P209535

ISSN-0771-3312

TROPICULTURA

2011 Vol. 29 N°4

Trimestriel (octobre- novembre- décembre)
Driemaandelijks (oktober- november- december)
Se publica po año (octubre- noviembre- diciembre)

Le lait des dromadaires est une source importante de nourriture pour les éleveurs turkanas au Kenya. Dans cette région aride, ces animaux peuvent rester plusieurs jours sans boire. © *Roger Job, 2009 (Vétérinaires Sans Frontières Belgium).*

Editeur responsable/Verantwoordelijke uitgever: J. Vercruysse
11, Rue d'Egmontstraat
1000 Bruxelles/Brussel

Avec les soutiens
de la Direction Générale de la Coopération au Développement (DGD) www.dg-d.be,
du Service public Fédéral Affaires étrangères, Commerce extérieur et Coopération au Développement, www.diplomatie.belgium.be
de l'Académie Royale des Sciences d'Outre-mer ARSOM, www.kaowarsom.be,
du Conseil interuniversitaire de la Communauté française de Belgique CIUF, www.ciuf.be,
du Vlaamse Interuniversitaire Raad VLIR, www.vlir.be, et de la Région Bruxelles Capitale

Met de steun van
De Directie-Generaal Ontwikkelingssamenwerking (DGD), www.dg-d.be, de Federale Overheidsdienst Buitenlandse Zaken,
Buitenlandse Handel en Ontwikkelingssamenwerking, www.diplomatie.belgium.be,
de Koninklijke Academie voor Overzeese Wetenschappen www.kaowarsom.be, de "Conseil interuniversitaire de la Communauté française
de Belgique (CIUF)", www.ciuf.be,
de Vlaamse Interuniversitaire Raad (VLIR), www.vlir.be, en van het Brusselse Gewest

BUREAU DE DEPOT- AFGIFTEKANTOOR
BRUXELLES X/ BRUSSEL X

**Avec le soutien de
LA COOPÉRATION
BELGE AU DÉVELOPPEMENT .be**

KAOW · ARSOM

**Met de steun van
DE BELGISCHE
ONTWIKKELINGSSAMENWERKING .be**

SOMMAIRE / INHOUD / SUMARIO

ARTICLES ORIGINAUX OORSPRONKELIJKE ARTIKELS

ORIGINAL ARTICLES ARTICULOS ORIGINALES

Qualité hygiénique du lait cru de vache dans les différents élevages de la Wilaya de Tiaret (Algérie)

Kheira Ghazi [1*] & A. Niar[1]

Keywords: Cow- Bacteria- Milk- Mastitis- Quality- Algeria

Résumé

Le lait, destiné à l'alimentation humaine, est le produit intégral de la traite totale et ininterrompue d'une femelle laitière bien portante, bien nourrie et non surmenée. Contaminé, il peut être un vecteur de transmission de germes pathogènes à l'homme et peut présenter un risque pour la santé humaine. L'évaluation de la qualité sanitaire et hygiénique du lait cru destiné à la consommation ou à la transformation est donc essentielle pour la protection du consommateur. Le lait est à la fois un aliment traditionnel et une boisson d'un grand intérêt nutritionnel, car il représente un aliment de base presque complet. Les microorganismes trouvent dans le lait un substrat idéal pour leur développement. La présence de nombreux facteurs de croissance permettra de satisfaire de nombreuses espèces microbiennes exigeantes et difficiles à cultiver dans un milieu moins complet. La présente étude porte sur l'évaluation de la qualité microbiologique de 155 échantillons de lait de mélange. Les résultats sont les suivants:-Une flore aérobie mésophile totale (FAMT) > 10^5 UFC/ml dans 81,2% des prélèvements. -Des coliformes fécaux présents dans 18,06% des prélèvements. -Des Staphylocoques aureus *et des streptocoques fécaux présents dans respectivement 81,93% et 80,64% des échantillons.*

Summary

Hygienic Quality of Cow Milk, in Various Bovine Breeds of Tiaret Area (Algeria)

The milk for human consumption is the full product of the total and uninterrupted milking of a female dairy healthy, well fed and not overworked. Contaminated, it may be a vector of transmission of pathogens to humans and may pose a risk to human health. Assessing the quality of health and hygiene of raw milk for consumption or processing is essential for consumer protection. It is both food and drink of great nutritional value, and is the traditional food of choice, providing for both humans and mammals a basic aliment almost complete. Microorganisms are also found in milk, which is considered as an ideal substrate for their development. The presence of many growth factors within this aliment will satisfy many of demanding microbial species, which are so difficult to grow in a less complete media. This study focuses on the evaluation of the microbiological quality of raw milk, results of a bacteriological analysis of 155 samples of bulk milk are:- A FAMT> 10^5 CFU/ml was found in 81.2% of our samples; - Fecal coli form bacteria are present within 18.06% of our samples; - Staphylococcus aureus *and fecal Streptococci are present in 81.93 and 80.64% respectively.*

Introduction

Dans les pays à élevage développé, les aspects de qualité sont devenus prépondérants.

Le producteur doit fournir à l'industrie un produit dont la composition est optimale pour la fabrication des produits recherchés par le consommateur.

Selon les prévisions de 2007, l'Algérie comme pays consommateur du lait, présente des besoins en lait de l'ordre de 3,2 milliards de litres par année, mais que 2 milliards de litres seulement sont produits localement (8). Le manque est donc énorme; ainsi, notre pays a adopté une politique d'importation des vaches laitières, mais celles-ci ne parviennent pas à donner les résultats escomptés. Ceci est sans doute dû à un ensemble de facteurs tels que les mauvaises conditions d'élevage et particulièrement une alimentation inadéquate en apports énergétiques, ainsi que la méconnaissance de sa conduite de la part de nos éleveurs.

Plusieurs facteurs de risques de contamination du lait aux différents stades de sa production entrent en jeu, ce qui nous a poussé à réaliser ce travail, dont l'objectif principal a été la mise en évidence de la qualité hygiénique et sanitaire du lait cru de vaches de la région de Tiaret.

[1]Institut des Sciences Vétérinaires, Université Ibn –Khaldoun de Tiaret , B.P. 72, Tiaret 14000, Algérie.
Reçu le 25.05.10 et accepté pour publication le 30.08.11

Matériel et méthodes

Cent cinquante-cinq échantillons de lait cru de vache (lait de mélange) ont été collectés à partir de différents élevages répartis dans la Wilaya de Tiaret.

Une fois la traite terminée le lait récupéré est stocké dans des tanks réfrigérés, milieu duquel ont été pris les échantillons à analyser dans des tubes à essai qui portaient le numéro de l'exploitation puis ils étaient acheminés vers le laboratoire dans des glacières.

Il faut noter que certains éleveurs utilisaient la traite mécanique.

Chaque échantillon a subi les analyses suivantes:

Le dénombrement de la flore aérobie mésophile totale (FAMT)

Ce dénombrement reflète la qualité microbiologique générale du produit.

-A partir de dilutions 10^{-1}, 10^{-2}, 10^{-3}, porter aseptiquement 1 ml de chaque dilution dans une boîte de Pétri vide.

-Ajouter 15 ml de gélose PCA (Plate Count Agar) fondue et refroidie à 45 ± 1 °C. Pour homogénéiser l'inoculum à la gélose, faire des mouvements circulaires et de va-et-vient. Laisser solidifier, puis incuber à 30 °C pendant 72 h.

-Le dénombrement des boîtes présentant des microorganismes à la dilution 10^{-3}, se fait sur la base de la norme fixée par la législation (2).

La recherche des coliformes fécaux

Verser 12 cm environ du milieu VRBL (Violet Red Bile Lactose Agar) en surfusion; mélanger et laisser prendre en masse. Recouvrir de 4 cm de milieu, incuber 24 heures à 44 °C.

Toutes les colonies rouges (lactose+) d'un diamètre de 0,5 mm minimum apparues en 24 heures sont considérées comme étant des coliformes fécaux.

Recherche de Staphylococcus aureus

Le milieu de Baird Parker solide (après l'avoir fondu) est coulé dans des boîtes de Pétri. On étale 0,1 ml de l'inoculum dilué, sur toute la surface de la boîte. L'incubation se fait pendant 24 à 48 heures à 37 °C.

Les colonies de *Staphylocoques aureus* apparaissent, noires, brillantes, convexes et entourées d'un halo clair d'environ 2 à 5 mm de diamètre (2)

Recherche des streptocoques fécaux

Introduire aseptiquement dans cinq tubes de milieu de Rothe, 1 ml de lait non dilué. Réaliser également des dilutions au 10^{-1}, 10^{-2}, et 10^{-3} et placer 1 ml de chaque dilution dans cinq tubes du milieu de Rothe.

L'incubation s'effectue à l'étuve à 37 °C pendant 48 heures; les contenus des tubes positifs, c'est-à-dire présentant un trouble, sont ensuite repiqués sur milieu Litsky avec une anse de platine et soumis à une incubation à 37 °C pendant 48 heures.

L'apparition d'un trouble homogène et celle d'une pastille violette au fond des tubes signent la présence de streptocoques fécaux.

Résultats

Les résultats ont révélé qu'au maximum 81,93% des échantillons sont contaminés avec un FAMT pour 81,2% (Tableau 1).

Il faut noter que plus de 80% ne présentaient pas du tout de coliformes. En revanche plus de 80% contenaient des streptocoques fécaux (125 échantillons sur 155).

En rapport avec les bactéries présumées pathogènes, 28 échantillons seulement sur les 155 analysés, soit 18% ne présentaient pas de contaminations par les staphylocoques.

La teneur moyenne pour les 127 échantillons contaminés a été de 2.10^2 pour les streptocoques fécaux.

Globalement les valeurs estimées sont représentées par le tableau 2.

Discussion -conclusion

La mise en évidence de la qualité du lait de mélange a permis de prouver que le produit mis sur le marché ou entre les mains des industriels est fortement contaminé. Certains prélèvements peuvent même

Tableau 2
Taux de contaminations des échantillons de lait analysés

Bactéries	Minimum	Moyenne	Maximum
Coliformes fécaux	$1,1.10^3$	1,7.10	$1,3.10^5$
Staphylocoques aureus	18	$2,10^2$	$1,7.10^3$

Tableau 1
Fréquences des contaminations microbiennes des échantillons

Bactéries	Flore aérobie mésophile totale	Coliformes fécaux	*Staphylocoques aureus*	Streptocoques fécaux
Nombre de cas positifs	126	28	127	125
Pourcentage	81,2	18,06	81,93	80,64

contenir une association de germes, ce qui a été déjà rapporté par Bind (6).

La totalité des échantillons ne répond pas à la norme recommandée dans ce domaine, ce qui signe des mauvaises conditions d'hygiène entre le moment de la traite et celui de la réception des échantillons par le laboratoire.

La qualité microbiologique du lait est importante pour sa conservation voire sa transformation (14).

La contamination par la FAMT est très importante car 81,2% des laits analysés montrent une flore supérieure à 10^5 UFC/ml. Cette situation est très inquiétante comparativement à celle rapportée à New York par Boor *et al*. (7) et en Bretagne par Raynaud (18) ou seulement 5% et 2% respectivement des laits des élevages comportaient une flore supérieure à 10^5 UFC/ml. Baazize (5) a rapporté, en Algérie une contamination de l'ordre de 91,78%.

La contamination des laits par une FAMT >$2,10^6$ UFC/ml a été rapportée par :

- Arimi *et al*. (4), au Kenya, où ils ont observé des taux de 86% et 88% à Nairobi et Nakuru, respectivement.
- Mwangi *et al.* (19), eux aussi, au taux de 82% dans ce même pays.

Les travaux de Kashifa *et al.* (18) montrent qu'à Faisalabad au Pakistan, seulement 24% des échantillons de lait présentent une flore <10^5 UFC/ml.

La contamination en coliformes fécaux est nettement moindre que celle en streptocoques fécaux, ceci est peut être dû au fait que les étables avec mécanisation de la traite et lavage systématique des mamelles en utilisant une vaisselle en métal présentent des faibles taux des coliformes fécaux, Les coliformes sont des témoins de mauvaises conditions hygiéniques pendant ou après la transformation du produit. Nos résultats (18,06%) pour les coliformes fécaux sont similaires à ceux rapportés par Baazize (5) qui sont de l'ordre de 17,80%. Ceci est purement la résultante d'une situation de négligence des plus simples règles d'hygiène dans certaines exploitations tel que: le lavage du pis avant et après la traite. La présence de coliformes fécaux signe le plus souvent une contamination exogène d'origine fécale. La traite manuelle augmente les possibilités de contamination du lait, en accroissant la surface de contact entre le lait et les microorganismes du milieu ambiant, surtout lorsque que ce dernier est souillé. Ngabet (20) dans son étude trouve que 57% des laits (lait caillé artisanal), au Cameroun sont contaminés par les coliformes. Dieng (11) ne trouve que 19% d'échantillons contaminés par les coliformes.

La présence de germes considérés comme pathogènes est probablement dû à la mauvaise qualité hygiénique des récipients utilisés dans la filière (3, 13).

La contamination du lait devient un problème majeur de la santé publique surtout avec la présence de *Staphylococcus aureus* qui est responsable des intoxications alimentaires.

La contamination par *Staphylococcus aureus* au taux de 81,93% des échantillons de lait est inquiétante; ces résultats se rapprochant de ceux publiés par Baazize (5).

Ce germe pathogène constitue un risque réel pour la santé publique dans les produits transformés comme il peut produire, dans certaines conditions, des entérotoxines thermostables qui peuvent résister aux traitements thermiques (3).

La contamination des laits de mélange par *Staphylococcus aureus*:

- D'une manière qualitative, de l'ordre de 12%, 62%, et 93,3% ont été rapportées respectivement par plusieurs travaux (1, 10, 18).
- D'une manière quantitative, avec un taux de 60% pour un dénombrement moyen de 12.10^3 UFC/ml, résultat rapporté par Fook Yee Chye *et al.* (12), et 93% pour une flore inférieur ou égal à 5.10^2 UFC/ml et 7% pour supérieure à 5.10^2 UFC/ml, résultat rapporté par De Reu *et al*. (10).

La présence des streptocoques dans le lait au taux de 80,64% semble être normale selon Baazize (5) car ce taux, quoique considérable, ne reflète que les mauvaises conditions d'hygiène des exploitations. Baazize (5) a rapporté un taux de 91,09%. Les entérocoques sont très répandus dans le milieu environnemental de l'animal mais ne sont que peu ou pas pathogènes. De ce fait, ils ne figurent pas parmi les critères retenus par la législation des laits crus.

La grande variabilité de la contamination des échantillons du lait dévoile une situation alarmante de la qualité de ce produit, au niveau de cette qualité hygiénique, tous les échantillons peuvent être qualifiés de mauvais car ils dépassent de loin la norme recommandée par le journal officiel (17) concernant les critères microbiologiques des laits et des produits laitiers.

Globalement la présence de cette diversité de flore, quelle soit fécale ou pathogène, n'est que le résultat logique d'un mauvais encadrement de nos éleveurs par les vétérinaires, l'absence des mesures d'hygiène, ainsi que le non-respect et la méconnaissance des conditions d'élevage, en particulier celles liées à la propreté des animaux et leur environnement et bien sûr les conditions de sécurité pour le stockage et la livraison de lait à mettre entre les mains du consommateur un produit de meilleure valeur nutritionnelle. Pour sortir du tunnel, nous proposons la mise en place de formations à destination des éleveurs, des convoyeurs et même des industriels, en vue d'améliorer l'hygiène du lait.

Références bibliographiques

1. Adesyun A.A., 1994, Bacteriological quality and associated public health risk of pre-processed bovine milk in Trinidad.
2. Afnor, 1980**,** Association française de normalisation, lait et produits laitiers, méthodes d'analyse.
3. Ashnafi M., 1996, Effect of container smoking and incubation temperature on the microbiological and ergo a traditional Ethiopian sour milk. International Dairy J., 6 pp. 94- Arimi S.M, Omare A.O, Dermot J.J, 2000, 5-104.
4. Arimi S.M, Omare A.O. & Dermot.J.J., 2000, Risk of infection from *E.Coli* O157 H7 through informally marketed raw milk in Kenya. Paper prepared for oral presentation at the 3 All Africa Conference on animal agriculture.
5. Baazize D., 2005, Qualité hygiénique et sanitaire du lait cru de vache. Mémoire de Magistère en hygiène et qualité du lait, Université Saad Dahleb de Blida (Algérie).
6. Bind J.L., Leplarte J. & Poutrel B., 1980, Les mammites: l'échantillon et son exploitation; mises aux points techniques, rôle du praticien et du laboratoire, Bull GTV. 1727.
7. Boor K.J., Brown D.P, Murphy S.C., Koslowski S.M. & Bandlar D.K., 1998**,** Microbiological and chemical band quality of raw milk in New York state. J. Dairy Sci. 81, 1743-1748.
8. Boudedja N., 2008, La production laitière soumise à de nombreux aléas. Journal El Watan. http://www.elwatan.com
9. Desmasures N., Bazin F. & Gueguen M., 1997, Microbiological composition of raw milk from selected farms the camembert region, J. Appl. Microbiol. V.83, n°**1**, 53-58.
10. De Reu K., Grijspeerdt K. & Herman L., 2004, A belgian survey of hygiene indicator bacteria and pathogenic bacteria in raw milk and direct marketing of raw milk farm products, J. of Food Safety, 24, 17-36.
11. Dieng M., 2001, Contribution à l'étude de la qualité microbiologique des laits caillés industriels commercialisés sur le marché Dakarois Th. Méd. Vét., Dakar, n°10, 91 p.
12. Fook yee, Amminah Abdullah & Mohd Khan Ayub., 2004, Microbiological quality and safety of raw milk in Malaysia, Food microbiology, **V21**, issue 5, 535-541.
13. Godefay B. & Molla B., 2000, Bacteriological quality of raw cow's milk from four dairy farms and a milk collection centre in and a round Addis Ababa. Berliner und Munchener Tierarztliche Wochenschrift. 113, 276-278. Storage conditions on the composition of raw milk. International Dairy J., S, 211-223.
14. Guinot Thomas P., Ammoury M. & Laurent F., 1995, Effects of storage conditions on the composition of raw milk. Int. Dairy J., 5, 211-223.
15. Guiraud J.P**.,** 1998**,** Microbiologie alimentaire. Paris: Dunod
16. Jayarao B.M. & Henning D.R., 2000, Prevalence of food borne pathogens in bulk tank milk, J. Dairy Sci. **84**, 10, 2157-62.
17. Journal officiel de la république algérienne N°35. 1998, Arrêté interministériel du 27 mai 1998.
18. Kashifa K., Ashfaque M., Hussain I. & Akhtar M., 2001, Bacteriological studies on raw milk supplied to Faisalabad city during summer months, Pakistan Vet. J. **21**, 2, 77-80.
19. Mwangi A., Kang Ethe E.K. & Omore A.O., 2000, Assurance of marketed milk quality in Kenya, Paper presented at the faculty of veterinary medicine Biennial Scientific Conference, University of Nairobi.
20**.** Ngabet Njassap V.H., 2001, Contribution à l'étude de la qualité microbiologique du lait fermenté « KOSSAM » commercialisé dans les rues de Yaoundé, Cameroun. Th. Méd. Vét., Dakar, n°11, 69 p.
21. Raynaut S., 2005, Etude sur la contamination du lait par les bactéries coliformes en Bretagne, Rapport final, Institut d'élevage.

Kheira Ghazi, Algérienne, Doctorat en Biologie de la reproduction, Maître de Conférences, Enseignante à l' Institut des Sciences Vétérinaires, Université Ibn-Khaldoun de Tiaret, B.P. 72, Tiaret 14000, Algérie.
A. Niar, Algérien, Professeur en Reproduction animale, Université Ibn-Khaldoun de Tiaret, B.P. 72, Tiaret 14000, Algérie.

Effet direct et résiduel de différents niveaux de fertilisation azotée sur la croissance et le rendement de *Brachiaria ruziziensis* à différents stades phénologiques

F. Tendonkeng[1*], B. Boukila[2], T.E. Pamo[1], A.V. Mboko[2] & N.E.F. Matumuini[2]

Keywords**:** *B. ruziziensis*- Height- Diameter- Nitrogen fertilization- Biomass

Résumé

L'effet direct et résiduel de différents niveaux de fertilisation azotée sur la croissance et la production de biomasse de Brachiaria ruziziensisa *été évalué à différents stades phénologiques à la Ferme d'Application et de Recherche de l'Université de Dschang en 2008 et 2009. Un dispositif factoriel comparant six doses d'azote (0; 50; 100; 150; 200 et 250 kg N/ha) et trois stades phénologiques (montaison, floraison et après grenaison) sur des parcelles de 8 m² (4 x 2 m) en quatre répétitions, soit un total de 72 parcelles expérimentales, a été utilisé. Aucune fertilisation n'a été apportée la deuxième année. A chaque stade phénologique au cours de la première et de la deuxième année, 120 plantes ont été prélevées par traitement pour les mesures des hauteurs et des diamètres. L'évaluation de la biomasse des tiges, des feuilles et de la plante entière s'est faite sur chaque parcelle en fonction du niveau de fertilisation azotée et du stade phénologique la première et la deuxième année. Les résultats obtenus ont montré que la fertilisation azotée et le stade phénologique ont influencé de manière significative (P< 0,05) la taille et le diamètre de* B. ruziziensis *lors de la première et de la deuxième année de fauche. Indépendamment du stade phénologique, la taille et le diamètre les plus élevés ont été obtenus la première année avec la dose de 200 kg N/ha alors qu'en deuxième année, ils ont été observés dans les parcelles ayant reçu la dose de 250 kg N/ha. Indépendamment de la fertilisation et de l'année de fauche, la biomasse des tiges et des plantes entières de* B. ruziziensis *a significativement (p< 0,05) augmenté avec le stade phénologique. La biomasse des feuilles la plus élevée a été obtenue à la floraison (8,42 ± 1,23 t MS/ha et 11,34 ± 0,32 t MS/ha respectivement la première et la deuxième année). Les résultats obtenus montrent que dans les conditions de réalisation de l'essai, l'effet direct de la fertilisation à la dose de 200 kg N/ha et l'effet résiduel de la fertilisation à la dose de 250 kg N/ha permettent d'obtenir la croissance la plus élevée des plantes de* B. ruziziensis *au niveau de la taille, du diamètre et de la biomasse produite.*

Summary

Direct and Residual Effect of Different Levels of Nitrogen Fertilisation on Growth and Yield of *Brachiaria ruziziensis* at Different Phenological Stage

The direct and residual effect of different levels of nitrogen fertilization on growth and biomass production of Brachiaria ruziziensis *at different phenological stages was assessed at the Research and Experimental Farm of the University of Dschang in 2008 and 2009. A factorial design comparing six doses of nitrogen (0, 50, 100, 150, 200 and 250 kg N/ha) and three phenological stages (bolting, flowering and after seed set) on plots of 8 m (4 x 2 m) in four replicates,(i.e. a total of 72 plots) was used. No fertilizer has been applied in the second year. At each phenological stage during the first and second year, 120 plants were harvested by treatment for measurements of heights and diameters. The evaluation of the biomass of stems, leaves and whole plant was made on each plot based on the level of nitrogen fertilization and phenological stage at the first and second year. The results obtained showed that nitrogen fertilization and growth stage influenced significantly (P< 0.05) height and diameter of* B. ruziziensis *during the first and second year of mowning. Regardless of growth stage, height and diameter, higher plants have been obtained with fertilization at the dose 200 kg N/ha while in the second year they were obtained with the dose 250 kg N/ha. Irrespective of fertilization and year of mowning, the biomass of stems and whole plant of* B. ruziziensis *significantly (p< 0.05) increased with phenological stage. The highest biomass of leaves was obtained at flowering (8.42 ± 1.23 t DM/ha and 11.34 ± 0.32 t DM/ha respectively in the first and second year). The results obtained show that under the conditions of the trial the direct effect of fertilization at the dose of 200 kg N/ha and the residual effect of fertilization at the dose of 250 kg N/ha allow to have the highest growth of* B. ruziziensis *in terms of height, diameter and biomass.*

[1]Laboratoire de Nutrition Animale, Département des Productions Animales, FASA, Université de Dschang, B.P. 222, Dschang, Cameroun. E-mail: ftendonkeng@yahoo.fr / pamo_te@yahoo.fr

[2]Institut National Supérieur d'Agronomie et de Biotechnologie (INSAB), Université des Sciences et Techniques de Masuku, B.P. 941, Masuku, Gabon.

*Auteur correspondant

Reçu le 26.10.10 et accepté pour publication le 13.09.11.

Introduction

La nécessité de nourrir une population sans cesse croissante dans les pays en voie de développement pousse de plus en plus les agriculteurs à étendre les surfaces cultivables au détriment des espaces pastoraux, ce qui a pour conséquence le développement de conflits pour l'utilisation de l'espace rural entre agriculteurs et éleveurs (15). Cette situation conduit au rétrécissement des parcours naturels (17), à leur déplacement vers les zones marginales et aux difficultés de satisfaire les besoins nutritionnels des animaux (14).

Une amélioration de la productivité animale peut passer non seulement par la maîtrise des systèmes de production, mais également par l'introduction et l'intensification de la production des espèces fourragères à haut rendement. Parmi les nombreuses espèces fourragères introduites au Cameroun, la graminée pérenne *Brachiaria ruziziensis*, présente les avantages de mieux s'adapter dans les différentes régions, d'avoir une bonne valeur nutritive et d'être bien appétée par les animaux (17). La culture de cette graminée et surtout l'intensification de son utilisation peuvent constituer un palliatif au rétrécissement des parcours ci-dessus évoqués. Les principaux facteurs influençant sa croissance, son rendement et sa valeur nutritive sont le climat, le sol et le mode d'exploitation. *B. ruziziensis* est proposé pour être utilisé avec profit non seulement dans l'amélioration des pâturages naturels mais également en fourrage vert ou conservé (4). Sans fertilisation, n'importe quelle forme d'exploitation des plantes fourragères conduit à la diminution du stock en nutriments du sol en général et de l'azote en particulier surtout quand il s'agit des graminées tropicales (12). Différentes études ont montré que la fertilisation azotée accélère la croissance des plantes, étale la production fourragère dans le temps et entraîne une importante augmentation de la biomasse (6, 11, 16). Si quelques travaux ont été menés au Cameroun sur la relation entre la fertilisation et le rendement de *B. ruziziensis* (16, 18), aucun n'a encore été réalisé pour la détermination du niveau optimal de fumure azotée, et ses effets immédiats et/ou lointains sur la croissance et la production de biomasse de cette plante à différents stades phénologiques dans les zones d'altitude.

L'objectif de cette étude est donc de déterminer l'effet direct et résiduel de différents niveaux de fumure azotée sur la croissance et le rendement de *B. ruziziensis* en fonction des stades phénologiques. De telles informations sont indispensables pour la formulation et la mise en place de meilleures stratégies de gestion de l'espèce.

Matériel et méthodes

Zone d'étude

L'étude a été conduite à la Ferme d'Application et de Recherche (FAR) de l'Université de Dschang entre mars et novembre 2008 pour la première année et entre mars et octobre 2009 pour la deuxième année. La FAR est située à 05°20' latitude Nord et 10°03' longitude Est et à une altitude moyenne de 1410 m. Le climat de la région est équatorial de type camerounien, modifié par l'altitude. Les températures oscillent entre 10 °C (juillet-août) et 25 °C (février) avec une insolation annuelle de 1800 heures et une humidité relative variant entre 40-97%. Les précipitations varient entre 1500 et 2000 mm par an. La saison sèche va de mi-novembre à mi-mars et la saison des pluies de mi-mars à mi-novembre correspondant à la période de cultures. La végétation originelle de cette région est une savane arbustive avec par endroit des forêts galeries.

Dispositif expérimental

Un dispositif factoriel comparant six doses d'azote (0; 50; 100; 150; 200 et 250 kg N/ha) sous forme d'urée (46 N) et trois stades phénologiques (montaison, floraison et après grenaison) sur des parcelles de 8 m² (4 x 2 m, espacées entre elles de 0,5 m) en quatre répétitions, soit un total de 72 parcelles expérimentales a été utilisé. Les échantillons du sol (n= 5) ont été prélevés sur le site expérimental dans l'horizon 0 - 20 cm avant la préparation du sol et la mise en place des éclats de souche la première année et sur les parcelles témoins (n= 5) après la fauche de régularisation la deuxième année. L'analyse a été effectuée au Laboratoire d'Analyse des Sols, de Chimie et de l'Environnement (LABASCE) suivant la méthode d'écrite par Pauwel et *al.* (19).

Préparation du sol, mise en place des plants et fertilisation

Le site expérimental a été labouré par un tracteur et la mise en place des parcelles a été faite manuellement. Le précédent cultural du site avant la mise en place de l'essai était composé principalement de maïs et de haricot. La même quantité (80 g) d'engrais phosphaté sous la forme de superphosphate triple a été appliquée sur toutes les parcelles (y compris les témoins) comme engrais de fond. Des éclats de souche (trois plantules) de *B. ruziziensis* ont été prélevés dans le parcours de la FAR. Ces éclats de souche ont subi une réduction de la taille des racines et des feuilles, et cent cinq éclats de souche ont été repiqués sur chaque parcelle à 4 cm de profondeur et suivant un écartement de 25 x 25 cm.

Deux mois après la plantation d'éclats de souche, une coupe de régularisation a été effectuée à 20 cm au-dessus du sol, et les parcelles ont été fertilisées une seule fois. La deuxième année, la fauche de régulation a été effectuée en mars et aucune fertilisation n'a été apportée.

Collecte des données

A chacun des trois stades phénologiques au cours de la première et de la deuxième année, trente plantes

ont été prélevées au hasard dans chaque répétition, pour un total de 120 plantes par traitement pour les mesures des hauteurs et des diamètres. La hauteur des plantes a été mesurée à l'aide d'un mètre ruban gradué au centimètre; leur diamètre a été mesuré à l'aide d'un pied à coulisse gradué au millimètre.
Pendant les périodes de coupe et pour éviter les effets de bordure, les plantes de *B. ruziziensis* étaient récoltées (coupe de la touffe à 5 cm du sol) au centre de la planche sur une parcelle utile d'une superficie de 2 m² (2 x 1 m) et pesées. Un échantillon représentatif (1 kg) de chaque répétition par traitement a été prélevé et séché dans une étuve à 60 °C jusqu'à poids constant pour la détermination de la matière sèche et l'évaluation du rendement.
Pour chaque stade phénologique et chaque niveau de fertilisation la première et la deuxième année, un échantillon représentatif de 1 kg des plantes entières, prélevés lors des mesures de biomasse ont été séparés en feuilles et tiges pour l'évaluation du poids relatif des différentes parties de la plante. Leur proportion a été exprimée en matière sèche (12).

Analyse statistiques

Les données sur la hauteur, le diamètre et la biomasse produite ont été soumises à une analyse de variance multifactorielle suivant le Modèle Linéaire Général (MLG). Lorsque les différences existaient entre les différents traitements, les moyennes ont été séparées par le test de Duncan au seuil de signification 5% (21).

Résultats

Composition chimique du sol

Les résultats de l'analyse du sol la première et la deuxième année sont présentés dans le tableau 1. D'après le triangle textural (FAO), la classe texturale moyenne de ce sol est limoneuse. C'est également un sol moyennement acide (5,4< pH-eau< 6,0) et ayant une acidité d'échange faible, ce qui réduit les risques de toxicité due à un excès d'aluminium et de manganèse. La teneur moyenne en azote total de ce sol était de 3,55 ± 0,10 g/kg de sol la première année et de 2,79 ± 0,18 g/kg de sol la deuxième année, ce qui est suffisant pour l'agriculture traditionnelle, mais nécessite un complément azoté pour une agriculture intensive. La teneur en carbone organique était moyenne (> 2,5%) la première année. Il s'agit par conséquent d'un sol riche en MO (> 6%) mais de mauvaise qualité car le rapport C/N était supérieur à 13 (22). Par contre à la deuxième année, le rapport C/N était compris dans la fourchette de l'équilibre idéal (8-12) traduisant une bonne minéralisation de la matière organique (22). Pour ce qui est des bases échangeables, le sol contient des quantités moyennes de calcium, magnésium et potassium, ce qui se traduit par un rapport Ca/Mg (3,24 la première année et 3,53 la deuxième année) et Mg/K (3,21 la première année et 7,32 la deuxième année) équilibré car compris entre 1 - 5 et 3 - 15 respectivement. Par contre, la teneur moyenne en sodium de ce sol est faible (0,27 ± 0,06 meq/100 g), et la teneur moyenne en phosphore assimilable est très faible (1,69 ± 0,28 mg/kg) au cours des deux années, ce qui nécessite un apport de phosphate.
Les teneurs en bases échangeables (SBE) ont été modérées (5 - 10 méq/100 g) au cours des deux années. D'après Beernart et Bitondo (2), la CEC à pH 7 serait qualifiée de faible (< 20 méq/100 g) au cours des deux années. Ces observations montrent que ce sol ne peut retenir les ions pour la nutrition des plantes, caractéristiques propres aux oxisols. Une amélioration de la CEC afin que tout engrais répandu y soit retenu pour être mis à la disposition des plantes est donc nécessaire. Pour *B. ruziziensis* qui exige un sol avec une fertilité élevée (4), les conditions de l'essai qui sont représentatives de celles des hautes terres de l'Ouest Cameroun sont acceptables.

Tableau 1
Analyse du sol la première et la deuxième année

Paramètres	Substrat sol	
	2008	2009
Profondeur (%)	0-20	0-20
Pente (%)	3	3
Horizon	Ap	Ap
Texture (%)		
Sable	14,67 ± 2,08	13,36 ± 1,72
Limon grossier	13,67 ± 2,08	12,52 ± 1,23
Limon fin	48,33 ± 3,21	41,87 ± 2,51
Limon total	61,67 ± 3,21	58,37 ± 2,45
Argile	23,67 ± 1,15	21,53 ± 1,23
Classe texturale	L	L
Réaction du sol		
pH-eau	5,40 ± 0,11	5,73 ± 0,14
pH-KCl	4,87 ± 0,08	4,76 ± 0,16
Matière organique		
CO (%)	4,79 ± 0,16	2,66 ± 0,15
MO (%)	8,27 ± 0,25	4,57 ± 0,25
N total	3,55 ± 0,10	2,79 ± 0,18
C/N	13,50 ± 0,57	9,57 ± 0,54
Cations échangeable (méq/100g)		
Calcium	5,93 ± 1,51	6,72 ± 1,13
Magnésium	1,83 ± 0,52	2,49 ± 0,73
Potassium	0,57 ± 0,51	0,34 ± 0,07
Sodium	0,27 ± 0,06	0,10 ± 0,01
Somme des bases (SB)	8,60 ± 0,10	9,65 ± 1,84
Capacité d'échange cationique		
CECeff	8,60 ± 0,10	9,65 ± 1,84
CEC à pH 7	19,07 ± 4,16	17,67 ± 0,32
Saturation en bases (%)	45,09 ± 3,79	54,61 ± 5,34
Acidité échangeable (cmol+/kg)	0,00 ± 0,00	0,00 ± 0,00
Phosphore du sol		
Bray-2 (ppm ou mg/kg)	1,69 ± 0,28	7,79 ± 0,48

Tableau 2
Effet direct et résiduel de différents niveaux de fertilisation sur la taille (cm) et le diamètre (cm) de Brachiaria ruziziensis à différents stades phénologiques

Fertilisation (kg N/ha)		Stades phénologiques					
		Montaison		Floraison		Après grenaison	
		Taille	Diamètre	Taille	Diamètre	Taille	Diamètre
Effet direct (2008)	0	100,47 ± 14,12^{a}	0,38 ± 0,03^{a}	155,55 ± 19,44^{a}	0,41 ± 0,03^{a}	176,72 ±18,29^{a}	0,41 ± 0,03^{a}
	50	112,77 ± 15,94^{bc}	0,46 ± 0,07^{c}	158,80 ± 23,02^{ab}	0,46 ± 0,06^{c}	187,80 ± 8,09^{b}	0,47 ± 0,07^{b}
	100	115,35 ± 23,06^{c}	0,46 ± 0,04^{c}	160,25 ± 11,95^{ab}	0,47 ± 0,05^{cd}	191,37 ± 19,89^{bc}	0,48 ± 0,07^{bc}
	150	118,10 ± 4,54^{c}	0,47 ± 0,06^{c}	163,67 ± 12,66^{b}	0,49 ± 0,07^{d}	194,27 ± 18,46^{bc}	0,50 ± 0,08^{c}
	200	123,90 ± 3,29^{d}	0,52 ± 0,06^{d}	172,37 ± 17,00^{c}	0,57 ± 0,06^{e}	197,52 ± 15,22^{c}	0,58 ± 0,06^{d}
	250	108,85 ± 14,67^{b}	0,42 ± 0,07^{b}	158,25 ± 21,23^{ab}	0,43 ± 0,05^{b}	178,70 ± 36,41^{a}	0,43 ± 0,05^{a}
Effet résiduel (2009)	0	75,90 ± 13,91^{a}	0,31 ± 0,06^{a}	147,85 ± 30,06^{a}	0,31 ± 0,04^{a}	186,00 ± 28,42^{a}	0,38 ± 0,07^{a}
	50	77,02 ± 20,17^{a}	0,32 ± 0,06^{a}	148,15 ± 23,67^{a}	0,34 ± 0,06^{ab}	187,27 ± 26,66^{a}	0,40 ± 0,07^{a}
	100	78,85 ± 11,27^{a}	0,32 ± 0,04^{a}	153,12 ± 38,19^{ab}	0,34 ± 0,05^{ab}	192,82 ± 35,52^{a}	0,41 ± 0,06^{a}
	150	81,42 ± 16,60^{a}	0,31 ± 0,05^{a}	152,27 ± 42,39^{ab}	0,36 ± 0,06^{bc}	190,75 ± 31,73^{a}	0,41 ± 0,09^{a}
	200	90,32 ± 16,50^{b}	0,32 ± 0,06^{a}	154,27 ± 42,39^{ab}	0,36 ± 0,06^{bc}	199,60 ± 33,03^{a}	0,45 ± 0,09^{b}
	250	93,50 ± 14,18^{b}	0,33 ± 0,06^{a}	168,82 ± 24,80^{b}	0,37 ± 0,07^{c}	201,77 ± 23,04^{a}	0,46 ± 0,11^{b}

^{a,b,c,d} : les moyennes portant les mêmes lettres dans la même colonne et dans la même année ne sont pas significatives au seuil de 5%.

Effets direct et résiduel de différents niveaux de fertilisation sur la taille et le diamètre de *Brachiaria ruziziensis* à différents stades phénologiques

La variation de la taille et du diamètre de *B. ruziziensis* en fonction de l'effet direct et résiduel de différents niveaux de fertilisation à la montaison, floraison et après grenaison est présentée dans le tableau 2. La taille de *B. ruziziensis* a augmenté avec la fertilisation jusqu'à 200 kg N/ha à la montaison, à la floraison et après la grenaison. A la montaison, la taille des plantes des parcelles fertilisées était significativement ($p< 0,05$) supérieure à celle des plantes des parcelles témoins. La fertilisation à la dose 200 kg N/ha a permis à la montaison et à la floraison d'avoir des plantes ayant une taille significativement ($p< 0,05$) supérieure à celle des autres plantes. La taille des plantes des parcelles non fertilisées (176,72 ± 18,29 mm) après grenaison n'était pas significativement différente ($p> 0,05$) à celle des plantes des parcelles fertilisées à la dose 250 kg N/ha. La fertilisation à la dose 200 kg d'N/ha a permis d'avoir des plantes avec une taille significativement ($p< 0,05$) plus élevée que celle des plantes des parcelles non fertilisées et celles fertilisées aux doses 50 et 250 kg N/ha.

