INTEGRATED PEST MANAGEMENT

Biological control agents of aphids (Homoptera: Aphididae) on potatoes (*Solanum tuberosum* L) in Kenya

Machangi J. M.[1,2], Gitonga L.M.[1], Nderitu J. H.[3], Maniania N.K.[2] and Kabira J. N.[4]

[1]Jomo Kenyatta University of Agriculture and Technology, Juja, P.O. Box 62000, Nairobi, Kenya
[2]International Centre of Insect physiology and Ecology, Duduville, P.O. Box 30772-00100, Nairobi, Kenya
[3]University of Nairobi, CAVS, P.O. Box 30197, Nairobi, Kenya
[4]National Potato Research Centre, KARI-Tigoni, P.O. Box, 57811 00200, Nairobi, Kenya
Contact e-mail: mainamachangi@yahoo.com or jmaina@icipe.org Telephone: +254-20-2373927

Abstract

A field survey was conducted in four major potato growing areas in Kenya (Kiambu, Nyandarua, Meru and Molo Districts) during January and February, 2008 (short rains crop) and June 2008 (long rains crop) to determine the occurrence of predators, parasitoids and pathogens of the aphids *Myzus persicae* Sulzer and *Aphis gossypii* Glover in potato crops. In each of the four areas, 30 potato farms distributed in different parts of the survey area were selected at random for the surveys. Insects collected were brought to the laboratories at the International Centre of insect physiology and Ecology (icipe), Nairobi, for identification and fungal infection. For isolation of fungal pathogens, dead aphids were transferred on moist filter paper placed on sterile Petri dishes to allow the growth of the fungus on the surface of the cadaver, after which fungus was transferred on artificial media for isolation. Four aphid species, *M. persicae*, *A. gossypii*, *Macrosiphum euphorbiae* Thomas and *Aulacorthum solani* Kaltenbach were identified in all the four survey areas. Sixteen predator species of the aphids were identified. The most prevalent were the ladybeetles *Harmonia axyridis* (Pallas) and *Hippodamnia convergens* Guérin-Méneville (Coleoptera: Coccinellidae), followed by the minute pirate bugs, Orius spp. (Heteroptera: Anthocoridae) and the aphid eating gall midge *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae). Three hymenopteran parasitoid species (Braconids followed by Chalcids then the Ichneumonids) and four fungal pathogen species, *Beauveria bassiana* Balsamo (Ascomycota: Hypocreales), followed by *Verticillium lecanii* Zimmermann (Hypocreales: Incertae sedis), Metarhizium anisopliae (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes) then *Pandora neoaphidis* (Remaudière &Hennebert) Humber (Zygomycetes:Entomophthorales) were identified in all four areas of the field survey.

Keywords: Biological control agents, Myzus persicae, Aphis gossypii, Solanum tuberosum

Introduction

Potato (*Solanum tuberosum* L) is the second most important food crop in Kenya after Maize (CIP, 1996; NPRC, 2010). The potato crop in Kenya earns farmers a total of 5.5 billion Kenya Shillings nationally (NPRC, 2010). The main problem facing potato production in Kenya is low yields due to diseases and insect pests (Kinyae *et al*., 1994). The average yield under small scale farmers' conditions where pests and diseases are prevalent is 10 tonnes per hectare while under research conditions with a lot of pest and disease control, yields of 30 to 40 t/ha are realised. Among the diseases, those caused by viruses are the least understood and the most difficult to control (Salazar, 1996).

Aphids (Homoptera: Aphididae) are the main vectors of potato viruses and their control is very important in the production of seed and ware potatoes. The common control measure currently used against the aphid pests in Kenya is spraying with chemical pesticides mainly Duduthrin (Lambda cyhalothrin 17.5 g/l a non systemic synthetic pyrethroid) and Dimethoate (Dimethoate 40% ww emulsifiable concentrate a systemic organophosphate). However this method, though sometimes effective, is both expensive to farmers and also lead to environmental pollution in addition to the development of resistance with prolonged use of the pesticides. Search for alternative control measures is therefore necessary. Biological control is both environmentally friendly and could be cheaper to the farmer if an appropriate natural enemy was identified. For instance, in Iran, a study showed that the cost of a biological control programme in cotton was three times lower than the cost of chemical treatments (Heydari *et al*., 1997).

This study was therefore intended to investigate and determine possible indigenous biological control agents for these aphid vectors in Kenya. A field survey was therefore conducted in the major potato growing areas in Kenya where all kinds of insects found on the potato crop were

collected. These were later identified and grouped into the different target categories i.e Aphid species, predator species, parasitoid species and other insects not known to fall under the above categories. Fungal pathogens that attack aphids were isolated from the dead aphids collected from the field by growing them on moist filter paper in a sterile petri dish in the laboratory at icipe. These were then grown on artificial media until pure cultures were isolated which were then identified to find out the aphid pathogenic species. This paper reports on the results of the survey and gives an overview of the indigenous biological control agents of the aphids as found in the potato fields in Kenya.

Materials and Methods

A field survey was conducted in all the four major potato growing areas in Kenya, Kiambu (Latitude S01°12.906' to S00°59.370', Longitude E036°45.055' to E036°37.188' and Altitude 1795m to 2417.5m a.s.l), Nyandarua (S00°52.178' to S00°02.311', E036°39.036' to E036°15.223' and Elevation of 2393m to 2770.5m a.s.l), Meru (S00°02.979' to N00°08.006', E037°35.569' to E037°17.059' and 1931m to 2490m a.s.l) and Molo (S00°20.670' to S00°16.434', E035°46.865' to E035°39.875' and 2496m to 2771m a.s.l) areas to determine the occurrence of predators, parasitoids and pathogens of *M. persicae* and *A. gossypii* in the potato crop. The survey was conducted in the months of January to February, 2008 (for the short rains crop) in Kiambu, Nyandarua and Meru Districts. A second survey was done in the month of June, 2008 for the long rains crop in all the four target areas, Kiambu, Nyandarua, Meru and Molo Districts. Another survey was done in the month of January, 2009 in Molo District alone to cover the short rains crop in the area which had been missed in the first survey in January/February, 2008 due to post election violence in the area. So, at the end the field survey, each of the four target areas had been surveyed twice, i.e. once for the short rains season crop and once for the long rains season crop.

In each of the four areas, 30 potato farms were surveyed distributed in the different potato growing regions (Agro-ecological zones) in the area. The farms selected for survey were those with potato crop planted in at least a quarter of an acre and at flowering stage as this is the stage where insect population is highest and hence the highest chance of getting all types of insects found in the survey area. The distance between the farms sampled was at least 500 metres. The farms in each region were randomly selected. At each farm, the survey started with the collection of basic data about the farm using a survey questionnaire. The data collected and recorded included physical and geographical location of the farm, GPS coordinates and data on cultural and agronomic practices on the farm e.g. potato variety grown, pesticide use, use of irrigation on the farm and other crops grown on the farm. This was used to correlate the occurrence or lack of occurrence of some insect species with the practices on the farm or the geographical positioning of the farms.

In each farm, 30 plants were randomly selected in a quarter acre of the potato farm and sampled for insects. The sampling was done by beating the plant on a white cloth placed underneath potato plant so that insects that fall from the plant are collected on the cloth. The insects collected here were then transferred to a plastic container of ½ litre capacity which was then closed with an aerated lid. In each container, a few potato leaves were enclosed as food for the insects being the host plant from which they had been collected. The container was then put in a cool box until the samples were later taken to a cold room at the International Centre of Insect Physiology and Ecology (icipe) at Duduville, Nairobi where they were preserved awaiting identification of the insect species collected. Apart from the 30 plants sampled by the beating method, another 10 plants were randomly selected in the ¼ acre potato field and from here, a whole potato stem with its branches and leaves was cut at the ground and put in a plastic paper bag. These were also put in a cool box for extraction of insects on the plants which were actively searched later in the day and put in the plastic container for that farm with the insects from the 30 plants collected by the beating method. This would capture insect species which are more stuck on the plant and might not have been captured by the beating of the plant. Insects collected from each farm were put in a different container. Farms sampled had potato plants at the flowering stage as this is the stage with highest number of insects (Machangi *et al*, 2003). After the completion of each survey, the insects collected were identified at the icipe laboratories using a telescopic dissecting microscope. The different species identified were preserved separately in a drop of glycerol placed on a glass slide and clearly labelled. Sampling for fungal pathogens was done by allowing the dead aphids to stay on a moistened filter paper placed in a sterile petri dish so that any fungus in the aphids could grow. The fungi were

then grown in Sabouraud (4%) Dextrose Agar (SDA) artificial media where the different isolates which grew here were picked and sub cultured several times in the same media until pure isolates were obtained. These were then observed under the light microscope and the different fungal species obtained from the pure isolates identified and recorded.

Data was recorded for the species diversity, abundance and distribution for the different areas covered in the four major potato growing areas. The insect species identified were classified to the different groups of interest in the study, viz. predators, parasitoids, aphid species and other insects. The fungi were also categorized to identify aphid entomopathogenic species. The data collected was subjected to analysis of variance (ANOVA) using the statistical package for social scientists (SPSS) version 12.0.1.308 and the statistical analysis system (SAS) version 9.2 computer programmes.

Results

Figures 1 to 4 show the overall means of the different insect counts in the survey areas as per the different categories.

Aphids

As shown in fig.1a, four aphid species were found in the survey areas. These were *Myzus persicae* Sulzer, *Aphis gossypii* Glover, *Macrosiphum euphorbiae* Thomas and *Aulacorthum solani* Kaltenbach. As the bar charts show, *A. gossypii* was the most abundant species overall in the four survey areas with a mean of 132 aphids per farm (30 plants). This was followed by *M. euphorbiae* (97 aphids) and *M. persicae* (85). The fourth species *A. solani* had negligible counts in the field with a mean of just about one aphid per farm which was significantly lower than the population of each of the other three species. This is mainly because this species is usually found in stored potato than in the field.

Fig. 1b shows that, on average, aphids were most abundant in Molo survey area with a mean of about 180 aphids per farm which was significantly higher than any of the other three areas. This was followed by Kiambu area with a mean of 62 aphids per farm and Meru area with an average of 58 aphids per farm which were not significantly different from one another though both significantly lower than Molo aphid population. Nyandarua area had the least abundance of aphids with a mean of just about 12 aphids per farm which was significantly lower than the mean aphid counts in all the other three survey areas.

Predators

Sixteen predator species were collected from the four survey areas as shown in fig. 2 (a). The most prevalent predators were the ladybeetle species *Hippodamnia convergens* with a mean of 7.2 per farm and *Harmonia axyridis* at 6.25 per farm. These were followed by the aphid eating gall midge *Aphidoletes aphidimyza* at 5.2 and the minute pirate bugs, (*orius* spp.) at 4.8 insects per farm. These four species had significantly higher population than all the other 12 species but their populations were not significantly different from each other. The other predators were present in low numbers and these included Spiders at 1.4 (mainly dwarf spiders *Erigone* spp.-White crab spider and brown crab spiders), Rove beetles at 1.1 (Staphylinidae, Genus *Paederus*), big eyed bug (*Geocoris* spp.) at 0.9, ladybird *Scymnus* spp. at 0.8, Lacewings (*Chrysoperla* spp.) 0.73 and Syrphid flies (Diptera: Syrphidae, *Syrphus* spp.) with a mean of 0.67 insects per farm. The populations of these six species were not significantly different from each other but were significantly lower than the first four species above. The other six species occurred at very low populations and included, Damsel bugs (*Nabis* spp.) 0.26, Tachnid fly (0.21), Assasin bugs (Hemiptera:Reduviidae, *Zelus* spp.) at 0.08, ladybug *Coccinela semptuncutata* (0.07), Lygus bugs (0.07), and Praying mantis (Mantodea: Mantidae) at 0.03. The populations of these species were not significantly different from each other but were significantly lower than the other 10 species above. The species classified as predators were those that have been recorded before as predators of aphids (CPC, 2006). Overall, the predators were highest in Kiambu with a mean of 2.71 per farm, followed by Nyandarua (2.13) then Meru (2.03) but these were not significantly different from one another (Fig. 2 b). The predators were lowest in Molo (0.58) which was significantly lower than the other areas above.

Parasitoids

Three hymenopteran parasitoid species were found in the survey areas. These were i)Braconids Hymenoptera: Braconidae ii)Ichneumonids Hymenoptera: Ichneumonidae and iii)Chalcids Hymenoptera: Chalcidae. The most abundant of these were the Braconids (mean of 1.4 per farm) followed by chalcids at 0.57 then the ichneumonids (0.17) in all the survey areas as shown in fig. 3(a) below. These differences were

all significantly different from one another. Fig. 3(b) shows that overall, the parasitoids were most abundant in Meru survey area (mean of 3.2 per farm) followed by Nyandarua (2.1) then Molo (1.4) and least in Kiambu area (0.6). These differences were also all significantly different from one another.

Entomopathogens of aphids

Four fungal entomopathogens of aphids were identified from aphids in the survey area. The most abundant species was *Beauveria bassiana* (a mean of 13.75 aphids per farm were infested by this fungus species) followed by *Verticillium lecanii* (6.75) then *Metarhizium anisopliae* (6.0). The fourth and least abundant species in the survey area was *Pandora neoaphidis (1.75)* as shown in fig. 4 below. The abundance of *B. bassiana* was significantly higher than *M. anisopliae* and *P. neoaphidis but not V. lecanii* which was also not significantly different from *M. anisopliae.* The abundance of *P. neoaphidis* was significantly lower than all the other three species above. Overall, the entomopathogens were highest in Nyandarua (mean of 12.5 per farm)followed by Molo (9.25) both of which were not significantly different from each other but were significantly higher than those in Kiambu (3.25) and Meru (3.25) survey areas whose pathogen abundance were lowest and not significantly different from each other.

Discussion

These results present the first survey of indigenous biological control agents of aphids on potatoes in Kenya as there are no published reports of any previous survey on these natural enemies in Kenya. The results obtained show that there are many biological control agents of aphids in Kenya that are known to be very effective in the control of aphids in studies done in other countries (CPC, 2006). For instance, the predators that ranked in the top two positions in terms of their prevalence and abundance in the field survey, i.e the ladybeetles *Harmonia axyridis* and *Hippodamia convergens* species followed by the minute pirate bugs, (*orius* spp.) and the aphid eating gall midge *Aphidoletes aphidimyza* have been recorded as very effective in the control of both *M. persicae* and *A. gossypii* aphid species on potatoes (CPC, 2006). The same is the case with the parasitoid that was the most abundant in the field survey. The braconid found in the survey was *Aphidius colemani* which has been recorded as being extremely effective in the search and control of the two aphid species on potatoes both in the field and in the screen house (CPC, 2006, Hagvar and Hofsvang, 1991). As for the entomopathogens, the most abundant species *Beauveria bassiana* followed by *Verticillium lecanii* then *Metarhizium anisopliae* have all been recorded as potential effective biological control agents of *M. persicae* and *A. gossypii* aphid species on potatoes (Kish *et al.* (1994), CPC, 2006. The Survey results have therefore, shown that, there are many potential biological control agents of aphids on potatoes in Kenya. Since these are indigenous in the country, there is need to assess their effectiveness in the control of the two target aphid species on potatoes with the aim of using the most potential ones in the control of these aphids on potatoes.

Acknowledgements

This field survey work was funded by the Ministry of Agriculture, Kenya for which we are grateful. We also wish to thank the National potato research centre (NPRC) Tigoni head office, for providing two field enumerators Mr. P. M. Kinyae and Mr.J. K. Kaburu for guidance in field survey areas.

References

CIP, 1996. International Potato Centre (CIP). CIP Circular Vol. 22, No 2, September1996
CPC, 2006. Crop Protection Compendium, CABI, 2006.

Hagvar EB, Hofsvang T, 1991. Aphid parasitoids (Hymenoptera, Aphidiidae): biology, host selection and use in biological control. *Biocontrol News and Information*, 12:13-41.

Heydari, H; Attaran, M.R and Daniali, M., 1997. Use of *Chrysoperla carnea* Steph. against cotton pests in the northern part of Iran. *Arab and Near-East Plant Protection Newsletter*. 1997, No. 25, 28.

Kinyae P.M., Lung'aho, C., Kanguha, E and Njenga, D.N. 1994. The status of Seed Potato in Meru, Nyambene, Nyandarua and Laikipia Districts. 2nd KARI/CIP technical workshop on collaborative research-January 1994, Nairobi, Kenya.

Kish, L.P; Majchrowicz, I. and Biever, K.D., 1994. Prevalence of natural fungal mortality of green peach aphid (Homoptera: Aphididae) on potatoes and non-solanaceous hosts in Washington and Idaho. *Environmental Entomology*, 23(5):1326-1330.

Machangi, J. M.; Olubayo, F. M.; Nderitu, J. H.; Njeru, R. W.; El-Bedewy, R. and Aura, J.,

2003. Aphid vectors of potato virus diseases in Kenya. Paper presented at the 15th African Association of Insect Scientists and the Entomological Society of Kenya conference at ICIPE Nairobi, Kenya. 9-13th June, 2003

NPRC, Tigoni, 2010. National Potato Research Centre, Tigoni. Personal communication. May, 2010.

Salazar, L.F., 1996. Potato Viruses and their control. Lima, Peru; International potato centre, 214pp

Illustrations - Figures

1. Aphids

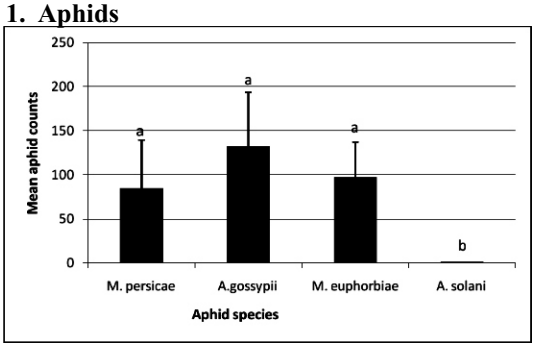


Figure 1a: Overall mean counts (± s.e) of the four aphid species found in the potato crop in the survey area. Means with the same letter are not significantly different

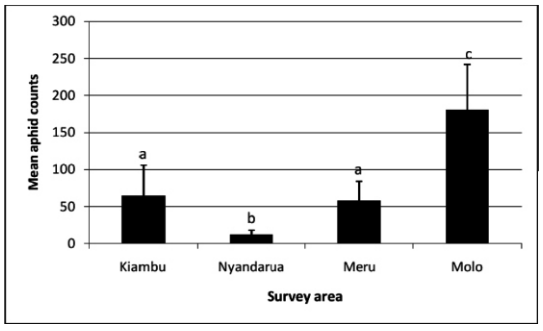


Figure 1b: Mean abundance (± s.e) of all the aphids per farm in the four survey areas. Means with the same letter are not significantly different

2. Predators

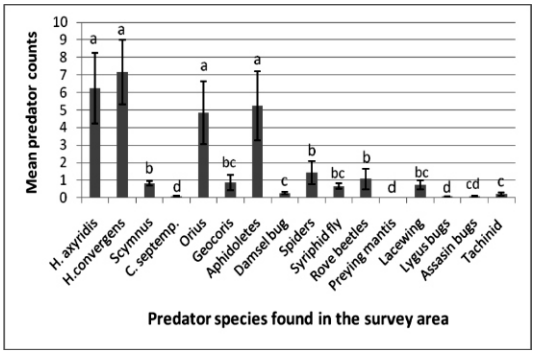


Figure 2a: Overall mean counts (± s.e) per farm for the different predators found in the potato fields in all survey areas. Means with the same letter are not significantly different

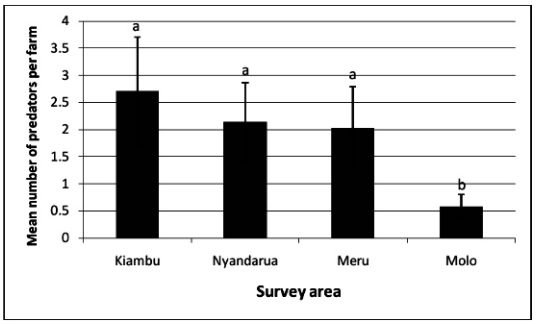


Figure 2b: Mean counts (± s.e) of all the predators per farm as found in the potato fields in the four survey areas. Means with the same letter are not significantly different

3. Parasitoids

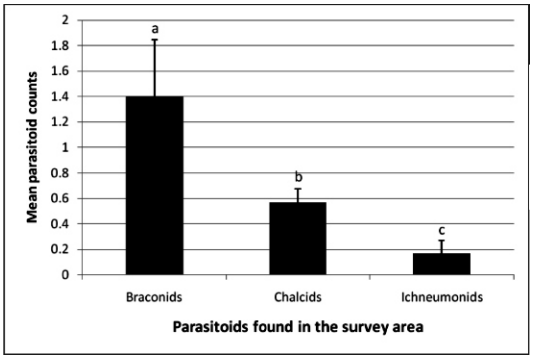


Figure 3(a): Overall mean counts (± s.e) per farm for the different parasitoids found in the potato crop in the survey areas. Means with different letters are significantly different

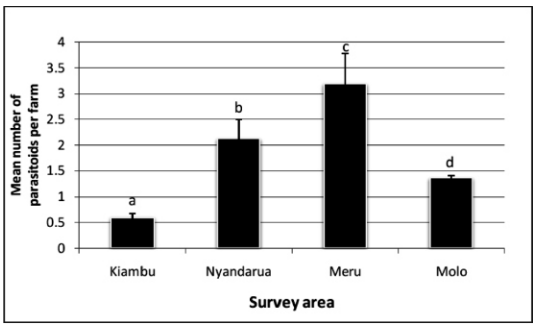


Fig. 3(b): Overall mean number (+ s.e) of parasitoids per farm in the four survey areas. Means with different letters are significantly different

4. Entomopathogens

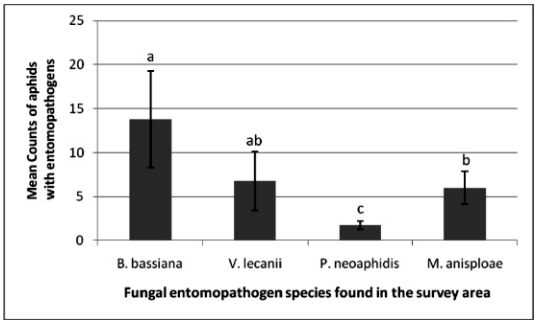
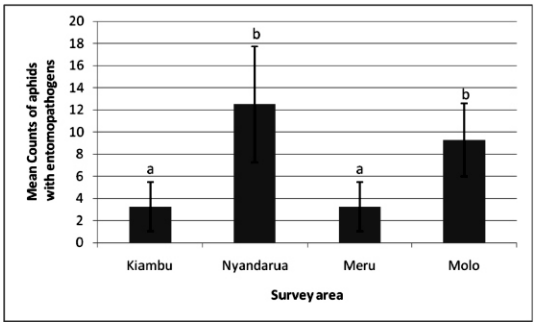


Figure 4a: Mean counts (± s.e) of aphids found infested with entomopathogens in the survey areas. Means with the same letter are not significantly different



4b: Mean counts (± s.e) of aphids found with entomopathogens in each of the four survey areas. Means with the same letter are not significantly different

Development des moyens de lutte contre le « Shimbu », cas de la culture du manioc au kasai oriental/RDC: Revue et contribution sur les moyens de lutte contre le complexe parasitaire shimbu

N. Mahungu[1], A. Frangoie[1], D. Mukendi[2]
A. Ndonda[3], T. Kayumba[4], K. Kasongo[4]
[1]IITA, Kinshasa, RDC; a.frangoie@iitadrc.org
[2]IITA, Kisangani, RDC
[3]IITA, Ngandajika, RDC
[4]INERA, Yangambi, RDC

Résumé

L'interaction des plusieurs contraintes, à savoir les maladies et ravageurs du manioc, les pratiques culturales inadéquates, la faible fertilité des sols, etc. contribuent à la baisse de la productivité du manioc. Il a été constaté à Luputa et Ngandajika que les champs des agriculteurs y compris les champs de manioc sont souvent infestés par un complexe maladie/ravageur localement appelée « shimbu ». Cette revue a pour objectif l'inventaire des connaissances et des différents moyens de lutte existants contre le « shimbu » afin de mieux orienter les nouvelles recherches et formuler certaines recommandations auprès des agriculteurs. Une enquête auprès des agriculteurs et l'inventaire de la littérature sur le « shimbu » ont été réalisés. La visite sur terrain a permis d'apprécier la perception du complexe « shimbu » par les agriculteurs, visualiser les symptômes ainsi que formuler quelques moyens de lutte. Il en découle que (i) « shimbu » qui signifie « déraciner en Ciluba» est un complexe des petits insectes ailés, vivant dans le sol, se reproduisant à partir des œufs et qui, en association avec les fourmis/champignons, s'attaquent aux plantes à partir des racines » ; (ii) les symptômes sont entre autres : l'apparition des insectes et des petits œufs lors du labour ; le faible taux de reprise après plantation ; la fanaison des plantes attaquées qui s'arrachent facilement ; (iii) les principaux moyens de lutte sont : bon choix du terrain, labour profond, plantation sur billons/buttes, enfouissement et semis des plantes odorantes, épandage de la cendre. La recherche sur le « shimbu » dans la province du Kasaï date des années 1950 par l'INEAC et le nom de « shimbu » était associé aux dégâts causés par l'association d'un coccide, voisin du genre

Gueriniella, de fourmis, appartenant aux genres *Camponotus* et *Crematogaster*, et d'un champignon, *Macrophoma phaseoli*. De 1950 à 1958, l'INEAC a menée des essais essentiellement sur la lutte à base des insecticides chimiques qui devraient être économiques, efficaces contre le « shimbu » et d'utilisation aisée en milieu rural. Toutefois, les résultats obtenus ne garantissaient pas forcément la rentabilité et outre le fait que les insecticides représentent un danger pour la santé et l'environnement, ils ne sont pas toujours à la portée des paysans. Cependant, certaines pratiques culturales (labour profond, plantation précoces, etc.), bien appliquées et de façon préventive, semblent être des moyens efficace de lutte contre le « shimbu ».Par conséquent, nous suggérons : (i) un approfondissement de la connaissance du complexe « shimbu » par des analyses appropriées au laboratoire et (ii) l'initiation des essais sur les pratiques culturales, les plantes insecticides ainsi que les variétés performantes.

Mots clés: Manioc, shimbu, moyens de lutte, RDC

Introduction

Le manioc est la culture principale en RDC et constitue l'aliment de base de plus de 80% de la population. Il couvre 50% des cultures emblavées. Les racines tubéreuses de manioc constituent une source principale d'énergie diététique et les feuilles de manioc qui sont largement consommées constituent une source végétale importante des vitamines et minéraux. Le manioc représente une source potentielle de génération de revenus et d'emploi dans les zones rurales et les centres urbains par la commercialisation des racines tubéreuses, feuilles et divers produits de transformation. De ce fait, cette culture constitue la base de l'économie du ménage agricole.

Cependant, l'interaction des plusieurs contraintes, à savoir les maladies et ravageurs du manioc, les pratiques culturales inadéquates, la faible fertilité des sols, etc. contribuent à la baisse de la productivité du manioc.

Dans le cadre de l'exécution du projet IITA/EJCSDJ (Institut International d'Agriculture Tropicale /Eglise de Jésus Christ des Saints de Derniers Jours) à Luputa dans le Kasaï Oriental en RDC, il a été constaté lors de la campagne agricole 2008/2009 que les champs des certains agriculteurs encadrés par le projet IITA/EJCSDJ ont été infestés par une maladie localement appelée « shimbu ». Alerté au mois de juin 2009, la Coordination Nationale de l'IITA avait dépêché sur place une mission qui avait fait le constat sur terrain et une intervention d'urgence sous forme de traitement chimique (insecticide + fongicide) pour tenter d'arrêter l'évolution du « shimbu » dans les champs concernés.

Lors de l'atelier de planification du plan de travail 2009/2010 sur le manioc organisé par la Coordination Nationale de l'IITA/RDC en étroite collaboration avec l'INERA (Institut National pour l'Etude et la Recherche Agronomique) du 03 au 08 août 2009 à Mbanza Ngungu dans le Bas Congo en RDC, un accent particulier était mis sur des études à mener pour approfondir la connaissance, les symptômes et la lutte contre le complexe « shimbu ». Ainsi, il a été préconisé au cours de cet atelier, de mener les études suivantes en rapport avec la lutte contre le « shimbu » : « Revue et contribution sur les moyens de lutte contre le complexe parasitaire shimbu » et « Etudes préliminaires sur le shimbu dans la province du Kasaï »

Cette revue constitue une contribution au développement des moyens de lutte contre le « shimbu » par les observations faites sur terrain, les connaissances tirées auprès des Agriculteurs et un aperçu sur la revue de la littérature sur le « shimbu » et permet par conséquent de faire quelques suggestions sur les études ultérieures à mener pour l'intensification de la lutte contre le shimbu.

