de la terre, en même temps que l'exploitation -malheureusement destructrice- de la rente forêt ; de même, dans certaines régions d'Indonésie où, comme le cacaoyer, le caféier est une culture pionnière, véritable outil de défrichement forestier.

Vieilles habitudes encore avec **le goût pour les cultures associées** comme par exemple en Indonésie où le caféier est en combinaison avec différentes autres cultures de rente, ou comme au Cameroun où le caféier arabica n'est qu'une des multiples composantes d'une association végétale complexe à base des vivriers les plus divers, et au sein de laquelle il fait figure de parent pauvre, dans des conditions qui rendent illusoire tout recours à des variétés améliorées et aux méthodes culturales capables d'en exprimer les potentialités.

Certes l'on comprend bien la démarche de ces caféiculteurs-là : s'approprier le sol en même temps que tirer parti du capital de fertilité offert par la forêt, utiliser le même sol pour satisfaire tous les besoins familiaux et assurer les rentrées d'argent minimum nécessaires à la survie de la famille, **autant de façons de faire dont on perçoit bien la logique dans l'ignorance d'autres possibles.**

Mais ce qui paraît au moins contestable est que certains analystes économiques donnent en quelque sorte leur bénédiction à ces pratiques.

Dans le cas particulier des associations végétales, la parcelle cumule certes les revenus procurés par tous les éléments qui s'y rencontrent, et, globalement, la rente obtenue peut paraître conséquente. Mais parler "d'intensification" dans ce cas sous prétexte qu'il y a plusieurs ressources donnant une certaine souplesse à l'ensemble face aux problèmes rencontrés par l'une ou l'autre, paraît abusif. Avant d'en louer les mérites, le schéma d'exploitation ainsi inventé par les paysans eux-mêmes devrait être expérimentalement comparé à ce que la même surface pourrait offrir si le caféier, n'y occupant qu'une moindre place mais dans les conditions idéales de culture, laissait aux autres plantes l'espace nécessaire pour qu'elles soient, de leur côté, cultivées dans les conditions adaptées à leur nature et à leurs besoins. Dans les mélanges décrits plus haut où chaque culture est plus ou moins en compétition avec les autres, les résultats de la recherche ne peuvent être appliqués ni pour le caféier ni pour les autres cultures ; ils pourraient l'être au contraire dans le cas d'une organisation de la parcelle, chaque production étant à même d'en exprimer tous les effets : polyculture n'est pas mélange.

Pour les pays où la disponibilité des terres de forêt a entraîné une extension des surfaces plantées plutôt qu'un minimum d'intensification, il y a maintenant une chance à saisir pour inverser la tendance. En Côte d'Ivoire par exemple où les terres à conquérir sont de plus en plus rares, les paysans n'auront plus la tentation d'étendre leur foncier. S'ils veulent continuer à produire, ce ne sera qu'au prix du rajeunissement du verger : la replantation des vieilles caféières pourra-t-elle se faire avec un matériel végétal de qualité, accompagné de la mise en pratique du référentiel technique disponible ? Il y a là un enjeu d'envergure, une opportunité à ne pas laisser passer.

Quelles lourdeurs et quelles carences ? Elles sont de nature structurelle.

Des analyses récentes montrent en effet que là où la caféiculture est la plus performante et où la crise récente a été le moins ressentie au moins en termes de production globale, les structures professionnelles qui ont un rôle important dans la diffusion des techniques et la professionnalisation des agriculteurs y sont ou y ont été les plus fortes. Pour se limiter à quelques cas, on peut citer la Colombie avec la Federacion de Cafeteros, le Costa Rica avec l'Institut du Café, par exemple.

A l'inverse, c'est dans les pays où de telles puissances corporatives n'existent pas ou ont mal fonctionné, que les performances de la caféiculture sont les plus faibles et que les effets de la crise ont été les plus forts ; ces analyses soulignent les difficultés que rencontreront ces pays, pour relancer une caféiculture digne de ce nom. C'est le cas de la Côte d'Ivoire qui aura une tâche immense pour reprendre en main son verger et le rajeunir, et du Cameroun qui s'élimine progressivement du marché de l'arabica, alors qu'il dispose plus que beaucoup d'autres pays producteurs, des conditions idéales de sol et de climat pour conserver et développer cette culture, et dont on dit aussi qu'il risque de stagner, pour le robusta, au tiers de son niveau d'avant la crise, alors que, pour cette variété aussi, il dispose de conditions de sol et de climat particulièrement favorables.

Il semble donc, si l'on veut que la recherche ait l'impact souhaitable, en termes d'amélioration de la productivité, que, non seulement, on bouscule ici et là des habitudes paysannes qui empêchent la mise en oeuvre des acquis scientifiques, mais que l'on crée, dans nombre d'endroits, les conditions institutionnelles indispensables à cette mise en oeuvre : groupements de paysans devant servir de points d'appui et de leviers pour le transfert des propositions de la recherche, établissements de crédit adaptés, structures de commercialisation liées directement à l'échelon de production, seront les éléments *sine qua non* de l'amélioration de la caféiculture.

Et ce d'autant plus que les Etats, ou bien se sont désengagés comme c'est le cas au Mexique, ou bien n'ont plus les moyens d'intervenir directement. On ne peut que souhaiter de la part des Etats un encouragement à la création ou au maintien de structures paysannes, et de la part des organismes internationaux les aides nécessaires à cette structuration. On ne peut par exemple qu'encourager l'OIAC ([1]) et l'OAMCAF ([2]) qui font déjà des efforts importants, à s'engager plus avant dans cette voie.

Résumé et Conclusion

Pour conclure, il apparaît clairement que les acquis de la recherche, en améliorant les rendements, valorisent d'autant le capital terre et le capital travail ; c'est ce qui justifie pleinement que des efforts importants soient faits en matière de transfert de technologie en milieu rural afin de donner au plus grand nombre de paysans la possibilité de profiter des progrès scientifiques et d'améliorer ainsi leur niveau de vie.

Ce transfert de technologie doit être l'affaire de tous, à commencer par les organismes de recherche pour qui **recherche utile doit être synonyme de recherche utilisée** ; ils sont en effet mieux placés que quiconque pour transmettre dans son intégralité leur propre message, et mettre au point les méthodes de pénétration du monde rural ; confrontés aux réalités multiples du terrain, il seront alors aussi à même de tester leurs résultats et de les affiner si besoin est ; les organismes de développement devront avoir le souci de s'associer à la recherche pour s'assurer de la pertinence du message à transmettre. Pour cette intégration de la recherche et du développement, on pourrait, en accentuant encore la tendance, s'inspirer du réseau PROMECAFE ([3]) qui, en Amérique Centrale, travaille en étroite liaison avec les organisations professionnelles des Etats membres et les centres de recherche régionaux et nationaux et conjugue ainsi les activités de recherche et les activités de transfert de technologie.

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[1] OIAC : Organisation interafricaine du Café.

[2] OAMCAF : Organisation africaine et malgache du Café

[3] PROMECAFE : Programa Cooperativo Regional para la Modernizacion y la Proteccion de la Caficultura en Centroamerica, Mexico, Panama y Republica Dominicana.

C'est en multipliant les opérations concrètes sur le terrain dans les situations les plus variées, que l'on enrichira le savoir-faire dans ce domaine difficile, et que l'on accélèrera la modernisation de la caféiculture -et des cultures qui l'entourent.

Il conviendrait que l'ASIC s'ouvre largement à la confrontation des expériences ainsi vécues ; en élargissant son champ de réflexion scientifique, elle favoriserait la mise en application des résultats des recherches plus traditionnelles.

Mais convaincre les paysans n'est pas suffisant. Il faudra aussi que, grâce à l'organisation de sociétés rurales plus aptes que les individus isolés à recevoir et utiliser le message scientifique, grâce à la création d'organismes de crédit à leur portée, grâce à l'essor d'institutions corporatives, ils soient placés dans un univers leur permettant de saisir et de mettre en pratique les résultats que les chercheurs et techniciens auront trouvé pour eux.

Sans cette structuration de la profession, la caféiculture ne changera pas de visage, et restera en majorité une activité rudimentaire, alors que l'on a en main tout ce qu'il faut pour en faire une industrie performante, pouvant apporter à ses acteurs de base, un légitime mieux-être.

On peut cependant s'interroger sur le bien-foncé des propositions qui sont faites ici.

Pourquoi en effet tant d'efforts pour convaincre les caféiculteurs à la traine ? Est-ce que les choses ne se feront pas d'elles-mêmes, en laissant faire le temps ? Comme pour beaucoup d'activités, agricoles ou autres, une certaine décantation ne s'opérera-t-elle pas en effet elle-même, les moins performants des caféiculteurs disparaissant progressivement pendant que les plus performants prendraient de plus en plus d'importance et s'amélioreraient de plus en plus ?

C'est vraisemblable, mais ce scénario n'est pas à souhaiter, car il aurait surtout pour effet d'affaiblir encore les plus faibles, avec pour conséquences des migrations vers les villes ou les ailleurs qui ne leur offriront qu'une autre misère.

La modernisation généralisée de la caféiculture avec toutes les mutations qu'elle suppose, comme on l'a vu plus haut, pourrait être au contraire un moteur pour l'amélioration des activités qui l'entourent, et par conséquent une source de mieux-être pour beaucoup.

A l'heure où, comme récemment à Copenhague, chacun affirme sa volonté de lutter contre la pauvreté et l'exclusion, la solidarité ne devrait-elle pas s'accroître pour aider ceux des pays qui en ont le plus besoin, à promouvoir la modernisation de leur caféiculture et du même coup leur capacité à diversifier leurs ressources ? Ne serait-ce pas aller dans le sens de ce qu'écrivait récemment dans "Coffee Annual" sous le titre de "New Directions for the ICO", le Directeur Exécutif de cette organisation, Monsieur Celsius A. LODDER, plaidant pour une nouvelle coopération entre pays consommateurs et pays producteurs ?

Car si l'on veut que la recherche profite au plus grand nombre, il faut s'en donner les moyens, en hommes, et financiers : un grand effort de mobilisation apparaît de plus en plus nécessaire.

COFFEE MICROPROPAGATION IN A LIQUID MEDIUM USING THE TEMPORARY IMMERSION TECHNIQUE

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1. INTRODUCTION

Genetic improvement of perennial crops such as *Coffea arabica* takes almost 35 years to produce a homozygous, stable line that can be distributed to producers in seed form.

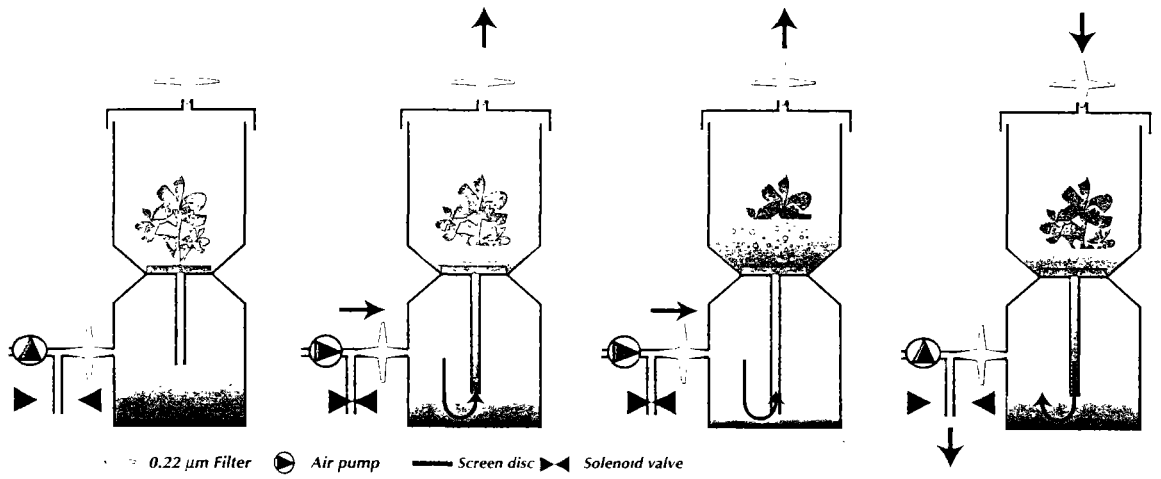
The progress made in *in vitro* micropropagation, particularly using liquid media, means that it is possible to multiply and use first generation (F1) or second generation (F2) hybrids obtained after only fifteen years' breeding or so (Van Der MOSSEN, WAYLYARO, 1981; BERTHOULY *et al.*, 1985).

The interesting results obtained on banana at the BIOTROP laboratory (ALVARD *et al.*, 1993) using temporary immersion in a liquid medium have opened up new possibilities for the *in vitro* multiplication of coffee, and the technique should be suitable for both *Coffea* sp. microcuttings and somatic embryogenesis.

2. EQUIPMENT USED

The apparatus used (Figure 1) is a standard autoclavable filtration unit with two compartments. It has been modified by fitting a small glass tube linking the two compartments and a selective mesh screen in the bottom of the upper compartment to hold the explants (microcuttings or calli) whilst letting the medium through.

Figure 1: **Commercial apparatus used at the BIOTROP laboratory (CIRAD, Montpellier) for temporary immersion in a liquid medium**



The explants are thus placed in the upper section and the medium in the bottom section, which is connected up to an air pump, itself linked to a timer so as to push the medium into the upper compartment through the glass tube at predetermined intervals, hence submerging the explants. When the pump switches off, the medium flows back down again due to the effect of gravity.

The immersion time and frequency depend not only on the species considered, but also on the technique used (microcuttings or somatic embryogenesis).

The air flow entering the bottom compartment is sterilized through a 0.22 M hydrophobic filter.

3. MATERIALS AND METHODS

3.1. Microcuttings

The plant material used comes from orthotropic stems already established *in vitro*. The stems are cut into orthotropic micronodes corresponding to the initial explants.

The basic medium used comprises Murashige and Skoog's minerals (MURASHIGE *et al.*, 1962), plus Morel's vitamins (MOREL *et al.*, 1951) and sucrose at 88 Moles. The gel control medium contains 2 g of Phytagel/litre. The pH is adjusted to 5.6.

Different doses of cytokinin (BAP: Benzylaminopurine) and different immersion times depending on the species were tested. The effect of adding gibberellic acid at different times was also studied.

Lastly, the temporary immersion technique was used for root induction and microcutting growth to prepare the *in vitro* plantlets for the acclimatization phase.

3.2. Somatic embryogenesis

Somatic embryogenesis in *Coffea* involves a double medium sequence (BERTHOULY, MICHAUX-FERRIERE, 1995) to produce a highly embryogenic friable callus. This friability enables its use in liquid media:

- either to maintain it at the undifferentiated stage;
- or to regenerate large quantities of somatic embryos.

Until now, these phases were completed in a stirred liquid medium (BERTHOULY *et al.*, 1991; ZAMARRIPA *et al.*, 1991); they can now be carried out using temporary immersion.

The maintenance and regeneration media used are the same as with the stirred liquid medium technique (BERTHOULY, 1991).

4. RESULTS

4.1. Microcuttings

In the case of coffee (*C. arabica* or *C. canephora*), multiplication by microcuttings in a semi-solid medium is of limited value, due to the slow growth of the orthotropic stems. The multiplication rate is around 6 or 7 every 3 months (SONDHAL *et al.*, 1989).

With temporary immersion, this same figure can be achieved after 5 or 6 weeks (Table 1).

In the case of temporary immersion, as nutrient absorption (minerals, growth regulators, etc.) is sporadic, it is clear that immersion time or frequency are of considerable importance.

Table 1: **Effect of immersion time on the *C. arabica* multiplication rate after 6 weeks' culture**

Immersion time	Number of initial explants	Number of micronodes produced	Multiplication rate
1 min/4 times/24 h	60	210	3.5
5 min/4 times/24 h	60	254	5.4
15 min/4 times/24 h	60	504	8.4

Table 1 shows that for a given dose of BAP (1 mg/l), the best results are obtained for an immersion frequency of 15 minutes 4 times in 24 hours.

Shorter times are not sufficiently effective, but prolonged immersion leads to explant vitrification.

However, immersion times and frequency, and the cytokinin dose, change depending on the species considered, as shown in Table 2.

Table 2: **Comparison of the multiplication rates for *C. canephora* and *C. arabica* after 6 weeks' culture**

Species	Number of initial explants	Immersion time and frequency	BAP dose in mg/l	Multiplication rate
C. arabica	60	15 min/4 times/24 h	1 mg	6.8
C. canephora	60	1 min/4 times/24 h	0.5 mg	7.2

Gibberellic acid can also be used for coffee microcuttings, but prolonged use in a gel medium to ensure efficacy often leads to a degree of toxicity. Temporary immersion means that it can be used briefly but just as effectively.

The dose added (0.5 mg/l) during the 5th week, whilst the axillary buds are in full growth, enables good internode elongation without being toxic to the plant (no leaf malformations).

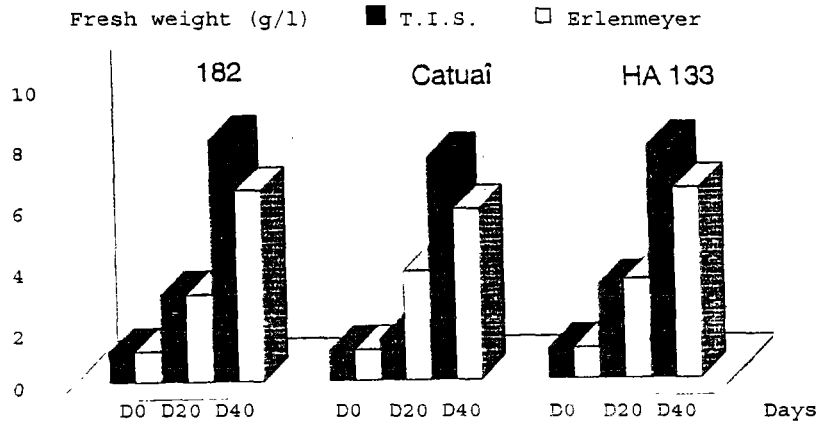
Lastly, the final phase (root induction and growth) can be carried out in the same container. Merely by changing the composition of the medium, root induction can be completed in 24 hours (medium with auxin) and a hormone-free medium can then be used to produce, within 5 weeks, a plantlet with 4 pairs of leaves, ready for direct acclimatization in the nursery.

4.2. Somatic embryogenesis

Somatic embryogenesis in a liquid medium can only be carried out using highly embryogenic friable calli (BERTHOULY, 1991).

The temporary immersion system makes it possible to maintain such calli in an undifferentiated state, just as effectively as the stirred liquid medium technique, and to regenerate somatic embryos right up to germination without changing the medium (Figure 2).

Figure 2: **Embryogenic callus growth on a medium in a temporary immersion system (TIS) or Erlenmeyer**



However, modifying the immersion time and frequency affects somatic embryogenesis expression. With four 15-minute immersion periods in 24 hours, the embryogenic cells develop into proembryos and then somatic embryos, eventually giving mature, germinated embryos ready for direct acclimatization in the nursery.

With the same medium, but with 1 minute's immersion in 24 hours, once the proembryo stage is reached, the proembryos break up to produce several other proembryos (4 or 5 each), which develop as far as the torpedo stage and then stop developing. The only way of ensuring continued development up to germination is to return them to four 15-minute immersion periods in 24 hours.

Merely modifying the immersion frequency and time is therefore enough to steer somatic embryogenesis without changing the medium or the container.

5. CONCLUSION

For coffee micropropagation, the results show that temporary immersion in a liquid medium offers several advantages over a semi-solid medium:

- it increases the multiplication rate for microcuttings;
- it improves the quality of *in vitro* plantlets, which are ready for acclimatization;
- it makes it possible to go as far as embryo maturation and germination in the case of somatic embryogenesis;
- as a result, it reduces production costs through reduced handling, medium consumption, etc.

The reasons why the system is more effective are not yet completely known as far as biological mechanisms are concerned, but they are surely linked to physical culture conditions:

- the explants are not in permanent contact with the liquid medium;
- the relative humidity level in the container is excellent and constant;
- the gaseous atmosphere is entirely renewed with each immersion;
- the "toxic substances" rejected into the medium remain in the bottom section of the apparatus;
- cellular sorting takes place each time the material is immersed.

Research has now begun in an attempt to obtain a better understanding of these nutrition and gas exchange phenomena.

The technique is due to be applied on a semi-industrial scale this year in the commercial tissue culture laboratory at the Kawanda coffee research station in Uganda (K.A.R.I.: Kawanda Agricultural Research Institute).

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7. ABSTRACT

In vitro micropropagation of coffee has been carried until now on semi solid medium. *In vitro* culture by the use of temporary immersion technic, developed on several crops in the laboratory of BIOTROP, has opened new prospects for improving micropropagation of coffee: by microcuttings, as well as somatic embryogenesis.

For microcutting orthotopics nodes measured 3-4 mn and possessed one leaf pair. A total of 10 explants was in each culture unit. After 6 weeks, multiplication rate in I.T. is equal to that obtained after 3 months on solid medium. Beside the I.T. system allows use o GA3 during a short period, for stimulation of internode elongation, without being toxic for the explant. The young buds (apex + 1 first node) undergo on auxin treatment (24 h) before transfer to hormonless medium. After 2 months, the plantlets (4 leaf pairs) are then directly acclimatized to nursery conditions.

The friable, highly callus is produced on semi solid medium. It is after transferee to liquid medium either for multiplication, or for regeneration. For several tested genotypes, growth of callus mass in I.T. system was more important than in erlen meyer flasks. This may be due to the fact that in erlen meyer, callus is continuously in contact with toxic substances excreted in the liquid medium (phenols,....). Optimal regeneration and development of somatic embryos in achieved by immersion of one minute per day stop the proembryos development. Under normal light conditions (50 μE), we are able to mature and germinate somatic embryos. At this stage, the embryos can be transferee to the nursery for hardencny into artificial soil substrate.

WATER STRESS EFFECTS ON LEAF TRANSPIRATION AND PHOTOSYNTHESIS OF *COFFEA ARABICA* L. UNDER DIFFERENT IRRADIANCE CONDITIONS

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INTRODUCTION

The genus *Coffea* originally evolved as understory woody species in African tropical forests and exhibit typical characteristics of shade obligatory plants. In some plantation area, the cultivation practices are established to simulate the conditions of its natural habitat, in other area, Brazil, coffee is traditionally planted in full sunlight but in the nursery plants are grown under shade. Exposure to full sunlight increases leaf transpiration and evaporation from the soil, which lead to high frequency of water stress on coffee cultivation. Stomatal closure in *C. arabica* as a result of drought stress was observed by several researchers (Nutman, 1937; Bierhuizen *et al.*, 1969; Kumar and Tieszen, 1980; Meguro and Magalhães, 1983). Soil moisture deficit increased the stomatal resistance cause to reduce leaf transpiration, which increased leaf temperature and leaf internal CO_2 concentration as a result of depressed photosynthesis (Nunes *et al.*, 1968; Bierhuizen *et al.*, 1969).

In the present study, we tried to explain how changes in stomatal movement of *Coffea arabica* leaves with water stress, in response to natural environmental conditions in a greenhouse on a sunny and on a cloudy day.

MATERIALS AND METHODS

Plant materials and growing conditions

One year old coffee plants (*Coffea arabica* L.; cv. Typica) with five to six mature leaf

pairs, established from seeds, were planted in 1/5000*a* Wagner pots containing a mixture of soil and leaf mold (3:1 by volume) with a single plant and maintained in a greenhouse.

We designed three different soil moisture conditions in the pots, well-irrigated, mild and severe water-stressed for about two months in summer after rooting. These soil water regimes were kept by adding the amount of water balanced to the loss of daily transpiration and evaporation from the soil surface measured by weighing a pot every day. Additionally, the recovery of the electric conductivity of the soil in each pot at early-morning was confirmed using a resistance block made of solid gypsum containing two embedded electrodes installed at 10-15 cm below soil surface.

The soil-leaf mold mixture for this experiment had 53% of field capacity (detected as soil water content after 24-h of free drainage) and 16% of temporary wilting point per maximum gravimetric water holding capacity (43.9% per dry weight). In the water-stressed treatments, soil water content per maximum gravimetric water holding capacity was in the range from 40 to 20% and from 20 to 16% (temporary wilting point) for mild and for severe drought conditions. In the well-irrigated treatment, it always over the field capacity because of the sufficient water supply to cause the pots to drain freely after irrigation on everyday.

Field measurements of transpiration and stomatal conductance in situ

Diurnal variations in transpiration rates, stomatal conductance, air temperature and humidity, and photosynthetic active radiation (PAR) were recorded on five coffee plants per soil moisture treatment plot. Transpiration rate and stomatal conductance were measured on the youngest fully expanded leaves attached on the third or fourth node from the stem top using a portable steady-state diffusion porometer (Li-1600, Li-Cor). All measurements were carried out from 09:00 to 17:00 every two hours under typically different climate conditions, on clear sunny days and on cloudy days. We paid attention to neglecting the obtained data for only patchy cloudy day, because the abrupt changes in climate conditions made a complex in response of intact leaves to the environment.

Gas exchange measurements under controlled conditions

Photosynthetic light response curves were measured in an open gas-exchange system as described in the previous paper (Kanechi *et al.* 1987) with a small, temperature controlled leaf assimilation chamber. The most recently fully expanded, attached leaf (second node from the apex), which was enclosed in a carbon assimilation chamber by clamping the petiole with soft rubber, and exposed to various PAR up to 800 μmol/m2/s, provided by metal halide lamps. The measurements were carried out at constant air temperature (25°C), CO_2 concentration of inlet air stream (350 μmol/mol) and VPD less than 5 hPa to desire widely uniform opening of stomata.

RESULTS AND DISCUSSION

Diurnal changes in PAR , VPD and leaf transpiration

Fig. 1 compares the diurnal courses of leaf transpiration rate measured on the three soil moisture plots, as well as the daily variations of PAR and calculated VPD as the pressure

difference between the water vapor in the air outside the leaf and the saturated water vapor at the leaf temperature for the representative sunny and cloudy days of the summer period. The diurnal variations of PAR and VPD were differ between the two typical climate conditions.

For typical sunny days in summer, the diurnal increasing pattern of solar radiation with a maximum on midday produced the similar increase in VPD throughout the day because of the expected increase in leaf temperature and decrease in ambient air relative humidity. The daily course of VPD closely paralleled that of PAR throughout the day. The daily transpiration flux of the well-irrigated plant was about two-times higher than that of the drought plant with a fairly constant value from early morning to mid-afternoon.

For cloudy days, PAR was higher between 09:00 and 13:00 than afternoon and VPD was fairly constant in a range from 10 to 15 hPa during the day. Transpiration rapidly increased after sunrise and reached maximum at 09:00, then decreased steadily during the day. The daily pattern of transpiration changes was emphasized for leaves without soil water stress, resulting in much higher daily transpiration rates in cloudy days than in sunny days. The same trends were shown for leaves with soil water stress, indicating that leaves always had higher transpiration rates on a cloudy day than on a sunny day.

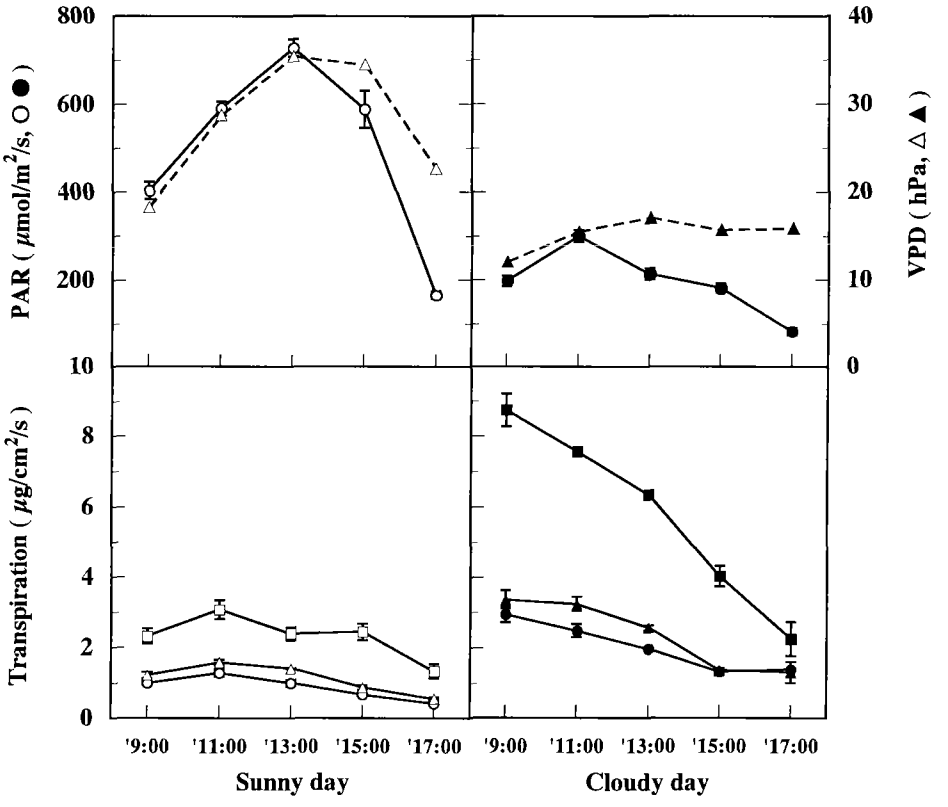

Fig. 1 Diurnal courses of PAR, VPD, and transpiration rate of well-irrigated (□■), mild droughted (△▲), and severe droughted (O●) coffee leaves in a sunny (open symbol) or a cloudy (closed symbol) day. Data recorded for attached leaves using a portable diffusion porometer (LI-1600) at 09:00, 11:00, 13:00, 15:00, and 17:00 under natural daylight conditions in a greenhouse.

Relationships between PAR, leaf temperature, VPD and stomatal conductance

Fig. 2 shows the relationships between PAR, leaf temperature, VPD and stomatal conductance (g_s) for well-irrigated and drought (mild and severe) leaves which were growing in a greenhouse. There were no direct relationships between PAR and g_s for all leaf water status under non-controlled environmental conditions, indicating another direct contributions to changes in leaf transpiration. The response of the drought leaves, with different soil water stress conditions, to the environment was similar, g_s for water-stressed and well-irrigated leaves decreased logarithmically with increasing leaf temperature and with increasing VPD. These decreases were remarkable above 30°C of leaf temperature and over 20 hPa of VPD. There seems to be threshold values of leaf temperature and VPD to close stomata tightly even in the turgid leaves with continuous irrigation. Stomatal conductance and VPD were not linearly related to each other, this indicates a change in water vapor diffusive conductance within the leaf occurred in response not only to changes in leaf temperature but also to changes in atmospheric humidity.

Although many reports showed a close relationship between them under only restrictedly controlled environmental conditions with a constant leaf temperature and low VPD (Kaufmann, 1982; Grantz *et al.*, 1987; Grantz and Meinzer, 1990), these observed relationships between g_s and leaf temperature or VPD show that the diurnal variations in g_s cannot be simply attributed to the influence of PAR.

Gutiérrez and Meinzer (1994) reported that for field grown coffee plants, high PAR, a major determinant of transpiration, reflected in higher transpiration, but low relative humidity may also have contributed to the reduction in transpiration by reducing stomatal conductance. Coffee stomata have previously been reported to exhibit a strong closing response to reduced atmospheric humidity (Fanjul *et al.*, 1985). The values of g_s obtained for cloudy days scattered widely in the ranges from 25 to 30 °C of leaf temperature and from 10 to 20 hPa of VPD. Under these climate conditions, the temperature differences between leaf and air were within about 1°C and relative air humidity was higher than about 70% around the leaves. As a consequence, coffee leaves showed stronger stomatal regulation on a sunny day than on a cloudy day. The detectable results show clearly that g_s changes with changes in the leaf internal factor such as leaf temperature and in the environment such as VPD, and that these responses do affect transpiration and photosynthesis.

Meinzer *et al.* (1990) reported that coffee was able to sustain relatively high levels of leaf gas exchange activity even under severe water deficit, suggesting a coffee's high degree of drought tolerance. Considering our findings in a sensitive regulation of coffee stomata to VPD even without water stress, coffee exhibited inherent high water use efficiency at the leaf gas exchange level, which might restrict yield when water supply was not limiting.

In several species, it has been observed that there was a sensitive response of stomatal movement to VPD, which was derived from the information available on the time course of the relationships between g_s or transpiration and the environmental factors (Sheriff, 1984; Cock *et al.*, 1985; Aphalo and Jarvis, 1991).

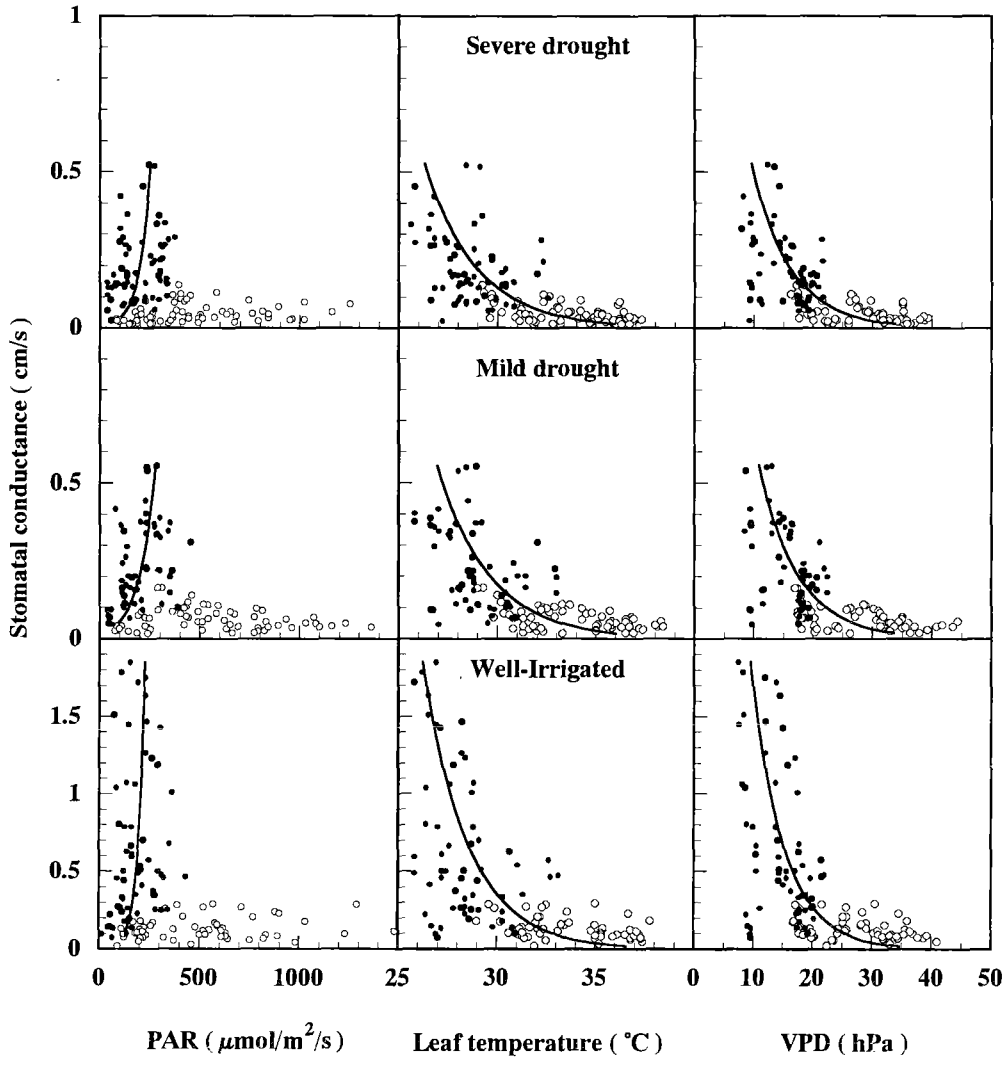

Fig.2 Relationships between PAR, leaf temperature, VPD and stomatal conductance of well-irrigated, mild droughted, and severe droughted coffee leaves in a sunny (O) or a cloudy (●) day. Deta recorded for attached leaves using a portable duffusion porometer (LI-1600) at 09:00, 11:00, 13:00, 15:00, and 17:00 under natural daylight conditions in a greenhouse.

Photosynthetic light response curves

Fig. 3 shows representative responses of intact leaf net photosynthesis with different soil moisture content to various PAR under restrict measurement conditions (leaf temperature varies within 25±1°C and VPD less than 5 hPa). The maximum net photosynthetic rate, which was obtained at saturate PAR compared to the sunny day, decreased due to water stress to about half of that for well-irrigated leaves. The similar 50% decrease in the net photosynthetic rate due to water stress was observed at lower PAR of 200-300 μmol/m2/s compared to the cloudy day. The shapes of the response curves were similar within water-stressed leaves (mild and severe). The net photosynthesis of leaves with wide open stomata increased with increasing PAR in the hyperbola response, in which maximum net photosynthetic rate reached at higher level of PAR. In the high-light environment of sunny days, the observed low g_s seemed to be a primary limiting factor of the photosynthetic carbon gain, which would probably inhibit photosynthesis due to a reduced CO_2 diffusion into leaf. The same situation occurred even in well-irrigated plants, with very low g_s less than one-third of those obtained for cloudy days, which might reduce net photosynthesis very much. The stomatal conductance increased sharply at lower VPD below 20 hPa in well-irrigated plants, this indicates the wide open stomata reflecting a obtained maximum net photosynthesis at high PAR prevailing on a sunny day in Fig. 3. Under microclimate conditions around coffee leaves growing in a greenhouse, VPD always exceeds 20 hPa on a sunny day. High sensitivity of stomatal regulation may play a major role in reduction of transpiration at high PAR for even well-irrigated coffee leaves. The sensitive closing response of coffee stomata to an increasing VPD could potentially cause to restricting photosynthesis in a hot sunny day.

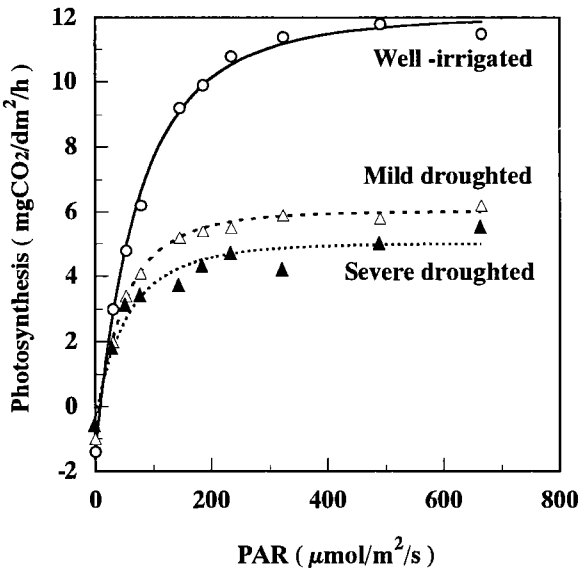

Fig.3 Photosynthetic PAR response curves of well-irrigated (O), mild droughted (△), and severe droughted (▲) coffee leaves measured under controlled environmental conditions : 25 °C, dark to 800 μmol PAR/m^2/s, 350 μmol/mol ambient CO_2 concentration, and VPD < 5 hPa.

ABSTRACT

Coffee trees are growing under typically different light conditions depending on their cultivated areas, shaded with shade trees and unshaded without them. The microclimate surrounding coffee plants become mild under shaded conditions compared with unshaded ones, which means decreases in both leaf temperature and vapor pressure deficit (VPD). We have been attempted to explain effects of chronic soil water depletion on the leaf transpiration and photosynthesis of *Coffea arabica* young plants growing under shaded or clouded and unshaded or sunlit conditions. Both turgid and wilted leaves had higher rates of transpiration on a cloudy day than on a sunny day. There was a positive proportional response of transpiration to light intensity on a clouded day, but sunlit leaves showed no significant increase in transpiration as a light intensity increased under drought conditions. This might be explained by the stomatal regulation in response to VPD which increased due to a raising leaf temperature when leaves were exposed to a direct sunlight.

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ORIGIN AND GENETIC DIVERSITY OF *COFFEA ARABICA* L. BASED ON DNA MOLECULAR MARKERS

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Coffee-trees belong to the tribe *Coffeae* in the family *Rubiaceae* (Bridson and Verdcourt, 1988). The subgenus *Coffea* consists of approximately 100 taxa so far identified in African and Madagascan intertropical forests. *Coffea arabica* L. is both the most widely cultivated species of *Coffea* and the only tetraploid species (2x = 44) in the genus. Arabica coffee has its primary centre of genetic diversity in the highlands of South West Ethiopia and the Boma Plateau of Sudan. Populations of *C. arabica* have been also reported (Berthaud and Charrier, 1988) in Mount Imatong (Sudan) and Mount Marsabit (Kenya). Carvalho (1952) suggested an allotetraploid origin since *C. arabica* presents a diploid meiotic behaviour and a centre of genetic diversity situated outside the distribution area of the diploid coffee species. According to Grassias and Kammacher (1975), and based on cytogenetic observation, *C. arabica* has to be considered as a segmental allotetraploid.

In recent years, DNA-based genetic markers have been developed which offer new potential in analysis of genetic diversity and in elucidating the evolutionary history of plants. In this report, recent results obtained with *C. arabica* are presented.

1-Evaluation of genetic diversity between cultivated and wild accessions of *Coffea arabica* through random amplified polymorphic DNA analysis

The large number of named varieties and selections of arabica coffee belies the actually very narrow genetic diversity of the base populations from which they were selected (van der Vossen, 1985). Historical evidence indicates that these base populations all descended from the few trees that survived various efforts to spread arabica coffee from Southern Arabia, now Yemen, into the main coffee producing areas in Latin America, East Africa and Asia. The coffee trees from Yemen gave rise to two distinct botanical types : 1) *C. arabica* var.

typica Cramer, which was the earliest grown coffee in Asia and Latin America, and 2) *C. arabica* var. *bourbon* (B. Rodr.) Choussy, which came to South America through the island of La Réunion, formerly called Bourbon. The genetic uniformity within these populations is further enhanced by the predominantly self-pollinating nature of *C. arabica*. Enlarging the genetic base has become a priority for further crop improvement and has prompted several collecting missions. In particular, two expeditions to explore and collect arabica coffee materials were undertaken in 1964 to South West Ethiopia under the auspices of the FAO (FAO, 1968), and in 1966 by ORSTOM in the Illubabor and Kaffa provinces of Ethiopia (Guillaumet and Hallé, 1978).

Random amplified polymorphic DNA (RAPD) analysis (Welsh and McClelland, 1990 ; Williams et al., 1990) was performed to estimate the level of genetic diversity within the germplasm collection and the relatedness between cultivated and subspontaneous accessions of *C. arabica*. Six varieties representing the two distinct cultivated coffee types (*typica* and *bourbon*), the cultivar K-7 resulting from a selection work in Kenya (Walyaro, 1983), 11 samples representing the different collecting sites of the ORSTOM mission in Ethiopia, and two accessions collected in Kenya (Berthaud et al., 1980), were included in this study.

As previously reported (Berthou and Trouslot, 1977 ; Lashermes et al., 1993), a low molecular diversity is detected in *C. arabica*. However, the RAPD method appeared to be effective in resolving genetic variation in arabica coffee and in grouping germplasm.

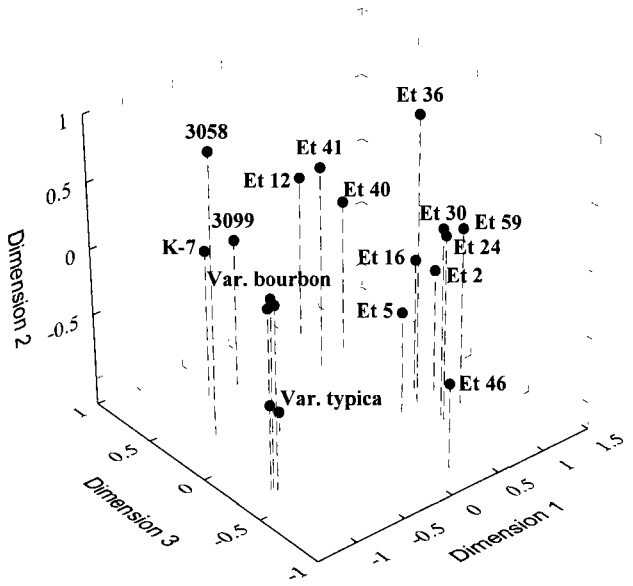

Figure 1. Non-metric multidimensional scaling applied to the matrix of RAPD-based genetic distances between 20 accessions of *C. arabica*.(Var. *bourbon* represents the accessions Caturra, Bourbon, Mbirizi and I-60 ; Var. *typica* represents the accessions Typica and Blue mountain).

This study indicated (Figure 1) a relatively large genetic diversity within the arabica germplasm collection and demonstrated the importance of collecting missions. As expected from their origin, we were not able to distinguished the cultivars belonging to the same type, either *bourbon* or *typica.* On the other hand, both *bourbon* and *typica* types showed important differences. The cultivar K7, which has been grown on a large scale in Kenya (Walyaro, 1983), appeared closely related to one of the accessions collected in the north of Kenya (Marsabit Mountain).

A clear separation was observed between the Ethiopian germplasm collected in the south west highlands of Ethiopia (Illubabor and Kaffa provinces), and the cultivated material spread world-wide from Yemen and the accessions collected in North Kenya. This result supports the hypothesis that the arabica plants transferred to Yemen for cultivation by the Arabs (Smith, 1985) originated from the south eastern part of the evergreen mountainous region of Ethiopia (Sidamo and Harar provinces). An east-west differentiation may exist in the primary centre of diversification of *C. arabica.* Similar observations have been reported from agro-morphological data (Bouharmont and Montagnon, 1995). Such differentiation may explain the large heterosis effect which has been noted in F1 hybrids resulting from crosses between indigenous cultivars from the south western and south eastern parts of Ethiopia (Bayetta-Bellachew et al., 1993), and between spontaneous Ethiopian accessions and *bourbon* type cultivars (Charrier, 1978). Therefore, the possibility of employing RAPD-based genetic distance measures for predicting hybrid performance should be considered.

2- Molecular genetic characterisation of *C. arabica*

Phylogenetic relationships inferred from chloroplast DNA variation

The low frequency of structural changes in the chloroplast molecule (cpDNA) together with a conservative rate of sequence evolution (Olmstead and Palmer, 1994) make it an ideal target for plant phylogenetic study. Maternal inheritance of cpDNA in coffee has been established in interspecific hybrids between *C. arabica* and *C. canephora* (4x) and in an intraspecific progeny of *C. canephora* (Berthou et al., 1983 ; Lashermes et al., in preparation).

CpDNA variations have been investigated in 27 coffee taxa representing the main species and undetermined taxa (Cros, 1994). RFLP (restriction fragment length polymorphism) analysis of cpDNA using homeologous probes from lettuce (*Lactuca sativa*) was accomplished. In addition, the sequence of the *trn*L-*trn*F intergenic region was established. The overall chloroplast genome showed a low level of polymorphism while the intergenic sequence (*trn*L-*trn*F) appeared more polymorphic. A phylogenetic analysis (Figure 2) using Wagner parsimony was performed.

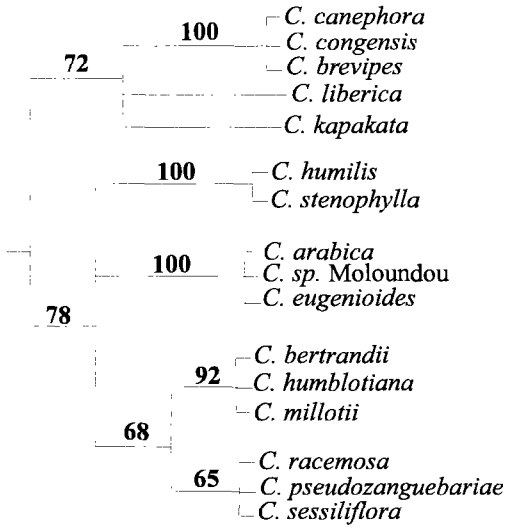

Figure 2. Phylogenetic tree of *Coffea* species based on chloroplast DNA variation. Strict consensus of the most parsimonious Wagner trees is represented. Values (%) on branches are bootstrap indices of support.

Several clades are revealed which are to some extent consistent with the classical biogeographical grouping (i.e. Madagascar, East Africa, West Africa). Results confirmed a monophyletic origin of *Coffea* species. CpDNA from *C. arabica* appeared similar to cpDNA from *C. eugenioides* and *C.sp.* Moloundou, suggesting that *C. arabica* could have diverged maternally from a species related to those species. Chloroplast genomes from *C. canephora* and *C congensis* were found to be identical, as previously reported by Berthou et al. (1983) following a total cpDNA RFLP analysis.

RFLP analysis using single-copy nuclear probes

A study was conducted to determine relationships among a series of *Coffea* species including *C. arabica* by comparing restriction fragment patterns.

Table 1. Distances (complement of the Jaccard index) between *C. arabica* and a representative panel of diploid *Coffea* species based on RFLP data obtained using nine nuclear single-copy probes.

Species	Distribution area	Distance to *C. arabica*
C. congensis	West and Central Africa	0.70
C. eugenioides	Central Africa	0.74
C. canephora	West and Central Africa	0.77
C. humilis	West Africa	0.77
C. sp. X	Unknown	0.80
C. sp. Moloundou	Central Africa	0.81
C. brevipes	Central Africa	0.87
C. kapakata	Central Africa	0.87
C. liberica	West and Central Africa	0.88
C. salvatrix	East Africa	0.88
C. stenophylla	West Africa	0.89
C. racemosa	East Africa	0.95
C. farafanganensis	Madagascar	1
C. humblotiana	Comores islands	1
C. millotii	Madagascar	1
C. pseudozanguebariae	East Africa	1

Probes from nuclear genomic arabica and arabusta libraries were selected to be single-copy using doubled haploid genotypes of *C. canephora*. RFLP-based distances between *C. arabica* and a large number of species were estimated (Table 1). When several accessions from the same species were analysed, the average distance is reported. *C. congensis*, *C.canephora* and *C. eugenioides* seemed to be the closest species to *C. arabica*. All distance values were higher than the expected one if *C. arabica* was an autotetraploid resulting from the duplication of one of the diploid species studied.

Nuclear ribosomal DNA sequence analysis

Among nuclear gene regions, the rDNA repeat unit is attractive for phylogeny reconstruction and genetic studies because of its ubiquity in all organisms, rapid concerted evolution, and the diverse rates of evolution observed within and among component subunits and spacers (reviewed in Jorgansen and Cluster, 1988). The internal transcribed spacer region ITS2 of 18-26S nuclear ribosomal DNA was sequenced for a number of *Coffea* species, including two genotypes of *C. arabica* (Caturra and Et 12).

No evidence of ITS length variants or major sequence variants within arabica accessions was found. *C. arabica* genotypes showed only one type of sequence although important ITS2 nucleotide sequence variations were observed between species.

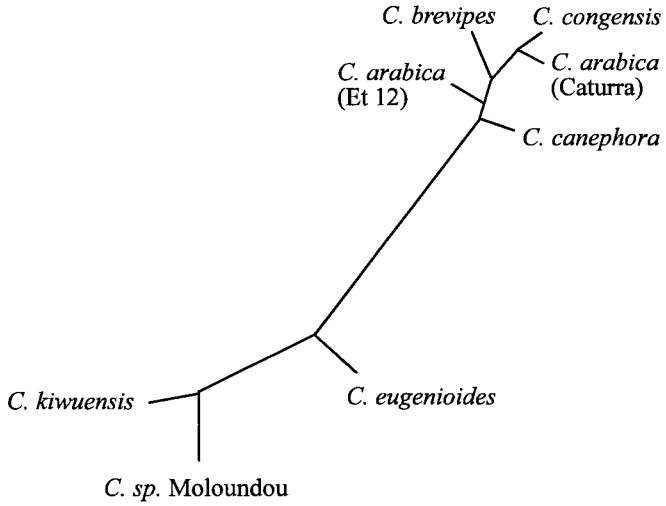

Figure 3. Parsimony analysis of ITS2 sequences of nuclear ribosomal DNA among *Coffea* species as putative ancestors of *C. arabica*. Branch lengths correspond to numbers of informative mutations.

Analysis for a restricted number of species showed (Figure 3) that the ITS2 region of *C. arabica* diverged markedly from the sequences of *C. eugenioides* and its sister-group (*C. kiwuensis* and *C. sp.* Moloundou), and appeared almost identical to the sequences of canephoroid species (*C. canephora*, *C. congensis* and *C. brevipes*).

3- Discussion of the origin of *C. arabica*

Earlier attempts to determine the genetic origin of *C. arabica* relied on analysis of meiotic behaviour of *C. arabica*, karyotyping (Bouharmont, 1959), chromosome pairing in hybrids with diploid species (Krug and Mendes, 1940 ; Kammacher and Capot, 1972) and in dihaploid plants of *C. arabica* (Vishveshwara, 1960 ; Berthaud, 1976 ; Kammacher, 1980). These studies have revealed marked chromosome affinity and the absence of substantial chromosome differentiation between the two constitutive genomes of *C. arabica*, and between *C. arabica* and the diploid *Coffea* species. The normal diploid behaviour of *C. arabica* is thought to be due to a genetic system (Grassias and Kammacher, 1975). Investigation of the origin of *C. arabica* can be based on the results of the different DNA sequence evolution studies.

The allotetraploid origin of *C. arabica* is corroborated by the extent of polymorphism observed by RFLP. In addition, hypothese involving intergeneric combination or association of distant *Coffea* species are improbable. Work on the chloroplast genome strongly supports the notion that a species close to *C. eugenioides* donated the maternal genome of *C. arabica*. Analysis of rDNA showed that the paternal parent was a species from the canephoroid group *(C. canephora, C. congensis)*. The very low divergence between ITS2 sequences of canephoroid species and *C. arabica*, as well as the similarity of the chloroplastic *trn*L-*trn*F intergenic sequences from *C. arabica*, *C. eugenioides* and *C. sp.* Moloundou, clearly indicate that formation and speciation of *C. arabica* are recent events and most likely occurred during the late quaternary period. Information on the origin of *C. arabica* is summarised in Figure 4.

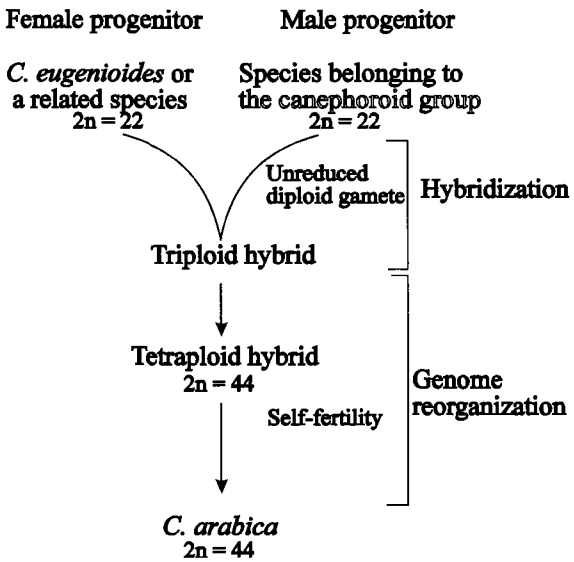

Figure 4. Proposed mode of speciation for *Coffea arabica*

Demarly (1975) has proposed sequences of events leading to the formation of *C. arabica*, such as unreduced gamete formation, breakdown of the self-incompatibility system, and adaptation to new habitats. These steps have little to support them and the described mode of speciation should be considered as one possibility. In particular, a mode of speciation involving association of unreduced gametes from both parental species and direct formation of a tetraploid hybrid, cannot be discarded.

C. arabica did not exhibit additivity of the ITS2 sequences of putative progenitors suggesting that homogenisation of rDNA or the elimination of a locus may have occurred. Similar results were observed for several RFLP loci (data not shown). Genome recombination could have been important among the various mechanisms which are likely to have played a major role in the progressive diploidisation from the archetype tetraploid to the present amphidiploid *C. arabica*. It seems evident that low, continued natural selection was necessary. The degree to which the ancestral genomes have recombined in the amphidiploid needs to be established by further experimentation.

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Abstract

Phylogenetic relationships between 25 *Coffea* species, including the only tetraploid species *Coffea arabica*, were inferred using DNA restriction length polymorphisms (RFLP) and DNA sequencing of both nuclear and chloroplastic genomes. The allotetraploid origin of *C. arabica* is corroborated by the extent of polymorphism observed by RFLP. In addition, hypothese involving intergeneric combination or association of distant *Coffea* species are improbable. Work on the chloroplast genome strongly supports the notion that a species close to *C. eugenioides* donated the maternal genome of *C. arabica*. Analysis of rDNA showed that the paternal parent was a species from the canephoroid group *(C. canephora, C. congensis)*. The very low divergence between ITS2 sequences of canephoroid species and *C. arabica*, as well as the similarity of the chloroplastic *trn*L-*trn*F intergenic sequences from *C. arabica*, *C. eugenioides* and *C. sp.* Moloundou, clearly indicate that formation and speciation of *C. arabica* are recent events and most likely occurred during the late quaternary period.

In addition, 20 coffee accessions of *C. arabica* representing the major types of cultivar and sub-spontaneous genotypes collected in Ethiopia were compared by RAPD (random amplified polymorphic DNA) analysis. This method appeared to be effective in resolving genetic variation in arabica coffee and grouping germplasm. An east-west differentiation may exist in the primary centre of diversification of *C. arabica*.

Résumé

Les relations phylogénétiques de 25 espèces de caféiers dont l'espèce tétraploïde *C. arabica* ont été abordées au niveau de leur ADN génomique nucléaire et chloroplastique par l'utilisation de la variation de la longueur des fragments de restriction (RFLP) et la comparaison de leur séquences. L'origine allotétraploïde de *C. arabica* est supportée par l'importance du polymorphisme RFLP. L'étude du génome chloroplastique indique *C. eugenioides* ou une espèce proche de *C. eugenioides* comme parent femelle de *C. arabica*. L'étude de l'unité nucléaire codant pour l'ARN ribosomique conduit à une espèce du groupe des canephoroides (*C. canephora, C. congensis, C. brevipes*) comme parent mâle. L'origine de *C. arabica* apparaît comme un événement récent de la fin du quaternaire et les conditions de sa formation sont en discussion.

De plus, 22 souches de *C. arabica* représentant les 2 grands groupes de cultivars - Typica et Bourbon - et des caféiers subspontanés collectés en Ethiopie ont été comparées par l'analyse du polymorphisme obtenu après amplification de fragments aléatoires d'ADN (RAPD). Elle met clairement en évidence une différenciation Est-Ouest dans le centre primaire de diversification en Ethiopie.

CLONAL PROPAGATION THROUGH SOMATIC EMBRYOGENESIS OF *COFFEA* SPECIES

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Introduction

Coffee is a perennial crop that is grown commercially in tropical areas. Various attempts at application of in vitro techniques for improvement of coffee have been made. Plant regeneration via tissue culture should be very effective for propagation and improvement of coffee plants. Staritsky (1970) reported development of embryos and plantlets on callus tissue derived from internode explants of *C. canephora* . Sondahl and Sharp (1977; 1979) established conditions for formation of somatic embryos from auxin-induced leaf callus of *C. arabica* using media with different combinations of auxins and cytokinins. Neuenschwander and Baumann (1992) described a protocol for somatic embryogenesis in liquid culture. Their procedure was very complicated, requiring many different media and multiple subcultures.

Generally, auxins are necessary for the onset of the growth of callus and for the induction of somatic embryogenesis (Ammirato 1983). We have reported that cytokinin is very important and auxins have inhibitory effects to *Coffea* somatic embryogenesis (Yasuda *et al* 1985, Hatanaka *et al* 1991).

We summarize a simplified culture system for somatic embryogenesis from the leaf explants of *Coffea* species using a defined medium containing cytokinin alone as the plant growth regulator and report the preparation of competent embryogenic callus proliferating on 2,4-D medium.

Somatic embryogenesis in *C. arabica*

Young leaves from plagiotropic branches of mature, greenhouse-grown trees of *C. arabica* cv Typica, were disinfested for 15 min in a 1% (v/v) solution of sodium hypochlorite containing a few drops of Tween 20, and then rinsed twice with sterile distilled water. The surface-sterilized leaves were cut into pieces in a 1% (w/v) solution of sodium ascorbate. The explants were then placed on the agar medium(A3 medium in Table 1) containing modified MS nutrients (Murashige and Skoog 1962) and B5 vitamins (Gomborg *et al* 1968) and supplemented with

combinations of 6-benzyladenine (BA) and 1-naphthaleneacetic acid (NAA). Cultures were incubated at 27C under the light (a 14 h photoperiod of 30 μ mol/m^2/s provided by cool-white fluorescent lamps).

Table 1. Composition of modified MS medium

Ingredients	MS (mg/l)	A3 (mg/l)
Macronutrients		
NH_4NO_3	1650	412.5
KNO_3	1900	475.5
$MgSO_4 \cdot 7H_2O$	370	92.5
KH_2PO_4	340	85.0
$CaCl_2 \cdot 2H_2O$	440	110.0
Micronutrients		
H_3BO_3	6.2	3.1
$MnSO_4 \cdot 4H_2O$	22.3	11.15
$ZnSO_4 \cdot 7H_2O$	8.6	4.3
$Na_2MoO_4 \cdot 2H_2O$	0.25	0.125
$CuSO_4 \cdot 5H_2O$	0.025	0.05
KI	0.83	-
$CoCl_2 \cdot 6H_2O$	0.025	-
Fe•Na•EDTA	42.11	21.0
Vitamins (B5)		
Inositol	100	100
Nicotinic acid	1.0	1.0
Pyridoxine HCl	1.0	1.0
Thiamine HCl	10	10
Sucrose	30 g/l	30 g/l
pH	5.7±0.1	5.7±0.1

In cultured with 5 μ M BA, yellowish friable callus was appeared from few points on the edges of the explants after 4 months of culture, and somatic embryos formed 4 weeks after initiation of callus (Table 2). An addition of NAA prevented somatic embryogenesis. Compact calli formed after 4 weeks on NAA containing medium, soon turned brown and stopped to grow. The yellowish friable callus were transferred the new medium containing 5 μ M BA. These calli proliferated and new somatic embryos and plantlets formed on the surface of the callus mass. The cultures consisted of yellowish callus, embryos and browned callus. The yellowish callus, which had embryogenic potential, was subcultured at 4-week intervals on the same medium and it has retained its embryo forming capacity for more than 4 years.

Embryogenic callus initiation needs a long time of culture on cytokinin medium, but, once induced embryogenic callus proliferates with producing a large amount of somatic embryos continuously. Generally, auxins are essential for callus induction and somatic embryogenesis. However, callus cultures grown in the presence of auxins are not suitable for maintaining embryogenic ability, and totipotency is lost soon during subculture. In the case of *arabica* leaf explants, cytokinin is essential on induction and maintenance of embryogenic callus.

Somatic embryos grew to plantlets in same medium. Plantlets 3-5cm high were transplanted in pots containing sandy loam and placed under high humidity condition for acclimatization. After 1-2 weeks, plants were transferred to greenhouse conditions and grew into mature plants which bore fruits after 3 years.

Table 2 Effect of NAA and BA on *Coffea arabica* leaf slices

NAA (μM)	BA	weeks after inoculation 4	8	12	16	20	24	28	embryogenic callus induced slices (%)	somatic embryos
-	0.5	-	b	b	b	b	b	b		
0.05	0.5	-	b	b	b	b	b	b		
0.5	0.5	-	b	b	b	b	b	b		
5.0	0.5	C	b	b	b	b	b	b		
-	5.0	-	-	b	C^+	C^+E^+	C^+E^+	C^+E^+	16/21(76)	185±93
0.05	5.0	-	-	b	b	C^+	C^+E^+	C^+E^+	2/21(9)	
0.5	5.0	C	b	b	b	b	b	b		
5.0	5.0	C	b	b	b	b	b	b		

Coffea arabica leaf slices were cultured on A3 medium with different combinations of NAA and BA, at 27C under the light conditions.
b: browned, C:hard compact callus, C^+:friable callus forming, E^+: somatic embryos

Somatic embryogenesis in *C. canephora*

C. canephora is a commercial crop and is known for its resistance to coffee rust. Since *C. canephora* is self-incompatible, the propagation of productive mature trees has to depend on vegetative propagation cutting or grafting. Clonal propagation system through somatic embryogenesis should be very useful for this crop.

The effect of plant growth regulators on somatic embryogenesis was investigated in leaf cultures of *C. canephora* (Hatanaka et al 1991). The maximum number of somatic embryos were obtained on media that contained only cytokinin as a plant growth regulator. Initially, small globular structures appeared on the cut edge of leaf disc, and the embryoids were observed on the all of cultured discs after 5 weeks of culture. As cytokinin, 2-isopentenyladenine(2iP) (specially at 5 μ M) was the most effective for embryogenesis. BA or kinetin also induced somatic embryos. Additional auxins (NAA, IBA, IAA or 2,4-D) to the medium contained 5 μ M 2iP, inhibited somatic embryogenesis. Auxin was not needed at all and cytokinin was essential factor somatic embryogenesis in leaf cultures of *C. canephora*.

Somatic embryos were formed only at the cut edges of the discs that were contact with the medium. When leaf disc was cultured as half of the disc immersed vertically in the [^{14}C]-BA medium. Radioactivity was detected only at edges of the discs that submerged in the medium. Somatic embryos were formed in the lower half of the cut edges, just same region that radioactivity of [^{14}C]-BA was detected. This indicates that cytokinins. are absorbed from cut edges in contact with medium, and not through the leaf epidermis, and that absorbed cytokinins. remains at the cut edges and are not transported into the leaf tissues(Hatanaka et al 1991).

Somatic Embryogenesis from Protoplasts

Embryogenic calli were collected from the mixture of embryogenic and browned calli and embryos in cytokinin medium. Approximately 15 mg of cells were incubated in 2 ml of an enzyme solution containing 0.2% Pectolyase Y23 , 1% Cellulase Onozuka RS and 0.5 M mannitol on a reciprocal shaker (60 rpm) at 27C. After incubation for 3 hr, the suspension was filtered through a 33 μ m nylon sieve , and protoplasts were sedimented by centrifugation at 100 x g for 3 min. Protoplast pellet was suspended and washed by 0.5 M mannitol solution with 2.5 mM $CaCl_2$. Protoplasts were cultured the medium solidified with 0.3% Gelrite in 35x10 mm plastic petri dishes. Protoplasts were cultured at a final density of 10^5/ml. Dishes were sealed with Sealon film. A3 medium was utilized as the basal medium and was supplemented with 3% (w/v) sucrose, 5É M BA, 10% coconut water (GIBCO) and mannitol (pH 5.7). The optimum concentration of mannitol for isolation of protoplasts was 0.5 M, but a lower level of the osmoticum(0.3M) was required for culture of protoplasts. (Tahara *et al* 1994)

Embryogenic calli were very friable and easily dispersed in the enzyme solution. Protoplasts were relatively small (10-30 μ m in diameter) and densely cytoplasmic. The first division of cells was observed during the third week of culture. Addition of liquid medium without mannitol accelerated growth of colonies. Some colonies produced somatic embryos in the petri dishes after 2 months of culture. Somatic embryos developed into plantlets on the medium, as did the original embryogenic calli.

Somatic embryos were regenerated from protoplasts isolated from embryogenic callus on young leaf explants from mature trees of *C. arabica*. Embryos were regenerated on A3 medium supplemented with 5 μ M BA or 2iP. Somatic embryos developed into intact plants. Sondahl et al. (1980) reported isolation of protoplasts from coffee, and Schopke et al. (1987) succeeded in regeneration of plants from protoplasts of *C. canephora*. Plantlets of *C. arabica* have been regenerated from protoplasts, but required elaborate procedures (Acuna and Pena 1991). The system reported herein is very simple and only one type of medium (A3 medium with 5 μ M BA or 2iP and gelling

agent) is used. This system may be useful for cell fusion and other genetic manipulations.

Embryogenic callus culture

The leaf explants of mature coffee tree (*Coffea arabica* cv. Cattura) cultured on A3 medium with 5 μ M 2-iP, produced yellowish calli that formed somatic embryos. The 2iP-induced and somatic embryo-producing callus was transferred to MS medium with 10 μ M 2,4-D and 0.3% Gelrite. From the 2iP induced callus, yellowish and white calli were proliferated. White callus grew rapidly over the yellow slow-growing callus. These two types of calli were picked up and separately subcultured on the 2,4-D medium. Yellow callus is friable and consists spherical and cytoplasmic dense cells. White callus has elongated and translucent cells. Yellow and white calli were examined the somatic-embryo forming ability by transferred to A3 medium with 5 μ M 2-iP and 0.9% agar, at every transplanting time for subcultures. White callus turned brown and stop to grow after transplanting to 2iP medium, and did not produced somatic embryos. Yellow callus produced somatic embryos vigorously (Figure 1). The potential of producing somatic embryos in the yellowish 2,4-D-callus has been kept more than 6 years.

The yellowish embryogenic callus can be produced by 2,4-D from 2iP induced callus from leaf explants. When the explants of coffee leaves or somatic embryos were cultured on the medium containing 10 μ M 2,4-D, embryogenic callus did not be obtained, while only white translucent non-embryogenic callus proliferated. Even if induced callus included embryogenic cells, rapidly growing white cells should dominated over a few embryogenic cells. When explants are cultured on cytokinin medium, embryogenic callus may be dominant in the callus mass.

The embryogenic callus cultured on 2,4-D is valuable materials for cell breeding by biotechnology and genetic engineering and for study on the mechanism of somatic embryogenesis.

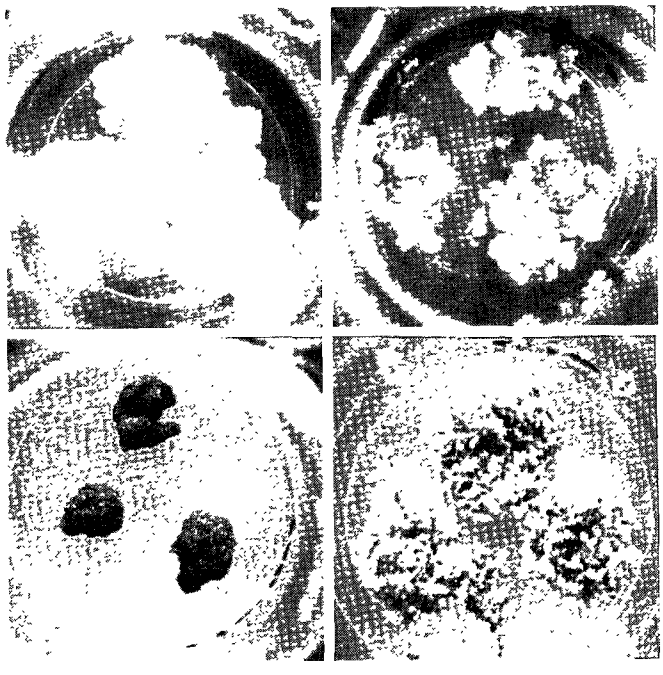

Figure 1 White and Yellow calli in 2,4-D medium. Embryogenic calli induced by 2iP from *C. arabica* leaf explants, were transferred to 10 μ M 2,4-D medium. White and yellow calli were proliferated. Each of them were picked up and subcultured. White (upper left) and Yellow (upper right) callus subcultured 3 years were transferred to 2iP medium. White callus (lower left) turned brown, and yellow callus (lower right) produced somatic embryos.

Conclusion

We established somatic embryogenesis in *Coffea arabica* and *canephora* ; commercially cultivated species, from leaf explants of mature trees using cytokinin as a sole plant hormone. Species; *arabica* and *canephora* react at different ways. In *canephora* , somatic embryos formed from the cut edges of cultured young leaf explants in contact with cytokinin of the medium. Auxins addition to cytokinin inhibited embryo formation. Somatic embryos were grown to young plants on the cytokinin medium. As *canephora* is is self-incompatible, this procedure is very valuable for its propagation. In *arabica* , embryogenic callus were induced after long time culture by cytokinin and then somatic embryo formed on embryogenic callus. Protoplasts of the embryogenic callus also produced somatic embryos.

The embryogenic callus produced by cytokinin were transferred to the 2,4-D medium and by visual selection, yellowish embryogenic callus were obtained. The embryogenic callus proliferating on 2,4-D medium, produced somatic embryos synchronously after transplanting to cytokinin or auxin-free medium. The embryogenic callus has been subcultured over 6 years with keeping embryo forming ability.

These system for somatic embryogenesis is valuable tools for the clonal propagation of *Coffea* species and for the breeding for disease resistant, stress tolerant, low-caffeine coffee *etc.*

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PRESERVATION OF COFFEE SOMATIC EMBRYOS THROUGH DESICCATION AND CRYOPRESERVATION

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INTRODUCTION

In vitro multiplication of coffee can be carried out by mass somatic embryo production in liquid medium (Zamarripa and Ducos, 1991). These somatic embryos are able to develop into plantlets with acceptable conversion frequencies. Coffee somatic embryogenesis can be achieved for various genotypes and the percentage of embryogenic explants depends on both the genotype and the inducing conditions (Zamarripa, 1993).

Somatic embryogenesis presents an evident interest for the clonal propagation of *Coffea canephora* or commercial hybrids of *Coffea arabica*. In order to optimize the advantage offered by the mass production of selected plants via somatic embryos, simple means for easy handling, storage delivery and sowing somatic embryos must be developed.

Moreover, coffee seeds are recalcitrant to traditional seed storage methods. However, Ellis (1990) has reported that some seeds can be stored at 15°C for 1 - 2 years when their water content was of about of 10%. But at the moment no efficient procedure is available for the long-term storage of coffee seeds.

Thus, the development of preservation methods answers two main objectives:

- The handling and delivery of embryos toward the plantations areas,
- The conservation of coffee genetic resources.

The potential techniques are based on either the reduction of the metabolism (deprivation of culture medium, partial dehydration) or the arrest of the metabolism by cryopreservation in liquid nitrogen (LN, -196°C). This latter method has already been envisaged with coffee somatic embryos. The authors obtained various types of success such as regrowth by secondary embryogenesis (Desbrunais *et al*, 1988, Tessereau, 1993) or the alteration of plant development in spite of direct germination of embryos after thawing (Hatanaka *et al*, 1994).

Our objective has been to develop various potential strategies for the preservation of coffee somatic embryos in order to obtain the best efficiency of preservation according to multiplication, diffusion or germplasm collection.

I. PLANT MATERIAL:

- Production of somatic embryos:

The somatic embryos are produced in liquid medium from an embryogenic cell strain of *Coffea canephora* according to the procedure previously described by Zamarripa *et al*, (1991).

Preservation experiments have been carried out using torpedo-shaped somatic embryos of 1.5 to 3.0 mm which have been selected after 8 to 12 weeks of culture.

In order to improve desiccation tolerance, a hardening treament consisting of subcultures of the embryogenic culture in a medium in which the sucrose concentration was progressively increased until the final concentration of 300 g/l was performed.

II. PRESERVATION METHODS :

- Preservation of hydrated embryos: Somatic embryos produced in standard embryogenic medium were isolated and transferred on filter paper supports imbibed with standard embryogenic medium to sterile Petri dishes.The Petri dishes are hermetically sealed and finally stored at 20°C in the dark. After storage, the embryos were directly cultured in standard culture conditions of plantlet development.

- Preservation of dehydrated embryos: Somatic embryos hardened for 5 weeks in liquid medium are directly transferred to environments of various controlled relative humidities (R.H.). For example, the following saturated salt solutions contained in desiccators were used to generate the respective R.H., NaCl, R.H. 75%, K_2CO_3, R.H. 43%.

- Preservation of frozen embryos: Freezing was carried out by direct immersion in liquid nitrogen of cryotubes containing the pre-dried embryos at 24°C for 7 d under 75% RH after hardening according the conditions described above. For recovery, the samples were rapidly thawed by agitation for 2-3 min in a +40°C water bath.

III. RESULTS

- Preservation of hydrated embryos:

Coffee somatic embryos which have been extracted from the culture medium are able to survive when they are placed in mimimal holding conditions. The experiment described in Table 1 shows that the preserved embryos are still able to develop into plantlets with embryo-to-plantlet conversion rates similar to those of the control. However, this rate dramatically decreases during the second month of storage although about 50% of embryos still germinate after 60 d of storage. The viable embryos keep their initial morphology and white color during the preservation phase and no germination appeared during this phase. Coffee somatic embryos are characterized by a low growth rate which seems to reduce the disappearance of the sugar embryo reserves. Thus, embryo death occurs although all the starch and sucrose contents have been consumed. The synchronism of the embryo population development and the relative embryo tolerance to minimal holding conditions are probably linked to the low coffee embryo metabolism and can be used as an advantage for the handling of cultures for these species.

- Preservation of dehydrated embryos:

From the dehydration point of view, coffee somatic embryos present at least two limits: The first is that somatic embryos generally have a low desiccation tolerance and, secondly coffee is naturally desiccation sensitive. The hardening of somatic embryos in the presence of a high sucrose concentration improves desiccation tolerance (Fig.1). Thus, after a 7-day drying period at 24°C under 75% RH, about 90% of dried embryos are able to develop into normal plantlets. Nevertheless, the water content has been decreased from 3.70 g H_2O/g^{-1}d. wt to 1.76 g H_2O/g^{-1}d. wt during the hardening treatment (Fig. 2), then to 0.35 g H_2O/g^{-1}d. wt after the desiccation phase (Fig. 2). Finally, in spite of total water loss of about 90% of the initial water of the

embryos, the germination capacity did not alter.
However, after a severe dehydration at 24°C under 43% RH, the germination ability of the embryos is dramatically affected although these embryos survive the drying treatment and are able to develop plantlets through a secondary embryogenesis process (Fig. 2). The residual water content was about 0.21 g $H_2O/g^{-1}d$. wt which means that 93% of the initial water of the embryos has been eliminated. Finally, the alteration of regenerating capacities and the death of coffee embryos are linked to extremely low variations in the residual water content.
The viability of dried coffee embryos is preserved depending on the relative humidity of storage (Fig. 3). After much more than a month of storage at 15°C, the biological response is qualitatively and quantitatively maintained for a range of RH from 53 to 43%. The medium- or long-term storage seems inefficient under higher RH although the results concerning the lower RH show the sentitivity of coffee embryos to severe desiccation.

- Preservation of frozen somatic embryos:

The freezing of large embryos like coffee somatic embryos is critical concerning the preservation of the germinative capacities during recovery. Somatic embryos hardened using the sucrose treatment described above do not withstand direct immersion in liquid nitrogen (Table 2). However, when these hardened embryos are dehydrated under 75% RH prior to freezing, they withstand direct immersion in LN and, most of them develop into plantlets after thawing (Table 2). The embryo-to-plantlet conversion rates are similar between the control and frozen embryos and plantlets formed from frozen embryos seem vigorous. Finally, freezing techniques based on the evaporation of the water before freezing seem better adapted for large embryos than techniques where a part of the dehydration occurs with the cooling of the specimen.

IV CONCLUSION

The preservation of coffee somatic embryos can be performed for at least three efficient techniques depending on the storage duration :
- The preservation of hydrated embryos and the partial dehydration are efficient to allow short-term conservation of embryos for both handling or delivery toward the plantation areas.
- The cryopreservation in liquid nitrogen is efficient and the extrapolation of this technique to other coffee genotypes is now under investigation. Zygotic embryos of coffee can be frozen using the same technique. The success of the cryopreservation opens the opportinity to develop frozen coffee germplasm bank in order to assume long-term preservation of germplasm.

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Coffee species are characterized by seeds which are short-lived and then exhibit responses intermediate between true chilling and desiccation tolerance and sensitivity. The development of storage methods for zygotic and somatic embryos offers a true opportunity for the conservation of genetic resources of this species. Three techniques have been evaluated with *Coffea canephora* somatic embryos. First, hydrated embryos can be preserved at 20°C for 1 to 2 months. Second, after partial dehydration, embryos can be stored in liquid nitrogen for an indefinite period of time. Frozen embryos are able to directly develop plantlets similar to the control. Moreover, zygotic embryos can been preserved by the same procedure. Finally, coffee embryos can be dehydrated and stored at 15 or 24°C under 43% of relative humidity for at least 1 month. The advantage of these various means of storage for both long-term storage of germplasm and the delivery of selected plants via somatic embryos will be discussed.

CONSERVATION DES EMBRYONS SOMATIQUES DE CAFEIER PAR DESSICCATION ET CRYOCONSERVATION

Le caféier est caractérisé par des semences qui se conservent mal en raison de leur comportement intermédiaire entre tolérance et sensibilité au froid et à la dessiccation. Le développement de méthodes de stockage des embryons zygotiques et somatiques constitue une réelle opportunité pour la conservation des ressources génétiques de cette espèce. Trois modes de stockage des embryons somatiques de *Coffea canephora* ont été évalués. En premier lieu, des embryons hydratés peuvent être maintenus en survie à 20°C entre 1 à 2 mois. Deuxièmement, aprés une déshydratation partielle, des embryons peuvent être stockés dans l'azote liquide pour une durée théoriquement indéfinie. Ces embryons congelés sont capables de développer directement en plantules, de façon similaire aux embryons non préservés. Des embryons zygotiques peuvent également être conservés selon la même méthode. Enfin, des embryons de caféier peuvent être déshydratés et stockés sous une humidité relative de 43% à 15 ou 24°C pendant au moins 1 mois. L'intêret de ces différents méthodes pour la conservation à long terme des ressources génétiques et la diffusion de plantes sélectionnées via les embryons somatiques sera discuté.

TABLE 1: *EFFECT OF HOLDING TIME AT 20°C ON THE VIABILITY OF HYDRATED SOMATIC EMBRYOS OF COFFEA CANEPHORA*

DURATION (Days)	SURVIVAL (%)	GERMINATION (%)	EMBRYO TO PLANTLET CONVERSION (%)
0	97 ± 3	50 ± 5	38 ± 3
15	100	89 ± 5	39 ± 7
30	100	86 ± 8	41 ± 9
45	93 ± 7	84 ± 4	28 ± 6
60	51 ± 9	44 ± 13	12 ± 10

Table 2: CRYOPRESERVATION OF SOMATIC EMBRYOS PRODUCED AND HARDENED IN LIQUID MEDIUM

	SURVIVAL (%)	GERMINATION (%)	EMBRYO TO PLANTLET CONVERSION (%)
CONTROL	100	50 ± 6	42 ± 2
HARDENED	100	97 ± 2	38 ± 14
HARDENED + LN	0	0	0
HARDENED + 75% RH + LN	96 ± 3	67 ± 13	31 ± 3

- HARDENING TREATMENT = SUCROSE 300 G/L
- DURATION OF DRYING = 7 d at 24°C
- LN = Liquid Nitrogen

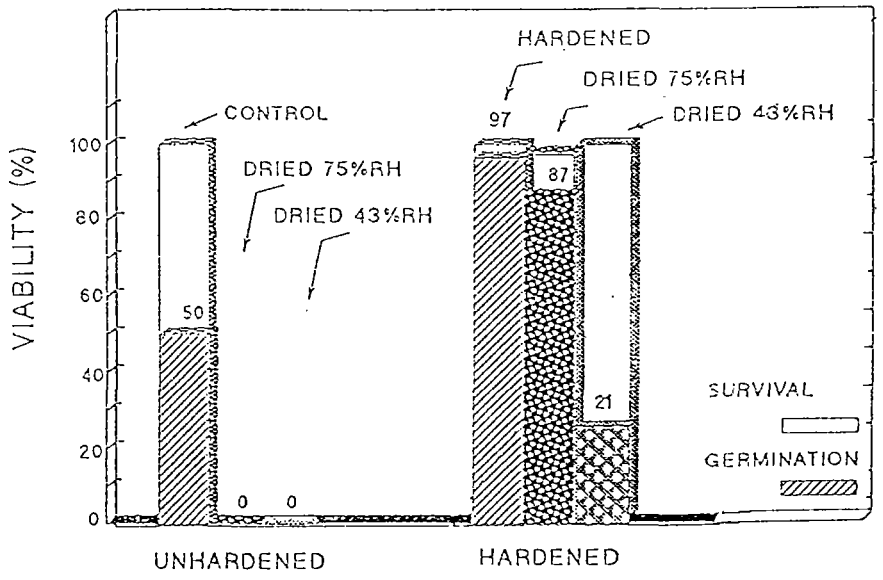

Fig.1 EFFECT OF ~~HARDENING~~ TREATMENT ON THE VIABILITY OF ~~DRIED~~ COFFEE EMBRYOS AFTER 7 DAYS OF STORAGE AT 24°C.

HARDENING TREATMENT = SUCROSE 300G/L

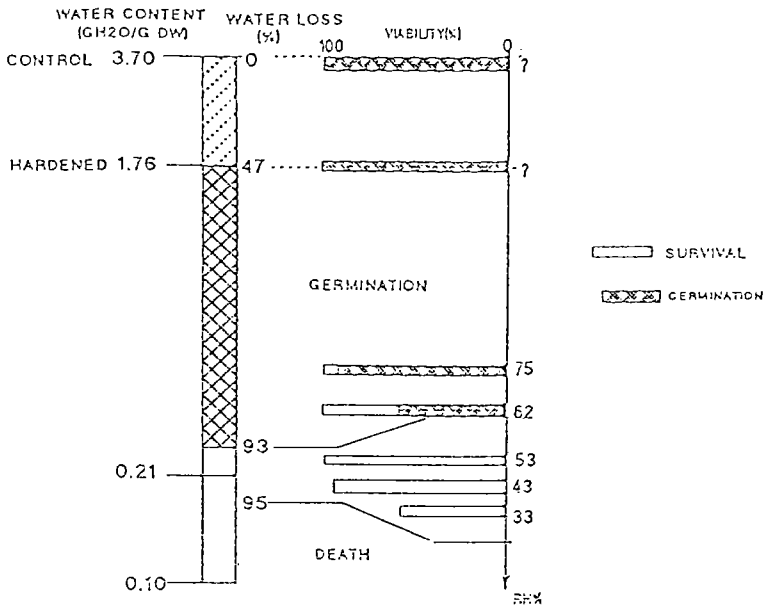

Fig. 2 *DESICCATION OF COFFEE SOMATIC EMBRYOS*
ASSESSEMENT OF WATER LOSS AFTER 7 DAYS UNDER 43% RH AT(24 °C

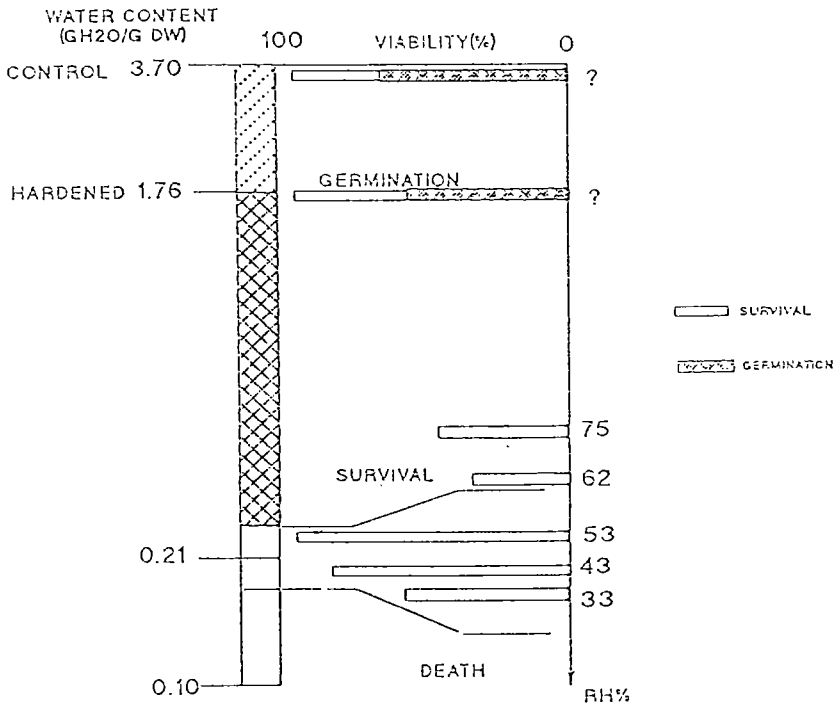

Fig. 3 *STORAGE OF DRIED COFFEE EMBRYOS 40 DAYS UNDER VARIOUS RH% at 15°C*

NEW DIMENSIONS IN UNDERSTANDING INHERITANCE OF COFFEE RUST RESISTANCE : A MENDELIAN PERSPECTIVE

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SUMMARY

Literature on inheritance of resistance to coffee leaf rust is re-examined and lacunae in understanding the mechanism of inheritance are identified as lack of sound genetic assumptions and distorted genetic analysis on the basis of manifestation of resistance or susceptibility rather than actual genotype testing. Assumptions for genetic analysis of inheritance of resistance are laid down and their importance highlighted. The credibility and viability of the suggested model is elucidated by applying it to some of the already published results. The possibility of hitherto unrecorded genotypes of considerable breeding value residing in the progenies of differentials A-, H- and G-types is indicated. Incomplete resistance is indicated to be a Mendelian phenomenon and a manifestation of gene dose effect rather than a quantitative trait.

INTRODUCTION

Inheritance of leaf rust resistance in coffee is one of the most confused research topics as can be seen from the literature on the subject which was well reviewed (Rodrigues *et al.*,1975; Eskes and Toma-Braghini, 1981; Eskes and Carvalho, 1983; Eskes, 1989). Even the most recent comprehensive treatise on rust did not explain

inheritance of resistance in a genetically understandable perspective (Kushalappa and Eskes, 1989). This situation appears to be a result of the general practice of assigning genotypes to coffee plants on the basis of their reaction to a variety of rust races (Bettencourt and Noronha-Wagner, 1971; Bettencourt *et al.*, 1980) rather than on sound genetic assumptions and testing. In this study an attempt is made to explain some of the observations recorded in literature in a Mendelian perspective which appears to give a satisfactory answer to many points of confusion.

The Assumptions

A basic set of assumptions to explain the observations was not laid down in coffee rust research with particular reference to inheritance of resistance. This is essential in view of the genetic fidelity of the higher plants and relative flexibility of fungi where somatic and parasexual recombinations are more frequent. Thus, for the coffee-leaf rust host-pathogen system, the following assumptions are laid down.

1. Coffee plant (*Coffea arabica* L) is an allotetraploid which can carry four allelomorphs (2 pairs) of any given gene, including S_H genes conditioning rust resistance.
2. The genotypes of various physiologic types need a redefinition in the light of above. Thus, genetic descriptions of all arabicas should include four allelic elements and dosage detail of individual alleles.
3. Leaf rust fungus (*Hemileia vastatrix* B. & Br.) is an heteroecious rust whose sexual phase is not known. Thus, the appearance of new biotypes of this fungus which infect the 'resistant' coffee types rendering them susceptible could be either due to mutation or heterokaryosis and somatic recombination. The latter situation allows for the accumulation of virulence genes/gene combinations without restriction (Horsfall and Dimond, 1960).
4. In the coffee-leaf rust system, leaf rust is in an advantageous position because every propagule produced by the fungus is subject to Natural selection leading to quick appearance of fit individuals which are new races. Coffee plant is at a relative disadvantage,because of the limitations on breeders' capacity to test a sufficiently large number of recombinants, and secondly,because any adapted cultivar produces crops over a long period making uniform material available for disease attacks.
5. Resistance classification into four classes- Resistant, Moderately resistant, Moderately susceptible and Susceptible (Rodrigues *et al.*, 1975; Eskes, 1989) is too vague to be a basis

for Mendelian interpretation. However, this can be a basis of incomplete resistance (Eskes, 1983) which also needs to be understood in a new dimension.

The Data

Primary data on the observations of resistance of coffee cultivars were published from CIFC, Portugal (Anonymous, 1965; Betten-court *et al.*, 1980,1992), IAC, Brazil (Eskes, 1983, 1989) and CCRI, India (Srinivasan and Narasimhaswamy, 1975; Ramachandran and Srinivasan, 1979). These publications formed the background data. The data states...

-that in *C. arabica* four dominant genes S_H1, S_H2, S_H4 and S_H5 control rust resistance.
-that S_H3 was added from *C. liberica*.
-that S_H genes exist in a multiple allelic series and manifest complementary gene action.
-that Hibrido de Timor (HDT) A-differential possesses resistance genes $S_H6,7,8,9$ all of which had their origin in *C. canephora*.
-that the genes S_H1 to S_H9 condition race-specific or vertical resistance.
-that horizontal or race non-specific resistance also exists in the gene pool of coffee.

The New Hypothesis

The new hypothesis envisages that leaf rust resistance is controlled by Mendelian genes. Mendelian tables were generated for the data recorded in literature. Appearance of segregant reaction groups is attributed to the recombination of S_H factors. The new theory strongly advocates the use of deductive genetic reasoning to understand the manifested reaction spectra in coffee progenies. Thus, physiologic type H- can segregate into H- and G-types or H-, G- and D-types or H-, G-, D- and E-types, depending on the parental genotype. Checkerboard (Table 1) indicates the possible derivation of genotypes $S_H(2,3)_2$, $S_H(2)_4$ and $S_H(3)_4$ which may have significance in breeding programmes. Ramachandran and Srinivasan (1979) reported the segregation of S.795 (H-type) into H- and D-types in a monohybrid ratio. It is possible that they are examining progenies of bulk pedigree descent where a large amount of sib-mating is usual. Their results are based on testing against race-I in which distinguishing H- and G-types is not possible. However, segregation of H- into H-, G-, D- and E-types is recorded at CIFC (Table 1). Similarly segregation of G-type giving rise to a large proportion of maternal types (Srinivasan and Narasimhaswamy, 1975) is also explained as a strict Mendelian dihybrid ratio (Table 2).

Hibrido de Timor and Derivatives

Hibrido de Timor is a putative spontaneous hybrid of *C. arabica* and *C. canephora*, known for its manifested highest vertical resistance. Recently, Bettencourt *et al.* (1992) identified the genotype of this differential (A) as S_H6,7,8,9. This is also shown to be a good general combiner with other arabicas (Sreenivasan and Ram, 1993; Bayetta-Bellachew *et al.*,1993). These qualifications of HDT made it very important in the World Coffee Breeding Programmes (Anonymous, 1965). Thus an analysis of the resistance of this material is very relevant. Since HDT-A is a quadruplex heterozygote random recombination of S_H factors results in the formation of six different genotypes of gametes. Their random union results in a wide array of parental and recombinant genotypes (Table 3). It can be seen that true A-type plants in any S_1 progeny of HDT-A can be of the order of 1/6. It is possible that the entire progeny may manifest resistance as rust races with appropriate virulence gene combinations are not known so far. An important objective of coffee breeding programmes is to shorten the gestation cycle leading to early yields and deriving dwarf/semi-dwarf types suitable for high density planting (Carvalho, 1988). Use of dominant mutants Caturra (Ct), San Ramon (SR) and Villasarchi (Vs) to derive such types is a significant development of recent times. These mutants of Bourbon are homozygous for S_H5 and are highly susceptible to leaf rust. However, the Catimor hybrids manifested high resistance. Dilution of resistance was observed under bulk pedigree selection and was attributed to the influence of Natural selection which appears to favour the archaic arabicoid factor S_H5 (Sreenivasan *et al.*,1994).A point of import is that resistance type-A is not possible in Catimor progenies which tend to manifest increased susceptibility as indicated in tables 4,5 and 6.

Incomplete Resistance

From the foregoing discussion, one point that emerges is that *C. arabica* is an allotetraploid and thus carry four genetic elements which can be responsible for the manifestation of resistance. This gives room for the possibility of cryptic genotypic variation within individual physiologic types which forms the basis for the on-coming proposal regarding the heredity of incomplete resistance. S_H5 factor is known to impart near immunity to the coffee plant in combination with other S_H genes indicating complementary gene action (Rijo *et al.*, 1991). Thus, in *C. arabica* S_H5 in various combinations could be responsible for the expression of resistance. Incomplete resistance was proposed to be imparted by the known major genes (Eskes and Carvalho, 1983; Eskes, 1989) as well as non-genetic parameters: lesion density, percent sporulating

lesions and spore production per lesion. Genotypic determination of latency period and leaf retention period is documented (Eskes, 1983,1989).

In the light of S_H5 being capable of imparting near immunity, other major genes imparting incomplete resistance and the complementary gene action, a model for explaining incomplete resistance is concieved. This envisages the dosage of S_H5 and/or other S_H genes to be responsible for the expression of incomplete resistance. Thus, the H-type plants $S_H2,2,3,5$; $S_H2,3,3,5$ and $S_H2,3,5,5$ possibly manifest differences in disease build-up leading to the appearance of resistant, moderately resistant, moderately susceptible and susceptible plants.

Horizontal Resistance

By definition this is a manifestation of disease resistance in the field and is non race-specific. It is known to be present in Rume Sudan collections of *C. arabica*. Residual expression of this resistance is possible in other arabicas also and is possibly the quantitative non-genetic component of incomplete resistance.

In the light of the foregoing discourse on the various types of rust resistance in coffee, the future breeding programmes should include the parents with right combinations of S_H genes to obtain complete/incomplete resistance and possibly those carrying horizontal resistance to afford durability to resistance.

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Table 1. SEGREGATION SPECTRUM OF PHYSIOLOGIC TYPE <H>

Segregation of $S_H2,3,5,5$
Possible Gametic Genotypes: $S_H2,3$; $S_H2,5$; $S_H3,5$; $S_H5,5$
Checkerboard of random recombinants

	$S_H2,3$	$S_H2,5$	$S_H3,5$	$S_H5,5$
$S_H2,3$	$S_H2,2,3,3$	$S_H2,2,3,5$	$S_H2,3,3,5$	$S_H2,3,5,5$
$S_H2,5$	$S_H2,2,3,5$	$S_H2,2,5,5$	$S_H2,3,5,5$	$S_H2,5,5,5$
$S_H3,5$	$S_H2,3,3,5$	$S_H2,3,5,5$	$S_H3,3,5,5$	$S_H3,5,5,5$
$S_H5,5$	$S_H2,3,5,5$	$S_H2,5,5,5$	$S_H3,5,5,5$	$S_H5,5,5,5$

H-type = 8 (50%); G-type = 3 (18.75%); Unknown type = 1 (6.25%)
These three constitute 75% of progeny resistant to race I
D-type = 3 (18.75%); E-type = 1 (6.25%)
These two constitute 25% of progeny susceptible to race I

Table 2. SEGREGATION SPECTRUM OF PHYSIOLOGIC TYPE <G>

Segregation of $S_H3,3,5,5$
Possible Gametic genotypes : $S_H3,3$ (25%); $S_H3,5$ (50%); $S_H5,5$ (25%)
and their frequencies
Checkerboard of random recombinants

	$S_H3,3$	$S_H3,5$	$S_H5,5$
$S_H3,3$	$S_H3,3,3,3$	$S_H3,3,3,5$	$S_H3,3,5,5$
$S_H3,5$	$S_H3,3,3,5$	$S_H3,3,5,5$	$S_H3,5,5,5$
$S_H5,5$	$S_H3,3,5,5$	$S_H3,5,5,5$	$S_H5,5,5,5$

G-type = 14 (87.5%), Unknown type = 1 (6.25%)
These constitute 93.75% of progeny resistant to races I & II
E-type = 1 (6.25%)
This constitutes 6.25% of progeny susceptible to races I & II

Table 3. SEGREGATION SPECTRUM OF PHYSIOLOGIC TYPE <A>

Segregation of S_H6,7,8,9
Possible Gametic Genotypes: S_H6,7; S_H6,8; S_H6,9; S_H7,8; S_H7,9; S_H8,9
Checkerboard of random recombinants

	S_H6,7	S_H6,8	S_H6,9	S_H7,8	S_H7,9	S_H8,9
S_H6,7	S_H6,6,7,7	S_H6,6,7,8	S_H6,6,7,9	S_H6,6,7,8	S_H6,7,7,9	S_H6,7,8,9
S_H6,8	S_H6,6,7,8	S_H6,6,8,8	S_H6,6,8,9	S_H6,7,8,8	S_H6,7,8,9	S_H6,8,8,9
S_H6,9	S_H6,6,7,9	S_H6,6,8,9	S_H6,6,9,9	S_H6,7,8,9	S_H6,7,9,9	S_H6,8,9,9
S_H7,8	S_H6,7,7,8	S_H6,7,8,8	S_H6,7,8,9	S_H7,7,8,8	S_H7,7,8,9	S_H7,8,8,9
S_H7,9	S_H6,7,7,9	S_H6,7,8,9	S_H6,7,9,9	S_H7,7,8,9	S_H7,7,9,9	S_H7,8,9,9
S_H8,9	S_H6,7,8,9	S_H6,8,8,9	S_H6,8,9,9	S_H7,8,8,9	S_H7,8,9,9	S_H8,8,9,9

This progeny remains largely resistant as rust races with requisite virulence gene combinations are not known.

Table 4. Origin of Catimor

Parents: Caturra (S_H5,5,5,5)
HDT (S_H6,7,8,9)

	S_H6,7	S_H6,8	S_H6,9	S_H7,8	S_H7,9	S_H8,9
S_H5,5	S_H5,5,6,7	S_H5,5,6,8	S_H5,5,6,9	S_H5,5,7,8	S_H5,5,7,9	S_H5,5,8,9

Table 5. Possible Segregation in Catimor

Segregation of Genotype $S_H5,5,6,7$ under inbreeding.
Possible Gametic Genotypes: $S_H5,5$; $S_H5,6$; $S_H5,7$; $S_H6,7$
(16.6%)(33.2%)(33.2%)(16.6%)

	$S_H5,5$	$S_H5,6$	$S_H5,7$	$S_H6,7$
$S_H5,5$	$S_H5,5,5,5$	$S_H5,5,5,6$	$S_H5,5,5,7$	$S_H5,5,6,7$
$S_H5,6$	$S_H5,5,5,6$	$S_H5,5,6,6$	$S_H5,5,6,7$	$S_H5,6,6,7$
$S_H5,7$	$S_H5,5,5,7$	$S_H5,5,6,7$	$S_H5,5,7,7$	$S_H5,6,7,7$
$S_H6,7$	$S_H5,5,6,7$	$S_H5,6,6,7$	$S_H5,6,7,7$	$S_H6,6,7,7$

Table 6. Segregation of Catimor

Interbreeding of genotypes $S_H5,5,6,7$ x $S_H5,5,8,9$
Gametic genotypes: $S_H5,5$; $S_H5,6$; $S_H5,7$; $S_H6,7$ and
$S_H5,5$; $S_H5,8$; $S_H5,9$; $S_H8,9$

	$S_H5,5$	$S_H5,6$	$S_H5,7$	$S_H6,7$
$S_H5,5$	$S_H5,5,5,5$	$S_H5,5,5,6$	$S_H5,5,5,7$	$S_H5,5,6,7$
$S_H5,8$	$S_H5,5,5,8$	$S_H5,5,6,8$	$S_H5,5,7,8$	$S_H5,6,7,8$
$S_H5,9$	$S_H5,5,5,9$	$S_H5,5,6,9$	$S_H5,5,7,9$	$S_H5,6,7,9$
$S_H8,9$	$S_H5,5,8,9$	$S_H5,6,8,9$	$S_H5,7,8,9$	$S_H6,7,8,9$

Origin and possible segregations of Catimor shown in the above tables indicate the gradual increase of the frequency of S_H5 factor in these lines with advancing generations either under inbreeding or inter breeding of sibs.

RÉSULTATS RÉCENTS SUR L'AMÉLIORATION GÉNÉTIQUE DE LA QUALITÉ À LA TASSE DE *COFFEA CANEPHORA* EN CÔTE D'IVOIRE

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Introduction

Le café produit par *Coffea canephora*, commercialement appelé Robusta, souffre d'une réputation de mauvaise qualité organoleptique par rapport au café produit par *C. arabica*. Ce dernier est souvent jugé plus doux et plus aromatique, présente une plus forte granulométrie et une plus faible teneur en caféine. Ceci se traduit au niveau du marché mondial par un plus faible prix payé aux producteurs de Robusta.
L'amélioration de la qualité du café par les méthodes technologiques a fait l'objet de nombreuses recherches. Il est établi que la qualité à la tasse dépend du mode de préparation (voie humide / voie sèche), du degré de maturation des cerises et de l'origine géographique, en particulier l'altitude (Clifford, 1985 ; Vincent *et al*, 1977 ; Carvalho, 1985 ; Clarke, 1985).
Très peu de recherches ont cependant porté sur la sélection variétale pour la qualité à la tasse. Pour *C. canephora*, Cramer (1957) avait cependant suggéré l'existence d'une variabilité pour ce caractère. Au Brésil, Teixeira *et al* (1979) ont observé des différences entre génotypes de *C. canephora* dont certains atteignaient le niveau du standard Arabica brésilien. L'absence de répétition interdisait cependant toute analyse statistique. Au niveau des programmes de sélection, et dans le meilleur des cas, la qualité "acceptable" des nouvelles variétés est testée (Charrier et Berthaud, 1985). Il a semblé nécessaire aux sélectionneurs travaillant en Côte d'Ivoire d'étudier les possibilités d'amélioration variétale de *C. canephora* pour la qualité à la tasse (Leroy *et al*, 1992 ; Moschetto *et al*, 1994). Cette communication décrit les principaux résultats obtenus sur ce sujet depuis le début des années 90.

I EST-IL INTERESSANT DE SE LANCER DANS L'AMELIORATION VARIETALE DU ROBUSTA POUR LA QUALITE A LA TASSE ?

On pressent facilement que l'expression de la qualité à la tasse d'un café relève de phénomènes nombreux et complexes. Parmi les facteurs importants, nous avons retenu le mode de préparation, le lieu de production (terroir) et la période de récolte (précoce ou tardive). Pour savoir si l'amélioration variétale de *C. canephora* pour la qualité à la tasse présentait un intérêt, nous nous sommes posé les questions suivantes :

* Est-il possible de détecter une variabilité au sein de *C. canephora* pour la qualité à la tasse ?

* Que représente cette variabilité par rapport à celle entraînée d'autres facteurs ?

* Existe-t-il des interactions entre le facteur variétal et d'autres facteurs ?

I-1 Détection d'une variabilité au sein de *C. canephora* pour la qualité à la tasse :

Un travail préliminaire a débuté en 1989. Dix clones génétiquement distants d'après les études de Berthaud (1986) ont été évalués pour la qualité à la tasse. Les résultats de ce travail ont été présentés à l'ASIC de San Francisco (Leroy *et al*, 1992). Nous rappelerons ici les principaux résultats.

A la suite de dégustations (ranking avec quatre répétions par clone), il a été possible de séparer les clones en fonction du corps, de l'amertume et de l'acidité. Les clones du groupe guinéen apparaissaient plus corsés et amers que ceux du groupe Congolais. Les hybrides intergroupes présentaient des caractéristiques intermédiaires. Le clone 126, hybride intergroupe, était particulièrement apprécié par les dégustateurs.

Il était donc possible de détecter une variabilité pour la qualité à la tasse au sein de *C. canephora*. Cependant, beaucoup de questions restaient posées. En effet, tous les clones avaient été préparés par la voie humide et provenaient de la station de Divo. Les différences entre clones seraient-elles toujours perceptibles avec une préparation en voie sèche ou dans une station (terroir) différente ? Les classements seraient ils les mêmes ? Une deuxième série d'analyse a donc été planifiée.

I-2 Importance du facteur variétal et existence d'éventuelles interactions :

* *Dispositif expérimental* :

Pour chaque essai étudié ci-après, un dispositif en blocs incomplets équilibrés a été utilisé. A chaque séance de dégustation, trois objets et un témoin constant étaient notés. Pour chaque séance, l'ordre (Or) de présentation des objets différait d'un dégustateur à l'autre pour contrôler l'effet de rang. Le témoin était un Robusta du commerce.

- Intensité de l'arôme (in)
- Qualité de l'arôme (qu ou ar)
- Corps (co)
- Acidité (ac)
- Amertume (am)
- Astringence (as)
- Note globale de préférence (pr)

Les données ont été traitées en Analyse Factorielle des Correspondances (AFC). Chaque variable posséde trois modalités identifiés de 1 à 3 dans l'ordre croissant d'importance ; par exemple, pr1, pr2 et pr3 pour une préférence de plus en plus marquée.

* *facteur variétal / mode de préparation* :

Trois clones (095, 126 et 182) ont été préparés en voie sèche et en voie humide avec quatre répétitions pour chaque combinaison clone/mode de préparation.

Les résultats de l'AFC sont présentés sur la figure 1. L'axe 1 est un axe de préférence globale liée à la qualité de l'arôme. L'axe 2 oppose acidité et amertume. Les deux modes de préparation sont différenciés mais restent proches de l'origine. La voie humide apporte plus d'arôme et d'acidité et est en moyenne préférée.

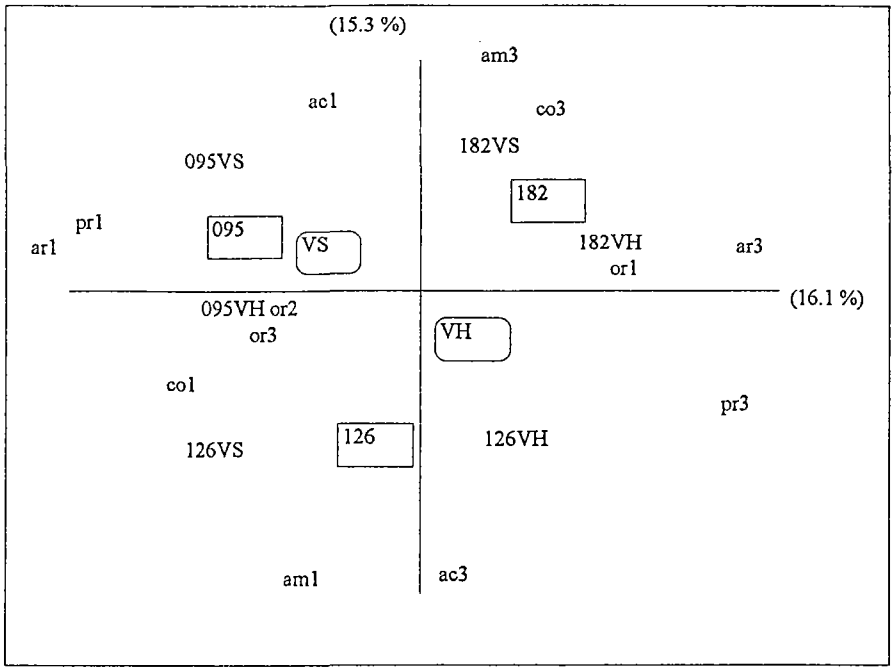

Figure 1 - Plan 1-2 de l'AFC concernant la comparaison des facteurs clone (095, 126 et 182) et mode de préparation (Voie sèche et Voie humide).

La différence entre les clones est beaucoup plus marquée. Le 182 et le 126 sont plus appréciés que le 095. Le clone 126 se distingue par son acidité élevée alors que le 182 a plus de corps et est plus amer. Le 095 a une mauvaise qualité aromatique.

Si l'on identifie chaque couple clone / traitement, on s'aperçoit que la voie humide tire le clone vers une meilleure appréciation et une plus grande acidité. Cette translation va dans le sens d'une amélioration quelque soit le clone. Le classement général des clones est le même avec la voie sèche ou la voie humide.

A l'occasion de cette analyse, nous avons pu vérifier l'influence de l'ordre de présentation qui justifie *a posteriori* son contrôle par randomisation du rang de présentation. Le café dégusté en premier, quel qu'il soit, est jugé plus aromatique et plus corsé que les suivants.

* *facteur variétal / terroir* :

Trois clones (126, 182 et 461) ont été récoltés dans trois terroirs différents (Abengourou, Bingerville et Divo). Il y a trois répétitions pour chaque combinaison clone / terroir.
Les résultats de l'AFC sont présentés sur la figure 2. L'axe 1 oppose les objets jugés aromatiques, acides aux objets corsés et amers peu appréciés. L'axe 2 identifie des appréciations intermédiaires pour l'ensemble des variables (effet Guttman).

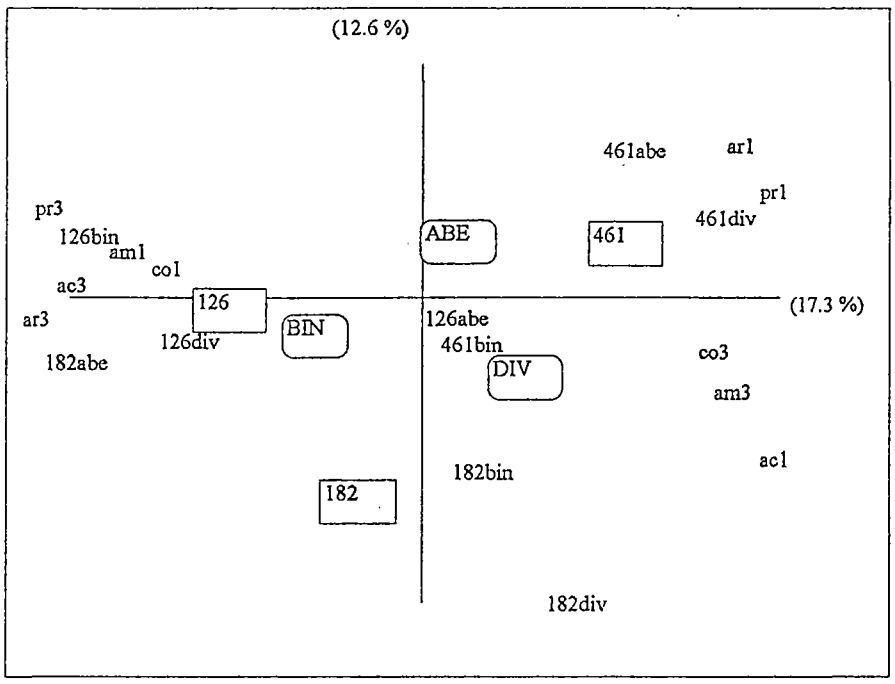

Figure 2 - Plan 1-2 de l'AFC concernant la comparaison des facteurs clone (126, 182 et 461) et terroir (ABEngourou, BINgerville et DIVo).

Les terroirs sont séparés bien que proches de l'origine. Les clones provenant de Divo sont dans l'ensemble moins appréciés que ceux venant de Bingerville. Abengourou est intermédiaire. Les clones sont mieux séparés que les terroirs. Le clone 461 est corsé, amer et peu apprécié. Le clone 126 est au contraire acide, peu amer, aromatique et bien apprécié.

En identifiant chaque couple clone / terroir, on note des différences semblables entre les clones 126 et 461 quel que soit le terroir. Le clone 182 semble toutefois réagir différemment par rapport aux autres clones en fonction du terroir. Il est beaucoup plus apprécié lorsqu'il est récolté à Abengourou. En fait, une analyse fine montre que les échantillons du 182 en provenance de Bingerville et Divo ont été trop torréfiés. Ceci entraîne une interaction qui ne modifie en rien les conclusions générales.

* *facteur variétal / date de récolte* :

Les cerises rouges de deux clones (126 et 461) ont été échantillonnées au premier passage (DA1) et au dernier passage de récolte (DA2). Les deux dates sont espacées d'environ un mois. Pour chaque couple, clone / date de récolte, on dispose de trois répétitions. Cet essai a été réalisé sur deux stations : Abengourou et Divo.

Les résultats de l'AFC sont présentés sur les figures 3. Dans les deux stations, l'axe 1 sépare nettement le clone 461 amer, peu acide, peu aromatique et peu apprécié du clone 126 qui a les caractéristiques opposées. Ceci

correspond aux résultats des essais précédents. A Divo, la date de récolte n'a aucun effet. A Abengourou, les échantillons prélevés lors du premier passage sont plus acides et plus appréciés. Cet effet est le même pour les deux clones.

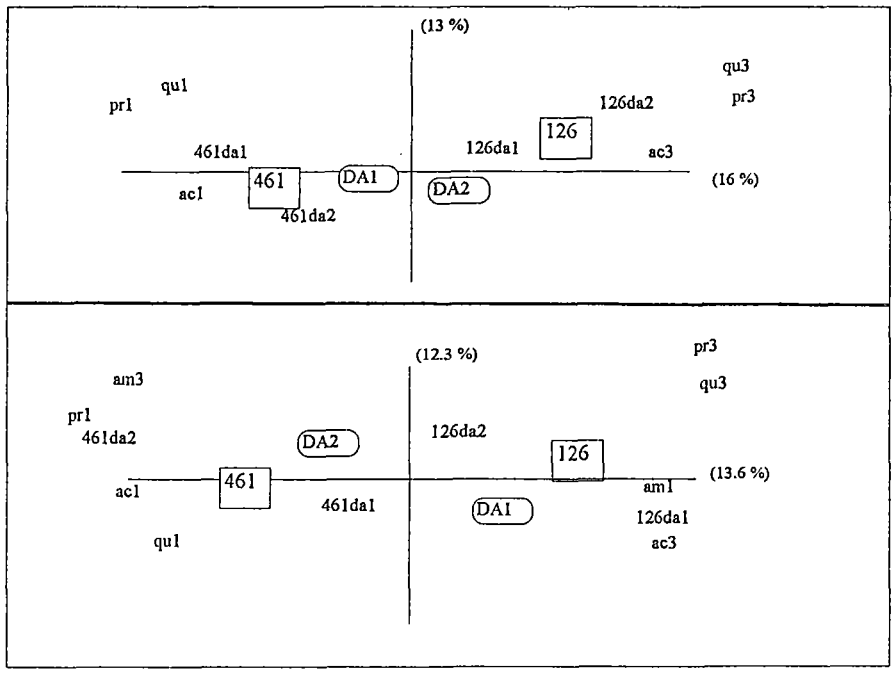

Figure 3 - Plan 1-2 de l'AFC concernant la comparaison des effets clone (126 et 461) et date de récolte (DA1 et DA2) à Divo (haut) et Abengourou (bas).

* *Conclusions* :

1) Des différences clonales pour la qualité à la tasse sont détectables au sein de *C. canephora.*

2) Ces différences sont plus importantes que celles observées entre des facteurs tels que le mode de préparation, le terroir ou la date de récolte.

3) Aucune interaction significative n'a été décelée entre le facteur clone et les autres facteurs étudiés.

Il est donc intéressant d'envisager des travaux d'amélioration variétale de *C. canephora* pour la qualité à la tasse.

II QUALITE ORGANOLEPTIQUE ET STRUCTURE DE L'ESPECE *C. CANEPHORA* :

Actuellement, la sélection de *C. canephora* en Côte d'Ivoire est basée sur un schéma de sélection récurrente réciproque utilisant les caractéristiques complémentaires des groupes guinéens et congolais et la vigueur hybride des descendances intergroupes (Leroy *et al*, 1993). Deux sous-groupes (SG1 et SG2) à l'intérieur du groupe

congolais (Montagnon *et al*, 1992) sont également identifiés. Il nous a paru intéressant de vérifier si des caractéristiques de qualité à la tasse différentes correspondaient à chacun de ces groupes ou sous-groupes.

Vingt huit clones ont été testés : 11 clones guinéens, 6 clones congolais du sous-groupe 1 et 11 clones Congolais du sous-groupe 2. Chaque clone a été considéré comme une répétition de son groupe ou sous-groupe d'appartenance. Les 28 échantillons ont été randomisés entre les différentes séances de dégustation.

Les résultats (tableau 1) montrent une grande variation à l'intérieur de chaque groupe. Toutefois, des différences significatives apparaissent pour toutes les variables étudiées. Le groupe guinéen est toujours différent du sous-groupe 2 congolais. Le sous-groupe 1 congolais est équivalent au groupe guinéen pour le corps et l'amertume et sous-groupe 2 Congolais pour la note de préférence, l'arôme et l'acidité.

Tableau 1 - Valeurs moyenne, minimum (m) et maximum (M) et différences statistiques des trois groupes génétiques de *Coffea canephora* pour les caractéristiques organoleptiques.

		Caractéristiques				
Groupe génétique	Nombre de clones testés	Préférence Moy. m/M	Arome Moy. m/M	Acidité Moy. m/M	Amertume Moy. m/M	Corps Moy. m/M
Congolais SG2	11	2.92 a* 1.7/3.7	2.66 a 1.7/3.3	3.34 a 3.0/3.6	2.30 b 2.1/3.4	2.60 b 2.5/3.7
Congolais SG1	6	2.89 a 2.6/3.5	2.76 a 2.0/3.0	3.47 a 2.9/4.0	2.91 a 2.4/3.7	2.96 a 2.5/3.7
Guinéen	11	2.40 b 1.7/3.1	2.31 b 1.7/2.9	2.98 b 2.6/3.6	3.16 a 2.8/3.8	3.15 a 2.6/3.9

* Les moyennes suivies de la même lettre ne différent pas d'après le test de Newman et Keuls au seuil de 5 %.

Ainsi, la structure de l'espèce *C. canephora* se retrouve également pour la qualité du café boisson. La variabilité observée devrait permettre une sélection à l'intérieur de chaque groupe et sous-groupe pour la qualité organoleptique.

III COMPARAISON DE CLONES CANDIDATS A LA VULGARISATION :

Cinq clones prometteurs au niveau de leurs performances agronomiques ont été confrontés entre eux avec les clones 126 et 461 comme témoin. Deux essais différents ont été réalisés avec une préparation en voie sèche et en voie humide.

Le tableau 2 résume les résultats obtenus. Une interaction est observée entre les deux essais pour les clones 588 et 126. Ces deux clones ont été très mal notés en voie sèche à cause d'une torréfaction trop faible. Leur note d'acidité témoigne de ce fait.

Tableau 2 - Moyenne et analyse de variance combinée pour les caractéristiques organoleptiques de sept clones de *Coffea canephora* préparés chacun en voie séche et en voie humide

Clone	Préférence		Qualité arôme		Acidité		Amertume	
628	3.23	a *	2.92	a	3.21	a b	2.50	b
539	2.73	b	2.54	a b	3.31	a b	2.92	a b
528	2.65	b	2.69	a b	2.94	b	3.00	a b
588	2.52	b	2.33	a b	3.42	a b	2.71	b
461	2.44	b	2.21	b	3.19	a b	3.35	a
636	2.35	b	2.54	a b	3.02	b	2.69	b
126	2.29	b	2.35	a b	3.58	a	2.96	a b

* Les moyennes suivies de la même lettre ne différent pas d'après le test de Newman et Keuls au seuil de 5 %.

On retrouve la forte amertume du clone 461. Le clone 628 est le plus apprécié et possède en particulier un arôme supérieur aux autres clones. Ainsi, même si sa productivité était moins forte que d'autres clones candidats à la vulgarisation, le clone 628 pourrait être vulgarisé pour sa qualité à la tasse. Il possède par ailleurs une excellente granulométrie. Une répétition des tests sera de toute façon nécessaire avant de prendre un décision définitive.

CONCLUSIONS GENERALES

Nous avons pu mettre en évidence l'intérêt de l'amélioration variétale pour la qualité à la tasse du café Robusta produit par *C. canephora*. Ni le mode de préparation, ni le terroir, ni la date de récolte ne masque ou interfère avec les effets génétiques.

De façon inattendue, les seuls problèmes rencontrées proviennent de la torréfaction. Il s'avère en effet difficile de contrôler son degré pour tous les échantillons et en particulier pour ceux dont la granulométrie est faible ou hétérogène (Moschetto *et al*, 1994). Cette étape de la préparation du café doit donc être étudiée de façon approfondie pour mieux la contrôler.

Malgré cela, des différences clonales ont été mises en évidence au niveau individuel mais aussi en relation avec la structure de l'espèce. L'intégration de critères de qualité, élargis aux nouveaux modes de consommation du café, (soluble, prêt-à-boire...), dans le programme de sélection récurrente réciproque est donc tout à fait pertinente et efficace. De prochaines études devraient permettre de définir l'héritabilité des caractéristiques du café boisson.

Il est possible que des variétés de faible qualité organoleptique soient par ailleurs très performante au niveau agronomique. Dans ce cas, éliminer ces variétés reviendrait à diminuer le potentiel de production. Une solution pourrait consister à mélanger les différentes variétés de telle sorte que qualité et productivité soient maintenues. L'évaluation de mélanges de cafés de qualité différente est prévue dans ce cadre.

L'amélioration variétale de la qualité du café-boisson n'est évidemment pas exhaustive des autres approches

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RESUME

L'importance relative de l'effet clonal par rapport au terroir, à la préparation et à la date de récolte est testée. Les éventuelles interactions entre le facteur clone et les autres facteur sont évaluées.

La part prépondérante du clone dans l'expression de la qualité du café boisson et l'absence d'interactions entre facteurs permettent d'envisager une sélection efficace pour la qualité à la tasse de *Coffea canephora*. Des clones de qualité supérieure sont identifiés.

L'étude d'un nombre important de génotypes du groupe Guinéen et des deux sous-groupes du groupe Congolais permet de relier les différences du café boisson à la structure de l'espèce.

SUMMARY

The relative importance of the clonal factor versus processing, location and harvesting date has been tested. The possible interactions between clones and other factors have also been assesed.

The preponderant effect of the clone for the cup quality and the lack of interactions make it possible to breed efficiently *C. canephora* for cup quality. Clones of superior quality are identified.

The study of a great number of clones belonging to the Guinean group and to both Congolese sub-groups shows that cup quality can be connected with the genetic structure of *C. canephora*.

PARCHMENT ARABICA COFFEE STORAGE

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INTRODUCTION

The quality of arabica coffee is intimately associated upon the processing system in the field and upon its moisture content during storage. Safe storage can be given in terms of water vapor pressure-in practice described in terms of the relative humidity of the microclimate around the bean (7,11,14,18). The vapor pressure of water is a function of temperature. For climatic conditions in which the water vapor content decreases the drying potential is increased (7,14). Hence long storage at relatively low humidities may cause bean moistures to fall. On the other hand, coffee bean is hygroscopic like many of agricultural commodities (3). Where longer storage occurs under high relative humidity in warm climates moisture may be absorbed from the atmosphere and rises coffee moisture. In either case, quality loss is inevitable in terms of raw appearance (color fading etc); mottled roasts; and deterioration of liquor due to tainting or introduction of unpleasent flavors (13,15,16).

Coffee stores at production area are intended as transit requirement which are meant for retention for few periods as a coffee passes from field to the market. Records of the farms and statistics (2,4,19), show that coffee is retained for longer period than it was generally supposed to stay in the primary stores. Many factors may be associated that coffee is being stored in excess (1,7,8,17,18), among which the most important are carry overs, capacity of central mills, and international market situations. This study was intended to identify storage conditions of parchment coffee at primary stores and to investigate the frequent reports that parchment coffee arriving at central mills tend to show high moistures than normally accepted.

EXPERIMENTAL

1. Store geometry and Equipments

Storage conditions of parchment coffee and microclimatic factors were investigated at experimental store at Melko-Jima Research Center and on farm coffee stores at Tepi. At Melko, a model of transit stores was constructed for the study in conjuction to the sample prepartion room at the washing station. Two side walls are concrete of the existing building and the remaining features are corrugated iron sheet walls, concrete floor, roof of corrugated iron sheet, eaves ventilation of mesh wire up to one meter below overhangs of the

sloping roof. Maximum-minimum thermometers were placed at central position of the side wall at about 1.50m above the floor level. A thermohygrograph recorder for maximum-minimum temperature and relative humidity of atmospheric air was installed in the outside measuring station in the Stevenson's screen. At Tepi, coffee plantation farms within altitude range of 1000m - 1500m, two major type of stores were encountered. Type I-rectangular wall and triangular roof, wall and roof made of iron sheet, concrete floor, about 20-50 cm mesh wire openings, and rarely ridge ventilation; total capacity 160 to 650 m^3. Type II - half cylinderical domes, walls and roofs continuous iron sheet, about 1-2m^2 mesh wire windows above and at both sides of the door (not standardized), concrete floor, total capacity of about 950 m^3. Overall assessment was made on both type of stores but continuous record was taken from type II store at one factory. Maximum-minimum thermometer was placed at 1.70 m above the floor level at the center line of the store.

2. Stacking and sampling

Source of coffee at both experimental centers was from the farms of respective organizations processed under normal wet processing techinques by the respective factories. At Melko, arrangements were made at peak harvesting and follow up was made at each stage of processing. Conditioning was performed at sample preparation room by manipulating the product under natural environment until desired moisture content was achieved before bagging. Generally, 60-kg Jute bags were used for storing parchment coffee. One layer hessian cloth and double layer jute bagswere used for dunnage. At stacking, single block with three bag layers (bottom, middle and top) and 3x4 bags area was done at the model store. Aisle space of about 50 cm was left along the walls. Three bags were sampled from each layer fortnightly throughout the year for a given entry. A representative sample of enough quantity was drawn by a sampler from each bag of a layer and mixed together to form a composite sample. The samples were subdivided for various tests. At Tepi where records were taken on the existing situation, bags are stacked to the left and right of the center line along the door, with aisle space at perpendicular bisector of this center line and between the stacks and along the walls. As a result, four blocks (pallets) were found with stack layers of 7 to 8 bags and 4x(7 to 12) bags area. Single to three layers hessian cloth dunnage was laid on concrete floor. The blocks were coded as; A=8 meters away immediate to the right of operational door, B=0.5 meters away immediate to the right of operational door, C=0.5 meters and to the left of operational door, D=8 meters further and to the left of the door. Stack layers of all blocks were numbered from 1 to 7; number starts at bottom layer.

3. Analytical

Moisture was determined by electrical moisture meters and by the oven method for each sampling. In the field and at mills there are two commercially available meters now fairly widely used. The one with the comparatively wider usage in the industry, earlier introduction and with the test range of 5% to 21% moisture content is referred to as Meter-A. The second, later introduction, but now in wide use, and test range of 9% to 30% moisture content is referred to as Meter-B. IS6673 was adapted as standard, in use with a gravity convection oven, analytical balance sensitive to one milligram, punched fire resistant paper bags, and maintaining the inherent procedures of the standard.

RESULTS AND DISCUSSION

The beans collected from drying tables may not have the same moisture and practically it is not possible to collect coffee at fixed moisture content at all times. In an intimate mixture of beans, the wet beans give off water to the air, from where it is absorbed by the dry beans and uniformity slowly develops (10). At Melko it took about a week on the average (Fig 1) for coffee collected within 10% to 11.5% moisture content from drying tables to even up; the differences in duration depending on the moisture content of the incoming bulk and the prevailing weather conditions. As a result all bagged coffee samples started at 10.4% which is considered as fully dry (16).

The meter readings and the standard method recording (Fig. 2 A,B,C) are average values of the three separate tests. Excellent duplication was recorded in most cases. The agreement between the standard and Meter-A was good. Meter-B reads 1% to 2% lower than the standard. Generally the results correlate closely with each other, and averages were used in subsequent reporting.

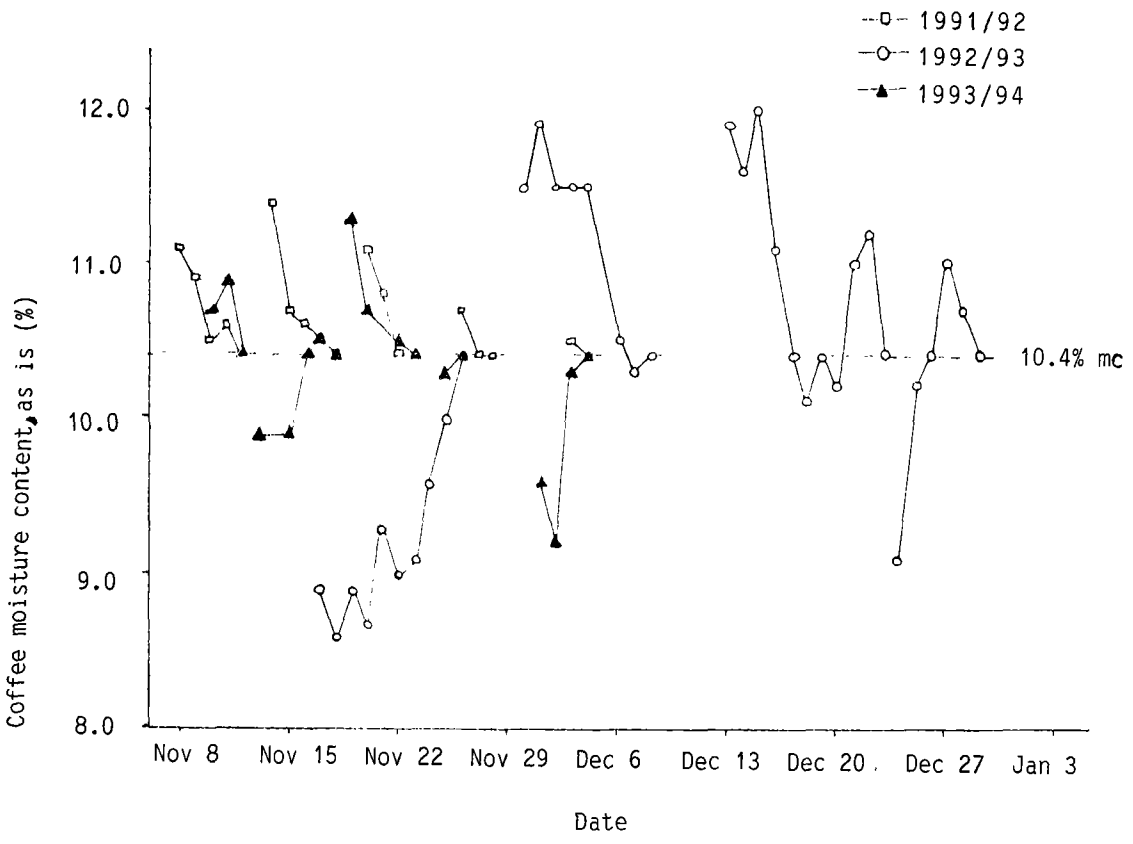

Figure 1. Dates and duration of bulk conditioning of parchment coffee (before being bagged) in the respective years at Melko.

Position of the block and stack bag layers (Fig 3,4,7) significantly affected moisture carrying conditions of the beans. Damp conditions were encountered at Block A (Fig 4) which was at far corner from operational door and lacks openings nearby and the lowest average moisture content at B was perhaps due to it proximity to operational door and access to direct solar says. First bottom layer moisture content was highest for all dates in all blocks at both trial sites (Fig 3, 7). The moisture content decreases as one goes from bottom to top layers, generally. Exception to this fact was Block C (Fig 4) where moisture decreases as one goes from bottom to middle layers and increases at top layer. This is due to moisture translocation or migration, characteristics of large stores. The over all average moisture content of the store which is around 10% is misleading in practical situations where small samples from elesewhere in the store are bulked for moisture test.