La deuxième année, la taille de *B. ruziziensis* à la montaison, floraison et après grenaison a augmenté

Tableau 3
Effet direct et résiduel de différents niveaux de fertilisation azotée sur la production de biomasse (t MS/ha) de *Brachiaria ruziziensis* à différents stades phénologiques

Fertilisation (kg N/ha)		Stades phénologiques					
		Montaison			Floraison		
		PE	F	T	PE	F	T
Effet direct (2008)	0	8,96±1,39^{a}	5,16±0,54^{a}	3,79±0,99^{a}	15,92±3,15^{a}	5,48±0,32^{a}	10,44±3,04^{a}
	50	10,46±0,70^{b}	5,89±0,77^{ab}	4,56±0,63^{ab}	18,12±0,97^{ab}	6,54±0,76^{ab}	11,57±0,58^{a}
	100	11,29±0,77^{bc}	5,85±0,62^{ab}	5,43±0,47^{bc}	20,27±1,16^{b}	7,39±0,42^{bc}	12,87±1,46^{ab}
	150	12,18±0,24^{cd}	6,78±0,44^{bc}	5,40±0,65^{bc}	19,51±0,77^{b}	7,38±1,21^{bc}	12,13±0,48^{a}
	200	13,24±0,71^{d}	7,13±0,77^{c}	6,11±0,87^{c}	23,96±2,22^{c}	8,42±1,23^{c}	15,54±1,96^{b}
	250	8,94±0,43^{a}	5,02±0,34^{a}	3,92±0,32^{a}	18,82±1,33^{b}	7,10±1,75^{abc}	11,72±2,14^{a}
Effet résiduel (2009)	0	4,73±0,85^{a}	2,83±0,31^{a}	1,89±0,58^{a}	10,24±0,42^{a}	5,04±0,52^{a}	5,19±0,77^{a}
	50	5,75±0,85^{ab}	3,25±0,48^{a}	2,50±0,38^{ab}	14,46±1,29^{b}	7,07±0,69^{b}	7,39±0,63^{b}
	100	5,93±0,72^{b}	3,40±0,39^{a}	2,53±0,39^{ab}	17,30±1,06^{c}	8,46±0,5^{c}	8,84±0,56^{c}
	150	6,67±0,73^{b}	3,63±0,57^{a}	3,04±0,18^{b}	18,81±1,62^{d}	9,04±0,94^{c}	9,76±1,01^{d}
	200	9,14±1,23^{c}	5,04±0,81^{b}	4,10±1,10^{c}	21,99±0,68^{e}	10,65±0,57^{d}	11,33±0,29^{e}
	250	9,48±0,74^{c}	5,01±0,68^{b}	4,47±0,50^{c}	23,17±0,69^{e}	11,34±0,32^{d}	11,82±0,41^{e}

^{a,b,c,d,e,f} : les moyennes portant les mêmes lettres dans la même colonne et dans la même année ne sont pas significatives au seuil de 5%.
PE: Plante entière; F: Feuilles; T: Tiges.

avec l'accroissement supposé de l'effet résiduel de la fertilisation azotée. A la montaison, l'effet résiduel résultant de la fertilisation aux doses 200 et 250 kg N/ha a permis d'obtenir des plantes ayant une taille significativement (p< 0,05) supérieure à celle des plantes des parcelles non fertilisées et celles résultants de la fertilisation aux doses 50, 100 et 150 kg N/ha. A la floraison, l'effet résiduel de la fertilisation sur la taille des plantes des parcelles fertilisées à la dose 250 kg N/ha a été significativement (p< 0,05) plus élevée que celui des plantes des parcelles non fertilisées et celui des plantes ayant reçu la dose de 50 kg N/ha la première année. Après grenaison, l'effet résiduel de la fertilisation sur la taille des plantes des parcelles fertilisées à la dose 250 kg N/ha a été le plus élevé (201,77 ± 23,04 cm) tandis que la taille la plus faible a été obtenue chez les plantes des parcelles non fertilisées. Aucune différence significative (p> 0,05) n'a été observée entre la taille des plantes à ce stade phénologique pour l'effet résiduel de la fertilisation.

Le diamètre des plantes a augmenté avec le niveau de fertilisation jusqu'à la dose de 200 kg N/ha avant de diminuer ensuite avec la dose de 250 kg N/ha (Tableau 2) la première année. A la montaison, la fertilisation a permis d'obtenir des plantes de diamètre significativement (p< 0,05) plus élevé que celui des plantes des parcelles non fertilisées. Les plantes des parcelles fertilisées à la dose 200 kg N/ha ont présenté un diamètre significativement (p< 0,05) supérieur à celui des autres plantes. A la floraison, le diamètre des plantes des parcelles fertilisées a été significativement (p< 0,05) supérieur à celui des plantes des parcelles témoins. Le diamètre des plantes des parcelles fertilisées à la dose 250 kg N/ha a été comparable (p> 0,05) à celui des plantes des parcelles témoins après grenaison. De manière générale, le diamètre des plantes le plus élevé a été obtenu au niveau des parcelles fertilisées à la dose 200 kg N/ha.

La deuxième année, le diamètre des plantes a augmenté avec l'effet résiduel (Tableau 2). A la montaison, le diamètre le plus élevé a été obtenu avec les plantes ayant reçu la dose de 250 kg N/ha la première année. Aucune différence significative (p> 0,05) n'a cependant été observée entre les diamètres des plantes à ce stade phénologique en fonction de l'effet résiduel de la fertilisation. A la floraison, l'effet résiduel de la fertilisation à la dose 250 kg N/ha a permis d'obtenir des plantes avec un diamètre significativement (p< 0,05) supérieur à celui des plantes des parcelles non fertilisées et celui des plantes des parcelles ayant reçu des doses de 50 et 100 kg N/ha la première année. Après grenaison, l'effet résiduel de la fertilisation sur le diamètre des plantes des parcelles fertilisées aux doses de 200 et 250 kg N/ha a été significativement (p< 0,05) supérieur à celui des autres plantes. De manière générale, le diamètre des plantes le plus élevé a été obtenu pour les parcelles fertilisées la première année à la dose 250 kg N/ha après grenaison.

	Après grenaison		
PE	F	T	
16,35±0,55[a]	4,85±0,65[a]	11,50±0,35[a]	
18,57±0,68[ab]	5,44±0,99[ab]	13,13±0,42[ab]	
21,67±0,73[cd]	6,00±0,6[ab]	15,67±0,77[cd]	
22,33±1,82[cd]	6,78±0,94[b]	15,54±1,93[cd]	
23,65±1,75[e]	6,28±1,48[ab]	17,36±0,84[d]	
19,68±3,55[bc]	5,66±1,14[ab]	14,02±3,09[bc]	
11,06±0,88[a]	4,79±0,15[a]	6,26±0,74[a]	
14,86±0,45[b]	6,32±0,33[b]	8,54±0,46[b]	
17,43±0,61[c]	7,34±0,17[c]	10,08±0,67[c]	
19,19±0,65[d]	8,17±0,71[d]	11,01±0,66[d]	
22,21±0,64[e]	9,65±0,57[e]	12,55±0,31[e]	
23,67±0,4[f]	10,09±0,76[e]	13,57±0,54[f]	

Effets direct et résiduel de différents niveaux de fertilisation sur la production de biomasse de *Brachiaria ruziziensis* à différents stades phénologiques

L'évolution de la biomasse de la plante entière, des feuilles et des tiges de *B. ruziziensis* en fonction des stades phénologiques est présentée au tableau 3. La biomasse a augmenté avec le niveau de fertilisation azotée pour atteindre la production maximale dans les parcelles fertilisées à la dose 200 kg N/ha à la montaison. A ce stade phénologique, la biomasse des feuilles était supérieure à celle des tiges, indépendamment du niveau de fertilisation azotée. La biomasse la plus élevée de la plante entière (13,24 ± 0,70 t MS/ha), des feuilles (7,13 ± 0,76 t MS/ha) et des tiges (6,11 ± 0,87 t MS/ha) a été obtenue dans les parcelles fertilisées à la dose 200 kg N/ha. Avec la fertilisation à la dose 250 kg N/ha, on a observé une baisse de la biomasse de l'ordre de 32,47% pour la plante entière; 29,73% pour les feuilles et 35,84% pour les tiges par rapport à la fertilisation à la dose 200 kg N/ha.

La biomasse des feuilles (8,41 ± 1,23 t MS/ha) et des tiges (15,54 ± 1,96 t MS/ha) des plantes de *B. ruziziensis* récoltées à la floraison sur la parcelle fertilisée à la dose 200 kg N/ha a été significativement supérieure (p< 0,05) à celle des plantes fauchées des parcelles non fertilisées (Tableau 3). La biomasse la plus élevée des plantes entières (23,96 ± 2,22 t MS/ha), des feuilles (8,41 ± 1,23 t MS/ha) et des tiges (15,54 ± 1,96 t MS/ha) a été obtenue avec la fertilisation à la dose 200 kg N/ha. A ce stade phénologique, la biomasse des tiges était supérieure à celle des feuilles, indépendamment du niveau de fertilisation. On a observé une baisse de la biomasse dans les parcelles fertilisées à la dose 250 kg N/ha de l'ordre de 21,45%; 15,57% et 24,58% respectivement pour

la plante entière, les feuilles et les tiges par rapport à celle des parcelles fertilisées à la dose 200 kg N/ha.
Après grenaison, la biomasse des tiges représentait plus du double de celle des feuilles (Tableau 3). La fertilisation à la dose 200 kg N/ha a permis d'obtenir la biomasse de la plante entière (23,65 ± 1,75 t MS/ha), des feuilles (6,28 ± 1,48 t MS/ha) et des tiges (17,36 ± 0,84 t MS/ha) la plus élevée après grenaison. Par contre, la biomasse des feuilles la plus élevée à ce même stade a été obtenue dans les parcelles fertilisées à la dose 150 kg N/ha. La fertilisation a permis de manière générale d'accroître la biomasse des tiges, des feuilles et donc de la plante entière de *B. ruziziensis*.
Ainsi, à la montaison et à la floraison, la fertilisation à la dose 200 kg N/ha, a permis d'obtenir la biomasse des plantes entières, des feuilles et des tiges la plus élevée. Par contre, la fertilisation à la dose 150 kg N/ha a permis d'avoir la biomasse des feuilles la plus élevée après grenaison, bien que cette biomasse n'ait pas été significativement différente ($p > 0,05$) de celle obtenue dans les parcelles fertilisées à la dose 200 kg N/ha.
La deuxième année de fauche, la biomasse a suivi la même tendance qu'en première année, mais le niveau maximal a été observé dans les parcelles ayant reçu la dose de 250 kg N/ha la première année (Tableau 3). A la montaison, la biomasse des feuilles a été supérieure à celle des tiges, quel que soit le niveau de fertilisation appliquée la première année. L'effet résiduel résultant de la fertilisation aux doses 200 et 250 kg N/ha a permis d'obtenir des biomasses significativement ($p < 0,05$) supérieures à celle obtenue dans les autres parcelles.
La biomasse des plantes entières, des feuilles et des tiges de *B. ruziziensis* récoltées à la floraison (Tableau 3) a augmenté en fonction de la dose d'azote appliquée la première année. A ce stade phénologique, la biomasse des tiges a été supérieure à celle des feuilles. La biomasse de la plante entière, des feuilles et des tiges de *B. ruziziensis* récoltées sur les parcelles ayant reçue de l'azote la première année a été significativement supérieure ($p < 0,05$) à celle obtenue sur les parcelles témoins.
Après grenaison, la biomasse de la plante entière et des tiges de *B. ruziziensis* a augmenté significativement ($p < 0,05$) pour toutes les parcelles fertilisées en première année. A ce stade phénologique, l'effet résiduel de la fertilisation à la dose 250 kg N/ha a permis d'obtenir la biomasse de la plante entière (23,67 ± 0,40 t MS/ha), des feuilles (10,09 ± 0,76 t MS/ha) et des tiges (13,57 ± 0,54 t MS/ha) la plus élevée. De manière générale, l'effet résiduel sur les parcelles fertilisées a permis d'accroître la biomasse des tiges, des feuilles et donc de la plante entière de *B. ruziziensis* après grenaison.
A la floraison et après grenaison, l'effet résiduel résultant de la fertilisation à la dose 250 kg N/ha, a permis d'obtenir la biomasse des tiges, des feuilles et de la plante entière la plus élevée. De même, l'effet résiduel de la fertilisation à la dose 250 kg N/ha a permis d'obtenir la biomasse la plus élevée de la plante entière à la montaison (9,48 ± 0,74 t MS/ha), à la floraison (23,17 ± 0,69 t MS/ha) et après grenaison (23,67 ± 0,40 t MS/ha).

Discussion

La taille et le diamètre des plantes ont augmenté avec le niveau de fertilisation azotée la première année de fauche pour atteindre la taille et le diamètre maximal avec la fertilisation à la dose de 200 kg N/ha. La taille et le diamètre ont ensuite baissé avec la fertilisation à la dose de 250 kg N/ha. L'azote étant le principal facteur limitant la croissance et la production des plantes (11, 12, 14), nous pouvons donc penser qu'au-delà de 200 kg N/ha, l'azote devient toxique pour la plante. En effet, l'azote apporté sous forme d'urée s'hydrolyse rapidement pour donner l'ammoniac (NH_3) et l'ammonium (NH_4^+). Les effets de ce dernier, nombreux et complexes lorsqu'il est en excès dans le milieu sont liés à une acidification du milieu racinaire et à des perturbations de la nutrition hydrominérale de la plante (10, 11), ce qui expliquerait la diminution de la taille et du diamètre observée.
Au cours de la deuxième année de fauche, la taille et le diamètre des plantes ont peu varié en fonction de l'effet résiduel de différents niveaux de fertilisation. L'analyse de la composition du sol la première et la deuxième année montre que ses propriétés, notamment le rapport C/N a évolué pour se trouver dans la fourchette de l'équilibre idéal pour une bonne minéralisation la deuxième année. La bonne minéralisation de l'azote par les microorganismes du sol expliquerait les tailles des plantes comparables à la floraison et après grenaison entre la première et la deuxième année malgré le fait qu'aucune fertilisation n'ait été apportée la deuxième année. En effet, la croissance et le changement de la composition chimique des plantes est un processus complexe où les mécanismes de régulation dans les plantes interagissent avec des facteurs environnementaux (23).
La fertilisation azotée a influencé positivement la production de biomasse de la plante entière de *B. ruziziensis* et de ses différentes parties. Cette observation est en accord avec celle de nombreux auteurs (5, 6, 7, 12, 14, 18, 20). La variation de la biomasse obtenue dans cette étude est semblable aux observations de Bogdan (3) et Cook *et al*. (4) qui ont montré que la biomasse de *B. ruziziensis* varie de 5 à 36 t MS/ha en fonction de la fertilité du sol, des précipitations et du niveau de fertilisation. En effet, la fertilisation accroît la vitesse de croissance, ce qui augmente la production pour un stade de développement donné, ou réduit le délai nécessaire pour atteindre un rendement défini (6, 7, 8, 20). La biomasse de la plante entière obtenue à la montaison

avec la dose 200 kg N/ha est inférieure à celle obtenue par Appadurai et Goonawardene (1) au Sri Lanka lorsqu'ils fertilisaient le *B. ruziziensis* avec 224 kg N/ha (22,03 t MS/ha) et 366 kg N/ha (25,6 t MS/ha). Par contre, la biomasse de la plante entière obtenue avec la fertilisation à la dose 150 kg N/ha à la montaison (12,18 t MS/ha) est semblable à celle obtenue avec la même espèce sur un oxisol au Brésil (12 t MS/ha) avec le même niveau de fertilisation (24).

Dans cette étude, nous avons observé au cours de la première année que la biomasse augmentait avec le niveau de fertilisation jusqu'à un seuil au-delà duquel elle commençait à baisser. Ces observations concordent avec celles de nombreux auteurs (6, 9, 10, 13). En effet, Olsen (13) obtient en Ouganda avec *B. ruziziensis* une production de biomasse de 26,5 t MS/ha avec une fertilisation maximale de 896 kg N/ha. Ce niveau maximal de fertilisation est largement supérieur à celui utilisé dans cette étude, qui est de 200 kg N/ha au cours de la première année. Cette différence peut s'expliquer par la fertilité des sols et les précipitations caractéristiques des deux essais. Par ailleurs, la baisse de rendement observée à partir d'un seuil de fertilisation est en accord avec les observations de Olsen (13) et Limani et De Vienne (9) qui ont montré qu'un apport d'azote à la dose qui excède les besoins de croissance potentielle de la plante ne permet plus d'augmenter le rendement fourrager car l'azote devient toxique pour la plante. Maurice *et al*. (10) ont montré qu'une fertilisation azotée à des doses élevées (qui excède les besoins de croissance potentielle de la plante) entraîne une baisse de production de la biomasse due à la toxicité à l'ion ammonium. De même, Lawlor *et al*. (6) ont montré que lorsque l'apport d'azote excède les besoins de la plante, l'efficacité de son utilisation par cette dernière baisse car elle en devient saturée. Au cours de la deuxième année, la biomasse des feuilles, des tiges et de la plante entière a augmenté avec l'effet résiduel de la fertilisation. L'analyse de la composition de ce dernier au cours des deux années montre une évolution de ses propriétés, notamment le rapport C/N qui, mauvais à la première année a évolué pour se trouver dans la fourchette de l'équilibre idéal pour une bonne minéralisation à la deuxième année. Ceci a donc permis une bonne minéralisation de l'azote par les microorganismes du sol, ce qui expliquerait l'augmentation des biomasses obtenues en fonction de l'effet résiduel de différents niveaux de fertilisation à différents stades phénologiques.

Conclusion

Il ressort de cette étude que l'effet direct de la fertilisation azotée a significativement ($P< 0,05$) influencé la taille et le diamètre et la production de biomasse de *B. ruziziensis* alors que l'effet résiduel a influencé de manière variable ces mêmes paramètres.

Références bibliographiques

1. Appadurai R.R. & Goonawardene L., 1973, Performance of three fodder grasses under high nitrogen applications in the mid-country wet zone of Sri Lanka. J. Nat. Agric. Soc. 8, 31-37.
2. Beernart F. & Bitondo D., 1992, Simple and practical method to evaluate analytical data of soil profiles. Soil Science Department. Belgian cooperation – University of Dschang. 66 p.
3. Bogdan AV., 1977, Tropical pastures and fodder plants (grasses and legumes). London, New York, Longman.
4. Cook B.G., Pengelly B.C., Brown S.D., Donnelly J.L., Eagles D.A., Franco M.A., Hanson J., Mullen B.F., Partridge I.J., Peters M. & Schultze-Kraft R., 2005, Tropical forages: an interactive selection tool., [CD-ROM], CSIRO, DPI&F(Qld), CIAT and ILRI, Brisbane, Australia.
5. Hyo W.L., Mu H.J. & Chang H.K., 1993, Effect of temperature, nitrogen fertiliser and cutting height on regrowth and dry matter production of orchagrass. Proceedings of the XVII International Grassland Congress 1993, Pp. 131-133.
6. Lawlor D., Lemaire G. & Gastal F., 2001, Nitrogen, plant growth and crop yield. *In*: Lea P.J. and Morot-Gaudry J.F. (eds). Plant Nitrogen. 2001. INRA. Pp. 343-367.
7. Lemaire G., Pablo C. & Jacque W., 1999, Ecophysiologie des plantes fourragères tropicales. *In*: Roberge G. et Toutain 1999. Cultures fourragères tropicales. CIRAD. Pp 19-51.
8. Lemaire G., Salette J. & Laissus R., 1982, Analyse de la croissance d'une prairie naturelle normande au printemps II. La dynamique d'absorption de l'azote et son efficience fourrage.
9. Limami A. & De Vienne D., 2001, Natural Genetic Variability in nitrogen Metabolism. *In*: Lea P.J. and Morot. Gaudry J.F. (eds) Plant Nitrogen. 2001. INRA. Pp. 369-378.
10. Maurice E.H., Robert F.B. & Darrel S.M., 1985, Forages: the science of grassland agriculture. Iowa State University press. Arnes, Iowa, USA. Pp. 318-325.
11. Morot-Gaudry J-F., 1997, Assimilation de l'azote chez les plantes: aspects physiologique, biochimique et moléculaire. Edition INRA. 422 p.
12. Obulbiga M.F. & Kaboré-Zoungrana C.Y., 2007, Influence de la fumure azotée et du rythme d'exploitation sur la production de matière sèche et la valeur alimentaire de *Andropogon gayanus* Kunth au Burkina Faso. Tropicultura, 2007, **25**, 3, 161-167.
13. Olsen F.J., 1982, Effect of large application of nitrogen fertilizer on the production and protein contents of four tropical grasses in Uganda. Tropical Agriculture, 49, 251-260.
14. Pamo T.E., Boukila B., Meduke C.N. & Tendonkeng F., 2008, Effect of nitrogen fertilisation and cutting frequency on the yield and regrowth of *Panicum maximum* Jacq. in West Cameroon. *In:* Xie Haining and Huang Jiehua (Eds). XXI International Grassland Congress / VIII International Rangeland Congress Hohhot, China, 29th June -5th July 2008. p. 354.
15. Pamo T.E., Tendonkeng F., Kana J.R., Boukila B. & Nanda A.S., 2006, Effect *Calliandra calothyrsus* and *Leucaena leucocephala* supplementary feeding goat production in Cameroon. Small Ruminant Research, 65, 31-37.
16. Pamo T.E., 1991, Réponse de *Brachiaria ruziziensis* Germain et Evard à la fertilisation azotée et à différents rythmes d'exploitation en Adamaoua, Cameroun. Revue Elev. Méd. Vét. Pays trop. **44,** 3, 373-380.
17. Pamo T.E., Boukila B., Fonteh F.A., Tendonkeng F., Kana J.R. & Nanda A.S., 2007, Nutritive values of some basic grasses and leguminous tree foliage of the Central region of Africa. Animal Feed Science and Technology, 135, 273-282.
18. Pamo T.E. & Pieper R.D., 1989, Effect of nitrogen fertilization in combination with potassium and phosphorus and cutting frequency on the yield of *Brachiaria ruziziensis* in Adamawa plateau, Cameroon. XVI International Grassland congress, 4-11 october 1989, Nice, France. Versailles, The french grassland society, 1989. pp. 111-112.
19. Pauwels J.M., Van Ranst E., Verloo M. & Mvondo Ze A., 1992, Méthode d'analyse de sols et de plantes, gestion de stock de verrerie et de

produits chimiques. Manuel de Laboratoire de Pédologie. Publication Agricoles. 28.

20. Peyraud J.L. & Astigarraga L., 1998, Review of effect of nitrogen fertilisation on the chemical composition, intake, digestion and nutritive value of fresh herbage: consequences on animal nutrition and N balance. Anim. Feed Sci. Technol. 72, 235-259.
21. Steel R.G. & Torrie J.H., 1980, Principles and procedures of statistics. New York, McGraw Hill Book C. 633 p.
22. Sys C., Van Ranst E., Debaveye J. & Beernaert F., 1991, Land evaluation part II. Methods for land evaluation. Belgium, General Administration for Development Cooperation.
23. Thorvaldsson G., 1987, The effects of weather on nutritional value of timothy in northern Sweden. Acta Agric. Scand. 37, 305-319.
24. Vincente-Chandler J., Silva S., Albrunia F. & Rodriguez J.A., 1972, Effect of two cutting heights, four harvest intervals and five nitrogen rates on yield and composition of Congo grass under humid tropical conditions. Puerto Rico Journal of Agriculture, 56: 280-291.

F. Tendonkeng**,** Camerounais, M.Sc., Enseignant au Département des productions animales, Faculté d'Agronomie et des Sciences Agricoles, Université de Dschang, Cameroun.

B. Boukila**,** Gabonnais, Ph.D, Professeur, Institut National Supérieur d'Agronomie et de Biotechnologie (INSAB), Université des Sciences et Techniques de Masuku, Gabon.

E.T. Pamo**,** Camerounais, PhD, Professeur, Département des productions animales, Faculté d'Agronomie et des Sciences Agricoles, Université de Dschang, Cameroun.

A.V. Mboko**,** Gabonnais, M.Sc., Doctorant, Département des productions animales, Faculté d'Agronomie et des Sciences Agricoles, Université de Dschang, Cameroun.

F.N.E. Matumuini, Gabonnais, M.Sc., Doctorant, Département des productions animales, Faculté d'Agronomie et des Sciences Agricoles, Université de Dschang, Cameroun.

Investigation of Plants Used for the Ethnoveterinary Control of Gastrointestinal Parasites in Bénoué Region, Cameroon

Christelle Mirande Djoueche[1], A.B. Azebaze[2] & A.B. Dongmo[1*]

Keywords: Helminthiasis- Medicinal plants- Benoué- Cameroon

Summary

This study was conducted in the localities of Laindé Karewa and Israël, in the Bénoué region of Cameroon. Carefully structured questionnaires and discussions were used to identify 25 plant species used for traditional veterinary treatment in the Mbororo settlements, in order to control internal worms and parasites in cows, sheep and goats. These plant species belong to 16 families. Combretaceae were most frequently used, while Mitragyna inermis *(14.07%) and* Anogeissus leiocarpus *(20.96%) were most frequently used for sheep and goats, respectively. Stem bark was the most frequently used part of the plant (58.33%), generally in decoction form (31.25%) or by mixing the whole plant with the animal's food (31.25%), and represented the majority of medicinal preparations. Medicinal plants are generally used on their own and rarely in combination with other plants. These medicinal plant remedies are almost always administered orally. Even if their safety and effectiveness have yet to be proven, they could represent an alternative strategy for managing helminthiases in the region.*

Résumé

Investigation sur les plantes à potentialités anthelminthiques utilisées dans la région de la Bénoué- Cameroun

Cette étude a été réalisée dans les localités de Laindé Karewa et d'Israël, situées dans la région de la Bénoué- Cameroun. Les conversations et les questionnaires bien structurés ont permis d'identifier 25 espèces végétales utilisées en médecine vétérinaire traditionnelle dans les campements Mbororos pour traiter les vers intestinaux, parasites des bovins, ovins et caprins. Ces espèces de plantes appartiennent à 16 familles. Les plantes appartenant à la famille des Combretacées sont les plus citées. Mitragyna inermis *(14,07%) et* Anogeissus leiocarpus *(20,96%) sont les plantes les plus utilisées respectivement chez les bovins, caprins et ovins. La partie végétale la plus utilisée est l'écorce (58,33%) généralement sous forme de décoction (31,25%) ou mélangée à l'alimentation animale (31,25%). Les plantes sont généralement utilisées seules donc rarement en association. L'administration à l'animal se fait presque toujours par voie orale. Même si l'innocuité et l'efficacité restent encore à prouver, elles constitueraient une alternative dans la gestion des helminthiases dans la région.*

Introduction

In the developing countries, demand for meat has increased rapidly over the last 20 years at the rate of 5.6% per year, due to an increasing tendency to consume animal products. This growing demand for animal products provides an opportunity for the world's 675 million poor people, who live in rural areas and rely on cattle, to improve their lives (11). However, parasitic diseases and other endemics represent a major barrier for the development of profitable animal production. Infections caused by nematodes (helminthiasis), particularly in small ruminants, create major problems in southern hemisphere countries, especially in areas affected by poor nutrition and hygiene (12, 23). These nematodes are responsible for the major fall in (milk and meat) production and sometimes even for mortality on goat and sheep farms (6, 24). The problem is even more noticeable in tropical countries and during the rainy season (22).

In Cameroon, it has been shown that nematodes and gastro-intestinal cestodes are responsible for high sheep and goat mortality (2, 19, 20). A post-mortem examination, conducted on sheep at the IRAD (Institute of Agricultural Research for Development) research station in Garoua in 1994, revealed the presence of nematodes (*Haemonchus contortus*, *Trichostrongylus colubriformis*, *Oesophagostomum columbianum*, *Gaigeria pachyscelis*, *Bunostomum trigonocephalum*, *Trichuris glubolusa)* and one cestode (*Moniezia expenza*). Over 75% of mortalities have been attributed to helminthiases, particularly

[1]Department of Animal Organism Biology, Faculty of Science, University of Douala, BP. 24157, Douala, Cameroon.
[2]Department of Chemistry, Faculty of Science, University of Douala, BP. 24157, Douala, Cameroon.
*Corresponding author: A. Dongmo, Department of Animal Organism Biology, Faculty of Science, University of Douala, BP. 24157, Douala, Cameroon.
Tel.: +237 76491950; E-mail: alainberd@yahoo.fr
Received on 18.01.11 and accepted for publication on 20.09.11.

haemonchosis and monieziosis.
In the developed countries, relatively successful results achieved in terms of combating helminthiases are linked to the availability and easy application of anthelmintics, as well as improved diagnostic tests and knowledge of parasite epidemiology in ruminants. However, the inadequate use of anthelmintics has rapidly led to increased resistance, thus limiting the effectiveness of current and future programmes aimed at controlling these parasites.
In the developing countries, livestock farmers do not have access to conventional medicines. They see the use of medicinal plants as an alternative due to ever-increasing impoverishment. Although the traditional use of some plants has been tried and tested, the effectiveness of most plants has not yet been demonstrated. This makes it necessary to conduct scientific studies aimed at efficient and appropriate use, without the risk of intoxication.
Veterinary research has also focused on the use of anthelmintic plants, particularly in western countries before the Second World War and subsequently in oriental countries and India. Data exists concerning the action of several plants and plant extracts, which are used to combat certain parasites in various African countries and Cameroon (1, 21).
The aim of this study is to analyse the traditional use of plants for the treatment of intestinal worms in ruminants by livestock farmers in the localities of Laindé Karewa and Israël, in the Bénoué region of Cameroon.

Methods

Study site

The localities of Laindé Karewa and Israël in Bénoué-Cameroon are located in the Sudano-Guinean region.

Table 1
Overview of plants used as anthelmintics for the treatment of cattle

N°	Scientific name	Local name (Peul)	Families	Parts used	Associated plants	Administered form	Administration route
1	*Anogeissus leiocarpus*	Godoli	Combretaceae	Bks		De	Or
2	*Bombax costatum*	Bouboli	Bombacaceae	Bks		De	or
3	*Boswellia dalzielii*	Anakehi	Burseraceae	Bks		Wo	or
4	*Bridelia ferruginea*		Euphorbiaceae	bks		De	or
5	*Calotropis procera*	Baladi	Asclepiadaceae	rts		Wo	or
6	*Carissa edulis*		Apocynaceae	fr		Wo	or
7	*Combretum glutinosum*	Dooki	Combretaceae	brch		Fu	in
8	*Daniella oliveri*	Karlahi	Caesalpiniaceae	bks		Wo	or
9	*Detarium macrocarpum*		Caesalpiniaceae	bks		De	or
10	*Entada africana*	Fadowandoki	Mimosaceae	bks		Ma	in
11	*Guiera senegalensis*	Gelohi	Combretaceae	brch		Fu	in
12	*Harungana madagascariensis*		Clusiaceae	bks and lvs		Ma	or
13	*Khaya senegalensis*	Dalehi	Meliaceae	bks		De	or
14	*Lannea acida*	Siibihi	Anacardiaceae	bks	*Lannea acida* *Guiera senegalensis*	Ma	or
15	*Maytenus senegalensis*	Yengotehi	Celastraceae	lvs		De	or
16	*Mitragyna inermis*	Kadioli	Rubiaceae	bks		If	or
17	*Parkia biglobosa*	Narehi	Mimosaceae	bks		If	or
18	*Piliostigma reticulatum*	Barkelehi	Caesalpiniaceae	bks		Ma	or
19	*Sbksuridaca longepedunculata*	Alali	Polygalaceae	bks		De	or
20	*Terminalia collinum*	Koulahi	Combretaceae	lvs and brch		Fu	in
21	*Terminalia laxiflora*		Combretaceae	bks		De	or
22	*Trianthema portulacartrum*		Aizoaceae	lvs		n-ins	n-ins
23	*Vitellaria paradoxa*	Kareji	Sapotaceae	bks	*Bombax costatum,* *Vitellaria paradoxa*	Ma	or

bks= barks; de= decoction; lvs= leaves; fr= fruits; if= infusion; in= inhalation; n-ins= nasal instillation; rts= roots; brch= branches; ma= maceration; wo= whole organ.

Table 2
Overview of plants used as anthelmintics for the treatment of sheep and goats

N°	Scientific name	Local name (Peul)	Families	Parts used	Associated plants	Administered form	Administration route
1	*Acacia seyal*	Badehi	Mimosaceae	fr and bks		wo	or
2	*Anogeissus leiocarpus*	Godali	Combrétacées	bks		wo	or
3	*Detarium macrocarpum*		Caesalpiniaceae	rts and bks	*Detarium macrocarpum* *Khaya senegalensis*	wo	or
4	*Gardenia ternifolia*	Dingali	Rubiaceae	rts		ma	or
5	*Khaya senegalensis*	Dalehi	Meliaceae	bks		wo	or
6	*Maytenus senegalensis*	Yengotehi	Celastraceae	lvs		de	or
7	*Parkia biglobosa*	Narehi	Mimosaceae	gr		wo	or
8	*Piliostigma reticulatum*	Barkelehi	Caesalpiniaceae	bks		wo	or
9	*Securidaca longepedunculata*	Alali	Polygalaceae	rts		de	or

bks= barks; de= decoction; lvs= leaves; fr= fruits; gr= grains; ma= maceration; or= oral; wo= whole organ; brch= branches; rts= roots.

It is characterised by a rainfall of 1000-1300 mm and dominated by ferruginous soils. These localities are situated between Touboro (7°30') and Garoua (9°30'). The vegetation consists essentially of forest and wooded Sudanian savannas, open dry Sudanian forests and Sudano-Guinean savanna shrublands with high-altitude formations. Vegetation in the Guinean-Sudanian zone consists of two floristic sectors, which are separated by the 9th parallel. The southern part consists of wooded savannas and dry open forests with *Isoberlinia doka, Monotes kerstingii* and *Uapaca togoensis*. The northern half is home to the same physiognomic types, dominated by *Boswellia papyrifera, Boswellia dalzielii, Sclerocarya birrea* and *Parkia biglobosa.* The savanna shrublands with combretaceae represent anthropogenic degradation types for these formations (13).

Ethnobotanical surveys

Ethnobotanical surveys were conducted during 2007. The plants used for the traditional treatment of intestinal worms in cattle, sheep and goats and chiefly nematodes have been identified. The method described by Jovel *et al.* (16) was used. It uses interviews, conversations and questionnaires to obtain information on plants used for the treatment of parasite worms in cattle, sheep and goats. The information was obtained from 73 people aged between 20-60, including 31 farmers and 19 livestock farmers.
Plants were selected, for which the information was consistent for several livestock farmers. The plants listed were collected, harvested and identified by Mr Tsobsala, who is a botanist at the Faculty of Science of the University of Ngaoundéré. The plant samples collected were deposited at the Biology Laboratory of the University of Douala.

Results

The surveys made it possible to identify a total of 32 potentially anthelmintic plants used for traditional medicine in the Mbororo settlements. Of this total, 23 plants are used for the treatment of intestinal worms in cattle and 9 plants are used to treat the same problem in sheep and goats. For each plant mentioned, we indicated the family, scientific name and popular/local name (Peul), the plant part(s) used, the administered form and method of administration to the animal (Tables 1 and 2). It emerged that 25 plant species from 16 families are used by the Bororo people (Tables 1 and 2) and that the combretaceae family is the most widely used (16.66%). Stem bark is the most commonly used plant part (58.33%). The parts used (roots, leaves, grains, fruits) are generally administered in decoction form (31.25%) or whole (31.25%), but may sometimes be macerated (18.75%), infused (6.25%) or inhaled (9.37%). Administration to the animal is generally by the oral route (84.37%). Table 3 shows the methods used to prepare plant extracts for administration to animals. It should be noted that some plant extracts administered to sheep and goats are first mixed with the animal's food, such as cereal bran, oil cakes and sometimes rock salt. The doses are not generally defined and no undesirable secondary effects have been reported.
The most frequently used plants are *Boswellia dalzielii*, *Daniellia oliveri*, *Mytragina enermis* for cattle (Figure 1) and *Gardenia ternifolia*, *Piliostigma thonningii* and *Anogeissus oleacarpus* for sheep and goats (Figure 2).

Discussion

This preliminary study made it possible to identify 25 plant species from 16 families, which are commonly used as anthelmintics for traditional veterinary

Table 3
Preparation of plants used as anthelmintics for the treatment of cattle

N°	Scientific name	Parts used	Methods for administration to the animal
			For cattle
1	*Anogeissus leiocarpus*	bks	Boil and administer bark to animal in any quantity until cured
2	*Bombax costatum*	bks	Boil and administer bark to animal in any quantity
3	*Boswellia dalzielii*	bks	Grind barks of *Boswellia dalzielii* with those of *Khaya senegalensis*, add rock salt and administer to the animal
4	*Bridelia ferruginea*	bks	Grind, boil roots and administer to the animal
5	*Calotropis procera*	rts	Grind roots, mix with rock salt and give to the animal in any quantity until it is cured
6	*Carissa edulis*	fr	Grind the fruits, mix with cereal bran and administer to the animal for 2 days (morning, noon and evening)
7	*Combretum glutinosum*	brch	Burn the branches and make the animal inhale the smoke in any quantity
8	*Daniella oliveri*	bks	Grind the roots, add cereal brans and administer to the animal within two days (morning, noon and evening)
9	*Detarium macrocarpum*	bks	Boil the barks and administer to the animal in any quantity
10	*Entada africana*	bks	Macerate the barks for one day and make the animal drink any quantity
11	*Guiera senegalensis*	brch	Burn the branches and make the animal inhale the smoke
12	*Harungana madagascariensis*	bks and lvs	Grind the leaves in water and make the animal drink any quantity
13	*Khaya senegalensis*	bks	Boil the roots and make the animal drink the liquid (morning, noon and evening) until it is cured
14	*Lannea acida*	bks	Macerate barks of *Lannea acida* with those from *Guiera senegalensis* and make the animal drink any quantity until cured
15	*Maytenus senegalensis*	lvs	Boil the leaves and the animal drink the liquid until it is cured
16	*Mitragyna inermis*	bks	Crush the roots, soak them in warm water for several hours and make the animal drink the liquid (morning, noon and evening) until cured
17	*Parkia biglobosa*	bks	Grind the roots, soak them in water and make the animal drink any quantity of the liquid
18	*Piliostigma reticulatum*	bks	Macerate the barks for one day and make the animal drink the liquid
19	*Securidaca longepedunculata*	bks	Boil the roots and make the animal drink the liquid until it is cured
20	*Terminalia collinum*	lvs and brch	Burn the leaves and/or branches and make the animal inhale the smoke
21	*Terminalia laxiflora*	bks	Boil the barks and make the animal drink the liquid in any quantity
22	*Trianthema portulacastrum*	lvs	Grind the leaves and make the animal inhale
23	*Vitellaria paradoxa*	bks	Macerate barks of *Bombax costatum* with those of *Vitellaria paradoxa* and make the animal drink any quantity until cured
			For sheep and goats
1	*Acacia seyal*	fr and bks	Mix the fruits and ground barks with rock salt and administer to the animal until it is cured
2	*Anogeissus leiocarpus*	bks	Grind the barks and administer to the animal, in rock salt, millet bran or oil cakes
3	*Detarium macrocarpum*	rts and bks	Mix ground barks of *Detarium macrocarpum* and *Khaya senegalensis* and administer to the animal
4	*Gardenia ternifolia*	rts	Wash and grind the roots, then administer ¼ litre of the solution via the animal's mouth every day until it is cured
5	*Khaya senegalensis*	bks	Burn the barks, mix with rock salts and administer to the animal in its food until cured
6	*Maytenus senegalensis*	lvs	Boil the leaves and administer in any quantity to the animal until it is cured
7	*Parkia biglobosa*	gr	Grind the grains and mix with rock salt before administering to the animal
8	*Piliostigma reticulatum*	bks	Grind the barks and administer to the animal, either in rock or cereal brans
9	*Securidaca longepedunculata*	rts	Boil the roots and administer in any quantity to the animal until it is cured

bks= barks; de= decoction; lvs= leaves; fr= fruits; gr= grains; ma= maceration; wo= whole organ; brch= branches; rts= roots.