Matériels et Méthodes

Pour atteindre l'objectif assigné à cette revue, nous avons procédé comme suit :

1) Observation sur terrain
2) Enquête auprès des agriculteurs
3) Revue de la littérature sur la lutte contre le « shimbu »

Observations sur terrain

Les visites des champs effectués en juin 2009 nous avaient permis de voir :

(i) Les symptômes attribués au « shimbu »
(ii) Quelques composantes supposées du complexe « shimbu »
(iii) Quelques moyens de lutte

Ces observations sont illustrées dans la partie annexe

Enquête auprès des agriculteurs

A l'occasion de la tenue d'une formation sur la multiplication rapide, le système de production et la gestion de la culture du manioc organisée par

l'IITA au profit des 47 Encadreurs et Agriculteurs de Luputa et ses environs du 14 au 16 octobre 2009 dans le cadre du projet IITA/EJCSDJ, les participants techniciens et agriculteurs - étaient répartis, lors de la première journée, en 9 groupes de 5 personnes et avaient répondus aux trois questions fondamentales suivantes relatives au complexe « Shimbu » :

1) Qu'est ce que le shimbu (définition) ?
2) Comment reconnaissez-vous le shimbu (symptômes) ?
3) Comment luttez-vous contre le shimbu (moyens de lutte) ?

Ci après la synthèse des réponses données par les 9 groupes aux trois questions :

a) La définition du « shimbu »

- *Origine du mot* : le terme « shimbu » vient du mot luba « shimbula » qui signifie « déraciner ». En kanyoka, langue locale de Luputa, « shimbu » est traduit par le terme « Kambumbu ».
- *Définition des agriculteurs* : en recoupant les informations recueillies par les agriculteurs interrogés, le « shimbu » a été défini comme suit : « Colonie des petits insectes ailés de couleur blanche ou noir, ayant des petites pattes et vivant dans le sol, se reproduisant à partir des œufs et qui, en association avec les fourmis, s'attaquent aux plantes à partir des racines ».

b) Les symptômes du « shimbu » Selon les agriculteurs interrogés, le « shimbu » se reconnait par les symptômes suivants :

(i) Présence des insectes noirs (ailés) et des petits œufs lors du labour, (ii) faible taux de reprise ou levée après plantation ou semis, (iii) constat des plages (espaces) vides sans herbes (l'herbe pousse de façon parsemée), (iv) jaunissement, perte de vigueur et/ou flétrissement (fanaison) des plantes attaquées qui s'arrachent facilement à la main libre, (v) fanaison des plantes pendant la période des fortes pluies (1 à 5 mois après la reprise), (vi) présence des herbes de petite taille, (vii) présence des galeries, des insectes, des fourmis et de poudre blanc sur le sol, (viii) présence des fourmis et des coccidies blancs (comparable à la farine) sur les racines des plantes arrachées, (ix) présence des petites termitières, (x) les racines des plantes attaquées ont la couleur rouge, (xi) constat de l'inexistence des racines chez certaines plantes, (xii) constat de destruction des poils absorbants des racines des certaines plantes attaquées.

c) Les moyens de lutte contre le « shimbu »

Pour faire face aux attaques de « shimbu », les agriculteurs utilisent et/ou préconisent les moyens de lutte suivant : (i) dans le choix du terrain, éviter le terrain où l'*Imperata Cylindrica* vire au jaune rouge ou dont la végétation est moins vigoureuse (taille) ; les herbes s'arrachent facilement ; existence des larves poudreuses et des fourmis ; etc., (ii) faire un labour profond et bien émietter le sol, (iii) plantation oblique (3/4 dans le sol) pour le cas du manioc et en début de pluies, (iv) forte densité de plantation, (v) planter le manioc entre les plates bandes ou billons (et une autre culture sur les plates bandes ou billons), (vi) planter sur des gros billons ou buttes, (vii) enfouissement et semis (à la volée) des plantes odorantes tel que « luenyi » (*Occimum viride*), « tshikota » (*Cymbopogon densiflora*), « kadiamba-diamba» (Plante à identifier), « kimbala» (Plante à identifier) mélangés si possible avec du poudre du « Naphtalène » (viii) trempage des boutures dans le mazout avant la plantation, (ix) association culturale du manioc avec les plantes (rampantes ou non) telles que le niébé, la patate douce et le soja, (x) épandage de la cendre aux pieds des plantes, (xi) sarclages précoces et (xii) laisser le terrain déjà infesté en jachère.

Revue de la littérature sur la lutte contre le « shimbu »

L'infestation du « shimbu » dans certaines contrées de la province du Kasaï Oriental en RDC et plus particulièrement dans les deux territoires voisins, à savoir Ngandajika et Luputa date de l'époque coloniale. Selon la littérature consultée, on donne le nom de « shimbu » aux dégâts causés par l'association d'un coccide, voisin du genre *Gueriniella*, de fourmis, appartenant aux genres *Camponotus* et *Crematogaster*, et d'un champignon, *Macrophoma phaseoli*.

La synthèse de la série des *essais de lutte contre le « shimbu »* est présentée dans l'article publié dans le bulletin d'information de l'INEAC, vol. VII, N°2, pages 117 124 en avril 1958 et écrit par P. DE FRANCQUEN, à l'époque chef du secteur de Léopoldville à l'INEAC.

Cette série des essais de lutte contre le « shimbu » remonte depuis l'époque de l'INEAC (Institut National pour l'Etude Agronomique du Congo) qui, de 1950 à 1958 (selon les archives retrouvées jusque là par l'équipe des Chercheurs INERA/IITA/RDC associés à ce travail) a menée

des essais essentiellement sur la lutte chimique à base des insecticides.

La recherche d'un insecticide économique, efficace contre le « shimbu » et d'utilisation aisée en milieu rural semble avoir été l'objectif principal visé.

Nous avons divisés ces séries d'essais en 3 phases suivantes :

- Phase 1 : impact du traitement à base d'un insecticide chimique
- Phase 2 : recherche d'un insecticide chimique économique et plus pratique en milieu rural
- Phase 3 : détermination des doses minimales à utiliser.

Au moins 7 essais avaient été conduits et repartis comme suit : phase 1 (1 essai) ; phase 2 (2 essais) et phase 3 (4 essais).

Les insecticides utilisés comme traitements dans ces essais sont : le D.D. (*Dichloropropane Dichloropropène*) ; le *Parathion* ; le *Chlordane* ; l'*Aldrine* et le *Dieldrine*. A part le D.D. qui était sous forme liquide, les quatre autres insecticides étaient des poudrages.

Les quantités des matières actives des insecticides en poudre utilisés dans les différents traitements ont varié entre 0,5 et 30,72 kg/ha. Les applications d'insecticides se sont faites tantôt par pulvérisation tantôt par enfouissement du poudre.
Dans tous les essais qui avaient été conduits, la méthodologie était basée sur le choix bien confirmé d'un terrain particulièrement infectée par le « shimbu ».

Résultats et discussions

l'issue des observations sur terrain, des interviews des agriculteurs et de la revue de la littérature sur le « shimbu », le constat ci-après se dégage :

1) Le « shimbu » est endémique au Kasaï et plus particulièrement dans les territoires voisins de Ngandajika et Luputa depuis l'époque coloniale (avant les années 1950) ;

2) Des études initiées à l'époque de l'INEAC, entre 1950 et 1960 se sont basées exclusivement sur la lutte chimique à base d'insecticide et bien que les résultats n'étaient pas toujours suffisamment nets, il était ressortit de l'ensemble des essais réalisés par l'INEAC que :

a) Les insecticides à base de *Dieldrine*, de *Chlordane* et d'*Aldrine*, avaient eu une action très nette sur l'intensité des attaques de « shimbu » lorsqu'ils étaient appliqués au sol sous forme de poudre, à la dose minimum de 1,25 kg/ha de produit actif pour le *Dieldrine* et l'*Aldrine* et de 2 kg/ha pour le *Chlordane*.
b) Il avait été également observé que ces produits, pulvérisés même à forte dose sur les plantes, n'avaient eu aucune action phytotoxique.
c) Le parathion, appliqué sous forme de poudre à la dose de 2,5 kg/ha n'avait pas exercé une action suffisante.
d) La désinfection du sol au moyen de poudre s'était montrée d'application très aisée et pouvait être utilisée sans difficulté.
e) Il était en outre ressorti des observations faites après traitements efficaces du sol que l'action de la désinfection se prolongeait pendant au moins un an. La persistance d'efficacité était cependant plus ou moins proportionnelle à l'étendue des zones traitées. La réinfection se produisait toujours par les bords et était centripète.
f) Cependant, les résultats obtenus ne garantissaient pas forcément la rentabilité et les insecticides n'étaient et ne sont pas toujours à la portée des paysans moyens, suite notamment à la rareté, au coût élevé ainsi qu'au danger que cela représente pour la santé des paysans et de l'environnement.

En outre, bien qu'aucune référence écrite des éventuelles études sur certaines pratiques culturales (labour profond, plantation précoces, etc.) menées n'a été retrouvée au stade actuel de nos recherches, il s'avère selon les observations effectuées sur terrain et l'interview avec les agriculteurs que, bien appliquées et de façon préventive, ces pratiques semblent être des moyens efficace de lutte contre le complexe « shimbu ».

Au vu de tout ce qui précède, nous suggérons ce qui suit :

1) Approfondir la connaissance du complexe « shimbu » en confrontant les différentes descriptions et observations avec des analyses plus élaborées dans le laboratoire ;
2) Reprendre et améliorer les essais de lutte à base d'insecticide chimique ;
3) Menés des nouveaux essais sur (i) les pratiques culturales, (ii) les plantes insecticides et (iii) les variétés performantes.

Remerciements.
Nous remercions vivement l'IITA pour avoir facilité les déplacements qui nos ont permis de recueillir le données sur terrain. Nos remerciements s'adressent également à tous ceux qui ont de près ou de loin participé à l'élaboration de cette revue pour leur disponibilité et les efforts fournis.

Références.

P. De Francquen. 1950 *Essai de lutte contre le « shimbu »*, Compte rendus des travaux scientifiques du Congrès scientifique du C.S.K., Communication 144, Elisabethville

P. De Francquen. 1952, *Essai de lutte contre le « shimbu »*, Trans.,IX, Int. Congr. Ent., pp. 751 756, Amsterdam

INEAC 1958. Bulletin d'information de l'Institut National pour l'Etude Agronomique du Congo Belge, VOL. VII, N° 2, pp 117 124

Figure 1 : quelques photos des observations effectuées sur terrain

(i) Symptômes attribuées au « shimbu »

De gauche à droite et de haut en bas :

- Début flétrissement d'un plant ;
- Galeries creusées au sol par (?) ;
- Pertes des feuilles latérales
- Un plant mort de manioc sectionné de ses racines ;
- Galerie creusé dans une tige de manioc à partir de la base.

Quelques composantes du complexe « shimbu »

- En haut à gauche : cochenille trouvé dans la partie souterraine d'un plant flétrit de manioc ;
- En haut au milieu : cochenille trouvé dans la partie souterraine d'un plant d'*Imperata cylindrica ;*
- En haut à droite : cochenille trouvé dans le plant d'*impérata cylindrica ;*
- En bas à gauche : morceau d'une termitière trouvé dans un champ de manioc infesté parle shimbu

Tableau 1 : Synthèse des essais de lutte contre le « shimbu »/ INEAC : 1950 - 1958

	Phase 1	Phase 2		Phase 3			
Buts	Impact du traitement à base d'un insecticide.	Recherche d'un insecticide économique et plus pratique en milieu rural		Détermination des doses minimales à utiliser.			
Essais	1er essai	2ème essai	3ème essai	4ème essai	5ème essai	6ème essai	7ème essai
Traitements (kg ou l/ha)	1)D.D. : 250 2)Sans insecticide	1)Parathion (2,5%) : 100 2)Chlordane (5%) : 100 3)Aldrine (2,5%) : 100 4)Dieldrine (2,5%) : 100 D.D. : 250	1)Parathion (25%) :32 2)Chlordane (96%) : 32 3)Aldrine (10%) : 100 4)Dieldrine (10%) : 100 5)D.D. : 250	1)Dieldrine (2,5%) : 100 2)Dieldrine (2,5%) : 75 3)Dieldrine (2,5%) : 50 4)Dieldrine (2,5%) : 25 5)Sans insecticide	1) Chlordane (5%) : 100 2)Chlordane (5%) : 75 3)Chlordane (5%) : 50 4)Chlordane (5%) : 25 5)Sans insecticide	1)Dieldrine (2%) : 100 2)Dieldrine (2%) : 75 3)Dieldrine (2%) : 50 4)Sans insecticide	1)Dieldrine (2,5%) : 80 2)Dieldrine (2,5%) : 40 3)Dieldrine (2,5%) : 20 4)Aldrine (? %) : 40 5)Sans insecticide
Méthodologie	1) Champs choisis dans une région particulièrement infectée. Chacun d'eux était dans plusieurs cas divisé en deux parcelles, l'une désinfectée, l'autre, non désinfectée, constituant le témoin ; 2) Semis très hâtifs ; 3) Un mois plus tard, prélèvement des poquets ne comportant plus aucune plantule (pertes dues principalement au « shimbu ») ; 4) Après prélèvement des données des poquets vides, les plantes restantes étaient arrachées Après arrachage, on appliquait l'insecticide uniquement dans les parcelles à désinfecter en fonction des différents traitements et on procédait au semis						
	Application insecticide au pal injecteur (250 l/ha) en injections de 2,5 cm3 répétées tous les 30 cm)	Le D.D. a été injecté. Les autres insecticides ont été épandus sur le sol puis enfouies par sarclage.	Application de la poudre en suspension ou en émulsion dans de l'eau	Les poudres des produits insecticides ont été simplement épandues sur le sol puis enfouies par un léger sarclage.			
Résultats	-Des rendements spectaculaires des parcelles traitées au D.D. avaient été obtenus par rapport aux parcelles non traitées, soit 153 % en fonction du témoin (840/550 kg/ha).	-Dans les deux séries, les différences des rendements ont été en faveur du traitement au D.D. -Les parcelles ayant reçues les autres types d'insecticides ont données des rendements oscillants entre 60 et 69 % de celui des parcelles pulvérisées par le D.D. Il avait été constaté une augmentation nette des rendements dans la 3ème série par rapport à la deuxième série.		- A toutes les doses étudiées, l'action du chlordane s'était avérée supérieure à celle du dieldrine.	- A raison de 50 kg/ha, les résultats donnés étaient déjà satisfaisants pour chlordane.	- Les comptages avaient montré une action très nette des applications de dieldrine à toutes les doses. - Rendements moins bons qu'essais précédents.	- Action produits pas suffisante même à la dose de 80 kg/ha. Pas de différences entre 80 et 40 kg/ha d'application.
Conclusion	- Augmentations production non rentables par suite du prix de revient trop élevé de l'insecticide (D.D.). - Méthode d'application du produit (appliqué au pal injecteur), non pratique pour le milieu paysan.	- Prix insecticides en moyenne 5 fois moins couteux que celui du D.D. Intéressant de voir les résultats en utilisant ces mêmes produits en quantité plus importante et en pulvérisation	-Augmentations nettes des rendements constatés attribuables à des variations de fertilité du sol. -Prix autres insecticides en moyenne 28 % moins couteux que prix du D.D. Application moins aisée et non économique	- Les résultats dont nous avons disposés concernent uniquement le comptage de poquets manquants. - Un certain pourcentage de plants légèrement atteints, quoique toujours présents, n'avaient cependant pas acquis un développement normal.		- Les résultats dont nous avons disposés concernent uniquement le comptage de poquets manquants. - L'époque trop tardive des semis aurait été à la base de bas rendements obtenus.	- La forme des parcelles dont la faible largeur (10 m), avait permis des interactions en tous sens. Ce qui n'aurait pas pu donner des résultats fiables.

Tableau 2 : Quantités matières actives des insecticides utilisés, modes d'application, proportion des poquets manquants et rendements obtenus (synthèse)

Phase	No Essai	Traitement		Résultats			Coût Insecticide (F/ha)
		Insecticide	Dose ma (kg/ha)	Proportion poquets manquants (%)		Rendement (kg/ha)	
				Avant traitement	Après traitement		
I	1	D.D.(1)	? (a)	41,97	18,43	840,3	7.500,00
		Sans Insecticide (2)		36,33	40,04	549,9	0,00
II	2	D.D.(3)	? (a)	31,30	9,70	743,0	7.500,00
		Parathion (4)	2,500 (b)	19,50	33,40	450,0	1.440,00
		Chlordane (5)	5,000 (b)	33,80	4,50	511,0	1.400,00
		Aldrine (6)	2,500 (b)	28,30	3,10	482,0	1.500,00
		Dieldrine (7)	2,500 (b)	32,00	3,60	480,0	1.700,00
	3	D.D(8)	? (a)	40,90	1,90	979,0	7.500,00
		Parathion (9)	8,000 (c)	36,00	2,70	682,0	4.710,00
		Chlordane (10)	30,720 (c)	47,20	1,90	577,0	3.520,00
		Aldrine (11)	10,000 (c)	40,40	2,20	645,0	5.926,00
		Dieldrine (12)	10,000 (c)	46,60	1,50	673,0	7.275,00
III	4	Chlordane (13)	5,00 (b)		11,20		
		Chlordane (14)	3,75 (b)		9,00		
		Chlordane (15)	2,50 (b)		11,40		
		Chlordane (16)	1,25 (b)		14,80		
		Sans Insecticide (17)			43,30		0,00
	5	Dieldrine (18)	2,500 (b)		12,90		
		Dieldrine (19)	1,875 (b)		14,10		
		Dieldrine (20)	1,250 (b)		17,90		
		Dieldrine (21)	0,625 (b)		23,80		
		Sans Insecticide (22)			36,60		0,00
	6	Dieldrine (23)	2,000 (b)	79,93	18,30		
		Dieldrine (24)	1,500 (b)	82,62	28,86		
		Dieldrine (25)	1,000 (b)	93,48	23,83		
		Sans Insecticide (26)		92,22	49,87		0,00
	7	Dieldrine (27)	2,000 (b)		17,50		
		Dieldrine (28)	1,000 (b)		17,90		
		Dieldrine (29)	0,500 (b)		22,20		
		Aldrine (30)	? (b)		18,80		
		Sans Insecticide (31)			24,80		0,00

- (a): Appliqué au pal injecteur, à raison d'environ 250 l/ha en injections de 2,5 cm^3 répétées tous les 30 cm
- (b): Poudres simplement épandues sur le sol puis enfouies par un léger sarclage.
- (c): Application en suspension ou en émulsion dans de l'eau à raison de 2.000 l/ha
- (T) : Témoin

N.B. : ce tableau est une compilation des plusieurs tableaux, certaines données ont été transformées pour raison d'uniformisation. Les cases vides s'expliquent par l'absence des données y relatives dans les sources auxquelles nous avons eu accès.

Figure 2 : Moyenne de poquets manquants après traitements (synthèse de 31 différents traitements

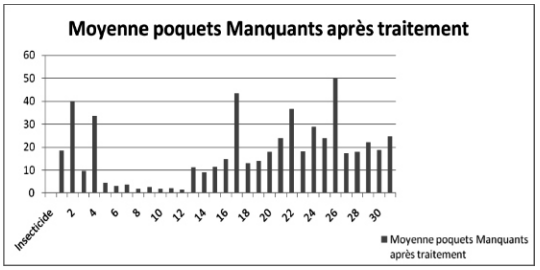
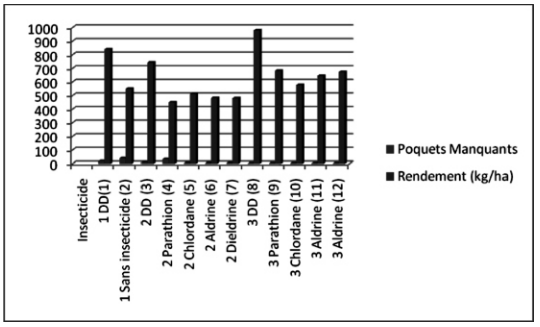


Figure 3 : Rendements des 12 premiers traitements et rapport avec les poquets manquants



Molecular epidemiology of cassava mosaic begomoviruses in Yangambi, Northeastern Democratic Republic of Congo

Monde G.[1,4], Walangululu J .[2], Winter S.[3] and Bragard C .[1]

[1]Université catholique de Louvain, Earth & Life Institute, Applied Microbiology - Phytopathology, Croix du sud 2bte3 B-1348 Louvain-la-Neuve, Belgium Email : claude.bragard@uclouvain.be Phone : +32 10 47 40 23 Fax +32 10 47 86 97

[2]Faculté des sciences agronomiques ; Université catholique de Bukavu B- 285 Bukavu, République Démocratique du Congo

[3]Plant Virus Collection; DSMZ Deutsche Sammlung von Mikroorganismen und Zellkuturen GmbH Messeweg 11/12 38104 Braunschweig, Germany

[4]Institut Facultaire des Sciences Agronomiques de Yangambi, Unité de production et défense des végétaux, B-1232 Kisangani, République Démocratique du Congo

Abstract

A molecular epidemiology study of *Begomoviruses* involved in Cassava Mosaic Disease (CMD) was conducted around Yangambi in Oriental Province, north-eastern Democratic Republic of Congo (DRCongo). Incidence and disease severity were systematically evaluated and compared to results from targeted PCR and sequences obtained from cassava, as well as legumes and whiteflies collected in the visited fields. The results showed that CMD-associated viruses are widely distributed throughout the investigated area. Dual infections were common (66%) in which both ACMV and EACMV species were present. EACMV-UG was predominant in highly infested fields. Sequence analysis showed a rather clonal distribution of the viruses, possibly related to the type of environment in which cassava is grown. The virus isolates were related to the principal ecosystems: moderate severity isolates were found close to the humid forest in which agricultural activity is not intensive and the severe isolates are limited to the secondary forest, in which the cassava is grown more intensively. EACMV isolates are randomly spread through each of the main agro-ecologies. Two virus species were frequently found in whiteflies (59%). The various whitefly biotopes and the season-long presence of cassava make it a region of special interest for studying the spread and development

of CMD. Therefore, knowing the constant evolutionary process of begomoviruses, our study focused on the potential contribution of forest plants species to the emergence of different viruses: ACMV and EACMV were detected in two leguminous *Fabaceae* species (*Centrosema pubescens* Benth and *Pueraria javanica* (Benth) Benth). The wide presence of EACMV-UG and the high incidence and severity of CMD demonstrates that this part of DRCongo continues to be affected by the CMD pandemic.

Keywords: Cassava mosaic begomoviruses, double infection, alternate host, Bemisia tabaci, distribution, agroecosystems

Introduction

Cassava mosaic disease (CMD) caused by African cassava mosaic viruses (Genus: *Begomovirus*, Family: *Geminiviridae*) is the most biotic constraint in cassava *Manihot esculenta* Crantz *(Euphorbiaceae)* growth in Democratic Republic of Congo (DRCongo). This viral disease affects at once cassava production (losses up to 95%) and farmers' subsistence (Thresh et *al*., 1994; Otim Nape et *al*., 2000) in Africa. CMD has reached a worrying epidemic level in East Africa by emergence of Uganda variant (Legg, 1999). CMD's spread is associated to the diversity of the strains present in cassava (Fondong *et al*., 2004 ; Ndunguru *et al.,* 2005); the influence of whiteflies vector (Legg et *al*., 2006) and naturals plants (Alabi et *al*., 2008; Ogbe et *al.,* 2006).

Indeed, the insecurity in DRCongo during the next decade didn't allow precise investigations on cassava mosaic disease epidemiology, like in other countries where CMBs are largely characterised (Legg, 1999; Legg and Thresh, 2000; Legg et *al*., 2006; Pita *et al.,* 2001; Were *et al.,* 2003; Ndunguru *et al*., 2005). However, the EACMV-Uganda variant was first reported by Neuenschwander et *al*. (2002) in Kinshasa province south-western region of DRCongo; by lacking of CMD epidemiology data in the eastern region of RDCongo all others estimations may be speculative.

The Agriculture Research Centre of Yangambi is a rainforest humid region at the altitude of 500 m where cassava selection and improvement programs were conducted since 1933; it was also an important cassava genotypes exchange Center.

Given the epidemic development of CMD in Eastern Africa, we postulate that virus involved in CMD development in Yangambi region may be similar to Uganda's because of neighbouringship and the previous rate of CMD epidemic propagation.

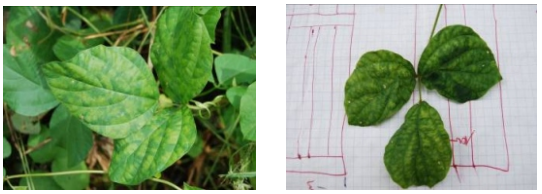
To consider the CMD pathosystem, CMBs strains in cassava plants might be similar to those identified in the host plants and in the insect vector. Hence, the present study aimed to determine the genetic diversity of associated viruses to CMD in Yangambi through cassava, in virus alternate hosts and in whiteflies vector. The finality is to have a better understanding of diversity of strains, to establish their phylogeny. A clear status and distribution of CMBs strains may contribute in global control strategies.

Materials and Methods

Sampling

Cassava leaves and stem cuttings (140 samples) were collected in 150 Km circle around Yangambi; up to 100 leaves of weeds or intercropping culture of species in Fabaceae family supposed to be alternate hosts of CMBs (Table 1) and 132 whiteflies (B. Tabaci; Aleyrodidae, Hemiptera) were collected in cassava fields investigated. Plants were selected on the basis of the presence of B. tabaci on their leaves or for the presence of mosaic symptoms on those leaves (Fig. 1) and their abundance in cassava fields as weeds or in intercropping systems.

Psophocarpus scandens (Endl.) Verdc,1968



Pueraria javanica (Benth.) Benth.1864

Centrosema pubescens Benth


Glycine max (L.) Merr. (Soja)1917


Vigna vexillata(L.) A. Rich 1845


Phaseolus vulgaris L. *1753*

Fig. 1: Symptoms of cassava mosaic disease are observed in the leguminous leaves. *P. javanica* (Benth) Benth and *C. pubescens* Benth both in the family of Fabaceae which have developed the same symptoms like cassava mosaic disease were CMBs double infected. These leguminous had been collected in cassava fields around Yangambi.

In cassava fields investigated, the incidence, disease severity and the whiteflies number on the three first leaves were systematically evaluated. The severity was evaluated by scale of IITA from 1 to 5; where 1: indicates no symptom and 5: presence of severe symptom. The incidence was obtained in ratio to virosis plants and the numbers of healthy plants. Samples were brought in Plant pathology laboratory, University catholic of Louvain (UCL), Belgium, for molecular diagnosis. Molecular data obtained after analysis were reported in the geographic map of Yangambi (Fig. 5) established in basis of general card of soil occupation of DRCongo provided by UCL-Geomatics, Louvain-la-Neuve, Belgium, 2006.

DNA extraction and quantification

DNA was extracted from cassava leaves and other vegetables species by using FastDNA® Kit with a FastPrep® Instrument (Qbiogene, Inc., CA). DNA was quantified by reading spectrophotometer absorbance (A) at 260/280 nm.

About whiteflies, DNA extraction has consist to crush into a tube of 200 µl in 50 µl of TE buffer (Tris HCl 10 mM, pH 8, EDTA 1 mM) with a tip and to heat at 90°C for 15 min. The tube was then centrifuged at 13,000 trs/min to precipitate the debris. The supernatant was directly used for PCR.

PCR amplification and sequencing

Primers used in PCR amplification were designed By Deng et *al*. (1994), Zhou et *al.* (1997) and Fondong et *al*. (2000), Were et *al.* (2003) were used to sequence the segment DNA-A. Specific nucleotides primers for AC2 and AC4 used were designed by Monde et *al*. (2010).

The mix PCR was prepared in a final volume of 50 µl using H20depec depec 26.25µl, MgCl2 25 mM 5µl, Go Taq® 5x flexi buffer 10µl, Dntp 100 mM 1.5µl, each upstream and downstream primer 1µl, Taq® DNA polymerase (Promega) 5 units/µl 0.25 µl, extract DNA 5µl.

The PCR was cycling at 94°C for 2 min for denaturizing, followed of 38 cycles of amplification 94°C for 30 sec for denaturizing; hybridization at 58°C for 30 sec; 72°C for 1 min for elongation. The final elongation has been at 72°C for 7 min. The revelation in agarose gel 1.2 % under UV was been made after electrophoresis in 120 V in ethidium bromide. The direct sequencing using biosequencer Genetic Analyser 3100 was made with PCR product diluted 10 fold in distilled water. Multiplex PCR was also used by mixing the above specifics primers together and by cycling PCR at 54°C for annealing temperature. Full length DNAA were obtained by assembling contiguous sequences (Vector NTI® Advance11). Phylogenetic trees were carried out using MEGA4.1 Software (Tamura et *al*., 2007). The sequences AC2 and AC4 from the following available accessions of ACMV and EACMV in NCBI GenBank were used in concatenation. Nucleotides sequence were translated in amino acids by a molecular toolkit (www.vivo.colostate.edu).

Results

Presence of cassava mosaic begomoviruses in plants weeds (Fabaceae)

The results showed the presence of viruses of CMD in some species of Fabaceae and the probability of their implication in CMD epidemiology in the Yangambi region. It may be due to the abundance of *Pueraria javanica* (Benth) Benth and *Centrosema pubescens* Benth in cassava fields as weeds. PCR diagnostic showed that two plants leguminous *C. pubescens* and *P.*

*javanica*were dually infected positive in ACMV and EACMV (Table 1) in respective proportions of 70 % and 80 %. Other vegetables species didn't react positively to PCR despite of the presence of CMD symptoms. The sequencing revealed double infection in those plants and let us think that those leguminous are also alternate host of cassava mosaic viruses.