Alarmingly high average moisture content of about 14% as compared to 10.4% intial was probably gained from atmospheric air, picked from floor and roof, or additional moisture averted from catabolism. Any external change of weather altered the internal conditions of the store (Fig 5, 6). The fluctuations in climatic conditions were different from year to year. About 50% to 80% relative humidities were encountened in the stores, though the relative humidities should be around 60% for safe storage for temperature similar to those encountered in the stores. During the rainy periods, the store retains high relative humidity and damp conditions. On the other hand, reports indicate that drying rate depends much more on the relative humidity of the air, and the relative humidity itself does depend markedly on dry bulb temperature (8,14). In this study, significant and negative correlation was observed between the minimum-maximum temperatures in the store and moisture content of the stack bag layer (Fig 3,5). Highly significant and positive correlation was observed between the relative humidity of the atmospheric air and moisture content in stored coffee (Fig 5).

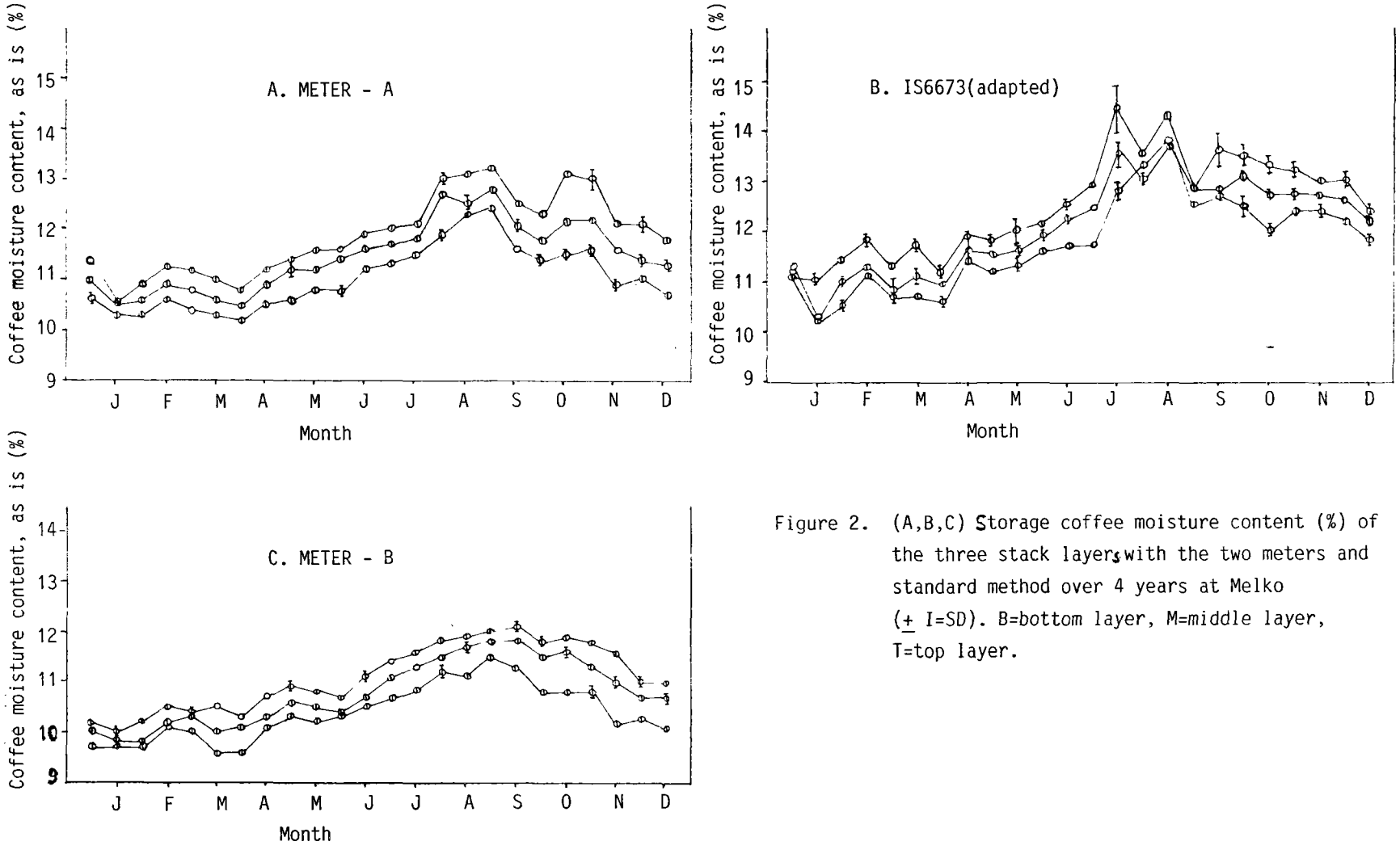

Figure 2. (A,B,C) Storage coffee moisture content (%) of the three stack layers with the two meters and standard method over 4 years at Melko (± I=SD). B=bottom layer, M=middle layer, T=top layer.

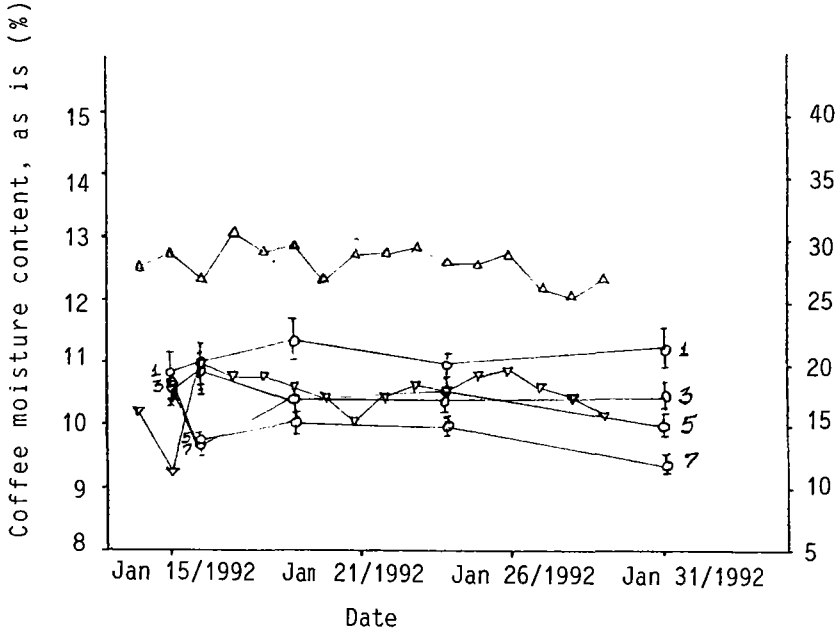

Figure 3. Storage coffee moisture content (%) of stack layers of bags 1,3,5 & 7 at Tepi (±I=SD). Δ-Max. temp., ∇-Min. temp..r value of mc. vs Min. temp. is ns for stack layers 1 & 3, and significant at P<0.05 for 5 & 7. r_5=-0.648, r_7=-0.670, n=10.

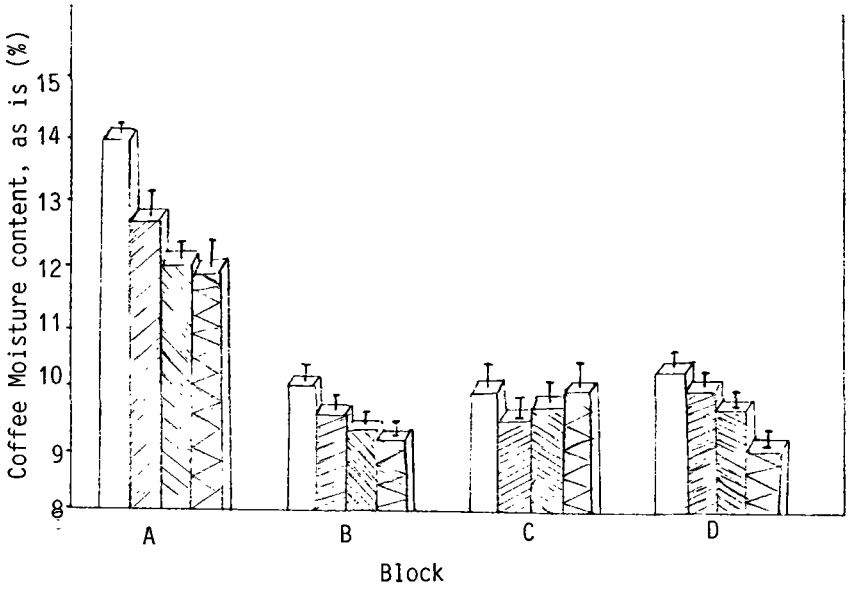

Figure 4. Average storage coffee moisture content (%) of blocks A, B, C and D of 1,3,5 and 7 layers at Tepi. Mean (± I=SD). Stack layers; □-1, ▨-3, ▨ -5, ▨ -7

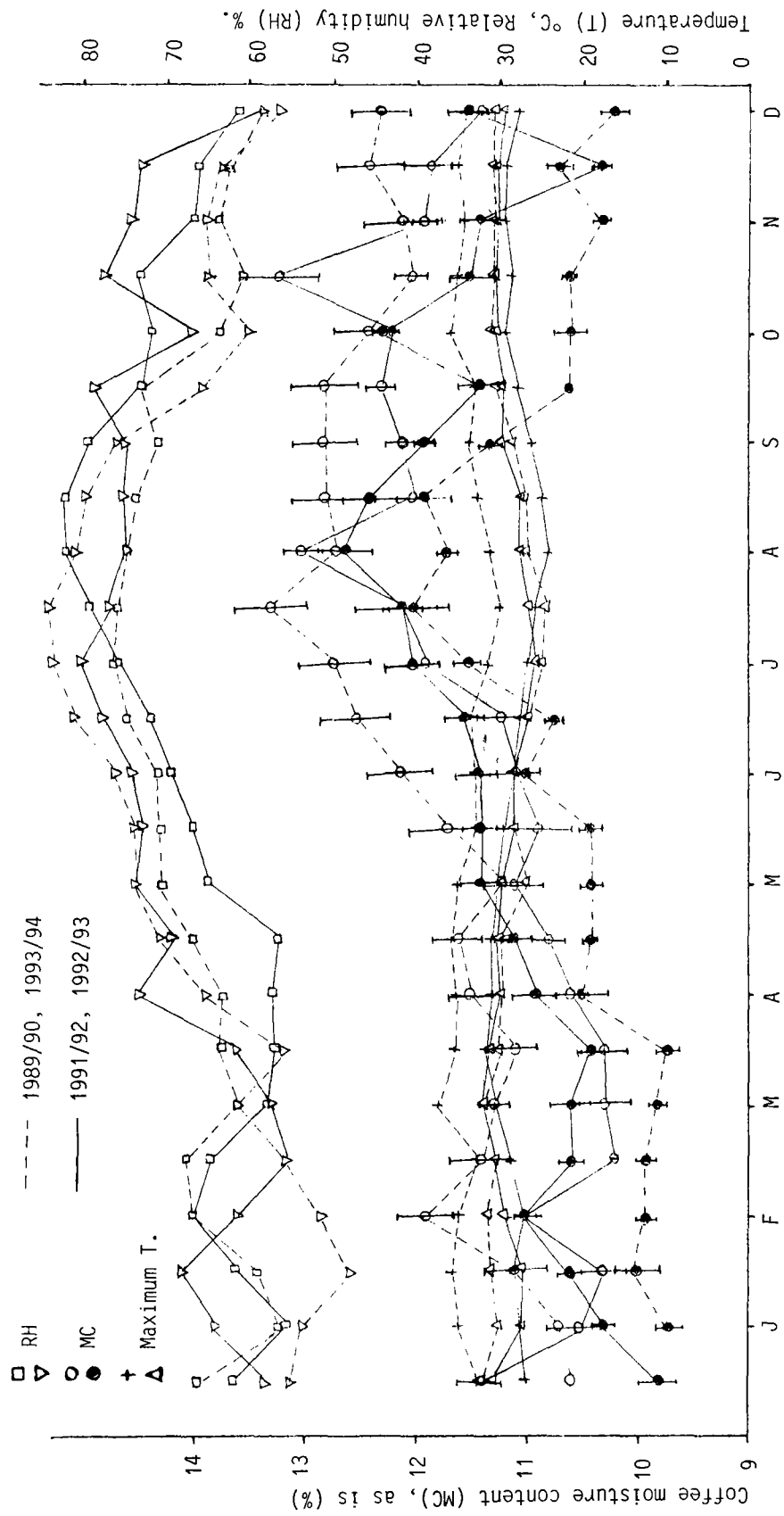

Figure 5. Storage moisture content (%) of parchment coffee averaged over layers and over meters and comparison of major climatic factors at different months of storage period in the respective years at Melko (±SD). r value between MC and RH is positive and highly significant (r_{RH}-89/90=0.637, r_{RH}-91/92=0.702, r_{RH}-92/93=0.584, r_{RH}-93/94=0.799). r value between MC and Maximum T is negative and significant at $P \leq 0.05$ (r_{max}-89/90=-0.590, r_{max}-91/92= -0.524, r_{max}-92/93=0.448, r_{max}-93/94= -0.809). n=24.

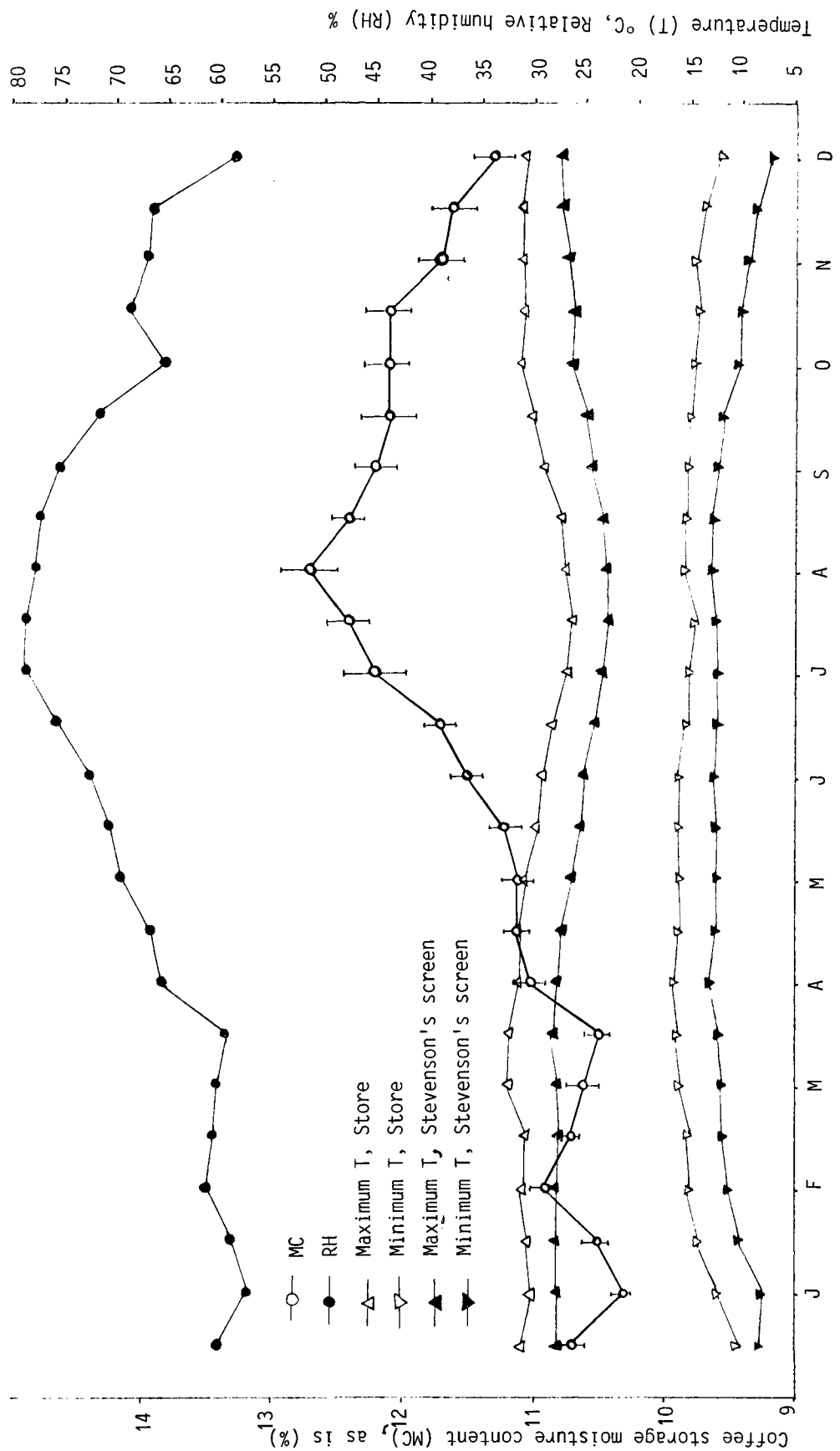

Figure 6. Storage moisture content (%) of parchment coffee and major climatic factors at corresponding storage period averaged over all the meters, layers and years at Melko (± I = SD).

In the view of the above, the moisture in coffee and storage conditions should be prescribed on the basis of the temperature and humidity conditions existing in the place of production as well as the extent of storage needed for sale or export. Higher temperatures and humid conditions in the production area do not warrant longer storage. The central mills are congested in the January-March peak arrival. Maximum increase of moisture was observed in the wet July-August months from 10.4% intial moisture in November-December harvesting. The results of the trend of moisture indicate that coffee despatches to the central mills should be shorter than this time or forced ventilation used otherwise.

SUMMARY

Storage conditions of parchment coffee, necessity of storage, and microclimatic impacts on stored coffee was investigated at experimental store at Melko and stores of coffee state farms at Tepi. Macroclimatic factors such as total rainfall, relative humidity, maximum-minimum temperatures with effect on WVC of the air were found to greatly influence storability and quality of stored parchment coffee. position of the tier and storage duration has also significantly affected the moisture content of the beans at the experimental conditions. At Melko, where two commonly used meters and IS6673 (adapted) were used for moisture determination, significant and positive correlation was observed with relative humidity of air. The result was inverse for maximum temperature. Similar results were observed at Tepi, warm humid climate and unconditioned on farm stores. From the initial moisture content of 10.4% in November-December, maximum increase of moisture of about 14% was observed in wet July-August months. It was shown that coffee cannot be stored in parchment form in the primary stores beyond 4 to 5 months.

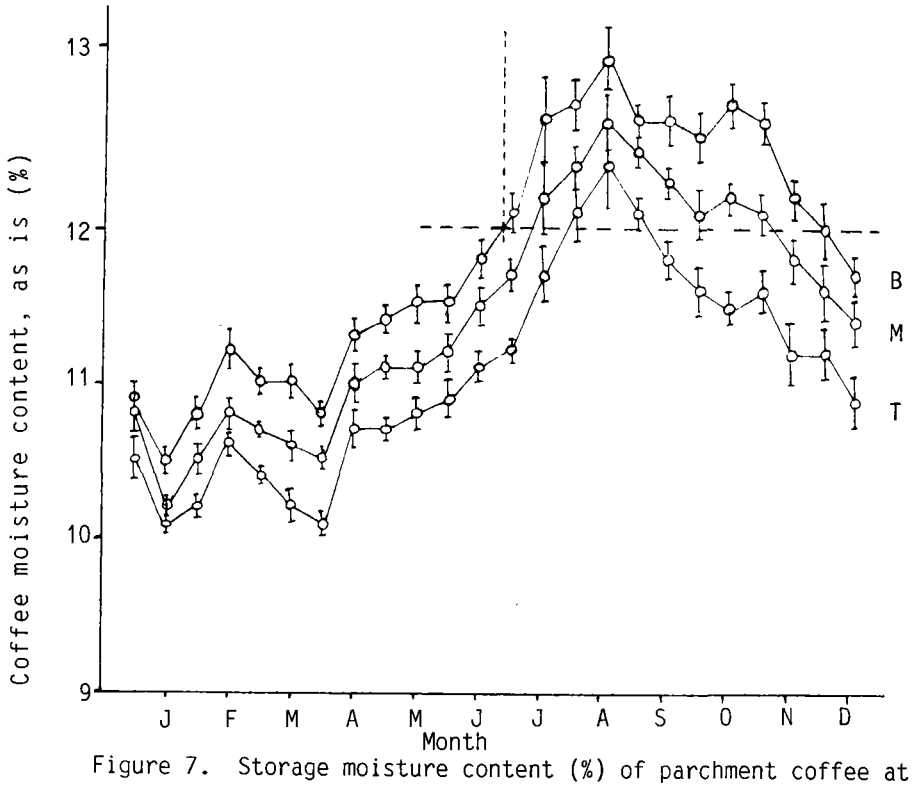

Figure 7. Storage moisture content (%) of parchment coffee at the three stack layers at Melko. B=Bottom layer, M=Middle layer, T=top layer.

ACKNOWLEDGEMENTS

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BEAN CHARACTERISTICS AND CUP QUALITY OF THE COLOMBIA VARIETY *(COFFEA ARABICA)* AS JUDGED BY INTERNATIONAL TASTING PANELS

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INTRODUCTION

Coffee leaf rust control (*Hemileia vastatrix* Berk & Br.) using resistant varieties has been the main research objective of a plant breeding programme in Colombia during recent decades. To achieve this objective, researchers at The National Coffee Research Centre - CENICAFE (of the National Coffee Growers Federation of Colombia) developed the Colombia variety which was released in 1982 as a result of research carried out over 20 years (Castillo y Moreno, 1986).

The variety "Colombia" is a composite, formed by the mixture of seeds from the best progenies of advanced generations (F5 and F6) of crosses between the Caturra variety (*C. arabica*) and the Timor hybrid. The Caturra variety, used as a basic progenitor in the crosses, is very well adapted to the Colombian coffee production area and possesses excellent agronomic and cup qualities. The Timor hybrid which contributes resistance to rust is a population with characteristics of a variety of the *C. arabica* species; it has 44 chromosomes, is self pollinated and when crossed with *C. arabica* varieties it produces fertile descendants free from important chromosome irregularities.

Colombian coffee is recognized in international markets for its high quality. Cup quality is a complex characteristic which depends on a series of factors such as the species or variety (genetic factors), environmental conditions (ecological factors) , agronomical practices (cultivation factors), processing systems (post-harvest factors), storage conditions, industrial processing, preparation of the beverage and taste of the consumer. All of these factors interact to form a complex in which it is difficult to determine the most important component. Bibliographic revisions on the factors affecting the quality has been done by Clifford (1985), Menchu (1966) and Wallis (1967).

In the development of the Colombia variety, the cup quality has been one of the characteristics most taken into account. On several occasions the components of the Colombia variety have been evaluated by national and international tasting panels, comparing its cup quality with the quality of the traditional varieties grown in the country. The objective of this paper is to present the main results obtained in these evaluations.

MATERIALS AND METHODS

The cup quality tests were performed at different times, using tasting panels from different national and international institutes. In each test, the Colombia variety was included and as a control another *C. arabica* variety of recognized cup quality was used. Control varieties were Typica, Bourbon and Caturra which are planted over large areas in Colombia. In some cases, samples of *C. canephora* were included, taken from Cenicafe's germplasm bank.

The samples for analysis were processed using the wet system as recommended by Cenicafe for taste tests: use of ripe beans only, depulping, fermentation, washing of samples several times with clean water and sun drying to 11 to 12 % humidity.

Once the samples were dry, they were sent to the different tasting panels for evaluation. The majority of these panels evaluate the quality by the physical characteristics of the bean (size, shape, color, uniformity) and sensorial characteristics of the beverage (acidity, body, flavor and the presence of strange odors or flavors) using descriptive methods and/or hedonic judgments. However, the procedures of evaluation used by the panels are different, and for that reason the results are not strictly comparable and cannot be analized using normal statistical methods.

The results presented nevertheless show coincidence in spite of the variation in the methodology used in each case.

RESULTS AND DISCUSSION

1. Beverage quality in the first components of the Colombia Variety.

Tables 1 and 2 show the results of the evaluations performed in 1980 and 1982, before the Colombia Variety was released to coffee growers. These evaluations were conducted by tasting panels in Colombia which analized a group of the F6 progenies of the Colombia variety, using other *C. arabica* varieties grown in the Colombia as controls.

The tables show that best qualifications are shared more or less equally between Colombia progenies and controls and that occasionaly either controls or progenies received negative judgements. These judgements however are due to the presence of strange odors or flavors, which cannot be attributed to the nature of the genotypes, but rather to irregularities during the processing of the samples. For example, the immature taste it is caused by the presence of immature beans in the samples. The astringent and fermented tastes, which indicate undesirable acidity, was due to overripening of the samples (Daget, 1980), whereas the dirty taste is caused by defects during the roasting process (Reymond, 1983).

A different case is the odour and taste of cereal, strongly detected in all the tests done with the *C. canephora* samples. This defect is absent in all the other samples, with the exception of a few cases where it was detected at low intensity. The constant presence of the cereal taste in the *C. canephora* samples is itself an interesting fact which could be useful to explore as a potential effective marker of the influence of this species in the descendants of crosses with *C. arabica*.

The above samples were also tested on two occasions by the tasting panel of the Hans Newman company of Germany. The main results from these evaluations are the similar markings given to the progenies of the Colombia variety as to samples of other export quality excelso coffee, in both bean quality and cup quality (see Table 3).

2. Cup quality of the present components of the Colombia variety.

The conformation of the Colombia variety has been modified due to the replacement of some of the initial components. The increase of the bean size has been one of the reasons for the replacement of these components. The first components had a bean size of 56% "supreme coffee" (coffee beans retain by a sieve of 17/64"). The present compo-

nents have a bean size equivalent to 70% in supreme coffee, similar to the size of the Typica variety beans, which is recognized as having one of the largest bean sizes of the *C. arabica* species. The percentages of supreme coffee of the Colombia variety obtained recently in several localities (see Figure 1), indicate that the large bean size obtained by means of selection between its components is a stable characteristic.

The cup quality of the present components of the Colombia variety was evaluated during 1994, using four tasting panels of a well-known international company from the USA, Canada, Colombia, and the UK as well as an experimental panel of the Coffee Chemistry Laboratory in Bogota. Tests included samples of the Colombia variety with red and yellow fruits collected in the seed propagation plots during two different seasons (samples 1 and 2). The varieties used as controls were grown in experimental plots in Cenicafe.

Table 4 shows a summary of the main observations done by the four international panels. Despite differences between concepts as regards the final product and in the procedure used, the results of the panels agree that the samples correspond to high quality coffees, without important defects in the cup.

The observations as a whole indicate that the genotypes of the red Colombia variety (samples 1 and 2) and Typica, are mild coffees, with good body and acceptable acidity. The Caturra variety displays a high acidity (citric acidity), while the Bourbon variety is considered as possessing a clean cup. The yellow Colombia variety received different evaluations. In one case, it was catagorized as a clean cup (sample 2), while in the other the beverage was considered of lower quality, (sample 1).

As one might expect, there are some differences in the tastes of the genotypes, a fact which one of the panels recommended might be used in specialty markets which are driven by demand for the final product. For example, the genotypes red Colombia, Caturra and Typica were recommended for very demanding markets, like those in Germany and United Kingdon. The other genotypes are recommended for less demanding markets, although all of them corresponded to high quality arabica type coffee.

Finally, the results of the experimental panel analyzed the previous genotypes plus a sample of the Robusta variety of *C. canephora*. This panel tested each genotype six times, quantifying the variables acidity, flavor, body and overall impression, by means of a 9 point ranking. With the averages of each genotype for each variable, two types of multivariant analysis were carried out (factorial correspondence analysis and ascendent classification by ranks), in order to objectively describe the behaviour of the genotypes as a function of the variables and to perform a grouping of these genotypes according to their behaviour.

The results of the analysis are presented in Figure 2. In this figure, the variables and the genotypes are located over the plane formed by the axes 1 and 2 representing 81% and 12% respectively of the total information in relation to the cup test. Axis 1 is formed mainly of the variables 'acidity' and 'overall impression', whilst axis 2 is formed by the variable 'body'.

The variables 'aroma' and 'overall impression' are closely related, as is shown by in their proximity in the plane of the diagram. In relation to these two, acidity is a distantly related variable.

Between the tested genotypes, Robusta shows a very different behaviour which is reflected in its distance from other genotypes in the plane of the diagram. The separation of the other genotypes is less evident, although it can be seen that sample 1 of yellow Colombia is removed somewhat towards the sector of higher acidity.

The ranking classification shows that the tested genotypes are divided into three groups. The first one formed by six genotypes (Typica, red Colombia samples 1 and 2, yellow Colombia sample 2, Bourbon and Caturra), between these there are no differences taking together all the variables as a group. The second group is formed only by the yellow Colombia variety sample 1 and the third by the Robusta variety (see Figure 2).

In general terms, the above results agree with those of the international panels in the similar scores given to the Colombia and control varieties. The exception is sample 1 of the yellow Colombia variety, which was scored differently in both cases. It is probable that these results are due to the yellow colouration of the fruits, making it difficult to know when they are well ripened; consequently the samples coming from the field may be too heterogeneus in respect to maturity of the berries.

Similar results to those presented in this paper have been obtained on other occasions when cup quality has been evaluated using other materials similar to those of the Colombia variety. Brazil (Texeira and Araujo 1976), India (Vishveshwara, 1971) and Kenya (Vosse and Walyaro, 1981) all confirmed that the products from the crosses of regional varieties of coffee with the Timor hybrid produced descendants with the beverage quality comparable to the quality of those varieties. Kenya's results analyzed by Vossen (1985) and later by Owuor (1988), have special interest because they demonstrate that the cup quality of the cultivar Ruiru 11, selected from some progenies of Caturra X Timor hybrid from Colombia, it is essentially equal to the quality of the K7 and SL28 varieties, considered as the standard for high quality coffee in that country.

CONCLUSIONS

The tests carried out by national and international tasting panels showed that there are no differences between the cup quality produced by the components of the Colombia variety and other varieties of *Coffea arabica* such as Typica, Bourbon and Caturra, which have always been qualified as producers of an excellent beverage.

In most of the cases the differences in acidity, body and flavor between these four varieties are not consistent, and when present, of little importance and could be due to mistakes during the prossesing of the beans.

The physical characteristics of the beans of the Colombia variety, specially in relation to the bean size, are equal to those of the Typica variety, recognized by international markets as one of the best varieties.

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ABSTRACT

Colombia is a composite type variety of coffee, resistant to leaf rust (*H. vastatrix*) and integrated by mixing seeds from advanced progenies (F5 and F6) resulting from the crossing of the Caturra variety and the Timor hybrid. These different components of the variety have been evaluated for bean characteristics and quality of beverage by tasting panels from five countries, including Colombia. The results indicated that the Colombia variety produced a beverage of the same quality as the Typica, Bourbon and Caturra varieties used as controls. These results are supported by the descriptive characteristics of cup-tasting according to multivariate statistical analysis. The physical characteristics of the bean showed that, specially with regard to bean size, the Colombia variety is similar or superior to the Typica variety regarded as one of the best in the coffee market.

TABLE 1. Sensorial characteristics of the beverage of progenies F6 of Caturra x Timor Hybrid, components of the Colombia variety and the control varieties, evaluated by the tasting panel of Federacafe, Bogotá.

Genotype	Acidity	Body	Flavor	Strange odours and taste
Caturra	good	regular	regular	
F6.321	regular to good	regular	regular	
F6.170	good	regular	regular	
F6.203	regular to good	regular	regular	Immature (+)
F6.206	regular to good	regular	regular	
Typica	regular to low	regular	regular	
F6.240	low to regular	regular	regular	Immature (+)
F6.219	good	regular	regular	
F6.404	low	regular	regular	
F6.192	low	regular	regular	
F6. 241	low to regular	regular	regular	Immature (+)
F6. 168	low to regular	regular	regular	
Canephora	The strange taste and odors interfer with the assesment of acidity, body and flavor.			Cereal (+++)

+ = ligh; ++ = little; +++ = intense

TABLE 2. Sensorial characteristics of the beverage of F6 progenies of Caturra x Timor Hybrid, components of the Colombia variety, and the control varieties, evaluated by the tasting panel of the Liophilized Coffee Factory, Chinchiná, Colombia.

Genotypes	Characteristics 1/			Intensity of strange tastes and odors 2/
	Acidity	Body	Flavor	
Caturra	7.8	8.5	8.5	Immature (3.0)
F6.321	7.4	8.4	8.4	
F6.170	7.0	8.5	8.7	
F6.203	6.0	8.1	8.4	
F6.206	6.8	8.5	8.4	
F6.293	6.8	8.3	8.4	
Typica (sample A)	6.5	8.3	8.4	Immature(2.5), sweet (2.0)
F6.240	5.8	8.2	8.2	
F6.219	5.2	8.1	8.2	
F6.404	4.6	7.5	8.0	
F6.192	5.2	7.8	8.1	Immature (2.7), Cereal (1.0)
F6.241	6.6	7.8	7.9	
F6.41	6.0	8.4	8.3	
Typica (sample B)	4.8	7.8	7.9	Immature (2.8), sweet (2.0)
Canephora	2.7	6.6	6.7	Cereal (8.0)

1/ Ranking of intensity from 1 to 10
2/ Ranking of intensity from 1 to 10

TABLE 3. Physical characteristics of the bean and quality of the beverage in progenies F6 of Caturra x Timor hybrid and in control varieties, evaluated by the tasting panel of the Hans Newman Company, Germany.

	Bean aspect		Cup quality	
Genotype	**Green**	**Roasted**	**Acidity**	**Body**
Results in 1980				
Typica(Check)	Flat	Good to very good	Medium	Medium to Complete
Bourbon (Check)	Borbon flat	Good to very good	Good to high	Complete
Progenie-41	Borbon	Good to very good	Good to high	Medium-complete
Progenie-206	Borbon	Good to very good	Good	Complete
Progenie- 240	Flat-borbon	Good to very good	Good to high	Medium-complete
Progenie-404	Borbon	Good to very good	Good to medium	Medium-good
Results in 1982				
Excellcel (Check 1)	Arabica	Good to very good	Intermediate	Good -complete
Excellcel (Check 2)	Arabica	Good to very good	Good to high	Good
Progenie-28	Flat	Good to very good	Intermediate to good	Complete
Progenie-168	Flat	Good to very good	Intermediate to good	Complete
Progenie-222	Borbon	Good to very good	Intermediate	Complete
Progenie-293	Flat	Good to very good	Complete	Complete
Progenie-404	Borbon	Good to very good	Intermediate	Complete

TABLE 4. Sensorial characteristics of the beverage of seven coffee genotypes evaluated by four international panels.

		PANELS			
Genotype	**Character**	**U.S.A.**	**CANADA**	**U.K.**	**COLOMBIA**
Red Colombia Sample 1	Acidity	greenish	medium		medium-high
	Body	high	medium	complete	medium
	Flavor				fragrant
	Other		typical taste (7.0)	good quality (01)	balanced taste
Red Colombia Sample 2	Acidity	greenish	low		medium-high
	Body	high			high
	Flavor				fragrant
	Other		(6.0)	good quality (01)	balanced taste
Yellow Colombia Sample 1	Acidity		low		medium-high
	Body				low
	Flavor	acceptable			poor
	Other		poor (6.0)	green wood (02/03)	ordinary taste
Yellow Colombia Sample 2	Acidity	balanced- delicate	medium-high	some greenish	high
	Body	medium	medium	thin.empty	high
	Flavor				fragrant
	Other		good roasting (7.5)	some hardness (01/02)	citric taste
Caturra	Acidity	balanced - delicated	medium-high	some greenish	very high
	Body	medium	medium		high
	Flavor				fruit
	Other		sweetness (8.0)	good quality (01)	citric taste
Bourbon	Acidity	balanced - delicated	low	satisfactory	medium-high
	Body	medium		lacking	high
	Flavor				herbal
	Other		poor roasting (6.0)	some hardness (01 borderline)	citric taste
Typica	Acidity	greenish	medium		medium-high
	Body	high	medium		high
	Flavor				fragrant
	Other		typical taste (7.0)	good quality (01)	aggresive acidity
Evaluation system		Descriptive	Hedonic judgment 7= standard <5=inaceptable	01= arabica coffee defects free 01 borderline= Clean coffee with no defects, but not optimun 02 = With some defect 03 = Undesirable	Descriptive

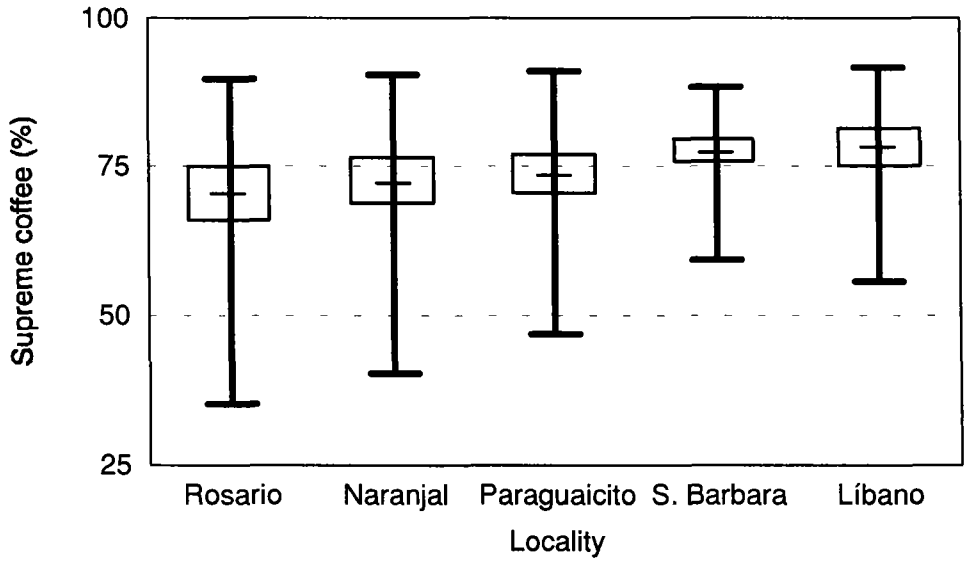

FIGURE 1. Average, confidence limits and variation intervals of the bean size of the actual components of the Colombia variety measured in five different locations in the Colombian coffee production area.

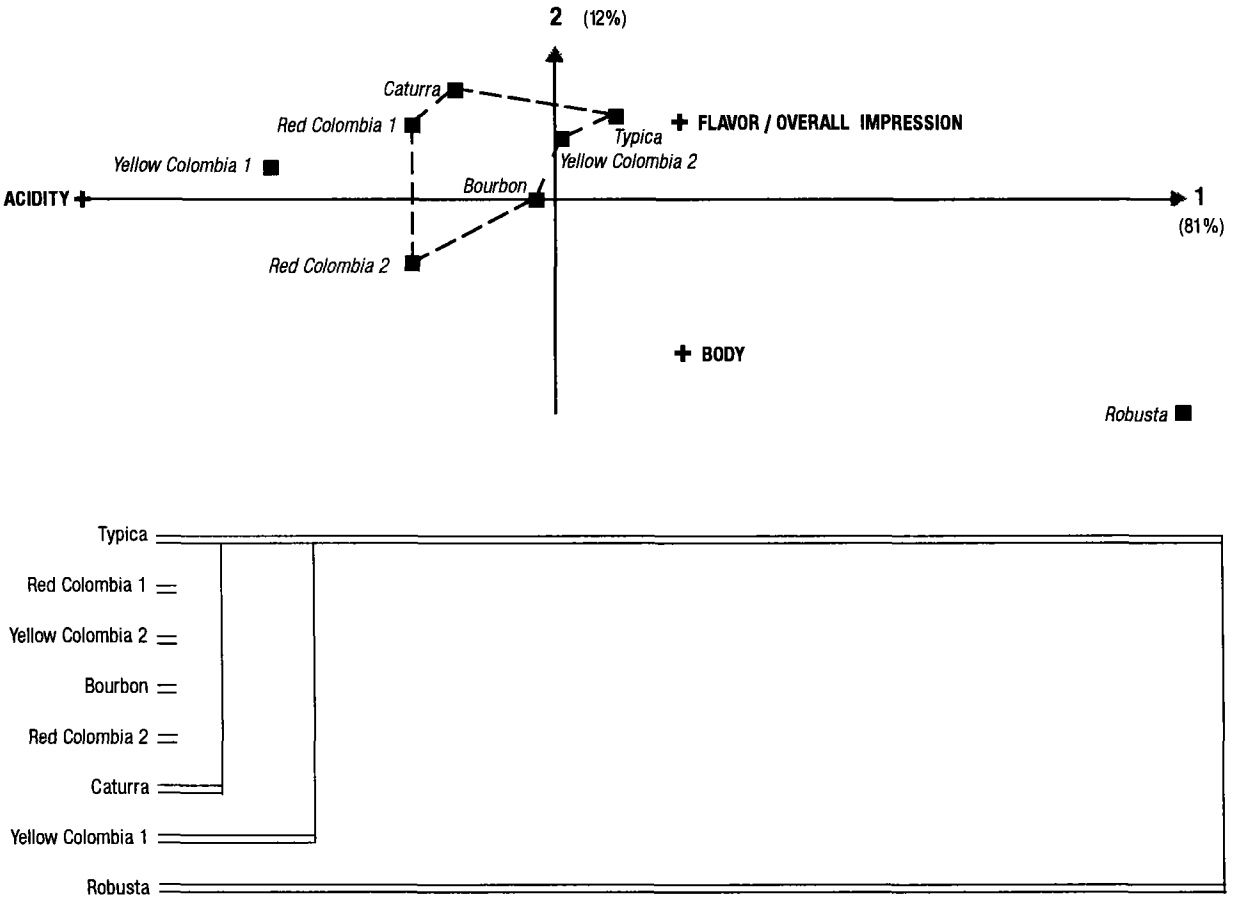

FIGURE 2. Representation of the genotypes (■) and variables (✚) used to measure the quality of the beverage, on the plane 1-2 of the factorial analysis of correspondence (up).
Grouping of the genotypes according to the classification by asscendent ranking (down)

COFFEE GENETICS AND QUALITY

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INTRODUCTION

Coffee (*Coffea arabica L.*) as been grown commercially in the State of Hawaii for over 150 years. For most of this time, production has been confined to the Kona District on Island of Hawaii. In recent years, coffee production has expanded to other islands in the Hawaiian chain, primarily onto land formerly cultivated in sugar cane. Along with this expansion has been the need to identify cultivars suitable for the new production areas.

Three major criteria have been used by planters in the screening of coffee cultivars: (1) agronomic adaptability, (2) suitability for mechanized production (especially harvesting), and (3) cup quality. Given the high cost of production in the State of Hawaii and the position of Hawaiian coffee in the growing specialty coffee trade, it is essential that cultivars meet accepted standards in all three of these categories. This paper will focus on the third of these categories, the relationship between cultivar and cup quality.

The influence of genetic and environmental factors on bean size and cup quality is well known (de Gialluly 1959, Cannel 1994). Cavaletto et al. (1991) demonstrated these relationships in Hawaii using traditional cultivar in sites throughout the state. This paper examines the relationship between quality and genetics within a commercial context on a single plantation on the Island on Maui. It highlights the importance of cultivar selection in meeting the demands of the growing U.S. specialty trade. It also illustrates the trade-off between quality and agronomic performance that the planter often faces.

MATERIALS

Test plantings of a wide selection of coffee cultivars (*C. arabica*) were established in 1986 above the Kaanapali district on the property of Pioneer Mill Company on the Island of Maui. The site utilized in this study is at a latitude of 21°55' and an elevation of 366 meters. It has an average annual

rainfall of 890 mm and an average annual temperature of 24°C. The trees were grown with the aid of supplemental drip irrigation. The soil in the test area is a well drained, Kahana silty clay with a pH of 6.0.

METHODS

Sample Preparation: During both 1991 and 1993, coffee cherries of selected cultivars were harvested at peak season (October through December), mechanically pulped and prepared uniformly using standard wet processing methods. Samples were dried to a moisture content of approximately 11 percent, hulled mechanically, and classified using standard screens.

Every effort was made to handle the different cultivars in a uniform manner at each step from harvesting through the final sample preparation. The names of the cultivars included in this study during the 1991 and 1993 harvest seasons are listed in the results section. Included are both traditional and newer cultivar selections.

Organoleptic Evaluation: Green coffee samples of the different cultivars were sent on a blind basis to cuppers within coffee companies throughout the U.S. specialty trade. Samples were roasted individually by each company and evaluated using a standardized format. Evaluators were asked to rate each cultivar sample on a scale of 1 to 10 (1 = lowest, 10 = highest) in each of the following five categories: acidity, body, aroma, flavor and overall. Written comments were included with the evaluation forms as appropriate. Duplicate samples of selected cultivars were included as checks. Fourteen different evaluators participated in 1991 and 19 in 1993. Data were normalized for each evaluator in order to utilize the full range of the evaluation scale.

RESULTS

Green Bean Size Grades: The distribution of green bean size grades varies among the different cultivars. Table 1 shows the grade distribution of the cultivars evaluated during the 1991 harvest season. The average for the test plot fell between a 16 and 18 standard screen size and most cultivars were in that range. Pretoria had the largest bean and was predominantly larger than an 18 screen size. On the other end of the spectrum, Mokka was the smallest, with most beans classified at less than a standard 16 screen size.

Cupping Results: Table 2 summarizes the overall ratings of the cultivars evaluated during the 1991 harvest season. The acidity, body, aroma, and flavor scores are shown as a ranking (from 1 to 17). It is important to note that bean size is not a good indicator of cup quality among varieties. Table 3 summarizes the ratings for the 1993 harvest season. The differences in the list of cultivars for the two seasons are due to the elimination from consideration of unacceptable cultivars and the addition of other promising candidates to the selection process.

There is a general consistency in the overall ranking of cultivars between the two seasons. In both cases, a selection from the plantation's screening program was the highest rated cultivar (Pioneer Selection). Similarly, a selection of Typica (P 502) was the lowest rated cultivar in both seasons. The relative rankings of cultivars between these two extremes were also fairly consistent.

Evaluators provided descriptive notes as appropriate for many of the samples. From these notes, it can be determined that the cup quality differences demonstrated among the different cultivars are of commercial significance and

would impact the price received for the coffee. The highest rated samples were generally regarded as "above average" to "excellent" against a commercial specialty coffee standard. The lower rated samples, while free of defects, were generally not acceptable for the U.S. specialty trade.

Table 1: 1991 Screen Size Distribution of Green Beans

	Screen Size				
Cultivar Name	> 18	> 16	< 16	< 14	Peaberry
Kent	47%	42%	3%	1%	6%
Pink Bourbon	11%	69%	14%	2%	4%
Blue Mountain	43%	41%	6%	2%	7%
Pretoria	89%	7%	1%	2%	1%
Red Caturra	21%	61%	9%	2%	6%
Yellow Catuai	11%	67%	13%	3%	6%
Guatemalan (Typica)	26%	60%	8%	3%	3%
Mokka	5%	22%	53%	15%	5%

Table 2: 1991 Cupping Results

	Overall	RANKINGS			
Cultivar Name	Rating	Acidity	Body	Aroma	Flavor
Pioneer Selection	7.9	1	4	3	1
Guadeloupe	6.9	3	5	2	2
Kent	6.8	2	6	7	5
Pink Bourbon	6.5	4	1	6	4
Yellow Caturra	5.6	9	7	11	3
Blue Mountain	5.4	6	10	1	6
Mundo Novo	5.4	8	16	8	12
Red Caturra	5.4	5	3	4	8
Mokka	5.2	15	9	9	9
Red Catuai	5.1	12	2	14	7
Red Catuai (BGR)	4.8	13	12	15	14
Pretoria	4.8	10	13	12	13
Guatemalan (Typica)	4.2	11	14	5	11
Yellow Catuai	4.0	14	11	13	16
P 502	2.0	17	17	17	17

Scale: 10 = Best, 1 = Worst. Samples Size = 14. Ratings normalized to utilize full range.

Table 3: 1993 Cupping Results

Cultivar Name	Overall	RATINGS Acidity	Body	Aroma	Flavor
Pioneer Selection	9.0	8.8	6.6	8.1	8.7
Red Catuai (89–8CR)	7.5	8.0	5.7	5.7	8.0
Guadeloupe	7.5	7.5	6.4	6.8	7.3
Red Catuai (89–7PN)	7.4	6.9	7.5	6.3	6.6
Mokka	7.1	5.7	6.0	7.0	6.7
Yellow Catuai (89–5PN)	7.0	6.5	7.1	6.2	6.4
Yellow Catuai (89–6)	6.9	7.1	5.3	5.2	7.2
Red Catuai (BGR)	6.5	6.2	5.7	6.7	6.3
Mundo Novo (89–3)	6.3	5.7	5.9	7.4	6.0
Yellow Caturra	6.0	5.5	5.1	5.7	6.1
Guatemalan (Typica)	6.0	5.6	4.6	5.9	5.3
Red Catuai	5.2	4.2	4.9	6.5	4.3
P502	4.2	4.5	5.1	4.3	4.1

Scale: 10 = Best, 1 = Worst. Sample Size = 19. Data normalized to utilize full range.

Table 4: Agronomic Rating Vs. 1993 Cup Quality Rating

Cultivar Name	Agronomic Rating	Overall Rating
Pioneer Selection	Med/Low	9.0
Red Catuai (89–8CR)	High	7.5
Guadeloupe	Medium	7.5
Red Catuai (89–7PN)	High	7.4
Mokka	Medium	7.1
Yellow Catuai (89–5PN)	High	7.0
Yellow Catuai (89–6)	High	6.9
Red Catuai (BGR)	High	6.5
Mundo Novo (89–3)	Med/High	6.3
Yellow Caturra	Med/High	6.0
Guatemalan (Typica)	Medium	6.0
Red Catuai	High	5.2
P502	Medium	4.2

Scale: 10 = Best, 1 = Worst. Data normalized to full range.

Agronomic Characteristics: Table 4 summarizes the agronomic ratings at Pioneer Mill Company of the different cultivars along with the overall quality scores from the 1993 harvest season. The agronomic rating combines several factors important within the Hawaiian production system. These include: overall plant vigor, yield potential, plant stature, and machine harvestability. As can be seen from the table, many of the better cultivars for cup quality (Pioneer Selection, Guadeloupe, and Mokka) received a medium or lower rating for their agronomic characteristics. Only one cultivar, a line of Red Catuai, combined high scores in both of these areas. Table 4 illustrates the difficulty of combining both excellent cup quality and superior agronomic performance in a single cultivar under Hawaiian conditions.

DISCUSSION

The coffee cultivars from the test plots at Pioneer Mill Company differ in both bean size and cup quality. The quality differences were great enough to be commercially significant within the context of the U.S. specialty trade. It is also important to stress that the quality differences are fairly consistent between years.

Bean size is not a good indicator of cup quality when comparing cultivars from a single production area. By combining the data from Tables 1 and 2, it is clear that the relationship between size and cup quality is weak. This finding is at odds with the commonly held market belief that larger bean sizes are superior in quality, irrespective of cultivar. It is more likely that bean size would be a useful indicator of cup quality within a single cultivar as it relates to horticultural practices and environmental conditions during a given growing season.

Cup quality is only one of the important factors that must be considered by the planter in selecting cultivars. Agronomic performance and production cost issues must also be considered. In Hawaii, the suitability of a cultivar for mechanized production is especially important. Cup quality and other important cultivar characteristics are often at odds with one another. It is important to determine the quality standards of the target market before final selections are made.

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BIOSYNTHETIC PATHWAYS OF CAFFEINE IN *COFFEA ARABICA* LEAVES

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Introduction

Caffeine (1,3,7-trimethylxanthine) and theobromine (3,7-dimethylxanthine) have been found in more than 60 sub-tropical plant species (see17). Many plants used for non-alcoholic beverages, such as tea (*Camellia sinensis* L.), coffee (*Coffea arabica* L), cocoa (*Theobroma cacao*) and maté (*Ilex paraguariensis*), contain these purine alkaloids (19). The biosynthesis pathway of theobromine and caffeine has been the subject of much study over the years. Although early investigations implied the involvement of nucleic acids as precursors in caffeine biosynthesis (15,18), more recent investigations with tea and coffee suggest that caffeine is produced from the purine nucleotides AMP, GMP and/or IMP, and that theobromine is the immediate precursor of caffeine (Fig. 1) (5,6,11,12,17). In coffee, young leaves that are not fully expanded, have the highest capacity for caffeine biosynthesis (5).

Recently, Nazario and Lovatt (9,10) have reported that theobromine is not converted to caffeine in coffee leaves and that two separate de novo and salvage pools are involved in the biosynthesis of theobromine. This raises serious questions on the validity of the caffeine biosynthesis pathway illustrated in Figure 1 and there is an urgent need for clarification. The current report describes an investigation of caffeine biosynthesis in coffee leaves in which metabolites originating from radiolabelled [8-^{14}C]adenine, [8-^{14}C]guanine, [8-^{14}C]xanthosine, [2-^{14}C]xanthine, [2–^{14}C]theobromine and [8-^{14}C]theophylline were analysed by HPLC-radiocounting (RC) and TLC. The data obtained enabled the the proposals of Nazario and Lovatt (9,10) to be scrutinized in detail.

Materials and methods

Plant material

Leaves were obtained in July and August 1994 from four-year old coffee plants (*C. arabica* L. cv. Kent) growing under a natural photoperiod in a greenhouse at the University of Glasgow. The developmental stages of the

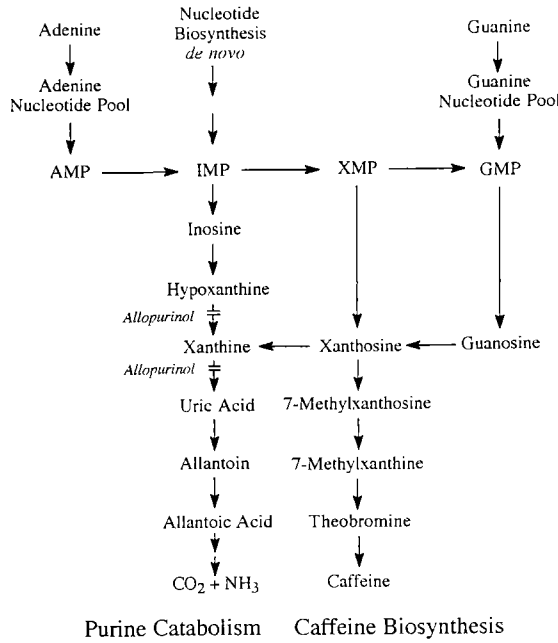

Figure 1. Metabolic pathways illustrating both the biosynthesis of caffeine from purine nucleotides and the catabolism of purine nucleotides to CO_2 and NH_3 in leaves of *C. arabica*.

leaves was categorised as (i) buds and young leaves, (ii) mature leaves and (iii) aged leaves. Young leaves were the most recently emerged, weighed ca. 25 mg (f.w.) and were ca. 20 mm long and 7 mm in width. Mature leavescomprised the fully expanded, second and third leaves below the apex (weight ca. 1.2 g) while aged leaves were dark green from near the base of the shoot and weighed ca. 1.3 g.

Chemicals

The following radiochemicals were purchased from the commercial sources indicated: [8-^{14}C]adenine (specific activity 1.96 MBq μmol^{-1}, Amersham International plc, Amersham, Buckinghamshire, UK), [8-^{14}C]guanine (2.1 MBq μmol^{-1}, Amersham International), [2-^{14}C]theobromine (2.07 MBq μmol^{-1}, Moravek Biochemicals Inc. Brea, CA, USA), [8-^{14}C]xanthosine (2.07 MBq μmol^{-1}, Moravek), [2-^{14}C]xanthine (1.94 MBq μmol^{-1}, Moravek) and [8-^{14}C]theophylline (2.04 MBq μmol^{-1}, American Radiolabelled Chemicals, Inc. St. Louis, Mo, USA). Radiolabelled uric acid, allantoin and allantoic acid were prepared in vitro from [2-^{14}C]xanthine using xanthine oxidase (buttermilk), uricase (*Candida utilis*) and allantoinase (peanut). These enzymes and all other chemicals were purchased from Sigma (St. Louis, MO, USA).

Extraction of endogenous xanthine derivatives

Segments of coffee leaves were boiled in water for 15 min and homogenised using a pestle and mortar. The resulting homogenate was centrifuged at 12000 g for 10 min after which the purine alkaloid content of an aliquot of the supernatant was analysed by reversed phase HPLC.

Metabolism of radiolabelled purine derivatives

Segments of *C. arabica* leaves (5 mm x 5 mm for mature and aged leaves; 5 mm strips of buds and young leaves) were incubated in 2 ml medium, comprising 30 mM potassium phosphate buffer, pH 5.6, 10 mM sucrose and a radiolabelled substrate, in a 30 ml Erlenmeyer flask, in a shaking water bath at 27°C. The Erlenmeyer flask

had a centre well containing a small glass tube into which was inserted a piece of filter paper wetted with 0.1 ml of a 20% potassium hydroxide solution. In pulse-chase experiments, after an appropriate period of time, the incubation medium was removed and replaced with fresh medium without the radiolabelled substrate.

At the end of the incubation period the glass tube and filter paper from the centre well were transferred to a 50 ml flask containing 10 ml of distilled water and, after thorough shaking, radioactivity in a 1 ml aliquot was determined by liquid scintillation counting in order to estimate the amount of $^{14}CO_2$ released during the metabolism period. The *C.arabica* leaf segments were separated from the incubation medium by filtering through a tea strainer, washed with 5 ml distilled water then mixed with 10 ml of extraction medium, comprising 80% methanol in 20 mM sodium diethyldithiocarbamate, and ground in a chilled pestle and mortar. The resultant tissue homogenate was centrifuged at 12000 g for 5 min and the supernatant and pellet separated. The pellet was resuspended in extraction medium and recentrifuged. The supernatant fractions, containing the methanol-soluble metabolites, were combined, reduced to dryness in vacuo and aliquots analysed by liquid scintillation counting, HPLC-RC and/or TLC. The pellet was treated with 1 ml 6% perchloric acid at 100° C for 20 min, to hydrolyse nucleic acids, after which the sample was centrifuged at 12000 g for 5 min. A 20 μl aliquot of the supernatant was removed and the solubilized radioactivity was estimated by liquid scintillation counting.

HPLC analysis of endogenous xanthine derivatives and radiolabelled metabolites

A Spectra Physics (San Jose, CA, USA) 8700 liquid chromatograph was used to deliver a 25 min, 0–40% gradient of methanol in 50 mM sodium acetate pH 5.0 at a flow rate of 1 ml min^{-1}, with samples being introduced off-column via a Rheodyne 7125 valve with a 500 μl loop. Reversed phase HPLC utilised a 250 x 4.6 mm i.d. universal ferruleless column (Capital HPLC Specialists, Broxburn, Lothian, UK), packed in-house with a 5 μm ODS Hypersil support (Shandon, Runcorn, Cheshire, UK). Column eluate was directed first to a Spectra Physics 8450 absorbance monitor operating at 270 nm, after which fractions were either collected or the solvent was mixed with liquid scintillant (10 g l^{-1} of 2,5 diphenyloxazole in Triton-X100/xylene/methanol [11:2:5, v/v]), pumped at a flow rate of 3 ml min^{-1} via a Reeve Analytical (Glasgow, UK) 9702 reagent delivery unit, and directed to a Reeve Analytical 9701 radioactivity monitor with a 500-μl spiral glass flow cell. Signals from both detectors were processed by a dual channel 2700 data handling system (Reeve Analytical). The reversed phase HPLC system successfully separated 11 different purine derivativesand was also able to resolve ^{14}C-labelled uric acid, allantoin and allantoic acid produced enzymically from [8-^{14}C]xanthine (4).

TLC analysis of radiolabelled metabolites

As nucleotides were retained on the reversed phase HPLC column, methanol-soluble radiolabelled metabolites were also subjected to TLC. Methanolic extracts extracts were subjected to TLC on 200 x 200 mm sheets of microcrystalline cellulose (Spotfilm, Tokyo Kasei Kogyo Co., Tokyo, Japan) using a solvent of n-butanol/acetic acid/water (4:1:2, v/v). Radiolabelled spots, detected after a one week exposure using Kodak X-OMAT AR film (Eastman Kodak Co., Rochester, NY, USA), were scraped off the TLC plates, eluted from the cellulose support with water and the radioactivity measured by liquid scintillation counting (1).

Results

Levels of endogenous xanthine derivatives

Only caffeine, theobromine and xanthine were present in detectable quantities when leaf and bud extracts were analysed by HPLC (Table 1). Buds and young leaves contained the highest concentration of caffeine with about half as much being detected in mature and aged leaves. Theobromine and xanthine were present in much lower

Table I. *Concentrations of endogenous caffeine, theobromine and xanthine in buds and young, aged and mature leaves of* C. arabica. *Similar data obtained in a duplicate experiment. Data expressed as mg g^{-1} fresh weight.*

Tissue	Caffeine	Theobromine	Xanthine
Buds	5.7	1.2	0.07
Young Leaves	6.5	0.98	0.17
Mature Leaves	2.6	0.13	0.09
Aged Leaves	3.1	0.09	0.02

concentrations than caffeine and the levels of theobromine, in particular, were markedly reduced in mature and aged leaves. Theophylline (1,3–dimethylxanthine) and paraxanthine (1,7-dimethylxanthine) were not detected in any of the leaf extracts.

Metabolism of [8-^{14}C]adenine

Figure 2 illustrates the results of pulse-chase experiments with [8–^{14}C]adenine and young leaves of *C. arabica*. Nucleotides, nucleic acids and theobromine were the most heavily labelled compounds after the 6 h pulse. The radioactivity associated with these compounds, as well as 7–methylxanthosine and 7–methyxanthine, declined after the leaves were transferred to a non–radioactive medium. In contrast, ^{14}C-labelled caffeine and CO_2 increased

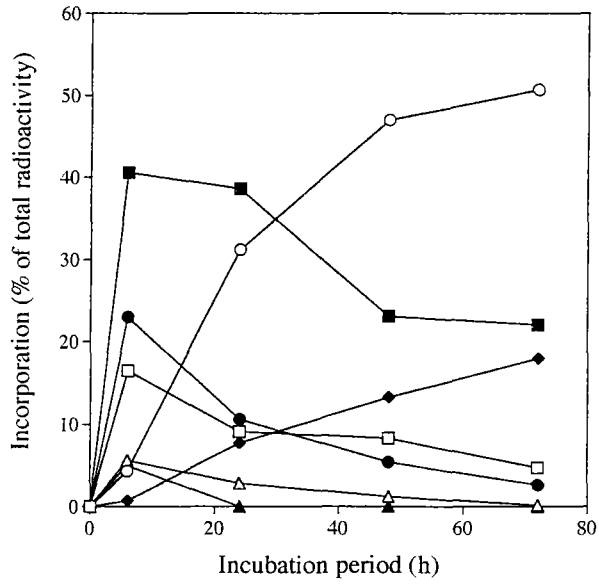

Figure 2. Distribution of radioactivity in metabolites from [8-^{14}C]adenine after a 'pulse-chase' experiment with young leaves of *C. arabica*. Leaves (50 mg f.w.) were incubated with 9.5 μM [8–^{14}C]adenine for 6 h after which the radioactivity was 'chased' for a further 66 h. Incorporation of radioactivity is expressed as a percentage of total radioactivity taken up by the leaves (7.1 $\pm$ 0.3 kBq 50 mg^{-1} f.w.). Symbols: —□— nucleotides, —●— theobromine, —○— caffeine, —△— 7–methylxanthosine, —▲— 7-methylxanthine, —◆— CO_2, —■— RNA.

after the pulse period. The accumulation of [^{14}C]caffeine in this manner is in keeping with the fact that the alkaloid is synthesized much more rapidly than it is catabolised in *C. arabica* shoots (4). The data presented in Table 2, which were obtained after an 18 h incubation with [8–^{14}C]adenine indicate that the capacity of leaves to convert adenine into theobromine and caffeine is highest in young leaves and decreases as the leaves mature and age.

Table 2. *Overall metabolism of [8- ^{14}C]adenine by young, mature and aged leaves of* C. arabica. *Leaf samples incubated with 9.4 µM [8–^{14}C]adenine (1.96 MBq µmol^{-1}) for 18 h. Total uptake of radioactivity expressed as kBq 100 mg^{-1} of leaf (f.w.) ± S.E. Incorporation of radioactivity into metabolites expressed as a percentage of total uptake ± S.E. n.d - not detected.*

	Leaf type		
Metabolites	Young	Mature	Aged
Xanthine	8.4±1.7	5.4±0.8	3.4±0.3
7-Methylxanthine	3.7±0.7	3.4±0.7	0.8±0.1
Theobromine	26.2±3.2	10.3±0.1	1.4±0.8
Caffeine	20.5±3.5	7.1±0.7	0.8±0.2
Theophylline	n.d	n.d	n.d
Other soluble compounds	4.4±0.5	13.5±0.6	7.8±0.8
CO_2	3.2±0.2	10.9±0.2	36.2±3.6
Nucleic acids	35.0±0.7	48.9±0.5	49.6±3.6
Total uptake of radioactivity	12.3±3.0	15.0±0.2	18.5±2.3

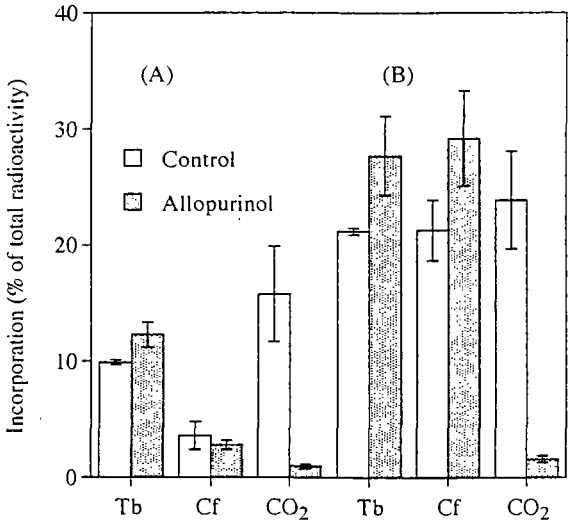

Figure 3. Effect of 5 mM allopurinol on the incorporation of radioactivity from [8-^{14}C]adenine into theobromine (Tb), caffeine (Cf) and CO_2 in buds and young leaves of *C. arabica*. Leaf samples (100 mg f.w.) were incubated for 18 h with 9.5 µM [8-^{14}C]adenine. In corporation of radioactivity is expressed as a percentage of the total radioactivity taken up by the young leaves and buds.

The effects of the hypoxanthine analogue, allopurinol, which an inhibitor of xanthine dehydrogenase/oxidase (14), on [8-^{14}C]adenine metabolism in buds and young leaves were investigated. The data obtained are presented in Figure 3. Although the release of $^{14}CO_2$ from [^{14}C]adenine was reduced greatly by 5 mM allopurinol, there was little or no effect on the incorporation of radioactivity into theobromine and caffeine. This observation contrasts with the findings of Nazario and Lovatt (9,10) who reported that allopurinol treatment of *C. arabica* leaves results in a marked reduction in the incorporation of [8-^{14}C]adenine in caffeine.

Metabolism of [8-^{14}C]guanine

Figure 4 illustrates the analysis of methanol-soluble metabolites of [8–^{14}C]guanine by HPLC–RC. In addition to the guanine substrate, the radiolabelled compounds detected included caffeine, theobromine, 7–methylxanthine, allantoin and allantoic acid. The incorporation of radioactivity into theobromine and caffeine was higher in young than in mature and aged leaves (Fig. 4, Table 3). This may be due partly to a decrease in guanine salvage activity as

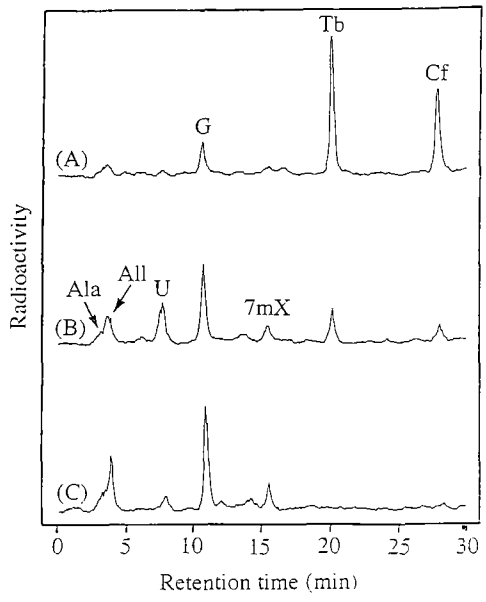

Figure 4. Reversed phase HPLC-RC analysis of methanol-soluble [8–^{14}C]guanine metabolites from (**A**) young, (**B**) mature and (**C**) aged leaves of *C. arabica*. Leaves (100 mg f.w.) incubated with 8.9 μM [8-^{14}C]guanine for 18 h prior to extraction. Ala - allantoic acid, All - allantoin, U - uric acid, G - guanine, 7mX - 7-methylxanthine, Tb - theobromine, and Cf - caffeine.

the leaf matures and ages. The decrease in the incorporation of radioactivity from [8-^{14}C]guanine into nucleic acids observed in the older leaves supports this possibility. In contrast, degradation of [8–^{14}C]guanine, as indicated by the release of $^{14}CO_2$, increased during leaf development (Table 3) and this was accompanied by an increase in the level of radioactivity associated with the purine catabolites, allantoin and allantoic acid (Fig. 4). Most of these degradation products appear to be produced directly from guanine by conventional purine catabolism pathways, independent of the production of purine alkaloids (see Fig. 1).

Metabolism of [8-^{14}C]xanthosine and [2- ^{14}C]xanthine

Figure 5 illustrates the HPLC-RC profiles obtained with the methanol-soluble fractions obtained from leaves of *C. arabica* after an 18 h incubation with [8–^{14}C]xanthosine. Incorporation of radioactivity into caffeine was detected only in young leaves. With all three types of leaf, most of the applied substrate was converted to allantoin and allantoic acid. Similar experiments carried out with [2-^{14}C]xanthine demonstrated that label was converted to

allantoin, allantoic acid and CO_2, but not theobromine and caffeine, even in young leaves (data not shown). This contrasts with the findings of Nazario and Lovatt (9,10) who reported that [8-^{14}C]xanthine is incorporated into both theobromine and caffeine.

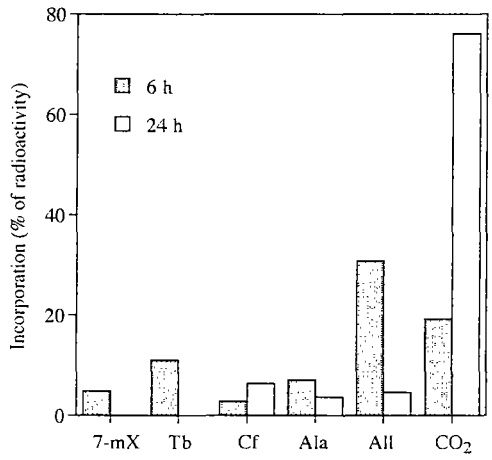

Figure 6. Distribution of radioactivity in metabolites from [8-^{14}C]xanthosine after a 'pulse-chase' experiment with young leaves of *C. arabica*. Leaves (60 mg f.w.) were incubated with 8.9 μM [8-^{14}C]xanthosine for 6 h after which the radioactivity was 'chased' for a further 18 h. Incorporation of radioactivity is expressed as a percentage of total radioactivity taken up by the leaves (0.75 $\pm$ 0.01 kBq 60 mg^{-1} f.w.). Ala - allantoic acid, All - allantoin, 7mX - 7-methylxanthine, Tb - theobromine, and Cf - caffeine.

In order to examine the caffeine biosynthesis pathway from [8-^{14}C]xanthosine, pulse-chase experiments were carried out with young leaves (Fig. 6). 7-Methylxanthine and theobromine were both labelled during the 6 h "pulse" but no radioactivity was associated with these compounds after a 24 h "chase". Some of the label was converted to caffeine but the majority appears to undergo degradation and be released as $^{14}CO_2$ (Fig. 6).

Metabolism of [2-^{14}C]theobromine and [8-^{14}C]theophylline

The metabolism profile of the methanol-soluble fraction obtained after feeding [2-^{14}C]theobromine revealed that theobromine is converted exclusively to caffeine by young, mature and aged leaves, after an 18 h incubation period (Fig. 7). The total amount of [^{14}C]caffeine to accumulate was 96,632 dpm (100%) in young leaves, 63,828 dpm (66%) in mature leaves and 36,828 (33%) in aged leaves. This implies that that the capacity of leaves to carry out this 1-N methylation step does not decline with age to the same extent as other sections of the caffeine biosynthesis pathway as indicted by the level of conversion of radiolabelled adenine, guanine and xanthosine to caffeine in young, mature and aged leaves (Tables 2 & 3, Fig. 6).

Unlike all the other labelled substrates investigated, with the exception of [2-^{14}C]xanthine, no incorporation of radioactivity from [8-^{14}C]theophylline into caffeine was detected (data not shown).

Discussion

There is strong evidence that the biosynthesis of caffeine is initiated by the degradation of purine nucleotides (1,26,12). In higher plants, as well as almost all other organisms, purine nucleotides are synthesized by both de novo and salvage pathways (see 7,16,20). The metabolic fate of purine nucleotides is usually investigated using radiolabelled purine bases and nucleosides (21). Since adenine is not readily degraded in higher plants as they lack adenine deaminase (8), it is relatively easy to follow the metabolic fate of adenine nucleotides that have been prelabelled with [8-^{14}C]adenine. Although guanine can be degraded by guanine deaminase, the activity of this enzyme in plants is low (13) so exogenous [8-^{14}C]guanine is usually converted to [8-^{14}C]GMP in significant amounts by hypoxanthine-guanine phosphoribosyltransferase (7).

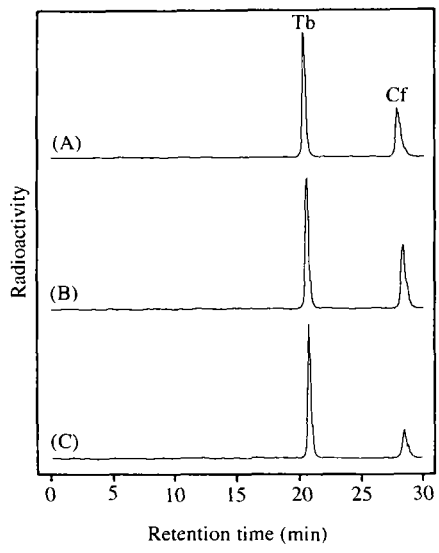

Figure 7. Reversed phase HPLC-RC analysis of methanol-soluble [2-^{14}C]theobromine metabolites from (A) young, (B) mature and (C) aged leaves of *C. arabica*. Leaves (100 mg f.w.) incubated with 8.9 μM [2-^{14}C]theobromine for 18 h prior to extraction. Tb - theobromine, Cf - caffeine.

In the present study, adenine and guanine nucleotides were prelabelled by feeding [8–^{14}C]adenine and [8–^{14}C]guanine, and the biosynthesis of caffeine from these purine nucleotides was investigated. Data obtained in pulse–chase experiments with [8–^{14}C]adenine (Fig. 2, Table 2) suggest strongly that caffeine is synthesized from adenine nucleotides via 7–methylxanthosine, 7–methylxanthine and theobromine, as illustrated in Fig. 1. The demonstrated conversions of [8–^{14}C]xanthosine to theobromine and caffeine (Figs. 5 and 6) and that of [2–^{14}C]theobromine to caffeine (Fig. 7) provide convincing evidence for the operation of this pathway. These findings clearly refute the proposal of Nazario and Lovatt (9,10) that theobromine is not an immediate precursor of caffeine in *C. arabica* leaves.

Nazario and Lovatt (9,10) presented data indicating that while allopurinol inhibited the conversion of [8–^{14}C]adenine to caffeine by more than 50% it had no effect on the incorporation of label into theobromine . This implies that the biosynthesis of caffeine from adenine involves reactions catalysed by xanthine dehydrogenase/oxidase and it was concluded that caffeine is synthesized from the degradation of purines via hypoxanthine, xanthine and paraxanthine, while theobromine is produced from adenine nucleotides (9,10). The data obtained in our studies do not confirm these proposals. In our experiments, although allopurinol strongly inhibited purine catabolism, it also brought about small increases in the incorporation of [8-^{14}C]adenine into both theobromine and caffeine (Fig. 3). This, presumably, was due to an increase in the size of the adenine nucleotide pool facilitated by the inhibitory effects of allopurinol on purine catabolism (see Fig. 1)

Biosynthesis of caffeine from [8-^{14}C]guanine in *C. arabica* leaves was also demonstrated in the current studies. The relatively high level of accumulation of [^{14}C]caffeine in young coffee leaves (Fig. 4A) implies that the applied guanine is converted to GMP (see Fig. 1). Since GMP reductase is not functional in plant cells (3), 5′–nucleotidase and guanosine deaminase (13) are probably responsible for the metabolism of GMP to guanosine and onto xanthosine which is incorporated into the caffeine biosynthesis pathway. Although [8–^{14}C]guanine was clearly converted to theobromine and caffeine, no incorporation of label into either theophylline or paraxanthine was observed (Fig. 4, Table 3). This finding does not lend credence to the hypothesis of Nazario and Lovatt (9,10) that theophylline is synthesized from guanine nucleotides.

The data obtained in the present study demonstrate that there is a marked decline in the rate of biosynthesis of caffeine from adenine, guanine and xanthosine as *C. arabica* leaves age, but because caffeine is catabolised very

slowly (4), there is not a concomitant reduction in the concentration of endogenous caffeine (Table 1). The reduced rate of incorporation into caffeine is probably an indirect consequence of enhanced purine catabolism in the mature and aged leaves which restricts the availability of xanthosine as a substrate for caffeine biosynthesis (see Fig. 1). In contrast to adenine, guanine and adenosine, the rate of conversion of theobromine to caffeine is not influenced greatly by leaf age (Fig. 7). Whether other committed steps in the caffeine biosynthesis pathway, behave in a similar manner, remains to be determined.

The major discrepancies between our findings and the data of Nazario and Lovatt (9,10) may result from the use of different plant material and experimental methods. Our data was obtained mainly with very young leaves of *C. arabica*, which have a high capacity for caffeine biosynthesis, and overall metabolism was examined by TLC and high resolution HPLC–RC after the application of radiolabelled precursors in doses that were usually less than 10 μM. In their studies, Nazario and Lovatt (9,10) used older, fully expanded leaves, much higher doses of radiolabelled substrates (2-5 mM) and the incorporation of label into individual metabolites was determined by co-crystalisation, isotopic dilution analysis. They also utilised a number of "non-committed" radiolabelled substrates, such as bicarbonate, formate and glycine, whose low level incorporation into purine alkaloids, especially in long-term experiments, could well be a consequence of the introduction of label into the purine ring and/or substituent methyl groups via indirect secondary pathways.

In conclusion, the data obtained with *C. arabica* leaves in the present study demonstrate that caffeine is synthesized via the routes illustrated in Figure 1 in which theobromine is the immediate precursor of caffeine rather than the end point of a separate pathway. Furthermore, theophylline and paraxanthine are not major intermediates in the in vivo biosynthesis of caffeine.

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Summary

The levels of endogenous caffeine and theobromine in buds and young leaves of *Coffea arabica* L. cv. Kent were much higher than in fully developed leaves. Biosynthesis of caffeine from ^{14}C-labelled adenine, guanine, xanthosine and theobromine was observed but there was no detectable incorporation of label into caffeine when theophylline and xanthine were used as substrates for in vivo feeds. The capacity for caffeine biosynthesis, especially from guanine and xanthosine, was reduced markedly in both fully-developed mature and aged leaves. Data obtained in pulse-chase experiments with young leaves indicate the operation of an AMP → IMP → XMP (or GMP → guanosine) → xanthosine → 7-methylxanthosine → 7-methylxanthine → theobromine → caffeine pathway. The data obtained provide strong evidence against recent proposals by Nazario and Lovatt (Plant Physiology 103, 1203-1210, 1993) on the independence of caffeine and theobromine biosynthesis pathways.

IN *VITRO* BIOSYNTHESIS OF CAFFEINE : THE STABILITY OF *N*-METHYLTRANSFERASE ACTIVITY IN CELL-FREE PREPARATIONS FROM LIQUID ENDOSPERM OF *COFFEA ARABICA*

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Introduction

Caffeine accumulates in more than 60 plant species, including tea (*Camellia sinensis* L.) and coffee (*Coffea arabica* L.) [1]. The many studies carried out on caffeine metabolism suggest that caffeine is synthesised from purines nucleotides via a xanthosine ->7-methylxanthosine -> 7-methylxanthine -> theobromine (3,7-dimethylxanthine) -> caffeine (1,3,7-trimethylxanthine) pathway [for reviews see 2 and 3]. *S*-Adenosyl-L-methionine (SAM) is the methyl-group donor for the methylation reactions involved in the pathway (7-*N* methylation of xanthosine, 3-*N* methylation of 7-methylxanthine and 1-*N* methylation of theobromine). The *N*-methyltransferase activities involved have been studied in cell-free preparations from tea leaves [4] and *C. arabica* seed [5]. However, in both instances the extracts lost activity rapidly . It has recently been reported that the *N*-methyltransferase which catalyses the *N*-1 methylation reaction in preparations from *C. arabica* endosperm has been purified, enabling the N-terminal polypeptide sequence to be determined [6]. Although the protein was apparently purified to homogeneity as determined by SDS-PAGE, the specific activity declined as enzyme activity deteriorated markedly during purification. There is some evidence from co-purification studies that the 7-methylxanthine *N*-3 and theobromine *N*-1 methylations are catalysed by the same enzyme in *C. arabica* [6].

Detailed characterisation of these *N*-methyltransferase activities requires intact, purified enzyme. The present investigation was aimed at stabilisation of methylxanthine *N*-methyltransferase activity prior to purification and characterisation. Enzyme activity was investigated in cell-free extracts from liquid endosperm of immature seed of *C. arabica* cv. Mundo Novo. Liquid endosperm was selected as suitable material for this programme of research because in many plant species it has been shown to be a rich and stable source of gibberellin biosynthesis enzymes which in extracts from other plant tissues had previously been too unstable to purify [see 7, 8, 9].

Materials and Methods

Plant material

Immature seeds of *C. arabica* cv. Mundo Novo were harvested in February and March 1994 from trees in the germplasm bank at the Agronomic Institute, Campinas, São Paulo, Brazil. Liquid endosperm was removed from the seeds and frozen immediately in liquid nitrogen. Lyophilised endosperm was stored at -20°C.

Chemicals

Paraxanthine was obtained from Sigma Chemical Co. (Poole, U.K.). S-Adenosyl-L-[methyl-^{3}H]-methionine (SAM) (specific activity 555 GBq mmol^{-1}) was purchased from Amersham International plc. (Little Chalfont, UK). Q Sepharose Fast Flow chromatography media and PD-10 gel filtration columns (Sephadex G25-M) were obtained from Pharmacia Biotech. (Uppsala, Sweden). Protease inhibitors were purchased from Boehringer Mannheim GmbH (Mannheim, Germany).

Extraction

Freeze dried endosperm was ground to a fine powder with acid washed sand using a mortar and pestle. The powder was suspended in 15 vols of chilled 50 mM Tris/HCl pH 7.5 containing 5 mM dithiothreitol, 5 mM EDTA, 1.5% (w/v) polyvinylpolypyrrolidone and 0.5% (w/v) sodium ascorbate. The extract was centrifuged at 20 000 g for 20 min at 4°C and the resulting supernatant filtered through eight layers of muslin. The filtrate was run through a PD-10 gel filtration column at 4°C equilibrated in 50 mM Tris/HCl pH 7.5, containing 5 mM EDTA and 5 mM dithiothreitol. Aliquots of desalted extract were frozen on dry ice and stored at -20°C. Protein was determined by the method of Bradford [10].

Q Sepharose chromatography

Chromatography was carried out at 4°C using a Gilson biocompatible liquid chromatograph with a Rainin UV-1 absorbance monitor (Anachem Ltd., Luton, U.K.). A 40 ml Q Sepharose FF anion-exchange column (2.2 cm x 10.5 cm) was equilibrated in starting buffer (see legend, Table 2 for buffer details). The pooled desalted extract was loaded onto the column and washed with starting buffer at 4 ml min^{-1} until the absorbance at 280 nm had fallen to zero. A 200 ml continuous linear gradient of 0-400 mM NaCl (or KCl) in starting buffer was run at 4 ml min^{-1}. Protein elution was monitored at 280 nm and successive fractions containing *N*-methyltransferase activity were combined and stored at -20°C. A 1 ml aliquot of the pool was desalted on a PD-10 column for *N*-methyltransferase activity and protein determination free of NaCl inhibition.

N-Methyltransferase assay

The *N*-methyltransferase assay is based on the transfer of the ^{3}H-methyl group from [^{3}H]SAM to the substrate, paraxanthine (1,7-dimethylxanthine), producing [^{3}H]caffeine [11]. The labelled caffeine partitions into chloroform facilitating its separation from the labelled SAM which remains in the aqueous phase. Paraxanthine was used as the methyl acceptor instead of theobromine because the *N*-methyltransferase has a lower apparent K_m for paraxanthine than theobromine, making it a more sensitive assay substrate [4, 5]. Assays were carried out at 25°C in a 300 μl total volume with final concentrations of 100 mM Tris/HCl pH 8.3 containing 0.75 mM paraxanthine, 20 nM [methyl-^{3}H]SAM (555 GBq mmol^{-1}) and endosperm extract. Incubation was terminated with the addition of 1 ml of chloroform which was followed by immediate vortexing. The chloroform extract

containing radiolabelled caffeine was dried, the residue resuspended in 1 ml distilled water, scintillant added and the radioactivity measured by liquid scintillation counting. Blank rates were taken as the radioactivity in chloroform extracts from samples incubated in the assay cocktail in the absence of the cell-free preparation.

Protease inhibitor studies

Endosperm extract was incubated with protease inhibitors at 4°C and aliquots were removed at hourly intervals and assayed for *N*-methyltransferase activity. The protease inhibitor was used at the final concentration suggested by the manufacturer: 4 mM Pefabloc®SC, 0.17 μM chymostatin, 28 μM E-64, 1 mM phenylmethylsulphonylfluoride (PMSF), 0.3 μM aprotinin, 74 μM antipain-dihydrochloride, 130 μM bestatin, 1 μM leupeptin, 1 μM pepstatin and 0.6 μM phosphoramidon.

Results

Protease inhibitors

A possible cause of the instability of the methyltransferases involved in caffeine biosynthesis is proteolysis by endogenous proteases. Figure 1 shows the decay of *N*-methyltransferase activity from *C. arabica* endosperm extract at 4°C over a 3 h period. The *N*-methyltransferase activity has a half-life of approximately 90 min. A similar pattern of decay was obtained for extracts prepared at both pH 7.5 and pH 8.3 (data not shown).

The incubation was repeated in the presence of protease inhibitors with varied specificity: phenymethylsulfonylfluoride, Pefabloc SC and aprotinin (serine protease inhibitors), antipain-dihydrochloride (papain, trypsin and cathepsin A and B inhibitors), bestatin (aminopeptidase inhibitor), chymostatin (chymotrypsin inhibitor), E-64 (metalloprotease inhibitor), leupeptin (serine and cysteine protease inhibitor), pepstatin (aspartate protease inhibitor) and phosphoramidon (metallo endopeptidase inhibitor). None of the

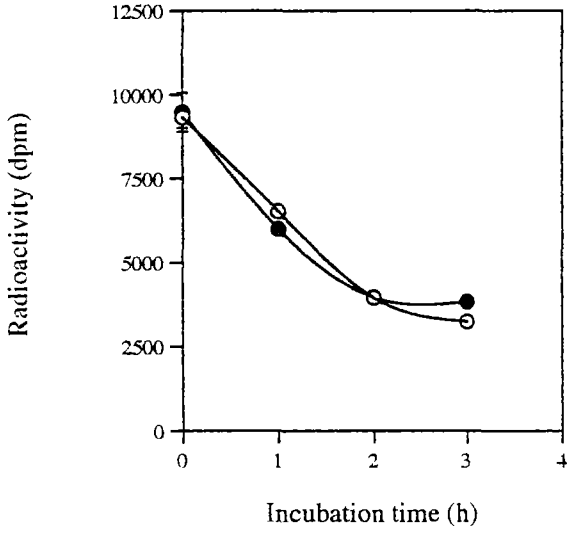

Figure 1. *N*-methyltransferase stability at 4°C. *C. arabica* endosperm extracts were incubated for 3 h in the presence and absence of pepstatin (1 μM). At hourly intervals 100 μl (54 μg protein) of the extract was removed and assayed for 5 min at 25°C for *N*-methyltransferase activity. Each assay was carried out in duplicate. o Extract, o Extract + pepstatin.

protease inhibitors tested had a stabilising effect on the *N*-methyltransferase activity and a typical decay curve in the presence of pepstatin is shown in Figure 1. Interestingly, the serine protease inhibitor Pefabloc SC inhibited the N-methyltransferase activity (Table 1) while none of the other serine protease inhibitors tested (aprotinin, phenylmethylsulphonylfluoride and leupeptin) had any effect.

Bovine serum albumin (BSA) (2mg/ml) also failed to have a stabilising effect on the *N*-methyltransferase activity in endosperm extracts (not shown). If proteases were present in the extract, the fall in *N*-methyltransferase activity would be expected to be slower due to the presence of BSA as an alternate substrate. This suggests that proteolysis of the *N*-methyltransferase is not the cause of instability in extracts.

Incubation with glycerol

The effect of glycerol on *N*-methyltransferase was examined. Figure 2 shows a time course of *N*-methyltransferase activity over 3 h at 4°C in the presence of 10% and 20% (v/v) glycerol. The data show that the stability of the enzyme is markedly improved in the presence of glycerol. The activity remaining after the 3 h incubation improved from 42% in the absence of glycerol, to 63% and 98% in the presence of 10% and 20% (v/v) glycerol respectively. The presence of 20% (v/v) ethylene glycol has a similar stabilising effect on activity (data not shown).

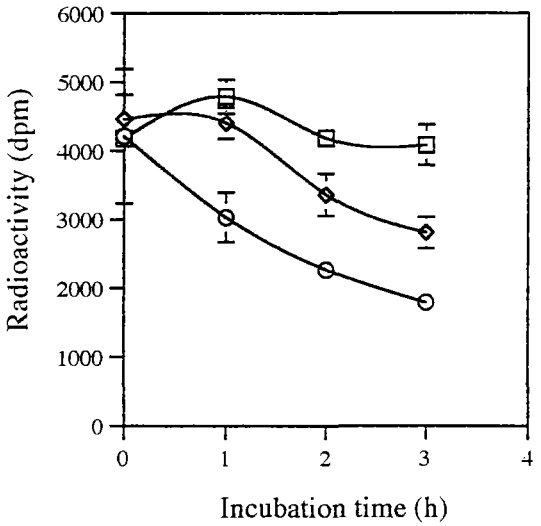

Figure 2. Effect of glycerol on *N*-methyltransferase stability. *C. arabica* endosperm extracts were incubated for 3 h at 4°C in the absence of glycerol and with 10% and 20% glycerol. 100 μl (52 μg protein) was removed at hourly intervals and assayed for N-methyltransferase activity for 5 min at 25°C. The standard deviation is shown for triplicate assays. o Extract, ◊ Extract + 10% (v/v) glycerol, □ Extract + 20% (v/v) glycerol.

Effect of inhibitors on N-methyltransferase activity

NaCl and KCl are commonly used salts for gradient formation during protein chromatography. Table 1 shows that both 0.5 M NaCl and 0.5 M KCl have an inhibitory effect on *N*-methyltransferase activity. This suggests that one of the reasons for the poor recoveries of *N*-methyltransferase activity following anion-exchange chromatography is due to an inhibition of the enzyme by the salt used in the gradient. Note that a NaCl gradient was used by Mazzafera *et al.* [6] to elute the *N*-methyltransferase activity from DEAE-cellulose. This would

result in an apparently poor yield and low specific activity for the enzyme. Interestingly, the serine protease inhibitor Pefabloc SC also inhibited the *N*-methyltransferase activity (Table 1)

Table 1. Effect of inhibitors on *N*-methyltransferase activity. *C. arabica* endosperm extracts (18 μg protein/assay) were assayed in duplicate for 5 min in the presence and absence of 1.7 mM Pefabloc SC. In a separate experiment endosperm extract (54 μg protein) was assayed in triplicate for 10 min alone or in the presence of 0.5 M NaCl or 0.5 M KCl. The radioactive caffeine produced for extract without inhibitors was taken as 100% activity, and the values in the presence of inhibitors are shown as a percentage of this value. The error is the standard deviation of the replicates.

Sample	Relative *N*-methyltransferase activity (%)
Extract	100
Extract + 1.7 mM Pefabloc SC	52 ± 7
Extract + 0.5 M NaCl	28 ± 1
Extract + 0.5 M KCl	35 ± 2

Effect of glycerol and desalting on recoveries from an anion exchanger

Table 2 shows the yields of *N*-methyltransferase from *C. arabica* endosperm following anion-exchange chromatography. The data shows that purification of *N*-methyltransferase in the absence of glycerol at pH 8.3 results in a low activity yield of 19%. Part of the reason for the poor recoveries is that the NaCl used to elute the enzyme was still present in the pooled fractions. The data also show that the *N*-methyltransferase yield resulting

Table 2. N-methyltransferase yield from Q Sepharose chromatography. The data show the purification of N-methyltransferase from C. arabica endosperm in the presence and absence of 20% (v/v) glycerol. The results of two separate experiments are shown for chromatography performed in the presence of glycerol. The yield is calculated against the activity in crude extract. The specific activity and purification is calculated for the desalted protein where applicable. All chromatography was carried out at 4°C. Buffer A: 50 mM Tris/HCl pH 7.5, containing 5 mM dithiothreitol and 5 mM EDTA. Buffer B: 20 mM Tris/HCl pH 8.3, containing 5 mM dithiothreitol and 5 mM EDTA. nd - not determined.

Anion exchanger	Buffer	Gradient	Specific activity (fkat mg^{-1} protein)	Purification (fold)	Yield before desalting (%)	Yield after desalting (%)
Without glycerol						
Q Sepharose FF	Buffer B	0-1M NaCl in buffer B	17.0	0.9	19	nd
With glycerol						
Q Sepharose FF	Buffer A containing 20% (v/v) glycerol	0-0.4M NaCl in buffer A containing 20% (v/v) glycerol	113 421	21 11	44 40	54 78

from chromatography at pH 7.5 when 20% (v/v) glycerol was present in all the buffers used, is considerably higher with 44 % and 54% recoveries even without desalting prior to assay. The yield was found to increase to 54 and 78 % respectively after desalting the pooled fraction by gel filtration using a PD-10 column. A direct comparison of the *N*-methyltransferase specific activities obtained by Mazzafera *et al.* [6] is not possible because theobromine was used as the methyl donor in assays. However, while the purification achieved by Mazzafera *et al.* [6] with anion-exchange chromatography fell to 0.23 fold, the purification in the present investigation, resulting from the addition of glycerol to buffers and desalting the pooled fractions was between 11 and 21 fold.

Discussion

The data show that *N*-methyltransferase activity is extremely labile in cell-free preparations from liquid endosperm of *C. arabica* which is comparable with observations in an earlier study where a 50% loss of activity was observed in 1 h in desalted extracts at 4°C [6]. This instability makes the purification of sufficient quantities of enzyme for characterisation extremely difficult. The present data indicate that the loss of activity in endosperm extracts does not result from proteolysis of the enzyme since incubation with a wide range of protease inhibitors failed to have a stabilising effect on activity.

The addition of 20% (v/v) glycerol to extracts was shown to have a marked stabilisation effect on the *N*-methyltransferase activity. The yield of *N*-methyltransferase is improved significantly by adding 20% (v/v) glycerol to the buffers used during anion-exchange chromatography. Mazzafera *et al.* [6] found that *N*-methyltransferase purified from *C. arabica* endosperm lost activity overnight at -20°C following anion-exchange chromatography. The addition of 20% (v/v) glycerol to the *N*-methyltransferase pool did not prevent the fall in activity and it was suggested it was due to the loss of a cofactor during purification on a DE52 anion-exchanger [6]. In the present study, there was no loss of activity when samples were incubated at -20°C for 24 h. The difference in stability may be due to the anion exchange media used.

The data obtained in this study indicate that the apparently low yields of *N*-methyltransferase observed during protein purification are due to the inhibition of the enzyme with salts, such as NaCl and KCl, which are commonly used to elute proteins during chromatography. The true yield can be determined by desalting an aliquot of chromatography fractions prior to assays.

A strategy for the purification of the *N*-methyltransferase activities involved in caffeine biosynthesis, incorporating glycerol and/or ethylene glycol in buffers, should facilitate the purification of sufficient quantities of the enzyme for detailed characterisation.

Acknowledgements

The authors would like to thank Professor Paulo Arruda, Centre for Molecular Biology and Genetic Engineering, Univesidade Estadual de Campinas, São Paulo, Brazil for arranging the collection of liquid endosperm and providing laboratory facilities for preliminary screenings of in vitro enzyme activity. This work is funded by a BBSRC grant to A.C. and G.I.J.

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Summary

A cell-free system has been developed from *Coffea arabica* that is a rich source of the *N*-methyltransferase activity which catalyses the transfer of the methyl group from S-adenosyl-L-methionine to methylxanthines producing caffeine. Purification of the enzyme by anion-exchange chromatography results in low activity yields. Part of the reason was found to be inhibition of the *N*-methyltransferase activity by KCl and NaCl which are used to elute the protein during anion-exchange chromatography. The enzyme was found to have a half life at 4°C of approximately 90 min. An extensive study of a wide range of protease inhibitors failed to show any effective stabilisation of activity suggesting that the losses are not due to the action of endogenous proteases in the extract. The stability of the enzyme has been shown to be improved substantially with the incorporation of 20% (v/v) glycerol or 20% (v/v) ethylene glycol in the buffers used. Incorporation of 20% (v/v) glycerol in buffers during anion-exchange chromatography resulted in 54 - 78% yield of *N*-methyltransferase activity and a *ca.* 10-20 fold purification.

PURINE ALKALOID CATABOLISM PATHWAYS IN *COFFEA ARABICA* LEAVES

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Introduction

The purine alkaloid, caffeine (1,3,7-trimethylxanthine) accumulates in leaves and fruits of coffee plants (*Coffea arabica* L.) in concentrations of ca. 1% dry weight. Young leaves and buds have a high capacity for caffeine biosynthesis but this declines markedly with age. Although endogenous caffeine levels decrease as leaves mature, substantial quantities remain in older leaves. The available evidence suggests this is because *C. arabica* leaves have a very limited capacity for caffeine catabolism and, as a consequence, most caffeine that is produced accumulates and is not subject to active turn over (2,3,4,10,13,16). A similar situation appears to exist in the endosperm of developing seed of *C. arabica* where a high rate of caffeine biosynthesis is accompanied by a slow rate of degradation. Other species of coffee, such as *C. dewevrei*, which are characterised by a low caffeine content, appear to be able to catabolise caffeine much more effectively than *C. arabica* (9,10).

Biodegradation of caffeine to xanthine, which is further catabolised by the purine catabolism pathway to CO_2 and NH_3, via uric acid, was first demonstrated in *C. arabica* leaves by Kalberer (6,7). Subsequently, Suzuki and Waller (13,14) showed that theophylline (1,3-dimethylxanthine) and theobromine (3,7-dimethylxanthine) were the first degradation products of caffeine in *C. arabica* fruits.

This paper reports on a detailed investigation of caffeine catabolism in which *C. arabica* leaves were incubated with a number of ^{14}C-labelled purine alkaloids. Incorporation of label into $^{14}CO_2$ was determined while methanol–soluble metabolites were analysed by reverse phase high performance liquid chromatography-radiocounting (HPLC-RC) and combined gas chromatography-mass spectrometry (GC-MS). Use was made of allopurinol, which inhibits xanthine oxidase activity (11) and thereby prevents degradation of xanthine by the purine catabolism pathway. It has been reported that allopurinol treatment results in the accumulation of xanthine in soybean (5) and black gram seedlings (1).

Materials and Methods

Plant material

Leaves were obtained in July and August 1994 from four-year old coffee plants (*C. arabica* L. cv. Kent) growing under a natural photoperiod in a greenhouse at the University of Glasgow. The developmental stages were categorised as (i) buds and young leaves, (ii) mature leaves and (iii) aged leaves. Young leaves were the most recently emerged, weighed ca. 25 mg (f.w.) and were ca. 20 mm long and 7 mm in width. Mature leaves comprised the fully expanded, second and third leaves below the apex (weight ca. 1.2 g) while aged leaves were dark green from near the base of the shoot and weighed ca. 1.3 g.

Chemicals

The following radiochemicals were purchased from the commercial sources indicted: [1-methyl-^{14}C]caffeine (specific activity 1.98 MBq mmol^{-1}, Moravek Biochemicals Inc. Brea, CA, USA), [3-methyl-^{14}C]caffeine (1.98 MBq μmol^{-1}, Moravek), [2-^{14}C]caffeine (2.07 MBq μmol^{-1}, Moravek), [2–^{14}C]theobromine (2.07 MBq μmol^{-1}, Moravek), [2–^{14}C]xanthine (1.94 MBq μmol^{-1}, Moravek) and [8–^{14}C]theophylline (2.04 MBq μmol^{-1}, American Radiolabelled Chemicals, Inc. St. Louis, Mo, USA). Radiolabelled uric acid, allantoin and allantoic acid were prepared in vitro from [2–^{14}C]xanthine using xanthine oxidase (buttermilk), uricase (*Candida utilis*) and allantoinase (peanut). These enzymes and all other chemicals were purchased from Sigma (Poole, Dorset, UK).

Metabolism of radiolabelled purine derivatives

Segments of *C. arabica* leaves (5 mm x 5 mm for mature and aged leaves; 5 mm strips of buds and young leaves), weighing 50 mg, were incubated in 2 ml medium, comprising 30 mM potassium phosphate buffer, pH 5.6, 10 mM sucrose and a radiolabelled substrate, in a 30 ml Erlenmeyer flask, in a shaking water bath at 27° C. The Erlenmeyer flask had a centre well containing a small glass tube into which was inserted a piece of filter paper wetted with 0.1 ml of a 20% KOH solution. Pulse-chase experiments were carried out in which leaves were incubated with ^{14}C-labelled substrate for 18 h at which point the radiotracer was removed and replaced with unlabelled substrate and the incubation continued for a further 24 h. At the end of the 42 h incubation period, $^{14}CO_2$ absorbed by the KOH solution was measured by liquid scintillation counting, and the leaves were separated from the incubation medium and extracted with methanol. Radiolabelled compounds in the methanol extract were analysed by reverse phase HPLC–RC (see 2.3).

HPLC-RC analysis of radiolabelled metabolites

Samples were analysed by reverse phase HPLC using a 250 x 4.6 mm i.d. 5μm ODS Hypersil column (Shandon HPLC, Runcorn, Cheshire, UK) with either a 25 min, 0–40% gradient, or a 10% mixture, of methanol in 50 mM sodium acetate pH 5.0, at a flow rate of 1 ml min^{-1}. Column eluate was directed first to an absorbance monitor operating at 270 nm, and then to a radioactivity monitor (Reeve Analytical, Glasgow, UK), with a 500 μl spiral glass flow cell, operating in the heterogeneous mode (12) Signals from both detectors were processed by a dual channel data handling system (Reeve Analytical). The gradient elution reversed phase HPLC system successfully separated 11 different purine derivatives (Fig. 1) and was also able to resolve uric acid, allantoin and allantoic acid produced enzymically from [8-^{14}C]xanthine (2).

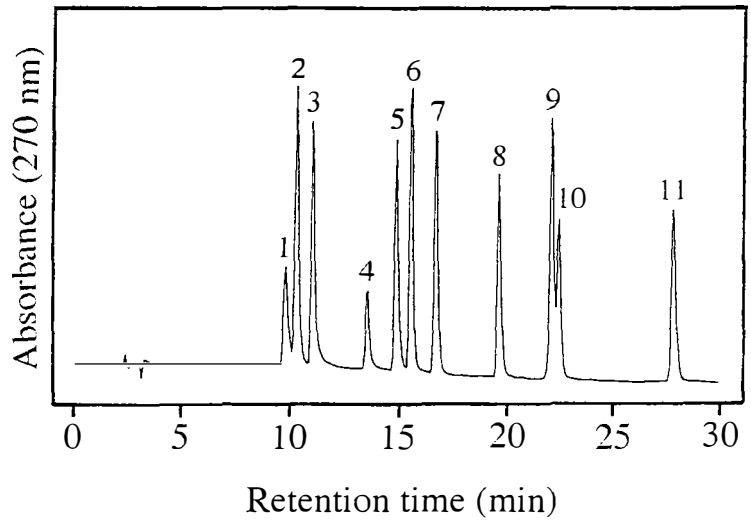

Figure 1. Gradient reverse phase HPLC of methylxanthines and related compounds. (1) hypoxanthine, (2) xanthine, (3) 7-methylxanthosine, (4) xanthosine, (5) 7-methylxanthine, (6) 3–methylxanthine, (7) 1–methylxanthine, (8) theobromine, (9) paraxanthine, (10) theophylline and (11) caffeine.

Gas chromatography-mass spectrometry

Dry, HPLC-purified samples and methylxanthine standards were trimethylsilylated in pyridine and N–methyl-N-trimethylsilyltrifluoroacetamide at 80°C for 30 min. The derivatization mixture was then reduced to dryness, dissolved in 20 μl dichloromethane and 1 μl aliquots injected, in the splitless mode, into an HP 5890 gas chromatograph (Hewlett Packard, Palo Alto, CA, USA) fitted with a fused silica glass capillary column (25 m x 0.25 mm i.d.) with a chemically bonded 0.25 μm SE-30 stationary phase (Quadrex, New Haven, Conn., USA). The injector temperature was 270°C. The linear flow rate of the helium carrier gas was 30 cm sec^{-1} and the split was opened 1 min after injection. The column temperature was held at 60°C for 1 min, then increased by 20°C min^{-1} to 160°C, and by 5°C min^{-1} to 230°C and held there for 6 min and then increased by 20°C min^{-1} to 260°C. The column effluent was introduced directly into the ion source of a Hewlett Packard 5970 mass selective detector. The interface temperature was 275°C and the electron energy was 70 eV. Samples were analysed in the full scan mode and 50-550 *m/z* electron impact spectra were processed by a Hewlett Packard 9133 data system.

Results

Metabolism of [1-methyl-^{14}C]-, [3-methyl-^{14}C]- and [2-^{14}C]caffeine

No metabolites were detected in methanolic extracts following incubation of mature *C. arabica* leaves with [3–methyl-^{14}C]caffeine (Fig. 2 A) and similar data were obtained with young and aged leaves. No methanol-soluble metabolites were detected in a study of the metabolism of [1-methyl-^{14}C]-, [3–methyl-^{14}C]- and [2–^{14}C]caffeine by mature leaves, in which the release of $^{14}CO_2$ from the three substrates, expressed as a percentage of total radioactivity taken up by the leaves, was 1.0, 0.1 and 0.03%, respectively. The latter figure for $^{14}CO_2$ release fell to 0.01% when 5 mM allopurinol was included in the incubation medium. Clearly, caffeine is catabolised very slowly by leaves of *C. arabica*. Although no intermediates accumulate in detectable quantities, even in allopurinol

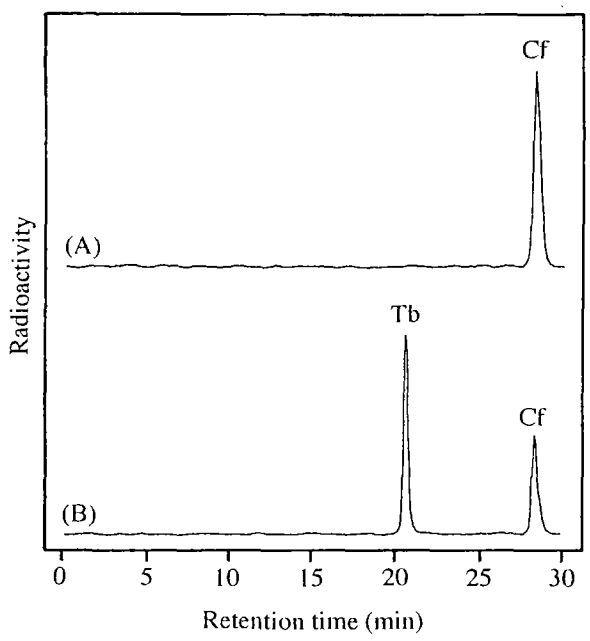

Figure 2. Gradient reverse phase HPLC-RC analysis of methanol extracts from mature *C. arabica* leaves following incubation with 36.7 kBq (**A**) [3-methyl-^{14}C]caffeine and (**B**) [2-^{14}C]theobromine. Caffeine (Cf), theobromine (Tb).

treated tissues, the likely route of this minor pathway is a series of demethylations leading to xanthine and entry into the purine catabolism pathway which results in degradation to CO_2 and NH_3 (6,7).

Metabolism of [2-^{14}C]theobromine

[2–^{14}C]Theobromine was converted to [^{14}C]caffeine and small quantities of $^{14}CO_2$ by young, mature and aged leaves of *C.arabica* (Fig. 2B, Table 1). Theobromine, thus, appears to act primarily as the immediate precursor of caffeine. Although relatively small, at 5% of the recovered radioactivity, the amount of $^{14}CO_2$ released from mature leaves incubated with [2–^{14}C]theobromine was much greater than the 0.03% observed in equivalent incubations with [2-^{14}C]caffeine. This implies that a minor portion of the applied [2–^{14}C]theobromine is not converted to

Table 1. *Summary of [2-^{14}C]theobromine metabolism by young, mature and aged leaves of* C. arabica. *Radioactivity recovered as CO_2 , in methanol-soluble extracts and as caffeine, expressed in kBq. Radioactivity associated with CO_2 also expressed as a percent of total radioactivity recovered.*

Leaves	CO_2	Methanol extract	Caffeine
Young	0.02 (0.5%)	4.11	1.61
Mature	0.14 (5.0%)	2.66	1.06
Aged	0.13 (4.7%)	2.56	0.62

caffeine but instead is probably subjected to demethylation, yielding xanthine, which is further catabolised to $^{14}CO_2$. Catabolism of theobromine to xanthine would be via either 3-methylxanthine or 7-methylxanthine, but as detectable levels of radioactivity were not incorporated into either compound after feeding leaves with [2–^{14}C]theobromine, no indications were obtained as to which pathway might be operating.

Metabolism of [8-^{14} C]theophylline

In contrast to the very slow metabolism of caffeine, [8-^{14}C]theophylline was catabolised very rapidly to a number of products as illustrated in Figures 3 and 4. More than 70% of the [8-^{14}C]theophylline taken up by young,

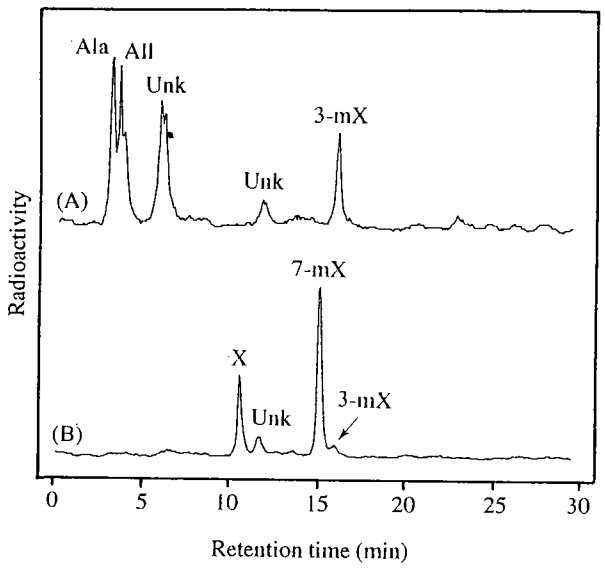

Figure 3. Gradient reverse phase HPLC-RC analysis of methanol extracts from mature *C. arabica* leaves following incubation with 36.7 kBq (**A**) [8-^{14}C]theophylline and (**B**) [8-^{14}C]theophylline and 5 mM allopurinol. Allantoin (All), allantoic acid (Ala), unknown (Unk), xanthine (X), 3–methylxanthine (3-mX), 7-methylxanthine (7-mX).

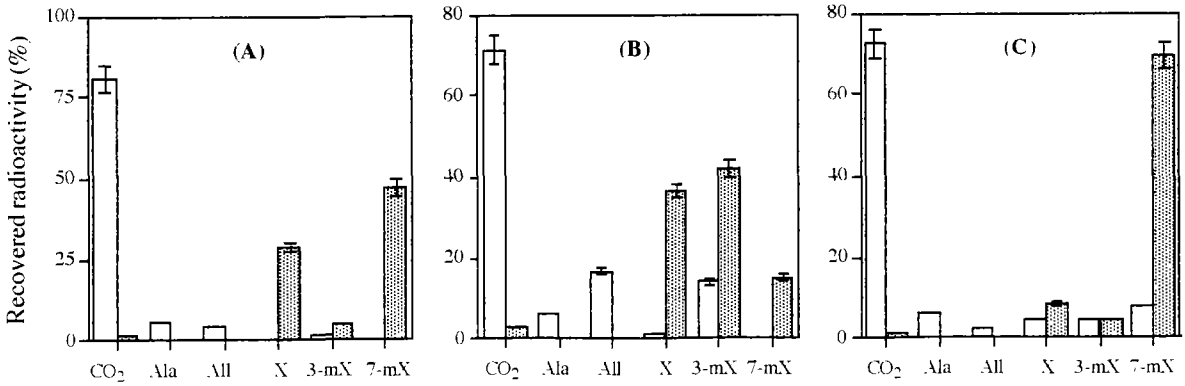

Figure 4. Metabolites produced following incubation of (**A**) young, (**B**) mature and (**C**) aged *C. arabica* leaves with 28.7 kBq [8-^{14}C]theophylline. Open bars - control; shaded bars - in the presence of 5 mM allopurinol. Allantoin (All), allantoic acid (Ala), xanthine (X), 3-methylxanthine (3-mX), 7–methylxanthine (7-mX).

mature and aged *C.arabica* leaves was recovered as $^{14}CO_2$. Radioactivity was also associated with allantoic acid, allantoin, xanthine, 3-methylxanthine and 7-methylxanthine. Incorporation of radioactivity into 3-methylxanthine was highest in young leaves while the accumulation of xanthine and 7-methylxanthine was greatest in aged leaves. The inclusion of 5 mM allopurinol in the incubation medium had major effects on [8-^{14}C]theophylline metabolism. The production of $^{14}CO_2$ declined dramatically, as a consequence of xanthine degradation being blocked, and there were concomitant increases in the incorporation of label into xanthine, 3-methylxanthine and 7-methylxanthine (Fig. 4). It is noteworthy that >70% of the radioactivity recovered from aged leaves treated with allopurinol was incorporated into 7-methylxanthine. While the presence of 3-methylxanthine and xanthine as catabolites of theophylline is to be anticipated, the detection of 7-methylxanthine, especially in such large amounts, is unexpected as its reported role in *C.arabica* is as a precursor of theobromine in the caffeine biosynthesis pathway (see 13).

Metabolism of [2-^{14}C]xanthine

Like [2-^{14}C]theophylline, [2-^{14}C]xanthine underwent extensive metabolism with >80% of the radioactivity recovered after incubation with young, mature and aged leaves being released as $^{14}CO_2$. The remainder of the radioactivity was in the form of residual [2-^{14}C]xanthine together with allantoin and allantoic acid (Figs. 5 and 6). Inclusion of 5 mM allopurinol in the incubation medium resulted in a large reduction in $^{14}CO_2$ production, a larger pool of unmetabolised [2-^{14}C]xanthine and a marked enhancement in the accumulation of radiolabelled 7–methylxanthine. This demonstrates that xanthine is the immediate precursor of 7-methylxanthine.

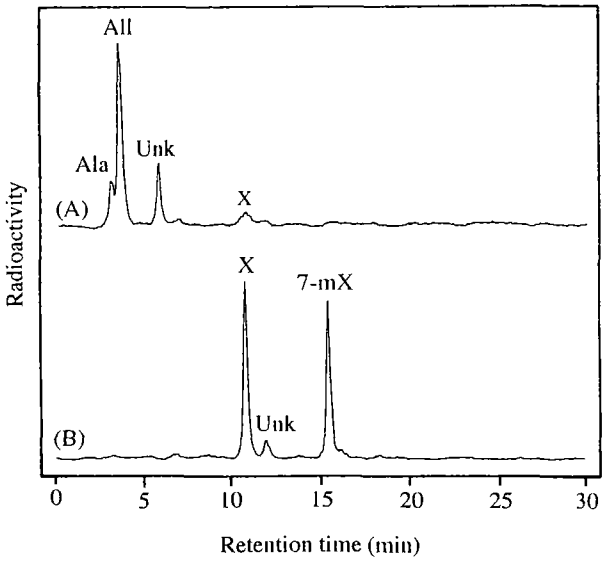

Figure 5. Gradient reverse phase HPLC-RC analysis of methanol extracts from young *C. arabica* leaves following incubation with 36.7 kBq (**A**) [2-^{14}C]xanthine and (**B**) [2-^{14}C]xanthine and 5 mM allopurinol. Allantoin (All), allantoic acid (Ala), unknown (Unk), xanthine (X), 7-methylxanthine (7-mX).

Confirmation of the identification of 7-methylxanthine as a catabolite of [2–^{14}C]theophylline and [2–^{14}C]xanthine

Small run-to-run variations in retention times are inherent in gradient reverse phase HPLC, and when this procedure is used to analyse 1-, 3- and 7-methylxanthine, the three compounds elute within a two minute time frame

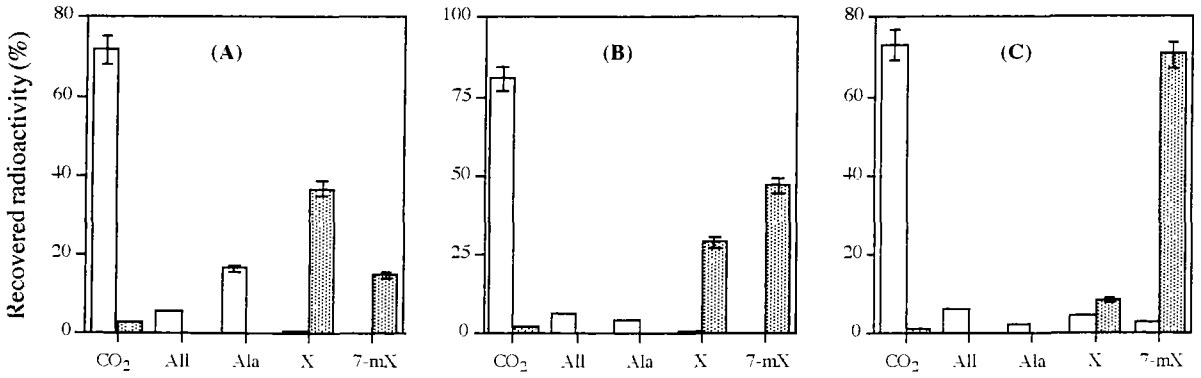

Figure 6. Metabolites produced following incubation of (**A**) young, (**B**) mature and (**C**) aged *C. arabica* leaves with 36.7 kBq [2-^{14}C]xanthine. Open bars - control, shaded bars - in the presence of 5 mM allopurinol. Allantoin (All), allantoic acid (ala), xanthine (X), 7-methylxanthine (7-mX).

(see Fig. 1). As a consequence, caution should be exercised when attempting to identify the mono–methylxanthines by reverse phase HPLC, especially in metabolism studies when radiolabelled standards are not available for co–chromatography. In the circumstances, it was deemed appropriate to obtain further analytical evidence on the identity of the ^{14}C-labelled 3- and 7-methylxanthine peaks obtained in feeds with [2–^{14}C]theophylline and [2–^{14}C]xanthine. All extracts referred to in the previous two sections were therefore reanalysed isocratically with a mobile phase of 10% methanol in 50 mM sodium acetate buffer, pH 5.0. This provides both a better separation of the three methylated xanthines (Fig. 7) and more reproducible retention times. Confirmation of all the identifications of 3-methylxanthine and 7-methylxanthine referred to above, were obtained in this manner. None of the extracts contained ^{14}C-labelled 1–methylxanthine.

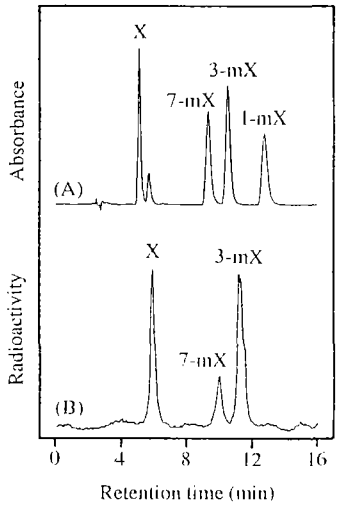

Figure 7. Isocratic reverse phase HPLC of (**A**) standards and (**B**) a methanol extract from young *C. arabica* leaves following incubation with 2.87 kBq [8-^{14}C]theophylline and 5 mM allopurinol. Xanthine (X), 1-methylxanthine (1–mX), 3-methylxanthine (3-mX) and 7-methylxanthine (7-mX) Note that the standards in trace A were detected with an absorbance monitor operating at 270 nm while the [^{14}C]metabolites in trace B were detected with a radioactivity monitor. There was 40 s delay in the response of the radioactivity monitor.

In addition, an extract from young leaves incubated with [2-^{14}C]xanthine in the presence of allopurinol (see Fig. 5B) was purified using a C_{18} cartridge prior to the separation of the labelled xanthine and 7-methylxanthine by preparative isocratic reverse phase HPLC-RC. The 7–methylxanthine metabolite peak was collected, reduced to dryness, trimethylsilylated and analysed by capillary GC-MS. The trimethylsilylated derivatives of 1-,3- and 7–methylxanthine, with retention times of 16.9, 16.3 and 16.7 min respectively, separated on the capillary GC column and, although all had an *m/z* 310 molecular ion and an *m/z* 295 base peak, the spectra were distinctive and distinguished between the three purine alkaloids. Both the retention time (16.7 min) and mass spectrum (m/z 310 [36% of base peak], 295[100%], 237[3%], 221[5%], 180[10%], 147[25%], 100[6%]) of the purified [2–^{14}C]xanthine metabolite were identical to that of 7-methylxanthine but because of dilution of the label with endogenous 7–methylxanthine, ^{14}C-labelled fragment ions were not evident.

Discussion

Little is known about the pathways that regulate the degradation of caffeine in *C. arabica*. Most previous investigations were based on analysis of metabolites by paper chromatography (6,7,14,15). In the present study, *C.arabica* leaves were incubated with a series of ^{14}C-labelled purine alkaloids and the metabolites analysed by reversed phase HPLC-RC and, in one instance, by GC-MS. The results obtained demonstrate that catabolism of caffeine in *C. arabica* leaves involves a caffeine -> theophylline -> 3–methylxanthine -> xanthine pathway. Xanthine is further degraded by the conventional purine catabolism pathway to CO_2 and NH_3 via uric acid, allantoin and allantoic acid (Fig. 8). The very slow degradation of [2–^{14}C]caffeine, compared with that of [8–^{14}C]theophylline, which is accompanied by a much higher incorporation of label into $^{14}CO_2$, indicates that the conversion of caffeine to theophylline is a major rate limiting step in the catabolism of caffeine and provides a ready explanation for the high endogenous caffeine content of *C. arabica* leaves.

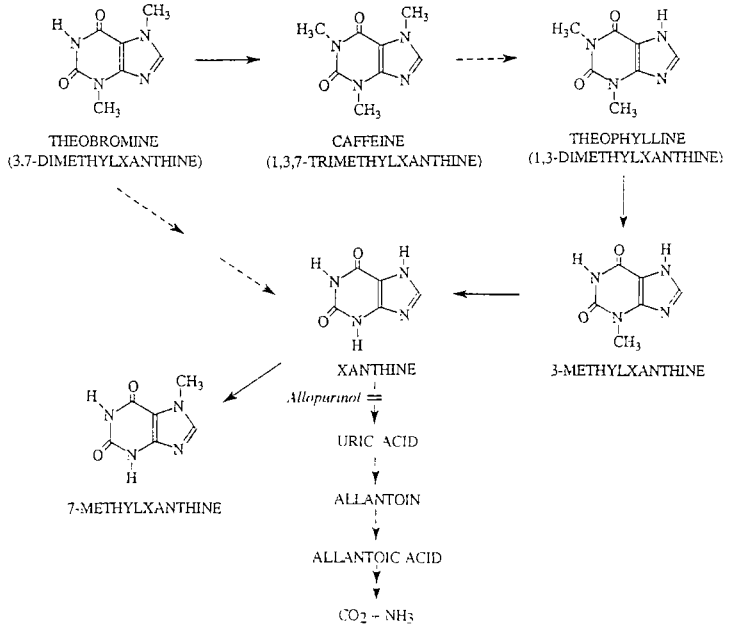

Figure 8. Purine alkaloid catabolism pathways operating in leaves of *C. arabica*.

Feeds with [2-^{14}C]theobromine to *C. arabica* leaves indicted that the main role of theobromine is in caffeine biosynthesis as the immediate precursor of caffeine. However, as there was more $^{14}CO_2$ production from [2–^{14}C]theobromine than from [2–^{14}C]caffeine, it would appear that a minor portion of the theobromine pool may be subjected to demethylation and catabolised to xanthine. In other species of coffee, such as *C. dewevrei*, where more extensive degradation of caffeine occurs, there is evidence implying that theobromine may play a more prominent role in caffeine catabolism than it does in *C. arabica* (8,9,10).

As well as being degraded to CO_2 via the purine catabolism pathway, xanthine was converted to 7–methylxanthine (see Fig. 8). This is the first reported instance of such a conversion. The function of 7–methylxanthine in purine alkaloid metabolism was previously thought to be limited to that of an intermediate between 7-methylxanthosine and theobromine in the caffeine biosynthesis pathway (see 13). In feeds with [8–^{14}C]theophylline in which xanthine degradation was inhibited by the addition of 5 mM allopurinol to the incubation medium, increased radioactivity was associated not only with xanthine but also with 3–methylxanthine and 7-methylxanthine. Metabolism of [2-^{14}C]xanthine by *C. arabica* leaves in the presence of allopurinol resulted in large increases in incorporation of label into 7-methylxanthine as breakdown of the substrate via the purine catabolism pathway was blocked.

On the basis of HPLC-RC metabolism profiles obtained after incubations with [8-^{3}H]caffeine in the presence and absence of allopurinol, it has been reported that 7-methylxanthine is not involved in caffeine catabolism in leaves and endosperm of *C. dewevrei* (8). This may well reflect genuine differences in caffeine degradation pathways operating in *C. arabica* and *C. dewevrei*. However, it could also be a consequence of erroneous identifications of [^{3}H]monomethylxanthine metabolites in the *C. dewevrei* extracts as there is no mention of appropriate precautions being taken to satisfactorily discriminate between 1-, 3- and 7-methylxanthine, which have almost indistinguishable retention times when analysed by gradient elution reversed phase HPLC.

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Summary

In a study of purine alkaloid catabolism pathways in coffee, ^{14}C-labelled theobromine, caffeine, theophylline and xanthine were incubated with leaves of *C. arabica*. Incorporation of label into $^{14}CO_2$ was determined and methanol-soluble metabolites were analysed by HPLC-RC. The data obtained demonstrate catabolism of caffeine –> theophylline –> 3-methylxanthine –> xanthine. Xanthine is degraded further by the conventional purine catabolism pathway to CO_2 and NH_3 via uric acid, allantoin and allantoic acid. The conversion of caffeine to theophylline is the rate limiting step in purine alkaloid catabolism and provides a ready explanation the high concentrations of endogenous caffeine that are found in *C.arabica* leaves. Although theobromine is converted primarily to caffeine, a small portion of the theobromine pool appears to be degraded to xanthine by a caffeine-independent pathway. In addition to being broken down to CO_2, via the purine catabolism pathway, xanthine is metabolised to 7–methylxanthine. Metabolism of [2–^{14}C]xanthine by *C.arabica* leaves in the presence of 5 mM allopurinol results in very large increases in incorporation of radioactivity into 7-methylxanthine as degradation of the substrate via the purine catabolism pathway is blocked. The identity of 7-methylxanthine in these studies was confirmed by GC-MS.

AFRICAN COFFEE : AN OVERVIEW

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PREAMBLE

Coffee is an important commodity of international trade in a number of countries of Africa. Both Arabica coffee (*Coffea arabica* L.) and Robusta coffee (*C. canephora*) are produced in appreciable quantities (Table 1) while other species such as *C. liberica* and *C. excelsa* are also grown in some countries such as Liberia, Côte d'Ivoire, Central African Republic and Benin, though in small quantities.

As a commercial crop, coffee is important to the continent of Africa for two reasons. First, it is an important foreign exchange earner to the producer nations (Table 2). In this respect, some countries such as Uganda, Rwanda and Burundi derive over 80% of their foreign currency earnings from coffee exports (Table 3). Secondly production of coffee provides an important source of employment for a large proportion of the rural populations in the producer nations.

Production of Arabica coffee in Africa has grown tremendously since the 1960s. The current levels of production stand at almost double the level of production in the early 1960s. Two factors contributed to this rapid growth. First and perhaps most important was the emergence of new producers in the southern part of Africa namely Malawi, Zimbabwe and Zambia. The second factor was the expansion of hectarage under coffee by the more traditional producers of coffee during the same period.

Unlike Arabica coffee, no appreciable growth in production has been seen in Robusta coffee since the 1960s. This stagnation was mainly due to the near-collapse of Angola's Robusta coffee production as a result of civil unrest.

Despite the significant role played by coffee in the economies of producer nations, an examination of long-term production levels of individual countries (Table 4) reveals output levels, which fluctuate from year to year and in some cases, with a declining trend, especially in Robusta producing countries. While civil unrest can be considered a major player in such fluctuations, it is obvious that shifts in both the world coffee prices as well as economic stability of producer nations are the more universal causes of production instability in most producer countries. Particularly affected by such market forces are usually the smallholder farmers who are the mainstay of coffee production in Africa. The African market share of coffee export has declined from 35% in 1970 to about 17% in 1995 (Figure 1).

Table 1: Countries producing both Arabica and Robusta coffee in Africa, and their 1990/91 production figures in millions of 60 kg bags

Producer	Arabica	Robusta	Total
Arabica Group	**33.799**	**7.253**	**41.052**
Burundi	0.535	0.015	0.550
Kenya	1.399	0.001	1.400
Tanzania	0.609	0.220	0.829
Robusta Group	**1.300**	**16.064**	**17.364**
Angola	0.015	0.155	0.170
Cameroon	0.175	1.000	1.175
Madagascar	0.020	1.072	1.092
Uganda	0.200	2.800	3.000
Zaire	0.310	1.385	1.695

Source: United States Department of Agriculture

Table 2: Total African Coffee Revenue (1988/89-1993/94)

Year	Exports in million bags	Revenue in billions US$
1988/89	16.0	1.94
1989/90	17.2	1.62
1990/91	16.4	1.45
1991/92	14.4	0.97
1992/93	15.6	1.27
1993/94	12.0	2.00

Source: International Coffee Organisation

Table 3: Coffee as a percentage of total exports by value in some African countries, 1985 - 1989

Producing Country	1985	1986	1987	1988	1989	Average
Uganda	94.2	96.9	95.4	91.5	96.2	94.9
Rwanda	87.6	90.2	93.0	84.0	98.9	90.7
Burundi	87.9	79.0	75.2	72.7	94.5	81.9
Ethiopia	33.6	74.4	56.7	61.9	69.5	59.2
Tanzania	46.7	53.2	37.8	34.2	40.5	42.5
Madagascar	35.4	47.8	27.0	23.1	22.1	31.1
Cameroon	41.9	43.6	24.8	21.9	15.3	29.5
Kenya	31.4	40.5	24.6	25.7	22.7	29.0
Central African Republic	44.8	22.2	21.7	26.0	20.8	27.1
Zaire	26.1	40.2	18.5	9.6	9.7	20.8

Source: International Coffee Organisation

Table 4: World coffee production, 1970-1990

	Average production of clean coffee (in thousands of 60 kg bags)			
	1970 - 1975	1975 - 1980	1980 - 1985	1985 -1990
Total Arabica	**55,703**	**55,119**	**69,446**	**71,974**
North & Central America	13,741	14,474	16,870	17,086
South America	34,247	31,608	42,301	44,066
Asia & the Pacific	2114	2755	3190	3655
Africa	5601	6282	7085	7167
Burundi	380	359	520	562
Ethiopia	2837	3126	3291	2918
Kenya	1140	1433	1659	1938
Malawi	3	3	25	82
Rwanda	339	437	521	652
Tanzania	872	843	932	802
Zambia	1	2	4	13
Zimbabwe	29	79	133	201
Total Robusta	**19,241**	**17,410**	**20,235**	**21,989**
America	73	72	57	27
Asia & the Pacific	3509	5309	7254	9020
Africa	15,659	12,029	12,924	12,941
Angola	3632	767	365	201
Ghana	57	35	13	13
Guinea	76	36	59	104
Liberia	80	142	152	63
Nigeria	48	49	33	56
Benin	35	58	44	27
Cameroon	1433	1490	1709	1686
Central African Republic	185	174	279	278
Congo	13	54	44	24
Côte d'Ivoire	3975	4478	4444	4252
Equatorial Guinea	81	0	7	16
Gabon	8	6	35	29
Madagascar	1121	1079	1036	1064
Togo	172	138	246	280
Sierra Leone	129	140	184	110
Uganda	3267	2118	2784	2852
Zaire	1347	1265	1490	1885
World Total	**74,944**	**72,529**	**89,681**	**93,963**

Source: International Coffee Organisation

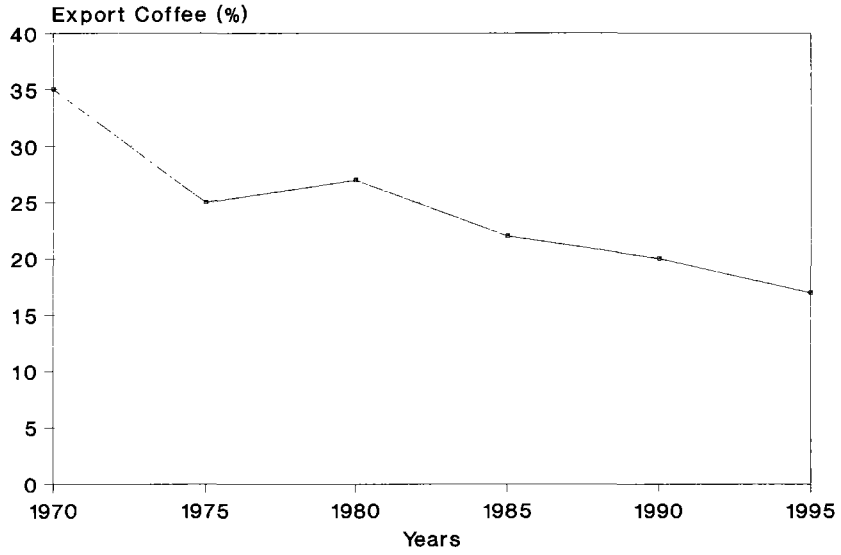

Fig 1. African market share since 1970

In order to safeguard against a declining trend in the future, it is necessary for African producer nations to take immediate steps to make coffee production cheaper, sustainable and more economical. In this respect, improved technologies have to be developed and adopted in order to increase productivity per unit area and to improve both bean and cup qualities. Improved technologies must include developing coffee varieties which combine disease resistance with high yield and good bean and cup qualities; emphasising the use of environmentally friendly methods such as biocontrol to control pests; and improving agronomic practices such as pruning, fertilization and weed control.