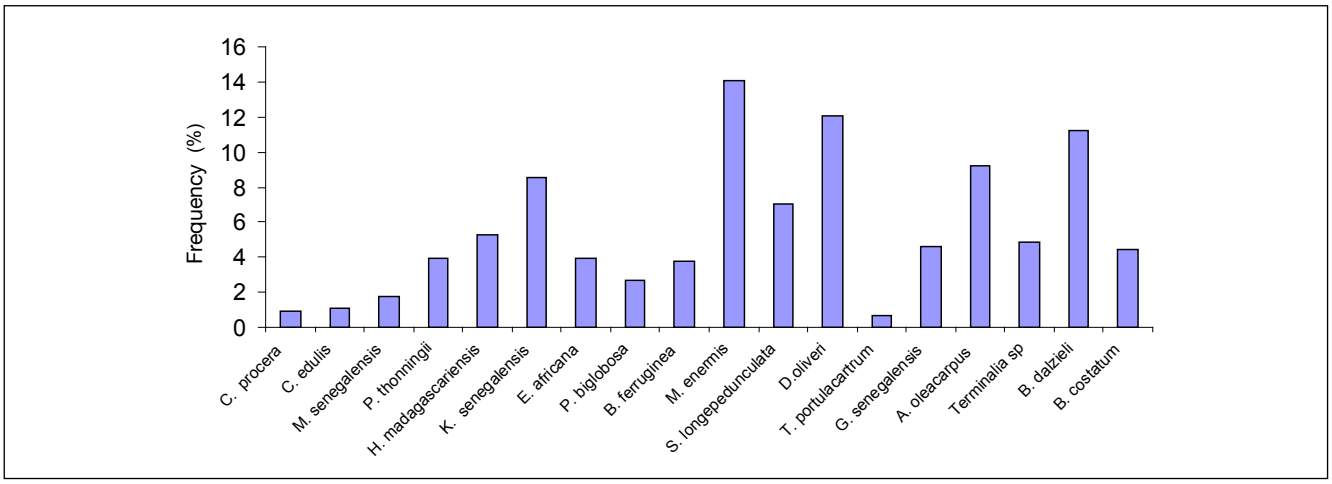


Figure 1: Frequency of response in plants used to combat cattle parasites in Northern Cameroon.

medicine by the Mbororo of Laindé Karewa and Israël in the Bénoué basin, in order to treat cattle, sheep and goats for intestinal worms. For many generations, these plants have been known to the local population as highly effective remedies and this knowledge is passed on by word of mouth between livestock farmers, who jealously guard it as family secrets (21).

Of the plant species identified, *Calotropis procera, Harungana madascariensis, Khaya senegalensis, Guiera senegalensis* and *Combretum glutinosum* were also recognised as having an anthelmintic effects on people (1). In addition, species such as *Carissa edulis, Maytenus senegalensis, Piliostigma thonningii, Entada africana, Parkia biglobosa, Bridelia ferruginea, Mitragyna inermis, Securidaca longepedunculata, Anogeissus oleocarpus, Boswellia dalzielii, Gardenia ternifolia,* and *Acacia seyal* have already been identified by Nsekuye (21) for treatment of the same parasites. The plants used to treat goats and sheep are identical to those used for cattle, except for *Gardenia ternifolia* and *Acacia seyal*.

The plants identified as being used by Mbororo livestock farmers are those used to combat intestinal worms. In Africa, and specifically in Sierra Leone, livestock farmers believe that intestinal problems are generally caused by worms (21), in other words helminths (platyhelminthes and nemathelminthes). As a parasite, nematodes are specific to a given host. When it comes to digestive or pulmonary strongyles, or even cestodes (tapeworms) in the digestive tract, the worms present in cattle are generally different from those encountered in sheep and goats. In contrast, trematodes appear to be far less restricted to a particular host (14). This tells us that the wide range of plant species used and particularly the variety of methods used to prepare extracts, for administration to cattle, sheep or goats, are due to parasite specificities and their similarities result from the treatment of similar worms (trematodes), or the wide spectrum of effects produced by these plant species.

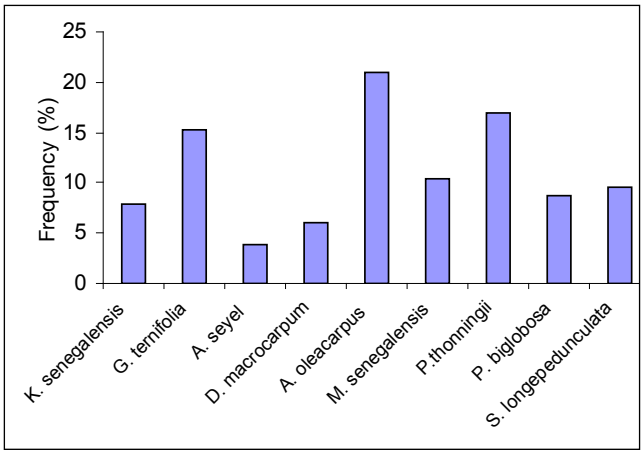


Figure 2: Frequency of response in plants used to combat sheep and goat parasites in Northern Cameroon.

The most widely used plant family for cattle are combretaceae. This family has also been observed in the treatment of helminths by the Baka pygmies in the Dja (Cameroon), a people who are known for their effective use of forest resources (5). Mimosaceae and caesalpiniaceae are used predominantly for sheep and goats.

Stem barks and decoction represent the most commonly used plant parts and preparation method. This observation confirms those made by Masika and Afolayan (18), Maphosa and Masika (17) who reported that, in the Cape region in South Africa, decoction is the preparation method most widely used by livestock farmers. Decoction is a method used to extract active substances and involves boiling part of the plant, as opposed to infusion, which means adding the plant material or plant itself to boiling water then allowing it to cool. The latter preparation method was used less by the Mbororo livestock farmers (6.25%). Decoction extracts water-soluble polar compounds. The high water temperature at the point of extraction using this method could lessen or alter the effects and even reduce the toxicity of thermolabile compounds. But the reasons for favouring decoction over other types of

preparation remain largely unknown. It would therefore be interesting to conduct a comparative study on the effectiveness of various types of preparation.
Similarly, the results show us that certain plants administered to sheep and goats are usually mixed with foodstuffs, such as cereal bran, rock salt and oil cakes, which means that the relevant organs (plant parts) are administered whole to the animals. The effects of the active substances are then influenced by the animal's food and other non-food substances (rock salt). In fact, mixing plants with the animal's food may influence the absorption of compounds contained in these plants. For example, as a fresh foodstuff, cereal bran could accelerate gastric emptying in ruminants. Due to its emulsifying properties, rock salt forms stable emulsions in the gastro-intestinal tract and also increases the solubilisation of alkaline compounds contained in the plant extracts, which thus increases their absorption. Due to their lipid value, oil cakes increase bile secretion, which in turn promotes the solubilisation of non-water soluble compounds (10). In addition, alkaline compounds are soluble if rock salt is present and could be absorbed in greater quantities.
In addition to these various preparation methods, some plants are burned before use, which means that the molecules contained in the inhaled smoke are absorbed and reach the blood circulation via the respiratory route. These preparations are almost always administered orally.
As the treatment methods used in the Mbororo settlements are empirical (traditional), it is not possible to identify the doses administered. At the same time, the effectiveness of a treatment depends on the administration of medication doses at very precise and regular intervals. It is therefore important to conduct studies aimed at standardising plant extracts, in order to make treatments more efficient and avoid problems of toxicity and resistance.
Previous phytochemical studies have shown the prevalence of secondary metabolites, such as triterpenoids (*D. oliveri, S. longipedunculata, M. senegalensis, A. leiocarpus*, etc.), flavonoids (*D. oliveri, S. longipedunculata, P. thonningii*, etc). The anthelmintic activity of these plants is therefore linked to the presence of some of these chemical compounds in the extracts, as it has been shown that pentacyclic triterpenoids (4) and flavonol glycosides (3) have anthelmintic properties. In addition, *in vitro* tests on eggs, using the method developed by Coles *et al.* (8), and on adult worms (*Haemonchus contortus)* using the method described by Hounzangbe-Adote *et al.* (15), have demonstrated the anthelmintic activity of some of these extracts (results not shown). The methanolic extract from the roots of *P. thonningii*, aqueous *D. oliveri* stem bark extract and root extract from *M. senegalensis* have shown major inhibitory effects on the egg hatching rate for *H. contortus*. At the same time, the methanol extracted from *P. thonningi* roots and that extracted from the stem bark of *S. longepedunculata* also inhibited, to a great extent, the activity of adult worms (*Haemonchus contortus)*.
It has been reported that most of the plants used by livestock farmers have other therapeutic effects, such as anti-inflammatory, immunoregulatory, analgesic, antimicrobial and purgative properties. For example, *Calotropis procera* is used for the treatment of boils and toothache; *Combretum glutinosum* is used for rheumatism (whole plant) (1); *Securidaca longepedunculata* is used for the treatment of inflammations (9).

Their use could equally be effective for the symptomatic treatment of helminthiases and for reinforcing animals' immune systems. This shows that alleviating clinical signs is also an objective for the treatment. Purgative effects help to flush out the gastro-intestinal contents, which is the desired effect when treating this type of parasite. In fact, most of the anthelmintics currently used also have purgative constituents, which expel worms (7).

Conclusion

The survey conducted in the localities of Laindé Karewa and Israël, in the Bénoué-Cameroon region, made it possible to identify 25 plants species used as anthelmintics for traditional veterinary medicine in the Mbororo settlements. Stem bark represents the most widely used plant part, generally in decoction form. The doses are not defined and no secondary or side effects have been reported. In order to complete this study, we plan:

- To continue to identify anthelmintic plants used in traditional veterinary medicine;
- To determine anthelmintic effects (*in vitro* and *in vivo*) of these plants and thus approve their use by the livestock farmers;
- To determine the most active extract fractions and isolate the molecules responsible for the activities observed.

Acknowledgements

This study was produced with financial support from the CORUS programmes (Project CORUS 6040-2). We would also like to thank Mr Tsobsala for his identification of plant species.

Literature

1. Adjanohoun J.E., Aboubakar N., Dramare K., Ebo M.E., Ekpere J.A., Enoworock E.G., Focho D., Gbile Z.O., Kamanyi A., Kamsukom J., Keila N.K., Nancy W.L., Nkongmeneck B., Sofowara A., Tamze V. & Wirmum C.K., 1996, Traditional medicine and pharmacopogia contribution to ethnobotanical and floristic studies in Cameroon. Edition CNPMS, Porto-Novo, 494-508.
2. Awa D.N., Achukwi M.D., Niba E., Manchang T.K., Wade A., Asongwed-Awa A., Dongmo A.L., 2006, Animal health in the traditional livestock

systems of North Cameroon: risk factors, health management and constraints. Working Document, IRAD/PRASAC, Garoua 12 p.

3. Barrau E., Fabr N., Fouraste I. & Hoste H., 2005, Effect of bioactive compounds from Sainfoin (*Onobrychis viciifolia* Scop.) on the *in vitro* larval migration of *Haemonchus contortus*: role of tannins and flavonol glycosides. Parasitol. 131, 531-538.
4. Begum S., Zehra S.Q., Siddiqui B.S., Fayyaz S. & Ramzan M., 2008, Pentacyclic triterpenoids from the aerial parts of *Lantana camara* and their nematicidal activity. Chem. Biodivers. 5, 1856-1866.
5. Betti J.L. & Nzooh D.Z., 1998, Les produits forestiers non ligneux (Article paru dans Canopée n° 12 – Août) chercheurs ECOFAC.
6. Chartier C. & Hoste H., 1994, Effect of anthelmintic treatments against digestive nematodes on milk production in dairy goats: comparison between high- and low-producing animal. Vet. Res. 25, 450-457.
7. Chidume F.C., Kwanashie H.O., Adekeye J.O., Wambebeand C. & Gamaniel K.S., 2002, Antinociceptive and smooth muscle contracting activities of the methanolic extract of *Cassia tora* leaf. J. Ethnopharmacol. 81, 205-209.
8. Coles G.C., Bauer C., Borgsteede F.H.M., Geerts S., Klei T.R., Taylor M.A. & Waller P.J., 1992, World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 44, 35-44.
9. Delaude C., 1992, Les Polygalaceae et leurs saponines. Bull. Soc. Chim. Belg. 61, 245.
10. Elghozi J.L. & Duval D., 1987, Aide-mémoire de pharmacologie. 1ère édition. Flammarion, Paris. P. 3-52.
11. FAO, 2002, Biological control of nematode parasites of small ruminants in Asia Final Proceedings of FAO Technical Co-operation Project in Malaysia TCP/MAL/0065 (T)
12. Faye D., Leak S., Nouala S., Fall A., Losson B. & Geerts S., 2003, Effects of gastrointestinal helminth infections and plane of nutrition on the health and productivity of F1 (West African Dwarf Sahelian) goat crosses in The Gambia. Small Ruminant Res. 50, 153-161.
13. Fondoun J.M., 1998, Situation des Ressources Génétiques Forestières du Nord Cameroun. Atelier sous-régional FAO/IPGRI/ICRAF sur la conservation, la gestion, l'utilisation durable et la mise en valeur des ressources génétiques forestières de la zone sahélienne (Ouagadougou, 22-24 sept. 1998).
14. Hoste H., Guitard J.P. & Pons J.C., 2004, Pâturage mixte entre ovins et bovins: intérêt dans la gestion des strongyloses gastrointestinales. 1 UMR 1225 INRA/DGER «Interactions Hôte Agents Pathogènes». 23 chemin des Capelles 31076 Toulouse Cedex pp. 1-12.
15. Hounzangbe-Adote M., Paolini V., Fouraste I., Moutairou K. & Hoste H., 2005, *In vitro* effects of four tropical plants on three life-cycle stages of the parasitic nematode, *Haemonchus contortus*. Res. Vet. Sci. 78, 155-160.
16. Jovel E.M., Cabanillas J. & Towers G.H.N., 1996, An ethnobotanical study of the traditional medicine of the Metizo people of Suni Mirano, Loreto, Peru. J. Ethnopharmacol. 53, 149-156.
17. Maphosa V. & Masika P.J., 2010, Ethnoveterinary uses of medicinal plants: a survey of plants used in the ethnoveterinary control of gastro-intestinal parasites of goats in the Eastern Cape Province, South Africa. Pharm. Biol., 48, 697-702.
18. Masika P.J. & Afolayan A.J., 2003, An ethnobotanical study of plants used for the treatment of livestock diseases in the Eastern Cape Province, South Africa. Pharm. Biol. 41, 16-21.
19. Ndamukong K.J.N., Sewell M.M.H. & Asanji M.F., 1986, Parasitic gastro-enteritis in sheep and goats in Bamenda, N.W. Province of Cameroon. Trop. Animal Health Prod. 2, 101-116.
20. Ndamukong K.J.N., Sewell M.M.H. & Asanji M.F., 1989, Disease and mortality in small ruminants in the North West Province of Cameroon. Trop. Animal Health Prod. 21, 191-196.
21. Nsekuye B., 1994, Traditional veterinary practice in Africa. Schriftenreihe der GTZ. 243, 42-46, 194-200, 398-405, 551-655.
22. Radostitis O.M., Leslie K.E. & Fetrow J., 1994, Health and production management for sheep. Herd Health: Food Animal Production Medicine. 2nd edition. W.B. Saunders Company.
23. Sharkhuu T., 2001, Helminths of goats in Mongolia. Vet. Parasitol. 101, 161-169.
24. Wanyangu S.W. & Bain R.K., 1994, The impact of helminths in small ruminant production in tropical Africa. The Kenyan Veter. 18,104-106.

Christelle Mirande Djoueche, Cameroonian, Undergraduate degree in Animal Organism Biology, option: Animal Physiology, 2nd year master's student in Organism Biology and Ecology (University of Liège).

A.B. Azebaze, Cameroonian, Master of Advanced of Studies in Organic Chemistry, at the University of Yaoundé I, Accreditation to supervise research, University of René Descartes-Paris V, Lecturer, Permanent Researcher, Department course leader at the University of Douala, Cameroon.

A. Dongmo, Cameroonian, State doctorate in Physiology and Phytopharmacology, accreditation to supervise research, University of Paul Sabatier Toulouse III, course leader, Associate Professor at the Department of Biology and Animal Physiology.

Phytochemical Composition and Insecticidal Effects of Aqueous Spice Extracts on Insect Pests Found on Green Beans (*Phaseolus vulgaris*) in Burkina Faso

G. Kambou[1*] & I.P. Guissou[2]

Keywords: Spices- Insects- Green bean-Yield- Burkina Faso

Summary

*This study focused on the insecticidal activity of aqueous spice extracts on pest insects (*Bemisia tabaci*,* Caliothrips impurus*,* Caliothrips occipitalis *and* Nisotra *spp.) found on green beans on farms in the Kou Valley, Burkina Faso. The pest insects were counted using transparent cylindrical cages, which were placed over 20 green bean plants per useful plot. The phytochemical analysis of aqueous extracts was based on the chemical method described by Ciulei I (1982). The spices contain chemicals (piperidine alkaloids, saponosides, anthraquinones and triterpenes), which have insecticidal properties. The differents extracts applied at the dose of 400 l/ha (100 g powder/1 l water) reduced total infestation populations of whitefly by 61-76%, thrips by 67-75% and coleoptera by 72-78%, compared to the untreated control. Aqueous extracts from black pepper (*Sinapis nigra*,* Xylopia aetiopica*), industrial tobacco (*Nicotiana tabacum*) make it possible to produce yields equivalent to those after the application of Deltamethrin and therefore represent an alternative to chemical insecticides.*

Résumé

Composition phytochimique et effets insecticide d'extraits aqueux de substances épicées sur les insectes ravageurs du haricot vert (*Phaseolus vulgaris*) au Burkina Faso

*Une étude de l'activité insecticide d'extraits aqueux de substances épicées sur les ravageurs (*Bemisia tabaci*,* Caliothrips impurus*,* Caliothrips occipitalis *et* Nisotra *spp.) du haricot vert a été réalisée en milieu paysan (Vallée du Kou) au Burkina Faso. Le dénombrement des ravageurs a été effectué à l'aide de cages cylindriques transparentes placées sur 20 plants du haricot vert par parcelle utile et l'analyse phytochimique des extraits a été réalisée par la méthode chimique de Ciulei I (1982). Les substances épicées renferment des constituants chimiques (Alcaloïdes pipéridiniques, saponosides, anthraquinones et triterpènes) à propriétés insecticides. Les différents extraits appliqués à la dose de 400 l/ha (100 g de poudre/1 litre d'eau) ont entraîné une réduction du cumul des populations infestantes de mouches blanches de 61-76%, des thrips de 67-75% et des coléoptères de 72 à 78% par rapport au témoin non traité. Les extraits aqueux du poivre noir (*Sinapis nigra*), du piment noir (*Xylopia aetiopica*), du tabac industriel (*Nicotiana tabacum*) assurent des rendements équivalents à la Deltaméthrine, constituants des alternatives aux insecticides chimiques.*

Introduction

Bemisia tabaci (Homoptera, Aleyrodidae) or whitefly cause considerable damage to vegetable crops (particularly green beans and tomatoes), which leads to significant or even total losses, due to the viruses (9, 16) that they transmit.

Losses are also caused by attacks of thrips and small coleoptera on green beans, which are an export crop for Burkina Faso.

In order to rectfiy this problem, agronomic methods used to combat pests include intercropping, biological control (8, 11, 18) by means of parasitoids, physical control (22) using cages to catch thrips and whitefly (24), while neem almond extracts have been considered (4, 13).

However, neem almonds are not easily available in all parts of Burkina Faso and a large number of farmers continue to use chemical insecticides, without always adhering to the relevant instructions due their being illiterate.

Based on the phytosanitary sampling that we conduct in vegetable crop fields every year, we have observed that onion leaves (purple Galmi variety) are not attacked by whitefly.

On the other hand, the growers themselves were producing spices, such as yellow and red chili peppers, ginger, garlic, etc for sale at market, for their families' consumption or for medicinal use.

In addition, it was considered necessary to develop a

*[1]INERA Farako-Bâ, CRREA de l'Ouest, Research Laboratory, BP 403, Dioulasso, Burkina. Faso.
Tel.: (226) 20 97 01 44/(226) 78 00 91 03 / (226) 76 88 57 34/(226) 70 39 01 76. E-mail: kambougeorges2000@yahoo.fr, Bobo-
[2]IRSS - MEPHATRA/PH - 03 BP 7192 Ouagadougou 03, Burkina Faso.
Tel.: 50 36 33 64 . E-mail: pierre.guissou@univ-ouaga.bf
Received 25.01.11 and approved for publication on 27.09.11.

control method that would be easily available to the farmers, by studying the insecticidal activity of spices on insect found on green beans, such as *Bemisia tabaci, Caliothrips impurus, Caliothrips occipitalis and Nisotra* spp. The objectives were to confirm that whitefly are repelled by the aromatic nature of the onion, to encourage growers to produce natural insecticides in response to the rising cost of synthetic pesticides and to minimise contamination risks caused by chemical insecticide residues in green bean pods, in order to meet the export quality standards required by the European Union (zero pesticide residues).

Materials and methods

Test environment

The tests were conducted on farming land with a ferruginous soil in the Kou Valley, which lies in the Sudanian zone. The green bean variety (Bravo), which is sensitive to pests, was sown in non-partitioned mounds, with 40 cm gaps between the pockets and 80 cm between lines. Two weedings were conducted on the 10th and 30th day after sowing (DAS). The recommended dose of 400 kg NPK mineral fertiliser (12.24.12) mineral fertiliser on the 14th DAS, followed by 200 kg urea/ha (100 kg/ha on the 30th day and 100 kg/ha after the 3rd harvest). The test method was a block of FISHER consisting of 10 treatments (repeated 4 times) on plots measuring 6.80 m x 4 m = 27.20 m². The spice extracts were prepared at a concentration of 100 g powder or homogenate/1 litre of water. The solutions were kept for 24 hours, filtered using muslin sheets and Adhesol was added at a concentration of 30 cc/hl. Four hundert l/ha of the extracts were applied using an OSATU regulated pressure knapsack sprayer. The various insect numbers (*B. tabaci, C. impurus, C. occipitalis* and *Nisotra* spp.) were obtained before and on the 3rd, 7th, 14th, 21st, 28th and 35th day after the extracts were applied using transparent cylindrical cages placed on 20 green bean plants and each useful plot. After each count was completed, the aqueous spice extracts were reapplied. The applications were halted 10 days after harvesting. The biological effectiveness coefficients were calculated using the following formula devised by Afanasseva *et al.* (1).

$$C = 100 \text{ X } \left(\frac{A - B}{A} - \frac{a - b}{a}\right)$$

C= Biological effectiveness coefficient (%)
A – Number of insects before application of extracts on the treated plot .
B – Number of insects after application of extracts on the treated plot.
a – Number of insects before application of extracts on the untreated control plot.
b – Number of insects on the untreated control plot after application of extracts on the treated plots.

The yield was assessed according to weight and per hectare. The data obtained was subjected to a variance analysis, followed by the Newman-Keuls test using STAT ITCF software and correlations using Origin 3.0 software.

Phytochemimcal analysis of spice extracts

Collection and treatment of samples
For this study, spice samples were collected from the market in Bobo-Dioulasso, a town in western Burkina Faso. These samples were dried at room temperature (approx. 30° C) on ventilated racks, which were sheltered from sunlight. After drying, the samples were pulverised using a mixer. The resulting powders were stored in bags suitable for phytochemical analyses.

Phytochemical analysis of samples
This involved chemical screening in order to determine the major active phytochemical groups present in extracts taken from the various samples.

The chemical screening was conducted using the qualitative method described by Ciulei *et al*. (3). The principle of this method is based on the affinity of the active substances according to their polarity and the capacity of the active substances to react with certain chemical reagents.

The presence of a substance or group of active substances is indicated by the development of a colour reaction or characteristic precipitation.

Preparation of extracts
Thirty grammes of each sample were placed in a Soxhlet cartridge. Each test sample was extracted semi-continuously from the Soxhlet with methylene chloride (DCM) and methanol diluted with distilled water according to a ratio of 80:20 v/v. The residual marc from each sample was infused with distilled water for 5 hours and filtered using absorbent cotton. The filtrates obtained were centrifuged at 2000 rpm for 5 minutes. The organic extracts were concentrated under reduced pressure in the Rotavapor and the aqueous extracts were dried in a ventilated oven at a temperature of 45-50 °C. The various extracts were stored in coloured flasks for phytochemical screening.

Phytochemical screening of extracts
The active phytochemical substances, such as flavonoids, base and salt alkaloids, sterols and triterpenes, coumarins and derivatives, saponosides and tannins were determined in the various extracts tested. For this purpose, the following characterisation tests were used. Shibata or cyanidin test, Dragendorff and Mayer, Liebermann - Burchard, Feigl's test and fluorescence at 254 - 366 nm, foam index and haemolytic test described by Paris *et al.* (17), 2% ferric chloride solution (m/v).

The qualitative assessment of the test results was

conducted by assigning the following ratings, according to the intensity of the reaction obtained: (++) = abundant, (+) = low quantity, (+/-) = trace, (-) = not detected.

Results

Phytochemical composition of spices

Phytochemical analysis of the substances studied reveals that saponosides, volatile oils, anthraquinones, triterpenes and alkaloids are present as chemical constituents (Table 1). Apart from garlic (*Allium sativum*), which does not contain saponosides, saponosides, volatile oils, sterols and triterpenes are common to all spices in abundant or significant quantities. Alkaloids are present in garlic, ginger and both types of tobacco. Tannins are present in black pepper (*Sinapis nigra*), as well as in traditional and industrial tobacco (cigarettes). Anthraquinones are present to a significant extent in the Jaune du Burkina (large yellow pepper) and virtually absent from extracts taken from other substances. The identification of active substances in the phytochemical groups, using organic solvents with different polarities, followed by fractionation and purification did not take place at this stage.

Effects of spices on the density of insect populations on green beans

As confirmed by the variance analysis, spices have influenced the density of *B. tabaci, C. impurus, C. occipitalis* and *Nisotra* spp. populations (Table 2).

With reference to whitefly, the mean effect of all products (19.46 flies/plant) is a reduction of 71.82% compared to the untreated control. Garlic, ginger, yellow pepper, red chilli, black pepper and traditional tobacco do not differ significantly from Deltamethrine. Nor is there any significant difference between spice extracts, which cause, however, a 61.72 - 76.15% reduction in whitefly infestation populations.

In terms of thrips, the mean effect of the products tested (8.18/plant) was a reduction of 73.76% compared to the untreated control (Table 2). No significant difference could be identified between extracts taken from different spices. These substances reduce *C. impurus* and *C. occipitalis* by 66.63 - 75.01%. Compared to Deltamethrin, a double – threefold increase could be observed.

The same trend could be seen in small coleoptera (*Nisotra* spp.). The mean effect of the products tested (10.87 coleoptera/plant) was a reduction of 77.15%. Deltamethrin caused a fall of 90.8% compared to the untreated control. The various spices, which are indistinguishable, caused reductions ranging from 71.8% to 77.91%. Compared to Deltamethrin, a double – threefold increase could be observed.

Biological effectiveness coefficients for spice extracts

After being evaluated using the formula described by Afanasseva *et al*. (1), compared to infestations on the untreated control, various treatments before the application of products and total infestations of treated plots after the application of products, the effectiveness coefficients for spices in terms of whitefly ranged from 46% - 69.15%, while the coefficient for Deltamethrine rose to 78.33% (Table 2).

In terms of small coleoptera, of the spices, only red chilli, black pepper, garlic and yellow peppers produced positive and low coefficients. Only Deltamethrine

Table 1
Phytochemical composition of spices

Spices	Chemical constituents					
	Alkaloids	Tannins	Anthraquinones	Saponosides	Volatile oils	Sterols and triterpenes
Garlic (*Allium sativum*)	+++	-	-	-	+++	+++
Black pepper (*Sinapis nigra)*	-	++	-	++	+++	+++
Ginger (*Zinziber officinale)*	++	-	-	++	+++	+++
Red chilli (*Capsicum fructescens*)	-	-	-	+++	++	+++
Large yellow pepper (*Capsicum* spp.)	-	-	+++	++	+++	+++
Senegal pepper (*Xilopia aetiopica*)	-	-	+/-	+++	+++	+++
Traditional tobacco (*Nicotiana tabacum*)	+++	++	-	+++	+++	+++
Industrial tobacco (*Nicotiana tabacum)*	+++	+/-	-	+++	++	+++

Key: - absent +/- doubtful presence ++ significant presence +++abundance.

Table 2
Effects of aqueous spice extracts on green bean infestations and their biological effectiveness coefficients (%)

Treatment	Total infestations (average/plant)			Biological effectiveness coefficients for extracts		
	Whitefly	Thrips	Coleoptera	Whitefly	Thrips	Coleoptera
Untreated control	69.08 a	31.17 a	47.56 a	-	-	-
Decis (Deltamethrine)	11.90 c	3.05 c	4.38 c	78.33	88.54	69.89
Garlic (*A. sativum*)	16.63 bc	8.01 b	12.33 b	69.15	82.49	15.84
Ginger (*Z. officinale*)	16.48 bc	9.86 b	12.89 b	62.60	80.80	-45.05
Yellow pepper (*C.* spp.)	19.56 bc	9.17 b	11.05 b	47.09	83.41	2.34
Red chilli (*C. fructescens*)	21.54 bc	7.79 b	10.51 b	67.95	83.50	34.54
Senegal pepper (*X. aetiopica*)	17.33 bc	8.48 b	11.14 b	64.30	97.10	-3.55
Industrial tobacco (*N. tabacum*)	26.45 bc	10.40 b	13.41 b	46.63	96.45	-28.02
Traditional tobacco (*N. tabacum*)	22.05 bc	8.59 b	10.96 b	50.31	97.81	-7.71
Black pepper (*S. nigra*)	23.22 b	8.26 b	11.23 b	61.39	94.41	26.57
Mean	24.42	10.51	14.52	N.B: The – symbol indicates an increase in pests.		
CV (%)	19.00	18.30	18.00			
RSD (ddl= 27)	4.64	10.51	14.52			
ETM (Sx)	2.32	5.26	7.26			

produced a coefficient as high as 79%. However, in terms of thrips, the spices have high biological effectiveness coefficients.
Senegal pepper (*X. aetiopica*), traditional tobaccos, industrial tobacco (*T. nicotiana*) and black pepper (*S. nigra*) have higher coefficients than Deltamethrine (94% - 97% compared to 87.5% respectively).

Effects of spice extracts on green bean yields
By modifying the infestation population of green bean pests, the application of spice extracts influenced pod quantities and green bean yields (Table 3). The mean effect of the products applied (812,340 pods/ha) represents an increase of 248.13% compared to the untreated control.
The spices caused pod increases of 208% - 296.57% compared to the untreated sample, but caused significant reductions compared to Deltamethrine.
In terms of yield factor, the mean effect of the products is an increased yield (215.51 kg/ha) of 222.78% compared to the control. Deltamethrine tripled the yield compared to the control. On average, the spices doubled the yield compared to the control. However, there is no significant difference between black pepper, Senegal pepper, industrial tobacco and Deltamethrine.

Table 3
Effects of aqueous spice extracts on green bean yields

Treatments	Quantity of pods (1000/ha)	% of control	Yield (kg/ha)	% of control
Untreated control	327.39 d	-	965.77 c	-
Decis (Deltamethrine)	1262.45 a	385.61	2995.06 a	310.12
Garlic (*A. sativum*)	682.86 c	208.83	1812.94 b	187.72
Ginger (*Z. officinale*)	726.07 c	221.78	1930.03 b	199.84
Yellow pepper (*C.* spp.)	716.07 c	218.72	2036.84 b	210.90
Red chilli (*C. fructescens*)	696.29 c	212.88	1748.49 b	181.05
Senegal pepper (*X. aetiopica*)	970.95 b	296.57	2350.70 ab	243.40
Industrial tobacco (*N. tabacum*)	833.50 bc	254.59	2328.69 ab	241.12
Traditional tobacco (*N. tabacum*)	660.40 c	201.72	1746.24 b	180.81
Black pepper (*S. nigra*)	762.45 c	232.84	2414.60 ab	250.02
Mean	763.84		2032.94	
CV (%)	14.10		18.30	
RSD (ddl= 27)	107.63		372.36	
ETM (Sx)	53.82		186.18	

Discussion

The antimicrobial activity of certain spices has been proven by authors such as Benkeblia (2), Erdogan *et al*. (6), Mahadi *et al.* (14) and Nikolic *et al.* (15), while their anti-carcinogenic activity has been demonstrated by Ejaz *et al.* (5) and Thomson and Ali (23).

Chemical analyses conducted using gas chromatography or mass spectrometry by Erdogan *et al.* (6) and Qin *et al.* (19) reveal that the active compounds contained in spices are found in the volatile oils. Alkaloids, aldehydes, ketones, esters and hydrocarbons are the main examples. In 1995, Robert *et al.* (20) showed that extracts of *Nicotiana gossei* controlled whitefly. Stephen *et al.* (21) confirmed that sugar esters from *Nicotiana glutinosa* 24, *N. langsdorffii* and *N. trigonaphylla* are biologically effective when applied to *B. tabaci*. Esters from *N. gossei* at a concentration of 10 at 0.05 mg/ml lead to high nymph mortality on *B. tabaci* (Gennadius) (Homoptera: Aleyrodidae).

In our study, the variations in yield were due to those seen to affect the population densities of green bean pests following the application of extracts, as witnessed by the different correlations determined between them and the yield (Figures 1, 2, 3).

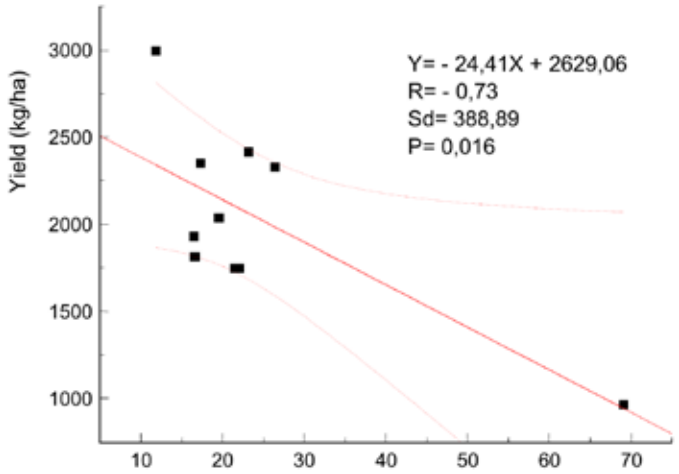


Figure 1: Correlation between thrips infestation and bean yields.

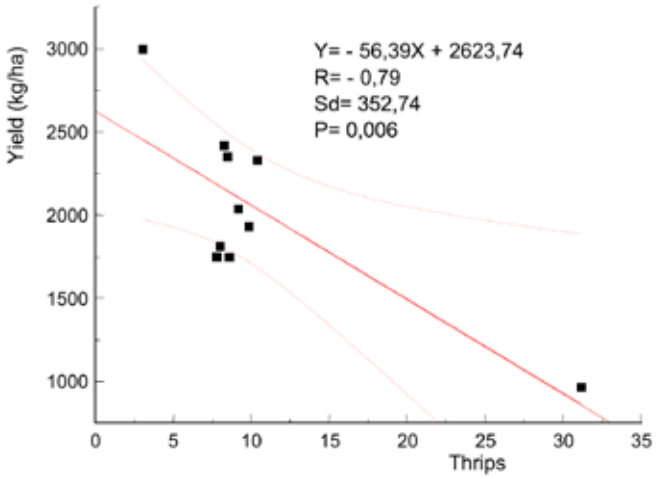


Figure 2: Correlation between thrips infestation and bean yields

The mathematical link between the total infestations of *B. tabaci* and the green bean yield is expressed by the following ratio: y= -24.41x + 2629.06 with r= - 0,73 (p= 0.016), that of thrip infestations and yield by y= -56.39x + 2623.74 with r= - 0.79 (p= 0.006) and that of coleoptera by the following regression equation: y= -35.44x + 2548.47 avec r= - 0.78 (p= 0.008).

This confirms the insecticidal effects produced by the chemical constituents contained in spice extracts. Piperidine alkaloids, tannins, anthraquinones, saponosides, sterols and triterpenes are chemical groups that are likely to have pesticidal properties (insecticides, vermicides, molluscicides, fungicides).

The high biological effectiveness of aqueous spice extracts on adult forms of whitefly (*B. tabaci*) and thrips (*C. impuris* and *C. occipitalis*) leads us to believe that some constituents have repellent properties. The low biological effectiveness on small coleoptera is probably linked to their protective shell, which hinders the penetration of extracts.

The yields produced using black pepper (*S. nigra),* Senegal pepper (*X. aetiopica)* and industrial tobacco are probably linked to the abundance of saponosides, volatile oils, sterols and triterpenes in these extracts. In addition, black pepper and tobacco appear to contain tannins, whose oxidant properties have been described by Field and Lettinga (7). Tobacco also contains alkaloids.

These factors have therefore helped make it possible to produce equivalent yields to those achieved when synthetic insecticides, such as Deltamethrine, are applied.

Conclusion

Spice extracts produce high biological activity on whitefly (*B. tabaci*), thrips (*C. impurus* and *C. occipitalis*) and low biological activiy on small coleoptera (*Nisotra* spp.). Applied at a concentration of 200-400 l/ha on a weekly basis, if attacks are observed, extracts of

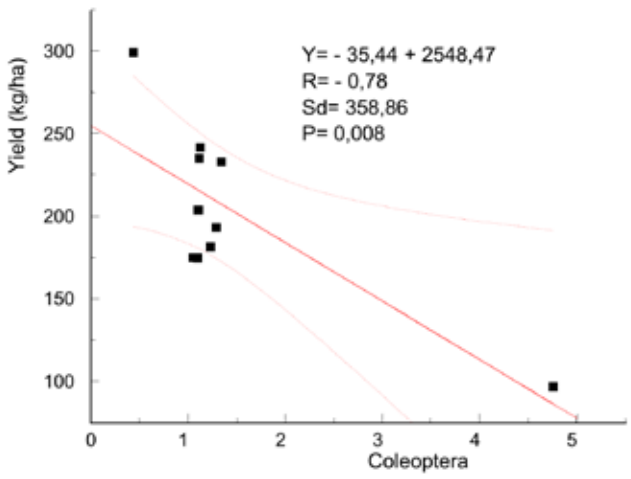


Figure 3: Correlation between coleoptera population density and bean yields

black pepper (*S. nigra*), Senegal pepper (*X. aetiopica*) and industrial tobacco make it possible to double green bean yields without harming the environment or consumer health.