Table 1: Weeds and intercropped leguminous plants (Fabaceae*)* collected around Yangambi area and tested by PCR for the presence of African cassava mosaic viruses.

No	Species	Family	Principal **sa**mpling locations	Abundance	Nature	Positives samples	ACMV/EACMV
1	*Arachis hypogaea L. 1753*	*Fabaceae*	Basoko	high	intercropping	0/10	-/-
2	*Centrosema pubescens Benth*	*Fabaceae*	Banalia	high	weed	7/10	+/+
3	*Glycine max (L.) Merr. (soja)1917*	*Fabaceae*	Banalia	high	intercropping	0/10	-/-
4	*Leucaena leucocephala (Lam.)De Wit, 1961*	*Fabaceae (Mimosaceae)*	Yatolema	low	weed	0/10	-/-
5	*Phaseolus vulgaris L. 1753*	*Fabaceae*	Kisangani	high	intercropping	0/10	-/-
6	*Psophocarpus scandens (Endl.) Verdc,1968*	*Fabaceae*	Kisangani	low	weed	0/10	-/-
7	*Pueraria javanica (Benth.) Benth.1864*	*Fabaceae*	Yangambi	high	weed	8/10	+/+
8	*Senna occidentalis (L.) Link1829*	*Fabaceae (Cesalpiniaceae)*	Opala axis	high	weed	0/10	-/-
9	*Vigna unguiculata (L.)Walp, 1843*	*Fabaceae*	Ubundu	high	intercropping	0/10	-/-
10	*Vigna vexillata(L.) A. Rich 1845*	*Fabaceae*	Bengamisa	low	weed	0/10	-/-

The similar observations were made by Alabi et *al*. (2008) in Nigeria, they have sequenced isolates ACMV and EACMV from some leguminous plants species (Fabaceae) like *Senna occidentalis L., Leucaena leucocephala* and *Glycine max L.*. Ogbe et *al*. (2006) have identified EACMCV isolates in *S. occidentalis L., L. leucocephala (Lam) De Witt (*Fabaceae*), Combretum confertum (Benth) M.A. Lawson (*Combretaceae*)* and *Manihot glaziovii Müll.arg (*Euphorbiaceae*)*.

Shonyika et *al*. (2001) reported the presence of ACMV in *Ricinus communis* (Euphorbiaceae*)* in Nigeria. It means that, CMBs are not largely restricted to a few vegetables species previously known p.e. *M. glaziovii Müll;* they can be spread in many plants in Africa as reservoir (Ogbe et *al*., 2006; Fauquet & Fargette, 1990).

As virus transmission from cassava to Fabaceae must be due primarily to *B. tabaci,* this suggests that whiteflies might infect non-cassava plants when there is high epidemic pressure.

Nature and diversity of virus strains isolated in *Fabaceae* species in Yangambi area

The present study has permit to identify 7 isolates of ACMV and 4 isolates of EACMV from natural leguminous in cassava fields in Yangambi (Fig. 2). Four isolates of ACMV had been identified from *P. javanica,* they have been listed in the following accessions (Ybi1[Pr]-FN435271, Ybi2[Pr]-FN435272, Ybi3[Pr]-FN435273, Ybi4[Pr]-FN435274) and 2 isolates of EACMV (Ybi1[Pr]-FN435278, Ybi2[Pr]-FN435279).

We have also listed 3 isolates of ACMV from *C. pubescens* Ybi1[Cm]-FN435275, Ybi2[Cm]-FN435276, Ybi3[Cm]-FN435277) and 2 isolates of EACMV (Ybi1[Cm]-FN435280, Ybi2[Cm]-FN435281).

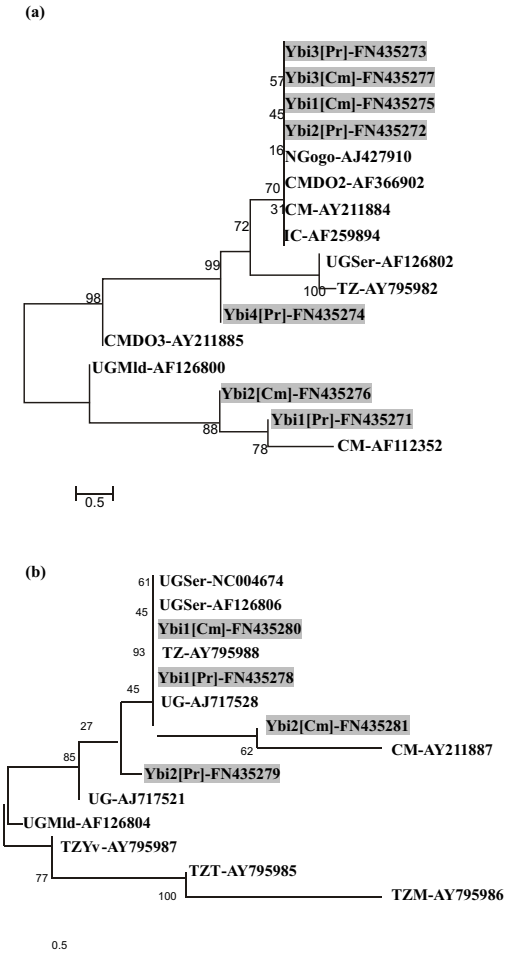


Fig. 2*:* Phylogenetic analyses were conducted using MEGA4 Software based on nucleotides of ACMV (a) and EACMV (b) with the complete DNA-A genome isolated in *Pueraria javanica* [Pr] and *Centrosema pubescens* [Cm] . Others referential accessions were provided by the NCBI GenBank. The evolutionary history was determined using the Neighbor-Joining method with the complete deletion option. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. To construct the phylogenetic tree for ACMV (a), sequences of the following origins were used: Cameroon [CM] AF366902, AF112352, AY211885, AY211884; Nigeria [NG] AJ427910; Uganda [UG] AF126800, AF126802; Ivory Coast [IC] AF259894; and Tanzania [TZ] AY795982. The strains isolated in the Yangambi [Ybi] are named using the following accessions: Ybi1[Pr]-FN435271, Ybi2[Pr]-FN435272, Ybi3[Pr]-FN435273, Ybi4[Pr]-FN435274, Ybi1[Cm]-FN435275, Ybi2[Cm]-FN435276, Ybi3[Cm]- FN435277. The two clusters obtained suggest the probability of two majors strains of ACMV in CMD epidemiology.

In the phylogenetic tree of EACMV (b), sequences of the following origins were used: Cameroon [CM] AY211887; Uganda [UG] AY795988, AF126804, AF126806, AJ717528; and Tanzania [TZ] AY795988, AY795985, AY795986, AY795987. The strains of EACMV isolated in the Yangambi [Ybi] were represented by the following accessions: Ybi1[Pr]-FN435278, Ybi2[Pr]-FN435279, Ybi1[Cm]-FN435280, Ybi2[Cm]-FN435281. All isolates formed a unique cluster, indicating the presence of one strain of EACMV.

Phylogenetic tree (Fig. 2a) shows that ACMV strains identified in *Fabaceae* presents two clusters. The first cluster is closely related to ACMV-NGogo (AJ427910), ACMV-CM (AF366902), ACMV-CM (AY211884), ACMV-IC (AF259894), ACMV-UGSer (AF126802) and ACMV-TZ (AY795982). The second cluster is related to ACMV-CM (AF112352) and ACMV-UGMld (AF126800). The results indicate that the mains ACMV strains isolated from *Fabaceae* in Yangambi environment present a high proximity with Cameroonian, Ugandan and Nigerian strains.

Furthermore, the phylogenetic tree with strains of EACMV (Fig. 2b) isolated in *P. javanica* and *C. pubescens* presents one cluster related to the variant of Uganda strains EACMV-UGSer (AF126806), EACMV-UG (Aj717528) and to an EACMV (AY211887) identified in Cameroon. The present study underscores the prevalence of like UgV and his largely spread in the North-eastern region of DRCongo. The spread of CMBs in double infection is a general phenomenon in this humid forest of Yangambi.

Diagnosis and virus diversity in whiteflies vector (*B. tabaci)* and in cassava from Yangambi

Multiplex PCR has revealed dual infection of CMBs in cassava and whiteflies (Fig. 3a). Up to 132 whiteflies collected in cassava fields indicates a high proportion of double infecting whiteflies by ACMV+EACMV (59%), EACMV (29%) and ACMV (8%). A few proportions (4%) of whiteflies tested were virus free (Fig. 3b). The same proportions were also obtained in cassava virus infested.

However, in a total of 140 cassavas diagnosed by PCR, 66% are in dual infection for ACMV+EACMV, 26% of cassava are EACMV

infected and 5% of cassava are ACMV single infected, only 3% of cassava is virus free (Fig.3c). To consider the preference of *B.tabaci* in the transmission of EACMV, we can assert the involvement of *B. tabaci* as a major factor in to CMD epidemiology in Yangambi.

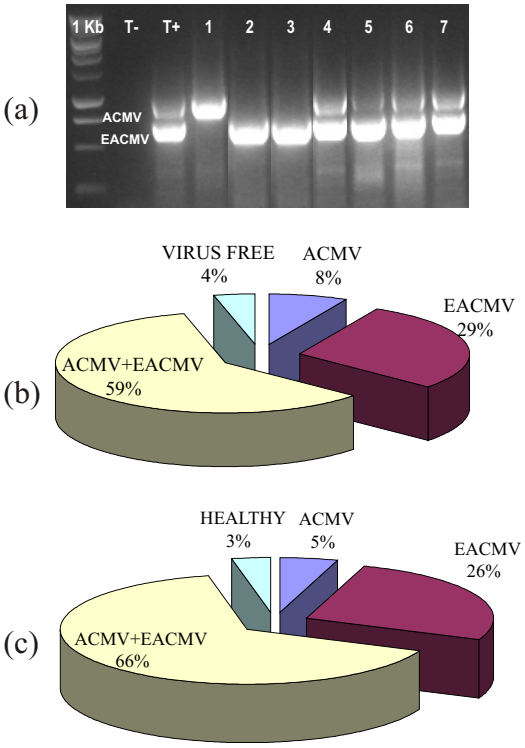


Fig 3: Amplification by multiplex PCR using whiteflies collected in Yangambi region (a). Samples of 132 whiteflies were diagnosed by multiplex PCR using primer AC4F/R for ACMV (750 bp) and EAC2F/R for EACMV (630 bp). The figure (a) designates the samples of virus positive whiteflies (lanes 1 to 7). T- : Negative control and T+: Positive control. Lanes 4-7 indicate the whiteflies with dual infection of virus; Lanes 2-3 are samples EACMV single infected, lane 1 shows ACMV single infected whiteflies.

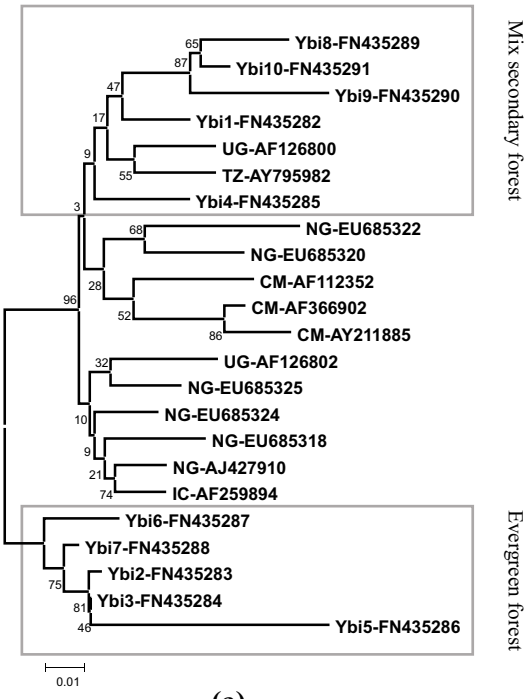
The proportion of B. tabaci infected (b) indicates a largely double infection by ACMV + EACMV (59 %), the proportions of infection by EACMV and ACMV are respectively 29 % and 8 %. Only 4 % of whiteflies diagnosed were virus free.

In the total of 140 cassavas sampled around Yangambi and diagnosed by PCR (c), the percentage of dual infection ACMV+EACMV is near 66 %; 26 % of cassava are EACMV infected and 5 % of cassava are ACMV infected single. Only 3 % of cassava is virus free.

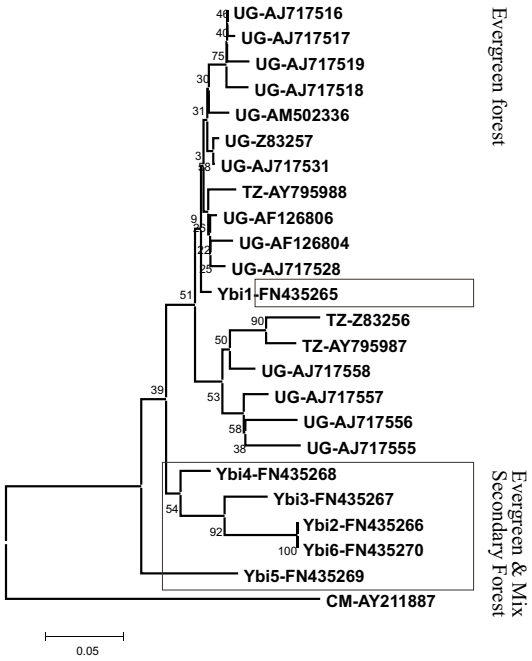
Diversity and phylogeny of CMBs by comparison to genes AC2 and AC4

The Phylogenetic trees established in basis of amino acids of gene AC4 and AC2 shows the presence of *10 isolates of ACMV clustered in* 3 majors strains (Fig. 4a) and *6 isolates of EACMV* clustered in 2 mains strains involved in epidemiology of CMD. The most prevalent EACMV strain in Yangambi is UgV. Phylogenetic tree (Fig. 4a) show that ACMV strains identified in Yangambi are closely related to ACMV-CM/DO3 (AY211885), ACMVCM (AF112352), ACMV-NGogo (AJ427910), ACMV-UGMld (AF126800), ACMV-UGSer (AF126802) and ACMV-IC (AF259894).

In the other hand, the strains of EACMV present a high proximity to variant of Uganda strains EACMVUG2Mld (AF126804) , EACMV-TZ (AY795988), EACMV-UGser (AF126806), (AJ717528) (Fig. 4b). Full length DNAA obtained by assembling contiguous sequences (vector NTI® Advance11) confirm this results basing on genes AC2 and Ac4.



(a)



(b)

Fig. 4: Phylogenetic tree based on concatenuous sequences of amino acids of genes AC2 and AC4 for ACMV (a) and EACMV (b) inferred from the Neighbour-joining method using MEGA version 4.1 with complete deletion option. Bootstrap values (1000 replicates) are shown as percentages in the branches (Tamura et *al*., 2007). For ACMV (a), isolates Ybi1-10 are the different isolates of ACMV identified from Yangambi in the accessions beginning by FN are clustering in 3 groups: the first cluster regroups 4 isolates (Ybi1,4,8,9,10) which have a short gene AC4 closely related to ACMV-UGMld(AF126800); the second consists to five isolates Ybi2,3,6,7 which have formed a apart cluster. It can be noted the presence of ACMV-Ybi5(FN435286) as an out group. IC strains (cluster 3). In basis on the analysis of amino acids of AC2/AC4, three various strains of ACMV are implied in cassava mosaic epidemiology in Yangambi. Basing on the analysis of gene AC2/AC4 in concatenation, we note that three major strains of ACMV are implied in epidemiology of cassava mosaic disease in Yangambi.

For EACMV (b), the isolates Ybi 1-6 are the EACMV isolates identified in samples from Yangambi have formed 2 clusters indicating the presence of 2 major strains of EACMV associated in the cassava mosaic disease in Yangambi region. Five isolates from Yangambi FN435266, FN435267, FN435268, FN43569 and FN435270 have presented a apart cluster. The isolate FN435265 has presented the proximity with Tanzanian and Ugandan's strains. It may be the presence of 2 main strains of EACMV which involve in epidemiology of CMD in Yangambi region.

Distribution of begomoviruses implied in the epidemiology of CMD in Yangambi

In the region of Yangambi, North-eastern DRCongo, we can note that begomoviruses implied in CMD are spread everywhere in region (Fig. 5). The high frequency (59-66%) of dual infected whiteflies and cassava plants is a dangerous epidemiology index in the Eastern of RDCongo. It can be indicated the important proportion of EACMV (26-29%). Those factors, related to the high emergence of whiteflies more than 5 per cassava plant (data not shown) present a worry pathosystem for epidemic explosion.

Our epidemiology investigations allow to note that begomoviruses implied in CMD are spread everywhere in the region of Yangambi, North-eastern DRCongo (Fig. 5). Cassava mosaic begomoviruses presents a largest proportion of dual infection (near 60%), followed by infection in EACMV (near 30 %) and 8 % by infection in ACMV as well in cassava as in whiteflies.

The distribution of ACMV isolates in Yangambi region is related to the principal ecosystems and to the main circulation ways. In the evergreen humid forest in which agricultural activity is not intensive; some isolates are restricted to the forest zone in which cassava cultivation is not very intensive. We can note (Table 2) the low severity score (2-3) and a low incidence (60-70%) of CMD in this zone indicating the presence of moderate strains in the locations in forest like Basoko, Mongandjo, Yambuya and Bakere.

However in the secondary forest, like in Banalia, Bengamisa, Kisangani, Yangambi, Yatolema, Yanonge and Yaolila locations, in which the cassava culture and anthropic activity are intensive, we note the presence of the main ACMV isolates as [1] FN435255; [8] FN435262; [9] FN435263 and [10] Fn435264.

Table 2*:* Expansion of the major CMBs isolates in relation to the principal agroecosystems and the epidemiologic index of CMD in the Yangambi region. High disease severity scores [3.100.32 to 4.110.33] and high incidence [85 - 95 %] of CMD is noted in the secondary forest and in savannah where cassava cultivation is intensive. In the deciduous forest or in the evergreen forest, the disease severity scores varied between [2.080.29 to 2.890.33] and the mean incidence of CMD was [60 - 70 %].

Principal locations	Ecosystems	Cropping system	CMD Viruses isolates		Proportion of virus (%)	Mean of whiteflies	Incidence mean (%)	Score severity
			ACMV	EACMV				
Banalia	Mix secondary forest and agriculture	spatial continuous	Ybi8	Ybi6,Ybi4, Ybi3	A : 9 E : 36 A+E : 55	7.72±1.35	90	3.73±0.65
Bengamisa	Mix secondary forest and agriculture	spatial continuous	Ybi10, Ybi8	Ybi3, Ybi4	A : 7 E : 29 A+E : 64	10.07±1.14	95	3.79±0.43
Yangambi	Mix secondary forest and agriculture	spatial continuous	Ybi10, Ybi9	Ybi2, Ybi4, Ybi6	A:11 E:33 A+E:56	8.00±1.11	85	4.11±0.33
Yaotonga	Mix secondary forest and agriculture	spatial continuous	Ybi9	Ybi2,Ybi6, Ybi3	A:10 E:30 A+E:60	8.10±1.10	85	3.87 ±0.35
Yanonge	Mix secondary forest and agriculture +Intensive agriculture	spatial continuous	Ybi10	Ybi6,Ybi2, Ybi3	A : 8 E :33 A+E : 59	9.17±1.03	90	3.25±0.45
Kisangani	Intensive agriculture	spatial continuous	Ybi9, Ybi1,Ybi10, Ybi 8	Ybi6,Ybi3	A : 10 E : 40 A+E : 50	10.30±1.17	95	4.05±0.39
Yatolema	Deciduous high forest + intensive agriculture	spatial continuous	Ybi9, Ybi10	Ybi6, Ybi3	A :11 E : 33 A+E : 56	9.33±1.93	90	3.55±0.52
Yaolila	Deciduous high forest + intensive agriculture	spatial continuous	Ybi9, Ybi10	Ybi3, Ybi6	A : 13 E : 25 A+E : 62	9.12±0.99	70	3.75±0.46
Basoko	Deciduous high forest	spatial discontinuous	Ybi5, Ybi7	Ybi2,Ybi3,Ybi4,Ybi6	A : 10 E: 30 A+E : 60	7.10±0.74	70	3.10±0.32
Mongandjo	Evergreen forest	spatial discontinuous	Ybi3, Ybi6	Ybi2, Ybi5, Ybi6	A : 8 E : 41 A+E : 51	6.33±1.07	72	2.08±0.29
Yambuya	Evergreen forest	spatial discontinuous	Ybi4, Ybi6	Ybi2, Ybi6	A : 13 E : 25 A+E :62	7.13±0.99	60	2.13±0.35
Bakere	Evergreen forest	spatial discontinuous	Ybi2,Ybi3, Ybi5	Ybi1,Ybi2	A : 11 E : 22 A+E : 67	6.11±0.78	65	2.89±0.33

A : single infection ACMV E : single infection EACMV A+E: dual infection with ACMV & EACMV
Score severity: 1 5 ; where 1: indicates no symptom and 5 : severe symptom

In Yangambi region, EACMV isolates are randomly spread as well in primary forest as in the mix secondary forest or savannah. We must note that our study underscores the prevalence of like UgV and his largely spread in the North-eastern DRCongo. The spread of CMBs in double infection is a general phenomenon in this humid environment around Yangambi. The CMD severity scores unregistered in these locations varied to 3-5 and the incidence 85-95% indicating that the strains involved in savannah and secondary forest are severe. These results are reinforced by Legg and Thresh's investigations made in East Africa in 2000.

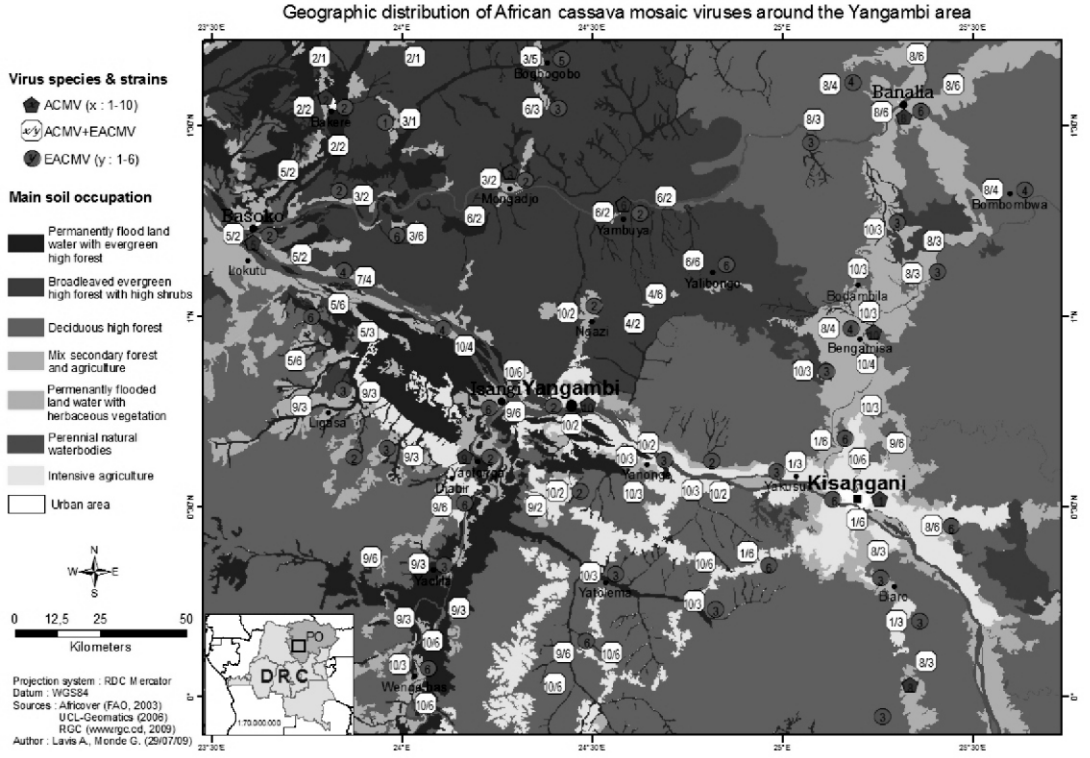


Fig. 4: Geographic distribution of viruses of CMD in function of soil occupation and anthropic activity in the Yangambi area.

The locations of sampling made in basis of the general card of Democratic Republic of Congo (©UCL-Geomatics, Louvain-la-Neuve, Belgium, 2006).

In a total of 140 samples of cassava leaves from Yangambi, 10 different isolates of ACMV (in base of gene AC4) and 6 isolates of EACMV (in base of gene AC2) were identified by molecular analysis. The main isolates of ACMV are represented in the card by numbers indicating the following accessions **1**: FN435255 **2** : FN435256 **3** :FN435257 **4** :FN435258 **5**: FN435259 **6**: FN435260 **7**: FN435261 **8**: FN435262 **9**: FN435263 **10**: FN435264 .

For isolates of EACMV, the following accessions were represented by numbers in the card **1**:FN435265 **2**:FN43526 **3**:FN435267 **4**:FN435268 **5**: FN435269 **6**: FN435270. Fractional numbers (**x/y**) represent the dual infection of ACMV+EACMV in the same sample collected in this location.

The distribution of CMBs in Yangambi environment has shown a largest proportion of dual infection (62%), followed by infection in EACMV (30 %) and 8 % by infection in ACMV as well in cassava as in whiteflies.

The distribution of ACMV isolates is related to the principal ecosystems and to the main circulation ways. In the evergreen humid forest of Yangambi in which agriculture activity is not intensive; the isolates **2** : FN435256 ; **3** : FN435257 ; **4** : FN435258 ; **5**: FN435259 ; **6**: FN435260 and **7**: FN435261 are restricted in the forest zone in which cassava cultivation is not very intensive. However in the secondary forest and savannah in which the cassava culture and anthropic activity are intensive, the main isolates spread are **1**: FN435255; **8**: FN435262; **9**: FN435263 and **10**: FN435264. In Yangambi region, EACMV isolates are randomly spread as well in primary forest as in the mix secondary forest or savannah.

Discussion

The present study showed the free movement of the same viruses infected cassava through some hosts alternate plants, insect vectors (*B. tabaci*) and cassava cultivars in Yangambi environment. The distribution of cassava and whiteflies dually infected by CMBs correlated to emergence of

insects vectors (>5 whiteflies per shoot) is a major epidemic index to explain a possible epidemic in the Eastern DRCongo.

Indeed, Alabi et *al*. (2008) concluded, without demonstrating the possibility of retro inoculation in cassava, that non-cassava hosts could play an important role in epidemiology of CMD in Nigeria because of presence of those plants as weeds in cassava cultivation. Since ACMV and EACMV isolates can be easily transmitted between cassava and non-cassava by whitefly vector, thereby facilitating the survival of viruses and their concentration in environment.

According Legg et *al.* (2006), the region of Yangambi in the North-eastern of RDCongo could be classified as a mature epidemic zone. Indeed, our field observations confirmed that virus transmitted by insect vector and cuttings is more than 70 % of CMD incidence average, the average CMD severity is > 3 and also we recorded more than 5 whiteflies per cassava shoot (data not shown).

Furthermore, the present study indicated a low diversity between viruses implied in CMD in Yangambi, the virus strains identified in Yangambi are randomly spread. It can be noted that there are some ACMV strains close to Cameroonian strain and Ugandan which cause moderate symptoms on cassava. EACMV isolate reported in Yangambi is specifically UgV.

Our results indicate that in 100 plants of cassava cultivation in Yangambi, virosis cassava are 66% ACMV+EACMV infected, 26% EACMV infected only, 3% ACMV infected only and 5% of cassava are virus free. Almost the same observations had been recorded in whiteflies during our investigation; this confirms the role of whiteflies in virus transmission.

All concordant analysis made on the nucleotides and amino acids indicate the presence of little strains of ACMV and EACMV (>98 identity of nucleotides of AC2 and AC4) which cause the CMD in Yangambi region. Contrary to some studies conducted in other parts of Africa (Ariyo et *al*., 2005; Were et *al.,* 2004; Ndunguru et *al*., 2005) which reported a high diversity of CMBs strains in CMD epidemiology. Our investigations showed through different sequences comparison that the diversity of CMBs in Yangambi is limited to some strains, the most spread is the Ugandan strain implied to severe symptom on cassava.

It can be reported also frequent recombination in coat protein between strains of ACMV and EACMV recorded in Yangambi (Monde, 2008; data unpublished). This phenomenon is largely due to the cassava doubly infected by ACMV and EACMV strains previously reported. The high frequency of mixte infection at once in vector, plant reservoir and cutting cassava may explain recombination in coat protein (Fondong et *al.,* 2000). The same phenomenon has been reported in cotton Geminivirus by Sanz et *al*., (2000).

Seal et *al.* (2006) and Zhou et *al*. (1977) had asserted that frequency of recombination could be explained by replication mechanism of begomovirus using plant host DNA polymerase. It may be the major cause of Uganda epidemic.