THE AFRICAN COFFEE RESEARCH NETWORK (ACRN)

The Inter-African Coffee Organization (IACO) has long been apprehensive of the decline in the African market share of coffee exports and, during a conference in Portugal in 1992, conceived the need to have a research body which could identify research areas of common interest to a number of African producing nations. This led to the launching of the African Coffee Research Network (ACRN), also known as Reseau de Recherche Cafeiere en Afrique (RECA), in London in March 1993. The goal of the ACRN is to revitalise and improve research in Africa so as to enhance the productivity and quality of African coffee. In order to realise this goal, ACRN is expected to promote the following activities among member states: (1) co-operation, concentration and exchange of scientific information; (2) formulation of common research objectives; (3) creation, co-ordination and development of regional research teams; (4) collaboration with international and regional coffee research institutes; (5) training and retraining of scientists and extension officers; (6) exchange of plant materials; and (7) mobilization of funds to finance the network's activities.

The current ACRN members are Angola, Benin, Cameroon, Côte d'Ivoire, Ethiopia, Guinea, Kenya, Madagascar, Malawi, Rwanda, Togo, Uganda and Zaire. Associate members are the Centre de Coopèration Internationale en Recherche Agronomique pour le Developpement (CIRAD) in France and the Centro de Investigacao Ferrugens do Cafeeiro (CIFC) in Portugal. Membership is open to all African coffee producing countries, and the participation of the countries not yet represented is greatly encouraged.

So far, three broad areas of research activities have been identified, namely, the establishment of coffee germ-plasm centres in Ethiopia (Arabica), Côte d'Ivoire (Robusta) and Madagascar (Mascaro coffee); the

formulation of research on development of Arabica coffee varieties resistant to Coffee Berry Disease (CBD) and the formulation of research on Robusta coffee varieties of good quality.

Coffee Berry Disease (CBD), caused by *Colletotrichum kahawae*, is a major problem in the African countries which produce Arabica coffee, especially in Kenya, Tanzania and Ethiopia. CBD was first reported in Kenya in 1922, but since then has slowly spread to Uganda, Zaire, Angola, Cameroon, Tanzania, Rwanda, Burundi, Ethiopia and, more recently, to Malawi, Zambia and Zimbabwe. CBD has not been reported outside Africa, and therefore, still remains solely an African problem. Losses due to the disease are enormous and despite success in establishing and using fungicide control regimes, crop losses have reached 20-30% during excessively wet weather conditions. Chemical control of the disease is expensive and imposes a continuing economic burden on the farmer. In Kenya, it is estimated that disease control constitutes about 30% of the total cost of coffee production which is currently about US$1500 per metric tonne.

An alternative long-term solution to the CBD problem is developing disease resistant varieties. Resistance to CBD is known to occur in some coffee varieties and has been used in Rwanda (Jackson Hybrid), Zaire (Bronze-tipped types) and Cameroon (Java variety). In Ethiopia, selection and multiplication of CBD-resistant varieties from semi-natural coffee populations was made possible because it is the centre of genetic diversity of Arabica coffee. Kenya, has opted to combine resistance to CBD and coffee rust with high yields and the good quality typical of Kenyan coffee in a well planned long-term programme. The programme which started in 1971, has produced a hybrid cultivar, Ruiru 11, which has been planted by Kenyan farmers since its official launching in 1985. This has been a milestone in the research on CBD in Kenya. Ruiru 11 is expected to cut down the cost of production by about 30%. The large scale adoption of Ruiru 11 has, however, been hampered by the slow - manual - production of hybrid seeds and the high cost of establishing the variety at close spacing. A research project on searching for or creating CBD-resistant coffee varieties to be carried out jointly between Cameroon, Kenya, CIFC and CIRAD has already been approved for funding by the European Union (EU). The studies scheduled under the CBD project, code-named STD III by the EU, are expected to enhance the current knowledge of the structure of the pathogen population and the genetic bases currently available for setting up breeding programmes in Arabica coffee growing countries in the whole of the African continent. Future possibilities of research within the framework of ACRN will include the following: (1) integrated management of all coffee pests (diseases, insects and weeds); (2) physiological studies on flowering, irrigation, fertilizers, photosynthesis and rooting systems; (3) studies on coffee technology; (4) soil and leaf analysis in order to make optimal and economical use of fertilizers; (5) studies on close spacing; (6) use of dwarf coffee plants in both Arabica and Robusta coffee production; (7) selection of improved Arabusta hybrids; (8) work on *Mascaro coffee* to create low caffeine Robusta coffee; and (9) classification of germplasm collections held in various member states.

CURRENT KEY AGRONOMIC PRACTICES

Both Arabica and Robusta are tree crops which come into production two to three years after planting and have an economic life of over 30 years, depending on local conditions and husbandry. Each species requires different growing conditions. *C. Arabica* prefers temperatures of 18-24° C while *C. canephora* prefers warmer conditions, 24-26°C, with less contrasting dry and rainy seasons. Both types of coffee require an average rainfall of 1500 mm per annum for healthy growth and satisfactory productivity. The best coffee growing areas of Africa receive more than 1800 mm of rainfall annually, well distributed throughout the year, with a drier period of two to three months during which growth slows, young wood hardens and flower buds develop. For optimum production, a number of basic agronomic practices are essential. To a great extent, the agronomic requirements for *C. arabica* and *C. canephora* are similar, and therefore the discussion will be based on Arabica coffee agronomy as a whole.

Establishment

Coffee seedlings are usually raised from seeds or as rooted cuttings in the nursery. After germination or establishment of the cuttings, the seedlings are planted into black polythene bags measuring 23 x 17 cm or 30 x 17 cm, previously filled with a regularly watered mixture of top soil, farmyard manure, sand, phosphatic fertilizer and a suitable insecticide. They become ready for field transplanting 9 - 18 months after potting.

In the field, the seedlings are planted in well prepared land, free of tree stumps and stubborn weeds such as couch grass. Soil erosion control measures are applied where necessary. Holes measuring 60 x 60 x 60 cm are

dug three months before planting and then re-filled one month before planting with top soil mixed with 13 kg of well decomposed cattle manure or any other organic manure, 100 g of double superphosphate (45% P_2O_5) and an appropriate insecticide. The seedlings are planted after the onset of the rains. Application of mulch along the planted coffee row or round the seedlings helps to preserve moisture and suppress weed growth.

The common coffee spacings are 2.74 x 2.74 m (1329 trees/ha), 2.74 x 1.37 m (2658 trees/ha) and 1.37 x 1.37 m (5320 trees/ha). For the compact cultivars like Ruiru 11, Catura or Catimor, the spacing usually adopted is 2 x 2 m (2500 trees/ha) or 2 x 1.5 m (3,333 trees/ha) (Njoroge, 1991). Large coffee estates sometimes use different spacing while maintaining the above tree densities, to facilitate the use of machinery for the various farm operations.

Nutrition

The soils on which coffee is grown in most African countries have low plant nutrients especially nitrogen. Hence fertilizers are needed for both the vegetative growth of the tree and the production of high quality coffee beans. In order to apply the correct type and rate of fertilizer, and thus avoid toxicity and nutrient imbalances, fertilizer recommendations are based on soil and coffee leaf analysis to determine the element deficiencies that require correction through fertilization.

Nitrogen is the most limiting element, and Arabica coffee responds positively to nitrogen application rates in the range of 50-100 kg N/ha/year (Njoroge, 1985). The proportion of grade 'A' sized beans has been shown to decrease with increased nitrogen rates (Njoroge, 1985). Better response to nitrogen have been observed with split applications than with single dose applications (Njoroge, 1985). Studies in Kenya have also shown no positive yield increases to phosphorus application alone despite observed low soil phosphorus (Keter, 1974). The soils are however well supplied with potassium. The types of fertilizers used include straight, compound and foliar fertilizers.

Organic manures, mainly cattle manure and sludge from methane gas plants have been used for a long time in Kenya. Organic manures increase the soil organic matter, thus improving the water holding capacity of the soil. They also release plant nutrients on decomposition (Oruko, 1977). Their use on very poor soils has been shown to result in increased coffee yields and quality (Mitchell, 1970). As the organic manures are formed from different sources they have varying nutrient composition, and their continuous use may lead to nutrient imbalances which may affect the coffee bean quality (Northmore, 1965). However, because they are cheap and readily available, organic manures can be used, to some extent, as substitutes for inorganic fertilizers and thus reduce production costs. More research should be geared towards evaluating the response of coffee to organic manures, organic - inorganic fertilizer substitution ratio and the use of green manures as sources of plant nutrition in coffee. Increased use of organic manures would improve soil fertility as a whole, especially of the exhausted soils in the coffee growing African countries. In addition, this would reduce over-reliance on imported fertilizers.

Weed Control

The weed species in coffee have been classified into annuals and perennials. Weeds have been shown to reduce coffee yields by over 50% in addition to reducing the coffee quality (Njoroge and Kimemia, 1989). Due to this reduction of yield and quality by weeds, various weed control methods are practised. The most common methods used are digging using forked hoes, slashing, mulching and the use of herbicides. There is a wide range of recommended herbicides (contact, systemic and soil-acting) for use in coffee plantations (Table 5).

The use of low rates and volumes of recommended herbicides can be effective in controlling annual weeds in coffee at the 1-3 leaf stage using low volume nozzles (Njoroge and Kimemia, 1992). Continuous use of one type of herbicide has led to the development of herbicide tolerance by some weeds, like Black Jack (*Bidens pilosa*) tolerance to paraquat observed in Kenya. To prevent this phenomenon, integrated weed control methods are recommended. Continued evaluation of new herbicide products, especially against difficult weeds such as *Oxalis* sp., *Cyperus* sp., and different methods of using the already existing ones, is emphasised.

Table 5: Common herbicides used in coffee

Soil acting	Contact	Systemic
Atrazine (50 and 80% WP)	Actril DS (70% EC)	Ametryne (80% WP)
	Mixture of loxynil and 2,4-D	
Candex (65% WP)	Amitrole (25 or 50% ML)	2,4-D amine
Mixture of asulam and atrazine		
Diuron (48% EC, (80% WP)	Diquat	Asulam (40% SL)
Fluometuron (80% WP)	Paraquat (20 WV)	Dalapon (74 and 85 WP
Linuron (50% WP)	Glufosinate - ammonium (20 and 14 SL)	Fluazifop-p-butyle (25% EC)
Oxyfluorfen (24 WV)		Glyphosate (various)
Simazine (50 and 80% WP)		Haloxyfop ethoxyethyl
		MCPA (Various)
		Tordon 101 (Picloran plus 2,4-D

Mulching

In Kenya, mulching is the covering of the soil with a layer of dry vegetative materials. Its benefits include preservation of soil moisture, control of soil erosion, improvement of soil structure, supply of mineral nutrients in cases of decomposition, regulation of soil surface temperature, suppression of weeds and reduction of thrips incidence. Due to these benefits, mulch contributes to increased coffee yields and quality. The main mulching materials are napier grass (*Pennisetum purpureum*), maize and banana stover, coffee prunings and other dry vegetative material. However due to declining land availability, the area for growing mulch material has been reduced considerably, and hence mulch is mainly used only in large estates. The mulch material is applied in alternate coffee interrows and then alternated in the following years.

Pruning

Coffee pruning involves the thinning out of unwanted branches and removal of old stems. The main reasons for pruning coffee are to maintain a suitable crop-to-leaf ratio to ensure good levels and a high proportion of large beans, to open the tree centres to light, to facilitate disease and pest control, and to make picking easier.

The pruning system depends on the type of training adopted. Training is the modification of the natural habit of coffee trees to suit the particular conditions under which they are grown. There are basically two training systems: single stem system and multiple stem system. The difference between the two is not based on the number of stems raised but rather on the tree framework on which bearing branches are borne, and on the method of renewal or replacement of these branches.

Under the single stem system, the coffee tree is restricted to a convenient height and has a permanent framework of main stems with primary branches which initiate secondary crop-bearing branches. Pruning is mainly cutting back of secondaries, tertiaries and laterals which have already produced two crops, in order to encourage the formation of new laterals. This is done immediately after the major crop is harvested. Routine removal of young shoots (handling) that flush out with the rains is also carried out.

For the multiple stem system, the permanent framework is restricted to the main stem. Renewal growth is obtained by the development of new top growth and eventual replacement of entire stems with new ones arising from the stump. The crop is largely borne on primary branches which are cut back after bearing two crops. Pruning consists of mainly cutting out lower primary branches. The change of cycle to raise new stems is carried out after six or seven years. In Kenya, the single stem system was the original practice, but now most growers have converted to the multiple stem system in which stems are replaced every 5-7 years. Most small-scale farmers allow their coffee to grow freely without capping, while most estates cap their coffee. The compact cultivar like the Ruiru

11 hybrid is currently recommended for the single stem system but require 'stumping' after every 5-7 years, to generate new stems (Njoroge, *et al.*, 1992). Little pruning is carried out in some countries in Southern Africa where foliar diseases pose no problem and farmers prefer replanting rather than raising new stems at the time of cycle change.

Intercropping and Shading in Coffee

Coffee is mainly grown as a monocrop in some countries, the main reason being that the quality of coffee might be affected adversely if farmers neglect coffee in favour of intercrops. This could be due to competition for nutrients, water and light between coffee and the intercrops. However, coffee farmers particularly the small scale farmers have been intercropping their coffee with various food, fruits and tuber crops especially at the establishment and change of cycle periods and even during the production phases. Large scale estates have also been observed to move in this direction.

Since coffee occupies a substantial amount of high potential land, available land for food crop planting is becoming limited, and hence more intercropping is expected to occur in most countries including Kenya where coffee was hitherto grown as a monocrop. This has led to research on intercropping with coffee in Kenya. Preliminary results have indicated that it is possible and economical to intercrop young arabica coffee with some food crops during the first two years after establishment (Njoroge, *et al.*, 1993). It is also possible to intercrop coffee with dry beans during the change of cycle phase (Mwakha, 1980). More studies are encouraged on this line in the Africa region in order to maximise available land, improve food availability and realise higher incomes. This would also help to sustain the coffee and farmers in periods of low coffee prices.

Several tree species have been grown in coffee orchards, mainly as shade trees or as wind-breaks, e.g. *Cordia* Spp., *Grevillea robusta, Albizzia* Spp, *Leucaena leucocephala*, and *Cypress* Spp. (Njoroge and Kimemia, 1993). Use of shade trees have been shown to help even out erratic yields caused by periodic overbearing, reduce crinkling of coffee leaves commonly known as 'hot and cold' disease and reduce hail damage. Shade has also been shown to reduce infection of Bacterial Blight of Coffee (BBC) in Kenya due to reduced hail damage which predisposes the coffee trees to BBC infection. Further research is needed into the use of trees of economic value and the effect of shade on coffee trees since the original introduction of shade trees in coffee was not preceded by such studies. It was taken that coffee needed shade as an understory plant in its centre of origin.

Diseases and Insect Pests

The major *Coffea arabica* diseases in Africa include Coffee Berry Disease (CBD) caused by *Colletotrichum kahawae*, Coffee Leaf Rust (CLR) caused by *Hemileia vastatrix* Berk et Br. and to a limited extent, Bacterial Blight of Coffee (BBC) caused by *Pseudomonas syringae* Van Hall mainly in Kenya. There are other diseases such as Fusarium root and bark disease which have tended to disappear with improved cultural practices on coffee farms (Masaba *et al.*, 1986). All the major diseases are controlled effectively by use of fungicides and bactericides. The new arabica cultivar developed in Kenya, Ruiru 11, is resistant to CBD and CLR. The introduction of this disease resistant coffee variety has contributed significantly in reducing coffee production costs.

There are a number of coffee insect pests, and most of these pests are controlled only when they exceed economic threshold levels. The control methods range from sanitary, cultural, use of insecticides and biological control. Use of insecticides to control coffee insect pests is not only expensive, but also insecticides destroy natural enemies of some of the coffee pests. Attention has now been focused on integrated pest management for many insect pests. The control of coffee mealybug (*Planococcus kenyae*) by rearing and dissemination of its natural parasite, *Anagyrus* Spp. is one of the classical examples of biological control in Africa (Masaba *et al.*, 1986). Other examples include control of coffee scales by use of ladybird bugs, and control of giant looper (*Ascotis selenaria reciprocaria*) by *Macroraphis acuta* and control of Antestia (*Antestiopsis spp*) by a parasitoid wasp (*Asolurs seychellensis* that parasites the eggs of the pest. More work is still in progress to find natural enemies of the common coffee pests. Currently the main thrust of research is on biological control within the integrated pest management concept.

PROCESSING

The majority of the Arabicas in the region, especially in Kenya and Tanzania, are carefully wet-processed to produce high quality coffee. This starts in the field where only the ripe cherry is picked. The harvested coffee is then sorted into ripe, over-ripes, under-ripes and others at the factory before pulping. Cherries that are over and under ripe, diseased or insect-damaged are removed, and foreign materials are also removed. Coffee is then graded into cherry 1 (slight yellow to an overall red) and cherry 2 (yellow, draughted, under and over-ripe cherry) and the two grades are pulped separately. The remainder, which includes the green, rotten, dry, diseased and insect-damaged cherry, is dried as 'mbuni'. The cherry is pulped as soon as possible after harvesting. During pulping the coffee parchment is graded by density into three classes; firsts, seconds, and lights. The pulped coffee is put into fermentation tanks for 16-20 hours, to break the mucilage covering. Thereafter, the coffee is washed with clean water and then soaked in water for 16-24 hours. Coffee drying is very vital and has a profound effect on quality. There are seven distinct stages of coffee drying:

i) Skin drying stage: In this stage moisture content is reduced from 55% to 45%. Drying should be done as quickly as possible (preferably 3 hours) otherwise the parchment develops sourness (onion flavour) which lowers quality.

ii) White stage: In this stage the moisture content is reduced from 44% to 33%. It requires slow controlled drying to avoid cracking of the parchment.

iii) Soft black drying stage: The moisture content is reduced from 32 to 22%. This MUST be done in the sun for 48 hours. The sun rays are believed to bring about some chemical changes that improve liquor quality.

iv) Medium black drying stage: The moisture content is reduced from 21% to 16%. This is a fairly stable stage, and the coffee can even be heaped together to 5.5 m or put in well ventilated bins to ease congestion at the drying tables.

v) Hard black stage: The moisture content is reduced from 15% to 12%. At this stage, the coffee bean is hard and can be dried quickly without any serious consequences.

vi) Fully dry and conditioning. The coffee is dried on wooden floors or ventilated conditioning bins and the moisture content is reduced from 12% to 10.5%. Dry air is fanned into the coffee to even up moisture.

The coffee is then stored in well-ventilated bins or on wooden floors for about 4 weeks, but not more than 6 months, to allow for the coffee to 'mature'.

The parchment is put into sisal bags, in predetermined grades as parchment 1 or parchment 2. It is then delivered to hulling stations. The parchment is hulled to clean coffee which loses about 20% of its weight. The clean coffee is graded by size and density into seven grades, namely AA, AB, PB, TT, C, T and E. Samples of the graded coffee are taken for cup quality testing by liquorers and exporters who also get samples for their own liquor tests before purchase. The coffee is sold to the coffee dealers in an auction or through direct sales in some countries. Coffee marketing in most of the countries is now being liberalised with the development of more free markets in the region, but at a controlled level to make sure the farmers reap maximum profits.

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SUMMARY

Coffee plays a leading role in the economies of a number of African countries. It is an important foreign exchange earner for the producer countries and a source of employment for the rural populations.

Its productivity and quality have fluctuated over the years due to shifts in the world coffee prices as well as in the social and economic stability of the producer nations. These have led to an overall decline in the quantity of coffee exported annually from Africa and to reduction of various coffee quality grades traditionally exported from the continent. Efforts are now being made to arrest the declining trend of quality and productivity per unit area. Joint research activities aimed at making coffee production cheaper and sustainable as well as those aimed at improving quality through better agronomic practices, processing and genetic research are being formulated by the African Coffee Research Network. The key agronomic practices for both *Coffea arabica* and *Coffea canephora* are discussed.

A PLAN OF ACTION FOR THE ACRN PROGRAMME ON PLANT GENETIC RESOURCES

L. GUARINO, African regional office IPGRI
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W. R. OPILÉ, Chairman of ACRN

I - Introduction

In early 1993 the African Coffee Research Network (ACRN) was established « to revitalize and improvise research so as to enhance the productivity and quality of African coffee. ». Article 4 of its Establishement Agreement states that to fulfill this objective, ACRN shall promote :

a. cooperation, concertation and exchange of information between Members
b. common research objectives
c. the creation, coordination and development of regional research teams
d. collaboration with international coffee research institutions, regional or international organizations and donors
e. the training and retraining of scientists and extension officials
f. the exchange of scientific information and plant materials
g. the mobilization of funds to finance the Network's activities

ACRN is coordinated by the Inter African Coffee Organization (IACO), based in Abidjan, Côte d'Ivoire. Fourteen countries (Angola, Benin, Burundi, Cameroun, Côte d'Ivoire, Ethiopia, Guinea, Kenya, Madagascar, Malawi, Rwanda, Togo, Uganda, Zaire) are currently members of ACRN.

At the first technical meeting of ACRN, held at Montpellier, France in June 1993, better conservation and use of genetic resources were seen as priority areas for action by ACRN. A small committee was therefore established to draft a five-year plan of action for the genetic resources programme of ACRN. Because the major germplasm collections in Africa are in Côte d'Ivoire (*C. canephora* and wild species), Ethiopia (*C. arabica*) and Madagascar (section *Mascarocoffea*), the committee was made up of representatives of these three countries, plus the Chairman of ACRN (Dr W. Opile of Kenya), Dr A. Charrier (ENSA, Montpellier) and IPGRI. The committee met in Nairobi, Kenya in May 1994 under the auspices of IPGRI's Regional Office for Sub-Saharan Africa.

The plan of action developed at the Nairobi meeting has the following principal objectives :

- Strengthening the infrastructure and personnel of existing field collections in Côte d'Ivoire, Ethiopia and Madagascar.
- Complementing the *ex situ* field collection approach with other types of conservation efforts.
- Improving documentation of the existing germplasm collections.
- Improving utilization of the collections.

II - Strengthening existing *ex situ* efforts

The *Coffea* field collections of IDEFOR/DCC in Côte d'Ivoire are located at research stations at Divo (6 ha), which is the main site, and at Man (4 ha), which is at a higher altitude (1100 m) more suitable for *C. arabica.* Together, the collections amount to some 8,000 accessions of over a dozen wild and cultivated species from all over Africa (see Table). Well funded until fairly recently, these collections are now under severe financial pressure, in particular after the devaluation of the CFA Franc. Though 3 technicians and 4 labourers work on the collections, there is no full-time researcher assigned to them.

The PGRC/E coffee collection in Ethiopia is located at Choche near Jimma and consists of about 4,000 accessions of *C. arabica* on about 10 ha. With almost no physical facilities at Choche, there is no staff working on the collection on a full time basis. The site is not suitable for all accessions, and some material has therefore not survived. However, it has not yet proved possible to implement a long-standing plan to establish smaller regional collections in other agro-ecological zones.

FOFIFA maintains coffee collections in Madagascar at three sites (see Table) : Kianjavato (800 accessions, mostly *Mascarocoffea* and *C. canephora*), Ilaka-Est (1,200 accessions, mostly *C. canephora*) ans Sahambavy (about 350 accessions, mostly *C. arabica*). With the bulk of the *Mascarocoffea* collection , Kianjavato is the main site. Its status is precarious. Funds are extremely limited, which means that maintenance work is minimal. Many of the accessions are old and should be replaced. The collection also needs to be duplicated, preferably at a better site on alluvium.

In summary, the main coffee germplasm collections in Africa, though not generally in imminent danger, are likely to be at risk in the near future because of lack of recurrent funds for day-to-day maintenance. They are also under-studied and under-used, because in no case are there researchers working on them on a full-time basis. Only in Ethiopa is further collecting being carried out regularly.

In view of this decidedly unsatisfactory situation, the first strand of this plan of action addresses the strengthening of the physical structure and personnel of the existing facilities for the *ex situ* conservation of the major coffee germplasm collections in Africa. This will require the following specific activities (throughout this document, letters in square brackets refer to budget line, see Section III) :

(1) Training of one scientists from each country to MSc level in plant genetic resources, to work full-time on the *Coffea* collections. [a]

(2) Short training of 3 technicians from Ethiopia and 2 from Madagascar in the maintenance of coffee germplasm collections, the former at the Coffee Research Foundation, Kenya, the latter at IDEFOR/DCC. The technicians will work full-time on the coffee collections on return to their country. [b]

(3) Provision of physical infrastructure at Choche to house full-time staff, plus the establishment of sub-stations at Harage and Sidano. Each of these will have a full-time technician. [c].

(4) The provision of funds for some essential equipment and recurrent costs for the day-to-day maintenance of the collections for five years. [d]

(5) Provision of PlantGeneCD and a CD-ROM reader to all three institutes. [e]

Once a strengthened infrastructure is in place, it will be possible to re-start adding to the collections. Three geographical areas have been identified as gaps in existing germplasm collections. These are : (1) various diploid wild species : central Africa (Gabon and Zaire) ; (2) *C. arabica* and others : Uganda and southern Sudan : (3) *Mascarocoffea* and others : northern Madagascar, southeastern Africa and the Zambezi basin. This plan of action envisages a series of cooperative collecting programmes involving the national programmes of the countries in the target areas listed above and the coffee genetic resources programmes of, respectively, Côte d'Ivoire, Ethiopia and Madagascar. [f]

III - Implementing complementary conservation strategies

Ex situ field collections is clearly not the only way to contribute to the conservation of coffee in Africa, nor in all cases even the best way. Complementary approaches need to be investigated.

One possibility is *in vitro* methods. These also have the potential to contribute to the solution of plant multiplication and germplasm transfer problems. Appropriate laboratories and trained staff exist in Côte d'Ivoire and Ethiopia, but there is a requirement for funds for recurrent costs, and for some equipment in Ethiopia [g]. The problem is different in Madagascar. Not only are trained staff and a laboratory not available there, but appropriate techniques have also not been perfected for *Mascarocoffea* species. This is a research problem that will be directly addressed by the complementary conservation strand of this plan of action in the form of a PhD studentship [h1]. This will be followed by the establishment of a coffee *in vitro* facility in the country [h2].

Another option is *in situ* conservation. Four specific areas are seen as being priorities in this context and will be the subject of research activities under this plan of action :

[i] Ethiopia (1) Survey of forest areas and mapping of *C. arabica* distribution (3 years).

(2) Genetic diversity study (using molecular markers) of geneflow between wild, semi-wild cultivated *C. arabica*, in particular the role of farmers (4 years).

[j] Madagascar (1) Botanical and ethnobotanical inventory of *Coffea* spp in protected areas (3 years).

(2) Development of molecular markers to differentiate among species (1 year)

IV - Improving documentation

One aspect of improved documentation of *Coffea* germplasm collections is being addressed independently of this plan of action but in concert with it by IPGRI. A descriptor list for *C. canephora* is being developed by IPGRI in collaboration with crop experts. A separate list will be developed for *C. arabica*. These will be produced in English, French and Spanish.

The documentation strand of this plan of action involves the wider application of the database software developed by ORSTOM to manage data from the Côte d'Ivoire collections. This software, BASECAFE, will be altered to reflect the descriptor lists under development, then distributed to documentation specialists from Ethiopia and Madagascar at a worshop, during which they will also be trained in its use. Data from collections in these countries will then be entered by the respective institutes. A BASECAFE expert will oversee the process. Data from collections in other countries will eventually be added. The result of the exercice will be a central *Coffea* database for Africa. [k].

V - Improving utilization

Various bottlenecks to improved utilization are addressed by this plan of action. One has already been alluded to. *In vitro* methods should improve plant multiplication and the production of healthy gerplasm. The issue of safe germplasm movement will be addressed directly by the preparation of « Guidelines for the Safe Transfer of *Coffea* Germplasm ». These guidelines will be developed at an international workshop and published in the FAO/IPGRI series [l]. There will be separate recommendations for *C. arabica* and *C. canephora*. The booklet will be published in English, French and Spanish.

Another aspect of improved utilization has to do with documentation. As already described, this plan of action will result in the development of a central database for wild and cultivated coffee in Africa. However, it will take this one step further by providing for the analysis of these data to designate intraspecific core collections for *C. arabica* and *C. canephora* and an interspecific core collection for the whole genus [m]. The methodology developed by Dr S. Hamon at ORSTOM will be used. This will be introduced to documentation experts at a workshop, and another workshop will be held after their analysis of the data to present the results more widely to coffee workers. The expectation is that regional agronomic and other trials using the core collections will follow.

VI - Summary

The five-year plan of action developed at the Nairobi meeting in May 1994 has the following principal objectives :

- Strengthening the infrastructure and personnel of existing field collections in Côte d'Ivoire, Ethiopia and Madagascar.
- Complemting the *ex situ* field collection approach with other types of conservation (*in vitro* methods, *in situ* conservation) and collecting programmes in target countries identified as gaps in existing germplasm collections.
- Improving documentation of the existing germplasm collections (descriptor lists, BASE CAFE Software).
- Improving utilization of the collections by coffee breeders with the expectation that regional agronomic trials will follow.

This draft plan has circulated at the IACO meeting in Lomé last november 94 ; the meeting supported the project proposal for funding by European Union and other possible donors.

VI - Résumé

Un plan d'action à 5 ans a été élaboré à Nairobi en mai 94, avec les objectifs suivants :

- Renforcer les infrastructures et le personnel assurant la conservation et collections de caféiers en champ en Côte d'Ivoire, Ethiopie et Madagascar.
- Développer des stratégies complémentaires de conservation *in situ* et *in vitro*, ainsi que des prospections dans les régions non représentées dans les collections existantes.
- Améliorer l'information sur les collections actuelles basée sur une liste de descriptions et la création d'une base de données (BASECAFE).
- Favoriser l'utilisation de germplasm par les sélectionneurs dans les essais agronomiques.

Le plan a été soumis aux membres de l'Organisation africaine du café à Lomé en novembre 94. Il a été approuvé et la recherche de fonds auprès de l'Union Européenne ou d'autres bailleurs est en cours.

GENETIC IMPROVEMENT OF COFFEE FOR RESISTANCE TO ROOT-KNOT NEMATODES (*MELOIDOGYNE* SPP.) IN CENTRAL AMERICA

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1. INTRODUCTION

The importance of the root-knot nematodes belonging to the genus *Meloidogyne* is now well known in Central America (ANZUETO, 1993 ; PENA, 1994). In certain areas, prevailing nematode populations are highly destructive, inducing death of coffee plants. In other areas, high levels of attack are found but coffee trees seem to develop normally though yields may be affected. All commercial varieties of *Coffea arabica* grown in Central America are susceptible. Chemical control is expensive and not always effective. Use of genetically resistant materials would be the most effective control measure. So far, only in a few countries unselected seed progenies of *C. canephora* are used as root-stock variety.

Resistance breeding needs to take into consideration the variation present among nematode populations. In Central America, taxonomic observations have identified different Meloidogyne species : eg. *M. exigua,* generally being less destructive, *M. incognita* and *M. arabicida,* which cause high damage where they occur. Application of isozyme markers seems to indicate that traditional taxomonic classification may have to be reviewed (SANTOS, 1992 ; see also poster presentation of HERNANDEZ *et al.* at this conference). Based on the type of root symptoms, one can identify species which cause intensive gall formation but little destruction (like *M. exigua*), whereas other species form fewer and smaller root-galls but appear to be more destructive (like *M. incognita* or *M. javanica)* .The first type of namatodes form egg- masses inside the roots , whereas the second type forms egg masses outside the roots (ARANGO et al., 1982 ; ANZUETO, 1993).

CARVALHO (1982) suggests that genetic resistance to one nematode species may not be related to resistance to other species. ANZUETO (1993) has shown similar resistance reactions of *C.canephora* and *C. arabica* towards nematode populations from Guatemala and Brazil. Both nematode populations resemble taxonomically *M. incognita* and form egg masses outside the roots.

In 1990, a breeding programme was inititiated in Central America through the joint effort of PROMECAFE, CATIE, national research organizations, CIRAD and ORSTOM. The main objective is creation of productive varieties with broad resistance to nematodes and coffee leaf rust. Results obtained with regard to resistance to local populations of root-knot nematodes are presented and breeding strategies discussed.

2. MATERIALS AND METHODS

Nematode populations. Results obtained with six nematode populations are presented. The origin and taxonomic classification, based on morphological observations, of these populations are incated below.

NEMATODE POPULATIONS	TAXONOMIC CLASSIFACTION	ORIGIN	EGG MASSES
1	M. arabicida	Juan Vinas, Costa	inside roots
2	M. exigua	Rica	" "
3	M. exigua	CICAFE, Costa Rica	" "
4	M. arenaria	Turrialba, Costa	outside roots
5	M. incognita	Rica	" "
		El Salvador	
		Guatemala	

Coffee genotypes. Commercial varieties of *C. arabica* were compared to ethiopian accessions (ORSTOM or FAO survey), to "Colombian" and "Portuguese" Catimor lines, to Sachimor lines and to crosses of eight ethiopian accessions with Catimor or Catuai. For *C.canephora*, open pollinated progenies were used as well as a factorial 3x4 mating design using parents available in the CATIE collection.

Experiments realized. In Costa Rica field and nursery experiments were carried out using local nematode populations. In one experiment, three months old seedlings of 70 coffee progenies were inoculated in a nursery at the CICAFE station in Heredia by applying 5 g infested roots per pot. Each progeny was presented by 20 seedlings, planted in sterile soil in plastic bags. Observations were made 5 months later, using a 0 to 5 point scale for gall index (GI) : 0 = zero, 1= 1 or 2 ; 2=3 to 10 ; 3 = 11 to 30 ; 4=31 to 100 and 5 = over 100 galls per root system. GI scores appeared to be well correlated to number of nematodes per g of roots (RAFINON, 1994). Field observations were made on adult Sarchimor progenies (40 plants per progeny), present in a highly infested plot at CICAFE. For each line, root systems of 40 plants were noted by using the GI scale. Furthermore, seedlings of seven progenies were planted in *M. arabicida* infested soil at the "Juan Vinas" plantation as well as at the "La Isabel" plantation in Turrialba (*M. exigua)* and inoculated each with 10g of infested roots. Observations were made 15 months after field planting using the GI scale.

In El Salvador, 3,5 months old seedlings of *C. canephora*, planted in plastic bags containing sterile soil, were inoculated each with 2.000 nematodes (eggs + larvae). The number of nematodes was evaluated 4 months later by the centrifugation - flotation extraction method, according to Coolen and D' HERDE (1972). The reproduction index (RI), as defined by TAYLOR (1967) was used to classify the plants : 0 = no reproduction, 1 = less than 1% reproduction ; 2 = 1 to 10% ; 3 = 10 to 25%, 4 = 25 to 50% and 5 = more than 50% reproduction in relation to the number of nematodes for the susceptible control. Nematode numbers were statistically analysed after transformation of data, as recommended by NOE (1985).

In Guatemala, 24 cross progenies of *C. arabica* were inoculated by similar methodology as described for El Salvador. Observations were made when plants were 10 months old. A 0 to 5 point scale was used for evaluation of root damage index (DI) : 0 healthy roots without galls or egg masses, 1 = healthy roots with very few galls or eggs masses, 2 = corky primary root, necrosis of up to 25% of secondary roots ; 3,4 and 5 = as 2, with respectively 25 to 50, 50 to 75 and more than 75% necrosis of secondary roots. Susceptible control varieties scored as 3,4 or 5.

3. RESULTS

3.1. Nematode populations from Costa Rica

In the nursery experiment carried out at CICAFE, Heredia (Table 1), the four control varieties showed high susceptibility (GI scores of 3 to 5). Ethiopian accessions were generally susceptible, only 3 out of 39 lines were segregating. Catimor origins were variable ; more resistance was found among Colombian Catimor lines. All plants of *C. canephora* tested were classified as resistant (GI scores of to 0 to 2). Six accessions tested in the nursery were also observed in the field in Heredia, as six years old plants. Five showed the same reaction type (three susceptible, one resistant and one segregating accession) whereas one accession (T18141) showed uniform resistance in the field, but segregated in the nursery.

Table 1 : **Nursery evaluation of resistance of 74 coffee accessions to a nematode population at CICAFE, Heredia, Costa Rica, (S = susceptible, Seg = segregating, R = resistant, as compared to reaction of control varieties).**

Coffee accession	Resistance reaction (n° of accessions)		
	R	Seg	S
C. arabica			
• Control varieties			4
• Ethiopian lines		3	36
HdT derivatives			
• CIFC 832/1	1	1	5
• CIFC 832/2	8	1	3
• CIFC 1343		6	4
C. canephora	5		
• o.p. progenies			

Seven different accessions, observed in the Heredia nursery (*M.exigua)*, were also tested as young plants in the field in the Juan Vinas plantation (*M. arabicida*) and in the La Isabel plantation (*M. exigua*) in Turrialba. Similar resistance responses were obtained at the three sites (table 2). One Colombian Catimor (T17939) was resistant and one Ethiopian accession segregated. The high level of resistance of T 17939 was confirmed by nematode counts after extraction from roots ; no nematodes were found with this accession whereas Caturra showed 144 to 890 nematodes per g root tissue.

Table 2 : **Evaluation of resistance of seven *C. arabica* accessions in relation to nematode populations from three sites in Costa Rica (S = susceptible, R = resistant, Seg = segregating).**

Accessions	Nematode Population		
	Heredia	Juan-Viñas	La Isabel
"Caturra"	S	S	S
"Catuai"	S	S	S
T17939	R	R	R
Anfilo (1-3)	S	S	-
T16733 (a2)	S	S	S
T4816 (1-3)	S	S	S
T4900 (1-2)	Seg	Seg	Seg

The above results show that resistance observed in the nursery can also be effective in the field. Furthermore, genotypes may be found that are simultaneously resistant to *M. exigua* and *M. arabicida*.

3.2. El Salvador

The results of the greenhouse inoculation of 12 cross combinations of *C. canephora* are shown in table 3. The susceptible control variety showed high nematode reproduction rates. Considering RI classes of 1 to 2 as resistant, the % of resistant plants varied between 0 and 79% for the cross combinations. The best parents are T3561/2-1 and T3751/1-2 ; progenies of these plants showed at least 40% resistant plants. Other parents, like T3757/2-1 and T3753/1-1 did not seem to contribute any resistance to their progenies. Statistical analyses of the number of nematodes indicated high general combining ability and high heritability for nematode resistance (PENA, 1994). The data on the % of resistant plants (table 3) suggest, in fact, that resistance of T3561/2-1 and T3751/1-2 could be mainly related to one dominant gene in each genotype, in heterozygous condition. Presence of intermediate levels of resistance (RI classes of 2 or 3) in some crosses suggests also action of minor genes.

Table 3 : **Evaluation of 12 cross combinations of *C. canephora* for resistance to *M. arenaria* in El Salvador, by estimating average number of nematodes, reproduction index (RI) and % of resistant plants (% R).**

Cross Combination		N° of nematodes ($\sqrt{x}$ +0.5)	RI						% R
			0	1	2	3	4	5	
Control (Pacas)		97.7 c*					5	19	0
T3561 (2-1)	x T3751 (1-2)	21.8 a	11	3	8	2	1	3	79
"	x T3752 (1-3)	35.3 ab	10		5	2	7	4	54
"	x T3753 (1-1)	43.9 ab	5	3	7	4	2	7	54
"	x T3755 (1-1)	30.9 ab	11	2	4	5	1	5	61
3757 (2-1)*	x T3751 (1-2)	56.2 b	6	3	4	1	4	9	39
"	x T3752 (1-3)	105.9 c			6	5	2	10	26
"	x T3753 (1-1)	95.5 c				6	8	13	0
"	x T3755 (1-1)	103.9 c	1			2	4	19	4
T3757 (2-2)*	x T3551 (1-2)	41.5 ab	6	1	10	3	4	4	61
"	x T3752 (1-3)	83.8 c			2	7	2	16	7
"	x T3753 (1-1)	85.6 c	1		3	2	2	15	17
"	x T3755 (1-1)	85.7 c		1	4	2	6	15	18

3.3. Guatemala

Seedlings of 24 cross combinations between *C. arabica* accessions were evaluated for resistance to a local population of *Meloidogyne* (population 5). All male parents (Catuai, Catimor T8667 and Sarchimor T5296) are susceptible to this nematode population, as shown in Table 4 for selfed progenies of Catimor and Sarchimor genotypes. The latter result contrasts to the high resistance of the same Sarchimor progeny in relation to nematodes of Costa-Rica (see 3.1). Resistance observed in the cross progenies should therefore be derived from the female parents. Two ethiopian parents (Et 59/a2 and Et 52/a2) transmit high resistance to most of their descendants, whereas others induce clear segregation for resistance. This result would indicate presence of oligogenic dominant resistance, generally in heterozygons condition, in the Ethiopian parental genotypes, which is in agreement with monogenic or di-genic complementary resistance observed earlier in this type of germplasm (ANZUETO *et al.*, 1994). Two male parents (Rume Sudan a8, and Et 2/a1) do not transmit any resistance to their progenies.

Table 4 : **Resistance reaction of plants of 24 cross progenies among *C. arabica* accessions, evaluated in relation to a nematode population from Guatemela (*M. incognita* ?) by estimating root damage (R = scores o to z and S = scores 3 to 5 for root damage index).**

Male parents	Female Parents					
	Catimor T8667		Sarchimor T5296		Catuai	
	R	S	R	S	R	S
Et 26-a1		10		10		6
Et 59-a2	8	1	7	3	10	
Et 52-a2	8	3	-	-*	9	
Et 47-a4	4	6	6	4	3	7
RS - a8		10	-	-		10
Et 1-a1	5	5	5	5	-	-
Et 6-a2	5	5	4	6	2	8
Et 57-a2	7	3	-	-	7	3
Selfings		9		10	-	-

"-" = not evaluated

4. DISCUSSION AND CONCLUSIONS

4.1. Resistance to nematodes in Costa Rica

Ethiopian accessions of *C. arabica* appeared to be generally susceptible to the *M. exigua* type. This was somewhat surprising, as the same type of germplasm had shown to be a good source of resistance to populations of *M.incognita* type from Guatemala and Brazil (ANZUETO *et al.*, 1993), but seems in accordance with results with *M. exigua* obtained in Brazil (CURY *et al.*, 1970). *C. canephora* showed high resistance to *M. exigua* type nematodes in Costa Rica, confirming results of Morera (1986). Similar results were also reported with regard to *M. exigua* in Brazil (FAZUOLI, 1986).

Resistance of Hybrid of Timor (HdT) derivatives in relation to *M. exigua* is variable ; resistance appeared most frequent with Colombian Catimor, less frequent with Sarchimor and rare with Portuguese Catimor lines. Less data are available with regard to *M. arabicida*. Present results indicate high resistance of a Colombian Catimor line, and segregation of an Ethiopian line (as observed with *M. exigua*). It was noted earlier that *C. canephora* progenies can be highly resistant to *M. arabicida* (CALDERON-VEGA, 1989). These results suggest that resistance to *M. exigua* may also be effective in relation to *M. arabicida*.

4.2. Resistance to nematodes from Guatemala and El Salvador

The nematode populations tested can be classified, morphologically, as *M. arenaria* in El Salvador and *M. incognita* like in Guatemala (see poster presentation of Hernandez *et al.* at this conference). Present results show 0 to 70% of resistance in *C. canephora*, depending on the cross combination, with regard to *M. arenaria*.The best parental plants were T3561 (2-1) and T3751 (1-2). This result is in good agreement with the high % of resistance observed in open pollinated progeny of T3751 (1-2), in relation to nematodes from Guatemala (ANZUETO *et al.*, 1993). Progeny of T3561 has been used with success to select the "Apoata" rootstock variety in Brazil, with good resistance to *M.incognita* in that country.

With regard to *C. arabica*, the present results confirm that Ethiopian accessions can be a good source of resistance to nematodes in Guatemala, as observed earlier (ANZUETO *et al.*, 1993). Little is known so far on resistance of derivatives of the hybrid of Timor with regard to nematodes in Guatemala and in El Salvador.

4.3. Stability of resistance

Table 5 summarizes results of the present investigation as well as of earlier research. Susceptibility of common cultivars of *C. arabica* in relation to *Meloidogyne* populations in Central America is confirmed. Ethiopian accessions are good sources of resistance to destructive populations in Guatemala and Brazil (ANZUETO et al. 1993), but appear generally susceptible to nematodes in Costa Rica, be it *M. exigua* or *M. arabicida* .

Table 5 : **Synthesis of results on resistance screening of coffee genotypes in relation to populations of *Meloidogyne* spp. (++ = high resistance frequent, + = resistance less frequent, ± = resistance rare, - = resistance generally absent).**

Coffee genotypes	Nematode populations			
	Costa Rica	Guatemala	El Salvador	Brazil
C. arabica				
• cultivars	-	-	-	-
• Ethiopian lines	-	++	?	++
C. canephora	++	+	+	+
• HdT derivatives	+	± (?)	?	±

On the other hand, resistance of *C. canephora* or of derivatives HdT is more easily found in relation to *Meloidogyne* populations in Costa-Rica than in relation to populations from Guatemala, El Salvador and Brazil. These results are in general agreement with findings in Brazil in relation to *M. exigua* and *M. incognita* types (CURI *et al.*, 1970 ; FAZUOLI, 1986 ;

GONCAVES, 1988). It is curious to note that the resistance reaction of coffee genotypes appear to be more related to the characteristic of the nematode in forming egg masses inside the roots (*M. exigua* and *M. arabicida*) or outside the roots (*M.arenaria, M. incognita* types) than to the species et *Meloidogyne* involved. This hypothesis would merit confirmation, as it suggests that resistance might be stable in relation to *Meloidogyne* species which cause similar root symptoms, hence simplification of testing for resistance may be possible.

4.4. Breeding strategies

The above presented results and discussion suggest that broad resistance to *Meloidogyne* populations in Central America can be obtained at short term by using a selected *C. canephora* rootstock variety. The best results were obtained so far with the cross between T3561 (2-1) and T3751 (1-2). This cross combination can be expected to give more than 80% resistant plants in relation to nematode populations from Guatemala and El Salvador and near to 100% resistant plants in relation to Costa Rican populations. Therefore, commercial use of this cross combination seems justified, although search for even higher resistance levels should be continued. On the other hand, a seedling variety of *C. arabica* with broad resistance may be obtained by combining the complementary resistance of selected Ethiopian accessions with that of derivatives of HdT, or by further selection within Catimor and Sarchimor germplasm. This strategy, in order to be successful, needs further testing of this germplasm in relation to nematode types forming eggs outside the roots (as *M. arenaria* and *M. incognita* types), as well as in relation to *M. arabicida*.

ABSTRACT

All cultivated varieties of *Coffea arabica* in Central America are susceptible to local populations of root-knot nematodes, belonging to the *Meloidogyne* genus. Chemical control of this important coffee pest is expensive and not always efficient, therefore genetic solutions are to be envisaged. So far, only unselected populations of *c. canephora* have been used in some areas in Central America as root-stocks for *C. arabica* varieties. The objective of this paper is to present recent results of the PROMECAFE breeding programme. The genetic material evaluated comprised *C. canephora* progenies, *C. arabica* accessions from Ethiopia and progenies derived from the hybrids of Timor (Catimor, Sarchimor), as well as susceptible control varieties (Caturra, Catuai). Laboratory or field observations were carried out with regard to nematode populations from Guatemala, El Salvador and Costa Rica.

The result show general susceptibility of most *C. arabica* accessions to nematodes from Costa Rica, whereas the same type of material had shown before high frequency of resistant plants to nematodes from Guatemala. With *C. canephora*, progenies were identified with high resistance to nematode populations from all three countries. Some Catimor and Sarchimor lines showed high resistance to nematodes from Costa Rica. These results suggest specific resistance responses with regard to nematodes from different countries. These differences seem to be related to the characteristic of the nematodes to form egg masses outside or inside the roots. Based on these observations strategies for breeding resistant root-stock varieties of *C. canephora* and seed varieties of *C. arabica* are proposed.

RESUMEN

Todas las variedades de cafeto cultivadas en America Central son susceptibles a poblaciones de *Meloidogyne* spp. El control guimico es caro y no es siempre eficaz, por esto soluciones geneticas deben ser procuradas. Hasta ahora, solo genotipos no seleccionados de *C. canephora* son utilizados en algunas areas en America Central como variedades de porta-injerto para *C. arabica*. El objectivo de este trabajo es de presentar resultados recientes del programa de mejoramiento genetico de PROMECAFE. El germoplasma evaluado consiste en descendencias de *C. canephora*, lineas de *C. arabica* de Etiopia, progenies derivadas del Hibrido de Timor (Catimor, Sarchimor) y variedades testigas (Caturra, Catuai). Observaciones fueren realizadas en el inverdanero y en el campo, utilizando poblaciones de nematodos de Guatemala, El Salvador y Costa Rica.

Se observo susceptibilidad generalizada en las lineas de Etiopia frente a los poblaciones de Costa Rica ; sin embargo este material anteriormente mostro ser resistente frente a nematodos de Guatemala. Progenies de *C. canephora* fueron identificadas con alta resistencia a nematodos de los tres paices. Algunas lineas de Catimor y Sarchimor mostraron alta resistancia a nematodos de Costa Rica. Estos resultados sugieren que respuestas especificas existen de los cafetos en relacion con los nematodos testados. Estas diferencias parecen relacionadas con las caracteristicas de los nematodos de poner las masas de huevos al interior o al exterior de las

raizes. Basando-se en estas observaciones, se proponen estrategias de obtencion de variedades porta-injerta de *C. canephora* o variedades hibridas de *C. arabica* con resistencia amplia a poblaciones de *Meloidogyne*.

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GENOTYPE BY ENVIRONMENT INTERACTION OF BEAN CHARACTERISTICS IN ARABICA COFFEE

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1. Introduction

Bean characteristics in coffee have been classified by Wormer (1964) as normal and abnormal beans. A normal bean is originated from a berry having two cavities, and the bean in each cavity develops normally, so that it will produce two beans which are normal in shape and size.

However, complicated growth sequence of bean formation often occurs. Common deviations are the formation of triage bean, elephant bean, round bean and empty locule as well as other abnormally shaped beans.

Some of bean abnormalities in coffee are estimated to be controlled by genetic factors, and some are due to environmental factors. Antunes-Filho & Carvalho (1954) reported that among the progenies of "Mundo Novo", it was observed that 42 per cent of them produce high quantity of empty fruit locules. Their observation indicated that the tendency to produce fruits with empty locules was a genetic characteristic. Sundar (1981) also reported that the percentage of empty locule formation in the variety differred from the other. Empty locules on S 795 was observed only 4,3 per cent, but that of S 945 reached 59,0 per cent.

The formation of abnormal beans in coffee, of course, will generate problems on productivity and physical quality of coffee bean. This study deals with the role of genotype by environment interaction in the formation of normal and abnormal beans in coffee.

2. Material and method

This study was conducted at three different locations in East Java, namely :

- Kalisat (+ 1.200 m asl., dry area, medium fertile soil)
- Pasewaran (+ 750 m asl., dry area, medium fertile soil)
- Malangsari (+ 625 m asl., wet area, fertile soil).

In each location the experiment was arranged at Randomized Complete Block Design with 4 replications. The treatments consisted of 12 Arabica coffee genotypes i.e. BP 415 A, BP 425 A, BP 426 A, BP 427 A, BP 428 A, BP 429 A, Caturra Red, Kartika 1, Kartika 2, USDA 762, AB 7 and S 1934. Each plot consisted of 20 plants.

The abnormal beans observed in this study were round bean, elephant bean, triage bean and empty locule as described by Wormer (1964). The observation on bean characteristics was done in 1993 and 1994 on coffee in the second and third crops, respectively. Observation was done by making cross section on 100 almost mature berries. The percentage of each bean characteristic was calculated to the total beans observed.

The effect of genotype by environment interaction was indicated from combined analysis of variance. The means of bean characteristic percentage were grouped by using cluster method according to Scott & Knott (1974). The stability parameters (bi and Sdi^2) of each bean characteristic on different environment was analysed according to Eberhart & Russell (1966) method.

3. Result and discussion

a. Variability of normal and abnormal beans

Variability between genotypes of each bean characteristic is presented in Table 1.

Table 1: Percentage of each bean characteristics

Genotypes	Bean characteristics				
	Normal	Round	Elephant	Triage	Empty Locule
BP 415 A	61.7 **B**	11.5 **B**	3.2 **B**	4.5 **D**	12.2 **C**
BP 425 A	79.9 **D**	5.1 **A**	1.1 **A**	5.6 **E**	5.3 **A**
BP 426 A	80.2 **E**	5.7 **A**	1.2 **A**	3.4 **C**	6.1 **A**
BP 427 A	75.7 **C**	6.4 **A**	1.3 **A**	5.9 **E**	6.3 **A**
BP 428 A	69.8 **C**	9.8 **B**	2.7 **B**	2.4 **B**	9.9 **C**
BP 429 A	65.7 **B**	6.1 **A**	9.6 **C**	5.8 **E**	7.3 **B**
Caturra red	75.9 **C**	5.9 **A**	2.1 **B**	5.5 **E**	5.5 **A**
Kartika 1	74.6 **C**	6.6 **A**	2.2 **B**	5.7 **E**	6.5 **A**
Kartika 2	72.6 **C**	6.9 **A**	2.3 **B**	5.6 **E**	8.2 **B**
USDA 762	87.9 **E**	4.1 **A**	0.5 **A**	0.9 **A**	4.1 **A**
AB 7	63.4 **B**	16.6 **C**	0.5 **A**	0.6 **A**	17.6 **D**
S 1934	52.7 **A**	9.2 **B**	14.0 **D**	8.7 **F**	10.7 **C**

Note : Figures in the same bean characteristics followed by the same letter were classified in the same cluster according to Scott & Knott (1974) method at 5 per cent level.

The lowest percentage of normal bean belongs to S 1934, because the variety produces high percentage of abnormal beans, especially elephant bean. Elephant bean is produced when one cavity contains more than one ovule. Consequently, more than one separate beans, normally two beans, are formed in one parchment. Usually the size of elephant bean is larger than the normal one. Problems of elephant bean occur on the depulping and dehulling processes since it produces not only split beans but also broken beans.

S 1934 was selected in India on the fifth progeny of S 288 x Kents (Srinivasan & Vishveshwara, 1978). The origin of S 288 itself was reported by Narasimhaswamy (1960) as a progeny of natural cross between *Coffea arabica* L. and *Coffea liberica* Hiern.

Under East Java condition, S 1934 performs very vigorous growth and resistant to leaf rust *(Hemileia vastatrix* B. *et* Br.*)* as well as high yielding capacity (Mawardi *et al.*,1990). However, the variety produces high percentage of abnormal beans, especially elephant beans.

A high percentage of elephant bean is also observed on BP 429 A (9.6 per cent). This variety was selected by the authors from the third progeny of S 288 x AB 3 in East Java. Therefore, it may be estimated that the progenitor of elephant bean characteristic is S 288. This abnormality might be inherited from *C. liberica.* Wormer (1964) mentioned that elephant bean was caused by a genetic aberration.

The highest percentage of empty locules belongs to AB 7, a typica type selected in Java. The disadvantage of high percentage of empty bean is the effect on yield reduction.

Empty locules are formed when integument expands normally but the endosperm fails to grow. Apparently a certain amount of empty locules come from physiological factors (Wormer,1964). However, an observation made by

Antunes-Filho & Carvalho (1954) on "Mundo Novo" indicated that the tendency to produce fruits with empty locules is a genetic characteristics.

By selection, Carvalho & Antunes-Filho (1955) had successfully reduced the percentage of abnormal plants, i.e. the plant that produced high percentage of empty beans, in Mundo Novo progenies.

Sometimes one of the two integuments fails to grow. The other one then has the opportunity to occupy the whole berry and in doing so becomes a **"pea berry"**. A "pea berry" then produces a round bean. In Arabica coffee, "pea berries" occur at a frequency of roughly 10 per cent, but differences exist between varieties. The major determining factor of "pea berry" formation is a genetic nature (Wormer,1964). This study showed that the highest percentage of round bean belongs to AB 7 (16.6 per cent), whereas the lowest one belongs to USDA 762 (4.1 per cent).

Sometimes one ovary of Arabica coffee flower contains three ovules in three cavities. The three ovules and their endosperms often grow together successfully to be triage seeds in one berry. Normally, the size of triage seed is smaller than the normal one. Wormer (1964) mentioned that the formation of triage bean is caused by the occurence of a genetic aberration. This study showed that the lowest percentage of triage bean belongs to AB 7. Progenies of *C. liberica* (S 1934 and BP 429 A) produce high percentage of triage beans.

b. Genotype by environment interaction on each bean characteristic.

The analysis of genotype by environment interaction of each bean characteristic is presented in Table 2.

Table 2 : Combined analysis of variance of each bean characteristic in Arabica coffee.

Sources of variation	df	Bean characteristics				
		Normal	Round	Elephant	Triage	Empty Locules
1. Varieties	11	**	**	**	**	**
2. Environment	5	**	**	**	**	**
- Year	1	ns	ns	ns	ns	ns
- Location/year	4	**	**	**	**	**
3. Replication/Env.	18	ns	*	ns	ns	*
- Replication/year	6	ns	ns	ns	ns	ns
- Rep. x (Loc./year)	12	ns	ns	ns	ns	ns
4. Varieties x Env.	55	*	*	**	**	**
- Var. x year	11	ns	ns	ns	ns	ns
- Var. x (Loc./year)	44	*	*	**	**	**
5. Var.x (Rep./Loc./Year)	198					

Notes : * : Significant at 5 per cent level
**: Significant at 1 per cent level
ns : Not Significant

The analysis of variance showed that the genotype by environment interaction is significantly different for each bean characteristic. This result indicates that the formation of both normal and abnormal beans are not only determined by genetic factor but also affected by environment where the genotype is grown. Information of the role of environment in determining coffee bean formation is still lacking.

From Table 2, it can be understood that the effect of location, mainly altitude (see Appendix 1), on the process of coffee bean formation is more important than the effect of year. Antunes-Filho & Carvalho (1954) also mentioned that the number of empty fruit locules on Mundo Novo nearly the same from year to year.

c. Genotype stability of each bean characteristic

The presence of genotype by environment interaction on coffee bean formation then gives an opportunity to analyse the stability of each bean characteristic per genotype. The result of the stability test is presented in Table 3.

Table 3 : Stability parameters of bean characteristics

Genotype	Stability parameter	Bean characteristics				
		Normal	Round	L-ephant	Triage	Empty
BP 415 A	bi	1.53 ns	1.92 *	0.64 ns	3.52 ns	2.09 *
	Sdi^2	13.33 *	9.90 *	-1.09 ns	-0.15 ns	-1.32 ns
		Unst	**Unst**	**Stab**	**Stab**	**Unst**
BP 425 A	bi	0.51 ns	0.56 ns	-0.03 ns	3.38 ns	0.86 ns
	Sdi^2	-1.30 ns	1.89 *	-0.97 ns	-0.03 ns	-1.36 ns
		Stab	**Unst**	**Stab**	**Stab**	**Stab**
BP 426 A	bi	0.43 ns	0.59 ns	0.15 ns	1.27 ns	0.91 ns
	Sdi^2	1.59 *	0.64 ns	-1.2 ns	0.89 *	-0.64 ns
		Unst	**Stab**	**Stab**	**Unst**	**Stab**
BP 427 A	bi	0.89 ns	0.99 ns	0.06 ns	1.85 ns	0.90 ns
	Sdi^2	40.17 *	-1.28 ns	0.16 ns	8.83 *	-1.41 ns
		Unst	**Stab**	**Stab**	**Unst**	**Stab**
BP 428 A	bi	1.10 ns	0.93 ns	0.44 ns	1.32 ns	1.38 ns
	Sdi^2	3.18 *	-0.88 ns	1.91 *	3.62 *	-1.37 ns
		Unst	**Stab**	**Unst**	**Unst**	**Stab**
BP 429 A	bi	0.86 ns	1.27 ns	3.31 *	0.11 ns	0.73 ns
	Sdi^2	71.82 *	0.54 ns	40.36 *	5.78 *	0.80 ns
		Unst	**Stab**	**Unst**	**Unst**	**Stab**
Caturra red	bi	1.08 ns	1.17 ns	0.62 ns	2.20 ns	0.82 ns
	Sdi^2	11.44 *	-1.41 ns	-0.86 ns	2.85 *	-0.63 ns
		Unst	**Stab**	**Stab**	**Unst**	**Stab**
Kartika 1	bi	0.51 ns	0.68 ns	0.35 ns	2.81 ns	0.92 ns
	Sdi^2	-1.35 ns	0.36 ns	-1.41 ns	-0.27 ns	-1.15 ns
		Stab	**Stab**	**Stab**	**Stab**	**Stab**
Kartika 2	bi	0.64 ns	0.95 ns	0.36 ns	3.27 ns	1.09 ns
	$Säi^2$	36.77 *	5.88 *	-0.37 ns	0.57 ns	0.92*
		Unst	**Unst**	**Stab**	**Stab**	**Unst**
USDA 762	bi	0.47 ns	0.36 ns	0.11 ns	-0.37 ns	0.60 *
	Sdi^2	1.56 *	-1.11 ns	-1.14 ns	0.01 ns	-0.58 ns
		Unst	**Stab**	**Stab**	**Stab**	**Unst**
AB 7	bi	1.83 *	1.99 *	0.07 ns	-0.57 ns	1.31 ns
	Sdi^2	20.94 *	-0.56 ns	-1.19 ns -	0.81 ns	0.82 *
		Unst	**Unst**	**Stab**	**Stab**	**Unst**
S 1934	bi	2.09 *	0.57 ns	5.89 *	-6.81 ns	0.36 *
	Sdi^2	8.33 *	24.76 *	-0.34 ns	3.52 *	24.0 *
		Unst	**Unst**	**Unst**	**Unst**	**Unst**

Notes : * : Significant at 0.05 level (for b=1; Sdi^2=0) ns : Not significant
Unst : Unstable
Stab : Stable

The stability of one genotype to produce normal and abnormal beans is different to the other one. For instance, BP 425 A and Kartika 1 perform good stability to produce normal bean at different environments, however, the other genotypes do not perform this stability.

The differences of stability performance on each genotype to produce each bean characteristic should be considered by coffee breeders in order to select and to release varieties or clones of Arabica coffee.

4. Conclusion

Arabica coffee varieties perform high variability of normal and abnormal beans. Some of them produce high percentage of abnormal beans. The effect of genotype by environment interaction is significantly different for each bean characteristic, so that the formation of both normal nad abnormal beans are not only determined by genetic factor but also affected by environment were the genotype is grown. The effect of location, mainly altitude, on the formation of abnormal beans is more important than the effect of crop year.

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Summary

This study is dealing with the effect of genotype by environment interactions on characteristics of Arabica coffee bean. The characteristics include normal bean as well as abnormal bean e.i. elephant bean, round bean (pea berry), triage bean and empty locule.

The experiments were conducted simultaneously at three estates in East Java, namely Malangsari estate (+ 625 m asl., wet climate), Pasewaran estate (+750 m asl., dry climate), and Kalisat estate (+1200 m asl., dry climate). In each site, the experiment was arranged in Randomized Complete Block Design with 12 genotypes and 4 replications. The observation of bean characteristics was done at the second and third crops.

The results showed that the coffee bean characteristics were significantly different from a genotype to the other one. The highest normal bean percentage belonged to USDA 762 (87.9), and the lowest one belongs to S 1934 (52.7). In contrast, the highest percentage of elephant bean belongs to S 1934 (14.0), whereas the lowest one belonged to AB 7 (0.5) and USDA 762 (0.5).

Genotype by environment interactions observed were highly significant different for each bean characteristics. Bean abnormalities are predominantly affected by the different altitude location rather than different crop year. The higher altitude, the higher percentage of abnormal beans.

APPENDIX 1 : Mean of each bean characteristic on each location (in per cent).

Normal bean

Genotype	Locations		
	Kalisat (+1200m)	Pasewaran (+750m)	Malangsari (+625m)
BP 415 A	53.0	49.1	74.7
BP 425 A	72.2	76.9	83.5
BP 426 A	72.8	80.3	87.0
BP 427 A	71.0	68.6	82.7
BP 428 A	61.0	62.3	74.1
BP 429 A	47.7	68.6	79.7
Caturra red	65.3	68.9	82.3
Kartika 1	64.6	71.3	79.6
Kartika 2	59.8	71.7	77.0
USDA 762	77.5	84.2	91.1
AB 7	63.2	48.7	80.4
S 1934	39.2	45.0	71.4

Round bean

Genotype	Locations		
	Kalisat (+1200m)	Pasewaran (+750m)	Malangsari (+625m)
BP 415 A	9.5	22.2	6.7
BP 425 A	8.8	7.9	3.2
BP 426 A	8.4	7.1	3.4
BP 427 A	9.0	10.3	3.3
BP 428 A	12.0	14.5	8.3
BP 429 A	7.9	11.2	2.9
Caturra red	9.8	10.4	3.9
Kartika 1	10.7	9.6	4.2
Kartika 2	11.8	10.8	4.3
USDA 762	7.6	6.2	3.5
AB 7	17.1	25.0	9.5
S 1934	5.0	15.3	8.7

Elephant bean

Genotype	Locations		
	Kalisat (+1200m)	Pasewaran (+750m)	Malangsari (+625m)
BP 415 A	10.8	3.1	3.2
BP 425 A	3.0	1.6	1.9
BP 426 A	3.1	1.5	1.1
BP 427 A	2.9	2.3	1.0
BP 428 A	6.2	3.1	2.4
BP 429 A	23.6	3.2	6.5
Caturra red	5.5	2.6	1.8
Kartika 1	5.5	2.1	1.9
Kartika 2	6.1	2.5	3.3
USDA 762	2.2	0.7	0.5
AB 7	0.5	0.6	0.1
S 1934	28.0	12.1	0.9

Triage bean

Genotype	Locations		
	Kalisat (+1200m)	Pasewaran (+750m)	Malangsari (+625m)
BP 415 A	2.2	2.5	6.7
BP 425 A	3.0	4.7	7.5
BP 426 A	1.6	2.8	3.5
BP 427 A	2.4	5.7	7.2
BP 428 A	0.9	2.7	4.1
BP 429 A	6.3	3.4	5.6
Caturra red	4.5	2.7	7.0
Kartika 1	3.3	4.0	7.6
Kartika 2	3.7	2.6	7.5
USDA 762	1.6	1.0	0.5
AB 7	1.3	0.3	0
S 1934	14.0	7.6	3.5

Empty locule

Genotype	Locations		
	Kalisat (+1200m)	Pasewaran (+750m)	Malangsari (+625m)
BP 415 A	24.5	23.1	8.6
BP 425 A	13.0	8.9	3.8
BP 426 A	14.0	8.3	5.0
BP 427 A	14.7	13.1	5.8
BP 428 A	19.8	17.4	11.1
BP 429 A	14.5	13.6	5.3
Caturra red	14.7	15.4	4.9
Kartika 1	15.8	13.0	6.6
Kartika 2	18.2	12.4	7.9
USDA 762	11.0	7.9	4.4
AB 7	17.8	25.4	9.9
S 1934	13.8	20.0	9.7

COMPARAISONS DE MÉTHODES D'ÉCHANTILLONNAGE DU SCOLYTE DU FRUIT DU CAFÉIER (*HYPOTHENEMUS HAMPEI* FERR.)

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INTRODUCTION

Le scolyte est un petit coléoptère qui s'attaque aux fruits du caféier. Il perfore la baie et sillonne de galeries l'albumen de la graine pour se nourrir et se reproduire. C'est l'un des déprédateurs du caféier les plus dommageables : il provoque des chutes de fruits et altère la qualité du café. La lutte contre le scolyte est culturale, chimique et biologique. La décision de traiter se fonde sur la connaissance des niveaux d'infestation établis par un échantillonnage et est prise lorsque le résultat dépasse un seuil lié au prix du café et au coût de la lutte.

La plupart des méthodes d'échantillonnage utilisées actuellement font appel à un choix "aléatoire" de fruits. Or le choix est en fait réalisé par l'observateur. Compte tenu des fortes hétérogénéités entre fruits (aspect, localisation dans l'arbre ...), nous avons émis l'hypothèse que l'observateur pouvait se laisser influencer par certains critères, ce qui pourrait biaiser le résultat de l'échantillonnage.

Nous avons donc réalisé une étude expérimentale comparative de différents types d'échantillonnages afin de détecter d'éventuels biais, ce qui nous permettra de déterminer dans quelle mesure l'observateur peut influencer l'estimation du taux d'attaque.

QUELQUES RAPPELS SUR L'ÉCHANTILLONNAGE

Présentation des techniques basiques d'échantillonnage

On peut définir 4 techniques basiques d'échantillonnage :

- L'échantillonnage aléatoire, où les unités échantillonnées sont choisies de manière totalement aléatoire dans l'ensemble de la population.
- L'échantillonnage systématique, où elles sont espacées de manière régulière.
- L'échantillonnage stratifié, dans lequel la population est découpée en strate. Dans chaque strate, on *échantillonne des unités au hasard.*
- Enfin, l'échantillonnage en grappe, pour lequel on divise la population en ensembles appelés grappes. Tous les individus de grappes choisies de manière aléatoire sont observés.

Des combinaisons de toutes ces méthodes sont évidemment possibles, c'est l'échantillonnage à plusieurs degrés.

L'échantillonnage fournit une estimation de la valeur que l'on désire connaître. La différence entre cette estimation et la quantité à estimer est l'erreur d'échantillonnage. Cette erreur est généralement la composition de diverses erreurs dont certaines sont aléatoires, d'autres liées aux conditions de l'expérience ou à l'expérimentateur. Gy (1992) distingue donc deux qualités que doit remplir la procédure d'échantillonnage (on considère des échantillons obtenus strictement dans les mêmes conditions) : la représentativité et la répétabilité.

- la représentativité : c'est la qualité qui correspond à l'absence d'une erreur systématique (ou biais). On la mesure en pratique par la moyenne des erreurs d'échantillonnage.
- la répétabilité : c'est l'étroitesse de l'accord entre les résultats des échantillonnages. On la mesure en général par la variance des erreurs d'échantillonnage (Multon, 1991). Lorsque les conditions d'échantillonnage ne sont pas totalement identiques, on l'appelle la reproductibilité.

Les différences entre différentes répétitions d'un même échantillonnage sont liées soient aux fluctuations d'échantillonnage, soit à des erreurs à caractère aléatoire dues, par exemple, à de mauvaises manipulations. Cependant, les erreurs liées aux conditions d'expérimentation ne peuvent être considérées comme aléatoires que si on échantillonne un grand nombre de cas.

Les méthodes actuellement les plus utilisées pour échantillonner les dégâts du scolyte

Diverses méthodes sont actuellement employées pour échantillonner les dégâts du scolyte. On peut les diviser en deux groupes : celles qui prennent en compte l'arbre comme unité de comptage et celles qui considèrent l'ensemble des fruits sans tenir compte de la structuration (arbre, glomérule ...).

Les plus simples préconisent l'examen de fruits choisis au hasard sur la parcelle (Bruneau de Miré, 1973). D'autres recommandent l'utilisation de grappes (de un ou plusieurs arbres). Les grappes sont choisies soit au hasard (Anacafé, 1989), soit de manière semi-systématique, c'est à dire que la ligne, dans lequel l'arbre est choisi, est prise de manière systématique, mais la position de l'arbre dans la ligne est tirée au hasard (Sanchez y Ramirez, 1984 ; Decazy et al, 1989). Sur chaque arbre de la grappe, des fruits sont examinés au hasard. Certains auteurs ont également préconisé d'examiner une branche choisie à mi-hauteur dans les arbres (Baker et al, 1989 ; Barrera, 1994).

MESURES EFFECTUÉES

Deux types de mesures ont été effectués : une comparaison de différentes techniques d'échantillonnage sur un arbre et un test de l'échantillonnage aléatoire sur une petite parcelle.

Échantillonnages sur un arbre

70 caféiers ont été retenus. Un observateur entraîné a examiné successivement sur chacun de ces arbres :

- 100 fruits choisis au hasard (échantillonnage aléatoire)
- les fruits de 30 glomérules choisis également au hasard (échantillonnage en grappe) soit environ 144 fruits
- les fruits de 1 glomérule sur 7 (échantillonnage systématique) soit en moyenne 105 fruits
- tous les fruits de l'arbre (recensement)

Il a dénombré les fruits sains et perforés, ce qui permet de calculer le taux d'attaque réel de l'arbre ainsi que les taux estimés par les trois méthodes. Toutes les baies mesurant plus de 0,5 cm ont été considérées comme des fruits. Le glomérule a été défini comme étant un ensemble de un ou plusieurs fruits rattachés au même noeud.

Nous nous sommes limités à l'étude des résultats d'un seul observateur afin de toujours nous placer dans des conditions expérimentales proches. De même, pour pouvoir considérer que la variance d'échantillonnage est constante, nous n'avons inclus dans l'étude que des caféiers ayant des taux d'attaque compris entre 1 et 7,5 %.

Comme nous disposons pour chaque mesure de la véritable valeur, nous utiliserons des régressions pour estimer le biais et la variance des erreurs d'échantillonnage (Grappin, 1991) :

Soit x_i la valeur mesurée pour un arbre par l'une des trois méthodes, v_i la valeur réelle, le modèle s'écrit :

$x_i = a\ v_i + b + \varepsilon_i$ où ε_i suit une loi normale $\mathcal{N}(0,\sigma^2)$,
a représente un biais multiplicatif (calibrage),
b un biais additif.

Ces deux termes de biais mesurent donc la représentativité des méthodes. Si *a* est significativement différent de 1 ou *b* différent de 0, il y a une erreur systématique. ε_i représente les erreurs aléatoires. Sa variance est une mesure de la reproductibilité des méthodes d'échantillonnage. On peut donc comparer la précision des différentes méthodes en comparant les variances associées par un test F (test de Fisher-Snedecor). Les différences de nombre de fruits par arbre engendrent des différences de variance théorique d'échantillonnage. Cependant du fait du nombre important de cas, nous considérons ces différences comme aléatoires et donc comprises dans ε_i.

Lorsque le biais additif, *b*, n'est pas significativement différent de 0, nous avons recalculé le biais multiplicatif, *a*, sous l'hypothèse d'un biais *b* nul (régression sans terme constant).

Une analyse complémentaire, le test des rangs signés de Wilcoxon pour des échantillons appariés (Siegel, 1956), a été effectuée pour tester l'hypothèse d'une différence nulle entre le nombre de fruits par glomérule échantillonné au hasard et le nombre moyen de fruits par glomérule dans l'arbre.

Échantillonnages sur une parcelle

Cinq types d'échantillonnages aléatoires (examen de respectivement 50, 100, 150, 250 et 400 fruits) ont été réalisés sur une parcelle comportant 15 lignes de 30 arbres. Ces échantillonnages ont été réalisés par 4 observateurs dont deux étaient entraînés (observateurs 1 et 2) alors que les deux autres n'avaient jamais réalisé ce type d'échantillonnage (observateurs 3 et 4). Pour trois des échantillonnages, 4 prélèvements ont été effectués, pour les deux autres (150 et 400 fruits) seuls 3 prélèvements ont été réalisés. Les prélèvements s'échelonnent sur deux mois mais il s'agit d'une période de faible évolution des attaques et le taux peut donc être considéré comme constant. Sur cette même parcelle, un échantillonnage très important (1/4 des glomérules de tous les arbres) a été réalisé. Cela nous fournit une valeur de référence que l'on considère comme représentant la valeur exacte du taux d'attaque.

Afin de déterminer l'influence des différents facteurs, une analyse de variance a été réalisée. En raison du déséquilibre du dispositif, le type Henderson III a été choisi pour tester les effets de facteurs.

RÉSULTATS

Échantillonnages sur un arbre

Les résultats obtenus sont représentés sur les figures 1 à 3. Sur chaque graphique nous avons représenté le taux d'attaque estimé par l'une des trois méthodes en fonction du taux réel ainsi que les régressions obtenues. La bissectrice correspond à que ce qu'on aurait obtenu si la méthode était exacte (erreurs d'échantillonnage nulles).

Le tableau I résume les résultats.

Tableau I : Résumé des régressions réalisées sur chaque méthode d'échantillonnage par arbre

méthode		moyenne	R^2 *	moyenne des erreurs	variance des erreurs	*a*	*b*
recensement		3,4459					
aléatoire	régression avec terme constant	6,4143	0,3752	-0,0000	13.26	**1,590**	0,935 ***
	régression sans terme constant		0,7885	0,1915	13,25	**1,806**	-
en grappe	régression avec terme constant	3,9765	0,4589	0,0000	4,45	1,094 **	0,205 ***
	régression sans terme constant		0,8181	0,0420	4,39	**1,142**	-
systéma tique	régression avec terme constant	3,4035	0,1868	-0,0000	6,50	0,689 **	1,030 ***
	régression sans terme constant		0,6623	0,2109	6,63	0,926 **	-

* Le R^2 associé à la régression sans terme constant n'est pas calculé de la manière habituelle car il n'y a pas ajustement par rapport à la moyenne. Il n'est donc pas comparable avec le R^2 associé à la régression avec terme constant.
** Valeur de *a* non significativement différente de 1 au seuil de 5%.
*** Valeur de *b* non significativement différente de 0 au seuil de 5%.

Figure 1 : fiabilité de l'échantillonnage aléatoire de 100 fruits sur un arbre

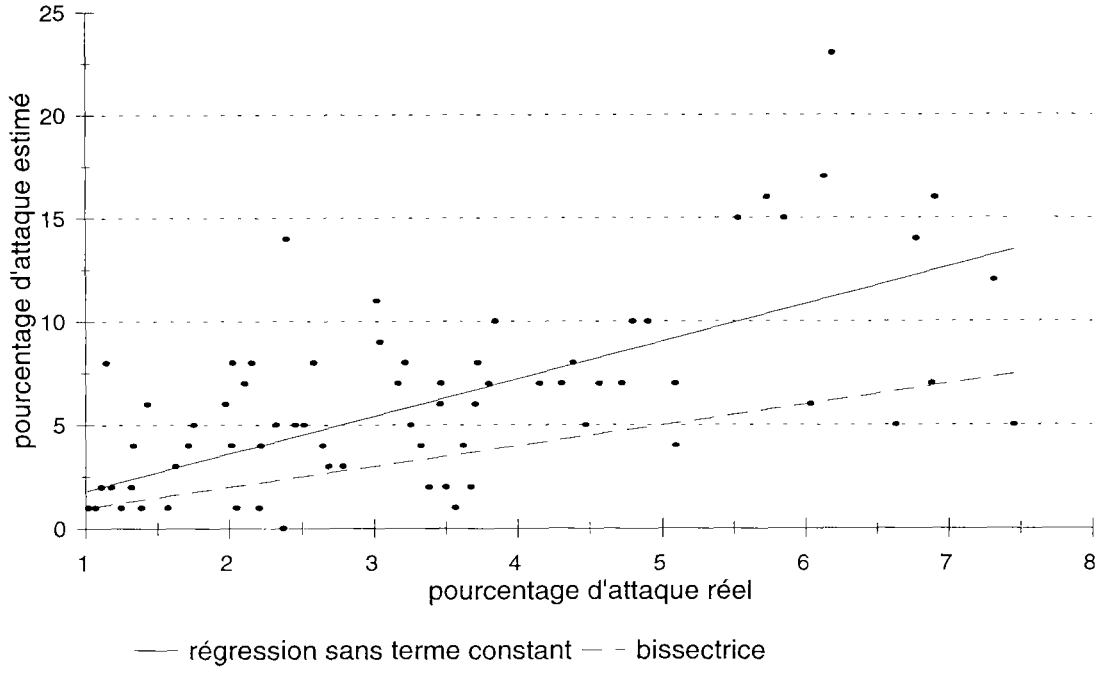


Figure 2 : fiabilité de l'échantillonnage aléatoire de 30 glomérules sur un arbre

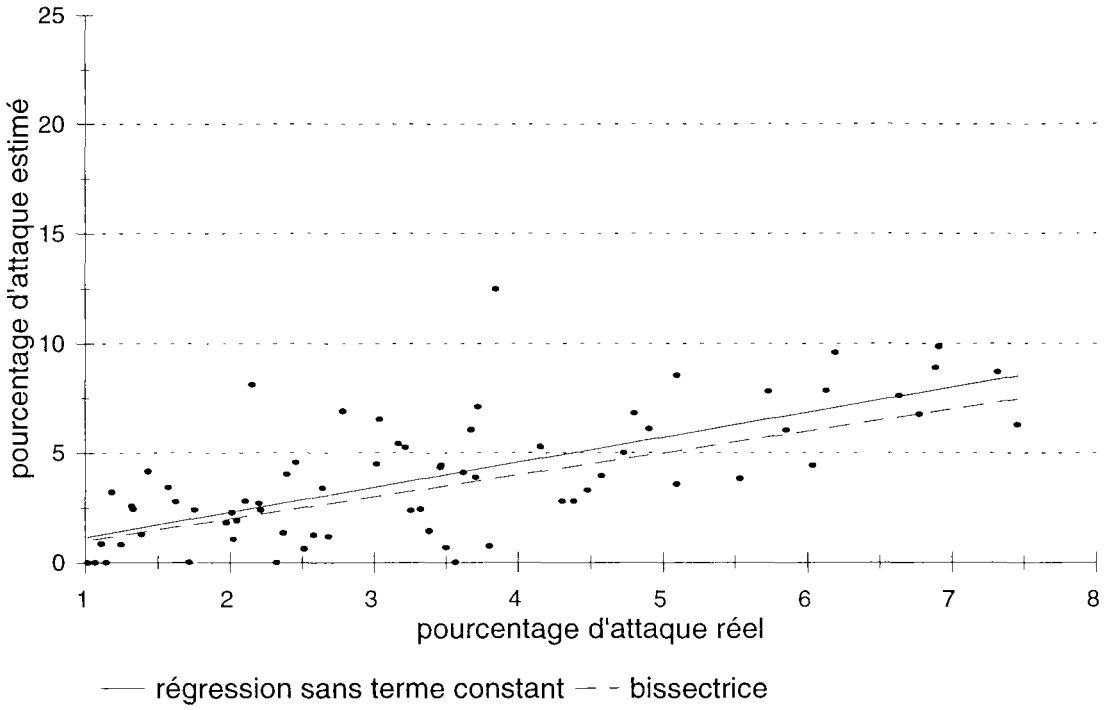
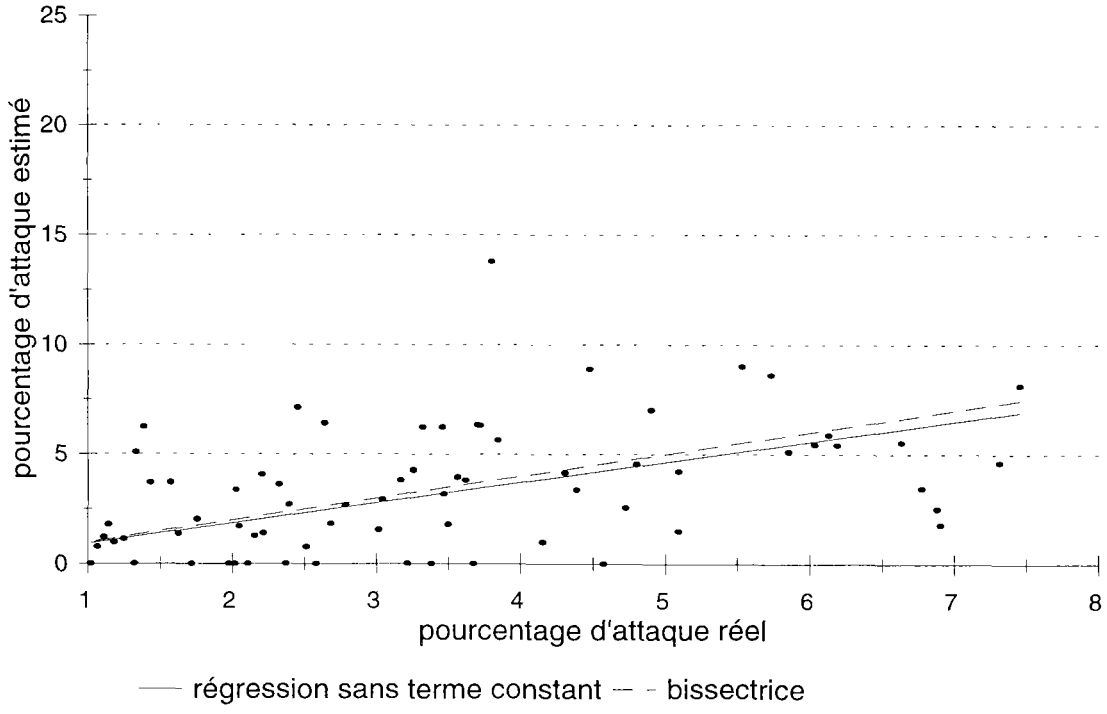


Figure 3 : fiabilité de l'échantillonnage systématique de 1 glomérule sur 7 sur un arbre



Ces graphiques permettent de visualiser d'importantes différences entre types d'échantillonnage.

Toutes les régressions donnent des résidus approximativement normaux (test d'ajustement à une loi normale de Kolmogorov). Dans le cas des régressions sans terme constant ces résidus sont de moyenne quasiment nulle (0,21 au maximum).

Pour les trois méthodes, le terme constant n'est pas significativement différent de 0. Il n'y a donc pas de biais additif sur l'intervalle étudié. Nous avons donc étudié les régressions sans terme constant. On constate alors que pour les échantillonnages aléatoires et en grappe, le terme multiplicatif est différent de 1 avec une probabilité de 95%.

La comparaison des variances d'échantillonnage se fait à l'aide d'un test de Fisher. La valeur du $F_{0,975}$ théorique, pour (69 ; 69) degrés de liberté, est comprise entre 1,48 et 1,63. Les valeurs du F observé sont :
échantillonnage aléatoire / en grappes : F_{obs} = **2,98**
échantillonnage aléatoire / systématique : F_{obs} = **2,04**
échantillonnage en grappes / systématique : F_{obs} = 1,46

Le test de Wilcoxon est un test non paramétrique qui permet de déterminer si deux échantillons appariés ont la même moyenne. Nous l'utiliserons pour comparer la moyenne du nombre de fruits par glomérule obtenue par l'échantillonnage en grappe (4,70) avec celle obtenue par le recensement (4,34). Sous l'hypothèse d'une différence nulle entre ces deux termes, la somme des rangs des différences négatives suit une loi normale et son intervalle de confiance à 95% est donc [901;1584]. Le calcul pour l'échantillonnage en grappe donne une somme de 590. L'hypothèse d'une différence nulle est donc rejetée avec un risque inférieur à 5%.

Échantillonnages sur une parcelle

L'échantillonnage d'un quart des glomérules fournit une estimation du taux d'attaque sur la parcelle de 12,2%. Les résultats de deux des observateurs sont présentés sur les figures 4 et 5. Le résumé des données et l'analyse de variance principale sont présentés dans les tableaux II et III.

Tableau II : Moyenne du pourcentage de fruits attaqués obtenue par chaque observateur pour chaque prélèvement (parcelle "El Morales")

observateur	prélèvement 1	prélèvement 2	prélèvement 3	prélèvement 4
1	13,75	15,48	14,34	11,23
2	18,38	17,24	**19,30**	**21,33**
3	19,28	19,03	**20,34**	10,87
4	21,38	13,38	12,78	12,47
moyenne	18,2	16,3	16,7	14,0

Tableau III : Analyse de variance réalisée sur le pourcentage de fruits attaqués estimé sur la parcelle "El Morales"

source	degrés de liberté	carré moyen	valeur de F	pr > F
modèle	19	0,00570515	4,23	0,0001
observateur	3	0,01022487	7,59	0,0003
prélèvement	3	0,00559308	3,66	0,0181
nombre de fruits échantillonnés	4	0,00372247	2,76	0,0371
observateur*prélèvement	9	0,00474675	3,52	0,0018
erreur	52	0,00134736		

Figure 4 : Résultats des échantillonnages aléatoires réalisés par l'observateur 2 sur la parcelle El Morales (Nicaragua, 1994)

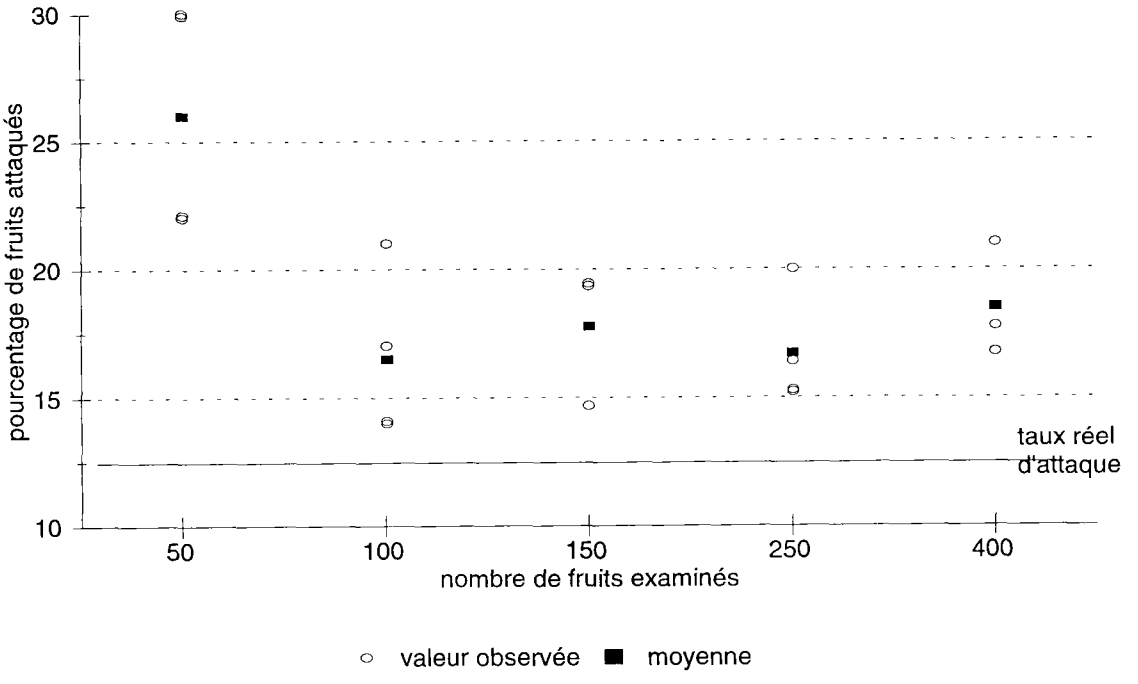
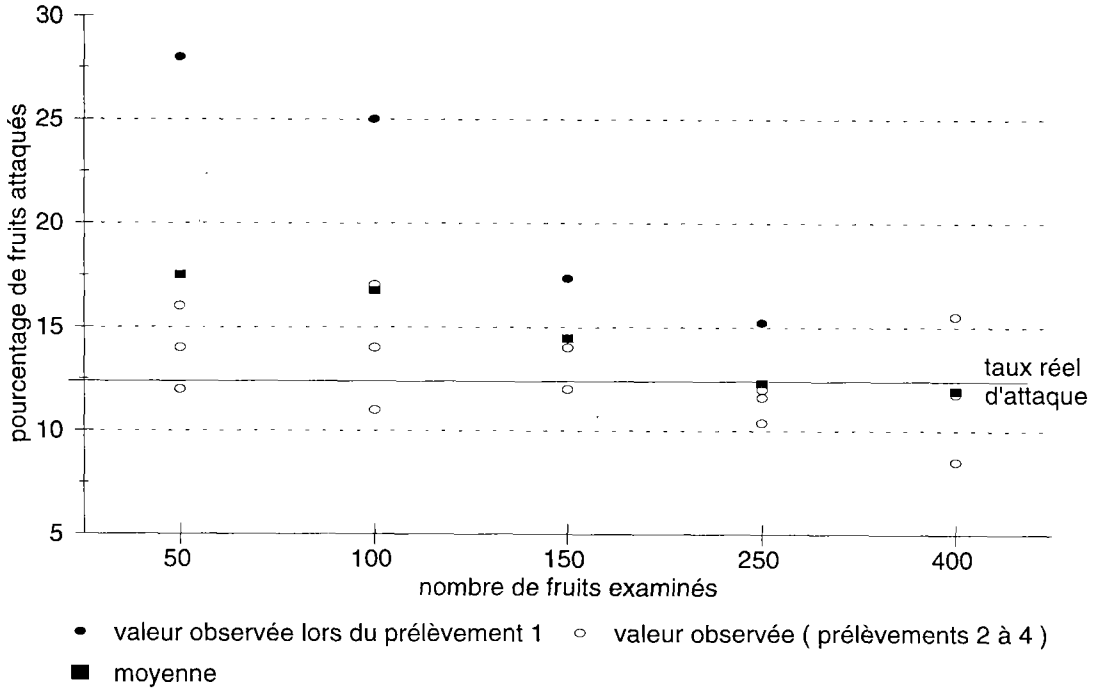


Figure 5 : Résultats des échantillonnages aléatoires réalisés par l'observateur 4 sur la parcelle "El Morales" (Nicaragua,1994)



L'analyse de variance montre que les facteurs "observateur", "prélèvement" et "nombre de fruits échantillonnés" ont un effet significatif au seuil de 5% sur l'estimation du pourcentage de fruits attaqués. L'échantillonnage de 50 fruits au hasard fournit une estimation significativement supérieure aux autres.

Les effets des observateurs et des répétitions sont étudiés séparément car une interaction forte existe entre les deux facteurs.

Pour les deux premières répétitions, il n'existe pas de différences significatives entre les observateurs. Par contre lors de la troisième, les observateurs 1 et 4 se différencient des observateurs 2 et 3. Lors de la dernière répétition, seul l'observateur 2 se distingue des autres.

Si chaque observateur est étudié séparément , il apparait que le facteur répétition n'est pas un facteur significatif pour les observateurs 1 et 2. Les observateurs 4 et 3 trouvent, au début, des taux élevés, puis significativement plus faibles respectivement à partir de la deuxième et quatrième répétition.

DISCUSSION

Échantillonnages sur un arbre

Les nombres moyens de fruits examinés par arbre pour chaque type d'échantillonnage sont relativement proches ce qui justifie les comparaisons effectuées. Les régressions donnent des résidus normaux, l'utilisation de régressions linéaires semble donc appropriée pour la validation des différentes méthodes.

Les coefficients de corrélations des régressions sont relativement faibles. Cela peut s'expliquer, en partie au moins, par l'importance de la variance des erreurs d'échantillonnage (entre 4,4 et 13,3%) devant l'intervalle de pourcentage d'attaque étudié [1 ; 7,5%].

La variance d'échantillonnage liée à la méthode aléatoire est significativement supérieure à celle des deux autres méthodes. Sa reproductibilité est donc moins bonne. Cela peut être imputé directement à la méthode d'échantillonnage : soit à un niveau théorique du fait d'une meilleure efficacité des méthodes systématique et en grappe, soit à un niveau pratique puisque la réalisation du choix aléatoire de fruits dépend entièrement de l'observateur.

L'examen de fruits au hasard dans un caféier engendre de plus un biais avec une surestimation du taux de fruits attaqués. Il s'agit apparemment d'un biais multiplicatif, c'est à dire qu'il augmente avec le taux à estimer. Cette surestimation est très importante puisque, sur l'échantillon d'arbres utilisés, l'estimation est presque le double du taux réel (6,4% au lieu de 3,4%). Deux raisons peuvent être avancées pour expliquer ce phénomène :

- Des différences d'accessibilité entre les fruits. Certains fruits ont peu de chance d'être sélectionnés : l'observateur privilégierait la zone directement accessible à sa vision et négligerait les autres : base ou sommet de l'arbre, fruits cachés par les branches ou les feuilles.
- Une attirance de l'observateur pour certains types de fruits : gros ou petit, rouge ou vert, perforé ou non. Des études ont montré que selon le degré de maturité du fruit, les taux d'attaque sont différents. Ainsi les fruits rouges sont plus attaqués par le scolyte que les fruits verts (Borbon-Martinez, 1989 ; Barrera, 1994).

Un léger biais est constaté pour l'échantillonnage de glomérules au hasard dans un arbre sous l'hypothèse d'un biais additif nul. Les mêmes phénomènes que pour l'échantillonnage aléatoire pourraient intervenir, bien que de manière atténuée puisque l'observateur ne choisi pas chaque fruit mais des ensembles de fruits. Le test non paramétrique de Wilcoxon a permis de montrer que le nombre de fruits par glomérule a été surestimé lors de l'échantillonnage en grappe. L'observateur a donc choisi de préférence des glomérules comportant plus de fruits que la moyenne des glomérules.

Cette étude n'a porté que sur les résultats obtenus par un observateur. Il est probable que d'autres observateurs trouveraient des biais différents pour l'échantillonnage en grappe et aléatoire.

L'échantillonnage systématique devrait donc être préféré à l'examen de fruits au hasard sur un caféier. Toutefois il est plus long à mettre en oeuvre et demande plus de technicité que les autres types d'échantillonnages.

Échantillonnages sur une parcelle

Dans l'ensemble, le taux d'attaque estimé par un échantillonnage aléatoire sur la parcelle étudiée a été surestimé. On peut expliquer ces résultats d'une part par l'inexpérience des observateurs 3 et 4 qui présenteraient une phase d'apprentissage au cours de laquelle ils surestimeraient les taux d'attaque. D'autre part, il semble exister des différences de comportement entre les observateurs : l'observateur 2 semble surestimer de manière importante le taux d'attaque puisqu'il trouve en moyenne 19,2 % au lieu de 12,2%. On a vu que de nombreux facteurs peuvent intervenir sur le choix des observateurs. L'observateur 2 se laisse probablement plus facilement influencer lors de la sélection des fruits que les autres observateurs.

Il apparait donc qu'à l'échelle de la parcelle, pour obtenir des résultats reproductibles et donc comparables, il faut non seulement entraîner les observateurs mais ensuite les sélectionner. Enfin, il semble souhaitable d'échantillonner plus de 50 fruits.

CONCLUSION

Que ce soit à l'échelle d'un arbre ou d'une parcelle, l'échantillonnage aléatoire semble donc entraîner des biais, dans le sens de la surestimation, qui semblent très variables en fonction des observateurs. Cette surestimation importante est probablement liée aux différences d'aspect et d'accessibilité des fruits. Lorsque l'échantillonnage est utilisé pour une prise de décision, on augmente le risque de décider de traiter alors que ce n'est pas nécessaire. Lors des expérimentations au champs, selon les observateurs et leur entraînement, on risque de surestimer le taux d'attaque de certaines parcelles. Les comparaisons n'auraient alors pas de sens. Pour éviter cette erreur, un échantillonnage systématique peut être recommandé car il ne laisse pas d'initiative de choix à l'observateur. Un entraînement et une sélection des observateurs devraient également permettre d'obtenir des estimations plus fiables.

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RÉSUMÉ : Comparaisons de méthodes d'échantillonnage du scolyte du fruit du caféier (*Hypothenemus hampeï* Ferr). RÉMOND F., CILAS C., DUFOUR B., BERNADETTE L, DECAZY B.

Diverses procédures d'échantillonnage du taux de fruits attaqués par le scolyte du caféier sont actuellement employées. Les plus simples préconisent un échantillonnage aléatoire sur la parcelle. D'autres recommandent un choix semi-systématique de caféiers ou de groupes de caféiers, chaque arbre étant sondé aléatoirement. Ces différents types d'échantillonnages, ainsi que l'échantillonnage systématique, ont été testés sur le terrain.

L'échantillonnage aléatoire sur un arbre entraîne un biais important dans l'estimation, variable en fonction des observateurs. Les taux d'attaque sont généralement surestimés car les observateurs semblent privilégier certains types de fruits (rouges, perforés) et favoriser les zones du caféier facilement accessibles (zone intermédiaire, extrémités des branches) qui sont souvent les plus attaquées. L'échantillonnage systématique est meilleur, mais il nécessite beaucoup plus de temps de travail.

A l'échelle de la parcelle, on retrouve le même phénomène. En effet, l'échantillonnage aléatoire sur la parcelle donne également des estimations différentes selon les observateurs. Le nombre de fruits examinés influence non seulement la précision de la mesure mais aussi l'estimation elle-même.

SUMMARY : Comparisons of sampling techniques of the coffee berry borer *(Hypothenemus hampeï* Ferr). RÉMOND F., CILAS C., DUFOUR B., BERNADETTE L., DECAZY B.

At the present time, various sampling techniques of the rate of attacked berries are used. The easiest ones advice a random sampling over the plot. Others recommend a half-systematic selection of coffee trees or groups of trees, every tree being randomly sampled. Those different sampling methods as well as the systematic one have been tested.

Random sampling on a tree gives an important bias, variable according to the observers. The rates of attack are generally over-estimated because the observers seem to privilege certain types of fruits (red, bored) and favour zones easily accessible (intermediate zone, end of branches) which are more often attacked. Systematic sampling is better, but it needs much more work.

To the plot level, the same phenomenon is observed. As a matter of fact, random sampling on a plot also gives different estimations according to the observers. The number of fruits examined has an influence on both measure accuracy and estimation itself.

AMÉLIORATION QUALITATIVE DE *COFFEA CANEPHORA* PIERRE PAR HYBRIDATION INTERSPÉCIFIQUE : EXPLOITATION D'UN NOUVEAU SCHÉMA DE SÉLECTION CHEZ LES ARABUSTA

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INTRODUCTION

L'amélioration du caféier *Coffea canephora* par hybridation interspécifique a été entreprise depuis plusieurs années et a permis de créer un nombre impressionnant d'hybrides interspécifiques de niveau différent de ploïdie (Berthaud, 1975, Le Pierres et Anthony, 1980, Van Der Vossen et Owuor, 1981, Louarn, 1992). L'arabusta tétraploïde (Capot, 1972) a été l'une des formes les plus étudiées.

Conçu pour pallier les insuffisances technologiques du canephora, l'arabusta présente un certain nombres de défauts dont le plus important constitue sa mauvaise fertilité. Elle se caractérise par une production élevée de grains caracolis (60%) et des taux importants de loges vides (De Reffye, 1975, Le Pierres et Charmetant, 1985, Yapo, 1987). Bien que vigoureux et possédant des qualités technologiques et organoleptiques appréciables, l'arabusta ne peut être vulgarisé en raison de sa faible productivité.

Différents programmes ont été élaborés pour l'améliorer. Après avoir fait un bref rappel des méthodologies utilisées antérieurement, le schéma de sélection en cours d'exploitation est décrit et quelques résultats de fertilité sont présentés.

1 - RAPPEL DES PROGRAMMES ANTERIEURS DE SELECTIONS

1. 1 - Restauration progressive de la fertilité par vagues successives

La duplication des chromosomes modifie la structure de l'espèce. Chez *C. canephora,* les autotétraploïdes présentent des défauts de fertilité (Capot, 1975). La méthodologie mise en place vise à restaurer leur fertilité par générations successives. Ainsi donc, chaque génération de canephora tétraploïde engendre-t-elle des hybrides arabusta qui constituent une vague (figure 1). Les autotétraploïdes (tétraploïdes directement obtenus par duplication chromosomique) produisent les arabusta de première vague; ceux de première génération donnent des hybrides de deuxième vague et ainsi de suite. On devrait s'attendre à un gain de fertilité d'une vague à l'autre.

L'analyse des résultats permet de constater que le niveau de fertilité n'a pas varié. En fait, six génotypes ont constitué le pool diploïde de départ dont deux seulement ont été géniteurs des arabusta sélectionnés (Charmetant et Le Pierres, 1985).

1. 2 - La diversification génétique des arabusta

Ce programme met l'accent sur l'élargissement de la base génétique et la diversification des géniteurs (figure 2). Du côté arabica, des souches mieux adaptées aux conditions de basse altitude et des génotypes originaux pour certains caractères ont été utilisés. Du côté canephora, de nouveaux géniteurs et des espèces qui lui sont proches ont été largement utilisés dans les croisements (Charmetant et Le Pierres, 1985). Plus de 6000 hybrides arabusta ont été créés et observés entre 1982 et 1989.

Les résultats ont montré une forte sensibilité des descendants à l'environnement (Charmetant et *a l,* 1991); de fréquents phénomènes de "die back" ont été observés. La sélection dans ces conditions est difficile et inefficace.

1. 3 - Les hybrides interspécifiques hexaploïdes

Le croisement direct de *C. canephora* diploïde (2n=22) par *C. arabica* (2n=44) produit des descendants triploïdes stériles (2n=33). La duplication des chromosomes (2n=33 à 2n=66) permet de restaurer la fertilité. Les hybrides hexaploïdes obtenus sont intéressants du point de vue de la fertilité et de la qualité du café. Mais la prédominance du parent arabica entraîne des problèmes d'adaptation aux conditions de basse altitude (Berthaud, 1978, Le Pierres et Anthony, 1980). Le programme n'a pas été poursuivi.

2 - LA METHODOLOGIE EN COURS D'EXECUTION: LE BRASSAGE ET LA SELECTION DES HYBRIDES DANS LES GENERATIONS SUCCESSIVES

Les arabusta de première génération sont caractérisés par une mauvaise fertilité qui se traduit par des taux élevés et relativement stable de grains caracolis et des taux de loges vides variables mais importants de l'ordre de 30%. Ils ont également une structure génétique non fixée qui se manifeste par une extrême hétérogénéité des descendances (Charrier, 1977). Cette mauvaise fertilité observée, est liée aux répartitions déséquilibrées des chromosomes, résultant d'appariements irréguliers lors de la méiose (Grassias, 1980). Cette mauvaise régulation traduit une mauvaise homologie des chromosomes parentaux impliqués. La restauration de fertilité passe donc par une régulation progressive de la méiose.

2. 1 - Le schéma de sélection

L'objectif recherché est de parvenir après plusieurs générations de brassage, à restaurer la fertilité des hybrides. Les meilleurs producteurs d'une génération sont sélectionnés et servent de géniteurs pour la génération suivante (figure 3). Ils peuvent également servir de têtes de clones pour la sortie variétale. Les descendances sont obtenus soit par croisements contrôlés, soit par fécondation libre. Tous les types d'arabusta de première génération sont exploitables (*C. arabica* x *C. canephora, C. arabica* x *C. congensis, C. arabica* x congusta, etc...).

2. 2 - Les critères de sélection

L'instabilité génétique des descendances arabusta nécessite un fort taux de sélection de manière à éliminer les combinaisons génétiques déséquilibrées et l'utilisation exclusive de la voie végétative pour la sélection des meilleurs individus.

Le premier critère à prendre en compte est la fertilité (mâle et femelle).
La fertilité mâle est exprimée par le taux de grains de pollen à cytoplasme coloré au carmin acétique à 2% . La fertilité femelle est mesurée par: le taux de grains caracolis
le taux de loges vides
le taux de remplissage des fruits
le taux d'utilisation des ovules

Le second critère est la production mesurée par le poids de cerises fraîches
Le troisième critère est la vigueur dont les paramètres sont:

le diamètre au collet
la hauteur
l'encombrement

Les autres critères (résistance aux maladies; qualités technologiques et organoleptiques) ne seront pris en compte que dans la dernière phase de la sélection: la sortie variétale.

Près de 20000 plants de deuxième, troisième et quatrième et une centaine de clones de première génération servant de témoins sont en observation au champ.

3. QUELQUES RESULTATS DE FERTILITE

3. 1 - Matériel et méthodes d'étude

Sur un échantillon de 182 individus répartis comme suit:

première génération (G1): 50
deuxième génération (G2): 55
troisième génération (G3): 38
quatrième génération (G4): 39

200 à 300 fruits immatures ont été récoltés coupés transversalement au scalpel et répartis suivant 5 catégories de cerises A,B,C,D,E.

A = 2 loges pleines
B = 1 loge pleine et 1 écaille
C = 1 loge pleine et 1 loge vide
D = 1 loge vide et 1 écaille
E = 2 loges vides

Ces différentes catégories de cerises permettent d'estimer:

- le taux de caracolis: (B/2A+B+C)*100
- le taux de loges vides: (C+D+2E/2A+B+2C+D+2E)*100
- le taux de remplissage des fruits: (2A+B+C/2A+B+2C+D+2E)*100.

ont été calculés

3. 2 - Résultats

Les résultats sont rapportés dans les tableaux ci-dessous:

- Taux de grains caracolis

G1	G2	G3	G4	effet	classement
m cv	m cv	m cv	m cv		PPDS à 5%
61 14	68 17	66 21	56 21	* *	G4<G1<G3=G2

- Taux de loges vides

G1	G2	G3	G4	effet	classement
m cv	m cv	m cv	m cv		PPDS à 5%
30 41	33 47	48 45	42 52	* *	G1=G2<G4<G3

- Taux de remplissage des fruits

G1	G2	G3	G4	effet	classement
m cv	m cv	m cv	m cv		PPDS à 5%
44 17	41 23	32 41	40 41	* *	G1=G2=G4>G3

m = valeur moyenne
cv = coefficient de variation

L'analyse des résultats montre qu'au niveau des taux de caracolis, la valeur moyenne qui était de 61% chez les individus de première génération (G1) subit une augmentation significative chez ceux de deuxième génération (G2). Ce surcroît de production de grains caracolis, conséquence d'une baisse de fertilité peut s'expliquer par le fait qu'en deuxième génération, il ya éclatement de la variabilité et apparition de descendants déséquilibrés et stériles. A partir de la troisième génération, il ya une légère baisse du taux moyen qui s'accentue à la quatrième génération. Cette observation déjà signalée par Le Pierres et Yapo (1993) indique l'amorce d'un réarrangement chromosomique: la régulation progressive de la méiose est entamée.

Au niveau des taux de loges vides, les fluctuations sont indépendantes des générations. Les individus de quatrième génération présentent des taux élevés par rapport à ceux de première ou de deuxième génération. Ces résultats montrent la prépondérance de l'effet du milieu sur celui du génotype.

Le dernier paramètre étudié est le taux de remplissage des fruits. L'examen du tableau montre qu'il n'y a pas de différence significative entre les individus de première, deuxième et quatrième générations. Ici également, l'effet de l'environnement est prépondérant.

Les deux derniers paramètres sont fortement liés aux capacités de production des arbres. Leur grande dépendance du milieu annihile les efforts mis en oeuvre pour améliorer les hybrides arabusta.

CONCLUSION

Le caféier arabusta est assez complexe en ce qui concerne sa structure génétique. Les différents programmes d'amélioration exploités non pas donnés de satisfaction. L'examen du programme en cours permet de relever deux facteurs liés à son amélioration: un facteur génétique qui peut être améliorée au cours des générations et un autre physiologique, peu maîtrisable dépendant du milieu, difficile à améliorer.

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RESUME

YAPO A. - **Amélioration qualitative de *Coffea canephora* Pierre par hybridation interspécifique: Exploitation d'un nouveau schéma de sélection chez les arabusta.**

Les hybrides arabusta sont caractérisés par une mauvaise fertilité et une production limitée qui empêchent leur diffusion à grande échelle. Les différentes méthodes d'amélioration étudiées n'ont pas donné de satisfaction.
Une méthode de sélection à l'intérieur des générations, mise en place à partir de 1988, permet d'espérer une amélioration significative de l'hybride. La fertilité de quatre générations d'hybrides a été observée. Les résultats sont présentés.

ABSTRACT

YAPO A. - **Qualitative improvement of *Coffea canephora* Pierre by interspecific hybridization: use of a new selection sheme on arabusta coffee.**

Arabusta hybrids are characterized by a low fertility and a reduced yield that do not allow their extension on a large scale. The use of different breeding methods has not allowed a substantial improvement of the fertility.

A within generation selection method laid out in 1988 would probably improve the hybrid. The fertility of four hybrid generations has been tested. The results are presented.

AMELIORATION DES ARABUSTA
RESTAURATION PROGRESSIVE DE LA FERTILITE
PAR VAGUES SUCCESSIVES

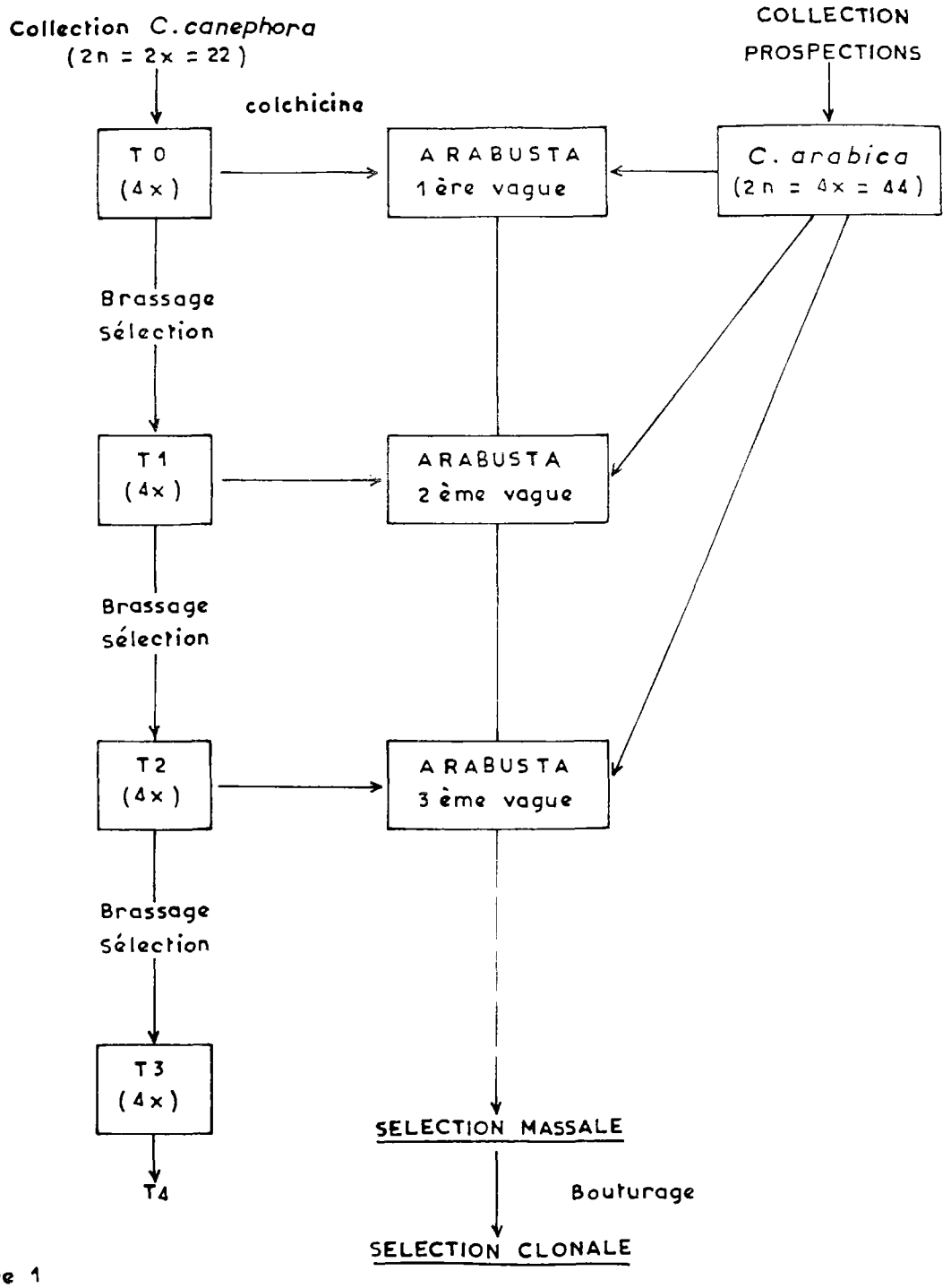

Figure 1

AMELIORATION DES ARABUSTA

DIVERSIFICATION DES GENITEURS

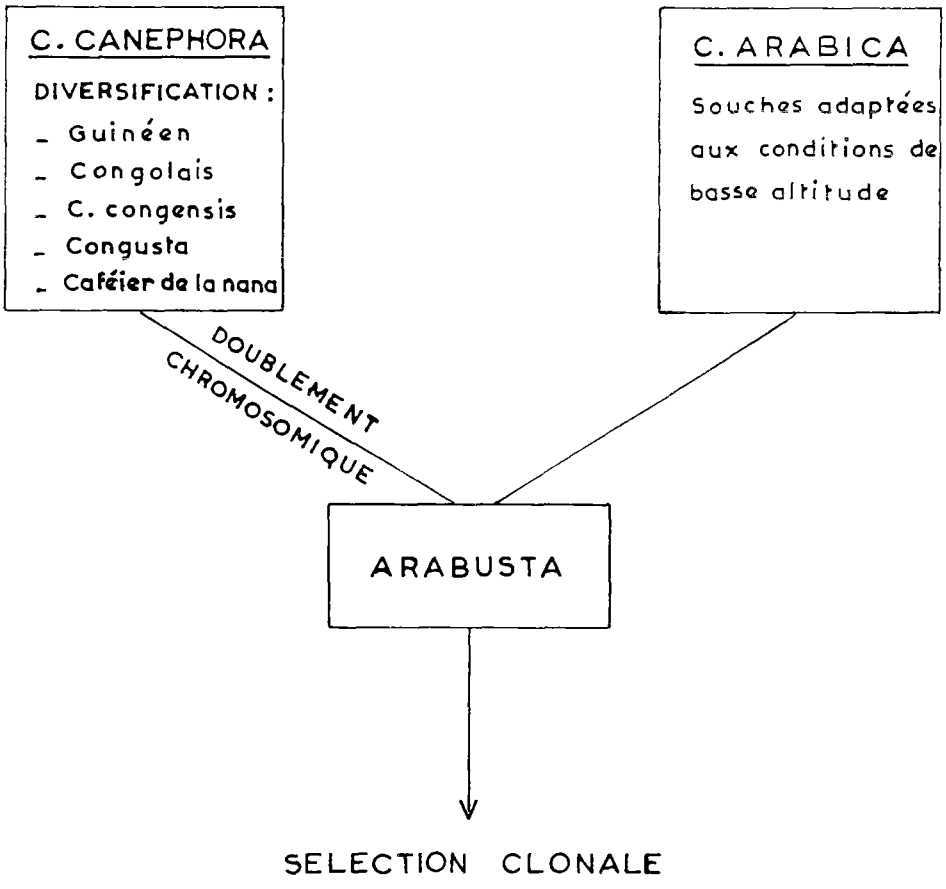

Figure 2

AMELIORATION DES ARABUSTA

BRASSAGE ET SELECTION DANS LES GENERATIONS SUCCESSIVES

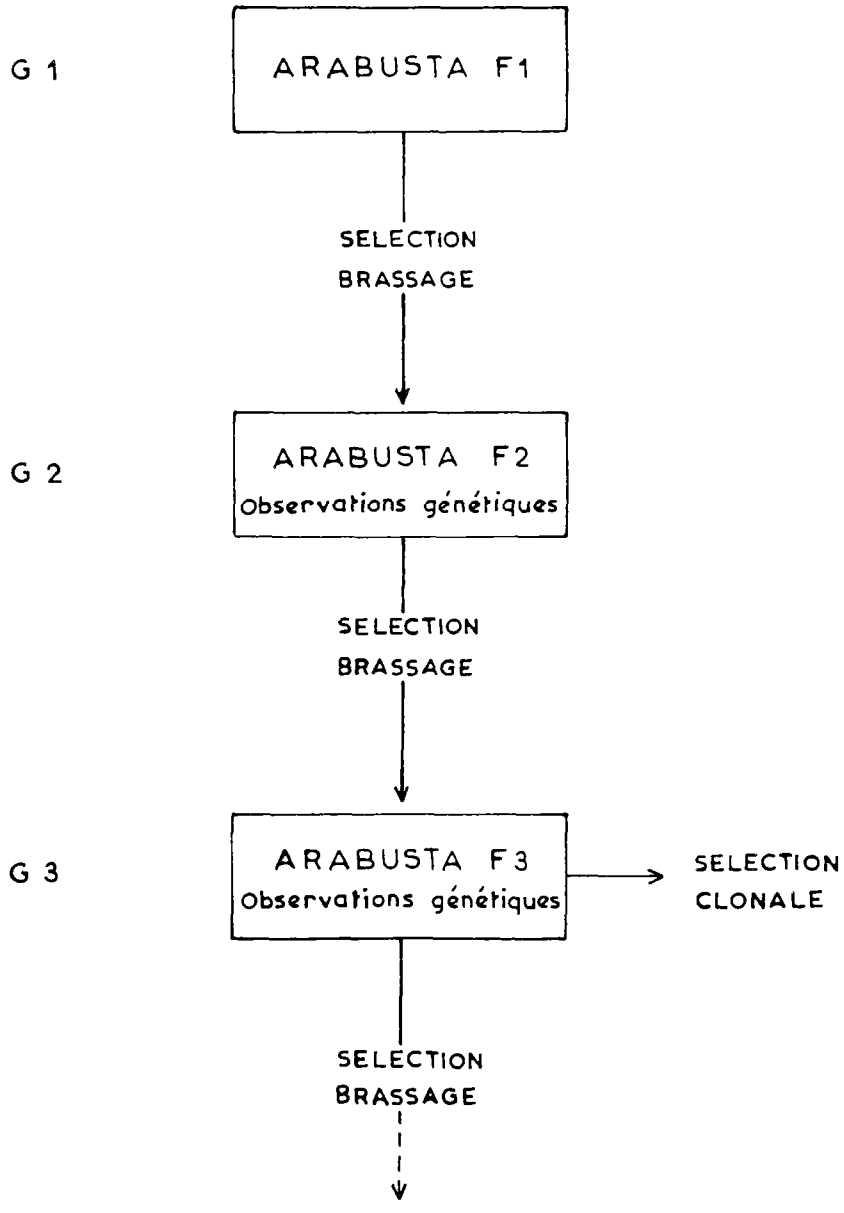

Figure 3

LA LUTTE BIOLOGIQUE CONTRE LE SCOLYTE DES BAIES DU CAFÉIER, *HYPOTHENEMUS HAMPEI* FERR. : ÉTUDE DES PARAMÈTRES ÉCONOMIQUES DÉTERMINANTS

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[2] ANACAFE, apto 1823, Guatemala, Guatemala

I - INTRODUCTION :

Le scolyte des fruits du caféier, *Hypothenemus hampei* Ferr. est considéré dans le monde entier comme le ravageur le plus dangereux pour la culture du café. Les pertes sont proportionnelles au pourcentage d'infestation et peuvent atteindre près de 35 % de la production (OCHOA-MILIAN *et al.*, 1989). Chacun des pays producteurs de café a dû mettre en place des programmes de lutte contre ce ravageur. Dans le passé, diverses méthodes ont été proposées, reposant essentiellement sur la lutte chimique raisonnée (DECAZY, 1985). Des méthodes de lutte complémentaires sont également recommandées, telles que certaines pratiques culturales (récolte sanitaire, taille des caféiers, désherbage des caféières) (DECAZY, 1989).

Depuis une dizaine d'années, a débuté une période correspondant à la fois à une baisse du prix du café, mais aussi à une prise de conscience des risques potentiellement engendrés par l'usage des insecticides (dégradation de l'environnement, résistance). Cette période a été favorable au développement de programmes de recherche sur la lutte biologique contre le scolyte en Amérique latine, à l'aide de deux parasitoïdes Bethylidae, *Cephalonomia stephanoderis* Betrem et *Prorops nasuta* Waterson), (BARRERA, 1994). Les résultats sont suffisamment encourageants pour que l'on puisse tenter de les valider au niveau des producteurs.

Cependant le principal problème est la production en masse des parasitoïdes. Afin de résoudre rapidement ce problème, une méthode participative a été mise en chantier. Initiée au Mexique (BARRERA *et al.*, 1992), cette méthode confie la production et les lâchers de parasitoïdes aux caféiculteurs eux-mêmes, lorsqu'ils se montrent intérressés.

L'objectif de cette étude est d'évaluer l'acceptabilité économique de la méthode de production du parasitoïde directement à la ferme dans une région du Guatemala où elle est mise en oeuvre.

II - MATÉRIEL ET MÉTHODES :

L'acceptabilité économique de la méthode s'établira essentiellement sur :
- l'étude de la logique du fonctionnement de chaque système de production, que l'on caractérisera par les itinéraires techniques suivis, et qui sera déterminant pour juger de l'intérêt du producteur à adopter cette innovation technique.
- l'établissement des coûts actuels de la lutte chimique, ainsi que du coût d'utilisation des parasitoïdes, dans chacun des systèmes de production.

La zone d'étude retenue après enquête préliminaire a été choisie dans une région à forte production de café, montrant une grande diversité des systèmes de production. Elle se situe dans la région sud-ouest du Guatemala, à cheval sur trois départements (Suchitepéquez, Retalhuleu, Sololá). Elle couvre une superficie de 300 km^2. Elle est située sur le versant sud de la chaine volcanique de Santa Madre. Elle est étagée entre 400 et 1300 m d'altitude. Les températures moyennes se

situent entre 20 et 25°C., la pluviosité, bien répartie au cours de l'année, se situe entre 2000 et 3000 mm.

Après enquête préliminaire semi-ouverte auprès des producteurs de café et des professionnels du secteur agricole, la zone est divisée en deux parties délimitées par l'altitude de 600 m, en fonction de la distribution spatiale des cultures. Jusqu'à 600 m d'altitude les cultures principales sont l'hévéa, la canne à sucre et le café; au dessus de 600 m, la terre est presque exclusivement utilisée pour la culture du café, accompagnée parfois par celle du maïs et de la banane.

En dessous de 600 m, mais aussi dans la partie ouest au dessus de 600 m, coexistent une population indienne et une population "ladino" (constituée des descendants métissés des Espagnols). Dans la partie est au dessus de 600 m, la population est presque à 100 % indienne (département de Sololá). A cette distribution de population se superpose une différence dans les systèmes agricoles : au niveau de Sololá, dominent de petites exploitations familiales de deux Manzanas (1,4 ha) en moyenne ; ailleurs cohabitent trois grands types d'exploitations agricoles (les grandes exploitations appelées "fincas" (dont le but est de rentabiliser le capital investi), les petites exploitations aux alautours des villages (qui tirent leurs revenus de la main d'oeuvre familiale), et une concentration de petits producteurs organisés en "Empresas Campesinas Asociativas" (ECA) résultant de la succession de diverses réformes agraires.

III - RÉSULTATS :

1) Les itinéraires techniques et les systèmes de production :

Dans cette partie sont donnés les divers systèmes de production ainsi que les divers itinéraires techniques utilisés pour la culture du café, qui s'y rattachent. Ce sont principalement les moyens existants (consommations intermédiaires et main d'oeuvre) qui permettent de caractériser ces systèmes, établis après enquête auprès des producteurs.

Les rendements par hectare présentés sont les rendements minimum et maximum ; Le coût de la main d'oeuvre inclut toutes les charges patronales ; les coûts en consommations intermédiaires incluent l'achât des engrais et des pesticides. La marge brute, exprimée ici est la différence entre le produit brut issu de la vente du café et le coût total de l'entretien de la caféière en production.

Le tableau N°1 résume les caractéristiques économiques de chaque itinéraire technique pour la conduite de la caféière en production.

Au dessus de 600 m d'altitude :

L'itinéraire technique intensif (ITO) est utilisé dans les exploitations de grande superficie consacrées entièrement à la culture du café et faisant travailler une importante main d'oeuvre (système de production intensif, SPO). Les productions (de café vert) sont élevées ainsi que les rendements.

L'itinéraire technique le plus économique en consommations intermédiaires (IT1) est utilisé dans des exploitations ayant parfois de grandes superficies consacrées exclusivement à la culture du café. Les rendements (café parche) y sont faibles.

L'itinéraire technique impulsé par les programmes de crédit-rénovation (ITR) est utilisé par les petits producteurs, ayant de petites exploitations de l'ordre de 0,7 à 2 ou 3 hectares. Les rendements en café cerise (ou parche, l'investissement dans un dépulpeur étant vite rentabilisé par ceux qui le font) sont moyens à élevés.

L'itinéraire technique pour vieux caféiers (ITV) est utilisé par les petits producteurs traditionnels ayant des variétés de caféiers de type bourbon agés de plus de vingt ans. Leur superficie mise en culture est de 0,35 à 5,6 hectares. Les rendements en café cerise sont très faibles.

Au dessous de 600 m d'altitude :

Cette partie de la zone d'étude comprend de grandes exploitations. Il n'y a pas de concentrations de petits producteurs, à la différence de la partie nord. Les systèmes de culture sont diversifiés, à base de café, d'hévéa et de canne à sucre. La main d'oeuvre y est moins abondante ; aussi les producteurs se sont-ils orientés vers des productions exigeant peu de main d'oeuvre, et offrant un prix correct et stable.

L'itinéraire technique complet (IT1') est utilisé dans les exploitations de grande superficie consacrées pour partie à la culture du café et pour partie à la culture de l'hévéa, nécessitant une main d'oeuvre permanente abondante (système de production complet, SP1'). Les productions (de café vert) sont élevées ainsi que les rendements.

L'itinéraire technique orienté vers l'abandon du café (IT3') est utilisé dans les exploitations ayant un système de production diversifié (IT3'). Situé sur sols peu profonds, il détient quatre productions : l'hévéa, la canne à sucre, l'élevage laitier et le café. Il fait travailler une main d'oeuvre permanente, peu abondante. Ce système de production s'oriente vers l'abandon progressif de la culture du café, en raison de la réduction de la disponibilité en main d'oeuvre, qui est mieux rentabilisée avec les autres cultures.

Tableau N°1 - Évaluation de divers itinéraires techniques (monnaie = Quetzal (Q))

Itinéraire	Rdt q/ha	Coût M.O.	Coût C.I.	Coût total	MO/CT %	MB/ha
IT0	*13	3300	2557	5857	56,3	21741
	23	4860	2157	7417	65,5	41392
IT1	5,1	1830	55	1885	97,1	6636
	11,4	2880	55	2935	96,4	13027
ITR	7,8	2940	2364	5304	55,4	11520
	16,3	3990	2364	6354	62,8	16170
ITV	1,3	840	0	840	100,0	1796
	3,2	1155	0	1155	100,0	5242
IT1'	13	3345	2631	5976	56,0	15428
	16	3870	2631	6491	59,6	20264
IT3'	1,3	645	0	645	100,0	700
	3,2	960	0	960	100,0	3602

* café vert ; Rdt/ha = rendement en café parche par hectare ; Coût M.O.= coût main d'oeuvre ; Coût C.I. = coût en consommations intermédiaires ; CT = coût total ; MB/ha = marge brute par hectare.

Il ressort du tableau N°1 que les systèmes de production économique en consommations intermédiaires et pour vieux caféiers ont une grande dépendance à l'égard de la main d'oeuvre (la part du coût de la main d'oeuvre se situe entre 97 et 100 %). Les marges brutes par hectare sont élevées pour les systèmes de production intensifs et pour les producteurs rénovateurs. un peu moins élevées pour le système économique, et basses pour les producteurs traditionnels. Le système de production complet (café + Hévéa) a une marge brute élevée et une abondante main d'oeuvre. Les systèmes de production orientés vers l'abandon de la culture du café consacrent leur main d'oeuvre aux autres spéculations agricoles.

2) Le coût de la lutte menée actuellement contre le scolyte selon les divers itinéraires techniques :

Dans l'itinéraire technique intensif (ITO), la lutte est exclusivement chimique et est effectuée lors de deux des trois applications de fertilisants. Dans l'itinéraire technique suivi par les petits producteurs rénovateurs (ITR), la lutte se fait par application d'insecticide mélangé aux fertilisants foliaires, comme dans le cas de l'itinéraire technique intensif. Chez les producteurs économisant dans les consommations intermédiaires (IT1), la lutte est culturale : après le dernier passage de récolte les fruits laissés sur les arbres et tombés au sol sont ramassés gratuitement par les habitants des villages voisins qui les vendent sur le marché local. Il n'y a aucune lutte contre le scolyte chez les petits producteurs traditionnels (ITV). Avec l'itinéraire complet (IT1'), la lutte est chimique et est effectuée lors des applications de fertilisants foliaires ; elle est aussi culturale (récolte sanitaire). Avec l'itinéraire technique orienté vers l'abandon du café, la lutte est essentiellement culturale (récolte sanitaire).

Le tableau N°2 résume les dépenses engagées pour la lutte contre le scolyte selon les divers itinéraires techniques :

- le prix de l'insecticide utilisé (endosulfan) est variable selon qu'il est acheté en fûts de 220 litres (35 Q/l) ou au détail (60 Q/l) ; la quantité utilisée par hectare chaque année est de deux fois 1,8 l, soit 3,6 litres.
- la main d'oeuvre nécessaire aux applications foliaires sur un hectare est de 3 journées pour deux applications (15 Q/journée) ; mais lors de ces applications certains producteurs (ITO et ITR) épandent à la fois les fertilisants foliaires et l'insecticide ; aussi doit-on savoir comment imputer les dépenses en main d'oeuvre ; trois solutions sont possibles:
  ▷ **0 journée/ha** du fait que les producteurs s'ils avaient à choisir entre une application de fertilisants et une application d'insecticide, choisiraient l'application de fertilisants, qui justifie donc à elle seule l'utilisation de la main d'oeuvre.
  ▷ **1 journée/ha** si trois travaux sont réalisés en même temps (fertilisation foliaire, lutte contre les maladies et lutte contre le scolyte).
  ▷ **1,5 journées/ha** si deux travaux sont effectués en même temps
  ▷ **3 journées/ha** si l'on impute la totalité de la main d'oeuvre à la lutte chimique contre le scolyte.
- l'amortissement de l'appareil d'application est compté en fonction de son coût (prix d'achât, 4000 Q, + maintenance,

4000 Q) ; il est amorti par une utilisation sur 300 ha, soit 27 Q/application/ha. (pour son amortissement on peut prendre les mêmes règles que pour l'utilisation de la main d'oeuvre).
- la récolte sanitaire nécessite **4 journées/ha**

Tableau N°2 : Coût de la lutte contre le scolyte pratiquée en fonction des divers itinéraires techniques (Quetzal)

Itinéraire technique	Lutte chimique			Récolte sanitaire M.O.	Total
	Endosulfan	M.O.	Amortis.		
ITO	126	0 à 45	0 à 54	0	126 à 225
ITR	216	0 à 45	0 à 54	0	216 à 315
IT1	0	0	0	0	0
ITV	0	0	0	0	0
IT1'	126	45	54	60	279
IT3'	0	0	0	60	60

Il ressort de ce tableau que :
- trois des quatre itinéraires techniques qui prévoient la lutte contre le scolyte adoptent une lutte chimique ; seul le système de production orienté vers l'abandon du café ne fait qu'une lutte culturale.
- le coût de la lutte chimique est très variable d'un système à un autre ; il s'étage de 126 Q à 315 Q, selon que cette pratique est obligatoire pour éviter de trop grosses pertes, et selon le prix d'achât de l'insecticide.
- les petits planteurs traditionnels n'ont pas les moyens financiers suffisants pour effectuer une lutte chimique, ni pour consacrer une partie de la main d'oeuvre familiale à la lutte culturale.