Literature

1. Afanasseva A.I., Gruzdiev G.C, Dimitriev L.B., Zintchenko V.A., Kalinin B.A. & Slotsov R.J., 1983, Practical manual of plant protection chemistry. Kolos, 226-227.
2. Benkeblia N., 2004, Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). Lebensmittel Winenschaft und- technologie, **37**, 2. 263-268.
3. Ciulei I., 1982, Methodology for analysis of vegetable drugs. Practical manuals on industrial utilization of medicinal and aromatic plants. Edited by the Ministry of chemicals Industry, Bucharest. 73 p.
4. Coudriet D.L., Prabhaker N. & Meyerdirk D.E., 1985, Sweet potato white fly (Homoptera; Aleyrodidae). Effects of neem seed extract on oviposition and unmature stages. Environmental entomology. V. **14**, 6, 776-779.
5. Ejaz S., Woonug L.C. & Ejaz A., 2003, Extract of garlic (*Allium sativum*) in cancer chemoprevention. Eksperimentalnaya ophologiya, **25**, 2, 93-97.
6. Erdogan F. & Daniel Y.C., 2004, Antimicrobial activity of spices. Journal of rapid methods and automation in microbiology, **12**, 1, 1-55.
7. Field J.A & Lettinga G., 1989, The effect of oxidative coloration on the methanogenic toxicity and anaerobic biodegradability of phenols. Biological Wastes, 29, 161-179.
8. Goolsby J., Ciomperir M.A., Legaspi B.C. Jr., Legaspi J.C. & Wendel L., 1998, Laboratory and field evaluation of exotic parasitoïds of *Bemisia tabaci* (Gennadius) (Biotype ''B") (Homoptera; Aleyrodidae). *In:* the lower Rio Grande Valley of Texas. Biological Control, 12, 127-135.
9. Honda Y., 1986, Mung bean Yellow Mosaic Virus. Ann. Phytopath. Soc. Japan. 52 , 553 p.
10. Jorge A., Marbot R., Rosado A. & Batista A., 2004, Chemical composition of the essential oil of *Zinziber officinale* roseoe L. from Cuba. Journal of essential oil research, **16**, 3, 186-188.
11. Legaspi J.C., Legaspi B.C. Jr., Carruthers R.I., Goolby W.A.J., Kirk A.A., Moonaw C., Poprawski J.J., Ruiz R.A., Talekar N.S. & Wacek D., 1996, Foreign exploration for natural enemies of *Bemisia tabaci* from south east Asia. Subtropical Plant Science, 48, 43-48.
12. Legaspi J.C., Nordlund D.A. & Legaspi B.C. Jr., 1996, Trophic interactions and predation rates in *Chrysoperea* spp. Attacking the silverleaf white fly. Southerwestern-Entomologist, **21**, 1, 33-42.
13. Leskovar D.T. & Boales A.K., 1996, Azadirachtin. Potential use for controlling lepidopterous insects and increasing marketability of cabbage. Hortscience, **31**, 3, 405- 409.
14. Mahady G.B., Pendland S.L., Yun G.S., Lu C.Z. & Stoia A., 2003, Ginger (*Zenziber officinale* Roseoe) and the gingerols inhibit the growth of cag at strains of *Helico bacter* pylori. Anticancer Research, **23**, 5A, 3699-3702.
15. Nikolic V.D., Stankovic L.B., Cvetkovic D.M. & Skala D.U., 2004, Anti microbial effect of raw garlic *Allium sativum* L. extracts, garlic powder and oil, and commercial antibiotics on pathogen microorganisms. Serbia an Montenegro Hemijka industria, **58**, 3, 109-113.
16. Nene A., 1972, Survey of viral diseases of pulse crops in Lettar Pradesh. Res. Bull. N°4. 191 p.
17. Paris R.R. & Moyse H., 1976, Précis de matière médicinale. Ed. Masson. Tome 1, 420-421.
18. Poprawski J.J., Legaspi J.C. & Parker P.E., 1998, Influence of entomopathogenic fungi on *Serangium parcesetosum* (Coleoptera: Coccinellidae) an important predator of white flies (Homoptera: Aleyrodidae). Environnemental Entomology, **27**, 3, 785-795.
19. Qin H., Lin C., Jiang L., Zhong Y. & Huang K., 2004, The GC/MS analysis of the chemical components of oil from *Capsicum*. *In*: Proceeding of the international conference on separation science and technology, 4th, Nanming. China. Editors: Tong Zhangha, Kim Sung Hyun. World science publishing Co. Pte Ltd Singapore. 485- 486.
20. Robert M., Hall E.K., Lambert A.L. & Herzog G.A., 1995, Effects of a natural pesticide from *Nicotiana gossei* on controlling white flies (Homoptera: Aleyrodidae) on a susceptible and resistant soybean line. Journal of Entomological Science, **30**, 3, 312-317.
21. Stephen F., Chortyk O.T. & Stephenson M.G., 1996, Sugar esters from *Nicotiona* species as potential insecticides against the sweet potato white fly (Homoptera: Aleyrodidae). Journal of Entomological science, **31**, 3, 331-339.
22. Swanberg K., 1995, The Kenya green bean revolution. Horticultural trade journal, 3, 3-4.
23. Thomson M. & Ali M., 2003, Garlic (*Allium sativum*): a review of its potential use as anticancer agent. Current cancer drug targets, **3**, 1, 67-81.
24. Toure G.K., Foster R., Thera M. & Diarra M., 2001, Development of disease and used pests on green beans. *In:* Eigh annual report of the Integrated Pest Management Collaborator Research Support Program (IPM CRSP). 500 p.

G. Kambou, Burkinabe, Ph.D in Ecotoxicology from the Stavropol State Agricultural Academy (USSR). Fulbrighter. Michigan State University (U.S.A.). Research associate at INERA (Environment and Agricultural Research Institute). Head of the Vegetable, Fruit and Tuber Crop (CMFPT) Programme. Ministry of Secondary, Higher Education and Scientific Research.

I.P. Guissou, Burkinabe, Doctor of Pharmaceutical Sciences. Specialist subject: pharmacology. DUESS in Toxicology. Professor at the University of Ouagadougou, Head of the Pharmaceutical Sciences Department, research associate of the IRSS (Institute of Health Science Research), head of the U/Pharma production unit. Ministry of Higher Education and Scientific Research.

Refinement of an *in vitro* Culture Technique for the Rescue of Globular Embryos Using Microcutting for *P. vulgaris* L. and *P. coccineus* L.

Eugénie Barikissou[1]* & J.-P. Baudoin[1]

Keywords: *Phaseolus*- Embryo culture- *In vitro* regeneration- Microcutting- Activated charcoal

Summary

Interspecific crosses between Phaseolus vulgaris *L. and* P. coccineus *L.(♀) almost always lead to embryo abortions at the globular stage. It is possible to rescue them using the pod culture technique, but this produces a very low regeneration rate (2.8%) due to poor root formation. In order to improve the regeneration of* P. coccineus *(♀) x* P. vulgaris *hybrid embryos, various tests were conducted with the aim of developing a micropropagation technique using microcuttings from cotyledonary nodes. The influence of the mineral composition of the culture medium and the addition of activated charcoal were evaluated as a means of promoting rooting. These tests served to evaluate the influence of the mineral composition of the culture medium and the addition of activated charcoal to the latter, in terms of encouraging rooting. The reaction of various genotypes to this technique was also evaluated. The use of a modified MS medium made it possible to produce plantlets with roots from 93% of cultivated explants of the NI16 variety of* P. coccineus*, while no regeneration took place when the plantlets were cultivated using a modified B5 Gamborg medium. It was possible to acclimate all plantlets produced on the modified MS medium. Micropropagation using microcuttings is more effective with* P. coccineus *(73.33% regeneration) than* P. vulgaris *(± 50%). The activated charcoal found in the modified MS medium made it possible to double the rate of regeneration from microcuttings in* P. vulgaris. *Pod culture combined with micropropagation using microcuttings from cotyledonary nodes achieved better results than regeneration methods using only pod culture. This new protocol opens up a new and promising method of rescuing embryos that would otherwise abort at the globular stage following interspecific hybridization using* P. coccineus *as the female parent.*

Résumé

Amélioration d'une technique de culture *in vitro* pour le sauvetage d'embryons globulaires par micro-bouturage chez *P. vulgaris* L. et *P. coccineus* L.

Les croisements interspécifiques entre Phaseolus vulgaris *L. et* P. coccineus *L.(♀) se soldent presque systématiquement par des avortements d'embryons au stade globulaire. Le sauvetage de ceux-ci est possible par culture de gousses mais avec un taux de régénération très faible (2,8%) suite à une mauvaise formation des racines. En vue d'améliorer la régénération d'embryons hybrides* P. coccineus *(♀) x* P. vulgaris*, différents essais visant à mettre au point une technique de micro-bouturage de nœuds cotylédonaires ont été réalisés. Ces essais ont porté sur l'évaluation de l'influence de la composition minérale du milieu de culture et de l'ajout de charbon actif dans celui-ci pour favoriser l'enracinement. La réaction de différents génotypes à cette technique a également été évaluée. L'emploi d'un milieu MS modifié a permis la production de plantules présentant des racines à partir de 93% des explants cultivés de la variété NI16 de* P. coccineus*; alors qu'aucune régénération de plantules n'a pu être obtenue en cas de culture des explants sur un milieu B5 de Gamborg modifié. Toutes les plantules produites sur le milieu MS modifié ont pu être acclimatées. Le micro-bouturage est plus efficace chez* P. coccineus *(73,33% de régénération) que chez* P. vulgaris *(± 50%). Le charbon actif dans le milieu MS modifié a permis de multiplier par deux le taux de régénération par micro-bouturage chez* P. vulgaris*. La culture de gousses combinée avec le micro-bouturage de nœuds cotylédonaires a permis d'obtenir des taux de régénération meilleurs que la culture de gousses seule. Ce nouveau protocole ouvre une nouvelle voie prometteuse de sauvetage des embryons qui avortent au stade globulaire lors des hybridations interspécifiques utilisant* P. coccineus *comme parent femelle.*

Introduction

Phaseolus vulgaris L. (common bean) is a leguminous crop that originated in Central and South America (10). It has the advantage of being a source of proteins (22% of seeds), cheap and contributing to food safety in high altitude regions in southern hemisphere countries (8). However, its average yield per hectare

Tropical Phytotechnology and Horticulture Unit, Gembloux Faculty of Agro-Bio Technology of the University of Liège, Passage des Déportés, 2 – B-5030 Gembloux, Belgium.
E-mail: ebarikissou@yahoo.fr / ebarikissou@student.ulg.ac.be Tel.: +32 (0) 499172856 / +32 (0) 81 62 21 10.
Received on 30.09.11 and accepted for publication on 04.10.11.

in 2008 was lower in Africa (0.61 t/ha) than in North America and the European Union (2 t/ha and 1.7 t/ha, respectively) (11). Biotic and abiotic constraints limit yields (18), particularly for small farmers in Africa and South America. Crossing *P. vulgaris* with two species from its secondary gene pool, *P. coccineus* L. and *P. polyanthus* Greenm., make it possible to improve its rusticity and resistance to diseases, but early abortions can be observed, especially if the common bean is used as a male parent (2, 6, 16). Embryo culture makes it possible to obtain hybrids from embryos at the late heart-shaped and cotyledonary stages (4, 9). In the majority of cases, abortion takes place at globular stage (23). Pod culture leads to the full development of globular embryos with *P. vulgaris* (NI 637 variety), but absence of root formation and stalled development of germinated embryos limits the proportion of growing plantlets to 2.8% of the total number of embryos produced by pod culture (14, 15). *In vitro* regeneration using explants other than the embryo has been reported in *P. vulgaris* and *P. coccineus* (3, 36, 37), using microcuttings from cotyledonary nodes from germinated seeds. However, this process is more effective with *P. coccineus* than *P. vulgaris* (36). The importance of the mineral solution (MS) (28) has been shown during regeneration from microcuttings of cotyledonary nodes with two *P. vulgaris* cultivars ("Lodino" and "Bico de Ouro") (3). On the other hand, the salts in the B5 medium developed by Gamborg *et al.* (12) are better than those found in the MS (28) used for regeneration from microcuttings of cotyledonary nodes with other *P. vulgaris* cultivars (Apetito G13637, Flor de Mayo Anita, ICA Palmar G4523 and Pinto Saltillo) (33). This difference could be explained by the existence of an interaction between the genotype and mineral composition of the microcutting medium for cotyledonary nodes. The addition of absorbent substances, such as PVP-360 (polyvinylpyrrolidone with a molecular weight of 360) or activated charcoal, encouraged rooting and *in vitro* seedling regeneration for *P. vulgaris* (5, 22). Based on these results, we aimed to develop an *in vitro* regeneration protocol from globular embryo by using microcuttings from cotyledonary nodes isolated from the stunted shoots found on *P. vulgaris* and *P. coccineus* germinated embryos. As part of this research, we tested the composition of the mineral solution used as a culture medium, the genotype and use of activated charcoal, for the regeneration of plants by micropropagation of microcuttings from cotyledonary nodes.

Materials and method

1. Plant material

The research was conducted at the Tropical Crop Husbandry and Horticulture Unit of the Gembloux Faculty of Agro-Bio Tech (GxABT) of the University of Liège. The *P. vulgaris* (NI 637 and X 484) and *P. coccineus* (NI 16) genotypes cultivated, which were used as plant material, were provided from the *Phaseolineae* collection maintained at GxABT – University of Liège.

2. Conditions for the culture of mother plants

The seeds are made to germinate in the dark in standard Petri dishes (Ø 9 cm), with 4-6 seeds in each dish. After 3-5 days of incubation, the germinated seeds are transferred to 1-litre pots, containing a mixture of 80% soil, 15% peat and 5% Rhine sand, to which 5-6 g of organic fertiliser are added (NPK)[1]. For 1-2 weeks, these pots are placed in conditioned cells until at least two trifoliate leaves appear and under the following climatic conditions: a day/night temperature of 24/20 °C, a relative humidity of ± 75%, a 12/12 hour day/night photoperiod with a light intensity of approx. 170 µmol. m^{-2}. s^{-1}. This measurement is taken 60 cm from the glass that separates the plants from 400-watt lamps. The young plants are then transferred to large polyethylene bags (3 L) containing the same substrate as the pots and placed in a larger growth chamber with identical climatic characteristics to those of the cells. The plants are watered twice per week with tap water, until they flower, which takes place after ± 30 days of culture for *P. vulgaris* and ± 45 days for *P. coccineus*. After flowering, nutrient solution is added each month (29).

Shoots are obtained from the parental genotypes after natural self-fertilisation for *P. vulgaris* or self-fertilisation for *P. coccineus*.

3. Pod disinfection

The freshly harvested pods are disinfected by immersion in an ethanol solution at 70° for 30 seconds, followed by calcium hypochlorite at 15 $g.L^{-1}$ for 1-3 minutes. They are then rinsed three times in sterile distilled water, in order to eliminate any trace of the disinfectant product. The part of the peduncle bleached by calcium hypochlorite is then removed prior to pod culture, in order to prevent the disinfectant spreading to the embryos.

4. Culture media

The germinated embryos, from which the cotyledonary nodes used as microcuttings are removed (Figure 1), were obtained from the young heart-shaped embryos isolated from pre-cultivated pods *in vitro*. For this purpose, the young pods (aged 5 days after pollination (DAP) for *P. coccineus* and 2 DAP for *P. vulgaris*), harvested from the plants and containing globular embryos, were cultivated in three media of decreasing molarity ($P_0$0 at 580 mosm, $P_0$1 at 450 mosm and $P_0$1 at 350 mosm) for one week, using the media described by Phillips *et al*. (31) and modified by Geerts (15). The young heart-shaped embryos isolated from pre-cultivated pods continue to develop until they

[1] Organic fertiliser NPK 6-3-12 + 2 MgO.

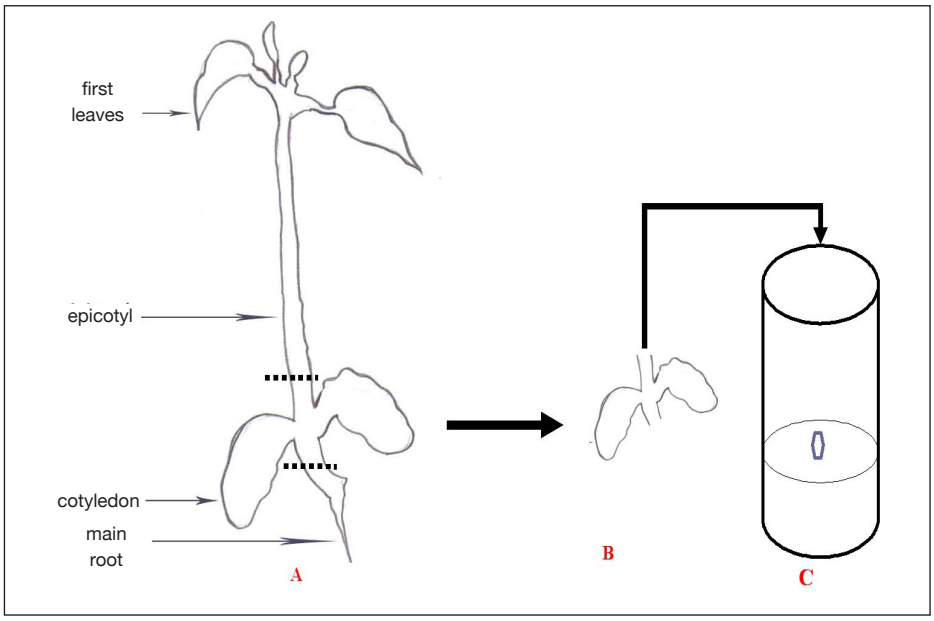


Figure 1: Schematic representation of microcutting.
Isolation of the cotyledonary node (A) followed by its transfer (B) to the culture tube containing a microcutting medium (C).

become germinated embryos (which should develop into plantlets) after being transferred successively to G1 media for maturation and germination (27) modified by the extraction of BAP, G6 for dehydration (19), G7g for root induction (15) and G7c for rooting or plantlet development (13, 15). These 4 culture media are prepared using the B5 mineral solution described by Gamborg *et al*. (12).
The appropriate culture medium for microcutting[2] was identified by evaluating the B5 mineral solution described by Gamborg *et al*. (12) in the G7c medium (13) and that of the MS (28) in a modified MS medium. The two media differ in terms of mineral salt composition, but are identical considering their vitamin, sucrose and hormone content and type of Agar used (13).
All the media were sterilised by autoclave at 121 °C for 20 minutes.

5. *In vitro* culture conditions

Conditions for the culture of pods and embryos isolated from the ovule have already been described in previous studies (13, 15, 27). For microcutting, culture tubes containing microcuttings and covered with transparent corks are placed in an incubator (Luminincube) under the following climatic conditions: temperature of 24 ± 2 °C, relative humidity of 100% inside the tubes, a 12-hour photoperiod and a light intensity of 60 $\mu mol.m^{-2}.s^{-1}$. These tubes are kept in the luminincube until the explants show vegetative growth.

6. Experimental protocol

6.1. Influence of the mineral solution

The culture of pods (13) harvested at 5 DAP was conducted with *P. coccineus* NI 16. This harvesting period corresponds to globular embryos (2). After one week in the G7c rooting medium, the explants are extracted from the tubes in order to remove the cotyledonary nodes (or microcuttings) (Figure 1). The microcuttings are cultivated separately in culture tubes using modified MS medium or G7c medium. After approx. 4 weeks of culture (the required time to regenerate a plantlet with one or more stems, at least one trifoliate leaf and many adventitious roots, each of the *in vitro* plants is transferred to a pot (Ø 6 cm) in order to be acclimated (27).
For each of the media tested, trial is made of 4 replicates and 55 microcuttings per replicate. The regeneration rates for *in vitro* plantlets and plants in acclimation are determined as a percentage of growing plantlets found in the total number of microcuttings transferred to tubes and the proportion of developed plants found in the total number of *in vitro* plants transferred to pots (Ø 6 cm), respectively.

6.2. Influence of the genotype

After the first microcutting test conducted using embryos of the NI 16 variety, a second test was conducted with two cultivated *P. vulgaris* genotypes (NI 637 and X 484). In this case, the pods are harvested 2 DAP, as this time corresponds to the globular stage of the embryos (16, 23). The *P. coccineus* genotype NI 16 acts as a control. After the cultivation of pods (15), all the microcuttings were transferred to the modified MS medium.
For each genotype, trials with three replicates were conducted with an average of 16 - 55 microcuttings per replicate according to the genotype. The numbers of plantlets generated *in vitro* are determined.

[2] The term “microcutting”, as used in this paragraph and the rest of the document, refers to the microcutting of cotyledonary nodes.

6.3. Influence of activated charcoal

On the basis of the immature heart-shaped stage reached by the embryos isolated from the pre-cultivated pods, we began this test with the culture of isolated young heart-shaped embryos (13). This makes it possible to overcome contamination problems affecting cultivated pods during this test period. For this reason, these embryos were isolated from the freshly harvested pods between 7-8 DAP for X 484 of *P. vulgaris* and between 9-10 DAP for NI 16 of *P. coccineus* (control). The isolated microcuttings (Figure 1) were transferred to tubes containing the modified MS medium, in the presence or absence of activated charcoal (0,5%). For each treatment, tests with five replicates were conducted on 50 microcuttings per replicate. The effectiveness of the activated charcoal was assessed according to the rooting rate and number of regenerated plantlets. The method used to determine the number of regenerated plantlets has already been described above. The rooting rate is determined according to the percentage of explants that developed adventitious roots with or without forming leafy stems.

7. Statistical analysis of results

The average variables observed are determined from the 3 to 5 replicates per trial, using Minitab software (version 14). The statistical analyses of the results are completed using the SAS programme (ANOVA) and the averages are classified using the Newman and Keuls test with a threshold of 5%.

Results

1. Influence of the mineral solution

The test used to determine the influence of the mineral composition of the modified MS medium and G7c medium on the success of the microcutting was conducted using cotyledonary nodes from embryos of the NI 16 variety of *P. coccineus*. Two to three weeks after the microcuttings were first cultivated, 100% bud break was observed in the latter with both media. For 204 out of 220 microcuttings (± 93%), the broken buds in the modified MS medium continued to grow and develop leafy and rooted stems after 4 weeks (Figure 2A). However, no plantlets were regenerated on G7c. This second medium caused a brown callus to appear on the part of the cutting that was in contact with the medium (Figure 2B). All the regenerated plantlets in the modified MS medium continued to develop into adult plants (producing flowers and shoots) after 45 days of acclimation.

2. Influence of the genotype

The regeneration of some cultivated microcuttings was observed in all the genotypes tested after 4 weeks (Table 1). However, the results obtained differ according to the genotype cultivated. The percentage of plantlets regenerated with the NI 16 variety of *P. coccineus* is greater (± 93%) than that obtained with genotypes X 484 and NI 637 of *P. vulgaris* (± 77%). A brown callus can be observed on the base of the *P. vulgaris* microcuttings that failed to develop into plantlets.

3. Influence of activated charcoal

The rooting problems encountered with *P. vulgaris* prompted us to test activated charcoal in the modified MS microcutting medium. The *in vitro* rooting and

Figure 2: Development of *P. coccineus* (NI 16) microcuttings after 4 weeks in the MS (A) and G7c (B) media.

A: Plantlet developed in the modified MS (28) medium; B: Stunted shoots with the formation of a brown callus on the root pole (blue stains) obtained using microcuttings cultivated in G7c (13) medium.

Table 1
Average number of regenerated plantlets as a percentage (%) according to genotype

Genotype	Total number of explants cultivated		Average number of developed plantlets (% of total)	
	Embryos	Microcuttings	Microcuttings	Embryos
NI 16	225	165	93.03 (± 3.60) a	73.33 (± 10.7) a
NI 637	108	48	76.59 (± 6.11) b	44.45 (± 4.81) b
X 484	108	60	77.32 (± 10.42) b	55.55 (± 4.81) b

The average numbers followed by identical letters are broadly equivalent to the probability threshold P≤ 0.05. The values between brackets refer to the standard deviations from the averages calculated after 3 replicate tests with a total of 16-55 microcuttings per replicate test depending on the genotype.

Table 2
Average rooting and plantlet development rates as a percentage (%) based on the presence of activated charcoal with *P. vulgaris* (X 484) and the control *P. coccineus* NI 16

Species	Culture medium	Total number of microcuttings	Average rate as a percentage	
			Rooted explant (*)	Regenerated plantlet
P. vulgaris (X 484)	MS+AC	250	89.17 a (± 6.8)	89.17 a (± 6.8)
	MS-AC	250	52.92 b (± 8.0)	43.43 b (± 4.5)
P. coccineus (NI 16)	MS+AC	250	88.33 a (± 4.8)	82.50 a (± 9.1)
	MS-AC	250	93.75 a (± 5.3)	85.83 a (± 3.5)

The average numbers followed by identical letters are broadly equivalent to the probability threshold P≤ 0.05. The values between brackets refer to the standard deviations from the averages calculated after 5 replicate tests with 50 microcuttings per replicate. (*): Rooted explant with or without leafy stems; MS: modified medium described by Murashige and Skoog (28). AC: activated charcoal.

regeneration rates obtained with *P. vulgaris* X 484 clearly show that the presence of activated charcoal in the modified MS medium produces better results in terms of *in vitro* rooting and regeneration (Table 2).
In fact, the average rooting rate with activated charcoal (± 90%) is significantly greater than that obtained if activated charcoal is absent (± 53%). If activated charcoal is present, the plantlets obtained present a large quantity of whitish adventitious roots (Figure 3A). However, if this constituent is absent, approximately half the microcuttings stopped growing after sprouting axillary buds and are characterised by the presence of a brown callus in place of roots (Figure 3B). The results from the control, *P. coccineus* NI 16, are equivalent and satisfactory with or without activated charcoal (± 90%). However, the presence of activated charcoal caused leaf chlorosis on NI 16, indicating physiological disorders, which appear after the formation of leaves after 3-4 weeks of cultivation.

Discussion

Our results concerning the influence of the medium's mineral composition, the addition of activated charcoal and the genotype on the success rate for the regeneration of *P. vulgaris* and *P. coccineus* varieties confirm those obtained by various other authors. The MS medium is used for the regeneration of plants using explants produced from germinated *P. coccineus* seeds (37). The importance of the MS mineral solution has also been shown for regeneration from microcuttings of cotyledonary nodes isolated from the germinated seeds with two *P. vulgaris* cultivars ("Lodino"and "Bico de Ouro") (3). These authors actually achieved better *in vitro* regeneration with the complete MS, compared to the MS medium, in which the nitrogen salts are reduced by one quarter. The latter medium ultimately causes necroses to appear and the formation of a callus at the base of the microcuttings, which come into contact with the medium, as we observed with the B5 salts in the medium described by Gamborg *et al*. (12). Developing shoots were seen on the axillary buds on cotyledonary nodes, which were kept intact on

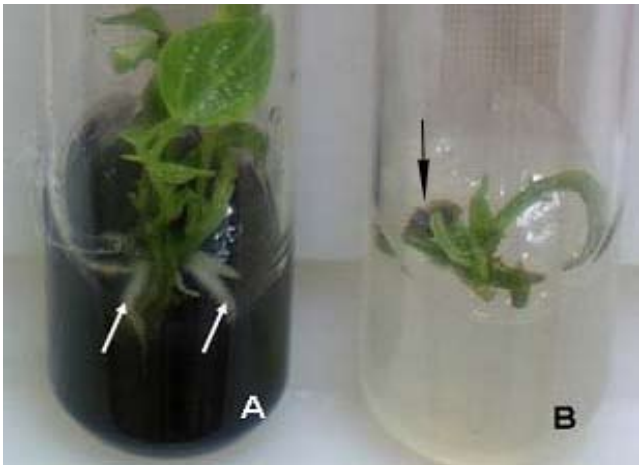


Figure 3: Plantlet (A) and explant (B) developed after 4 weeks of the microcutting of cotyledonary nodes using *P. vulgaris* (X 484), respectively, with or without activated charcoal.

A: Development of adventitious roots (indicated by white arrows) in a medium containing activated charcoal; B: Absence of regenerated plantelts without activated charcoal. The asterisk (*) indicates browning on the section of the explant, which comes into contact with the culture medium.

germinating embryos in an MS medium, using various leguminous food crops. This was the case with *Lens culinaris* Medik, taken from a culture of immature heart-shaped embryos (32), *Cajanus* (25, 26) and *Trifolium* (34, 35) taken from a culture of embryos at the mature heart-shaped or cotyledonary stages. In our test, the culture was initiated using globular embryos present in the shoots, which are cultivated *in vitro* after being removed from the plant. The sound growth of the shoots associated with the mineral salts contained in the MS medium could be linked to its nitrogen compound content (NH_4NO_3), which is higher than that of the B5 described by Gamborg *et al.* (12). The nitrogen is used for the synthesis of vital molecules for plant growth and development, such as amino acids, proteins, nucleic acids, nucleotides, chlorophylls and coenzymes (17). On the other hand, the opposite results were reported for the Flor de Junio Marcela, Apetito G13637, ICA Palmar G4523 and Pinto Saltillo *P. vulgaris* cultivars, for which plantlets could be regenerated *in vitro* from microcuttings of cotyledonary nodes (from germinated seeds) in B5 medium, but not in the MS medium (33). It is therefore possible that an interaction exists between the genotype and mineral composition of the culture medium for successful micropropagation from microcuttings. It is, however, certain that the genotype influences the success rate of plant regeneration using microcuttings. In our studies, the *in vitro* regeneration rate was significantly greater for *P. coccineus* than for the two *P. vulgaris* genotypes cultivated. The superiority of *P. coccineus* over *P. vulgaris* had already been reported in various regeneration studies focusing on cotyledonary nodes isolated from germinated seeds (36). This genotypic variation has also been reported for the regeneration of plants using direct organogenesis from the apex of the embryonic axis of 10 *P. vulgaris* cultivars (22). According to these authors, the formation of calluses depends on the capacity of each cultivar to heal, during *in vitro* culture, the wounds caused when the explant was excised. These same authors (22) were able to reduce the formation of calluses *in vitro* by optimising the composition of the culture media, mainly in terms of the appropriate cytokinin and auxin content for each cultivar. This reduced appearance of calluses contributed to the development of adventitious shoots, rooting and regeneration for all the *P. vulgaris* cultivars tested (22). However, it must be noted that, in many studies, the formation of calluses is a precursor to rhizogenesis induction, as reported for olive trees (*Olea europaea* L.) (7), cotton plants (*Gossypium hirsutum* L.) (30) and *P. vulgaris* (5). According to these authors (5), rhizogenesis only takes place when an absorbent substance, in particular PVP-360, is added to the media before rooting, i.e. the callus induction and adventitious shoot development medium. The same result is obtained if another absorbent is added - activated charcoal (22). The beneficial effects of these two absorbent substances on rooting can be explained by their ability to absorb inhibitory substances produced and rejected by the explant into the medium. We evaluated the effect of activated charcoal in the microcutting medium in terms of promoting rooting. This substance enabled us to double the regeneration rate for rooted *in vitro* plantlets to ± 90%, compared to ± 43% without the addition of activated charcoal. In this latter case, explants that fail to regenerate (± 57%) are characterised by the presence of a brown callus and the medium in direct proximity to the explant takes on a brownish colour. The beneficial effects of activated charcoal during *in vitro* regeneration have been reported by many authors, particularly in the case of explants from germinated *P. vulgaris* (22) and *Vicia faba* L. seeds (1). The absence of rooting or shoot growth is due to the production by the explant of toxic and inhibitory chemical substances, such as 4-hydroxybenzyl alcohol, which has been identified during somatic and zygotic embryogenesis in carrots (20, 21). A second effect, demonstrated with the NI 16 variety of *P. coccineus*, is that activated charcoal can cause physiological disorders by absorbing useful elements for plantlet development, such as magnesium and iron needed for chlorophyll synthesis (24). Total shoot growth inhibition after the addition of 0.5% activated charcoal to the microcutting medium has been observed during the *in vitro* regeneration of *V. radiata* L. (39). In view of the problems likely to be caused by the addition of activated charcoal (17, 38), it is vital to determine in each case the optimum concentration that should be added.

Conclusion

The large-scale regeneration of *P. coccineus* and *P. vulgaris* plants has been achieved by using microcuttings from cotyledonary nodes removed from germinated embryos obtained from a preliminary phase of pod culture. The study of the effects of the mineral composition of the culture medium on microcuttings of the NI 16 variety of *P. coccineus* has made it possible to show that a medium containing MS salts is favourable for *in vitro* regeneration via microcutting. However, this process is more effective with *P. coccineus* than with *P. vulgaris*. The cessation of growth of sprouted buds and the absence of rooting in this second species are linked to the formation of a brown callus at the base of the explants. The extent of this problem is reduced by adding activated charcoal at the dose of 0.5% to the medium. This new protocol, which combines embryoculture and micropropagation using microcuttings, has permitted to increase considerably the plantlet regeneration rate obtained by cultivating pods of the NI 637 genotype of *P. vulgaris* (15). Pod culture followed by micropropagation using microcuttings from cotyledonary nodes could be applied for the rescue of embryos produced by interspecific crosses of *P. coccineus* (♀) and *P. vulgaris*, which abort at globular stage. As the beneficial effect, which we observed,

of adding activated charcoal for *in vitro* rooting and shoot growth has not been reported systematically in studies on the use of this substance for promoting successful development of microcuttings, it is necessary to determine the appropriate concentration of this product on a case-by-case basis.

Literature

1. Abdelwahd R., Hakam N., Labhilili M. & Udupa S.M., 2008, Use of an absorbent and antioxidants to reduce the effect of leached phenolics in *in vitro* plantlet regeneration of faba bean. African J. of Biotechnol. **7**, 8, 997-1002.
2. Abid G., 2011, Molecular characterization of embryogenesis in *Phaseolus*. Thèse de doctorat: Gembloux Agro. Bio Tech. Université de Liège, Belgium.
3. Allavena A. & Rossetti L., 1983, Efforts in somatic embryogenesis of *Phaseolus vulgaris* L. Acta Hort. 131, 239-246.
4. Alvarez H.N., Asher P.D. & Davis W., 1981, Interspecific hybridization in *Euphaseolus* through embryo rescue. Hortscience, **16**, 4, 541-543.
5. Arellano J., Fuentes S.I., Castillo-España P. & Hernández G., 2009, Regeneration of different cultivars of common bean (*Phaseolus vulgaris* L.) via indirect organogenesis. Plant Cell Tiss. Org. Cult. **96,** 1, 11-18.
6. Baudoin J.P., Silue S., Geerts P., Mergeai G. & Toussaint A., 2004, Interspecific hybridization with *Phaseolus vulgaris* L.: Embryo development and its genetics pp. 349-364, *in*:, Pandalai S.G. (editor.), Recent Research Developments in Genetics and Breeding, Trivandrum, 180 p.
7. Benderradji L., Bouzerzour H., Ykhlef N., Djekoun A. & Kellou K., 2007, Réponse à la culture *in vitro* de trois variétés de l'olivier (*Olea europaea* L.). Sciences & Technologie, 26, 27-32.
8. Broughton W.J., Hernandez G., Blair M., Beebe S., Gepts P. & Vanderleyden J., 2003, Beans (*Phaseolus* spp.) model food legumes. Plant and Soil, 252, 55-128.
9. Camarena F. & Baudoin J.P., 1987, Obtention des premiers hybrides interspécifiques entre *Phaseolus vulgaris* et *Phaseolus polyanthus* avec le cytoplasme de cette dernière forme. Bull. Rech. Agron. Gembloux, **22**, 1, 43-55.
10. Chacón M.I., Pickersgill S.B. & Debouck D.G., 2005, Domestication patterns in common bean (*Phaseolus vulgaris* L.) and the origin of the Mesoamerican and Andean cultivated races. Theor. Appl. Genet. **110**, 3, 432-444.
11. Faostat. [en ligne], 2005, Consulté 07/04 2010. Disponible sur World Wide Web: <http://faostat.fao.org/site/567/default.aspx#ancor, (07/04/10)>.
12. Gamborg O.L., Miller R.A. & Ojima K., 1968, Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. **50**, 1, 151-158.
13. Geerts P., Mergeai G. & Baudoin J.P., 1999, Rescue of early heart-shaped embryos and plant regeneration of *Phaseolus polyanthus* Greenm. and *Phaseolus vulgaris* L. Biotechnol. Agron. Soc. Environ. **3**, 3, 141-148.
14. Geerts P., Sassi K., Mergeai G. & Baudoin J.P., 2000, Development of an *in vitro* shoot culture technique for young shoots of *Phaseolus vulgaris* L. *In vitro* Cell. Dev. Biol.-Plant, 36, 481-487.
15. Geerts P., 2001, Study of embryo development in *Phaseolus* in order to obtain interspecific hybrids. Thèse de doctorat: Faculté Universitaire des Sciences Agronomiques de Gembloux, Belgium.
16. Geerts P., Toussaint A., Mergeai G. & Baudoin J.P., 2002, Study of the early abortion in reciprocal crosses between *Phaseolus vulgaris* L. and *Phaseolus polyanthus* Greenm. Biotechnol. Agron. Soc. Environ. **6***,* 2, 109-119.
17. George E.F., Hall M.A. & De Klerk G.J., 2008, Plant propagation by tissue culture: Volume **1**. The Background, 501 p.
18. Hillocks R.J., Madata C.S., Chirwa R., Minja E.M. & Msolla S., 2006, *Phaseolus* bean improvement in Tanzania, 1959-2005*.* Euphytica, **150**, 1-2, 215-231.
19. Hu C.Y. & Zanettini M.H.B., 1995, Embryo culture et embryo rescue for wide cross Hybrdis pp. 129-141, *in*:, Gamborg O.L. & Phillips G.C. (Editors), Plant cell, tissue and organ culture, fundamental methods, Berlin Heidelberg, 360 p.
20. Kobayashi T., Higashi K. & Kamada H., 2001, 4-Hydroxybenzyl alcohol accumulates in suspension-cell cultures and inhibits somatic embryogenesis in carrot. Physiol. Plant, 112, 280-284.
21. Kobayashi T., Higashi K. & Kamada H., 2003, 4-Hydroxybenzyl alcohol accumulates in flowers and developing fruits of carrot and inhibits seed formation. Journal of Plant Physiol. 160, 713-716.
22. Kwapata K., Sabzikar R., Sticklen M.B. & Kelly J.D., 2010, *In vitro* regeneration and morphogenesis studies in common bean. Plant Cell Tiss. Org. Cult. 100, 97-105.
23. Lecomte B., 1997, Etude du développement embryonnaire *in vivo* et *in vitro* dans le genre *Phaseolus* L. Thèse de Doctorat: FUSAGx Gembloux, Belgique.
24. Lucena J.J., 2006, Synthetic iron chelates to correct iron deficiency in plants pp.103-128, *in*: Abadía J. & Barton L.L. (Editors). Iron Nutrition in Plants and Rhizospheric Microorganisms, Mexico, 477 p.
25. Mallikarjuna N. & Moss J.P., 1995, Production of hybrids between *Cajanus platycarpus* and *Cajanus cajan*. Euphytica, 83, 1, 43-46.
26. Mallikarjuna N., 1998, Ovule culture to rescue aborting embryos from pigeonpea (*Cajanus cajan* L. Millspaugh) wide crosses. Indian J. Exp. Biol. **36**, 2, 225-228.
27. Mergeai G., Schmit V., Lecomte B. & Baudoin J.P., 1997, Mise au point d'une technique de culture *in vitro* d'embryons immatures de *Phaseolus*. Biotechnol. Agron. Soc. Environ. **1,** 1, 49-58.
28. Murashige T. & Skoog F., 1962, A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant, **15,** 3, 473-497.
29. Otoul E. & Le Marchand G., 1974, Contribution à l'étude de l'influence de l'équilibre minéral sur la composition en amino-acides de *P. vulgaris* L. Bull. Rech. Agron. Gembloux, **9**, 1, 72-93.
30. Ozyigit I.I., Kahraman M.V. & Ercan O., 2007, Relation between explants age, total phenols and regeneration response of tissue cultured cotton (*Gossypium hirsutum* L.). Afr. J. Biotechnol. **6**, 1, 3-8.
31. Phillips G.C., Collins G.B. & Taylor N.L., 1982, Interspecific hybridization of red clover (*Trifolium pratense* L.) with *T. sarosiense* Hazsl. Using *in vitro* embryo rescue. Theor. Appl. Genet. **62**, 1, 17-24.
32. **Polanco MC.** & **Ruiz ML.,** 2001, Factors that affect plant regeneration from in vitro culture of immature seeds in four lentil cultivars. Plant Cell. Tiss. Org. Cult. 66**,** 133- 139.
33. Quintero-Jiménez A., Espinosa-Huerta E., Acosta-Gallegos J.A., Guzmán-Maldonado H.S. & Mora-Avilés M.A., 2010, Plant Cell Tiss. Org. Cult. 102, 381-386.
34. Repkova J., Jungmannova B. & Jakesova H., 2006, Identification of barriers to interspecific crosses in the genus *Trifolium*. Euphytica, **15**, 1, 39-48.
35. Roy A.K., Malaviya D.R., Kaushal P., Kumar B. & Tiwar A., 2004, Interspecific hybridization of *Trifolium alexandrinum* with *T. constantinopolitanum* using embryo rescue. Plant Cell. Rep. **22**, 9, 705-710.
36. Santalla M., Power J.B. & Davey M.R., 1998, Efficient *in vitro* shoot regeneration responses of *Phaseolus vulgaris* and *P. coccineus*. Euphytica, **102**, 2, 195-202.
37. Vaquero F., Robles C. & Ruiz M.L., 1993, A method for long-term micropropagation of *Phaseolus coccineus* L. Plant Cell. Rep. 12, 395-398.
38. Thomas D.T., 2008, The role of activated charcoal in plant tissue culture. Biotechnol. Adv. **26**, 6, 618-631.
39. Tivarekar S. & Eapen S., 2001, High frequency plant regeneration from immature cotyledons of mungbean. Plant Cell Tiss. Org. Cult. **66**, 3, 227-230.