By making relation between the high frequency observed of dual infecting cassava (66%), the proportion of dual infection in natural plants (70-80%) and in his insects vector (59%), the presence of UgV well known in Uganda as epidemic agent (Zhou et *al*., 1997, Deng et *al*.; 1997), the sensitive genotypes of cassava (Ngeve et *al*., 2003) and the emergence of whiteflies, the region of Yangambi in the North-eastern DRCongo may be considered as a potential CMD epidemic zone (Legg et *al*., 2006). This observation confirms that UgV has supplanted the ACMV in the Eastern Africa by him preferential transmission by whitefly (Were et *al*., 2003). By considering the distribution of African cassava mosaic viruses, UgV is the most spread strain in Yangambi region. It is also the most prevalent and the largest distributed strain in the Eastern RDCongo. The double infected virus cases are also systematically distributed.

In conclusion, Yangambi region may be considered as a potential CMD epidemic zone. The free movement of virus associated to the emergence of vector should be considered as a precursor signal of epidemic in spite of the restricted diversity of virus strains.

Acknowledgements

This study was supported by Belgian Technical Cooperation (BTC) scholarship, the Applied Microbiology Phytopathology Unit of UCL in Belgium and the Agricultural Institute Faculty of Yangambi IFA-Yangambi in the DR Congo.

References

Alabi J.O., Ogbe F., Bandyopadhyay R. & Lava Kumar P. 2008: Alternative hosts of African cassava mosaic virus and East African cassava Cameroon virus in Nigeria. *Archives of Virology* 153: 1743-1747

Ariyo O.A., Koerbler M., Dixon A.G.O., Atiri G.I. & Winter S. 2005: Molecular variability and distribution of cassava begomoviruses in Nigeria. *J. Phytopathology* 153: 226-231

Deng D., McGrath D F., Robinson D J., & Harrison B D. 1994: Detection and differentiation of Whitefly-transmitted geminiviruses in plants and vector insects by the polymerase chain reaction with degenerate primers. *Annals of Applied Biology* 125:327-336

Deng D., Otim-Nape G W., Sangare A., Ogwal S., Beachy R.N. & Fauquet C.M. 1997: Presence of a new virus closely related to *East African cassava mosaic geminivirus* associated with cassava mosaic outbreak in Uganda. *African Journal of Root and Tuber Crops* 2: 23-28

Fauquet C.M. & Fargette D. 1990: *African cassava mosaic virus*. Etiology, epidemiology and control. *Plant Disease* 74: 404-411

Fondong V.N. , Pita J.S. ; Rey M., De Konchko A., Beachy R.N. & Fauquet C.M. 2000: Evidence of synergism between *African cassava mosaic virus* and a new double-recombinant geminivirus infecting cassava in Cameroon. *Journal of General Virology* 81:287-297

Fondong V.N., Pita J.S., Rey & Fauquet C.M., 2004 : Molecular caractérisation of viruses associated with the cassava mosaic disease epidemic in Cameroon. Unpublished.

Legg J P. 1999: Emergence, spread and strategies for controlling the pandemic of cassava mosaic virus disease in east and central Africa. *Crop Protection* 18: 627-637

Legg J P. & Thresh J.M. 2000: Cassava mosaic virus disease in East Africa: a dynamic disease in a changing environment. *Virus Research* 71: 135- 149

Legg, JP, Owor,B., Sseruwagi P. & Ndunguru J. 2006: Cassava mosaic viruses disease in East and Central Africa: epidemiology and management of a regional pandemic. *Advances in Virus Research* 67: 355-418

Monde G., Walangululu J., Winter S., Bragard C. 2010: Dual infection by cassava begomoviruses in two leguminous species (Fabaceae) in Yangambi, North-Eastern Democratic Republic of Congo. *Archives of Virology*, doi 10.1007/s00705-010-0772-3.

Ndunguru J., JP Legg, TAS Aveling, G.Thompson & CM Fauquet 2005: Molecular biodiversity of cassava begomoviruses in Tanzania: evolution of cassava geminiviruses in Africa and evidence for East Africa being a center of diversity of cassava geminiviruses. *Virology Journal* 2: 21 doi:10.1186/1743-422X-2-21

Neuenschwander P., Hughes J., Ogbe F., Ngatse,J.M. & Legg,J.P.2002: The occurrence of the Uganda variant of East African cassava mosaic virus (EACMV-UG) in western Democratic Republic of Congo and the Congo Republic defines the western-most extent of the CMD pandemic in East/Central Africa. *Plant Patholology* 51:384

Ngeve J.M. , Nukenine E.N. & Dixon A.G.O. 2003 : Reaction of genotypes to the cassava mosaic disease in three district agroecologies in Nigeria. *J.Phytopathology* 151: 101-107

Ogbe F.O., Dixon A.G.O., Hughes J.A., Alabi O.J. and Okechukwu R. 2006: Status of cassava begomoviruses and their new natural hosts in Nigeria. *Plant Disease* 90:548-553

Otim-Nape G.W., Bua A., Thresh J.M., Baguma Y., Ogwal S., Ssemakula G.N., Acola G., Byabakama B., Colvin J., Cooter R.J. & Martin A. 2000: The current pandemic of cassava mosaic virus disease in East Africa and its control. Natural Ressources Institute, Chatham, UK

Pita J.S., Fondong V.N., Sangare A., Kokora R.N.N.& Fauquet C.M. 2001: Genomic and biological diversity of African cassava geminiviruses. *Euphytica* 120:115-125 Ed. Kluwer Academic Publishers, Netherlands 2001

Sanz A. I., Fraile A., Gracia-Arenal F., Zhou X ., Robinson D.J., Khalid S., Butt T. & Harrison B. 2000: Multiple infection, recombination and genome relationships among begomovirus isolates found in cotton and other plants in Pakistan. *Journal of General Virol*ogy 81: 1839 - 1849

Seal S.E. , VanderBosch F. & Jeger M.J. 2006 : Factors influencing Begomovirus evolution and their increasing global significance: Implications for sustainable control. *Critical Reviews in Plant Sciences* 25: 23 46

Thresh J.M., Fargette D. & Otim-Nape G W. 1994: Effects of *African cassava mosaic virus*

on the yield of cassava. *Tropical Science* 26:34-37

Tamura K., Dudley J., Nei M. & Kumar S. (*2007*) *MEGA4*: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24:1596-1599.

Were H.K., Winter S. & Maiss E. 2003: Distribution of begomovirus infecting cassava in Africa. *Journal of Plant Pathology* 85(3):145-151

Were H.K., Winter S. & Maiss E. 2004: Occurrence and distribution of cassava begomoviruses in Kenya. *Annals of Applied Biology* 145: 175-184

Zhou X., Liu Y., Calvert L., Munoz C., Otim-Nape G.W., Robinson D & Harrison B.D. 1997: Evidence that DNA-A of a geminivirus associated with severe cassava mosaic disease in Uganda has arisen by interspecific recombination. *Journal of general Virology* 78: 2101-2111.

Les facteurs déterminant l'abondance du vecteur mouche blanche (*Bemisia tabaci*, Homoptera : Aleyrodidae) et les types des virus distribués sur le manioc en R.D. Congo

Z. Bakelana [1,*], J.P. Legg [2,3], K.M. Lema [4], S. Hauser [5], N.M. Mahungu [5].

[1]Institut National pour l'Etude et la Recherche Agronomiques, INERA, Centre de Recherches Agronomiques de Mvuazi. Bas Congo, RD. Congo.

[2]International Institute of Tropical Agriculture, Dar es Salaam, Tanzanie.

[3]Natural Resources Institute, University of Greenwich, Chatham Maritime, Kent ME4 4TB, UK

[4]Université de Kinshasa, Faculté des Sciences Agronomiques, Kinshasa, R.D. Congo.

[5]International Institute of Tropical Agriculture, Kinshasa, R.D. Congo

* Adresse de l'auteur : Tél : + 243 9 98 70 60 87
Email : bakelanatony@yahoo.fr

Résumé

La mouche blanche (*Bemisia tabaci*) est le vecteur de la mosaïque et de la striure brune du manioc. Sa population a toujours été faible dans les champs de manioc. En plus, cet insecte trés polyphage, n'avait jamais constitué d'une manière directe, une menace réelle à la culture du manioc en Afrique. Depuis quelques années, cependant, on constate une pullulation de la mouche blanche sur le manioc. En plus du rôle lui réconnu comme vecteur des virus responsables de la CMD et de la CBSD, la mouche blanche est devenue un véritable ravageur de la culture de manioc, en induisant des dégâts bien spécifiques sur les jeunes feuilles apicales et l'apparition de la fumagine sur les vieilles feuilles. Plusieurs facteurs ont été évoqués être impliquées dans la pullulation des populations de la mouche blanche en Afrique de l'Est, à savoir l'apparition du recombinant EACMV-Ug, qui augmente la fécondité des mouches blanches qui se nourrissent sur les plantes malades et l'apparition en Ouganda d'un nouveau biotype des mouches blanches plus fécond que l'espèce autochtone. Il a été confirmé la susceptibilité génétique de certaines variétés de manioc à héberger des populations élevées de la mouche blanche. Les pics des populations les plus élevés ont été enregistrés à Mvuazi et Mulungu, respectivement en mai 2008 (21,91 mouches par

plante) et juillet 2008 (35,31 mouches par plante). Le site de Mvuazi a présenté le nombre de cas d'infections mixtes ACMV et EACMV le plus élevé (31,4%), comparativement aux sites de Gimbi (3,2%), Luki (7,6%) et Mulungu (15%), augmentant par consequent la sévérité et l'incidence de la CMD sur les variétés sensibles de manioc à Mvuazi. La striure brune a été rencontrée dans tous les sites et sur toutes les variétés en place, à raison de 10% d'incidence en moyenne. La sévérité variant entre les côtes 2 et 3.

Mots clés: Mosaïque du manioc (CMD), striure brune (CBSD), mouche blanche, sévérité, incidence.

Introduction

Le manioc (*Manihot esculenta* Crantz) est l'une des cultures les plus largement cultivées en Afrique Sub-saharienne et sa production totale de plus de 121 millions de tonnes est plus élevée que celle de n'importe quelle autre culture en Afrique (FAO, 2006). Le manioc est particulièrement très important pour les petits fermiers à cause de son rôle dans la sécurité alimentaire et comme source de revenus (Fauquet et Fargette, 1990 ; Harrison et al.,1997).

La production de manioc en Afrique constitue environ 54% de la production mondiale (FAO, 2003), mais le rendement moyen en Afrique (8,9 tonnes/ha) représente 70% de ceux obtenus en Amérique du Sud et 61% de ceux d'Asie (Legg et Thresh, 2003). En RDC, la production totale du manioc dépasse 14 millions de tonnes, et s'est chiffré en 2006 à 14.974.470 (FAO, 2006)

La faiblesse de rendement en Afrique pourrait être attribuée aux maladies et ravageurs (Hahn et Keyser, 1985). La maladie la plus répandue du manioc en Afrique et d'importance économique est sans doute, la mosaïque du manioc (CMD). Les épidémies sont particulièrement très dévastatrices, avec des pertes de rendement de l'ordre de 20 à 90%, qui sont très fréquentes à travers l'Afrique Sub-saharienne (Hahn et al.1980 ; Muimba et Phuti, 1987). On retrouve souvent la CMD dans les champs de manioc de petits fermiers qui ne peuvent pas mettre en place de bonnes pratiques phytosanitaires tels que la plantation des boutures saines et l'arrachage des plantes malades.

La mosaïque du manioc (CMD) est causée par au moins quatre geminivirus du genre *Begomovirus* (Famille Geminiviridae), et est transmise par la mouche blanche *Bemisia tabaci* (Gennadius) (Hemiptera : Aleyrodidae) biotype A. (Swanson et Harrison, 1994 ; Zhou et al., 1997).

Plusieurs pays d'Afrique Centrale et de l'Est ont été affectés par la pandémie des geminivirus (CMGs) de la mosaïque du manioc (Fauquet et Fargette, 1990 ; Harrison et al., 1997). Plusieurs espèces des geminivirus existent en Afrique, parmi lesquels les plus prévalents, à la base de la propagation de la pandémie sont "l'African cassava mosaic virus" (ACMV) et "l'East African Cassava Mosaic Virus" (EACMV). Les deux geminivirus sont transmis non seulement par la mouche blanche, *Bemisia tabaci* mais sont aussi disséminés à travers le matériel utilisé pour la plantation (Storey et Nichols, 1938 ; Dubern, 1994).

Le premier rapport d'une forme très sévère de la CMD affectant de vastes superficies de manioc fut établit en 1988 au nord de l'Ouganda, dans le District de Luwero (Otim-Nape et al.,1997, 2000). Le problème s'est ensuite propagé vers le sud, dévastant les champs de manioc et entrainant la famine. Plus récemment, la pandémie a affecté de vastes zones de la Tanzanie, du Soudan, du Kenya, du Burundi, du Rwanda et de la RD. Congo (Legg, 1999 ; Otim-Nape et al., 2001).

Un nouveau begomovirus recombinant désigné EACMV-Uganda (EACMV-Ug) (Deng et al.,1997 ; Pita et al., 2001), considéré aussi comme étant la variante ougandaise (UgV) (Harrison et al., 1997 ; Zhou et al., 1997), était associé à la pandémie. L'EACMV-Ug est issu de la recombinaison entre l'ACMV et l'EACMV (Zhou et al., 1997). La propagation rapide de ce virus à partir de l'Ouganda vers les pays environnants (Legg et Ogwal, 1998) et spécialement les rapports récents confirmant la présence de l'EACMV-Ug en RD. Congo et l'Est du Gabon (Neueuschwander et al., 2002), témoignent son mouvement vers les pays de l'Afrique de l'Ouest où l'ACMV est prédominant.

Les infections mixtes des virus avaient été démontrées importantes à l'expansion du "front" de la pandémie et contribuèrent à l'accroissement de la sévérité des symptômes. L'évidence avait aussi démontré une interaction synergique entre les plantes sévèrement attaquées et le vecteur mouche blanche, B.tabaci, ayant pour résultat, des populations amplifiées des mouches blanches à l'avant de la pandémie.

L'augmentation sans cesse du nombre des geminivirus identifiés à ce jour, met en évidence l'importance de la détermination de leur distribution géographique. Le test ELISA est disponible dans plusieurs pays africains et a l'avantage d'être rapide, robuste et bon marché. Le TAS ELISA est quelque peu plus sensible que le

DAS-ELISA, mais les deux techniques ont des limites par le fait qu'elles détectent rarement les geminivirus dans les feuilles sans symptômes. En plus, ELISA ne détecte pas l'EACMV en cas d'infection mixte avec l'ACMV, ou ne distingue pas l'ACMV et l'EACMV-Ug, qui ont des épitopes des capsides protéiques similaires. Les limites d'ELISA expliquent l'utilisation croissante des méthodes basées sur l'ADN, la "polymerase chain reaction" (PCR) et la "restriction fragment length polymorphisms" (RFLPs) pour détecter et distinguer les différents begomovirus et déterminer leur distribution. Ces techniques ont été utilisées dans très peu de laboratoires en Afrique et par des collaborateurs en Europe et aux USA (Fondong et al., 2000 ; Pita et al., 2001 ; Fauquet et Stanley, 2003 ; Ogbe et al., 2003).

La striure brune du manioc est une autre maladie virale transmise aussi par *B. tabaci* (Maruthi, 2005) dont l'agent pathogène est un potyvirus. Cette maladie a été reconnue depuis longtemps comme étant endémique dans les zones côtières de culture de manioc en Afrique de l'Est et limitée aux zones d'altitudes basses et moyennes en dessous de 1000 m (Nichols, 1950).

La récente recrudescence de la striure brune en Ouganda et les rapports non confirmés de la maladie en RD. Congo (Mahungu et al., 2003) et l'Ouest du Kenya soulèvent l'inquiétante hypothèse selon laquelle les populations abondantes des mouches blanches seraient en train de propager une double pandémie (Legg et al., 2006).

Matériels et Méthodes

Choix des variétés.
Quatre variétés de manioc, à différentes sensibilités vis-à-vis de la CMD avaient été utilisées. Toutes les boutures étaient supposées être apparemment saines au départ, pour éviter une éventuelle infection primaire.

- Une variété locale (témoin) , très sensible à la CMD, la variété Ngamanza, locale du plateau des Batéké dont le matériel de plantation avait été trié dans un champ paysan au plateau des Batékés.

- Deux variétés tolérantes, Mvuama et RAV, anciennes variétés améliorées de l'INERA, très répandues et obtenues d'un champ de multiplication de l'INERA avec phyto-sanitation.
- Une variété résistante, Butamu, figurant dans le lot de nouvelles variétés de l'INERA / IITA, résistantes à la CMD.

Choix des sites.
Nous avions restreint notre zone d'investigation dans les différents centres et stations de recherche de l'INERA à travers la RD.Congo, principalement suite à la disponibilité des données climatiques. Ces stations ont été sélectionnées en fonction de leurs environnements contrastés.

C'est ainsi que dans la zone de savane et de basse altitude, le Centre de Mvuazi a été retenu. Dans les zones de transition savane-forêt, en basse altitude, nous avions choisi Gimbi et Kiyaka. La zone forestière est représentée par la Réserve de Luki et celle de haute altitude par le Centre de recherches de Mulungu.

Dispositif expérimental, traitements et échantillonnage
Nous avons utilisé le dispositif des blocs complètement randomisés (BCRD) avec quatre répétitions. Les traitements sont constitués des variétés en place. Les parcelles élémentaires de 5m x 5m étaient séparées de part et d'autre par des allées de 2m de largeur. Les écartements de plantation du manioc étaient de 1m x 1m, ce qui fait que chaque parcelle élémentaire comptait 25 plants au total. Tous les 25 plants de la parcelle élémentaire avaient constitué notre échantillon de travail. Les plantes centrales et les plantes de bordure avaient été évaluées concomitamment pour les données entomologiques (spécificité entomologique), tandis que les plantes centrales avaient concernés les autres données à caractère agronomique.

Paramètres et fréquence de prise des données:
Les différents paramètres qui ont fait l'objet de nos observations sont :

Le nombre des mouches blanches par plante:
La méthode la plus courante pour évaluer la population de *B. tabaci* sur les plantes individuelles de manioc implique le comptage direct des adultes sur les cinq jeunes feuilles apicales (Fargette, 1985). Cela, parce que les adultes se nourrissent et pondent de préférence sur les feuilles immatures (Khalifa et El-Khider, 1965 ; Fargette, 1985).

La feuille de manioc est donc tenue par le pétiole et doucement renversée et par conséquent les mouches blanches présentes à la surface inférieure peuvent être facilement comptées (Fargette, 1985 ;

Fargette et al., 1985 ;Fishpool et al., 1995).

Dans le contexte de nos investigations, les adultes et les larves étaient régulièrement dénombrés, toutes les deux semaines, à partir de 1 MAP (mois après plantation). Les adultes étaient dénombrés sur les cinq jeunes feuilles apicales tandis que les larves étaient comptées sur les vieilles feuilles basales.

Il est reconnu que les données de comptage d'insectes ne suivent pas la distribution normale. Les données de comptage suivent généralement la distribution de Poisson et pour corriger l'hétéroscedasticité (différence de variance), la non-normalité et la non-additivité en vue d'appliquer une analyse de la variance, les données ont été transformées. La transformation logarithmique (n'importe quelle base) a été recommandée. Pour le cas d'espèce, la transformation x' = log x était propice. Lorsque certaines données sont inférieures à 10 ou égales à 0, la transformation

$x' = \log (x + 1)$ est préférable. (Zar, 1974).

Les données sur le nombre d'adultes et des larves des mouches blanches ont été transformées sur base de la fonction $x' = \log (x + 1)$.

La sévérité de la CMD: Sur base d'une échelle de cotation (1-5), le niveau d'attaque de la CMD était régulièrement évalué (toutes les 2 semaines à partir de 1 map) sur chaque plante individuelle.

L'incidence de la CMD: Les données sur l'incidence de la mosaïque africaine renvoient aussi aux données de comptage évoquées ci-haut et ont été exprimées en pourcentage. Pour des raisons d'analyse statistique, ces données ont été convertit en proportion puis transformées sur base de la fonction $x' = \arcsin\sqrt{x}$ (Zar, 1974).

La proportion des plantes affectées par la CMD a été déduite des données de la sévérité de la CMD.

Le poids des racines fraîches ou le rendement: évalué à la récolte, à 12 map.

L'incidence et la sévérité de la CBSD: évalué à la récolte, à 12 map. Toutes les racines de manioc des plantes centrales avaient été découpées en cinq tranches transversales et cotées suivant une échelle de sévérité allant de 1 à 5. Les symptômes foliaires de la CBSD n'avaient pas été pris en compte suite à la diversité de ces symptômes et par manque de certitude de présence de virus dans ces feuilles affectées (Legg,2009).

- **La distribution de différentes espèces et/ou virus de la CMD en RDC**.

Résultats et discussion

Nombre d'adultes des mouches blanches : L'analyse de la variance a révélé une différence hautement significative entre les différents sites sur le nombre d'adultes des mouches blanches. Les sites de Mulungu et Luki ont présentés le plus grand nombre d'adultes des mouches blanches, suivi des sites de Gimbi et Mvuazi. Tandis que le site de Kiyaka a présenté le nombre le plus faible (Figure 1).

L'analyse de la variance a révélé une différence hautement significative entre les différentes variétés sur le nombre de mouches blanches adultes. Les variétés Mvuama et Ngamanza ont présenté le plus grand nombre de mouches blanches adultes, suivi de la variété RAV. La variété résistante à la CMD, Butamu a présenté la moyenne la plus faible, contrairement aux résultats obtenus en Ouganda par Sserubombwe et al. en 2000, selon lesquels la variété résistante à la CMD, la SS4 avait hébergé la population la plus élevée en mouches blanches adultes tandis que la variété locale sensible à la CMD, Ebwanateraka, avait hébergé la population la plus faible en mouches blanches adultes (Sserubombwe et al., 2000).

Les résultats obtenus dans nos investigations confirment la préférence variétale due à la mouche blanche tel que évoqué précédemment par Fauquet et al. en 1987, par Otim-Nape et al. en 1996 et par Sserubombwe et al. en 2000.

Cependant, certains facteurs dus à la croissance de la plante et au microclimat peuvent être déterminant pour la différenciation variétale (Sserubombwe et al., 2000).

Les différences sont hautement significatives entre les périodes d'observation, la figure 2 montre que les populations les plus élevées ont été observées à Mvuazi entre avril et juin 2008, avec le pic en mai 2008 et à Mulungu, les populations les plus élevées ont été observées entre juin et août 2008, avec le pic en juillet 2008.

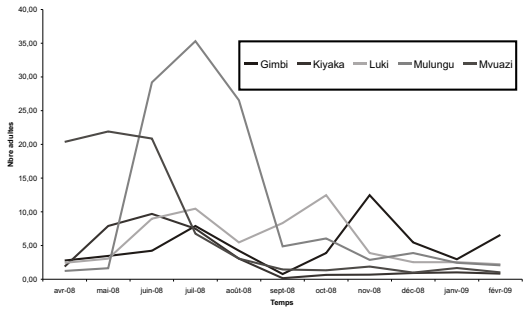


Figure 1: Evolution moyenne de la population des mouches blanches adultes dans les différents sites selon l'âge de la culture (temps).

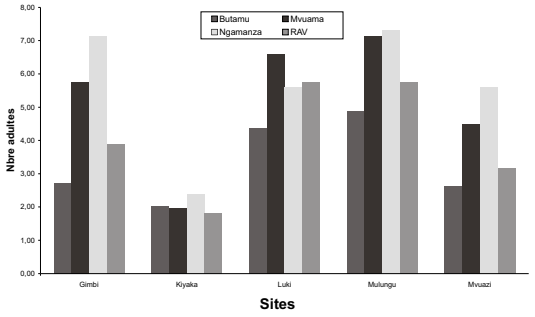


Figure 2: Distribution moyenne de la population des mouches blanches adultes sur les différentes variétés dans chaque site.

La figure 2 ci-dessus confirme la prévalence des mouches blanches adultes à Mulungu et à Luki. Dans presque tous les sites, il a été observé que c'est la variété Butamu qui s'est révélée comme étant la moins sensible aux mouches blanches adultes.

Une gamme des variétés de manioc élites avaient été évaluées en 1999/2000 et 2000/2001 face à l'infestation des mouches blanches dans les parcelles expérimentales de l'IITA au Nigéria. Il avait été observé des différences hautement significatives entre les variétés élites, entre les périodes d'observations et entre les sites, en terme d'infestation de la mouche blanche. Les interactions entre variétés et sites, variétés et périodes d'observations, sites et périodes d'observations, sites et périodes d'observations et variétés étaient aussi hautement significatives et indiquèrent qu'il existait aussi différentes réponses variétales face à l'infestation de la mouche blanche et cela variait aussi en fonction des sites (Ariyo et al., 2005).

Deux hypothèses avaient été émises sur la super abondance des populations de *B.tabaci* associées avec la pandémie de la CMD en Ouganda. La première hypothèse suggère un changement dans la qualité de l'hôte dû à l'infection du plant de manioc par le nouveau virus associé à la pandémie, l'EACMV-Ug, amplifiant ainsi le taux de reproduction de *B.tabaci*. La seconde hypothèse suppose que l'accroissement de la population de *B.tabaci* est associé à l'apparition d'un nouveau biotype plus fécond de *B.tabaci* dans les zones affectées par la pandémie (Legg et al., 2002).

Les données préliminaires avaient confirmées la première hypothèse. Il avait été observé que *B. tabaci* colonisant la variété sensible à la CMD, Ebwanateraka infectée par l'EACMV-Ug , se reproduisait plus rapidement que le *B. tabaci* appartenant à la même source mais élevé sur des plantes saines de la même variété (Colvin et al. 1999).

Les changements dans la composition relative des acides aminés résultant d'une sévère infection de la CMD avaient été proposés comme étant la cause possible de cette apparente interaction synergique (Colvin et al. 1999).

Cependant, une contradiction apparente à cette hypothèse était la très forte pullulation des mouches blanches rapportée dans le matériel végétal sain et résistant à la CMD planté dans le cadre du programme de lutte contre la pandémie dans les zones précédemment affectées.

Les investigations menées par Legg et al., en Ouganda 2002, présentèrent des informations additionnelles et complémentaires supportant la deuxième hypothèse, selon laquelle un nouveau biotype de *B.tabaci* était associé à la pandémie de la mosaïqué du manioc en Ouganda (Legg et al., 2002).

Deux génotypes de *B.tabaci* avaient été identifiés colonisant le manioc en Ouganda, considérés Ug1 et Ug2. Le génotype envahisseur Ug2 avait été identifié exclusivement dans les localités en arrière du front de l'épidémie et dans la zone du front, bien qu'une faible proportion avait été trouvée en avant du front ensemble avec Ug1. Au contraire, le génotype local Ug1, avait été fréquemment détecté en avant du front et dans certains endroits au niveau du front, bien qu'il n'avait jamais été identifié en arrière du front. Ces données avaient fournies une indication sur la très forte association entre Ug2 et la sévère pandémie de la mosaïque. Ce fût la première démonstration de l'association d'un génotype de *B.tabaci* avec la récente pandémie de la mosaïque en Ouganda. Ces études avaient aussi fourni la première information quantitative suggérant que *B.tabaci*

était plus abondant au niveau du front et en arrière, qu'en avant de celui-ci (Legg et Ogwal, 1998).

Nombre de larves des mouches blanches: L'analyse de la variance a révélé une différence hautement significative entre les différents sites sur le nombre de larves des mouches blanches. Le site de Mvuazi a présenté le nombre le plus élevé suivi de Gimbi, Luki et Mulungu. Kiyaka a enregistré une fois de plus, le nombre de larves le plus faible.

L'analyse de la variance a révélé des différences hautement significatives entre les variétés sur le nombre de larves des mouches blanches. La variété Ngamanza a hébergé le nombre le plus élevé des larves des mouches blanches suivi de la variété Mvuama tandis que très peu de larves ont été observées sur la variété RAV.

Les différences sont hautement significatives entre les périodes d'observation, la figure 3 montre que les populations les plus élevées ont été observées à Mvuazi entre mai et juillet 2008 avec un pic très marqué en mai 2008 pendant que dans les autres sites, l'évolution de la population larvaire de la mouche blanche a été assez stable au courant de l'année.

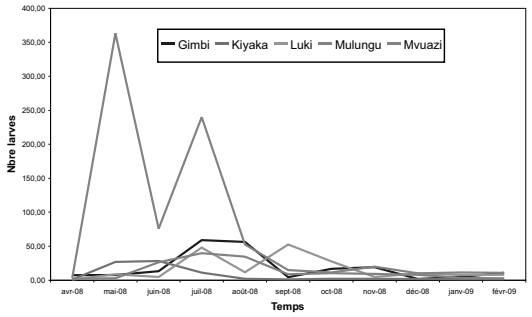


Figure 3: Evolution moyenne de la population larvaire des mouches blanches dans les différents sites selon l'âge de la culture (temps).

La figure 4 confirme la prédominance de larves des mouches blanches à Mvuazi et une très faible pullulation à Kiyaka. On observe une certaine tendance générale des variétés affectées par la CMD, Mvuama et Ngamanza à héberger beaucoup de larves.