3) Mesure du coût de la lutte biologique contre le scolyte dans quelques élevages ruraux :

Les productions de parasitoïdes étant confiées aux producteurs de café au sein des élevages ruraux, ceux-ci doivent accomplir les nombreuses tâches leur permettant de produire et de libérer un grand nombre d'individus au cours de cette période. La première des tâches est soit de collecter en plantation des fruits hébergeant les populations de scolyte nécessaires aux élevages de *Cephalonomia stephanoderis*, soit de faire un élevage préliminaire de scolyte à partir de fruits sains ou de semences. La seconde tâche est l'élevage proprement dit du parasitoïde. La troisième tâche est la libération en caféière d'une partie des parasitoïdes (l'autre partie servant à la poursuite de l'élevage).

l'investissement de base :

Il est le suivant quelle que soit la superficie de la caféière, :
▷ un local contenant table et étagères (en réalité un local initial existe souvent)
▷ un peu de matériel consommable, tel que boites en plastique, flacons, tissu, canif, brosse, etc.
Cet investissement évalué à partir de plusieurs élevages ruraux, dont certains ne disposent pas de local, se situe de ce fait entre **650 et 2000 Q.**
L'amortissement de ce matériel se fait sur 10 ans pour le local et le mobilier, soit **135 Q/an**, sur 5 ans pour le matériel consommable, soit **130 Q/an**.

Les consommations intermédiaires :

Elles correspondent en grande partie aux produits d'entretien (savon, produits anti-acridiens, et anti-champignons), mais aussi à de petits articles tels que cahier, stylo, bracelets élastiques, peinture, etc..Elles ont été évaluées à **500 Q/an** dans l'ensemble de sélevages ruraux.

Les ressources humaines :

Les élevages ruraux visités sont confiés à une personne responsable ; un ou deux assistants peuvent l'aider dans ses tâches journalières. Le responsable doit être organisé, motivé et formé. Deux remarques sont à faire :

▷ l'élevage préliminaire éventuel de scolytes est fort consommateur en main d'oeuvre et constitue de ce fait un facteur limitant la production de parasitoïdes.
▷ il est nécessaire d'accroître la quantité de main d'oeuvre en fin de récolte du café, de manière à intensifier les élevages, dans le but d'avoir une production maximum après la récolte

Coûts d'utilisation de *C. stephanoderis* :

Ces coûts sont résumés dans le tableau N°3, dans lequel :
- les amortissements sont calculés en fonction de l'existence ou de la construction d'un local destiné à l'élevage rural.
- les consommations intermédiaires, de l'ordre de 500 Q/an, subissent peu de variations.
- l'élevage conduit par les petits producteurs rénovateurs (ITR) sera difficile à interpréter dans la mesure où l'on divisera le coût total de production par la superficie totale de l'ECA, sachant que la production de *C. stephanoderis* (bien qu'inconnue) est très loin de satisfaire l'ensemble des besoins des associés.

Le tableau montre que :
- le coût de la main d'oeuvre représente 90 à 95 % du coût total de la lutte biologique ; les futures estimations du coût de celle-ci pourront donc se réaliser rapidement sans erreur importante, à partir du coût de la main d'oeuvre, sans qu'il soit nécessaire de déterminer le coût des produits et des amortissements.
- la production de parasitoïdes est d'autant plus élevée qu'il est aisé de se procurer des fruits infestés par le scolyte pour les élevages (ITO).
- l'élevage pratiqué par ITO est le plus économique ; il représente le coût minimum que pourrait avoir un élevage rural produisant toute l'année avec peu de main d'oeuvre ; son efficacité sera cependant rapidement limitée en raison du faible nombre de parasitoïdes libérés par unité de surface.
- le coût par unité de surface est très hétérogène en raison de surfaces différentes dans chacun des exemples d'exploitations analysés ; en effet IT1' détient le coût par hectare le plus élevé, mais un faible coût par parasitoïde. Or on sait par les échantillonnages de populations de scolytes effectués régulièrement dans cette "finca" que les libérations ont donné des résultats significatifs : le niveau d'infestation des fruits a baissé de 6 % à 3,5 %.

Tableau N° 3 : Exemples de coûts annuels de production de *C. stephanoderis* (unité de monnaie = Quetzal (Q))

Itinéraire	# *C.s.** produits	Amortis.	Cons. Inter.	M.O.	Total	Total /ha	Coût /*C.s.*
ITO	184 040	130	500	7 400	8 030	36	0,04
IT1	134 725	130	500	9 900	10 530	92	0,08
ITR	?	265	500	9 900	10 665	19	?
IT1'	306 057	265	500	10 700	11 465	181	0,04

* *ratio* population libérée /population produite = 70 à 90 % selon la période

IV - DISCUSSIONS :

Il est encore impossible de définir sans risque d'erreur un coût par unité de surface pour l'utilisation du parasitoïde *Cephalonomia stephanoderis*, en raison des données trop fragmentaires sur leur efficacité.

On sait cependant que la lutte biologique inondative se fait par libération d'un grand nombre de parasitoïdes après la fin de la période de récolte. Dans ces conditions, au Mexique (GARCIA *et al.*, 1994) on obtient expérimentalement une baisse des taux d'infestation des fruits pouvant attenidre 20 %, lorsque la quantité de parasitoïdes libérés est élevée (6000 individus par hectare). Par ailleurs DUFOUR (1995, communication personnelle) abaisse de moitié environ le nombre de fruits infestés de la fructification suivante en libérant un parasitoïde pour cinq fruits infestés abandonnés de la récolte précédente.

1) Évaluation du coût de la lutte biologique :

Dans les conditions actuelles, il est possible de tenter une extrapolation pour évaluer grossièrement le coût de la lutte biologique (tableau N° 4) :
- les productions de fruits sont calculées en fonction des rendements figurant dans le tableau N° 1.
- par rapport à ce nombre, le pourcentage de fruits abandonnés après la récolte (sol et plante) est de 0,2 à 1,0 % des

fruits produits. 80 % des fruits abandonnés sont infestés par le scolyte (REMOND, communication personnelle).
- le calcul des coûts de production par hectare se fait en considérant que le coût de production d'un individu est de **0,04 Q** (cette donnée semble la plus logique à considérer : à noter que BARRERA 1994) donne une valeur similaire de l'ordre de 5,2 x 10^{-3} U.S.$.).

Tableau N° 4 : paramètres de production annuelle de café et de coût de la lutte biologique par hectare

Itinéraire	# fr.produits x 1000	# fr.aband. x 1000	# *C.s.* à prod. x 1000	Coût de prod. (Q)
ITO, IT1'	5000 à 9000	10 à 90	1,6 à 14,4	64,0 à 576,0
IT1	1000 à 3700	2 à 37	0,31 à 5,92	12,8 à 236,8
ITR	2500 à 5300	5 à 53	0,8 à 8,48	32,0 à 339,2
ITV, IT3'	80 à 200	* 1,6 à 20	0,3 à 3,2	12,0 à 128,0

* on peut estimer que le pourcentage de fruits abandonnés et infestés est 10 fois supérieur à ce que l'on observe avec les autres itinéraires techniques, car aucun contrôle cultural du scolyte ne se fait.
 # fr.produits = nombre de fruits produits ; # fr.aband.= nombre de fruits abandonnés ; # C.s. à prod.= nombre de *C. stephanoderis* à produire ; Coût de prod.= coût de production.

A la lecture du tableau N°4, on constate que :
- la production d'un élevage rural peut être suffisante pour des libérations sur plusieurs dizaines d'hectares, ce nombre étant variable en fonction des niveaux d'infestation par le scolyte.
- la lutte biologique a un coût relativement élevé, d'autant plus élevé que la production de café et le niveau d'infestation sont hauts.

2) Comparaison des coûts de la lutte biologique et de la lutte actuelle :

Le tableau N° 5 reprend les données des tableaux N° 2 et N° 4 et montre pour chaque itinéraire technique la différence de coûts entre les deux méthodes de lutte.

Il ressort de ce tableau que :
- le coût actuel de la lutte contre le scolyte, lorsqu'elle se fait, est situé entre le coût minimum et le coût maximum de la lutte biologique
- le coût de la lutte actuelle a peu d'incidence sur le coût total de production pour chacun des itinéraires techniques (faible pourcentage) ; le coût de la lutte biologique se situe dans un intervalle dont la valeur minimum est certes peu élevée, mais dont la valeur maximum représente un fort pourcentage du coût total de production, puisqu'il peut dépasser 10 %, voire atteindre 20 %.

Tableau N° 5 : Comparaison des coûts/ha de la lutte actuelle (chimique et culturale) à ceux de la lutte biologique (Q).

Itinéraire Technique	Lutte actuelle (Q)	Lutte biologique (Q)	% coût total It. Tec. lutte .act.	lutte. bio.
ITO	126 à 225	64,0 à 576,0	1,7 à 3,8	0,9 à 9,9
ITR	216 à 315	32,0 à 339,2	3,4 à 5,9	0,5 à 6,4
IT1'	279	64,0 à 576,0	4,3 à 4,7	1,0 à 9,6
IT3'	60	12,0 à 128,0	6,3 à 9,3	1,3 à 19,8
IT1	0	12,8 à 236,8	0	0,4 à 12,6
ITV	0	12,0 à 128,0	0	1,0 à 15,2

L'interprétation de ce tableau se fera avec prudence dans la mesure où trop d'inconnues subsistent :
- on sait que la lutte actuelle chimique et culturale est efficace
- on ne connaît pas avec certitude l'efficacité de la lutte biologique
- on peut penser que l'on arrivera à diminuer peu à peu le coût de la lutte biologique, par amélioration de la technique de production des parasitoïdes, par abaissement progressif des niveaux de population de scolyte en raison des lâchers successifs de parasitoïdes.

3) L'adoption de la lutte biologique dans les divers systèmes de production :

Dans l'état actuel des connaissances, l'adoption de la lutte biologique contre le scolyte constitue un espoir pour l'avenir. La motivation des producteurs dépend beaucoup de leur état d'esprit : certains pensent diminuer ainsi progressivement les niveaux d'infestation par le scolyte, d'autres espèrent avoir une meilleure production, d'autres y voient de substantielles économies, d'autres pensent à la préservation de l'environnement, d'autres enfin pensent produire ainsi un café "biologique" qu'ils espèrent pouvoir négocier à meilleur prix. Selon GARCIA *et al.* (1994), au Mexique dans l'état du Chiapas, 87 % des caféiculteurs ayant entrepris des élevages ruraux affirment vouloir les poursuivre.

Dans la région de l'étude un ensemble d'hypothèses peuvent être émises sur l'acceptabilité de la méthode de lutte biologique dans chacun des systèmes de production.

- Les producteurs à itinéraire intensif (ITO) ont un taux de profit du capital important. Ils ne devraient donc pas prendre de risques inutiles, mais pourraient mettre progressivement en place cette innovation technique, tout en l'associant à la lutte chimique, d'autant que cette dernière est compatible avec l'utilisation des parasitoïdes BRUN *et al.*,1992).
- Les producteurs économes en consommations intermédiaires (IT1) peuvent affecter plusieurs de leurs employés à la conduite de la lutte biologique, sans les remplacer dans leurs tâches antérieures.
- Les producteurs suivant un itinéraire technique complet (IT1') et effectuant des dépenses en main d'oeuvre et en consommations intermédiaires pour la lutte contre le scolyte devraient être favorables à la lutte biologique.
- Les petits producteurs rénovateurs (ITR) ont un intérêt particulier à adopter la lutte biologique, car elle n'est pas plus chère pour eux que la lutte actuelle et leur permet une meilleure gestion de leur trésorerie, grâce aux économies en en consommations intermédiaires. Il se pose cependant un problème d'organisation pour déterminer quelles seront les personnes responsables des élevages au sein des ECA.
- Les petits producteurs traditionnels ne vont probablement pas accepter les contraintes d'un élevage régulier.
- Les producteurs se tournant vers d'autres spéculations (IT3') ne devraient pas être prêts à innover.

V - CONCLUSIONS

L'étude conduite dans une zone de la région sud-ouest du Guatemala a mis en évidence l'existence de trois ensembles d'exploitations agricoles, les grandes fermes, les petits producteurs rénovateurs, et les petits producteurs traditionnels.

Pour chacun de ces types d'exploitation, l'étude a déterminé les coûts de la lutte menée actuellement contre le scolyte, ainsi que ceux d'une éventuelle lutte biologique à l'aide de parasitoïdes de l'espèce *Cephalonomia stephanoderis*. Elle a montré que l'acceptabilité de cette lutte biologique ne peut être comprise qu'en incluant des paramètres économiques liés au fonctionnement global de l'exploitation agricole et aux contraintes régionales qui entourent celle-ci, sachant que la lutte biologique ne peut être rentabilisée que si les autres pratiques culturales sont correctes.

La lutte biologique ainsi envisagée a un coût réel qui doit être pris en compte par les producteurs. Mais bien que les paramètres économiques actuels ne soient pas tous favorables au développement de cette innovation technique, des arguments prenant en compte à la fois le maintien d'une agriculture durable, l'environnement et le consommateur devraient intervenir pour une rapide adoption de la lutte biologique par une majorité de producteurs.

Ainsi conduite, cette étude a permis d'établir une méthodologie pour de futures analyses de faisabilité de la lutte biologique contre le scolye à l'aide de parasitoïdes élevés directement par les producteurs, lorsque celle-ci sera devenue une pratique agricole courante.

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RÉSUMÉ

L'importance des pertes dues au scolyte des baies justifie la mise en oeuvre de campagnes de lutte. La possibilité d'utiliser un parasitoïde, *Cephalonomia stephanoderis* Betrem pour contrôler ses populations ayant été démontrée sur le plan technique, l'un des facteurs limitants pour le développement de cette technique peut être d'ordre économique.
Une étude économique conduite dans le Sud-ouest du Guatemala évalue les possibilités d'application de cette innovation technique à l'échelle des exploitations caféières de cette région, dans les grandes exploitations, ainsi que dans les petites exploitations familiales.
La rentabilité économique de la lutte biologique est analysée, en comparaison avec celle de la lutte chimique, dans les différents systèmes de production existants. Il en ressort que cette innovation technique ne représente pas un investissement financier élevé, et que son coût, comparable à celui de la lutte chimique, est tout au plus égal à 7 % du coût total de l'itinéraire technique, quel que soit le système de production. Ce coût représente essentiellement la main d'oeuvre nécessaire au maintien des élevages du parasitoïde. Dans un système basé sur la monoculture, cette main d'oeuvre peut être détachée d'un autre poste de travail dans lequel elle n'est pas remplacée en cas de crise du café ; dans un système de polyculture, cette main d'oeuvre peut ne pas être disponible pour l'innovation technique. Les petits exploitants qui rénovent leur caféière, et pour lesquels l'achat des intrants est plus lourd à supporter que l'apport de main d'oeuvre, sont très favorables à l'adoption des techniques de lutte biologique, car ils peuvent se grouper pour les mettre en oeuvre.

SUMMARY

The extent of the damage caused by the coffee berry borer calls for control measures. As it has proved technically possible to use a parasitiod, *Cephalonomia stephanoderis* Betrem, to control these populations, one of the limiting factors for the development of the technique may well be economic.
An economic study carried out in southwest Guatemala assessed the prospects for applying this technical innovation in coffee plantations in the region, from large estates to family-run smallholdings.
The cost-effectiveness of biological control compared to chemical control was analyzed in the existing farming systems, which revealed that this technical innovation does not involve heavy financial investments, and that its cost, which is comparable to that of chemical control, is at the most 7% of the total cost of the technical management sequence, irrespective of the farming system. This cost essentially represents the necessary manpower for parasitoid rearing. In a monoculture-based system, staff can be released from another work post in wich they will not be replaced in the event of a crisis in the coffee sector ; in a polyculture system, staff may not be available for the technical innovation. Smallholders who are renovating their plantations, and for whom input purchases are more of a problem than providing manpower, are largely in favour of adopting biological control techniques, as they can work together with other growers to implement them.

INTEGRATED PEST MANAGEMENT TO CONTROL THE COFFEE BERRY BORER, *HYPOTHENEMUS HAMPEI*, IN COLOMBIA

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Introduction

The coffee berry borer (**cbb**), ***Hypothenemus hampei*** (Ferrari), is the most important pest of coffee in Colombia. Since its introduction into Colombia in 1988 the National Federation of Coffee Growers through Cenicafé, its research center, is conducting studies aimed to develop biological alternatives compatibles in an IPM program to control the **cbb**.

In Africa the **cbb** has several natural enemies (Le Pelley 1968), but so far the parasitoids ***Cephalonomia stephanoderis*** and ***Prorops nasuta*** and the entomopathogens ***Beauveria bassiana*** and ***Metarhizium anisopliae*** are seriously considered in our research programs (Bustillo 1990). Studies are in progress to implement the use of these bioregulators in conjunction with cultural practices and chemical control (Bustillo *et al.* 1991, Bustillo 1993; Cárdenas 1993; Morales *et al.* 1991; Antia *et al.* 1992; Orozco 1993; Posada 1993; González *et al.* 1993; Bernal *et al. 1994*). The final objective is to provide useful information to the farmers to control the **cbb** in an efficient and economical way, avoiding the environmental contamination by an abuse of insecticides. In this report information is provided on the mass production of parasitoids and fungi, alternatives on the use of insecticides of low environmental impact and effect of insecticides and entomopathogens on parasitoids under field conditions and how are incorporated into IPM recommendations.

Methodology

Parasitoid studies

Specimens of ***C. stephanoderis*** and ***P. nasuta*** were introduced from Africa via quarantine in England and through Ecuador during 1990. Laboratories were set up to establish the colonies and initiate studies to develop a rearing technique that could

allow the mass production of these parasitoids. Initial studies were conducted with ***C. stephanoderis*** under field conditions to determine its effect on ***cbb*** populations.

Initially the laboratory production of ***C. stephanoderis*** was used for field studies to measure the impact of releases in ***H. hampei*** infested plots. Field plots of 200 trees at two different farms, each with a control plot, were stablished. Three releases of parasitoids were made at the beginning of the study at a ratio of 1 parasitoid per 3 infested berries, this was estimated by an initial census. Evaluation was made by monthly samples of about 30% of the population for three years. Plots were not harvested during the study and **cbb** population was allowed to grow freely.

Beauveria bassiana studies

Selection of isolates of ***B. bassiana*** (**Bb**) were made from a large stock (>50) maintained at Cenicafé's laboratories. Two approaches have been investigated for the **Bb** production, one is at the industrial level. Production of **Bb** is initiated with cultures passed through insects, then the fungus is grown in petri dishes containing SDA for conidia production, after that flasks containing a liquid nutritive medium are aseptically inoculated with conidia in a submerged culture that is kept under continuous agitation at 110 rpm for 72 hours. This culture will produce blastospores which are used to inoculate trays containing a nutritive liquid substrate to produce aerial conidia. After 15 to 20 days, depending on temperature, the fungus is ready to be harvested, homogenized, formulated and dried into a dust form.

Also a technique to produce the fungus at the farm level has been developed (Antia ***et al.*** 1992). The substrate used is rice in water poured into disposable bottles plugged with cotton. This medium is sterilized in a "mary bath" using large bowls that support about 200 bottles. The sterile bottles are inoculated with the fungus in a clean environment with the aid of burners. Production in these bottles is about $5x10^{11}$ conidia/100g of substrate and development time is 23.5 days at 25°C. The production of a bottle is enough to spray 500 trees at a rate $1x10^{8}$ conidia/tree. Quality control of formulations is performed through a protocol specially developed for this purpose (Cenicafé 1994). Production of fungus in this way has been used since 1991 for further studies and borer control.

Epizootiology

The epizootiology of **Bb** was followed in a cbb infested farm planted with colombia variety at a density of 10000 plants/ha. CBB infestation was initiated in the central part of the plot in june 1991 and at that time the borer population was sprayed with **Bb** at a rate of $1x10^{7}$ conidia/tree, then population of **cbb** was allowed to grow and disperse in the field and **Bb** infection and dispersion was evaluated by taking monthly random samples of 300 branches with berries. On each branch total number of berries, infested berries and infested berries with signs of fungus growth on beetle body were recorded.

Insecticide trials to control *H. hampei*.

Insecticide formulations were tested to control ***H. hampei*** under field conditions. two different experiments were conducted during 1993 and 1994 and efficacy and residual effects were analized. Experiments were organized in a complete randomized design with 7 repetitions. The experimental unit was a coffee plant branch with 150-days old berries (50 berries/branch). Artificial infestation of cbb (>50%) was achieved with the aid of sleeve cages. For efficacy tests, insecticides treatments were sprayed after 1, 3, 8 and 15 days after borer infestation. For residual effects, the insecticide treatments were sprayed before the infestation, and infestation was made at 1, 3, 7, 15 and 21 days after spray time. Evaluation was made three days after treatment cutting off the branches and dissecting all the infested berries to record total number of adult borers and number of death adult borers.

Active ingredients and dosage per hectare of commercial products tested were as follows:

1. endosulphan: Thiodan 35EC 1.7 l/ha, Thionil 35 1.7 l/ha, Endosulfan 1.7 l/ha.
2. fenitrothion: Sumithion 1.5 l/ha, Fenothion 1.2-1.5 l/ha
3. pirimiphos-methyl: Actellic 1.5 l/ha
4. clorpiriphos: Pirifos 48E 1.8
5. diazinon: Basudin 60% 1.6 l/ha
6. fenthion: Lebaycid 1.5 l/ha
7. malathion: Malathion 57E 5 l/ha
8. isazophos: Miral 1.6 l/ha

B. bassiana* and *M. anisopliae* effect on *C. stephanoderis

The entomopathogens **Bb** and **Ma** were studied to determine their effects on ***C. stephanoderis*** populations under field conditions. Fungi were sprayed at a rate of $1x10^9$ per tree, before (21, 14 and 7 days), during and after (2, 4 and 7 days) field release of parasitoids. The experimental unit was a branch with enough berries (>30) which was infested with adults of **cbb** 30 days before releasing the parasitoids, using sleeve cages. A complete randomized design with seven repetitions was used, evaluations were made collecting 30 infested berries per branch to determine total number of parasitoids and recording those killed by these fungi.

Insecticide effects on *C. stephanoderis*

Four insecticides endosulphan 0.6 l a.i. /ha, clorpiriphos 0.8 l a.i. /ha, pirimiphos-methyl 0.7 l a. i. /ha were tested for their effects on the parasitoid ***C. stephanoderis*** under field conditions. A similar methodology for the above experiment was used. Selected branches of experimental plots were infested with **cbb** 30 days in advance to the release of parasitoids. One part of the experiment consisted in insecticide treatments applied to the plots 1, 3, 9, 15, and 21 days after release of parasitoids. The other part was the release of parasitoids 1, 3, 9, 15, 21 and 27 days after treatment application. A control plot was kept in both experiments. Evaluations were made by collecting the selected branches per plot and dissecting the infested berries to register the wasp mortality.

RESULTS

Parasitoid studies

The biology of both species ***P. nasuta*** and ***C. stephanoderis*** is very similar. Upon entering into the infested berry, the female stings and paralizes the adult ***cbb***, then feeds on eggs and small larvae, and lay eggs on mature larvae in the case of ***P. nasuta*** and on pupae in the case of ***C. stephanoderis.*** This last parasitoid is easier to rear in laboratory and currently is the only species that is mass produced in Colombia.

The ***cbb*** is reared using parchment coffee with a 45% humidity content. The infested grains are kept in cabinets on trays at 80% RH and 27°C and after 20 days they have enough immature stages for parasitoid reproduction. Development of ***C. stephanoderis*** takes about 18-21 days at 25°C in a darkroom. Emergence of parasitoids is enhaced by ligth and higher temperatures

Field parasitism studies

Results are shown in figures 1 and 2. Parasitism is dependent on pest density and although levels of parasitism are relatively low at the beginning, they are higher as time progresses. These results showed that ***C. stephanoderis*** can be establised under colombian coffee ecosystems, however alone it is not able to reduce the **cbb** populations to economical levels.

Mass production of ***C. stephanoderis*** has been initiated with the cooperation of private laboratories to introduce them in all coffee berry borer infested areas. Releases of parasitoids were initiated in October 1994 and about 50 millions have been released.

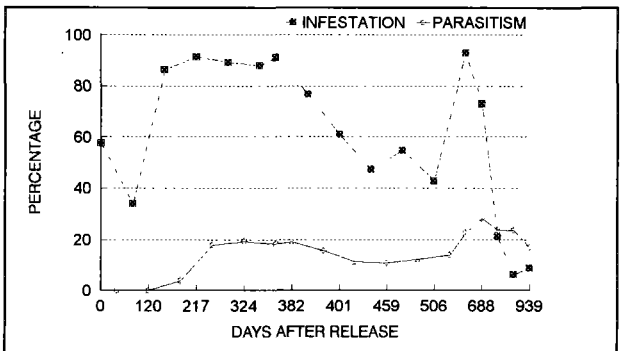

Figure 1. Parasitism of *Cephalonomia stephanoderis* on *Hypothenemus hampei* (1080 m.a.s.l Vereda Dorada-Guaitara, Sandoná, Nariño, Colombia, 1991 - 1994)

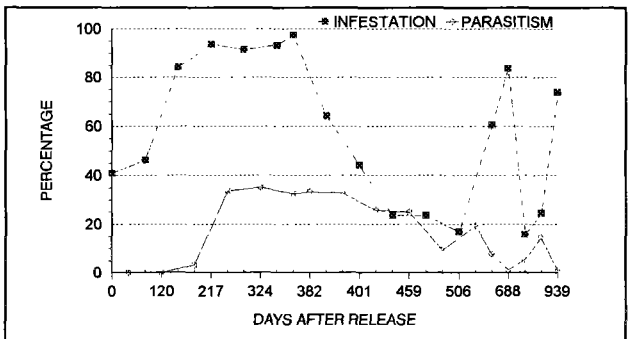

Figure 2. Parasitism of *Cephalonomia stephanoderis* on *Hypothenemus hampei* (1240 m.a.s.l. Vereda Dorada-Guaitara, Sandoná, Nariño, Colombia, 1991 - 1994)

Fungus production

During 1992, with the cooperation of private laboratories, 5 tons of **Bb** were produced to control **cbb**, this amount was increased to 60 in 1993 and 100 tons in 1994, including industrial production and farmers production. So far there are 5 laboratories with license to produce this fungus in Colombia.

Epizootiology

Results (Fig. 3) indicate that a high proportion of borer population can be infested by the fungus as time passes causing a reduction on its population, however the reduction in population using only fungus is not enough to keep populations of **cbb** below economic thresholds, p.e. less than 5%. Other measures are needed such as good harvesting, post-harvest control of borer populations and release of parasitoids.

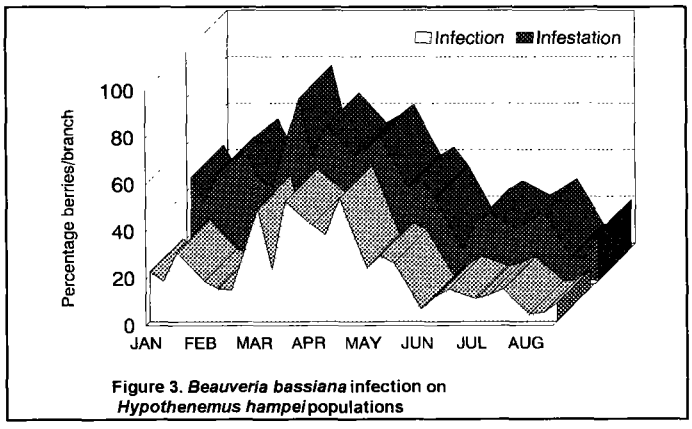

Figure 3. *Beauveria bassiana* infection on *Hypothenemus hampei* populations

Insecticide trials to control *H. hampei*.

Efficacy and residual effects of some of the insecticides tested are shown in tables 1, 2, 3 and 4. It is evident from the data that the insecticide efficacy for all treatments decreased as the **cbb** gets inside the berry. Besides formulations of endosulfan it could be demostrated that other products like pirimiphos-methyl, fenitrothion, clorpiriphos and fenthion are as effective as endosulfan with the advantage that they are less toxic and have antidotes for intoxication treatment to humans. All these insecticides did not show biological activity after 15 days, which is desirable under colombian coffee conditions. Use of insecticides of low environmental impact (categories III, IV) could be included in an IPM program for treatments of localized infested areas, only when cbb infestations are high enough, the borer is in the entrance tunnel and no interference is caused to other control measures.

Effect of *B. bassiana* (Bb) and *M. anisopliae* (Ma) on *C. stephanoderis*.

Results are shown in figures 4 and 5. Both fungi were pathogenic to the parasitoids. Mortality diminished as time between spray and released was greater. Highest rate of mortality ocurred when fungi and parasitoids were applied simultaneously (24.0% for **Bb** and 4.6% for **Ma**), however very low mortalities (less than 6.8% for **Bb** and 4.6% for **Ma**) at other elapsed times studied were recorded. These results showed that both biological agents can be used in an IPM program by leaving at least time intervals of ten days between spray of pathogens and release of parasitoids.

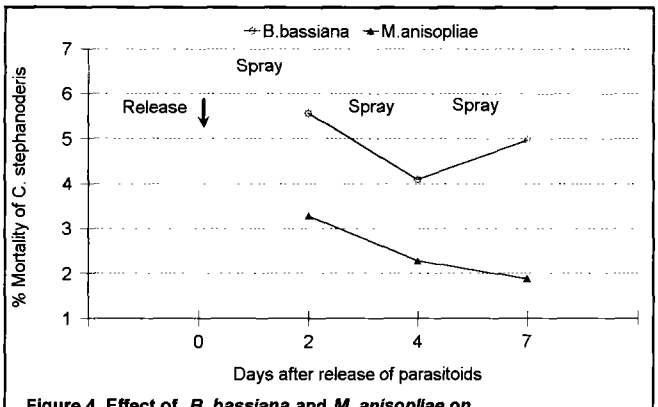

Figure 4. Effect of *B. bassiana* and *M. anisopliae* on *Cephalonomia stephanoderis*

Tabla 1. Efficacy of insecticides to control *Hypothenemus hampei*

Hacienda La Zulia, San Joaquín, Pereira, 1993.

	% Mortality* days after borer infestation			
	1	3	8	15
MIRAL (1,6)	65.9	42.0	32.9	41.3
THIODAN EC 35 (1,7)	37.2	46.9	63.2	74.7
ENDOSULFAN (1,7)	49.2	93.3	58.5	75.9
THIONIL (1,7)	74.7	97.7	64.6	44.9
SUMITHION (1,5)	79.1	79.5	74.4	37.2
ACTELLIC (1,5)	84.6	55.9	76.7	28.6
PIRIFOS 48 (1,8)	33.7	72.3	72.0	71.2
BASUDIN (1,6)	73.7	82.9	36.8	51.5

*Data corrected according Schneider-Orelli

Table 2. Residual effect of insecticides to control *Hypothenemus hampei*

Hacienda Bonanza, vereda El Pomo, Pereira ,1994.

	% Mortality* days after insecticide spray				
	1	3	7	15	21
MIRAL (1,6)	95.6	99.3	95.9	93.0	33.1
THIODAN 35 EC (1,7)	37.1	30.7	22.9	23.3	1.3
ENDOSULFAN (1,7)	26.9	7.9	24.4	4.9	0.0
THIONIL (1,7)	23.0	14.4	6.0	1.9	5.0
SUMITHION (1,5)	74.7	27.9	3.9	1.9	0.7
ACTELLIC (1,5)	44.0	29.7	11.7	1.5	0.0
BASUDIN (1,6)	44.1	4.7	5.3	2.8	1.5
PIRIFOS 48 (1,8)	10.9	0.0	14.7	16.8	2.8

*Data corrected according Schneider-Orelli

Table 3. Efficacy of insecticides to control *Hypothenemus hampei*

Hacienda Bonanza, El Pomo, Pereira, 1994.

	% Mortality* days after borer infestation			
	1	3	8	15
FENOTHION (1.2)	98.5	100	82.7	49.5
FENOTHION (1.5)	100	100	100	84.4
MALATHION (5.0)	83.6	89.9	70.1	49
THIODAN 35 SC (1,5)	98.1	97.9	99.7	83.7
LEBAYCID (1,5 + 0,75 Cosmoflux)	98	96.5	84.9	31.4
FUMIBROCA (5,0)	45.7	21.9	21.1	5

*Data corrected according Schneider-Orelli

Table 4. Residual effect of insecticides to control *Hypothenemus hampei*

Hacienda La Pastorita. Armenia 1994

	% Mortality days after insecticide spray				
	1	3	7	15	21
FENOTHION (1.2)	88.1	65.5	30.4	3.0	0.7
FENOTHION (1.5)	99.0	68.0	57.2	3.7	4.4
MALATHION (5,0)	94.0	23.8	25.9	7.1	5.8
THIODAN 35 SC (1,5)	72.7	6.1	0.0	0.3	0.3
LEBAYCID (1,5 + 0,75 de Cosmoflux)	63.9	19.3	0.7	2.0	2.2

*Data corrected according Schneider-Orelli

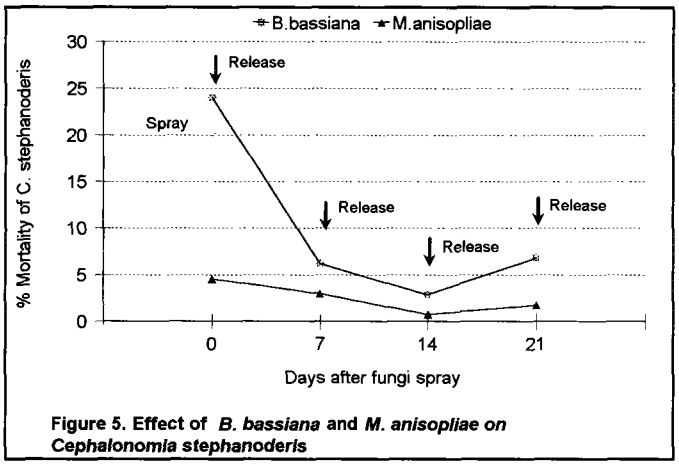

Figure 5. Effect of *B. bassiana* and *M. anisopliae* on *Cephalonomia stephanoderis*

Effect of insecticides on *C. stephanoderis*.

Results are shown in figures 6 and 7. All the insecticides tested caused mortality to the parasitoid population at the different time intervals tested. There were significant differences (P=0.001) with the control plot, but not among treatments. Effect of insecticides were more evident when the parasitoids were released after spraying the insecticides (Fig. 7) than the other way around (Fig. 6). This is explained because parasitoids upon finding a suitable host can enter rapidly into the infested berry avoiding the effect of insecticides. These results indicate that use of parasitoids in an IPM program can only be accomplished if sufficient time is allowed (i.e., 30 days) between the use of these control measures.

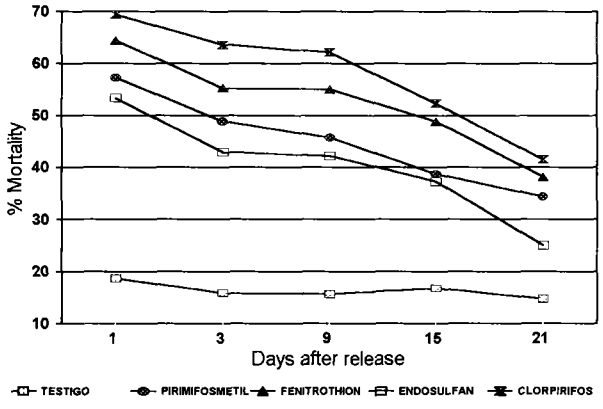

Figure 6. Mortality of Cephalonomia stephanoderis by insecticides

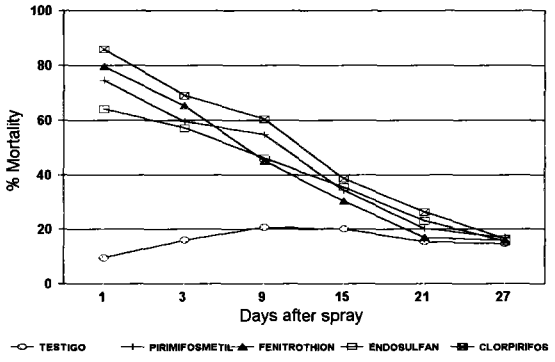

Figure 7. Mortality of Cephalonomia stephanoderis by insecticides

CONCLUSIONS

Since ***H. hampei*** is an introduced pest with no natural enemies present in the invading region, the first strategy based on initial results with parasitoids and entomopathogens, was to introduce them in the coffee ecosystem. The final aim is to implement an IPM program that can include different methods of control such as cultural practices, post-harvest control, chemical control when needed using low impact insecticides and biological components based on parasitoids and entomopathogens.

SUMMARY

Hypothenemus hampei is the main pest of coffee in Colombia. Since its introduction in 1988, Cenicafé, the research center of the National Federation of Coffee Growers of Colombia, has been conducting studies toward the implementation of an IPM program against this pest. Main emphasis has been given to the development of biological control using parasitoids and entomopathogens. The bethylids, ***Cephalonomia stephanoderis*** and ***Prorops nasuta*** and the mycopathogens ***Beauveria bassiana*** and ***Metarhizium anisopliae***, are now being mass produced.

The first step has been the introduction of these biocontrol agents to all the borer infested areas and the enhacement of natural enemies by discouraging the use of chemical insecticides. A second step is the integration of these biocontrol agents with cultural practices which can reduce borer populations both in the field and at the parchment coffee processing. So far 60 million parasitoids have been released in coffee plantations and during 1994 about 100 tons of ***B. bassiana*** and ***M. anisopliae*** were produced to control the borer. The extension service is also guiding farmers on the correct implementation of Cenicafé's recomendation against this pest.

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DISTRIBUTION DE *ANTESTIOPSIS ORBITALIS* WESTWOOD *(HEMIPTERA, PENTATOMIDAE)* DANS PLUSIEURS CAFÉIÈRES DU BURUNDI. IMPLICATION SUR L'ÉCHANTILLONNAGE

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INTRODUCTION

La production de café constitue la principale source de devises du Burundi. Les tonnages exportés atteignent près de 40 000 tonnes par an, et les recettes procurées par cette denrée représentent 80 % des rentrées de devises de l'état (Costes, 1989). La caféière burundaise, essentiellement constituée de l'espèce *Coffea arabica*, occupe environ 80 000 ha et est morcelée en plantations familiales de 100 à 300 caféiers.

Parmi les ravageurs de cette culture, la punaise bigarrée, *Antestiopsis orbitalis* Westwood, provoque d'importants dégâts sur les fruits et constitue l'un des plus sérieux problèmes phytosanitaires de la caféière burundaise. La diminution de la production en café en fonction de divers niveaux d'infestation a été quantifiée (Foucart, 1960), et le seuil d'intervention contre ce ravageur a été fixé à une moyenne d'une punaise par arbre. Les piqûres de cet insecte sur les baies du caféier peuvent provoquer la chute des jeunes fruits, l'arrêt du développement des fèves ou des nécroses sur les fèves. La présence de fèves piquées par cette punaise dans les lots de café marchand représente un défaut majeur et entraîne une diminution du prix. L'intervention de cet insecte dans la propagation du goût de pomme de terre a par ailleurs été suggérée (Bouyjou *et al*, 1993).

Le contrôle de cette punaise requiert une bonne connaissance des niveaux d'infestation dans les parcelles. Il est donc nécessaire de disposer de procédures d'échantillonnage fiables, c'est à dire de définir le type de sondage et la taille des échantillons, pour évaluer avec une précision satisfaisante le niveau des populations.

Le principal objectif de cette étude est de caractériser les distributions et les répartitions spatiales de cet insecte dans plusieurs caféières du Burundi. Cette étude devrait permettre de proposer des procédures d'échantillonnage adaptées aux expérimentations sur ce ravageur ou à son contrôle.

MATERIEL ET METHODES

Des dénombrements exhaustifs de la punaise *Antestiopsis orbitalis* ont été réalisés durant deux années consécutives dans plusieurs caféières de la région du Buyenzi, principale région caféicole du Burundi. Ces dénombrements ont porté sur une parcelle en 1991 (a) et cinq parcelles en 1992 (b), (c), (d), (e) et (f), chacune de ces parcelles étant constituée de 100 caféiers.

Les coordonnées de chaque arbre ont été notées par un repérage du numéro de ligne et du numéro de colonne. La connaissance de l'écartement entre les lignes et entre les colonnes permet de positionner chaque caféier dans un plan représentant la parcelle. Les nombres de punaises présentes sur chaque arbre au moment de l'observation ont également été notés. Ce dénombrement a été réalisé par la méthode du test d'inventaire : chaque caféier est pulvérisé avec 0,5 litre d'une bouillie insecticide de Pyréthrum à 6 %, soit 0,12 ml de pyréthrine. Les insectes (larves et adultes) sont collectés sur des bâches préalablement étendues au sol sous la frondaison.

Sur les différentes parcelles, une étude de la distribution du nombre de punaises par caféier est réalisée et des ajustements à des lois connues sont tentés.

L'étude des répartitions spatiales de cette punaise est envisagée selon plusieurs méthodes :

- une cartographie des différentes parcelles en fonction des quantités d'insectes permet de visualiser ces répartitions,
- des analyses de variance permettent de tester l'effet de différentes échelles d'hétérogénéité sur le nombre de punaises par arbre.

Une étude de la précision sur la moyenne du nombre de punaises par arbre en fonction du nombre d'échantillons (nombre d'arbres observés) est proposée. La méthode robuste du bootstrap est utilisée pour cette étude (Rémond *et al*, 1993) ; elle permet de calculer des intervalles de confiance sur la moyenne en fonction du nombre d'échantillons observés et du type de sondage choisi (aléatoire, stratifié ou systématique), (Cochran, 1977).

RESULTATS

Etude des distributions

Paramètres statistiques

Les principaux paramètres statistiques des distributions du nombre de punaises par arbre sont présentés pour chacune des six parcelles étudiées (Tableau 1).

Tableau 1 : Distribution d'*Antestiopsis orbitalis* - Paramètres statistiques

	Parcelles					
	a (91)	b (92)	c (92)	d (92)	e (92)	f (92)
médiane	8	6	2	1	3	4
moyenne	9,59	7.01	2,67	1,16	4,10	6,38
variance	44,24	18,41	4,00	1,81	17,36	41,59
Γ_1 *	0,646	0,904	0,939	1,501	1,943	1,619
Γ_2 **	- 0,541	0,498	0,925	2,901	5,551	3,742

* coefficient de symétrie de Fisher ** coefficient d'aplatissement de Fisher

Pour toutes les distributions observées, la variance d'échantillonnage est très supérieure à la moyenne, la médiane est inférieure à la moyenne, et les coefficients de dissymétrie sont significativement différents de 0. Il s'agit donc de distributions surdispersées, caractéristiques de phénomènes agrégatifs. Des ajustements à des lois de Poisson sont donc impossibles. En revanche, il semble raisonnable d'ajuster ces distributions à des lois binomiales négatives, souvent utilisées pour paramétrer des phénomènes agrégatifs.

Ajustements à des lois binomiales négatives

La loi binomiale négative a souvent été utilisée pour modéliser des distributions d'insectes (Anscombe, 1949 ; Bliss, 1956). Plusieurs écritures de cette loi sont possibles et la formulation de Anscombe (1950) sera retenue, car elle permet d'estimer des paramètres prenant des valeurs dans l'ensemble des réels ($\mathbb{R}$).

Soit X la variable aléatoire : nombre de punaises observées par caféier, la probabilité d'observer u punaises sur un caféier est :

$$P[X = u] = \frac{\Gamma(k+u)}{\Gamma(k).\Gamma(u+1)} \alpha^k . (1-\alpha)^u$$

avec ,

Γ : fonction gamma

$k \in \mathbb{R}^+$ (paramètre de dispersion),
si $k \to 0$, alors la distribution est très agrégative
si $k \to +\infty$, alors la distribution est aléatoire (ou poissonienne)

$\alpha = \frac{k}{m + k}$, où m est la moyenne de la distribution

Cette loi est donc définie par deux paramètres : α et k (ou m et k) qui peuvent être estimés simplement par la méthode des moments :

$$E(X) = \frac{k.(1-\alpha)}{\alpha} \quad \text{et} \quad Var(X) = \frac{k.(1-\alpha)}{\alpha^2}$$

E(X) et Var(X) représentent respectivement l'espérance et la variance de la distribution.

on en déduit :

$$k = \frac{[E(X)]^2}{Var(X)-E(X)} \quad \text{et} \quad \alpha = \frac{E(X)}{Var(X)}$$

Ces paramètres étant estimés, il est ensuite possible de déduire les probabilités (donc les effectifs) associées à chaque classe.

Ces probabilités sont estimées par la formule de récurrence suivante:

$$P[X=0] = \alpha^k$$

$$P[X=1] = k.P[X=0].(1-\alpha)$$

.

.

$$P[X=i] = \frac{i-1+k}{i} P[X=i-1].(1-\alpha)$$

Les ajustements, réalisés sur les données des parcelles d'étude, sont résumés dans le tableau 2.

Une comparaison des effectifs observés et des effectifs théoriques, calculés selon l'hypothèse d'une distribution binomiale négative, permet d'accepter les ajustements au risque de 5 % (tableau 2).

Tableau 2 : Paramètres d'ajustement des distributions à des lois binomiales négatives

	Parcelles					
	a (91)	b (92)	c (92)	d (92)	e (92)	f (92)
α	0,217	0,381	0,667	0,640	0,236	0,153
k	2,654	4,309	5,356	2,062	1,267	1,156
valeur du χ^2 observé	8,061	2,266	2,576	0,914	1,409	2,312
d.d.l. après découpage en classes	4	5	4	2	5	5
probabilité* (%)	9,04	85,03	56,05	61,24	96,07	85,03

* il s'agit de la probabilité d'obtenir une valeur inférieure ou égale à celle du χ^2 observé

Sur ces parcelles, le coefficient d'agrégation k varie entre 1,156 et 5,356 ; il ne peut donc pas être considéré comme constant sur l'ensemble des parcelles. Il serait donc nécessaire d'observer un plus grand nombre de parcelles pour identifier des valeurs de k représentatives de l'espèce en fonction de différents milieux considérés.

Etude des répartitions spatiales

Cartographies des parcelles

Dans les cartographies des parcelles, chaque arbre est représenté par une barre dont la hauteur est proportionnelle à la quantité d'*Antestiopsis orbitalis* qu'il héberge. Seules sont représentées deux parcelles hébergeant des quantités très différentes d'insectes (figures 1 et 2, pour les parcelles b et d respectivement).

Aucune régionalisation ne semble intervenir dans la répartition des punaises pour les différentes parcelles.

Figure 1 : Repartition spatiale des punaises – parcelle b

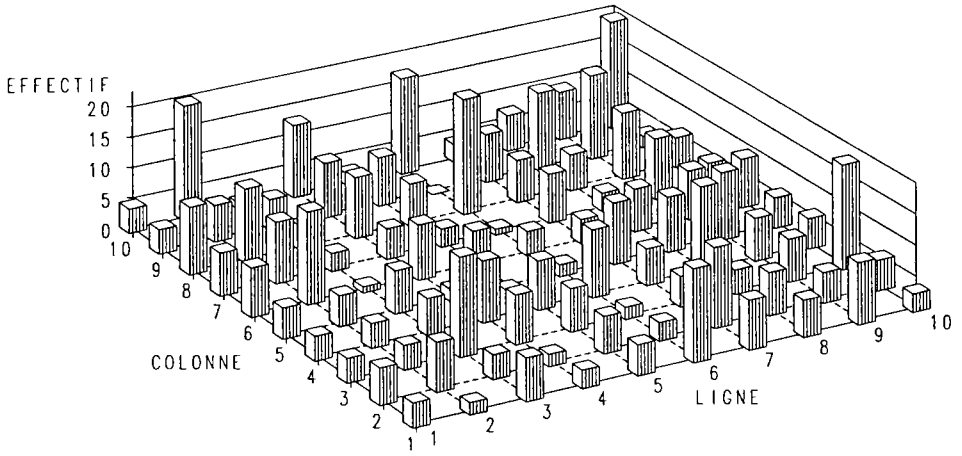
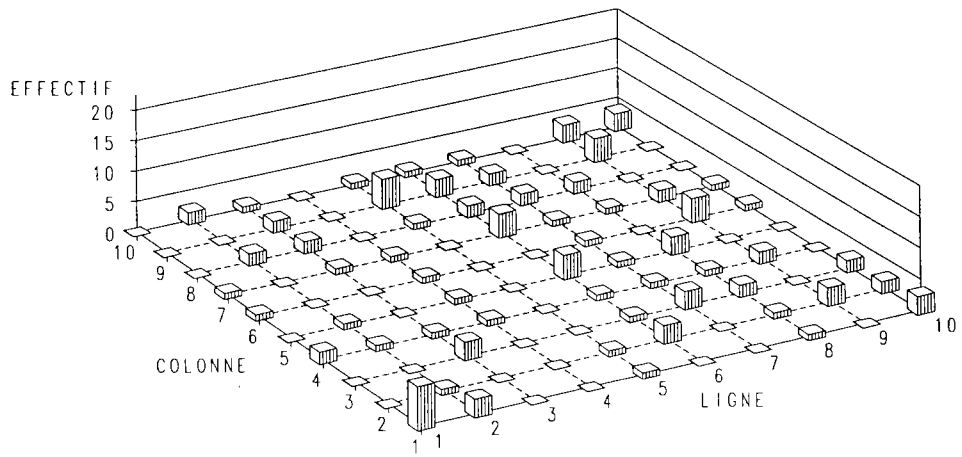


Figure 2 : Repartition spatiale des punaises – parcelle d



Analyses de variance

Les effets lignes et colonnes sont testés par analyse de variance, d'abord sur les effectifs observés (tableau 3), puis sur les effectifs transformés ($\sqrt{x+0,5}$) (tableau 4), cette transformation permettant d'obtenir une distribution normale des résidus.

Tableau 3 : Effets lignes et colonnes sur données brutes, tests de Fisher et probabilités associées

	Parcelles					
	a (91)	b (92)	c (92)	d (92)	e (92)	f (92)
F (ligne)	1,30	0,86	1,47	0,95	1,68	1,84
α (%)	27,85	56,49	17,40	48,79	10,77	7,35
F (colonne)	0,64	0,89	0,34	0,29	2,44	0,79
α (%)	85,92	53,82	95,94	97,64	1,66	63,05

Tableau 4 : Effets lignes et colonnes sur données transformées, tests de Fisher et probabilités associées

	Parcelles					
	a (91)	b (92)	c (92)	d (92)	e (92)	f (92)
F (ligne)	1,55	1,02	1,61	0,90	1,37	2,14
α (%)	19,52	42,77	12,54	52,84	21,48	3,54
F (colonne)	0,69	0,82	0,54	0,20	1,94	0,66
α (%)	81,79	59,82	84,41	99,37	5,70	74,63

Pour la plupart des parcelles, il n'existe aucun effet ligne ou colonne sur la répartition des punaises. Un effet colonne apparaît sur la parcelle e au seuil de 1,66 % pour les données brutes et un effet ligne sur la parcelle f au seuil de 3,54 % sur les données transformées. Ces effets ne correspondent cependant pas à des gradients continus d'après la comparaison des moyennes (tableau 5).

Tableau 5 : Moyennes des lignes et colonnes (effets significatifs) et tests de Newman et Keuls

Parcelle e , effectifs brutes			Parcelle f , effectifs transformés		
Colonne	moyenne	N&K (5 %)	Ligne	moyenne	N&K (5 %)
6	7,7	a	9	2,93	a
7	6,9	a b	5	2,68	a
10	4,9	a b	6	2,66	a
1	4,2	a b	1	2,53	a
9	3,4	a b	3	2,37	a
3	3,2	a b	2	2,12	a
5	3,1	a b	4	1,88	a
8	3,0	a b	7	1,51	a
2	2,8	a b	8	1,39	a
4	1,8	b	10	1,14	a

D'autres découpages en blocs ont été testés, mais aucune régionalisation significative n'a pu être mise en évidence.

Conséquences sur l'échantillonnage

La répartition spatiale des arbres infestés par les punaises semble donc aléatoire dans les parcelles. En revanche, il existe une agrégation de ces insectes au niveau des arbres, sûrement pour des raisons liées à la biologie de cette espèce. Aucune régionalisation n'apparaissant au niveau des parcelles, le type de sondage à effectuer n'a pas grande importance ; il peut être

aléatoire ou systématique, en fonction de critères de faisabilité et de coût.

La taille de l'échantillon, c'est à dire le nombre de caféiers à observer dans les parcelles, doit être fonction de la précision souhaitée. La distribution d'*Antestiopsis orbitalis* étant surdispersée, avec un coefficient d'agrégation variable en fonction des parcelles, la précision sur la moyenne de punaises par arbre en fonction de la taille de l'échantillon est étudiée par la méthode robuste du bootstrap. Les intervalles de confiance en fonction du nombre de caféiers échantillonnés sont présentés pour les parcelles b et d (figures 3 et 4). Pour connaître le niveau des populations à un instant donné, l'observation de 8 à 10 caféiers par parcelle permet de déterminer le moyenne du nombre de punaises par arbre avec une précision de l'ordre de 40 % de la moyenne, pour un seuil de probabilité de 95 % . Cette précision reste toutefois dépendante du nombre d'individus détectés dans la parcelle et devient assez mauvaise pour des moyennes très faibles.

Figure 3 : Intervalle de confiance sur la moyenne, estimé par bootstrap. Parcelle b

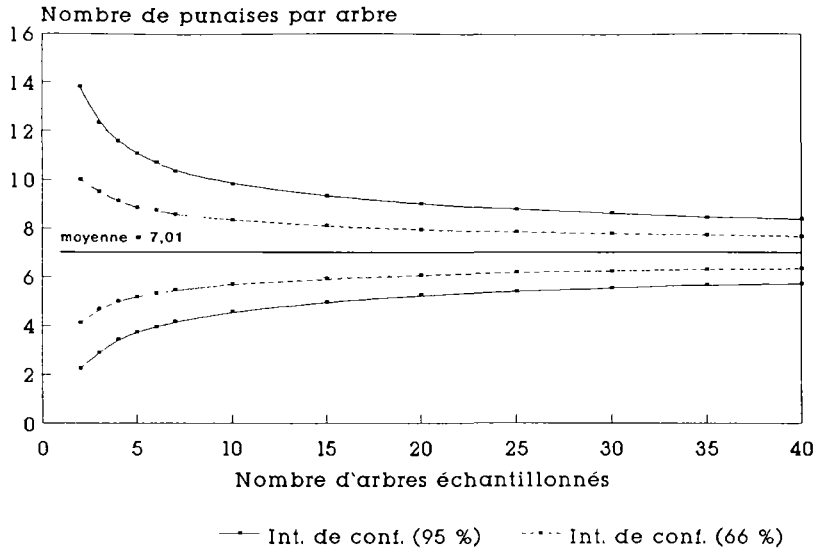
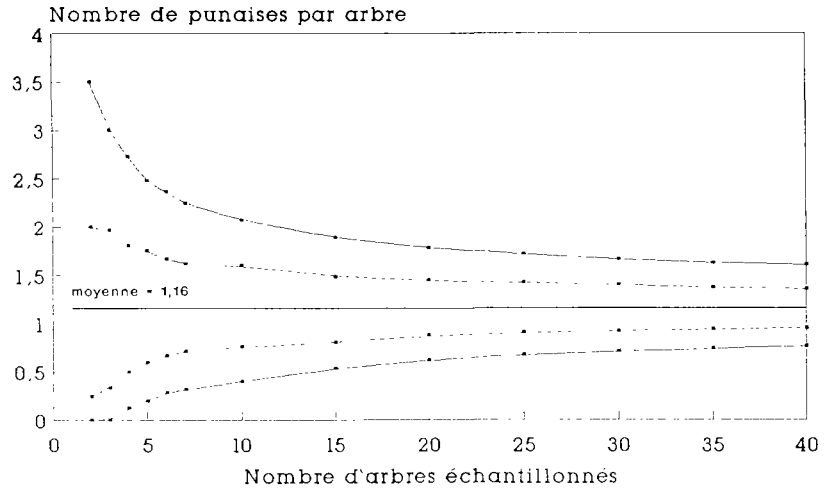


Figure 4 : Intervalle de confiance sur la moyenne, estimé par bootstrap. Parcelle d



DISCUSSION ET CONCLUSION

La répartition d'*Antestiopsis orbitalis* est agrégative mais aucune régionalisation n'apparaît sur les différentes parcelles ; l'unité d'agrégation est donc l'arbre et aucune corrélation entre arbres voisins n'a pu être mise en évidence.

Différentes hypothèses peuvent être formulées quant à la répartition spatiale particulière de cette espèce :

- des arbres plus attractifs que d'autres sont répartis aléatoirement dans les parcelles ; cette attractivité différentielle pourrait provenir de différences de production et/ou d'aspect phénologique des arbres d'une parcelle donnée.
- la première phase de colonisation des punaises adultes pourraient être de type aléatoire ; les pontes entraîneraient ensuite une répartition groupée des larves, ce qui impliquerait une distribution globale agrégative au niveau des arbres, suivant une loi binomiale négative.

L'aspect aléatoire de la répartition spatiale implique que le type de sondage à effectuer pour évaluer le niveau des populations peut être choisi uniquement en fonction d'impératifs techniques ou économiques.

L'agrégation des punaises sur les arbres nécessite des taux de sondage élevés, de l'ordre de 10 %, pour estimer avec une précision satisfaisante le niveau des populations.

Si l'objectif d'un échantillonnage est de décider ou non de l'opportunité d'un traitement, des procédures séquentielles pourraient être développées pour diminuer les coûts de sondage.

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Résumé :

La punaise, *Antestiopsis orbitalis* Westwood, cause d'importants dégâts dans la caféière burundaise. Les piqûres de cet insecte peuvent provoquer la chute des jeunes fruits, le non développement des fèves et la présence de fèves piquées pour les fruits arrivant à maturité. Pour lutter efficacement contre cet insecte, il est nécessaire de disposer de procédures d'échantillonnage fiables afin d'avoir une bonne estimation du niveau des populations dans les parcelles de production.

Dans cet objectif, la distribution de cette punaise a été étudiée dans six parcelles de 100 caféiers chacune. Le nombre de punaises par arbre s'ajuste bien à une loi binomiale négative, ce qui implique que cette espèce se répartit de manière agrégative sur les caféiers. Aucun gradient ou foyer d'infestation n'est mis en évidence sur les différentes parcelles. L'unité d'agrégation est donc l'arbre et aucune corrélation entre arbres voisins n'est détectée. Plusieurs hypothèses sont avancées pour expliquer cette répartition spatiale particulière.

La répartition spatiale de cette punaise étant aléatoire, le type de sondage à effectuer pour estimer le niveau des populations peut être choisi uniquement en fonction de critères techniques ou économiques. Des sondages simples peuvent donc être mis en oeuvre. Les intervalles de confiance sur la moyenne du nombre de punaises par arbre ont été calculés par la méthode robuste du bootstrap. En raison de la nature agrégative des distributions, des taux de sondage élevés sont nécessaires pour estimer les moyennes avec une précision satisfaisante.

Summary :

The Antestia bug, *Antestiopsis orbitalis* Westwood, is responsible for important damage in Burundi coffee plantations. The stings of this insect may be the cause of the fall of young fruits, the stop of beans development and the presence of pricked beans in fully mature fruits. To fight effectively against this insect, it is necessary to dispose of reliable sampling procedures in order to have a good estimation of population levels in production plots. In that aim, the distribution of this bug has been studied in six plots, each containing 100 coffee trees. The number of Antestia bugs per tree fits well to a negative binomial distribution, which implies that this species distributes on coffee trees in an aggregative way. No gradient or infestation focus could be detected in any plot. The aggregation unit is hence the tree, and no correlation between neighbour trees has been found. Several hypothesis are proposed to explain this particular spatial distribution.

As this bug spatially distributes randomly, the type of sampling to use to estimate population levels, can only be chosen according to technical or economical criteria. Simple sampling techniques can hence be used. Confidence limits for mean of the number of Antestia bugs per tree, have been calculated by a robust method : the bootstrap . Because of the aggregative nature of the distributions, high sampling rates are necessary to estimate the means with a satisfactory accuracy.

SITUATION PATHOLOGIQUE DES ARABICA EN NOUVELLE-CALÉDONIE, CORRÉLATIONS ENTRE PATHOGENÈSE ET ENVIRONNEMENT

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INTRODUCTION

Les recherches épidémiologiques sur *Coffea arabica* développées en Nouvelle-Calédonie ont comme objectif la compréhension du fonctionnement du pathosystème associant au caféier, son cortège parasitaire (*Hemileia vastatrix, Colletotrichum gloeosporioides,Cercospora coffeicola*) et l'environnement. La prise en compte des caractéristiques pathologiques et écologiques des sites étudiés, puis des traitements statistiques de ces ceux catégories de données ont constitué l'approche retenue pour identifier et hiérarchiser les paramètres environnementaux pouvant influer sur l'émergence puis le développement des maladies fongiques du caféier.

La finalité générale de ces recherches consiste à élaborer un outil épidémiologique moderne permettant, après modélisation, d'effectuer une prévision du risque épidémique pour une gestion raisonnée de l'environnement agricole.

Dans ce contexte, une première approche épidémiologique (Lamouroux et al. 1993, 1995), en privilégiant l'aspect spatial, avait montré l'existence d'une importante diversité pathologique au sein des multiples sites de l'enquête. Tout particulièrement, il est apparu que la variabilité intersites était très supérieure à la variabilité intrasite (i.e. au sein des parcelles constituant un site).

Sur ces bases, de nouvelles investigations ont essentiellement pris en compte l'aspect temporel des phénomènes étudiés, tant au plan de la dynamique des maladies que de la caractérisation mésologique. Dans ce cadre, des méthodes d'analyses multivariées complexes, élaborées par Chessel et al. (1993) pour une problématique écologique, ont été adaptées afin de pouvoir interpréter un cube de données du type "variables x sites x dates d'observation". Le recours aux outils informatiques et statistiques a permis également d'élargir sensiblement le champ des paramètres environnementaux classiquement utilisés pour décrire le contexte dans lequel s'expriment les maladies.

Cette approche a tout d'abord débouché sur une typologie pathologique détaillée des sites retenus pour les suivis épidémiologiques. Dans un second temps, les corrélations existant entre variables pathologiques et environnementales ont été déterminées, identifiant de ce fait les principaux facteurs environnementaux favorisant les épidémies. Sur la base de cette modélisation, des essais de prévision à partir des seules caractéristiques environnementales ont abouti à la détermination des conditions maximisant, dans une parcelle donnée, les risques d'épidémie.

MATÉRIELS et MÉTHODES

a. Dispositif de l'enquête

Il est apparu nécessaire de prendre en compte simultanément les trois agents pathogènes qui affectent *C. arabica* (var. Typica et Bourbon). Leur chronologie respective d'apparition sur l'hôte, la gravité des lésions, leurs interrelations (compétition/synergie) sur l'organe infecté, sont autant de facteurs qui participent à la compréhension des processus infectieux.

Le dispositif expérimental repose sur un suivi épidémiologique mensuel réalisé pendant trois années dans des sites traditionnels de caféiculture représentatifs de la Nouvelle-Calédonie (NC). La grande hétérogénéité écologique de ce Territoire a permis d'optimiser la diversité des situations environnementales retenues pour ces enquêtes.

Les procédures d'enquête épidémiologique et de caractérisation de l'environnement dans chaque parcelle ont été détaillées dans Lamouroux et al. (1993, 1995).

b. Gestion des données

Pour l'analyse des données, une partition préalable a été faite -au sein du patrimoine de feuilles étudiées- entre les feuilles présentes au premier relevé (soit en moyenne 90 % de feuilles de moins de deux mois et 10 % de feuilles datant du cycle précédent) et les catégories de nouvelles feuilles observées lors des relevés suivants.

La gestion des données pathologiques et environnementales caractérisant chaque cycle cultural annuel a été effectuée avec la base de données ORACLE au moyen d'un ensemble de requêtes permettant d'extraire les informations de manière sélective pour générer des tableaux. Afin de parvenir à une analyse détaillée de la pathologie, plusieurs paramètres synthétiques ont été élaborés :

- note sanitaire : elle intègre d'une part, le niveau moyen de gravité des attaques foliaires dans une parcelle à un moment donné et d'autre part, l'historique pathologique relatif aux feuilles disparues. Selon le cas, la note sanitaire [NS] définit soit le niveau d'infestation, tous pathogènes confondus, de la parcelle soit celui imputable à chacune des trois maladies [rouille =NSRou, anthracnose =NSAnth, cercosporiose =NSCerco].
- pourcentages cumulés de feuilles devenues malades [%cFmal], de feuilles malades tombées i.e. défoliation pathologique [%cFTmal], de feuilles saines tombées i.e. défoliation physiologique [%cFTsain], pourcentage de rameaux morts, etc.
- durée moyenne du cycle infectieux : cet indice exprime, pour une parcelle et pour un pathogène donnés, le nombre moyen de jours s'écoulant entre l'infection et la chute des feuilles ; il mesure l'incidence de chacun des pathogènes par rapport à la durée de vie moyenne des feuilles saines.
- indice d'aggravation : intégrant l'accroissement moyen de la gravité des lésions due à une même maladie et la durée moyenne du cycle infectieux, il met en évidence les sites où les conditions sont particulièrement favorables à l'extension des lésions foliaires.

c. Interprétation des données.

A partir de ces indices, les cinétiques épidémiologiques de chaque site ont été comparées pour établir des bilans annuels du niveau des infections.

L'interprétation statistique des données a été réalisé avec le logiciel ADE 3.7 (Chessel et Dolédec 1993). Outre des analyses multivariées classiques et de multiples possibilités d'interprétations graphiques, il autorise la réalisation de statistiques plus complexes qui sont particulièrement bien adaptées à la problématique de cette étude spatio-temporelle :

- l'analyse triadique d'un cube de données (variables x sites x dates d'observation). Cette approche réalisée par Thioulouse et Chessel (1987) repose sur trois étapes : détermination préalable d'une "interstructure" qui propose une ordination des dates d'échantillonnage, établissement d'un "compromis" exprimant les structures communes aux différentes dates pour les variables pathologiques sur les différents sites et enfin, caractérisation d'une "intrastructure" décrivant pour chacune des dates les écarts par rapport aux structures communes du compromis.
- la costructure de deux cubes de données par analyse de co-inertie. Cette approche récemment mise au point par Chessel et Mercier (1993) et Franquet et Chessel (1994) réalise simultanément un couplage d'analyses interdates (effets temporels) et d'analyses intradates (effets spatiaux). Elle recherche les combinaisons de variables (ou axes de co-inertie) qui expriment la covariation temporelle

et la costructure spatiale existant entre les nuages de points représentant les données. La projection de ces variables sur ces axes définit des plans précisant la costructure entre les cubes.

- l'analyse discriminante. Pour chaque cycle cultural, les différentes parcelles sont classées en fonction du niveau d'infestation maximal (note sanitaire) atteint par la maladie considérée. L'analyse discriminante sur cette partition donne une équation composée des moyennes annuelles des variables environnementales retenues. Sur la base de cette modélisation, des prévisions de la classe d'infestation sont réalisées.

RÉSULTATS

A- Caractéristiques pathologiques

a) Dynamiques épidémiques annuelles

Les enquêtes épidémiologiques confirment l'existence d'une mosaïque de situations pathologiques très diverses au sein du dispositif expérimental. Cette diversité est particulièrement perceptible au travers des courbes représentant les cinétiques d'infection et de mortalité de chacune des trois maladies (Figure 1). Les graphes obtenus révèlent une partition entre parcelles selon la précocité de l'apparition de la maladie et sa vitesse de développement.

Au bilan, la rouille demeure la maladie la plus représentée au sein du Territoire, toutefois son incidence varie sensiblement. En moyenne, près de 60 % des parcelles ont au moins 80 % de feuilles infectées dès le cinquième mois de suivi, alors que 20 % des parcelles finissent le cycle avec moins de 20 % de feuilles atteintes. Les gravités de ces attaques (note sanitaire) sont fortement corrélées aux effectifs de feuilles malades.

En moyenne, près de 65 % des parcelles présentent des attaques de *C. gloeosporioides* et, pour moins de la moitié d'entre elles, les infections concernent 70% des feuilles du patrimoine initial. Les cas de cercosporiose se limitent à 20 % des parcelles et seules deux d'entre elles présentent plus de 40% de feuilles infectées.

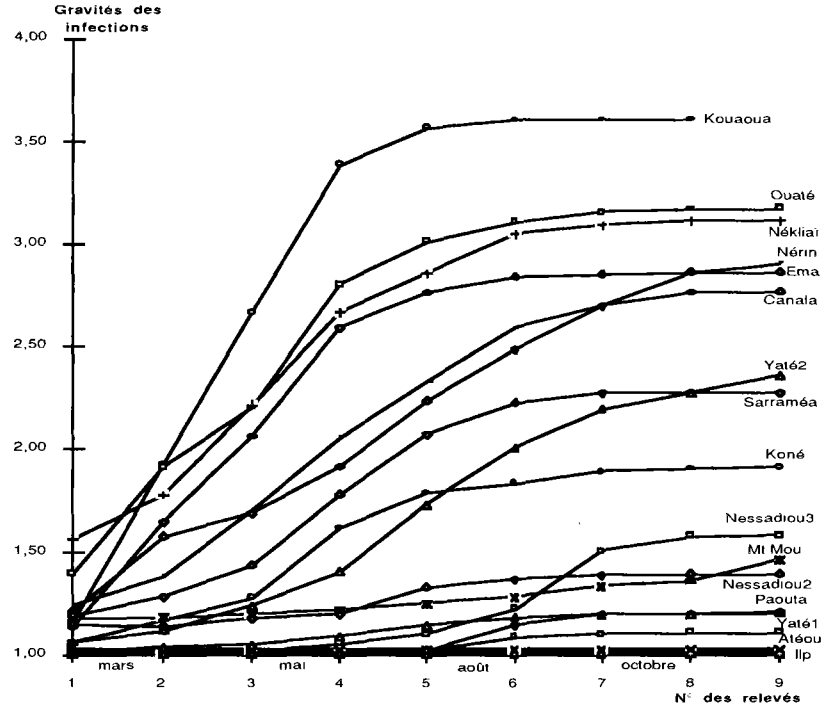

Figure 1 : Infestation par la rouille en 1993, Notes Sanitaires des parcelles.

Pour les feuilles malades, la prise en compte des dates d'infection et de disparition, des gravités initiales et finales des lésions révèle de fortes disparités interparcelles dans les valeurs moyennes de la durée du cycle infectieux. En fonction des différents sites, de la nature des pathogènes et de l'existence d'infections simples ou multiples, les valeurs correspondantes s'échelonnent pour la rouille de 35 à 120 jours, pour l'anthracnose de 35 à 181 jours et pour la cercosporiose de 35 à 70 jours. Pour un site donné, des analyses par catégories d'âge de feuilles indiquent que les pentes des cinétiques d'infection demeurent semblables tout au long du cycle cultural. Il semble donc que, dans les conditions de NC, l'âge des feuilles infectées ne soit pas corrélé à la sévérité des attaques.

Un phénomène de mortalité de rameaux est observé en fin de cycle. Il est particulièrement notable dans 26% des parcelles où les taux de rameaux morts peuvent alors dépasser 50%. Ceci hypothèque, de fait, l'état sanitaire global de la parcelle et par conséquent la récolte suivante. On notera qu'à chaque fois, *Colletotrichum* a été associé à ces lésions apicales.

b) Typologie des parcelles

L'analyse triadique intègre l'évolution temporelle de la pathologie (suite chronologique de tableaux correspondant à chaque ronde d'observation). On aboutit ainsi à une véritable typologie des différentes parcelles constituant le dispositif de l'enquête. Après normalisation par variable et par date du cube de données, l'"interstructure" donne pour chaque parcelle, l'agencement des variables en fonction du facteur temporel et pour chaque variable, la disposition des parcelles en fonction du temps. Cependant, le plan 1/2 du "compromis de date" demeure l'étape la plus explicite pour une analyse globale ; il indique en effet clairement la position respective des parcelles et des vecteurs décrivant la pathologie (Figure 2). Le nuage de points/parcelles peut alors être partagé en sous-ensembles cohérents ayant en commun par exemple, soit un niveau élevé de rouille, soit de fortes infestations par l'anthracnose, soit une forte défoliation pathologique, etc. L'"intrastructure" réalise, pour chaque parcelle et sur la base de ce compromis, une chronique des dates permettant de rechercher des événements temporels marquants. Ces informations sont directement utilisables dans la recherche des facteurs déclenchants afin de focaliser les analyses sur des périodes particulières.

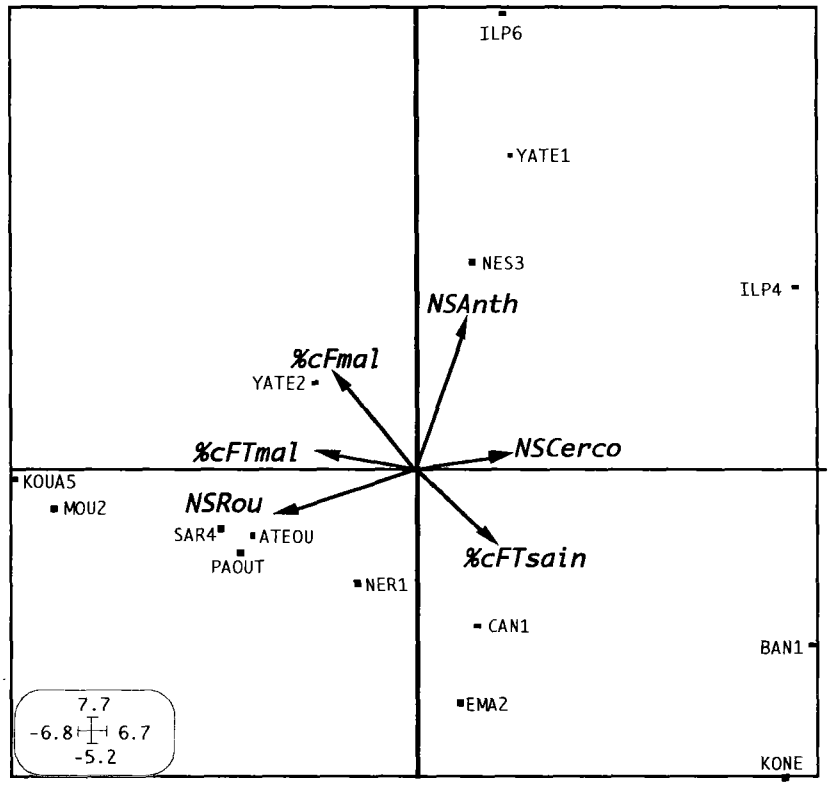

Figure 2 : Plan 1-2 du compromis de dates (1994).

c) Fluctuations interannuelles des épidémies

Après avoir pris en compte le facteur temps au sein d'un cycle cultural donné, les variations annuelles de ces phénomènes ont été étudiées. A cet effet, la comparaison graphique des signatures épidémiques correspondant pour chacun des sites aux différents cycles culturaux révèle l'existence de deux catégories de situations (Figure 3).

Dans près de 35 % des parcelles (Canala, Kouaoua, Ouaté, Nérin, i.a.) les profils des courbes sont comparables puisque la pente et le niveau maximal des infections demeurent similaires d'une année sur l'autre. En revanche, pour d'autres parcelles (ILP6, Koné, Mt Mou, Nessadiou,...) on constate de fortes variations interannuelles. Cette observation originale est particulièrement intéressante pour appréhender les corrélations pouvant exister entre signatures pathologiques et caractéristiques du milieu. En effet, dans les cas d'instabilités interannuelles, ces fluctuations de la sévérité des attaques peuvent être reliées aux caractéristiques climatiques qui, par définition, sont variables d'une année à l'autre. A l'opposé, la stabilité interannuelle des signatures épidémiques de certains sites est à rapprocher des paramètres édaphiques (e.g. pH, relief, fertilité, ...) qui sont considérés comme stables à l'échelle de temps de cette étude.

Cette mise en évidence de catégories de parcelles aux comportements antagonistes constitue certainement une voie à privilégier pour préciser les liens existant, entre sol et climat, avec la pathologie.

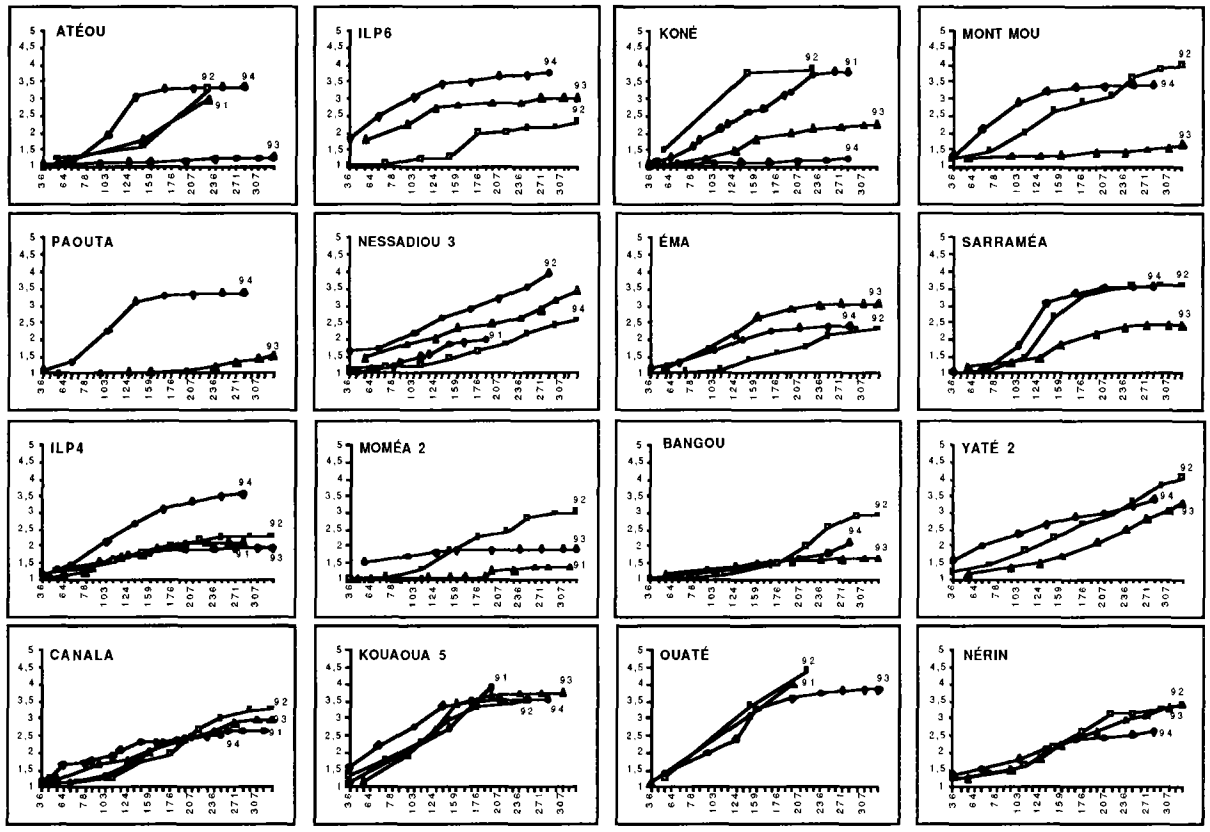

Figure 3 : Variations interannuelles des Notes Sanitaires des parcelles.

B- Corrélations Pathologie-Environnement

Des analyses de co-inertie entre les cubes de données épidémiologiques et environnementales ont permis d'identifier, pour chaque année, des combinaisons de variables de chacun des tableaux qui expriment d'une part, la covariation temporelle et d'autre part, la costructure spatiale des nuages de points correspondant aux données.

Dans un premier temps, la part relative des effets temporels (chronologie des dates de notation) et des effets spatiaux (diversité des parcelles) a été estimée sur le cube 'pathologie' (implicitement dans ce cas, l'effet temporel a été associé aux variations météorologiques et l'effet spatial, principalement, aux facteurs édaphiques caractérisant les parcelles). Ainsi, pour chaque année, les niveaux d'infestation de l'anthracnose et de la cercosporiose trouvent dans les "effets parcelles" une part importante d'explication, alors que l'effet temporel a peu d'incidence sur l'évolution de ces deux maladies. En conséquence, les facteurs édaphiques apparaissent déterminants dans le comportement de ces deux pathogènes. En ce qui concerne la rouille, on peut distinguer selon les années des situations contrastées, avec un "effet date" mineur en 1993, ou équivalent à l'effet parcelle en 1992.

Dans un second temps, la comparaison des trois plans résultant de la co-inertie définit la nature des corrélations (Figure 4). A nouveau, les résultats sont contrastés selon la maladie considérée.

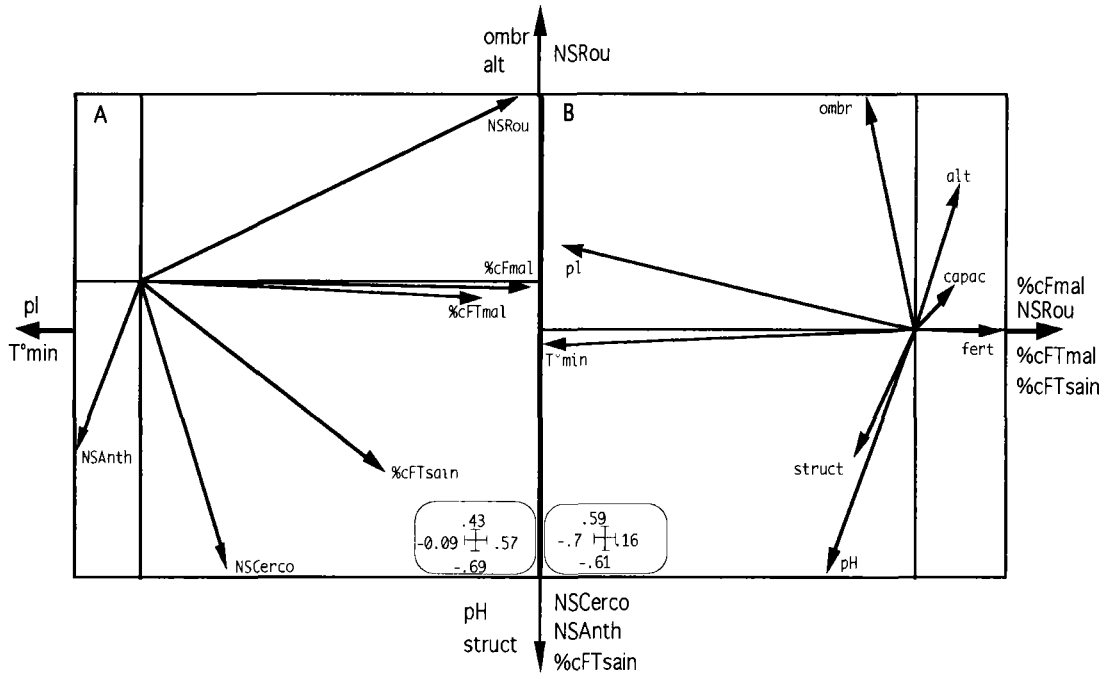

Figure 4 : Exemple de costructure entre les cubes Pathologie et Environnement (1994)

Des tendances majeures sont communes aux trois années. Ainsi, l'anthracnose (NSAnth) apparaît toujours fortement liée à des facteurs édaphiques tels que fortes valeurs de pH, bonne structure du sol (struct) et faible taux d'ombrage (ombr). En ce qui concerne la cercosporiose (NSCerco), des valeurs de pH et d'ombrage importants et dans une moindre mesure, une altitude (alt) faible constituent les principaux facteurs influant sur son comportement.

La position de la rouille (NSRou) s'oppose systématiquement aux deux précédents pathogènes mais, selon l'année considérée, cette maladie n'est pas corrélée aux mêmes variables environnementales. En 1992 et 1994, elle s'exprime préférentiellement dans des sites caractérisés par de faibles valeurs du pH du sol, de faibles pluies (pl), des températures minimales (T°min) relativement fortes, un taux d'ombrage important et une altitude (alt) élevée. A l'opposé, en 1993 (année significativement plus sèche que 1992 et 1994), les liens avec ces variables environnementales sont nettement plus faibles alors qu'émergent d'autres facteurs environnementaux comme la capacité de rétention en eau (capac) et la fertilité du sol (fert).

Les pourcentages cumulés de mortalité des feuilles infectées (%cFTmal) et de feuilles saines (%cFTsain) ainsi que le pourcentage de feuilles devenues malades (%cFmal) sont fortement liés à des variables météorologiques (faibles précipitations, températures élevées). Par ailleurs, le taux de mortalité des feuilles saines se signale du fait de sa corrélation négative avec le taux d'ombrage.

C- Prévisions des niveaux d'infestation

Les tendances majeures résultant de ces précédentes interprétations ont servi de base à la modélisation du risque de maladie imputable soit à la rouille soit à l'anthracnose (la cercosporiose étant trop peu représentée *in situ* pour permettre ce type d'analyse). Des prévisions du niveau d'infestation de chaque parcelle en fin de cycle cultural ont été effectuées en tenant compte des seuls paramètres environnementaux retenus pour chacune des années étudiées (Tableau 1). Pour l'anthracnose, dont le

		ROUILLE		ANTHRACNOSE	
		Prévision en %	Proba/hasard	Prévision en %	Proba/hasard
1992	pl, T°min, struc, pH	57	3.10-4	86	9.10-5
1993	T°min, struct, capac, pH	63	7.10-3	78	1,5.10-2
1994	pl, struct, pH, alti	47	2.10-1	80	1,8.10-2

Tableau 1 : Scores correspondants aux différentes prévisions du risque épidémique.

déterminisme est apparu essentiellement lié à des caractéristiques édaphiques, les pourcentages de parcelles bien classées (i.e. niveau d'infestation théorique identique au niveau observé) sont satisfaisants puisqu'ils oscillent, sur trois ans, entre 78 et 86%. En revanche, les chiffres obtenus pour la rouille sont nettement en retrait. La qualité des prévisions demeure cependant acceptable en 1992 et 1993 car la probabilité d'obtenir ces taux par le seul fait du hasard est très faible. Pour cette maladie au cycle biologique particulier, la forte liaison mise précédemment en évidence avec les facteurs météorologiques intervient certainement pour beaucoup dans ce médiocre taux de prévision.

CONCLUSIONS

L'originalité de l'approche expérimentale suivie en Nouvelle-Calédonie pour étudier le fonctionnement du pathosystème-caféier repose sur les points suivants : existence sur le caféier *arabica* d'un cortège parasitaire ayant impliqué la prise en compte des trois principaux agents pathogènes et de leurs interactions ; dispositif pluri-local (avec notation mensuelle de chaque feuille de caféier) dans le cadre d'une enquête réalisée en parcelles traditionnelles ; nombre des paramètres descripteurs intégrés dans les analyses afin de mieux décrire les signatures épidémiques et les caractéristiques environnementales d'une parcelle donnée ; prise en compte, avec un protocole identique, d'enquêtes annuelles successives.

Dans ce contexte, l'élaboration d'un schéma cohérent et fonctionnel des mécanismes mis en jeu dans le pathosystème, nécessite de comprendre les causes des fluctuations annuelles enregistrées dans certaines signatures pathologiques.

En premier lieu, cet objectif repose sur l'analyse de sous-fichiers de données plus homogènes (e.g. partition entre parcelles "stables" et "instables" au fil des années, sélection de rameaux de même âge physiologique, focalisation sur les périodes critiques du développement épidémique, ...).

En second lieu, une meilleure perception des mécanismes étudiés est subordonnée à l'évaluation de la part de variabilité revenant à la diversité génétique des populations des agents pathogènes ainsi qu'à une éventuelle diversité de l'hôte. Dans cette optique, des études complémentaires sont actuellement en cours :

* une analyse des populations de *Colletotrichum gloeosporioides* (isolé sur les sites de l'enquête) est réalisée au Laboratoire de Phytopathologie de l'ORSTOM à Montpellier à l'aide de marqueurs moléculaires (RAPD), enzymatiques ou liés à la compatibilité végétative. En première analyse, une forte différenciation intersouche ressort de ces expériences (Kohler et al. 1995).

* une analyse des populations d'*Hemileia vastatrix* est en cours à Nouméa afin de typer par des marqueurs moléculaires les différentes provenances de rouille. Dans ce cas, l'analyse comparée des ADN fongiques par la méthode PCR-RFLP est l'approche retenue (Kohler et al. 1995). Une séquence génomique (ITS) spécifique de la rouille vient d'être obtenue ce qui va faciliter les expérimentations ultérieures. De manière complémentaire, les races de rouille existant dans les sites de l'enquête, sont

en cours d'identification au CIFC d'Oéiras (Portugal).

Pour être complètes, ces analyses de la diversité des populations sont couplées avec des études sur le pouvoir pathogène.

* l'homogénéité intraspécifique des plants d'arabica étudiés devrait être prochainement évaluée dans le cadre d'un programme plus général sur la diversité génétique des caféiers en NC.

Ces résultats sur la diversité des agents pathogènes et de l'hôte ainsi que l'amélioration des fichiers de données constituent autant de facteurs de clarification qui permettront, après intégration dans le modèle, d'augmenter d'une part, les coefficients de corrélation entre les caractéristiques pathologique et environnementales et d'autre part, le taux de résolution des prévisions du niveau de sévérité des attaques fongiques sur caféier.

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RÉSUMÉ

Situation pathologique des arabica en Nouvelle-Calédonie, corrélations entre pathogénèse et environnement

F. PELLEGRIN, D. NANDRIS, S. WESTRELIN, F. KOHLER

Les recherches épidémiologiques développées en Nouvelle-Calédonie ont comme objectif la modélisation du fonctionnement du pathosystème multiple caféier afin d'effectuer une prévision du risque épidémique. Après mise en évidence d'une diversité spatiale dans la distribution des maladies, une étude des cinétiques d'infection révèle également des différences significatives intersites dans la précocité d'émergence et la gravité des attaques. La comparaison des cinétiques correspondant aux trois années consécutives d'enquêtes différencie deux catégories de sites, selon que leurs signatures épidémiques demeurent comparables ou non d'une année à l'autre.

Des méthodes d'analyses multivariées particulières ont été utilisées afin d'exploiter ces résultats. En premier lieu, l'analyse triadique (logiciel ADE 3.7) des cubes de données "sites x paramètres épidémiologiques x dates d'observations", en réalisant la typologie détaillée des parcelles, précise la diversité annuelle des comportements intersites. En second lieu, des analyses de co-inertie entre cube de données pathologiques et cube de données environnementales (climat, sol) révèlent l'existence de corrélations entre ces deux catégories de variables, en mettant particulièrement en relief le rôle de certains facteurs édaphiques ou climatiques dans l'évolution des maladies.

Sur la base de ce modèle, il est possible de réaliser des prévisions du risque épidémique en fonction de ces seuls facteurs environnementaux. Selon les années et les pathogènes, ces prévisions sont plus ou moins significatives.

ABSTRACT

Pathological status of *arabica* in New-Caledonia, correlations between epidemics and environment

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Epidemiological researches are carried out in New-Caledonia in order to model the overall running of the coffee multiple pathosystem then to forecast the epidemic risk. According to the observed disease spatial diversity, current studies on annual infection curves showed definite differences in the emergence and the severity of the diseases. Comparisons of curves corresponding to each of the annual surveys sorted the sites into two categories according to the similarity or the non-similarity of the infection kinetics.

Based on these results, new statistical methods were used first to interprate the three-dimensional data tables (disease parameters x sites x dates). A partial triadic analysis of this data cube led to an annual typology of the sampling plots. Then, coinertia analyses between pathology and environnement data tables identified the main parameters influencing the diseases. Using these featured parameters, a predictive model was developed to quantify the potential risk of a disease in a given environmental context.

STUDIES ON GROWTH AND YIELD PERFORMANCES OF COFFEE VARIETY IMY UNDER DIFFERENT TRAINING AND PRUNING SYSTEMS AT TWO DIFFERENT LOCATIONS IN SRI LANKA

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1. INTRODUCTION

The adoption of an appropriate training and pruning technique is very important for the profitable cultivation of Canephora type coffee because of its fast vegetative growth (Purseglove, 1982). Nevertheless, specific requirements and advantages of each training method are yet to be studied under local conditions (Vishveshwara and Jacob, 1982). By having the above objectives in mind, an experiment was started in 1989 using coffee Canephora (variety IMY) at two locations, Matale and Nillambe in the mid-country of Sri Lanka.

2. MATERIALS AND METHODS

The growth and yield of Coffee bushes under different pruning practices were investigated in this study. The experiment was repeated in two locations; Matale (in the border of mid country intermediate-wet zone) on a flat land with deep soils and Nillambe (in the mid country wet zone) on a marginal tea land. Seedlings of the variety IMY were planted at the spacing of 3mx3m. Pruning methods had been imposed at six months after field planting by means of bending whenever multiple stems were required (Robinson, 1964; Clifford and Willson, 1985). Treatments were single-stem one-tier (T1), single-stem two-tiers (T2), two-stems system (T3), three-stems system (T4), four-stems system (T5), main stem unchecked and without water shoots (T6) and the control, neither training nor pruning (T7). The experimental design was a Randomized Complete Block with three replicates at each location. Ten plants were taken as one plot. Canopy diameter, stem diameter at 15 cm above the ground, number of primary branches and secondary branches were recorded periodically at two monthly intervals. The yield data were collected at each plant whenever available. Additionally, soil texture and soil bulk density of the top 25cm soils were also studied for the each location.

3. RESULTS AND DISCUSSION

Total annual rainfall and weekly rainfall distribution pattern of each location were almost similar. There was a relatively long inter-monsoonal dry spell between January and April and comparatively short dry spell was observed during August-September. However, there were slight year to year variations.

For example, 1992 was a relatively dry year and January-April dry spell was about 90 consecutive days long for both the locations and August-September dry spell was very short (2 weeks). Inter-monsoonal dry spell was appeared from the first week of December for Nillambe at the end of 1992 while Matale received continuous rain until the end of December. There were few intermittent showers during January-April dry spell in 1993, but there was an additional short dry spell of about 3 weeks during April in Nillambe. Furthermore, second inter-monsoonal dry spell (August- Sept.) was relatively long in 1993 when compared to 1992.

Table 1 Soil bulk density (gm/cm^3) distribution of the experimental site.

Profile Depth	Matale	Nillambe
15 cm	1.30	1.56
30 cm	1.36	1.56
45 cm	1.37	1.43
60 cm	1.37	1.50
75 cm	1.42	1.54

Soil bulk density of the root-zone of each location is shown in the Table-1. The soil bulk density of each layer of the soil at Nillambe found to be significantly higher than that of Matale. The reason may be the presence of comparatively higher percentage of sand and gravel in the profile of Nillambe. The top soils of the experimental area of Matale was clay-loam and total available soil water storage was 17.9cm/100cm profile. While Nillambe soil was sandy loam and total available soil water storage was only 10.5cm/100cm of the profile.

3.2 Growth Performances

Data showed that there was a clear location effect on plant growth. All the growth parameters observed namely plant height, canopy and stem diameters, number of branches showed significantly increased values for Matale when compared to Nillambe (Table -2).

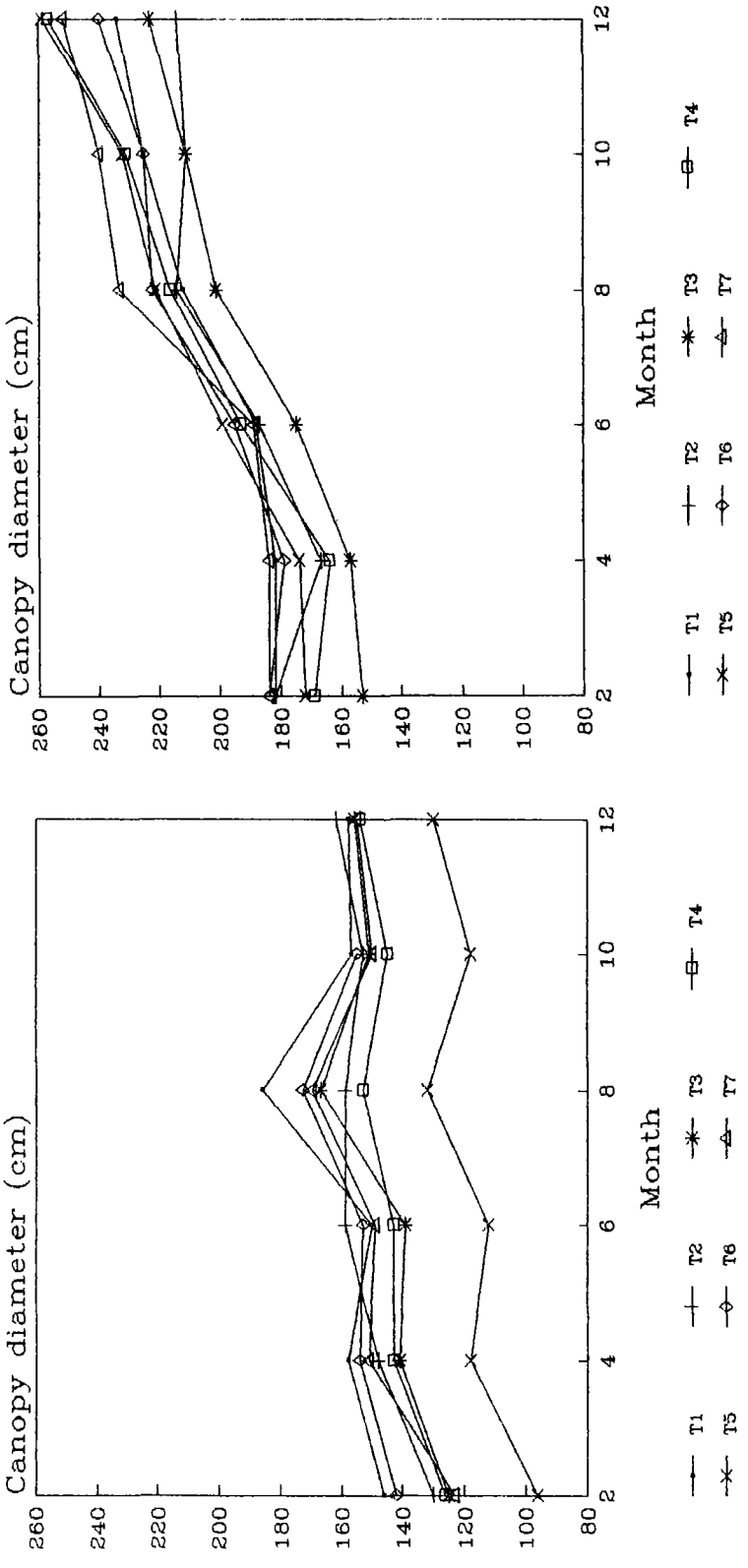
Table 2 Location effects on mean plant growth parameters.

Growth parameter	Nillambe	Matale
Plant height	129cm	187cm
Canopy diameter	126cm	202cm
Stem diameter	2.6cm	3.9cm
Number of primary branches	23.3	34.5
Number of secondary branches	7.6	27.6

A. Canopy diameter

There was an increase in canopy diameter during the third year after field planting at both the locations (Fig.-1). However, rate of elongation was higher at Matale when compared to Nillambe. As Fig. 1a indicates T5, four-stems system showed significantly ($P \geq 0.05$) lower canopy diameter when compared to the other treatments. All the treatments showed the simillar rate of diameter growth.

Fig.-1 Mean canopy diameter during the third year



However, there were some fluctuations in canopy diameter observed during the year at Nillambe and reason could be the intermittent loss of some branches due to twig borer damage. In contrast, data of Matale showed no clear difference between treatments in canopy diameter. However, rate of diameter growth appeared to be consistent and high at Matale when compared to Nillambe. This suggests the requirement of 3mx3m spacing for Matale.

B. Stem Diameter

The mean stem diameter growth for each location during the third year of field planting are shown in Table-3. Substantially slow growth at Nillambe when compered to Matale was prominent. Stem diameter of T1, T2, T6 and T7 were significantly higher when compared to other treatments at the end of the third year. The diameter increment showed a variable results. These treatments were almost similar in physical condition up to the third year as all the plants contain only one stem and any difference could be seen only in due course.

Table 3 Mean stem diameter and diameter increment of a plant at 10cm above the ground in the third year

Growth parameter	T1	T2	T3	T4	T5	T6	T7
A. Nillambe							
Stem diameter(cm)	3.8	3.5	2.9	2.4	2.2	3.5	3.7
Diameter increment (cm/yr)	0.8	0.3	0.4	0.5	0.6	0.5	0.6
B. Matale							
Stem diameter(cm)	5.0	4.7	3.2	3.2	2.4	4.9	4.2
Diameter increment (cm/yr)	0.8	1.3	0.4	0.8	0.2	0.6	1.2

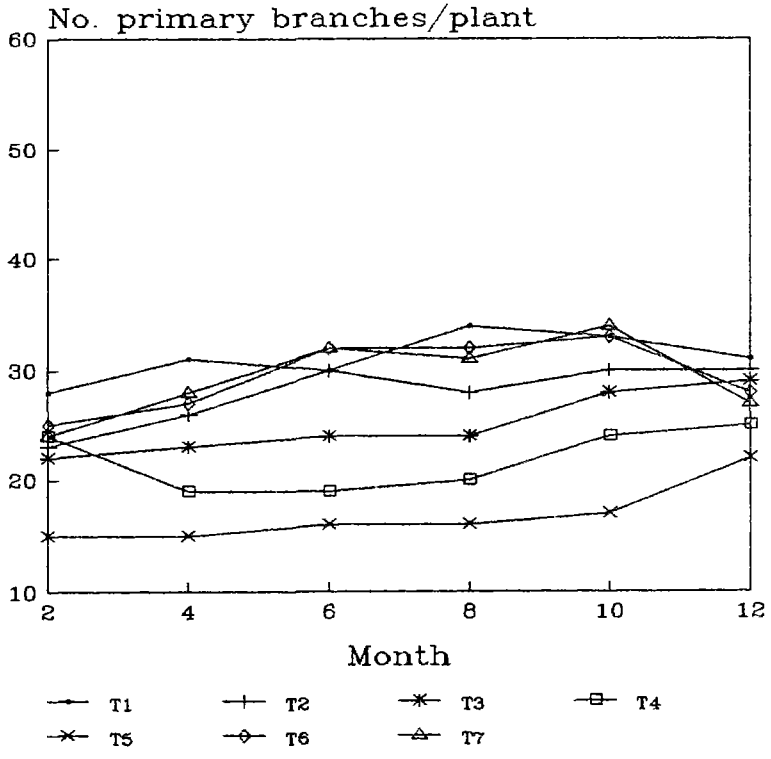
Only one main stem could be seen in T7 and mean stem diameter became smaller due to substantial contribution of food reserve for the growth and development of several water shoots at Matale, but this was not evident in Nillambe. It is interested to note that the differences among T7, T2, T1, and T6 were not significant ($P \geq 0.05$) at Nillambe and T4 and T5 have shown small stem diameters. This situation is associated with the very poor growth of water-shoots in this location and therefore, formation of multiple stems were also strenuous at Nillambe.

C. Number of primaries

There were some fluctuations in the mean number of primary branches per plant within the year and the reason was the fluctuation of twig borer outbreaks over the year. In general, mean number of primaries per plant at Matale was almost double when compared to Nillambe irrespective of the treatment (Fig.-2). The maximum number of primaries were observed through out the third year in T6 and the second largest number was seen under T1 in spite of the fluctuations at Matale. Usually there was a smaller increment in number of primaries during the first five months of the year due to the first inter-monsoonal dry spell occurred in February and March. Nevertheless, a relatively severe twig borer out break was observed with the high rainfall experienced during April, May 1992 and as a result number of primaries were severely dropped. However, there was a progressive recovery and development of primaries in the later part of the year with the beginning of the North-eastern monsoonal rains.

Fig.–2 Mean number of primary branches per plant during the third year.

A. Nillambe



B. Matale

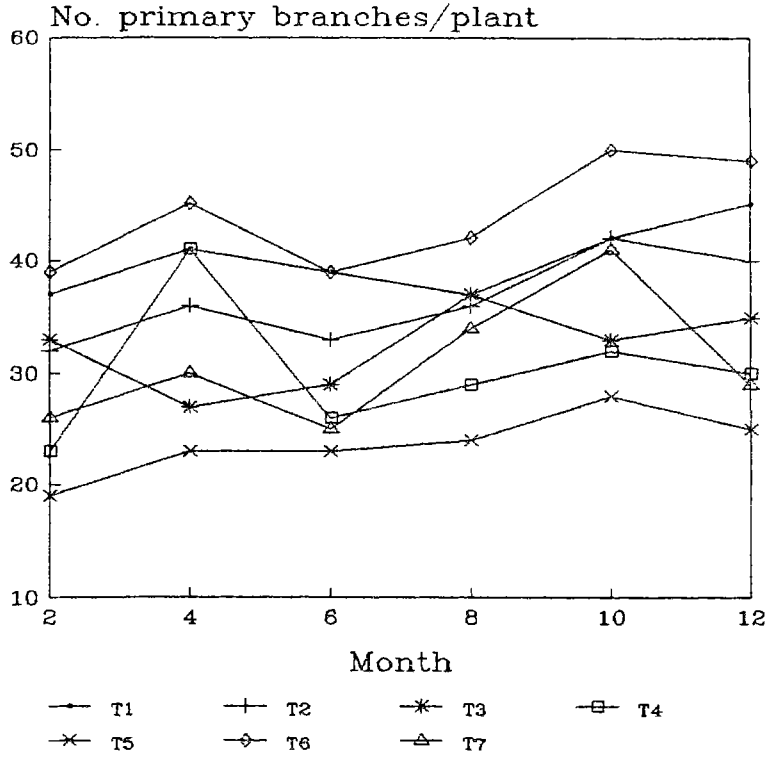
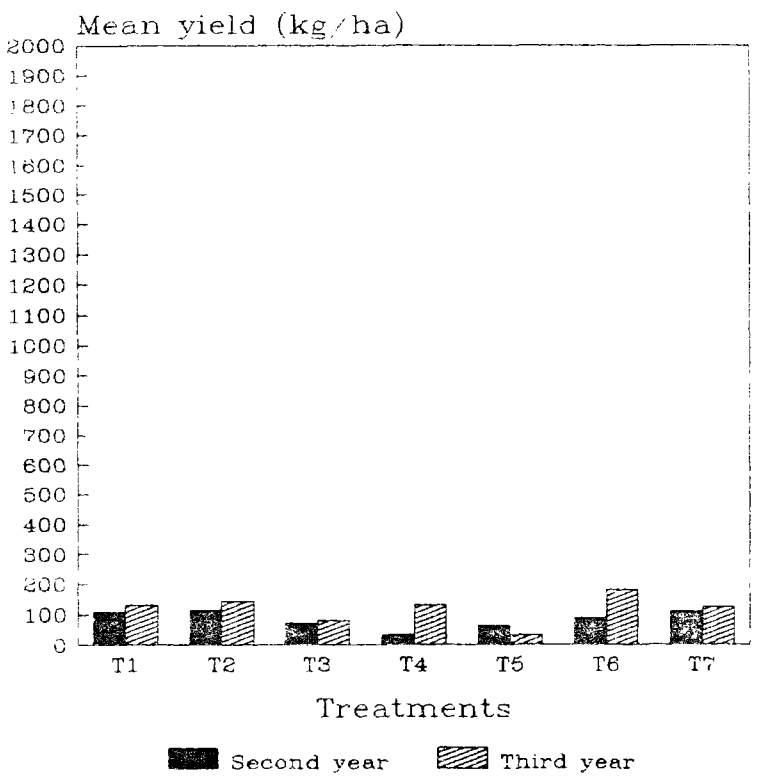
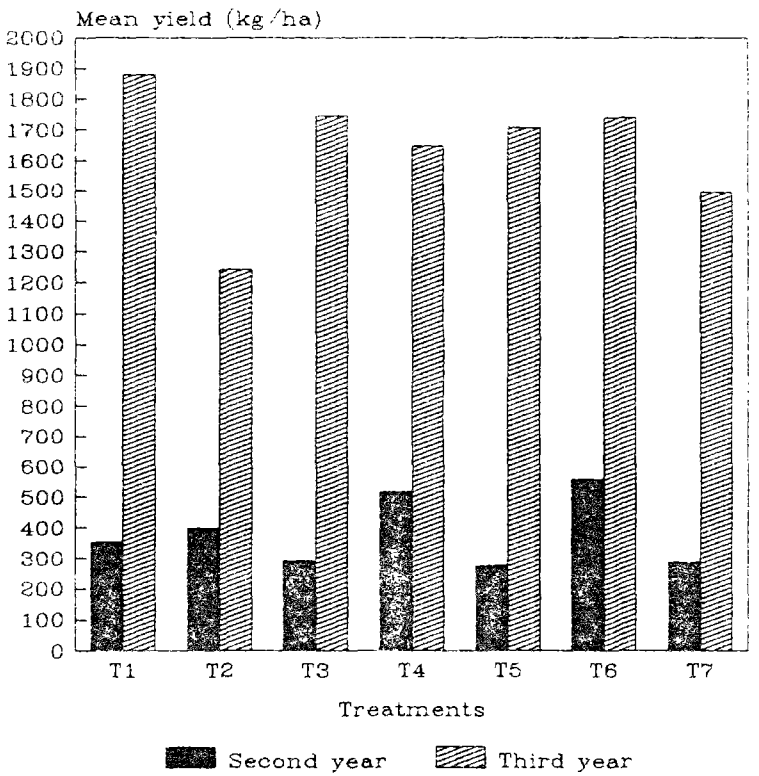


Fig.-3 Yield performance of processed coffee beans under different treatments.

A. Nillambe



B. Matale



Lower number of remaining primaries were observed at T4, T5 and T7 at the end of the year and this suggests that the multiple stem pruning methods i.e. T4 and T5 are not appropriate even for Matale. Similarly, a significantly ($P \leq 0.05$) lower number of primaries were observed in T4 and T5 at Nillambe (Fig. 2a). It is important to note that a sudden out break of twig borer damage was observed in November with a new flush at Nillambe and the same phenomenon was observed in May at Matale, throughout the study period.

3.3 Yield performances

Overall statistical analysis revealed that the treatment effect was significant ($P \leq 0.05$) for each location (Fig.-3). The highest yield (559kg/ha of dry coffee) was recorded at T6 in the first year at Matale and the yield at T5 and T7 were significantly lower recording less than 289kg/ha/year. In contrast to the first crop, the second crop was very high at each treatment and the yield at T2 was the lowest (1241kg/ha/year) at Matale compared to the other treatments. The total yield analysis showed that T1, T3 and T6 were in high magnitude and the highest cumulative yield of 2298kg/ha was recorded at T6. In contrast, Clifford and Wilson (1985) reported that no significant difference in crop yield between capped and uncapped single stem coffee in Colombia.

In general, yield of Nillambe was very low compared to Matale after three years of establishment. For example, overall mean dry coffee yields of Nillambe and Matale were 118kg/ha/yr and 1631kg/ha/yr respectively. The lowest yield of 32kg/ha/yr was observed in Four stems pruning system (T5) at Nillambe. This may be attributed to the lower water holding capacity of the soil at Nillambe and the frequency of short dry spells in that location. The highest yield observed in T6 at Nillambe was 182kg/ha/yr. No significant difference was observed among T1, T2, T6 and T7 for the total yield in Nillambe. It indicates that multiple stems pruning systems with more than two stems are not appropriate for Nillambe where the environmental conditions are marginal. In contrast, Mitchell (1974) reported that coffee is allowed to grow freely in Brazil so that old coffee with 4-6 trees per planting point (*COVA*) will carry up to 25 stems resulting increased yield. The growth and yield trends of Nillambe revealed that coffee variety IMY with more than two stems training systems are not suitable.

4. Conclusions

Although, overall yield was higher in the treatment with a main stem unchecked and without water shoots (T6), harvesting would be more labour intensive in the later years because of the continuous upright growth. Therefore, single-stem system (T1) or two-stems system (T3) would be more appropriate for Matale. Growth and yield data proved successful cultivation of IMY coffee at Matale. However, the results revealed that cultivation of coffee Canephora variety IMY is not profitable under particular environmental conditions at Nillambe. Continuation of this study is necessary to draw a firm recommendation.

ACKNOWLEDGEMENTS

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INDONESIAN COFFEE ; PERFORMANCE, PROBLEMS, AND PROSPECTS

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I. INTRODUCTION

Coffee has a typically important roles in Indonesian economy and unique features for several reasons. **First**, amongst tree crops, planted area of coffee is the third largest; after the coconut and rubber. The industry continue to grow and survive although for several times the government has attempted to reduce the planted area, and the prices of coffee always fluctuate. In the past, during the Dutch colonization, coffee was also cultivated by large commercial plantations but, nowadays, it cultivated mostly by smallholders. **Second**, many coffee smallholders are also being "accused" as illegal forest squatters who cultivate coffee in sloping lands of forest conservation and protection areas. Practicing clean weeding systems and thin canopy of shade trees lead to erosive farming and fastly deteriorating productivity as well.

Third, share of coffee is also very important to the export of agricultural primary commodities; especially to the 1980's or prior to the era of oil boom when the prices of oil and natural gas were relatively very low. In terms of values, due to the changing prices of world coffee prices, the export of coffee also fluctuate from the lowest of about US $ 250 million per year to the highest of about US $ 800 million.

Fourth, Indonesia has been known as the major producer and exporter of robusta coffee. In the domestic market, the production share of robusta coffee is 90 percent of total production of coffee; whereas, in the world market, it reachs almost 95 percent or more. For several reasons, Indonesian's robusta coffee is very competitive in the world market; and various barriers that have been set up so far are unable to stop the commodity from overflowing the world market.

This paper exposes some very important features of the biology, agronomy, cultivation practices, and post-harvest product handling of coffee in Indonesia, in the attempts to

understand the problem of Indonesia's coffee production. The discussion will focus on robusta coffee, as the major coffee production of Indonesia. The nature of the problems partly could be understood by a long-term observation on the performance of the industry and it will be the subject of the discussion on the next section.

The nature of the problems will be elaborated in the third section; which it focuses on some biological and agronomic aspects of the robusta coffee in Indonesia. The fourth section presents the future prospect or outlook of Indonesian coffee.

II. THE PERFORMANCE

As mentioned before, the coffee farmings in Indonesia is marked by millions of smallholders which average farm sizes, range from less than one to two hectare. The smallholder share in planted area is about 95 percent (of about 1.16 million hectares in 1993), and share in production is 93 percent (of about 449.8 thousand metric tons in 1993) as indicated in Table 1. The proceed from coffee bean sales are not necessarily as the major source of income to the smallholders especially when the prices are low. However, coffee is one of most important source of cash income to them. Therefore, they will continue to cultivate and harvest the coffee beans; regardless of how much of its prices.

The level of production, planted area and productivity of the coffee fluctuate (Figure 1 to 6 and Table 1). Between 1969 to 1993 the planted area of coffee showed negative growth nine times. But the rate of the decrease was more significant in the part of commercial plantations, especially for the state-owned plantation. Table 3 shows the remining state-owned coffee plantation is only in East Java which the size of the farm is approximately 22,000 hectares. The one and only coffee research institute is also located in that province, precisely in Jember.

On the other hand, during the same period, the coffee's planted area of smallholders continue to growth; except in 1972 and 1973, there were decreases in the planted area, which for other several years, the rate of changes are nearly zero percent per annum. On the whole period, however, it could be said that the planted area of the smallholders increased.

Figure 1 and 2 also show that the significant increases of the planted area, especially the smallholders, were not necessarily followed by significant increases in the (robusta) coffee production. It is interesting to note that in 1974 there was a sharp increase of production of coffee plantation. This could be connected with the sharp increase of the area of coffee plantation in 1972; by new planting and rehabilitation efforts which gave the results after three years. The sharp decrease in the area of the plantation in 1973 may also be part of the rehabilitation.

The patterns continue to exist between 1980 to 1981, 1982 to 1984, 1986 to 1988, and 1989 to 1990. After those times, the production of coffee of the plantations continue to drop, even the annual changes reach zero percent. The same is true with the planted area of coffee of those plantations. The data, however, do not show the effect of the severe attack of nematode parasite (*Pratylenchus coffeae*) in 1986; which probably have caused a significant decrease in the coffee production range from 39 to 79 percent (Wiryadiputra, 1990).

The productivity of the smallholders are relatively low; i.e., in average 515 kilogram per hectare in the period of 1969-1993. The productivity fluctuate in the range of plus and minus 10 percent per annum (see Figure 6). The highest increase in the productivity of the smallholders was in 1977; i.e., an increase about 18 percent. Compare to the previous period the productivity of the smallhoders after 1977 tended to be better. In 1977 to 1993, the productivity ranged from 484 to 621 kilogram per hectare, whereas, in the period of 1969-1976, the productivity ranged from 380 to 484 kilogram per hectare (Table 1).

The productivity of large scale plantations is relatively better than smallholders. In average the productivity of the plantations are 558 kilogram per hectare in the period of 1969- 1993. For examples, in 1993, the average productivity are 542 and 727 kilogram per hectare for smallholders and large scale plantations, respectively. Both the smallholders and plantations in the period of 1977-1993, show considerably higher productivity compared to previous period (1969-1976). Yet, the productivity in 1977-1993 show more fluctuation; i.e., from the lowest 501 kilogram per hectare in 1983 to the highest 727 kilogram in 1992 and 1993.

The result of the discussion on the fluctuation of production and productivity above indicate two important features of coffee industry. **Firstly**, coffee is an agronomically risky crops. One could easily locate documents and reports on various pest (or parasite) and diseases of either robusta or arabica coffee. Irregularity of the tropical seasons also contribute to the risks and uncertainties.

Secondly, in the period of 1969-1993, the average rate of productivity changes of the smallholders and large scale plantations are 0.8 and 6.8 percent per year, respectively. In the same period, the average rate of changes in the planted area of the two types of plantaions are 1.7 and 2.4 percent per annum, respectively, whereas, for the smallholders rate is 5.1 percent per annum. This is an interesting situation; suggesting that compare to the large scale plantations there have been little technical progress amongst the smallholders. Increased production of the smallholders, therefore, have been primarily contributed by the increase in planted area or extensification rather than intensification.[1]

III. THE PROBLEM

3.1. On the Farm Level

Coffee (*Coffea* sp.) is not indegenous to Indonesian flora. There were three species of coffee introduced to Indonesia, i.e., Arabican Coffee (*Coffea arabica*), firstly introduced in 1696; Liberican Coffee (*C. liberica*), introduced in 1875; and, Robusta Coffee (*C. canephora*), introduced in 1900. For more than two centuries, during Dutch colonization, Arabican coffee was the only one cultivated commercially in Indonesia, but the cultivation drastically deteriorated due to leaf stain disease (*Hemileia vastatrix*) which came to Indonesia in 1876. This caused the cultivation of arabican coffee moved to the land areas with elevation more than 1,000 meter above means sea levels where the disease have less effect on the cultivation (Cramer, 1957, and Huttema, 1935, as cited in Yahmadi, 1979).

[1] If Production (Y) is defined as Y = A * y; where A is planted area and y is yield or productivity (Ton/Ha); then clearly dY = dA + dy, where d denotes annual rates of changes. Thus, if dy is nearly zero, then, the increased in the production (denoted by dY) will be solely cuased by the increased in planted area (denoted by dA).

To replace the losses, Liberican coffee was brought in from Sri Lanka, but later it was found that the species was also not resistant to the leaf stain disease. Morevover, the taste was not quite acceptable and, therefore, the species was no longer exist in Indonesia (Yahmadi, *ibid*).

When robusta coffee firstly introduced, it was found that robusta coffee was quite resistant to the disease and it needed a very light requirements for growing and cultivation, but the productivity was relatively high. The robusta coffee rapidly replaced other species, and now it becomes the major coffee production in Indonesia (about 95% of planted area is robusta coffee).

In the meantime, the Arabican coffee in Indonesia generally belong to *C. arabica var. typica*, which is best grown on the high lands of more than 1,000 m amsl. On the other hand, robusta coffee is best grown in the lowland up to 800 m amsl. To fill the gap, a new *cultivar* (i.e., *C. arabica var. abyssinica*) was introduced in 1929 which was more resistant than the *typica* and it could be cultivated commercially in the high land of 700 m amsl or more.

Table 2 shows the composition of the planted area of robusta and arabica coffee in Indonesia. The table also shows that the area of robusta coffee continue to increase considerably during 1985-1989, as well as to present time, whereas in the same period, the area of Arabica coffee shows only a slight increases. Perhaps, this is simply because of one could hardly find suitable farm lands for arabica coffee in Indonesia. Besides, the suitable farm land for coffee with good soil physical and chemical conditions are only a small part of Indonesia's land. With the existing low capacity of coffee in generating income to the smallholders, coffee farming may be less competitive especially if intensive farming practices are to be adopted. At present, most of good farm lands have been utilized for other tree crops which can generate better incomes such as oil palm, rubber and coconut. Coconut can grow in marginal lands where coffee is less suitable.

In Indonesia, coffee is basically planted in orchard farms. Normally some banana clusters, chilly, egg plants, vanili, pepper and papaya are grown together amongst the coffee trees and shade trees such as *Erythrina* spp as well. In general, the smallholders perform clean-weeded system in their orchard farm; regardless of the slope condition of their farm lands. In sloping farm lands, such practices would generate considerable productivity degradation due to soil erosion. Because of such practises, the productivity will decline significantly after five years. Usually, the smallholders who adopting such practices would have no incentive to rehabilitate the farm and prefer to abandon it. In some manner, shifting cultivation of coffee is in process. Some of those smallholders are actually cultivating in the protected and conservation forest areas.

In addition to this crop diversification, across the region and provinces in Indonesia, the availability of suitable lands where coffee has the comparative advantage show high variability. At present, Lampung Province is known as the major production area of coffee in Indonesia (mostly robusta coffee). In fact, the largest area of coffee farms is in South Sumatra Province (Table 3). It is interesting to note that while the area of coffee farm in South Sumatra more than twice of those in Lampung Province, the coffee production in South Sumatra was considerably lower compare to Lampung Province. It seems that the coffee production in South Sumatra (1989) was underrecorded. This may be connected to the fact that the major market center of coffee (not in physical terms) is in Lampung

Province, especially for the export. In a regional perspective, part of South Sumatra and Bengkulu Provinces are actually **the hinterland** of Lampung Province.

Coffee is a tree crop or perennial crop, which normally has small main stem and diameter is not more than 30 cm even when the age of the tree is more than 50 years. In Lampung Province, as the major coffee producing area in Indonesia, coffee is primarily planted in the lowland with rich and deep soils with slope range from 3 to 10 percent, or in some gently undulating areas.

In the smallholders, the majority of the trees (robusta coffee, and arabica as well) were more than 50 years old; many of which also reaching nearly hundred years; which had been existed or planted since the Dutch colonization. Of course, besides those old trees, newly planted trees also exist but the percentage might be quite low.

But so far, there is no significant problem associated with productivity maintenance; at least from the smallholders point of view. The problems become more significant when the prices of coffee are going up and the smallholders found that they are not ready to exploit the golden opportunity as experienced in the last three or four years. The government and the association of coffee exporter concern with such situation because they could not tap the benefit from the price increases maximally.

Many smallholders especially who cultivate on good farm lands continue to maintain the farm productivity, but this was only during the last decade. The practice which adopted by the smallholders in Lampung Province is simple grafting. The branch which has only a little beans and clusters (unproductive branches) would be replaced through the grafting. First, they go for highly productive branches than bend them to the earth. After some time, when the branch start budding it would be utilized as the upper part or *entrees* in the grafting. The lower shoot would be the bud of unproductive branches. All unproductive branches would be gradually replaced more with other productive ones.

Another problem at farm level is the coffee fruit borer, *Hypothenemus humpei*, which frequently degrades the quality of the beans. In fact, one criteria of the quality of coffee production is the percentage of how much damages has fruit borer caused to the beans. The local agency of estate crop suggests to utilize fungi *beauvaria bassina* as the control of borer but the reproduction of this borer parasite become one of the constraints.

Also another problem is associated with absentism. While many hardworking coffee smallholders put a lot of efforts to improve and enhance productivity, a considerable part of the coffee farms have not been taken care properly by the owners. The farms were just left to some sharecroppers who have little incentive to improve the productivity. Or, the owners would visit the orchard very occasionally such as during harvest time and leave the coffee improperly cultivated. Unfortunately, the absentee-landowners who are rich farmers or urban rich who have many other sources of incomes also control over quite a large area of unproductive coffee farms.

3.2. The Harvest and Post-Harvest or Product Handling

The appropriateness of the practices during the harvest and post-harvest would significantly lead to the quality of the coffee beans sold to the market. To some extent, this would also be associated with the agronomic practices adopted.

One inappropriate practice is to harvest the coffee fruits when it is not sufficiently and

homogeneously ripe. Because of desire for saving labor hours, many smallholders just pick up the fruits without proper selection. The percentage of ripe fruits may be too low to give a certain desired quality. Also, bored fruits are likely be included in a considerable percentage which deteriorate the quality.

Another important aspect is the practice to remove the fruit flesh from the bean adopted by smallholders. Usually, traditional practice which is considered appropriate is to pound the fresh fruit first, and then sundry the beans. Then, the dried flesh could be easily removed from the beans. In the past, many smallholders sometimes just put the fruits along the busy roads and let the passing cars (and trucks) pound them to remove the flesh from the beans. This practice is very poor and inappropriate, making the quality of the coffee very low. There is no clear empirical evidence why the smallholders adopt such practices. Whether they are lazy or probably they have a thought that adopting appropriate practice would not pay the opportunity costs of their labor hours, it has no explanation.

In Lampung Province particularly, where coffee is one major economic bases to push up the regional incomes and employment, the government has more concern about the practice. The governor himself has encouraged and joined in the campaign to promote a programme which they call **the red harvest**, i.e., requesting all coffee smallholders in the province to adopt the harvesting of ripe fruit. The term **red harvest** has come from the red color of ripe fruits of coffee. The government staffs are requested to visit the smallholders during harvest time for the inspection. They also provide information about the significant roles and benefits of appropriate harvest and post-harvest handling to the smallholders.

3.3. The Marketing System

Many also believe that marketing system is one of the most important handicap to promote productivity. However, little has been known how the marketing systems could provide disincentive to the smallholders. This is because part of the system is not transparent or tighly closed. Also, marketing study is not an easy work in Indonesia because the situation can actually be dictated by a few large traders and or exporters. However, one is sure that the marketing of coffee in the local market as well as for export is a long channel. This can be noted in Diagram 1.

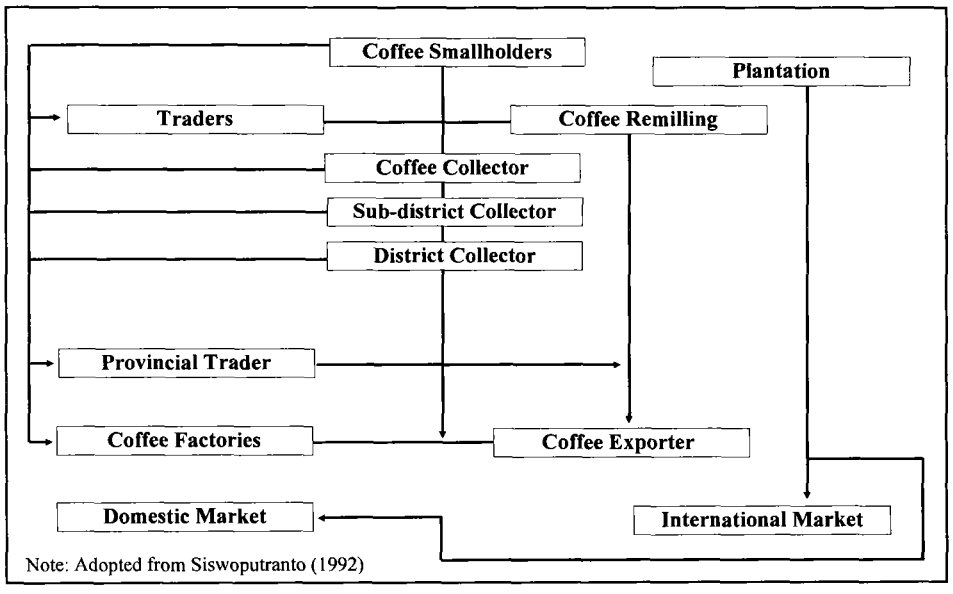
From the diagram, many speculate that the coffee smallholders are under the control of the coffee factories, provincial traders, or exporters. This speculation arise because many smallholders have a financial problem. Most of them, also has a debt and they do not know when they can repay. Unfortunately, the systematic studies about it has not been done yet.

IV. FINAL REMARK; THE PROSPECT

Despite the performance in the productivity is still relatively poor, many people believe that robusta coffee of Indonesia will continue to have the comparative advantage in the world market. This is because of the following considerations:

- The production cost of smallholders' coffee which dominate the farming is very low and the proceed obtained from the sales is not the major source of their incomes.

Diagram 1.
The Marketing System of Coffee in Indonesia



Coffee is the only source of cash incomes in the attempts of farm diversification as to smooth out the flow of cash around the year.

- In such situation, the present level of productivity would be considered sufficient and the smallholders could still survive with the old coffee trees. The trees, as observed in the field, may still productive even if the age is more than a hundred years.

- The existing pest and disease such as leave stain, nematode and fruit borer are found attacking only sporadically. The level of production and the quality of bean may be damaged but they would not spred in very large areas. The adopted farming systems, i.e., the orchard, while as part of crop diversification, may also have performed as an effective integrated pest management.

Thus, in order to provide better incentive to the smallholders to promote production and productivity, improved marketing systems would be the best feasible choice. In other words, agribusiness development of the coffee industry should be encouraged. Amongst the alternatives are to shorten the market channel, improve practices in harvest and post-harvest product handling. In order to improve productivity and income of smallholders, the introducing of high yielding cultivar is not necessary an obstacle anymore, if all those alternatives are done. This would need the strengthening and provision of infra-structures and facilities, including the farmers' cooperative associations, financial institutions, and extension and information.