Eugénie Barikissou, Gabonese, Master of Advanced of Studies in Agronomic Sciences and Bio-Engineering, Research Associate at the Technological Research Institute (IRT) in Libreville. Address: 8, Rue Monseigneur Heylen, 5030 Gembloux, Belgium.

J.-P. Baudoin, Belgian, Full Professor at GxABT, Head of the Department of Tropical Plant Husbandry and Horticulture, specialising in the teaching and research of phytogenetic resources, genetic plant modification, tropical plant science, applied plant biotechnology.

Insecticidal Effect of *Jatropha curcas* Oil on the Aphid *Aphis fabae (Hemiptera: Aphididae)* and on the Main Insect Pests Associated with Cowpeas *(Vigna unguiculata)* in Niger

Z. A. Habou[1,2,3], A. Haougui[4], G. Mergeai[2], E. Haubruge[1], A. Toudou[2] & F.J. Verheggen[1*]

Keywords: *Jatropha curcas* oil- Toxicity- Insect pests- Beans- Cowpeas- Niger

Summary

The insecticidal activity of Jatropha curcas *has been evaluated on various crop pests. Oil concentrations of 0.5, 1, 2.5, 5, 10 and 15% were first tested on the black bean aphid (*Aphis fabae *Scop.) as part of an efficacy test conducted in the laboratory. During a second stage, the insecticidal efficacy of 5 and 7.5% oil concentrations was evaluated on the main pests infesting cowpea crops (*Vigna unguiculata *L.) as part of a field test conducted at the University of Niamey (Niger). The results obtained in the two tests demonstrate the biocidal effect of the treatments applied, which increases with the concentration. On the black bean aphid, the biocidal effect increases during the hours following the application of oil before reaching a peak after 4 days. On cowpeas,* J. curcas *oil concentrations of 5% and 7.5% make it possible to reduce the level of attack by aphids (*Aphis craccivora*) by 10 and 50% respectively compared to the control. A 50% and 75% fall in the number of thrips (*Megalurothrips sjöstedti*) and bugs (*Anoplocnemis curvipes*), respectively, can be observed under the same conditions. This treatment made it possible to greatly increase yields compared to the untreated control.*

Résumé

Effet insecticide de l'huile de *Jatropha curcas* sur le puceron *Aphis fabae (Hemiptera: Aphididae)* et sur les principaux ravageurs du niébé (*Vigna unguiculata*) au Niger

L'activité insecticide de l'huile de Jatropha curcas *a été évaluée sur divers ravageurs des cultures. Des concentrations de 0,5; 1; 2,5; 5; 10 et 15% d'huile ont été testées sur le puceron noir de la fève (*Aphis fabae *Scop.) dans un essai d'efficacité réalisé en laboratoire. Dans une seconde étape, l'efficacité insecticide des concentrations de 5 et 7,5% d'huile a été évaluée sur les principaux ravageurs du niébé (*Vigna unguiculata *L.) dans un essai en plein champ réalisé à l'université de Niamey (Niger). Les résultats obtenus dans les deux essais mettent en évidence un effet biocide des traitements appliqués qui augmente avec la concentration. Sur le puceron de la fève, l'effet biocide augmente au cours des heures qui suivent l'application de l'huile pour atteindre un niveau maximum après 4 jours. Sur le niébé, les concentrations de 5% et 7,5% d'huile de* J. curcas *permettent de réduire le niveau d'attaque des pucerons (*Aphis craccivora*) de 10 et 50% respectivement par rapport au témoin. Une diminution du nombre des thrips (*Megalurothrips sjöstedti*) et des punaises (*Anoplocnemis curvipes*) de 50 et 75% respectivement est observée dans les mêmes conditions. Ce traitement a permis une augmentation importante du rendement par rapport au témoin non traité.*

Introduction

Jatropha curcas L. is a shrub of the Euphorbiaceae family, which originated in Central America. This succulent plant is highly resistant to drought. The *Jatropha* genus is widespread in the tropical countries (3). In several West and Central African countries, *J. curcas* is used as a means of delimiting fields, in order to protect cereal crops against the wind and grazing by animals (4). *J. curcas* seeds are rich in an oil used as biofuel, which makes this plant an important subject for research into renewable energies. In addition to its use as a biofuel, Jatropha oil can also be used as a bio-pesticide (10). In fact, several authors have tested the use of oil emulsions against insects that attack stored maize grains, *Sitophilus zeamais*, and mung beans, *Callosobruchus chinensis,* at concentrations of 0.5, 1, 2.5 and 10%. After being stored for 2 months, damage to the grains was reduced to 10% when doses of 10% and 5% were applied to *S. zeamais* and *C. chinensis,*

[1]Department of Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, Liege University, Passage des Déportés 2, B-5030 Gembloux, Belgium.
[2]Department of Tropical Crop Husbandry and Horticulture, Gembloux Agro-Bio Tech, Liege University, Passage des Déportés 2, B-5030 Gembloux, Belgium.
[3]University Abdou Moumouni of Niamey, Faculty of Agronomy, BP 10960, Niger.
[4]National Institute of Agronomic Research of Niger, BP 429, Niamey, Niger.
E-mail address for correspondence: entomologie.gembloux@ulg.ac.be, fverheggen@ulg.ac.be
Tel: 0032 (0) 81 62 22 86, Fax: 0032 (0) 81 62 23 12
Received on 04.10.11 and accepted for publication on 04.10.11.

respectively. In 2000, the same authors also tested the effect of Jatropha oil on various aggressive bio-agents affecting cotton plants (*Amrarsca biguttula*, *Aphid gossypii* and *Helicoverpa armigera*). Doses of 800 ml and 250 ml/ha were compared against commonly used insecticides (Profenofos at 400 g/ha and Deltametrine at 12.5 g/ha). Jatropha oil showed itself to be more effective than Deltamethrine on *A. gossypii,* while the opposite effect could be observed on A. *biguttula*. For *H. armigera*, synthetic insecticides were more effective than Jatropha oil at the start of treatment, as the oil affects only insect growth and its effect is therefore slower (9).

Our study focuses on the toxic effect of *J. curcas* oil on crop predators. It aims to determine the biocidal effect of different oil concentrations on black bean aphids (*Aphis fabae* Scop.) and the main pests affecting cowpea crops (*Vigna unguiculata*).

Materials and method

Jatropha curcas seeds

Jatropha curcas seeds were harvested in Gaya (Niger) by IBS Agro Industrie in October 2010. The grinding was conducted by means of a "Superior" Deklerck hammer mill with a 4 mm sieve. In order to limit the pressing temperature and based on the equipment available locally, a manual hydraulic press of the ADMGA brand from Burkinabé was used.

1. Breeding of aphids

Broad beans (*Vicia faba* L.) were used as a host plant for breeding *A. fabae*. The seeds were sown in 30 cm × 20 cm boxes, which contained a mixture (1:1) of Vermiculite and Perlite. The plants were infested with aphids at two-leaf stage. The aphids were bred under controlled conditions (22 ± 2 °C, 60 - 80% relative humidity and a photoperiod based on 16 hours of light and 8 hours of darkness).

***J. curcas* oil-based formulation**

The Jatropha oil used in the experiments was formulated using 50% oil, 30% pure ethanol as a stabiliser and 20% gum arabic as an adjuvant in order to fix active molecules on the plant. Using this standard solution, oil concentrations of 0.5, 1, 2.5, 5, 10 and 15% were prepared and the insecticidal activity of these formulations on *Aphis fabae* was evaluated and compared in the laboratory to a control (water + alcohol + gum arabic) and a positive control (insecticide: KB Multisect, dosed at 0.05 g/l of Acetamiprid, $C_{10}H_{11}Cl_N4$, systemic).

Sprayer calibration

A trigger-pump sprayer is used to conduct this test. It is suitable for the application of small quantities of specific products. The number of pressings required to wet a young bean plant was pre-determined as being equal to 7. The volume of these seven pressings is measured using a tare flask in order to determine the weight of the sprayed product. This procedure is conducted five times and the average weight of the products collected in the flask is calculated. The average obtained was 13.7 ml.

Infestation of plants and evaluation of mortality

Twenty aphids at the third larval stage were placed in each cage. The aphids were placed on the plants using a moistened brush. The spraying was conducted after the aphids were left to acclimate for one hour. The number of dead aphids was counted after 24, 48, 72, 96 and 120 hours. An aphid is considered dead if it fails to react when touched by the brush. The average mortality of the aphids (M0) is expressed as a corrected mortality (Mc), taking into account the natural mortality observed on the control (Mt) (Table 1) according to Abbott's formula (1):

$$Mc = \frac{M0 - Mt}{100 - Mt} x100$$

In order to calculate lethal concentrations 50 and 90, a binary logistic regression is produced:

(2)

$$\ln\left(\frac{p_i}{1-p_i}\right) = \beta_0 + \beta_1 X_i$$

(pi: probability, β_0 and β_1 predictors, and Xi as doses in our case).

2. Field test conducted on cowpea pests in Niger

J. curcas oil concentrations of 5 and 7.5% were compared to a negative control (water) and a positive control (Decis: Deltamethrine). The cowpea variety used is TN5-78, as selected by the National Institute of Agronomic Research of Niger (INRAN). This is an early and productive variety (cycle duration lasting 40 days and potential yield of 1.5 - 2 t/ha). The test materials consisted of a Fisher block with four repeat tests. Each simple plot is made up of 10 cowpea plants separated by 1 m between the lines and 0.5 m on the lines. The product was applied at three stages of the plants' development: initiation of flowering (35 days after sowing), 50% flowering (45 days after sowing), shoot initiation (60 days after sowing). The product is applied at floral initiation stage, 50% flowering and shoot formation using a ULV sprayer from 6 am or between 4 pm – 5 pm on 10 plants for each repeat test. The quantities of Jatropha oil applied per ha are 800 ml/ha and 1,200 ml/ha, respectively (10). The total volume of spray mixture applied is 22.5 litres/ha. Observations are made twice per day (8 am – 10 am and 4 pm – 6 pm) the day before treatment and the 3rd, 7th and 11th day after treatment. The insects are collected according to the following methods:

- Thrips: three flowers are harvested per plant and placed in a flask containing ethanol at 70° C. The number of thrips is determined using a binocular magnifier
- Bugs: the number of bugs is counted directly on the plants

Table 1
Corrected mortality of aphids

	Observation periods				
Concentrations	24h	48h	72h	96h	120h
0%	0	0	0.6 ± 0.3	0.6 ± 0.3	0.6 ± 0.3
0.50%	13.1 ± 2.4	38.1 ± 2.6	39.4 ± 2.3	43.1 ± 2.8	43.1 ± 2.8
1%	30.0 ± 2.6	38.7 ± 2.5	55.6 ± 1.7	57.0 ± 1.3	57.0 ± 1.3
2.50%	38.1 ± 2.4	50.6 ± 1.5	60.0 ± 2.9	63.1 ± 2.9	66.2 ± 1.2
5%	56.9 ± 2.3	70.0 ± 1.9	76.2 ± 1.7	78.7 ± 1.3	80.0 ± 1.1
10%	81.2 ± 1.2	83.7 ± 1.4	89.3 ± 1.3	89.4 ± 0.8	89.4 ± 0.8
15%	88.1 ± 1.3	95.6 ± 1.2	97.0 ± 1.1	100.0 ± 0	100.0 ± 0
Acetamiprid	100.0 ± 0	100.0 ± 0	100.0 ± 0	100.0 ± 0	100.0 ± 0

Table 2
Binary logistic regression

Logit	24h	48h	72h	96h	120h
Probability	p< 0.001	p< 0.001	p< 0.001	p< 0.001	p< 0.001
Constant (βo)	-1.77	-1.09	-0.84	-0.77	-0.75
Regression coefficient (β1)	0.36	0.32	0.35	0.37	0.38
Odds ratio	1.44	1.39	1.43	1.46	1.47
Wald's test (z)	14.08	12.49	12.04	11.95	11.91
Probability ratio (G)	282.282	223.95	227.51	236.65	239.95

- Aphids: a scale is used to estimate the number of aphids per leaf: 0: 0 - 50 aphids; 1: 51 - 100 aphids; 2: 101 - 200 aphids; 3: 201 - 300 aphids; 4: 301 - 400 aphids; 5: over 400 aphids

Results

1. Insecticidal efficacy of Jatropha oil on *Aphis fabae*

The corrected mortality of *A. fabae* aphids, which were subjected to different treatments of Jatropha oil is shown in table 1. The higher the Jatropha oil concentration, the greater the mortality (p< 0.001).

The mortality of the aphids varies significantly according to the *J. curcas* oil concentrations (p< 0.001).

The mortality also increases with the duration of the treatment. The number of dead aphids rises as time passes after treatment for all concentrations up to 72 hours. It becomes stable between 96 - 120 hours after treatment.

The results of the binary logistic regression are shown in table 2.

At the 5% threshold required for Tukey's test, the tests are significant, regardless of the time of observation. The (positive) regression coefficient sign β_1 shows that aphid mortality increases according to the concentrations and observation times. The odds ratio shows that, if the *J. curcas* oil concentration is increased by one unit, aphid mortality increases by 14.4% for 24 hours, 13.9% for 48 hours, 14.3% for 72 hours, 14.6% for 96 hours and 14.7% for 120 hours after treatment. Observations conducted after 24 and 48 hours provide more detailed information on the resulting regression model. Based on this binary logistic regression, 50 and 90 lethal doses were estimated (Table 3).

Table 3
Lethal concentration estimates

Hours	CL50	CL90
24h	4.85	10.86
48h	3.33	10.01
72h	2.36	8.53
96h	2.06	7.84
120h	1.95	7.61

The 50% and 90% lethal concentrations decrease according to the duration of treatment. The lethal concentration that makes it possible to kill 50% of aphids is approximately 5% after 24 hours and 3.5% after 48 hours. The lethal concentration that makes it possible to eliminate 90% of aphids is approximately 11% of *J. curcas* oil after 24 hours and 10% for 48 hours.

3.2 Insecticidal efficacy of Jatropha oil against cowpea pests in the fields

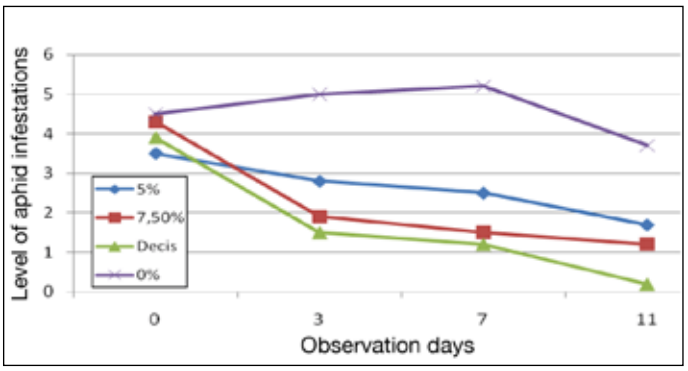


Figure 2: Increased levels of attack by aphids per plant depending on the day of observation after the treatment applied at floral initiation stage

Three categories of insect were evaluated according to development stages of the culture. The aphids were evaluated at floral initiation stage, thrips at 50% flowering stage and bugs at shoot formation stage. Observations were made at each stage: before treatment, and on the 3rd, 7th and 11th days after treatment. Statistical analysis of the data shows that the greater the dose of Jatropha oil, the greater the insecticidal efficacy of this oil ($p < 0.001$).

Increased numbers of aphids were determined depending on the days of observation (Figure 2). Before treatment, the level of aphid infestation was 3.5 – 4.5 for all the plants studied. Three days after the product was applied, reduced aphid infestation was observed with all the treatments. However, for the control sample, the level of infestation increased. Figure 2 shows how the number of thrips increases according to the day of observation.

Flowers were infested by 4 - 5 individual thrips per flower before the products were applied. A fall in the number of thrips is observed until the 11th day after treatment (Figure 3).

Bugs appeared 64 days after sowing, which coincides with the formation of shoots. A fall in the number of bugs per plant can be observed for all the treatments, except for the control sample, for which this number increases (Figure 4).

Table 4 shows average numbers of insects collected during the 3 product applications: aphids at floral initiation stage (35 days after sowing), thrips at 50% flowering stage and bugs at short formation stage. For treatments containing Jatropha oil, a fall in the number of cowpea pests can be observed compared to the control sample, together with an increased yield per plot. This reduction is proportional to the oil content of the formulation applied.

Discussion

Many studies have shown that the toxicity of *J.curcas* oil is due to the presence of phorbol esters (6). These natural organic compounds are tiglianes from the diterpene family. Tiglianes were discovered by Bohm et *al.* in 1934, but their structure was determined in 1967 by Hecker *et al.* [quoted by Mahaela (5)]. According to the model obtained using the binary logistic regression, a 5% *J. curcas* oil concentration would be needed to kill 50% of aphids (*A. fabae*) after 24 hours and 11% to eliminate 90%, whereas an oil concentration of 4% would be required to kill 50% of aphids after 48 hours and 10% to eliminate 90%. These results are comparable to those obtained by Solsoloy *et al.* (10) who tested oil emulsions against insect pests attacking stored maize grain, *Sitophilus zeamais*, and bean weevils, *Callosobruchus chinensis*.

Studies conducted by Ratnadass *et al.* (8) focusing on *Busseola fusca* and *Sesamia calamistis Hampson* (*Lepidoptera: Noctuidae*) on sorghum have shown that the raw oil extracted from *J. curcas* seeds has a larvicidal effect, at concentrations of 0.01% and 1% on *B. fusca* and *S. calamistis*, respectively, and its phorbol ester content at the concentration of 0.025% on *S. calamistis*.

For this reason, the effectiveness of Jatropha oil (1% of the nutrient medium) was compared to that of phorbol esters at 0.025, 0.05 and 0.1% added to the nutrient medium for *S. calamistis* and 0.01, 0.1 and 1% to the

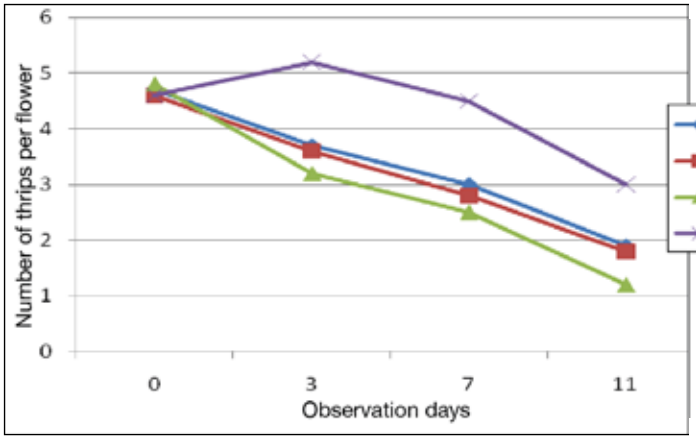


Figure 3: Increased number of thrips per plant depending on the day of observation after application of the treatment at 50% flowering stage

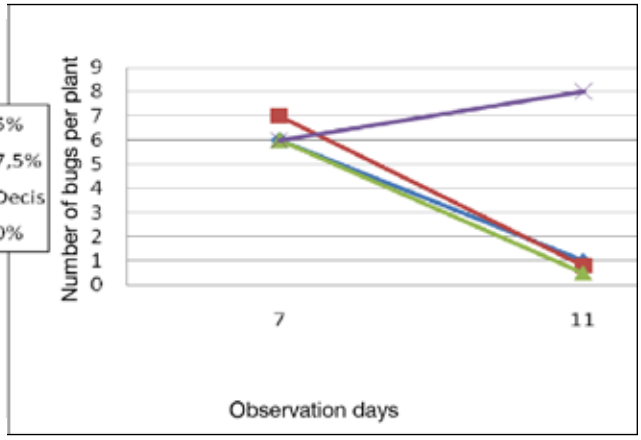


Figure 4: Increased number of bugs per plant depending on the days of observation after the treatment is applied at shoot formation stage on the 56th day after sowing.

Table 4
Average numbers of main pests and plot yields according to *J. curcas* oil concentration

Concentrations	Aphids	Bugs	Thrips	Yield in grains (g/24m²)
0%	4.16 ± 1.30a	6.26 ± 0.20a	6.90 ± 0.29a	30.05 ± 0.96d
5%	3.96 ± 2.73a	3.04 ± 0.15b	3.38 ± 0.18b	160.10 ± 4.16c
7.5%	2.08 ± 1.71b	2.07 ± 0.28c	1.34 ± 0.15c	260.53 ± 8.05b
Decis	1.67 ± 1.67bc	1.23 ± 0.24c	0.85 ± 0.17c	410.10 ± 9.52a

nutrient medium for *B. fusca*. Levels of nymphosis were zero for *S. calamistis* for all doses of the product, for *B. fusca* for treatments using 0.1 and 1.0% oil, while it was 55% for the treatment supplemented by 0.01% and 70% on the control.

Insects represent the major hindrance to cowpea cultivation in semi-arid tropical regions. Effectively combating these pests will make it possible to increase cowpea yields by 10-30% (7). In total, over 100 insect species have been identified as cowpea pests in the world, but only about ten of them have real economic importance in Niger. These pests include aphids (*Aphis craccivora)*; thrips (*Megalurothrips sjöstedti)*; bugs (*Anoplocnemis curvipes)*; stem borers; blister beetles (*Mylabris senegalensis, Coryna argentata* and *Decapotoma affinis)* and weevils (*Bruchidius atrolineatus and Callosobruchus maculatus*) (7). Our study focused on the first three pests. A *J. curcas* oil concentration of 5% makes it possible to reduce aphids by 10% and thrips and bugs by 50%. The 7.5% *J. curcas* oil concentration makes it possible to reduce aphids by 50% and thrips and bugs by 75%. At the same time, increased grain yields of 60-75% were observed when 5 and 7.5% *J. curcas* oil concentrations were used. However, other biological and physical factors may help reduce these insect populations. For example, ladybirds and heavy rain considerably reduce aphids, while blister beetles are also thrips predators (10).

Conclusion

An insecticidal effect by contact has been demonstrated for *J. curcas* oil on aphids (*Aphis fabae*) that attack broad beans (*Vicia fabae*). This toxicity increases as the dose of oil is increased. Insect mortality increases during the hours following the treatment and reaches a peak after 96 hours. *J. curcas* oil has also shown itself to be effective against cowpea (*Vigna unguiculata*) insect pests. On cowpeas, *J. curcas* oil concentrations of 5% and 7.5% make it possible to reduce the level of attack by aphids (*Aphis craccivora)* by 10 and 50%, respectively, compared to the control. A 50 and 75% fall in the number of thrips (*Megalurothrips sjöstedti*) and bugs (*Anoplocnemis curvipes*), respectively, can be observed under the same conditions. This made it possible to significantly increase grain yields compared to the untreated control sample.

Literature

1. Abbot W.S., 1925**,** A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18, 265-267.
2. Duyme F. & Claustriaux J.J., 2006, Notes statistique et d'informatique, FUSAGx, Gembloux, Belgique, 24 pages.
3. Heller J., 1996, Physic nut, *Jatropha curcas* L., Promoting the conservation and use of underutilized and neglected crops (IPGRI). Biodiversity International, (1) 66 pages
4. Henning R.K., 2008, *Jatropha curcas* L. in Africa: assessment of the impact of the dissemination of "the Jatropha System"on the ecology of the rural area and the social and economic situation of the rural population (target group) in selected countries in Africa golbal facillition for undertilizer specices, 49 pages, http://www.underutilized-pecies.org/Documents/jatropha_curcas_africa.pdf, 09/10/209).
5. Mahaela C., 2008, Approche générale et efficace des terpénoïdes possédant le squelette bicyclo [5.3.0] décane: synthèse des sesquiterpènes (Aromadendranes, guaianes et tri nor guaianes) et approche de di terpènes 173 pages, Thèse de doctorat en chimie organique, Université Joseph Fourier de Grenoble (France).
6. Makkar *et al*., 1997, Studies *on* nutritive potential and toxic constituents of different provenances of *Jatropha curcas,* J. Agric. Food. Chem. 45, 3152-3157.
7. Pollet A., 1995, Insectes ravageurs et parasites des légumineuses a graines en Afrique de l'ouest, Rapport scientifique final, ORSTOM, 83 pages.
8. Ratnadass A., Cissé B., Diarra A., Mengual L., Taneja S.L. & Thiéro C.A.T., 1997, Perspectives de gestion bio intensive des foreurs des tiges de sorgho en Afrique de l'Ouest, Insect Sci., **17,** 2, 227-233.
9. Solsoloy A.D. & Solsoloy T.S., 2000, Insecticide resistance management in cotton in the Philippines. Philippine Journal of Crop Science, 25: p. 26.
10. Solsoloy A.D. & Solsoloy T.S., 1997, Pesticidal efficacy of formulated product *J. curcas* oil on pests of selected field crops *In*: Gubitz G.M., Mithelbach M. and Trabi M., Symposium on Biofuel and Industrial Products from *Jatropha curcas* and other Tropical Oil Seed Plants, February 23-27, Managua, Nicaragua.

Z.A. Habou, Niger, Master of Advanced of Studies in Agronomy and Crop Protection from the ENSA of Thiès, Senegal. Registered for a doctorate thesis in Agronomic Sciences and Bio-Engineering at the University of Liège/Gembloux Agro-Bio Tech (Belgium) since 2009. Researcher at the National Institute of Agronomic Research, Niger. INRAN/DCI BP 429, Niamey, Niger.

Haougui, Niger, researcher at the National Institute of Agronomic Research, Niger (INRAN). Head of Plant Protection (DPV). INRAN/DCI BP 429, Niamey, Niger.

G. Mergeai, Belgian, Doctorate in Agronomic Sciences, professor at the Department of Tropical Plant Husbandry and Horticulture of the University of Liège/ Gembloux Agro-Bio Tech (Belgium).

E. Haubruge, Belgian, Doctor of Agronomic Sciences, Professor at the University of Liège, Head of the Functional Entomology Unit at Gembloux Agro-Bio Tech. Vice-chancellor of the University of Liège and President of Gembloux Agro-Bio Tech.

A. Toudou, Niger, agronomist/plant pathologist, lecturer and researcher, full professor, Dean of the Faculty of Agronomy of the University of Abdou Moumouni de Niamey, Niger.

F.J. Verheggen, Belgian, qualified chemical and bio-engineer – Faculty of Agronomic Sciences, Gembloux.
Doctorate in Agronomic Sciences and Bio-Engineering – Faculty of Agronomic Sciences, Gembloux.
First assistant at the University of Liège, Gembloux Agro-Bio Tech
Department of Functional and Evolutionary Entomology, Passage des Déportés, 2, 5030 Gembloux. E-mail: fverheggen@ulg.ac.be

AVIS DE CHANGEMENT D'ADRESSE **CHANGING OF ADDRESS**

ADRESVERANDERING **CAMBIO DE DIRECCION**

Tropicultura vous intéresse! Dès lors signalez-nous, à temps votre changement d'adresse faute de quoi votre numéro nous reviendra avec la mention "N'habite plus à l'adresse indiquée" et votre nom sera rayé de la liste.

You are insterested in Tropicultura! Make sure to inform us any change of your address in advance. Otherwise your issue will be sent back to us with the postal remarks "Addresse not traceable on this address" and then you risk that your name is struck-off from our mailing list.

U bent in Tropicultura geïnteresseerd! Stuur ons dan uw adresverandering tijdig door, anders riskeert U dat uw nummer ons teruggezonden wordt met de vermelding "Woont niet meer op dit adres" en uw naam wordt dan automatisch van de adressenlijst geschrapt.

Si Tropicultura se interesa, comuniquenos a tiempo cualquier cambio de dirección. De lo contrario la publicación que Ud. recibe nos será devuelta con la mención "No reside en la dirección indicada" y su nombre será suprimido de la lista de abonados.

Capacité de développement de trois espèces zooplanctoniques d'intérêts aquacoles (*Brachionus calyciflorus*, *Moina micrura* et *Thermocyclops* sp.) élevées en condition monospécifique en aquariums avec la fiente de volaille

H. Agadjihouede [1]*, C.A. Bonou [2], E. Montchowui[3], A. Chikou [1] & P. Laleye [1]

Keywords: Zooplankton- *Brachionus calyciflorus*- *Moina micrura*- *Thermocyclops* sp.- Development capacity- Benin

Résumé

La capacité de développement des populations du zooplancton à partir d'un ensemencement initial a été étudiée chez trois espèces zooplanctoniques, proies potentielles d'intérêt aquacole: un rotifère Brachionus calyciflorus, *un cladocère* Moina micrura *et un cyclopide du genre* Thermocyclops. *Les taux d'accroissement journaliers sont beaucoup plus élevés chez les herbivores* B. calyciflorus *(moyenne= 1,14) et* M. micrura *(moyenne= 1,07). Chez ces deux espèces, l'évolution est très rapide et marque un pic le 6ème jour avec de très grandes densités de production qui sont de 18 857 ind/L (3 582,77 µg PS/L) pour* B. calyciflorus *et de 11 323 ind/L (24 118 µg PS/L) pour* M. micrura. *Elle est plus lente chez* Thermocyclops. *La courte durée de développement exponentiel des herbivores* B. calyciflorus *et* M. micrura *favorise plusieurs cycles de production facilement contrôlables de ces espèces. Dans la perspective d'une culture des espèces zooplanctoniques pour l'élevage larvaire des poissons zooplanctonophages, le fort taux d'accroissement et la courte durée de production massive de* B. calyciflorus *et* M. micrura *peuvent être considérés comme des facteurs de leur choix.*

Summary

Development Capacity of Three Species of Aquacol Interests Zooplankton *(Brachionus Calyciflorus*, *Moina Micrura* and *Thermocyclops* sp.) Breeding in Monospecific Condition in Aquariums with Poultry Droppings

The development capacity of the populations of zooplankton from an initial sowing was studied at three species of zooplankton, potential aquacol interest preys: a rotifer Brachionus calyciflorus, *a cladoceran* Moina micrura *and a cyclopid* Thermocyclops *sp. The most daily rates of increase are obtained at* B. calyciflorus *(average= 1.14) and* M. micrura *(average= 1.07). Evolution of the populations of those two species is very fast and marks a peak the 6th day with very high densities of production which are 18 857 ind / L (biomass: 3 582.77 µg PS/ L) for* B. calyciflorus *and 11 323 ind/ L (biomass: 24 118 µg PS/ L) for* M. micrura. *But the evolution of the populations of* Thermocyclops *sp. is slow. The short duration of exponential development of* B. calyciflorus *and* M. micrura *favours several easily controllable cycles of production of these species. In the perspective of a culture of the species of zooplankton for the breeding the larvae of zooplanctonophagous fish, the strong rate of increase and the short duration of mass production of* B. calyciflorus *and* M. micrura *can be considered as their choice factors.*

Introduction

En Afrique, l'aquaculture est un élément important des politiques d'autosuffisance alimentaire longtemps préconisées, notamment dans les études de la FAO. Le développement de celles-ci permettrait de couvrir d'immenses besoins en protéines animales et réduirait la sortie massive de devises. Mais ce développement reste lié à la disponibilité de l'aliment qui est un facteur capital pour la réussite de tout élevage.

L'importance du zooplancton en général pour la nourriture des larves de poisson a été déjà prouvée à travers plusieurs études (3, 15). En dehors de l'artemia qui est très coûteux et non disponible surtout dans les zones tropicales rurales, l'utilisation d'autres proies vivantes à fort potentiel de production comme les rotifères, les cladocères et certains copépodes est moins répandue mais peut s'avérer plus facile et moins coûteuse. Dans l'étude de la possibilité de remplacer l'artemia par les espèces zooplanctoniques

[1]Laboratoire d'Hydrobiologie et d'Aquaculture/ Faculté des Sciences Agronomiques/ Université d'Abomey- Calavi, 01 BP 526, Cotonou, Bénin.
[2]Laboratoire de Recherche en Biologie Appliquée/ Ecole Polytechnique d'Abomey-Calavi/ Université d'Abomey-Calavi. 01 BP 2009, Cotonou, Bénin.
[3]Ecole Nationale Supérieure des Sciences et Techniques Agronomiques de Kétou, BP95, Kétou, Bénin.

Auteur correspondant : H. Agadjihouede; BP: 465, Abomey-Calavi - Bénin.
Tél: (00229) 97 26 57 04 e-mail: agadjihouede@yahoo.fr
Reçu le 05.07.11 et accepté pour publication le 06.10.11

locales (rotifères, cladocères et copépodes), la maîtrise des techniques simples de production de ces organismes constitue actuellement un enjeu capital dans les fermes piscicoles privées. Dans le cadre des recherches initiées à cet effet, un des objectifs importants est d'étudier la dynamique des organismes du zooplancton élevée avec la fiente de volaille. La présente étude s'inscrit dans cette perspective et est complémentaire à celle effectuée sur le développement et croissance du zooplancton produit en mélange avec la fiente de volaille (Agadjihouèdé *et al.,* soumis). Les espèces retenues sont *Brachionus calyciflorus* (rotifère), *Moina micrura* (cladocère) et *Thermocyclops* sp. (copépode). Ces espèces sont choisies en raison de leurs caractéristiques biologiques différentes d'une part et de leur abondance relative dans nos milieux aquatiques continentaux et infrastructures piscicoles d'autre part. Cette étude vise à évaluer les performances de croissance de ces organismes élevés avec la fiente de volaille en condition monospécifique afin d'établir un critère de choix d'ordre aquacole pour ces espèces.

Matériels et méthodes

L'étude repose sur des suivis d'élevage qui débutent à l'ensemencement en phytoplancton et se terminent après le maximum d'abondance de l'espèce cible de manière à couvrir la phase d'installation et de développement de cette espèce. Les trois espèces zooplanctoniques ont été élevées dans six aquariums de 0,60 m x 0,30 m x 0,30 m, pris 2 par 2. Ces aquariums sont remplis avec 20 litres d'eau (soit 16 litres d'eau de consommation locale et 4 litres d'eau d'étang filtrés sous une soie de 50 µm pour ensemencer le milieu en phytoplancton). Les aquariums sont ensuite fertilisés avec la fiente de volaille sèche tamisée à raison de 600 g/m^3. Le mélange a été laissé pendant 72 heures pour permettre au phytoplancton de se développer avant d'ensemencer chaque type de zooplancton. Les densités de mise en charge sont de 200 ind/L pour *B. calyciflorus,* 115 ind/L pour *M. micrura* et de 90 ind/L pour *Thermocyclops sp.* Ces différences espèces sont retenues à cause de leurs abondances relatives dans nos structures d'élevage (étangs, bassins).

Collecte des données

Paramètres physico-chimiques et nutritifs de l'eau

Les paramètres de l'eau tels la température, le pH et l'oxygène dissous sont relevés chaque jour.

Les sels nutritifs (N-NO3, P-PO4) et la chlorophylle-a ont été dosés tous les 3 jours sur des échantillons d'eau de 100 ml prélevé par aquarium après homogénéisation du milieu d'élevage. Ces échantillons d'eau prélevés sont filtrés sous une soie de 55 µm pour être débarrassés du zooplancton et sur filtre Whatman GF/C pour le dosage de la chlorophylle.

Les données collectées ainsi que les méthodes utilisées sont consignées dans le tableau 1

Zooplancton

Dans chaque aquarium, 1 litre d'eau est prélevé en 5 points (4 points répartis au 4 coins de l'aquarium et 1 point situé au centre) puis mélangés dans un seau. Le volume d'eau ainsi prélevé est filtré sur une soie de 55 µm qui retient la quasi-totalité du zooplancton. L'eau débarrassée du zooplancton après filtration est remise dans l'aquarium. Les microorganismes du filtrat sont fixés par ajout de formol à 5% environ. Un sous-échantillon du filtrat est prélevé au moyen d'une pipette Eppendorf (capacité: 1000 µl) et systématiquement compté au microscope optique (type MOTIC, G 10X).

Des données obtenues sont déterminées la densité (D), la biomasse et le taux d'accroissement (a) des populations. La densité (D) est déterminé à travers la formule suivante: $D= (n/v_1) \times (v_2/v_3)$ avec n= nombre d'individus comptés, v_1= volume (ml) du filtrat prélevé, v_2= volume (ml) du filtrat concentré, v_3= volume (ml) d'eau filtrée (1).

La biomasse, exprimée en poids sec (PS) (µg PS/l) est évaluée connaissant la densité et le poids individuel moyen correspondant à chaque espèce. Ces poids individuels fournis par la littérature est de 0,19 µg PS pour *B. calyciflorus* (8); 3,5/2,7/0,2 µg PS pour respectivement les femelles adultes, les juvéniles et les néonates de *Moina micrura* (16) et 0,08/1,36 µg PS pour les nauplies et adultes de *Thermocyclops* sp. (8).

Le taux d'accroissement retenu est égale à la pente de la droite de régression s'ajustant aux couples (temps; *ln* Biomasse) qui s'inscrivent dans la phase d'accroissement de l'espèce, délimitée par le jour d'ensemencement (t_0) et le jour du maximum de biomasse (t_x) (13).