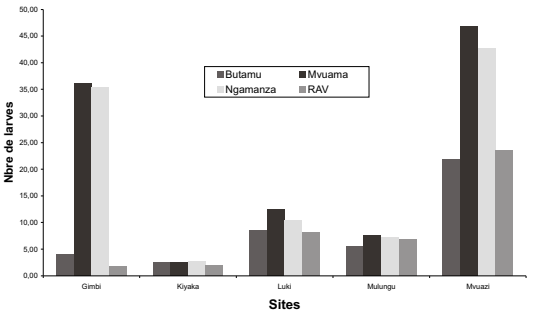


Figure 4: Distribution moyenne de la population de larves des mouches blanches sur les différentes variétés dans chaque site.

Les études préliminaires menées en Ouganda ont suggéré une interaction synergique entre les plantes de manioc sévèrement attaquées et les populations de *B.tabaci*. Les composantes de cette interaction, incluent l'accroissement de la colonisation des plantes affectées par la CMD, la concentration d'œufs sur les portions saines des plantes malades et le taux élevés de fécondité et de développement larvaire sur les plantes malades (Omongo, 2003).

En ce qui concerne le coefficient de corrélation entre la population d'adultes et la population des larves des mouches blanches, les deux séries des données ont permis de calculer à l'aide du tableur Excel le coefficient de corrélation, soit r = 0,33. La corrélation est positive mais faible. L'adulte de la mouche blanche étant mobile, la larve étant sessile, les adultes et les larves n'occupent pas toujours la même niche écologique.

Incidence de la mosaïque africaine de manioc: L'analyse de la variance a révélé des différences hautement significatives entre sites en ce qui concerne l'incidence de la mosaïque en R.D. Congo. Le site de Mvuazi a été le plus attaqué avec 43,48 % de CMD, suivi de Luki, Gimbi, Kiyaka et Mulungu.

L'analyse de la variance a montré des différences hautement significatives entre variétés. La variété Butamu confirme son niveau de résistance à travers les cinq sites tandis que la RAV s'est manifesté comme étant la plus sensible. La variété locale Ngamanza du Plateau des Batékés que nous avions considéré au départ comme étant la plus sensible suite à son comportement affiché à Mvuazi, s'est bien comporté dans les sites expérimentaux ainsi que la Mvuama.

La figure 5 montre que la variété Butamu s'est comportée comme étant résistante dans les différentes zones agro-écologiques choisies de la R.D. Congo, la variété Ngamanza a été très sensible à Luki et à Mvuazi où elle a atteint le maximum d'incidence possible, soit 100%.

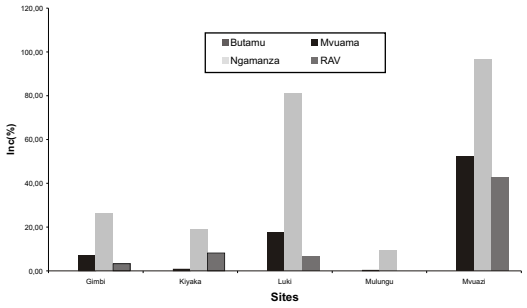


Figure 5: Distribution moyenne de la CMD sur les différentes variétés dans chaque site.

Les variétés RAV et Mvuama que nous considérons comme étant tolérantes selon les conditions de Mvuazi, se comportent encore mieux dans les autres zones du pays, où elles peuvent encore être davantage cultivées à défaut des variétés résistantes.

La figure 6 confirme en premier lieu qu'aucune infection primaire de la CMD due au matériel de plantation n'a fait l'objet dans nos observations. La figure confirme en plus que par rapport à l'âge de la culture (temps), le site de Mvuazi a manifesté l'incidence la plus élevée de la mosaïque (70,81%), suivi de Luki, Gimbi, Kiyaka et Mulungu.

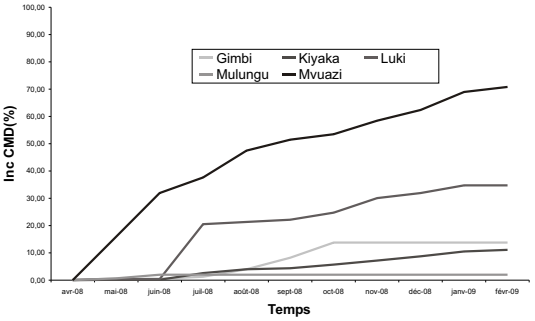


Figure 6: Evolution de l'incidence de la CMD dans les différents sites selon l'âge de la culture.

Les résultats obtenus dans nos investigations n'ont pas montré des relations entre les populations des mouches blanches adultes et l'incidence de la CMD dans les différents sites.

D'une manière générale, les relations entre la propagation de virus et les populations des vecteurs sont complexes. Par exemple, le type de plante, la proportion des vecteurs virulifères et l'activité au vol des vecteurs ont une influence critique sur le taux de développement des épidémies. Selon Ariyo et al., 2005, il n'existe pas de corrélations entre l'infestation de la mouche blanche et l'incidence de la CMD, car la résistance au virus et au vecteur sont déterminées par deux mécanismes génétiques différents (Fargette et al., 1996).

Il a été observé à Mvuazi (figure 7) une très forte proportion des plantes affectées par la CMD et plus des plantes saines à Mulungu.

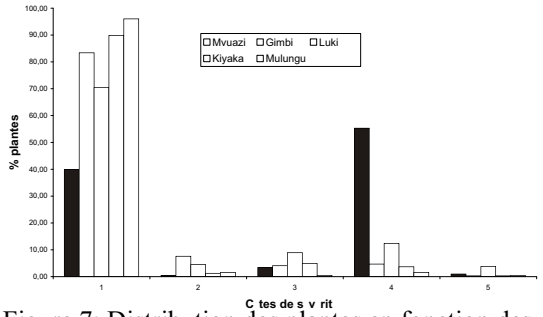


Figure 7: Distribution des plantes en fonction des côtes de sévérité de la CMD dans chaque site.

Il faut noter que le Centre de Recherche de Mulungu a bénéficié ces dernières années de plusieurs actions et projets de développement sous-régionaux sur la lutte contre la pandémie de la mosaique en RD. Congo, visant à améliorer les conditions de vie dans les zones post-conflits. Ce qui explique à suffisance cette réduction sensible de l'incidence de la maladie dans cette zone qui avait été fortement affectée les années passées par la pandémie.

Incidence et sévérité de la striure brune: L'analyse de la variance n'a pas révélé des différences significatives entre variétés. D'une manière générale, l'incidence des tranches de manioc affectées par la CBSD avoisine 10%.

L'analyse de la variance n'a pas révélé des différences significatives entre sites. D'une manière générale, l'incidence des tranches de manioc affectées par la CBSD dans chaque site avoisine 10%. Les côtes de sévérité variant entre 2 et 3.

Les symptômes racinaires semblables à ceux de la CBSD ont été observés dans tous nos sites d'investigations, ce qui confirme les observations faites par Mahungu et al. en 2003 qui avaient observé des symptômes similaires à Kinshasa et au Bas-Congo.

Rendement en racines fraiches: L'analyse de la variance a révélé des différences hautement significatives entre sites. Les rendements les plus élevés ont été obtenus dans les sites de Mvuazi et Kiyaka, suivi de Luki. Le rendement le plus faible a été obtenu à Mulungu.

L'analyse de la variance a démontré que les variétés RAV et Mvuama ont procuré les rendements les plus élevés, suivis de Butamu. Ngamanza a fourni le rendement le plus faible.

En dépit de leur tolérance à la CMD, les variétés RAV et Mvuama présentent un potentiel de production élevée comparativement à la variété résistante, Butamu. Suite à sa sensibilité élevée à la CMD à Mvuazi et à Luki, la variété locale Ngamanza a procuré les rendements les plus faibles (figure 8).

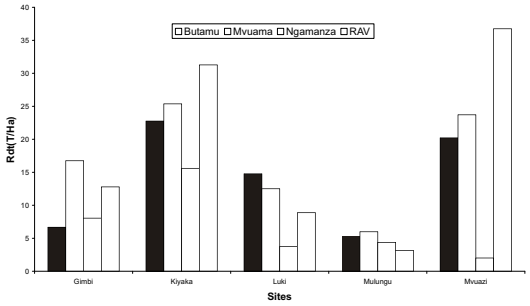


Figure 8: Distribution du rendement des différentes variétés selon les sites.

Essai au laboratoire: A travers les différents sites expérimentaux, nous remarquons une recrudescence du virus EACMV comparativement au virus ACMV qui est reconnu exister dans nos zones depuis longtemps. L'EACMV est reconnu appartenir aux zones de l'Afrique de l'Est (Owor et Legg, 2000). L'EACMV a été signalé pour la première fois au Cameroun en 1998 (Fondong et al., 1998), au Nigéria (Ogbe et al., 1999) et au Ghana (Offei et al., 1999) en 1999 et au Gabon en 2003 (Legg, 2003).

Aucune trace d'EACMV-Ug n'avait été identifié dans nos échantillons et pourtant il a déjà été identifié en RD. Congo dans plusieurs sites, à l'instar de Kisangani en 1998 (Owor et Legg, 2000) et à Kinshasa et au Bas Congo par Neueusch-wander et al., en décembre 2000.

Les données synthétiques sur la distribution des geminivirus exprimées en pourcentage de concentration sont présentées dans les figures 9 et 10.

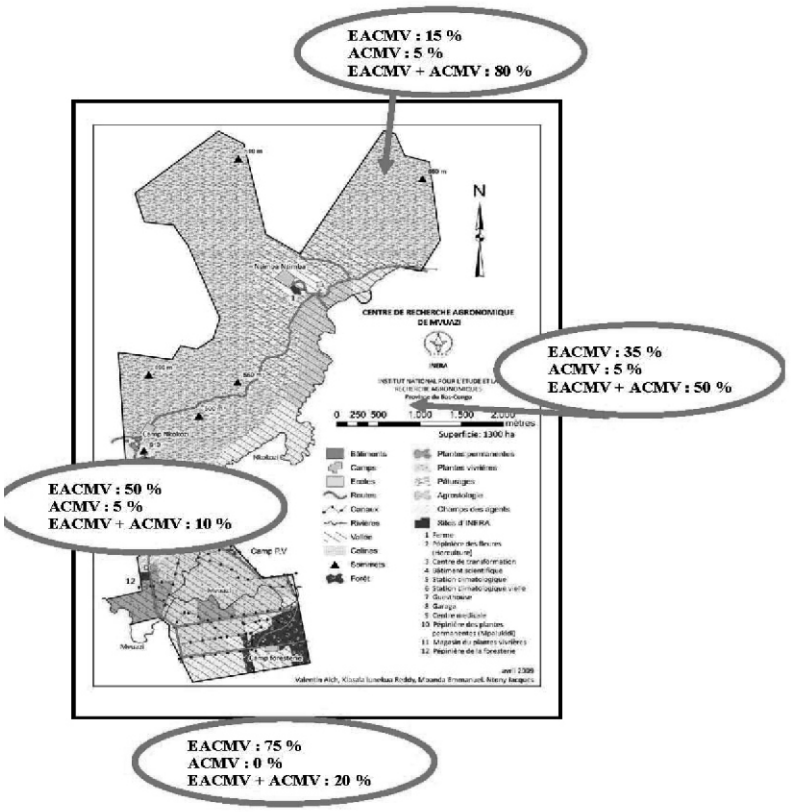


Figure 9 : Distribution de différents geminivirus à Mvuazi

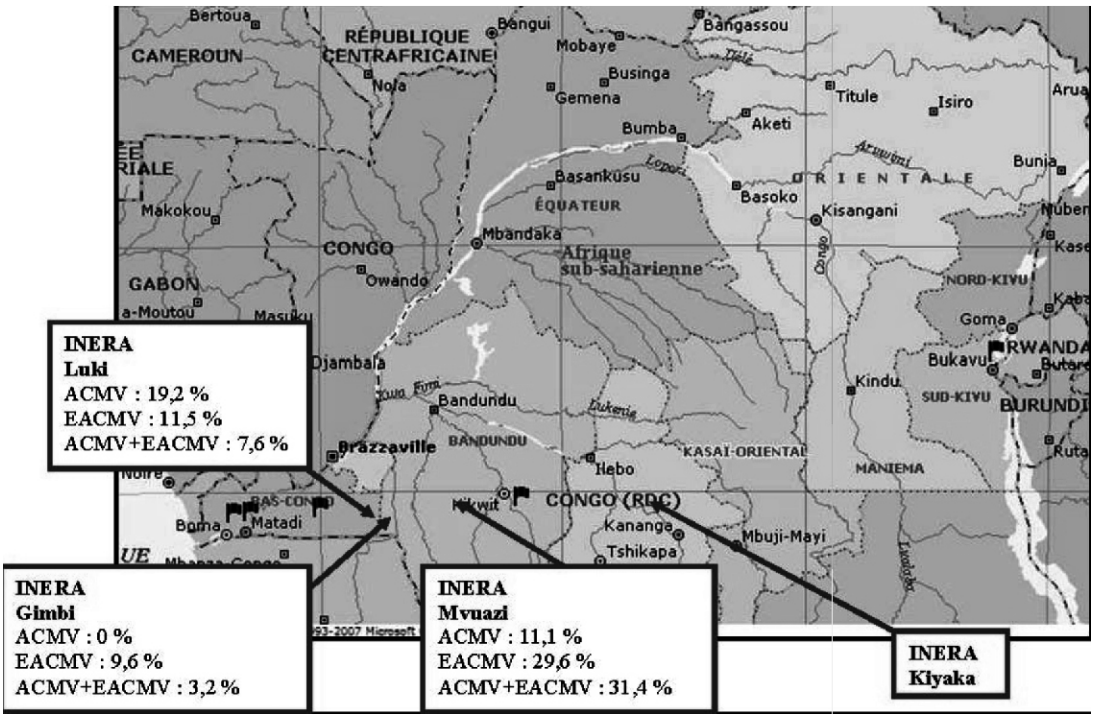


Figure 10 : Distribution des geminivirus en RD. Congo

Le site de Mvuazi a présenté le nombre de cas d'infections mixtes le plus élevé (31,4%), ce qui explique la prévalence de la CMD dans ce site. Les infections mixtes d'ACMV et d'EACMV ont joué un rôle déterminant dans l'évolution de la pandémie déclarée en Ouganda et dans les pays environnants (Harrison et al., 1997 ; Legg, 1999 ; Pita et al., 2001a).

Une enquête de diagnostic conduite en 2002-2003 pour déterminer le statut des begomovirus du manioc au Nigeria, avait démontré que les symptômes de la CMD étaient plus sévères chez les plantes doublement infectées d'ACMV et d'EACMV par rapport aux plantes avec infection simple (Ogbe et al., 2006).

Conclusion

Ce travail s'était assigné comme objectifs , de ressortir les facteurs à la base des variations saisonnières observées d'avril 2008 à avril 2009 sur les populations des mouches blanches, de ressortir les différences variétales en termes de sensibilité à la mosaïque et à la striure brune, de déterminer la distribution spatiale des geminivirus associés à la propagation de la mosaïque ainsi que de déterminer leurs proportions relatives dans différentes zones agro écologiques de la RD. Congo.

C'est ainsi qu'au terme de ce travail, il se dégage:

- Qu'il existe une certaine préférence variétale génétique des mouches blanches adultes ; les variétés sensibles et/ou tolérantes Mvuama et Ngamanza ont hébergé le plus grand nombre des mouches blanches adultes tandis que la variété résistante Butamu a hébergé moins de mouches blanches adultes.

- Les populations les plus élevées de mouches blanches adultes ont été observées à Mvuazi entre avril et juin 2008, avec le pic en mai 2008, tandis qu'à Mulungu, les populations les plus élevées ont été enregistrées entre juin et août 2008, avec le pic en juillet 2008. Le site de Mvuazi s'est particulièrement distingué en rapport au nombre de larves avec des populations très élevées entre mai et juillet 2008, avec le pic en mai 2008.

- Ce site s'est aussi distingué en rapport à l'incidence et à la sévérité de la mosaïque à cause de la prévalence des cas d'infections doubles (mixtes) des geminivirus ACMV et EACMV. La souche ougandaise (EACMV-Ug) n'a pas été identifiée dans nos échantillons et pourtant elle avait été identifiée en 1998 à Kisangani (Owor et Legg, 2000) et en décembre 2000 à Kinshasa et au Bas Congo (Neueuschwander et al., 2000).

- Aucune corrélation n'a été trouvée entre le niveau de populations des mouches blanches dans les différents sites avec la propagation de la mosaïque. D'une manière générale, les relations entre la propagation des virus et la population du vecteur sont très complexes. Le type de plante, la proportion des vecteurs virulifères, l'activité au vol des vecteurs ont une influence très critique sur le développement des épidémies (Fargette et al., 1985).

- En ce qui concerne les rendements obtenus en racines tubéreuses fraiches, les variétés tolérantes Mvuama et RAV se sont mieux comportés dans l'ensemble des sites comparativement à la variété résistante Butamu. Ce qui revient à dire que les variétés tolérantes peuvent néanmoins être cultivées (avec phytosanitation) à défaut des variétés résistantes dans les sites à faible pression de la mosaïque tandis que les variétés résistantes, compte tenu de leur disponibilité assez limitée, seraient mieux indiquées pour les sites à très forte pression de la mosaïque (Legg, 2009) , le cas des sites de Mvuazi et de Luki, pour ce qui concerne nos investigations.

- La striure brune a été retrouvée dans tous nos sites d'expérimentation et sur toutes les variétés mais à un niveau faible d'incidence et de sévérité, soit 10% d'incidence des racines fraiches affectées. Ce qui confirme la présence de la maladie dans les différentes zones agro écologiques du pays.

D'une manière générale, les facteurs à la base de la pullulation des mouches blanches dépendent des caractéristiques génétiques des variétés en culture, des caractéristiques génétiques des populations des mouches blanches, et des caractéristiques environnementales et épidémiologiques en rapport à chaque site.

En guise de recommandations, nous suggérons :

- Que des enquêtes de diagnostic plus étendues à l'échelle nationale sur le statut des geminivirus soient réalisées à travers le pays. L'apparition des variantes d'EACMV, d'EACMV-Ug associées à la détection d'ACMV et les informations sur leur distribution en RD. Congo pourraient être utilisé pour la sélection des variétés de manioc dans les programmes de sélection pour la résistance à la mosaïque du manioc. Ces informations serviraient aussi à mieux orienter la diffusion des variétés résistantes et tolérantes à travers le pays.

- Que l'identification génétique des mouches blanches soit réalisée pour compléter les informations disponibles sur les facteurs à la base de la pullulation des mouches blanches à travers le pays.

Remerciements

Les auteurs remercient l'Institut International de l'Agriculture Tropical (IITA) et l'Institut National pour l'Etude et la Recherche Agronomiques dont l'appui technique et matériel a rendu cette étude possible.

Cette étude a été exécutée grâce au Projet REAFOR (Relance de la Recherche Agricole et Forestière en RD. Congo), financé par l'Union Européenne et mis en œuvre par la FAO.

Références bibliographiques

Ariyo, O.A., A.G.O, Dixon, G.I., Atiri (2005) : Wthitefly *Bemisia tabaci* (Homoptera : Aleyrodidae) Infestation on Cassava Genotypes Grown at Different Ecozones in Nigeria, Plant resistance, Journal of Economy Entomology. 98(2) : 611-617.

Colvin, J. , G.W., Otim Nape, J., Holt (1999) : Factors driving the current epidemic of severe cassava mosaic disease in East Africa. Proceedings of the VIIth International Plant Virus Epidemiology Symposium, Aguadulce (Almeria), Spain, April 11-16, 1999, Abstract 76-77.

Dubern, J., (1994): Transmission of African cassava mosaic geminivirus by the whitefly (*Bemisia tabaci*). Tropical Science 34, 82-91.

FAO, Food and Agriculture Organization of the United Nations, 2006. Cassava production statistics. http: // www.fao.org/ es/ess/top/country.htm

Fargette, D. (1985) : Epidémiologie de la Mosaïque Africaine du Manioc en Côte d'Ivoire. University thesis, USTL de Montpellier. pp 203.

Fargette,D., L.T., Colon, R., Bouveau, and C., Fauquet, (1996): Components of resistance of 3cassava to African cassava mosaic virus. Eur. J. Plant Pathol. 102: 645-654.

Fargette, D., C.,Fauquet, and J-C., Thouvenel, (1985) : Field studies on the spread of African cassava mosaic. *Annals of Applied Biology* 106: 285-294.

Fargette, D., C., Fauquet, et J-C.,Thouvenel, (1986) : African cassava mosaic virus : The virus, the vector, the plant and the reservoirs. In Proceedings of the Third International Workshop on Epidemiology of Plant Virus Disease, 1986. Orlando, Florida, USA : University of Florida.

Fauquet, C., J., Dejardin, F.,Leylavergne, L., Colon, et J-C., Thouvenel, (1986) : Multicomponent resistance of cassava to African cassava mosaic virus. Dans Proceedings of the Third International Workshop on Epidemiology of Plant Virus Diseases,1986. Orlando, Florida, USA : University of Florida.

Fauquet C., D., Fargette and J-C., Thouvenel (1987) : The resistance of cassava to African cassava mosaic. In Proceedings of the International Seminar on African Cassava Mosaic Disease and its Control, 4-8 May 1987, pp. 183-188. Yamoussoukro, Côte d'Ivoire : CTA/FAO/ ORSTOM/IITA/IAPC.

Fauquet, C.,and D.,Fargette, (1990): African cassava mosaic virus: etiology, epidemiology and control. Plant disease 74, 404-411.

Fauquet, C.M, and J., Stanley, (2003): Geminiviruses classification and nomenclature: progress and problems. Ann. Appl. Biol. 142, 165-189.

Fondong, V.N., J.S., Pita, , C., Rey, R.N., Beachy, , C.M., Fauquet, (1998) : First report of the presence of East African cassava mosaic virus in Cameroon, Plant Diseases.

Fondong,V.N., J.S., Pita, , M.E.C., Rey, A., Kochko, R.N.,Beachy, C.M.,Fauquet,

(2000): Evidence of synergism between African cassava mosaic virus and a new double-recombinant geminivirus infecting cassava in Cameroon. J. Gen. Virol. 81, 287-297.

Hahn, S.K., A.K., Howland, E.R., Terry (1980) : Correlated resistance of cassava to mosaic and bacterial blight diseases. Euphytica 29 : 305-311.

Hahn, S.K., and J., Keyser, (1985) : Cassava, a basic food of Africa. Outlook Agric.14 : 95-99.

Harrison, B.D., X., Zhou, G.W., Otim-Nape, Y., Liu, D.J., Robinson (1997): Role of a novel type of double infection in the geminivirus-induced epidemic of severe cassava mosaic in Uganda. Annals of Applied Biology 131, 437-48.

Legg, J.P., (1999) : Emergence, spread and strategies for controlling the pandemic of cassava mosaic virus disease in East and Central Africa. Crop Protection 18, 627-637.

Legg, J.,(2009): "Communication personnelle".

Legg, J.P., P., Assimwe, P., Sseruwagi, and J.K.,Brown, (2006): Changes in the Biology of *Bemisia tabaci* on cassava in Africa and their impact on Virus Disease Pandemics. In Proceedings of 4th International Bemisia Workshop, Dec 3-6,2006, Florida, USA.

Legg J.P., R., French, D., Rogan, K.,Okao-Okuja et Browns, (2002) : A distinct *Bemisia tabaci* (Gennadius) (Hemiptera : Stenorrhyncha : Aleyrodidae) genotype cluster is associated with the epidemic of severe cassava mosaic virus disease in Uganda, Molecular Ecology (2002) 11, 1219-1229.

Legg,J.P. and S., Ogwal, (1998) : Changes in the incidence of African cassava mosaic virus disease and the abondance of its whitefly vector along south-north transects in Uganda. J. App. Entomol 122 : 169-178.

Mahungu N.M, M., Bidiaka , H., Tata, S., Lukombo, and S., N'luta, (2003) : Cassava brown streak disease-like symptoms in Democratic Republic of Congo, ROOTS ; Newsletter of the Southern Africa Root Crops Research Network (SARRNET) and the East Africa Root Crops Research Network (EARRNET).

Maruthi,M.N., R.J.,Hillocks, K., Mtunda, M.D., Raya, H., Muhanna, H., Kiozia, A.R., Rekha, J.,Colvin (2005) : Transmission of Cassava brown streak virus by Bemisia tabaci (Gennadius), Natural Resources Institute, University of Greenwich, Kent, UK

Muimba-Kankolongo,A., K., Phuti (1987): Relationship of cassava mosaic severity in planting material to mosaic development, growth and yield of cassava in Zaire. Exp. Agric. 23: 221-225.

Neuenschwander,P., J A., Hughes, F., Ogbe, J.M., Ngatse, J.P.,Legg (2002): Occurrence of the Uganda variant of East African cassava mosaic virus (EACMV-Ug) in western Democratic Republic of Congo and the Congo Republic defines the westernmost extent of the CMD pandemic in East Central Africa. Plant Pathol 51: 385.

Nichols,R.F.J.,(1950): The brown streak disease of cassava: distribution climatc effects and diagnostic symptoms. East African Agricultural Journal 15, 154-160.

Offei,S.K., K.M.,Owuna, G.,Thottappilly (1999) : First report of East African cassava mosaic begomovirus in Ghana, Plant Diseases.

Ogbe,F.O., A.G.O.,Dixon, J.A., Hughes O.J., Alabi Okechukwu R.,(2006) : Status of cassava begomovirus and their new natural hosts in Nigeria, Plant Disease, 2006.90(5). 548-553.

Ogbe, F.O., Thottappilly, G., Dixon, A.G.O., G.I., Atiri, H.D.,Mignouna, 2003: Variants of East African cassava mosaic virus and its distribution in double infections with African cassava mosaic virus in Nigeria. Plant Dis. 87, 223-229.

Omongo, C.A.(2003) : Cassava whitefly, *Bemisia tabaci* , behaviour and ecology in relation to the spread of the cassava mosaic pandemic in Uganda. Ph.D. Thesis, University of Greenwich, UK, 227 pp.

Otim-Nape,G.W., T., Alicai, J.M.,Thresh, (2001): Changes in the incidence and severity of cassava mosaic virus disease, varietal diversity and cassava production in Uganda. Annals of Applied Biology 138, 313-27.

Otim Nape, G.W., J.M., Thresh, D.,Fargette (1996) : *Bemisia tabaci* and cassava mosaic virus disease in Africa. In *Bemisia* 1995 ; Taxonomy, Biology, Damage, Control and Management, pp.

319-350. Eds D Gerling and R T Meyer. UK : Intercept Ltd.

Owor B. and J.P. , Legg (2000). Cassava mosaic virus training notes, International Institute of Tropical Agriculture, Eastern and Southern Africa Regional Centre, Namulonge, Uganda.

Sserubombwe,W.S., J.M., Thresh, G.W., Otim-Nape(2000) : Progress of cassava mosaic virus disease and whitefly vector populations in single and mixed stands of four cassava varieties grown under epidemic conditions in Uganda, Ann.appl.Biol.

Zar H.J., 1974 : Biostatistical Analysis, Prentice Hall Biological Sciences Series, Prentice-Hall, Inc., Englewood Cliffs.

Zhou, X., Y., Liu, L., Calvert, C., Munoz, G.W., Otim-Nape, D.J., Robinson, B.D., Harrison, (1997) : Evidence that DNA-A of a geminivirus associated with severe cassava mosaic disease in Uganda has arisen by interspecific recombination. J Gen Virology 78 : 2101-2111.

Preliminary results of screening IITA improved germplasm for resistance to Cassava Brown Streak Disease (CBSD) in Uganda

Ntawuruhunga P[1,3], Kiryowa M.[1], Okechukwu R.[2], Otim-Okello F.[1], and Kanju E.[4]

[1] International Institute of Tropical Agriculture (IITA), IITA-Uganda,

[2] International Institute of Tropical Agriculture (IITA), IITA-Ibadan, Nigeria,

[3] Eastern Africa Roots Crops Research Network (EARRNET), c/o IITA-Uganda

[4] International Institute of Tropical Agriculture (IITA), IITA-Tanzania

Abstract

For quite some time, cassava breeding activities of the International Institute of Tropical Agriculture (IITA) in Uganda were mainly focused on developing genotypes resistant to cassava mosaic disease (CMD), which was the major biotic production constraint since the early 1990s. Although cassava brown streak disease (CBSD) was reported some time back in 1940s, it had been dormant in Uganda. However, in early 2004, cases of CBSD were reported and re-confirmed both on-station (Namulonge) and in farmers' fields around the country. The symptoms of CBSD were observed on cassava in some locations in central Uganda this time in relatively higher incidence, albeit localized, on some of the popular CMD-resistant varieties being grown country wide by farmers. This has caused serious concern in the country since cassava production has just been restored through use of CMD-resistant varieties following devastation by the unusually severe CMD epidemic in the 1990s. This, therefore, raised a need to re-screen all the improved germplasm under conservation for resistance to the new disease. Re-screening began in 2005 with a total of 963 improved genotypes initially conserved in situ at Serere research station. The trial was established in two sets (one with 528 and the second with 435 genotypes) at Namulonge, Central Uganda, a CBSD hotspot, using a check-plot design with single row plots of 1 x 10 M. Cultivar TME 204 was used as a spreader and check. Biotic data was taken at 2, 4, 6, 7, 8 and 9 MAP for CMD, CBSD, cassava bacterial blight (CBB), cassava anthracnose disease (CAD) and cassava green mites (CGM). The first set was

evaluated at 15 months after planting while the second set was evaluated at 12 MAP. At harvest data was collected on number of tubers, CBSD storage roots score and yield. The preliminary results indicated that five hundred and sixty six (566) genotypes were resistant to CMD; four hundred and ten (410) genotypes did not show any foliar and storage roots CBSD symptoms though they could have been susceptible to CMD. Six hundred and twenty four (624) genotypes showed no foliar CBSD but may have showed root symptoms, while six hundred and five (605) showed no CBSD storage root symptoms. Two hundred and sixty (260) showed resistance to both CBSD (foliar and storage roots) as well as CMD. The study showed that CBSD severity increases over time and negatively affects fresh storage roots yield. These preliminary results showed hope for possible sources of CBSD resistance.