Table 1.
Planted Area, Production and Productivity of Coffee
in Indonesia; By Type of Holdings (1969–1993)

Year	PLANTED AREA							PRODUCTION							PRODUCTIVITY (Kg/Ha)	
	Total	Smallholder		State Plantation		Private Plantation		Total	Smallholder		State Plantation		Private Plantation			
	(000 Ha)	(000 Ha)	(%)	(000 Ha)	(%)	(000 Ha)	(%)	(000 Ha)	(000 Ha)	(%)	(000 Ha)	(%)	(000 Ha)	(%)	Small	LSP
1969	379.6	337.1	88.8	20.1	5.3	22.4	5.9	186.4	173.6	92.6	7.8	4.5	5.0	2.9	447.0	307.0
1970	394.8	351.0	88.9	20.5	5.2	23.3	5.9	200.1	185.1	91.9	8.9	4.8	6.1	3.3	484.0	349.0
1971	407.7	366.1	89.8	20.8	5.1	20.8	5.1	199.9	180.9	89.5	10.9	6.0	8.1	4.5	442.0	450.0
1972	405.1	356.9	88.1	29.2	7.2	19.0	4.7	199.4	178.7	88.4	12.3	6.9	8.4	4.7	443.0	430.0
1973	381.2	340.4	89.3	21.0	5.5	19.8	5.2	160.2	150.2	93.4	5.6	3.7	4.4	2.9	412.0	242.0
1974	386.6	346.8	89.7	20.5	5.3	19.3	5.0	167.2	149.8	88.4	10.2	6.8	7.2	4.8	382.0	434.0
1975	399.9	361.5	90.4	20.0	5.0	18.4	4.6	185.4	170.4	91.2	9.5	5.6	5.5	3.2	430.0	385.0
1976	440.0	402.2	91.4	19.8	4.5	18.0	4.1	208.9	193.4	92.0	9.9	5.1	5.6	2.9	443.0	401.0
1977	497.8	454.5	91.3	20.4	4.1	22.9	4.6	210.1	194.0	91.7	10.3	5.3	5.8	3.0	513.0	559.0
1978	520.6	477.4	91.7	21.9	4.2	21.3	4.1	239.8	222.7	92.3	9.8	4.4	7.3	3.3	574.0	536.0
1979	624.1	577.9	92.6	20.6	3.3	25.6	4.1	290.7	273.7	93.8	11.5	4.2	5.5	2.0	621.0	526.0
1980	707.4	663.6	93.8	21.2	3.0	22.6	3.2	314.1	294.9	93.6	13.3	4.5	5.9	2.0	616.0	677.0
1981	796.8	749.8	94.1	23.1	2.9	23.9	3.0	339.5	314.9	92.2	16.1	5.1	8.5	2.7	588.0	726.0
1982	803.1	758.9	94.5	24.1	3.0	20.1	2.5	300.4	281.3	93.2	13.2	4.7	5.9	2.1	524.0	599.0
1983	814.9	766.1	94.0	24.4	3.0	24.4	3.0	324.0	305.6	94.0	10.1	3.3	8.3	2.7	562.0	501.0
1984	894.3	837.9	93.7	22.4	2.5	34.0	3.8	339.8	315.5	92.3	14.8	4.7	9.5	3.0	513.0	759.0
1985	931.1	874.3	93.9	23.3	2.5	33.5	3.6	334.5	311.4	92.6	12.8	4.1	10.3	3.3	484.0	589.0
1986	935.1	888.4	95.0	24.3	2.6	22.4	2.4	383.9	356.8	92.4	17.8	5.0	9.3	2.6	538.0	733.0
1987	961.5	908.7	94.5	24.0	2.5	28.8	3.0	409.7	388.7	94.6	13.2	3.4	7.8	2.0	526.0	543.0
1988	1025.9	969.5	94.5	25.6	2.5	30.8	3.0	420.0	391.1	92.6	16.0	4.1	12.9	3.3	564.0	692.0
1989	1036.6	984.7	95.0	21.8	2.1	30.1	2.9	425.4	401.0	93.9	13.6	3.4	10.8	2.7	566.0	702.0
1990	1070.0	1014.3	94.8	25.7	2.4	30.0	2.8	441.3	412.8	93.1	15.7	3.8	12.8	3.1	558.0	660.0
1991	1118.7	1062.7	94.9	25.8	2.3	30.2	2.7	457.4	428.3	93.2	16.7	3.9	12.4	2.9	529.0	695.0
1992	1143.6	1087.6	95.1	26.3	2.3	29.7	2.6	471.5	440.3	92.9	17.6	4.0	13.6	3.1	537.0	727.0
1993	1160.9	1105.2	95.2	25.5	2.2	30.2	2.6	481.2	449.8	93.0	17.5	3.9	13.9	3.1	542.0	727.0

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Table 2.
The Area of Robusta and Arabica Coffee in Indonesia, 1979–1989 (Ha)

YEAR	ROBUSTA	ARABICA
1979	603584	20640
1985	899001	32128
1987	928368	33272
1989	1008806	33335

Source : CBS and DGEC of MOA

Table 3.
Planted Area and Production of Coffee in Indonesia,
By Province and Type of Holdings, 1989

No	PROVINCE	SMALLHOLDER		STATE-OWNED PLANTATION		PRIVATE PLANTATION		TOTAL			
		Area (Ha)	Production (Ton)	Area (Ha)	Production (Ton)	Area (Ha)	Production (Ton)	Area (Ha)	Area % *0	Production (Ton)	Production % *0
1	D.I. Aceh	59958	43793	6	0			59964	5.86	43793	9.96
2	North Sumatera	66716	43751	43	42			66759	6.52	43793	9.96
3	West Sumatera	21279	9241	64	0	71	12	21414	2.09	9253	2.10
4	Riau	11148	6266					11148	1.09	6266	1.42
5	Jambi	30441	7772					30441	2.97	7772	1.77
6	South Sumatera	239031	79038					239031	23.36	79038	17.97
7	Bengkulu	91887	49939					91887	8.98	49939	11.35
8	Lampung	108372	81749	61	52	128	16	108561	10.61	81817	18.60
9	West Java	22667	5276			73	4	22740	2.22	5280	1.20
10	Central Java	30737	7398	3113	2793	1321	534	35171	3.44	10725	2.44
11	D.I. Yogjakarta	1540	502					1540	0.15	502	0.11
12	East Java	47199	20941	22206	13660	18915	8918	88320	8.63	43519	9.89
13	Bali	31473	9235			511	31	31984	3.13	9266	2.11
14	Nusa Tenggara Barat	5570	1929			369	69	5939	0.58	1998	0.45
15	Nusa Tenggara Timur	38075	9756			195	40	38270	3.74	9796	2.23
16	West Kalimantan	8642	2600					8642	0.84	2600	0.59
17	Central Kalimantan	7607	792					7607	0.74	792	0.18
18	South Kalimantan	8289	1872					8289	0.81	1872	0.43
19	East Kalimantan	8233	1937					8233	0.80	1937	0.44
20	North Sulawesi	4100	2200					4100	0.40	2200	0.50
21	Central Sulawesi	12119	2251					12119	1.18	2251	0.51
22	South Sulawesi	46090	11299			519	368	46609	4.55	11667	2.65
23	South-East Sulawesi	12093	2961					12093	1.18	2961	0.67
24	Maluku	4782	676			10	9	4792	0.47	685	0.16
25	Irian Jaya	966	145					966	0.09	145	0.03
26	D.K.I. Jakarta							0	0.00	0	0.00
27	Timor Timur	49037	9456			7664	533	56701	5.54	9989	2.27
INDONESIA		968051	412775	25493	16547	29776	10534	1023320	100.00	439856	100.00

Source : Directorate General of Crops, Ministry of Agriculture and Central Bereau of Statistics *) of Indonesia

Figure 1.
Coffee Planted (000 Ha) in Indonesia
Smallholder and Plantation, 1969 - 1993

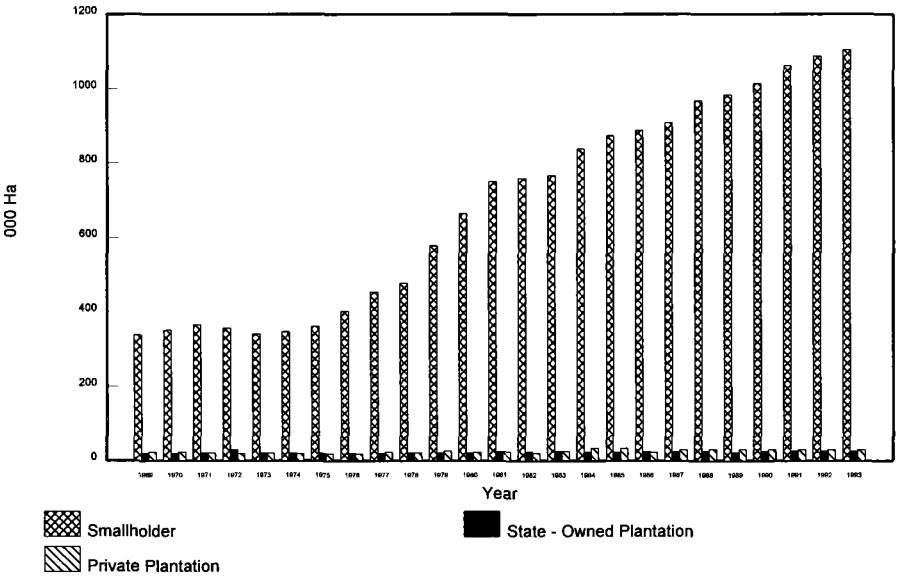


Figure 2.
Coffee Productions (000 Ton) in Indonesia,
Smallholder and Plantation, 1969 - 1993

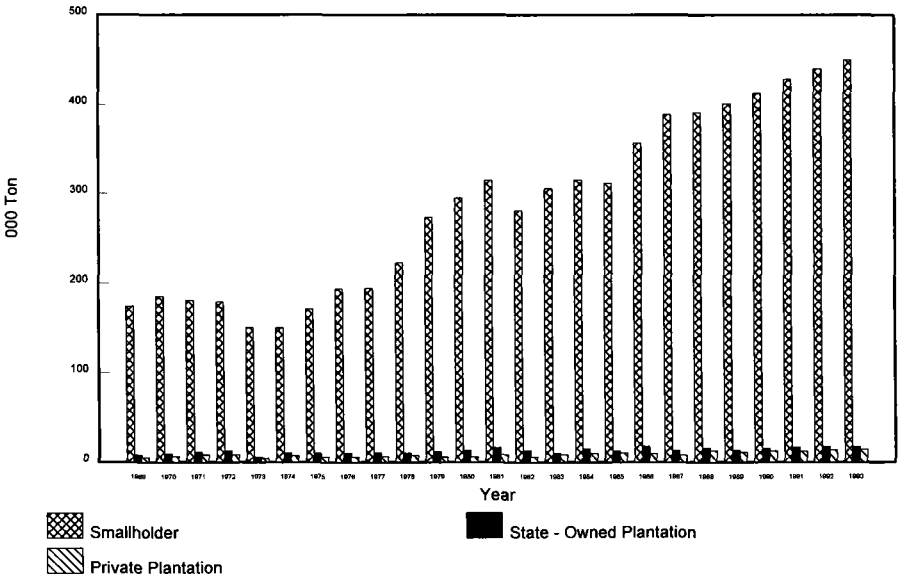


Figure 3.

Coffee Productivity (Kg/Ha) in Indonesia,
Smallholder and Plantation, 1969 - 1993

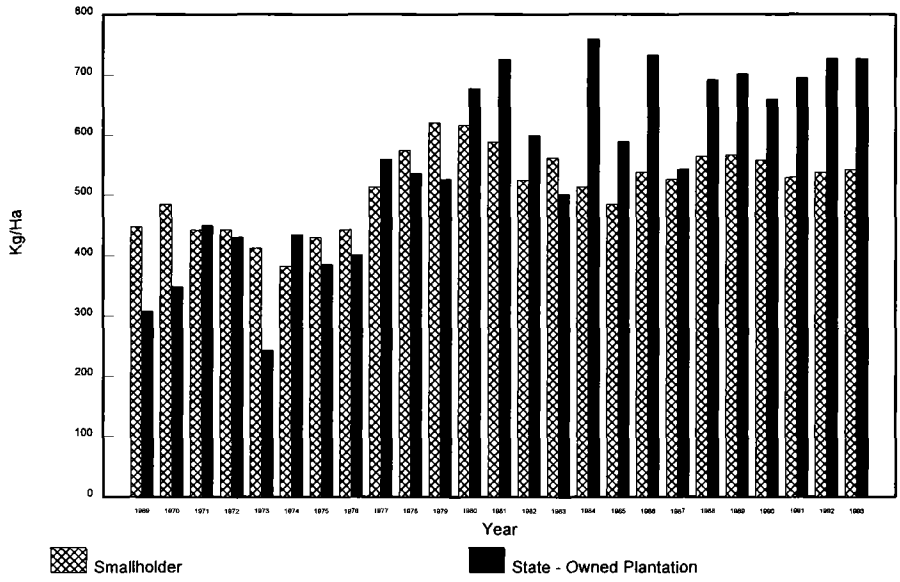


Figure 4.

Changes in Planted Area of Coffee (%/Yr) in Indonesia,
Smallholder and Plantation, 1969-1993

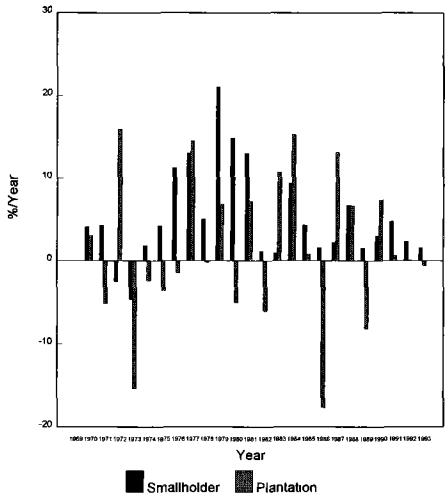


Figure 5.

Changes of Coffee Production (%/Yr) Indonesia,
Smallholder and Plantation, 1969-1993

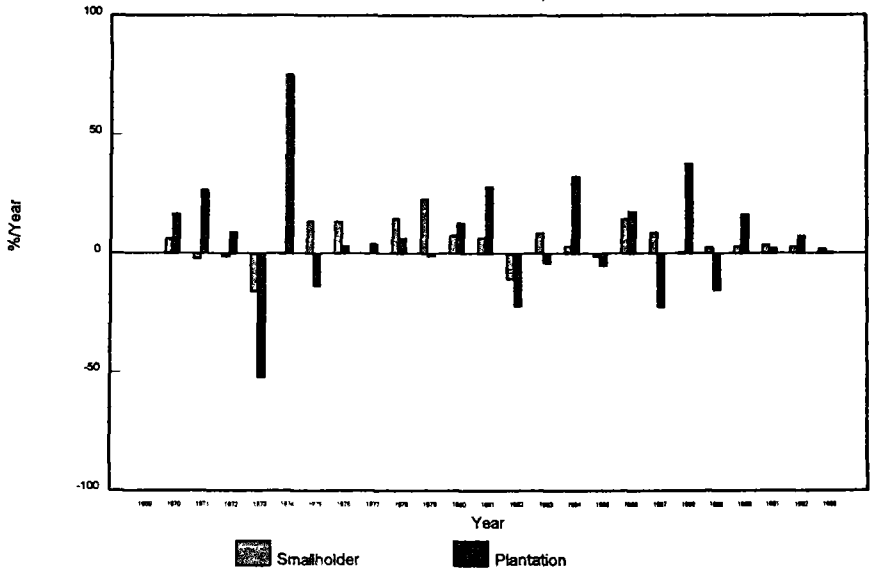
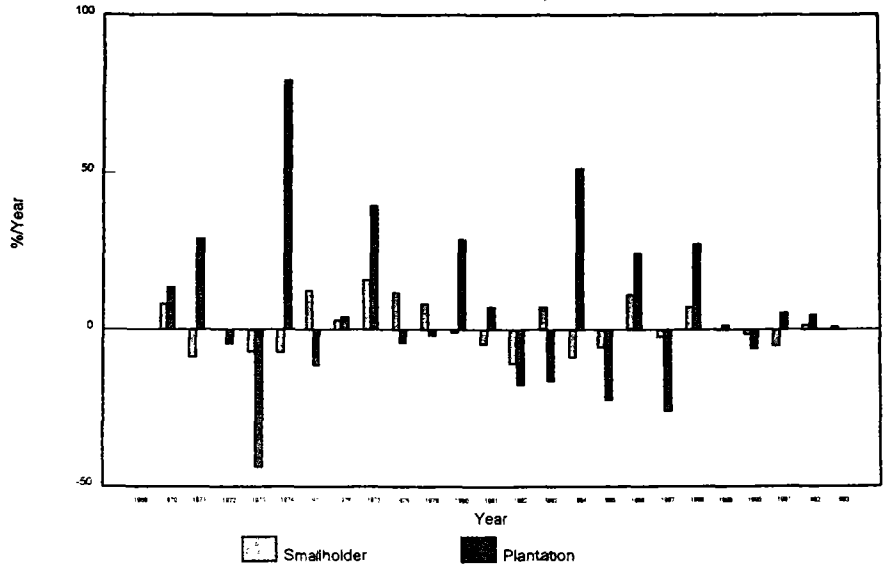


Figure 6.

Changes in Yield of Coffee (%/Yr) in Indonesia,
Smallholder and Plantation, 1969-1993



PRODUCTION ET TRANSFORMATION DE CAFÉ AU VIÊT-NAM : CONTRAINTES ET TRANSFERTS DE TECHNOLOGIES

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1. Introduction

Le Viêt-nam est un pays en pleine mutation politique, économique et sociale depuis l'adoption en décembre 1986 par le Parti Communiste Vietnamien d'une résolution visant la rénovation de l'économie (doi moi).

La politique de réforme menée depuis cette date vise aussi le secteur agricole. Elle inclut un certain retour aux exploitations familiales ainsi qu'une libération des prix. Les grandes fermes d'Etat non rentables sont progressivement démantelées. Depuis ces réformes, la production agricole a cru en moyenne de plus de 5 % par an.

C'est dans ce contexte que le Viêt-nam a connu un accroissement remarquable de sa production caféière. Il est devenu le 6ème exportateur mondial en café robusta et l'objectif de production pour l'horizon 2000 est de 200 000 tonnes par année.

Des opportunités s'offrent aux opérateurs étrangers de consolider la filière café et d'accroître la valeur ajoutée locale par le biais d'accords de coopération qui prennent le plus souvent la forme de "joint-ventures".

Le but de notre communication est de présenter l'expérience que nous avons initiée dans la province de Dong Nai, deuxième province caféière du Viêt-nam, afin d'améliorer la compétitivité et l'efficience de cette importante filière-produit.

2. Aperçu de l'économie vietnamienne

En 1994 le Viêt-nam se place parmi les pays les plus pauvres avec un PNB estimé à environ 214 USD/hab.. Néanmoins, depuis le début des années '90, le PNB croit à un rythme soutenu d'une moyenne annuelle de 7,8 %. L'objectif du gouvernement est de doubler le PNB/hab. d'ici à la fin du siècle pour atteindre 400 USD/hab.

Le Viêt-nam est un pays agricole en voie d'industrialisation, comme l'illustre la structure de son PIB. Le secteur agricole domine, assurant 27,7 % du PIB et employant plus de 60 % de la population active.
Depuis 1990 on assiste à une croissance sensible du secteur industriel et de la construction, dont les parts sont respectivement de 22 % et de 7,6 % en 1994. Le secteur tertiaire contribue pour environ 38 % à la formation du PIB.

Les échanges commerciaux du Viêt-nam se concentrent surtout en Asie, preuve d'une bonne intégration régionale, et les principaux partenaires sont Singapour, le Japon et Hong Kong. La balance commerciale est déficitaire, mais le déficit courant est faible, se situant entre 6-7 % du PIB.

Le Viêt-nam exporte principalement du pétrole brut, du charbon, du riz et du café, des produits de pêche, du textile et de la confection. Les importations sont dominées par des produits pétroliers, des biens de construction et d'équipement.

La dette extérieure du Viêt-nam s'est élevée à 17,7 milliards de USD en 1993 avec un ratio du service de la dette (en % des exportations) de 28,5 %. Ce ratio est estimé à 12 % pour l'année 1994.

Le taux d'inflation a été considérablement réduit au cours des dix dernières années, il était de 5,2 % en 1993 et de 9 % en 1994.

Le Viêt-nam est aujourd'hui bien avancé dans la phase de transition passant d'une économie planifié vers une économie de marché. Suite à l'apurement des arriérés auprès du FMI en octobre 1993, le pays a de nouveau accès aux prêts des principales organisations internationales. En vue de renforcer le rôle du secteur privé, le Viêt-nam s'est également doté d'une loi sur les investissements étrangers qui est parmi les plus libérales de la région.

3. La production caféière vietnamienne

3.1. Historique

Le café est cultivé au Viêt-nam depuis près de deux siècles. Les premières grandes plantations avaient été créées par les colonisateurs français au début de ce siècle.

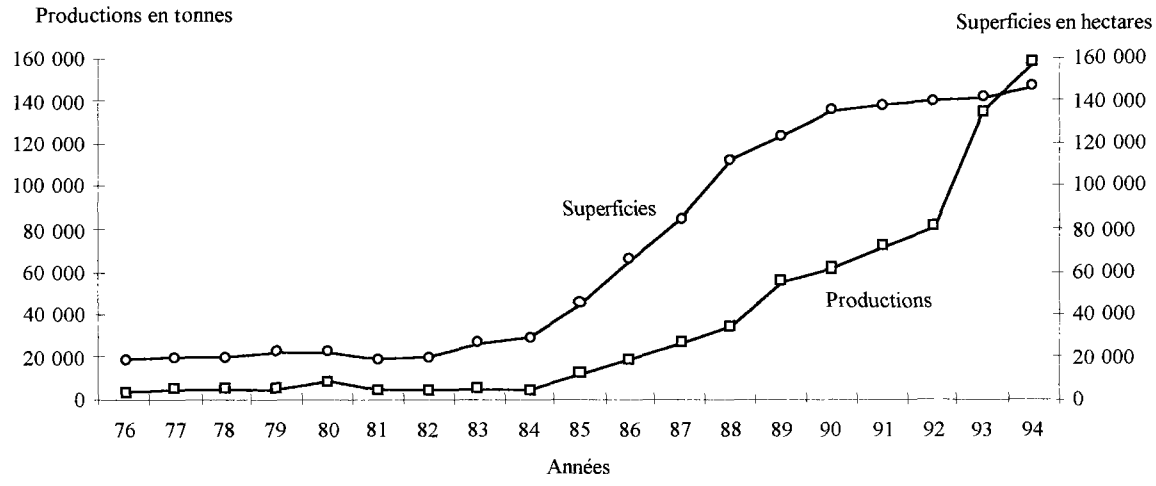
Après la réunification du Viêt-nam en 1976, la culture de café a été collectivisée dans un premier temps en coopératives et grandes fermes d'Etat. Avec la réforme du secteur agricole intervenue en 1986, la caféiculture est partiellement cédée au secteur privée. Aujourd'hui elle est conduite à 60 % par des petites exploitations familiales cultivant en moyenne 0,5 à 1 ha de café et à 40 % par les grandes fermes d'Etat d'une superficie moyenne de 800 à 1 000 ha.

3.2. Production

La grande majorité du café planté au Viêt-nam est le caféier Robusta (Coffee Canephora P.). En 1993 la production d'arabica (espèce Coffea arabica L.) couvre environ 2 % de la production, soit 2 000 ha dans la province de Dak Lak. L'évolution des surfaces plantées est présenté dans la figure ci-après.

Depuis 1976, les surfaces caféières vietnamiennes ont pratiquement décuplé, passant de 18 850 ha en 1976 à environ 147 000 ha en 1994. L'objectif du gouvernement vietnamien est d'atteindre 200 000 ha à la fin du siècle.

Evolution des productions et superficies caféières vietnamiennes



La production présente une croissance exponentielle. Outre l'augmentation des superficies, l'accroissement de la production est également due à l'augmentation significative des rendements qui sont passés de 0,6 tonnes/ha en 1985 à 1,1 tonne en 1993. Au cours de la campagne 93/94, le Viêt-nam a produit environ 158 000 tonnes de café marchand. La récolte 94/95 est estimée à 180 000 tonnes. D'après le Ministère du commerce, la valeur des exportations de café ont représenté pour l'année 1994 environ 100 millions de dollar US.

La grande majorité des plantations ont été réalisées au cours de la période 85-89. L'augmentation des superficies a stagné depuis la campagne 90/91 en raison du contexte de bas prix sur le marché mondial. Depuis l'année 1994, on assiste à nouveau à une extension des superficies caféières.

3.3. Commercialisation

Le café est commercialisé sous la forme de café vert ou café marchand. Depuis 1988, le commerce intérieur a été libéralisé et le secteur privé est habilité à acheter du café aux planteurs. Jusqu'à cette date, cette activité était réservée aux institutions étatiques.

Actuellement deux circuits de commercialisation existent :

- un circuit public : VINACAFE est responsable de l'achat de la totalité de la production des fermes d'Etat, soit en direct soit par l'intermédiaire des compagnies publiques de district et/ou de province.

- un circuit mixte qui couvre le café produit par les petits producteurs qui vendent au plus offrant, soit à des commerçants privés, soit également aux compagnies publiques de district et de province.

Depuis 1991 de très nombreux commerçants privés provinciaux existent. Ceux-ci réalisent un triage grossier du café marchand pour éliminer les principales impuretés et font l'ensachage. Certains commerçants privés sont en relation directe avec ceux de Ho Chi Minh Ville qui leur accordent des avances pour financer l'achat de café. Ces commerçants provinciaux n'exportent pas le café mais le revendent soit à des commerçants de Ho Chi Minh Ville, soit à VINACAFE, soit à des compagnies provinciales.

Les compagnies publiques provinciales ont généralement des activités d'import-export de produits agricoles et d'intrants agricoles (essentiellement des engrais). Elles possèdent une licence d'import-export et disposent d'une certaine expérience du marché international.

Il n'existe pas de système de fixation de prix au producteur. Ce prix dépend des prix à l'exportation et donc du marché mondial, des taux de change et des coûts de commercialisation. Il dépend également de l'offre et de la demande locale ainsi que de la période de l'année.

Le "Comité d'Etat pour les prix et la finance" est responsable de la collecte de données statistiques sur l'évolution des prix et de la publication de ces informations par les média (radio, télévision, journaux).

Depuis la flambée des prix en 1994, un projet de caisse de stabilisation est en cours d'élaboration.

Le gouvernement a adopté un décret, en juillet 1994, créant un fond de stabilisation. Les exportateurs payent un montant de 200 USD par tonne de café exporté. Ces montants alimentent un fond de stabilisation qui devrait soutenir les prix sur le marché intérieur, lorsqu'ils descendent sous le prix de 20 000 VND/kg (1,82 USD/kg).

3.4. Exportation

Contrairement à d'autres produits d'exportation vietnamien, les exportations de café ont toujours été davantage dirigées vers les pays non socialistes, et cette proportion s'est encore accrue au cours des années 90.

En 1991, 80 % des exportations allaient vers Singapour pour y être retraitées et réexportées.

La France, l'Autriche, l'Algérie, l'Australie et Hong Kong et plus récemment l'Allemagne constituent un groupe de clients stables qui importent en moyenne 2 500 tonnes par an. Parmi les nouvelles destinations, on peut citer l'Italie, le Japon, Taiwan, la Belgique et l'Angleterre.

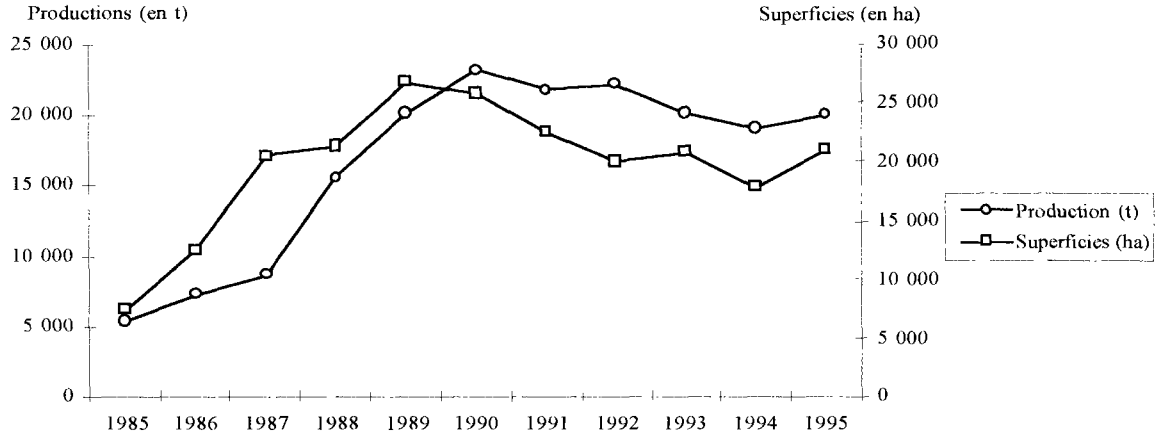
La valeur des exportations vietnamiennes de café (Ministère du Commerce) a évolué comme suit :

Année	Valeur en mio USD
1985	9,75
1990	49,20
1992	91,40
1993	85,00
1994	97,50

4. La caféiculture dans la province de Dong Nai

La culture de café dans la province de Dong Nai est relativement récente. Elle a débuté après la libération de 1975, ce qui explique la relative jeunesse du verger. En outre, la culture du café est exclusivement pratiquée par des petits producteurs dans cette province. L'évolution de la production de café de la province depuis 1985 est donnée dans la figure et le tableau ci-après :

Evolution des productions et superficies caféières dans la province de Dong Nai



Année	Production (t)	Superficies (ha)
1985	5 397	7 533
1986	7 328	12 602
1987	8 703	20 476
1988	15 650	21 327
1989	20 112	26 816
1990	23 180	25 886
1991	21 797	22 531
1992	22 270	20 094
1993	20 120	20 900
1994	19 000	17 900
1995	20 000	21 000

Source : Dong Nai Province

Suite à la réforme administrative de 1991, une partie du territoire de la province de Dong Nai a été transférée à celle de Vung Tau. Ce changement administratif a entraîné une diminution fictive des superficies emblavées de café de l'ordre de 3 000 ha.

Les producteurs sont tous des exploitants familiaux qui supportent la totalité des charges de l'exploitation (foncier, plantation, entretien de la caféière, engrais, irrigation, récolte et fonds de roulement). Dans un tel environnement, la flexibilité des producteurs est grande. Les années répétées de bas prix du café ont entraîné un arrachage important de caféiers dans la province au début des années 90.

A l'inverse, avec la reprise des cours, des surfaces importantes ont à nouveau été plantées. Elles ont été estimées par les services techniques de la province de Dong Nai à plus ou moins 4 000 ha qui entreront en production dans 3 ou 4 ans. Les extensions se poursuivent actuellement. Nous avons pu observer l'existence de pépinières (sélection massale) dans les caféières afin d'autoriser les extensions.

L'utilisation des engrais est généralisée au niveau des producteurs. Les recommandations des centres de recherche vietnamiens sont les suivantes :

- 200 kg N;
- 150 kg P205;
- 250 kg K20.

Ces quantités d'engrais visent à obtenir des rendements de l'ordre de 1 250 kg de café marchand avec une variation de 20 %. Les coûts des engrais sont de 10 USD le sac de 50 kg de produit simple ou 15 USD le sac d'engrais composé N.P.K.

L'irrigation est généralisée à l'aide de petites pompes achetées ou louées. Hormis dans les grandes plantations, l'irrigation se fait par remplissage d'eau dans les fosses de plantation des caféiers, plus ou moins 200 litres d'eau par passage et par fosse, un passage d'eau tous les 10 jours en période sèche, durant 3 à 4 mois. L'effet de l'eau est évidemment immédiat. Il permet aux producteurs de moduler leur récolte et, associé aux engrais, il représente un élément majeur dans la productivité de la caféière vietnamienne.

La récolte se fait usuellement en quatre passages décidés en fonction de la maturité des fruits. En fait les trois premiers passages ne visent que la cueillette des fruits arrivés à parfaite maturité. Le quatrième et dernier passage consiste à enlever la totalité des fruits, pour clôturer la campagne de récolte et éviter les nids à parasites sur les cerises qui arriveraient à maturité par la suite. La totalité des fruits tombés au sol sont enlevés.

5. Méthodes de transformation du café en milieu paysan

Le traitement du café au niveau de la plantation se fait exclusivement par voie sèche.

Sur les plantations les plus petites, elle se résume à la cueillette et au séchage des cerises sur aires bétonnées ou même le long des routes, sur le goudron. Le café séché est décortiqué à l'aide de décortiqueurs à main ou de petits décortiqueurs montés sur les moteurs polyvalents qui équipent les motoculteurs largement diffusés dans le monde rural vietnamien, puis vanné et trié à la main.

Dans les exploitations qui possèdent un motoculteur équipé d'un moteur polyvalent, on observera la présence de petits dépulpeurs très simples. Ceux-ci permettent de casser l'exocarpe de la cerise sans dépulper réellement le café, qui est alors mis à sécher. La fracture de l'exocarpe réduit largement le temps de séchage, et facilite ultérieurement le travail de la décortiqueuse actionnée par le moteur du motoculteur.

La contrainte majeure du secteur caféier vietnamien réside dans la mauvaise qualité des lots à l'exportation.

Le séchage du café se fait à même le sol et les lots sont automatiquement souillés de terre et de pierres. Le taux d'humidité des cafés commercialisés reste le plus souvent élevé. Il est estimé à 15-16 %. Seul un vannage et un triage manuel extrêmement rudimentaire sont réalisés à la ferme avant commercialisation.
Peu d'opérateurs économiques publics ou privés intervenant dans la filière en aval des producteurs ne procèdent à un usinage ou retraitement du café.

En conséquence, les lots à l'export sont très hétérogènes quant à la taille et la granulométrie des grains.

Les lots comportent également une part significative d'impuretés (pierres, poussières, etc.) et de déchets (coques, brisures, grains défectueux etc.).

6. Transfert de technologie : retraitement du café

Le retraitement consiste en un ensemble d'opérations mécaniques de cafés décortiqués, tels qu'ils sont commercialisés au Viêt-nam, fournissant des lots de café de calibre homogène, dépourvu d'impuretés. Le retraitement est une opération indispensable dans le cadre d'une politique de café de qualité à l'exportation.

Le procédé comprend principalement les 4 étapes suivantes : prénettoyage - séchage - calibrage - triage.

Le prénettoyage permet d'éliminer les corps étrangers et grosses impuretés, notamment les objets métalliques. Ce nettoyage est complété d'un épierrage. Le machines les plus utilisées dérivent du brevet Egelberg.

Le calibrage sert à classifier le café en fonction de la granulométrie. Il permet d'éliminer le reste des impuretés fines et grossières. Il se fait généralement à l'aide de tamis plans oscillant. Le calibrage du café est indispensable pour procéder ultérieurement à un triage efficace. Ce dernier est nettement amélioré sur des fèves de même granulométrie.

Le triage éléctronique consiste en un processus de tri par couleur en vue d'éliminer les grains défectueux : noirs, blanchis, décolorés ou altérés.

La valeur ajoutée induite par ce process justifie pleinement les investissements à consentir. Dès lors, des opportunités concrètes de joint ventures apparaissent pour les opérateurs économiques étrangers possédant la technologie et les capitaux nécessaires.

7. Conclusion

La production de café au Viêt-nam est très performante. D'une manière générale les producteurs maîtrisent parfaitement la culture tant au niveau des méthodes culturales que de l'usage des intrants et sont en mesure de fournir du café de très bonne qualité avec de très bon rendements.

D'importantes faiblesses existent en aval de la filière : transformation - usinage - commercialisation. A l'heure actuelle la quasi absence d'usinage constitue la contrainte majeure du secteur. Les lots à l'export sont de mauvaise qualité, les défauts majeurs à éliminer sont les coques, les brisures, les grains noirs et les pierres.

C'est la raison pour laquelle 80 % du café est exporté à Singapour pour y être retraité. Il s'agit là d'une perte importante de valeur ajoutée pour le pays. Les importateurs européens (Madrid, Hamburg, Genève) estiment la moins value de l'ordre de 350 USD par tonne.

Un usinage simple permet de remédier à ce problème. Sur base de nos estimations, les investissements s'élèvent à environ 1 million de USD pour l'usinage de 5 000 tonnes de café vert.

THE PRESENT STATUS OF ROBUSTA COFFEE GROWING IN INDONESIA

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1. Introduction

Talking about Indonesian coffee is almost the same as talking about Robusta coffee. Presently, Indonesia is not only well-known as the third biggest coffee producer, after Brazil and Colombia, but the country is also well-known as the biggest Robusta producer in the world.

Robusta coffee (*Coffea canephora* Pierre var. *Robusta* Cheval.) was introduced to Java in 1900 when Arabica coffee grown in Indonesia was seriously attacked by leaf rust disease. The introduction of the species, of course, was first aimed to solve the disease problem.

At the moment coffee growing areas in Indonesia are predominantly covered by Robusta. This paper is meant to give brief information about the present status of Robusta coffee growing as well as its development, especially during the last five years.

2. Robusta coffee growing areas

By 1993 the total coffee growing areas in Indonesia was estimated 1,161,196 ha. These areas consisted of Robusta, Arabica, and other types (Excelsa and Liberica). However, the areas of each coffee type have not been decribed very well yet.

In Indonesia coffee is cultivated by smallholders, government estates and private estates. The recent development of each holding type is shown in Table 1.

As shown in Table 1, up to 95 per cent of the total coffee area is held by smallholders. The increase of coffee growing area over recent years is also the result of the own expansion by smallholder. This expansion is, of course, very difficult to control. The area of government and private estate had no significant changes over the recent years.

Robusta coffee is grown by farmers in 26 out of 27 provinces in Indonesia. Most of the coffee, however, is produced in Sumatera. The main Robusta producing areas in the island are South Sumatera, Lampung and Bengkulu. In 1993 they produced 42 per cent of the total national production. Other significant Robusta producing province out of Sumatera are East Java, South Sulawesi and East Nusa Tenggara (Figure 1).

Table 1 : Coffee Growing Areas in Indonesia by Type of Holding (1989 - 1993)

Year	Type of Holding			Total (ha)	Change (%)
	Smallholder (ha)	Government Estate (ha)	Private Estate (ha)		
1989	984,234	21,800	30,516	1,036,550	-
1990	1,014,125	25,834	29,889	1,069,848	3.2
1991	1,083,289	25,891	30,674	1,139,854	6.5
1992	1,087,421	25,881	30,364	1,143,666	0.3
1993	1,104,951	25,881	30,364	1,161,196	1.5
% in 1993	95.0	2.3	2.7	100	12.0

Source : Directorate General of Estate, Ministry of Agriculture.

The condition coffee growing in Indonesia is classified as immature crop, productive crop and damage or old crop. In 1993, the percetage of each condition is 20.1, 70.2 and 9.7 respectively (Table 2).

Since 1983 the government has stopped program on Robusta coffee expansion. The program is then stressed on intensification by introducing high yielding clones as well as better farming techniques. However, the introduced technologies have not been adopted by the farmers very well. Consequently, the average national productivity is still low, e.i. 552 kg/ha in 1993. The adaption of technology is apparently highly effected by coffee prize condition. Ussualy, when the prize going down most of farmers don't care their coffee husbundary very well.

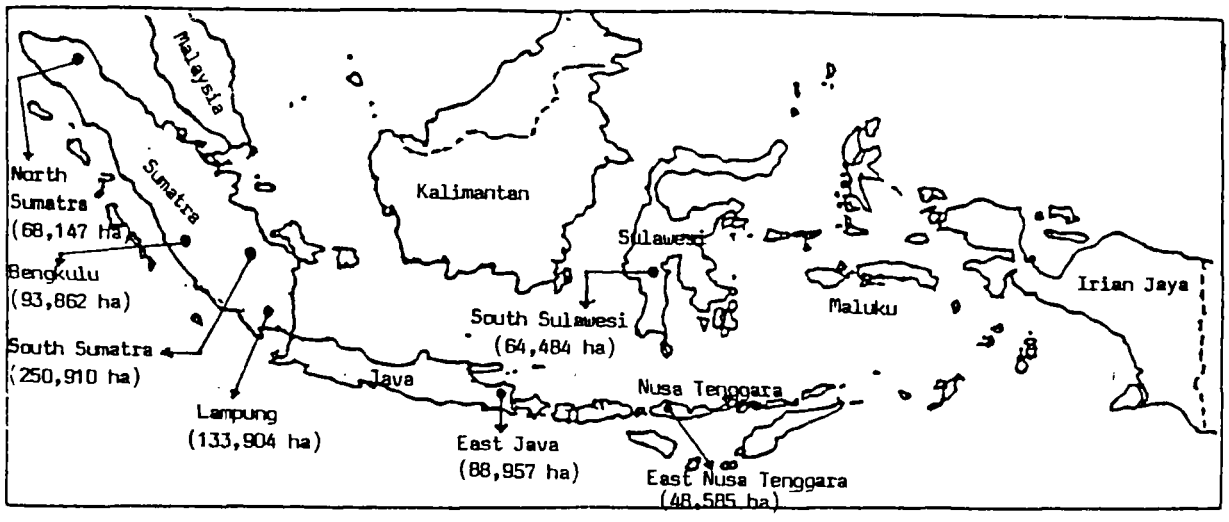

Figure 1. The Main Robusta Producing Areas in Indonesia (1993).

Table 2 : Coffee Growing Areas by Crop condition

Crop conditions	Year			Total 1993	% in 1993
	1991	1992	1993		
Immature crops(ha)					
Smallholders	191,360	218,949	224,843		
Government estates	4,914	2,616	2,616	233,704	20.1
Private estates	7,934	6,245	6,245		
Productive crops(ha)					
Smallholders	753,829	762,412	772,401		
Government estates	20,558	22,674	22,674	815,260	70.2
Private estates	17,832	20,185	20,185		
Damage/Old crops(ha)					
Smallholders	118,100	106,060	107,617		
Government estates	419	591	591	112,142	9.7
Private estates	4,908	3,934	3,934		
Total				1,161,206	

Source : Directorate General of Estate, Ministry of Agriculture.

3. Production and Exportion

During the last five years coffee production in Indonesia increase 5.6 per cent. The increase on Robusta type, however, only 5.0 per cent. By 1993 national coffee production reached 449,799 tonnes, it consisted of Robusta 419,662 tonnes (93 per cent) and Arabica 30,137 tonnes (7 per cent). The development of coffee production by type is presented in Table 3.

Table 3 : Coffee Production by Type (1989 - 1993)

Year	Coffee type		Total (tonnes)	Change (tonnes)
	Arabica (tonnes)	Robusta (tonnes)		
1989	27,209	398,612	425,821	-
1990	29,644	416,347	444,991	4.5
1991	27,840	400,465	428,305	(3.8)
1992	27,300	413,016	440,316	2.8
1993	30,137	419,662	449,799	2.2
% in 1993	6.7	93.3	100	5.6

Source : Directorate General of Estate, Ministry of Agriculture.

Export of coffee bean from Indonesia is, of course, dominated by Robusta type (90.7 per cent). The proportion of export volume by coffee type as shown in Table 4.

Table 4 : Volume of Coffee bean exportation from Indonesia (1992)

Coffee bean	Volume (tonnes)	%
1. Arabica	22,939	9.2
2. Robusta	226,796	90.7
3. Other type	1.1	0.1

4. Quality Improvement of Robusta Coffee Export from Indonesia

By 1983 the Government of Indonesia introduced a new system of coffee bean grading. The government emphasized on the importance of the new system in order to facilitate quality improvement of coffee bean and its marketing. That is to ensure consumers preferences in term of quality as well as to reflect the price to be received by the farmers accordingly.

The new grading system is based on the number of physical defects in the bean. The system is also called "defect system". The kinds of defect and their description had been standardized by the Ministry of Trade. According to the system, coffee beans are classified into six grades, namely grade I,II,III,IV,V, and VI. Grade I and II are mentioned as high quality, grade III and IV as medium quality, whereas grade V and VI as low quality.

By applying the system, the composition of coffee bean export from Indonesia by quality has changed during the last ten years (Table 5). Proportion of low bean quality decreased significantly i.e. 37.9 per cent in 1984/1985 to be 13.7 per cent only in 1993/1994. On the other hand, a significant increase was observed on the proportion of medium quality i.e. 56,4 per cent in 1984/1985 to be 76.9 per cent in 1993/1994. A slight increase was also observed on high quality i.e. from 5.7 per cent in 1984/1985 to be 9.4 per cent in 1993/1994.

Therefore, it can be concluded that the introduction of a new grading system is quite effective to reduce the proportion of low quality export.

Table 5 : Composition of Coffee export from Indonesia by quality (in per cent of total export volume)

Group of qualities	Coffee years		
	1984/1985	1988/1989	1993/1994
High quality (Grade I & II)	5.7	9.6	9.4
Medium quality (Grade III & IV)	56.4	71.8	76.9
Low quality (Grade V & VI)	37.9	18.6	13.7

Source : Ministry of Trade and Association of Indonesian Coffee Exporter.

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Summary

By 1900 Robusta coffee was introduced to Indonesia in order to combat coffee leaf rust. At that moment the disease severely attacked Arabica coffee and significantly reduced yield.

Nowadays the total area of coffee planting in Indonesia has reached 1.1 million ha, and 84 % of which is Robusta coffee. The main Robusta coffee producing areas (42%) are South Sumatera, Lampung, and Bengkulu provinces. Robusta coffee has been also widely grown at high altitude.

Since 1983 the government had stopped the expansion of Robusta coffee, and its development program was emphasized on intensification and quality improvement. Thus, high quality bean export increased from 5.7 % in 1984/1985 to 9.4 % in 1993/1994, whereas low quality bean export decreased from 37.9 % in 1984/1985 to 13.7 % 1993/1994.

A NEW METHOD FOR GREEN COFFEE BEAN PROCESSING AND SOME FLAVOUR CHARACTERISTICS OF THE BEVERAGE

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INTRODUCTION

Among the factors affecting coffee quality, the type of harvesting and green coffee bean processing are also used as main descriptors for exportations from producing countries. The literature about overall aspects in coffee production and trade reports the general concept that the harvesting of mature cherries and the depulping phase in fermentation tanks in the wet method, lead to high acidity and aroma beverage, whereas the stripping method of harvesting and the direct transportation of the beans to the drying places (dry method) induce a beverage with less aroma and less acid, with a characteristic known as hardness. (SMITH, 1985; CLIFFORD, 1985).

A new method for green coffee bean processing - the semi-dried coffee - is now being introduced in Brazil, combining the use of water for washing, selection and depulping of the mature cherries (as in the wet method) with their rapid transportation to the drying places. Still with their adherent mucilage, the selected fraction of mature cherries is laid out in thin layers and continuously turned, the rapid dehydration hindering the occurrence of undesirable fermentations. In this report, some sensory attributes of the beans processed by this method are presented, as well as the results of the same sensory tests after an application of isolated microrganisms, aimed at improving the yield of mature cherries and protecting the coffee fractions against undesirable fermentations, both before harvesting or during drying.

MATERIALS AND METHODS

Samples of semi-dried coffee were obtained from coffee properties in Minas Gerais, Sao Paulo and Parana States. A screen selection was made (flat beans over 17/64 inches) to ensure size homogeneity during roasting and a sample of dry processed beans was used in comparing the processing systems used in Brazil.

A proportion of 100 g / L of roasted and ground coffee was analysed by experienced judges in the coffee trade, using grades from 0 (absence of attribute) to 5 (strongest attribute) for green coffee bean appearance (aspect) and beverage characteristics of acidity, aroma, body and occurrence of fermentation (here understood as rotten, harsh, fermented, chemical, etc.).

APPLICATION OF MICROBIAL ISOLATES

Microbial isolates were applied to hinder the occurrence of undesirable fermentations and to improve the yield of mature cherries, acting either as a "repellent" or as a physical barrier over the coffee cherry surface. Some aspects were taken into account in selecting the isolated microorganisms, for example:

1) their common use in food preparations, especially in oriental cooking;
2) their classification as G.R.A.S. (Generally Recognized As Safe) for food preparations by the F.D.A. - USA ; (DEMAIN & SOLOMON, 1985) and
3) the impossibility of causing any damage to the coffee cherry surface.

Three isolates were obtained from Hayashibara Biochemicals, Okayama, Japan - identified as FQ/A 1, FQ/A2 and FQ/T - and prepared in Brazil to be sprayed over the coffee cherries still on the plants. Normally, three concentrations (6 kg/ha, 4 kg/ha and 3 kg/ha) were tested, and applied before harvesting. In one plantation, three intervals between applications and the moment of maximum cherries ripeness (30, 60 and 90 days) were also studied. One sample of dry processed coffee was used for comparison between the dry and semi-dried processing methods.

RESULTS AND DISCUSSION

Table 1 lists some coffee properties using the semi-dried method, their geographical conditions, main cultivated arabica variety and the percentage of beans originating from the second blossoming (normally the most significant in Brazil). Some factors affecting coffee quality were also selected, such as the use of organic fertilization, partial shade and less-cultivated varieties.

Table 2 shows the sensory characteristics of two selected properties, displaying the effects on the sensory properties when the matures cherries were not isolated and when the semi-dried coffee processing method was used. A comparison with the results of the same tests made on samples from a farm with very suitable geographical condition for quality - the Cerrado Coffee - and therefore using the dry method - is also shown. It can be seen that the immature and over-ripe cherries normally induce a note of unpleasant flavour (fermented taste and/or aroma), under some climatic conditions

when these fractions are not removed. Depending on the coffee varieties cultivated - and the variable point of maximum ripeness among them - the influence of the immature or the over-ripe fractions will prevail.

Table 3 shows the results of the sensory attributes of the beverage from selected coffee properties using the semi-dried coffee processing method. It is interesting to note that a strong relationship was found between low altitudes + high latitudes and high altitudes + low latitudes (properties 8 and 7 as against property 9 - dry processed coffee) with respect to sensory characteristics. Normally, the results for acidity are low, whereas the body values are a little higher and the aroma grades can be considered very satisfactory. The low value for aspect in property 1 must be due to high moisture content during storage.

Table 4 shows the results of the application of microbial isolates on the beverage characteristics in two selected properties. In Prop. 3 there was an increase in grades for aroma, acidity and aspect and a decrease in body grades with all the products. In Prop. 9 applications of product FQ/A 1 cannot be recommended, nor products FQ/A 2 and FQ/T in higher concentrations, due the unpleasant flavours associated with fermentations. These two products showed antagonic behaviours in relation to acidity and aroma grades; nevertheless, the increase in body and aspect grades was remarkable.

CONCLUSIONS

The adoption of the semi-dried coffee processing method offers many advantages to coffee farmers, especially where the climatic conditions are not so naturally suited to coffee quality. It was observed that many of the green bean fractions showed prevailing characteristics, such as harsh, rotten, fermented, "stinker" etc. in the beverages where the immature and over-ripe cherries were not separated. The semi-dried coffee method can recover the original desirable characteristics of the mature cherries, offering brazilian coffee of high quality with distinctive characteristics when compared with the milds coffees and other dru-processed coffees.

Due to the phenological behaviour of the arabica plants in Brazil, there is a natural desire amongst coffee farmers to increase the yield of the mature cherries fraction. There are some methods aimed at attaining this, like closed spacings, different varieties, partial shade, etc.. One of these methods involves prolongating the mature cherry stage on the tree and their protection against natural fermentations and consequent formation of "off-flavours". The application of isolated microrganisms, under certain concentrations, was not only successful in these aspects but even modified some of the characteristics of the beverage.

There are some hypotheses about the role of microbial products as biocontrol agents in fungal processes, some of them refering to the formation of antibiotics or the simple physical occupation of the vegetable surface (DAMAIN & SOLOMON, 1985). In any case, the improvement of the quality in brazilian arabica coffee batches can assist the change of established concepts.

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Table 1 - Coffee properties : Geographical conditions, main cultivated variety and percentage of main blossoming

Prop. 1 - Ouro Fino (MG) - Faz. Ouro Verde - Lat.: 22º17'S Long.: 40º27'W - Alt : 1.000 m - Var.: Mundo Novo (40%)

Prop. 2 - S. Gonçalo Sapucaí (MG) - Sítio Leblon - Lat : 21º54'S Long : 45º36'W - Alt. 900 m - Var.: Mundo Novo (40%) - Organic fertilization

Prop. 3 - Carmo Rio Claro (MG) - Faz. Vitoria - Lat .20º58' S Long.: 46º07'W - Alt.. 760 m - Var.: Catuaí (30%) - also partial shade on Mundo Novo Acaia variety

Prop. 4 - Alfenas (MG) - Faz Espigão - Lat.: 21º21'S Long.: 45º54'W - Alt : 874 m - Var.: Mundo Novo (40%)

Prop. 5 - Nova Resende (MG) - Sítio Cafundó - Lat.: 21º10'S Long.. 40º27'W - Alt.: 1.000 m - Var.: Bourbon (30%)

Prop. 6 - Mocóca (SF) - Faz. Itaçoé - Lat.: 21º18'S Long 45º49'W - Alt : 850 m - Var.: Catuai (30%)

Prop. 7 - Ribeirão Claro (PR) - Faz. Jamaica - Lat.. 23º10'S Long.: 49º50'W - Alt.: 700 m - Var.: Catuaí (30%)

Prop 8 - Tomasina (PR) - Faz Jaboticabal - Lat 23º45'S Long : 50º00'W - Alt. 570 m - Var.: Catuaí (30%)

Prop. 9 - Carmo do Paranaíba (MG) - Faz. Paraiso - Lat.: 19º00'S Long.. 46º18'W - Alt 1.100 m - Var.: Mundo Novo (50%) - dry-processed coffee cherries

TABLE 2 - Influence of the stage of green coffee bean maturity on the sensory characteristics of the beverage. Prop. . 1, 3 and 9 (See Table 1)

		AROMA	BODY	ACIDITY	ASPECT	FERMENT
PROP 1	M + IM	3.5	3.5	2.0	1.5	2.0
	M	3.5	3.0	2.0	1.5	0.0
	M + O.R	4.0	3.5	1.0	2.0	2.0
PROP 3	DRY-PROC	2.0	4.0	4.0	3.0	1.0
	CATUAI M	3.0	4.0	3.0	3.0	0.0
	CATUAI O.R	2.0	3.0	3.0	2.0	2.0
	ACAIA M	4.0	4.0	3.0	4.0	0.0
	ACAIA O.R.	3.0	3.0	3.0	4.0	3.0
PROP 9	M. NOVO	4.0	3.5	2.0	3.0	0.0

CODES: M = Mature cherries
IM = Immature cherries
O.R.= Over ripe cherries

TABLE 3 - Sensory characteristics of semi-dried coffee beverages, compared to those of the dry method (Prop 9), geographical conditions, main cultivated varieties and other cultural practices shown in Table 1

	AROMA	BODY	ACIDITY	ASPECT	FERMENT.
Prop. 1	3.5	3.0	2.0	1.5	0.0
Prop. 2	4.5	4.0	3.0	4.0	0.0
Prop. 3	3.0	4.0	3.0	3.0	0.0
Prop. 4	3.0	2.5	4.0	3.5	0.0
Prop. 5	4.0	3.5	2.0	3.0	0.0
Prop. 6	4.0	3.5	2.0	3.0	0.0
Prop. 7	4.0	4.0	3.0	3.0	0.0
Prop. 8	5.0	4.0	3.0	4.0	0.0
Prop. 9	4.0	3.5	2.0	3.0	0.0

Table 4 - Sensory aspects of arabica coffee beverages after application of isolated microorganisms at different concentrations in properties 3 and 9 (See Table 1)

			AROMA	BODY	ACIDITY	ASPECT	FERMENT.
Prop. 3	FO/A 1	4 kg/ha	5.0	3.0	5.0	4.0	0.0
	FO/A 2	4 kg/ha	5.0	3.0	5.0	4.0	0.0
	FO/T	4 kg/ha	5.0	3.0	5.0	4.0	0.0
Prop. 9	FO/A 1	6 kg/ha	5.0	3.0	3.5	3.5	2.5
	FO/A 2	6 kg/ha	3.5	3.5	3.5	2.0	1.0
		4 kg/ha	3.5	5.0	2.5	4.0	0.0
		3 kg/ha	3.5	4.5	1.0	4.0	0.0
	FO/T	6 kg/ha	3.0	3.5	3.0	3.0	1.0
		4 kg/ha	4.5	5.0	1.0	4.0	0.0

RESUME

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A new method for green coffee beans processing and some flavour characteristics of the beverage.

In this study are shown the physical aspect of the green coffee beans and the sensory characteristics of the beverage of samples obtained from several coffee properties in Brazil, using a new method for green coffee bean processing - the semi-dried coffee.

By this method, the selected fraction of mature cherries shows different grades of aroma, acidity, body and aspect according to the geographical and climatic conditions, system of fertilization, cultivated variety and other cultural practices. Where these conditions are so suited for quality, even the dry method can be utilized, with very distinctive results.

Due to the natural desire among coffee farmers to increase the yield of mature cherries, it was also studied the application of microbial isolates products - designed as FQ/A 1, FQ/A 2 and FQ/T - on the coffee cherries still in the plants. Acting as a "repellent" or as a physical barrier against formation of "off-flavours", the applications in certain concentrations were not only successful but even modifed some of the characteristics of the beverage.

FERTILIZER USE IN COFFEE FARMING IN INDONESIA, PRESENT AND FUTURE. A case study in Java

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1. Introduction

Coffee growing areas in Indonesia are spread out mainly in Sumatera, Java, Nusa Tenggara, Sulawesi and Irian Jaya. The dominant soils in those areas are volcanic origin and have undergone a varying degree of weathering. The soil types are young volcanic ash; juvenile, brown and reddish brown lateritic clay from sedimentary materials; young, gray, alluvial; and black, humic mountain soils.

Due to the large area and high variability in their soil, climate, variety, age, as well as the cultivation method, the kind and doses of fertilizer used are also varied.

During last eight years there was a tendency of decreasing for inorganic fertilizer use. The cause of the change in fertilizer use possibly were the increasing inorganic fertilizer price, increasing wage, decreasing coffee price, and the increasing consumer's demand on natural or organic coffee.

This paper report a study of fertilizer use in some coffee plantations in Java from 1983 to 1994.

2. Material and method

Type of coffee observed in this study was robusta located in six main plantations spread out in East as well as Central Java provinces. The parameters measured were inorganic fertilizers used, i.e. urea, triple superphosphate, muriate of potash, dolomite, and kieserite in g/tree/semester, and yield (kg/ha/yr). This observation was conducted since 1983 to 1994.

3. Results and discussion

Fertilizer application on coffee plantation from 1983 to 1987 was increased, but after that until 1994 there was a tendency to decrease (Figure 1). This took place for all of the fertilizers usually used by planters, i.e. urea, triple superphosphate, muriate of potash, kieserite, and dolomite.

When the decrease of fertilizer application was related to fertilizer price in the same period, it could be concluded that there was a negative correlation between fertilizer application and the fertilizer price. The fertilizer price in 1987-1994 is presented in Figure 2.

Besides the increase of fertilizer price, the increase of worker wage (as presented in figure 3) was also a factor in reducing the fertilizer rate applied by the planters.

The rate of fertilizer application was negatively correlated with coffee price in the world market, as showed in figure 4.

One factor which also possibly caused in decreasing fertilizer application was the increasing consumer's demand on organic coffee, but the data related to this possibility was difficult to find. This problem can be seen from data of yield in which the tendency was not decrease although the inorganic fertilizer rate was decrease. From yield data, it could be presumed that there was an addition of nutrients besides those originated from inorganic fertilizer, which derived from organic fertilizer. Many planters suggested that they apply cow manure as well as compost to their coffee plants, but unfortunately data of the rate of application was not well recorded.

Yield in 1994 was significantly dropped compared to the yield in 1993. It was caused by the rainy season in 1993 which came late.

Because of the world conditions which is tend to the globalisation era, so it stimulate the planters to increase their efficiency in management of plantation. Impact of this is that there is no increase of fertilizer rate applied on coffee in the future, although in 1994-1995 the coffee price was increased. The reason was also supported by the fact that the consumers who prefer natural or organic product of agriculture continuously going up.

5. Conclusion

From 1987 to 1994 there was a decrease in inorganic fertilizer rate applied to robusta coffee in Java. The decrease possibly related with the increase of fertilizer price, the increase of wage, the decrease of coffee price, and the increase of natural or organic coffee consumers.

It is presumed that the application of little inorganic fertilizer will continued, although the coffee price in 1994-1995 tends to go up.

6. Reference

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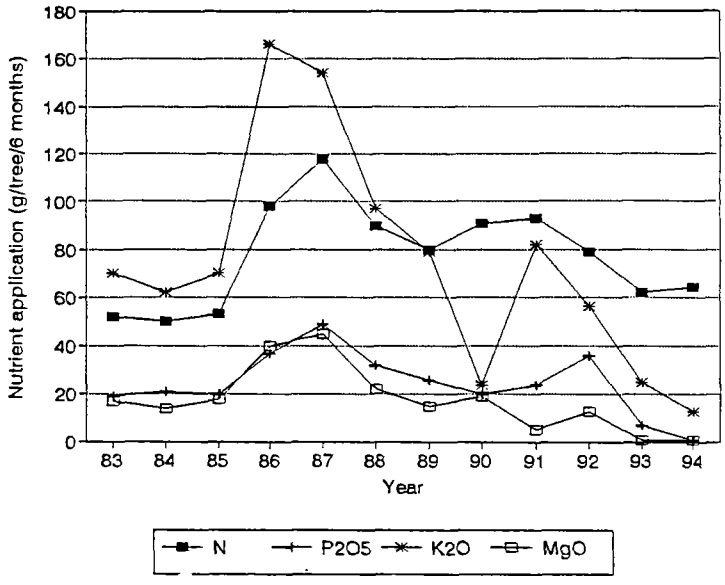

Figure 1. The amount of nutrients applied (g/tree/semester) for coffee in Java since 1983 until 1994.

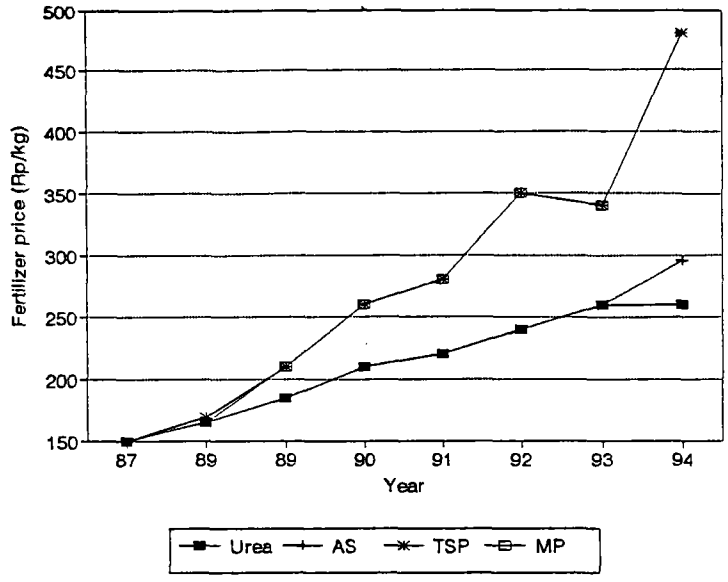

Figure 2. Price of several fertilizer used for coffee in Java since 1987 until 1994.

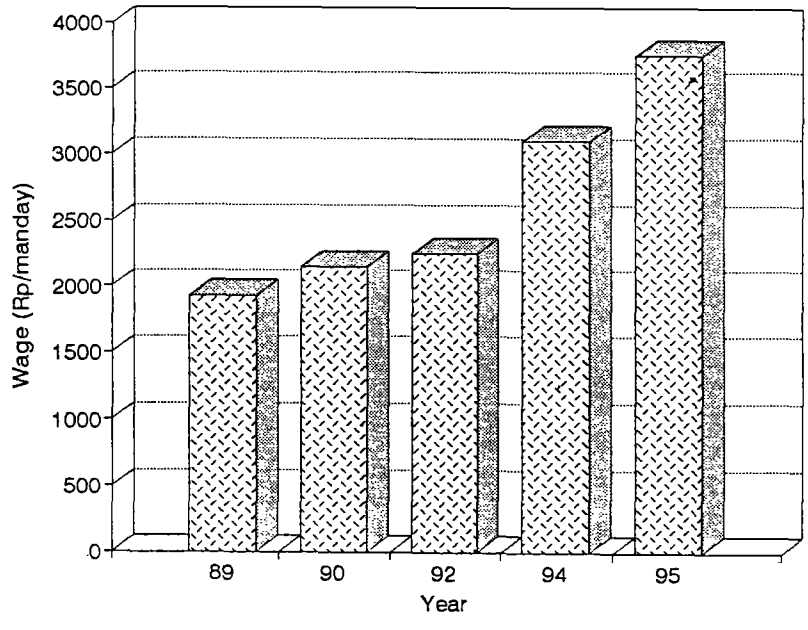

Figure 3. Wage of labour (Rp/manday) in coffee plantations in Java since 1989 until 1995.

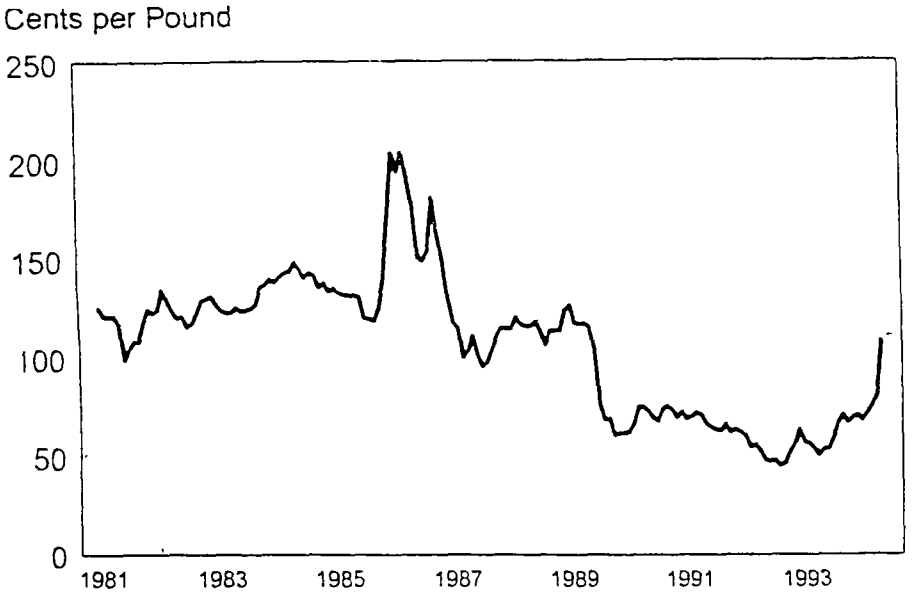

Figure 4. World coffee price (cents/pound) based on New York market since 1981 until 1994.

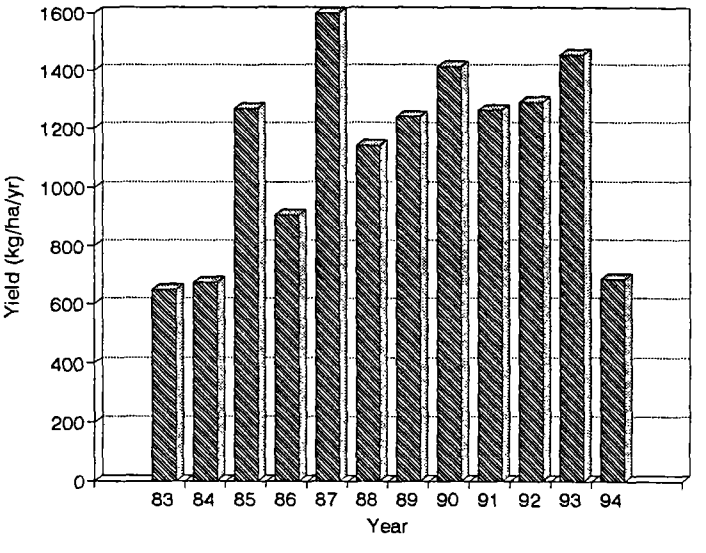

Figure 5. Coffee yield (kg/ha/yr) in Java plantations since 1983 until 1994.

A TECHNOLOGY TRANSFER OPERATION : A COMMERCIAL *COFFEA CANEPHORA* MICROPROPAGATION LABORATORY IN UGANDA

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INTRODUCTION

The Ugandan Ministry of Agriculture, via the FSSP (Farming System Support Programme), has launched a project for the multiplication and distribution of six *Coffea canephora* clones to coffee producers.

The programme, funded by the European Union, is in two parts:

- setting up of clone gardens and clone production using the conventional horticultural cutting technique,
- construction and equipping of a tissue culture laboratory at the Kawanda Agricultural Research Institute (KARI) station 20 kilometres from Kampala, technician training and scientific follow-up.

LABORATORY

The laboratory has 10 rooms and a total floor space of 14 m x 13 m. The transfer and culture rooms are at constant overpressure, to prevent contamination from outside as far as possible. A 100 kVA electricity generating set outside the building takes over in the event of national grid power cuts.

With a fully equipped media preparation room and two transfer rooms with 6 work stations, large-scale *Coffea canephora* clone production can be envisaged.

Two temperature-controlled (to within a 10th of a degree) culture rooms at constant overpressure will be equipped with a temporary immersion system in 1995 for the multiplication and growth of *in vitro* plantlets.

Its highly qualified staff, trained in coffee micropropagation techniques by CIRAD-CP in Montpellier, comprises 3 technicians, 1 person in charge of

glassware maintenance, 1 technician for general laboratory maintenance and a laboratory manager.

A greenhouse with all the standard equipment has been built behind the laboratory by the FSSP for *in vitro* plantlet acclimatization.

MICROPROPAGATION OF COFFEE

Coffea canephora clones resistant to coffee leaf rust have been introduced at the laboratory. As the material has been brought in from the field, the first step is to place the orthotropic cuttings on sterile sand for 15 days.

Once the axillary buds have opened, the explants are transferred to a semi-solid medium, on which the orthotropic stems continue to develop.

When the stems have 3 or 4 pairs of leaves, they are cut into as many micro-nodes and transferred to a liquid medium for multiplication by the temporary immersion system.

Somatic embryos have been produced in a liquid medium by temporary immersion, in order to set up field trials in 1996 to test *in vitro* plantlet conformity.

In vitro plantlet acclimatization is carried out in polybags directly in the nursery, with a 90 to 95% striking rate.
The substrate is a mixture of soil, sand and coffee pulp.

CONCLUSION

Two culture rooms are to be equipped with temporary immersion equipment in 1995 (2,000 units in all).

With this system, it should initially be possible to produce 0.6 to 0.7 million microcuttings per year. Once the conformity of *in vitro* plantlets obtained from somatic embryos has been tested in trials, the laboratory will have a production capacity of 2 to 2.5 million germinated somatic embryos per year.

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DIVERSITÉ GÉNÉTIQUE ET VARIABILITÉ DU POUVOIR PATHOGÈNE CHEZ *COLLETOTRICHUM KAHAWAE*, AGENT DE L'ANTHRACNOSE DES BAIES DE *COFFEA ARABICA*

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1. OBJECTIFS

L'anthracnose des baies du caféier arabica (Coffee Berry Disease) est signalée pour la première fois dans sa forme typique sur jeunes fruits vert au Kenya en 1922. Elle s'est répandue progressivement à toutes les zones d'arabicaculture du continent africain.

La classification de l'agent pathogène a été soumise à de multiples controverses. En 1973, HINDORF décrit cinq formes rencontrées sur caféier et occasionnellement la forme parfaite *Glomerella cingulata*, parmi lesquelles il note une seule forme pathogène des baies ayant une stricte spécificité d'hôte, un mycélium gris-verdâtre à croissance lente, une absence d'acervules en culture. L'auteur réserve le nom de *C. coffeanum* NOACK *sensu* HINDORF à la forme pathogène.

Récemment WALLER *et al* (1993), en se basant sur des caractères morphologiques, la nutrition carbonée et en reprenant largement les critères d'HINDORF, ont proposé l'introduction d'une nouvelle espèce : *C. kahawae* pour désigner l'agent responsable du C.B.D.

Toutefois, au delà des classifications proposées, une certaine confusion demeure due à la présence de plusieurs espèces de *Colletotrichum* rencontrées sur les différentes parties aériennes du caféier (feuilles, rameaux, fleurs) sur lesquelles elles provoquent des dégâts plus ou moins importants. Une des préoccupations actuelles concerne la variabilité du pathogène dans les différentes zones de culture.

L'étude a pris en compte les interactions hôte-pathogène et l'analyse de la diversité génétique à l'aide des Groupes de Compatibilité Végétative d'une population d'isolats géographiques prélevés sur différentes parties aériennes de la plante.

L'objectif étant d'analyser la diversité de la population des *Colletotrichum* pathogènes sur baies en vue :

- de définir les isolats représentatif de cette population qui seront pris en compte dans l'évaluation des caractères de résistance du matériel végétal sauvage ou en cours de sélection,
- d'évaluer l'éventualité de l'existence de pathotypes.

2. CARACTERISATION DES ISOLATS

Seuls les isolats provenant de baies vertes infectées se sont révélés pathogènes à la fois sur baies vertes et jeunes semenceaux. Les autres isolats prélevés sur feuilles, rameaux ou feuilles ne sont pas responsables de l'anthracnose des baies. La souche 790 D est considérée comme saprophyte. La grande spécialisation trophique du *Colletotrichum kahawae* détermine l'occupation d'une niche écologique très étroite et soulève le problème de sa forme de conservation en zone de culture à saison sèche marquée.

SOUCHES	ORGANES VEGETAUX	ORIGINE GEOGRAPHIQUE	PATHOGENIE SUR BAIES
		CAMEROUN	
636D	rameau non aouté	Santa	-
638A	pédoncule floral	"	-
675A	baie verte	Baham	+
680B	feuille robusta		-
711A	baie verte infectée	Santa	+
712B	"	"	+
712F	"	"	+
731	"		+
732A	"	Babadjou	+
737	"	Foumbot	+
785A	rameau d'un an	Santa	-
790D	baie verte*		-
A1	baie verte infectée	ANGOLA	+
Q2	baie verte infectée	KENYA	+
KHW1B	baie verte infectée	KENYA	+
M2	baie verte infectée	MALAWI	+
042.1A	baie verte infectée	BURUNDI	+
ZW1	baie verte infectée	ZIMBABWE	+
EQ1	baie verte	EQUATEUR	-
EQ6	feuille	"	-
EQ8	rameau	"	-
F1	feuille	N. CALEDONIE	-
F2	"	N. CALEDONIE	-

* 2ème floraison

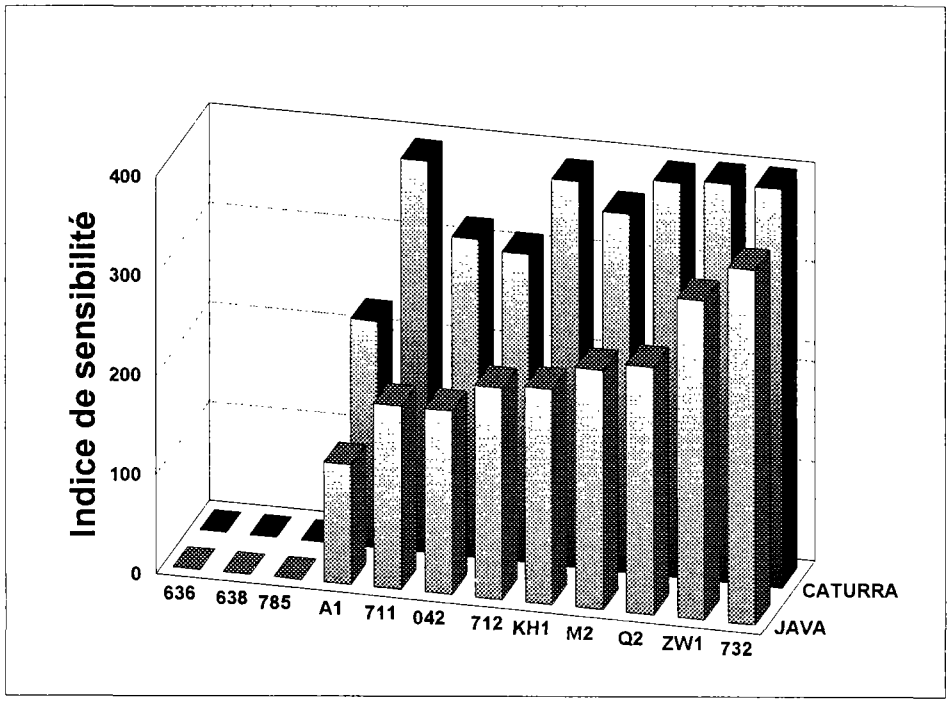
3. VARIABILITE DU POUVOIR PATHOGENE

La pathogénie des isolats a été étudiée par inoculation sur jeunes semenceaux de deux variétés de caféiers, le Caturra amarillo variété sensible et la variété Java considérée tolérante selon la technique d'inoculation modifiée de Van der Vossen, et l'échelle de lecture décrite par Van der Graaf. L'indice de sensibilité obtenu par analyse des classes de distribution, s'étend de 0, variété immune (cas jamais observé) à 400 qui correspond à une sensibilité maximum.

La variété Caturra amarillo montre un niveau de sensibilité très élevé vis à vis de la quasi totalité des isolats à l'exception de A1 où la réaction de sensibilité est intermédiaire.

Avec la variété Java, différents niveaux de réactions de sensibilité sont observés allant d'une réaction de tolérance avec la souche A1 à une réaction de forte sensibilité avec 732 A.

Sur ces deux variétés mais principalement avec la variété "Java", l'expression de la résistance/sensibilité montre un continuum de réactions et ceci indique une certaine variabilité du pouvoir pathogène des isolats.



Réactions de résistance de deux variétés Caturra et Java vis à vis d'une gamme d'isolats

4. ETUDE DES INTERACTIONS HOTE-PATHOGENE

L'analyse des interactions hôte-pathogène a porté sur 20 lignées hybrides Sarchimor et Catimor fournies par le CATIE. Les tests précoces d'inoculation sur jeunes semenceaux ont été réalisés avec 5 isolats d'origines géographiques différents.

Réactions différentielles

	732	KHW1B	042.1A	M2	ZW1
CR.198	TS	MS	R	MR	MS
CR.200	TS	R	R	R	R
CR.203	TS	R	TS	MS	-
CR.208	TS	-	-	R	-

Avec quatre lignées hybrides, des réactions de résistance spécifique sont observées avec un spectre d'hôte différent pour chaque isolat. Ces résultats tendent à montrer l'existence de réactions différentielles mais l'expérimentation devra être renouvelée pour confirmation.

5. ETUDE DES GROUPES DE COMPATIBILITE VEGETATIVE

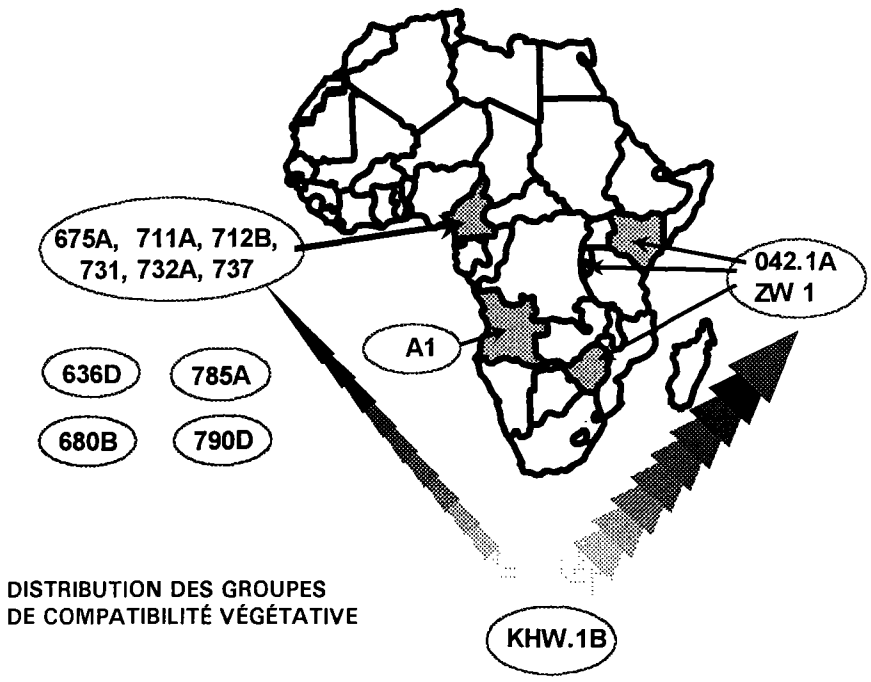
L'analyse génétique de la diversité de la population des *Colletotrichum kahawae* a pu être réalisée à l'aide des GCV car ce sont des champignons imparfaits pour lesquels la reproduction sexuée n'existe pas.

Cependant, des échanges génétiques peuvent se produire grâce au phénomène de l'hétérocaryose, c'est-à-dire la fusion éventuelle par combinaison mitotique de deux cellules de deux filaments mycéliens. Deux souches réalisant une telle fusion sont compatibles entre elles.

La mise en évidence de la compatibilité est facilitée à la suite des travaux de PUHALA (1985) chez *Fusarium oxysporum*. Elle est fondée sur la recherche de mutants spontanés dont la mutation porte à différents niveaux des gènes de régulation de la voie de l'assimilation des nitrates. Les mutants en culture présentent un mycélium ras et sont reconnus à leur incapacité à se développer sur un milieu contenant la source d'azote pour laquelle le gène en question est affecté. Trois types de mutants obtenus sur différentes sources d'azote sont identifiés. La confrontation de deux mutants réalisant l'hétérocaryose indique que les fonctions déficientes sont restaurées et un mycélium aérien fonctionnel est observé.

	MILIEU		
Mutant	NO_3	NO_2	Hypoxanthine
Nit 1	--	+	+
Nit 3	--	--	+
Nit M	--	+	--

+ mycélium aérien
-- mycélium ras



DISTRIBUTION DES GROUPES
DE COMPATIBILITÉ VÉGÉTATIVE

CONCLUSIONS

1. Etude des interactions hôte/pathogène

Une spécificité étroite entre l'origine tissulaire de la souche et son pouvoir pathogène a été confirmé. Seuls les isolats provenant de baies vertes infectées sont responsables du CBD. Toutefois, des isolats *Colletotrichum sp* saprophytes sont aussi présents sur baies.

L'analyse de la pathogénie des isolats induisant des symptômes sur semenceaux de différents génotypes montre un continuum de réactions de sensibilité (de résistantes à très sensibles). Ceci met en évidence la différence du niveau d'agressivité des isolats et souligne leur diversité.

L'analyse des interactions hôte-pathogène a révélé, parmi les sélections Sarchimor et Catimor du CATIE, des génotypes présentant des réactions différentielles. Ceci amène à supposer chez *Colletotrichum kahawae* l'existence de races physiologiques et pourrait permettre à terme de créer une gamme de-- plantes différentielles.

2. Etude de la diversité génétique

La diversité génétique de la population de *Colletotrichum kahawae* révélée par l'utilisation des Groupes de Compatibilité Végétative a permis de montrer en premier lieu qu'au sein des souches camerounaises, les isolats pathogènes sur baies et non pathogènes sur baies appartiennent à des G.C.V. différents. De plus, tous les isolats pathogènes se retrouvent dans le même G.C.V.. En second lieu, il a pu être distingué dans l'ensemble des isolats pathogènes étudiés, deux groupes. Le premier correspondant aux souches d'origine camerounaise et le second englobant des souches originaires d'Afrique de l'Est dans lequel semble coexister plusieurs sous-groupes.

Les compatibilités notées entre la souche du Kenya, KHW1B, et des souches appartenant à deux G.C.V. différents, fait que KHW1B est considérée comme une souche intermédiaire entre ces deux G.C.V., ou "souche-pont" selon la dénomination de KATAN (1991).Ceci est un indice d'une certaine proximité génétique et laisse supposer que la population de *Colletotrichum kahawae* signalée pour la première fois au Cameroun en 1955 a pu se diversifier localement dans les conditions de l'arabicaculture camerounaise. En conclusion, l'évaluation des caractères de résistance devra prendre en compte la diversité de la population du pathogène et la pression de sélection exercée devra être effectuée avec une gamme d'isolats représentative de cette diversité.

RESUME

L'anthracnose des baies de caféier arabica (ou CBD) provoque des dégâts sur fruits pouvant atteindre 80 % de la production en l'absence de traitements. Le champignon responsable de la maladie est le *Colletotrichum kahawae.*

Toutefois, une grande diversité des populations de *Colletotrichum* spp existe sur les différents organes du caféier arabica et leur niveau de spécialisation est peu connu. L'étude de la variabilité du pathogène a été conduite avec une gamme d'isolats d'origines géographiques différentes prélevée sur divers organes du végétal. Le test de pathogénie sur baies et sur semenceaux a permis de séparer les souches pathogènes sur baies, des souches non pathogènes et de mettre en évidence la variabilité du pouvoir pathogène sur deux variétés.

Ensuite, l'analyse de la diversité génétique par la méthode des Groupes de Compatibilité Végétative a permis de distinguer deux groupes parmi les souches pathogènes : le premier correspondant aux isolats camerounais et le second aux isolats d'Afrique de l'Est, ce dernier pouvant regrouper plusieurs populations. L'existence d'une "souche-pont", selon la définition de KATAN (1991), a aussi été observée. Enfin, l'étude des interactions hôte-pathogène sur des Sarchimor et des Catimor a montré des réactions de spécificité vis-à-vis de quelques souches. Ainsi, quelques plantes pourraient être considérées comme plantes différentielles.

STUDY OF PEROXIDASE PATTERN CHANGES DURING THE MICROSPOROGENESIS OF TWO COFFEE CVS. CATUAI AND CATIMOR

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INTRODUCTION

Cultivars Catuaí and Catimor are very important due to its agronomic traits, like productivity, uniformity, vegetative vigor and resistance to coffee rust (Catimor).

It is well known the importance of the production of haploid plants in the coffee breeding program. The importance of the stage of the microspore development in the success of androgenesis has already been studied (Sunderland, 1974; Nitsch,1970a,b; 1977,1983; Dunwell,1976; Carneiro,1987,1991,1992,1993).

Concerning coffee, the role of the enzymes in tissue organ differentiation, namely in reproductive organs has not been studied. These kind of studies are of the great importance to correlate the different stages of anther development related with tissue differentiation during microsporogenesis.

In previous work (Carneiro and Guedes, 1989), esterase isoenzyme patterns and histochemical localization during the microsporogenesis was studied.

In this report, the authors attempted to detect the relation between the changes in isoenzyme peroxidase patterns and soluble protein patterns during the microsporogenesis of cvs. Catuaí and Catimor.

MATERIAL AND METHODS

Plant Material

Floral buds cvs. Catuaí (CIFC 8223/61-124) and Catimor (CIFC 7963/212), growing in glasshouses at an annual temperature varying from a minimum 13-14°C to a maximum 28-29°C were used.

Methods

Anthers of flower buds belonging to seven developmental stages (premeiosis, 1st meiosis, 2nd meiosis, tetrad, tetrad releasing microspores, young microspores and young pollen), were excised and homogenised (Carneiro and Guedes, 1989).

Protein and Peroxidase Isoenzyme Patterns

Polyacrilamide focusing of the samples extracts was carried out in Phastsystem Apparatus Pharmacia (Carneiro and Guedes, 1989). Thin-layer polyacrilamide gels in the pH ranges at 3-9 were used. The peroxidases were stained using the procedure of Nadolny & Sequeira (1980).

Histochemical Localization of Peroxidases

The anthers of cvs. Catuaí and Catimor collected from the same seven development stages, ranging from premeiosis to young pollen were prepared for cryosectioning at 10μ using the Knox technique (1970).

The sections were incubated in peroxidase reaction solution and were stained according the technique described by Nave and Sawhney (1986). Control sections for this enzyme were incubated in reaction solution without H_2O_2.

RESULTS

The results are summarized in fig. 1, 2, 3, 4 and 5 and in table 1.

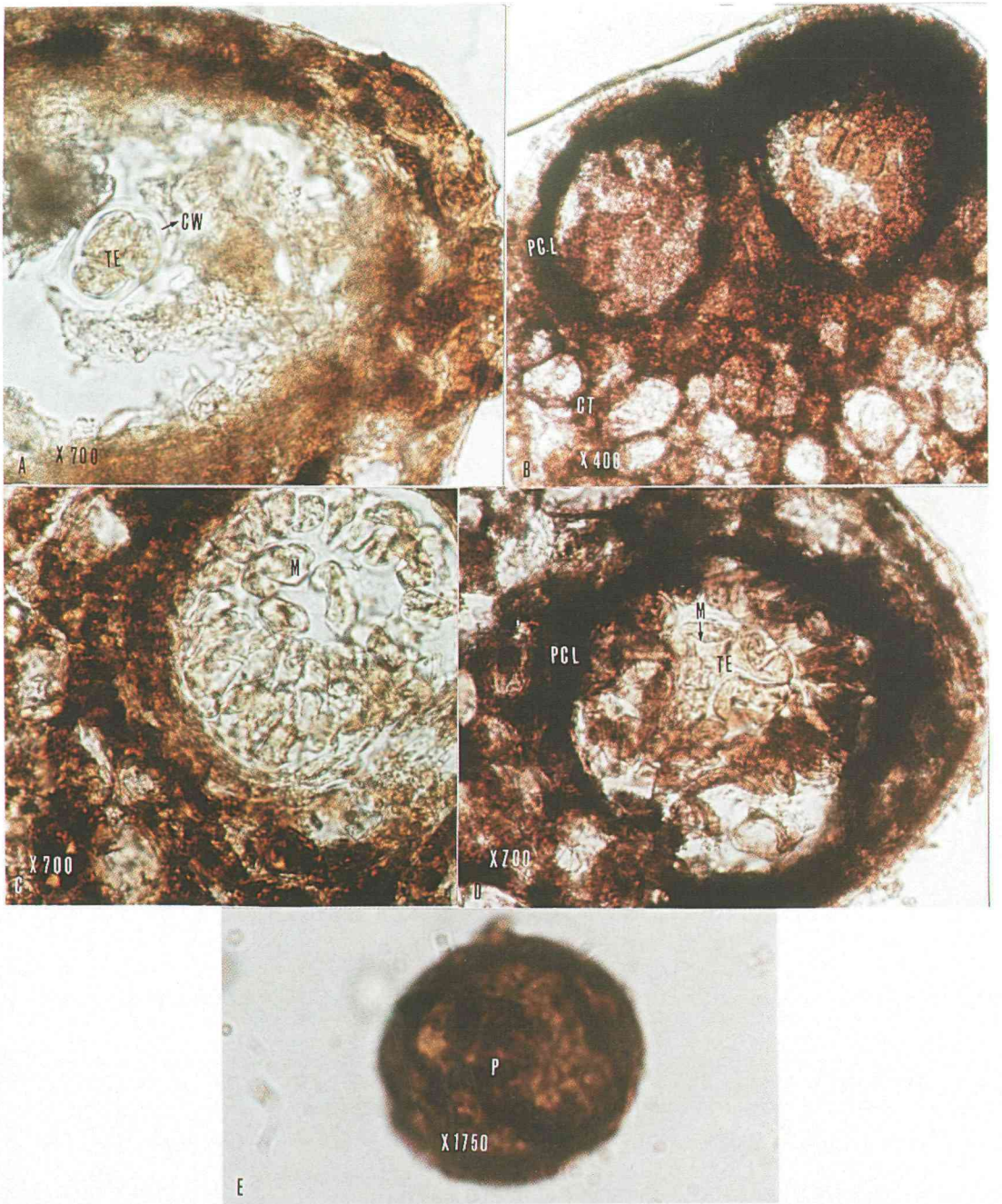

Fig 1. Different aspects of cv Catuai microsporogenesis. A. Cross section of an anther locule incubated in control staining solution for peroxidase activity where tetrads (T) and callose wall (CW) can be observed. B. Cross section of two anther locules at meiosis stage, stained for peroxidase activity. The enzyme reaction product has concentrated in the 2-3 parenchymatous cells layers (PCL) and also in the walls of parenchymatous cells of connective (CT). C. Cross section of an anther locule at young microspore stage (M), incubated in the control staining solution for peroxidase activity. D. Cross section of an anther locule at young microspore stage (M), stained for peroxidase activity. Enzyme reaction product has concentrated in 2-3 parenchymatous cell layers (PCL) and in cell walls of parenchymatous connective tissue. E. Young pollen (P) stained for peroxidase activity.

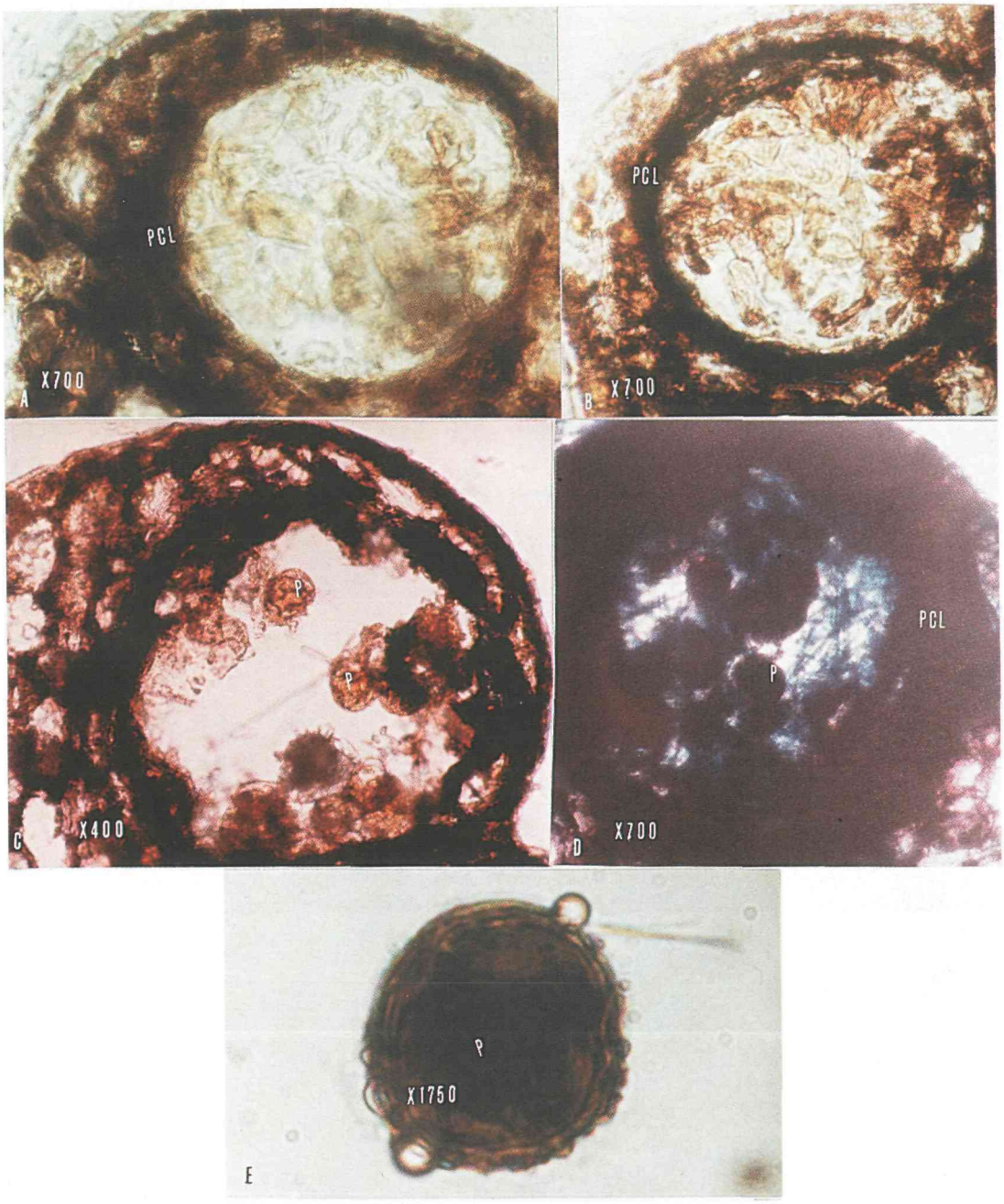

Fig 2. Different aspects of cv Catimor microsporogenesis. A. Cross section of an anther locule at meiosis, incubated in control staining solution for peroxidase activity. B. Cross section of an anther locule at meiosis stage, stained for peroxidase activity. The enzyme reaction product has concentrated in the 2-3 parenchymatous cell layers (PCL). C. Cross section of an anther locule at young pollen stage (P), incubated in the control staining solution for peroxidase activity. D. Cross section of an anther locule at young pollen stage (P), stained for peroxidase activity. Enzyme reaction product has concentrated in 2-3 parenchymatous cell layers (PCL) and in young pollen (P). E. Young pollen (P) stained for peroxidase activity.

Table 1 - Weight and soluble protein contents from cvs. Catuai and Catimor anthers

Stages	Number of anthers	Weight anthers (mg)	Weight/ anther (mg)	Protein anthers (µg/ml)	Cultivar
Premeiosis	175	43.0	0.25	77.0	
1st meiosis	80	25.0	0.31	22.2	C
2nd meiosis	125	60.7	0.49	137.2	a
Tetrad	125	79.7	0.64	126.4	t
Tetrad releas.					u
microspores	125	92.1	0.74	75.6	a
Young micro.	100	68.0	0.68	113.2	i
Young pollen	50	159.4	3.2	26.1	
Premeiosis	150	34.0	0.23	105.5	
1st meiosis	80	40.0	0.50	47.9	C
2nd meiosis	130	78.9	0.61	175.4	a
Tetrad	125	72.5	0.58	155.8	t
Tetrad releas.					i
microspores	125	76.5	0.61	131.5	m
Young micro.	125	109.3	0.87	155.8	o
Young pollen	50	100.0	2.00	69.80	r

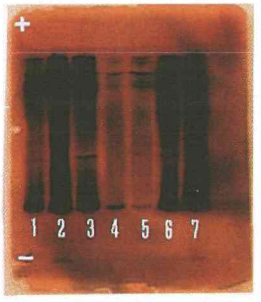

Fig 3. Isoelectric focusing gel with total protein contents of cv Catuai anther

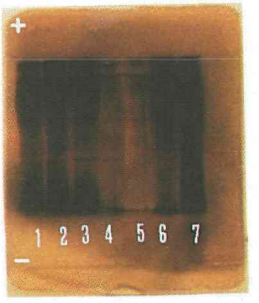

Fig 4. Isoelectric focusing gel with total protein contents of cv Catimor anther

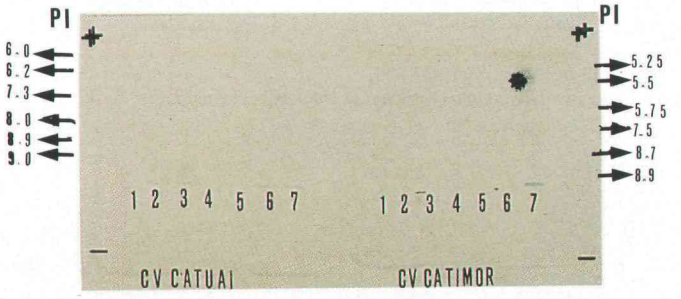

Fig 5. Peroxidase isoenzyme patterns obtained on isoelectric focusing gels from cvs Catuai and Catimor. Bands of peroxidase isoenzymes PI 8.9 have a similar pattern to those of the peroxidase in cultivars in 3rd and 7th stages. The bands PI 5.75, 5.5, 5.25 were detected in cv Catimor and they were not observed in cv Catuai.

DISCUSSION

Peroxidases are known to have an important role in the cell lignification process (Harkin and Obst, 1973). Sawhney and Nave (1986) and Nave and Sawhney (1986) related the peroxidase activity to the thickening of cell walls of endothecium in *Petunia hybrida* anthers. The positive peroxidase reaction observed by these authors was indicated by the formation of a rust brown precipitate in those cell

In previous work, Carneiro and Guedes (1989), studied the role and the histochemical localization of esterase isoenzyme in seven developmental anther stages and related them with important phenomenons that occur during microsporogenesis like tapetum degeneration and pollen grain formation in the cultivars Catuaí and Catimor.

A cross section of a typical anther of *Coffea arabica* L. var. *Typica* Cramer (Dedecca, 1957) shows an outer epidermis of one layer of big cells, one parenchymatous tissue of big cells, constituting the connective, containing frequently tannin which get dark with staining. Each pollinic sac presents, one external epidermis, 2-3 concentric layers of small parenchymatous cells and one layer of tapetum cells which enclose sporogeneous tissue.

Weight and soluble protein contents as well as the peroxidase isoenzyme patterns were studied in seven developmental anther stages, premeiosis, 1^{st} meiosis, 2^{nd} meiosis, tetrad, tetrad releasing microspores, isolated microspores and young pollen.

Peroxidase isoenzyme patterns were observed in 2^{nd} ,3^{rd}, 6^{th} and 7^{th} developmental stages in cv Catuaí and in 3^{rd} and 7^{th} in cv Catimor (fig 5).

Histochemically, they were localized in the 2-3 concentric layers of parenchymatous cells which involve the sporogeneous tissue and in pollen grain (fig 1 and 2).

Considering the results presented it seems that the peroxidase activity is related with the rigidification of the parenchymatous cell walls, (Gaspar *et al.*, 1991).

Further work will be done to clarify the peroxidase role in the process of cell wall lignification.

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GROWTH RESPONSE OF COFFEE (*COFFEA ARABICA* L.) SEEDLINGS TO PHOSPHORUS NUTRITION IN NUTRIENT SOLUTION

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INTRODUCTION

Plants vary in their degree of response to levels of phosphorus nutrition. Fox et al. (1974) found significant variability in the relative yield of maize, sweet potato and head lettuce at the same level of phosphorus (P) fertilization. The response of *Coffea arabica* L. to P fertilization has been reported from almost nil to high (Müller, 1966; Aduayi, 1978; Cooil et al. 1961, Paulos Dubale, 1988). Significant increase in dry weight of seedlings was observed to increasing concentration of P in sand culture (Aduayi, 1978).

This investigation was undertaken to examine the effects of a range of phosphorus (P) concentrations on the growth and distribution of dry mass and P and N in the seedlings of *Coffea arabica* L. in a nutrient solution. The relationship between growth and P concentration in photosynthetic tissue and in the external solution was assessed.

MATERIALS AND METHODS

Experiment I

Seeds of *Coffea arabica* L. were germinated in vermiculite in a growth cabinet at Wye College and kept in pots filled with peat medium for three weeks before being transferred into a 5.5 litre pots filled with perlite. The seedlings were irrigated with distilled water for five days before being supplied with nutrient solution. Phosphorus (P) was applied at the rate of 0.5, 1.0, 5.0 and 10.0 x (10^{-4} M) as KH_2PO_4, in four replications. The composition of the nutrient solutions other than phosphorus is as fellows: KNO_3 10^{-3}M, $MgSO_4.7H_2O$ 5 x 10^{-4} M, $CaCl_2.2H_2O$ 10^{-3}M,

$MnSO_4.4H_2O$ 5 x 10^{-6}M, $CuSO_4.5H_2O$ 5 x 10^{-7}M, $ZnSO_4.7H_2O$ 5 x 10^{-7}M, H_3BO_3 2.5 x 10^{-5}M, $NH_4Mo_7O_{24}.4H_2O$ 3.5 x 10^{-8}M and FeEDTA 2.5 x 10^{-5}M. The deficiency in potassium at the lower P concentrations, or nitrate at the higher K concentration was balanced using potassium nitrate (KNO_3) or sodium nitrate ($NaNO_3$).

The nutrient solutions were applied on alternate days after flushing the perlite with distilled water to prevent the possible accumulation of salts in the pots. Each experimental unit was divided into four harvests at an interval of 40 days. This experiment was carried out between 19 January and 21 May 1990.

Experiment II

This experiment in additon had 0 and 20 x 10^{-4}M P, replicated 5 times and a different variety was used. It also had 6 harvesting dates at an interval of 20 days including t=0 harvest. The experiment was carried out between 6 March and 9 June 1990.

The growth data collected at each harvest were: the fresh and dry weights of shoots and roots, leaf area (Delta-T Device leaf area meter) and root length (Delta-T Device). The fresh roots were assumed to have a specific density of 1.0 g cm^{-3} in order to calculate root volume. Root radius was determined using the following relations.

$$r_o = (V/\pi L)^{0.5} \quad (1)$$

and root surface area (RSA) when determined is

$$RSA = 2\pi r_o L \quad (2)$$

and substituting for r_o in (2) and rearranging

$$RSA = (4\ V/\pi L)^{0.5} \quad (3)$$

Where r_o = mean root radius; V = root volume
L = root length

Phosphorus concentrations in the samples where determined using the molybdenum blue method after wet ashing the samples in the sulphuric acid-hydrogen peroxide procedure and N by indophenol blue method (Allen, 1989; Rowland, 1983).

The least squares method was used in experiment II to fit polynomials to the regressions of the natural logarithms of the growth parameters on time. The exponential regression model was found adequate to fit the data.

The data in experiment II was further examined by running analyses of growth of the primary data. Derived characteristics of fresh and dry weights, leaf area, root length and root surface area, from the fitted growth curves, were compared by relating each to percent P in shoot (% P_s). Phosphorus distribution into the shoot and root in relation to % P_s and phosphorus uptake from the solution was also studied by relating it to root fresh weight, root surface area and root length respectively.

RESULTS

Experiment I

Changes in plant growth with external P supply, for whole seedlings at 120 DAT, are given in Table 1. There was no significant response of any of the growth parameters recorded to the increasing supply of P. The growth parameters were also not significant for the 40 and 80 DAT.

The concentration of P in shoots and roots of coffee seedlings for each harvesting is given in Table 2. In the 120 DAT there were significant differences in P concentrations in the plant between the low (0.5 and 1.0 x 10^{-4}M) and medium to high (5.0 and 10.0^{-4} x 10^{-4}M) P concentrations in the external solution. In the 40 and 80 DATs there was also a slight increase in P concentration in the tissue although this was not consistent in the root and the concentration also tended to fall at the highest level of P. The concentrations of N and K were non-significant with a tendency of decline at the second harvest (80 DAT).

There was very little difference between the means of individual P treatments for each of the growth parameters but the difference between the control and all the P plants, though small increased with time. Comparisons of the regression lines of 0.5, 1.0, 10.0, and 20.0 x 10-4M P against time indicated that there was no significant difference between them.

Table 1. Growth response of coffee seedlings to varying concentrations of phosphorus in nutrient solutio.

P 10^{-4}	Fresh weight(g)	Dry weight(g)	Leaf area (cm^2)	Root length (cm)	RSA cm^2
0.5	8.67	2.12	184.50	447.50	129.40
1.0	8.18	2.01	190.80	414.00	118.00
5.0	10.35	2.50	222.00	482.20	146.70
10.0	8.98	2.18	204.00	456.00	134.80
Mean	9.06	2.20	200.30	449.90	132.20
se(±)	0.71	0.18	13.60	32.70	8.50
P<0.05	n.s	n.s	n.s	n.s	n.s

The duration of experiment II was shorter and therefore, the incremental growth of the seedlings during the period was small. The fresh and dry weights of the shoots and roots of the seedlings are shown in Figs. 1a to 1d. A high percent variance of the error mean square of the weights on time was observed for both the -P and +P plants. There was no significant difference

Table 2. Effect of P supply on the P concentration of coffee seedlings (mg g^{-1} dry weight).

P 10^{-4}M	40 DAT		80 DAT		120 DAT	
	shoot	root	shoot	root	shoot	root
0.5	0.91	1.00	0.98	0.74	1.35	1.14
1.0	0.85	0.76	1.00	0.92	1.48	1.17
5.0	1.24	1.35	1.26	0.71	1.68	1.42
10.0	0.78	1.08	1.22	1.14	1.68	1.42
Mean	0.91	1.05	1.12	0.88	1.54	1.29
Se (±)	0.096	0.24	0.17	0.12	0.05	0.07
P<0.05	*	0.43	0.56	0.12	**	*

(P > 0.05) in the fresh and dry weights of shoots and roots between the P treatments. However, the growth of the check plant declined with the elapse of time.

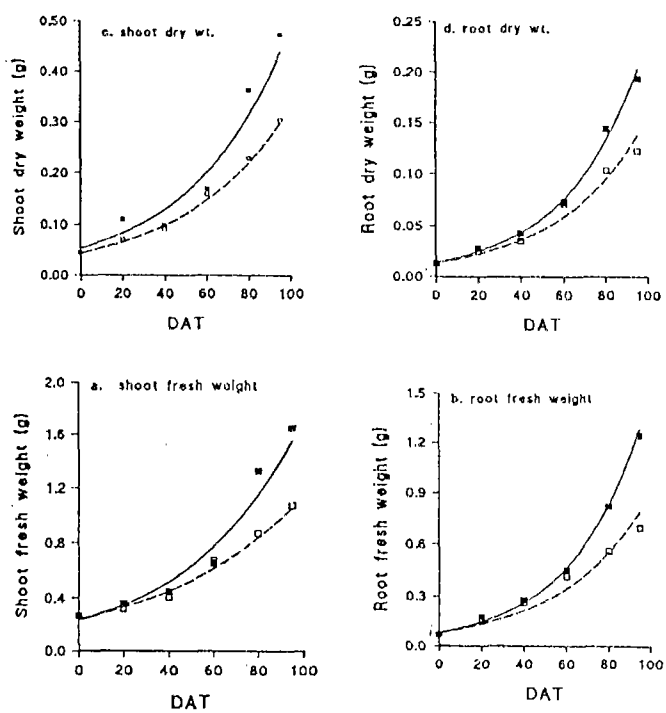

Fig. 1 Increase in fresh and dry weights of shoots and roots. The □ and ■ are -P and +P plants respectively.

Root growth was slow and the seedlings only required a very small amount of phosphorus at this stage as there was no marked effect ($P > 0.05$) on root dry weight by the increasing concentration of P in the nutrient solution.

The supply of phosphate to seedlings eventually resulted in a significantly greater leaf area per plant than on the plants that received no P. Little variation was observed, however, between the phosphate treatments (Fig 2). The decline in length and surface area of the roots of the no P plants was faster in the last harvesting interval (Fig. 2b,c)

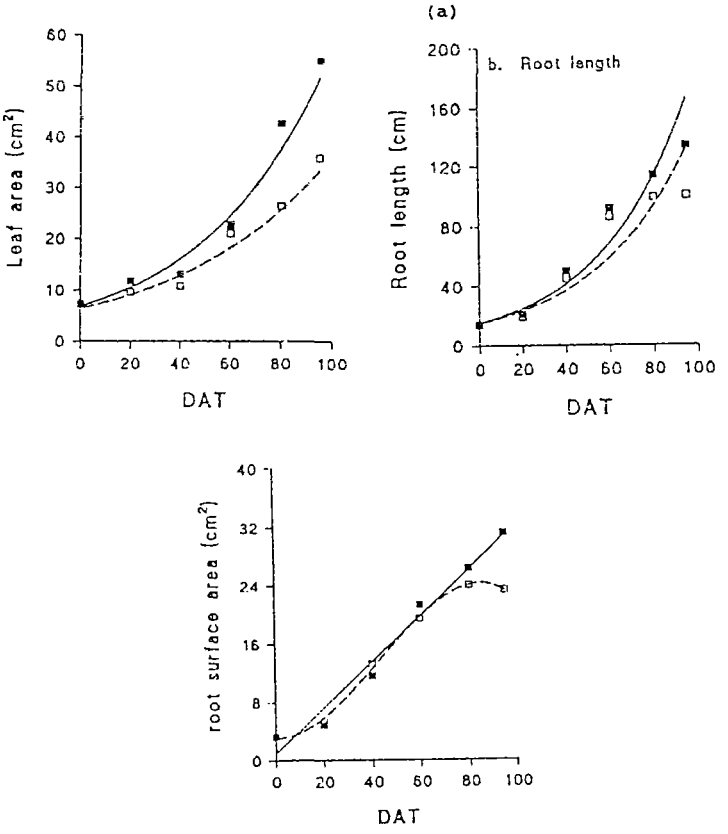

Fig. 2 (a) Effect of P supply on leaf area expansion; (b) root length and root surface area. The legends are same as in Fig 1.

Plant Nutrient Concentration

There was a significant increase ($P < 0.01$) in the concentrations of P in the shoots between plants which received P and those did not. The no phosphate plants survived much longer than expected probably by transporting P from the older leaves to the top younger ones. In general, the check plants had a low P concentration throughout the experimental period (Table 3). The seedlings turned pale as they grew older. The P concentrations

in the shoots also increased significantly with the increasing concentration of P in the nutrient solution.

When growth is described by an exponential function of the form 'a * exp [bt]' relative growth rate (RGR) is constant with 'a * exp [Rt]' where R = b. Parameter 'a' is the weight of the seedling ($\log_e$ W) at time 't' = 0 and parameter b is the rate of increase of logarithm of weight of the seedling, $\log_e$ W with time. The RGR of the check plant is about 83% of the values at

Table 3. Phosphorus concentration of the coffee seedlings (mg g^{-1} DW) grown in nutrient solution.

P 10^{-4}M	20 DAT		40 DAT		60 DAT		80 DAT		95 DAT	
	shoot	root	shoot	root	shoot	root	shoot	root	shoot	root
0.0	1.14	1.37	0.76	1.00	0.60	1.32	0.39	0.95	0.970	1.03
0.5	1.16	1.44	1.82	1.20	0.98	0.99	1.174	1.05	1.216	1.21
1.0	1.46	1.08	1.08	1.10	1.32	1.08	1.312	1.24	1.392	1.20
5.0	1.72	1.93	1.40	1.20	1.48	1.02	1.39	1.34	1.490	1.35
10.0	1.66	1.46	0.98	1.30	1.78	1.91	1.432	1.51	1.554	1.87
20.0	1.40	2.29	1.34	2.30	1.84	2.38	1.904	1.84	1.880	1.32
Mean	1.42	1.60	1.06	1.33	1.33	1.45	1.36	1.32	1.42	1.33
Sem(±)			0.13	0.119	0.119		0.072		0.073	
P<0.05			**	***	***		***		***	

the higher rate of P and the RGR of the root was apparently greater than shoot RGR so that the root: shoot ratios increased with time (Table 4).

Table 4. Relative growth rate of the seedlings (g g^{-1} d^{-1})

P 10^{-4}M	Total	shoot	root
0.0	0.0214 ± 0.0013	0.0202 ± 0.0013	0.0244 ± 0.0015
0.5	0.0246 ± 0.0018	0.0234 ± 0.0019	0.0280 ± 0.0018
1.0	0.0258 ± 0.0015	0.0249 ± 0.0016	0.0279 ± 0.0015
5.0	0.0250 ± 0.0013	0.0240 ± 0.0014	0.0272 ± 0.0012
10.0	0.0256 ± 0.0013	0.0246 ± 0.0014	0.0283 ± 0.0013
20.0	0.0257 ± 0.0015	0.0248 ± 0.0013	0.0273 ± 0.0015

The net assimilation rate (NAR) increased markedly ($P < 0.01$) with increasing concentration of shoot P ($\%P_s$) but with a slight drop at the highest concentration which was produced by 20 10^{-4}M solution P (Fig. 4a). The relationship between unit shoot rate (USR) and the $\%P_s$ was highly significant (Fig. 4b) mainly because of the variations between the control plants and those receiving P. The difference between the two treatments widened as the plants got larger.

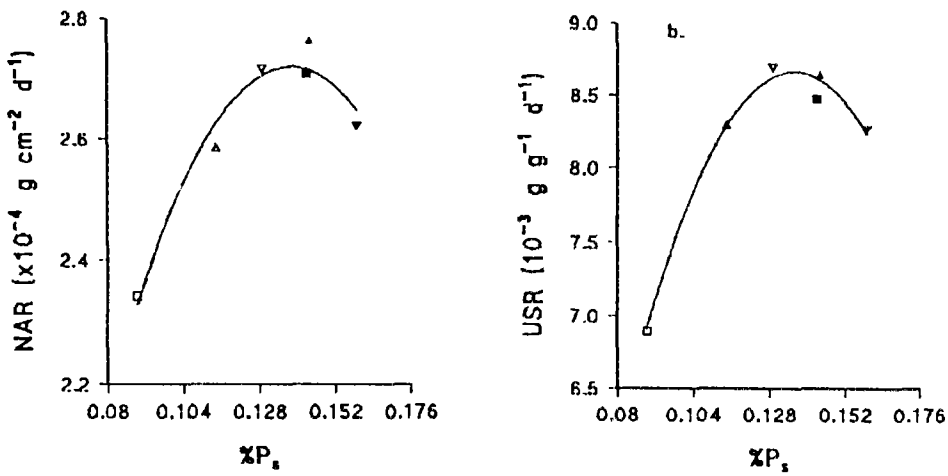

Fig. 3 (a) Relationship between net assimilation rate (NAR) and (b) unit shoot rate (USR) and $\%P_s$. The legends are same as in Fig 1.

P uptake

The uptake of P by coffee seedlings in relation to root fresh weight (unit absorption rate, UAR), root surface area (flux) and root length (inflow), was examined using P concentrations in the shoot and in the nutrient solution. Concentrations of P in the nutrient solution might not have been constant because of depletion with time but it is assumed to be so for the purpose of this analysis. Means of UAR, flux, and inflow of the inter-harvest period were related to the P concentration in the nutrient solution for and plotted in Fig. 4a,b,c.

The P uptake using UAR, flux and inflow was highly correlated with P concentration in the shoot (% P_s) and it also increased with time. The uptake also increased significantly with increasing concentrations of P in the nutrient solution (Fig. 4a,b,c). In the last two intervals (60-95 days) the uptake kept pace with growth. At the earlier stages uptake was not enhanced by the ample supply of P and must have been limited, probably by the root size or all the plant requirement must have been met and hence, the plant could still do well at the lower concentration.

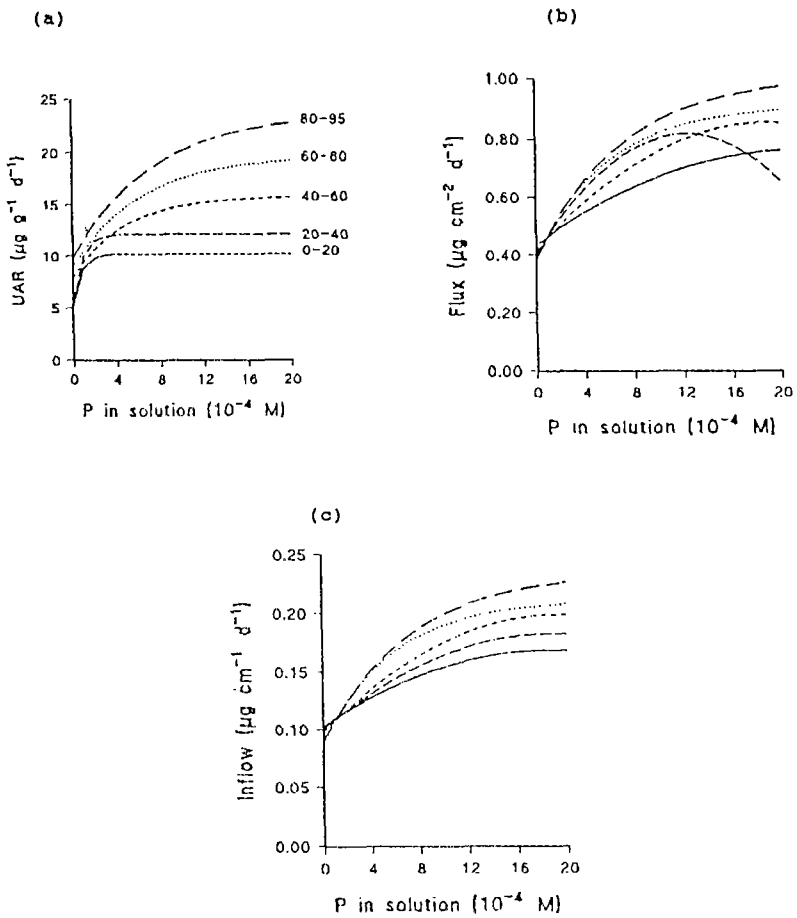

Fig. 4 Relationship between P uptake and P concentration in nutrient solution. The fitted lines are exponential function of the form $Y=a+b*r^x$ where $r=\exp(-c)$. The legends for the lines in (b) and (c) are the same as in (a).

DISCUSSION

The lack of significant growth response in Experiment I suggests that (i) either the lowest concentration of P (0.5×10^{-4}M) was just sufficient to meet the P demand of the plant at this stage of seedling growth, (ii) that the highest concentration of P(10×10^{-4}M) was not enough to increase seedling growth significantly of (iii) that this experiment was terminated before the plant started to respond to treatments. Although all of them might have affected the out come, it is most likely that the low P

concentration was sufficient to meet the P requirement of the seedlings in the short duration of the experiment, and therefore, has more influenced than the others.

The response in growth between the check and +P plants, in Experiment II, was numerically apparent and the means of the plants receiving different levels of P nearly over-lapped with each other. Other works on the response of coffee seedlings older that two years, to P in nutrient solution culture in sand medium, has indicated that it does respond to the application of P (Aduayi, 1978).

When P and N limit plant growth the roots become the stronger sink (Wild et al, 1987, Loneragan and Asher, 1967) and hence, the ratios root weight: shoot weight of the seedlings were higher for low phosphate plants in both experiments.

Increased supply of P in the external solution induced increased uptake of P by coffee plants and this resulted in a significantly higher P concentration in the shoot than in the root. There must also have been greater transport of P from the root to the shoot. The increased concentration of P in the tissue with increasing concentration of P in the external solution presumably suggests that the coffee plant accumulated the orthophosphate in the cytosol when it was supplied in excess in the medium (Lawlor, 1991). However, the lack of marked response in growth by the seedlings may suggest an inefficiency by the coffee plant to utilize P or that it adapts to a low concentration of P in the medium. The evolution of arabica coffee in south west Ethiopia where the plant available soil P is low may suggest the possibility of the latter. Another possible reason for the lack of a response may be associated with the availability of other nutrients below an optimal concentration in the tissue.

Coffee seedlings which already have an accumulation of P in the tissues may not develop a sign of P hunger for months when transferred to a no P medium (Muller, 1966). The root parameters-fresh weight, length and surface area gave similar curves for the uptake of P form the nutrient solution and their relationship with P concentration in the shoot. Since root fresh weight is easier no measure than root length or root surface area it might be a good tool to model nutrient uptake from a medium.

SUMMARY

Coffee (*Coffea arabica* L.) seedlings were grown in nutrient solutions having concentrations of 0, 0.5, 1.0, 5.0, 10.0 and 20.0 ($x10^{-4}$ M) P. Distributions of dry mass and P were measured regularly by destructive harvesting. Growth analyses of the primary data were performed to test the response. No significant response ($P > 0.05$) was observed on the growth parameters between the P receiving plants. The uptake of P measured by unit absorption rate, flux and inflow was highly correlated with P concentration in shoot ($\%P_s$) and with nutrient concentration in the solution.

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HAPLOMETHODS : FACTORS CONTROLLING CALLUS OBTENTION ON *COFFEA ARABICA* ANTHERS

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1. Introduction

Cultivated varieties of *C. arabica* possess a narrow genetic base, and in Latin America these varieties are susceptible to the main diseases.

The coffee vegetative cycle (seed-to-seed) can take three years; progenies must be evaluated on a minimum of three harvests (i.e. three years) and at least six backcross cycles are necessary in order to attain an acceptable homozygosity level; therefore, development and release of a new variety requires about thirty years.

In Central America, a programme has been set up with the aim of increasing genetic variability of cultivated coffee. It is based on classical plant breeding but relies on tools offered by the biotechnologies : molecular biology (RAPD markers for description of variability) and *in vitro* culture.

Research on haploid plants fits well in this scheme. After duplication of the chromosome set of the haploids, the obtention of homozygous coffee would allow us, in case of crossing, to obtain an homogeneous F1 population. Moreover, in a genealogical selection program, development of new lines would be expedited. Finally, genetic studies would be easier, as for example, the identification of recessives genes. The amphidiploid characteristic of coffee ensures us that plants obtained through haplomethods will behave as true haploids, being in fact dihaploids.

After preliminary studies, we chose to work on induced androgenesis using isolated microspores and anther culture. Results on isolated microspores have already been presented (Neuenschwander *et al*, 1993); we describe here some results obtained with anther culture on several genotypes.

2. Antecedents.

Spontaneous dihaploid plants of *C. arabica* have been obtained through polyembryony (Dublin and Parvais, 1975). In comparison with other species, polyembryony is relatively frequent in the genus *Coffea*, especially in the tetraploid forms. The authors studied 2000 plantlets of *C. arabica* coming from double embryos. Nineteen of them were dihaploids.

Induced androgenesis studies on coffee began in 1973 when Sharp and his team (Sharp *et al*, 1973) obtained dihaploid callus from anthers of the varieties Bourbon Amarelho and Mundo Novo. The callus was described as embryogenic, but no embryo developed.

In 1977, Monaco *et al* showed a relationship between three different stages of microsporogenesis and the amount of callus produced by the anther after introduction *in vitro*. No regeneration was obtained.

Only two years ago, a work was presented about isolated microspores and anther culture of varieties Catuai and Catimor and haploids were reported (Carneiro, 1993). Unfortunately this work has not been published.
Recently, an article stressed the importance of a cold pretratment for anther culture of *C. arabica* var. Garnica (Ascanio and Arcia, 1994). The anthers were pretreated at 5°C or left at room temperature for 24 or 48 hours. The percentage of anthers forming callus was significantly higher after cold pretreatment, which was necessary to obtain embryos of which a large proportion were dihaploid. Their chromosome set has been duplicated and about a hundred of plants are currently in the field for further studies.

3. Materials and methods

3.1 Preliminary study.

Plant material. This study has been undertaken on three genotypes : T-2308 (Caturra), T-12855 (Garnica) and T-8927 (Catimor).
Anther stage. According to information found in the literature, the propitious microsporogenesis stage for an androgenic response of the microspores is the early to mid-uninucleate stage. In coffee, meiosis is triggered by a heavy rain following a dry period. In order to study the different stages able to respond to androgenesis, it is important to understand *in situ* microsporogenesis. Anthers from the Caturra genotype were fixed, sectioned and stained according to techniques previously described (Neuenschwander *et al*., 1993). We also found it was sometimes very difficult to find an exact correlation between uninucleate stage and a phenological stage of the flower. A study has been undertaken to try to link the size and the colour of the buds to a specific microspore stage in the studied genotypes.
Desinfection. In order to find a good compromise between an efficient desinfection and a good survival of the microspores, four methods have been tried :

- A : 1% Calcium hypochlorite solution for 5 min.; last rinse with 10 mg/l cysteine
- B : 0.1 % $HgCl_2$ for 4 min.; last rinse with 1mg/l cysteine
- C : 7% Sodium Hypochlorite for 15 min.; last rinse with 50 mg/l cysteine.
- D : 25 % Calcium hypochlorite for 5 min., last rinse with 50 mg/l cysteine and 500 mg/l Benomyl.

The percentage of contaminated anthers was observed for each treatment, as well as the level of oxidation (browning) and the viability of the microspores assessed by staining with Alexander stain.
Pretreatments. A temperature pretreatment was applied to the flower buds before culturing : 5°C for 2, 4 and 8 days, in comparison with buds kept at 25°C.
In another experiment, the same temperature pretreatment was also combined with centrifugation (300 rpm) for 20, 40 and 60 min.
Finally, an osmotical shock was studied, placing the dissected anthers in 20% and 40% sterile sucrose solution for 1 hour before culturing.
In all cases, the anthers were then cultured on Murashige and Skoog or Gamborg B-5 medium, in the dark and at a temperature of 28°C. After two and four weeks, the percentage of living microspores was recorded, as well as the number of microspores in division and the percentage of anthers forming callus.

3.2 General study

Plant material. In this study, five genotypes were investigated : T-16781 (Catuai), T- 15859 (Caturra Rojo), T-12855 (Garnica), T-15869 (Catimor), T-16786 (Sarchimor F3).

Flower bud stage. Based on the results of the preliminary study, the flowers were collected with a bud size between 7 and 9 mm and a light green colour. They were desinfected with the Method C previously described.
Pretreatments. Only cold pretreatments were studied, at a temperature of 5°C for 0, 2, 3, 5 and 7 days.
Callogenesis. Several media were studied; the basal medium was that of Gamborg (B5), supplemented with Linsmaier and Skoog vitamin solution, 50 mg/l cysteine, 30 g/l sucrose, and 2 g/l gelrite, at pH 5.8. Four hormonal combinations were tried : A : 2 mg/l AIB + 8 mg/l BAP (Ascanio and Arcia, 1994); B : 2 mg/l ANA + 5 mg/l BAP; C : 2 mg/l ANA + 2 mg/l BAP; Ascanio : 0.5 mg/l ANA + 2 mg/l 2i-P (Ascanio, 1987). A minimum of 125 anthers were cultured per treatment. Anthers were cultured in the dark at 28°C.
Regeneration. Calli obtained on the above media were transferred to Murashige and Skoog based media containing 2,4-D 2 mg/l + Kinetin 6 mg/l or 2,4-D 2 mg/l + BAP 6 mg/l or Kinetin 0.5 mg/l + ANA 0.05 mg/l. Calli were cultured at 26°C, in diffuse light.

4. Results

Microsporogenesis. About 36 hours after meiosis, it is possible to observe spherical tetrads, with the tetraspores linked by callose. One can observe a large nucleus and a cytoplasm containing a lot of proteins. At that point, the tapetum consists of meristematic cells, sometimes vacuolized. Soon after liberation of the microspores, it is possible to observe formation of the exine. Forty-eight hours after meiosis, the early uninucleate stage is caracterized by the presence of a vacuole, a lateral nucleus, and the occurence of both polysaccharides and proteic storage compounds. A few hours later, the vacuole splits into several small vacuoles that gather around the periphery of the cell. The nucleus is then at the center and is surrounded by a lot of starch grains (mid-uninucleate stage). By the end of the third day, the nucleus has moved again towards the side of the cell (late uninucleate stage) where the first haploid mitosis will take place, giving rise to an oval reproductive nucleus and a round vegetative nucleus.

Anther stage. In general we found that in each flower bud the anthers had microspores at different stages of development; the same phenomenon exists within one anther. For Garnica we found that the majority of uninucleate microspores was present in 8 mm long buds. For Caturra, the best size was 7 mm and for Colombia 8 or 9 mm.

Desinfection. Method A was discontinued very early as it resulted in 95% contamination. For Caturra, there were no significant differences between the three other techniques for the percentage of contaminated anthers, but the C technique was the best for the two other genotypes (75.3% non contaminated anthers for Catimor and 83.9 % for Garnica). The C technique was the one we chose since it resulted in the least anther oxidation for the three cultivars and the best survival rate of the microspores for Catimor and Garnica.

Pretreatments.
a) *Cold pretreatment.*
-Preliminary study. We did not find any significative differences between the pretreatments. The control gave a similar percentage of anthers forming callus compared to the 5°C treatments, but the callus seemed to come out of the anther in only a few cases. The best medium for callus production was Gamborg B5. Analyzing the interactions, the best treatment for the three introductions was the treatment at 5°C for two days cultured on B5 medium.

- *General study.* The best pretreatment for the five genotypes under study was two days at 5°C. Together with the three days pretreatment, those are the only times for which all the genotypes responded. It is clear that media C and Ascanio (NAA 2mg/l + BAP 2 mg/l and NAA 0.5 + 2-iP 2 mg/l) gave the best results for callus formation. The best percentages are obtained with Catuai. A light response is observed without cold pretreatment.

b) *Cold plus centrifugation pretreatment (preliminary study).* The three introductions responded in the same way: the control gave the best percentage of callus in comparison with all the treatments. Analyzing the viability of the anthers, it was higher when the cold was combined with centrifugation than only with the cold pretreatment; likewise, the microspores were in better condition after 40 and 60 min of centrifugation than after 20 mn.

c) *Osmotic pretreatment (preliminary study).* No callus formation was observed with that type of pretreatment, but it is important to note that after 30 days, there are still 32 % viable microspores in comparison with the control. For Garnica, about 16% of the microspores showed a high nucleo-cytoplasmic balance and the occurrence of two nucleoli in the nucleus, sign of high metabolism.

d) *Culture medium influence (general study).* The highest percentages of callus are obtained with medium C (NAA 2mg/l + BAP 2 mg/l). Together with medium B, those are the only media where all the studied genotypes responded. Medium A gave the worst response. It is interesting to note that on medium C, Catuai and Catimor seem to respond better to a shorter pretreatment (2 and 3 days), whereas Caturra needs a longer pretreatment and Garnica responds to all times between 0 and 5 days.

e) *Genotypes.* Caturra responded the best to all treatments (media and temperatures). Catimor gave the worst results and Garnica an average response.

In some cases, the calli came directly out of the cell wall, in some other cases it was obvious that it came from the filament scar, but in several cases, especially for Garnica, the anther opened and the calli came directly out of the loculae. The histological studies did not show any important changes within the anthers. In general the calli were formed with large cells, some of them containing starch. In a few instances, an equal division of the nucleus of a microspore was observed, but apparently was not followed by cell wall regeneration.

All the calli were transferred to embryo regeneration medium as described by Sondhal and Sharp (1977). So far, no embryos have been obtained but some calli aquired a nodular structure together with a green colour. The majority of the calli remained soft .

5. Discussion and conclusion

It was possible to obtain a large amount of callus from several genotypes. Cultivar Garnica has already been described in the literature as responding to a 48 hr cold pretreatment at 5°C (Ascanio and Arcia, 1994). However, contrarly to those authors, we did not observe any embryos, even with the medium they described. This might be due to a difference in the physiology of the mother plants that were not cultured at the same altitude and latitude.

In the different cultivars under study, several microsporogenesis stages have been observed within the same flower bud or the same anther. This has been described in other plants, for example *Hyoscyamus niger* (Raghavan, 1978).

In this study we could determine a good desinfection method that not only provides an acceptable contamination percentage, but also gives a high level of viability of the microspores, an effect that is not always considered by the different authors.

Gamborg's B5 medium gave better callus formation than MS medium. The total ionic concentration of B5 medium is 60.2 mM in comparison with 93.3 mM for MS. Also, it is important to note that the NO_3^-/NH_4^+ balance is 12.5 for B5 medium and 1.91 for MS, since it is known that the ammonium can be toxic for the microspores (Amari, 1994). Also, MS medium contains a higher proportion of Chlorin (6 mEq/l) which could be detrimental to the cultures.

Finally, we obtained different responses according to the genotype, Caturra being the most responsive in regard to callus formation. One must keep in mind the genotypic effect when studying a new technique, and it is advisable to consider a wide range of varieties.

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Résumé

Après étude de la microsporogenèse dans nos conditions de travail, des anthères de *Coffea arabica* var. Catuai, Catimor, Caturra, et Garnica ainsi que d'une sélection F3 de Sarchimor ont été mises en culture au stade uninucléé médian.

Dans le but de détourner le développement gamétophytique mâle vers la voie sporophytique, diverses durées, de 0 à 7 jours, de prétraitement au froid (5°C) ont été essayées. Ces prétraitements ont été réalisés avant la mise en culture.

De même, plusieurs milieux ont été choisis : quatre milieux différents pour la callogenèse et trois milieux pour la régénération.

Le prétraitement au froid le plus efficace a été celui de deux jours, ce qui confirme les résultats rencontrés dans la littérature. Tous les génotypes étudiés ont réagi par la formation de cal à 2 et trois jours de prétraitement.

Le plus grand nombre de cals a été atteint avec une combinaison hormonale de 2 mg/l d'ANA et 2mg/l de BAP.

La réponse est variable selon les génotypes : Caturra nous a donné les meilleurs résultats et Catimor, les plus mauvais. Le comportement des cals sur les milieux de régénération a été varié, mais jusqu'à présent un seul embryon a été régénéré à partir de 400 cals. Des analyses histologiques ont été réalisées.

Abstract
After studying *Coffea arabica*'s microsporogenesis in our climatic conditions, anthers from the varieties Catuai, Caturra, Catimor and Garnica, as well as from a F3 selection of Sarchimor were cultured at the mid-uninucleate stage.
With the aim of disturbing the male gametophytic development towards a sporophytic pathway, 0 to 7 days of cold pretreatment (5°C) were tried. This was applied to the flower buds before culturing. Also, several media were chosen : four different media for callogenesis and three for regeneration. All explants were cultured in the dark for callogenesis or at 12hrs photoperiod for regeneration experiments.

The best cold pretreatment was that of two days, which confirms previous results from our team or from the literature. All the studied genotypes formed calli with 2 or 3 days cold pretreatments. The highest number of calli was obtained with an hormonal combination of 2 mg/l ANA and 2 mg/l BAP. The reponse varied according to the genotype : Caturra was the most responsive and Catimor the least. Calli's behaviour on regeneration media varied and up to now, only one embryo has been regenerated among 400 calli. Histological analysis were carried out.

EVALUATION OF OPTIMUM SPRAY VOLUME APPLICATIONS AGAINST COFFEE BERRY DISEASE ON *COFFEA ARABICA* IN HARERGE REGION

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INTRODUCTION

The ways of controlling coffee berry disease (CBD) with chemicals is often highly appreciated by coffee growers in Harerge region, Ethiopia, but their use is restricted by the scarcity of water for spraying purposes and these restrictions often result in the cancellation of applications altogether.

When taking into account the large quantity of water required to spray coffee trees in Harerge, ways have to be found to reduce the water quantity which can be achieved by employing special reduced rate of application. The conventional high volume application used in the region requires over 1.5 liters of water per tree. Therefore it seems plausible that the efficacy of different spray volumes like high (HV), low (LV) and ultra low volumes (ULV), using knapsack, motorized and micron ULVA-8 sprayers respectively, have to be compared and a suitable one be adopted in the region. HV, LV. ULV, are expressions which normally refer to the quantity of liquid applied per hectare of land. The choice of spraying equipment is normally governed by the ease of use, availability of diluent, labour and other factors (Logan; 1987). ULV application refers to the spraying of a very highly concentrated solution of active ingredient.

This study was intended to provide coffee growers in Harerge with optimum spray volume applications which are suitable and require minimum quantity of water for spraying against CBD.