Tableau 1
Méthodes de collectes des données physico-chimiques et trophiques

Paramètres	Méthodes
Température et oxygène dissous	Electrométrie par un Oxythermomètre de terrain (type DO-100 Oxygen Gauge, Voltcraft, Hirschau, Germany; précision: 0,01 mg/l et 0,1 °C près) plongé à 10 cm de la surface de l'eau
pH de l'eau	Electrométrie par un pH-mètre de terrain (type pHScan 10, Oakton, Eutech Instruments, Vernon Hills, USA; précision: 0,1 près) plongé à 10 cm de la surface de l'eau
N-NO3	Dosage par colorimétrie au bleu d'indophénol (AFNOR T90-015); (unité: mg/l; précision: 0,03 mg/l)
P-PO4	Dosage photométrique d'un complexe phosphomolybdique après réduction à l'acide ascorbique (AFNOR T90-023); (unité: mg/l; précision: 0,01 mg/l)
Chlorophylle-a	Spectophotométrie de Lorenzen, 1967

Analyses statistiques
L'analyse de variance à un critère de classification (ANOVA 1) et le LSD (Least Significant Difference) de Fisher a été utilisée pour comparer les différentes moyennes. Elle a été réalisée à l'aide du logiciel statistique Statview (Version 6, SAS Institute Inc.). L'hypothèse nulle est chaque fois rejetée au seuil de 5%.

Résultats

Evolution des facteurs physico-chimiques de l'eau
L'évolution au cours des observations est illustrée dans la figure 1. Les températures sont relativement faibles et oscillent entre 24,9 °C et 26,6 °C dans les aquariums à *B. calyciflorus*, entre 25,5 °C et 27,5 °C dans les aquariums à *M. micrura* et enfin entre 25,2 °C et 27,2 °C à *Thermocyclops* sp. Ces faibles valeurs de température se justifient pleinement par l'ombrage où sont déposés les aquariums. Les valeurs du pH varient peu et se situent entre 8,8 et 10,1; entre 8,4 et 9,6 et puis entre 8,3 et 9,8 respectivement dans les aquariums à *B. calyciflorus*, *M. micrura* et *Thermocyclops* sp. Les plus fortes valeurs sont enregistrées au début de l'expérimentation. On notera donc que ces paramètres sont a priori soumis aux conditions de l'environnement.
Les valeurs de l'oxygène dissous sont élevées et varient entre 8,72 mg à 14,09 mg/l dans les aquariums à *B. calyciflorus*, entre 12,56 à 16,37 mg/l dans les aquariums à *M. micrura* et entre 11,03 à 14,22 mg/l dans les aquariums à *Thermocyclops* sp. Les plus fortes valeurs de l'oxygène dissous sont enregistrées les premiers jours de l'expérimentation et seraient liées à l'abondance relative de phytoplancton dans les milieux pendant cette période.
Les teneurs en sels minéraux (N-NO3 et P-PO_4) varient fortement du début jusqu'à la fin de l'expérimentation (Figure 2). Les plus fortes concentrations (N-N03 compris entre 0,5 et 0,7 mg/l; P-PO4 compris entre 0,5 et 0,61 mg/l) notées au début ont probablement pour origine une forte minéralisation de la fiente.
Dans les aquariums à *B. calyciflorus* et à *M. micrura*, l'évolution du phytoplancton (chl-a) est également caractérisée par de fortes concentrations au début jusqu'au 9ème jour et une chute progressive pour atteindre les plus faibles concentrations à la fin de l'expérience (Figure 2). Le broutage joue probablement un rôle non négligeable dans les variations observées. La diminution progressive des concentrations en chlorophylle qui intervient ensuite est vraisemblablement liée à la baisse des teneurs en sels nutritifs. Dans les aquariums à *Thermocyclops* sp., les concentrations de chl-a sont restées fortes du début jusqu'à la fin de l'expérimentation (Figure 2).

Evolution du peuplement zooplanctonique
L'évolution du peuplement des trois espèces cibles est illustrée dans la figure 3. Globalement, elle se

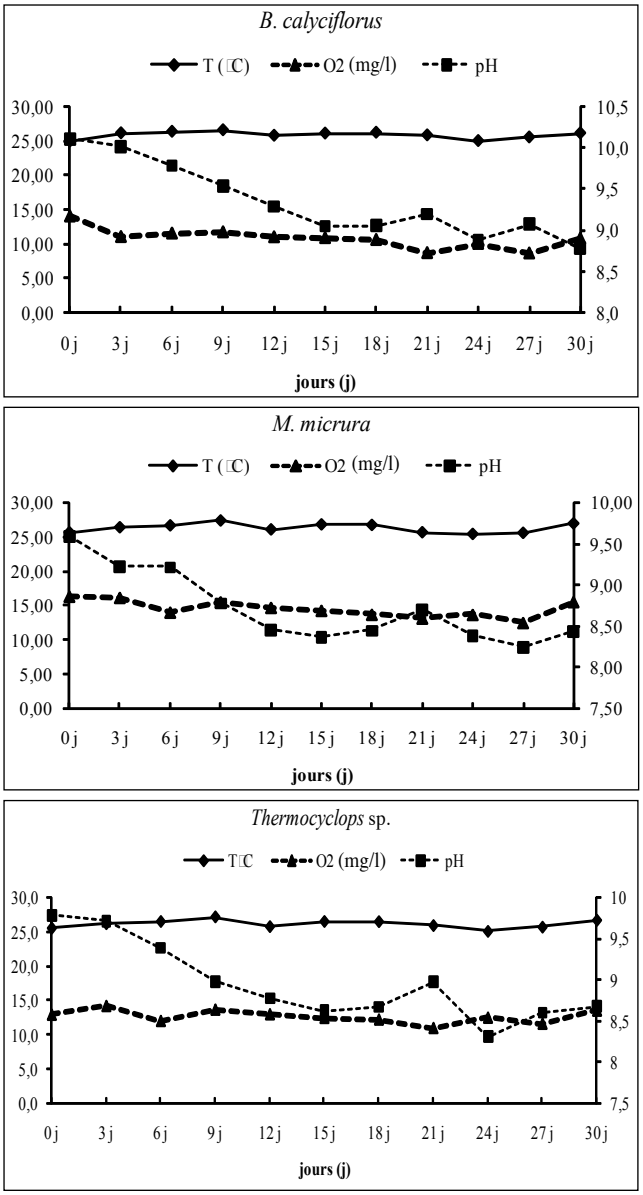


Figure 1: Evolution des paramètres physico-chimiques de l'eau dans les aquariums.

caractérise par une phase initiale de développement exponentiel des populations, suivie d'une phase de décroissance puis de relative stabilisation, surtout dans le cas de *B. calyciflorus* et de *M. micrura*. Chez ces dernières espèces, la phase initiale appelée phase de colonisation est très rapide allant du début au 6ème jour. Pendant cette phase, leurs populations ont atteint 18 857 ind/l (3 582,77 µg PS/l) et 11 323 ind/l (24 118 µg PS/l) pour respectivement *B. calyciflorus* et *M. micrura*. La phase de décroissance s'étend du 6ème jour au 18ème jour pour *B. calyciflorus* et au 15ème jour pour *M. micrura*. On notera qu'au cours de cette phase, les populations de ces espèces sont restées à des niveaux relativement importants. Comparativement à ces deux espèces, *Thermocyclops* sp. présente une phase de colonisation plus lente qui va jusqu'au 21ème jour. A cette date, le maximum d'abondance de l'espèce est de 2 020 ind/l soit une biomasse de 1 814,08 µg PS/l.
Le taux d'accroissement de la biomasse (a_1) calculée pendant la période de développement des espèces

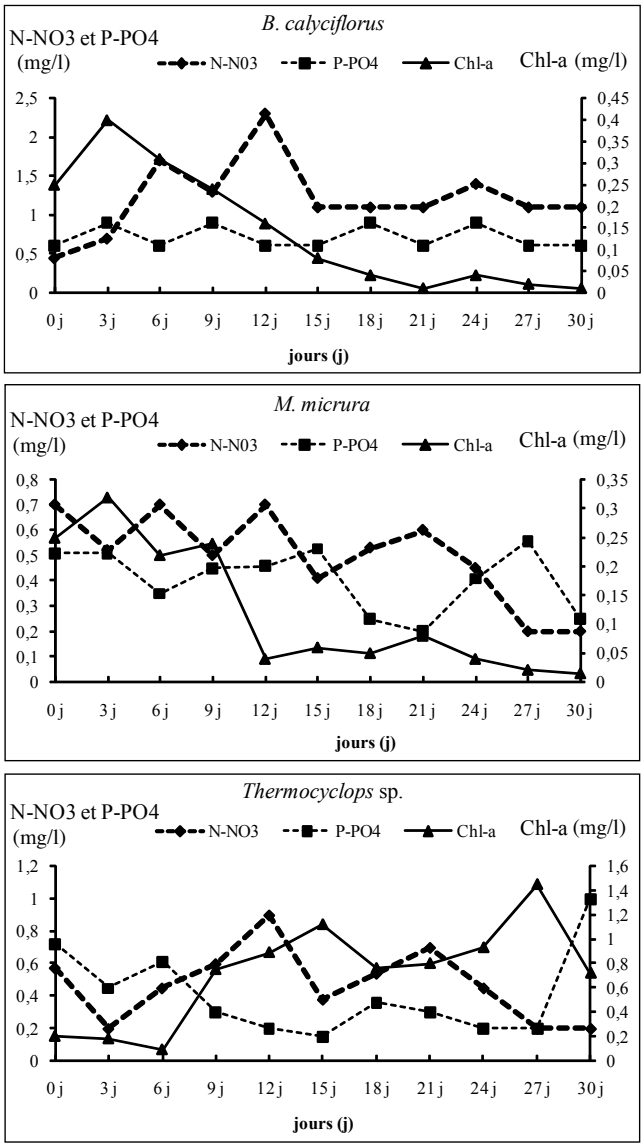


Figure 2: Evolution des sels nutritifs et de la chlorophylle-a dans les aquariums.

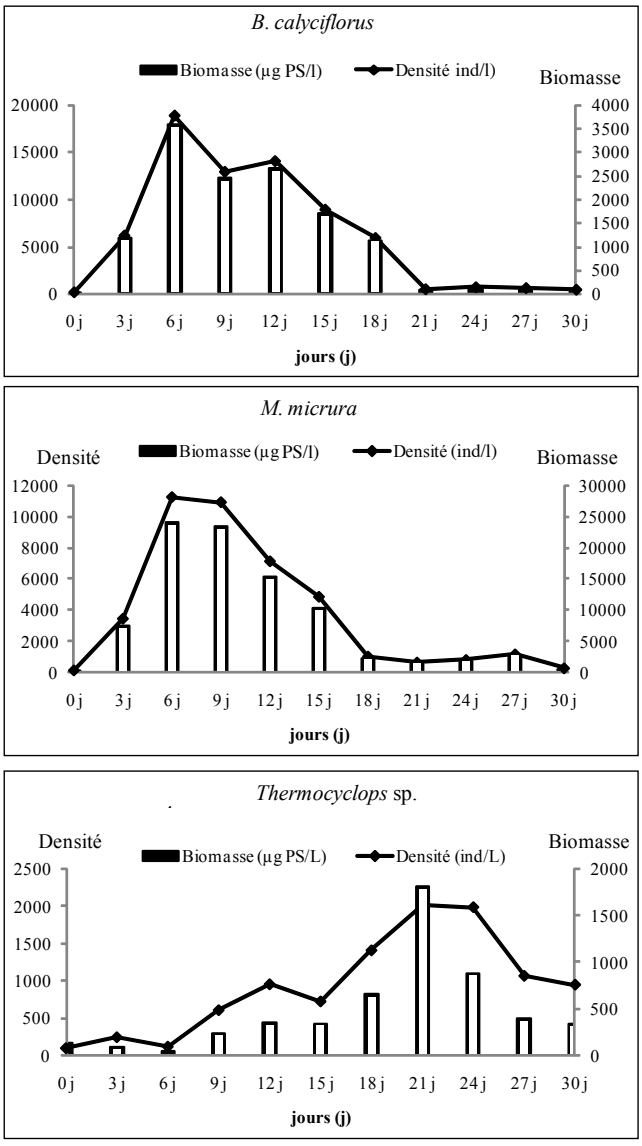


Figure 3: Evolution des densités et biomasses des trois espèces au cours de l'expérimentation.

décroît depuis *B. calyciflorus* jusqu'au *Thermocyclops* sp. (Tableau 2). Quoique la différence ne soit pas considérable, on observe des taux systématiquement plus élevés avec *B. calyciflorus* (a_1 allant de 0,92 à 1,36; moyenne= 1,14) et *M. micrura* (a_1 allant de 0,95 à 1,19; moyenne= 1,07). Mais ce taux est nettement plus faible avec *Thermocyclops* sp. (a_1 allant de 0,42 à 0,47; moyenne= 0,44).

Les taux de décroissance (a_2) des populations calculés pendant la phase de décroissance sont faibles (Tableau 3) chez toutes les espèces sauf chez *Thermocyclops* sp. Ces taux sont en moyenne de 0,28; 0,34 et 0,52

Tableau 2
Taux d'accroissement journalier (a_1) des droites d'ajustement *Ln* Biomasse= f (temps) des populations de *B. calyciflorus, M. micrura* et de *Thermocyclops* sp. calculés pendant la phase de colonisation. t_0, t_1= début et fin de la période de colonisation; r= coefficient de correlation, significatif à p< 0,05

Espèces	Aquarium	t_0	t_1	n	r	a_1
B. calyciflorus	1	0	6	3	0,717	1,36
	2	0	6	3	0,604	0,92
	Moyenne	0	6	3	0,815	1,14
M. micrura	1	0	6	3	0,99	1,19
	2	0	6	3	0,686	0,95
	Moyenne	0	6	3	0,872	1,07
Thermocyclops sp.	1	0	21	8	0,735	0,42
	2	0	24	9	0,97	0,47
	Moyenne	0	24	9	0,834	0,44

Tableau 3
Taux de décroissance journalier (a_2) des droites d'ajustement *Ln* Biomasse= f (temps) des populations de *B. calyciflorus, M. micrura* et de *Thermocyclops* sp. calculés pendant la phase de décroissance. t_1, t_2= début et fin de la période de décroissance; r= coefficient de correlation, significatif à p< 0,05

Espèces	Aquarium	t_1	t_2	n	r	a_2
B. calyciflorus	1	6	18	5	0,901	-0,27
	2	6	18	5	0,833	-0,28
	Moyenne	6	18	5	0,826	-0,28
M. micrura	1	6	15	4	0,913	-0,3
	2	6	15	4	0,99	-0,41
	Moyenne	6	15	4	0,904	-0,34
Thermocyclops sp.	1	21	30	4	0,936	-0,58
	2	24	30	3	0,857	-0,47
	Moyenne	21	30	4	0,911	-0,52

respectivement pour *B. calyciflorus*, *M. micrura* et *Thermocyclops* sp.

Discussion

Le démarrage du processus de développement des populations après la mise en charge semble être étroitement lié aux conditions favorables que présentent ces milieux. En effet, au début de l'expérimentation, les concentrations initiales en sels nutritifs sont fortes et conduisent ainsi à la richesse des milieux en phytoplancton. L'assimilation de ces sels nutritifs par les microalgues justifierait leur diminution progressive notée par la suite dans les milieux comme l'ont prouvé plusieurs études (1, 8). Parallèlement à la diminution des sels, on observe une diminution du phytoplancton et du zooplancton surtout dans les milieux à *B. calyciflorus* et *M. micrura*. Legendre *et al*. (8) ont observé des phénomènes similaires dans les étangs de pisciculture lagunaire à Layo (Côte d'Ivoire) lors d'une étude de recolonisation après la mise en eau.

En ce qui concerne la dynamique de production des trois espèces cibles, les résultats d'élevage démontrent que ces organismes zooplanctoniques présentent deux phases: une phase de colonisation suivie d'une phase de décroissance. Au cours de la première phase, le rotifère *B. calyciflorus* et le cladocère *M. micrura* présentent un développement très rapide et massif dans les 6 premiers jours. Alors que le copépode *Thermocyclops* présente un développement plus lent. Ces observations rejoignent celles faites par Pagano (12) et Agadjihouèdé *et al*. (*soumis*). Legendre *et al*. (8) sont parvenus vraisemblablement à la même conclusion en signalant lors d'une étude de la recolonisation des étangs de Layo qu'elle s'effectue tout d'abord par les rotifères dans les 10 premiers jours et ensuite par les cladocères et les copépodes dans les 13ème et 20ème jours. L'extension de la comparaison à la culture d'autres espèces de cladocères montre que *Moina rectirotris et M. macropora* présentent respectivement une phase de colonisation de 5-6 jours et de 7 jours (2). Ces résultats semblent liés au cycle de développement de ces différents organismes. En effet, les rotifères et les cladocères, qui sont strictement herbivores ont un cycle de développement court (13, 14), tandis que les copépodes ont un cycle de développement long (8, 13).

Pendant cette phase de colonisation, les taux d'accroissement obtenus ici pour les trois espèces dépassent ceux obtenus par Agadjihouèdé *et al.* (*soumis*) lors de l'élevage en mélange (condition plurispécifique) de ces mêmes espèces. Pour ces auteurs, les taux d'accroissement sont compris entre 0,71-0,74 pour *B. calyciflorus*; entre 0,85-0,92 pour *M. micrura* et entre 0,2-0,22 pour *Thermocyclops* sp. Dans ces conditions d'élevage en mélange de zooplancton, il y a généralement une compétition alimentaire entre les espèces associées surtout entre les herbivores (4) d'une part et d'autre part, les copépodes adultes exercent une action de prédation sur les autres (13). D'autres études en prouvant l'existence de cette compétition entre *B. calyciflorus, M. micrura* et *Diaphanosoma excisum* ont précisé que cette compétition entre ces espèces est faible (7, 12). Ce qui pourrait influer les taux d'accroissement des populations de ces espèces associées.

Les densités atteintes au terme de cette période de colonisation (18 857 ind/l pour *B. calyciflorus*, 11 323 et 2 020 ind/l pour *M. micrura* et *Thermocyclops*), comme les biomasses (jusqu'à 24, 118 mg/l) sont élevées. Ces densités sont largement supérieures à celles obtenues dans les cultures en mélange du zooplancton (inférieures à 13 000 ind/l pour les rotifères et 2000 ind/l pour les cladocères et copépodes (1). Chez le rotifère elles sont comparables aux valeurs habituellement rencontrées dans des cultures *in vitro*, mais il convient de signaler que des valeurs supérieures ont déjà été observées aussi bien en milieu naturel (6)

qu'en élevage (5). Pour *M. micrura*, des densités plus faibles sont enregistrées aussi bien en milieu naturel (8) qu'en monoculture de cette espèce en bacs (12, 13). Les biomasses sèches atteintes sont environ 3,5 mg/l, 24 mg/l et 2 mg/l pour respectivement *B. calyciflorus*, *M. micrura* et *Thermocyclops* sp.; soient des biomasses fraîches respectives estimées environ à 33 mg/l, 230 mg/l et 19 mg/l. Cette estimation est faite en considérant une teneur en matière sèche moyenne de 9,61% pour un mélange de zooplancton produit en étang en Inde d'après Mitra *et al.* (10). Ces biomasses sont importantes surtout avec *M. micrura*. Signalons quand même que, l'estimation des biomasses zooplanctoniques à travers les masses zooplanctoniques individuelles produites par la littérature pourrait donner des valeurs sous-estimées ou surestimées de la biomasse. Puisque selon Agadjihouèdé *et al*. (*sous presse*), il est tout aussi possible d'observer une différence de poids pour une même espèce produite dans des conditions différentes.

Les très faibles densités que présentent les herbivores après le 18ème jour résulteraient de l'épuisement des nutriments dans les milieux de culture. Ce qui montre la nécessité d'apport des fertilisants d'entretien afin de garder les milieux de culture riches et favorables pour le maintien des fortes densités de ces organismes zooplanctoniques.

Conséquences en aquaculture

Examinés dans la perspective d'une culture de zooplancton pour l'élevage larvaire des poissons zooplanctonophages, les présents résultats permettent de dégager quelques conclusions très générales.

Tout d'abord, la courte durée de la phase de développement exponentiel des espèces *B. calyciflorus* et *M. micrura* peut être considérée comme un facteur de choix de ces espèces. En effet, avec cette courte durée, la production est brève et facilement contrôlable. Ainsi, plusieurs cycles de production de ces organismes peuvent être réalisés.

Les densités de *B. calyciflorus* et *M. micrura* obtenues sont favorables à une nurserie intensive en bacs avec des densités intéressantes de stockage de prédateurs (larves de poisson). Car, avec les nauplii d'artémia de taille (400 à 450 µm) une densité minimale de 1000 ind/l est indicative pour une nurserie intensive; en-dessous de cette densité les larves ont du mal à trouver leur proie. En considérant les tailles moyennes des différentes espèces élevées dans la présente étude, cette densité minimale serait de 2 500 ind/l pour *B. calyciflorus* (taille: 88 µm - 255 µm), 700 ind/l pour *M. micrura* (taille: 345 - 702 µm) et moins de 500 ind/l pour *Thermocyclops* (taille: 388 - 1061 µm).

Les biomasses correspondantes permettraient surtout avec *M. micrura* de stocker des larves de 30 mg à des densités atteignant 60 ind/l en supposant une ration journalière de 100% de poids corporel. Puisque, à titre indicatif, avec 3 g de zooplancton (crustacée notamment), on pourrait élever selon Legendre *et al*. (8) des larves de 30 mg à une densité de 100 ind./l avec une ration journalière de 100% de poids corporel.

La grande taille, la longue phase de colonisation et le comportement alimentaire de *Thermocyclops* tendant vers le cannibalisme aux stades adultes et copépodites limiteraient son utilisation par les larves de poisson surtout lorsque ces larves sont dans leurs premiers jours de vie. Mais, au cours de la larviculture de certaines espèces de poissons telles que *Clarias gariepinus* et *Heterobranchus longifilis*, *Thermocyclops* pourrait être utilisé sans inquiétude une semaine après leur éclosion (11).

Conclusion

Au terme ce cette étude, nous pouvons retenir qu'en culture monospécifique, les 3 espèces cibles présentent de très bonnes performances. Le taux d'accroissement et la production sont meilleurs par rapport à une culture en mélange de ces espèces. Les herbivores donnent de très fortes densités en très courte durée. Ce qui favoriserait leur culture pour la larviculture.

Remerciements

Les auteurs tiennent à remercier tous les examinateurs anonymes pour leur contribution à la qualité scientifique de ce manuscrit.

Références bibliographiques

1. Agadjihouèdé H., Bonou C.A., Chikou A. & Lalèyè Ph., 2010, Production comparée de zooplancton en bassins fertilisés avec la fiente de volaille et la bouse de vache. International Journal of Biological and Chemical Sciences **4**, 2, 432-442.
2. Alva-Martinez A.F., Sarma S.S.S. & Nandini S., 2007, Effect of mixed diets (cyanobacteria and green algae) on the population growth of the cladocerans *Ceriodaphnia dubia* and *Moina macrocopa*. Aquat. Ecol. 41, 579-585.
3. Barnabé G., 1986, Les collectes de zooplancton. *In*: Aquaculture, 1. Barnabé G., Lavoisier, Paris: 260-269.
4. Bonou C.M., Pagano M. & Saint-Jean L., 1991, Développement et croissance en poids de *Moina micrura* et de *Mesocyclops ogunnus* dans un milieu saumâtre tropical: les étangs de pisciculture de Layo (Côte d'Ivoire). Rev. Hydrobiol. trop. 24, 287-303.
5. Gatesoupe F.J. & Luquet P., 1981, Practical diet for mass culture of the rotifer *Brachionus plicatilis*: application to larval rearing of sea bass, *Dicentrarchus labrax*. Aquaculture, 22, 149-163.
6. Iltis A. & Riou-Duwat S., 1971, Variations saisonnières du peuplement en rotifères des eaux natronées du Kanem (Tchad). Cah. ORSTOM Sér. Hydrobiol. **5**, 2, 101-112.
7. Kâ S., Pagano M. & Bâ N., 2006, Zooplankton distribution related to environmental factors and phytoplankton in a shallow tropical lake (Lake Guiers, Senegal, West Africa). Int. Rev. Hydrobiol. 91, 389-405.
8. Legendre M., Pagano M. & Saint-Jean L., 1987, Peuplements et

biomasse zooplanctonique dans des étangs de pisciculture lagunaire (Layo, Côte d'Ivoire). Etude de la recolonisation après la mise en eau. Aquaculture, 67**,** 321-341.

9. Lorenzen C.J., 1967, Determination of chlorophyll and phaeopigments: spectrophotometric equations. Limnol. Oceanogr. 12, 343-346.

10. Mitra G., Mukhopadhyay P.K. & Ayyappan S., 2007, Biochemical composition of zooplankton community grown in freshwater earthen ponds: nutritional implication in nursery rearing of fish larvae and early juveniles. Aquaculture, 272, 346-360.

11. Otémé Z.J., Hem S. & Legendre M., 1996, Nouvelles espèces de poissons-chats pour le développement de la pisciculture africaine. *In*: Legendre M. & Proteau J.P. (eds), The Biology and Culture of Catfish, Aquatic Living Resources volume **9**, Hors Série, Paris: 207-217.

12. Pagano M., 2008, Feeding of tropical cladocerans (*Moina micrura*, *Diaphanosoma excisum*) and rotifer (*Brachionus calyciflorus*) on natural phytoplankton: effect of phytoplankton size–structure. Journal of Plankton Research, **30**, 4, 401-414.

13. Saint-Jean L. & Bonou C.A., 1994, Growth, production and demography of *Moina micrura* in brackish tropical fishponds (Layo, Ivory Coast). Hydrobiologia, 272, 125-146.

14. Seyer J., 2002, Le chant de l'eau. Production de proies vivantes *Brachionus plicatilis* et *Artemia salina*. Ifremer, station Merea, 83-84.

15. Schlumberger O. & Bouretz N., 2002, Réseaux trophiques et production piscicole en étangs fertilisés (Dordogne, France). Rev Sci Eau, 15/1, 177-192.

16. Sipaúba-Tavares L.H. & Bachion M.A., 2002, Population growth and development oftwospecies of Cladocera, *Moina micrura* and *Diaphanosoma birgei*, in laboratory. Braz. J. Biol. 62, 701-711.

H. Agadjihouede, Doctorant en Hydrobiologie et Aquaculture au Laboratoire d'Hydrobiologie et d'Aquaculture de la Faculté des Sciences Agronomiques de l'Université d'Abomey-Calavi.

A.C. Bonou , Maître Assistant des Universités (CAMES) à l'Ecole Polytechnique d'Abomey-Calavi de l'Université d'Abomey-Calavi.

E. Montchowui, Maître Assistant des Universités (CAMES) à l'Ecole Nationale Supérieure en Sciences et Techniques Agricoles de Kétou.

A. Chikou, Maître Assistant des Universités (CAMES) à la Faculté des Sciences Agronomiques de l'Université d'Abomey-Calavi.

P. Laleye, Professeur, Maître de Conférences des Universités (CAMES) à la Faculté des Sciences Agronomiques de l'Université d'Abomey-Calavi.

Buying Attitude of Yam Consumers in Southeastern, Nigeria

I.N. Nwachukwu[1], O.U. Oteh[1], C.C. Ugoh[1] & C. Ochomma[2]

Keywords: Buying attitude- Yam- Consumer- Demand- Nigeria

Summary

Buyer attitude is a complex and dynamic subject. It differs from consumer group to another. This study focused on the buying behavior of yam consumers in Imo state, Nigeria and sought to identify the major factors that influence buyer attitude as well as the effects of different variable on consumer decision to purchase yam. In order to achieve this mandate, the study adopted a multistage sampling technique, which was employed in the selection of location and respondents. Descriptive statistics, logistic regression and paired t test were the tools of analysis adopted for this study. The results showed that household income, education, taste and age were statistically significant and therefore influence demand for and willingness to buy yam. Based on the findings, it was recommended that there is the need for increased consumer education especially to enlighten parents to include yam in the diet of their children. This is necessary because the findings showed that adult consumers were dominant. Breeders are also challenged to develop new varieties that have desirable qualities in addition to good and palatable taste.

Résumé

Attitude d'achat des consommateurs d'igname au Sud-Est du Nigeria

L'attitude des acheteurs est un sujet complexe et dynamique. Il diffère d'un groupe de consommateurs à l'autre. Cette étude a porté sur le comportement d'achat des consommateurs de l'igname dans l'État d'Imo et cherché à identifier les principaux facteurs qui influencent l'attitude d'acheter ainsi que les effets de différentes variables sur la décision des consommateurs d'acheter l'igname. Afin de réaliser ce mandat, l'étude a adopté une méthode d'échantillonnage, qui a été employée dans la sélection de l'emplacement et les répondants. Les statistiques descriptives, la régression logistique et le test t apparié ont été les outils d'analyse adopté pour cette étude. Les résultats montrent que le revenu du ménage, l'éducation, le goût et l'âge sont statistiquement significatives, et donc influent sur la demande et la volonté d'acheter de l'igname. Sur la base des conclusions, il a été recommandé la nécessité de l'éducation des consommateurs en particulier pour éclairer les parents de l'importance de l'igname dans l'alimentation de leurs enfants. Cela est nécessaire car les résultats ont montré que les consommateurs adultes ont été dominants. Les sélectionneurs sont aussi mis au défi de développer de nouvelles variétés qui ont les qualités désirées en plus du bon goût agréable.

Introduction

One of the basic human needs for his continued existence in life is food. Human's need for food goes beyond fulfillment of his biological necessities and meeting with their cultural and social needs (4, 23). Importantly, it gulps a large percentage of his expenditure (23).

Yam (*Dioscorea* species of family Dioscoreaceae) is a (staple) food; it serves the above highlighted purposes and more for man. According to IITA (12), it has a rich nutritional content providing more than 200 dietary calories per capita daily for more than 150 million people in Nigeria; vitamin B6 and potassium. Information on the nutritive value of yam has been highlighted by several authors in their work (1, 5, 7, 21). Yam also has ritual, medicinal and socio-cultural significance. For instance, it is a choice food during ceremonies and festivities (11).

Approximately 95 percent of the 51.4 million metric tons of yams produced in 2009 in the world are grown in the yam belt of the West Africa. Nigeria is the largest producer with a total production of 38.7 million metric tons accounting for 75 percent of total world output (10, 13). According to the Nigerian Export Promotion Council (NEPC) report, Nigeria realized N70 billion from yam export in 2009 as against N56 billion in 2008 and N37 billion in 2007 (20). In terms of yam consumption, the quantity consumed in Nigeria (258 kcal) is low when compared with Benin (364 kcal), Ivory Coast (342 kcal), and Ghana (296 kcal) (13).

The consumption patterns and taste in food are often shaped by the family life cycle and the number, age, and gender of people in the household besides their occupation (14). Moreover, despite the fact

[1]College of Agribusiness and Financial Management, Michael Okpara University of Agriculture, Umudike, PMB 7267, Umuahia, Abia State, Nigeria.
[2]Dept of Agricultural Economics, Abia State University, Umuahia Campus, PMB 7010, Umuahia, Abia State, Nigeria.
*Email: ifnwachukwu@yahoo.com; nwachukwu.ifeanyi@mouau.edu.ng
Received on 26.05.11 and accepted for publication on 14.10.11

that economists had in time past developed series of models explaining consumer choice in terms of changing utilities, or demand preference according to the variations in price, income and quantity, recent empirical evidence suggests that consumer decision making processes involve some unseen relative cognitive values that include taste, species, durability, weight etc.

Consumer behavior with respect to food has not received much attention in Nigeria. At least part of the difficulty in conducting research in this important area lies in the complexity and diversity in the influence at work in food choice and consumption. Based on the foregoing, this study assesses the socio-economic profile of yam consumers; analyze and compare the consumption pattern of yam with other staples and identifies the major factors that influence buyers' attitude towards yam. To guide the study in achieving meaningful results, the following hypotheses were formulated:

H_1: there is a significant relationship between price and consumption of yam;

H_2: age, income, education, household size, weight, colour, taste are positively related to purchases and rate of consumption of yam while price and price of substitutes are negatively related to its purchase and rate of consumption.

Materials and methods

The Study Area

The study area is Imo State, Nigeria. Imo State is situated in the south eastern geographical zone of Nigeria. It lies between longitudes 06^0 35' and 07^0 28' E and latitudes 05^0 00 and 05^0 37' N, covering an area of 3,289. 49 km^2. It is bounded on the east by Abia State, on the west by Delta State, on the north by Anambra and Ebonyi State and on the west by Rivers State. The state falls within the tropical rainforest zone with an average annual rainfall of up to 2550 mm. It has estimated population of 3.7 million with a growth rate of 2.8% per annum.

For ease of administration of agricultural programmes, the State is divided into 3 geographical zones namely: Owerri, Orlu and Okigwe; farming is the major occupation of the people and yam is one of the major root and tuber crops produced in the area.

Method of data collection

Multi-stage sampling technique was employed in the selection of location and respondents. In the first stage, Owerri main town was chosen purposively for this study due to its cosmopolitan nature, in addition to being the state capital. The second stage involved a random selection of ten streets from the town. Finally, ten (10) households were randomly selected from each street. This aggregated one hundred households and constituted the sample for the study.

Method of data analysis

Data were analyzed using descriptive statistics, logistic regression and t-test statistic to realize the objectives. Specifically, the socio-economic profile was analyzed using descriptive statistics while logistic regression was employed in the assessment of the factors that influence the buyer attitude towards yam. The paired t-test statistic however was employed for the last objective.

The logistic regression for the estimation of the factors that influence buyer attitude is stated thus:

$$Y= b_0+b_1X_1+b_2X_2+b_3x_3+b_4X_4+b_5X_5+b_6X_6+b_7X_7....b_nX_n+\varepsilon_1$$

Where Y= Dummy: (1= demand for and willingness to buy yam and 0= Otherwise)

X_1= Age (years)
X_2= Household Income (Naira)
X_3= Education (years)
X_4= Price of substitute (Naira)
X_5= Price of yam (Naira)
X_6= Household size (No)
X_7= Taste (dummy: Good= 1, otherwise= 0)
X_8= Weight (dummy: sizeable = 1, otherwise= 0)
X_9= Color (dummy: white= 1, yellow= 0)
ξ_1 = Composite error term

In the comparison of consumption for yam with other carbohydrate foods, the paired t-test statistic will be employed and thus stated as:

$$t = \frac{X_1 - X_2}{\sqrt{\frac{S^2_1}{n_1} + \frac{S^2_2}{n_2}}}$$

Where t= t - test statistic
X_1= mean value of consumption of yam by regular consumers.
X_2= mean value of consumption of other staples by regular consumers.
S^2_1= sample variance of consumption of yam by regular consumers.
S^2_2= sample variance of other staples by regular consumers.
n_1= sample size of regular consumers of yam.
n_2= sample size of regular consumers of other staples.

The above methodologies are consistent with Nwachukwu *et al.* (18) and Ariyawardana and Prathiraja (2) who employed same in their studies.

Results and discussion

Analysis of socio-economic profile

This study analyzed the socio-economic characteristics of the respondents under the following: age, household income, education, price of yam and substitute, household size. The data are presented in table 1. Table 1 showed that a dominant proportion of

Table 1
Socio-economic statistics of yam buyers (n= 100)

Variables	Frequency (Percentage)
Age (Years)	
18 - 20	7.00
21 - 25	5.00
26 - 30	13.00
31 - 35	18.00
36 - 40	23.00
41 - 60	34.00
Education (Years)	
School Certificate	7.00
WAEC/NECO	25.00
OND/NCE	14.00
B.Sc/BA/HND	49.00
Others	5.00
Household Income (Naira)	
30,000 - 49,999	11.00
50,000 - 69,999	16.00
70,000 - 89,999	40.00
90,000 - above	33.00
Sex	
Female	69.00
Male	31.00
Price of Yam (Naira)	
100 - 199	28.00
200 - 299	40.00
300 - 399	20.00
400 - 499	6.00
500 - 599	4.00
600 - 699	2.00
Price of Substitute (Naira)	
100 - 299	49.00
300 - 499	18.00
500 - 699	19.00
700 - 899	7.00
900 - above	7.00
Household Size (No)	
1 - 4	31.00
5 - 8	60.00
9 - 12	9.00

Source: Field Survey (2010)
NB: The frequencies are the same as the percentages and N150 is equivalent to $1.

the respondents are adults. This is possible because adults decide most their respective household purchases. Also, majority of the household has an appreciable income.

While other staples are less expensive, it takes an individual with high income to demand and consume more quantity due to cost and availability caused by variability in season. On the other hand, the high mean for other staples depicts a higher demand/willingness and availability of these staples than yam.

The frequency distribution in table 1 also revealed that about more than 50% of the respondents have attained their tertiary education, meaning that majority of the respondents are literate and rational buyers. This high literacy level demonstrates that our respondents have a wide knowledge of food nutritional content. Their level of exposure academically influences the kind of food and willingness to buy more quantity of their choice product despite economic pressure.

More so, the table 1 shows that an average yam (about 2 kg -3 kg) costs between N250- N300. This is validated by about 40% of the respondent. About 60% of the households that participated in the study have between 5-8 people in a household. The size of a household determines the quantity of a food item purchased hence, the willingness to buy more.

The consumer profile showed that the majority of the respondents are educated, female adults in their middle ages with appreciable income and relatively large households. This is plausible given the fact that adults decide most their respective household purchases and yam is not readily affordable. High education level of the buyers implies that they have a wide knowledge of nutritional values of foods and are able to choose the type of food that will suit their nutritional needs cum preferences despite economic pressures (Table 1).

Analysis and comparison of the consumption pattern of yam with other staples

Yam, garri and rice were the major staple foods selected for this study. As such, to assess and compare the quantity and consumption rate for yam and other staples, paired t-test statistic was employed and the results are presented in table 2.

Table 2 shows a higher mean value for garri and rice and as such confirms that these staples are consumed more both in quantity and frequency than yam. According to Daniel and Kormawa (8), yam is the most expensive among the tubers and roots crops.

Table 2
Paired t-test comparison of consumption pattern for yam and other staples

Variables	Individual mean(kg)	Paired difference		
		Mean	standard deviation	t
Quantity				
Yam	2.70			
		-23.12	10.39258	-22.24
Garri	25.39			
Yam	2.70			
		-17.71	9.08245	-19.49
Rice	19.98			
Consumption rate				
Yam	1.26			
		-3.42	1.80448	-18.95
Garri	4.68			
Yam	1.26			
		-3.43	1.9448	-17.63
Rice	4.69			

Source: Field Survey, 2010.

This also lend credence to the fact that average yam consumption per capita per day is low when compared with other nations that produce yam (13). Moreover, available quantity is not adequate to feed the growing population, after export. In Nigeria, yam is becoming more expensive and relatively unaffordable in urban areas as production has not kept pace with population growth leading to demand exceeding supply (15).

Also from table 2, it could be deduced that an average quantity of yam consumed in one month per household was 2.7 kg at the cost of N100 - N300. When compared with other staples (rice and garri), the quantity and rate of consumption of yam is less. Garri appeared to be the most consumed but a little less than rice in terms of rate. This is consistent with the findings of IITA (12) and Nigeria Food Consumption survey (17) and which averred that other staples are consumed far more than yam because they are more affordable and available.

Factors influencing demand and willingness to buy yam

To identify the factors that influence the demand and willingness of consumers to buy yam and the effect of these different variables on consumer purchase decision, logistic regression was employed. The variables used for this analysis include: age, household income, household size, taste, weight, color, education, price of yam and substitute. The result of the analysis is presented in table 3.