Keywords: Cassava germplasm, screening, resistance, CMD and CBSD

Introduction

Following its initial introduction by the Arab traders, Cassava (*Manihot Esculentum* Cranz) quickly spread to other areas of Uganda and today it is one of the most important staple crops in Uganda; ranked second to bananas in terms of area occupied, total production and per capita consumption (Otim-Nape, 1990). It is grown by over 75% of all households in the country. Unfortunately, severe epidemics of cassava mosaic disease (CMD) have traversed the country since 1988 from north to south and caused devastating losses and food shortages. A more severe form of African cassava mosaic virus eliminated cassava in many parts of the country (Otim-Nape, 1990). The National cassava program together with the International Institute of Tropical Agriculture (IITA-ESARC) embarked on vigorous breeding and selection for mosaic-resistant varieties which were then multiplied and distributed to farmers and introduced in the East Africa region.

As success was being registered in combating CMD in Uganda, Cassava Brown Streak Disease (CBSD), a new threat to cassava production, was reported in 2002 and re-confirmed in 2004. The disease, caused by a whitefly-transmitted virus, *Cassava Brown Streak Virus* (*CBSV*) was first reported in Uganda in 1945 (Nichols, 1950) at Bukalasa experimental station, central Uganda. It was assumed to have been introduced in 1934 in cassava stems from Amani, Tanzania. An eradication campaign was carried out between 1945 and 1950 and since then there has been no report of this disease (Emechebe, 1976) until recently when its re-emergence has been confirmed through field surveys and laboratory protocols such as reverse transcriptase-polymerase chain reaction (RT-PCR) and nucleotide sequence analysis (Alicai *et al.*, 2006).

It was believed that the distribution of CBSD is delimited by altitude and is rarely found above 1000 m absl, although symptoms can be expressed at altitudes above this through infected cuttings (Nichols, 1950). The reappearance of CBSD in Uganda, which on average is above 1000 masl, raises great concern for food production and monetary income for the country and the entire region (Alicai *et al.,* 2006, Ntawuruhunga and Legg, 2007). There are several possibilities for the origin of the current outbreak in Uganda. Alicai *et al.* (2006) suggested two possibilities namely; firstly that the CBSD might have been present for many years at a low and unnoticed level, since it was once accidentally introduced in Uganda in the 1930s, although all infected plants in the affected sites were destroyed; secondly, that the new outbreak may be due to the emergence of a new and more aggressive strain of CBSV. The high whitefly numbers observed was believed to aid the spread of the disease.

The disease, reported to be endemic in all East African coastal cassava-growing regions (Hillocks and Jennings, 2003), is known to cause serious losses. Above 40% yield losses were reported in Malawi (Gondwe, *et al*., 2002); while in Tanzania, Tanga region, yield losses up to 64% were reported in susceptible cultivars leading to farmers to abandon cassava for other crops (Mtunda *et al*., 2002).

Following the 1990s devastation of cassava production in Uganda due to CMD, the re-emergence of CBSD in the country will require quick and rigorous measures to combat the disease. In response, IITA-Uganda in collaboration with the cassava national program initiated the breeding and screening activities for CBSD. One of them was the re-screening of all the IITA conserved germplasm with the aim of identifying sources of CBSD resistance.

Materials and Methods

The conserved improved germplasm at Serere was transferred and planted at Namulonge, the hotspot for CBSD, in Central District of Uganda at

National Crop Resources Research Institute (NaCRRI) in 2005 and 2006. These genotypes had earlier-on been evaluated for CMD resistance more than eight years. The trial was established, in two sets. Set 1 was established in November 2006 with 528 genotypes while set 2 was established in April 2006 with 435 genotypes making a total of 963 genotypes. The genotypes were planted in single row plots of 1 x 10 M non-replicated using a check-plot design with TME 204 as the check/spreader planted after every 10 genotypes. Weeding was carried out using hand hoe and no fertilisers were applied. Data was collected on emergence (Emerg), biotic stresses (CMD, CBSD, CBB and CGM) at 2, 4, 6, 8 and 10 months after planting (MAP). At harvest data was collected on the following aspect; plants harvested number of marketable and non-marketable storage roots (notuber), weight of marketable (wtmkt) and non-marketable (wtnmkt) storage roots, number of storage roots with CBSD symptoms (Nopcbsdr) and CBSD severity on storage roots (CBSDRS). The biotic stresses scores were converted as index, which was a cross product of their mean severity over the assessment periods by the disease incidence. For CBSD, the final index was a mean of the disease index on the leaves and disease index on the roots. Fresh yield (Fyld), in tones/hectare, was derived from sum of marketable and non-marketable weight in 1 x 10 M extrapolated to 1 ha.

Biotic stresses were evaluated using IITA (1990) scale of 15. Genotypes were categorized into five classes namely; '*resistant*', '*moderately resistant', 'moderately susceptible', 'susceptible'* and '*highly susceptible'*. Below is a classification of disease resistance based on disease incidence, severity and index.

Disease incidence (Count of infected plants)

0%:	Resistant
>0-10%:	Moderately resistant
>10-25%:	Moderately susceptible
>25-50%:	Susceptible
>50%:	Highly susceptible

Disease severity (Extent of damage)

1.0-1.3:	Resistant
>1.3-2.0:	Moderately resistant
>2.0-2.3:	Moderately susceptible
>2.3-3.0:	Susceptible
>3.0:	Highly susceptible

Disease index (Cross product of incidence and severity)

0:	Resistant
>0-20:	Moderately resistant
>20-57.5:	Moderately susceptible
>57.5-150:	Susceptible
>150:	Highly susceptible

CBSD was evaluated both on leaves and on the storage roots using a scale of 15 as described above. Scores of leaf and storage roots symptoms were averaged to derive CBSD disease index (CBSDMT), a variable used to reflect damage on the whole plant due to CBSD.

Results

The results indicated that the CBSD incidence and severity for both sets increased over the six assessment growth periods (2, 4, 6,7,8,9 MAP) (Figure 1 and Figure 2). The severity of the highly susceptible genotypes continued to increase in a sigmoid manner over maturity period implying that more symptoms developed as plants matured.

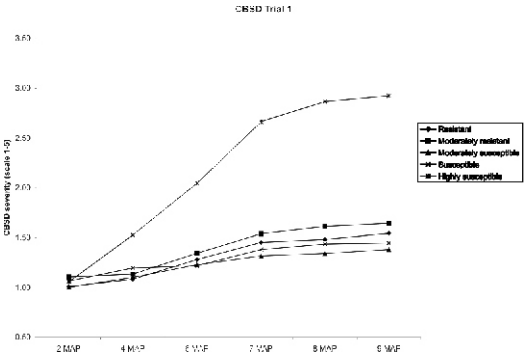


Figure 1. CBSD disease severity over 6 assessment growth periods for 5 categories of genotypes (Trial 1).

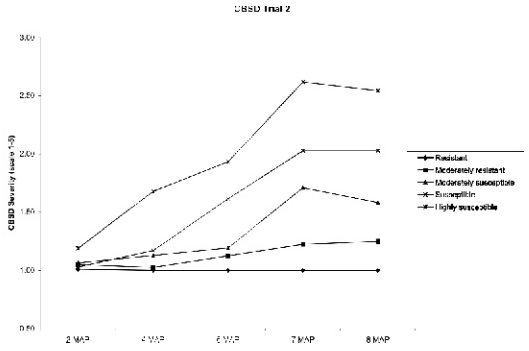


Figure 2. CBSD disease severity over 5 assessment growth periods for 5 categories of genotypes (Trial 2)

Cassava Mosaic Disease (CMD) ratings and grouping of set 1 and 2 genotypes is shown in Table 1. Five hundred and sixty six genotypes (58.8%) were 'resistant' to CMD, 110 (11.4%) were 'moderately resistant', while 170 (17.6%) were either susceptible or highly susceptible to CMD. The 17.6% was considered to have completely succumbed to CMD, since earlier evaluations had qualified all the 963 genotypes as either resistant or moderately resistant.

Table 1: CMD ratings of 963 genotypes

CMD Disease index[a] rating	Group	No. genotypes in set 1	No. genotypes in set 2	Total	Percentage (%)
0	Resistant	322	244	566	58.8
> 0-20	Moderately resistant	76	34	110	11.4
> 20-57.5	Moderately susceptible	72	45	117	12.1
> 57.5-150	Susceptible	44	46	90	9.3
> 150	Highly susceptible	14	66	80	8.3

[a] *Disease index = incidence x severity;*

CBSD index ratings and grouping of genotypes are presented in Table 2. The genotypes were grouped using the CBSD index for the whole plant (CBSDMT). Four hundred and ten (410) genotypes were resistant to both storage root and leaf symptoms, 172 (17.9%) were moderately resistant while 186 (19.3%) were moderately susceptible. One hundred and ninety five (195) genotypes or 20.3% were either susceptible or highly susceptible to CBSD.

Two hundred and thirty six (236) genotypes showed leaf symptoms but were free from the storage roots symptoms and of these 175 were resistant to CMD, 118 genotypes had storage root symptoms but lacked leaf symptoms, while 126 genotypes had storage roots as well as leaf symptoms for CBSD. Two hundred and sixty (260) genotypes from both sets were found to be resistant to both CMD and CBSD storage roots and leaf infections. These materials are of particular interest and further evaluations will be carried out on them. The top 100 of these genotypes together with the check are presented in Table 3.

Table 2: CBSD ratings of 963 genotypes in both set 1 and set 2

CBSD Disease index[a] rating	Group	No. genotypes (set 1 and 2)	Percenta ge (%)
0	Resistant	410	42.6
> 0 -20	Moderately resistant	172	17.9
> 20-57.5	Moderately susceptible	186	19.3
> 57.5-150	Susceptible	118	12.3
> 150	Highly susceptible	77	8.0

[a] *Disease index = average of CBSD index on leaves and CBSD index on roots;*

Table 3: Disease indices and yield performance of the top 100 genotypes from both sets of materials

Clone	[a]MCMDT	[b]MCBBT	[c]MCGMT	[d]MCBSDT	[e]CBSDRT	[f]CBSDMT	Tubers/plt	**F**Yld/plt
MM02/1806	0.0	0.0	10.0	0.0	0.0	0.0	8.2	10.4
MM02/0258	0.0	26.7	0.0	0.0	0.0	0.0	12.8	10.0
MM98/0637	0.0	88.9	11.1	0.0	0.0	0.0	11.5	11.8
MM96/7128	0.0	103.7	0.0	0.0	0.0	0.0	7.8	4.5
MM02/0123	0.0	0.0	45.0	0.0	0.0	0.0	7.8	9.5
MM98/4252	0.0	111.1	0.0	0.0	0.0	0.0	12.7	12.0
MM02/0782	0.0	106.7	0.0	0.0	0.0	0.0	7.0	7.2
MM96/2946	0.0	0.0	0.0	0.0	0.0	0.0	4.5	8.8
MH97/1200	0.0	140.0	0.0	0.0	0.0	0.0	5.4	3.8
MM02/0807	0.0	26.7	45.0	0.0	0.0	0.0	7.0	6.8
MH98/0982	0.0	116.7	0.0	0.0	0.0	0.0	8.7	4.7
MH97/1630	0.0	13.3	0.0	0.0	0.0	0.0	4.8	6.6
MM02/0175	0.0	50.0	0.0	0.0	0.0	0.0	11.7	10.7
MM01/0622	0.0	53.3	0.0	0.0	0.0	0.0	11.0	10.3
MM96/3920	0.0	133.3	0.0	0.0	0.0	0.0	6.9	4.4
MM01/3004	0.0	166.7	0.0	0.0	0.0	0.0	9.0	5.6
MM01/1029	0.0	26.7	20.0	0.0	0.0	0.0	11.2	5.6
97/4045	0.0	116.7	0.0	0.0	0.0	0.0	9.3	6.9
MM01/0786	0.0	133.3	0.0	0.0	0.0	0.0	5.3	6.8
MM02/0106	0.0	26.7	30.0	0.0	0.0	0.0	7.8	6.5
MM96/7580	0.0	100.0	0.0	0.0	0.0	0.0	9.2	5.1
MM01/0802	0.0	88.9	0.0	0.0	0.0	0.0	9.8	5.0
MH96/1262	0.0	60.0	45.0	0.0	0.0	0.0	5.0	8.3
MM97/0681A	0.0	133.3	0.0	0.0	0.0	0.0	11.0	6.0
MH97/1226	0.0	133.3	0.0	0.0	0.0	0.0	7.3	3.3
MM96/3249	0.0	33.3	0.0	0.0	0.0	0.0	11.2	4.6
MM96/7394	0.0	66.7	0.0	0.0	0.0	0.0	5.0	5.8
MM98/2312	0.0	133.3	0.0	0.0	0.0	0.0	11.6	4.6
MM98/2316	0.0	44.4	0.0	0.0	0.0	0.0	9.7	3.8
MM02/0596	0.0	26.7	0.0	0.0	0.0	0.0	9.3	7.7
MM02/1594	0.0	66.7	0.0	0.0	0.0	0.0	4.6	4.6
MH97/1509	0.0	103.7	0.0	0.0	0.0	0.0	6.5	2.8
MM01/1491	0.0	33.3	0.0	0.0	0.0	0.0	9.0	7.2
MM96/7745	0.0	133.3	0.0	0.0	0.0	0.0	9.8	4.3
MM01/0414	0.0	133.3	0.0	0.0	0.0	0.0	4.1	2.3
MM96/3762	0.0	116.7	0.0	0.0	0.0	0.0	9.5	5.3
MH96/0871	0.0	0.0	0.0	0.0	0.0	0.0	7.0	5.3
MM02/2022	0.0	0.0	0.0	0.0	0.0	0.0	7.4	4.2
81/01610	0.0	25.0	0.0	0.0	0.0	0.0	7.7	3.3
MM96/8364	0.0	57.1	0.0	0.0	0.0	0.0	8.3	5.0
MM02/2209	0.0	0.0	80.0	0.0	0.0	0.0	6.5	5.0
MM97/2143	0.0	26.7	0.0	0.0	0.0	0.0	7.3	6.7
MM96/3075B	0.0	83.3	0.0	0.0	0.0	0.0	5.0	4.9
MH971229	0.0	133.3	0.0	0.0	0.0	0.0	3.5	3.2
MM01/1282	0.0	118.5	0.0	0.0	0.0	0.0	6.0	3.2
MM02/2212	0.0	0.0	0.0	0.0	0.0	0.0	6.5	4.8
I81/01635	0.0	91.7	0.0	0.0	0.0	0.0	9.0	3.6
MM02/0754	0.0	40.0	0.0	0.0	0.0	0.0	4.8	4.5
MM02/2101	0.0	0.0	0.0	0.0	0.0	0.0	6.7	6.0
MM96/5267	0.0	0.0	75.0	0.0	0.0	0.0	7.3	6.0
MM98/3944A	0.0	133.3	0.0	0.0	0.0	0.0	6.2	2.9
MM96/5940	0.0	133.3	0.0	0.0	0.0	0.0	4.0	2.8
MM02/0138	0.0	0.0	45.0	0.0	0.0	0.0	5.4	3.4
MM96/2606	0.0	22.2	16.7	0.0	0.0	0.0	8.0	8.5
MM01/1661	0.0	0.0	0.0	0.0	0.0	0.0	7.0	5.3
MM02/0499	0.0	0.0	45.0	0.0	0.0	0.0	3.4	3.2
MM02/1495	0.0	0.0	0.0	0.0	0.0	0.0	7.5	8.0

Clone	[a]MCMDT	[b]MCBBT	[c]MCGMT	[d]MCBSDT	[e]CBSDRT	[f]CBSDMT	Tubers/plt	FYld/plt
MM02/1855	0.0	0.0	0.0	0.0	0.0	0.0	5.8	3.2
MM96/4653	0.0	33.3	0.0	0.0	0.0	0.0	3.7	5.3
MM98/0155	0.0	0.0	0.0	0.0	0.0	0.0	9.0	5.3
MM98/5136	0.0	106.7	0.0	0.0	0.0	0.0	7.3	5.2
MM02/0075	0.0	26.7	0.0	0.0	0.0	0.0	4.3	3.8
MM02/1616	0.0	0.0	70.0	0.0	0.0	0.0	4.5	3.8
MM96/6753	0.0	0.0	0.0	0.0	0.0	0.0	7.5	7.5
MM96/5057	0.0	133.3	0.0	0.0	0.0	0.0	5.8	2.8
MM96/8447	0.0	103.7	0.0	0.0	0.0	0.0	3.3	2.3
MM98/1632	0.0	88.9	0.0	0.0	0.0	0.0	5.2	2.3
MM02/0865	0.0	53.3	0.0	0.0	0.0	0.0	10.0	7.0
MM02/1257	0.0	26.7	0.0	0.0	0.0	0.0	4.3	4.7
MM02/1615	0.0	0.0	60.0	0.0	0.0	0.0	5.7	4.7
MM01/0563	0.0	116.7	0.0	0.0	0.0	0.0	5.5	1.6
MM98/6004	0.0	46.7	0.0	0.0	0.0	0.0	8.2	2.6
92/0067	0.0	0.0	0.0	0.0	0.0	0.0	3.3	3.3
MH97/3069	0.0	33.3	50.0	0.0	0.0	0.0	12.0	13.0
MM02/1036	0.0	66.7	0.0	0.0	0.0	0.0	5.0	3.3
MM02/1643	0.0	26.7	10.0	0.0	0.0	0.0	6.8	3.3
MM96/4800	0.0	0.0	30.0	0.0	0.0	0.0	7.0	6.5
I95/0097	0.0	104.8	0.0	0.0	0.0	0.0	5.0	2.1
MH98/0105	0.0	44.4	0.0	0.0	0.0	0.0	6.0	6.3
MM96/5775	0.0	59.3	0.0	0.0	0.0	0.0	6.0	2.5
MH96/0105	0.0	0.0	37.5	0.0	0.0	0.0	2.5	6.0
MH97/1127	0.0	0.0	15.0	0.0	0.0	0.0	7.0	12.0
MM01/0904	0.0	0.0	12.5	0.0	0.0	0.0	5.3	4.0
MM02/2269	0.0	40.0	40.0	0.0	0.0	0.0	4.3	3.0
MM96/2615	0.0	26.7	45.0	0.0	0.0	0.0	4.5	6.0
MM96/6173	0.0	0.0	0.0	0.0	0.0	0.0	3.5	3.0
MM96/7940	0.0	22.2	0.0	0.0	0.0	0.0	1.8	3.0
MM97/0422	0.0	0.0	0.0	0.0	0.0	0.0	3.8	3.0
97/2412	0.0	26.7	0.0	0.0	0.0	0.0	3.4	2.3
MM96/5011	0.0	123.8	0.0	0.0	0.0	0.0	5.6	2.3
I96/0529	0.0	133.3	0.0	0.0	0.0	0.0	4.5	1.8
MM01/0715	0.0	66.7	0.0	0.0	0.0	0.0	5.3	3.7
MM98/3947	0.0	66.7	0.0	0.0	0.0	0.0	16.0	11.0
MH95/0420	0.0	44.4	75.0	0.0	0.0	0.0	5.5	5.5
MM02/0098B	0.0	0.0	0.0	0.0	0.0	0.0	3.3	2.8
MM02/1187	0.0	26.7	0.0	0.0	0.0	0.0	3.0	3.7
MM02/1593	0.0	26.7	20.0	0.0	0.0	0.0	3.3	2.8
MM02/1728	0.0	0.0	30.0	0.0	0.0	0.0	1.5	2.8
MM96/0106	0.0	26.7	0.0	0.0	0.0	0.0	10.0	5.5
MM96/1729	0.0	0.0	0.0	0.0	0.0	0.0	5.4	2.2
TME 204	4.9	61.8	1.4	87.0	397.7	237.8	3.4	2.7
Mean	0.1	54.2	9.6	0.9	3.9	2.4	28.3	20.0
Se	0.1	5.0	2.0	0.9	3.9	2.4	1.5	0.9

[a] MCMDT = average of the cross product of cassava mosaic disease severity scores and incidences over the five assessment periods; 2, 4, 6,8 & 10 MAP

[b] MCBBT = average of the cross product of cassava bacterial blight severity scores by incidences over the five assessment periods

[c] MCGMT = average of the cross product of cassava green mite severity scores by incidences over the five assessment periods

[d] MCBSDT = average of the cross product of cassava brown streak leaf severity scores by incidences over the five assessment periods; 2, 4,6,8 & 10 MAP

[e] CBSDRT = cross product of CBSD root severity scores by proportion of infected roots at harvest

[f] CBSDMT = average of MCBSDT and CBSDRT

Effect of CBSD on the fresh storage root yield using whole plant index is presented graphically in Figures 3a and effect of CBSD on fresh storage roots yield using storage roots index only is presented in Figure 3b and 4 for set 1 and 2 data respectively. The graphs show a weak negative relationship between CBSD index (whole plant index and root index) and fresh yield in all the three graphs. Therefore, CBSD in both situations did not affect significantly the yield in terms of weight (tones/ha). Whole plant index did not show any stronger relationship than storage root index. The simple correlation analysis shoed a very significant ($p<0.01$) relationship between CBSD index and fresh yield at 8 ($r = 0.83$) and 10 ($r = 0.91$) month after planting (MAP).

Effect of CBSD on fresh storage roots yield

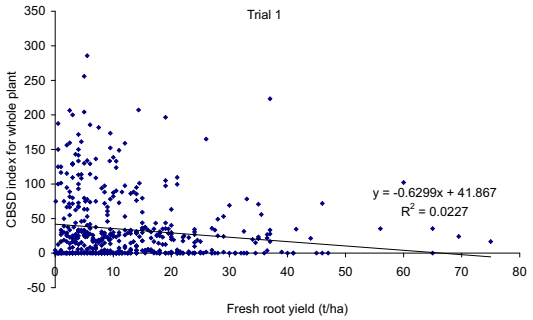


Figure 3a. Effect of CBSD on fresh storage roots yield from trial 1 using whole plant index

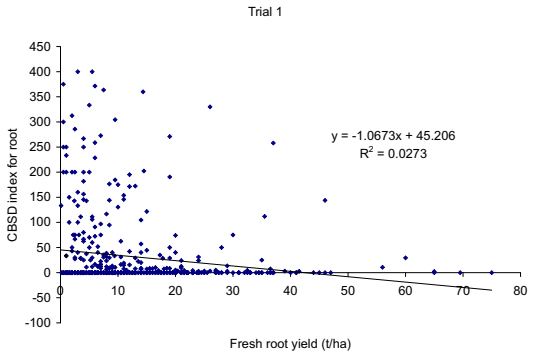


Figure 3b. Effect of CBSD on fresh storage roots yield from trial 1 using storage roots index only

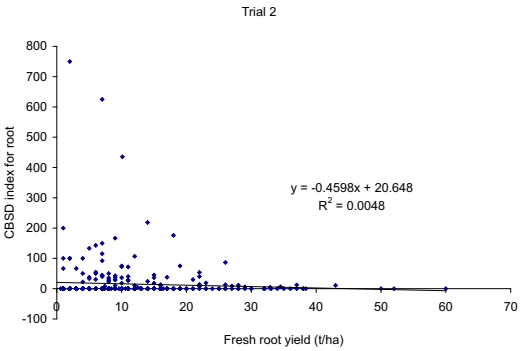


Figure 4. Effect of CBSD on fresh storage roots yield from trial 2 using storage roots index only

Discussion

The results showed an increase in severity following a sigmoid trend during the plant growth. This was probably due to build of inoculum or the vector populations. Recent study indicated that the vector transmitting CBSD is *Bemisia tabaci* (Maruthi et al., 2005). Hillocks (2003) found a very close association between new incidences of CBSD and fluctuations in whitefly populations although results from another study by Muhanna and Mtunda (2003) gave no evidence of transmission of CBSD by whiteflies.

Results also showed that there was little or no effect of CBSD, whether on roots or leaves or both, on root weight (Fyld in tons/ha). This is supported by Nichol's (1950) findings who found no significant difference in root weights of diseased and healthy plants although roots from the diseased plants were rendered of little economic value. On the other hand Hillocks (2003) found up to 50% root weight loss using the most susceptible cultivars although the loss was attributed to removal and discarding of the necrotic areas after peeling and chopping for drying. However, in very severe cases of CBSD and with highly susceptible cultivars like TME 204, significant weight losses are possible.

The study identified 260 genotypes resistant to both CMD and CBSD as possible sources of resistance. The materials may be tested on-farm for selection, multiplication and release to farmers. This can serve as a temporary mitigation to the CBSD threat. The genotypes may also be used in breeding programs as donors of resistance to other susceptible genotypes. The genotypes that had leaf symptoms but had clean roots are considered tolerant although a more in depth tolerance study using these materials should be carried out.

References

Alicai, T., Omongo, C., Maruthi, M.N., Hillocks, R.J., Baguma, Y., Kawuki, R., Bua, A., Otim-Nape, G.W. and Colvin, J., 2006. Re-emergence of Cassava Brown Streak Disease in Uganda. *Plant Diseases.* Vol. 91. No. 1. Pp. 24-29.

Emechebe, A.M. 1976. Some Aspects of Crop Diseases in Uganda. Makerere University press, Kampala. 86 pp.

Gondwe, F.M.T., Mahungu, N.M., Hillocks, R.J., Raya, M.D., Moyo, C.C., Soko, M.M., Chipungu, F.P. and Benesi, I.R.M. 2003. Economic losses by small-scale farmers in Malawi due to cassava brown streak disease. Pp. 28-36. In: Cassava Brown Steak Virus Disease: Past, Present and Future. Proceedings of an international workshop, Mombasa, Kenya, Oct. 2002. Legg, J.P. and Hillocks, R.J. (Eds).

Hillocks, R.J. 2003. Cassava Brown Streak Virus Disease: Summary of present knowledge on distribution, spread, effect on yield and methods of control. Pp. 23-27. In: Cassava Brown Steak Virus Disease: Past, Present and Future. Proceedings of an international workshop, Mombasa, Kenya, Oct. 2002. Legg, J.P. and Hillocks, R.J. (Eds).

International Institute of Tropical Agriculture (IITA). 1990. Cassava in tropical Africa, Ibadan, Nigeria.

Maruthi, M.N., Hillocks, R.J., Mtunda, K., Raya, M.D., Muhanna, M., Kiozia, H., Rekha, A.R., Colvin, J. and Thresh, M. 2005. Transmission of cassava brown streak virus by *Bemisia tabaci* (Gennadius). Phytopathology 153:307 - 312.

Mtunda, K.J., Muhanna, M., Raya, M.D. and Kanju, E.E. 2002. Current status of Cassava Brown Streak Virus Disease in Tanzania. Pp. 7-11. In: *Cassava Brown Steak Virus Disease: Past, Present and Future. Proceedings of an international workshop, Mombasa, Kenya, Oct. 2002*. Legg, J.P. and Hillocks, R.J. (Eds).

Muhanna, M. and Mtunda, K.J. 2003. Cassava Brown Streak Virus Disease transmission studies in Tanzania. Pp. 39-40. In: *Cassava Brown Steak Virus Disease: Past, Present and Future. Proceedings of an international workshop, Mombasa, Kenya, Oct. 2002*. Legg, J.P. and Hillocks, R.J.(Eds).

Nichols, R.F.J. 1950. The brown streak disease of cassava: distribution climatic effects and diagnostic symptoms. East African Agriculture 15: 154-160.

Ntawuruhunga P. and Legg J.P. 2007. New Spread of Cassava Brown Streak Virus Disease and its Implications for the Movement of Cassava Germplasm in the East and Central African Region. http://c3project.iita.org

Otim-Nape, G.W. 1990. Root crops in Uganda. In: Agricultural Research in Uganda: Five year Research Plan. Vol 1. Hartmans(ed) USAID.