MATERIALS AND METHODS

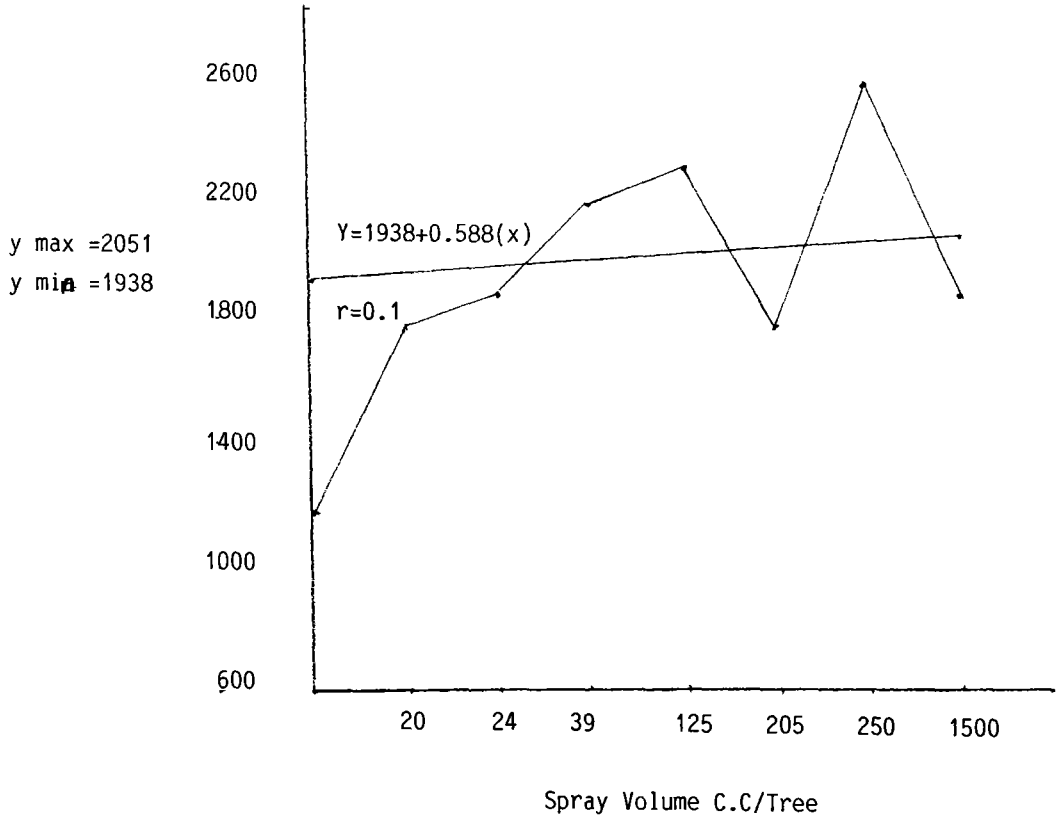
Chlorothalonil at the rate of 4.4kg/ha was suspended in different volumes, 0.02, 0.024, 0.039, 0.125, 0.205, 0.25 and 1.5 l/tree which has concentrations of 33% 28%, 17.5%, 5.4%, 2.7% and 0.4% respectively. Unsprayed plots served as control. The varying volumes of application were achieved by using different kinds of sprayers. The conventional high volume was applied by knapsack sprayer, the low and very low volumes were attained by motorized knapsack sprayer while the ultra low volume was applied by micron ULVA-8 sprayer (Maithia; 1988).

Table 1. The Effect of Spray Volume on the Control of CBD at Mechara.

Treatments	Conc. in%	% CBD			Clean Coffee kg/ha (1600 Trees)					
					1989		1990		X	
		1989	1990	x	Per tree	Per ha	Per tree	Per ha	Per tree	Per ha
High volume (1500 c.c/tree)	0.4	0.17	0.13	0.15	0.764	1222.4	1.602	2563.2	1.183	1892.8
Low volume (250 c.c/tree)	2.0	0.17	0.00	0.09	0.729	1166.4	2.492	3987.2	1.611	2576.8
Low volume (250 c.c/tree)	3.3	0.84	0.41	0.63	0.755	1208.0	1.615	2584.0	1.185	1896.0
Very low volume (125 c.c/tree)	5.4	4.53	2.30	3.42	0.725	1160.0	2.141	3421.6	1.433	2292.8
Ultra low volume (39 c.c/tree)	17.5	1.27	1.30	1.29	0.628	1004.8	2.101	3361.6	1.365	2183.2
Ultra low volume (24 c.c/tree)	28.0	1.97	1.50	1.74	0.494	790.4	1.86	2976	1.177	1883.2
Ultra low volume (20 c.c/tree)	33.0	1.83	2.65	2.24	0.127	843.2	1.699	2718.4	1.113	1780.7
Control (untreated)	-	11.65	3.29	7.47	0.152	243.2	1.312	2099.2	0.732	1171.2
Arth. mean		2.80	1.45	2.13	0.597	954.8	1.813	2964.4	1.225	1959.59
Sd		1.87	3.08	1.64		0.26		22.16		11.32
LSD at 5%		5.68	NS	NS		NS		NS		NS
1%		7.889	NS			NS		NS		NS

Fig. 1

Estimated Linear regression(r) between coffee yield (y) and spray volume (x)



Treatments were laid down in a randomized complete block design in four replications and conducted at Mechara on Farmer coffee holdings. Each plot consisted of 6 trees. Effect of the treetments was assessed by recording CBD from all the trees per plot. CBD pressure was rated using the berry count and vissual assessment. Yield was collected from each experimental unit and then converted to clean coffee k.g/ha. All data were transformed using the Angular or arcsine transformation before being analyzed.

RESULTS AND DISCUSSION

In 1989, all the different volume applications were significanly better than the untreated check, both at 5% and 1% level, in controlling the disease (Table 1). But the different spray volumes did not vary in yield or in the degree of CBD control when compared to the conventional high volume application. CBD incidence was generally lower and yield was relatively higher for the 1990 crop season than for the previous season (Table 1). Moreover, no significant differences among treatments were observed both in disease control and in yield.

The regression of coffee yield (y) on different spray volumes (x) for the experimental seasons was Y=1930 + 0.059 (x) and was not significant (Fig.1).

This result may suggest that the low and ultra low volume applications of fungicides on Coffea arabica in Harerge are as effective, for the control of CBD, as the high volume application. On the contrary, ULV applications produce spray-mixture droplets of approximately 70 microns (Matthews; 1985) and since water was used as the carrier, the spray solutions remain suspended in the air and do not reach their targets, instead they drift into the general environment.

This constitutes a major serious element of risk for Harerge coffee growers who usually intercrop food crops with coffee or plant khat, catha edulis, in the vicinity. Apart from this the excessive tallness (upto 8 mts) of Harer coffee has made the transport and deposition of spray solution on top of the tree very difficult when using the ULVA-8 sprayer, which theoretically should be held at least one metere above the top of the coffee tree being sprayed. Consequently, when considering these drawbacks, which seem to have significant impact on the adoption of this technology, the use of ULV applications on tall coffee trees seems to be very controversial.

In this study the use of low volumes of spray liquid using motorized knapsack sprayer was found to be useful when spraying tall coffee trees and are supposed to have an advantage in removing much of the human involvement. Besides, the vertical throw or the height to which droplets can be carried by the air stream achieved by using motorized sprayer during low volume application was very high and thus spray solutions can reach the top of the coffee tree there by serving the purpose for which it was intended (Frohlich and Rodewald; 1970).

Conclusion

When using water as a carrier, the use of ULV applications on tall coffee trees seems to be very controversial. On the other hand the use of low volume applications using motorized knapsack sprayer, despite the

heavy weights and higher prices when compared with other types of sprayers should be advocated and encouraged to control the infamous coffee berry disease in Harerge region where coffee trees are very tall.

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SUMMARY

The ways of controlling coffee berry disease (CBD) with chemicals is often highly appreciated by coffee growers in Harerge region-Ethiopia. But its use is restricted by the scarcity of water for spraying purposes. Considering this problem a trial was conducted at mechara to provide coffee growers in Harerge region with optimum spray volume applications which are suitable and require minimum quantity of water for spraying against CBD and thus to increase coffee production in the region. Chlorothalonil at the rate of 4.4 k.g/ha was suspended in various volumes (high, low, very low and ultra low) and variable concentrations. Applications were achieved by using manually operated knapsack sprayer, motorized knapsack sprayer and Micron ULVA-8 sprayers respectively. The low and ultra low volume applications of fungicides on Coffea arabica in Harerge were as effective for the control of CBD as the high volume application. However, because of the unmanageably tall size of Harer coffee trees and exo-drift, which occurs mainly from small droplet sizes and since water was used as a carrier, the use of ULV application against CBD on Harer coffee seems to be controversial. On the other hand, the low volume application in the region should be encouraged.

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PROTEIN AND ISOZYME PATTERNS OF SIXTEEN RACES OF *HEMILEIA VASTATRIX* BERK. & BR.

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INTRODUCTION

Coffee leaf rust (*Hemileia vastatrix* Berk. et Br.) is a limiting factors in coffee culture in many areas of the American, African and Asiatic continents.
The knowledge of the chemical constituents of coffee rust may be of great value in developing strategies for the protection of the coffee plant from this fungus.
Many plant pathogen species exist as a group of races that are distinguished from each other by their unique patterns of infection on select host cultivars (Anderson, 1978).
The physiologic specialization of *Hemileia vastatrix* in rust sample collected in different regions of the world has been object of continuos investigation in the Centro de Investigação das Ferrugens do Cafeeiro (CIFC).
Cell-wall composition of uredospores of the *Hemileia vastatrix* was reported in order to understand the plant-fungus interaction (Tavares *et al.*, 1985; Pamplona *et al.*, 1988; Pamplona *et al.*, 1993).
At present the biochemical differences between the races that account for their host specifies are unknown.
In the present work we study, the relation between the changes in the esterase and peroxidase enzymes patterns and the virulence of sixteen physiologic races of *H. vastatrix,* by polyacrilamide gels electrophoresis.

MATERIALS AND METHODS

Sixteen physiologic races of *H. vastatrix* were used.

Race I	(Culture 1285)
Race III	(Culture 995)
Race IV	(Culture 32)
Race VI	(Culture 71)
Race VII	(Culture 130a)
Race X	(Culture 137a)
Race XV	(Culture 70)
Race XIX	(Culture 264)
Race XX	(Culture 394)
Race XXI	(Culture 256)
Race XXIII	(Culture 292a)
Race XXVII	(Culture 264a)
Race XXIX	(Culture 1321)
Race XXX	(Culture 1326)
Race XXXI	(Culture 1302)
Race XXXII	(Culture 256a)

These races were differentiated on the basis of their pathogenicity (table 1).
Differentiation of races of *H. vastatrix* was made from samples sent from different coffee growing regions (table 2).
The rust cultures, usually purified through successive single pustule isolations from the differentials were done by method of Oliveira (1957).
The protein contents and enzyme patterns were evaluated on the basis of number, position (pI value), density and width of the bands.

Preparation of cell-free extracts

The soluble proteins and isozymes (about 1g) of the uredospores were prepared by ultrasonic disintegration with a (Sonics & materials, Inc., model VC 250B) suspended in 0.1M Tris-HCL, pH 7.6, were exposed to intermittent treatments of 2 min. duration several times until breakage was observed microscopically (about 60-70%).
Crud extracts were centrifuged at 15 000 rpm (Sorval SM-24 Rotor) for 60 min. at 4°C. The supernatant fractions were use for enzyme assays.

Table 1 - Races of *Hemileia vastatrix*, differentials, coffee physiologic groups, genes for resistance and phenotypic expressions of the interactions coffee : rust*

Physiologic races of *H. vastatrix* and number of each type culture	Postulated rust genotypes	Host factors for rust resistance																																		
			S_H 1	S_H 4	S_H 5	S_H 6	S_H 1,4	S_H 1,5	S_H 1,6	S_H 2,5	S_H 3,5	S_H 4,5	S_H 5,6	S_H 1,2,5	S_H 1,3,5	S_H 1,4,5	S_H 1,4,6	S_H 1,5,6	S_H 2,3,5	S_H 2,4,5	S_H 2,5,6	S_H 3,4,5	S_H 3,5,6	S_H 4,5,6	S_H 1,2,3,5	S_H 1,2,4,5	S_H 1,3,4,5	S_H 2,3,4,5	S_H 2,3,5,6	S_H 1,2,3,4,5	S_H -	S_H 5,9	S_H 5,8	S_H 5,6,7,9	S_H 5,6,7,8,9,?
		Coffea arabica and Tetraploid Interspecific hybrids																																		
		849/1 - Matari	128/2 - Dilla & Alghe	635/2 - S 12 Kaffa	63/1 - Bourbon	1343/269 - H. Timor	134/4 - S 12 Kaffa	87/1 Geisha	H 468/23	32/1 - DK 1/6	33/1 - S 288-23	110/5 - S 4 Agaro	H 440/7	1006/10 - KP 532 (pl 31)	H 153/2	635/3 - S 12 Kaffa	H 539/8	H 538/29	34/13 - S 353 4/5	H 152/3	H 537/18	H 151/1	H 581/17	H 583/5	H 150/8	HW 17/12	H 148/5	H 147/1	H 535/10	HW 18/21	644/18 - H. Kawisari	H 419/20	H 420/2	H 420/10	832/1 - H. Timor
		β	α	γ	E	R	I	C	5	D	G	J	4	L	Z	W	8	7	H	Y	6	X	10	11	V	O	U	T	9	S	M	3	2	1	A
I (1285)	v 2,5	S	-	-	S	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
III (995)	v 1,5	S	S	-	S	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IV (32)	v ?	MS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VI (71)	v ?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VII (130 a)	v 3,5	S	-	-	S	-	-	-	-	-	MS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
X (137 a)	v 1,4,5	S	S	MS	S	-	MS	S	-	-	-	MS	-	-	-	MS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
XV (70)	v 4,5	S	-	S	S	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
XIX (264)	v 1,4,?	MS	MS	MS	-	-	MS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
XX (394)	v ?	MS	MR	MR	-	-	MR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
XXI (256)	v ?	MS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
XXIII (292 a)	v 1,2,4,5	S	S	MS	S	-	MS	S	-	S	-	MS	-	S	-	MS	-	-	-	MS	-	-	-	-	-	MS	-	-	-	-	-	-	-	-	-
XXVII (264 a)	v 1,4,6,?	MS	MS	MS	-	MS	MS	-	MS	-	-	-	-	-	-	-	MS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
XXIX (1321)	v 5,6,7,8,9	S	-	-	S	S	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	MS	S	-	-
XXX (1326)	v 5,8	S	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	-	-	-
XXXI (1302)	v 2,5,6,9	S	-	-	S	S	-	-	-	S	-	-	S	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	S	-	-	-	-
XXXII (256 a)	v 6,?	MS	-	-	-	MS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* Traces = resistance reactions, S = susceptible, MS = moderately susceptible, MR = moderately resistant

Table 2 - Geographical distribution of differentiated physiologic races of *Hemileia vastatrix*

Origin	Physiologic races of *Hemileia vastatrix*															
	I	III	IV	VI	VII	X	XV	XIX	XX	XXI	XXIII	XXVII	XIX	XXX	XXXI	XXXII
Angola	9	15			1		15					15				
Brazil	6	4					4									
Cambodia				1												
Cameron	1	1														
Rep. Central African			6					1				1				
Ceylon	6						4									
Cuba	3															
Democ. Rep. Congo	1															
Ethiopia	1	38														
India	13	2					1				4				1	
Indonesia	5	9					3				3					
Ivory Coast	1															
China	2					1	1			1	1					
Kenya	73	2			1	1	2		3							
Madagascar	1															
Malawi		1														
Mozambique				14												
Nigeria		4														
New Caledone	2															
Rep. Vietnam		1														
Philippines	2					1										
São Tomé	1	1			1		1									
Sri-Lanka	3						3									
Tanzania	6	8				1			1							
Timor (Portuguese)	4	1					3						4	2		
Uganda	4	2	3			1			2	1						1
Zambia	2															
Rwanda	1															

Proteins and isozymes patterns

Polyacrilamide isoelectric focusing of the samples extracts was carried out in the Phastsystem separation and development apparatus (Pharmacia). Thin-layer polyacrilamide gels in the pH ranges 3-9 were used.
Silver staining was used to visualize proteins, according Heukeshoven & Dernik (1985).
Staining for esterases - 0.6% of Fast Red TR salt, in 0.1M phosphate buffer, pH 6.8, was mixed with 1.5 ml of 1% α-naphthyl acetate (in acetone) and 0.5 ml of 1% β -naphthyl acetate (in acetone). The bands appear after about 30 min. (Nave & Sauhney, 1986).
Staining for peroxidase - 1mM o-dianisidine in 0.1M sodium acetate buffer, pH 4.5. After 1h, the gels were rinsed in distilled water and placed in 1% H2O2 to visualize the peroxidase isozyme bands (Hammerschmidt *et al.*, 1982)

RESULTS

The results of isozymes esterase, peroxidase and protein contents the separation in thin layer polyacrilamide gels of the sixteen races of *H. vastatrix* are presented in figs. 1, 2 and fig. 3.
The two densely colored bands (pI 6.65, 6.35) of esterase isozymes were found in race III. In races I, VI, X, XIX, XXI and XXX we found two light colored bands (pI 6.65, 6.35) (fig. 1). It was not detected any isozymes in races IV, VII, XV, XX, XXIII, XXVII, XXIX, XXXI and XXXII.
The peroxidase isozymes densely colored bands were found in race I, III, VI and X (pI 7.0, 6.85,. 6.65, 6.35, 6.0). We found light colored bands in races , XIX, XXI, XXVII and XXX (pI 6.85, 6.5, 6.35). It were not detected any isozymes in races IV, VII, XV, XX, XXIII, XXIX, XXXI and XXXII (fig. 2).
The soluble protein patterns of all the sixteen races of the *H. Vastatrix* were closely identical qualitatively and quantitative variation in the relative amounts of various proteins contents, they were common when compared by the various thickness and color intensity of bands (fig. 3).

DISCUSSION

One possible explanation of host specificity is that the resistant interaction reaction occurs only when it is triggered through a recognition event in the plant (Anderson, 1978).
To understand the plant fungus interaction it was studied the cell-wall composition of uredospores of the *H. vastatrix* which it was found as being

constituted by sterols and fatty acids (Tavares *et al.*, 1985; Pamplona *et al.*, 1988; Pamplona *et al.*, 1993). Although it is well known that steroids are required for growth and sexual reproduction of many species of fungi (Hendrix, 1970).Little is known about their role in host-parasite interactions between fungi and higher plants.

Isozyme analysis will soon become a standard tool for plant pathologists and mycologists and it will help solve many mysteries of the fungal kingdom.

Based on our results we show that the races where we found isozymes of esterase and of peroxidase, (table 3) they have a common gene of virulence the V5 (table 1).

In conclusion, the results of this study seem to indicate that the electrophoretic patterns of protein, esterase and peroxidase it may be a useful complementary tool for the identification of races of the *H. vastatrix.*

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Table 3 - pI values from races of *Hemileia vastatrix* isozymes

Races	Esterases		Peroxidases					
	6.65	6.35	7.0	6.85	6.65	6.5	6.35	6.0
I	+	+	+	+	+	0	+	+
III	+	+	+	+	+	0	+	+
IV	0	0	0	0	0	0	0	0
VI	+	+	0	0	+	0	+	0
VII	0	0	0	0	0	0	0	0
X	+	+	+	+	+	0	+	+
XV	0	0	0	0	0	0	0	0
XIX	+	+	0	+	0	+	+	0
XX	0	0	0	0	0	0	0	0
XXI	+	+	0	+	0	+	+	0
XXIII	0	0	0	0	0	0	0	0
XXVII	0	0	0	+	0	+	+	0
XXIX	0	0	0	0	0	0	0	0
XXX	+	+	0	+	0	+	+	0
XXXI	0	0	0	0	0	0	0	0
XXXII	0	0	0	0	0	0	0	0

Bands of multiple forms of esterase and peroxidase from races of *H. vastatrix:* I (culture 1285); III (culture 995); IV (culture 32); VI (culture 71); VII (culture 130a); X (culture 137a); XV (culture 70); XIX (culture 264); XX (culture 394); XXI (culture 256); XXIII (culture 292a); XXVII (culture 264a); XXIX (1321); XXX (culture 1326); XXXI (culture 1302); XXXII (culture 256a).

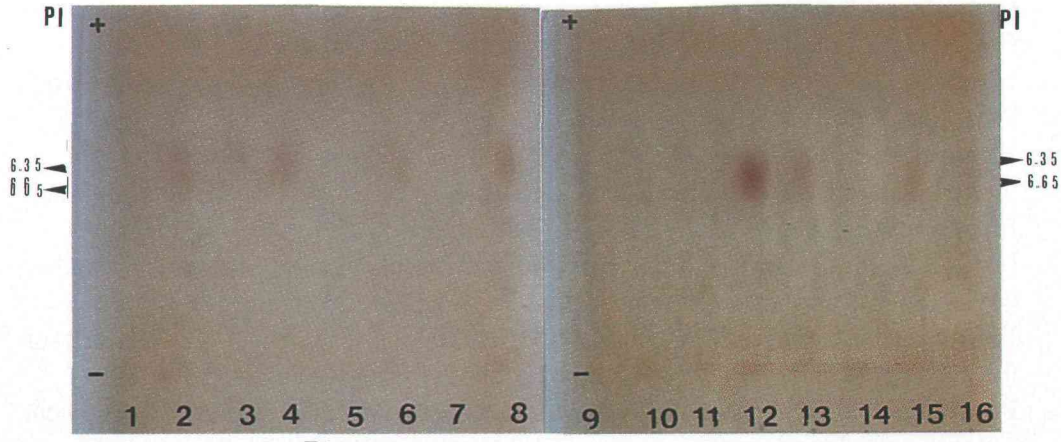

Fig. - 1 Isoelectric focusing gel patterns of esterase from races of *H. vastatrix*: **1** - race IV (culture 32); **2** - race VI (culture 71); **3** - race X (culture 137a); **4** - race XXI (culture 256); **5** - race XXXII (culture 256a); **6** - race XIX (culture 264); **7** - XXVII (culture 264a); **8** - race XX (culture 394); **9** - race XV (culture 70); **10** - race VII (culture 130a); **11** - race XXIII (culture 292a); **12** - race III (culture 995); **13** - race I (culture 1285); **14** - race XXXI (culture 1302); **15** - race XXIX (culture 1321); **16** - race XXX (culture 1326).

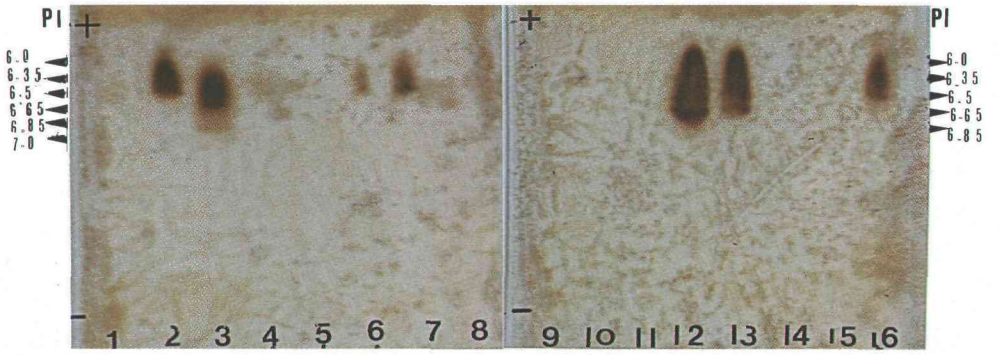

Fig. 2 - Isoelectric focusing gel patterns of peroxidase from races of *H. vastarix*: **1** - race IV (culture 32); **2** - race VI (culture 71); **3** - race X (culture 137a); **4** - race XXI (culture 256); **5** - race XXXII (culture 256a); **6** - race XIX (culture 264); **7** - XXVII (culture 264a); **8** - race XX (culture 394); **9** - race XV (culture 70); **10** - race VII (culture 130a); **11** - race XXIII (culture 292a); **12** - race III (culture 995); **13** - race I (culture 1285); **14** - race XXXI (culture 1302); **15** - race XXIX (culture1321); **16** - race XXX (culture 1326).

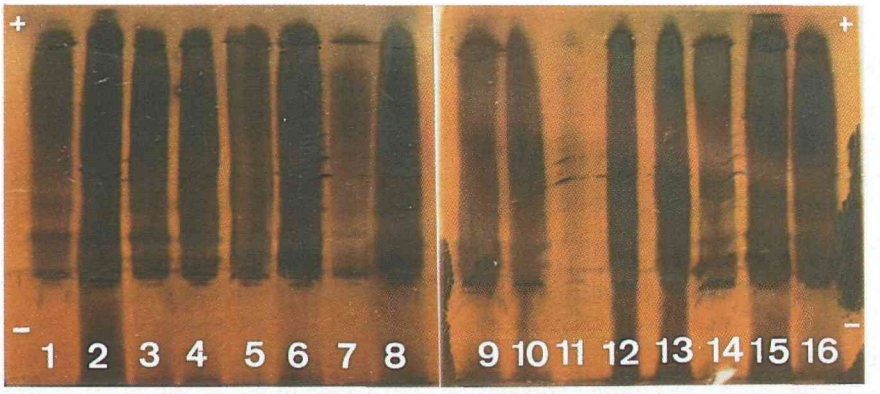

Fig. 3 - Isoelectric focusing gel patterns of soluble proteins from races of *H. vastatrix*: **1** - race IV (culture 32); **2** - race VI (culture 71); **3** - race X (culture 137a); **4** - race XXI (culture 256); **5** - race XXXII (culture 256a); **6** - race XIX (culture 264); **7** - XXVII (culture 264a); **8** - race XX (culture 394); **9** - race XV (culture 70); **10** - race VII (culture 130a); **11** - race XXIII (culture 292a); **12** - race III (culture 995); **12** - race III (culture 995); **13** - race I (culture 1285); **14** - race XXXI (culture 1302); **15** - - race XXIX (culture 1321); **16** - race XXX (culture 1326).

CAFFEINE CONTENTS IN SOME COFFEE-ORANGE RUST INCOMPATIBLE INTERACTIONS

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INTRODUCTION

Coffee is the most popular beverage among people and represents an important source of income for millions of people.

The two most damaging coffee diseases are orange rust and coffee berry disease (CBD).

Caffeine (1,3,7-trimethylxanthine) is a predominant purine alkaloid of coffee (*Coffea* spp.) and it is synthesized from some precursors utilized for purine and methyl group synthesis in other systems.

Caffeine effect in coffee plants in incompatible reaction when inoculated at different times, was studied, showing correlation between high quantity of caffeine and resistance to pathogens (Medeiros *et al.* 1989).

Coffea arabica and tetraploid interspecific hybrids of Híbrido de Timor (HDT), were used in all experiments. These genotypes are used as differentials.

Híbrido de Timor appeared spontaneously in Timor and began to be cultivated in this territory in a private farm by the second half of decade 1940-1949.

The objective of this study was the quantification of caffeine, from leaves in incompatible reaction and to correlate it with different times of infection .

MATERIALS AND METHODS

Plant material

All the experiments were performed with cv Matari (CIFC 849/1), without any genes of resistance to *H. vastatrix* and tetraploid interspecific hybrids, Híbrido de Timor (HDT) CIFC 4106 and CIFC 832/1, possessing the genes SH 5,6,7,8,9,?; CIFC 420/2 [Mundo Novo x HW 26/14 (Caturra x Híbrido de Timor)], possessing the genes SH 5,8 CIFC H 420/10 [Mundo Novo x HW 26/14 (Caturra x Híbrido de Timor)], possessing the genes SH 5,6,7,9; CIFC H 419/20 [Mundo Novo x HW 26/13 (Caturra x Híbrido de Timor)], possessing the genes SH 5,9 (table 1).

All these plants were grown under the CIFC's greenhouses condition, in Oeiras.

The plants were inoculated with Race III (Culture 995) except in Matari (CIFC 849/1) it was with race VI (Culture 71) of *H. vastatrix*.

The young leaves were inoculated by spreading 0.25 mg of the uredospores with a camel hair brush over the lower surface of each leaf and then distilled water was sprayed on the inoculated surface, the plants were kept in a moist chamber for 48 hours (Oliveira, 1957).Leaves of the terminal and subterminal nodes were harvested 3.5, 5, 7, 10 and 15 days after inoculation, in incompatible reaction. Uninoculated leaves were used as control.

The extracts were prepared from fresh leaf tissue and the extraction of caffeine were done by method of Guedes *et al.* (1982) and Medeiros *et al.*(1989).

Caffeine quantification

The caffeine was quantificated by gas-liquid chromatography (GLC) with a 5790 Hewllet-Packard Gas Chromatograph

equipped with a 6' x 1/4" x 2mm glass column packed with 3% OV-17 on a granular particle (Mesh Size 100-120) Gaschrom Q (Chrompack) and flame ionization detector (FID).

The quantities of caffeine were determined on the basis of peak areas relative to that of the external standard, caffeine (Medeiros *et al.*,1989).

RESULTS

The results presented in fig. 1, showed caffeine levels in infected plants in 3.5, 5, 7, 10 and 15 days after inoculation. The accumulation of caffeine was much higher at 15 days after inoculation in Híbrido de Timor (CIFC 4106) and it was constant in Híbrido de Timor (CIFC 832/1), when compared with control (uninoculated leaves). In Matari (CIFC 849/1) the concentration of caffeine was higher at 3.5 days after inoculation and then declined. In H 420/2 and H 420/10 the concentration of caffeine was higher at 3.5 and 5 days after inoculation and then declined. The contents of caffeine in H 419/20 are more or less constant when compared with control (table 2).

Table 1 - Coffee physiologic groups, genes for resistance and phenotypic expression of the interaction coffee - rust.

Physiologic races of *H. vastatrix*	Host factors for rust resistance					
		S_H 5,6,7,8 9,?	S_H 5,6,7,8 9,?	S_H 5.8	S_H 5.6,7.9	S_H 5,9
	Coffea arabica	Tetraploid Interspecific hybrids				
	849 / 1*	4106	832 / 1	H 420 / 2	H 420 / 10	H 419 / 20
I	S					
II	S					
III	S					
IV	MS					
VI						
VII	S					
VIII	S					
X	S					
XI	MS					
XII	S					
XIII	S					
XIV	S					
XV	S					
XVI	S					
XVII	S					
XVIII						
XIX	MS					
XX	MS					
XXI	MS					
XXII	S					
XXIII	S					
XXIV	S					
XXV	S					
XXVI	S					
XXVII	MS					
XXVIII	S					
XXIX	S			MS	S	S
XXX	S			S		
XXXI	S					S
XXXII	MS					

Blanks = resistance reaction: S = susceptible; MS = moderately susceptible

* Universal Susceptible

Table 2 - Caffeine content (mg/g fr. wt.) of coffee leaves inoculated with uredospores of *H. vastatrix* in incompatible interaction

Days after inoculation	849/1	4106	832/1	H420/2	H420/10	H419/20
Control	0,885±0,023	1,086±0,014	0,563±0,012	0,943±0,034	0,915±0,010	1,287±0,068
3,5	1,700±0,026	0,283±0,041	0,498±0,010	1,938±0,029	0,735±0,018	0,833±0,040
5	1,022±0,026	0,783±0,107	0,886±0,021	0,505±0,033	1,467±0,095	1,167±0,049
7	0,460±0,009	0,836±0,021	0,447±0,031	0,693±0,063	0,370±0,006	1,003±0,013
10	0,540±0,015	0,173±0,009	0,527±0,012	1,252±0,046	0,447±0,006	0,789±0,025
15	0,375±0,026	2,750±0,143	0,377±0,007	1,155±0,132	0,497±0,006	0,500±0,007

Data is the mean of six replications ± SE
Control - uninoculated leaves

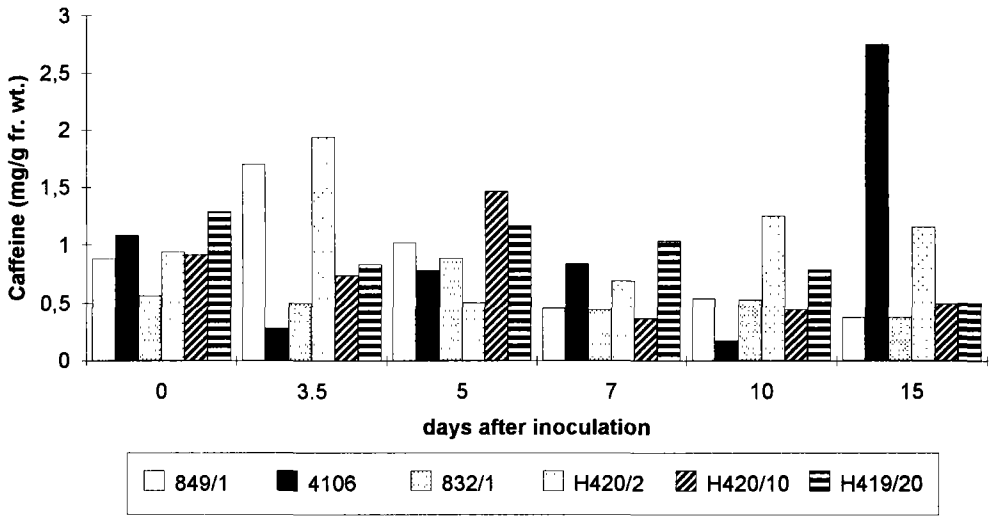

Fig. 1 - Caffeine content is expressed as mg/g fresh wt. Control - uninoculated leaves. Coffee leaves were inoculated in incompatible reaction, and each value is the mean of 2 samples repeated at least 3 times.

DISCUSSION

According Kihlman (1977) more than 60 plant species throughout the world contain caffeine: the most common are from the genera *Coffea, Camellia, Colla, Paullinia, Ilex* and *Theobroma.*

As in *C. arabica* fruits, the metabolism of theobromine and caffeine in the leaves appears to be closely associated with leaf development and ageing. In coffee plants the rate of caffeine synthesis differs markedly among species (Waller *et al* ,1993).

Nazario and Lovatt (1993) report that theobromine (3,7-dimethylxanthine), theophylline (1,3-dimethylxanthine) and paraxantine (1,7-dimethylxanthine) are the three methylated bases proposed as immediate precursors and/or catabolic products of caffeine. However, they showed that theobromine, theophylline and caffeine are the products of different pathways.

Medeiros *et al.* (1989) reported that caffeine removed from the leaves of *Coffea arabica* L. cv Caturra (CIFC 1637), possessing the gene SH5, at different times after infection with *H. vastatrix* was presented in large quantities in resistant plants ,in some days, after inoculation, when compared with control and that there is positive correlation between high caffeine and resistance to pathogens.

The plants possess numerous defense mechanisms conferring disease resistance against potential pathogens, among these several inducible biochemical responses including the production of antimicrobial products like phytoalexins, lignification and increase of enzymatic activity (Rodrigues *et al.*, 1975; Guedes, 1981; Guedes *et al.*, 1982; Guedes, 1984; Rijo & Vasconcelos, 1984; Guedes, 1988; Guedes *et al.*, 1992; Guerra-Guimarães & Guedes, 1993; Guerra-Guimarães & Guedes, 1994; Guedes *et al.*, 1994).

The results in fig. 1 and table 2, indicated that the infection was açcompanied by a change in contents of caffeine, at 3.5, 5 and 15 days after inoculation with races of *H. vastatrix*. In interspecific hybrid H 420/2 and H 420/10 the contents of caffeine are different and the reaction with *H. vastatrix* are also different which indicate that they bear different genes for resistance. In Híbrido de Timor (832/1) the contents of caffeine did not showed any difference at the different times of the inoculation. The role of caffeine as another factor in the dynamics of resistance, remains then as a possibility in coffee.

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EFFECT OF PLANT HORMONES ON SOMATIC EMBRYOGENESIS OF *COFFEA CANEPHORA*

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Key words :
coffee (*Coffea canephora*), cryopreservation, plant hormones, somatic embryogenesis

Abbreviations :
2-iP : iso-pentenyladenine, BA : benzyladenine, KI : kinetin, ZE : zeatin,
IAA : indole-3-acetic acid, IBA : indole-3-butyric acid,
NAA : 1-naphthaleneacetic acid, 4-FA : 4-fluorophenoxyacetic acid,
2,4-D : 2,4-dichlorophenoxyacetic acid, ABA : abscisic acid

Introduction

Coffea canephora is a commercial crop used for the manufacture of instant coffee and is known for its resistance to coffee leaf rust. *C. canephora* is the first species in the genus *Coffea* in which regeneration by tissue culture has been successful (Staritsky, 1970). Pierson et al. (1983) reported embryogenesis in *C. canephora* when a combination of 1 mg/l 2-iP and 5 mg/l IBA was used, but they did not discuss the effects of the kind and concentration of the plant growth regulators on embryogenesis. We previously developed techniques for somatic embryogenesis using leaf cultures of *C. canephora*. Formation of somatic embryos is easily induced directly from leaves of *C. canephora* in primary culture, in the presence of a cytokinin as the sole plant growth regulator (Hatanaka et al., 1991). In the present study, we examined the effects of various plant hormones on somatic embryogenesis in leaf cultures of *C. canephora*.

Furthermore, this paper describes a procedure for cryopreservation of coffee somatic embryos that ensures the direct development of shoots and roots from cryopreserved somatic embryos without formation of callus or adventitious embryogenesis.

Materials and Methods

Plant materials and culture of leaf discs

Leaf discs of *Coffea canephora* were prepared from leaves of young trees, as described previously (Hatanaka et al., 1991). The leaf discs were cultured on agar-solidified MS medium (Murashige and Skoog, 1962) that had been supplemented with 5 μM cytokinin, by the method of Hatanaka et al. (1991). Cultures were maintained with 14 hours of light and 10 hours of darkness daily, under fluorescent light at an intensity of 30 μmol m^{-2} s^{-1} at 25 °C. For treatment with ethylene, six culture vials (35 mm in diameter x 175 mm in height), capped with aluminum foil, were maintained in a sealed Lucite box (10 cm x 17.5 cm x 10 cm in height) with a silicone stopper for injection and sampling of gases with a syringe (Fig. 1). The air in the box was replaced by fresh air after analysis of gases, which was performed twice a week. Somatic embryogenesis and the growth of explants were quantitated by counting the number of embryoids and measuring the fresh weight of explants, respectively, after two months of culture.

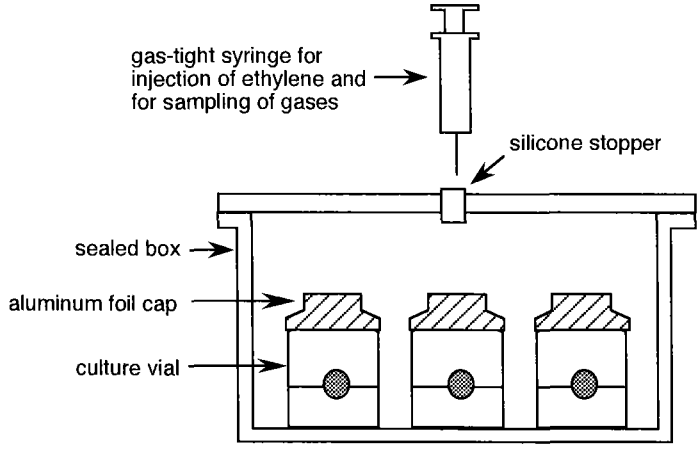

Fig. 1. The culture system for examination of the effects of ethylene on somatic embryogenesis. Culture vials, capped with aluminum foil, were placed in a sealed box with a silicone stopper in its lid.

Treatment with plant hormones

The leaf discs were cultured on media that contained various plant hormones at different concentrations, namely, the cytokinins 2-iP, KI, ZE and BA, the auxins IAA,

IBA, NAA, 4-FA and 2,4-D, and ABA. For treatment with ethylene, ethylene gas was injected into the sealed box. The concentrations of each are given in the legends to Tables 1, 2 and 3, and Fig. 5.

Cryopreservation

Alginate-coated beads containing somatic embryos were prepared by the techniques of Dereuddre et al. (1990) and Niino and Sakai (1992). Precultured somatic embryos were suspended in calcium-free culture medium supplemented with 3% (w/v) sodium alginate and 0.5 M sucrose (Niino and Sakai, 1992, Hatanaka et al., 1994). The mixture was dispensed from a sterile glass pipette into culture medium that contained 0.1 M calcium chloride and 0.5 M sucrose and allowed to stand at room temperature for 20 minutes for polymerization of alginate. Beads of about 5 mm in diameter, each containing one somatic embryo, were precultured in liquid medium supplemented with 0.8 M sucrose for one day at 25 °C. Then, any solution adhering to the beads was wiped off and then beads were subjected to dehydration on sterilized filter paper (Whatman no.2) in a petri dish (6 cm in diameter).

The petri dish was placed in a second Parafilm-sealed petri dish (12 cm in diameter) that contained 35 g of dry silica gel at room temperature (Fig. 2). Dehydration was allowed to proceed for different lengths of time up to 24 hours.

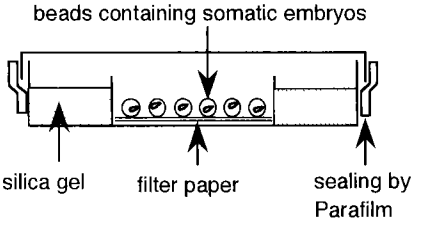

Fig. 2. Methods of dehydration of alginate-coated beads.

After dehydration, about 15 beads were placed in a 1.5-ml Eppendorf tube and plunged into liquid nitrogen for 1 hour (cooling rate : about 190 °C/min.).
Samples were rewarmed by placing the tubes in water at 30 °C (warming rate : 140 °C/min.). This rapid cooling in liquid nitrogen did not cause additional loss of water beyond that occurring during dehydration. Treated beads were transferred onto agar-solidified basal medium without plant hormones under the standard culture conditions.

Results and Discussion

The effects of plant hormones on somatic embryogenesis were examined in *Coffea canephora*. In our previous study, we demonstrated that all explants cultured with 5 μM cytokinin formed embryoids and almost all of them developed to plantlets, and that cytokinins clearly play an important role in somatic embryogenesis in coffee (Hatanaka et al., 1991). When half of the leaf disc was immersed vertically in the medium, embryoids

were formed only at the cut edges of the discs that were in contact with the medium (Fig. 3, 4).

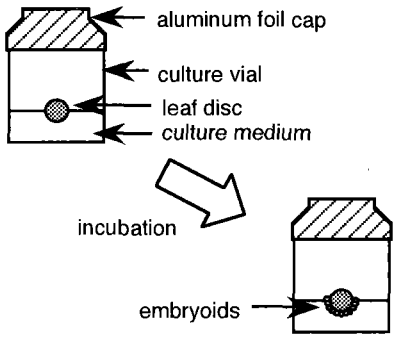

Fig. 3. Formation of embryoids. Embryoids were formed only at edges in the lower half of the disc that was half-immersed in the medium.

Fig. 4. Example of formation of embryoids along to the edge of the lower half of the disc after 2 months of culture. The bar represents 5 mm.

When media that contained various cytokinins at concentration of 5 μM were used, effective embryogenesis was observed on the media that contained 2-iP (Table 1).

Table 1. Effects of various cytokinins at concentration of 5 μM on somatic embryogenesis from leaf discs of *Coffea canephora*.

Kinds of cytokinins (5 μM)	Number of embryoids (N / explant)	Fresh weight (mg / explant)
2-iP	68.1 ± 4.5	70.2 ± 6.5
ZE	13.9 ± 1.8	29.9 ± 2.2
KI	9.4 ± 1.2	31.6 ± 3.3
BA	11.3 ± 2.4	26.1 ± 2.7

The numbers of embryoids and fresh weights were measured after 2 months of culture. Values represent the means ± S.E. of results from 9 replicates.

Table 2. Effects of various auxins at different concentrations on somatic embryogenesis from leaf discs of *Coffea canephora*.

Kinds and concentrations of auxins		Number of embryoids (N / explant)	Fresh weight (mg / explant)
IAA	0	56.2 ± 9.3 (100.0)	34.0 ± 2.6 (100.0)
(μM)	0.5	48.9 ± 3.1 (87.0)	35.2 ± 2.1 (103.5)
	5.0	20.7 ± 3.6 (36.8)	27.6 ± 1.6 (81.2)
	50.0	24.1 ± 3.5 (42.9)	36.2 ± 3.1 (106.5)
IBA	0	79.6 ± 9.5 (100.0)	37.4 ± 5.7 (100.0)
(μM)	0.5	59.4 ± 7.2 (74.6)	44.2 ± 5.9 (118.2)
	5.0	32.8 ± 6.1 (41.2)	30.4 ± 1.8 (81.3)
	50.0	29.9 ± 4.7 (37.6)	43.6 ± 7.9 (116.6)
NAA	0	72.3 ± 5.0 (100.0)	33.8 ± 3.3 (100.0)
(μM)	0.05	80.0 ± 9.3 (110.7)	49.2 ± 5.9 (145.6)
	0.50	50.4 ± 2.1 (69.7)	43.0 ± 2.7 (127.2)
	5.00	11.6 ± 1.8 (16.0)	29.8 ± 1.4 (88.2)
4-FA	0	90.9 ± 4.8 (100.0)	38.4 ± 1.6 (100.0)
(μM)	0.05	91.0 ± 5.0 (100.1)	46.7 ± 1.6 (121.6)
	0.50	38.5 ± 4.2 (42.3)	31.4 ± 2.6 (81.8)
	5.00	17.8 ± 2.2 (19.6)	67.1 ± 6.1 (174.7)
2,4-D	0	53.3 ± 5.3 (100.0)	25.2 ± 1.9 (100.0)
(nM)	5	28.5 ± 3.4 (53.5)	24.3 ± 2.1 (96.4)
	50	9.4 ± 2.1 (17.6)	24.3 ± 3.1 (96.4)
	500	6.2 ± 1.1 (11.6)	72.0 ± 1.5 (285.7)

The culture media contained 5 μM 2-iP and various concentrations of IAA, IBA, NAA, 4-FA and 2,4-D. The numbers of embryoids and fresh weights were measured after 2 months of culture. Values represent the means ± S.E. of results from 9 replicates. The values in parentheses represent percentages relative to controls.

Auxins inhibited somatic embryogenesis in proportion to its concentration, but it increased fresh weights of explants because of the formation of adventitious roots and non embryogenic callus (Table 2). ABA was inhibitory even at concentration as low as 5 μM (Table 3). Auxins and ABA were not needed at all and cytokinin was the essential factor for somatic embryogenesis in leaf culture of *C. canephora*.

Next, we tested the effects of exogenous ethylene on somatic embryogenesis. The addition of ethylene at 6 μl/l had no effect on embryogenesis, while ethylene at 12 μl/l increased the number of embryoids by 45%, as compared with the control (Fig. 5). Higher concentration of ethylene, such as 24 μl/l, inhibited somatic embryogenesis. The concentration at which ethylene promoted embryogenesis was limited to a narrow range

around 12 μl/l. It is important to note that ethylene had both promotive and inhibitory effects on somatic embryogenesis, depending on its concentration. From our results and those of others (Hatanaka et al. 1995), it seems possible that promotion of growth in other plant species, in which growth and differentiation have been found to be limited by ethylene in other studies, is also associated with a particular optimal and limited range of concentrations of ethylene in each case.

Table 3. Effects of ABA on somatic embryogenesis from leaf discs of *Coffea canephora.*

Concentration of ABA (nM)	Number of embryoids (N / explant)	Fresh weight (mg / explant)
0	80.7± 8.9 (100.0)	29.2 ± 2.4 (100.0)
5	65.2± 7.5 (80.8)	31.2 ± 4.8 (106.8)
50	34.6± 5.3 (42.9)	19.3 ± 1.6 (66.1)
500	8.8± 1.3 (10.9)	13.2 ± 0.8 (45.2)

The culture media contained 5 μM 2-iP and various concentrations of ABA. The numbers of embryoids and fresh weights were measured after 2 months of culture. Values represent the means ± S.E. of results from 9 replicates. The values in parentheses represent percentages relative to controls.

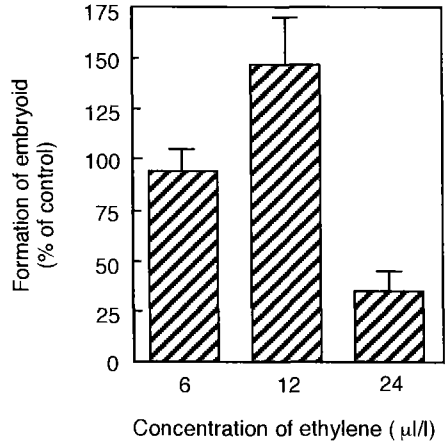

Fig. 5. Effects of various concentrations of ethylene on embryogenesis. The formation of embryoids was estimated after 2 months of culture. Control cultures were maintained without treatment with ethylene. Vertical bars represent the standard errors of means for 12 replicates.

Lastly, we tried to cryopreserve of coffee somatic embryos. The survival rates of alginate-coated embryos before and after cooling to -196 °C at various water contents (of beads) are shown in Fig. 6. In the case of unfrozen coated embryos dehydrated to 13%, the survival rate was 77% but it decreased at water contents below 13% as a result of desiccation injury. In the case of coated embryos cooled to -196 °C after dehydration, the maximal survival (about 63%) was obtained at a water content of 13%. Subsequently, the survival rate rapidly decreased. This decrease was probably due to desiccation injury, since the same trend was observed in dehydrated embryos that were not cooled to -196 °C.

More than half of the revived somatic embryos (63%) after cryopreservation developed shoots and roots directly within 50 days after recultured and normal growth continued even 8 months after reculture (Fig. 7). During recovery growth, formation of callus and secondary embryogenesis were not observed. The remaining cryopreserved embryos ceased their growth after rooting.

The drying technique using alginate-coated somatic embryos is easy to perform and simplifies the dehydration process. Thus, this method may allow the establishment of germplasm banks of coffee using somatic embryos.

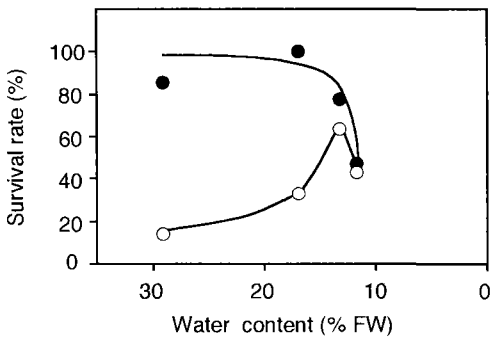

Fig. 6. Survival rates of encapsulated somatic embryos at different degrees of dehydration before (●) and after (O) exposure to liquid nitrogen.

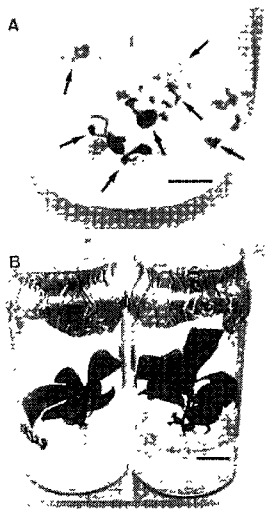

Fig. 7. Recovery of growth of encapsulated somatic embryos that had been cooled to -196°C in alginate-coated beads. A: 50 days after reculture. Arrows indicate the development of shoots. B: 8 months after reculture. Bars represent 1 cm.

Summary

The effects of plant hormones on somatic embryogenesis were studied in leaf cultures of *Coffea canephora*. Embryogenesis occurred only in the regions where cytokinin was in contact with cut edges of coffee leaves. The optimal concentration of cytokinin for somatic embryogenesis was 5 μM. All of the auxins tested (IAA, IBA, NAA, 4-FA and 2,4-D) inhibited the formation of embryos. ABA markedly inhibited somatic embryogenesis in proportion to its concentration, too. When cultures were exposed to ethylene at concentration of 12 μl/l, somatic embryogenesis were promoted. The somatic embryos obtained from culture with cytokinin were similar to zygotic embryos. Encapsulated somatic embryos were successfully cryopreserved, and ensured the direct development of shoots and roots from cryopreserved them without formation of callus and adventitious embryogenesis.

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CARACTÉRISATION BIOCHIMIQUE, BIOLOGIQUE ET MORPHOLOGIQUE DE DIFFÉRENTES POPULATIONS DE *MELOIDOGYNE* SP. PARASITES DU CAFÉIER EN AMÉRIQUE CENTRALE

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INTRODUCTION

Les nématodes du genre *Meloidogyne* sont d'importants ravageurs de la caféiculture en Amérique Centrale. Au niveau mondial, quatorze espèces ont été répertoriées dont les plus communes sont *M. incognita et M. exigua* Elles ont été reconnues comme faisant de graves dégâts sur le caféier en Amérique du Sud et Centrale, en Afrique et en Asie. En ce qui concerne l'Amérique Centrale, les deux espèces principales sont rapportées du Guatemala, Salvador, Nicaragua et du Costa Rica, (Campos et. al 1990). Une autre espèce très pathogène a été décrite au Costa Rica sous le nom de *M. arabicida*, (Lopez et Salazar, 1989). En général les espèces de *Meloidogyne* développent des grosses galles sur les racines des plants qu'elles parasitent. Les galles envahissent tout le système racinaire et les plantes attaquées perdent leur vigueur.
De nombreux travaux ont montré que les variétés de *Coffea arabica* sont sensibles aux attaques de *Meloidogyne* spp ; par contre quelques variétés de *C. canephora* sont résistantes. En Amérique Centrale se déroulent actuellement des programmes d'amélioration de *C. arabica* pour leur résistance à *Meloidogyne* spp. La caractérisation précise des espèces de nématodes parasites est primordiale dans de tels programmes. C'est l'objectif du travail presenté ici

MATERIEL ET METHODES

Les travaux ont été réalisés au laboratoire de nématologie du CIRAD-FLHOR à Montpellier. Ils incluent des études de caractérisation morphologique, biochimique et biologique sur 25 populations de *Meloidogyne* spp prélevées e Amérique Centrale.

Neuf populations ont été caractérisées biologiquement et morphologiquement ; toutes les populations ont été caractérisées biochimiquement.

Les caractérisations biochimiques ont été faites selon la méthode de Esbenshade et Triantaphyllou (1985), basée sur l'étude électrophorétique des isoenzymes des estérases.

Les études de caractérisation biologique ont été effectuées par la mesure du pouvoir pathogène de neuf populations sur trois variétés de *Coffea arabica* : ET-28 d'origine éthiopienne (résistante), Catuai (sensible) et

Sarchimor C-1669-20 (résistante).Des plantes âgées de deux mois ont été inoculées avec 300 juvéniles (J2). Les paramètres relevés 90 jours après l'inoculation sont: la hauteur des plantes, le poids frais des racines, le poids frais des parties aériennes, la quantité de nématodes par plante et par gramme de racine (l'extraction des nématodes est faite dans la totalité des racines), la densite de galles par plant notée de 0 à 5.

La caractérisation morphologique a été effectuée en étudiant les plaque périnéales des femelles, pour cela on a utilisé la technique de Harmant et Sasser (1985).

RESULTATS ET CONCLUSIONS

Les estérases sont les isoenzymes les plus utilisées pour le diagnostic biochimique des différentes espèces du genre Meloidogyne.
Neuf phénotypes différents (Esbenshade et Triantaphyllou, 1985) ont été trouvés sur les vingt-cinq populations étudiées (Tableau 1).

Le phénotype A2 a été trouvé dans deux populations du Salvador et correspond à l'espèce *M. arenaria.*
Les populations du Guatemala se caractérisent par les phénotypes F1 et VS1-S1. Le premier, trouvé sur six populations, correspond à un phénotype déjà signalé dans une population isolée du caféier au Brésil, le deuxième à une espèce décrite à Puerto Rico comme *M. mayaguensis.*
Trois populations du Costa Rica présentent le phénotype S1 qui est associé aux espèces *M. incognita*, *M. chitwoodi* et *M. platani.*
Les phénotypes J3 et "CR" ont été trouvés sur deux populations du Costa Rica, le premier correspond à l'espèce *M. javanica*, le deuxième est un phénotype inconnu trouvé pour la première fois.
La population du Brésil présente un phénotype nouveau qui a été nommé "Br".
Le phénotype VF1 correspond à *M. exigua*, il a été observé pour une population du Honduras. Quatre populations du Costa Rica, quatre du Nicaragua et trois du Honduras ne révèlent aucun profil estérasique (profil "blanc" ou "O").

Les tableaux 2, 3 et 4, montrent le taux de multiplication de neuf populations sur trois variétés de caféier.
Les trois populations du Guatemala se multiplient intensemment sur Catuai, moyennement sur Sarchimor et quasiment pas sur ET-28 ; elles développent des petites galles et les femelles pondent leurs masses d'oeufs à l'extérieur des galles.
Les trois populations du Costa Rica se comportent de façon similaire à celles du Guatemala sur Catuai et Sarchimor. Toutefois elles parviennent à se devélopper significativement sur ET-28 ; l'une d'entre elle, originaire de Juan viñas, s'y multiplie fortement. Ces populations développent des grosses galles sur les trois variétés et les femelles pondent leurs masses d'oeufs à l'interieur des galles.
Les deux populations du Salvador (*M. arenaria*) se multiplient fortement sur Catuai et Sarchimor mais très faiblement sur ET-28. Elles développent des petites galles sur les racines et pondent leurs masses d'oeufs à l'extérieur des galles.
La population du Brésil se devéloppe moyennement sur Catuai et très faiblement sur Sarchimor et ET-28 ; elle induit des petites galles sur les racines, et pond ses masses d'oeufs à l'extérieur des galles.

L'examen des plaques périnéales des femelles montre une grande variabilité inter et intra populations. Toutefois les deux populations du Salvador ont pu être identifiées grâce à ces critères : il s'agit de l'espèce *M. arenaria.*

Ces résultats, démontrent que l'analyse des estérases est une méthode fiable pour l'identification des espèces de Meloidogyne, et permet d'oserver la diversité spécifique des nématodes à galles, parasitant les caféiers en Amérique Centrale.

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Tableau 1: Liste des populations caractérisées par isoenzymes des estérases

Code Pop.	Pays	Lieu de prelevément.	Espèce supposée	Phen. Estérase	Espèce
S1- M-9	Salvador	Canton C. grande	*M. incognita*	A2	*M. arenaria.*
S2-M-10	"	Finca M. Bello.	*M. incognita*	A2	*M. arenaria.*
B1-M-2	Brésil.	Sao Paulo.	*M. incognita*	Br	*M. sp*
G1-M-3	Guatemala	Finca la Libertad.	*M. sp*	F1	*M. sp*
G2-M-5	"	Finca Providencia.	*M. incognita*	F1	*M. sp*
G3-M-6	"	Finca Providencia.	*M. sp*	F1	*M. sp*
G4-M-20	"	Finca Elviras.	*M. sp*	F1	*M. sp*
G5-M-21	"	Finca Don Bosco.	*M.exigua*	VS1-S1	*M. mayaguensis*
G6-M-22	"	Finca Panorama.	*M. incognita*	F1	*M. sp*
G7-M-23	"	Finca Manaques	*M. incognita*	F1	*M. sp*
C1-M-8	C. Rica	Finca CICAFE.	*M. arabicida*	CR	*M. sp*
C2-M-4	"	Finca Juan Viñas.	*M. arabicida*	O	*M. sp*
C3-M-1	"	H. Finca CICAFE	*M. sp*	O	*M. sp*
C4-M-7	"	Region Turrialba.	*M. exigua*	O	*M. sp*
C5-M-18	"	Region Turrialba.	*M. incognita*	J3 et S1	*M. javanica et M. incognita.*
C6-M-19	"	Finca CICAFE	*M. arabicida*	O	*M. sp*
C7-M-24	"	CATIE.	*M. arabicida*	S1	*M. incognita.*
C8-M-25	"	CATIE.	*M. incognita*	S1	*M. Incognita.*
H1-M-15	Honduras	Fincas D. Paraiso.	*M. sp*	VF1	*M. exigua.*
H2-M-16	"	Fincas D. Paraiso.	*M. sp*	O	*M. sp*
H3-M-17	"	Fincas D. Paraiso.	*M. sp*	O	*M. sp*
N1-M-11	Nicaragua	Fincas. P. Carazo.	*M. sp*	O	*M. sp*
N2-M-12	"	Fincas. P. Carazo.	*M. sp*	O	*M. sp*
N3-M-13	"	Fincas. P. Carazo.	*M. sp*	O	*M. sp*
N4-M-14	"	Fincas. P. Carazo.	*M. sp*	O	*M. sp*

Tableau 2 : Effet de neuf populations de Meloidogyne spp sur la variété ET-28. (Moyenne de cinq répétitions.)

Pays	Code Pop.	phen. Estérase.	Echelle Galles. 0-5	Taille Galles. g/p	Poids plante (grs).	N/g racines.	Masse d'oeufs. Int/Ext	Necrose +/-
C. Rica	C-2	0	3	g	9,6	3434	Int	0
	C-3	0	2	g/p	10	570	Int	0
	C-4	0	3	g	9,6	512	Int	0
Brésil	B-1	Br	0	-	7,6	0	Ext	0
Guat.	G-1	F1	0	-	7,7	440	Ext	0
	G-2	F1	0	-	10,8	23	Ext	0
	G-3	F1	0	-	10.05	19	Ext	+ -
Salv.	S-1	A2	3	p	5,85	557	Ext	+
	S-2	A2	1	p	8,17	40	Ext	0
	Temoin	----- -	------	------	9,8	--------	-------	--------

Tableau 3 : Effet de huit populations de Meloidogyne spp sur la variété Catuai. (Moyenne de sept répétitions).

Pays	Cod. Pop.	Phen. Estérase.	Echelle Galles. 0-5	Taille Galles. g/p	Poids plante (g).	N /g racines.	Masses d'oeufs Int/Ext	Necrose +/-
C. Rica	C-3	0	2,1	g/p	11,27	1744	Int	0
"	C-4	0	2,7	g	12,45	1409	Int	0
Brésil	B-1	Br	2,7	p	10,79	1207	Ext	+
Guat.	G-1	F1	2	p	9,71	2245	Ext	+
"	G-2	F1	2,6	p	8,33	2272	Ext	+
"	G-3	F1	3,7	p	10,11	1690	Ext	+
Salv.	S-1	A2	3	p	9,85	2833	Ext	+
"	S-2	A2	2,4	p	10,6	1856	Ext	+
	Temoin	--------	-------	-------	9,58	-------	-------	--------

Tableau 4 : Effet de neuf populations de Meloidogyne spp sur la variété Sarchimor (C-1669-20), (Moyenne de sept répétitions).

Pays	Cod. Pop.	Phen. Estérase	Echelle Galles. 0-5	Taille Galles. g/p	poids plante (g)	N/g racines	Masses d'eoufs Int/Ext.	Necrose +/-
C. Rica	C-2	0	1.3	p	11,96	347	Int.	0
	C-3	0	1.3	g	11	1125	Int.	0
	C-4	0	1,6	g	11,62	700	Int.	+
Brésil	B-1	Br	0	-	12,27	70	Ext.	0
Guat.	G-1	F1	1	p	12.98	614	Ext.	+
	G-2	F1	1	p	12,74	452	Ext.	+
	G-3	F1	1.1	p	13,80	225	Ext.	+
Salv.	S-1	A2	2.7	p	10.80	3723	Ext.	+
	S-2	A2	3	p	11.5	2402	Ext.	+
	Temoin	--------	-------	------	10.29	--------	-------	--------

WATER STRESS EFFECTS ON LEAF PHOTOSYNTHESIS AND DIFFUSIVE CONDUCTANCES OF THREE *COFFEA* SPECIES *(C. ARABICA, C. CANEPHORA, C. LIBERICA)*

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INTRODUCTION

There are three economically important species of *Coffea*, *arabica*, *canephora*, and *liberica* for agricultural production in the world. The former two are widely cultivated in the sub- and tropical countries, whereas the production area of *liberica* is comparatively too small in all species because of its low seed quality.

The correlations between productivity, ecological adaptability and physiological characteristics have been useful for a selection criterion in coffee cultivating. Comparative studies of growth analysis and photosynthetic rates under laboratory controlled conditions and in field conditions provided many important informations about growing coffee, especially for *arabica* species. However, there have been few studies giving physiological explanations for adaptability of other two species, *canephora* and *liberica,* to environmental injury such as water stress. Some earlier knowledges of *C. canephora* were reported for net photosynthesis under controlled conditions (Munes *et al.*, 1969; Sondahl *et al.*, 1976), for growth analysis (Huxley, 1967), and for leaf temperature and stomatal resistance under sun and shaded conditions (Butler, 1977). Meinzer *et al.* (1990a, 1990b) reported on variation in water use efficiency and other photosynthetic characteristics among five *Coffea arabica* cultivars under different soil moisture regimes and a increasing pattern of leaf internal CO_2 concentrations independent of stomatal limitations on the decline in leaf photosynthesis. Generally speaking, coffee plants seem to be rather resistant to drought although the growth may be depressed to a large extent. Comparative research on the effects of water stress on plant productivity, transpiration and photosynthesis rates under controlled environmental conditions among these

economically important species of *Coffea*, *arabica*, *canephora*, and *liberica* , however, is scanty.

In the present study, we compared photosynthetic responses of three coffee species, *arabica*, *canephora*, and *liberica,* to water stress in order to discuss how importance of non-stomatal contribution to the reduction in their leaf photosynthesis.

MATERIALS AND METHODS

Plant materials and growing conditions

One year old coffee plants (*Coffea arabica* L.; cv. Typica, *Coffea canephora* P. ex F.; Robusta-1 line and *Coffea liberica* B. ex H.; Liberica-1 line) with five to six mature leaf pairs, established from seeds, were planted in 1/5000*a* Wagner pots containing a mixture of soil and leaf mold (3:1 by volume) with a single plant per pot and maintained in a temperature controlled glasshouse. Culture conditions were 25/20°C day/night temperature, 60/80% day/night ambient relative humidity and about 70% full sunlight with a 12 to 13-h photoperiod. All plants were well watered until the water deficit treatment began.

Water stress treatment and measurements of leaf water potentials

Water deficit, imposed by withholding water supply allowed leaf water potentials (ϕ) to fall from -1.0 to -4.0 MPa over one week. The ϕ of the opposite leaf that used for gas exchange measurements was determined with a C-52 sample chamber and thermocouple psychrometer (HR-33(T), Wescor Inc.) soon after measuring gas exchange each day. Three 6 mm diam. leaf segments were placed for 3 h in a sample chamber to reach equilibrium, and then the ϕ was measured.

Gas exchange measurements

Uniform, most recently expanded leaves (second node from the apex) were chosen for measurements of gas exchange. Transpiration and CO_2 dependent rate of net photosynthesis of single attached leaves were determined in an open gas-exchange system with a plexiglass, temperature controlled leaf assimilation chamber (Kanechi *et al.* 1991). The measurements were carried out at constant air temperature (25°C) and at vapor pressure deficits (VPD) less than 5 hPa to avoid stomatal closure due to leaf desiccation. CO_2 concentrations of inlet air stream varied from 30 to 1300 μmol/mol using a massflow controller system. Light from metal halide lamps was filtered through 10 cm water to reduce leaf heating, giving 800 μmol/m2/s of photosynthetic active radiation (PAR) at the leaf surface inside the assimilation chamber.

Calculations of leaf intercellular CO_2 concentration (C_i) and other gas exchange parameters (stomatal and mesophyll conductances) were made according to von Caemmerer and Farquhar (1981). We calculated the contribution of the non-stomatal component to the decline in photosynthesis during leaf dehydration using the changes in the CO_2 dependent photosynthesis response curves and details for its estimation was mentioned in a previous report (Kanechi *et al.*, 1991) according to Matthews and Boyer (1984).

RESULTS AND DISCUSSION

Net photosynthesis under different ϕ conditions

Fig. 1 shows leaf net photosynthetic rates of three coffee species with different ϕ. With decreasing ϕ, net photosynthetic rate decreased progressively but in different patterns within species. *Canephora* showed a most sensitive decrease in net photosynthetic rate with decreasing ϕ, whereas it for the dehydrated *arabica* leaves decreased curvilinearly against the ϕ. *Liberica* leaves had a intermediate response of photosynthesis to decreasing ϕ between other two species. The 50% reduction in photosynthesis due to leaf dehydration occurred at approximately -2.6, -1.7 and -2.1 MPa for *arabica*, *canephora* and *liberica*, respectively.

This half reduction in photosynthesis was observed for *arabica* coffee leaves with same range of ϕ in earlier work (Kumar and Tieszen, 1980). In droughted coffee leaves, even at their bulk leaf turgor levels between 0 and 0.1 MPa (-2.0 MPa > ϕ > -2.5 MPa), a approximately 50% of the average maximum photosynthetic rate was recorded, which has contributed to the substantial drought resistance shown by coffee plants (Meinzer *et al.*, 1990b). Also, the complete inhibition of photosynthesis of dehydrated leaves occurred at about -3.0 and -3.5 MPa for *canephora* and *liberica*, but *arabica* leaves had a still positive photosynthetic rate at about -4.0 MPa of ϕ.

Stomatal and mesophyll conductances under different ϕ conditions

Changes in leaf conductances (stomatal and mesophyll) of dehydrated leaves with various ϕ were also shown in Fig. 2. Stomatal and mesophyll conductances decreased sensitively with decreasing ϕ, indicating that both conductances contributed to decline in leaf photosynthesis under water stress conditions for all three coffee species. The decrease in stomatal conductance in the range from ϕ of -1.0 MPa down to -1.5 MPa was more sensitive to decreasing ϕ than that of mesophyll conductance. This different response between stomatal and mesophyll conductances suggested that in mild water-stressed leaves photosynthetic rates decreased primary due to loss of CO_2 availability, which attributed to the low CO_2 diffusive supply into leaves by quick closure of stomata.

There are several reports (Wormer, 1965; Bierhuizen *et al.*, 1969; Kumar and Tieszen, 1980) in which a close relationship between stomatal closure and soil water depletion in *arabica* coffee, indicating an efficient water control of coffee leaves. Also, Meguro and Magalhães (1983) showed a significant increase in leaf diffusive resistance in the range of ϕ down to -2.5 MPa. Meinzer *et al.* (1990) showed the decreasing relative symplast volume and Ci with both decreasing ϕ and bulk leaf turgor of dehydrated coffee leaves, suggested that stomatal limitation was not responsible for the decline in photosynthesis.

Canephora showed the most quickly decrease in both conductances among three species, indicating the most susceptible to water stress leading to severe inhibition of photosynthesis. *Arabica* might be the most tolerant to leaf dehydration because of positive both conductances at about -4.0 MPa which inhibited photosynthesis completely in other two species.

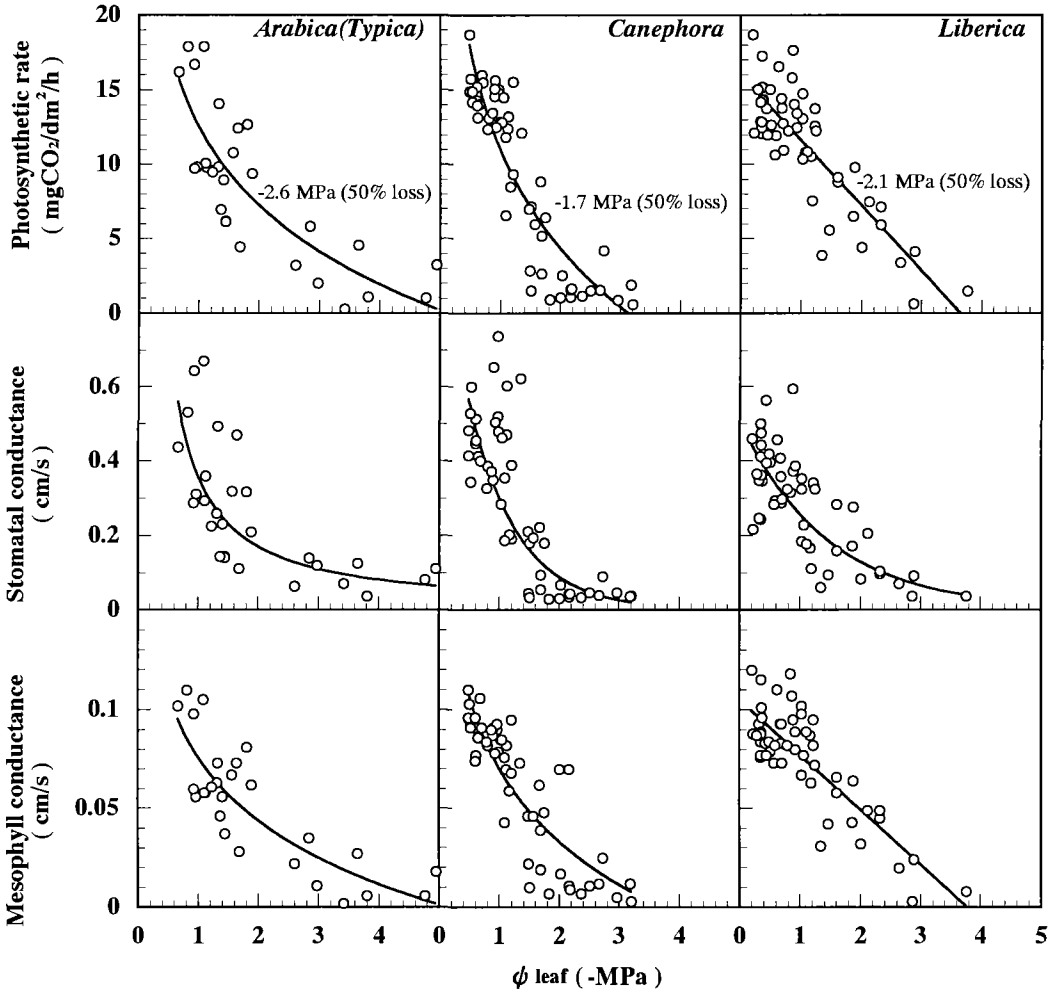

Fig.1 Photosynthetic rates, stomatal and mesophyll conductances of dehydrated coffee leaves with various ϕ. Measurement conditions: 25 °C, 800 μmol PAR/m^2/s, 350 μmol/mol ambient CO_2 concentration, and VPD < 5 hPa. At values of ϕ in the upper graph, photosynthetic rate reduced by 50%.

Photosynthetic rate-C_i relationships and non-stomatal limitation at ambient air

Fig. 2 shows the depression in photosynthetic CO_2 response curves of intact leaves with decreasing ϕ of three coffee species. The CO_2 dependent rates of photosynthesis increased curvilinearly as Ci increased for leaves of all species at ϕ > -1.0 MPa. As leaves dehydrated, photosynthesis of all species saturated at lower Ci than each respective control without water stress and the CO_2 saturated rate of photosynthesis decreased for all species. Water deficit

reduced the carboxylation efficiency (CE: the initial slope of photosynthetic CO_2 response curve) by 75%, 67% and 71% in the dehydrated leaves with about -2.0 MPa ϕ of *arabica*, *canephora* and *liberica*, respectively. This was probably related to the deactivation of the ribulose-1,5-bisphosphate carboxylase of dehydrated leaves *in vivo*.

A well watered leaf which is photosynthesizing at ambient CO_2 level (350 μmol/mol) has a Ci less than ambient because of the inevitable stomatal component. This Ci value was about 300, 320 and 310 μmol/mol for *arabica*, *canephora* and *liberica*, respectively, suggested that a biggest decrease in Ci from 350 μmol/mol in *arabica* leaves had a substantially large stomatal limitation without water stress. Change in Ci was dependent on partitioning between biochemical (non-stomatal) and diffusion (stomatal) factors in photosynthesis under environmental stress. An increase in stomatal resistance at ambient photosynthetic rate usually observed for dehydrated leaves lower the Ci less than that of respective control. This could be derived from that stomatal closure primary occurred when the leaves began to wilting. The Ci at ambient conditions initially decreased with decreasing ϕ, but later increased when leaf dehydration became sever (arrows in Fig. 2).

Munes *et al.* (1968) reported that an increase in leaf internal CO_2 concentration could mainly account for the lower rate of photosynthesis recorded at high leaf temperature. Thus, this change in Ci might be associated with stomatal and non-stomatal limitation of photosynthesis under water stress.

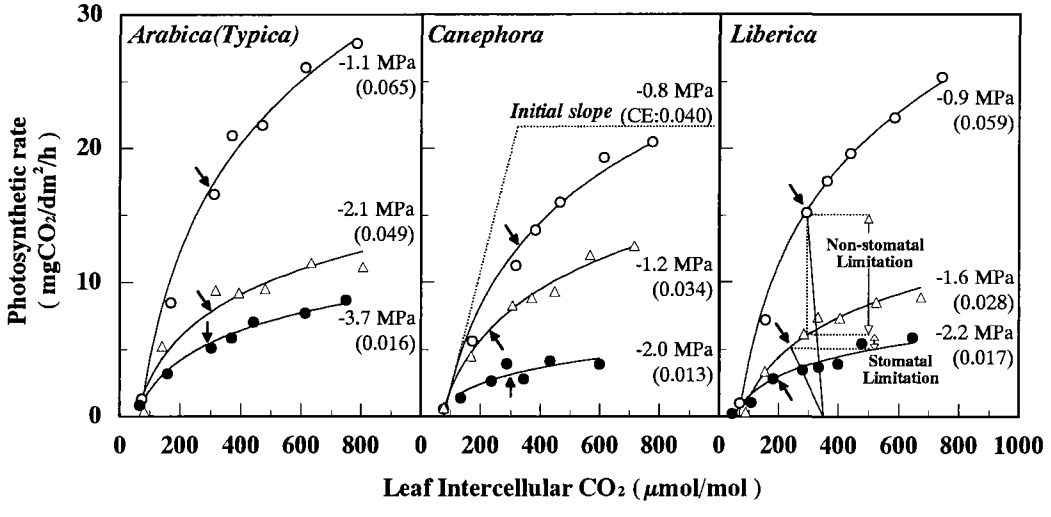

Fig.2 The CO_2-dependent photosynthetic rates of dehydrated coffee leaves with various ϕ. Measurement conditions: 25 °C, 800 μmol PAR/m^2/s, VPD <5 hPa. Arrows indicate the estimated intercellular CO_2 concentrations of photosynthesizing leaf at 350 μmol/mol ambient air conditions. The carboxylation efficiency (CE) was shown as a initial slope of the response curve.

At near -1.0 MPa leaves of all species (Fig. 3), non-stomatal limitations were about 50-60%, indicating that the lightly dehydrated leaves decreased photosynthesis equally due to stomatal closure and due to lost biochemical activity of mesophyll photosynthesis. At ϕ below -1.5 MPa, the non-stomatal limitation increased up to 80% in *canephora* and 90% in *arabica*, whereas it was about 70% in *liberica*, indicating that the chloroplast activity of dehydrated leaves might be most susceptible for *arabica* and would be most tolerant to progress in water stress for *liberica* among three species. Under more severe leaf dehydration (ϕ < -2.0 MPa), the non-stomatal limitation approached 100% for all species, indicating that most of the decline in leaf photosynthesis was solely due to loss of intrinsic capacity in the mesophyll photosynthesis.

Our previous report (Kanechi *et al.* 1991) explained that the primary cause of the decrease in photosynthesis of *Coffea arabica* leaves exposed to chronic drought was a inhibition of mesophyll photosynthetic capacity. In the case of rapidly decreasing ϕ in present study, stomatal conductance and mesophyll photosynthetic capacity appeared to be affected independently. Decreasing ϕ under water stress led to a sharp decline in photosynthetic rates primarily due to low stomatal conductance for CO_2 diffusion, and further decrease in ϕ dramatically inhibited mesophyll photosynthetic capacity in three coffee species.

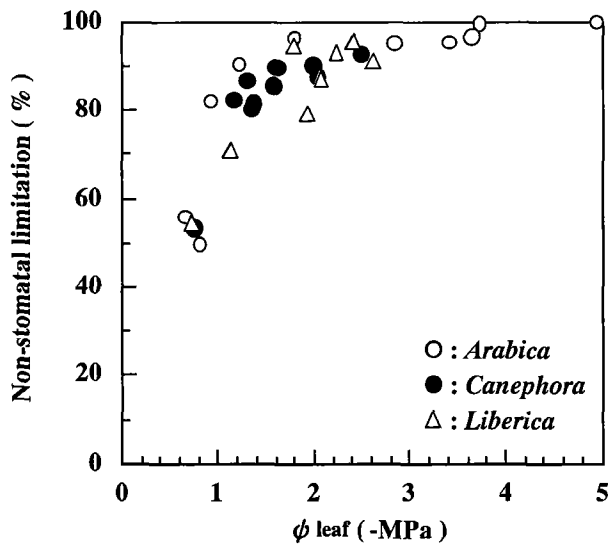

Fig.3 The non-stomatal contribution to the reduction in photosynthetic rates measured at 350 μmol/mol ambient air conditions of dehydrated coffee leaves with various ϕ. The percentage of the limitation was estimated graphically from the photosynthetic rate-intercellular CO_2 response curves shown in Fig. 2.

ABSTRACT

A comparative study of leaf water relations to photosynthetic rates was conducted on three economic coffee species (*arabica*, *canephora*, *liberica*) to investigate their CO_2 diffusive limitations on leaf photosynthesis under water stress conditions. Photosynthetic rates of *arabica* leaves reduced gradually with decreasing leaf water potentials, while in both *canephora* and *liberica* leaves there were sharp declines in photosynthetic rates as their leaves wilted due to dehydration. A 50% inhibition of normal photosynthetic rates was measured at leaf water potential of about -2.6 MPa, -1.7 MPa and -2.1 MPa in *arabica*, *canephora* and *liberica*, respectively. Decreases in photosynthesis of all three species were associated with lowered stomatal and mesophyll conductances by wilting their leaves, which could suggest a functional linkage between mesophyll photosynthesis and stomata. We also graphically estimated non-stomatal limitations on decreases in photosynthesis from leaf internal CO_2 concentration (Ci)-photosynthetic rate response curves measured with turgid and wilted leaves. In all species the inhibition of light-saturated photosynthesis at leaf water potentials around -1.0 MPa, measured at ambient CO_2 concentration, was equally attributable to stomatal and non-stomatal limitations, but the further inhibition below -2.0 MPa was caused solely by non-stomatal limitation which led to a lowered activity of mesophyll photosynthesis.

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STUDIES ON THE MANAGEMENT OF RUST RESISTANT CATIMOR VARIETIES IN PAPUA NEW GUINEA

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1- Introduction

The majority of coffee varieties grown in Papua New Guinea are tall and rust susceptible. The smallholder sector produces approximately 70% of the annual crop. A small proportion of the estate sector has been planting rust susceptible, dwarf Caturra since the early 1980's. Due to an outbreak of coffee leaf rust (*Hemileia vastatrix)* in 1986 (Muthappa & Kokoa 1989), compact, rust resistant Catimor varieties were introduced and evaluated for their adaptability (Kiara, 1992; Charmetant, et al, 1993). In a country like Papua New Guinea, with no definite dry season (McAlpine, et al 1983), high tree density can be a deterrent to cherry picking due to continuous wetness. A trial was set up with low and medium densities, and the canopy trained as single or multiple bearing heads to optimize yields. It has been established that, multiple bearing stems compensate for low density in tall varieties (Kiara and Stolzy, 1986).

2- Materials and Methods

A trial was established with rust resistant Catimor seedlings, in March 1990, at the Western Highlands Province, Coffee Research Institute Substation. The objective of the trial was to study the influence of three field configurations, three tree densities and two canopy structures on green bean yields. The three field configurations used were square, rectangle and triangle. The densities were 2600, 3900 and 5200 trees/ha. Half of the trees were capped in the field to produce multiple bearing stems. The bearing heads per hectare were 2,600 to 10,400. The treatment combinations were 3 x 3 x 2 in a factorial design. Coffee yields on plot basis, were recorded over a three year period between 1991/92 and 1993/94. The harvested yields were converted to green bean yields per hectare. It was observed that, field capping delayed cropping in multiple stem bearing trees.

3- Results

The first trees started flowering in January 1991. Cherry harvesting started in November 1991. In 1991/92, most of the crop was harvested between July and September 1992. As the trees matured

during the second and third years, the peak cherry picking shifted to between May and June. During the first year 1991/92, mean yield was 1824 kg gb/ha. The highest yield of 3180 kg gb/ha was produced in 1992/93. The highest tree density of 5200 trees/ha produced the highest yield of 3958 kg gb/ha. In 1993/94, the yield dropped to 2032 kg gb/ha. The yield drop was probably due to the well known biennial cropping in tall coffee varieties.

The effects of field configuration and tree density on green bean yields are presented in Figure 1. Over the three year period, the yield differences between the three field layouts were very small. The respective yields were 2273, 2305 and 2440 kg gb/ha for square, rectangle and triangle arrangements. The lowest tree density gave the lowest green bean yields. The yield increases due to density in 3900, and 5200 trees/ha were 36.6% and 50.3% respectively over 2600 trees/ha. A combination of triangular planting with the highest tree density (5200 trees/ha), produced the highest green bean yields (Fig.1).

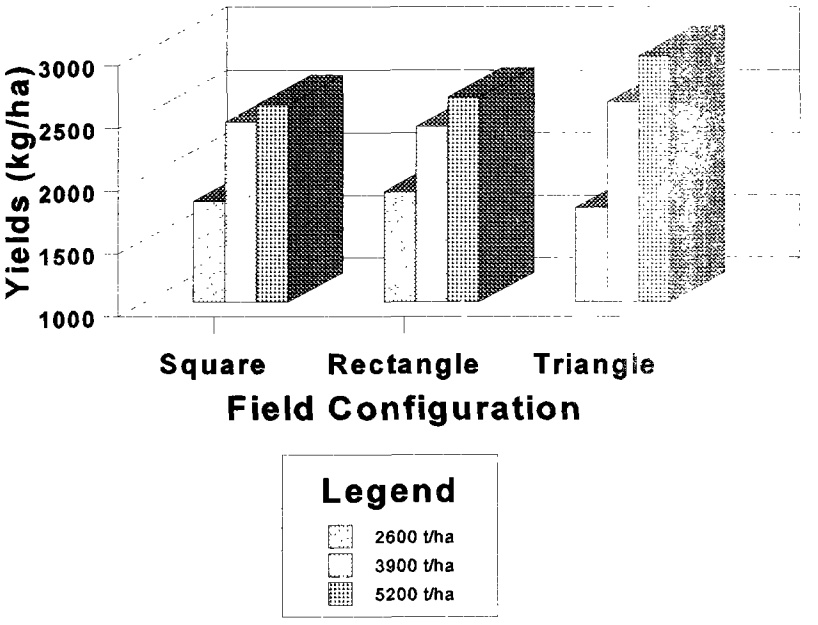

Figure 1: Effects of field configuration and tree density on green bean yields.

The effects of tree density and the number of bearing stems per tree are presented in Figure 2. The observable yield increases were mainly due to tree density. The highest tree density gave the highest green bean yields. The yield increase due to the range of tree density studied, could be explained by a linear regression equation:- $Y = 970 + 0.351X$, $r = 0.417$, where Y = yield, X = tree

density and r = coefficient of regression. The number of bearing stems per tree did not increase the green bean yields. At the lowest tree density, the multiple bearing stems produced less than the single stems. Similarily, at the highest tree density of 5200 trees/ha, multiple bearing stems produced lower yields than the single stems. It appears then, that in compact coffee growth, single bearing stems are more productive than multiple stems, although the differences were not statistically significant. Therefore, the effort spent in producing multiple bearing stems in this trial was not worthwhile.

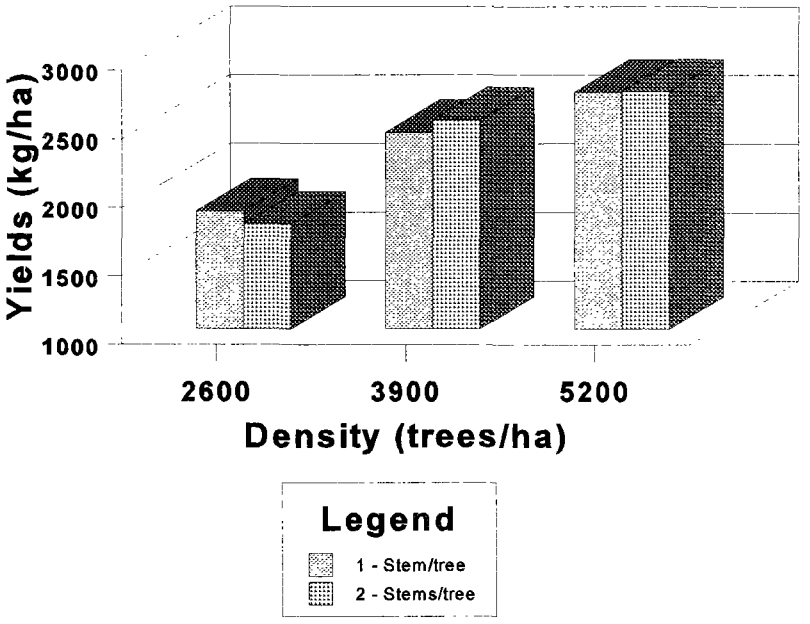

Figure 2: Effects of tree density and stems per tree on green bean yields.

4- Discussion and Conclusions

The suitable coffee growing conditions of Papua New Guinea, with regular rainfall and warm temperatures, allowed coffee to establish and start bearing early. Capping of young coffee trees to produce multiple bearing heads delayed production. The results indicated that, the best method of growing compact rust resistant varieties is planting trees at high density and in triangular configuration. The best tree density appeared to be 5200 trees per hectare, the highest density planted in this trial. Ten thousand four hundred (10,400) bearing stems per hectare produced the same as five thousand two hundred (5,200) stems. The training of the tree canopy, to produce multiple bearing stems in compact varieties, is not a practical proposition. The findings are in agreement with Kenyan results on Ruiru 11, where training on multiple stems depressed yields over the first production cycle (Njoroge, et al, 1993).

While more attention should be paid to high density plantings, it should be borne in mind that

cherry pickers do not like getting wet. They tend to skirt the high density blocks, harvesting the bushes at the edges. A compromise density will probably lie somewhere between 5000 and 6000 trees/ha, although higher densities may give higher yields. The best management appears to be a combination of high tree density with triangular field arrangement.

5- Acknowledgements

The authors are thankful for the assistance rendered by the staff of the Agrophysiology Department of the Coffee Research Institute in accomplishing the work. The Coffee Industry Corporation LTD, funded the project. Ms D Nad assisted with data processing and typing of the manuscript.

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7- Abstract

A trial was established in Papua New Guinea in March 1990 with rust resistant compact Catimor, to study the effects of field layout, tree density and canopy structure on the green bean yields. The trees were planted in three field configurations, viz:- square, rectangle and triangle. The three tree densities were 2600, 3900 and 5200 trees/ha. Half of the trees were capped to produce multiple bearing stems, while half of them were left on single stems. Yield data were recorded over a period of three years between 1991/92 and 1993/94 coffee seasons. It was found that triangular tree layout was the best field configuration. Coffee yields increased linearly with the increasing tree density. The highest tree density of 5200 trees/ha produced the highest green bean yields. Multiple bearing stems failed to increase green bean yields over the single stems, in the rust resistant compact coffee cultivar.

ESTABLISHMENT OF RUST RESISTANT ARABICA COFFEE CULTIVAR UNDER TEMPORARY SHADE AND INORGANIC FERTILIZER REGIMES IN PAPUA NEW GUINEA

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1- Introduction

In Papua New Guinea, approximately 70% of the coffee is produced by smallholders who hardly use agricultural chemicals. The majority of the smallholders and some plantations grow coffee under shade. In young coffee, temporary shade is provided by shrubs like Crotalaria anagyroides, Tephrosia candida, Cordyline spp and Justicea gendarussa, which are alternate hosts to Pink Disease (Phanerochaete salmonicolor, Berk & Br.) that attacks coffee (Kimber, 1977). In mature coffee, shade is provided by perennial trees like Albizzia stipulata, Casuarina oligodon and Leucaena spp. (Bourke, 1984). Shade protects coffee from strong sun rays, winds, minimizes the effects of hot and cold, and provides plant nutrients. It has been shown in Costa Rica that nutrient recycl'ng is rapid in coffee under Erythrina popeppigiana, resulting in high coffee yields (Fassbender, et al 1985). An agroforestry farming system in the highlands of Papua New Guinea, with food crops, Coffea arabica and Casuarina as the main components, has been practised for many years (Bourke ,1978).

With the outbreak of coffee leaf rust (Hemileia vastatrix) in 1986 (Muthappa and Kokoa, 1989) and the introduction of rust resistant Catimor (Kiara, 1992), it became necessary to carry out investigations on shade requirements for the new cultivars.

2- Materials and Methods

A trial was started in March 1993 at the Highlands Agricultural Experiment Station, Aiyura to determine the benefits of temporary shade, and the level of shade required to provide beneficial effects. Coffee trees were planted at 2.00m x 1.00m (5000 trees/ha). Tephrosia seeds were planted between coffee rows. Five shade treatments were combined with two fertilizer treatments in a 5 x 2 factorial design. The ***Fertilizer treatments:-*** F0 - No fertilizer;
F1 - Normal inorganic fertilizer. The ***Shade treatments:-*** S0 - No shade; S1 - Complete row of shade every three coffee rows; S2 - Shade spaced at 50 cm every three coffee rows; S3 - Complete row of shade every two coffee rows; S4 - Shade spaced at 50 cm every two coffee rows.

Shade hedges were pruned every 2-3 months and prunings were left between the coffee rows as mulch. Soil samples were taken from the top and subsoils for nutrient analysis before coffee planting. Further

soil and coffee leaf samples were taken in July 1994. The samples were analyzed for major and trace elements. Tree growth parameters were monitored every six months on stem diameter, tree height, total and bearing branches.

3- Results

The effects of temporary shade and inorganic fertilizers on growth parameters and plant nutrients are presented in Figures 1and 2, and Table 1 below. Results of stem diameter, bearing branches and plant nutrients are discussed in detail.

3.1- Growth Parameters

The coffee trees with no shade had the thickest stem diameters. Temporary shade every two coffee rows produced the thinnest stem diameters. Fertilized plots produced trees with thicker stem diameters. Shaded coffee trees were taller than the unshaded ones. The coffee trees at closer shade spacings, were much shorter than those at wider spacings. Fertilized coffee trees were much taller than the unfertilized ones. The number of primary branches per coffee bush decreased as the number of temporary shade rows increased. Regular fertilizer applications increased the number of the primary branches per coffee bush. The highest number of bearing branches was realized in coffee trees with temporary shade planted every three coffee rows. In zero shade and shade every two coffee rows, fertilizer greatly increased the number of the bearing branches. The greatest response to fertilizer was obtained where a complete row of shade was planted every two coffee rows (S3). Those were the plots with maximum competition for plant nutrients between the coffee trees and the shade shrubs. Figures 1 & 2 show the treatment effects on stem diameter and bearing branches.

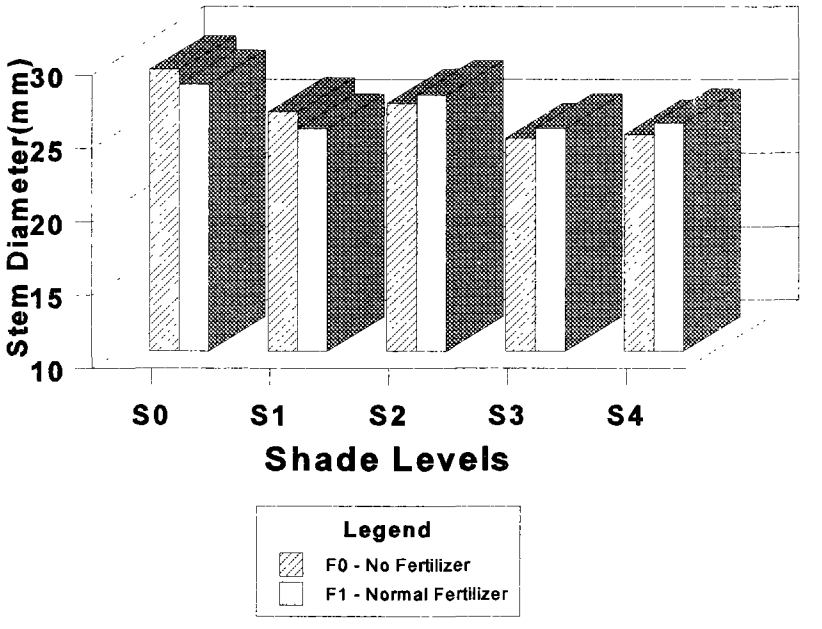

Figure 1: Effects of shade and fertilizer on Stem Diameter

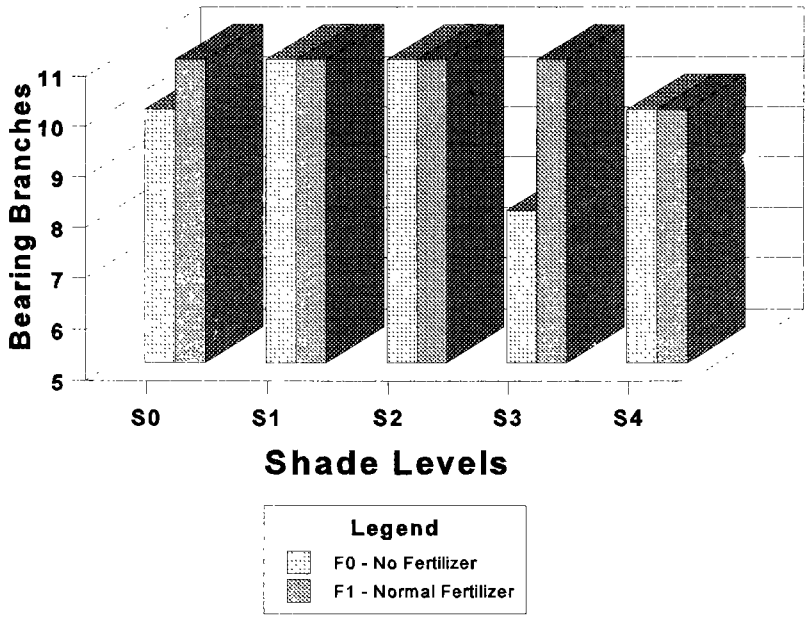

Figure 2: Effects of shade and fertilizer on Bearing Branches

3.2- Soil and Plant Nutrients

Soil and plant analyses prior to and during coffee establishment revealed no deficient or excessive nutrient levels. Soil results in July 1994, showed that fertilized plots were more acidic, had more extractable potassium, and increased phosphorus and cation exchange capacity. The effect of temporary shade and inorganic fertilizers on the coffee leaf nutrients percentages (%) and parts per million (ppm) are presented in Table 1. There were significant differences in N, P, K, Ca, Mg and Mn.

Table 1: Effects of temporary shade and inorganic fertilizers on plant nutrients in young coffee

Treatments		%					ppm				
Fertilizer	Shade	N	P	K	Ca	Mg	Mn	Fe	Zn	Cu	B
F0	S0	1.98	0.15	0.96	0 49	0.23	134	149	6	7	22
F0	S1	2.09	0 14	0.91	0.63	0.26	138	116	7	10	17
F0	S2	1.86	0.16	0 85	0.56	0.23	131	140	7	11	20
F0	S3	1 82	0.16	0.93	0.72	0.25	162	169	5	10	25
F0	S4	1.92	0.16	0 80	0.74	0.29	159	187	6	12	24
F1	S0	2.34	0.14	1.17	0.47	0.22	169	92	5	6	18
F1	S1	2.16	0.14	1.07	0.59	0.26	205	152	7	8	16
F1	S2	2.11	0.13	1.13	0.53	0.22	157	176	8	8	17
F1	S3	2.04	0.14	1.08	0.63	0.24	170	186	9	9	14
F1	S4	2.05	0 13	1.12	0.58	0.22	151	203	8	9	12
Significance											
Fertilizer		***	***	***	**	*	***	NS	NS	***	***
Shade		**	NS	NS	***	*	*	**	NS	***	NS
C. of V. %		7.8	7.1	15.8	15	12.5	19.8	56.3	67.7	16.7	24.3

Note: Significance levels: - * = 10%, ** = 5% and *** = 1%

4- Discussion and Conclusions

Coffee trees received adequate nutrient supplies from the soil for normal establishment. Tephrosia temporary shade planted every three coffee rows had less detrimental effects on coffee growth. Shade planted every two coffee rows was more competitive for soil nutrients and sunlight. Inorganic fertilizers appeared to alleviate the competition for soil nutrients and sunlight. Coffee trees established better under fertilizer regime. Coffee can establish successfully with and without fertilizers or shade. Fertilizer increases growth vigour. The ultimate test of successful establishment will be the future yields. The current findings help to explain why the agroforestry farming system (Bourke 1978) practised by Papua New Guinea farmers has been so successful over the past four decades. The system of recycling nutrients in a food crops - coffee - shade trees continuum is the nearest one can get to "organic coffee farming".

5- Acknowledgements

The authors are grateful for the technical inputs of the Agrophysiology Department field staff. Ms D Nad's assistance with data processing and typing of the manuscript are acknowledged. Papua New Guinea coffee industry, through Coffee Industry Corporation, research division, provided the facilities and funding for the work.

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7- Summary

A trial was established at the Highlands Agricultural Experiment Station, Aiyura in Papua New Guinea in March 1993. The objectives of the trial were to determine the benefits of temporary shade, and the level of the shade required to produce the beneficial effects. Five shade and two fertilizer treatments were combined in a factorial design. The treatment effects were assessed over the first one-and-half years of coffee establishment by monitoring tree growth parameters, and soil and plant nutrient contents. It was found that young coffee can establish successfully with and without fertilizer or temporary shade. Temporary shade every two coffee rows adversely affected coffee growth. Shade competition was minimized by the application of inorganic fertilizers.

STUDY ON THE AGRICULTURAL UTILIZATION OF COFFEE RESIDUE. UTILIZATION OF COFFEE RESIDUE FOR WEED CONTROL

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1- Introduction

The volume of coffee residue has been increasing because of the increase of the demand of "COFFEE". Most of coffee residue, however, is dealt with a industrial waste similar to a discharge issued from the other food industry. In order to make environment save, the attention has been focused on the establishment of safety and sustainable methods for their disposal.

From this point of view, a study on agricultural utilization of coffee residue, as a deal with industrial waste is very important. A portion of coffee residue is made up compost in Japan. However, composting coffee residue needs a much longer period than the general materials for compost, such as crop residue or cattle feces.

The purpose of this research was to determine, 1) if fresh coffee residue is useful material for mulch compared to a polyethylene film, and 2) if coffee residue has a allelopatic potential for weed control.

2- Materials and Methods

Experiment 1.

Four kg air dried soils, added with 1g urea, 3g suprephosphate, and 1g potassium chloride, were filled into a 1/5000a wagner's pot, and the pot was mulched with (CR) or without (NM) 80g coffee residue. The chemical properties of the soil used and the coffee residue were shown in Table 1 and 2, respectively.

Winter crops (wheat and broadbean) and summer crops (corn and soybean) were seeded in those pots on October 18, 1990, and on May 15, 1991. These crops were cultivated for 27 weeks, 26 weeks, 5 weeks, and 10weeks, respectively. Thereafter, the growth of each crop was investigated. Simultaneously, nitrogen fixing activity of soybean plant was measured by acetylene reducing method. The two treatments (CR and NM) of 4 species consisted of 3 replications.

To measure the soil chemical properties, tree soil cores of the 0-2.5 cm (upper layer), 2.5-5.0 cm (middle layer), and 5.0-10.0 cm (lower layer) soil layers were collected from each uncultivated pot at 2 weeks, 6 weeks, and 10 weeks after sowing of soybean. At the soil sampling times, weeds were cut at ground level, oven dried at 75°C for 2 days, and reared as weed biomass.

Table 1. Chemical Properties of the Coffee Residues (Exp. 1)

Total Carbon (g kg^{-1})	503
Total Nitrogen (g kg^{-1})	18.9
C/N	26.7
P (g kg^{-1})	0.6
K (g kg^{-1})	1.2
Ca (g kg^{-1})	0.2
Mg (g kg^{-1})	0.9
Na (g kg^{-1})	0.2
800mL L-1 Ethanol Soluble Fraction (g kg^{-1})	293
Hot Water Soluble Fraction (g kg^{-1})	103
Lignin Fraction (g kg^{-1})	184
Hemicellulose Fraction (g kg^{-1})	266
Cellulose Fraction (g kg^{-1})	154

Table 2. Chemical Properties of Used Soil (Exp. 1)

pH	5.43
EC (dS m^{-1})	0.13
Total Carbon (g kg^{-1})	13.4
Total Nitrogen (g kg^{-1})	1.0
C/N	14.2
NH_4^+-N (mg kg^{-1})	50.8
NO_3^--N (mg kg^{-1})	14.9
Available P_2O_5 (mg kg^{-1})	691
Exchangeable K^+ (cmol(+) kg^{-1})	0.42
Exchangeable Ca^{2+} (cmol(+) kg^{-1})	1.06
Exchangeable Mg^{2+} (cmol(+) kg^{-1})	0.20

Experiment 2.

40L fresh soils applied with 4.35g urea, 29.45g suprephosphate, and 5.00g potassium chloride, were put in to containers (61×37×25cm).These containers were mulched with 2.5kg coffee residue or polyethylene black film. Those containers are referred to CR (mulched with 2.5 kg coffee residue) and BF (mulched with polyethylene black film), respectively. In addition, container treated with no mulch was prepared in the same way as control. The properties of soil and coffee residue used in this experiment are given in Tables 3 and 4.

Eight seeds of soybean were sown on April 19, 1994 in the container. After emergence, the seedlings were thinned to 2 plants per container, and cultivated during July 12, 1994. The harvest was carried out by cutting the plants at the root-shoot transition zone, and weights of leaves, stems, pods, roots, and nodules were measured after oven dried.

At 5 and 10 weeks after sowing, weed species, the number, and biomass in each container were investigated. Besides, soil temperature at 5 cm depth, and soil pF at 10 cm depth of each container were monitored on alternate days during the duration of the experiment. For each treatment, 3 replications were performed.

Table 3. Chemical Properties of Used Soil (Exp. 2)

pH	4.52
EC (dS m^{-1})	0.04
Total Carbon (g kg^{-1})	10.37
Total Nitrogen (g kg^{-1})	0.59
C/N	17.57
NH_4-N (mg kg^{-1})	23.5
NO_3-N (mg kg^{-1})	17.6
Inorganic-N (mg kg^{-1})	41.1
Total P_2O_5 (mg kg^{-1})	1062
Ca-P_2O_5 (mg kg^{-1})	7.9
Al-P_2O_5 (mg kg^{-1})	154.4
Fe-P_2O_5 (mg kg^{-1})	177.8
P_2O_5 Absorption coefficient (mg kg^{-1})	6457
Exchangeable K^+ (cmol(+) kg^{-1})	0.32
Exchangeable Ca^{2+} (cmol(+) kg^{-1})	0.36
Exchangeable Mg^{2+} (cmol(+) kg^{-1})	0.34
Exchangeable Na^+ (cmol(+) kg^{-1})	0.04
Exchangeable Mn^{2+} (cmol(+) kg^{-1})	0.041
Exchangeable Al^{3+} (cmol(+) kg^{-1})	1.35

Table 4. Chemical Properties of Used Coffee Residue (Exp. 2)

Total Carbon (g kg^{-1})	550
Total Nitrogen (g kg^{-1})	18.7
C/N	29.5
P (g kg^{-1})	0.8
K (g kg^{-1})	0.3
Ca (g kg^{-1})	0.7
Mg (g kg^{-1})	1.7
Na (g kg^{-1})	0.2

Experiment 3.

Dried coffee residue powder was extracted by shaking 0.5g of the powder with 50 mL of distilled water for 1 hour at room temperature. The ratio of weight of powder extracted to water added was 1: 100. The extract was centrifuged at 10,000×g for 10 minutes (4℃). The supernatant was filtered through filter paper (Toyo Roshi No. 1). The extract was then employed to bioassay to measure

germination and early root growth in 13 species of plants.

In germination test, 10 seeds of each plant were placed in each of 6 plastic dishes (diameter 35mm), containing a disk of filter paper (diameter 30mm) and 0.8mL of the extract or distilled water. However, 15 wheat seeds and 10 soybean seeds were placed on 5 glass dish (diameter 85mm), containing a disk of filter paper (diameter 80mm) and 4mL of the extract or distilled water. All experiments were run in the continuous dark condition at 25℃, and the number of germinated seeds were counted daily.

On the other hand, in root growth test, approximately 100 Italian ryegrass, lettuce, komatuna, alfalfa, and white clover seeds were placed on a 80mm disk of filter paper in the lid of a 85mm glass dish. The filter paper was soaked with 4mL distilled water, and the seeds were incubated for 24-48 hours at 25℃. After the incubation, 5 plant seedlings were transferred to each of 6 plastic dishes, containing a disk of 30mm filter paper and 0.8mL of the extract or distilled water, and were incubated in continuous dark condition for 24 hours at 25℃. Thereafter, the elongation of plant root was measured.

3- Results and Discussion

Experiment 1. Effect of mulched with coffee residue on the plant growth and the soil chemical properties.

Mulching effects of coffee residue on the growth of wheat, broad bean, corn, and soybean grown under pot condition are presented in Fig. 1.

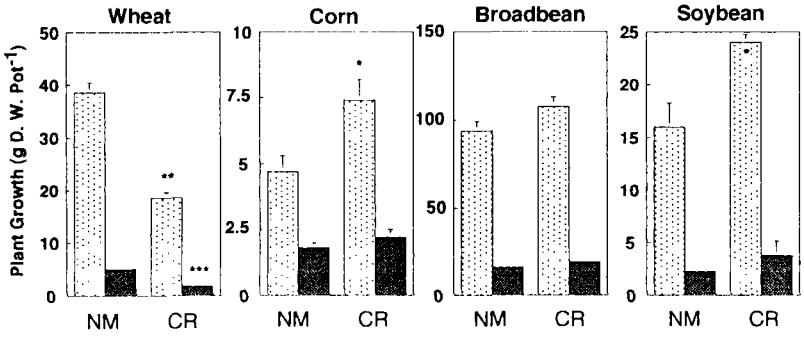

Fig. 1. Effects of Mulched with Coffee Residue on the Growth of Wheat, Corn, Broadbean and Soybean

*, **, *** = significante differrence at p=0.05, 0.01 and 0.001, respectively

Top Root

It is well known that the plant growth are decreased by nitrogen immobilization and allelopatic effect, when fresh organic matters, such as crop residue green manure, are applied. In particular, these effects could conceivably severe the microorganism activity in winter season. In this experiment, both aerial and underground parts of wheat growth were significantly decreased in CR compared to NM.

However, the growth of leguminous crop such as broad bean and soybean, and corn, grass crop, were not retarded irrespective of mulched with coffee residue. Especially, aerial parts growth of soybean and corn were significantly promoted in CR compared to NM. This is probably due to the short period of nitrogen immobilization and degradation of allelopatic compounds from coffee residue, and to having different respond to allelopatic compounds on each crop. Although, the difference in the nitrogen fixing activity of soybean between NM and CR was not significant as shown in Fig. 2, another potential reason is that leguminous crops held nitrogen fixing activity.

Fig. 3. shows the weed biomass in both NM and CR uncultivated pots during the growing period of soybean growing period.

Weed biomass in the pot was significantly lower in CR than in NM at 2, 6, and 10 weeks after treatment. This phenomenon is probably due to shield effect of coffee residue mulched on the soil surface. Another reason is that allelo-chemicals originated from coffee residue retarded the emergence and the growth of weeds.

The chemical properties of soil from uncultivated pots are shown in Fig. 4-6.

Soil water contents and pH values were higher in CR than in NM at 6 and 10 weeks after treated.

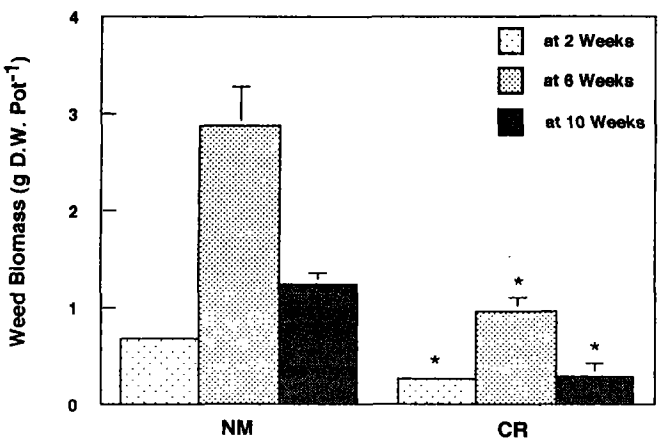

Fig. 3 Effect of Mulched with Coffee Residue on the Weed Biomass during soybean Growing Period

*= significant difference at p= 0.05

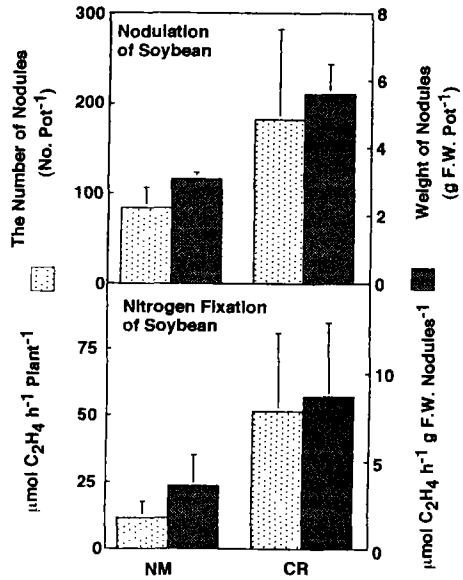

Fig. 2. Effects of Mulched with Coffee Residue on the Nodulation and Nitrogen Fixation of Soybean Plant

In particular, the effect was more pronounced in upper layer. There was not a significant difference in EC values between NM and CR.

There was not a significant difference in the inorganic nitrogen content between NM and CR soils at 6 and 10 weeks after treated. However, the content of CR soils was decreased in comparison to the NM soil for nitrogen immobilization. Furthermore, the content of available phosphate in soil was higher in CR than in NM at 2 weeks after treated, and was lower in CR than in NM at 6 and 10 weeks after treated.

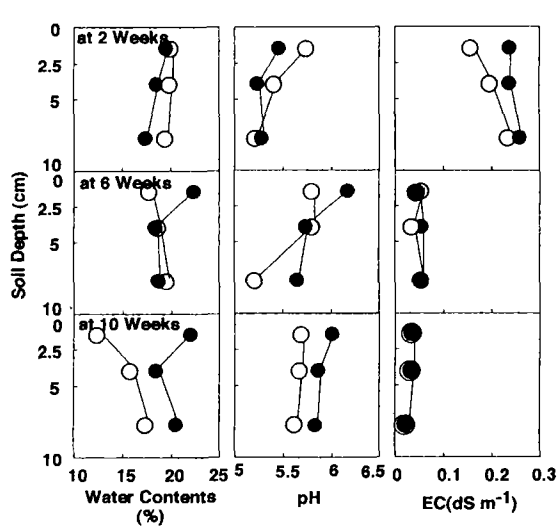

Fig. 4. Effects of Mulched with Coffee Residue on the Water Content, pH, and EC Values of the Soil

○ NM ● CR

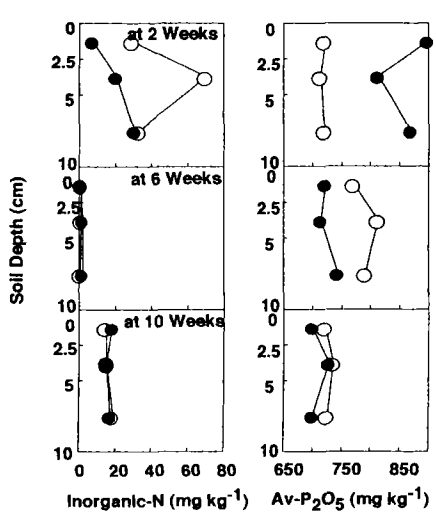

Fig. 5. Effects of Mulched with Coffee Residue on the Contents of Inorganic N and Available P_2O_5 of the Soil

○ NM ● CR

Except for the exchangeable calcium content, the exchangeable cations contents in CR were increased compared to NM one, especially at upper layer.

From these results, it was clear that the growth of crops (corn, broad bean, and soybean), were increased by mulching with coffee residue. This phenomenon is partially due to the allelopatic

effects of coffee residue on suppressing the weed growth. The soil chemical properties were not significantly affected by mulching with coffee residue, since the decomposition of coffee residue was slow in comparison to some fresh organic matters, such as green manure and crop residue.

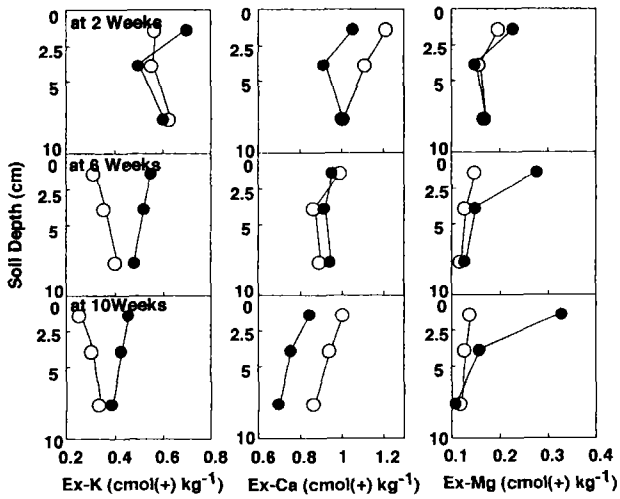

Fig. 6. Effects of Mulched with Coffee Residue on the Contents of Excahngeable Cation of the Soil

Experiment 2. Mulching effects of coffee residue and polyethylene film on soybean growth and soil physical properties.

Figure 7 presents the growth of soybean plant grown under mulching with coffee residue (CR) and polyethylene film (BF).

Similar to Experiment 1, soybean growth was increased in mulching with coffee residue. Namely, the growth of aerial parts was increased in the order : NM <BF <CR, and the growth of under ground parts was increased in the order : NM <CR <BF.

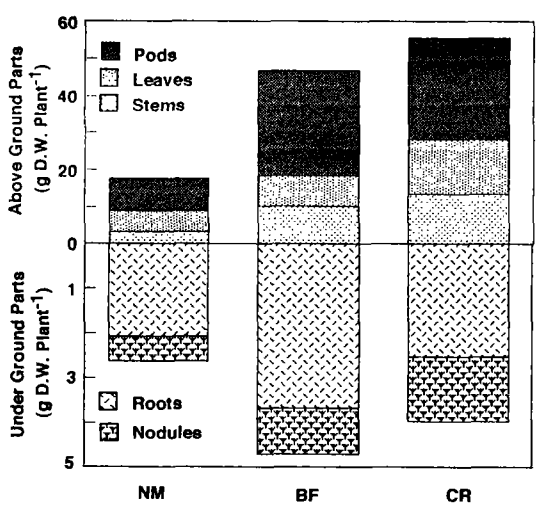

Fig. 7. Mulching Effects of Black Polyethylene Film or Coffee Residue on the Growth of Soybean Plant

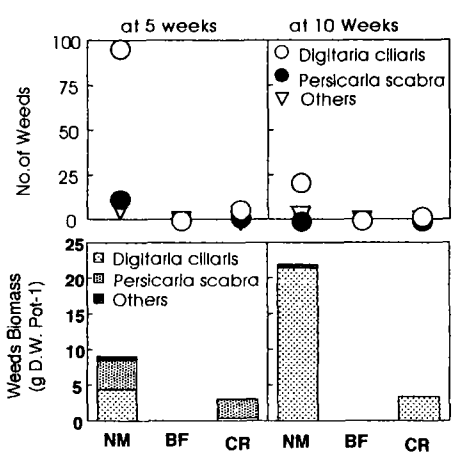

Fig. 8. Mulching Effects of Black Polyethylene Film or Coffee Residue on the Weeds Control

The number and biomass of weeds emerged in each container at 5 and 10 weeks after seeded are shown in Fig. 8. It was observed that Digitaria ciliaris was dominant weed during the experiment.

Although weed control was less affected by coffee residue than by polyethylene film, it was observed that the weed biomass was outstandingly decreased in mulching with coffee residue.

The changes in pF values and soil temperature in each container soil during the duration of the study are presented in Fig. 9 and Fig. 10.

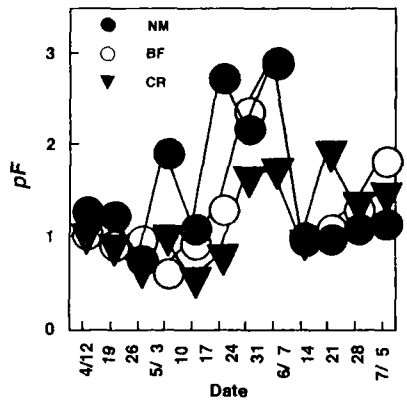

Fig. 9. Mulching Effects of Black Ploye thylene Film or Coffee Residue on The Soil pF Values

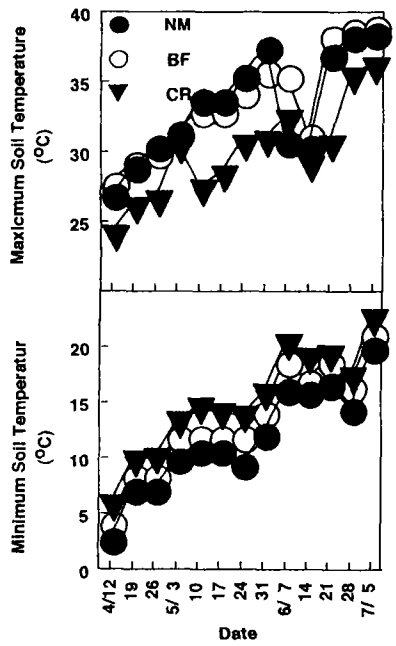

Fig. 10. Mulching Effects of Black Ploye thylene Film or Coffee Residue on The Soil Temperature

The pF values of CR and BF were lower than that of NM during most of experiment,particularly the values of CR were consistently low in comparison to the values of NM and BF at drought periods (24 May to7 June).

Maximum soil temperature was higher in the order : NM > BF >CR, and minimum soil temperature was lower in the same order. It is well known that soybean growth is increased with the increase of minimum soil temperature at early spring. Therefore, it seems that the soybean growth was increased due to the increase of minimum soil temperature by mulching with coffee residue. Similarly, it was assumed that the decline of maximum soil temperature by mulching with coffee residue is probably due to the protection of soybean plant from high temperature stress during summer.

Based on the results, it was confirmed that the growth of soybean was markedly increased in mulching with coffee residue, as a result of the rise of the minimum soil temperature and soil water potential. Moreover, weed biomass in the container cultivated with soybean was reduced by mulching with coffee residue. Accordingly, it was assumed that coffee residue was useful materials for mulching compared to polyethylene film.

Experiment 3. Bioassay for allelopathy of coffee residue

Figure 11 presents the effects of water extract from coffee residue on germination of wheat, Italian ryegrass, timothy, tall fescue, orchard grass, Kentucky bluegrass, lettuce, chrysanthemum, komatuna, soybean, white clover, red clover, and alfalfa seed.

As previously described, the growth of grass crop, wheat and corn, was decreased in applying with coffee residue. In this experiment, however, the seed germination of 6 grass crops, wheat, Italian ryegrass, timothy, tall fescue, orchard grass, Kentucky bluegrass, was not affected by the extract from coffee residue. Moreover, the seed germination of other crop was also less affected by the extract, except for white clover. The germination number of white clover was significantly lower in the extract from coffee residue than in the control up to 48 hours after seeded.

In contrasts with seed germination, the difference in root elongation of Italian ryegrass, lettuce, komatuna, alfalfa, and white clover between the control and the extract from coffee residue was

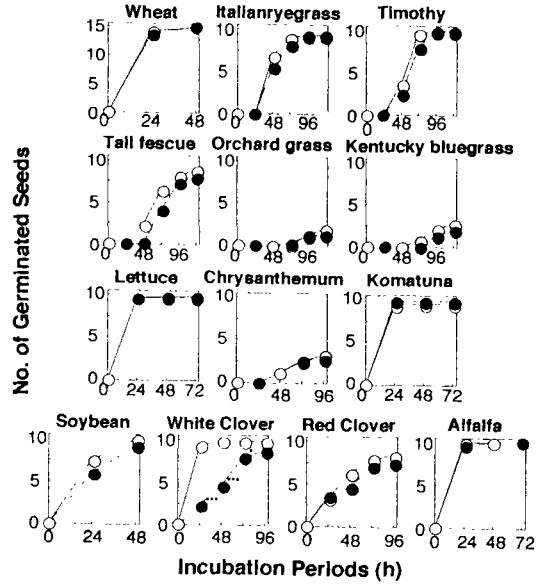

Fig. 11. Effects of Water Extracts from Coffee Residue on the Seed Germination of Some Plant Species

○ **Distilled Water**

● **Water Extracts from Coffee Residue**

significant. Namely, the root elongation in the extract from coffee residue treatment was shorter than control. Root elongation of white clover was significantly inhibited by the extract from coffee residue.

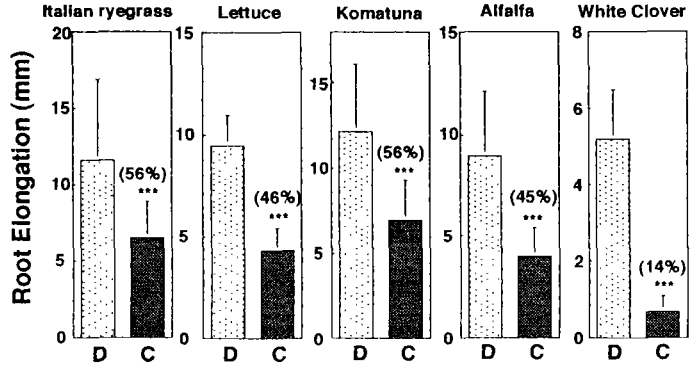

Fig. 12 Effects of Water Extracts from Coffee Residue on the Root Elongation of Some Plant Species

D: Distilled Water

C: Water Extracts from Coffee Residue

***= significant difference at p=0.001

From these results, it was conceived that the water soluble allelo-chemicals of the coffee residue affected the root elongation, and white clover was the most sensitive to the compound extracted from coffee residue.

Based on the foregoing result, we are considered that coffee residue is beneficial as mulching material instead of a polyethylene film, because of improving soil physio-chemical properties, and controlling weed to allelopathy.

4. Summary

1) While the growth of wheat was decreased in mulching with coffee residue, the growth of broad been was not effect of mulching with coffee residue. Moreover, the growth of corn and soybean was outstandingly increased in mulching with coffee residue.

2) The weed biomass was significantly decreased in mulching with coffee residue during the period of soybean grown.

3) The content of exchangeable K and Mg, and pH value of the soil mulched with coffee residue were higher than the non-mulched soil. In addition, those effects were clearly observed at surface soil.

4) The minimum soil temperature and soil water potential were risen by mulching with coffee reside

5) Based on the bioassay experiment, it was clear that the seedling growth of lettuce alfalfa, white clover, komatuna, and Italian ryegrass was inhibited by the extracts from coffee residue.

DIVERSITÉ PHÉNOTYPIQUE DE *COFFEA ARABICA* OBSERVÉE EN COLLECTION AU CAMEROUN

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Le centre principal de diversité de *C. arabica* se situe en Ethiopie (Sylvain, 1958). Des caféiers spontanés de cette espèce ont également été trouvés sur le plateau de Boma au Soudan (Thomas, 1942) et sur le mont Marsabit au Kenya (Berthaud *et al*, 1977). L'histoire montre (Chevalier, 1929) que l'ensemble des variétés de *C. arabica* cultivées à travers le monde proviennent toutes d'un nombre réduit de génotypes. La base génétique de ces variétés cultivées est donc très faible. Ceci se traduit par une grande difficulté à améliorer cette espèce, pour la résistance aux maladies en particulier.

Des prospections ont été réalisées en Ethiopie afin d'élargir cette base génétique. Parmi les plus récentes, on note la prospection FAO de 1964-65 (FAO, 1968) et la mission IRCC-ORSTOM de 1966 (Charrier, 1978). Plusieurs collections vivantes de *C. arabica* de grande importance existent à travers le monde : en Ethiopie, en Tanzanie, en Côte d'Ivoire, à Madagascar, au Cameroun, au Costa Rica, au Brésil... Malgré cela, peu d'études de la diversité phénotypique de *C. arabica* basées sur l'observation en collection ont été réalisées. Carvalho (1959 et 1962) observe une grande variabilité des caractères quantitatifs chez des variétés éthiopiennes. Charrier (1978) donne les premières analyses de la variabilité au sein de la prospection IRCC-ORSTOM de 1966.

Nous présentons dans ce travail l'analyse de la diversité phénotypique de *C. arabica* à partir d'observations réalisées au Cameroun.

MATERIEL ET METHODES

Matériel

Sur plus de 320 variétés en collection au Cameroun, 148 ont été analysées dans ce travail (Tableau 1). Parmi celles-ci, 50 proviennent de la prospection IRCC-ORSTOM de 1966. Les variétés de cette mission avaient été plantées à l'origine dans une collection (COC) appartenant à un privé. En 1975, elles ont été transférées à la station de Fumbot. Pour les variétés de la prospection IRCC-ORSTOM, la descendance plantée à Fumbot provient d'un seul pied de la collection COC. Dans cette collection, la variabilité à l'intérieur des lignes était beaucoup plus faible qu'entre les lignes (Bouharmont, obs. pers.).

Les variétés sont plantées en ligne à raison de dix pieds par ligne. Toutes les observations à l'exception de la sensibilité à l'anthracnose des baies ont été réalisées à Fumbot (1000 mètres d'altitude). La sensibilité à l'anthracnose des baies a été évaluée à Santa (1800 mètres d'altitude).