Table 3 indicates that the coefficients of age, income, education were significant at 99% and while taste was significant at 95% confidence level, as represented by the wald statistics. It is equally important to note that the positive signs in the statistics depict that the observed variables have a direct relationship with demand and willingness of consumers to purchase yam against other staples, despite the cost implications and quantity that can satisfy a particular household. This result is consistent with the findings of Nwachukwu *et al*., (18) in their study on buyers' attitude for made in Nigeria shoes. This is equally consistent with the views of Kotler and Keller (14), which observed that consumption patterns and taste in food are often shaped by the family life cycle, and the number, age and gender of people in the household and occupation (income).

The effect of economic circumstances such as disposable income and borrowing power on demand is consistent with Olanyinka and Aminu (19). According to them, income and other economic factors affect purchases, and by extension affect availability, quantity purchased and subsequently consumed. This justifies the first hypothesis that price is significantly related to consumption of food. Also, In Nigeria, the overall expenditure elasticity of demand for yam was greater than one. Therefore, increased urban income is likely to boost the sale of yam without affecting the prices. Also, yam was shown to have a positive price elasticity, improved production or storage methods which increase the supply of yam will lower the price and increase quantities at low expenditure levels (3).

The positive signs for price(s) show that price is an important determinant of consumer purchase intentions/choice for goods/service. The higher value for price of substitutes indicated that given consumers budget constraint, demand will shift downwards to alternative foods, due to the effect of price. Each individual wants to maximize utilities, but due to budget constraints, the individual faces a dilemma. It is to solve this problem of choice that economists turn to the theory of indifference curve. Price changes affect willingness to buy, purchasing power and quantity demanded, unless the food is a necessity in the households and has to be bought, no matter the price. Previous studies show garri-to-yam cross price elasticity positive depicting that they are strong substitute goods. This is consistent with the finding of Daniel and Kormawa (8).

It could be observed that two factors stood out as the most important determinants of yam purchase attitude and rate of consumption. They are income of individual/

Table 3
Binary logit estimates of factors that influence buying behavior for yam

Variables	Co-efficient (B)	Standard error	Wald	Exp (B)
Constant	7.002	3.186	4.833	1102.073***
HH income (N)	0.001	0.002	7.790	1.000***
Education (yrs)	-0.331	0.139	5.692	0.718***
Price of Sub.(N)	0.002	0.001	1.666	1.002
Price of yam(N)	-0.001	0.002	0.167	0.999
HH size (No)	-0.146	0.127	1.323	0.864
Weight (dummy)	-0.088	0.162	0.293	0.916
Color (dummy)	0.610	0.136	0.201	1.063
Taste (dummy)	0.291	0.189	2.369	0.748**
Age (yrs)	0.051	0.031	2.660	1.052***
Cox-Snell R^2	0.215			
Nagelkerke R^2	0.322			

Source: Field Survey, 2010.

*** and ** denoted statistical significance at 1% and 5% risk level respectively.

household and price of goods/service. This is consistent with the views of Deaton and Muelbauer (9).

The positive signs of color, weight and taste confirm consumer perception of quality with respect to food. It equally confirms that consumer perception of quality involves some unseen relative cognitive values that include taste, species, weight, color etc. Taste in consumer food quality assessment is referred to as the hedonic quality dimension of food (6). Quantitative studies on important predictors of food choice usually conclude, not surprisingly, that taste and pleasure are among the most important predictors of food choice (22). Perception of quality as an important determinant of buyer's preference has been highlighted by many authors by previous studies (4, 6, 16).

The Cox-Snell and Nagelkerke R^2 values are attempts to provide a logistic analogy to coefficient of multiple determination, R^2 in OLS regression. The Nagelkerke measure adapts the Cox-Snell measure so that it varies from 0 to 1 as does R^2 in OLS. At 21% and 32% for Cox-Snell and Nagelkerke respectively, the regression line fits data to up to the stipulated level. As such, they imply the extent of explanation of variation in the dependent variable, lending to credence to the fact that age, income, education and household size are positively related to purchase and consumption rate of yam.

Conclusion

Consumers have been recurrently the unit of analysis in all economic activities. Each individual want to maximize utilities but due to budget constraint must resorts to alternatives. Alternatives in terms of whether the alternatives will satisfy their need or not must be evaluated. From the analysis of the buying behavior of consumers of yam, this study reveals the major determinants of demand and willingness to buy yam. The major determinants are age, education, income and taste. The effect of these variables on their willingness to buy is direct. Therefore, this study suggests the need for increased consumer education especially to enlighten parents to include yam in the diet of their children. This is necessary because the findings show that adult consumers are dominant. Breeders are also challenged to develop new varieties that have desirable qualities in addition to good and palatable taste.

Literature

1. Afoakwa E.O. & Sefa-Dedeh S., 2001, Chemical composition and quality changes in trifoliateyam *Dioscorea dumetorum* tubers after harvest. Food Chemistry, **75**, 1, 88-91.
2. Ariyawarda A. & Prathiraja P.H.K., 2003, Impact of nutritional on consumer buying behavior, Sri Lankan Journal of Agricultural Economics, **5**, 1, 5-11.
3. Asumugha G.N., Njoku M.E., Okoye B.C., Aniedu O.C., Ogbonna M.C., Anyaegbunam H.N., Akinpelu O.A., Ibeagi O. & Amaefula A., 2009, An analysis of the supply for seed yams in Nigeria, African Journal of Business Management, **3**, 1, 028-031.
4. Aycan N., Turkoguz S. & Torkusoglu O., 2005, Food attitude towards food safety concept among Turkish university students, Journal of Social Science, **1**, 3, 156-161.
5. Bradbury J.H. & Holloway W.D., 1988, Chemistry of tropical root crops, Australian Centre for International Agricultural Research. Canberra. Australia pp. 101-119.
6. Brunso K., Fjord T.A., Klaus G.G., 2002, Consumers food choice and quality perception, The Aarhus School of Business working paper, N° 77, ISSN 0907 2101.
7. Coursey D.G. & Aidoo A., 1996, Ascorbic acid Level in Ghanaian yam. Journal of the Science of Food and Agriculture, Vol. **17**, 446-449.
8. Daniel T. & Kormawa P., 2002, Determinants of urban households' demand for cassava and cassava products in Kaduna, Northern Nigeria: the application of AIDS model. A paper presented at Conference on International Agricultural Research for Development, Deutscher Tropentag – Witzenhausen, 9-11 October.
9. Deaton A.S. & Muellbauer J., 1980, An almost ideal demand system. American Economic Review, 10, 312-326.
10. FAOSTAT, 2009, Food and Agricultural Organisation. Statistical Report. www.fao.org/stat
11. Hahn S.K., Osiru D.S.O., Akoroda M.O. & Otoo J.A., 1987, Yam production and its future prospects. Outlook on Agriculture, **16,** 3, 105-110.
12. IITA, 2007, International Institute of Tropical Agriculture. Annual Report. www.iita.org
13. IITA, 2009, Yam production in Africa. International Institute of Tropical Agriculture (IITA), Nigeria. Available at http://www.iita.org/cms/details/yam_project_details.aspx?zoneid. (Accessed on 6th April 2010).
14. Kotler P. & Keller K.L., 2007, Marketing Management, 13th edn, New Delhi: Pearson Educational Inc.
15. Kushwaha S. & Polycarp I.M., 2001, Economics of small scale yam production in Qua'an Pau LGA of Plateau. *In*: Abubakar M.M., Adegbola T.A. and Butswat I.S.R. (Eds), The role of agriculture in poverty alleviation. Proc. 34th Ann. Conf. of Agric. Soc. of Nigeria, held at Abubakar Tafawa Balewa University (ATBU), Bauchi, Oct., 15-19, 2001, pp. 69- 74.
16. Myae A.C. & Goddard E., 2010, Consumer's behavior with respect to meat demand in the presence of animal disease concerns: the special case of consumer who eat Bisonm Elk, and Venison. A paper presented at the Agricultural and Applied Economics Association Joint Annual Meeting, Denver, Colorando, July 25-27.
17. NFC, 2003, Summary of Nigeria food consumption and nutrition survey 2001-2003, published by IITA. www.iita.org
18. Nwachukwu I.N., Udeh O.C. & Nwachukwu C.N., 2009, Determinants of buyer attitude towards Made in Nigeria Shoes. The Nigeria Journal of Economic and Management Studies, **4**, 1, 107-116.
19. Olayinka K. & Aminu S.A., 2006, Marketing management planning and control:text and insight, Lagos: Sundoley Press.
20. Osibo O., 2009, Nigeria: country realizes N56 billion from yam export. Vanguard, Nigeria. Available at http://allafrica.com/stories/200903230071.html (Accessed in May 2009).
21. Pius C.I. & Odjuvwuederhie E.I., 2006, Determinants of yam production and economic efficiency among small-holders farmers in Southeastern Nigeria. Journal of Central European Agriculture, **7**, 2, 337-342.
22. Roininen K., Lähteenmäki L. & Tuorila H., 1999, Quantification of consumer attitudes to health and hedonic characteristics of foods. Appetite, **33**, 1, 71-88.
23. Wierenga B., Van T., Klaus G. & Steenkamp E.M., 1997, Agricultural marketing and consumer behavior in a changing world, New York: Kluwer Academic publishers.

I.N. Nwachukwu, Nigerian, Ph.D, Lecturer in the Department of Agribusiness & Management, Michael Okpara University of Agriculture, Umudike, Nigeria.
O.U. Oteh, Nigerian, Graduate Student of the Dept of Marketing, Michael Okpara University of Agriculture, Umudike, Nigeria.
C.C. Ugoh, Nigerian, Graduate Student of the Dept of Marketing, Michael Okpara University of Agriculture, Umudike, Nigeria.
C. Ochomma, Nigerian, Assistant Lecturer, Dept of Agricultural Economics & Extension, Abia State University, Umuahia Campus, Nigeria.

Challenges of Agricultural Adaptation to Climate Change: Empirical Evidence from Southeast Nigeria

A.A. Enete* & A.N. Onyekuru

Keywords: Climate Change- Agricultural Adaptation- Challenges- Nigeria

Summary

Climate change has direct impact on agricultural production, because of the climate-dependent nature of agricultural systems. This impact is particularly significant in developing countries where agriculture constitutes employment and income sources for the majority of the population. This paper, based on primary data collected within the auspices of the African Technology Policy Studies Network (ATPS) examines the challenges faced by farmers (Southeast Nigeria), in applying indigenous climate change adaptation practices in their farms. The study was conducted in two randomly selected states of the region namely Imo and Enugu, and in four randomly selected agricultural zones, two from each state. The data was analyzed using descriptive and inferential statistics. The result of the analysis shows that in the face of extreme weather events occasioned by climate change, and apparently because of its tolerance to these conditions, cassava has become the dominant food crop in the area. Virtually all the respondents were reportedly aware of the effect of climate change on agriculture, but were not aware that some of their agricultural practices could exacerbate climate change. The most often practiced farm activities that could contribute to climate change were, in order of importance, burning of wood fuel, the use of fertilizers and bush burning. The major household level socioeconomic factors identified to be driving farmers' investment in climate change adaptation practices were age, level of formal education and level of awareness of climate change issues. At the societal level, the major factors constraining them from adapting to climate change were poverty, farmland scarcity and inadequate access to more efficient inputs, lack of information and poor skills, land tenure and labour constraints. The findings underscore the need for farmers' education, awareness creation, poverty alleviation and increased access to more efficient inputs as potent tools for climate change adaptation in the area.

Résumé

Les défis de l'adaptation agricole au changement climatique: données empiriques provenant du Sud-Est du Nigeria

Le changement climatique a un impact direct sur la production agricole, car de la nature du climat dépendent des systèmes agricoles. Cet impact est particulièrement important dans les pays en développement où l'agriculture constitue la source d'emploi et de revenus pour la majorité de la population. Cet article, basé sur des données primaires collectées dans le cadre des études de l'African Technology Policy Network (ATPS) examine les défis auxquels sont confrontés les agriculteurs (Sud-Est du Nigeria), en appliquant les pratiques autochtones d'adaptation au changement climatique dans leurs exploitations. L'étude a été menée dans deux états choisis au hasard de la région, à savoir Imo et Enugu, et dans quatre zones agricoles choisies au hasard, deux de chaque Etat. Les données ont été analysées en utilisant des statistiques descriptives et inférentielles. Le résultat de l'analyse montre que, face à des phénomènes météorologiques extrêmes causés par les changements climatiques, et apparemment en raison de sa tolérance à ces conditions, le manioc est devenu la principale culture vivrière dominante dans la région. Pratiquement tous les répondants auraient été conscients de l'effet du changement climatique sur l'agriculture, mais ne savaient pas que certaines de leurs pratiques agricoles pourraient aggraver le changement climatique. Les activités agricoles les plus souvent pratiquées qui pourraient contribuer aux changements climatiques ont été, par ordre d'importance, la combustion de combustibles ligneux, l'utilisation des engrais et les feux de brousse. Des principaux facteurs socio-économiques identifiés au niveau des ménages dans les pratiques d'adaptation aux changements climatiques sont l'âge, le niveau de scolarité et le niveau de sensibilisation aux questions de changement climatique. Au niveau sociétal, les principaux facteurs les contraignant à l'adaptation aux changements climatiques sont la pauvreté, la rareté des terres agricoles et l'accès inadéquat à des facteurs plus efficaces, le manque de d'information et de faibles capacités, le régime foncier et les contraintes du travail. Les résultats soulignent la nécessité pour l'éducation des agriculteurs, la sensibilisation, la lutte contre la pauvreté et un accès accru à des outils plus puissants pour l'adaptation au changement climatique dans la région.

*Department of Agricultural Economics, University of Nigeria, Nsukka, Nigeria.
Received on 31.05.11 and accepted for publication on 14.10.11

Introduction

Climatic change, which is attributable to natural climate cycle and human activities, has adversely affected agricultural productivity in Africa (25). As the planet warms, rainfall patterns shift, and extreme events such as droughts, floods, and forest fires become more frequent (26), which results in poor and unpredictable yields, thereby making farmers more vulnerable, particularly in Africa (23). Farmers (who constitute the bulk of the poor in Africa), face prospects of tragic crop failures, reduced agricultural productivity, increased hunger, malnutrition and diseases (26). It is projected that crop yields in Africa may fall by 10-20% by 2050 or even up to 50% due to climate change (14), particularly because African agriculture is predominantly rain-fed and hence fundamentally dependent on the vagaries of weather. As the people of Africa strive to overcome poverty and advance economic growth, this phenomenon threatens to deepen vulnerabilities, erode hard-won gains and seriously undermine prospects for development (11, 26). There is therefore the need for concerted efforts toward tackling this menace.

Much of climatic change agricultural research has tended to concentrate on assessing the sensitivity of various attributes of crop systems (e.g. crop/ livestock yields, pest, diseases, weeds etc) - the bio-physical aspects of food production, with little or no regard to the socioeconomic aspects. These partial assessments most often consider climatic change effects in isolation, providing little insight into the level of awareness of the farmers on the issue, what and how they are doing to cope with climate change, etc. To better address the food security concerns that are central to economic and sustainable development agenda, it is desirable to also address these aspects of climate change and agriculture. Wisner *et al.* (24) report that the vulnerability of agriculture is not determined by the nature and magnitude of environmental stress like climate change per se, but by the combination of the societal capacity to cope with and/or recover from environmental change. While the coping capacity and degree of exposure is related to environmental changes, they are both also related to changes in societal aspects such as land use and cultural practices. This could be at the root of the much talked about poverty alleviation and food security for the vulnerable groups in Africa, who are most at risk when agriculture is stressed.

This paper, based on primary data collected within the auspices of the African Technology Policy Studies Network (ATPS) examines the challenges faced by farmers of Southeast Nigeria in applying indigenous climate change adaptation practices. Ozor *et al.* (21) studied barriers to climate change adaptation among farm households of southern Nigeria, dwelling mostly on societal constraints thereby ignoring farmers' level of awareness, household level factors and farm practices, all of which could pose challenges to agricultural adaptation to climate change. In addition, Enete and Amusa (7) presented a literature survey of challenges of agricultural adaptation to climate change with no empirical information. The present study attempts to fill these gaps.

Method of the study

The study area

Southeast Nigeria is located within longitudes 5° 30' & 9° 30' E and latitudes 4° 30' & 7° 00' N. It occupies a land area of 75,488 km^2 and comprises nine states namely Abia, Akwa Ibom, Anambra, Bayelsa, Cross River, Ebonyi, Enugu, Imo, and Rivers. These states fall into two geopolitical zones in Nigeria namely the south-south and southeast. While Akwa Ibom, Bayelsa, Rivers and Cross River are in the south-south, Abia, Anambra, Ebonyi, Enugu and Imo are in the southeast.

The region has a total population of 31,371,941 and an average population density of 416 persons per square kilometer. This average however conceals the true picture of population pressure in the region as Madu (17) has shown that population pressure is the most important problem of rural development in the region. The effects of population pressure in the area have been recognized in a broad spectrum of livelihood activities such as intensive agriculture, engagement in non-farm activities, migration and ecological problems.

Sampling procedure and the data

For logistical reasons, the study was restricted to southeast geo-political zone, comprising Abia, Anambra, Ebonyi, Enugu and Imo. Two states were randomly selected from these for the study. These were Enugu and Imo states. In each selected state, two agricultural zones were then randomly selected. These were Owerri and Okigwe in Imo state and Enugu and Nsukka in Enugu state. In each agricultural zone and with the assistance of the extension services Department, farming communities were compiled, from which two communities were randomly selected making a total of eight communities for the study. These were Ugwuene in Agwu and Amaechi in Nkanu, all in Enugu agricultural zone; Umualumo in Okigwe and Okwe in Onuimo, all in Okigwe agricultural zone; Ovoko and Akpa-Edem in Nsukka agricultural zone; Amaigbo and Okpuala in Owerri agricultural zone. In each selected community, a list of farm households was compiled, also with the assistance of extension agents, from which fifty farmers were randomly selected, bringing the total sampled respondents to four hundred for the study.

A structured survey instrument was then developed and pre-tested in a pilot survey/focus-group discussion. This was to help validate the questions

and check the information to be supplied later by the individual farmers. A farmer to farmer visit was next undertaken to collect the data, which included farmer's opinion on the trend of change of climate change variables in the last ten years, farming practices, climate change adaptation practices and estimated costs and returns from these strategies, the area of land where the adaptation practices were applied, etc. The data were analyzed using descriptive statistics, Ordinary Least Squares regression and Factor Analysis.

Results and discussion

Crops and Animals grown/reared in the area

The first most important food crop in the area was cassava, as ranked by 64% of the respondents. Cassava is not only a major staple but also a major source of farm income for the Nigerian farmers (20). And compared to other crops, cassava is the most resistant to extreme weather events. It is therefore most often described as a hardy crop and may in this sense be the most adaptable crop to climate variations (8). Benhin (3) reports that one of the strategies which serve as an important form of insurance against rainfall variability is increasing diversification by planting crops that are drought tolerant and/ or resistant to temperature stresses. Cassava was followed by Yam and Cocoyam with about 23% and 4% respectively of the respondents ranking them as the first most important crop. Yam is the second most important root crop after cassava, especially in southeast Nigeria, where there is generally an annual celebration in honour of the crop. This was followed by vegetables as ranked by 3%, maize and rice by 2% and 1% respectively, oil palm by 1% and other unspecified crops ranked by 2% of the respondents as the first most important crop. From the second to the fourth most important crops, the respondents just listed variations of the above crops, hence discussions were limited to the first most important crop.

On the animals reared in the area, 50% of the respondents ranked goat as the first most important animal domesticated. About 38% ranked poultry, 6% ranked sheep, 5% ranked pig, while 1% each ranked cattle and fish as the first most important animal reared in the area. From the second through the fourth most important animals in the area, the respondents also listed various combinations of the above animals.

Awareness of climate change and its link with agriculture

The respondents were asked whether they have heard of climate change before. About 96% of them responded in the affirmative. This suggests a high level of awareness of the subject matter in the area. The awareness of climate problems and the potential benefits of taking action is an important determinant of adoption of agricultural technologies (10). Maddison (16) argued that farmer awareness of change in climate attributes (temperature and precipitation) is important to adaptation decision making. For example, Anim (1) and Araya and Adjaye (2) reported that farmers' awareness and perceptions of soil erosion problem as a result of changes in climate, positively and significantly affect their decisions to adopt soil conservation measures. On the source of such information, majority (36%) of the respondents indicated that they hear from friends, about 26% of them hear from extension workers, 24% from radio/ television, 2% from researchers, 1% from farmers' cooperatives, while 6% hear from other sources not specified in the survey instrument.

Similarly, on the question of whether climate change will affect agriculture, the respondents overwhelmingly (97%) said yes. Most governments in Nigeria already have agencies charged with environmental issues including climate change and they most often sensitize the people through the radio and television. This may explain the high level of awareness of the respondents. However, majority (52%) of the respondents do not agree that farming contributes to climate change. Thus, suggesting that the farmers, though aware of climate change and its effect on agriculture, were

Table 1
Mean responses of farmers on extent of practice of activities that could cause climate change

	N	Minimum	Maximum	Mean	Std. Deviation
Bush burning	381	1.00	3.00	2.0682	.70380
Continuous cropping	348	1.00	3.00	1.7126	.69012
Over grazing	323	1.00	3.00	1.1393	.40467
Extent to which swamprice is produced	286	1.00	3.00	1.3986	.73642
Extent to which crop wastes are burnt	357	1.00	3.00	1.8235	.70289
Burning of woodfuel	389	1.00	3.00	2.6967	.54727
Use of fertilizers	389	1.00	3.00	2.1568	.76562
Use of insecticids/ pesticides	317	1.00	3.00	1.5710	.74131
Use of herbicides	316	1.00	3.00	1.5222	.74075
Deforestation	312	1.00	3.00	1.5705	.55689

Cut off mark= 2.0

unaware that some of their agricultural practices could exacerbate climate change. This underscores the need for educating the farmers on the consequences of some of their actions. However, FAO (12) reports that although climate change affects agriculture and vice versa, a lot of uncertainties pervade each step of the logic from economic activity to climate change.

Activities of farmers that contribute to climate change

The respondents were asked to indicate the extent to which they practice some suggested farm related activities that could contribute to climate change on a 3-point LSR. The information collected (Table 1) shows that the most often practiced activities by the respondents were burning of wood fuel (mean= 2.70), the use of fertilizers (mean= 2.16), and bush burning (mean= 2.07). With the widely reported rising poverty in Nigeria, especially among farming households, and the also rising prices of cooking gas and kerosene, burning of wood fuel as cooking energy has become the predominant practice, not only in rural farming communities but also among the urban poor (7).

Moreover, decreasing soil fertility is one of the extreme weather events that nearly all the farmers (84%) said has been on the increase in the past ten years. The natural tendency would therefore be to increase the application of fertilizer in order to maintain soil fertility, which contributes to greenhouse gases. In addition, bush burning is generally the preferred traditional means of clearing farmland for seedbed preparation, which increases the concentration of greenhouse gases and particulate matter in the atmosphere. The International Federation of Organic Agriculture Movement (IFOAM) (13) reports that conventional agricultural activities of farmers contribute to climate change because they apply excessive amounts of nitrogen fertilizer that is released as nitrous oxide and mines the earth of the nutrients needed to sustain production through rainforest clearing. Slash and burn techniques reduce carbon storage and release huge amounts of carbon dioxide from burning vegetation.

Household level factors affecting investment in adaptation practices

In assessing the factors that influence the level of investment in climate change adaptation practices, we assume the utility maximization theory, where the household maximizes utility in farm income, which is assumed dependent on the household's level of adaptation to climate change, *ceteris paribus*. This in turn is a purely farm management decision that is related to the household's socioeconomic characteristics (9) such as age, level of education, awareness of climate change related issues. The household's level of adaptation to climate change was indexed by the amount of money (Nigerian Naira) spent on adaptation practices per hectare of farmland.

In doing this, the ordinary least squares regression analysis was used. The result of the analysis (Table 2) show that the explanatory powers of the specified variables seem low (24%), but this is not uncommon in cross sectional analysis. Other works with similar coefficient of determination include Nweke (20) and Enete (8). The overall goodness of fit as reflected by the F-value of (2.93) was however highly significant at ($p< 0.01$).

Four of the nine explanatory variables were significant. Age of the farmer was positively and highly significantly related with the level of investment in climate change adaptation practices by the farmers. This is surprising because older farmers are more likely to be risk averse, especially regarding climate change matters, than younger ones. However, age may likely endow the farmers with the requisite experience that will enable them make better assessment of the risks involved (22) in climate change adaptation investment decisions. Enete *et al.* (9) noted that older farmers have more experience and are able to take healthier production decisions than younger ones.

The farmer's number of years of formal education was also positive and highly significantly related with the level of investment in indigenous climate change adaptation practices. This is to be expected as educated farmers may better understand and process information provided by different sources regarding new farm technologies, thereby increasing their allocative and technical efficiency (22).

The two variables on level of awareness of climate change effects were all positive and significantly related with the level of investment in adaptation practices. These were "*whether the farmer knows that climate change will affect agriculture*" and "*whether the farmer knows that agriculture contributes to climate change*" This underscores the importance of awareness in adaptation measures. The awareness of climate problems and the potential benefits of taking action is an important determinant of adoption of agricultural technologies (12). Maddison (16) argued

Table 2
OLS Regression result on factors affecting the farmers level of investment in adaptation practices

s/n	Variables	Coefficients	t
i.	Age (years)	2716.444	2.55***
ii.	Gender of household head (male=1, female= 0)	-5998.05	-0.26
iii.	Level of education (years)	6453.965	2.68***
iv.	Profit from adaptation practices	25760.96	0.93
v.	Av. Annual income from farming	0.0418673	0.66
vi.	Farm size (ha)	3145.585	0.38
vii.	Household size	1482.901	0.27
viii.	Climate change affect agriculture (yes=1, No= 0)	49687.81	2.01**
ix.	Farming contributes to climate change (yes= 1, No= 0)	91423.52	1.72**

No of observations= 94; R^2= 0.2391; F= 2.93; Prob> F= 0.0045, *** = significant at p≤ 0.01, ** = significant at 0.01≤ p≤ 0.05.

that farmer awareness of change in climate attributes (temperature and precipitation) is important to adaptation decision making. For example, Anim (1) and Araya and Adjaye (2) reported that farmers awareness and perceptions of soil erosion problem as a result of changes in climate, positively and significantly affect their decisions to adopt soil conservation measures.

Societal constraints to climate change adaptations

In this context, we define societal constraints as those that result from the shortcomings of the society at large. In economic terms they are factor constraints resulting from market failure *ceteris paribus*. In this sense therefore they are not within the control of the farm households. The applicable economic theory here is that of externalities and public goods (15). Our focus in this section therefore is to sieve out, from among these constraints, those that are hindering the farm households from adapting to climate change. This was done using factor analysis.

Table 3 presents the result of the factor analysis. It shows the varimax-rotated factors constraining farmers in the area from climate change adaptations. From data in the table, five factors were extracted based on the responses of the respondents. Only variables with factor loadings of 0.40 and above at 10% overlapping variance were used in naming the factors. Variables that have factor loading of less than 0.40 and those that loaded in more than one factor were not used (18). The next step was to give each factor a denomination according to the set of variables or characteristics it was composed of. In this regards, the variables were grouped into five major factors as: factor 1 (poverty constraints), factor 2 (land and more efficient input constraints) and factor 3 (information and training factor), factor 4 (land tenure constraint), and factor 5 (labour constraints).

Under factor 1 (poverty constraints), the specific constraining variables against climate change adaptation include high cost of farmland (0.580), high cost of irrigation facilities (0.483), non-availability of storage facilities (0.648), limited income (0.782), non-availability of processing facilities (0.668), high cost of processing facilities (0.808), and lack of access to weather forecast technologies (0.751). With limited income (poverty), the acquisition of necessary facilities will be difficult. They may not only be costly, but may also appear scarce for poor farmers. In addition, the farmers may not also have the necessary facilities for current information like radio and television to obtain weather forecasts. This underscores the problems of under capitalization of farmers (6) and suggests the need to improve the availability of credit to them. Benhin (3) reports that lack of access to credit or saving and adequate information about climate change are some of the major problems encountered by farmers in adapting to climate change in Africa. Deressa (4) reported that most of the problems or constraints encountered by farmers in adaptation to climate change are associated with poverty.

Under factor 2 (Land and more efficient input problem), the constraining variables against climate change adaptation were: limited availability of land for farming (0.558), non-availability of improved seeds (0.598), high cost of fertilizer (0.761), and high cost of improved varieties (0.725). Benhin (3) noted that farm size is a major determinant of speed of adoption of adaptation measures to climate change. Moreover, Downing *et al.* (5) reported that high yielding and fast growing crops can easily escape the vagaries of climate change by completing their growth cycle before storm and drought sets-in, thereby checking the impact of climate change. The use of heat tolerant and drought resistance crops is also effective adaptation practices.

The factors that loaded under factor 3 (information and training constraints) include poor access to information sources (0.588) and inadequate knowledge of how to cope (0.771). In the present information age, information problems could pose serious challenges to the farmers' coping strategies as they may not be aware of recent developments regarding climate change adaptations and the necessary readjustments needed. Mark *et al.* (19) argued that a lack of adaptive capacity due to constraints on resources like information may result in further food insecurity. In addition, Benhin (3) noted further that farmers' level of education and access to extension service are major determinants of speed of adoption of adaptation measures to climate change.

Under factor 4 (land tenure constraints), the constraining variables were inherited system of land ownership (0.786) and communal system of land ownership (0.775). In traditional societies, individual farmers do not usually have title to farmland but enjoy user rights, which could be withdrawn at any time by the custodian of the communal land. One of the factors identified by Benhin (3) as determining the speed of adoption of climate change adaptation measures is land tenure status.

Under factor 5 (labour constraints), only one variable loaded – high cost of farm labour (0.743). Previous analyses of barriers to climate change adaptation show that shortage of farm labour is one of the major constraints to adaptation by farmers (4).

Conclusion

The foregoing shows that cassava has become the dominant crop in the area, essentially because it is a hardy crop and hence relatively tolerant to the harsh conditions occasioned by climate change. Virtually all the respondents were reportedly aware of the effect of climate change on agriculture, but were

Table 3
Constraints to adaptation (Rotated Component Matrix)

Variables	Factors				
	1	2	3	4	5
Limited availability of land for farming	.152	.558	.283	.384	-.010
High cost of farmland	.580	-.002	.388	.287	.105
Inherited system of land ownership	.086	.249	-.113	.786	-.178
Communal system of land ownership	-.110	.310	-.042	.775	-.139
Poor access to information sources	.353	.030	.588	-.045	-.354
Non-availability of credit facilities	.559	.378	.007	.141	-.496
High cost of irrigation facilities	.483	.244	.222	-.109	.357
Non-availability of farm inputs e.g. improved seeds	.255	.598	.329	-.002	.368
High cost of fertilizers and other inputs	.158	.761	.003	.040	.043
Inadequate knowledge of how to cope or build resilience	.050	.267	.771	-.025	.091
high cost of improved varieties	.013	.725	-.076	.203	.021
Non-availability of farm labour	.404	.413	.371	.129	.219
High cost of farm labour	.042	.201	-.150	-.075	.743
Lack of access to weather forecast technologies	.751	.146	-.267	-.200	.086
Government irresponsiveness to climate risk management	.408	-.206	.683	.006	-.131
Non-availability of storage facilities	.648	.017	.348	.311	-.150
Limited income	.782	.146	.145	.139	-.002
Non-availability of processing facilities	.668	.196	.284	-.150	-.089
High cost of processing facilities	.808	.030	.278	.109	.109
Traditional beliefs/ practices e.g. on the commencement of farming season etc	.204	-.154	.179	.663	.408

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.

not aware that some of their agricultural practices could contribute to climate change. Some of the farm practices in the area that contribute to climate change were the burning of wood fuel, the use of chemical fertilizers and bush burning, in order of intensity. The major household level factors identified to be driving farmers' investment in climate change adaptation practices were age, level of formal education and level of awareness of climate change issues. At the societal level, the factors constraining them from adapting to climate change were poverty, farmland scarcity and inadequate access to more efficient inputs, lack of information and poor skills, land tenure and labour constraints. These findings underscore the need for farmers' education, awareness creation, poverty alleviation and increased access to more efficient inputs as potent tools for climate change adaptation in the area.

Acknowledgement

This paper was produced as part of the implementation of the African Technology Policy Studies Network (ATPS) Phase VI Strategic Plan, 2008-2012 funded by ATPS Donors including the Ministerie van Buitenlandse Zaken (DGIS), the Netherlands, Rockefeller Foundation, amongst others. The authors hereby thank the ATPS for the financial and technical support during the implementation of the program. The Authors specially thank the ATPS Climate Sense Program Director, Dr. K. Urama (ATPS), and the program coordinators including Prof. F. Mutua (ATPS Kenya), Dr. G.O. Essegbey (Council for Scientific and Industrial Research (CSIR), Ghana, Prof. E. Eboh (ATPS Nigeria), Dr. N. Ozor (ATPS), and Ms Wairimu Mwangi (ATPS) for their technical support during the research process.

Literature

1. Anim F.D.K., 1999, A note on the adoption of soil conservation measures in the Northern Province of South Africa. Journal of Agricultural Economics, vol. **50**, 336-345.
2. Araya B. & Adjaye J.A., 2001, Adoption for farm level soil conservation practices in Eritrea. Indian Journal of Agricultural Economics, vol. **56**, 239-252.
3. Benhin J.K.A., 2006, Climate change and South African agriculture: impacts and adaptation options. CEEPA Discussion paper N° 21. CEEPA, University of Pretoria, South Africa.
4. Deressa T., 2008, Analysis of perception and adaptation to climate change in the Nile Basin of Ethiopia. An unpublished Ph.D. thesis, Centre for Environmental Economics and Policy for Africa (CEEPA), University of Pretoria, South Africa.
5. Downing T.E., Ringius L., Hulme M. & Waughray D., 1997, Adapting to climate change in Africa. Mitigation and adaptation strategies for global change Vol. **2**, 19-44.
6. Enete A.A. & Achike I.A., 2008, Urban agriculture and food insecurity/ poverty in Nigeria; the case of Ohafia-Southeast Nigeria. Outlook on agriculture, **37**, 2, 131-134
7. Enete A.A. & Amusa T.A., 2010, Challenges of agricultural adaptation to climate change: a synthesis from the literature. FACTS Reports, vol. **4,** http://factsreports.revues.org/
8. Enete A.A., 2003, "Resource use, marketing and diversification decisions in cassava producing household of Sub-Saharan Africa". A Ph.D dissertation presented to the Department of Agricultural Economics, Catholic University of Louvain, Belgium.
9. Enete A.A., Nweke F.I. & Tollens E., 2002, Determinants of cassava cash income in female headed households of Africa. Quarterly Journal of International Agriculture, **41**, 3, 241-254
10. F.A.O., 2000, Two essays on climate change and agriculture. A developing country perspective. FAO, Rome, Italy.
11. German Advisory Council on Global Change (WBGU), 2004, World in transition fighting poverty through environmental policy. Earthscan, London Gregory, P.J., Ingram, J.S.I and Brklacich M., 2005. Climate change and food security. Philosophical transactions of the Royal Society B. vol. **36**: 2139-2148.
12. Hassan R. & Nhemachena C., 2008, 'Determinants of African farmers' strategies for adapting to climate changes: multinomial choice analysis. AFJARE, **2**, 1, 85-104.
13. IFOAM, 2007, Organic agriculture's role in countering climate change. IFOAM, Germany.
14. Jones P.G. & Thornton P.K., 2002, Croppers to livestock keepers: livelihood transition to 2010 in Africa due to climate change. Global Environmental Change, World Health Organization, Geneva, Switzerland.
15. Kolstad C.D., 2000, Environmental Economics. Oxford University Press, Inc. New York, USA.
16. Maddison D., 2006, The perception of and adaptation to climate change in Africa. CEEPA Discussion paper N° 10, CEEPA, University of Pretoria, South Africa.
17. Madu I.A., 2005, "Population and settlement constraints to sustainable agricultural land use in southeastern Nigeria" *in*: Okoko, E. Adekunle, V.A.J and Adeduntan, S.A. (eds). Environmental sustainability and conservation in Nigeria. Environmental Conservation and Research Team, Federal University of Technology, Akure Nigeria pp. 163-171.
18. Madukwe M.C., 2004, "Multivariate analysis for agricultural extension research". *In*: Terry. A.O. (ed) Research Methods in Agricultural Extension. Pp. 206-236.
19. Mark W.R., Mandy E., Gary Y., Lan B., Saleemul H. & Rowena V.S., 2008, Climate change and agriculture: threats and opportunities. Federal Ministry for Economic Cooperation and Development, Germany.
20. Nweke F.I., 1996, Cassava: a cash crop in Africa. COSCA working paper N° 14, COSCA, IITA, Ibadan, Nigeria.
21. Ozor N.S., Madukwe M.C., Enete A.A., Amaechina E.C., Onokala P., Eboh E.C., Ujah O. & Garforth C.J., Barriers to climate change adaptation among farming households of Southern Nigeria. Journal of Agricultural Extension, 14, 1.
22. Panin A. & Brummer B., 2000, Gender differentials in resources ownership and crop productivity of smallholder farmers in Africa: a case study. Quarterly Journal of International Agriculture, **39**, 1, 93-107.
23. United Nations Framework Convention on Climate Change (UNFCCC), 2007, Climatic change impact, vulnerabilities and adaptation in developing countries UNFCCC Secretariat, Martin-Luther-King-Straat 8 53175 Bonn, Germany. www.unfccc.int
24. Wisner B., Blaikie P., Cannon T. & Davis I., 2004, At risk: natural hazards; people's vulnerability and disasters. 2nd Edition. Routledge, London.
25. Ziervogel G., Nyong A., Osman B., Conde C., Cortes S. & Dowing T., 2006, Climate variability and change: implications for household food security. Assessments of Impacts and Adaptations to Climate Change (AIACC) Working Paper N° 20, January 2006. The AIACC Project Office, International START, Secretariat, Washington DC, USA.
26. Zoellick R.B.A, 1993, Climate smart future. The Nation Newspapers. Vintage Press Limited, Lagos, Nigeria. Page 18

A.A. Enete, Nigerian, Ph**,** Lecturer at the Department of Agricultural Economics, University of Nigeria, Nsukka (UNN), Nigeria.

A.N. Onyekuru, Nigerian, PhD student, Department of Environment, University of York, UK.

NOTES TECHNIQUES TECHNISCHE NOTA'S

TECHNICAL NOTES NOTAS TECNICAS

An Alternative View of Deforestation in Central Africa Based on a Boserupian Framework

I. Bamba[1*], Marjolein Visser[1] & J. Bogaert[2]

Keywords: Deforestation- Central Africa- Demographic pressure– Boserup- Agricultural intensification

Summary

Deforestation remains a real concern for environmental managers. The main causes cited in a wide range of studies are: (i) agricultural practices, especially shifting cultivation, (ii) timber exploitation and (iii) charcoal and firewood production. All of these causes are exacerbated by population growth. This rather pessimistic view of the effect of population growth on the environment corresponds to the Malthusian theory. While there is unanimous agreement on the fact that these forests and their resources are invaluable not only for local populations but also for the entire planet, statistical predictions confirm that the population will place increased pressure on forested areas over the next few decades. Given the lack of clear policy at government level aimed at managing this heritage, it is vital that action is taken while there is still time. In order to help guide the activities of non-government organisations and institutions, which often fill the gaps left by governments with regard to agrarian issues, we propose an alternative approach to tackling the problem of tropical deforestation: the Boserupian vision. This alternative approach, in addition to increasing environmental awareness of the local populations by NGOs, sees population growth as an asset for development. It offers a new view of the required conditions for agricultural intensification based on techniques, which have been tried and tested elsewhere. The fight against deforestation must be initiated by means of agrarian transition in tropical rainforest areas.