Thrips (Tripidae; Thysanoptera) infestation on cassava (*Manihot esculenta* Crantz) at Mvuazi INERA Research Station, Bas-Congo Province in the Democratic Republic of Congo

Tata-Hangy K. W.[1]; K. Mfuti[2]; M. Tuwizana[2]; N. Mahungu[1] and K.M. Lema[2]

[1]International Institute of Tropical Agriculture (IITA) Cassava Project, Kinshasa, RD Congo
[2]Institut National pour l'Etude et la Recherche Agronomiques (INERA) ; Programme National Manioc BP 2037 Kinshasa 1, RD Congo.
[3]Université de Kinshasa, Faculté des Sciences Agronomiques; Kinshasa XI, RD Congo

Abstract

In the dry season 1997-1998, unusual damage was observed on cassava at M'vuazi INERA research station (Lat. 5°25'S, Long. 14°54'E, Alt. 465m asl) in the Bas-Congo province, DR Congo (DRC). The damage was first attributed to cassava green mite (CGM), but subsequent observations and literature search revealed later that such damage is likely to be due to thrips feeding on cassava. Thrips on cassava had been reported from Latin America. Samples collected on cassava in DRC were sent to IITA curator, who identified these tiny insects as thrips (Thysanoptera; Tripidae) belonging to the genus *Frankliniella*. The species is not known yet.

Preliminary investigations consisted in learning about the field characteristics of the pest and to understand the conditions that lead to high infestations. They indicated that all cultivated cassava varieties were infested in most of cassava farms around the station in a radius of over 50 Km. The incidence and severity of damage varied between variety and sites. Population developments as well as the types of damage were monthly assessed on the predominant varieties and results indicated the pest and its damage to cassava are most prevalent during the dry season, like most other cassava pests. Furthermore, the impact of this arthropod pest on the crop was assessed in a classical exclusion trial using a systemic insecticide CONFIDOR 050 EC. The results showed a yield reduction of 10 to 64.8% depending on the variety. These preliminary results revealed that thrips could be a potential threat to cassava production and attention needs to be given to its control methods

Introduction

Unusual damage was observed on cassava at M'vuazi INERA research station in the Bas-Congo province, DR Congo from 1998. The damage was first attributed to cassava green mite (CGM), but subsequent literature search revealed later that such damage is likely to be due to thrips feeding on cassava. Thrips on cassava had been reported from Latin America (Belloti, A.C. and van Schoonhoven, A. 1978; Lazano et al. 1981). Samples collected on cassava in DRC were sent to IITA curator in Cotonou, Benin Republic , , who identified these tiny insects only to the genus level as *Frankliniella*. The species is yet to be determined

Thrips as cassava pests are more frequent in the Latin america where 3 species including *Frankliniella williamsi, Corynathrips stenopterus, Caliothrips masculines,* are known to be more important (Lazano *et al*. 1981; Silvestre et Arraudeau, 1983). In Africa, two species of thrips have been reported on cassava. They are; *Scirtothrips manihoti*, reported from Uganda and *Bolothrips marshalli* from Sierra Leone (Silvestre et Arraudeau, 1983). Appert and Deuse (1982) however reported *Retithrips syriacus* on cassava, but did not tell the country where they occur.

In DRC, thrips were observed for the first time at Mvuazi research station of the

"Institut National pour l'Etude et la Recherche Agronomiques" (INERA) in 1998. Damage observed start from simple silvery discoloration of the leaves along the veins, followed by the perforation of the leaf surfaces (Plate 1). Further infestations cause the plant to produce lateral shoots growing in a characteristic disorder followed by the shortening of the internodes. Visible brown wounds on the stems (Plate 2) and stem distortion are observed as the infestations increase (Plate 3). All these symptoms correspond to those described by Lozano *et al*. (1981). Discoloration on leaves probably reduces photosynthesis. Plant's ability to recover from stress increases the metabolic demand on the stored photosynthate and ultimately reduces root yield (Cock, 1978)

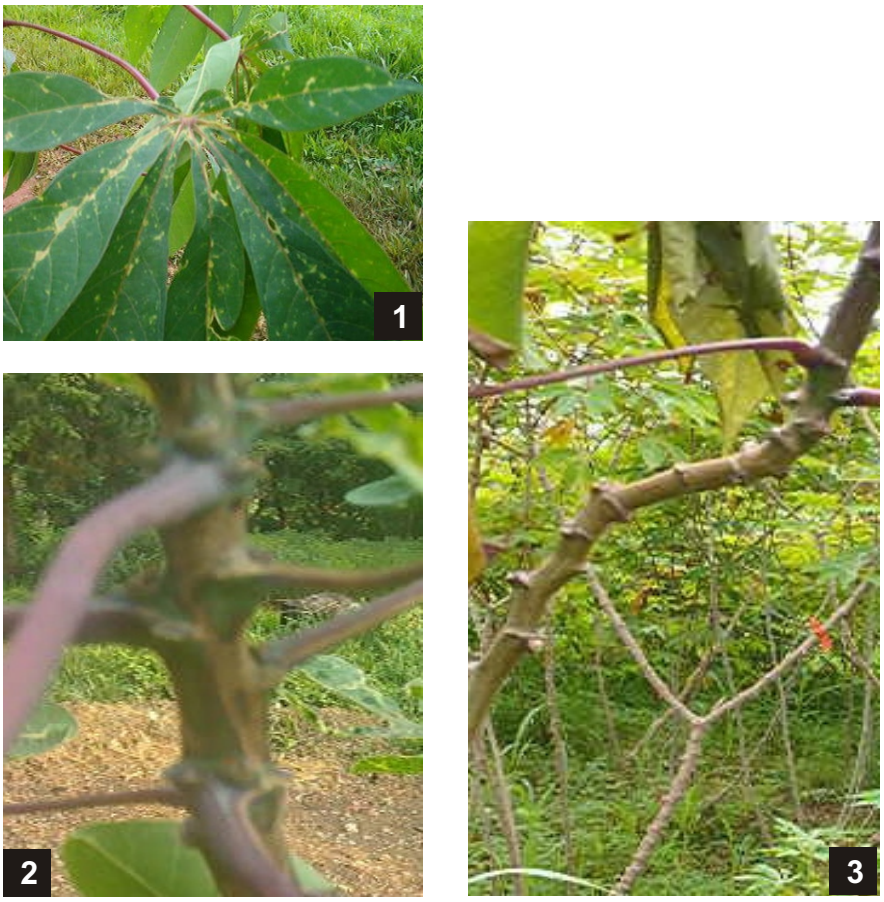


Plate 1, 2 and 3: 1. Silvery discoloration of the leaf. 2. Brown wounds on the green parts of the stem and 3. Stem distortion

This paper presents preliminary observations made on this newly observed pest and describes its phenology on the crop as well as the impact on cassava yield.

Material and Methods

Investigations on the thrips were carried out in 2002 through 2004 cropping seasons at Mvuazi (Latitude 5°25'S, Longitude 14°54'E, Alt. 465m asl.). Two sites were chosen for the follow up of the infestations; Mvuazi Poste on the plateau with sandy poor soil, and Mankewa on an alluvial rich soil with good moisture retention. Samples of the thrips were collected and put in vials with 70% alcohol then sent to IITA-Benin for identification. They were identified only to the genus level as *Frankliniella*.

For the evaluation of the development of the thrips infestations in the two locations, cassava plants were sampled at monthly basis in farmer's fields in the two locations and symptoms recorded. The incidence of infestation was estimated as percentage of affected plants in the sampling unit whereas the severity of damage was rated on 1 5 scale; with 1 = no symptoms, 2= silvery spots on the leaves along the nerves; 3 = slight perforations of the leaf limbs; 4 = severe perforation of the leaves and reduction in the leaf surface; and 5 = wounds on the stems followed by the shortening of the internodes and distortion of the stems. The varieties used in the study were Mvuama, Sadisa and RAV, all improved varieties among the best at that time to control the cassava mosaic disease (CMD).

In addition, the impact of *the thrips* on cassava production was assessed in a classical yield loss trial in Mankewa site during 2002 2003 and 2003-2004 cropping seasons. Three improved varieties with different levels of resistance to thrips were used in this trial. Two of these varieties (Mvuama and Sadisa) were widely used by farmers in the

province, while Nsasi was among the new released varieties highly resistant to CMD. The trial was implanted in November 2002 for the 1st evaluation and October 2003 for the second.

The study was designed as a factorial experiment with variety and thrips infestations as factors arranged in a randomised complete blocks with four replications. Two levels of thrips infestations consisted of protected and unprotected plots. In the protected plots, plants were sprayed with a systemic insecticide CONFIDOR 050 EC in order to prevent from thrips infestation. Protected plots comprised plots where the insecticide was sprayed every month, and plots sprayed every 15 days, thus twice a month. The first insecticide treatment was applied at 3 MAP. Data were collected monthly by a direct counting of the thrips abundance and scoring the severity of damage on the plants of the varieties under study, starting at 5 months after planting.

In the first year of the study, the plants in the protected plots showed a very low level of infestation. At the harvest time, treatments were harvested separately. In the second year, it was observed that the effects of the insecticide was not uniform on plants in the protected plots, due to probably to some uncontrolled factors. Thus, plants in protected plots were categorised into 3 different classes of damage and marked according to the level of thrips damage. The 3 classes were as follow; 1 = plants free from thrips damage, 2 = plants with damage of score 2 and 3 or mild to moderate damage, and 3 = plants with score 4 and 5 or severe damage, as described on the scale above. It is only these selected plants which were used to assess the impact of the pest on the varieties tested. From each variety (plot), 30 plants in these 3 classes of damage were harvested separately. The trials were harvested at 12 month after planting. At harvest, the number and the weight of marketable roots were recorded for each treatment for the 1st year trial, whereas these same data were recorded from each of the marked plants only for the second year trial, as the effect of the insecticide was not uniform. In the first year, data were submitted to the analysis of variance and the significance of treatment effects on thrips severity and yield parameters were evaluated using means and LSD. In the second year, data were submitted to a t-student test.

The percent of the yield loss was computerised as follow: **% Loss = © T)*100/C**

Where C= data from the protected plots and T = data from the unprotected plots; when data from different treatments were used. When data from the marked individual plants were used, C= data recorded from the symptomless plants (class 1) and T = data recorded from class 2 or class 3.

Results and Discussion

During the follow up of the thrips infestation on cassava in 2002-2003, no thrips were observed on the crop in the rainy season. Figure 1 shows data between March to September 2003 on the 3 varieties sampled at Mankewa in Mvuazi. Population density of thrips on cassava started to increase as the rain frequencies decreased and reached the peak in the dry season in August and September (Figure 2), implying thrips to be a dry season pest like most of the other cassava pests such as cassava green mite, cassava mealybug and the african root and tuber scale (Yaninek *et al.* 1989; Le Ru and Fabres, 1987; Lema *et al*. 2000). There was no difference in the pattern of thrips infestation between the two sites (Mankewa and Mvuazi Plateau), though thrips density was numerically higher at Mvuazi Plateau. The figure shows the variety effect with SADISA harboring more thrips than RAV and Mvuama.

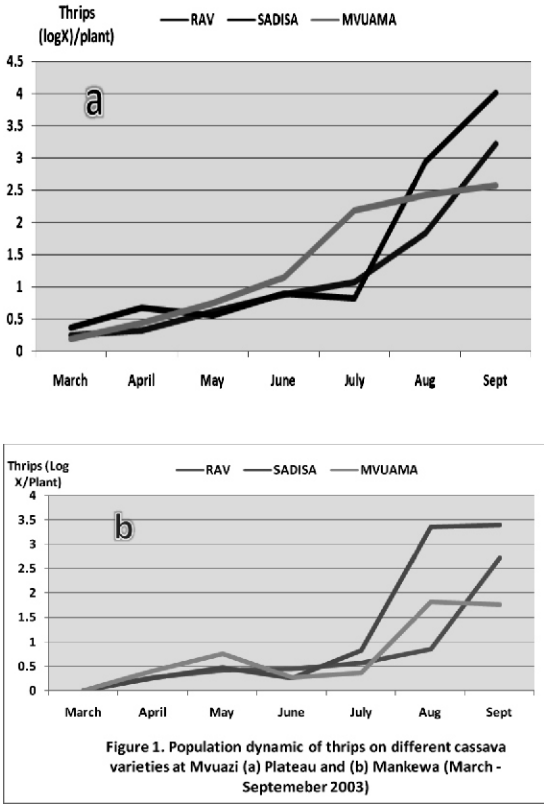


Figure 1. Population dynamic of thrips on different cassava varieties at Mvuazi (a) Plateau and (b) Mankewa (March - Septemeber 2003)

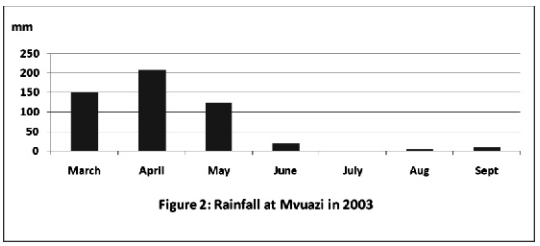


Figure 2: Rainfall at Mvuazi in 2003

Thrips damage on cassava was also determined, and data showed damage to be more severe in the site of Mvuazi Poste on the plateau with sandy poor soil, than at Mankewa with rich soil and good moisture retention.

Results from the exclusion trial did not show any statistical difference between the treatments neither in the severity of damage (Figure 2) nor in the fresh root yield, indicating that insecticide application did not show any effect. Significant difference was observed, however, between varieties in the severity of damage. Nevertheless, protected plots yielded numerically higher than no protected ones (Table 1). The yield reduction calculated on the data from the different treatments varied from 4.7 to 26.1%.

The no significance of the treatments effects in this trial may be justified by the single prediction model of infestation level. Teng & Bissonnette (1985) working on the early blight in potato documented the inadequacy of single prediction model. Data from a single assessment of the exclusion of the pest may not serve as a very useful criterion for the yield loss estimation. Some other factors not examined could have influenced the yield.

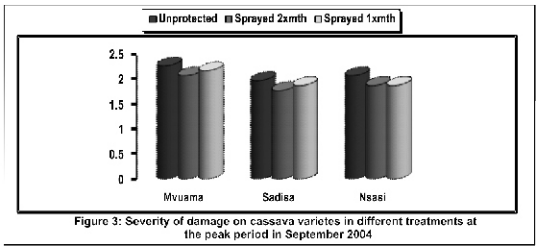


Figure 3: Severity of damage on cassava varietes in different treatments at the peak period in September 2004

Table 1: Number, diameter and mean weight of tuberous roots in protected and non protected plots against thrips infestation at Mvuazi, 2003

Variety	Treatment	Mean number of Tuberous root	Mean diameter of tuberous root	Mean weight (Kg/plant)	% Yield loss
Sadisa	No spray	5.3	4.7	3.3	-
	Sprayed	5.3	5.0	4.0	17.5
	1X/Month	6.0	5.3	4.3	23.3
	Sprayed 2				
Mvuama	X/Month	3.7	4.6	1.7	-
		3.3	5.0	2.3	26.1
	No spray	4.3	4.7	2.3	26.1
	Sprayed				
Nsasi	1X/Month	4.0	4.6	2.0	-
	Sprayed 2	4.7	5.3	2.1	4.7
	X/Month	4.0	5.3	2.7	25.9
PPDS	No spray	NS	NS	NS	
(Pd0,05)	Sprayed				
CV (%)	1X/Month	30,0	9	35,9	
	Sprayed 2				
	X/Month				

Based on data on the level of damage, t-test analysis was used to detect the significance of the yield loss. T-test on data from the marked plants in the 3 classes of damage as classified above, revealed highly significant differences between class 1 and the other two classes in the number, the diameter and the weight of the fresh roots. Significant differences were observed between class 2 and class 3 in the same parameters (Table 2). Yield loss calculated on the basis of the 3 classes of damage on the data from the marked plants varied between 10.8-49% between class 1 and 2; 51.6-64.8% between class 1 and class 3 and 10-58.1% between class 2 and class3.

Table2: Average number and weight of tuberous roots from plants in the classes of damage of 3 varieties at Mvuazi in 2004.

Variety	Class of Severity	Mean number of tuberous roots	Mean weight of tuberous roots	% Yield loss
Sadisa	Class 1	7,4	5,1	
	Class 2	5,7	4,3	
	Class 3	2,4	1,1	
	t (class 1 2)	1,87	1,07	16,0
	t (class 1 3)	4,68**	3,14*	64.8
	t (class 2 3)	4,40**	4,96*	38.1
Mvuama	Class 1	6,7	4,1	
	Class 2	4,1	2,1	
	Class 3	3,9	1,9	
	t (class 1 2)	2,87*	2,65*	49,0
	t (class 1 3)	2,46*	2,83*	54,0
	t (class 2 3)	0,42	0,69	10,0
Nsasi	Class 1	6,2	4,1	
	Class 2	5,5	3,1	
	Class 3	2,5	1,3	
	t (class 1 2)	3,16*	3,24**	24,0
	t (class 1 3)	7,42**	7,55**	51.6
	t (class 2 3)	5,20	4,08**	26.2

NB : * = Significant at 5%, ** = Significant at 1%

These results show the pest status of the thrips on cassava and indicate that they require attention. They can induce more losses in the yield in the case of outbreak.

Acknowledgements

This work was done under the IITA-Cassava Project funded by USAID. The authors acknowledge INERA management and all Cassava Program staff for technical assistance.

References

Appert. J et Deuse, 1986.-Les ravageurs des cultures vivrières et maraîchers sous les tropiques.

Belloti, A.C. and van Schoonhoven, A. 1978. Mite and insect pests of cassava. Annual Review of Entomology 23:39-67.

Cock, J. H. 1978. A physiological basis of yield loss in cassava due to pests, pp. 9-16. In I. Brekelbaum,

A. C. Bellotti & J. C. Lozano (eds.), Proc. Cassava production workshop, Cali, Colombia, 7 - 12 Nov.1977. Centro Internacional de Agricultura Tropical, Series CE-14.

Lema K.M., Tata-Hangy K., Bidiaka M. et Ndambi N. 2000. Distribution, importance et dynamique

des populations de la cochenille radicole du manioc (Stictococcus vaysierrei,

Homoptera Stictococcidae) en République Démocratique du Congo. Annales de la Faculté des Sciences Agronomiques, Vol 1 No 1 : 40-49

Le Ru B. and Fabres G. 1987. Influence de la température et de l'hygrométrie relative sur la capacité d'accroissement et le profile d'abondance des populations de la cochenille du manioc. Acta Oecologica, Oecologica Applicata 8 : 165-174.

Lozano, J.C., Bellotti, A., Reyes, J.A., Howeler, R., Leihner, D., Doll, J. 1981. Field Problems in Cassava. Cali, Colombia. CIAT.208 p.

Silvestre.P et M. Arraudeau, 1983. Le manioc. Techniques Agricoles et Productions Tropicales. Edition Maisonneuve et Larose. ACCT, Paris

Teng, P.S. and Bissonnette, H.L. 1985. Estimating potato yield responses from chemical control of early blight in Minnesta. American Potato Journal 62, 595-606.

Yaninek J. S., Herren H.R. and Gutierrez A.P. 1989. Dynamics of Mononychellus tanajoa (Acari; Tetranychidae) in Africa: seasonal factors affecting phenology and abundance. Environ. Entomol. 18(4):625-632.

Scale insects on yams in Benin: diversity, incidence and farmers' knowledge and perceptions

Georg Goergen[1], Romuald Dossou[2], Robert Asiedu[3] and Rachid Hanna[4]

[1]International Institute of Tropical Agriculture (IITA), Cotonou, Benin;
[2]Institut National des Recherches Agricoles du Bénin (INRAB), N'Dali, Benin,
[3]IITA , Ibadan, Nigeria;
[4]IITA, Yaoundé, Cameroon,

Abstract

Yam (*Dioscorea*spp.) is a primary agricultural commodity and a traditional staple food over much of West and Central Africa, where it is highly profitable both for producers and traders especially when production and storage strategies are successful in securing the supply of urban markets throughout the year. Insect pests such as mealybugs of the genus *Planococcus* Ferris (Hem.: Pseudococcidae) and the scale *Aspidiellahartii* (Cockerell) (Hem.: Diaspididae) can become major constrains causing significant yield losses both in the field and in storage. Detailed information on the pest status of the scale insects on yams and farmers' knowledge and perceptions of these insects were assessed in two surveys carried out in central and southern Benin in May and December 2009. In the May survey, covering134 sites and 68 villages, scale infestations were found in 68% of yam stores and sales points with in average 41% of the tubers infested. Lower incidence and infestation rates were observed at the main harvest period in December when scale and mealybug infestations were found in 56% of the 102 fields from 68 villages with an average of 23% infested tubers, indicating that scale and mealybug infestations are initiated in the field. *Aspidiellahartii* tended to become proportionally more important in stores increasing from 9 to 40% of the recovered scale insect pests. In both surveys the percentage of mixed infestations remained relatively low - at 13 and 5% for the first and second survey respectively. Structured questionnaires performed at every site showed that almost all farmers and traders were able to recognize yam scales and mealybugs and ranked both groups equally in importance as pests. The presence of the scale insects was generally regarded as having a negative impact on the quality of stored tubers, especially their germination rate when used as seeds for planting new fields. Whereas all cultivars seemed to be at risk from scale insects, late varieties, appeared to be more susceptible than other varieties. Farmers estimated an average of 22% reduction of the total yield and 23% reduction in planting material due to scale insect infestations. Tuber rot, theft, cattle feed and bush fire were recurrently cited as the most important problems of stored yams. Interestingly, 66% of the questioned farmers believed scale infestations have been on the rise over the last few years, while at the same time the length of fallow periods were on the decline primarily because of increasing pressure on the land which might suggest a relationship between scale infestation and fallow period. Farmers, however, differed considerably in their admission that such a relationship is real. Taken together, both field evidence and farmers perceptions of scale insect infestations on yams are a first and valuable step in the development of appropriate management practices to reduce yam losses due to scale insect infestations. Control options are presently limited to preventive actions such as early harvest, culling of infested tubers, selective early consumption, and production of yam chips.

Introduction

Yams (*Dioscorea* spp.) are one of the traditionally important components of the human diet for large parts of West and Central Africa (Asiedu&Sartie, 2010). The productivity of yam cultivation can be significantly impaired by high tuber infestations with pests insects such as the scale *Aspidiellahartii* (Cockerell)(Hem.: Diaspididae) and mealybugs species of the genus *Planococcus (Hem.: Pseudococcidae) (Braimahet al., 2007;* Manyong*et al., 2001).* The problem can be aggravated by viruses vectored by mealybugs and by the general action of scale insects in the induction of tuber rot both during production and post-harvest processes (Kenyon et al., 2001). In smallholder farming systems, where most yams are produced, the maintenance of healthy plantingpropagules is particularly important since yam propagation is vegetative and thus infested seed yams may become the source for further spread of pests and diseases. Several other factors such as changes in land use patterns, pest management practices, varietal susceptibility, etc. may contribute to an increase of the problem in future. The main objective of this study is therefore to provide baseline information on the diversity and incidence of yam scale insects in rural Benin together with related farmer's knowledge and

perceptions on the status of scale insects on yams.

Material and Methods

Two separate diagnostic surveys were carried out in central and southern Benin in five provinces including the Borgou, Donga, Atacora, Collines and the Zou. A first survey comprising 68 villages and 134 sites was conducted in May 2009, i.e., two months following the tardiest planting time to assess the presence of scale insects from 10 randomly selected tubers stored under various conditions in the field, villages and at sales points. To appraise the situation in the field during the main yam harvest time, a second survey covering 43 villages and 102 fields, was performed in the same areas at the beginning of December 2009. Here, farmers were asked at every visited site to harvest 10 randomly selected yam mounds preferably of a same variety. In both surveys all examined tubers were individually measured, weighed, inspected for pest presence and degree of its infestation, and tubers assembled for a photograph. Samples of scale insects and of attending ants if present - were removed from every infested yam and preserved in 70% alcohol for identification in the laboratory.

At every site, the collection of agronomic data was followed by structured interviews of about 30 min each. Farmers, storekeepers or traders were individually questioned about their knowledge status concerning yam scale insects, perceived infestation severity, effects on quality criteria and storage, varietal susceptibility, pest incidence, management measures and to rank three of the most important yam storage problems. Yam producers were additionally interrogated about fallow length change in yam fields over the last decade, effects on the occurrence of scale insects, associations with other insects and pest origin, and to give an estimate of experienced losses due to scale insect infestations.

Results and Discussion

Our study shows that, from a total of 136 villages and 236 sites studied during the two surveys, yams were found free of insect pests in 37% of all sites. One diaspidid species i.e. *Aspidiellahartii* (Cockerell) and at least two species mealybugs, *Planococcus* spp., often attended in the field by the ant *Crematogaster* sp. (Hym.: Formicidae), were recovered from infested yam tubers. Data gained during the December survey from freshly harvested tubers showed a much lower scale insect incidence and pest population densities compared with the infestations found in stores and sale points in May (Table 1). These changes in infestation patterns strongly suggest the proliferation of the scale and the mealybugs during post-harvest storage. This is particularly true for *A. hartii*which became the dominant species in stores and sales points during, as found during the May survey. The two surveys tended to demonstrate that although most farmers recognized scales and mealybugs as pests and were aware of their general negative impact on yam culinary properties, storage longevity, market value, and germination, relatively few measures were developed to-date to control them or to prevent new infestations. Similarly comparatively few solutions were available for theft, bush fire, cattle damage and tuber rot, which were recognized as the most important storage problems. Despite possible interactions, a general perception by farmers of a pattern of increasing scale and mealybug infestations over the last few years was infrequently perceived as being related to dramatically reduced fallow length in recent years.

Table 1. Infestation incidence of scale insects on yams as found on 236 sites and 2.380 tubers in two surveys in May and December in Southern and Central Benin.

	Survey 1 (May)		Survey 2 (Dec)	
	Sites	Tubers	Sites	Tubers
No infestation	43 (32 %)	430 (-)	45 (44 %)	450 (-)
Infested with *A. hartii*	54 (40 %)	540 (69 %)	9 (9 %)	90 (41 %)
Infested with *Planococcus* spp.	49 (37 %)	490 (42 %)	54 (53 %)	540 (37 %)
Infested with both pests	10 (13 %)	100 (45 %)	6 (5 %)	60 (30 %)

Conclusion

Field evidence and farmers' perceptions underscore the importance of scale insects in central and northern Benin. These insects, when taken from the field, proliferate on yam tubers under prevailing traditional storage conditions. Further shortening of fallow lengths and a relative high incidence of infested seed yams may well contribute to a rapid aggravation of the situation presently assessed if adequate control options are not timely developed.

Acknowledgements

This research was supported by IITA core donors and special project funds to IITA from the International Fund for Agricultural Development (IFAD).

References

Asiedu, R. & A. Sartie, 2010. Crops that feed the World 1. Yams. Yams for income and food security. Food Security 2(4): 305-315.

Braimah, H., Anchirinah, V. M. &J.,Adu-Mensah, *2007.* Yam pests in the Ashanti and BrongAhafo regions of Ghana: A study of farmers' indigenous technical knowledge and control practices**.** Ghana Journal of Agricultural Science 40: 33-42.

Kenyon, L., Shoyinka, S. A., Hughes, J.&B. O., Odu, 2001. An overview of viruses infecting Dioscorea yams in Sub-Saharan Africa. In Plant Virology in SubSaharan Africa, pp 432-439. Eds. J.d'A. Hughes, and B.O. Odu.

Manyong, V.,Asiedu, R.&G. O, Olaniyan, 2001. Farmers' perceptions of, and actions on resource management constraints in the yam-based systems of western Nigeria. Root Crops in the 21st Century, Government of Benin, pages 156-167.

Management strategy for spiralling whitefly, *Aleurodicus dispersus* RUSELL (Hemiptera: Aleyrodidae) in Tanzania

Beatrice Pallangyo[1], R. Hanna[2], E. Nsami[1], V. Mgoo,[1] O.Mfugale[1,] Bokonon Ganta A[3] and O. Ajuonu[3]

[1]National Biological Control Programme (NBCP) P.O.BOX 30031, Kibaha Tanzania beatricepallangyo@yahoo.com,

[2]Internatonal Institute of Tropical Agriculture (IITA) BP 2008 (Mesa) Yaounde, Cameroon,

[3]IITA Benin , 08 BP 0932, Cotonou, Benin.

Abstract

Several species of whiteflies, including Spiralling whitefly (SWF), *Aleurodicus disperses* Russell (Hemiptera: Aleyrodidae) pose severe threat to many agricultural crops throughout the world. The SWF, which is of South American origin, was observed for the first time in Tanzania in Unguja Island in 2002 and in the mainland in 2003. A survey conducted in 2004 reported occurrence of SWF in Morogoro, Tanga and Coast regions. Another survey conducted in 2005 reported occurrence of the pest in Lindi and Kilimanjaro in addition to previously reported areas. By 2006, the pest had already spread to Mtwara, Ruvuma and Dodoma regions covering a distance of 600 km within a year. At present, SWF is found in all regions of Tanzania mainland**.** Based on host range and spread mechanism, chances to spread to other countries are high. Depending on type of crop and damage severity, the pest can cause up to extensive yield losses leading to household food and income insecurity. Crops of economic importance attacked by SWF include cassava, banana, cashewnut, fruits & vegetables and roses. These crops are major staple foods and source of income to majority of Tanzanian population. SWF has been also shown to transmit the Cassava Brown Streak Virus, the causal agent of Cassava Brown Streak Virus Disease (CBSD), a serious disease of cassava in much of Eastern and southern Africa. Two parasitoid species, *Encarsia dispersa* (Polaszek) and *E. guadeloupae were i*ntroduced in the country from IITA Benin to manage the pest. The parasitoids were released in 15 sites in Tanga, Morogoro, Coast and Dar Es Salaam regions. Follow up surveys reported recovery of *E. guadeloupae i*n 6 sites in Morrogoro, Coast and Dar Es Salaam regions. More releases are planned to cover other affected regions.