Tableau 1. Inventaire et origine du matériel végétal.

pays d'importation (Cameroun)		pays d'exportation			origine			observations
variété	n°	appellation	n°	appellation	loc	pays	pays	
	(1)				(2)	(3)	(4)	
Ab 1	91	*Abyssinie 1*	5749	Lignée M	RU	RW	ET	
Ab 2	103	Abyssinie 2	5719		IL	MA		
Am	72	Amphillo		Amphillo	RU	RW		
Ao	71	Amarello		Amarello	RU	RW		
BmJm 1	44	Blue Mountain Jamaïque 1	Blue Mountain Jamaïque 13		RU	RW		type Blue Mountain Jamaïque, sélection du Kenya
BmKe 1	87	Blue Mountain Kenya 1		Blue Mountain Kenya 168	RU	RW		
Bo 2	43	Bourbon 2		Bourbon 72	RU	RW		type Bourbon, originaire du Guatemala
Bo 3	88	Bourbon 3		Bourbon 72-1529	RU	RW		type Bourbon, originaire du Guatemala
BoMz 1	40	Bourbon Mayaguez 1		Bourbon Mayaguez 139	RU	RW		type Bourbon, originaire de Mayaguèz (Porto Rico)
BoMz 2	49	Bourbon Mayaguez 2		Bourbon Mayaguez 71-2147	RU	RW		type Bourbon, originaire de Mayaguèz (porto Rico)
BoSa 1	28	Bourbon Salvadoreno 1	5718	Bourbon Salvadoreno	RU	RW	ET	
Ca 1	39	Caturra 1		Caturra 140	RU	RW		
Ca 2	76	Caturra 2		Caturra 58	RU	RW		
Ca 3	77	Caturra 3		Caturra 34	RU	RW		
Ca 4	78	Caturra 4		Caturra 142	RU	RW		
Ca 5	97	Caturra 5	B14	Caturra	IL	MA	BR	
Co	37	Coorg	62 bis	Coorg	RU	RW		
Cr 1	9	Costa Rica 1		Costa Rica	DG	CA		introduit d'Amérique du Sud en 1936
Dg 1	1	Dschang 1		S 288	DG	CA		
Dg 2	12	Dschang 2		A2	DG	CA		⎫ proviennent de 3 pieds élites :
Dg 3	13	Dschang 3		A3	DG	CA		⎪ sélection massale en 1935
Dg 4	14	Dschang 4		A4	DG	CA		⎭ *à partir de Blue Mountain Jamaïque*
Dg 5	15	Dschang 5		n°2	DG	CA		provient de sélection massale locale
Dg 6	16	Dschang 6		n°3	DG	CA		provient de sélection massale locale
Dg 7	17	Dschang 7		n°7	DG	CA		provient de sélection massale locale
Dg 8	18	Dschang 8		n°14	DG	CA		provient de sélection massale locale
Dg 9	19	Dschang 9		n°33	DG	CA		provient de sélection massale locale
Dg 10	20	Dschang 10		n°37	DG	CA		provient de sélection massale locale
Dg 11	21	Dschang 11		n°77	DG	CA		provient de sélection massale locale
Dg 12	22	Dschang 12		n°104	DG	CA		provient de sélection massale locale
Gu 1	81	Guatemela 1		Guatemala 26	RU	RW		
Gu 2	99	Guatemala 2	B3	Guatemala	IL	MA		
Ha 1	27	Harrar 1	5716	Harrar Dugda Lemita Arussi	RU	RW	ET	
Ha 2	48	Harrar 2		Harrar	RU	RW		
Ha 3	55	Harrar 3	5714	Harrar Dugda Lemita Arussi R1	RU	RW	ET	
Ho 1	7	Honduras 1		Honduras	DG	CA		introduit d'Amérique du Sud en 1936
Il 3	194	Illulabor 3		Illulabor 1	FO	CA	ET	caféier repéré hors-type (fruits jaunes) parmi les Il 1
Il 4	195	Illulabor 4		Illulabor 2	FO	CA	ET	caféier repéré hors-type (fruits rouge clair) parmi les *Il 1*
Jk 2	41	Jackson 2		Jackson 2	RU	RW		sélection réalisée sur Jackson, au Kenya
Jk 3	50	Jackson 3		Jackson 2-1257	RU	RW		
Jm 1	53	Jamaïque 1		Jamaique	KO	CA		
Ka	82	Kabare		Kabare 18	RU	RW		
Ke 1	3	Kenya 1		Kenya	DG	CA		
Ke 2	104	Kenya 2		SL 34	RR	KE		
Ke 3	105	Kenya 3		K 7	RR	KE		
Ke 4	106	Kenya 4		Geisha	RR	KE		
Ke 5	107	Kenya 5		K 20	RR	KE		
Kf 1	24	Kaffa *1*	5691	Ainamba Babaca Kaffa	RU	RW	ET	
Kf 2	25	Kaffa 2	5718	Babaca Kaffa	RU	RW	ET	
Kf 3	56	Kaffa 3	5692	Agaro Gimma Kaffa	RU	RW	ET	
Kf 4	57	Kaffa 4	5694		RU	RW	ET	
Kf 5	58	Kaffa 5	5693	Tanabo A Buna Mia Limmu Kaffa	RU	RW	ET	
Kf 6	93	Kaffa 6	5717	Ennarea Limmu Gimma Kaffa	RU	RW	ET	
Kf 7	94	Kaffa 7	5953	Agaro Kaffa	RU	RW	ET	
Kt 1	45	Kent 1		Kent 170	RU	RW		

Lb 1	42	Local Bronze 1		Local Bronze 183	RU	RW		
Lb 2	80	Local Bronze 2		Local Bronze 8	RU	RW		
Lp	38	Las Palmas	60	Las Palmas	RU	RW		
Mi 1	83	Mibirizi 1		Mibirizi 68	RU	RW	GU	
Mi 2	84	Mibirizi 2		Mibirizi 49	RU	RW	GU	
Mi 3	89	Mibirizi 3		Mibirizi 68-1284	RU	RW	GU	
Mi 4	90	Mibirizi 4		Mibirizi 49-1848	RU	RW	GU	
Mn 1	98	Mundo Novo 1	B15	Mundo Novo	IL	MA	BR	
Mn 2	112	Mundo Novo 2		Mundo Novo LCP 391	CP	BR	BR	
Mn 3	113	Mundo Novo 3		Mundo Novo LCP 379-19	CP	BR	BR	
Mn 4	114	Mundo Novo 4		Mundo Novo LCP 390	CP	BR	BR	
Mo 1	11	Mokka 1		Mokka	DG	CA		
Mo 2	47	Mokka 2		Mokka (BE 145)	RU	RW		
MoAd 1	70	Mokka d'Aden 1		Mokka d'Aden	RU	RW		
MoT	36	Mokka de Tahiti	947	Mokka de Tahiti	RU	RW		
Mu 1	32	Mulungu 1		Mulungu 1	RU	RW	CB	
Mu 2	33	Mulungu 2		Mulungu 2	RU	RW	CB	
Mu 3	34	Mulungu 3		Mulungu 3	RU	RW	CB	
Mu 4	35	Mulungu 4		Mulungu 4	RU	RW	CB	
Mu 5	64	Mulungu 5		Mulungu 4	RU	RW	CB	
Mu 6	65	Mulungu 6		Mulungu 8	RU	RW	CB	
Mu 7	66	Mulungu 7		Mulungu 11	RU	RW	CB	
Mu 8	67	Mulungu 8		Mulungu 16	RU	RW	CB	
Mu 9	68	Mulungu 9		Mulungu 24	RU	RW	CB	
Mu 10	69	Mulungu 10		Mulungu 25	RU	RW	CB	
Mu 11	197	Mulungu 11		Mulungu 1	FO	CA	CB	caféier hors-type (fruits allongés, bouts verts) dans les Mu 1
My 1	46	Mysore 1		Mysore	RU	RW		
My 2	51	Mysore 2		Mysore 175	RU	RW		
My 3	96	Mysore 3	1105	Mysore	RU	RW	ET	
Ni 1	8	Nicaragua 1		Nicaragua	DG	CA		introduit d'Amérique du Sud en 1936
Pr 2	75	Porto Rico 2 (Caturra)	61 bis	Porto Rico	RU	RW		
Pu 1	59	Purpurascens 1		Purpurascens	RU	RW		
Sa 1	10	Salvador 1		Salvador	DG	CA		introduit d'Amérique du Sud en 1936
Si 1	26	Sidamo 1	5712	Sidamo	RU	RW	ET	
Si 2	29	Sidamo 2	5902	Garbito Irgalem Sidamo	RU	RW	ET	
Si 3	30	Sidamo 3	5898	Irgalem Kella Sidamo	RU	RW	ET	
Si 4	60	Sidamo 4	5895	Irga Ciaffe Sidamo	RU	RW	ET	
Si 5	61	Sidamo 5	5896		RU	RW	ET	
Si 6	62	Sidamo 6	5901	Wondo Sidamo	RU	RW	ET	
To	74	Tonkin		Tonkin	RU	RW		
Vy	79	Vyumvuhore (erecta)		Vyumvuhore	RU	RW		

NB.
(1) n° d'introduction à l'IRA-IRCC
(2) localité de provenance :
BB : Babadjou DG : Dschang KO : Koutaba RR : Ruiru
BI : Bingerville FO : Foumbot NG : Ngouendam RU : Rubona
CP : Campinas IL : Ilaka OE : Oeiras SC : Santa Coffee Estate
VI : Viscosa
(3) pays de provenance
(4) pays d'origine
BR : Brésil CI : Côte d'Ivoire Gu : Guatemala OU : Ouganda
CA : Cameroun CR : Costa Rica Ke : Kenya PO : Portugal
CB : Congo Belge Et : Ethiopie MA : Madagascar RW : Rwanda

Tableau 1 (suite) Variétés provenant de la prospection ORSTOM en Ethiopie (1966)

pays d'importation (Cameroun)		pays d'exportation et d'origine			
variété	n°	appellation	région	pays	Station (5)
Et 1	116	Ethiopie 1	Godgeb Valley, Ati Tadese Farui (savane), alt.1420 m	ET	a
Et 2	117	Ethiopie 2	Godgeb Valley, Ati Tadese Farui (savane), alt.1420 m	ET	a
Et 3	118	Ethiopie 3	Bonga, 3 km vers Gommerea, plantat.J.Aroya,	ET	c
Et 4	119	Ethiopie 4	Bonga, 3 km vers Gommerea, plantat.J.Aroya, forêt de montagne, alt.1730 m	ET	c
Et 5	120	Ethiopie 5	Wush-Wush, plant.M.Buchholz, alt.1860 m, forêt montagne.	ET	a
Et 6	121	Ethiopie 6	Wush-Wush, plant.M.Buchholz, alt.1860 m, forêt montagne.	ET	a
Et 7	122	Ethiopie 7	Wush-Wush, plant.M.Buchholz, alt.1860 m, forêt montagne.	ET	a
Et 8	123	Ethiopie 8	Wush-Wush, plant.M.Buchholz, alt.1860 m, forêt montagne.	ET	a
Et 9	124	Ethiopie 9	Bonga, mission catholique, alt.1600 m, forêt montagne.	ET	b
Et 10	125	Ethiopie 10	Bonga, mission catholique, alt.1600 m, forêt montagne.	ET	b
Et 11b	126	Ethiopie 11b	Limu, plateau Ato Getahun Birke, alt.1660 m, savane	ET	b
Et 11c	127	Ethiopie 11c	Limu, plateau Ato Getahun Birke, alt.1660 m, savane	ET	b
Et 13	129	Ethiopie 13	Limu, plateau Ato Getahun Birke, alt.1660 m, savane	ET	b
Et 14	130	Ethiopie 14	limu-Gimma, 30 km de Gimma sur route Limu, alt.1530 m, forêt montagne	ET	d
Et 15	131	Ethiopie 15	Gimma-Gore, route Gimma-Gore (70 km de Gore), alt.1350 m, forêt montagne	ET	d
Et 16	132	Ethiopie 16	Gimma-Gore, route Gimma-Gore (80 km de Gore), alt.1420 m, forêt montagne	ET	d
Et 17	133	Ethiopie 17	Gimma-Gore, route Gimma-Gore (70 km de Gore), alt.1350 m, forêt montagne	ET	d
Et 18	134	Ethiopie 18	Gore, ; pl. Keurcshurcsh HadjidAbush, alt.1700 m, forêt de montagne	ET	b
Et 19	135	Ethiopie 19	Gore, ; pl. Keurcshurcsh HadjidAbush, alt.1700 m, forêt de montagne	ET	b
Et 20	136	Ethiopie 20	Tippi, aéroport, alt.1300 m, forêt semi-décidue,	ET	d
Et 21	137	Ethiopie 21	Tippi, aéroport, alt.1300 m, forêt semi-décidue,	ET	e
Et 24	140	Ethiopie 24	Tippi, vallée Baco, alt.1205 m, forêt semi-décidue,	ET	f
Et 25	141	Ethiopie 25	Tippi, vallée Baco, alt.1205 m, forêt semi-décidue,	ET	f
Et 26	143	Ethiopie 26	Tippi-Gore, 2 km aéroport, alt.1230 m, forêt semi-décidue,	ET	f
Et 27	144	Ethiopie 27	Tippi-Gore, W. de la Baco, alt.1240 m, forêt semi-décidue,	ET	f
Et 28	145	Ethiopie 28	Tippi-Gore, W. de la Baco, alt.1240 m, forêt semi-décidue,	ET	f
Et 29	146	Ethiopie 29	Tippi-Gore, W. de la Baco, alt.1240 m, forêt semi-décidue,	ET	f
Et 30	148	Ethiopie 30	Tippi, aéroport, alt.1300 m, forêt semi-décidue,	ET	f
Et 31	149	Ethiopie 31	Tippi, aéroport, alt.1300 m, forêt semi-décidue,	ET	f
Et 32	150	Ethiopie 32	Tippi, aéroport, alt.1300 m, forêt semi-décidue,	ET	f
Et 33	152	Ethiopie 33	Mizan-Teferi, aéroport, alt.1465 m, forêt semi-décidue,	ET	f
Et 34	154	Ethiopie 34	Mizan-Teferi, alt.1500 m, forêt semi-décidue,	ET	e
Et 35	156	Ethiopie 35	Mizan-Teferi, alt.1500 m, forêt semi-décidue,	ET	f
Et 36	160	Ethiopie 36	Kollo, alt.1400 m, forêt claire de moyenne altitude,	ET	f
Et 40	165	Ethiopie 40	Wush-Wush, alt.2000 m, forêt de montagne, mission catholique,	ET	e
Et 41	166	Ethiopie 41	Decchia, alt.2000 m, forêt de montagne,	ET	e
Et 42	167	Ethiopie 42	Decchia, alt.2000 m, forêt de montagne,	ET	e
Et 43	168	Ethiopie 43	Decchia, alt.2000 m, forêt de montagne,	ET	e
Et 47	172	Ethiopie 47	Bonga, alt.1700 m, forêt de montagne,	ET	f
Et 49	174	Ethiopie 49	Bonga, alt.1700 m, forêt de montagne,	ET	f
Et 50	175	Ethiopie 50	Bonga, alt.1700 m, forêt de montagne,	ET	f
Et 51	176	Ethiopie 51	Bonga, alt.1700 m, forêt de montagne,	ET	f
Et 52	177	Ethiopie 52	Bonga, alt.1700 m, forêt de montagne,	ET	f
Et 53	178	Ethiopie 53	Bonga, alt.1700 m, forêt de montagne,	ET	f
Et 54	179	Ethiopie 54	Bonga, alt.1700 m, forêt de montagne,	ET	f
Et 55	180	Ethiopie 55	Bonga, alt.1700 m, forêt de montagne,	ET	f
Et 56	181	Ethiopie 56	Bonga, alt.1700 m, forêt de montagne,	ET	f
Et 57	182	Ethiopie 57	Bonga, alt.1700 m, forêt de montagne,	ET	f
Et 58	183	Ethiopie 58	Bonga, alt.1700 m, forêt de montagne,	ET	f
Et 59	184	Ethiopie 59	Bonga, alt.1700 m, forêt de montagne,	ET	f
Et 60	185	Ethiopie 60	Bonga, alt.1700 m, forêt de montagne,	ET	f
Et 61	186	Ethiopie 61	Bonga, alt.1700 m, forêt de montagne,	ET	f

5) a: plantation industrielle b: plantation semi-industrielle c: plantation sauvage enrichie d: plantation sauvage e: plantation de case f: plantation familiale g: sauvage

Observations

Architecture, Botanique :
(Observations sur un arbre représentatif de la ligne)

ANPRI : Angle (°) formé par les branches à leur point d'insertion sur la tige (Moyenne de huit mesures)
DIBRA : Diamètre (1/10 mm) des branches à leur cinquième entre-noeud (Moyenne de huit mesures)
LGENP : Longueur (cm) des entre-noeuds plagiotropes (15 entre-noeuds à partir de l'extrémité de huit branches)
LGFEU : Longueur (cm) des feuilles (20 feuilles)
LAFEU : Largeur (cm) des feuilles (20 feuilles)
LOLAF : Rapport Longueur sur Largeur des feuilles (20 feuilles)
SUFEU : Surface (cm^2) des feuilles estimée par le produit Longueur par Largeur divisé par 1.5 (20 feuilles).
ANNER : Angle (°) formé par les nervures secondaires à leur point d'insertion sur la principale (20 feuilles).
NBNER : Nombre de nervures secondaires des feuilles (20 feuilles)

Technologie :
(Echantillons prélevés sur la récolte de la ligne de dix arbres)

CAFEI : Teneur (% ms) en caféine (de 1 à 5 années de test)
CARAC : % de caracoli (en poids)
GRANU : Poids (g.) de 100 grains (sans les caracoli) à 10 % d'humidité (500 grains)
GRADE : Grade A + B : % de refus en poids au tamis 17,18,19 et 20 (sans les caracoli)
LGGRA : Longueur des grains (100 grains)
LAGRA : Largeur des grains (100 grains)
EPGRA : Epaisseur des grains (100 grains)
LOLAG : Rapport Longueur sur Largeur des grains (100 grains)
LOEPG : Rapport Longueur sur Epaisseur des grains (100 grains)

Phytopathologie :
(Observations de tous les caféiers)

MAXRO : % maximum de feuilles atteintes par la rouille orangée estimé visuellement sur 4 années de notation
%RF : % de feuilles atteintes par la rouille farineuse (sur trois rameaux de huit caféiers pendant huit mois).
MAXCB : % maximum de fruits anthracnosés estimé visuellement sur 4 années de notation.

Production :

PRO1C : Production en Kg cm/ha/an sur les 5 premières années de production (1er cycle)
PRO2C : Production en Kg cm/ha/an sur les 3 premières années de production du second cycle
PRODU : PRO1C + PRO2C

Croissance (Prospection IRCC-ORSTOM uniquement) :

HTPEP : Hauteur (cm) à 6 mois en pépinière
NBENO : Nombre d'entre-noeuds orthotropes à 6 mois en pépinière
HAUTE : Hauteur (cm) à deux ans (Moyenne de dix arbres en lignes)
DIAME : Diamètre au collet à deux ans (Moyenne de dix arbres en lignes)
NBPRI : Nombre de primaires à deux ans (Moyenne de dix arbres en lignes)
NBETA : Nombre d'étages à deux ans (Moyenne de dix arbres en lignes)

Méthodes d'analyse :

Les données ont été traitées en Analyse en Composantes Principales (ACP). A partir des coordonnées factorielles sur les trois premiers axes de l'ACP, une Classification Ascendante Hiérarchique (CAH) a été réalisée avec comme résultat une partition des individus en groupes. Les logiciels CSTAT du CIRAD et STATITCF de l'INRA ont été utilisés.

RESULTATS :

Analyse de l'ensemble des variétés (148) :

Après plusieurs ACP, les variables retenues comme actives, c'est à dire qui expliquent le mieux la variabilité de l'ensemble sont les suivantes : ANPRI, LAFEU, SUFEU, MAXCB et MAXRO.
Les variables technologiques ont été mises en variables supplémentaires directement car le nombre d'observations diffère d'une variété à l'autre et les observations ne sont pas toutes faites les mêmes années.
Les trois premiers axes de l'ACP expliquent respectivement 40.5, 36 et 11.8 soit au total 88.3 % de la variabilité globale de l'ensemble de la variabilité. Les variables sont représentées sur les deux premiers axes de l'ACP (Figure 1).

Après une CAH à partir des coordonnées factorielles sur les trois premiers axes, les 8 groupes suivants ont été définis :

Groupe 1 (53 variétés) : Ab1, Am, Bo2, Et1, Et3, Et4, Et5, Et6, Et7, Et8, Et9, Et10, Et11B, Et14, Et15, Et17, Et18, Et19, Et20, Et21, Et24, Et25, Et26, Et28, Et29, Et31, Et33, Et34, Et35, Et36, Et40, Et41, Et42, Et43, Et47, Et49, Et50, Et52, Et53, Et54, Et55, Et57, Et58, Et59, Et61, Il3, Kf1, Kf2, Kf4, Kf6, Kf7, Mo1, MoAd1.

Groupe 2 (78 variétés) : Ab2, Ao, BmJm1, BmKe1, Bo3, BoMz1, BoMz2, BoSa1, Ca1, Ca2, Ca3, Ca5, Cr1, Dg2, Dg3, Dg4, Dg5, Dg6, Dg7, Dg8, Dg9, Dg10, Dg11, Dg12, Et2, Et16, Et60, Gu1, Gu2, Ha1, Ha2, Ha3, Ho1, Jk2, Jk3, Jm1, Ka, Ke1, Ke2, Ke3, Ke4, Ke5, Kf3, Kt1, Lb1, Lb2, Lp, Mi1, Mi2, Mi3, Mi4, Mn1, Mn2, Mn3, Mn4, Mo2, Mot, Mu1, Mu2, Mu3, Mu4, Mu5, Mu6, Mu8, Mu9, Mu10, Mu11, My1, My2, My3, Pr2, Sa1, Si1, Si3, Si4, Si5, Si6, To.

Groupe 3 (5 variétés) : Et11C, Et13, Et27, Et30, Et32

Groupe 4 (5 variétés) : Co, Dg1, Et51, Et56, Ni1

Groupe 5 (2 variétés) : Pu1, Si2

Groupe 6 (2 variétés) : Ca4, Mu7

Groupe 7 (1 variété) : Il4

Groupe 8 (2 variétés) : Kf5, Vy

Six de ces groupes ont un faible effectif. Le groupe 8 est composé de deux mutants Erecta présentant un très faible angle des primaires. Les groupes 4, 5, 6 et 7 sont composés de phénotypes extrêmes respectivement pour un port de branche presque horizontal, de très petites feuilles, de très grandes feuilles et des feuilles très fines. Les deux groupes principaux (1 et 2) contiennent respectivement 53 et 78 variétés, soit 88.5 % de l'ensemble des variétés étudiées. Il y a une claire séparation entre les deux groupes (Figure 2). Les variétés du groupe 1 ont des branches relativement érigées, des feuilles fines et une certaine résistance à la rouille orangée et au CBD. Au contraire, les variétés du groupe 2 ont des primaires plus horizontales, des feuilles larges et sont plus sensibles aux maladies. De plus, les variétés du groupe 2 présentent en moyenne une granulométrie, un pourcentage de caracoli et une productivité plus importants que le groupe 1.

La répartition des variétés dans les groupes 1 et 2 n'est pas aléatoire. Figurant dans le groupe 1, on trouve presque tous les génotypes de la prospection IRCC-ORSTOM et les génotypes prospectés par Lejeune dans les provinces éthiopiennes de Kaffa et Illubabor. Dans le groupe 2, on rencontre pratiquement toutes les variétés cultivées à travers le monde en compagnie de génotypes prospectés par Lejeune dans les provinces éthiopiennes de Harrar et Sidamo.

Analyse de la diversité des variétés de la prospection IRCC-ORSTOM :

Une ACP a été réalisée sur 52 variétés de la mission IRCC-ORSTOM. Les variables de départ sont les mêmes que pour l'analyse globale de la collection, avec en plus des variables de croissance. Les variables actives retenues comme les plus explicatives sont les suivantes : DIBRA, DIAME, LAFEU, LOLAF et MAXCB.
Les trois premiers axes expliquent respectivement 40.0, 31.8 et 13.2 soit au total 85 % de la variabilité. Le premier axe est un axe de caractéristiques foliaires, le deuxième axe est un axe de vigueur. Les variables et individus sont représentés sur la figure 3.

On remarque sur l'axe 1 une nette opposition entre les variétés de la région de Tippi, aux grandes feuilles rondes et peu sensibles au CBD, et les variétés de la région de Bonga aux petites feuilles fines et plus sensibles au CBD.

Sur l'axe 2 témoin de la vigueur des variétés, sont différenciées la majorité des variétés ne provenant ni de Tippi ni de Bonga. Toutefois, une partie des variétés de Bonga montre également une certaine vigueur. Il est remarquable que cette différenciation correspond à la station d'échantillonnage (Tableau 1). On ne retrouve dans la partie négative de l'axe 2 presque que des variétés prospectées en plantation familiale ou à l'état sauvage.

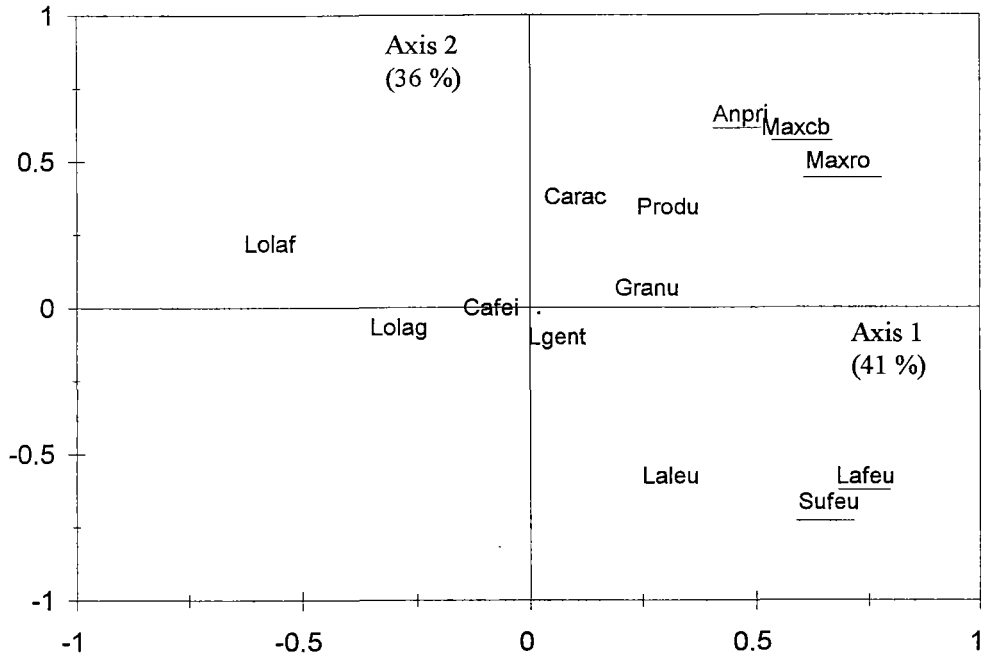


Figure 1 - Variables actives (soulignées) et variables supplémentaires les mieux représentées après une analyse en composantes principales basées sur des données phénotypiques de 148 génotypes de Coffea arabica

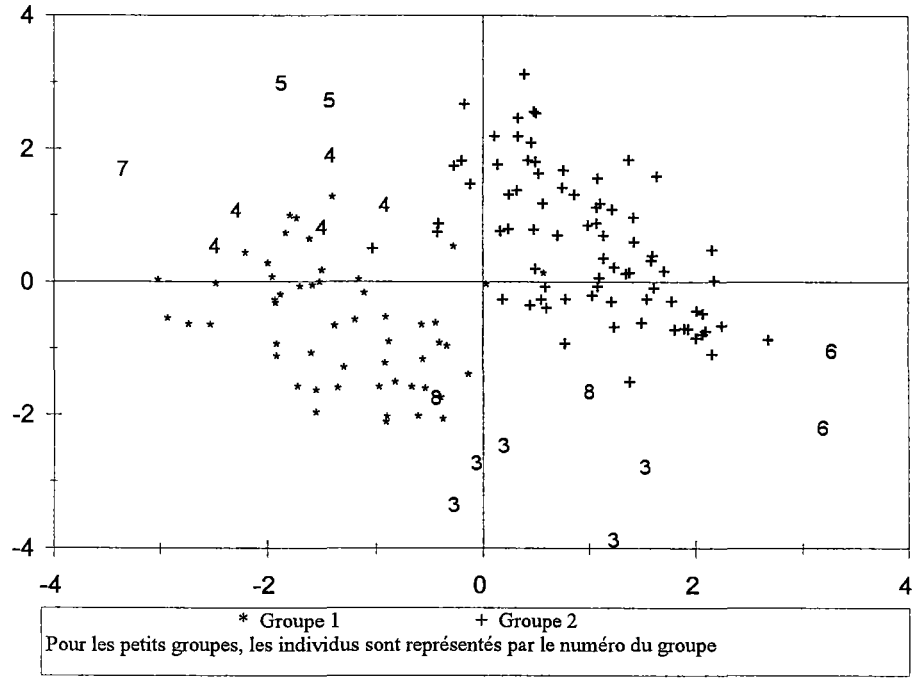
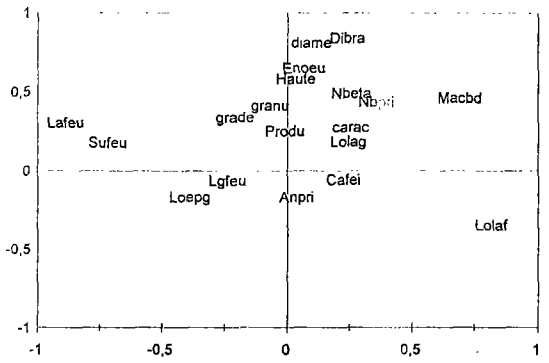
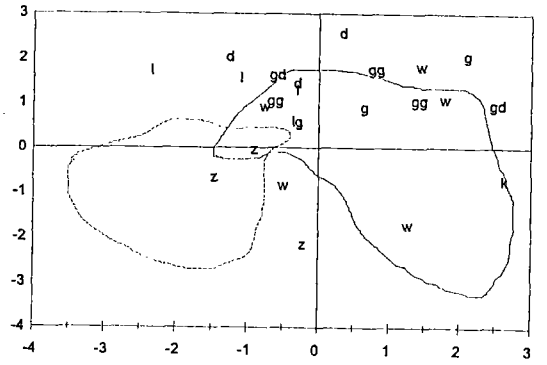


Figure 2 - Représentation de différents groupes de C. arabica aprés une analyse en composantes principale utilisant des données phénotypiques





Variables actives et supplémentaires les mieux représentées (voir Matériels et Méthodes pour codes)

Origines géographiques
——— : Bonga
--------- : Tippi
d: Decchia
gd: Godgeb Valley
gg: Gimma-Gore
w: Wush-Wush
l: Limu
lg: Limu-Gimma
g: Gore
z: Mizan-Tefferi

Figure 2 - Plan 1-2 de l'ACP menée sur 52 variétés de *Coffea arabica* en collection au Cameroun issues de la prospection ORSTOM de 1966.

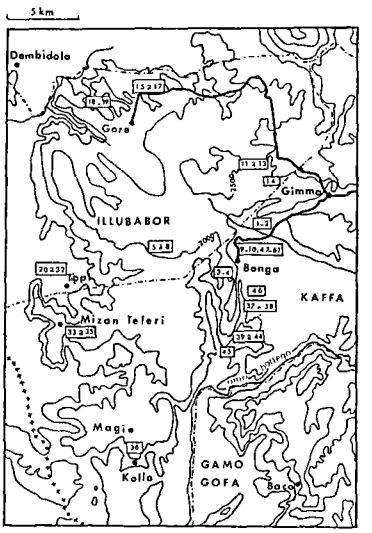


Figure 3 - Lieux de récoltes de *C. arabica* dans le sud-ouest de l'Ethiopie lors de la mission ORSTOM de 1966 (d'après Guillaumet et Halle, 1978)

En ce qui concerne les variables supplémentaires, on note logiquement une production et une granulométrie plus forte pour les variétés les plus vigoureuses. Les grains du type Tippi sont plus plats (fort Loepg) que ceux des autres variétés. Les variétés du type Bonga semblent présenter un plus fort taux de caféine et de caracoli avec des grains plus allongés.

DISCUSSION-CONCLUSIONS

Une diversité structurée a pu être mise en évidence chez *C. arabica*, au niveau de l'ensemble d'une collection d'une part et au niveau d'une prospection régionale en Ethiopie d'autre part. Ceci nous amène à trois niveaux de réflexions.

La base génétique de *Coffea arabica* :

Les résultats de nos travaux confirment la faible variabilité des variétés cultivées avec la présence de phénotypes rares, parfois mutants (Carvalho, 1988). Cependant, notre étude montre la base génétique de *C. arabica* est remarquablement élargie par les génotypes provenant des provinces éthiopiennes de Kaffa et Illubabor, en particulier ceux de la prospection IRCC-ORSTOM de 1966. Les prospections de caféiers dans ces régions du sud-ouest de l'Ethiopie s'avèrent donc extrêmement efficace. Même à l'intérieur de la prospection IRCC-ORSTOM, une structuration de la variabilité a été mise en évidence en accord avec les premiers résultats obtenus par Charrier (1978) :

* géographique entre le type Bonga et le type Tippi + Mizan-Tefferi
* de degré de sélection entre les types sauvages ou semi-sauvages et les arbres ayant subi une certaine sélection.

Le type morphologique des variétés vigoureuses est proche du type Bonga. Il est probable que les variétés cultivées dans la région de Gimma, Gore, Limu et Decchia proviennent de la région de Bonga et non de Tippi (Figure 4).

La domestication de *Coffea arabica* :

Les provinces de Kaffa et Illubabor se situent à l'ouest de la Rift Valley alors que les provinces de Harrar et Sidamo se situent à l'est de cette vallée. Les deux principaux groupes observés semblent donc correspondre à une discontinuité géographique. Les principaux caractères séparant nos deux groupes (sensibilité aux maladies, pourcentage de caracoli, productivité...) sont souvent liés à un phénomène de domestication. Ici, le groupe 2 semble représenter un groupe domestiqué alors que le groupe 1 renferme des génotypes sauvages ou semi-sauvages. L'apparition de quelques variétés de l'ouest de la Rift Valley dans le groupe 2 peut s'expliquer par des introductions récentes. La question se pose de savoir si le groupe 2 dérive directement du groupe 1 après une domestication qui ne se serait produite qu'à l'est de la Rift valley ou bien si le groupe 2 dérive d'une autre source de caféiers sauvages située à l'est de la Rift Valley. Une telle source de caféiers sauvages à l'est de la vallée n'a jamais été mentionnée dans la littérature. Pourtant, différents arguments témoignent en faveur de l'existence d'une telle source aujourd'hui disparue :

* White (1983) rapporte que les conditions écologiques sont les mêmes des deux cotés, est et ouest, de la vallée. Toutefois, l'auteur précise que la partie est aurait pu subir des incendies de forêts, naturels ou causés par l'homme, dont la présence d'une seule espèce dominante (*Juniperus procera*) dans les forêts de l'est serait le témoignage.
* l'histoire de l'Ethiopie montre que ses régions du sud-ouest ont été complètement isolées jusqu'à la fin du 19ème siècle (Meyer, 1965 ; Harlan, 1969)
* Les premiers résultats obtenus par les techniques de marquages moléculaires sur un nombre limité de variété indiquent une séparation entre les variétés cultivées à travers le monde et les génotypes du sud-ouest de l'Ethiopie (Orozco-Castillo *et al*, 1994 ; Lashermes, communication personnelle)

Conséquences pour la sélection :

Si cette assertion concernant l'existence d'une source de caféier sauvage devait s'avérer exacte, ce serait d'une extrême importance pour les programmes de sélection. En effet, un heterosis serait attendu avec des croisements entre génotypes de l'ouest de la Rift Valley et les variétés cultivées provenant de l'est de cette vallée. Or, l'évaluation d'hybrides à Madagascar et au Cameroun semble confirmer l'existence de cette vigueur hybride (Charrier *et al*, 1978 ; Boccara *et al*, 1994).
L'intérêt des génotypes du sud-ouest de l'Ethiopie comme source de résistance aux principales maladies du caféier est amplement confirmé.

La différenciation historique entre Typica et Bourbon

Cette différenciation n'apparaît pas clairement dans notre étude même si des variétés typiques bien identifiées (Typica ou Bourbon) sont séparées en accord avec d'anciennes descriptions (Krug *et al*, 1939). Le continuum observé entre de tels génotypes suggèrent que beaucoup d'intermédiaires ont pu apparaître à travers des croisements entre les types originaux. Ceci est en accord avec les observations de plusieurs auteurs (Sylvain, 1958 ; Meyer, 1965 ; Eskes et Muckred, 1990) pour qui les caractéristiques Bourbon et Typica ne sont pas nécessairement des critères botaniques majeurs.

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RESUME

148 variétés d'une collection de *Coffea arabica* ont été évaluées pour 31 critères botaniques, architecturaux, technologiques, phytopathologues et de productivité. Pour la première fois, une structure importante est observée au sein de cette espèce avec l'identification de deux groupes principaux. Le groupe 1 a des primaires relativement érigées, des feuilles dines et une certaine résistance à la rouille orangée et au CBD. Le groupe 2 possède des primaires plus horizontales, des feuilles larges et presente une plus grande sensibilité aux maladies. Le groupe 1 ne contient presque que des génotypes éthiopens provenant de l'ouest de la Rift Valley, alors que le groupe 2 contient la plupart des variétés cultivées à travers le monde et des génotypes éthiopiens proventant de l'est de la Rift Valley. Cette structure phénotypique ainsi que des évidences historiques laissent supposer que le groupe 1 n'a pas participé à la domestication de *C. arabica*. Les résultats confirment que les prospections en Ethiopie à l'ouest de la Rift valley sont trés efficaces pour l'enrichissement de la base génétique de *C. arabica*. L'intérêt de ces résultats en matière de sélection est discuté.

A l'intérieur même des génotypes d'une prospection IRCC-ORSTOM effectuée à l'ouest de la Rift Valley, une structuration phénotypique forte est décelée en fonction de la géographique entre les caféiers sauvages, et en fonction du degré de sélection.

SUMMARY

Thirty one different morphologic and agronomic traits observed in a field collection of 148 varieties of *Coffea arabica* were analyzed using multivariate analysis. For the first time, a clear structure is observed within this species with the identification of two main groups. Accessions of group 1 have got a relatively erect branching habit, narrow leaves and are in average resistant or moderately resistant to coffee leaf rust and coffee berry disease. Accessions of group 2 have got a relatively horizontal branching habit, broad leaves and are moderately or highly susceptible to coffee leaf rust and coffee berry disease. Group 1 mostly contains ethiopian accessions collected west of the rift valley, whereas group 2 mostly contains commonly cultivated varieties throughout the world and ethiopian accessions collected east of the great rift valley in Ethiopia. This phenotypic structure together with historical evidences suggest that group 1 did not participate in the domestication of *C. arabica*. The present results confirm that the surveys of sub-spontaneous genotypes west of the rift valley are highly valuable for enriching the genetic basis of *C. arabica*. The potential value of this material in breeding of *C. arabica* is discussed. Within the genotypes collected by an IRCC-ORSTOM mission west of the Rift Valley, a strong phenotypical structure is found in relation with geography, between wild or semi-wild varieties, and in relation with the level of selection.

AMINO ACIDS ON SOMATIC EMBRYOGENESIS IN *COFFEA ARABICA*

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INTRODUCTION

Levels and forms of nitrogenous compounds, especially reduced nitrogens, in media are important factors in controlling somatic embryogenesis (Halperin 1964, Halperin and Wetherell 1965, Wetherell and Dougall 1976, Yatazawa and Furuhashi 1968, Sangwan and Harada 1976). The addition of amino acids, reduced organic nitrogenous compounds, to medium promotes somatic embryogenesis (Stuart *et al.* 1984a, Stuart *et al.* 1984b, Kamada and Harada 1979). Glutamine generally promotes somatic embryogenesis in many plant materials (Stuart *et al.* 1984b, Wetherell and Dougall 1976, Kamada and Harada 1979). We examined the effects of amino acids on somatic embryogenesis from *Coffea arabica* embryogenic callus and found that asparagine had a promotive effect on somatic embryo formation, while glutamine and glutamate strongly inhibited.

In this report, the effect of asparagine on *Coffea* somatic embryogenesis was further investigated. We described that asparagine was very effective for somatic embryogenesis and induced somatic embryos in the presence of 2,4-D that promoted cell proliferation without embryogenesis.

MATERIALS AND METHODS

Embryogenic callus of coffee

The explants of young leaves of a mature coffee tree (*Coffea arabica* cv. Caturra), cultured on modified MS medium (Yasuda *et al.* 1985) with 5 μ M N^6-[2-isopentenyl] adenine (2-iP) as plant growth regulator, produced friable calli that formed somatic embryos. When the 2iP-induced callus was transferred to MS medium (Murashige and Skoog 1962) with 10 μ M 2,4-dichlorophenoxyacetic acid (2,4-D), two types of calli, yellowish and white

calli, proliferated. These calli were picked up and subcultured on 10 μ M 2,4-D containing MS medium every 30 day. When transplanted to 5 μ M 2-iP containing modified MS medium, only yellowish callus produced somatic embryos. The yellowish embryogenic callus were used in the following experiments and were subcultured on MS medium with 10 μ M 2,4-D (growing medium) every 30 day.

Culture conditions

We used modified MS medium as basal medium for regeneration of somatic embryos, which contain 1/4 strength in macro elements except phosphate, 1/2 in phosphate and micro elements, supplemented with 5 μ M 2-iP (basal regeneration medium). This medium were used as control for all the experiments. B5 organic elements (Gamborg *et al.* 1968) and 3 % sucrose are common in both MS and modified MS medium. The pH was adjusted at 5.7$\pm$0.1. The media were autoclaved at 120℃ in 1.2 atm for 12 min. The solutions of amino acids and MSX (methionine sulfoximine) were sterilized by membrane filteration (0.2 μ m) and were added to the autoclaved media. About 5 mg of embryogenic callus were inoculated at 3 spots on the 15ml culture media in 50ml vessel. The culture conditions were at 26 ℃ in dark. The ability to form somatic embryos were evaluated by the number of formed somatic embryos per callus spot. Data show the average of the number of formed somatic embryos per callus spot. Three replicates were done for each experiment.

RESULTS

When embryogenic callus subcultured on growing medium (2,4-D medium) were transferred to regeneration medium (2-iP medium), a lot of somatic embryos were always formed. The addition of glutamine, glutamate or aspartate to the regeneration medium (basal regeneration medium containing both 10 mM nitrate and 5 mM ammonium ions) inhibited somatic embryogenesis (data not shown), but asparagine did not so (Table 1-(a)). In the regeneration medium with only nitrate as nitrogen source, somatic embryos were not observed. The addition of 10 mM asparagine perfectly recovered somatic embryogenesis (Table 1-(b)). In no nitrogen-containing regeneration medium in which somatic embryos were not observed, the additions of 10 and 20 mM asparagine partly recovered somatic embryogenesis (Table 1-(c)). MSX added to basal regeneration medium completely inhibited somatic embryogenesis. The addition of 10 mM asparagine to the MSX-inhibited medium recovered somatic embryogenesis (Table 1-(d)). Addition of glutamine, glutamate or aspartate instead of asparagine did not recovered somatic embryogenesis.

Coffee yellowish embryogenic callus cultured on 2,4-D medium (growing medium) proliferated vigorously without somatic embryogenesis. Surprisingly, asparagine added to the 2,4-D medium induced somatic embryogenesis were formed at 1 - 60 mM concentrations (Table 2-(a)). In the growing medium with only nitrate as nitrogen source and in the growing medium added 10 μ M MSX, the additions of asparagine produced somatic embryos (Table 2-(b),(c)). In each asparagine-treated medium, the number of produced somatic embryos was less than in 2-iP regeneration medium, the usual proliferation of callus was suppressed strongly. Additions of glutamine, glutamate or aspartate to 2,4-D medium did not induce somatic embryogenesis.

Interaction between asparagine and 2,4-D on somatic embryogenesis were examined (Table 3.). As the concentration of 2,4-D decreased and the concentration of asparagine increased, somatic embryogenesis accelerated. In the treatments with 100 μ M 2,4-D, the growth of calli were strongly inhibited. The lower the concentration of 2,4-D, the better the development of somatic embryos produced by asparagine.

Table 1. The effect of asparagine in the regeneration medium with various nitrogen conditions on somatic embryogenesis.

Treatment	Number of formed somatic embryos ± S.E.	
(a) 5 μM 2-iP medium	86.0 ± 3.3	(100)*
+asparagine 10 mM	83.0 ± 22.2	(100)
(b) 5 μM 2-iP medium	55.7 ± 11.3	(100)
- NH_4	0	(0)
+asparagine 10 mM	57.0 ± 2.6	(100)
(c) 5 μM 2-iP medium	86.7 ± 2.0	(100)
- NH_4, - NO_3	0	(0)
+asparagine 10 mM	14.0 ± 7.5	(100)
20 mM	20.7 ± 10.3	(100)
(d) 5 μM 2-iP medium	65.7 ± 4.3	(100)
+MSX 1 μM	0	(0)
+asparagine 10 mM	72.3 ± 18.2	(100)

The values represent the average number of formed somatic embryos per callus spot ± the standard error (S.E.) in 3 replicates. Embryogenic callus in each treatment was cultured for 30 days. 5 μM 2-iP medium contain nitrate and ammonium (basal regeneration medium).

* percentage of calli spot producing somatic embryos.

DISCUSSION

In the regeneration media with only nitrate as nitrogen source or nitrogen-free, the additions of asparagine recovered somatic embryogenesis. Asparagine seemed to be a substitute for nitrogen sources in the media (Table 1-(b), (c)). When asparagine was added to MSX-inhibited regeneration medium, somatic embryos were formed as well (Table 1-(d)). MSX is glutamine synthetase (GS) inhibitor and GS is the key enzyme for the assimilation of ammonia with glutamate into glutamine. This is major pathway for incorporation of inorganic N into organic N in higher plants. Nitrogens derived from glutamine are utilized in nitrogen metabolism in a living thing, endogenous generated-ammonia from nitrogenous compounds is reassimilated via GS/GOGAT. Therefore, we could not conclude that ammonia released from asparagine is nitrogen source. The addition of asparagine to nitrogen-free medium (Table 1-(c)) recovered somatic embryogenesis partly, while to only nitrate containing medium or MSX-inhibited medium perfectly (Table 1-(b),(d)). The difference seemed to be due to nitrate. But nitrate in MSX-inhibited medium cannot be utilized in cells because MSX blocked the assimilation pathway. The effect of nitrate was not due to nitrogen source. The coexistance of nitrate and reduced nitrogen (asparagine in the coffee cells), as described by Wetherell and Dougall (1976), seemed to be important for somatic embryogenesis.

Table 2. The effect of asparagine in the growing medium on somatic embryogenesis.

Treatment		Number of formed somatic embryos ± S.E.	
(a) 5 μM 2-iP medium		61.7 ± 7.9	(100)*
10 μM 2,4-D medium		0	(0)
	+Asn 1	11.0 ± 3.5	(100)
	5	38.0 ± 4.9	(100)
	10	41.0 ± 7.6	(100)
	15	27.7 ± 3.8	(100)
	60 mM	23.3 ± 3.3	(100)
(b) 10 μM 2,4-D medium			
$-NH_4$		0	(0)
	+Asn15	15.0 ± 0.2	(100)
	60 mM	13.3 ± 1.8	(100)
(c) 10 μM 2,4-D medium			
+ MSX 10 μM		0	(0)
	+Asn15	10.3 ± 1.5	(100)
	60 mM	12.7 ± 2.4	(100)

The values represent the average number of formed somatic embryos per callus spot ± the standard error (S.E.) in 3 replicates. Embryogenic callus in each treatment was cultured for 60 days. 5 μM 2-iP medium contain nitrate and ammonium (basal regeneration medium). 10 μM 2,4-D medium is basal growing medium.
* percentage of calli spot producing somatic embryos.

Table 3. Somatic embryogenesis in different combinations of 2,4-D and asparagine concentrations in MS medium.

2,4-D concentration (μM)	Asparagine (mM)			
	0	1	5	15
0	73.3 ± 4.6	86.7 ± 2.3	87.0 ± 11.3	96.0 ± 15.6
1	5.7 ± 1.7	15.0 ± 2.0	24.0 ± 2.3	32.5 ± 4.5
10	0	0	9.0 ± 2.5	22.7 ± 4.5
100	0	0	0	0

The values represent the average number of formed somatic embryos per callus spot ± the standard error (S.E.) in 3 replicates. Embryogenic callus in each treatment was cultured for 60 days.

The addition of asparagine to 2,4-D medium for cell proliferation induced somatic embryogenrsis and inhibited normal callus growth. Asparagine appeared to have some interactions to 2,4-D, besides of the promotive effect for somatic embryogenesis. Callus did not grow by the removal of NH_4 from basal growing medium, consequently at least, reduced nitrogen is necessary for normal callus growth. If asparagine is merely a substitute for reduced nitrogen, callus must grow. Indeed callus did not grow. Also, the results of asparagine addition to MSX-inhibited medium indicates the recovery of somatic embryogenesis was not due to reassimilation of free ammonia from asparagine. In mulberry (Suzuki 1982), asparagine and arginine are considered to be the storage substances and they were utilized for germination. Asparagine seemed to have the important role for new generation like initiation of somatic embryos or germination.

The ratio of the concentration of 2,4-D to asparagine seemed to be critical for somatic embryogenesis, asparagine was considered to have the action similar to a plant growth regulator and to repress the action of 2,4-D. The conjugation of 2,4-D with amino acids (Montague *et al.* 1981) may reduce the endogenous 2,4-D content in the cells and influence to somatic embryogenesis. Asparagine, itself or its metabolites must be concerned as the trigger or signal with somatic embryogenesis.

In conclusion, even in the presence of 2,4-D, asparagine led to somatic embryogenesis and asparagine has an important role for somatic embryogenesis from coffee callus.

SUMMARY

Embryogenic cell lines of *Coffea arabica* were maintained on 10 μM 2,4-D medium (growing medium) and were transferred to 5 μM 2-iP medium (regeneration medium) for somatic embryogenesis. While the addition of glutamine, glutamate or aspartate to 2-iP medium, strongly inhibited *Coffea* somatic embryogenesis, asparagine did not inhibit and rather promoted embryogenesis. Although no somatic embryos were formed on 2-iP medium containing nitrate only as N source, nitrogen-free 2-iP medium or MSX-added 2-iP medium, the addition of asparagine induced somatic embryogenesis. Moreover, asparagine surprisingly induced somatic embryos on callus cultured on 2,4-D medium. These results suggest that asparagine has an important role for somatic embryogenesis in *Coffea arabica*.

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ÉTUDE DE LA DIVERSITÉ DES CHAMPIGNONS PATHOGÈNES DU CAFÉIER A L'AIDE DE MARQUEURS MOLÉCULAIRES, ENZYMATIQUES ET MORPHOLOGIQUES

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INTRODUCTION

Un programme de recherche ayant pour but d'étudier le fonctionnement du pathosystème associant au caféier, ses pathogènes fongiques et l'environnement, est actuellement développé en Nouvelle-Calédonie (NC). Au terme de trois années d'enquêtes pluri-locales, des différences importantes ont été observées entre les sites d'étude, tant au plan de la distribution spatiale des principaux champignons pathogènes (*Hemileia vastatrix, Colletotrichum gloeosporioides*) que de la sévérité de leurs attaques. Complétant les études épidémiologiques axées sur la dynamique des maladies foliaires en fonction des conditions de milieu, une analyse de la diversité des populations des deux parasites a été entreprise.

De nombreux travaux ont été consacrés à la diversité du pouvoir pathogène chez *Hemileia vastatrix,* agent de la rouille orangée (Rodrigues et al. 1975). Mais, s'agissant d'un parasite strict impossible à cultiver au laboratoire, l'utilisation de marqueurs de polymorphisme génétique autres que les facteurs de virulence est restée très limitée. Cependant, des travaux récents conduits au CIFC portent sur la caractérisation de 16 races du parasites à l'aide de profils protéiques et isozymiques (Guedes 1995). L'analyse du polymorphisme génomique à l'aide de marqueurs moléculaires, constitue également une voie à explorer pour la caractérisation de la diversité génétique et l'étude de la structure des populations de ce parasite.

Colletotrichum gloeosporioides, champignon parasite très polyphage, présente une grande variabilité génétique (Sutton 1992). Chez les caféiers, où il exite plusieurs types d'anthracnoses, les diagnostics ont souvent été rendus difficiles du fait de cette variabilité. L'analyse du polymorphisme génomique a permis d'évaluer cette variabilité en relation avec la plante hôte chez des souches *C. gloeosporioides* isolées de plantes fruitières tropicales (Hodson et al. 1993). Pour le caféier, les travaux de Hindorf (1973, 1975) puis ceux plus récents de Waller et al. (1993) ont permis de caractériser l'espèce *Colletotrichum kahawae* (ex. *C. coffeanum* sensu Hindorf) responsable de l'anthracnose des baies et de la distinguer de *C. gloeosporioides* responsable des anthracnoses de feuilles et de rameaux. Sreenivasaprasad et al. (1993) ont, de plus, étudié la structure génétique de l'espèce *C. kahawae* et ses relations avec *C. gloeosporioides* par l'analyse du DNA (RFLP et RAPD). D'autres marqueurs tels que la compatibilité végétative (Brooker et al. 1991), le polymorphisme enzymatique (Bonde et al. 1991) et le polymorphisme génomique (Mills et al. 1992 ; Manners et al. 1992) ont par ailleurs été utilisés pour étudier la structure de l'espèce *C. gloeosporioides.*

Ce document présente les premiers résultats obtenus sur l'étude du polymorphisme des deux principaux parasites foliaires du caféier en Nouvelle-Calédonie. Divers marqueurs ont été utilisés pour

comparer les isolats représentatifs des différents sites. Ce sont les profils isoenzymatiques, les groupes de compatibilité végétative (VCG) et le polymorphisme de l'ADN (RAPD & PCR-RFLP).

MATÉRIELS & MÉTHODES

1) Analyse moléculaire d'*Hemileia vastatrix*

La rouille étant un parasite strict qui ne se cultive pas en milieu axénique, l'extraction de l'ADN a présenté des contraintes particulières. En effet, dans le cas d'urédospores récoltées sur des sores, des contaminations par d'autres parasites tel *Colletotrichum*, par l'hyperparasite *Verticillium hemileiae* ou par d'autres champignons saprophytes sont à craindre. Par ailleurs, dans le cas de mycélium de rouille colonisant les parenchymes foliaires, la présence d'ADN du caféier est inévitable. Néanmoins, ces deux types de matériels ont été utilisés pour amplifier l'espaceur interne transcrit (ITS) de l'ADN ribosomique des isolats par la méthode PCR dont les étapes sont les suivantes :

* prélèvement de mycelium à partir de feuilles infectées : après nettoyage des feuilles à l'eau additionnée de Twin 80, lavage à l'alcool à 70°C et séchage avec du papier absorbant, les pustules de rouille sont découpées au plus près de leur front de croissance. Leur conservation se fait à -20°C.

* prélèvement de spores : la récolte se fait par grattage de jeunes sores fructifiés, les spores ainsi obtenues sont conservées à -20°C.

* extraction de l'ADN : les pustules ou les spores sont broyées au piston Pellet Blue dans du tampon d'extraction (CTAB) contenant du PVPP. Après une incubation d'une heure à 65°C, l'extraction de l'ADN est réalisée dans un mélange (24/1) de chloroforme-alcool isoamylique puis par précipitation dans l'alcool absolu. Le culot est conservé au froid après dissolution dans du TE à pH 7,5.

* amplification de l'ITS : elle est effectuée soit avec les amorces ITS1-ITS4 universelles pour les champignons, soit avec les amorces spécifiques résultant du séquençage de l'ITS. Cette dernière opération a été réalisée au laboratoire de pathologie végétale de l'INRA à Versailles.

Pour les amorces ITS1-ITS4, les réglages du thermocycleur sont les suivants : 1 cycle à 94°C/4 min; 35 cycles (94°C/1 min, 55°C/1 min, 72°C/1 min) ; 1 cycle à 72°C/3 min. Pour les amorces spécifiques à la rouille du caféier, les réglages sont les suivants : 1 cycle à 94°C/4 min ; 35 cycles (94°C/1 min, 55°C/1 min, 72°C/1 min) ; 1 cycle à 72°C/3 min.

* l'électrophorèse des amplifiats est effectuée sur gel d'agarose à 2% coloré au BET.

* pour la RFLP, les enzymes de restriction utilisés sont : EcoR I, Cfo I, Rsa I, Hae III, Msp I, Hind III, Hpa II, Sau96 I.

2) Méthodes mises en oeuvre pour l'étude de *Colletotrichum gloeosporioides*

L'étude du polymorphisme de ce pathogène a fait appel à trois techniques différentes.

a. Polymorphisme enzymatique

L'électrophorèse des protéines est effectuée à partir d'extraits bruts de mycélium lavé à l'eau stérile, essoré, broyé à froid avec du sable de Fontainebleau et centrifugé 10 min à 12.000g. Celui-ci provient de cultures âgées de 6 jours sur milieu pomme de terre-dextrose liquide. Les migrations sont réalisées en cuve Biorad sous un courant continu de 100 volts pendant 3 heures. Les différentes activités sont révélées selon les méthodes de Second et Trouslot (1980).

b. VCG

Des groupes de compatibilité végétative ont été recherchés en utilisant la technique des mutants nitrate "nit" (Puhalla, 1985; Correll et al. 1987). La compatibilité entre deux souches cultivées sur milieu minimum se traduit par la formation d'un hétérocaryon de phénotype sauvage au point de contact entre les deux thalles.

c. RAPD

Les amorces RAPD utilisées proviennent du kit F, Operon Technologies (Alameda, CA, USA). Les conditions d'amplification décrites par Williams et al. 1990 ont été modifiées de la façon suivante : les réactions d'amplification sont réalisées dans un volume total de 25 µl contenant 10 mM Tris-HCl (pH 8,3), 50 mM KCl, 1,5 mM MgCl2, 0,001% gélatine, 50 µM de chaque dATP, dCTP, dGTP et dTTP, 15 pmoles d'amorce, 25 ng d'ADN génomique, et 1U de Taq polymérase. Des contrôles négatifs sont réalisés pour chaque expérience d'amplification afin de tester la présence d'éventuelle contamination. Les réactions d'amplification sont effectuées à l'aide d'un thermocycleur programmé de la façon suivante : 1 cycle de 5 min à 95°C, suivi de 30 cycles d'1 min à 94°C, 1 min à 34°C, 2 min à 72°C et

d'un cycle final de 15 min à 72°C. Les produits d'amplification (20 µl de l'amplifiat) sont séparés par électrophorèse en gel d'agarose à 1,4%, colorés au bromure d'éthidium puis photographiés sous lumière UV.

RÉSULTATS & DISCUSSION

A) Diversité génétique de la rouille.

L'approche retenue à Nouméa privilégie la méthode de la PCR-RFLP dont les protocoles ont été progressivement mis au point sur de l'ADN extrait à partir de spores ou de pustules.

Dans un premier temps, l'ITS des isolats a été amplifié à l'aide d'amorces universelles. Les essais de digestion (RFLP) de ces amplifiats ont été effectués avec 8 enzymes de restriction différents. Pour 3 de ces enzymes Cfo I (=Hha I), EcoR I et Rsa I, des polymorphismes de restriction (RFLP) ont été détectés ce qui suggère l'existence d'une diversité génétique entre les isolats de rouilles.

Dans un second temps, un séquençage de l'ITS a été réalisé récemment à partir d'un extrait d'ADN d'urédospores, ce qui a permis d'élaborer des amorces spécifiques à la rouille dont les séquences sont les suivantes :

HEM1 = 5' GTA GTC CCA CCT GAT TTG AG 3' ; HEM4 = 5' CGT CTT CGG GAC ACT GCG 3'

Ces amorces permettent des amplifications plus performantes et spécifiques quels que soient la provenance ou le degré de pureté de l'ADN (Figure 1).

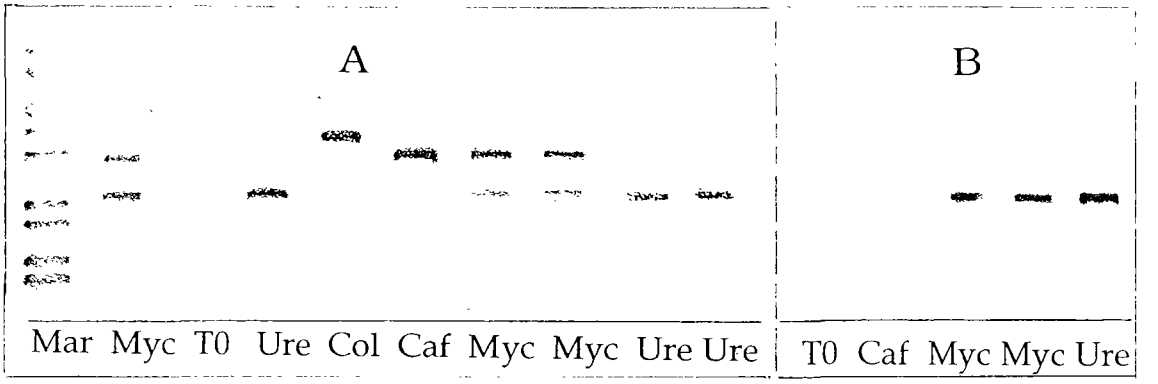

Figure 1 : comparaisons entre amplifications avec amorces universelles (A) ou spécifiques (B)
Marqueur (Mar), Témoin (T0), Mycélium dans pustule (Myc),
Urédospores (Ure), *Colletotrichum* (Col), Café (Caf).

Ce séquençage donne également la possibilité, grâce à la connaissance précise des sites de restriction sur l'ITS, d'optimiser le choix des enzymes. Sur ces bases méthodologiques, de nouvelles RFLP vont être réalisées afin d'évaluer la diversité génétique des isolats de rouille correspondants à chacun des sites de l'enquête épidémiologique. Parallèlement, la détermination des races correspondant à ces mêmes échantillons est en cours d'identification au CIFC (Portugal).

La synthèse de ces deux approches permettra d'évaluer la diversité des populations de rouille afin de la relier aux différents profils épidémiques obtenus.

B) Diversité génétique de *Colletotrichum gloeosporioides*

a- Polymorphisme enzymatique

Le polymorphisme enzymatique a été évalué à partir de 7 activités enzymatiques sur une collection de 29 isolats. Les résultats des électrophorèses ont permis de générer une matrice de similarité (indice de Sokal et Michener) qui montre une grande diversité génétique au sein de l'espèce

parasitaire entre isolats de feuilles, de rameaux et de baies. Néanmoins, le dendrogramme de synthèse des profils obtenus (Figure 2) fait apparaître des regroupements entre certaines souches, par exemple F1, F4, R1 et R4 qui ont été isolées dans des sites d'altitude.

Figure 2 : dendrogramme de synthèse des profils pour 7 activités enzymatiques
Souches isolées à partir de feuille (F), pétiole (RF), rameau (R), baie (B), sites (1 à 20)

b- Groupe de compatibilité végétative

Dans le cas d'un champignon comme *Colletotrichum,* l'étude des groupes de compatibilité végétative est citée comme particulièrement intéressante puisque cette méthode permet de mettre en ,évidence, au sein des populations, les individus capables de former des hétérocaryons, donc potentiellement capables d'échanger de l'information génétique via la parasexualité (Brooker et al. 1991). Aussi, après réalisation de mutants nitrate-déficients, toutes les souches étudiées ont été confrontées entre elles pour rechercher les compatibilités et identifier d'éventuels groupes (VCG).

Les résultats montrent une extrême diversité des isolats dont la plupart, bien qu'auto-compatibles, sont incompatibles entre eux (Figure 3). Chaque souche (à l'exception des 4 souches compatibles) constitue donc une entité indépendante sans aucune possibilité d'anastomose avec les autres.

	F1	F2	F3	F4	F6	F9	F10	F11	F14	F15	F16	F18	F19	F20	R4	R6	R15	Rf10	Rf14	B9	B13
F1	+																				
F2	-	+																			
F3	-	-																			
F4		-	-	+																	
F6	-	-	-	-	+																
F9	-	-	-	-	-	+															
F10	-			-			+														
F11	-	-	-	-	-	-	-	+													
F14	+	-	-	-	-	-	-	-	+												
F15	-	-		-	-	-	-	-	-	+											
F16	-	-	-	-	-	-	-	-	-	-											
F18	-		-	-	-	-	-	-	-	±	-	+									
F19	-	-		-	+				-	-		-	+								
F20	-	-		-	-	-		-	-	-	-			+							
R4	-	-	-	-	-		-	-	-	-	-	-	-	-	+						
R6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+					
R15	-	-	-	-				-	-	-	-	-			-	-	±				
Rf10	-	-	-	-				-	-	-	-	-			-	-		+			
Rf14	-	-	-	-				-	-	-	-	-			-	-			+		
B9	-	-	-	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	
B13	-	-	-	-				-	-	-	-	-			-	-				-	

Figure 3 : matrice des confrontations entre souches
Confrontations partiellement compatibles (±), compatibles (+), non compatibles (-)

c- Analyse moléculaire par RAPD

Les premières analyses de l'ensemble du génome à l'aide de marqueurs moléculaires (RAPD) semblent confirmer l'existence d'une diversité intersites. Néanmoins, certains regroupements sont possibles car on constate l'existence (Figure 4) de profils similaires ou très proches pour certaines souches (F1, R1, F4, R4, par exemple), similitudes confirmées pour au moins 4 amorces (OPF5, OPF9, OPF10 et OPF19) parmi les 20 testées. Ces résultats confirment, en partie, les regroupements observés à partir des profils isoenzymatiques, ce qui montre que ces souches sont assez proches génétiquement.

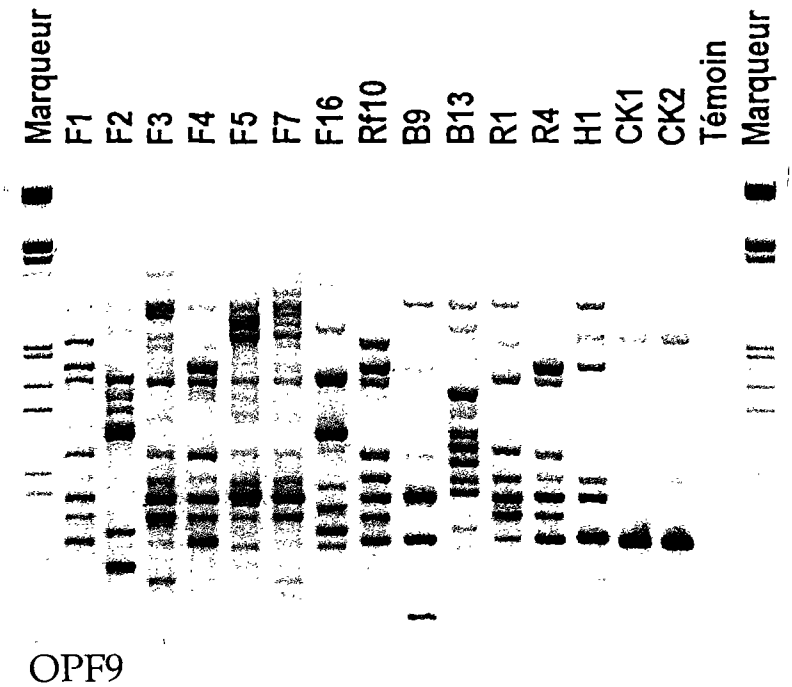

Figure 4 : exemple de profils obtenus avec l'amorce OPF9
Souches isolées à partir de feuille (F), pétiole (RF), rameau (R), baie (B), sites (n° 1 à 20)

Il est intéressant de noter également que les profils CK1 et CK2 correspondant à deux souches de *Colletotrichum kahawae* responsable du CBD du Cameroun, sont extrêmement proches et qu'ils se démarquent nettement des profils B9 et B13 issus d'isolements de *C. gloeosporioides* en NC sur baies infectées. A titre de comparaison, le profil H1 provient d'une souche isolée sur hévéa en Indonésie.

En revanche, une première analyse portant sur 14 isolats issus de différents arbres d'une même parcelle (site Yaté) montre des profils RAPD très voisins avec l'amorce OPF-9, ce qui indiquerait que la diversité génétique intrasite est beaucoup plus faible que celle observée chez les isolats provenant de sites différents. En réalité, il est vraisemblable que chaque population, c'est à dire l'ensemble des individus d'une même communauté génétique (individus susceptibles d'avoir des échanges génétiques), a une aire de répartition extrêmement réduite qui se limite probablement à une plantation, voire à un ou quelques arbres. L'analyse des profils RAPD sur un échantillon plus important d'isolats de chaque site devrait permettre de préciser l'échelle de variation inter-population.

En définitive, deux des trois techniques utilisées semblent plus adaptées à l'analyse des populations de ce pathogène. Paradoxalement la VCG, considérée par différents auteurs (Brooker et al. 1991, i.a.) comme fiable pour la classification des souches de *Colletotrichum*, ne montre aucun regroupement entre les isolats pour ce caractère. Dès lors, on peut considérer, en première analyse, que l'espèce *Colletotrichum gloeosporioides,* parasite des feuilles, des rameaux et des baies du caféier arabica en NC, est distribuée selon une mosaïque de souches différant d'un site à l'autre.

CONCLUSION

La synthèse de ces diverses approches complémentaires, couplée avec l'étude du pouvoir pathogène, devrait permettre d'évaluer la diversité génétique des populations de rouille et d'anthracnose en Nouvelle-Calédonie. La finalité de ces études réside d'une part, dans la possibilité de cartographier la distribution des souches de chaque pathogène. D'autre part, il est indispensable d'associer pathogénèse, diversité génétique et environnement afin d'expliquer les différences de comportement épidémique enregistrées entre les différents sites étudiés.

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RÉSUMÉ

ETUDE DE LA DIVERSITÉ DES CHAMPIGNONS PATHOGÉNES DU CAFEIER A L'AIDE DE MARQUEURS MOLÉCULAIRES, ENZYMATIQUES ET MORPHOLOGIQUES.

F. KOHLER, M. LOURD, E. BRESSON, D. FERNANDEZ, D. NANDRIS, F. PELLEGRIN

Un programme d'épidémiologie est en cours en Nouvelle-Calédonie depuis 1991, afin d'identifier les mécanismes de fonctionnement du pathosystème "caféier-pathogènes-environnement". Au terme de 3 années consécutives d'enquêtes pluri-locales, de fortes différences ont été observées dans les signatures pathologiques des sites d'étude, ceci tant au plan de la distribution des pathogènes (*Hemileia*, *Colletotrichum*, *Cercospora*) que de la sévérité de leurs attaques. De ce fait, une étude de leur polymorphisme a été initiée afin de quantifier la variabilité intra-spécifique des souches fongiques isolées dans les différentes plantations de caféier.

En ce qui concerne *Hemileia vastatrix*, l'espaceur interne transcrit (ITS) de l'ADN ribosomique a été analysé par la technique PCR-RFLP. Les techniques d'extraction et d'amplification de l'ADN de spores de rouille et de lésions foliaires sont désormais au point. Des essais de digestion (RFLP) ont été réalisés à l'aide de huit enzymes différents. L'analyse des profils obtenus a révélé l'existence d'un polymorphisme entre certains isolats de rouille. Le séquençage de l'ITS a permis de définir des amorces spécifiques de la rouille, ce qui permettra d'optimiser l'analyse sur un plus grand nombre de souches. En parallèle, la détermination des races des différents isolats est en cours au CIFC.

Les analyses de populations des souches de *Colletotrichum gloeosporioides* sont réalisées à Montpellier. Trois techniques sont confrontées pour optimiser le niveau de discrimination. En premier lieu, l'étude des activités enzymatiques (ß Est., GDH, LDH, MDH, ICD, PGD, PGM) a abouti à une matrice de similarité (indice de Sokal et Michener). Dans un second temps, après réalisation de mutants nitrate, chacune des souches étudiées a été confrontée par la technique VCG. Une importante hétérogénéité intersites est apparue à l'issue de ces deux analyses mais certains regroupements sont possibles entre ces souches. La méthode RAPD confirme cet état de fait et révèle une variabilité intrasite beaucoup plus faible.

La synthèse de ces différents éléments sur la diversité génétique a pour but de réaliser, pour chaque pathogène, une cartographie détaillée de sa distribution dans les sites, puis d'associer pathogénèse, diversité génétique et environnement, afin d'expliquer les différences de comportement enregistrées au cours des enquêtes épidémiologiques.

SUMMARY

INVESTIGATIONS ON THE DIVERSITY OF THE COFFEE FUNGAL PATHOGENS USING MOLECULAR, ENZYMATIC AND MORPHOLOGICAL TOOLS.

F. KOHLER, M. LOURD, E. BRESSON, D. FERNANDEZ, D. NANDRIS, F. PELLEGRIN

As part of a study on the multiple pathogenic system occurring on *Coffea* in New Caledonia, the spatial repartition and the relative importance of the pathogens on the plant's aerial parts have been assessed over a 3-year period in several locations which were representative of distinct climatic and

cultural conditions. Two fungal species, *Hemileia vastatrix* causing orange rust disease, and *Colletotrichum gloeosporioides* causing foliar and stem anthracnose, are the main observed pathogens. Genetic diversity analyses of these species have been undertaken in order to determine the relationships between their population structure and the distinct observed epidemiological conditions.

Analysis of the ribosomal internal transcribed spacer (ITS) in *H. vastatrix* isolates was conducted by extracting the fungal DNA from spores and the total (fungal and plant) DNA from *Coffea* foliar lesions. PCR-RFLP analyses conducted with universal primers and 8 restriction enzymes allowed to detect polymorphisms between the rust strains. The ITS region was sequenced and some specific primers were designed. Future studies using these primers will allow to enhance further analyses. The race designation of the isolates is currently undertaken at CIFC.

Study of *C. gloeosporioides* genetic diversity has been conducted on 40 strains originating from 3 distinct locations. Several markers were used : vegetative compatibility, isozymes and RAPD. Vegetative compatibility of the isolates, assessed by using "nit" mutants, showed that each isolate constituted a single vegetative compatibility group (VCG). Seven enzymatic systems were tested for isozyme polymorphism : ß Est., GDH, LDH, MDH, ICD,PGD, PGM. A high level of polymorphism was obtained between most of the isolates but a few strains show similar zymogram patterns. For now, no definite correlation between the genetic diversity, the geographic origin and the parasitic specificty (stem or leave) of the isolates has been observed. Analyses are currently undertaken with RAPD markers in order to assess intra-location variability and the genetic population structure of *C. gloeosporioides* in each geographic location.

TRANSFORMATION OF COFFEE WITH *AGROBACTERIUM RHIZOGENES*

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Introduction

Coffee is one of the most favorite soft drinks in the world and this becomes an extremely important economic crop in tropical areas. Arabica(*Coffea arabica* L.), Robusta(*C.canephora*), and Liberica(*C.liberica*) are known as main cultivated varieties for productions of coffee beans. Arabica that is widely cultivated as the highest quality among three varieties has a weak point on the susceptibility to the rust leaf disease. Therefore, changing from Arabica to Robusta, resistant to the rust leaf disease, has been reported in various places. For those back grounds, various research institutions in many countries cultivating coffee are giving their efforts for breeding resistant varieties against the rust leaf disease. By those efforts, several varieties in the resistance to the disease were produced by pollination and selection, but still rooms are left for improvements.

By previous pollination breeding and the cell fusion method, adding a specific characteristic, like the disease resistance, to the existing varieties takes long time and is quite difficult. However, recent gene recombination technology made it possible that aimed useful characteristics can be added. The production of plants added the disease resistance by this technology already became practical steps rather than experimental steps.[1) 2)]

Studies on the gene recombination technology in coffee are progressing. Ocampo *et al.*[3)] and Freire *et al.*[4)] made studies on the possibilities of the gene transformation in Ti plasmid of *Agrobucterium tumefaciens* as a vector. Barton *et al.*[5)] examined the transformation of the foreign genes by an electro-polation method. This study suggests that the transformation in Ri plasmid of *Agrobucterium rhizogenes* strain IFO 14554 is applicable to the production of plantlets with a phenotype of foreign genes. The transformed plantlets were

obtaind, which confirmed the transformation of rol gene group in T-DNA area by a PCR method. Those results are discussed in this report.

Materials and Methods

Plant materials

Cotyledon, hypocotyl and leaf of *Coffea arabica ver. typica* were used in this study. Cotyledons and hypocotyls were gained by the following method. Surface of seeds were sterilized with 0.5% hypochlorite for 20 minutes, and germinated on modified Murashig and Skoog(MS)medium[6] without sucrose under 16/8 light/dark by turn at 25℃. Leaves were gained from the plants, which were regenerated on hormone free modified MS medium from callus subcultured on medium with 2 μM N^6-(2-Isopentenyl)adenine(2-ip).

Bacterial strain

A.rhizogenes strain IFO 14554, harboring mikimopin type Ri plasmid, was grown at 28℃ on YEB medium for 24 hours.

Inoculation and hairy root culture

The tissues were dipped into 5ml of modified MS medium with 100ml of the bacterial suspension. After cocultivation at 25℃ under the dark condition for 4 days, the inoculated tissues were transfered onto modified MS medium with 0.9% ager, 0.25μM 1-Naphthylacetic Acid(NAA), 0.1μM 6-Benzylaminopurine(BAP), and 200mg/l cefotaxine.

Adventitious roots generated from inoculated tissues were excised and transplanted to same medium. After 2 months, these growing roots were subcultured in hormone free ager or liquid medium at an interval of one month. Adventitious embryos appeared from the hairy roots were excised and regenerated on hormone free medium under 16/8 light/dark by turn at 25 ℃.

DNA isolation and PCR

Total DNA was isolated from leaves by CTAB method.[7] PCR was performed with 1724A-B primer[8] using the following condition[8] for 30 cycles:93℃ for 30 seconds, 55℃ for 30 seconds, 73℃ for 2 minutes. One half of the PCR reaction was analyzed on a 1.5% agarose gel and visualized by ethidium bromide staining.

Results and Discussion

Within 2 months after inoculation, several adventitious roots similar to hairy roots and callus appeared at the inoculation site. As shown in Table 1 and Fig. 1, callus were formed in a frequency of 48.5% and hairy roots were produced in 39.4% from fragments of the cotyledon. The hypocotyl formed callus in 95.0%, but no hairy roots were produced. Subcultured leaf fragments produced only small numbers of hairy roots and callus.

In control experiments, no hairy roots were observed and formation of callus was recognized only.

Table 1. Frequency of callus and hairy root formation from explant source following inocuration culture.

explant source	callus %	hairy root %
subcultured leaf	4.5 (0.0)*	0.8 (0.0)
cotyledon	48.5 (0.0)	39.4 (0.0)
hypocotyl	95.0 (16.7)	0.0 (0.0)

*;control(non-inocuration culture)

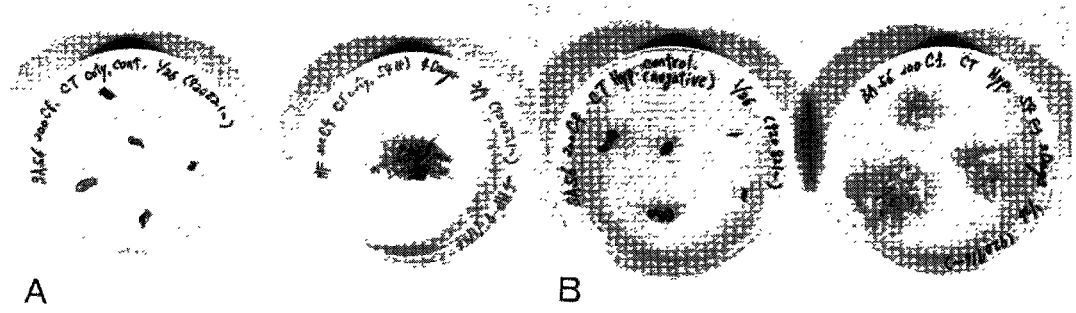

Fig. 1 Callus and hairy root formation from cotyledon and hypocotyls inoculated with *A.rhizogenes*
A:Transformed (right) and non-transformed(left) cotyledon after 6 months of inoculation.
B:Transformed (right) and non-transformed(left) hypocotyl after 6 months of inoculation.

The obtained callus were non-embryogenic in every cases. Because an infection of *A.rhizogenes* was easy to juvenile plant seedling just after germination[9], it was presumed that subcultured leaf fragments showed low rates of infections by *A.rhizogenes* and also low rates of callus and hairy roots formation.

Then, a liquid culture showed better results, when it was examined the propagation of hairy roots on the different culture medium, such as liquid and agar. In many species of plants, it is known the propagation rates of hairy roots are most effective in the liquid medium. And some plants show the propagation in several thousands times[10], if suitable conditions were existed. Even though the same results showed in coffee, poor rations were recognized on its propagation, because the culture medium with cefotaxine was used for its propagation. From now on, it is necessary to examine suitable conditions to the propagation of hairy roots.

After 6 months of the subculture, small numbers of adventitious embryos were produced from hairy roots directly(Fig. 2). Formation of adventitious embryos were recognized in callus, but they were not normal. It is presumed that they were produced accidentally during the subculture, but the frequency of formation was low. If transplantings to the subculture medium suitable for the regeneration were made, the rates of formation should be obtained higher.

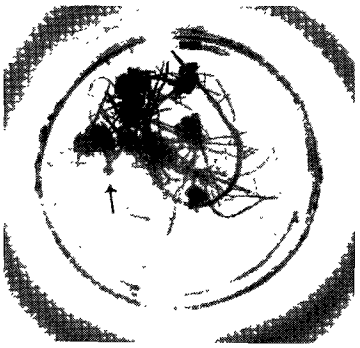

Fig. 2 Adventitious embryos appeared from hairy roots directly. (→)

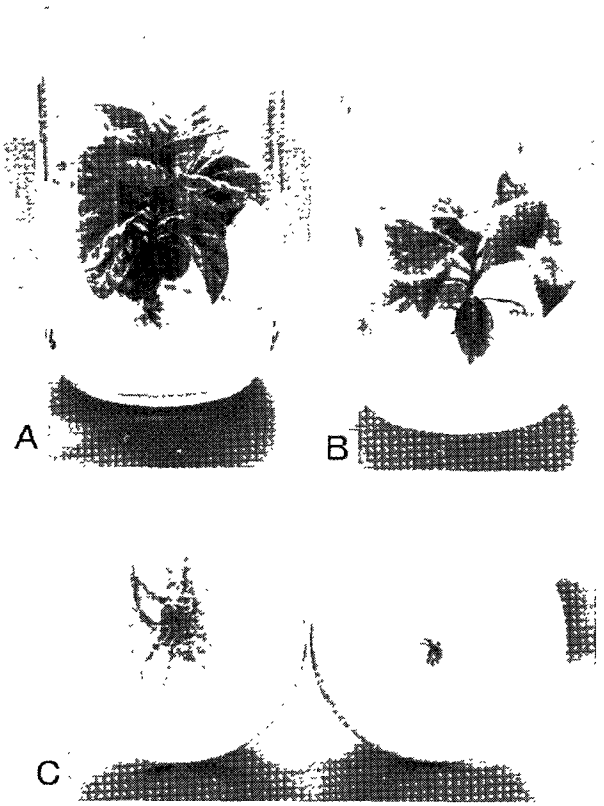

Fig. 3 Regenerated plantlets from hairy roots.
A: The plant regenerated from hairy roots transformed with *A. rhizogenes*.
B: Control plant regenerate from non-transformed leaves.
C: Roots of regenerated plants.
Right is non-transformed plant.
Left is transformed plant.

Furthermore, the regeneration of adventitious embryos may become to the normal situation.

When obtained adventitious embryos were transplanted to hormone free medium, plantlets appeared as shown in Fig. 3. Plantlets regenerated from hairy roots showed phenotype of short internodes and remarkable development of roots, compared with non-transformed plants. Generally, the plants transformed by *A.rhizogenes* were shown remarkable characteristics called as the hairy root syndrome[11][12], such as wrinkled leaves, wider leaves, dwarf with short internodes, non-dominance of terminal buds, remarkable differentiation of adventitious roots, non-hypertrophy of roots and remarkable development of roots. And the same characteristics were found in coffee.

It is well known these characteristics are caused by A, B, C, D, genes in the T-DNA area[13], and those rol gene groups of transformed plantlets are able to detect by the PCR method of Kiyokawa *et al.*[8]. Therefore, we isolated total DNA from transformed and non-transformed plantlets, and treated with the PCR method. With those results, the rol genes were amplified in the transformed plantlets(Fig. 4). In addition, clonal plantlets were propagated by the internode culture from transformed plantlets which were confirmed their transformation of rol genes. Those clonal plantlets showed short internodes and remarkable development of roots, as the mother plant. When the DNA, isolated from leaves of clonal plantlets was teared by the PCR method as the same with the mother plant, the amplification of the rol gene group was found.

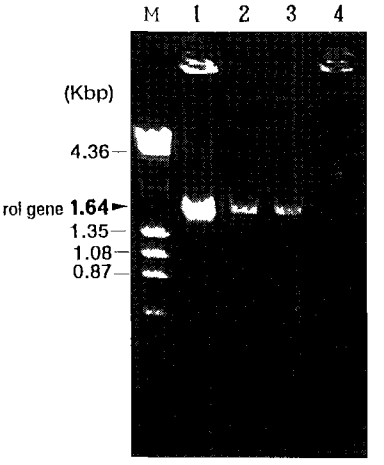

Fig. 4 PCR analysis with 1724A-B primers in coffee.
M:Molecular weight marker of ϕ174/HaeIII and λ/HindIII.
1:Bacterial DNA as a control.
(*A.rhizogenes* strain IFO 14554)
2:DNA of transformed plant.
3:DNA of clonal plant.(from transformed plant)
4:DNA of a non-transformed plant.(control)

For those results written above, the plantlets regenerated from adventitious hairy roots were included the rol gene group in T-DNA area of Ri plasmid containing by *A.rhizogenes* into coffee genes, and appeared transformed phenotype. It is necessary to prove that the detected rol gene group is from DNA of transformed plantlets, not from Agrobacterium, because the PCR method is an extremely acute detection method of DNA. In this study, we kept on subculturing by the medium with cefotaxine, therefore it may be impossible that Agrobacterium is survival. But the recognition on the sterilization was performed with a method discussed by Kiyokawa *et al.*[8]. Now, we are applying with their method.

Thus, these study suggests that the transform of foreign genes by *A.rhizogenes* is applicable to the production of transformed coffee. Therefore, it may be possible to produce resistant varieties, after transformation resistant genes against the rust leaf disease. In the case of productions on varieties of resistance to the rust leaf disease, we must produce many transformed plants and select from them. Because remarkable differences might be appear in every transformed plants. For those purposes, it is necessary to make clear effective conditions for developing excellent transformed plants. In the future, we will continue our studies on those subjects and observe characteristic of transformed plants.

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Summary

With using the wildtype *A.rhizogenes* strain IFO 14554, we examined it is a possibility to transform foreign genes into *C. arabica*, susceptible to the rust leaf disease. As the results, adventitious hairy roots were obtained, and small numbers of adventitious embryos from them were produced directly in subculture by the propagation medium with cefotaxine. After transplanting to a hormone free medium, those embryos grew plantlets. Regenerated plantlets showed their characteristic, such as short internodes and excellent development of roots. It was recognized that the rol gene group in the T-DNA area of Ri plasmid was amplified by the PCR method. Those results suggested that foreign genes were transformed coffee plants by *A.rhizogenes*, and there was applicableto the production of transformed coffee. Therefore, we may be possible to produce resistant varieties after transformation resistant genes against the rust leaf disease.

HISTOLOGICAL AND BIOLOGICAL ASPECTS IN SOMATIC EMBRYOGENESIS OF *COFFEA ARABICA*

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INTRODUCTION

Somatic embryogenesis of coffee has been described in several instances. The first description of regeneration of plantlets by the route of somatic embryogenesis was reported by Staritsky (1970) on callus tissue derived from internode explants of *Coffea canephora*. In *C. arabica*, Sondahl and Sharp obtained plantlets via somatic embryos (1977), and many subsequent studies have demonstrated the embryogenic potential of this genus from various explants; leaves (Dublin 1981, Pierson et al. 1983, Yasuda et al. 1985), stems (Nassuth et al. 1980), hypocotyls and cotyledons (Sondahl 1984) and ovules (Lanaud 1981). Recently histological (Nakamura et al. 1992) and biochemical (Yuffa et al. 1994) studies of somatic embryogenesis on coffee have been reported. However little is known about histological and biological events on early stage of somatic embryogenesis as well as the characters of embryogenic cells.

In this study, embryogenic callus was characterized by histological feature to compare with non-embryogenic calli. Additionally, the effect of 2,4-dichlorophenoxyacetic acid (2,4-D) on somatic embryogenesis, which was regulated by 2,4-D, was estimated.

MATERIALS AND METHODS

Tissue culture

Three types of calli were obtained through two steps culture system. On the first step, callus was derived from leaf explants of mature coffee tree (*Coffea arabica* L. cv. Caturra) on modified Murashige and Skoog (MS) (Murashige and Skoog 1962) basal medium which was composed of 1/4 strength macro salts and half strength micro salts of MS medium, organic constituents of B5 medium (Gamborg et al. 1968), 30 g/l sucrose and 0.9 % (w/v) agar, supplemented with 5 μM benzylaminopurine (BAP) (Yasuda et al. 1985). On the next step, the callus was transferred onto MS medium with 10 μM 2,4-D, which was solidified with 0.3 % (w/v) Gelrite, after 4 weeks culture, three types of calli, one embryogenic callus (EC) and two non-embryogenic calli(NYC, NWC), arose. EC regenerated into somatic embryos after 4-8 weeks culture on the modified MS medium with 5 μ M

iso-pentenylaminopurine (2-iP) (regeneration medium). All calli were subcultured on MS medium with 10 μM 2,4-D every one month.

All media were adjusted to pH 5.7 before they were autoclaved for 12 minutes at 120°C. All cultures were incubated under dark conditions at 25°C.

Histological procedures

For light microscopic observation, calli were fixed in a solution of 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2 for 2h. After fixation, calli were rinsed twice with 0.1 M phosphate buffer, pH 7.2, and dehydrated in an ethanol series. Sample tissues which were immersed in Technovit 7100 (Kulzer) overnight before embedding, were placed in polyethylene capsules with fresh Technovit 7100 and polymerized at room temperature for 2h. Embedded tissues were sectioned into 1 to 2 μm and mounted on glass slides. Tissues were stained with 1% toluidine blue. The cryo-system (Hitachi S-2460N) was adopted for scanning electron microscopic observation.

For measurement of cell size, protoplasts were prepared in each callus. Protoplasts were derived from enzyme treatment and purified (Tahara et al. 1994). The enzyme solutions were consisted of 1.0% (w/v) cellulase Onozuka RS (Yakult Pharmaceutical, Tokyo), 0.2 % pectolyase Y23 (Seishin Pharmaceutical, Tokyo) and 0.5 M mannitol for EC and NYC, for NWC, 2.0 % driselase (Kyowa Hakko, Tokyo), 1.5 % cellulase Onozuka RS, 1.5 % cellulase YC (Seishin Pharmaceutical, Tokyo), 1.0 % pectolyase Y23, 1.0 % macerozyme R-10 (Yakult Pharmaceutical, Tokyo), 0.5 % meiselase P-1 (Meiji Seika, Tokyo) and 0.5 M mannitol.

Effect of 2,4-D on somatic embryogenesis

EC was cultured on MS medium supplemented with 10 μM 2,4-D (2-^{14}C) (specific activity 37 KBq/μ mol ; Amersham Japan, Tokyo) for 2 weeks. After transferring to regeneration medium, EC was harvested every week. Radioactivity of samples were counted by scintillation counter.

To examine the effect of 2,4-D concentration on somatic embryogenesis, EC was cultured on MS medium with several concentrations (0-100 μM).

RESULTS AND DISCUSSION

Three types of calli were compared in their histological characters. EC was yellow and friable in appearance (Fig. 1) and consisted of isodiametric spherical cells which average 18.6 μm in diameter of protoplast (Table. 1). Each cell connected tightly and made small clumps, but they didn't organize any

Table 1 Characters of embryogenic (EC) and non-embryogenic (NYC, NWC) calli of *Coffea arabica*.

	outlook	cell shape	DW/FW (%)	diameter.of protoplast (μm)	protein content (mg/g FW)
EC	yellow	spherical	12	18.6	1.57
NYC	yellow translucent	spherical	11	21.4	1.03
NWC	white translucent	elongated swollen	8	62.9	0.27

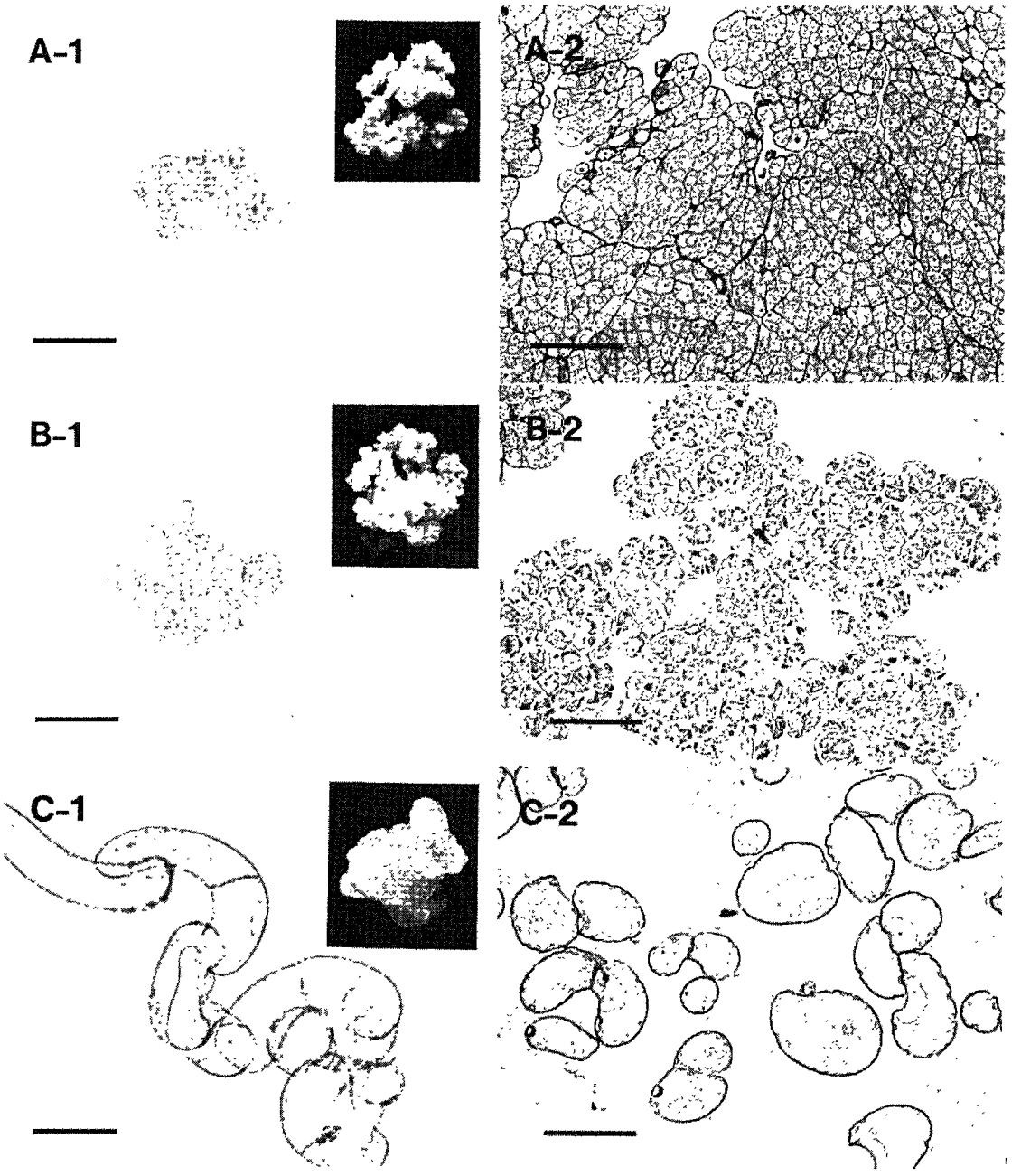

Fig. 1 Comparative structure of *C. arabica* calli cultured on MS medium with 10 μM 2,4-D for 4 weeks. A: embryogenic callus, B: non-embryogenic yellow callus, C: non-embryogenic white callus. Bars in A-1, B-1 and C-1 represent 50 μm. Cross sections (1 μm in thickness) were stained with 1.0% toluidine blue. Bars in A-2, B-2 and C-2 represent 100 μm.

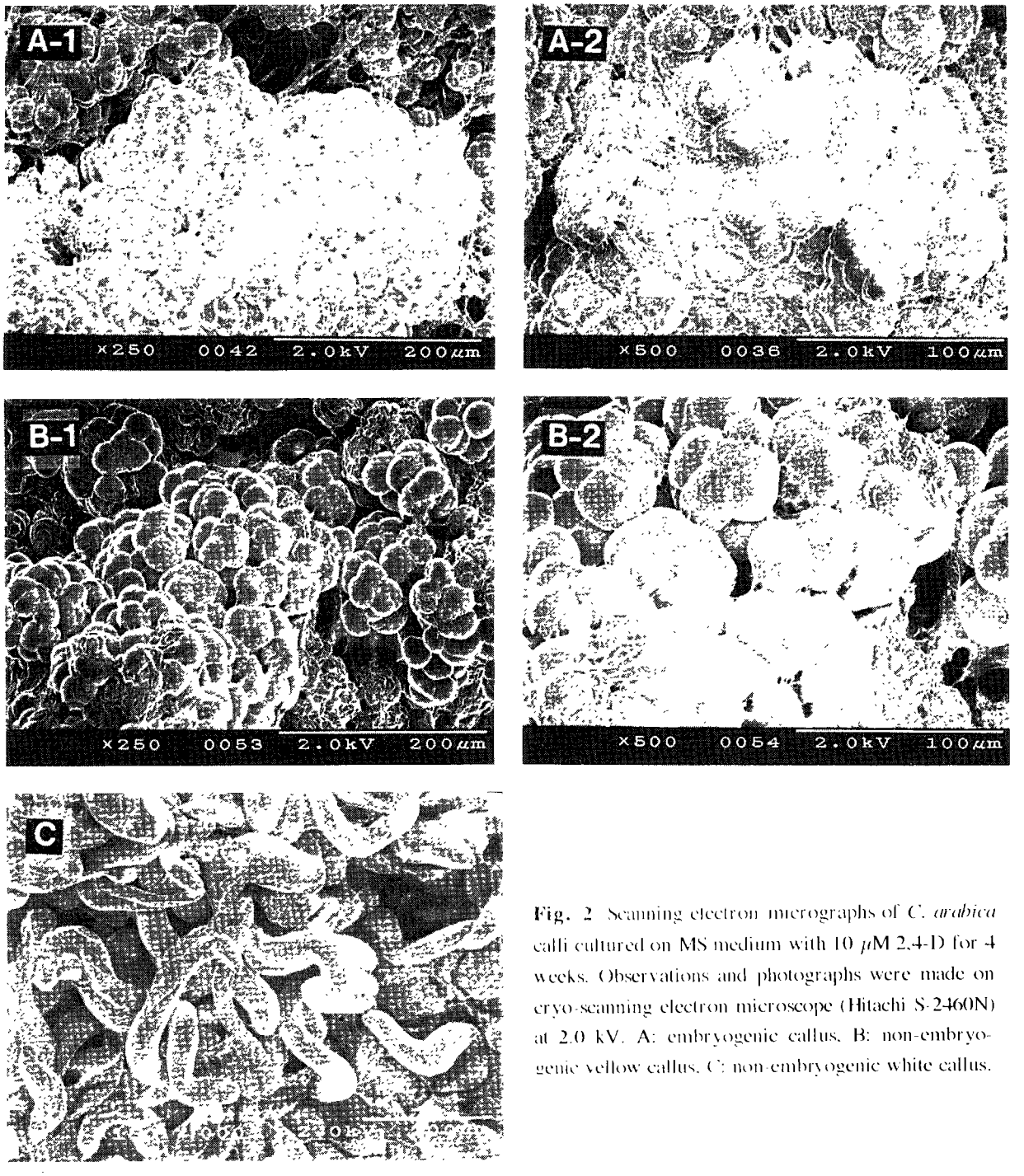

Fig. 2 Scanning electron micrographs of *C. arabica* calli cultured on MS medium with 10 μM 2,4-D for 4 weeks. Observations and photographs were made on cryo-scanning electron microscope (Hitachi S-2460N) at 2.0 kV. A: embryogenic callus, B: non-embryogenic yellow callus, C: non-embryogenic white callus.

somatic embryos even early globular stage embryos (Fig. 2). NYC was translucent yellow but nearly same as EC in its appearance, cell shape (Fig. 1) and size which average 21.4 μm in diameter of protoplast (Table. 1). NYC consisted of a small mass of cells, but each mass aggregated loosely (Fig. 2) so that NYC was dispersible. NWC which was different from other two calli distinctly was translucent white, and composed of larger, elongated or swollen cells (Fig. 1, 2). The diameter of protoplast in NWC was 62.9 μm in average (Table. 1) and covered a wide range, whereas those in EC and NYC were considerably uniform (Fig. 3).

Protein content in EC was the highest (1.57mg/g F.W.) and that in NWC was extremely low (0.27mg/g F.W.)(Table.1).

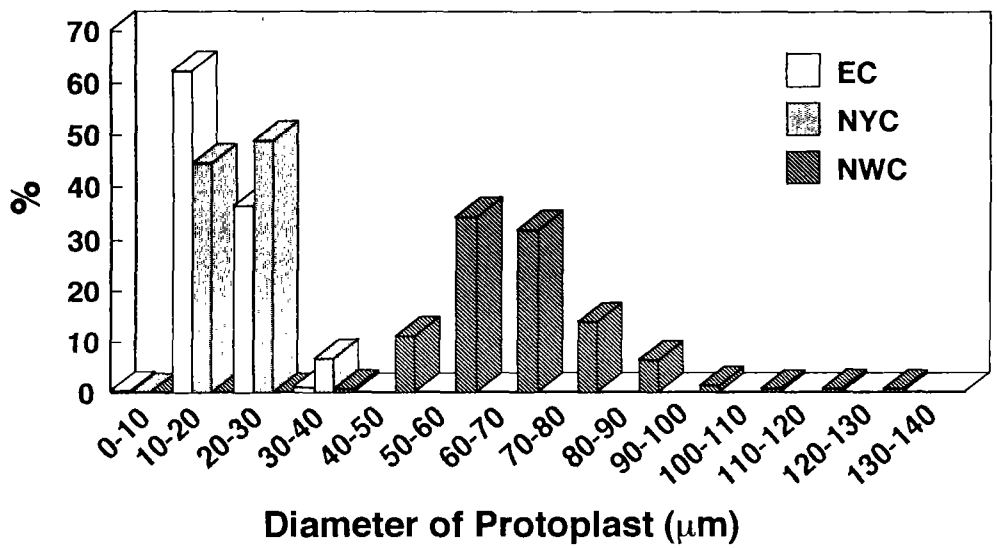

Fig. 3 Diversity of cell size in embryogenic (EC), non-embryogenic yellow (NYC) and non-embryogenic white (NWC) calli of *Coffea arabica*. Cell size was estimated as a diameter of protoplasts, which were prepared from the calli culturedon MS medium supplemented with 10 μM 2,4-D for 4 weeks. In each callus, 250 protoplasts were measured.

EC which was unorganized cell mass on MS medium with 10 μM 2,4-D regenerated into somatic embryos for 4-8 weeks after transferring to 2,4-D free medium (regeneration medium). The effect of 2,4-D on embryogenesis was examined. Somatic embryogenesis occurred on low concentrations of 2,4-D media, but less than that on regeneration medium (Table 2). A few embryos were rarely derived on 5 μM 2,4-D medium. EC on 10 or 50 μM 2,4-D medium showed vigorous growth but never formed any embryos. 100 μM 2,4-D suppressed growth of callus; thus, EC turned into brown without any growth. After 3-5 weeks culture, small amount of compact and yellow callus arose on the browned callus, but proliferated very slowly. Additional effect of cytokinin to MS medium with 10 μM 2,4-D was also examined. Calli were cultured on MS medium supplemented with 10 μM 2,4-D and 2-iP (0.1-100 μM) for 1 month. On each concentration of 2-iP, calli didn't show any different growth from on 2-iP free mediumand never regenerated any somatic embryos. After EC was cultured on MS mediumwith 10 μM 2,4-D (2-14C), 37KBq/μmol) for 2 weeks, EC was subcultured onto regeneration medium. The specific activity in EC was counted by a liquid scintillation counter. The specific activity decreased considerably within a week (Fig. 4). Globular stage embryos were observed after 2-3 weeks culture on regeneration

Table 2 The effect of 2,4-D concentration on somatic embryogenesis of *Coffea arabica*. 2,4-D was added to MS medium in each concentration. Calli were cultured on each medium for 6 weeks.

	Concentration of 2,4-D (μM)						
	0	0.1	1.0	5.0	10	50	100
Degree of embryo formation	++	++	+	±	–	–	–

The relative frequency of the embryo formation is indicated by plus signs (+); the absence of embryo formation is indicated by a minus sign (–).

medium. As additional experiment, radioactivities of calli were analyzed with thin layer chromatography during culture with labeled 2,4-D. Calli were collected every 2 days and extracted with 80 % ethanol, and specific activities were also counted at the same time. The concentrated extractions were applied on cellulose plate (20 cm X 20 cm, 0.1 mm thick) and developed with n-butanol : acetone : water (5:2:3). Specific activity in growing callus increased until 6 days and reached a plateau. Most of radioactivities in each extraction were detected on the same position as 2,4-D (data not shown).

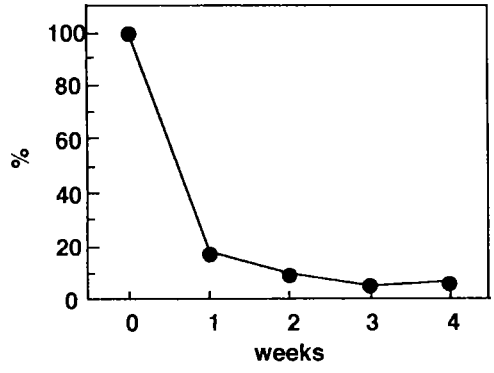

Fig. 4 Changes in radioactivity in EC fed on 2-[^{14}C] 2,4-D during somatic embryogenesis. EC that had been cultured on MS medium with 10 μM 2,4-D (2-[^{14}C]; specific activity 37 KBq/mmol) for 2 weeks, was transferred onto regeneration medium (2,4-D free). Some pieces of the EC were collected every week and the radioactivities were counted.

Since somatic embryogenesis was first recognized by Steward et al. (1958) and Reinert (1958) in *Daucus carota* (carrot), many advanced works were carried out on carrot, because of the relatively simple procedure with which massive amounts of somatic embryos can be produced under controlled conditions in liquid cultures. In carrot cultures, somatic embryos develop exclusively from small clusters of cells, designated proembryogenic masses (PEM) by Halperin (1966). PEM which is the first detectable embryonal stage consists of 10-20 tightly adhering and cytoplasm-rich cells and can only advance up to globular stage in presence of auxin.

In coffee cell culture which was established in our study, some similarities to carrot cell culture were recognized. Embryogenesis was controlled by auxin and embryogenic cells were densely cytoplasmic and adhered each other tightly. Also elongated cells didn't have embryogenic potential. In our observations on coffee callus, embryogenic cells were adhered each other tightly, but they didn't composed any PEM-like structures on subculturing medium. This must be caused by relatively high

concentration of auxin. Namely, vegetative growth only occurred with 10 μM 2,4-D because of entire block embryogenesis by 10 μM 2,4-D. As the results, the first event on embryogenesis occurred after transferring to regeneration medium.

Cultured carrot cells adapted to moderate auxin condition in the subculture medium diminished gradually and lost their embryogenic potential within a year, while coffee embryogenic callus maintained with 10 μM 2,4-D preserved embryogenic potential for 6 years.

These observations have led to the conclusion that once 2,4-D (10 μM) has made the cells competent to initiate somatic embryogenesis, it will then inhibit somatic embryogenesis and the permissive condition such as the reduction of auxin content in cells has been necessary to complete embryo formation.

These results suggest that embryogenic callus cultured on the conditions which inhibit embryogenesis completely, maintained embryogenic potential for long time.

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SUMMARY

Three types of coffee (*Coffea arabica* L.) calli, one embryogenic callus (EC) and two non-embryogenic calli (yellow callus, NYC; white callus, NWC) were maintained on Murashige and Skoog (MS) medium supplemented with 10 μM 2,4-dichlorophenoxyacetic (2,4-D) acid and examined for their histological characters and structural differences. EC consisted of yellow, spherical cytoplasm -rich cells and cell size was uniform. NWC consisted of elongated or swollen translucent cells. NYC was similar to EC on its appearance but more dispersible than EC.

On the SEM observation, EC on MS medium with 10 μM 2,4-D didn't compose any somatic embryos. After transferring to 2,4-D free medium, intracellular 2,4-D concentration decreased immediately and globular stage embryos arose. Somatic embryos were derived on low concentration 2,4-D medium. These results suggest that 10 μM 2,4-D suppress somatic embryogenesis on this cell line.

EVALUATIONS AND EXPERIMENTS ON FERMENTATION AND DRYING OF JAVA COFFEE

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Introduction

Java coffee has been produced since 19 centuries and well known for its specialty taste. The coffee grown on the high-land of East Java where the environment is very suitable for its growth.

Java coffee produced from Typica type coffee is processed by wet method. Traditionally the coffee is processed under tight control starting from fruit picking and sortation, pulping, fermentation, washing, sun drying and finally hand sortation of the product. At first the coffee was dried under sun drying procedure. Due to the higher yield, and for efficiency recently the drying procedure has been modified to mechanical drying.

Evaluations on the existing production process and experiments on fermentation and drying procedures were done in order to improve the quality of the Java coffee as well as the production process.

Material and Methods

The evaluations and experiment as mentioned in Figure 1 were conducted at Kalisat estate-East Java in 1992-1994. The bean layer during sun drying and artificial drying process was 7 cm. The artificial drying temperature was 45 ^{o}C. Sensory evaluation was done by SCAA (Specialty Coffee Association of America) and CIRAD (Center for International Cooperation in Agricultural Research for Development).

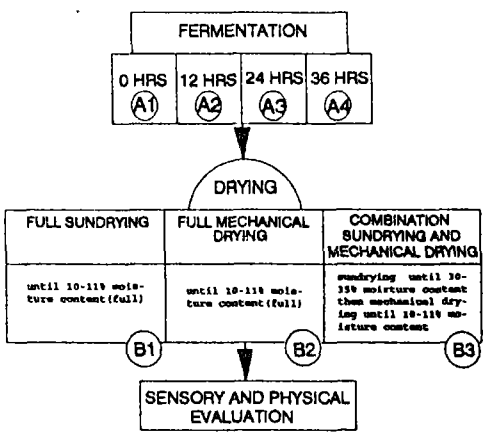

Figure 1. The procedure of fermentation and drying experiment

Results and Discussion

Evaluations on the existing process showed that some problems encountered especially on the sun drying procedure, which are characterized by its low capacity, difficult to control, and depend on weather conditions. Normally the drying process on a drying terrace with 7 cm bean layer needs 7 to 24 days. The slow drying rate is due to the low ambient temperature and relatively high RH (75-90 %). After the fourth day of sun drying, the beans still have moisture content of 30-35 % (Figure 2), that condition promoting the sourish flavour is due to post-fermentation during the drying process. Some micro-organisms can cause off-flavours to develop, particularly in prolonged fermentation (2)

The fermentation and drying trials interactively showed an effect on the bean quality. The trials in which the condition has low daily temperature and high RH showed that the effect of drying trials on the coffee flavour were more dominant compared to the fermentation trials, although longer fermentation trials produced beans with more sourish flavour.

Table 1. Sensory and physical analysis results

Treatment Code *	Particular Taste					Taste (overall)	Cup	Physical Appearance
	Body	Acidity	Bitterness	Astringency	Flavour			
A1 B1	net good	weak good	net	very weak		good cup		very good colour in green,roast good
A2 B1	net	weak	net	weak		good cup	good	excelent colour
A3 B1	x	x	x	x		x	good	very good colour, roast good, excelent break
A4 B1	net good	net no acid	net	weak		good cup		excelent colour, roast good
A1 B2	net	weak	net	weak		metallic	good	very good colour, roast good
A2 B2	net	net	net	weak		sourish	good	very good colour, roast good
A3 B2	x	x	x	x		x	good	good colour, roast good
A4 B2	net	net	net	weak		sourish fruity tone	very good	very good colour, roast good, excelent break
A1 B3	net good	weak	net	very weak	good	grassy sharp undertone		very good colour, uneven preparation, roast fair
A2 B3	net good	weak	net	weak	good	sourish		very good colour, fair preparation, roast fair
A3 B3	x	x	x	x		not balance	good	unique foxy style,poor preparation, borehole roast fair
A4 B3	net	weak	net	weak		sourish	good with acid	excelent colour, roast good

Note : * : see Figure 1 for explanation
x : not analyzed

An effort to accelerate the drying rate by applying fully mechanical dryer might produce beans with good taste, unless the drying temperature was controlled at 40-45 °C (Figure 3). Overheating during drying produce sour or cooked flavours in the brewed coffee. As an approximate guide, it may be stated that coffee will tolerate temperature of 40 °C for a day or two, 50 °C for a few hours, and 60 °C for less than an hour without damage (2). The mechanical drying process might produce beans with metallic, sourish and fruitytone flavours, more astringency, and bitterness (Table 1).

The drying process on a drying table could reduce the drying time to 6 days to reach the bean moisture content of 10-11 %. The coffee bean product has a better flavour with no off flavour.

Combined process of drying on table for 4 days to reach of 30-35 % moisture content, then by mechanical drying for 24 hours to final of 10-11 % moisture content, produced better flavour compared to the fully mechanical drying, although some off-flavour like grassy, sharp undertone and sourish were still present and its taste not balance.

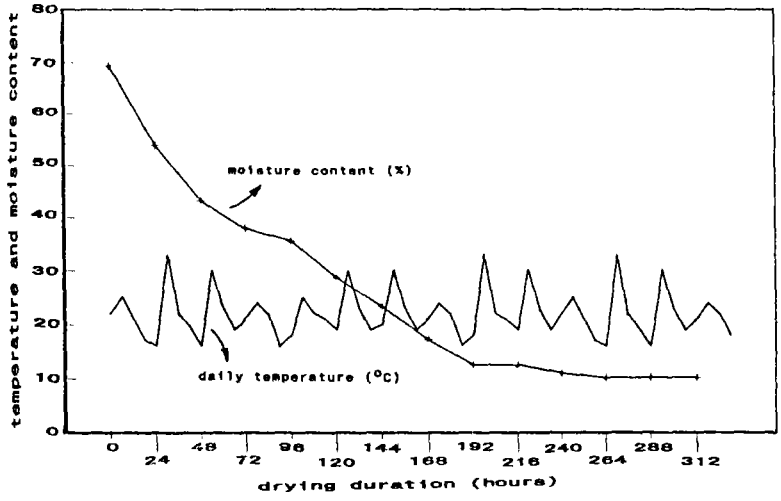

Figure 2. Daily temperature and moisture content during drying trials on terrace

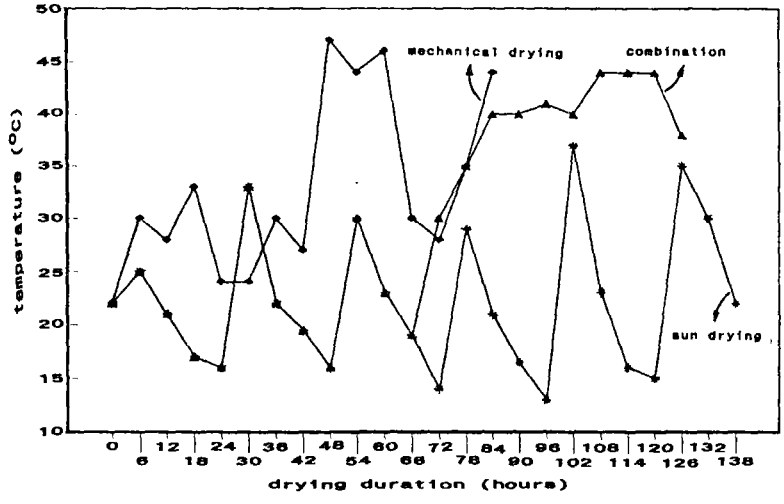

Figure 3. Beans temperatures during sun, mechanical, and combined drying trials

Conclusion

Coffee beans dried on the drying table produced beans with better taste compared to that dried on the terrace. Trial in which the ambient condition with low temperature and high RH, was carried out proved that the drying process had more dominant effect on the coffee product quality compared to the fermentation trials. Therefore, efforts to improve the Java Coffee should be more emphasized on the drying process.

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Summary

Arabica coffee (JAVA COFFEE) plantations in East Java have environmental characteristics i.e. high rainfall, high relative humidity, and low daily temperature. These conditions are considered to be an interferent in coffee processing, especially on sundrying. Condition standard for sundrying cannot be reached in such an environment. An evaluation of the existing method and an effort to optimize fermentation and drying stages are required in order to produce green coffee having good flavour.

The evaluation results of the existing processing method showed that fermentation for 36 hours followed by sundrying until it reached 30-35% moisture content and mechanical drying until 10-11% moisture content produced green coffee having acceptable flavour. Meanwhile 36 hour fermentation followed by sundrying until it reached 20-25% moisture content and mechanical drying until 10-11% moisture content as well as full mechanical drying process produced lower quality than the first. Full sundrying on unsatisfied condition such as high humidity and low daily temperature caused post fermentation to produce sourish taste. On the other hand full mechanical drying with rapid drying rate produced worse quality with highly sourish, astringent and bitter taste.

The experiment results showed that drying process had more dominant role on flavour compared to fermentation process. Full sundrying on drying table under sunny-condition as well as the combination process of sundrying until 30-35% moisture content followed by mechanical drying until 10-11% moisture content produced green coffee having better flavour than the bean which was full mechanical dried.

CONSTRAINT AND SUPPORTING FACTORS OF THE DEVELOPMENT OF ORGANIC ARABICA COFFEE GROWING IN CENTRAL ACEH, INDONESIA

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INTRODUCTION

One of the main growing area of arabica coffee in Indonesia is located in Gayo highlands. Recently, the total coffee area in Gayo highlands, district of Central Aceh, the widest smallholder arabica coffee area in Indonesia, reaches 62.000 ha.

Before 1986, the main problem of the smallholder arabica coffee in this area was the marketing. To overcome the problem, since 1986 the Aceh province goverment's company (PD Genap Mupakat) have bought coffee cherries from the farmers. The coffee was processed, and exported to Japan, USA, Canada, Germany, Netherlands, Switzerland, etc. Since 1992 the coffee received the organic certificate from Skal, The Netherlands. Certificate issued by Skal confirm that product and production method meet with the requirements of Council Regulation (EEC) No. 2092/91 (Junker,1991), and it can be marketed in Europe as organic.

The demand of organic arabica coffee produced by PD Genap Mupakat increased dramatically in the last three years. The organic coffee demand in 1992, 1993, and 1994 were 39, 197, and 445,5 tons respectively. To anticipate the increasing demand, the author had conducted a survey to evaluate and to know the constraint and the supporting factors in enlarging the acreage of organic arabica coffee in central Aceh and the improvements needed.

MATERIAL AND METHOD

The survey had been carried out in 1994 in 6 subdistricts of Central Aceh which were not included in the organic program yet, i.e. Bebesen, Bintang, Kota, Pegasing, Silih Nara, Timang Gajah. Three villages were taken from each subdistricts and 11-15 farmer samples were taken from each villages by random sampling method. The total number of the samples was 218.

The data collected by filling the questionaires, than the surveyors checked the data by direct observation to the coffee field. The collected questionaires were evaluated, tabulated, and analysed base on the basic standard of organic agriculture (IFOAM, 1994) and organic coffee standard developed by Indonesian Coffee and Cocoa Research Institute and Skal, The Netherlands (Winaryo, *et al*, 1994).

RESULT AND DISCUSSION

1. Constraints of Organic Coffee Growing.

The survey results showed that only 12.3% of the farmer used chemical fertilizer to the coffee trees. The chemical fertilizer used were Urea, TSP and KCl. 87.7% of the farmers used other methods, like natural litter, compost, or nothing for manuring.

The main pests of arabica coffee in Central Aceh are berry borer and jumping lice, while the main disease is root disease. 1.0% of the farmers controlled the pest or the disease by chemical pesticide. Pesticide used to control berry borer was Supracide. 14.4% of the farmers controlled pest or disease manually or by biological method, for instance controling berry borer by spraying *Beauveria basiana* and controling jumping lice by *Curinus coeroleous*. The rest (84.6%) was without pest and disease control.

Weeds community in Central Aceh was vary, consist of wide leaf and grass. The wet climate and the fertile soil in Central Aceh support the weeds growth in the coffee field, and it needs weeding continuesly. The farmer who control the weed by herbicide was 38.6%, organic control for instance by slashing 60.5%, and no weeding 0.9%.

Most of the coffee farmers (89.5%) grow coffee as a monoculture (single crop), and 10.5% grow coffee by intercroping system. As intercrop are annual crops i.e. chilly, tomato, potato, corn, and they are grown in between young coffee trees, in the bank of terrace, or in the area of some coffee died. In the intercrop system 13.3% of the farmers used chemical fertilizer (Urea, TSP, KCl) and 2.9% of them used pesticide (Supracide, Dithane-M45).

2. Supporting factors of organic coffee growing

In case of organic material used, 38.9% put organic material for instance cow/buffalo dung, compost, and natural litter. Actually, the potency to produce organic material in central Aceh is high. The average farmer has 0.5 big cattle (cow, buffalo, horse), 0.1 small cattle (ship), and 14.5 poultry (chicken, swan, duck). However, the farmer do not keep their cattle in a certain place, so the cattle dung is spread out anywhere and it is not so easy to collect and to make compost for coffee.

Most of the pruning method of coffee in Central Aceh is single pruning. Formation pruning conducted by cut of the top of coffee trees at 1.5-1.6 m high. Maintenance/production pruning carried out by picking orthotroph bud, dried branch, pest/disease infected branch. Based on the pruning standard of Indonesia, the pruning conducted by the farmer could be classified as follows : good (14.1%), fair (48.5%), bad (27.7%), no pruning (9.7%). Bad and no pruning usually caused by the lower skill and knowledge of the farmer.

Most of coffee field in Central Aceh use shade. Leucaena is used as a shade tree. Since 1986 the Leucaena attacked by the jumping lice, so the condition of the shade is vary. The good shade classification covered 49.5%, bad shade 44.4%, and no shade 6.1%. The bad shade means sporadic shade and the canopy of the shade trees is also bad.

The slope condition in every villages is vary. Hilly and steep slope 38.7%, flat and undulating slope 61.3%. Erosion control percentage especially for the hilly and steep slope area is only 21.6% and 78.4% of the coffee plantation are without erosion control activity. The lower percentage of erosion control activity in hilly and steep slope is a consquence of the lack of experience and knowledge on the effect of erosion on the soil fertility and on the damage of environment. There are many kinds of erosion control method for instance terracing, contour planting, and planting vetiver on the lip of the terrace.

All the farmer were willing to joint to the organic coffee program. They will stop using pesticide or fertilizer. The willingness of the farmers is a supporting factors of the organic program.

3. Organic Classification.

The arabica coffee field in 6 subdistricts which were not included in the organic program yet, could be classified as follows. Fully organic 44.3 %, consisted of Organic with good management 17.8 % and with poor management 26.5 %. Organic inconversion 55.7 %, consisted of Organic inconversion first year 45.8 %, second year 6.8 %, and third year 3.1 %. Non organic 0%.

Based on the classification above, if the organic program applied directly in the year of survey (1994) only 44.3% (fully organic) of the farmer field could be included in the organic program. The arabica coffee cherries from these field could be processed and exported as organic coffee.

The remain field, the organic inconversion third year (3.1%) needs one year (no chemical fertillizer and no pesticides spraying) to be fully organic. The organic inconversion second year (6.8%) and the organic inconversion third year (45.8%) could be classified as fully organic after 2 years and 3 years respectively without chemical fertillizer application and pesticides spraying.

The non organic percentage is zero (0%), because all the farmer were willing to joint the organic coffee program.

CONCLUSION

1. Theere are 44.3% of the arabica coffee fields in 6 subdistricts of Central Aceh which could be included in the organic arabica coffee program directly, and further 3.1%, 6.8%, and 45.8% need 1, 2 or 3 years to be fully organic respectively.
2. The recent constraints and the improvements needed of the area to meet fully organic with good management are in case of manuring, pest and disease control, weed control, intercropping, organic material application, coffee pruning, shade tree, and erosion control. The improvement can be done by training and education of the farmers, and well organised extension service.

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SUMMARY

The demand of organic arabica coffee of Central Aceh (PD Genap Mupakat) increased dramatically in the last three years. To meet the increasing demand, a survey focused on the growing technic of arabica coffee had been conducted in 1994. The aim of the survey is to know the constraint and supporting factors in enlarging the acreage of organic arabica coffee and the improvements needed.

There were 218 samples of coffee farmers taken from 6 subdistricts which were not included in the organic coffee program yet. Three villages from each subdistricts and 11-15 farmers from each villages were taken by random sampling method. The questionaire and the evaluation method were based on the standard of organic coffee developed by Indonesian Coffee and Cocoa Research Institute and requirements of Skal, The Netherlands.

The results showed that 44.3% of the arabica coffee field in 6 subdistricts of Central Aceh could be included in the organic arabica coffee program directly, and the rest (3.1%, 6.8%, 45.8%) needs 1, 2, and 3 years to be fully organic respectively. The recent constraints and the improvements needed of the area to meet fully organic with good management are in case of manuring, pest and disease control, weed control, intercropping, organic material application, coffee pruning, shade tree, and erosion control. The improvement can be done by training of the farmer and effective extension service.

ISOLATION AND CHARACTERIZATION OF BACTERIA STRAINS FROM SOIL WITH ABILITY TO DEGRADE CAFFEINE

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1- INTRODUCTION

The alkaloid caffeine, the major alkaloid in coffee, is indicated as a potent allelopatic agent, inhibiting seed germination of different species, or even coffee seeds. Caffeine is incorporated in the soil by leaching from the coffee tree canopy, as well as from litter and lost coffee beans. In the soil, caffeine is strongly absorbed by the clay-silica layers due to its planar structure. However, since readly usable non-humic substrates are scarce in the soil, keeping microorganisms under constant starvation, it would not be surprising to find bacteria degrading caffeine in soil under coffee cultivation, before its absorption.

A few reports in the literature have already described the isolation of bacteria strains from soil with the ability to degrade caffeine (Bucher and Lingens, 1977; Gluck and Lingens, 1987; Mazzafera et al., 1995). Nevertheless, caffeine enrichment was made during the isolation. Here we communicate the one-step isolation of several bacteria strains from soil under coffee cultivation. The predominant bacteria was Pseudomonas, and some strains were able to grow in very high caffeine concentrations (50g/L), as the sole source of carbon and nitrogen.

2- MATERIALS AND METHODS

Soil samples presenting different clay composition were collected under coffee trees growing at three Brazilian localities (sandy = Marília/SP, clay = Londrina/PR, mixture = Campinas - SP).

Isolation of bacteria strains followed the scheme below:

10g soil/90ml of 300mg/L caffeine liquid medium overnight (100rpm 28°C)

↓ (serial dilution)

1ml plated on Nutrient Agar medium (30°C)

↓

Individual colonies transferred to 300mg/L caffeine solid medium (30°C)

↓

Growth on caffeine confirmed on caffeine liquid medium

Caffeine medium: 430mM Na_2HPO_4, 22mM KH_2PO_4, 60mM NaCl, 100mM $MgSO_4.7H_2O$, 100mM $CaCl_2$, supplemented with caffeine. Solid medium were supplemented with bactoagar 1,5%. The isolates were maintained in caffeine solid medium.

Characterization and identification of isolated cultures were made by morphological and biochemical tests according the Bergey's Manual of Determinative Bacteriology (1993) and MacFadding (1980).

Determination of DNA base composition (mol% G+C). DNA was obtained from log-phase cultures, treated with RNAase and digested with 88% formic acid at 120°C/2h. Guanine and cytosine were analysed by HPLC (Maniatis et al., 1982; Tamaoka, 1994; Klaas et al., 1989).

Caffeine degradation by the isolates growing in (2g/L and 20g/L) caffeine liquid medium was monitored by means of HPLC analysis in samples taken at different incubation periods.

Electron microscopy of the isolates was performed using negative staining with 1% sodium phosphotungstate.

3- RESULTS AND DISCUSSION

Sixteen bacteria strains were isolated from soil samples. We did not observe any correlation between soil texture and bacteria isolates. No bacteria was found exclusively in a specific soil. Four isolates were identified as gram-positives (coryneform), 2 Flavobacterium, 1 Acinetobacter, 2 Moraxela, and 7 Pseudomonas (6 Pseudomonas putida, 1 Pseudomonas fluorescens). One isolated was not identified.

The more efficient group for caffeine degradation (Fig.1 A and B, Table 1) were identified as Pseudomonas .Table 2 and 3, and Fig.2 show the results of the morphological and biochemical tests used for the complete identification of the Pseudomonas isolates.

Due to re-crystallization of caffeine in higher concentrations than 25g/L in liquid medium, inoculations of the Pseudomonas were made in solid medium from 30g/L up to 50g/L. Caffeine also crystallized in solid medium, however, bacterial growth could be observed after 96h of incubation at 28°C (Fig.3). For all concentrations tested, strain L showed more intense growth than the other Pseudomonas isolates.

Our results clearly show that active caffeine degradation by microorganisms in the soil may occur before its absorption by minerals. Therefore, the effect of caffeine as an allelopatic agent in coffee plantations should be reconsidered.

Due to the high ability to degrade caffeine, the Pseudomonas isolates can be considered as a valuable tool for technological applications, such

as production of caffeine derivatives, as well as for studies on molecular biology towards a naturally decaffeinated coffee plant. The metabolic control of caffeine degradation in the Pseudomonas strains is under investigation.

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Table 1. Caffeine concentration in the liquid medium after 96h cultivation of the bacteria strains. Caffeine initial concentrations in the medium were 2g/L and 20g/L

Bacteria Strains	Caffeine Concentration (mg/L)*	
	2g/L	20g/L
19o	0.035	3.797
19x	0.005	1.992
21	0.003	0.934
L	0.152	0.467
20b	0.009	3.745
dob2	0.003	0.642
dob5	0.010	3.731
jo2	1.900	17.810
o	1.347	20.110
nam	1.398	20.892
x/10	1.929	15.444
20a	1.776	20.464

*- Values higher than the initial concentration were due to medium evaporation.

Table 2. Tests for identification of Pseudomonas species. Pp = Pseudomonas putida; Pf = Pseudomonas fluorescens

Identification ⟶	**Pp**	**Pp**	**Pp**	**Pp**	**Pp**	**Pp**	**Pf**
Isolate ⟶	**19o**	**19x**	**21**	**L**	**dob2**	**dob5**	**20b**
Fluorescent pigment (medium King B)	+	+	+	+	+	+	+
Growth 4°C	+	+	+	+	+	-	+
Growth 41°C	-	-	-	-	-	-	-
PHB accumulation	-	-	-	-	-	-	-
Levan production	-	-	-	-	-	-	-
Gelatin hydrolysis	-	-	-	-	-	-	+
Starch hydrolysis	-	-	-	-	-	-	-
Tween 80 hydrolysis	-	-	-	-	-	-	+
Denitrification	-	-	-	-	-	-	-
Nitrate reduction	+	-	+	-	-	-	-
ARG dehydrolase	+	+	+	+	+	+	+
PHE deaminase	-	-	-	-	-	-	-
Oxidase	+	+	+	+	+	+	+
O/F	Ox	Ox	Ox	Ox	Ox	Ox	Ox
MacConkey	+	+	+	+	+	+	+
Motility	+	+	+	+	+	+	+

Ox = Oxidative in O/F Hugh-Leifson medium

Table 3. Mol %(G+C) of the Pseudomonas putida isolates. Control strains of P. putida were purchased from CNPMA - Embrapa[1] and Instituto Biológico de Campinas[2].

Bacteria Strains	%(G + C)
Control Strain PSB[1]	75.6
Control Strain PC11B[1]	82.6
Control Strain 1806[2]	77.1
Isolate 19x	79.7
Isolate dob5	86.7
Isolate G2	84.6
Isolate L	84.2
Isolate 21	76.9
Isolate 19o	88.4
Isolate 20b	61.3

Figure 1. Growth of the isolated strains in caffeine liquid medium at 2g/L (A) and 20g/L (B).

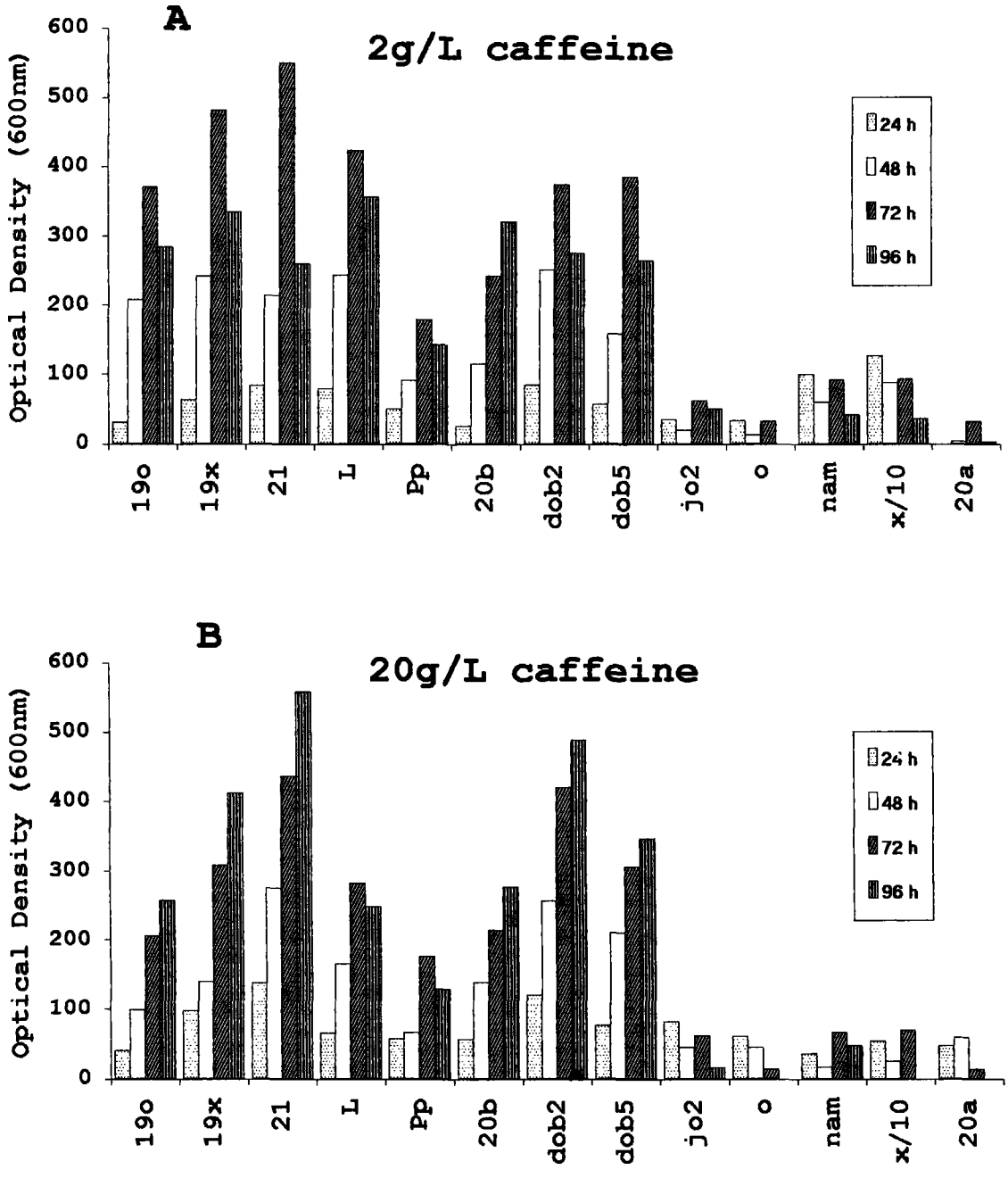


Figure 2. Electron microscopy of a isolated strain of Pseudomonas putida.

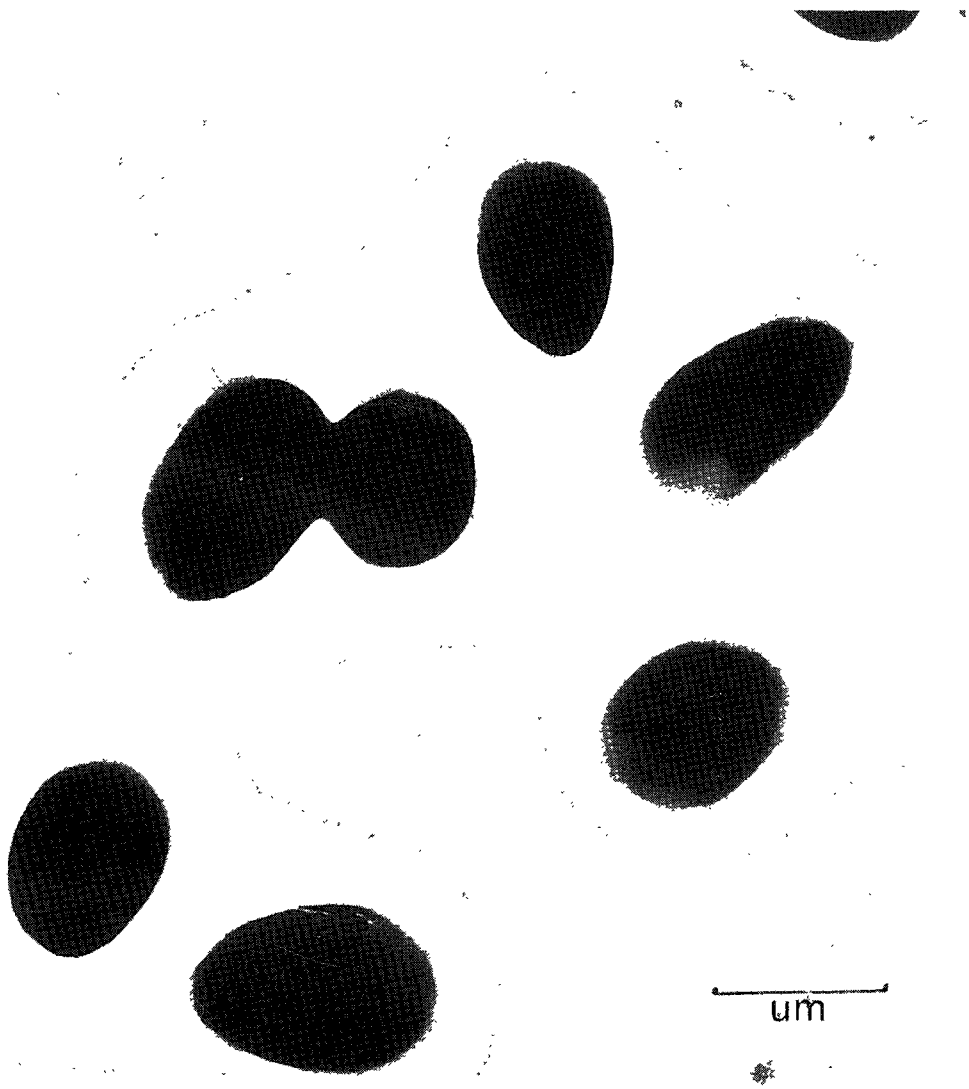
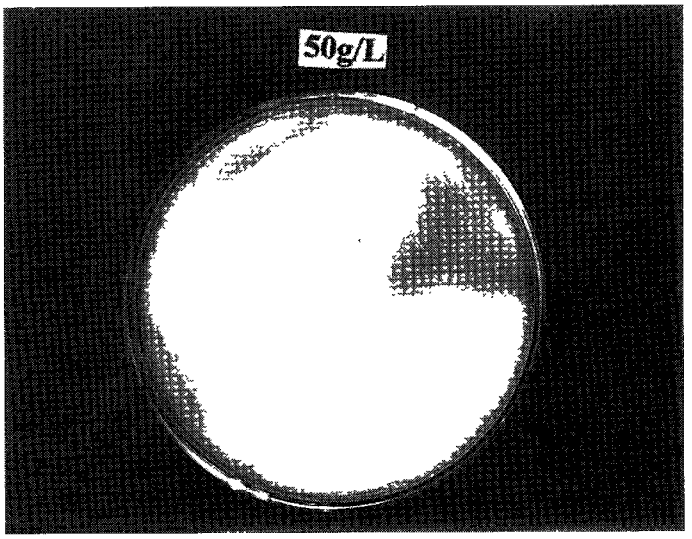


Figure 3. Isolate L growing on 50g/L caffeine solid medium.