Résumé

Une vision alternative de la déforestation en Afrique centrale par l'approche Boserupienne

La lutte contre la déforestation tropicale demeure une réalité préoccupante pour les gestionnaires de l'environnement. De nombreuses études désignent comme causes: (i) les pratiques culturales, essentiellement l'agriculture itinérante sur brûlis, (ii) l'exploitation du bois d'œuvre et (iii) la production de charbon de bois et de bois de feu; le tout sous la pression de la croissance démographique. Cette vision plutôt pessimiste de l'effet de l'augmentation de la population sur son environnement correspond à la thèse Malthusienne. Alors que l'unanimité est faite sur le fait que ces forêts et leurs ressources sont d'une utilité inestimable non seulement pour les populations locales mais pour la planète, les prédictions statistiques confirment l'augmentation de la pression démographique dans les zones forestières pour les années à venir. Face à l'inexistence de politique claire de gestion de ce patrimoine par les états, il s'avère urgent de réagir pendant qu'il est encore temps. C'est pour contribuer à l'orientation des activités des organisations et institutions non gouvernementales qui ont pris le relais des états que nous proposons une approche alternative dans la lutte contre la déforestation tropicale : la vision Boserupienne. Cette alternative Boserupienne, en plus de la conscientisation de la population par les ONG, considère la croissance démographique comme un atout pour le développement. Elle pose les bases d'une réflexion plus poussée sur les conditions initiant une intensification agricole via l'adaptation aux conditions locales de techniques issues d'expériences déjà vécues ailleurs. La lutte contre la déforestation devra passer par le pilotage d'une transition agraire dans les zones forestières tropicales.

Introduction

When it comes to man's relationship with his environment, the thorny problems caused by the reduction of tropical rainforest cover remain at the forefront, not only for the conservation of biodiversity,

[1]Department of Landscape Ecology and Plant Production Systems of the Free University of Brussels, 50 Av. F.D. Roosevelt, C.P. 169, B-1050 Brussels, Belgium.
[*]bambisso@yahoo.fr, Tel.: +32-2-650-21-32, Fax: +32-2-650-48-97.

[2]University of Liège/ Gembloux Agro-Bio Tech. Biodiversity and Landscape Unit, Passage des Déportés 2, B-5030 Gembloux, Belgium.
Received on 03.09.10 and accepted on 13.09.11.

but also due to its probable influence on the current global climate changes (19). The causes of deforestation are wide-ranging but essentially of anthropological origin (2, 7). While some studies attempt to classify these causes, according to their level of intervention, into direct and underlying causes (17), the most widely accepted view identifies population density as the key variable, which explains the loss of forest areas at global level (12). This rather pessimistic view of the effect of population growth on the environment corresponds to the Malthusian theory. According to this theory (20), the human carrying capacity of natural environments is not only limited but also set in stone, while major population growth leads to an imbalance between the environment's productive capacities and the needs of its populations. In fact, the population increases exponentially if this growth is not halted by any limiting factor, whereas livelihoods, especially agricultural production, develop according to a linear progression. This prompted Malthus to conclude that, regardless of the actual livelihoods involved, it is therefore inevitable that they will rapidly fail to meet the needs of a growing population. In the short term, this leads to the institutionalisation of abortion and infanticide practices in the absence of effective methods of contraception and, in the long term, famines, epidemics or large-scale armed conflicts, which make it possible, in a negative way, to re-establish the balance between population density and the human carrying capacity of its environment. Malthus even argued in favour of positively controlling population growth, by means of preventive measures aimed simply at reducing birth rates. His theory was later adopted by the neo-Malthusians (15, 24), who believe that it is rather by means of emigration and pushing back agricultural frontiers that it will be possible to redress this balance. In other words, demographic pressure causes the decline of natural resources and, in order to avoid famine, the population moves to new unoccupied areas, while such areas still exist. Many studies, including even some very recent research, continue to confirm this theory. In tropical zones, according to Williams (28), the degradation of areas covered by forest is in close negative correlation to population densities. At regional level, in Central Africa, Achard *et al.* (1), Bogaert *et al.* (7), and Mayaux *et al.* (21) have shown that a negative causality exists between demographic pressure and forest cover. At local level, the studies conducted by Bamba *et al.* (5, 6) in the forest zones of the Eastern Province of the Democratic Republic of Congo confirm this tendency and essentially point to the following as practices that cause deforestation: shifting cultivation, timber exploitation, and charcoal/firework production, all of which are exacerbated by pressure from demographic growth, which is the ultimate cause of forest decline.

Certainly, forests have long been used to produce goods and services for local populations. In Central Africa, over 90% of households in forested areas are involving in agricultural practices; but with population growth of 2-3% per year, the need for food, space, fuel wood and cultivable land increase pressure on forest resources (10). In order to tackle this problem, the population of these forest regions clears even more forest by practising shifting slash-and-burn agriculture. This traditional agrarian system involves burning an area of forest before using it for discontinuous cultivation, with fallow periods that are many times longer than the cultivation period. Fallowing has the primary purpose of restoring soil fertility and the secondary purpose of preventing the build-up of weeds. However, constraints linked to the reduced availability of land and population growth negatively affect the sustainability of this agrarian system. This dysfunction leads to shorter fallow and extended cultivation periods (13). Over a period of time, the original forest vegetation does not have time to recover by means of the ecological succession following cultivation, which has a whole series of consequences, such as reduced soil fertility, lower yields per area unit and, due to this retroactive system, the obligation to cultivate more and more land in order to obtain the same yield per inhabitant. If it is practised in an unregulated way, as described by Bamban *et al.* (5, 6), deforestation caused by shifting cultivation represents a threat for the future existence of forest habitats and populations. In addition, it has been shown that, beyond a human density threshold estimated at 30 - 60 inhabitants per km², the shifting cultivation system is no longer sustainable (13) and is becoming devastating for the forest. The population density remains low in most forest areas (16). If we consider, for example, the Eastern Province of the Democratic Republic of Congo, the average population density is 15 inhabitants/km², which is rather low compared to the estimated national average of 25 inhabitants/km² (3). But, with average annual population growth estimated at 2.4%, within about thirty years, this density will double and reach the commonly agreed limit for the sustainability of shifting cultivation. Therefore, if this neo-Malthusian tendency continues, what can we expect in terms of the future of existing forests? Will we have to wait for them to disappear altogether before the affected populations react? Before answering this question, it would be useful to explore another approach: Boserup's theory (8).

Boserup's theory: an alternative to deforestation

An alternative theory to the purely Malthusian vision of relationships between populations and agrarian systems was developed by Danish agro-economist Ester Boserup (1910-1999), who worked in the field of economic and agricultural development for the United Nations and other international organisations. She was the author of many works, the most famous of which is entitled "The Conditions of Agricultural Growth:

the Economics of Agrarian Change under Population Pressure" (8) and translated into French under the title "Evolution Agraire et Pression Démographique" (9). This work adopts exactly the opposite of Malthusian theory, by considering that, in the non-industrialised countries, rural population growth is a favourable factor for agricultural intensification. It is seen as increasing the quantity of work and/or capital per cultivated area unit in order to increase the yield of each area unit. It would therefore be an illusion to expect an intensification of agricultural production if the population density remains low (18). In her analysis of agrarian dynamics based on increased land pressure, Boserup (8) identifies various stages of this development mainly according to the fallow duration[1].

These stages range from shifting cultivation to permanent agriculture systems, which may include several agricultural cycles within the same year (18). Following problems caused by reducing the fallow period, populations are compelled to adapt their agrarian systems for the purpose of intensification.

Therefore, based on this Boserupian approach, demographic growth, instead of being negative, should be considered rather as a catalyst for change in terms of reorganising agricultural production and working towards intensification. The more population density increases, the more population will use its capacity for invention and technological innovation (26, 27). Man is equipped for creativity and rationality. This means that he is faced with constraints; he adapts and progresses technologically depending on the risk linked to his survival. But he will not do this if this need fails to make itself felt. Based on this argument, Boserup lays the foundations for a more advanced study of the conditions that initiate agricultural intensification, which has the merit of nuancing the Malthusian theory and reflecting on the possibilities of agricultural intensification in terms of slowing down the pace of deforestation currently observed in the forest region of Central Africa. The challenge is that of knowing the time scale, within which this intensification may take place in relation to the threat of relatively rapid environmental degradation following deforestation.

Agricultural intensification manifests itself in relation to changes affecting the relative contributions of three traditional factors for agriculture activities: land, labour and physical capital. The yield can therefore be expressed not only per area unit but also per labour unit (man/day) or per unit of invested capital. The increasing scarcity of land due to the effects of demographic growth leads to an intensification, which may be reflected in increased labour investment per cultivated area unit, as observed in the agricultural history of the non-industrialised countries, or capital investment (acquisition of equipment, use of inputs, building construction) as currently observed in the industrialised countries.

In most African forest regions today, we are still faced mostly with a pre-industrial agrarian system, virtually without capital (primitive tools), but (still) equipped with a huge reserve of land. In this context, the shifting cultivation system optimises the yield per labour and capital unit ("rare" factors) at the expense of yield from the land, which is the less rare factor. However, we must remember that agricultural intensification achieved by increasing the amount of labour invested per area unit, based on this cultivation system, carries with it the heavy price of reduced labour productivity. In other words, it is necessary to work for longer in order to obtain the same productivity from the land. Under sustained demographic growth conditions and without alternative ways forward, sooner or later the population will have to learn to survive a crisis caused by the scarcity of land, as explained so well by Boserup (9). However, it may take centuries before we react in order to overcome this crisis. In this way, the experience gained in Europe should be highly useful. In fact, Mazoyer and Roudart (22) describe how this crisis phenomenon has repeatedly been followed by an agrarian revolution in the history of Europe. These crises have had explicitly Malthusian consequences (wars, famines and epidemics, such as the plague), which acted as negative controllers of demographic growth until the agrarian system was fundamentally transformed in the direction of intensification.

In reality, agricultural intensification, combined with the processes associated with pushing back the frontiers of agriculture, also depends on a whole series of social, political and economic factors (11). In fact, in forest areas, most land is customary land. For example, in the Eastern Province of the Democratic Republic of Congo, which, with over 73% forest cover, is now considered a *hot spot* for deforestation in the Congo Basin (1), customary land rights take priority over the rights of the state. Land rights are held and exercised by the clan or community, to which individuals belong, which have rights and duties (3). On the land belonging to his village, the individual or more precisely the household can choose freely where to place its fields, provided this does not infringe the

[1]Boserup's classification consists of five categories:
- Forest fallow or shifting cultivation (land is cleared in the forest, cultivated for one or two years then left fallow for a sufficiently long period, so that the forest grows back after a minimum of 15-20 years);
- Shrub fallow agriculture (during a fallow period of 8-10 years, the land is covered with bushes and shrubs, the duration of the cultivation period ranges from one year to a duration equivalent to that of the fallow period);
- Short-term fallow cultivation (the fallow period lasts only one or two years and the land is invaded by grasses during this period);
- Annual harvesting (the land is harvested every year but it is still possible to refer to it as fallow for the few months between the harvest and next sowing);
- Multiple harvests (the same field produces several consecutive harvests per year).

rights of other individuals, and can farm as much land as he wishes without restriction (3). In addition, the increased poverty, inadequate road infrastructures for the transport of harvests from fields situated far from the roads, combined with funding deficiencies for crop production, mean that agricultural potentials cannot be put to optimum use. In order to obtain food product supplies from the towns or imported from other countries, the rural population has to supply even more of the forest products demanded by the town dwellers (charcoal, firewood, etc.), which increases pressure on the forests. It must be noted that the lack of a clear integrated policy for managing the forests and agriculture means authorities in the relevant countries can take advantage of the financial windfall created from the exploitation and export of natural resources (wood and mines). This phenomenon is known as the "Dutch disease" (14). As long as the forest is available and accessible, left to itself, the population cannot understand why it should be deprived of this resource.

According to Boserup, agricultural intensification, combined with the reduction or even abandonment of fallowing, involves developing alternatives techniques for the maintenance and restoration of soil fertility using local resources. Various possibilities can be envisaged depending on the pedoclimatic zones. In areas highly affected by deforestation or low forest cover, intensification can be achieved by integrating livestock farming increasingly closely with agriculture, based on the agrarian history of temperate Europe (22).

As this option would not be realistic in many densely forested areas (25), we currently believe that the only other realistic option is that of agroforestry. Agroforestry refers to a range of techniques and practices, in which ligneous species are intentionally combined with crops in an organised way when it comes to space and time. In a context, in which fields are increasingly far from the forest, this mixture of trees and crops may be more productive than their spatial separation (17, 29). In other words, agroforestry makes it possible to increase productivity per area unit, while providing fuel wood, fodder and fruit. But this set of techniques will not be adopted without the provision of participative research and development programmes aimed at demonstrating its possibilities.

The development of village forests, which are managed and administered by the village authorities, could also be beneficial for the collection of forest products. In fact, replanting with slow-growing forest species (high forest) and fast-growing species for firewood (coppices) could be integrated in the management of these forests in each village. This set of techniques must form the basis for the intervention of organisations and NGOs, aimed at providing a real alternative to populations faced with the loss of these natural resources, whose importance for the production of goods and services is recognised by the populations themselves (4, 23). These research and development programmes must be accompanied by non-contradictory and visionary agricultural, environmental and food policies. The current "Dutch disease" policy, which is to the detriment of agricultural development, leads to increased food product imports. Ultimately, this can only result in a major crisis, due to the population's growing dependence on the outside world.

Boserup's message (8) was that the human carrying capacity of an eco-region is not set in stone (Malthusian theory) but flexible, as man is able to transform an original eco-system in order to make it more productive for his needs, while preserving the forests required in order to supply a whole series of ecosystem services in deforested areas. Obviously, this flexibility also has its limits and beyond these limits, Malthus will be right again.

Our message is that it must be possible to reduce the pace of deforestation in Central Africa. This deforestation is caused essentially by an agrarian system, which has become anachronistic due to growing population densities. For this reason, we believe that the reduction of deforestation involves the provision of a new agricultural and environmental policy that is coherent and takes into account the need to accelerate the transition towards a new and more intensive agrarian system, which is therefore more in tune with the current population densities (18), while respecting limitations in terms of the flexibility of human carrying capacity. At the same time, this agrarian transition and resulting global economic development (22) will enable positive birth rate control and population stabilisation, which is the final phase of the demographic transition anticipated by the demographers (18).

Literature

1. Achard F., Eva H., Glinni A., Mayaux P., Richards T. & Stibig H.J., 1998, Identification of deforestation hot spot areas in the humid tropics. Luxembourg, European Commission, TREES Publications Series B, N°4.
2. Allen J. & Barnes D., 1985, The causes of deforestation in developing countries. Annals of the Association of American Geographers, **75**, 2, 163-184.
3. Anonyme, 2005, Monographie de la Province Orientale. Ministère du plan, RDC. Kisangani, 134 p.
4. Bahuchet S., 2000, Les systèmes de production des peuples forestiers pp. 43-63. *In*: Bahuchet S., De Maret P. (Editors). Avenir des peuples des forêts tropicales, 2, Approche thématique. Commission Européenne, Direction Générale VIII, Bruxelles.
5. Bamba I., Barima Y.S.S. & Bogaert J., 2010a, Influence de la densité de la population sur la structure spatiale d'un paysage forestier dans le bassin du Congo en République Démocratique du Congo. Tropical Conservation Science, **3**, 1, 31-44.
6. Bamba I., Yedmel M.S. & Bogaert J., 2010b, Effets des routes et des villes sur les forêts denses dans la province de la République Démocratique du Congo. European Journal of Scientific Research, **43**, 3, 417-429.
7. Bogaert J., Bamba I., Koffi K.J., Sibomana S., Kabulu Djibu J.P., Champluvier D., Robbrecht E., De Cannière C. & Visser M.N., 2008,

Fragmentation of forest landscapes in Central Africa: causes, consequences and management. pp. 67-87. *In*: Lafortezza R., Chen J., Sanesi G. & Crow T.R. (Editors) Patterns and processes in forest landscapes, Springer Verlag, London.

8. Boserup E., 1965, The economics of agricultural growth: the economics of agrarian change under population pressure. George Allen and Unwin Ltd. London, 124 p.
9. Boserup E., 1970, Evolution agraire et pression démographique. Flammarion. Paris, 222 p.
10. CARPE., 2001, Deforestation in Central Africa: Significance and Scale of the Deforestation, Congo River Basin Information Series. Issue Brief #6.
11. Carr D.L., 2004, Proximate population factors and deforestation in tropical agricultural frontiers. Population and Environment, **25**, 6, 585-612.
12. Contreras-Hermosilla A., 2000, The underlying causes of forest decline. Indonesie: CIFOR, occasional paper n°30.
13. Dounias E., 2000, La diversité des agricultures itinérantes sur brûlis. pp. 65-106. *In*: Bahuchet S., De Maret P. (Editions). Avenir des peuples des forêts tropicales, 2, Approche thématique, Commission Européenne, Direction Générale VIII, Bruxelles.
14. Ebrahim-Zadeh C., 2003, Back to basics. Dutch disease: too much wealth managed unwisely. Finance and Development. A quarterly magazine of the IMF. 40, 1-4.
15. Ehrlich P., Ehrlich A. & Daily G., 1993, Food security, population and environment. Population and Development Review, 19, 1-32.
16. FAOSTAT, 2009, Disponible *via* http://faostat.fao.org/site/452/default.aspx
17. Geist H.J. & Lambin E.F., 2001, What drives tropical deforestation? A meta-analysis of proximate and underlying causes of deforestation based on sub-national case study evidence. LUCC, Report serie N°4, Louvain-la-Neuve. Belgium, 116 p.
18. Jouve P., 2004, La croissance démographique, frein ou opportunité pour une intensification agricole durable en Afrique subsaharienne? Transition agraire et résilience des sociétés rurales. Courrier de l'environnement de l'INRA, 52, 101-106.
19. Locatelli B. & Merckx V., 2005, Changement climatique : les négociations de Buenos Aires. Bois et Forêts des Tropiques, **286**, 4, 73-80.
20. Malthus T., 1798, Essai sur le principe de population. Éditions Gonthier, 1963. Paris, 236 p.
21. Mayaux P., Gond V., Massart M., Pain-Orcet M. & Achard F., 2003, Evolution du couvert forestier du bassin du Congo mesurée par télédétection spatiale. Bois et Forêts des Tropiques, **277**, 3, 45-52.
22. Mazoyer M. & Roudart L., 1997, Histoire des agricultures du monde, Points, Paris, 534 p.
23. McKey D., 2000, Tropical forest peoples and biodiversity. pp. 12-32. *In*: Bahuchet S., De Maret P. (Editors). Avenir des peuples des forêts tropicales, 2, Approche thématique, Commission Européenne, Direction Générale VIII, Bruxelles.
24. Meadows D.H, Meadows D.L., Randers J. & Behrens W.W., 1972, The limits to growth. Universe Books : New York, 205 p.
25. Ruf F., 2009, Les zones forestières et la lente intégration de l'élevage dans les exploitations de culture pérenne. Ghana, Côte d'Ivoire et Indonésie. pp. 171-192. *In*: Thibaud B., François A. (Editors). Systèmes de production et durabilité dans les pays du Sud. Khartala Editions.
26. Simon J., 1985, L'homme, notre dernière chance. Presse universitaire de France. Paris, 424 p.
27. Simon J., 1989, On aggregate empirical studies relating population variables to economic development. Population and Development Review, **15**, 2, 323-332.
28. Williams M., 2000, Dark ages and dark areas: global deforestation in the deep past. Journal of Historical Geography, **26**, 1, 28-46.
29. Whitmore J.L. & Brumel B., 1986, Industrie et agroforesterie. *In*: FAO (Editions). L'évolution de l'industrie forestière, UnaSylva 153.

I. Bamba, Ivorian, Doctor of Science, Research professor in the field of landscape ecology, member of the Department of Landscape Ecology and Plant Production Systems of the Free University of Brussels, 50 Av. F.D. Roosevelt, C.P. 169, B-1050 Brussels, Belgium, bambisso@yahoo.fr,
Tel.: +32-2-650-21-32, Fax: +32-2-650-48-97.

Marjolein Visser, Belgian, Professor of agro-ecology and agrarian systems, member of the Department of Landscape Ecology and Plant Production Systems of the Free University of Brussels, 50 Av. F.D. Roosevelt, C.P. 169, B-1050 Brussels, Belgium.

J. Bogaert, Belgian, Professor of landscape ecology, University of Liège/ Gembloux Agro-Bio Tech. Biodiversity and Landscape Unit, Passage des Déportés 2, B-5030 Gembloux, Belgium.

LES ACTIONS DE LA DGD

DGD'S ACTIVITIES

DE ACTIVITEITEN VAN DE DGD

LAS ACTIVIDADES DEL DGD

Research into Biodiesel from Jatropha among the Winners but Jatropha is not a Wonder Plant

On 16 November 2010 researcher Wouter Achten received the FWO McKinsey & Company Science Prize for his lifecycle-oriented study into the sustainability of tropical biodiesels. He did research in India, among other places, for which he received a Flemish PhD scholarship (VLADOC) from VLIR-UOS. Wouter Achten is currently a doctoral research fellow at the Forest, Nature and Landscape Section (K.U. Leuven).

The study in five questions

What did you want to find out in your study?
I wanted to evaluate whether biodiesel from tropical *Jatropha curcas* is a sustainable alternative to fossil diesel and whether it attains a better sustainability performance than other biofuels.

Why did you feel that was important to study?
Because a great many investments and land conversions were planned without adequate knowledge of the plant itself, never mind the sustainability aspects of such a system. These investments and land conversions were predominantly planned in the South. I felt that big risks were being taken due to the lack of knowledge, mostly in the South.

What do you feel is the study's most important discovery and why?
Despite Jatropha's status and the attention and investments it has been given, it's not a wonder plant. Sustainability will only be attained in a small set of situations where the conditions are right. That's why Jatropha seems to us to be an option for small-scale production in rural conditions for local consumption.

What is the link with developing countries in your study?
The Jatropha hype is mainly about application in warm dry countries. Jatropha comes from Central America, but has been and continues to be primarily promoted in India and Africa.

Why is your study relevant to development in the South?
It shows what conditions are needed if the Jatropha biodiesel system is to function. It identifies a number of gaps in knowledge. It shows where we might be able to improve the process and lastly it shows the opportunities for small-scale production in rural areas for local consumption.

Read more about the study at
http://tinyurl.com/JatrophaArchives

Top expertise thanks to VLADOC

Professor Bart Muys (K.U. Leuven) has supervised a number of PhD students who have received a VLADOC grant from VLIR-UOS.
"Linking VLADOC grants to existing VLIR-UOS projects in the South generates advantages with regard to project coherence and reinforcement. In my own group, the PhDs of Raf Aerts and Bert Reubens are good examples of that. They built the capacity of the VLIR-UOS projects in the South and even resulted in spin-offs, such as Ma'ar and Trees for Farmers. Spin-offs can be a source of continuity when VLIR-UOS financing ends.

One thing linking a VLADOC grant to a VLIR-UOS project in the South does not permit is exploring innovative ideas or strengthening international networks. Let me use Wouter Achten as an example. His study was about Jatropha, its use in biodiesel production and the connection with sustainability and food security. By choosing a very innovative subject, in association with ICRAF, the international Research Centre, as a research group we were able to acquire top expertise in a short space of time.
The VLADOC study generated a multiplier effect in all areas:

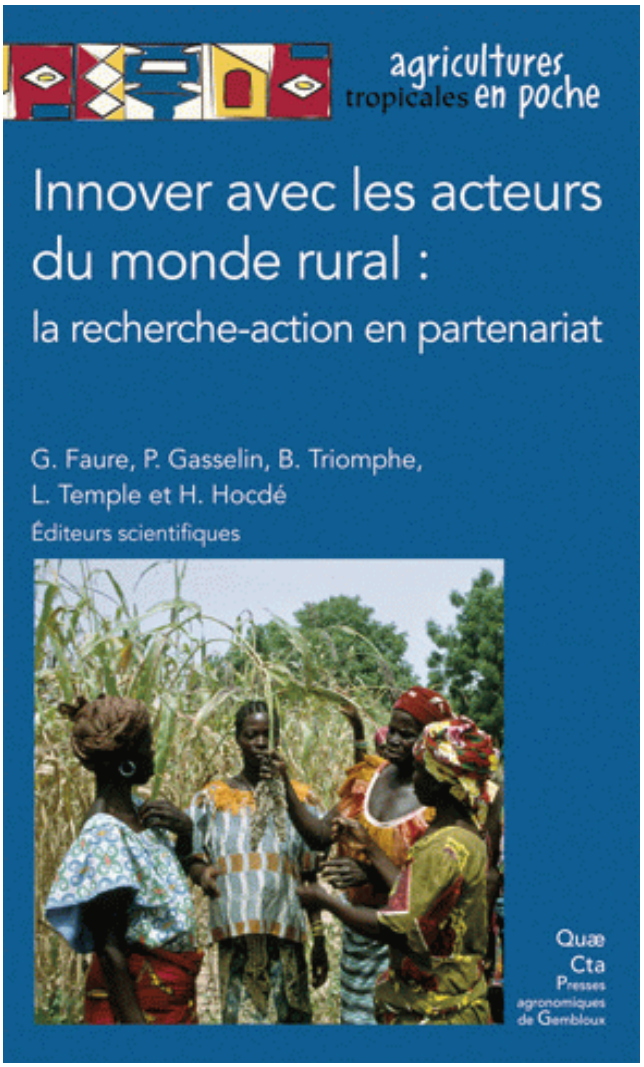
• 10 articles about Jatropha in international periodicals
• International recognition as Jatropha experts
• 3 additional PhDs on this subject, including the PhD of Aklilu Negussie, a researcher from Mekelle who started out as a technician in our Own Initiative project in 2000, earned a master's degree under the supervision of VLADOC scholarship recipient Raf Aerts and is now doing a PhD at K.U. Leuven in Zambia with logistical support from Belgian company D1 Oils Plant Science.
• 2 additional projects: an EU FP6 ERA ARD project on Jatropha sustainability in Mexico and India and a Belgian ERA ARD project (DGD/Africa Museum) on Jatropha sustainability in Mali."

This text was published in the 2010 annual report of VLIR.

BIBLIOGRAPHIE BOEKBESPREKING

BIBLIOGRAPHY BIBLIOGRAFIA

Innover avec les acteurs du monde rural: la recherche-action en partenariat



Edition 2010
Editeur: Quae/ Cta/ Presses agronomiques de Gembloux
Collection: Agricultures tropicales en poche
221 pages, 3 tableaux, 8 figures.

ISBN 978-2-87016-095-4

Prix Belgique: 25 euros (TVAC)
Export: 25 euros (HTVA)

Contact: presseagro.gembloux@ulg.ac.be
Commande site: www.presseagro.be

La recherche-action en partenariat combine production de connaissances, transformation des réalités sociales et renforcement des compétences individuelles et collectives. L'ouvrage donne les fondements de la recherche-action en partenariat en agriculture et des éléments pour mettre en pratique une telle démarche. Il souligne non seulement les questions théoriques mais aussi les questions pratiques que soulève ce triple objectif. L'ouvrage s'appuie sur un large éventail d'expériences dans l'agriculture des pays du Sud, qui illustrent comment des praticiens ont répondu aux défis d'une démarche qui est toujours à réinventer selon les contextes. Des exemples concrets sont donnés, repris d'expériences menées tantôt au Brésil, tantôt au Cameroun, au Vietnam, au Sénégal, ... Ce manuel de plus de 200 pages est destiné aux chercheurs, aux techniciens du développement rural et aux représentants d'organisations du monde rural confrontés à la résolution des problèmes complexes que pose le développement rural dans les agricultures des pays du Sud.

Les auteurs

Guy Faure, économiste au Cirad, mène des recherches sur l'accompagnement des producteurs et de leurs organisations dans le cadre de partenariats en Afrique et en Amérique latine.

Pierre Gasselin, agronome et géographe à l'Inra, conduit ses recherches en Amérique latine et en France dans des situations de pluriactivité et de crises sectorielles ou territoriales.

Bernard Triomphe, agronome au Cirad, travaille sur les interfaces entre systèmes techniques et processus d'innovation, notamment la conception d'innovations de la recherche-action en partenariat en Amérique latine et en Afrique.

Ludovic Temple, économiste au Cirad, focalise ses recherches sur les déterminants institutionnels et organisationnels des changements technologiques dans les filières alimentaires, notamment en Afrique subsaharienne et en Amérique latine.

Henri Hocdé, agronome-système au Cirad, engagé pendant plusieurs années en Amérique latine dans des actions de renforcement des capacités d'innovation des agriculteurs, se consacre actuellement aux dispositifs de recherche en partenariat.

ORGANIZACIÓN

Naturaleza de la entidad responsable de la publicación y el asunto de la revista TROPICULTURA.
Agri-Overseas a.s.b.l. es una asociación creada con el objetivo de establecer relaciones profesionales de interés común entre todos aquellos quienes obran para el desarrollo rural en los países del Sur. Esta asociación publica la revista científica y de información "Tropicultura" dedicada a los problemas rurales en los países en desarrollo.Esta revista es publicada trimestralmente con el apoyo financiero de la Dirección General de la Cooperación al Desarrollo (DGD), Servicio Público Federal de Asuntos exteriores, Comercio Exterior y Belgas Cooperación al Desarrollo, y aquel de la región de Bruselas-Capital; quienes reciben auspicio científico de la Academia Real de Ciencias de Ultra Mar (ARSOM) y del apoyo del Consejo Interuniversitario de la Comunidad Francesa de Bélgica (CIUF) y del Consejo Interuniversitario Flamenco (VLIR).

Agri-Overseas a.s.b.l. se compone de miembros individuales y de miembros de las instituciones belgas siguientes: la Academia Real de Ciencias de Ultra Mar (ARSOM), la Comisión universitaria para el Desarrollo del Consejo Interuniversitario de la Comunidad Francesa de Bélgica (CUD-CIUF), la instancia de Cooperación Universitaria al Desarrollo del Consejo Interuniversitario de la Comunidad Flamenca (VLIR-UOS), las cuatro Facultades en Ciencias agronómicas de Bélgica (Gembloux, Gent, Leuven y Louvain- La- Neuve), las dos Facultades en Medicina veterinaria (Gent y Liège), el Departamento de Salud animal del Instituto de Medicina tropical de Emberes (Antwerpen), la Sección *interfacultaria* de Agronomía de la Universidad Libre de Bruselas, las Facultades Universitarias de Nuestra Señora de la Paz (Namur), El Departamento de Ciencias y gestión del medio ambiente de la Universidad de Liège (Arlon), la Dirección General de la Cooperación al Desarrollo (DGD).

Consejo de administración

El consejo de administración de Agri–Overseas se compone del Profesor Dr J. Vercruysse, Presidente; del Profesor Dr Ir G. Mergeai, Administrador Delegado; del Dr E. Thys, Secretario; del Profesor Dr B. Losson, Tesorero; del Profesor J. Bogaert, miembro representante de la CUD-CIUF; del Profesor Ch. De Cannière, miembro representante de la ULB, del Profesor Honorario Dr S. Geerts, miembro representante de la ARSOM; del Profesor R. Merckx, miembro representante de VLIR y del Profesor Honorario Dr Ir J. Hardouin, miembro.

Comité de redacción

El comité de redacción se compone del Profesor Dr Ir G. Mergeai, Redactor en jefe, y de los siguientes redactores delegados: el Profesor Ch. De Cannière «la Selvicultura, la ecología del paisaje y de los sistemas de la producción vegetal" , el Profesor Dr J.-P. Dehoux de «la Producción pecuaria y la Gestión de la fauna», el Dr D.de Lame de «la Sociología», el Profesor Honorario Dr F. Malaisse de «Ciencias forestales y la Ecología», el Profesor Emérito Dr J.-C. Micha de «la Pesca y la Piscicultura», el Profesor Dr Ir E. Tollens de «Economía rural», el Profesor Dr Ir P. Van Damme de «Agronomía y la «, el Profesor Dr E. Van Ranst de «Ciencia del suelo «, el Profesor Dr J. Vercruysse y Dr E. Thys para «la Salud animal» y Ir. F. Maes, colaborador científico. La secretaría trata directamente los otros temas en lo que posee competencia (economía, sociología,...).

Secretaría de redacción

11, Rue d'Egmont, B- 1000 Bruxelles – Bélgica
Teléfono: ++32.(0)2.540 88 60/ 61; Fax.: ++32.(0)2.540 88 59
Email: ghare.tropicultura@belgacom.net, mjdesmet.tropicultura@belgacom.net
Website: http://www.tropicultura.org

Distribución

La distribución de la revista TROPICULTURA es gratuita y puede ser obtenida con un simple pedido escrito, dirigido a la secretaría de redacción.

ALCANCE DE LA REVISTA

TROPICULTURA publica artículos originales, informes de investigación y síntesis, resúmenes de libros y tesis, así como informes de películas y soportes audiovisuales en lo que concierne a todas las áreas vinculadas al desarrollo rural: producciones vegetales y animales, ciencias veterinarias, ciencias forestales, ciencias del suelo y de la tierra, Ingeniería rural, ciencias del medio ambiente, bioindustrias, industria agro-alimentaria, sociología y economía.

INSTRUCCIÓN A LOS AUTORES

Los temas de los artículos publicados en la revista Tropicultura conciernen todo lo es vinculado al desarrollo rural y la gestión sostenible del medio ambiente de las regiones cálidas del planeta. Se dará la prioridad a los artículos que presentan asuntos originales, abarcando un ámbito lo más amplio posible, es decir cuyo contenido concierne sobre todo aspectos metodológicos transferibles en un conjunto amplio de medios ambientes y regiones del mundo.
De igual manera, se dará una atención particular en la fiabilidad de las informaciones publicadas, es decir, cuando se trata de resultados experimentales, en el número de repeticiones de los ensayos, en el tiempo y en el espacio, que son al origen de los datos obtenidos.
Los manuscritos serán inéditos y no habrán sido sometidos a una publicación anteriormente o simultáneamente. Se pueden redactar en uno de los cuatro idiomas siguientes: ingles, español, francés y holandés. Los manuscritos están dirigidos al redactor en jefe a través del servicio postal, en tres ejemplares, en forma de documento en papel o directamente a la dirección electrónica de la secretaría de redacción, en forma de archivos adjuntos. Se redactarán en cara simple, en doble espacio (27 líneas de 60 caracteres por página en formato DIN A4), con un margen de 3,5 cm mínimo alrededor de la superficie imprimada. Ellos contendrán un máximo de diez páginas de texto (excluyendo la primera página, los resúmenes y las referencias bibliográficas).
La primera página llevará: el título, el título abreviado (máximo 55 caracteres), los apellidos y nombres completos de los autores, la dirección profesional completa de cada uno, los agradecimientos eventuales. El apellido del autor corresponsal será marcado mediante un " * " y su dirección completada por sus números de teléfono y telecopia y de su dirección electrónica.
Las páginas siguiendo la primera página presentarán: (i) los resúmenes (max 200 palabras) en el idioma del manuscrito y en ingles, precedidos del título traducido y seguidos de un máximo de seis palabras claves dentro de cada uno de los dos idiomas; (ii) el texto principal; (iii) la bibliografía; (iv) se admitirán solamente tres cuadros numerados por medio de cifras árabes; (v) las ilustraciones identificadas sin ambigüedad por un número al verso; (vi) las leyendas de los cuadros y las ilustraciones. Todas las páginas serán numeradas en continuo. Se aceptarán tres figuras, dibujadas de manera profesional. Las fotografías serán proporcionadas no montadas, bien contrastadas sobre papel brillante.
Solamente los coautores, quienes habrán manifestado por escrito su acuerdo para que su nombre figura en un manuscrito, aparecerán en la versión final del articulo publicado en Tropicultura. Los acuerdos escritos de los coautores concerniendo este punto podrán ser transmitidos al Comité de redacción en forma de correo postal o electrónico. La aprobación del organismo de tutela de los autores es supuestamente adquirida para todo artículo que se publica en Tropicultura. Agri-Overseas declina toda responsabilidad en esa matera.
El primer depósito de un articulo a la redacción podrá hacerse en forma impresa o en forma electrónica. En la medida de lo posible, después de la aceptación del artículo para publicación, el autor dará su ultima versión, revisada y corregida, sobre un disquete (o en forma de archivo adjunto). El software Word es recomendado pero una versión ASCII o RTF de los archivos es aceptada.
El texto estará generalmente dividido en introducción, material y método, resultados, discusión, conclusiones. La subdivisión del texto no sobrepasará de dos niveles. Los subtítulos, muy concisos serán conformados en minúsculas y jamas no serán subrayados.
Las referencias estarán citadas dentro del texto por medio de números ubicados entre paréntesis. En caso de citación de varias referencias, sus números se sucederán por orden ascendente.
Las referencias bibliográficas serán clasificadas por orden alfabético de apellidos de autores y por orden cronológico para un autor dado. Ellas estarán numeradas en continuo , comenzando por la cifra 1.
Para los artículos de revistas, las referencias comprenderán: los apellidos de los autores seguidos de las iniciales de los nombres, el año de publicación, el título completo del artículo en el idioma de origen, el nombre de la revista, el número del volumen subrayado, los números de la primera y de la ultima página separadas por un guión.
Ejemplo: Poste G., 1972, Mechanisms of virus induced cell fusion. Int. Rev. Cytol. 33, 157-222.
Para las monografías, los elementos siguientes son esenciales: los apellidos de los autores seguidos de las iniciales de los nombres, el año de publicación, el título completo de la obra, el apellido del editor, el lugar de edición, la primera y la ultima página del capítulo citado, el número total de páginas de la obra. Los informes de conferencias se tratan como monografías, además, ellos mencionarán si es posible el lugar, la fecha de la reunión y el (los) editor(es) científico(s).
Ejemplo: Korbach M.M. & Ziger R.S., 1972, Heterozygotes detection in Tay-Sachs disease a prototype community screening program for the prevention of recessive genetic disorders pp 613-632, i*n*: B.W. Volks & S.M. Aronson (Editors), Sphingolipids and allied disorders, Plenum, New-york, 205 p.
Una comunicación personal será mencionada en el texto por las iniciales del nombre, seguidas del apellido, comunicación personal y el año. Ejemplo (W.R. Allan, comunicación personal, 1988). Esta referencia no se volverá a tomar dentro de las referencias bibliográficas.
Las referencias anónimas serán mencionadas numéricamente en el texto y en las referencias bibliográficas. Anónimo (año). Título. Fuente (donde las informaciones pueden ser encontradas.)
El comité de redacción se reserva el derecho de rechazar todo artículo no conforme a las prescripciones mencionadas en la parte superior.
Los artículos están sometidos a uno o a varios lectores escogidos por la redacción y esos lectores se mantendrán anónimos por los autores.
En caso de aceptación del artículo, la redacción exigirá un compromiso de los diferentes autores a ceder sus derechos de publicación a TROPICULTURA.

TROPICULTURA

2011 Vol. 29 N° 4
Four issues a year (October, November, December)

CONTENTS

TROPICULTURA IS A PEER-REVIEWED JOURNAL INDEXED BY AGRIS, CABI, SESAME AND DOAJ

LITHO-OFFSET J.F. DE JONGHE • 696 CHSSEE DE GAND B1080 BRUSSELS • +32 (2) 465 77 17

With the support of THE BELGIAN DEVELOPMENT COOPERATION .be

KAOW - ARSOM

Con el apoyo de LA COOPERACIÓN BELGA AL DESARROLLO .be