CONTRIBUTION A L'AMÉLIORATION DE LA QUALITÉ DU CAFÉ PAR LE CHOIX D'UNE TORRÉFACTION OPTIMALE

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1 INTRODUCTION

Le degré de torréfaction du café est jusqu'à ce jour assez mal défini. On parle de torréfaction légère, moyenne, et forte (Café d'Afrique, 1993 "OIAC"). Ces notions étant liées à la couleur des grains torréfiés et à l'expérience du spécialiste de la torréfaction. C'est donc une notion assez subjective. A. F. MABROUK. F.E. DEATHERAGE, Food Techn., 10 (1956), 194 et M. BLANC ont étudié la variation de la teneur des acides en fonction de la durée de torréfaction. Ces mêmes auteurs définissent le degré de torréfaction en fonction de la perte en poids du café. Le degré de torréfaction peut être aussi mesuré par la densité des fèves (Nestec Ltd, 1988).

Plusieurs auteurs se sont intéressés aux acides présents dans les cafés torréfiés, ces études sont soit qualitatives ou quantitatives. Par ailleurs, nous savons que, pendant les premiers moment de la torréfaction, les fèves de café se déshydratent c'est alors que commencent les pertes organiques. L'apparition d'arômes volatiles devient importante au delà de 150 °C. Pendant la dernière phase de la torréfaction, la réaction devient exorthermique, les fèves éclatent (Nestec Ltd 1998). L'ensemble de ces données nous informe sur le comportement des acides du café vert au cours de la torréfaction sans tenir compte de la classification, c'est-à-dire du grade en fonction duquel (entre autres) le prix du café est fixé.

Nous nous proposons dans ce travail d'étudier l'influence de la durée de torréfaction sur le corps et l'acidité globale de la liqueur de café en fonction du grade et de l'espèce, afin de fournir des indications de torréfaction pour qu'un café donne le meilleur de lui-même (torréfaction optimum).

2 MATERIELS ET METHODES

a) la matière première

Le café vert utilisé est du Robusta et de l'Arabusta provenant des plantations de l'IDEFOR-DCC.

Le Robusta (dont la teneur en eau est de 13 %) est calibré pour obtenir les grades 1, 2, 3, le grade 4 étant essentiellement constitué de brisures, nous n'avons pas retenu cette fraction. Mais, quant à l'Arabusta (teneur en eau de 14,3 %), nos analyses ont porté essentiellement sur les grades 1 et 2, car les grades 3 et 4 sont en très faible proportion.

b) Le Torréfacteur

Le torréfacteur utilisé est un mini torréfacteur électrique de laboratoire de type PROBAT. (U = 220 v, I = 5,7 A) = 886,7 w.

c) Torréfaction

La torréfaction est conduite à la température de 250 °c, àdes durées de torréfaction variant de 2 à 9 min.

La masse initiale de chaque échantillon de café est de
155,8 g correspondant à un taux des remplissage du torréfacteur 67,39 kg/m^3.

La durée de torréfaction dépend de la capacité du torréfacteur, de sa puissance de chauffe, de la température, du taux de remplissage en café de ce dernier, de l'humidité et de la taille des grains.

d) Le moulin

Le moulin utilisé est un appareil à meules Ditting qui dispose d'un réglage de la granulométrie de la mouture.

Pour l'analyse de nos échantillons, nous avons choisi le réglage fin qui correspond à la graduation "5" du dispositif pour avoir une mouture de dimension standard. (90 % de la mouture àune taille inférieure à 1,5 mm).

e) Pesée

Toutes les pesées ont été réalisées sur une balance électronique de type Mettler P1200 (n=361845).

f) Mesure du pH de la liqueur

La liqueur de café est préparée à partir d'échantillons de 20 g de mouture de café torréfié, placés dans des bêchers de 600 ml.

A l'aide d'un bouilloire électrique Téfal, on porte une certaine quantité d'eau distillée à 70-90 °c ; 250 ml de cette eau sont versés directement sur la poudre de café. La solution obtenue est homogénéisée à l'aide d'un agitateur magnétique pendant 30 min. pour permettre une bonne solubilisation des substances de café. Le pH est ensuite mesuré. Le pH de l'eau distillée utilisée est noté avant chaque analyse. Celui-ci varie entre 4,3 et 5.7.

g) Analyse organoleptique

La liqueur de café utilisée pour les analyses organoleptiques est préparée de la même manière que celle qui a servi pour la mesure des pH.

Les échantillons de café sont dégustés par grade de même espèce et en double. Les valeurs figurants dans ce rapport sont une moyenne des notes attribuées par chacun des 5 dégustateurs.

Le barème utilisé est le suivant :

échelle

0-	néant	5-6-	net
1-2-	très faible	7-8-	fort
3-	4 faible	9-10-	très fort.

3) RESULTATS ET DISCUSSIONS

3.1) Influence de la durée de torréfaction sur l'acidité de liqueur.

3.1.1. Acidité organoleptique

Cas du ROBUSTA

On constate une croissance de l'acidité en fonction de la durée de torréfaction.

Toutes les courbes des trois grades 1, 2 et 3 indiquent une croissance de l'acidité de la 2ème à 6,5 min. de torréfaction suivie d'une diminution très rapides. Le maximum d'acidité est atteint à la 6ème min. de torréfaction. La phase décroissante montre que lorsque la durée de torréfaction est trop longue, l'acidité diminue et le café perd ainsi une de ses qualités les plus importantes (fig. 1, tableau 1).

Tableau 1 : Acidité et PH de la liqueur des échantillons en fonction de la durée de torréfaction

Temps min.	ARG 1	ARG 2	ARG 3	AAG 1	AAG 2	PHRG 1	PHRG 2	PHRG3	PHAG1	PHAG2
2	0,5	0,5	0,5	1,25	1	5,55	5,5	5,7	5,4	5,4
3	0,83	0,83	0,67	2,17	1,53	5,35	5,35	5,5	5,3	5,35
4	1,5	1,5	1,5	2,67	2,29	5,15	5,25	5,45	5,15	5,05
5	4,33	4,33	3,67	4,5	5,38	4,85	4,9	5,1	4,65	4,6
6	6,33	6	5,83	6,17	5,75	5,05	5,05	5,7	4,65	4,65
6,5	7	6,83	6,83	5,5	5,57	5,5	5,5	5,8	4,8	4,8
7	5	5	4	5	4,27	5,95	5,95	5,95	4,9	5,5
8	2,33	1	2	4	3,69	6,75	6,8	6,8	6,22	6,4
9	2,33	0,33	0,33	3,25	0,93	9,3	8,95	8,95	7,7	8,2

La plage de 5 à 7 min. nous donne une acidité moyenne supérieure à 5 (échelle de 0 à 10) quelque soit le grade du café. (fig. 1 et 2, tableau 1).

Les équations ci-dessous donnent une corrélation de l'acidité organoleptique en fonction de la durée de torréfaction

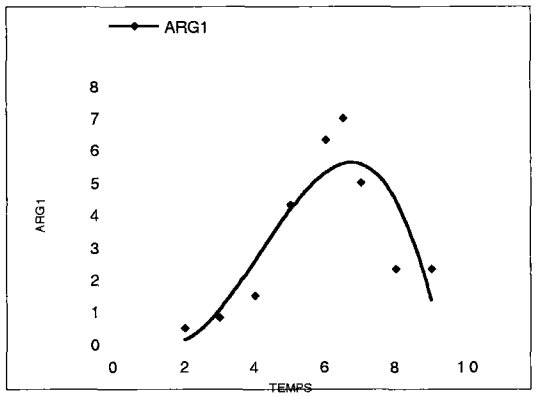


Fig.1 : Acidité du Robusta grade 1 en fonction de la durée (min.) de torréfaction.

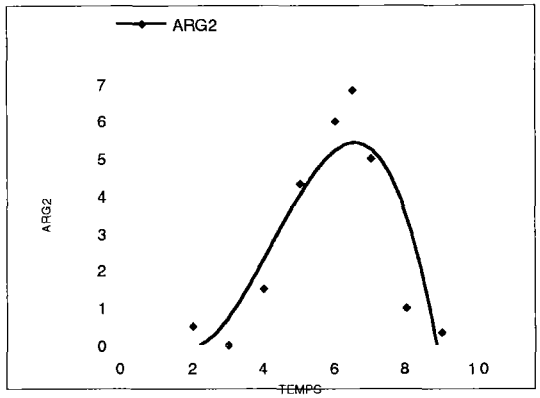


Fig. 1.2. Acidité du Robusta grade 2

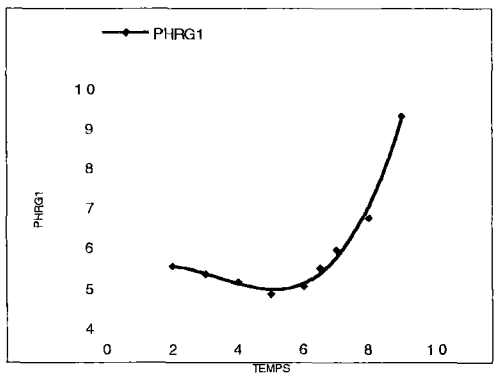


Fig. 1.3 PH. Robusta grade 1

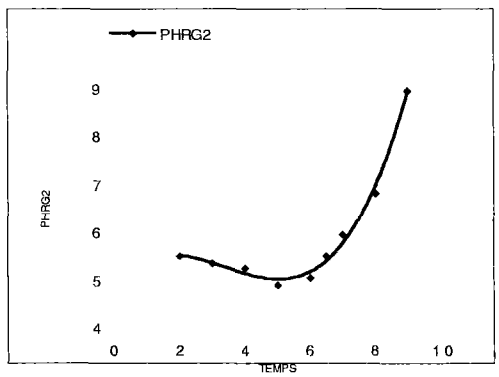


Fig. 1.4. PH du Robusta grade2

Fig. 1 : Acidité et PH du Robusta grades 1 et 2 en fonction de la durée (en min.) de torréfaction.

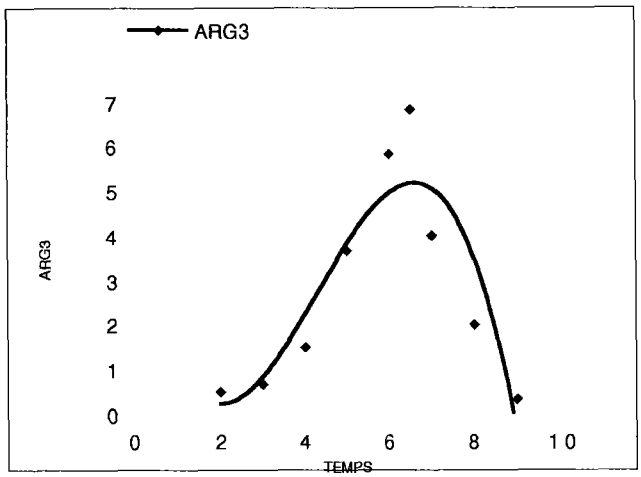


Fig. 1.5. : Acidité du Robusta grade 3

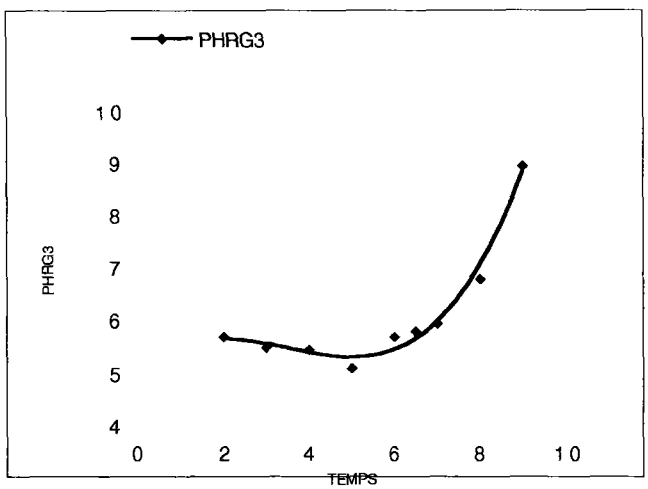


Fig. 1.6. PH. du Robusta grade 3

grade 1 : $Y = 2,037 - 2,700\ t + 1,040\ t^2 - 0,083\ t^3$ Eq.1
avec $r^2 = 0,88$

grade 2 : $Y = 2,814 - 3,398\ t + 1,232\ t^2 - 0,1\ t^3$ Eq.2
avec $r^2 = 0,80$

grade 3 : $Y = 3,941 - 4,108\ t + 1,333\ t^2 - 0,103\ t^3$ Eq.3
avec $r^2 = 0,83$

b) Cas de l'Arabusta

Nous observons la même tendance que dans le cas du robusta. Les deux courbes (fig. 2, tableau 1) présentent chacune deux
phases, une croissante de la 2ème à la 6ème min. de torréfaction, suivi d'une décroissante de la 6ème à la 9ème min.

L'intervalle de temps de 5 à 7 min., nous garantie une acidité de la liqueur supérieure ou égale à 5 (échelle de 0 à 10) avec un maximum à 6 min. correspondant à une acidité de 6,17 et 5,7 respectivement pour les grades 1 et 2 (fig. 2).

Les équations ci-dessous donnent une corrélation de l'acidité organoleptique en fonction de la durée de torréfaction

grade 1 : $Y = 0,712 - 0,583\ t + 0,482\ t^2 - 0,069\ t^3$ Eq.4
avec $r^2 = 0,90$

grade 2 : $Y = 1,456 - 1,675\ t + 0,800\ t^2 - 0,069\ t^3$ Eq.5
avec $r^2 = 0,91$

Conclusion

Dans les deux cas, l'acidité organoleptique passe par un maximum à un moment donné de la torréfaction.

Les grades 1 procurent une plus forte acidité que les grades 2 et ainsi de suite (Fig. 3 et 4).

3.1.2. Influence de la torréfaction sur le pH de la liqueur (acidité réelle).
Cas du Robusta

Les pH obtenus en fonction de la durée de torréfaction présentent toutes, deux phases : Une décroissance presque linéaire jusqu'à la 5ème min.

Une croissance d'abord très progressive (de 5 à 7 min.) puis galopante par la suite.
Cette évolution des pH de la liqueur corrobore les résultats des dégustateurs sur l'acidité organoleptique.

Les pH les plus bas sont observés entre 4 et 5,5 min. et sont compris entre 4,8 et 5,4.

L'augmentation du pH s'explique par la calcination du café, d'où diminution de l'acidité. Le pH devient très alcalin et ne reflète aucune qualité de la liqueur de café.

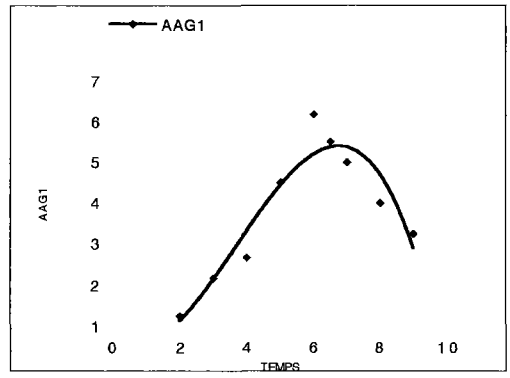


Fig. 2.1. : Acidité de l'Arabusta grade 1

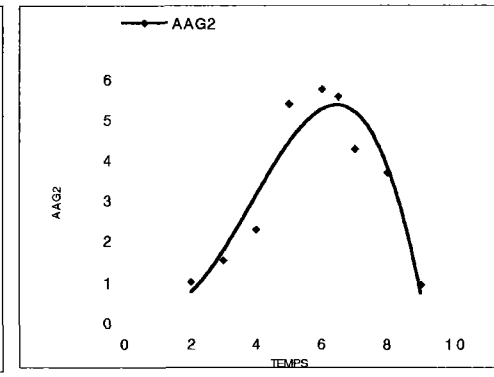


Fig.2.2. : Acidité de l'Arabusta grade 2.

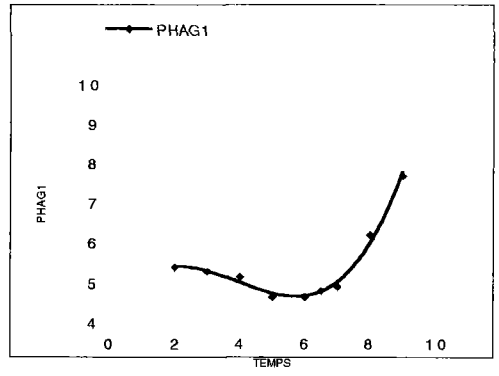


Fig. : 2.3 - PH de l'Abusta grade 1

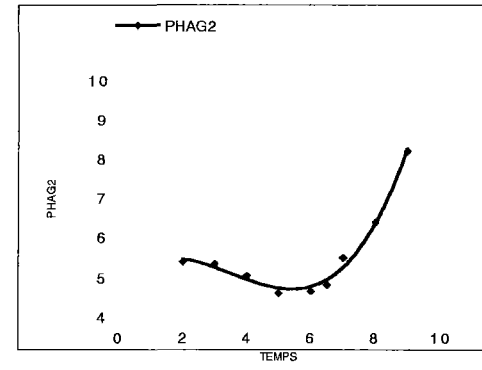


Fig. 2.4. : PH de l'Arabusta grade 2

Fig. 2. : Acidité et PH. de l'Arabusta grade 1 et 2 en fonction de la durée (en min.) de torréfaction.

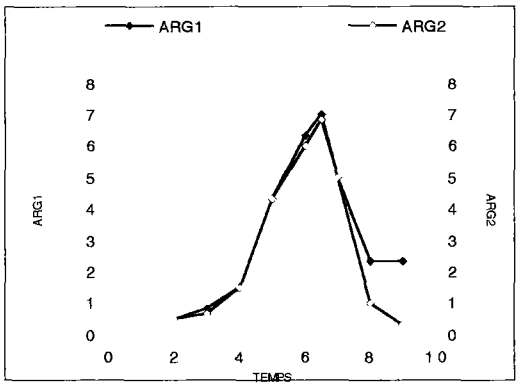


Fig. 3.1. : Acidité du Robusta grades 1 et 2.

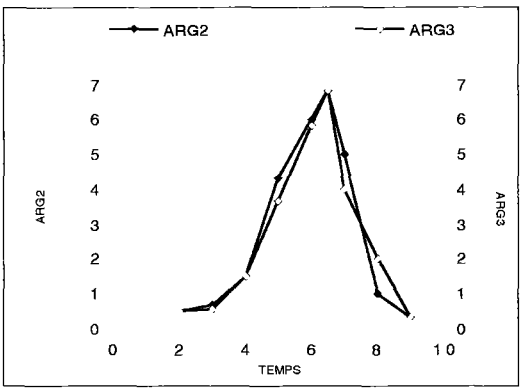


Fig. 3.2. : Acidité du Robusa grades 2 et 3.

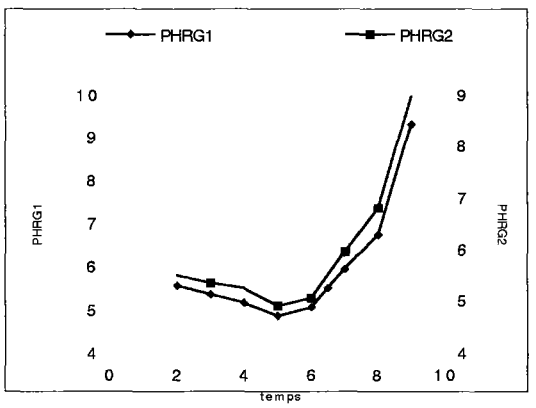


Fig. 3.3. : PH du Robusta grades 1 et 2.

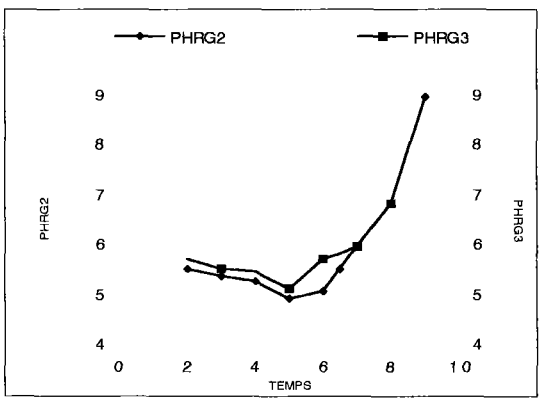


Fig. 3.4. PH. du Robusta grades 2 et 3.

Fig. 3 : Comparaison des acidités et des PH de la liqueur du Robusta grades 1, 2 et 3.

Fig. 4.1. : Acidité de l'Arabusta grades 1 et 2.

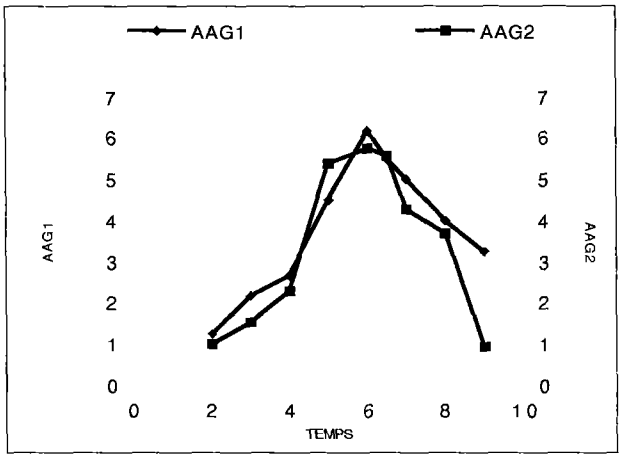


Fig. 4.2. : PH. de l'Arabusta grades 1 et 2.

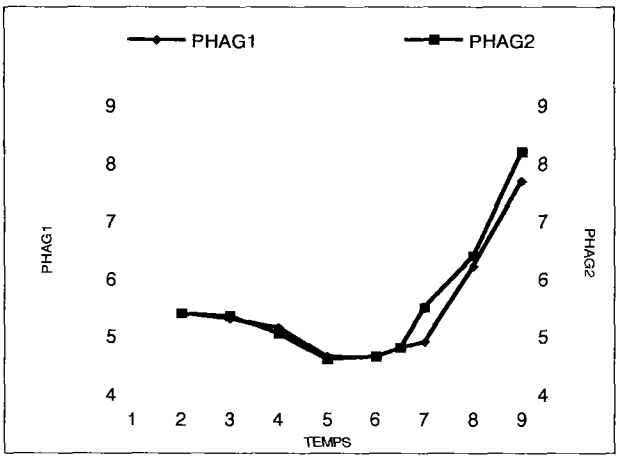


Fig. 4 : Comparaison des acidités, des PH de la liqueur d'Arabusta grades 1 et 2.

Le café de grade 3 a un pH plus élevé que ceux des grades 2 et 1. Ceci corrobore également les résultats des dégustateurs qui indiquent que le café de grade 1 est plus acide que celui de grade 2 et du grade 2 plus que celui de garde 3. Fig. 1, Tableau 1.

Les équations ci-dessous donnent une corrélation du PH de la liqueur en fonction de la durée de torréfaction

grade 1 : $Y = 4,924 + 0,819\ t - 0,317\ t^2 + 0,031\ t^3$ Eq.6
avec $r^2 = 0,98$

grade 2 : $Y = 4,944 + 0,740\ t - 0,284\ t^2 + 0,028\ t^3$ Eq.7
avec $r^2 = 0,99$

grade 3 : $Y = 4,994 + 0,770\ t - 0,270\ t^2 + 0,026\ t^3$ Eq.8
avec $r^2 = 0,98$

Cas de l'Arabusta :

Nous observons la même allure que dans le cas précédent : une phase décroissante et une autre croissante.

Le pH va descendre jusqu'à 4,6 pour les deux courbes à la 5ème min.. La phase croissante commence à la 6ème et la 5ème, les plus fortes valeurs de pH observées sont de 7,7 et 8,2 respectivement pour les grades 1 et 2 (fig. 2 Tableau 1).

Les équations ci-dessous nous donnent une corrélation du PH de la liqueur en fonction de la durée de torréfaction.

grade 1 : $Y = 4,289 + 1,216\ t - 0,392\ t^2 + 0,033\ t^3$ Eq.9
avec $r^2 = 0,98$

grade 2 : $Y = 4,890 + 0,800\ t - 0,316\ t^2 + 0,030\ t^3$ Eq.10
avec $r^2 = 0,99$

Conclusion

Toutes ces courbes de pH présentent les mêmes allures. Une phase décroissante et une croissante. Elles atteignent toutes leur point optimum à la 5ème min. de torréfaction. En outre, depuis la 2ème min. jusqu'à la 6,5 min. de torréfaction, nous avons une confirmation de l'acidité de l'Arabusta plus élevée que celle du Robusta, le pH du Robusta étant élevé que celui de l'Arabusta. De plus, on note un pH plus faible pour les grades 1 ; ce qui indique une plus forte acidité de la liqueur de café de grade 1 que celui de grade 2 et ainsi de suite.

3.2- Influence de la torréfaction sur le corps de la liqueur

Cas du robusta et de l'Arabusta

Les courbes présentent deux phases :
- Une croissance de la 2ème à 6,5ème min.
- Une décroissance à partir de la 6,5ème min. (fig 5, 6 tableau 2).

La croissance des courbes indique une augmentation du corps de la liqueur avec la durée de torréfaction. Le corps maximum est obtenu à 6.5 min. de torréfaction.

Tableau 2 : Corps de la liqueur en fonction de la durée de torréfaction

Temps	CRG1	CRG2	CRG3	CAG1	CAG2
2.2	0.330	0.5	0.5	0.152	0.75
3	0.830	1	0.83	0.01	1.56
4	2.50	2	1.75	0.007	2.25
5	4.00	4	4	0.01	4.38
6	6.00	5.67	5.2	0.009	5.38
6.5	6.33	6.33	6	0.01	6.25
7	5.30	4.33	4	0.008	5.63
8	2.33	1	2	0.009	3.88
9	2.00	0.5	0.5	0.003	1.38

Les grades 1 procurent plus de corps que les grades 2 et ces derniers plus que les grades 3. (fig.7)

Il convient donc d'en déduire qu'autant pour le Robusta que pour l'Arabusta, la meilleure tasse de café s'obtient au bout d'une durée de torréfaction de 5,5 à 7,8 min. à 250°c ; avec un taux de remplissage du torréfacteur égal à 67,39 kg/m^3.

Les équations ci-dessous sont une corrélation du corps de la liqueur en fonction de la durée de torréfaction.

- Cas du Robusta

grade 1 : $Y = 4{,}436 - 4{,}195\ t - 1{,}379\ t^2 - 0{,}105\ t^3$ Eq.11
avec $r^2 = 0{,}81$

grade 2 : $Y = 0{,}706 - 1{,}634\ t + 0{,}828\ t^2 - 0{,}007\ t^3$ Eq.12
avec $r^2 = 0{,}80$

grade 3 : $Y = 1{,}980 - 2{,}500\ t + 0{,}972\ t^2 - 0{,}080\ t^3$ Eq.13
avec $r^2 = 0{,}87$

- Cas du l'Arabusta

grade 1 : $Y = 0{,}078 - 1{,}283\ t + 0{,}758\ t^2 + 0{,}67\ t^3$ Eq.14
avec $r^2 = 0{,}87$

grade 2 : $Y = 4{,}025 - 3{,}688\ t + 1{,}212\ t^2 - 0{,}093\ t^3$ Eq.15
avec $r^2 = 0{,}97$

3.3 Choix de la durée de torréfaction optimum

Nous avons noté au cours de cette étude que l'acidité, et le corps de la liqueur passaient pas un maximum avant de décroître. Nous allons dans ce paragraphe choisir par le calcul les durées de torréfaction qui procurent une acidité et un corps élevés.

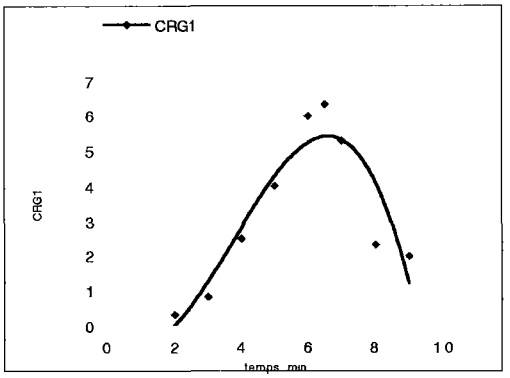


Fig. 5.1. : Corps du Robusta grade 1

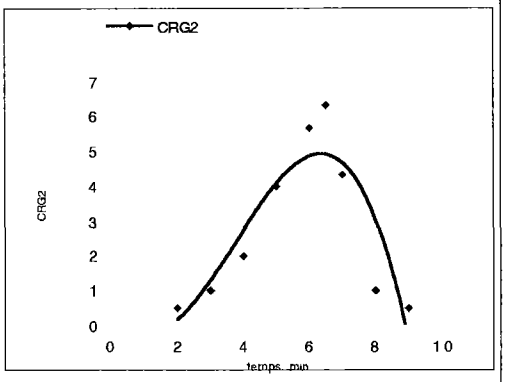


Fig. 5.2. : Corps du Robusta grade 2

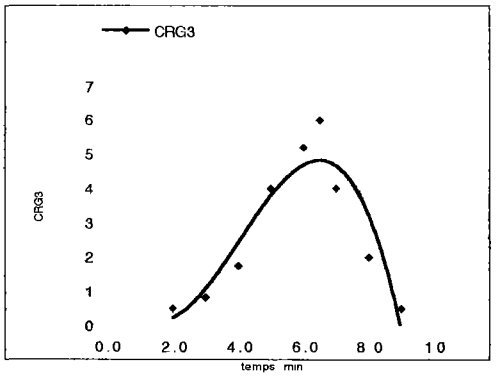


Fig. 5.3. : Corps du Robusta grade 3

Fig. 5 : Corps de la liqueur de Robusta grades 1, 2, et 3 en fonction de la durée de torréfaction.

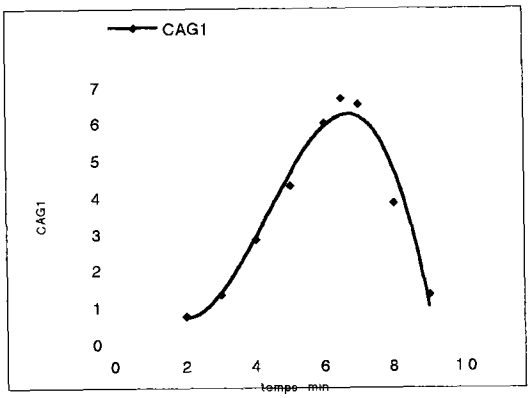


Fig. 6.1 : Corps de l'Arabusta grade 1

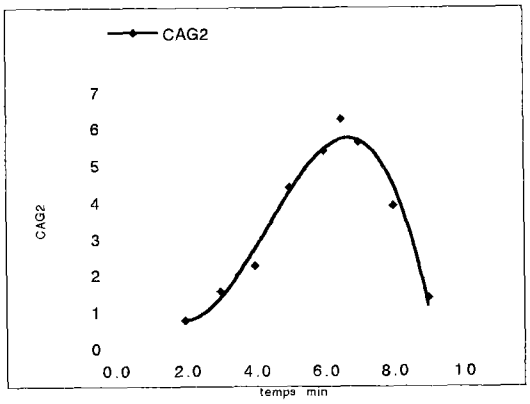
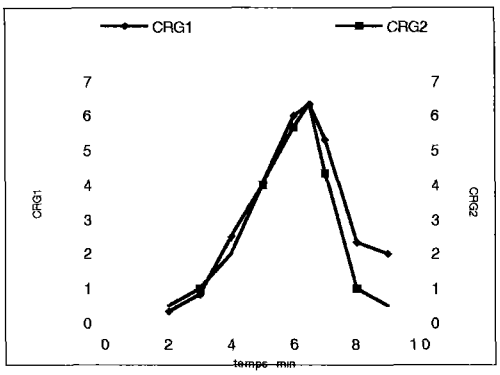
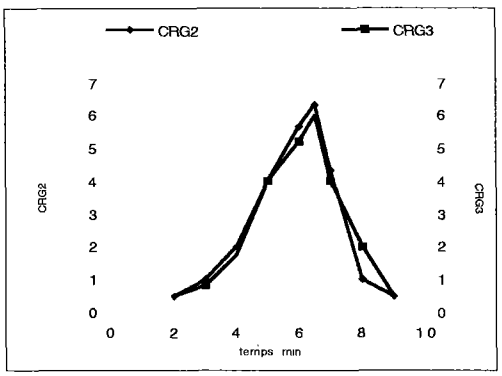


Fig. 6.2. : Corps de l'Arabusta grade 2

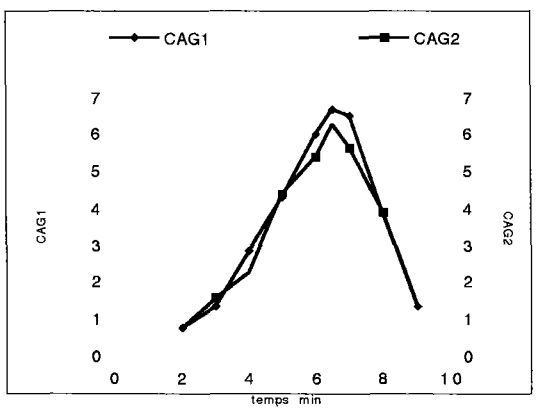
Fig. 6. : Corps de la liqueur d'Arabusta grades 1 et 2 en fonction de la durée de torréfaction.



F.7.1. : Comparaison du corps du Robusta de grades 1 et 2.



F7.2. : Comparaison du corps du Robusta de grades 2 et 3.



F.7 .3. : Comparaison du corps de l'Arabusta de grades 1 et 2.

Fig. 7 : Comparaison en fonction des grades du corps de la liqueur de Robusta et de l'Arabusta.

Exemple du Robusta grade 1

Par dérivation, des équations 1 et 11 donnant respectivement, l'Acidité et corps de la liqueur de grade 1, on obtient :

grade 1 : $Y'_1 = -2,7 + 2,080\ t - 0,249\ t^2$

grade 2 : $Y'_{11} = -4,195 - 2,758\ t - 0,315\ t^2$

Sachant que les durées maximum sont données pour Y'_1 et Y'_{11} égales à zéro, on obtient ces durées par la résolution de ces quations du second degré :

$t_1 = 6,75$ min.
$t_{11} = 6,8$ min.

On en déduit que l'intervalle de temps compris entre t_1 et t_{11}, nous procure une liqueur d'une bonne acidité et d'un bon corps.

CONCLUSION

Cette étude nous permet de choisir la durée nécessaire pour une bonne torréfaction en fonction du grade et de l'espèce de café. Elle nous indique une meilleure qualité du grade 1 par rapport au grade 2 et du grade 2 par rapport au grade 3.

On a pu noter que toutes les propriétés organoleptiques (acidité, corps, goût, arôme) du café se développent pratiquement dans le même laps de temps (5,5 à 7 min.)

Cette étude nous permet en outre d'affirmer que :

1 - l'acidité et le corps du café peuvent être rehaussés si nous observons une torréfaction optimum (de même, certains défauts pourront être également minimisés).

2 - Les cafés de grade supérieur ont un degré de corps et d'acidité plus élevé que ceux de grade inférieur.

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NOTATIONS

ARG1 : Acidité du Robusta grade 1
ARG2 : Acidité du Robusta grade 2
ARG3 : Acidité du Robusta grade 3
AAG1 : Acidité du l'Arabusta grade 1
AAG2 : Acidité du l'Arabusta grade 2
PHRG1 : PH du Robusta grade 1
PHRG2 : PH du Robusta grade 2
PHRG3 : PH du Robusta grade 3
PHAG1 : PH de l'Arabusta grade 1
PHAG2 : PH de l'Arabusta grade 2

LA NUTRITION POTASSIQUE DU CAFÉIER ROBUSTA EN CÔTE D'IVOIRE

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I.- INTRODUCTION

Parmi les cartions majeurs, seul le potassium est apporté en quantités plus ou moins importantes selon le potentiel chimique du sol. Comme l'indique Loué (7), le calcium et le magnésium ne font l'objet d'apport qu'en cas de déficiences identifiées dans les sols latéritiques. Ces deux éléments représentent dans le sol près de 95 % des bases échangeables, par rapport au potassium qui n'est préférentiellement fixé que sur des sites peu nombreux du complexe absorbant (2).

Sous caféier, des travaux ont montré que le potassium issu des engrais s'accumule dans le sol et améliore le taux de cet élément dans la somme des bases échangeables malgré une exportation excessive par les cérises : 44 kg de K20 contre 5 à 6 kg de Ca0 et Mg0 par tonne de café marchand. (9)

Il s'en suit des phénomènes de déséquilibre et d'antagonisme entre ces trois cations. Or un équilibre optimum est nécessaire entre eux pour assurer de meilleures productions. Dans cette optique, plusieurs expériences notamment celles de Forestier ont été conduites soit en utilisant le diagnostic foliaire comme guide, soit en établissant des relations entre les caractéristiques chimiques de la plante et du sol (4) (8).

Pour la présente étude, l'objectif est de déterminer une teneur optimum de potassium dans le sol sous des caféiers canephora, varieté robusta en tenant compte des interactions avec les autres cations notamment le magnésium. Elle porte sur des doses de potassium exprimées en meq/100 g de terre (meq %) àatteindre et à maintenir dans les 20 premiers centimètres du sol dans un essai NP-K. Ces doses sont calculées à partir des analyses de terre grâce au programme informatisé pour la détermination des besoins en engrais minéraux mis au point pour le cacaoyer et le caféier par Jadin et Snoeck en Côte d'Ivoire (6) (11)

II.- MATERIELS ET METHODES

1.- Quelques caractéristiques du milieu

L'essai a été conduit de 1987 à 1993 sur la station expérimentale de Divo où la pluviométrie est de 1320 mm avec deux saisons de pluies qui s'étalent respectivement de mars à juillet et de septembre ànovembre, et deux saisons sèches dont la plus grande se situe entre novembre et mars.

Les sols sont de type ferrallitique issus de granite. Le tableau 1 n'indique aucune différence significative, en début d'essai, entre les données analytiques du sol : la teneur en potassium total est de 2,70 meq % contre 0,19 meq % pour la partie échangeable ; la saturation est de 71 % en moyenne.

Tableau 1 : Potentiel chimique du sol avant l'essai - 1986 -

Traitements	N %	K	Ca	Mg	CEC	pH	V%
T1 = Temoin	1,15	0,20	4,37	0,78	7,40	6,25	72,6
T2	1,19	0,20	6,19	0,85	8,82	6,42	75,8
T3	1,42	0,18	5,38	0,77	8,63	6,01	72,0
T4	1,31	0,21	4,61	0,75	7,95	6,05	71,0
T5	1,25	0,19	6,29	0,90	9,57	6,23	75,0
T6	1,43	0,17	4,87	0,68	8,40	5,79	61,8
Moyenne	1,29	0,19	5,28	0,79	8,46	6,12	71,37
CV%	23,16 NS	33,9 NS	40,35 NS	22,9 NS	22,4 NS	4,9 NS	16,87 NS

Sur échantillons composites						
Traitements	P.total ppm	P.ass. ppm	K total meq %	C	M.O	A + L %
T1	242	12	3,24	1,63	2,81	33,8
T2	246	9	2,72	1,86	3,21	34,0
T3	255	9	2,34	1,54	2,65	39,2
T4	250	9	2,50	1,51	2,60	39,6
T5	269	10	3,08	1,81	3,12	37,0
T6	243	9	2,34	1,53	2,64	33,6
Moyenne	250,8	9,67	2,70	1,65	2,84	36,2

2.- Les doses étudiées

L'essai NP-K compare 6 doses croissantes de potassium dans un dispositif de bloc Fischer comportant 5 répétitions de 6 parcelles élémentaires de 28 caféiers utiles. En se basant sur les analyses faites sur les échantillons de sol, les niveaux potassiques recherchés sont obtenus à partir de la méthode de calcul utilisée pour déterminer les engrais pour le cacaoyer en vue de corriger le sol sur une profondeur de 20 cm dans une couronne de 0,60 m à 1 m autour des caféiers. Les exportations n'ont pas été prises en compte.

Les besoins en chlorure de potassium et en magnesium sont donc calculés en fonction des niveaux actuels dans le sol, des niveaux théoriques recherchés lorsque le rélèvement était possible et du rapport Mg/k compris entre 2 et 5. Des contrôles chimiques ont été réalisés tous les 2 ans pour réajuster les besoins en engrais minéraux (1986, 1988, 1991 et 1993).

La plus forte dose retenue est de 0,42 meq % parce que des essais factoriels NPK à 3 niveaux antérieurs sur la même station faisaient apparaître des effets dépressifs de la potasse sur tous les clones vulgarisés à partir de cette dose (10).

L'azote et le phosphore sont apportés uniformément dans tous les traitements sous forme d'urée et de superphosphate triple dans les proportions N2P1. Les doses de ces types d'engrais ont été déterminées grâce aux mêmes essais factoriels sus-mentionnés : la dose d'urée est de 100 unités N/hectare et celle du superphosphate triple 50 unités P205/hectare. Il n'y a pas d'apport de phosphore quand le niveau de P assimilable atteint 50 ppm dans le sol.

Tableau 2 : Les doses étudiées en meq %

Traitements	K recherché	Mg recherché	Mg/k actuel	Mg/k calculé	K % BE calc.
T1	0,20	-	3,9	3,9	4
T2	0,22	1,0	4,3	4,5	3
T3	0,27	1,0	4,3	3,7	4
T4	0,32	1,0	3,6	3,1	5,4
T5	0,37	1,0	4,7	2,7	5
T6	0,42	1,0	4,0	2,4	6,7

K et Mg exprimés en meq %.

3.- Les méthodes d'analyse

Deux modèles de regression ont été utilisés pour étudier la dependance entre les productions de café de 1988 à 1993 (Y) et les doses d'engrais (X). Seules les récoltes de cette période ont été considérées. En effet, les premiers épandages d'engrais ayant debuté en 1987, la production de cette année n'a pas été prise en compte dans les analyses.

Le premier modèle est un modèle curvilinéaire de type $Y = C + B X + A X2$. Le second modèle est non linéaire de type exponentiel $Y = A.e.BX$ (modèle monomoléculaire). Ils sont programmés par l'Institut Technique des Ceréales et de Fourages (ITCF) (13).

L'objectif est de mettre en évidence le modèle qui permet de mieux décrire le phénomène. Ces deux modèles sont comparés ensuite à un méthode de calcul mis au point par Forestier puis Snoeck.

III.- RESULTATS ET DISCUSSIONS

1 - Evolution chimique du sol

Les analyses de sol sont effectuées entre les mois de novembre et d'avril, période caractérisée par la grande saison sèche où les caféiers sont en repos physiologique et où les activités microbiennes dans le sol sont presque inhibées.

Les analyses de variance (tableau 3) ne décèlent aucune différence significative entre les cations, le pH et la saturation. Les rapports Mg/K ont varié de 1988 à 1993 entre 2,4 et 6 dans tous les traitements valeurs considerées comme favorables sous caféier par Forestier.

Les teneurs en magnésium sont en moyenne de 1 meq %, le niveau recherché. Il est atteint au cours de l'expérimentation parce que les caféiers utilisent moins cet élément, et aussi parce qu'il est solidement fixé par le complexe du fait d'un taux de calcium élevé, de l'ordre de 80 % en moyenne dans la somme des 3 cations, et de sa faible vitesse de diffusion.

En revanche, les teneurs pour le potassium sont restées en deçà du niveau recherché. Malgré les apports d'engrais potassiques, les valeurs dans les parcelles fertilisées sont équivalentes à celle du témoin. On peut toutefois noter une amélioration du taux de potassium dans les traitements à fortes doses par rapport à la somme des bases. Les analyses de sol de 1991

et de 1993 indiquent une différence significative en faveur des traitements recevant les doses élevées de potassium. Le sol aurait certainement fourni du potassium issu de la réserve non échangeable à la solution du sol (2) ; et le potassium amené par les engrais minéraux aurait servi à assurer les récoltes des caféiers.

L'utilisation importante de cet élément par le caféier pour son métabolisme, la capacité du sol à libérer du potassium à partir de sa réserve mobile (5), et le lessivage éventuel de K dû aux apports simultanés d'azote n'ont pas permis d'atteindre aisément les seuils théoriques fixés dans le sol. La saturation est restée proche de 70 % en 1993 soit sept ans après le début de l'essai.

Tableau 3 : Valeurs moyennes des données analytiques par traitement

Traitements	K meq %	Ca meq %	Mg meq %	pH	Mg/K	K % BE	V%	A+L (*)
1988								
T1	0,24	6,19	1,06	5,99	4,40	3,25		
T2	0,22	5,41	1,32	5,88	6	3,20		
T3	0,24	6,19	1,16	5,68	4,8	4,01		
T4	0,25	5,85	1,28	5,79	5,10	3,97		
T5	0,27	5,09	1,03	5,75	3,8	4,24		
T6	0,24	3,89	1,09	5,50	4,5	5,32		
Moy.	0,24	5,44	1,16	5,77	-	4,0		
CV%	27,5 NS	53,5 NS	27,9 NS	7,99 NS		42,2 NS		
1991								
T1	0,25	5,56	0,81	5,72	3,2	3,95	90	
T2	0,26	6,38	1,15	5,78	4,4	3,49	101	
T3	0,29	5,69	1,07	5,56	3,7	4,74	90,8	
T4	0,26	5,48	0,88	5,62	3,4	4,19	79,3	
T5	0,36	4,91	0,99	5,53	2,8	5,94	82,4	
T6	0,33	4,10	0,79	5,36	2,4	7,26	75,0	
Moy.	0,29	5,35	0,95	5,59	-	4,93	86,4	
CV%	22,3 NS	41,5 NS	25,5 NS	6,54 NS		31,9 S		
1993								
T1	0,29	4,65	1,07	5,86	3,7	4,91	63	34,7
T2	0,24	6,75	1,16	6,24	4,8	2,96	81,6	31,7
T3	0,25	6,0	1,25	5,89	5,0	3,55	67,4	37,6
T4	0,24	5,55	1,12	5,83	4,7	3,63	70,2	34,2
T5	0,29	6,0	1,21	5,99	4,2	3,97	78,6	34,3
T6	0,31	4,50	1,22	5,70	3,9	5,55	57,6	33,7
Moy.	0,27	5,58	1,17	5,92	-	4,06	69,7	34,37
CV%	27,9 NS	33,6 NS	17,6 NS	NS		26,5 S	NS	14,7 NS

(*) limon grossier non compris ; NS = non significatif ; S = significatif.

2 - Analyse des rendements

A partir de 1988, deuxième année d'épandage des engrais, les traitements recevant les fortes doses de potassium ont les productions les plus élevées par rapport au témoin ; les différences sont statistiquement significatives (Tableau 4). L'effet conjugué des engrais potassiques et azotés a permis une meilleure assimilation des éléments et substances élaborés favorables à la formation du rendement par les caféiers (1).

Le potassium issu de la réserve mobile ou du potassium non échangeable n'a pas influencé positivement le rendement dans les parcelles témoins. Les doses d'engrais apportées et les équilibres retenus semblent adaptés aux conditions du milieu d'étude.

Le rapport Mg/K compris entre 2 et 5 et l'amélioration du taux du potassium dans la somme des bases constituent des indicateurs favorables pour de bonnes productions des caféiers; ils confirment des résultats de plusieurs travaux antérieurs (4) (6) (9).

Tableau 4 : Production en kg café marchand/ha en fonction des traitements

Traitements	1987	Cumul 1988-91	Cumul 1988-93
T1 : Témoin	2023,8	8 221	10 901
T2	2132,2	9 463	13 293
T3	2199,2	9 852	13 760
T4	2389,6	9 569	13 236
T5	2267,0	9 806	13 748
T6	1998,2	10 211	14 214
CV%	17,6	13,3	11,6
	NS	T1 <T2 à T6	T1<T2 à T6

3.- Les modèles de regression

Les ajustements ont porté sur les récoltes de 1988 à 1991, soit 4 récoltes (fig.1) et sur celles de 1988 à 1993, soit 6 récoltes (fig.2). L'allure générale des courbes est identique sur les deux périodes quel que soit le modèle utilisé.

- Regression curvilinéaire :

Le maximum de production est atteint à la teneur de 0,37 meq % de K dans le sol avec un coefficient de détermination R2 = 0,675 dans la figure 1 et R2 = 0,621 dans la figure 2. Les probabilités sont respectivement de 18,55 % et 23,36 % de sorte que les liaisons ne sont pas significatives.

Cependant la table des valeurs de coefficient de corélation R indique une liaison significative pour la figure 1 à P = 0,05 avec 4 dégrés de liberté. Aussi les productions tendent-elles à baisser lorsqu'on atteint des doses supérieures à 0,42 meq %, résultat déjà observé dans des essais factoriels.

Les équations sont :

$$Y = 4435{,}08 + 28700{,}1\ X - 36652{,}75\ X2 \quad \text{(fig.1)}$$

et $$Y = 4024{,}47 + 53076{,}29\ X - 70246{,}38\ X2 \quad \text{(fig.2)}$$

- Modèle monomoléculaire :

Dans ce modèle, la courbe rencontre l'asymptote à la teneur 0,37 meq % de K dans le sol mais les productions à la teneur 0,32 meq % sont très voisines. Par conséquent ce modèle donne des niveaux de K favorables entre 0,32 meq % et 0,37 meq % aussi bien dans la figure 1 que 2.

Fig.1 : **REPONSE AUX DOSES DE POTASSIUM**

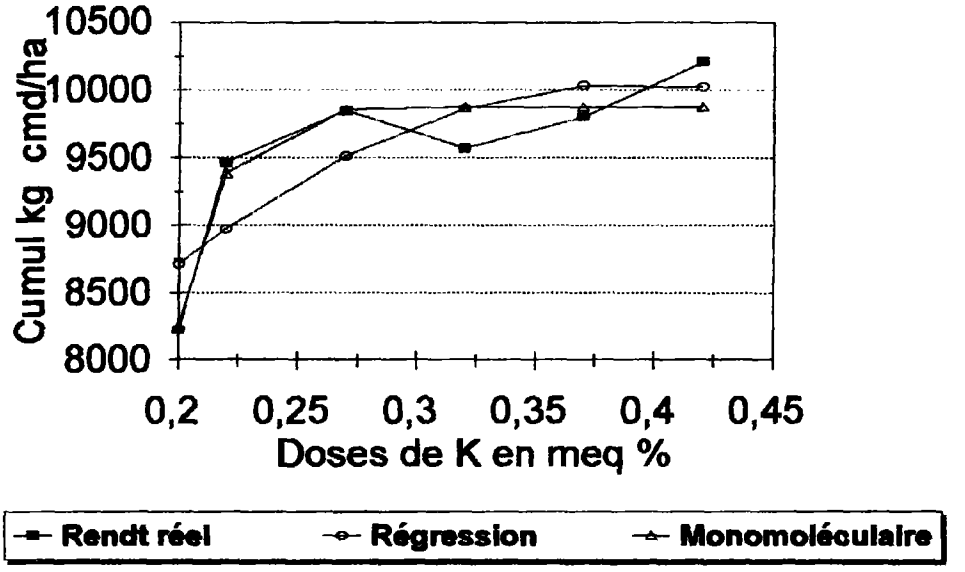
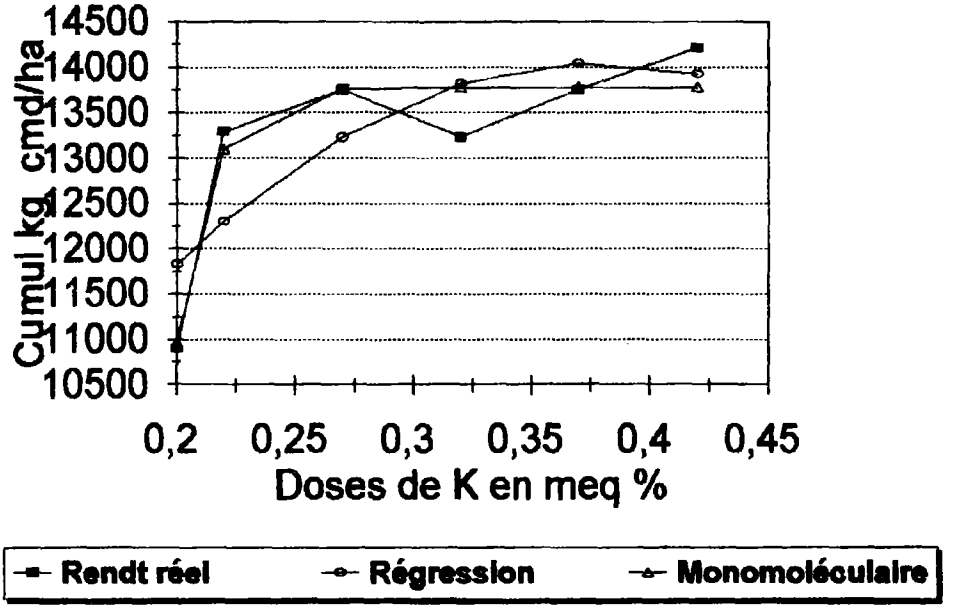


Fig.2: **REPONSE AUX DOSES DE POTASSIUM**



L'équation des courbes obtenues est respectivement :

$$Y = 10570 - 12957{,}14 \text{ Exp.}^{(-8{,}54.X)}$$

et $$Y = 14668 - 25786{,}68 \text{ Exp.}^{(-9{,}62.X)}$$

Si par les deux modèles, nous arrivons à la teneur 0,37 meq % pour assurer de bonnes récoltes, le modèle monomoléculaire donne une courbe plus proche de celle obtenue à partir des rendements observés en plein champ. Ce modèle explique donc mieux les phénomènes intervenus.

4 - La méthode de calcul

A partir des travaux de Forestier en République Centrafricaine basés sur les relations entre les taux d'éléments fins du sol (argile + limon) et la teneur en K. échangeable (4), Snoeck a établi une formule pour calculer les teneurs optimales, fortes et faibles de K dans le sol. Le limon grossier n'est pas pris en compte.

La teneur optimale est obtenue par la formule :

$$K.\ optimum = A\ (argile + limon) + B$$

où $A = 11{,}39.10^{-3}$ et $B = -1{,}449.10^{-3}$

L'application de cette formule à notre milieu avec 34,37 % d'argile et de limon fins, nous donne une teneur de 0,39 meq %. Bien que supérieure à la teneur obtenue des courbes ci-dessus, cette méthode simple peut permettre d'apprécier l'ordre de grandeur des niveaux de K dans le sol à ne pas dépasser, y compris les engrais potassiques, en fonction du taux d'éléments fins (argile + limon).

A titre d'exemple, la teneur optimum de K. pour la station de Bingerville qui a un taux moyen d'éléments fins de l'ordre 15 % se situe entre 0,1 et 0,2 meq %.

IV.- CONCLUSION

Bien que les teneurs du potassium dans le sol ne soit pas connues de façon précise à partir des analyses de sol, l'essai de doses exprimées en meq % nous a permis de déterminer un niveau favorable aux caféiers robusta en Côte d'Ivoire.

Ce niveau qui est de 0,37 meq % doit nécessairement tenir compte des équilibres entre les cations majeurs ; le rapport Mg/K doit être compris entre 2 et 5 mais proche de 3.

Parmi les modèles de regression utilisés, le modèle monomoléculaire explique mieux le phénomène. Par ailleurs, la formule établie par Forestier puis Snoeck à partir des taux d'éléments fins du sol (argile + limon) permet d'avoir des indications utiles sur la teneur de potassium échangeable àpréserver sous les caféiers afin d'assurer de bons rendements.

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NATURE OF COFFEE RESISTANCE TO TWO COSTA-RICAN *MELOIDOGYNE* POPULATIONS

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INTRODUCTION

Creation of coffee varieties resistant to root-knot nematodes started little time ago in Central America, through a project financed by European Union. Usually, resistance phenomenons are more likely studied through the point of view of their influence on nematode populations. The histological study here presented allows to link macroscopic symptoms (number and size of galls) observed during the screening of several C. arabica and C. canephora introductions, with microscopic characteristics that tell about the nature of the encountered coffee resistance towards Meloidogyne arabicida and Meloidogyne exigua.

MATERIALS AND METHODS

Nematodes under study

Population # 1 comes from Costa Rica. Possibly, it would be a new species described by Lopez and Salazar (1991) under the name of Meloidogyne arabicida sp. n. The population we used comes from "Hacienda Juan-Viñas" in Turrialba county (altitude : 1000 m).
Population #2 comes from CICAFE farm in Heredia (altitude 1100 m) and is identified as Meloidogyne exigua.
Although those two populations belong to apparently different species, they induce a same genotipic reaction (see presentation of Bertrand et al., at this conference).

Plant material

During different resistance trials, we tested several types of plant material towards populations 1 and 2. C. arabica is represented by control varieties (Caturra, Catuai), by Ethiopian cultivars from ORSTOM and FAO field collections and by several Catimor lines. C. canephora is represented by five accessions coming from Congo.

Control varieties and Catimors were analyzed in the field (6 years old adult plants) and at the nursery (8 months old plants). The other ones were studied at a young stage (between 8 and 18 months) and came from the field or the nursery.

Macroscopic observations
We adopted a classical qualitative scale called Gall Index (GI) and adapted from Arango et al (1982) as follow :

GI 0: no gall
GI 1: 1 or 2 very small galls
GI 2: 3 to very small to small galls
GI 3: 11 to 30 small and medium galls
GI 4: 31 to 100 small, medium and large galls
GI 5: more than 100 small, medium and large galls

- Very small galls are barely visible bumps on thin roots. The difference of diameter between a healthy and an affected cylinder vary between 0.2 and 0.5 mm (measured with a caliper).
- Small galls are galls which relative diameter can reach 0.5 to 3 mm
- Medium galls are galls which relative diameter vary between 3 and 5 mm
- Large galls are galls which relative diameter is superior to 5 mm

This scale has been verified by Rafinon (1994) and is highly correlated with the number of nematodes (eggs and larvae) for those types of nematodes.

Microscopic observations
For each GI used in the macroscopic observations we sampled representative roots. In every case, they are young white roots without any apparent necrosis. The sections were made at the spot of the gall. In case of no visible gall (it is the case for resistant varieties), the sections were made randomly. For each section we studied : the presence of nematodes, the presence of well developped or mishaped giant cells, the presence of egg masses.

Histological techniques
The samples were fixed in FAA (Formaldehyde, alcohol, acetic acid and distilled water) for 48 hours. They were then dehydrated in a series of ethanol (70%, 80%, 90%, 95%, 100%, 100%), one hour in each bath, embedded in HistoresinÔ at 4°C overnight, and then molded. Sections 3 mm thick were cut and stained with a quadruple stain (CIRAD, 1989).
Fresh samples were also stained in acidic fushin-lactophenol in order to evaluate internal of external presence of egg masses.

RESULTS

Observed symptoms in susceptible plants
We did not find any susceptible plant in C. canephora. All the susceptible plants described all come from C. arabica (Catimors, Ethiopians and control varieties). Staining of fresh and sectionned material shows that we are in presence of two species which sare the characteristic of burying most of their egg masses inside the roots (Pictures). This is different from the symptoms observed by Anzueto (1993) or Peña (1994) with Guatemalan populations (M. sp) and Salvadorian populations (M. incognita) in which egg masses are mostly external.
Within susceptible plants (GI 3 to 5), the histological patterns are identical, being with M. arabicida or with M. exigua. The sectionned galls reveal several females deeply buried in the root tissues at the vasular bundle level. These females are connected to large to very large egg masses. The giant syncitial cells are well formed and

very aboundant. It is possible to observe a dense cellular content and the presence of several nuclei. The cortex is devoid of nematodes and is constituted of living cells (Figs. 1, 2, 3).

Observed symptoms in very resistant plants (GI 0).
The very resistant plants are found within C. canephora and principally within Catimor varieties. It is most likely that the Catimors resistance genes come from C. canephora. However, we observed one Ethiopian plant classified as resistant (T4900-E531).
As there is no gall, we decided to observe certain points where the root is naturally (or not) thicker. In some cases, nematodes fragments are observed only at the periphery of the root (Fig. 4), but in most of the cases, no nematode is observed (Fig. 5). No difference is noted between resistant Canephoras, Catimors or the E531 accession.

Observed symptoms in resistant plants (GI 1 or 2).
As above, those plants are found within C. canephora and Catimor varieties. On the chosen scale, the GI is 1 or 2. Small galls were sampled and sectionned.

Case 1 : In some plants it is possible to observe a normal development of the female. The difference with the susceptible controls is that the average number of female per gall is down to 1 or two. The egg masses are often reduced. For those trees, there would be a simple reduction of the female proliferation. This phenomenon is observed in C. canephora as well as in C. arabica (Catimor varieties) (Fig. 6).

Case 2 : In a few plants, one can observe a small number of syncitial cells (sometimes, just one is left). However, the female seems to develop. Nevertheless, we did not see any egg mass (Figs. 7, 8).

Case 3 : Contrarily to case 2, the symptoms are localised in the periderm cells. One can see that the cellular enlargement process was stopped since those cells are empty, without any cellular content. Remains of nematodes can be observed (Fig. 9).

CONCLUSION

The qualitative scale used to classify the plants according to the gall number index (GI) matches with characteristical microscopic symptoms.

For a GI from 3 to 5, we confirm that the plants are equally susceptible. The differences in the gall numbers are to be connected with the variations unavoidable when an artificial inoculation method is used and with the differences of growth of the root systems.

For a GI =0, two resistance phenomenons are possible:
- A post-infection resistance mechanism; in this case, a penetration is observed since fragments of nematodes were detected, but they don't penetrate deeply. Since the nematode cannot reach its nutritional site, it cannot complete its normal development cycle.
- An hypersensibility mechanism; in some cases, a necrose is observed in the periderm, that could be explained by a tentative penetration of the nematode. More accurate studies must be undertaken in order to define this mechanism.

For a GI of 1 and 2, several phenomenons coexist:
- A normal development of the nematode, but with a reduction in the number of the females formed. It would be a case of partial resistance.
- An abnormal nematode development due to the presence of mishaped giant cells.
- An interrupted development of the nematode due to the failure in the formation of giant cells.

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Fig. 1. Presence of females and development of egg masses and giant cells in roots of a coffee plant susceptible to nematodes.
f, female; **em**, egg mass; **gc**, giant cells.

Fig. 2. Development of giant cells, close to the vascular tissue.
n, nematodes; **vt**, vascular tissue; **gc**, giant cells.

Fig. 3. Presence of female *Meloidogyne* in roots of coffee plant. Note the concentration of phenols in the cortical cells.
f, female; **em**, egg mass; **ph**, phenols.

Fig. 4. Presence of nematodes inside coffee root cortex.
c, cortex; **n**, nematode; **v**, vascular cambium.

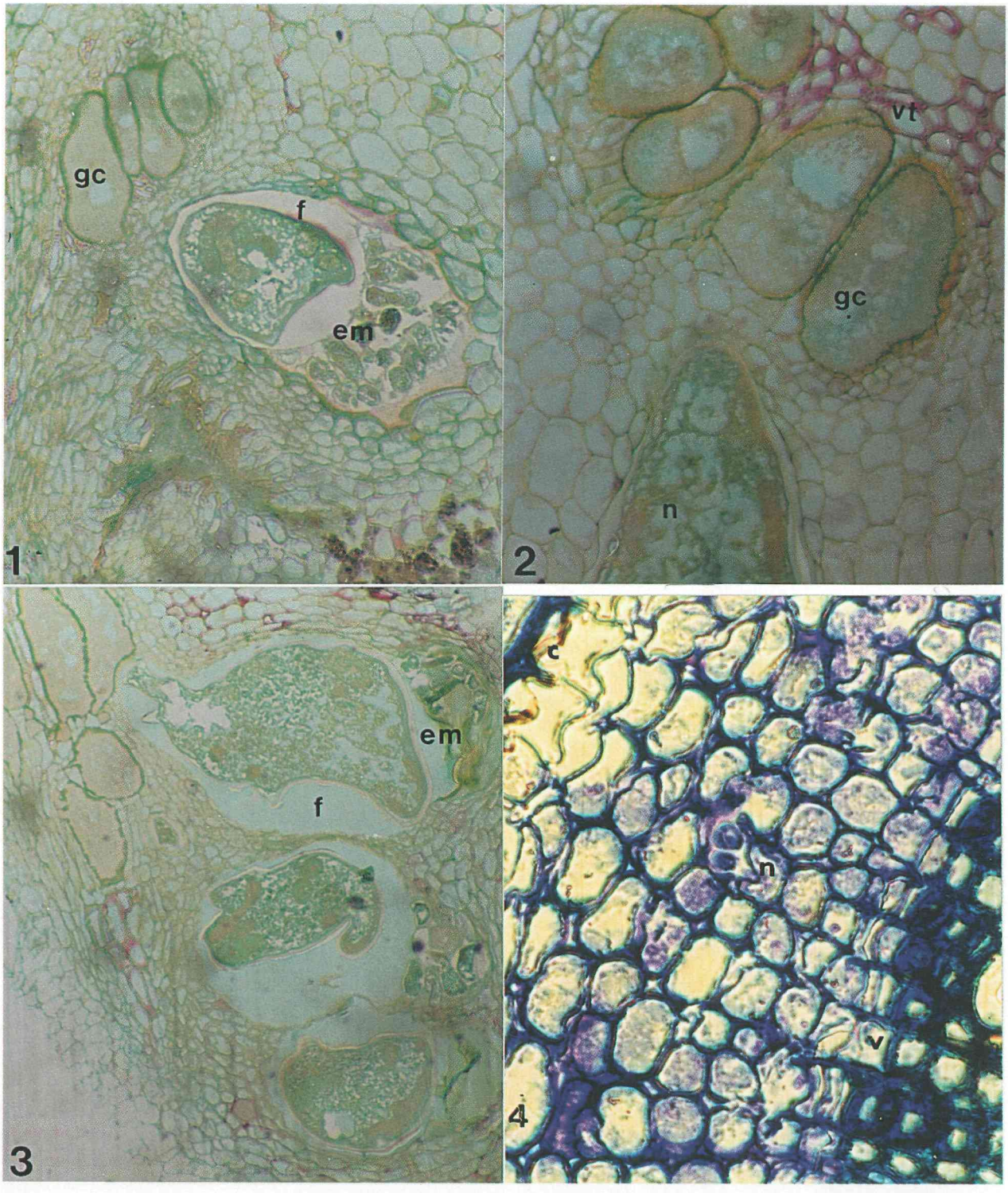
Fig. 5. Transversal section of a coffee root from a resistant cultivar.
c, cortex (back); **vt**, vascular tissue.

Fig. 6. Development of egg masses inside coffee root cortex. Note the development of giant cells.
f, female; **em**, egg mass; **gc**, giant cells.

Fig. 7. Presence of a female with a small egg mass and development of a unique giant cell.
f, female; **em**, egg mass; **gc**, giant cells.

Fig. 8. Development of atypical giant cells, surrounded by a thick cell wall and with little cytoplasm.
f, female; **gc**, giant cells.

Fig. 9. Development of atypical giant cells in the cortex area.
n, nematode; **vt**, vascular tissue; **gc**, giant cells.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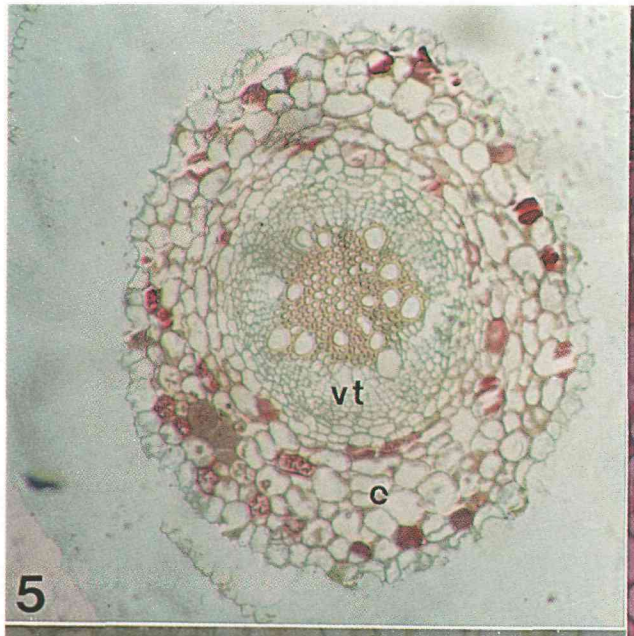
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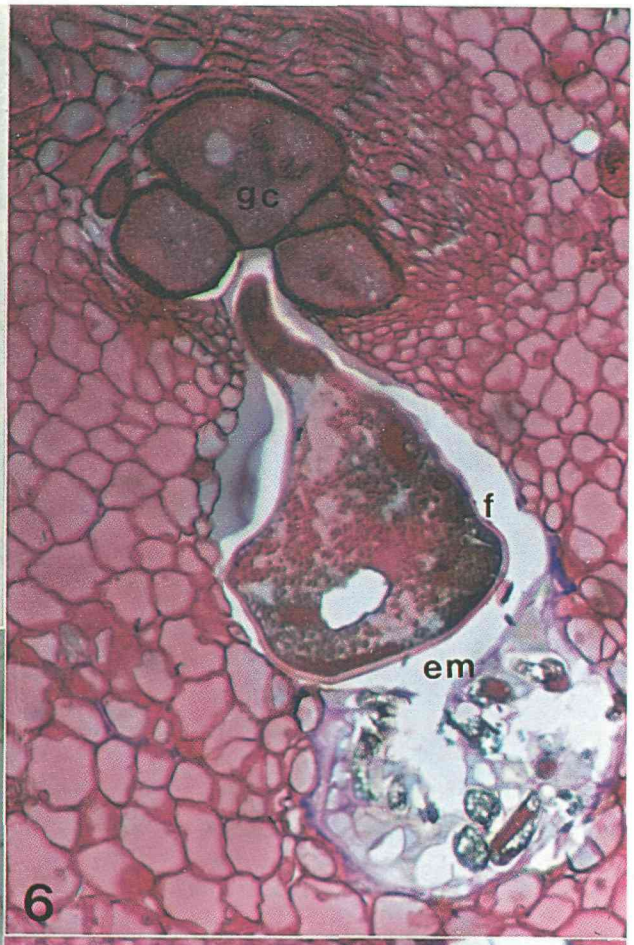
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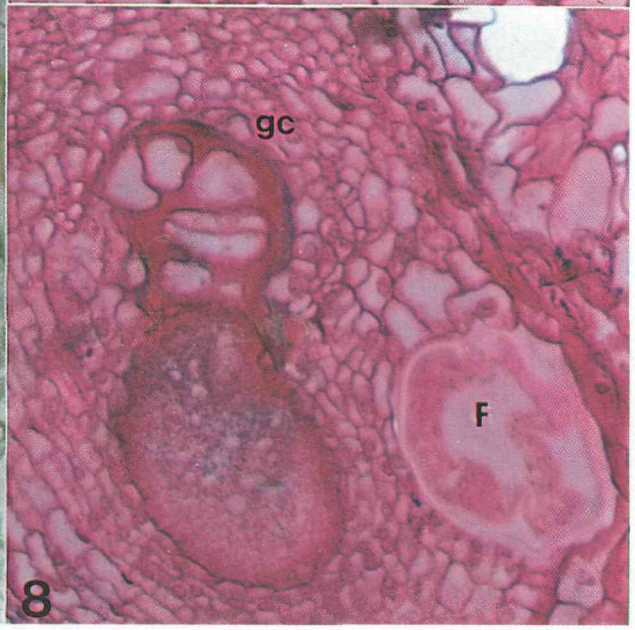
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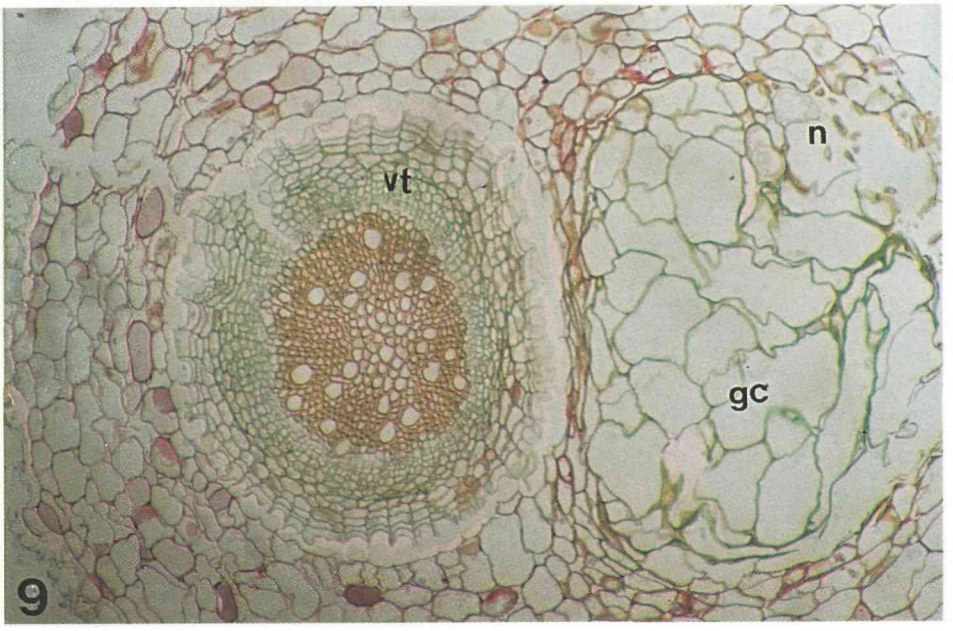
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RAPPORT DE SYNTHÈSE / SUMMARY REPORT

Rapport de synthèse

Effets physiologiques

Les exposés relatifs aux effets physiologiques du café ont, dans l'ensemble, traité de trois sujets principaux : (1) fonction du cerveau et aspects psycho-physiologiques ; (2) métabolisme des lipides et effets sur le système cardiovasculaire ; (3) mécanismes de protection contre le cancer.

A l'aide de techniques très sophistiquées, le professeur Y. Koga a montré que l'arôme du café augmente le flux sanguin dans certaines régions du cerveau chez l'homme. Les régions situées du côté droit du cerveau, siège de l'expression de l'émotion et du traitement des sensations olfactives, étaient particulièrement affectées. L'arôme du café peut induire une variété d'effets potentiellement bénéfiques sur la fonction de la connaissance et sur le comportement, et peut présenter un intérêt dans le traitement de troubles émotionnels légers. Le professeur T. Yamamoto a démontré que la consommation de café améliore le traitement de l'information par le cerveau et amplifie la sensation de détente chez les sujets qui aiment le café. Ces effets résultent d'une interaction complexe entre l'arôme, le goût et la caféine, chacun des éléments contribuant à l'effet général. Le docteur M. Lorist a examiné les effets de la caféine sur la perception et l'attention, dans le cas de stimuli visuels. Ces stimuli peuvent être classés en "quoi" (caractéristiques de l'objet) et en "où" (situation). Il ressort que la caféine améliore particulièrement le traitement des caractéristiques "où" de l'information visuelle.

Dans une revue très claire des effets du café sur les maladies cardiovasculaires, le professeur M. Katan a montré que le café a un effet négligeable sur la pression sanguine. L'influence du café sur l'augmentation du cholestérol, qui a été mentionnée, dépend de la méthode de préparation du breuvage, de telle sorte que seules les boissons ayant les teneurs les plus élevées en lipides ont cette influence. L'agent de l'hypercholestérolémie a été identifié au diterpène cafestol, que l'on trouve essentiellement dans le café bouilli, le café dit "turc" et celui donné par la cafetière à piston française. Il est présent en très faible quantité dans les boissons de café communément absorbées, telles que les cafés filtrés et les cafés solubles. La torréfaction, la décaféination et le temps de préparation de la boisson n'ont que peu d'influence sur la teneur en cafestol. Si l'ingestion chronique de grandes quantités de café boisson contenant de fortes teneurs en cafestol peut accroître l'éventualité d'une maladie cardiaque, la consommation modérée de café n'est pas liée à des répercussions négatives sur le système cardiovasculaire. Le docteur T. Ito a montré que l'ingestion de café filtre peut être une protection contre les maladies cardiovasculaires. L'augmentation du HDL-cholestérol chez les femmes, une réduction de l'aptitude du LDL-cholestérol à l'oxydation et une augmentation de la lipoprotéine anti-athérogénique ont été enregistrées chez des sujets consommant ce type de café. Il semble de plus en plus évident que la consommation de café peut protéger du cancer du colon et du rectum. Un certain nombre de composants du café peut être impliqué. Le docteur A. Huggett a montré que les diterpènes du café, le cafestol et le kahweol, peuvent altérer le métabolisme du cancer en empêchant la réaction d'intermédiaires activés avec l'ADN, qui constitue le premier stade du processus du cancer. Ces diterpènes peuvent provoquer ce phénomène en modulant les enzymes cytochromes P450 qui catalysent l'activation carcinogène et également en induisant une forme spécifique de glutathione S-transférase qui peut ditoxiquer les intermédiaires génotoxiques. Le docteur T. Tanaka a signalé que les acides chlonogéniques et caféique réduisent ou empêchent la formation de tumeurs du colon, du foie et de la cavité buccale chez un grand nombre d'animaux cobayes. Le professeur V. Nguyen a présenté des effets antimutagènes de l'acide chlorogénique et de composants du café qui apparaissent après la torréfaction. Un extrait de café torréfié ainsi que des produits issus de la torréfaction, l'acide chlorogénique et la trigonelline, étaient particulièrement antimutagènes et le mécanisme semble comporter une activité de capture des radicaux libres.

Dans une étude historique, le professeur T. Namba a fait ressortir que des propriétés médicinales sont depuis longtemps reconnues au café en Asie, essentiellement en tant que diurétique et que stimulant. Ces effets peuvent être attribués à la caféine. De récentes études ont montré qu'un extrait aqueux de café présente une activité de capture des radicaux libres et peut empêcher la peroxydation des lipides et supprimer l'antigène de surface du virus de l'hépatite B. Le professeur S. Garattini a donné une large vue d'ensemble sur les effets du café et de la caféine sur la santé. Les disterpènes, les 5-hydroxytryptamides et les purines, notamment la caféine, sont les principaux composants du café biologiquement actifs. L'interprétation des effets du café sur la santé a été rendue difficile en raison de facteurs de confusion tels que la consommation de tabac, notamment. Il est maintenant possible de rejeter les assertions antérieures relatives à la relation entre la consommation de café et les maladies cardiovasculaires et le cancer des voies gastro-intestinales. On est donc arrivé à la conclusion, étayée scientifiquement, que la consommation modérée de café n'a pas d'effets nocifs sur la santé.

Affiches :

Les effets du café et de ses composants sur une variété de processus physiologiques potentiellement importants ont été traités dans quelques affiches bien présentées.

Les effets antioxydants et anticancéreux du café ont été rapportés dans plusieurs affiches. L'équilibre entre la production de peroxyde d'hydrogène et l'effet de capture des radicaux libres du café dépend de la concentration en café, d'après Y. Araki et V. Nguyen. Le potentiel des acides chlorogéniques et de l'acide caféique dans l'activité de capture des radicaux libres, comparé à celui des vitamines antioxydantes, a été démontré par H. Morishita et R. Kido. Un extrait de cerises de caféier empêche le développement de

tumeurs chez les souris comme l'a montré H. Nagasawa ; S. Furusawa a montré que la caféine peut protéger contre l'apoptose (mort programmée d'une cellule) par les anthracyclines. K. Yagasaki rapporte que le café inhibe la fonction du TGFB (facteur β de transformation de la croissance). Cet effet indique que le café peut avoir une action protectrice potentielle contre la formation de tumeurs et contre les néphrites (lésion des reins).

D'importants effets potentiels de la caféine ont été présentés dans d'autres affiches. T. Fushiki a montré que l'absorption de café augmente l'activité du système nerveux autonome ; N. Sakane, que la caféine est capable d'induire une thermogenèse qui entraîne une diminution du poids du corps et de la graisse chez certains sujets obèses. Cependant, un autre groupe de sujets obèses n'a pas réagi, ce qui laisse à penser que les tissus adipeux bruns ne sont pas activés dans ce groupe de personnes et que des thérapeutiques de remplacement sont nécessaires pour le traitement de certains sujets obèses.

R. Viani, A. Huggett
(traduction)

Chimie

Les propriétés antioxydantes des composants du café sont un des sujets traités au cours de ce colloque de l'ASIC ; ce sujet a déjà été évoqué dans la session des "effets physiologiques". La caractérisation et la détermination de ces propriétés ont également été soulignées dans la session consacrée à la chimie. Plusieurs isomères de l'acide phénolique et leurs esters, communément décrits comme acides caféique et chlorogéniques, ont le pouvoir d'absorber, ou mieux de capturer, les radicaux libres. Les anti-oxydants ont également une telle aptitude. Ces radicaux libres, qui pour la plupart réagissent à l'oxygène, ont été présentés comme des causes majeures du cancer. H. Sakuray et ses collaborateurs analysèrent par ESR (spectroscopie par résonance de spin électronique) la suppression, par l'action d'extraits de café, des radicaux libres. Ils déterminèrent l'activité éliminatrice de l'anion superoxyde (SSA).

Dans une affiche, T. Nakayama a rapporté que, *in vitro*, les esters de l'acide caféique pouvaient inhiber les ruptures dans les cellules d'ADN, induites par le superoxyde. Ceci confirme les effets antioxydants de l'acide caféique observés *in vivo*. H. Hirazawa et T. Okada présentèrent leur expérience dans laquelle ils avaient utilisé les propriétés antioxydantes d'extraits de café pour maintenir la fraîcheur d'aliments comme le poisson.

Dans une étude exhaustive, R. Stadler, J. Richoz et L. Fay examinèrent la disparition des radicaux due à l'action de la caféine : ils purent prouver que la caféine a aussi des propriétés antioxydantes ; son produit d'oxydation est caractérisé comme étant la 8-oxocaféine.

Plusieurs exposés traitèrent des propriétés et de l'analyse des acides du café.

V. Leloup, A. Louvrier et R. Liardon explorèrent le mécanisme de la rupture chimique de cette partie des acides chlorogéniques qui est réduite pendant la torréfaction ; des réactions d'estérification purent être déterminées au cours des premiers stades de la torréfaction.

K. J. Balyaya et M. N. Clifford présentèrent dans une affiche l'analyse quantitative de différents isomères d'acides chlorogéniques et caféique dans des cafés Arabica et Robusta moussonés ; ces cafés ont un arôme neutre, doux, caractéristique ; leur teneur en acides diffère significativement de celle des cafés de la même région de l'Inde préparés par voie sèche ou par voie humide.

Dans une autre affiche, F. Kusu, T. Fuse et K. Takamura ont décrit la détermination électrochimique des acides carboniques inférieur et supérieur, ainsi que des acides chlorogéniques dans le café.

A. Bradbury et ses collègues montrèrent qu'il était possible d'appliquer la moderne électrophorèse capillaire à la détermination des acides aliphatiques et phosphorique dans les cafés Arabica et Robusta.

Au cours d'un exposé magistral, S. Homma a fait part de son étude exhaustive sur la chélation de métaux provoquée par les composants du café, notamment les acides chlorogéniques et leurs dérivés : il a pu isoler des complexes de zinc et de fer II, après avoir ajouté les sulfates et chlorures correspondant à des solutions de café ; il a également isolé pour la première fois la coumaroyl-tryptophane. L'évaluation quantitative des résultats ne permet pas de penser que les composants du café puissent être à l'origine de déficiences en fer chez les humains. M. Murata, H. Okada et S. Homma décrirent la détermination et l'isolement de onze acides chlorogéniques, ainsi que l'élucidation de la structure du nouveau composant du café Robusta, le p- coumaroyl - (L) - tryptophane.

Le complexe de l'arôme du café a été largement discuté dans deux exposés magistraux au cours desquels les derniers développements de la technique ont été examinés en détail.

W. Grosch a décrit les derniers progrès réalisés au cours des années 1990 dans la combinaison des analyses instrumentales et sensorielles des éléments volatils de l'arôme du café. A l'heure actuelle, dans les aliments comme le café, des éléments odorants puissants peuvent être répertoriés en humant avec le nez à la sortie d'un chromatographe en phase gazeuse, puis par une analyse ultérieure d'une solution de l'arôme, la signification sensorielle de tels composés peut être déterminée. Il présente de nouveaux modes de quantification qui utilisent des dérivés deutérés des composés de l'arôme : même de très faibles quantités, comme celles des thiols, par exemple, peuvent être détectées en les liant à du mercure organique immobilisé sur des colonnes de gel. Une description détaillée des différences dans les composants puissants de l'arôme entre l'Arabica et le Robusta est donnée.

Le rôle de la réaction de Maillard dans la formation des arômes du café était le thème de l'exposé magistral de G. A. Reineccius. Dans une revue convaincante, il présenta le stade des connaissances sur les mécanismes chimiques du processus de torréfaction, les précurseurs concernés, le rôle des lipides et les études cinétiques de la réaction, conduites jusqu'à présent. Il insista également sur la nouvelle théorie de la transition vitreuse, qui donne quelques explications aux réactions physiques et chimiques dans les aliments secs et comment la mélanoidine, à l'origine de la réaction de Maillard, peut être affectée.

D. Hinman a montré la relation entre l'espace de tête et la concentration en composants volatils légers du café ; ceci autorise une évaluation plus précise des mesures de l'espace de tête, obtenue par chromatographie.

C. Gretsch et ses collègues décivirent dans une affiche la détermination des coefficients de partition des composants volatils du café pour les systèmes air/eau, air/huile de café et huile/eau.

Y. S. Ko et H. J. Baik ont montré dans leur affiche l'analyse des espaces de tête d'arômes de différents cafés.

Y. Kawakami et ses collègues se sont aussi référés dans leur affiche à la détermination des composants volatils de l'arôme du café à partir de neuf cafés torréfiés différemment : ils utilisèrent les techniques ICP-AES d'analyse.

K. J. Kim, J. M. Rho et S. Y. Kim firent des recherches sur l'effet de la "pénétration à travers le lait". Ils ont étudié

la rétention de l'arôme par le lait, en analysant l'espace de tête de l'arôme de différentes boissons lait/café.

A l'aide d'une affiche, H. Steinhart, C. Grötzbach et J. Wilkens présentèrent leurs recherches sur la stabilité des boissons de café maintenues à 80°C pendant quatre heures. Ils notèrent des changements dans la couleur, la turbidité et la concentration des composants volatils dans l'espace de tête, en relation avec la teneur en oxygène.

E. Ludwig, N. Raczek et T. Kurzrock montrèrent dans une affiche l'isolement de protéines à partir de cafés verts et comment, ensuite, caractériser par différents procédés les structures complexes obtenues.

H. Steinhart et A. Lüger présentèrent la structure des acides aminés des cafés dits traités à la vapeur. Ces cafés sont considérés comme ayant un effet positif sur la santé, par comparaison avec les cafés non traités. Un procédé analytique permettant de distinguer les divers cafés Arabica a été donné.

K. Higuchi, T. Suzuki et H. Ashihara montrèrent dans une affiche comment détecter l'acide pipécolique parmi d'autres acides aminés dans des feuilles en développement et des fruits non mûrs de caféiers Arabica. Il est avancé que ce composant pourrait jouer un rôle dans la biosynthèse de la caféine.

Des recherches sur les glucides ont été rapportées par S. Oestreich-Janzen, qui a décrit les méthodes de détermination des mono-, di- et trisaccharides dans le café et les produits qui y sont liés, par HPLC.

A. Lüger et H. Steinhart ont décrit dans leur affiche les changements provoqués dans les glucides par les traitements à la vapeur des cafés. Ces oligosaccharides ont été déterminés par chromatographie en phase gazeuse après silylation.

R. M. Noyes a montré dans son exposé que le rendement du café vert dans la fabrication du café soluble doit être calculé comme étant la résultante du rendement du nettoyage, de la torréfaction et du procédé. Un système d'extraction en autoclave pour déterminer le rendement est décrit.

Dans trois exposés, il a été décrit les différences entre les variétés et la détermination des mauvais arômes et des adultérations des cafés.

K. Speer a décrit sa méthode d'analyse de la teneur en Robusta de mélanges de café : en utilisant les techniques d'isolement par chromatographie préparative, il peut en outre détecter douze esters d'acides gras supplémentaires du 16-0- méthylcafestol.

W. Holscher et ses collègues élucidèrent l'origine d'un mauvais arôme apparu dans des cafés du Kenya ; il s'agit du TCA, connu dans les cafés riotés du Brésil, produit résultant de la dégradation d'un pesticide.

D. R. White a rapporté ses recherches sur les adultérations du café et décrit les méthodes d'identification appliquées aux échantillons dans le cadre d'un contrôle de qualité.

Un autre domaine, abordé au cours de la session sur la chimie de ce colloque de l'ASIC, était la présentation de systèmes d'évaluation sensorielle permettant de caractériser rapidement les échantillons de café par des méthodes globales.

K. Toko et ses collaborateurs ont décrit un système de capteurs de goût, employant des doubles couches de lipides pour caractériser des sensations gustatives comme l'amertume, l'âpreté, l'astringence, etc. Des électrodes comportant jusqu'à huit canaux avec des doubles couches de lipides différentes et bien reproductibles donnent un signal quand ils sont immergés dans la boisson de café. On a pu ainsi démontrer des différences entre les cafés.

H. Komai et ses collègues présentèrent des recherches plus précises effectuées avec un tel appareil de détection sensorielle.

Remarque générale : si la reproductibilité, la répétabilité, le pouvoir de discrimination et la durée de conservation en rayon peuvent continuer à être améliorés, de tels instruments pourraient devenir des outils intéressants dans l'avenir pour les mesures de contrôle de la qualité.

Un autre type d'appareil d'évaluation sensorielle a été présenté par T. Fukunaga et ses collègues qui décrivent les expériences faites avec un "nez électronique", qui détermine l'odeur du café. Basé sur des détecteurs constitués d'oxydes métalliques, la caractérisation de mauvais arômes critiques, comme l'odeur de médicament riotée bien connue au Brésil, peut être effectuée.

B. Guyot et ses collègues étudièrent trois techniques de traitement primaire du café vert Robusta : par voie sèche, par voie humide et par voie humide avec addition d'enzymes. Les tests chimiques montrèrent que le traitement par voie humide avec addition d'enzymes donne des grains ayant une teneur plus élevée en sucres et en acides chlorogéniques. Les tests de dégustation donnent des résultats similaires à ceux obtenus avec les fèves préparées par voie humide.

O. G. Vitzthum
(traduction)

Technologie

Dans cette revue de la session de technologie, les thèmes seront traités dans l'ordre suivant : torréfaction, conservation du café, café soluble, séchage, espresso, café vert.

G. Lührs a présenté un appareil assurant la torréfaction du café par lot, qui, grâce au système de refroidissement auquel il est associé, en combinaison avec un brûleur catalytique, permet une réduction des émissions d'odeurs et de gaz indésirables dans l'atmosphère. Dans une étude complète, T. Kino et T. Takagi ont donné les résultats obtenus avec la torréfaction par infra-rouges, comparée à la torréfaction conventionnelle : l'évaluation a été faite en mesurant la teneur en eau pendant le chauffage, en faisant des mesures ESR et RMN pour les radicaux et l'eau libres, en comparant la couleur interne et externe des grains, en déterminant les acides par HPLC et les composants volatils de l'arôme par chromatographie en phase gazeuse et en déterminant la qualité par des capteurs d'odeur et de goût.

V. D. Nagaraju, K. Ramalaxmi et P. Srinivasa Rao proposèrent un torréfacteur en flux continu destiné à traiter les cafés indiens de bas de gamme (noir ; marron ; brisures).

T. Matsushima, N. Oguro et S. Ichiyanagi rapportèrent que des absorbants d'oxygène conviennent très bien pour prolonger le temps de conservation en rayon du café torréfié et moulu ; une absorption complémentaire du gaz carbonique contribue à stabiliser le produit.

C. Severini et ses collègues étudièrent un traitement pour stabiliser la boisson de café en appliquant une pression élevée, pouvant atteindre 7 000 bar. Tandis que la stabilité biologique était atteinte à 40°C, la stabilité de la boisson n'a pu être améliorée.

M. Grønlund a présenté, vues par un industriel, de nouvelles améliorations dans la fabrication de cafés solubles de meilleure qualité. Ceci peut être obtenu en mouillant le café moulu avant l'extraction et en faisant des extractions de plus courte durée.

En se basant sur des études poursuivies sur des modèles, S. Yamamoto a décrit les processus particuliers qui se produisent pendant le séchage de solutions, en mettant l'accent sur l'arôme et la rétention d'enzymes dans les liquides contenant du sucre.

N. Ohtani et ses collègues étudièrent le séchage par atomisation des cafés solubles à basse température. La rétention de l'arôme par différents procédés de concentration a été déterminée.

Dans une étude approfondie, O. Fond a examiné la dynamique fondamentale du type d'extraction espresso, en insistant sur l'influence de la composition de l'eau d'extraction et de l'origine du café sur le processus de compaction.

H. Kumori et ses collègues présentèrent dans une affiche la dégradation des polysaccharides solubles et insolubles du café et des marcs de café à l'aide de *Trichoderma viride* No 8. La dégradation qui conduit à des mono- et à des oligosaccharides était de l'ordre de 90 % dans le cas des cafés ougandais.

Z. R. Lopez, J. E. Sanchez et R. Bello traitèrent de l'utilisation des eaux résiduaires du traitement du café par voie humide, pour la culture de champignons comestibles.

H. C. de Menezes et L. R. Romeiro ont étudié l'altération de la croissance lorsque de la pulpe de café est ajoutée à la ration de l'animal en proportion supérieure à 10 %. La fermentation de la pulpe de café par *Lactobacillus plantarum* et l'addition de 1 % de sucrose pourraient permettre de porter cette adjonction dans la ration à 20 %.

O. G. Vitzthum
(traduction)

Agronomie

La session Agronomie du 16ème colloque de l'ASIC a permis d'avoir une vue générale de l'avancement des recherches pour l'amélioration de la caféiculture des différentes régions du monde grâce à trois exposés généraux, vingt-cinq présentations orales et vingt-neuf présentations par affiche.

Dans son exposé introductif, le Dr Muller a fait part de ses réflexions sur l'avenir de la caféiculture. Il a analysé les causes de la chute de la production, en particulier des *Robusta* africains, en rapport avec les difficultés socio-économiques de ces dernières années. Son message, "produire mieux sur des surfaces limitées, dans des conditions plus favorables aux planteurs", s'appuie sur un effort de transfert des acquis scientifiques et techniques de l'ASIC vers les pays producteurs de café.

Le Dr Opilé (CRF, Kenya) a insisté sur la place prépondérante du café dans l'économie et le développement des producteurs d'*Arabica* et de *Robusta* en Afrique. Dans le même temps, la quantité et la qualité de cette production ont décliné fortement. Les pays africains se sont groupés au sein d'un réseau de recherche créé en 1993 (RECA) sous l'égide de l'Organisation Inter-Africaine du Café (OIAC) pour développer des programmes coopératifs : la lutte contre l'anthracnose des baies (CBD), l'amélioration de la qualité du *Robusta* et la conservation des ressources génétiques. Un plan à cinq ans de soutien aux trois principales collections de caféiers de Côte d'Ivoire, d'Ethiopie et de Madagascar a été établi pour améliorer leur gestion et leur utilisation, avec l'appui du Centre International des Ressources Génétiques (IPGRI).

Enfin, le Dr Saragih (Bogor, Indonésie) a présenté la production du café dans un des rares pays où elle est en croissance rapide, l'Indonésie. Cette production est principalement basée sur le *Robusta* et pratiquée par des petits planteurs. Le système traditionnel de culture du caféier en verger, associé à des plantes vivrières et des arbres fruitiers explique la faible productivité et le vieillissement des caféiers ; la pression parasitaire paraît limitée dans ces conditions. L'amélioration de la production par des pratiques agricoles adaptées ne pourra devenir effective qu'avec le développement des infrastructures, des coopératives, du crédit et des techniques favorisant l'intensification et l'amélioration qualitative.

Les **biotechnologies appliquées aux caféiers** se développent dans plusieurs directions :

1 - Le Dr Yasuda *et al.* (Kobé, Japon) a présenté une synthèse des différentes voies de multiplication végétative *in vitro* - embryogenèse somatique, protoplastes. Le développement semi-industriel de la multiplication par microboutures et embryons somatiques a progressé significativement grâce à la culture en milieu liquide avec immersion temporaire selon un procédé mis au point par le CIRAD (France). Il est en cours d'essai dans un laboratoire mis en place à Kawanda (Ouganda). Trois posters en rapport avec ces approches concernaient l'effet des hormones (Dr Hatanaka et Tahara *et al.*, Japon) et des acides aminés (Dr Nishibata *et al.* Japon).

2 - Les conditions de conservation des embryons somatiques par déshydratation et à basses températures dans l'azote liquide ont été rapportées par le Dr Deshayes *et al.* (Francereco, France). Des applications multiples sont proposées : conservation plus d'un mois pour transfert et semis d'embryons, conservation à long terme (cryoconservation) des souches embryogènes originales.

3 - L'étude de la diversité génétique des caféiers est développée avec les marqueurs RFLP, RAPD et le séquençage de l'ADN par les généticiens ORSTOM (Montpellier, France). Ainsi, les variétés Typica et Bourbon de *C. arabica* sont distinguées, ainsi que les souches subspontanées d'Ethiopie. Les progéniteurs de l'espèce allotétraploïde *C. arabica* sont précisés : génome chloroplastique maternel de *C. eugenioides,* Moloundou ; ADN ribosomique paternel de *C. congensis*.

4 - Quelques informations complémentaires rapportées par affiches concernaient les tentatives d'obtention d'haploïdes par Dufour (CATIE, Costa Rica) et l'évaluation des peroxydases au cours de la microsporogenèse (Guedes, CIFC, Portugal). En outre, la transformation génétique du caféier par *Agrobacterium rhizogenes* a été appliquée avec succès par le Dr Sugiyama (Pokka Co, Japon).

Les **présentations orales sur la création variétale** témoignent de l'importance accordée à la sélection du café pour la qualité à la tasse. Montagnon *et al.* (CIRAD, France) ont démontré l'impact des différences génétiques entre clones Robusta par rapport au terroir et à la préparation du café en Côte d'Ivoire. Le Dr Cadena *et al.* (CENICAFE, Colombie) ont rapporté les résultats des tests de dégustation au niveau national et international de la variété Colombia ; ses caractéristiques physiques ont été améliorées (70 % de café "suprême") et ses qualités organoleptiques sont comparables à celles des variétés courantes d'Arabica.

La caféiculture industrielle décrite par Roche (PIONEER Co. Hawaii) est fondée sur le choix de variétés de *C. arabica* à maturité groupée permettant la récolte mécanique, une qualité à la tasse supérieure et de bons résultats agronomiques. L'amélioration qualitative de *C. arabica* dans différentes localités à l'est de Java a été discutée par le Dr Mawardi (Jember, Indonésie) sur la base des anomalies des grains nettement affectés par l'interaction variété x environnement.

L'amélioration du café Robusta a aussi été tentée par la création d'hybrides Arabusta. Yapo (IDEFOR, Côte d'Ivoire) a indiqué l'amélioration limitée de leur fertilité au cours des générations de sélection et un effet marqué des conditions climatiques de basse altitude.

Une étude extensive de la collection de *C. arabica* du Cameroun par Bouharmont et Montagnon (CIRAD) pré-

sentée par affiche donne une évaluation de la variation des caractères botaniques et agronomiques.

La résistance génétique aux maladies et parasites reste une préoccupation majeure pour les sélectionneurs. Santa Ram (CCRI, Inde) a proposé une nouvelle hypothèse explicative du déterminisme génétique de la résistance à *Hemileia vastatrix*. Bertrand *et al.* (PROMECAFE - CIRAD, Costa Rica) ont entrepris une sélection pour la résistance aux nématodes à galles (meloïdogynes) d'Amérique centrale : utilisation à court terme, comme porte-greffe, d'un croisement contrôlé de *C. canephora* ; création à long terme de variétés de *C. arabica* tolérantes.

La spécificité de ces résistances nécessite d'approfondir les différences de réaction des souches de nématodes par inoculation et connaissance des pathotypes. La caractérisation biochimique et biologique des différentes populations de méloïdogynes parasites des caféiers en Amérique centrale est en cours (affiche de Hernandez *et al.* CIRAD-ORSTOM).

La physiologie des caféiers soumis à des contraintes hydriques a fait l'objet d'études précises par le Dr Kanechi *et al.* (Kobé, Japon) sur la transpiration et la photosynthèse foliaires, en fonction de l'ombrage et de l'éclairement de jeunes plants de *C. arabica* en pot. La décroissance de la photosynthèse des trois espèces cultivées, associée à la réduction de la conductance stomatique des feuilles sous contrainte hydrique, a été précisée par affiche.

La **lutte contre les principales maladies et parasites** a fait l'objet de nombreuses communications, en particulier sur un parasite majeur, le scolyte des grains qui envahit l'ensemble des régions de production. Cilas *et al.* (CIRAD) ont présenté les méthodes d'échantillonnage adaptées à l'évaluation de l'impact du scolyte et à la décision des traitements. Un échantillonnage stratifié systématique pour détecter les foyers est mis en pratique avec des observateurs formés en Amérique centrale. Une étude de même nature concernant la punaise bigarrée (*Antestiopsis*) au Burundi, responsable d'infections bactériennes et de goûts désagréables, a conduit Cilas *et al.* (CIRAD) à des pratiques différentes par sondage simple, arbre par arbre.

Decazy et al. (CIRAD) ont présenté l'impact agroéconomique et l'acceptation au Guatemala de la **lutte biologique** contre *Hypothenemus hampei* par le parasitoïde *Cephalonomia stephanoderes*, comparativement à la lutte chimique et à la disponibilité en main-d'œuvre.

Le Dr Bustillo *et al.* ont décrit l'important programme de lutte biologique entrepris par CENICAFE (Colombie), fondé sur différents entomopathogènes et champignons pathogènes (*Beauveria*) produits en masse. Les services de vulgarisation favorisent l'intégration de cette approche biologique aux pratiques agronomiques et la réduction des traitements chimiques.

Quant à la pathologie de *C. arabica*, Pellegrin *et al.* (ORSTOM) ont décrit la situation des plantations de Nouvelle-Calédonie en fonction des composantes de l'environnement. Des tentatives de prédiction par modélisation épidémiologique des infections pour la rouille, l'anthracnose des rameaux et la cercosporiose. En parallèle, l'étude de la diversité des champignons parasites en Nouvelle-Calédonie présentée par affiche (Kohler, ORSTOM) est réalisée par marqueurs enzymatiques, RAPD et compatibilité des souches.

D'autres présentations par affiche ont complété nos connaissances sur le CBD et la rouille :

— diversité génétique et variabilité du pouvoir pathogène de *Colletotrichum coffeanum* (Bieysse *et al.* CIRAD),

— méthodes de traitement chimique à basse pression du CBD (Derso, Ethiopie),

— caractérisation biochimique de seize souches d'*Hemileia vastatrix* et influence de la teneur en caféine sur l'infection (Guedes, CIFC, Portugal).

Les **systèmes et pratiques agronomiques dans les différents pays producteurs de café** ont été illustrés par plusieurs exemples. Pour le Sri Lanka, le Dr Wickramasinghe a présenté les performances de *C. canephora* variété IMY cultivé dans différentes conditions de milieu, de culture et de taille. Les conditions de l'important développement de la production de Robusta au Vietnam (objectif 200 000t/an) ont été résumées par MM. Michel et Lebailly (Belgique). Les contraintes de ce transfert de technologie, en particulier la préparation de cafés de qualité, l'usinage et la commercialisation doivent être rapidement surmontées.

Diverses expériences de fertilisation minérale et organique ont été présentées sous forme d'affiche :

— en Indonésie (Dr Abdoellah - Dr Winaryo) sur l'accroissement de l'utilisation de la fumure organique,

— en Ethiopie (Dr Dubale) sur la nutrition minérale phosphatée de jeunes plants,

— en Côte d'Ivoire (Dr Koffi NGoran) sur la nutrition minérale potassique de caféiers Robusta,

— en Papouasie-Nouvelle-Guinée (Dr Kiara) sur la combinaison de densités de plantation, l'utilisation d'ombrage et la fertilisation,

— au Japon (Dr Kito) sur l'utilisation des résidus du café en compost pour le contrôle des mauvaises herbes.

Le **traitement après récolte** est une préoccupation majeure pour obtenir un café de qualité. Le Dr Woelore (Ethiopie) a décrit les conditions d'entreposage et de conservation du café en parche à la ferme, et par les entreprises. Ce stokage doit être de durée limitée et le café transféré vers les entrepôts centraux. Les Dr Cortez et Menezes (Campinas, Brésil) ont présenté une méthode de préparation du café améliorant significativement les caractéristiques organoleptiques par rapport à la voie sèche, ainsi que l'intérêt d'appliquer des souches microbiennes particulières sur les cerises avant récolte.

Les conditions de fermentation et de séchage du café Arabica produit à Java ont fait l'objet d'une affiche (Dr Wahyudi et Ismayadi, Indonésie). Les conditions optimales de torréfaction ont été mises au point en Côte d'Ivoire pour les cafés Robusta et Arabusta (Yate, IDEFOR).

L'étude de la biosynthèse de la caféine a été abordée en collaboration par les équipes de recherche de l'Université Ochanomizu (Japon) et de Glasgow (U.K). Le Dr Ashihara *et al.* ont démontré sur les feuilles de *C. arabica* une voie préférentielle de synthèse *in vivo* de la caféine à partir de la théobromine. M. Gillies *et al.* ont étudié la stabilisation d'une enzyme clé de la biosynthèse de la caféine, le N-méthyl-transferase ; une méthode de purification sur colonne a été mise au point grâce à l'utilisation du glycérol dans le tampon d'extraction. Le Dr Crozier *et al.* ont abordé la voie de dégradation des alcaloïdes dérivés des purines en xanthine ; l'identification de la 7-methylxanthine est confirmée par ces travaux.

Enfin, des bactéries telluriques échantillonnées dans les plantations de caféiers au Brésil ont été criblées pour leur aptitude à dégrader la caféine. L'identification de ces souches de bactéries par les Dr Yano et Mazzafera est en cours (Campinas, Brésil).

A. Charrier

Summary report

Physiology

The presentations concerning the physiological effects of coffee were generally focused in three major areas : (1) brain function and psycho-physiological aspects ; (2) lipid metabolism and effects on the cardiovascular system ; (3) cancer-protecting mechanisms.

Using very sophisticated techniques, Professor Y. Koga showed that coffee aroma increased regional cerebral blood flow in human subjects. Areas on the right side of the brain involved in emotional expression and olfactory input processing were particularly affected. Coffee aroma has a variety of potentially beneficial effects on cognitive function and behavior and may be of value in the treatment of moderate emotional disorders. Professor T. Yamamoto demonstrated that coffee consumption resulted in improved information processing in the brain and an enhancement of a relaxed feeling in subjects who enjoy coffee. These effects are due to a complex interaction of aroma, taste and caffeine, each of which contribute to the overall effect. Dr. M. Lorist examined the effects of caffeine on perception and attention with regard to visual stimuli. These stimuli can be separated into "what" (object characteristics) and "where" (location) aspects. Caffeine was found to specifically improve the processing of the "where" characteristics of visual information.

In a very clear review of the effects of coffee on cardiovascular disease, Professor M. Katan showed that coffee has a negligible effect on blood pressure. The reported cholesterol raising effects of coffee are dependent upon the method of brewing, such that only brews containing the highest levels of lipid produce this effect. The hypercholesterolemic constituent has been identified as the diterpene cafestol which is found mainly in boiled, Middle-East-type and French press coffees. It is present in very low amounts in commonly consumed brews such as filtered and instant coffees. Roasting, decaffeination and brewing time have little influence on cafestol content. Although chronic ingestion of large quantities of coffee brews containing high levels of cafestol may result in an increased likelihood of heart disease, the consumption of coffee in moderate amounts is not associated with adverse effects on the cardiovascular system. Dr. T. Ito showed that ingestion of filtered coffee may protect against cardiovascular disease. Increased HDL-cholesterol in females, a reduced susceptibility of LDL-cholesterol to oxidation and an increase in anti-atherogenic lipoproteins were measured in subjects consuming this type of coffee brew.

There is increasing evidence to indicate that coffee consumption may protect against colorectal cancer. A number of different coffee components may be involved. Dr A. Huggett demonstrated that the coffee diterpenes cafestol and kahweol may alter carcinogen metabolism to prevent the reaction of activated intermediates with DNA, the first step in the cancer process. They can do this by modulating cytochrome P450 enzymes catalyzing carcinogen activation and also by inducing a specific form of glutathione S-transferase which can detoxify the genotoxic intermediates. Dr. T. Tanaka reported that chlorogenic and caffeic acids reduce or prevent tumor formation in the colon, liver and oral cavity in a number of different animal models. Professor V. Nguyen, showed antimutagenic effects of chlorogenic acid as well as of coffee components produced after roasting. An extract of roasted coffee as well as products formed by roasting, chlorogenic acid and trigonelline, were particularly antimutagenic and the mechanism appears to involve a radical scavenging activity.

In a historical review, Professor T. Namba showed that coffee has long been recognised within Asia for its medicinal properties, mainly as a diuretic and stimulant. These effects are attributable to caffeine. Recent studies have shown that a water extract of coffee displays radical scavenging activity and can prevent lipid peroxidation and suppress hepatitis B virus surface antigen. Professor S. Garattini provided a comprehensive review of the health effects of coffee and caffeine. Diterpenes, 5-hydroxytryptamides and purines, particularly caffeine, are the major biologically active components of coffee. The interpretation of health effects of coffee has been complicated by confounding factors such as smoking, so that previous associations of coffee with cardiovascular disease and gastrointestinal tract cancer can now be discredited. It was concluded that the scientific evidence supports that moderate consumption of coffee does not result in adverse health effects.

Posters

The effects of coffee and its components on a variety of potentially important physiological processes were covered in some clearly presented posters.

Antioxidant and anti-cancer effects of coffee were reported by several presenters. The balance between hydrogen peroxide production and radical scavenging effects of coffee was shown by Y. Araki and V. Nguyen to be dependent upon the coffee concentration. The potency of chlorogenic acids and caffeic acid as radical scavengers compared to antioxidant vitamins was demonstrated by H. Morishita and R. Kido. Coffee cherry extract was shown to prevent tumor development in mice by H. Nagasawa, and S. Furusawa showed that caffeine may protect against apoptosis (programmed cell death) by anthracyclines. K. Yagasaki reported that coffee inhibits the action of transforming growth factor-beta. This effect indicates that coffee may have potentially protective effects both against tumor formation and nephritis (kidney damage).

Potentially important effects related to caffeine were presented in other posters. T. Fushiki showed that coffee drinking augments the activity of the autonomic nervous system. N. Sakane showed that caffeine is able to induce thermogenesis resulting in a reduction in body weight and fat in some obese subjects. However, another group of subjects did not respond suggesting that brown adipose tissue may not be activated in this group and that alternative therapies may be required for the treatment of certain obese subjects.

R. Viani, A. Huggett

Chemistry

Antioxidant properties of coffee ingredients are one of the topics of this ASIC conference ; this already has been mentioned in the Physiology part.

Their characterisation and determination were stressed in the Chemistry session as well.

Several phenolic acid isomers and their esters, commonly described as caffeic and chlorogenic acids, have the power to catch - or better scavenge - so called free radicals. Antioxidants have such capbilities as well. Those free radicals - mostly reactive oxygen species - have been suggested as major causes of cancer.

H. SAKURAI and coworkers analysed the free radical suppressing effects of coffee extracts by Electron Spin Resonance Spectroscopy (ESR) ; they determined the superoxide anion scavenging activity (SSA).

In a poster contribution T. NAKAYAMA reported that caffeic acid esters *in vitro* could inhibit superoxide induced DNA breaks in cells ; this is a confirmation of the *in vivo* antioxidant effects of caffeic acid.

H. HIRAZAWA and T. OKADA reported on their experiences using the antioxidant properties of coffee extracts for freshness preservation of foods, like fish. In a comprehensive study R. STADLER, J. RICHOZ and L. FAY investigated the radical scavenging effects of caffeine ; they could give proof that caffeine also has antioxidant properties : its oxidation product could be characterized as being 8-oxocaffeine.

Several papers dealt with the properties and analysis of acids in coffee. V. LELOUP, A. LOUVRIER, and R. LIARDON explored the chemical breakdown mechanism of that part of the chlorogenic acids that is reduced during roasting ; in the first stages of roasting esterification reactions could be characterized.

K. J. BALYAYA and M. N. CLIFFORD in a poster reported on the quantitative analysis of various chlorogenic and caffeic acid isomers in monsooned Arabica and Robusta coffees ; these coffees have a distinguished neutral mellow flavor ; the content of those acids differs significantly from dry and wet processed coffees out of the same region in India.

In another poster F. KUSU, T. FUSE and K. TAKAMURA described the electrochemical determination of lower and higher carbonic acids as well as chlorogenic acids in coffee.

A. BRADBURY and colleagues showed the applicability of modern capillary electrophoresis to the determination of aliphatic acids and phosphoric acid in Arabica and Robusta coffees.

In a plenary paper S. HOMMA presented his comprehensive work on metal chelating effects of coffee ingredients, especially chlorogenic acids and derivatives : he could isolate zinc and iron II complexes after having added the corresponding sulfates and chlorides to coffee solutions. First time isolation of cumaroyl-tryptophan he could report as well. Quantitative evaluation of the results gave no reasons that coffee ingredients could cause iron deficiency reactions in humans.

M. MURATA, H. OKADA and S. HOMMA described the determination of eleven chlorogenic acids and isolation as well as structural elucidation of the new p-coumaroyl - (L) - tryptophan compound in Robusta coffee.

The aroma complex of coffee was thoroughly discussed in two plenary papers where the latest state of the art was considered in a very comprehensive way.

W. GROSCH described the new developments of the 90s with regards to instrumental and combined sensory analysis of the volatile coffee aroma. Nowadays potent odorants in foods like coffee may be screened by sniffing with the nose at the outlet of a gaschromatograph and how by subsequent aroma dilution analysis the sensory significance of such compounds may be determined. He discussed new ways of quantification using deuterated aroma impact compounds ; even very tiny amounts, as those of thiols for example, can be traced by binding them to immobilized organic mercury containing gel columns. A detailed description of the different potent aroma compounds between Arabica and Robusta coffees was given.

The role of the Maillard reaction for the formation of coffee flavors was the theme of G. A. REINECCIUS' plenary paper. In a convincing survey he described the state of the art knowledge on chemical mechanisms of the roast process, which precursors are relevant, the role of the lipids herein and reaction kinectic studies done so far. He also stressed the new glass transition theory that gives some explanations for physical and chemical reactions in dry foods and how the melanoidin forming Maillard reaction may be affected.

D. HINMAN showed the relationship between headspace and bulk concentrations of light aroma volatiles in coffee ; this allows a more precise evaluation of the gaschromatographic headspace measurements.

C. GRETSCH and colleagues in their poster described the determination of partition coefficients of coffee volatiles for the systems air/water, air/coffee oil and oil/water.

Y. S. KO and H. J. BAIK showed in their poster the gaschromatographic headspace analysis of aromas from various coffees.

Y. KAWAKAMI and colleagues in their poster also referred to the determination of coffee aroma volatiles from nine differently roasted coffees ; they used ICP-AES techniques for analysis.

K. J. KIM, J. M. RHO and S. Y. KIM investigated the "milk penetration" effect ; they studied the aroma retention of milk creamers, analysing the headspace aroma over different milk/coffee beverages.

In a poster contribution H. STEINHART, C. GROTZBACH and J. WILKENS investigated the aroma stability of coffee beverages kept at 80 °C for up to 4 hours. They found changes of color, turbidity and headspace volatiles concentration in dependence of the oxygen content.

In their poster E. LUDWIG, N. RACZEK and T. KURZROCK reported on the isolation of proteins from green coffees and on ways how to further characterize the complex structures, obtained by different procedures.

The amino acid pattern of so called steam treated coffees was shown by H. STEINHART and A. LUGER ; such coffees are claimed to have some positive health effects over untreated products ; an anlytical way of distinction for Arabica coffees was offered.

K. HIGUCHI, T. SUZUKI and H. ASHIHARA presented in a poster the detection of the amino acid pipecolic acid besides other free amino acids in developing leaves and unripe fruits of Arabica coffees. It is postulated that this compound be involved in the caffeine biosynthesis process.

Investigations of carbohydrates were reported by S. OESTREICH - JANZEN. She described methods for determining mono-, di-, and trisaccharides in coffee and related products by HPLC methodology.

A. LUGER and H. STEINHART in their poster described the carbohydrate changes that are related to steam treatment of coffees. Oligosaccharides were determined by GC after silylation.

R. M. NOYES showed in his presentation that green yield in instant coffee processing has to be calculated as the product of cleaning-, roasting- extraction- and process-yield. An autoclave extraction device for yield determination was described.

Three papers were given for describing differences for varieties and determination of off flavors respectively adulteration in coffees.

K. SPEER described his method for analysing the Robusta content in coffee blends ; by applying preparative

chromatography isolation techniques he further could detect 12 more fatty acid esters of 16-0-methylcafestol.

W. HOLSCHER and colleagues reported the elucidation of an off flavor that appeared in Kenyan coffees ; it could be identified as the pesticide degradation product TCA, which is known from Rioy Brazil.

D. R. WHITE reported on his experiences discovering adulterations in coffee. Ways were described how to identify such samples for quality control purposes.

Another topic of this ASIC symposium in the Chemistry session was the presentation of sensor systems to fast characterize coffee samples by integral methods.

K. TOKO and colleagues described a taste sensor system, that used lipid bilayers for the characterization of taste sensations like bitter, sour, astringent, etc. Electrodes having up to 8 channels with specifically different but well reproducible tuned organic lipid bilayers give signals if dipped into the coffee beverage. The differentiation between various coffees thus could be demonstrated.

H. KOMAI and colleagues reported on more precise investigations with such sensor systems.

A general comment here : If reproducibility, repeatability, discrimination power and shelf life further can be improved, such instruments could be interesting tools for future quality control measurements.

Another type of sensor was presented by T. FUKUNAGA and colleagues.

They described the experiences with an "electronic nose" detector, determining the smell of coffee. Based on metaloxide sensors a characterization of critical off flavors — like the medicinal one known from Rioy Brazil - could be determined.

B. GUYOT and colleagues studied 3 ways of green Robusta coffee processing : dry - wet - and wet processing with added enzymes. Chemical tests yielded for enzyme/wet processed beans higher sucrose and elevated chlorogenic acid compounds. Sensory results showed similarity with wet processed beans.

O. G. Vitzthum

Food engineering

In this Food Engineering review the themes will be treated in the ranking order of roasting/coffee stabilization/soluble coffee/ drying/ espresso/green coffee.

G. LÜHRS presented a batch roaster which due to its attached cooler system in combination with a catalytic burner guarantees a reduction of smell and undesirable emission gases to the environment.

In a comprehensive study T. KINO and T. TAKAGI presented the results of infrared roasting vs conventional roasting ; evaluation was carried out by measuring the moisture during heating, doing ESR and NMR measurements for radicals and free water, analysing internal vs external bean color, determining acids by HPLC and volatile aroma compounds by GC as well as doing quality evaluations by smell and taste sensors.

V. D. NAGARAJU, K. RAMALAXMI, and P. SRINIVASA RAO proposed a spouted bed technology roaster for processing of low grade Indian coffee (black, brown, bits).

Oxygen adsorber as T. MATSUSHIMA, N. OGURO and S. ICHIYANAGI reported are well suitable to prolong the shelf life of roast and ground packages ; additional absorption of carbon dioxide helps in stabilizing the product.

C. SEVERINI and colleagues investigated a stabilization treatment of coffee brews by applying high pressures of up to 7.000 bar. Whereas biological stability was reached at 40° centigrade, the brew stability could not be improved.

M. GRØNLUND presented new developments from the side of an equipment vendor for the processing of instant coffees with improved quality ; this could be achieved by prewetting the ground coffee prior to extraction and applying shorter extraction times.

By model studies S. YAMAMOTO described the single processes that occur during drying of solutions with special emphasis to aroma and enzyme retention in sugar containing liquids.

N. OHTANI and colleagues studied the spray-drying process of instant coffees at low temperatures. The aroma retention by different concentration processes was determined.

In an extensive study O. FOND investigated the fundamental dynamics of espresso type extraction, emphasizing the influence of the composition of extract water and coffee origins for the compaction process.

H. KUMORI and colleagues reported in a poster on the degradation of soluble and insoluble polysaccharides in coffee and spent grounds by *Trichoderma viride* No. 8. The degradation leading to mono- and oligosaccharides was about 90 % for Uganda coffees.

Z. R. LOPEZ, J. E. SANCHEZ and R. BELLO reported on the utilisation of waste water from wet coffee processing for cultivation of edible mushrooms.

H. C. MENEZES and L. R. ROMEIRO investigated the fact of growth impairment if coffee pulp is added to animal feed in higher level than 10 %.

Fermentation of coffee pulp with *Lactobacillus plantarum* and addition of 1 % sucrose could elevate the feed level to 20 %.

O. G. Vitzthum

Agronomy

The three general talks, 25 oral presentations, and 29 posters of the Agronomy session of the 16th Colloquium of the ASIC provided an overview of advances in research into improvement in coffee growing in different parts of the world.

In his introductory talk, Dr. Muller shared his thoughts on the future of coffee growing. He analysed the causes of the drop in production, in particular of African *Robusta*, due to the socioeconomic difficulties of recent years. His message of "improve production over limited areas under conditions more favourable to growers" is based on scientific and technological transfer from the ASIC to the coffee-producing countries.

Dr Opilé (Kenya) stressed the major role of coffee in the economy and the advances made by *Arabica* and *Robusta* producers in Africa. At the same time, the quantity and quality of this production have declined sharply. In 1993, the African countries created a research network (RECA) under the auspices of the Inter-African Coffee Organisation (IACO) to foster cooperation in the control of anthracnosis of coffee beans (CBD), improvement in quality of *Robusta*, and the conservation of genetic resources. With the support of the International Plant Genetic Resources Institute (IPGRI), a five-year plan was set up to improve the management and use of the three major coffee-tree collections of Côte d'Ivoire, Ethiopia and Madagascar.

Dr. Saragih (Bogor, Indonesia) described coffee production in Indonesia, one of the few countries where it is growing rapidly. Production is mainly based on *Robusta* grown by small producers. The traditional system of growing coffee-trees in orchards alongside food crops and fruit trees explains the low productivity and the ageing of

the coffee-trees. Parasite pressure seems low under these conditions. Production can only be improved by means of suitable agricultural practices, with the development of infrastructures, cooperatives, credit, and techniques favouring intensification and improvement in quality.

Biotechnologies applied to coffee-trees are developing in several directions :

1 Dr. Yasuda *et al.* (Kobe, Japan) reviewed the different methods of *in vitro* vegetative multiplication - somatic embryogenesis, protoplasts. The semi-industrial development of multiplication by micropropagation and somatic embryos has advanced significantly due to culture in liquid medium with temporary immersion according to a process developed by CIRAD (France). This is currently being tested in a laboratory set up in Kawanda (Uganda). Three posters dealing with these approaches described the effects of hormones (Hatanaka and Tahara *et al.*, Japan) and of amino acids (Nishibata *et al.*, Japan).

2 The conditions for storage of somatic embryos by dehydration, and in liquid nitrogen, were reported by Dr. Deshayes *et al.* (Francereco, France). Many applications were proposed : storage for more than one month for transfer and direct development of embryos into plantlets, long-term storage (cryopreservation) of original embryogenic strains.

3 Geneticists at ORSTOM (Montpellier, France) have studied the genetic diversity of coffee-trees using RFLP and RAPD markers, and DNA sequencing. The Typica and Bourbon varieties of *C. arabica* have been distinguished, as have the subspontaneous strains of Ethiopia. The progenitors of the allotetraploid species *C. arabica* have been specified : maternal chloroplastic genome of *C. eugenioides*, Moloundou ; paternal ribosomal DNA of *C. congensis*.

4 Some complementary information reported in posters related to production of haploids by Dufour (CATIE, Costa Rica) and changes in peroxidases during microsporogenesis (Guedes, CIFC, Portugal). The genetic transformation of the coffee-tree by *Agrobacterium rhizogenes* has been successfully applied by Dr. Sugiyama (Pokka Co., Japan).

The oral presentations on varietal creation reflect the importance of the selection of coffee for the quality of the drink. Montagnon *et al.* (CIRAD, France) have demonstrated the impact of genetic differences between *Robusta* clones in terms of the soil and the treatment of coffee in Côte d'Ivoire. Dr. Cadena *et al.* (CENICAFE, Colombia) reported the results of national and international tastings of the Colombia variety, whose physical characteristics have been improved (70 % of high quality coffee) and whose organoleptic qualities are comparable to those of the common *Arabica* varieties.

Roche (PIONEER Co., Hawaii) described industrial coffee growing based on the choice of *C. arabica* varieties that ripen at the same time, thus allowing mechanical harvesting, a better quality drink, and good agricultural results. Dr. Mawardi (Jember, Indonesia) discussed the qualitative improvement of *C. arabica* in different localities east of Java on the basis of anomalies in beans clearly affected by the interaction between the variety and the environment.

Attempts have also been made to improve *Robusta* coffee by the creation of *Arabusta* hybrids. Yapo (IDEFOR, Côte d'Ivoire) indicated the limited amelioration in their fertility through generations of selection, and a marked effect of the low-altitude climatic conditions.

In their poster, Bouharmont and Montagnon (CIRAD) described an extensive study of the collection of *C. arabica* from Cameroon and evaluated the variation in botanical and agronomic characteristics.

Genetic resistance to diseases and parasites remains a major concern for selectors. Santa Ram (CCRI, India) proposed a new hypothesis to explain the genetic determinism of the resistance to *Hemileia vastatrix*. Bertrand *et al.* (PROMECAFE - CIRAD, Costa Rica) have selected for resistance to the root-knot nematodes (meloidogyne) of Central America : short-term use of a controlled cross of *C. canephora* as root-stocks for *C. arabica* varieties, long-term creation of tolerant *C. arabica* varieties.

Because of the specificity of these resistances, the differences in reaction of the nematode strains should be increased by inoculation, and understanding of pathotypes needs to be improved. The biochemical and biological characterisation of the different populations of meloidogynes parasites of coffee-trees in Central America is under way (poster of Hernandez *et al.*, CIRAD-ORSTOM).

Dr. Kanechi *et al.* (Kobe, Japan) described the physiology of coffee-trees subject to water stress studied in terms of leaf transpiration and photosynthesis in shaded and exposed, young potted *C. arabica* plants. The photosynthesis of the three cultivated species decreased, as did the stomatic conductance of the leaves subjected to water stress.

Disease and parasite control was examined in various reports, particularly in terms of the major parasite the scolytoid bug, which affects all producing regions. Cilas *et al.* (CIRAD) presented sampling methods suitable for evaluation of the *Hypothenemus hampei* and decisions regarding treatments. Routine stratified sampling to detect infestation foci is practised with observers trained in Central America. A similar study of *Antestiopsis orbitalis* in Burundi, which causes bacterial infections and unpleasant taste, has led Cilas *et al.* (CIRAD) to different practices by simple tree-to-tree testing.

Decazy *et al.* (CIRAD) described the agrieconomic impact and acceptance among growers in Guatemala of the biological control of *Hypothenemus hampei* by the parasite *Cephalonomia stephanoderes*. They compared biological to chemical control, and considered the relative labour requirements.

Bustillo *et al.* described the large programme of biological control undertaken by CENICAFE (Colombia) based on different mass-produced entomopathogens (*Cephalonomia stephanoderes*) and mycopathogens (*Beauveria*). They favour the integration of this biological approach into agronomic practices and a reduction in the use of chemical treatments.

Pellegrin *et al.* (ORSTOM) described the pathology of *C. arabica* in plantations in New Caledonia as a function of environmental factors. Epidemiological modelling was used in an attempt to predict the infections for rust, anthracnosis of the branches, and *Cercospora*. A poster (Kohler, ORSTOM) described the study of the diversity of parasitic fungi in New Caledonia using enzymatic markers, RAPD, and strain compatibility.

Other posters completed the review of CBD and rust :

— genetic diversity and variability of the pathogenic potency of *Colletotrichum coffeanum* (Bieysse *et al.*, CIRAD),

— methods of low-pressure chemical treatment of CBD (Derso, Ethiopia),

— biochemical characterisation of 16 strains of *Hemilea vastatrix* and the influence of the caffeine content on infection (Guedes, CIFC, Portugal).

Several examples were given of the agronomic systems and practices employed in different coffee-producing countries. Dr Wickramasinghe reported the performances of the IMY variety of *C. canephora* grown in Sri Lanka under different conditions (environment, culture, size of plantation). The conditions of the rising production of

Robusta in Vietnam (aim 200,000 tonnes/year) were summarised by Michel and Lebailly (Belgium). It is necessary to overcome rapidly the limitations of this technology transfer, in particular the preparation of quality coffees, mechanisation and commercialisation.

Various posters describe experiences with mineral and organic fertilisation :

— in Indonesia (Dr. Abdoellah - Dr. Winaryo) : increasing use of organic manuring,

— in Ethiopia (Dr. Dubale) : the phosphate requirements of young plants,

— in Côte d'Ivoire (Dr. Koffi NGoran) : the potassium requirements of *Robusta* coffee-trees,

— in Papua New Guinea (Dr. Kiara) : the combination of planting density, the use of shading, and feritilisation,

— in Japan (Dr. Kito) : the use of coffee residues in compost for weed control.

Post-harvest treatment is a major concern in production of quality coffee. Dr. Woelore (Ethiopia) described the conditions of warehousing and parchment Arabica coffee storage at the farm, and by companies. This storage should be of limited duration and the coffee should be transferred to central warehouses. Cortez and Menezes (Campinas, Brazil) presented a method of coffee treatment which significantly enhances its organoleptic characteristics compared with the dry process, and the value of applying particular microbial strains on cherries before harvest.

The conditions of fermentation on drying of *Arabica* coffee beans grown in Java were described in a poster (Wahyudi and Ismayadi, Indonesia). The optimal conditions for roasting were developed in Côte d'Ivoire for the *Robusta* and *Arabusta* coffees (Yate, IDEFOR).

Caffeine biosynthesis was examined in a collaborative research effort of the Universities of Ochanomizu (Japan) and Glasgow (U. K.). Using *C. arabica* leaves, Ashihara *et al.* demonstrated a preferred pathway for *in vivo* synthesis of caffeine from theobromine. Gillies *et al.* studied the stabilisation of a key enzyme in caffeine biosynthesis - N - methyltransferase - which was purified by column chromatography, using glycerol in the extraction buffer. Crozier *et al.* studied the degradation into xanthine of alkaloids derived from purines, and confirmed the identification of 7-methylxanthine.

Lastly, bacteria sampled in the soil of coffee-tree plantations in Brazil were screened for their ability to degrade caffeine. Drs. Yano and Mazzafera (Campinas, Brazil) are currently identifying these bacterial strains.

A. Charrier
(translation)

CLOSING SPEECH
by Tatsushi Ueshima, president of ASIC

Ladies and Gentlemen,

It is now time to officially close the ASIC'95 conference. I would like to express my sincere gratitude to all of you for your cooperation and support. We have achieved some important results.

The scientific findings have been summarized by Dr. Illy, the First Vice President, Dr. Viani, the Administrative Secretary, Dr. Vitzthum, the Scientific Secretary and by Dr. Charrier, of the ASIC Board. For me, the most important point is that this was the first ASIC conference to be held in Asia. Asian green coffee production and coffee consumption have recently sky-rocketed. The Asia region certainly merits attention as a region of great potential.

Although the International Coffee Agreement, that became effective last September, was passed without the economic clause, the Japanese coffee industry strongly desires to import green coffee at a reasonable, and reasonably stable price.

Furthermore, although the consumer countries and the producer countries have different positions, a mechanism that allows people in the coffee business to share information and ideas, and to reach a consensus is especially vital for the Asia region. ASIC provides a very responsible scientific forum for coffee researchers and others interested in the science of coffee. Holding an ASIC conference in Asia is a good first step towards developing such a mechanism, for ASIC has a major role to play in it.

The ASIC Kyoto conference is drawing to an end. I would like to urge you, the world's coffee technologists, to work even harder to elevate science to a higher plane in order to ensure rising prosperity for the international coffee industry, from the rural producer to the urban consumer.

In your conference case there was a short paper (it is yellow) explaining how ASIC survives. May I encourage you to read it and respond positively.

Last, let me repeat that this conference has been a success thanks to the members of the ASIC Board, the speakers, the participants, the ASIC Secretariat, especially one person who has been efficiently contributing for a very long time -, - I am referring to Miss Collot - -, thanks also to the ASIC'95 organising committee, Kyodo Senden Agency, Meiko service, ISS the Inter-language Service System and the excellent interpreters. My gratitude also goes to the Kyoto Grand Hotel for these lovely premises and for the delicious food. It is my pleasure to repeat this expression of gratitude to you all ; thank you for making ASIC'95 such a wonderful success. I wish you a good trip home and hope very much to see you again, two years from now, in Kenya.

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