Keywords: Classical biological control, Aleurodicus disperses, Encarsia dispersa, E. guadeloupae

Introduction

Whiteflies including the spiraling whitefly (SWF) *Aleurodicus dispersus* Rusell (Hemiptera: Aleyrodidae), pose severe threat to many agricultural crops throughout the world. The species which is of South American origin is now reported to occur in Hawaii (Waterhouse and Norris, 1989), Trinidad (Kumashiro, 1983), Indonesia (Kajita et al., 1991) and in India (Leg *et al*., 2003, Mani and Krishnamoorthy, 2002). In Africa *A. dispersus* was first reported in Nigeria in 1992 (Akinlosotu, 1993). In Tanzania the pest was reported for the first time in Unguja Island, Zanzibar in late 2002 (Pallangyo & Otema unpublished survey report).

The nymphs and adults of spiraling whitefly suck the sap from the surface of the leaves, stems and fruits. The nymphs secrete sticky honey dew which serves as a substrate for dense growth of sooty moulds which interfere with photosynthesis. Severe infestation may result in defoliation and death of the attacked plant. The copious white waxy material secreted by nymphs is readily spread elsewhere by flying adults, wind, planting materials and fruits. The pest attacks a broad range of horticultural crops including fruits vegetables, ornamentals, shade trees and weed species. In India alone, *A. disperses* attack 72 plant species belonging to 38 genera (Mani & Krishnamoorth 2003). SWF presents a serious phytosanitary risk to tropical and subtropical areas on the edges of its current range. Quarantine areas have been declared in Queensland, Australia. Severe damage due to spiraling whitefly has been reported on banana in Costa Rica (Coto and Metzie, 1989) guava and pawpaw in Hawaii (Annon, 1981), cassava and capsicum in Nigeria (Akinlosotu, 1993), and coconut in Papua New Guinea (Waterhouse and Norris, 1989). *The pest* has also been implicated in transmission of: Lethal yellowing virus of coconut palms in Florida (Weems, 1971) (Cherry, 1979) and Cassava brown streak virus in Kenya (Mware *et al*..; 2009).

Biological control by using *Encarsia dispersa* (Polaszek) and *E. guadelupae* (Viggiani) have been reported to be effective in suppressing SWF population in Hawaii (Waterhouse and Norris, 1989) and West Africa (Neuenschwander, 1994, D' Almeida *et al*,.. 1998). Based on success reported from other countries, National Plant Protection Advisory *Committee* (NPPAC) approved the introduction of the two parasitoids in May 2005. Baseline surveys were conducted, prior to the introduction of the bio agents to determine incidence, distribution, abundance, damage severity and host range of SWF; and composition of natural enemies. The status of SWF and introduced bio agents are presented and the way forward recommended.

Methodology

Baseline surveys

Surveys were conducted during the dry season to determine incidence, distribution and damage severity of spiraling whitefly in Tanzania mainland. The surveys were conducted in August 2004 in the Eastern zone (Morogoro, Tanga and Coast regions) and June 2005 in Eastern (Dar Es Salaam region), Southern (Lindi region) and Northern (Kilimanjaro region) zones. In September 2006 survey was conducted in Eastern (Morogoro region) and southern zone (Lindi, Mtwara and Ruvuma regions). In November 2008, countrywide survey was conducted. Sampling was conducted at 10km interval in rural areas, while in township areas sampling was conducted at 2km interval. At each sampling site various crops known to be hosts of SWF including cassava, guava, pawpaw, banana and Indian almond were sampled. Abundance and damage caused by SWF were determined using a 0 4 scale (D'Almeida *et al*.., 1998). For SWF density, 0 = no infestation, 1 = < 25% of leaf covered by SWF eggs and waxy filaments, 2 = 25% of leaf covered 3 = 25 - 50% of leaf covered, 4 = > 50% of leaf covered. Damage score was quantified according to sooty mould cover whereby 0 = no infestation, 1 = < 25% of leaf covered by SWF eggs and waxy filaments, 2 = 25% of leaf covered 3 = 25 - 50% of leaf covered, 4 = > 50% of leaf covered. At least 10 SWF infested leaves were collected in paper bags for parasitoid emergence. GPS readings for each field were recorded. Analysis of variance (ANOVA) using the proc GLM (SAS 1997) was used to compare damage and infestation ratio between hosts and regions.

Release and follow up of SWF natural enemies

Based on success reported from other countries on management of *A. dispersus* by using *E. dispersa* and *E. guadeloupae*, the introduction of bio agents was approved by National plant Protection Advisory Committee (NPPAC) in May, 2005. Pre release survey was conducted to identify suitable

release sites. Shipments of *E. dispersa* and *E guadeloupae* were received from IITA Benin in December 20008 and 2009 and released in Coast, Dar es Salaam, Morogoro and Tanga regions. Follow up survey was conducted in release sites to determine recovery of the parasitoids. Ten leaf samples were taken on five different hosts in each site. The samples were kept in paper bags, and taken to laboratory where they were carefully examined under microscope to determine number of parasitized larvae. Mummies were collected in vials to allow emergence of parasitoids.

Results

A. dispersus distribution and spread

Survey conducted in Eastern zone in 2004 established occurrence of SWF in Tanga (Muheza and Korogwe districts), Coast (Kisarawe, Bagamoyo and Mkuranga districts) and Morogoro (Morogoro, Kilosa and Mvomero districts) regions. The spreading limit towards Northern part of the country was Korogwe town about 300 km North from Kibaha (Coast region) where the pest was observed in 2003. In a survey that was conducted in March 2005, the pest was found in Kilimanjaro region (Moshi and Hai districts), Dar es Salaam and Lindi region. Spreading limit towards the northern part of the country was Bomang'ombe town, about 300 km North from Korogwe town. Towards Southern part of the country, spreading limit was Kilwa Masoko. By September 2006, *A disperses* was found in Dodoma, Mtwara and Ruvuma regions. The spreading limit towards Southern part of the country was Tunduru town which is about 600 km South from Kilwa Masoko that was the spread limit in 2005, suggesting that the pest had spread to cover a distance of 600km within a year (Fig 1). Countrywide survey conducted in December 2008 established occurrence of SWF in Arusha (Mto wa mbu), Mara, Mwanza and Mbeya regions in addition to previously reported areas (Fig 2).

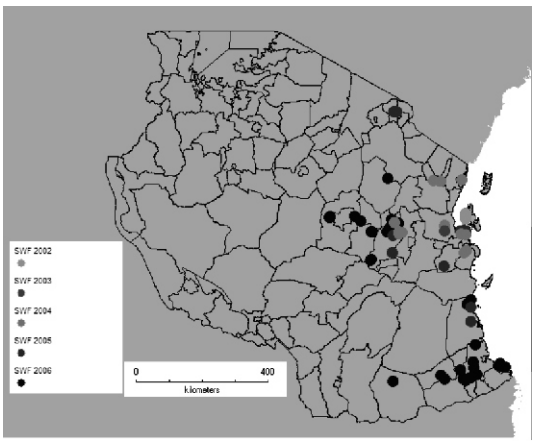


Fig 1. SWF distribution in Tanzania 2004 2006

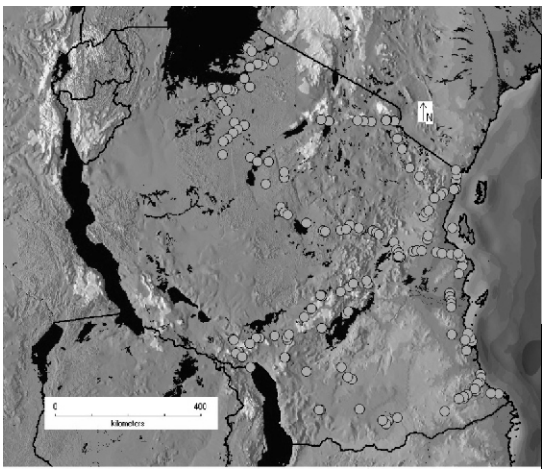


Fig 2. SWF distribution in Tanzania, 2008

SWF density and damage severity

Pest densities

Crops that recorded high density of both SWF adults and nymphs include pepper, guava, banana, cassava and pawpaw. The lowest densities were recorded on mango crop while only eggs were found on tomato crop. Although coconut has been reported as suitable host of SWF (Akinlosotu, 1993), during the survey the crop was found to be free from infestation (Table 1)

Damage severity

Damage score ranged between1 and 3. Highest scores were recorded on cassava, guava, pawpaw, and banana crops. These are the same crops which recorded highest density of SWF egs, nymphs, and adults. Citrus and mango had low damage suggesting that they are not preferable hosts of SWF. No damage was found on phaseola bean despite that the crop had highest density of SWF eggs. (Table 1

Table 1. Average density (eggs, nymphs, adults) and damage severity by SWF on different crops sampled in Coast Tanga and Morogoro regions, August 2004

Crop	Adults	Nymph	Eggs	Damage
Citrus	0.095	0.079	0.049	1
Banana	0.54	0.68	0.81	3
Capsicum	0.83	1.15	1.2	1
Pawpaw	0.25	0.65	0.6	3
Okra	0.12	0.1	0.77	1
Cassava	0.47	0.48	0.64	3
Guava	0.56	0.87	1.02	3
Mango	0.02	0.02	0	1
Tomato	0	0	0.4	1
Cashew nut	0.04	0.16	0.56	1
Coconut	0	0	0	0
Phaseola beans	0.05	0	1.35	0

Percentage of infested plants and damage severity were significantly higher ($f = 6.50$, $p < 0.0001$) on *Manihoti* spp. compared to *Musa* spp, *Carica papaya* and *Terminalia catapa* (Table 2).

Table 2. SWF infestation and damage severity on five hosts in Lindi, Mtwara, Ruvuma and Morogoro regions, 2006

Host Species	No. of sampled plants	INFESTATION (%)	LEAF DAMAGE
Manihot spp	49	56.77 ± 6.44	
Psidium guajava	60	47.43 ± 5.88	1.47 ± 0.18ab
Mussa spp	60	31.13 ± 4.77	0.80 ± 0.12b
Carrica papaya	67	34.09 ± 4.57	0.80 ± 0.11b
Terminalia catappa	9	54.44 ± 13.93	1.22 ± 0.36ab

Release and recovery of SWF Natural enemies

In December 2008, two parasitoid species, *E. haitiensis* and *E. guadeloupae* were shipped from IITA Benin and released in 1 location at Kibaha. In December, 2009, more shipments of natural enemies were received from IITA Benin and released in 15 sites in 4 regions including Tanga, Morogoro, Coast and Dar Es Salaam. During post release survey that was conducted in May 2010, *E. guadeloupae* was recovered in 50% of release sites (Table 3).

Table 3. Release and recovery sites for SWF natural enemies in the Eastern zone of Tanzania.

Region	Release Sites	Recovery Sites
Ta Nga	3	0
Morogoro	3	2
Coast	6	4
Dar Es Salaam	5	5
Total	17	11

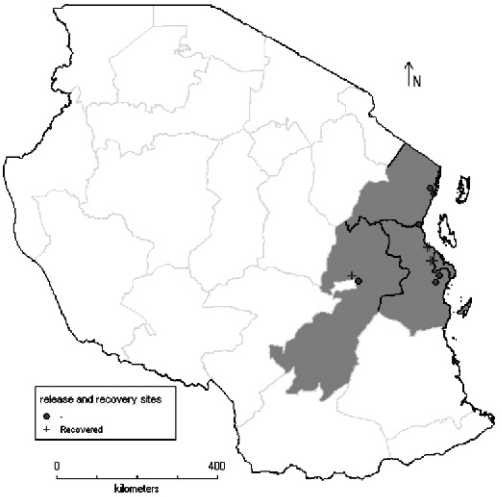


Fig 3. Location of recovery sites for SWF Natural enemies in Tanzania, 2010

Discussion and the way forward

SWF is widespread in Tanzania, highest infestation levels are found in low altitude areas especially along the Coast. Based on host range and spread mechanism, chances to spread to other countries are high. Crops of economic importance attacked by SWF include cassava, banana, cashewnut, fruits & vegetables and roses. These crops are major staple foods and source of income to majority of Tanzanian population. Depending on type of crop and damage severity, the pest can cause up to extensive yield losses leading to household food and income insecurity. Among the introduced parasitoids, *E. guadeloupae* has been recovered in more than 50% of release sites while no *E. dispersa* has been recovered so far. Recovery of *E. guadeloupae* in release sites indicates that the parasitoid has adopted to the environment therefore more releases should be conducted to cover other SWF affected regions. Post release monitoring will be required to determine recovery establishment and persistence of the parasitoid. Monthly population dynamic are planned to establish the impact of the introduced parasitoid. To ensure sustainability of the project, public awareness creation is crucial to enable farmers participate in conservation of environment including the released bio agents.

Acknowledgement

The reported work was funded by the Ministry of Agriculture Food Security and Cooperatives (MAFSC) and International Institute of Tropical Agriculture (IITA) Cassava IPM Project. Map for SWF distribution in Tanzania 2002 - 2006 and location of release and recovery sites for SWF natural enemies were prepared by Mr. Simon Boniface and Mr. Claude Maeda of IITA, Kibaha.

References

Akinlosotu T, Jackal L, Ntonifor N, Hassan A, Agyakwa G, Odebiyi J, Akingbohungbe A, and Rossel H (1993) Spiralling whitefly, *Aleyrodicus disperses*, in Nigeria *FAO Plant Protection Bulletin 41, 127 129*

D'Almeida Y.A, Lys J.A, Neuenscwander P and Ajounu O (1998) Impact of two accidentally introduced *Encarsia* species (Hymenoptera:Aphelinidae) and other biotic and abiotic factors on the spiraling whitefly *Aleurodicus disperses* Russel (Homoptera: Aleyrodidae) in Benin West Africa *Biocontr. Sci. Technol. 8,* pp163 -175

Esguerra N M (1987) The spiraling whitefly, *Aleyrodicus disperses* Russell *Entomology Bulletin no 1* pp1

Kajita H, Samudra I and Naito A (1991) Discovery of the spiraling whitefly *Aleurodicus disperses* Russell (Homoptera: Aleyrodidae) inform Indonesia, with notes on its host plants and natural enemies *Applied Entomol. Zool. 26, 397 400*

Kumashiro, B, Lai P, Funaki Gand Teramoto K (1983) Efficacy of Nephasis and Encarsia? Haitiensis in controlling Aleurodicus dispersus in Hawaii. Proceedings of Hawaiian Entomology Society 24: 261 - 270

Legg J. P, Gerling D, Neuenschwander P, (2003) Biological Control of Whiteflies in Sub Saharan Africa In Biological Control in IPM Systems in Africa. *CAB International 2003* (Eds) P. Neuenschwander, C. Borgemeister and J. Langewald pp 93 -95

Mani M & Krishnamoorty A (2002) Classical Biological Control of the Spiralling Whitefly, *Aleyrodicus dispersus* Russell - An Appraisal. *Insect Sci. Applic. Vol 22 No 4* pp 263 273

Mware B, R. Naria, F Olubayo, J. Songa, S Kyamanywa, E. Ateka, (2009) Efficiency of Cassava Brown Streak transmission by two whitefly species in Coastal Kenya. *Journal of General Molecular Virology* Vol 1 (4)

Neuenschwander P (1994) Spiralling whitefly *Aleyrodicus disperses,* a recent invader and new cassava pests in Africa *African Crop Sci. J. 2*, pp 419 421

Pallangyo B, Nsami E, Mfugale O, Manahiri F (2004) Survey for cashewnut mealybug (Pseudococcus longispinus), Mango mealybug (Rastrococcus iceryoides) and spiraling whitefly (Aleurodicus dispersus) in Tanga, Coast, Lindi, and Mtwara regions. *In* Documents of the 7th National Plant Protection Advisory Committee (NPPAC) Meeting held at PHS, Dar es Salaam Tanzania on 15th 16th April 2004

Pallangyo B, Nsami E, Mfugale O (2004) Distribution, host range and damage severity of Spiralling whitefly Aleurodicus dispersus Rusell (Hemiptera: Aleyrodidae in the Eastern zone of Tanzania. *In* Documents of the

8th National Plant Protection Advisory Committee (NPPAC) meeting held at Sugarcane Research Institute Kibaha, Tanzania 24th November 2004 pp 113 125

Waterhouse D.F and Norris K.R(1989) *Aleyrodicus disperses* Russell, pp 13- 14 *In Biological Control: Pacific Prospects- Supplement* 1, *ASIAR Monograph* No 12, ACIAR Canberra, Australia

Towards the development of sustainable control options for the African root and tuber scale on cassava in Central Africa understanding the ecology of the associated ant *Anoplolepis tenella*

Apollin Fotso Kuate[1], Rachid Hanna[1*], Maurice Tindo[2] and Peter Nage[3].

[1]International Institute of Tropical Agriculture, B.P. 2008 Yaoundé-Messa, Cameroon

[2]Department of Animal Biology, Faculty of Science, University of Douala BP 24157, Douala

[3]Department of Environmental Sciences, Biogeography Section, University of Basel, St. Johanns-Vorstadt 10, 4056 Basel, Switzerland

*Corresponding author: r.hanna@cgiar.org; Ph. +237 2 223 3474

Abstract

Anoplolepis tenella Santschi is an afrotropical ant widely distributed in the forest zones of Central Africa. It is the most frequent species associated with the African root and tuber scale (ARTS) *Stictococcus vayssierei* (Hemiptera: Sticto-coccidae), an afrotropical insect that infests a wide range of root and tuber plants, particularly cassava. We investigated the biology and ecology of *A. tenella* which is one of the factors favoring the proliferation of the scale in the field. *A. tenella* was more abundant in the mixed-crop fields. It was rare or absent in the lowland where the scale was also absent. *A. tenella* is actively engaged in the transport and dispersal of scale crawlers. Boric acid-sucrose solution significantly killed *A. tenella* workers and queens under laboratory conditions. This result was later demonstrated in a farmer participatory trial and reduced scale infestation on cassava. These results demonstrate that control of *S. vayssierei* in cassava fields is contingent to the reduction of the density of the associated ant *A. tenella*.

Keywords: Anoplolepis tenella, Boric acid, cassava, pest, Stictococcuss vaysseirei.

Introduction

The African root and tuber scale (ARTS) *Stictococcus vayssierei* Richard (Hemiptera, Stictococcidae) is an emerging major pest of cassava in Central Africa (Hanna et al., 2004; Tatat-Hangy et al. 2006). ARTS is a subterranean

insect indigenous to the humid forest zone of Central Africa, home to some of the poorest human populations on the continent, where this insect has increasingly become a major pest of cassava since the mid-1970s. This pest, previously confined to several indigenous plants with tuberous roots has moved onto cultivated crops such as cassava, yams, cocoyam, and groundnut, with the greatest abundance occurring on cassava (Tindo et al., 2009). The pest has been reported from Cameroon, Gabon, Central African Republic, Democratic Republic of Congo (DRC), Equatorial Guinea, Republic of Congo, and extreme western Uganda (Lutete et al., 1997; Ambe et al., 1999; Bani et al., 2003; Ngeve, 2003; Hanna, unpublished data). Yield loss trials from the Bas-Fleuve district of DRC and from Central Cameroon showed that high scale densities could lead to losses of over 60% of cassava root yield and possibly an increase in losses due to cassava root rots (Hanna et al., 2004; Tata-Hangy et al., 2006). A Farmer survey in IITA's Forest Margins Benchmark in Cameroon indicated that ARTS ranked very high as a major pest concern, and is thought to be contributing to approximately 30% of cassava losses (J. Gockowski, Economist, IITA-HFS, unpublished data).

Scale abundance in cassava fields is positively related to the degree of disturbed forest cover, and to the frequency of occurrence of a closely associated ant, *Anoplolepis tenella* Santschi (Hymenoptera, Formicidae). The ant builds its nest preferably immediately adjacent to crop plants infested with ARTS (Fotso Kuate *et al.* 2006). As the scale depends on *A. tenella* for its survival, developing options to disrupt ant-scale association require adequate understanding of the biology and ecology of *A. tenella* and its impact on ARTS life cycle and ecology. In the present study, we evaluated the distribution of *A. tenella* in the dominant vegetation types in southern Cameroon, assessed its role in ARTS dispersal and tested control options using ant baits.

Figure 1: *S. vayssierei* adult female surrounded by its progeny on cassava stem.

Material And Methods

Study area

Four locations were selected in the humid forest zone of southern Cameroon based on previous trials and surveys conducted within the framework of the cassava IPM project of the International Institute of Tropical Agriculture (IITA). These four locations were all in the Center region. They included locations (1) Awae II (03° 35'37"N; 011° 36'40"E) which is administratively called Ndangueng in the Mefou-et-Afamba Division; (2) Matomb (03° 48'11"N; 011° 03' 27" E), (3) Boga (03° 53' 15" N; 010° 46' 25" E); and (4) Sombo (03° 53' 36"N; 010° 42' 26" E). Both Boga and Sombo are in the Nyong-et-Kéllé Division. Awae II and Matomb are upland areas (615 m a.s.l. < elevation < 680 m a.s.l.), while Boga and Sombo are lowland areas (201 m a.s.l. < elevation < 245 m a.s.l.)

Ant survey in the field

Ant diversity was monitored in the four locations using three methods: Pitfall traps, quadrat and baits. Sampling was done at monthly interval for 12 months in three vegetation types: mixed crop field, fallow and forest. Ant samples were sorted to genus and species. Specimen were mounted and stored at IITA Cameroun museum. Undetermined species were sent to Brian Taylor for assistance in the identification. *A. tenella* abundance (total occurrence in the samples), activity (total numbers

on individuals in pitfall traps) and density (number of individual per square meter) were determined for each vegetation type and season.

Monitoring scales dispersal by *A. tenella* worker and by queens

Anoplolepis tenella colonies were collected from the fields and maintained in artificial nest in the laboratory and used in different trials. Colonies are polygynous with several reproductive queens per nest. Ants were fed with artificial diet made with honey and a supplement of lab reared grasshoppers.

We use a set up adapted from Way (1954) to monitor the role of ant scale dispersal (Figure 2). The set up consist of basins with cassava plants with scales and *A. tenella* colony, connected by a bridge to another basin with cassava plants but free from scale. The bridge has a gap in the middle that cannot allow the crawlers to cross but ants could easily move from one side to the other. Basin borders were coated with Tanglefoot to prevent crawlers and ants to move out of their basin except through the bridge. The set up was replicated 5 times. One and two months after the set up, crawlers were check on the initially clean cassava across the bridge.

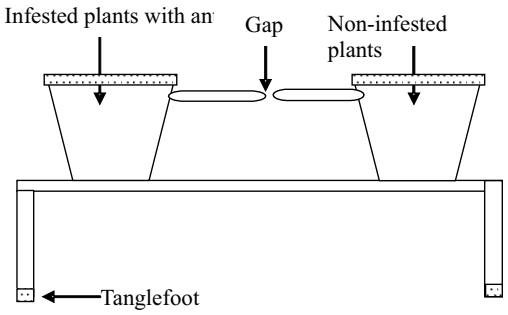


Figure 2: Experimental set up to study the dissemination of *scale* crawlers by *A. tenella* workers (Adapted from Way (1954))

Queens were also collected during nuptial flight and check for the presence of crawlers on their body parts (phoresis).

Effect of Boric acid on *A. tenella* workers

Twenty ant workers from laboratory colonies were starved for 3 days and then placed in 1L disposable plastic boxes 24 hrs preceding the test. This pretreatment holding period allowed the ants to adjust to the experimental condition prior to the start of treatments. Boric acid solution was filled in 1.5 ml-plastic vials with a cotton wool as a stopper. One gram of Amdro and Borax were offered in a Petri dish and placed in the plastic box containing the ants in the respective treatments. Workers were allowed to feed solely on it for 48 hrs. Thereafter, new Petri dishes containing cotton soaked with laboratory liquid diet were placed in the boxes and replaced at two day-interval for the remainder of the test period. Twenty replicates were set for each treatment. Control boxes were not exposed to the toxicants. Distilled water was offered to control workers in the Boric acid solution trials. All replicates were set in an incubator (Percival Scientific, USA, temperature: 24.8 ± 0.1 oC; relative humidity 75.8 ± 2.8%). Mortality was recorded daily for 10 days after initial treatment. Experiment was repeated with a mix of Boric acid (1%) and sucrose solution (10%). This was offered to A. tenella colonies in the laboratory.

The experiment was repeated in a participatory trial in Awae II where Boric acidsucrose solution was set in treated fields using homemade bait stations. Results of scale infestation on cassava in treated fields were compared to those of the control fields in which only sucrose solution were offered.

Results

Ant survey

A total of 237 ant species were recorded during the survey. Myrmicaria opaciventris was the most abundant ant species followed by Anoplolepis tenella. A. tenella abundance, density and activity were affected by vegetation types and season. Its density and activity were higher in the mixed-crop fields (Figure 3). Workers were also rare or absent at low altitude (Boga and Sombo) where S. vayssierei was also absent.

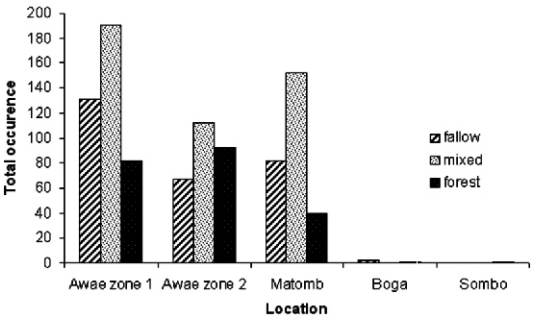


Figure 3: Occurrence of *A. tenella* in the three vegetation type

Scale dispersal

Anoplolepis tenella workers were found to be actively engaged in transport and dispersal of *S. vayssierei* crawlers. One month after the beginning of the experiment, scale crawlers were found on the initially clean cassava plants over the bridge and the number increased with time. Some workers were observed transporting scale crawlers between their mandibles over the bridge. No case of phoresis was recorded on *A. tenella* queen.

Effect of Boric acid on *A. Tenella*

The results indicated that each of the three toxicants displayed delay toxicity with less than 15% mortality during the first day. Unlike for Amdro and Borax, 1% Boric acid proved to be more effective in reducing the ant workers (Figure 4).

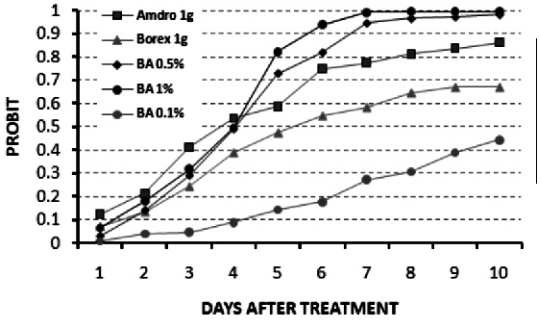


Figure 4. Cumulative proportion of workers mortality

In colony test, Boric acid -sucrose solution reduced workers and brood by 90% at 6 weeks. In field test, boric acid sucrose bait significantly reduced *A. tenella* population in treated field compared to control field ($F_{(1,94)}$=4.49; p= 0.04). However, the impact of treatment was appreciable only during the first five months after cassava planting. There were a significant difference in scale infestation on cassava between treated and control field ($F_{(1,570)}$ =6.22; p= 0.01). Consequently, cassava yield (kg per plant) was 16.11% higher in treated fields (1268.69±213.62) compared with control fields (1092.68±213.62).

Discussion

Many ant species are attracted to honeydew which is a source of carbohydrates and amino acids. The ants of the sub-families Dolichoderinae, Formicinae, Myrmicinae and some Ponerinae exploit honeydew of homopterans belonging to Aphididae, Cercopidae, Cicadellidae, Coccidae, Fulgoridae, Membracidae, Pseudococcidae, Psyllidae and Stictococcidae's families. This trophobiotic relationship is established at the detriment of the host plant. Considering the association between *A. tenella* and *Stitococcus vayssierei*, the ant benefits from scale in terms of energy derived from the honeydew, which is needed for colony maintenance and growth (Degen et al. 1986; Buckley 1987; Hölldobler and Wilson 1990; Cushman and Addicot 1991; Delabie 2001; Pierce et al. 2002). The benefit derived by hemipterans from ant attendance is classified under four main categories: (1) protection from natural enemies though their predatory behavior, (2) building shelters around Hemiptera aggregations, (3) removal of contaminated honeydew, and (4) dispersal by the transport of juvenile stages and adults to new feeding sites (Way 1963). In the experiments where the colonies of Aphids are reared in the presence/absence of their mutualistic ants, the presence of the ants increases the reproductive performances of the Aphids (Banks 1958), the developmental rate of individuals (El-Ziady 1960) and the growth of the colony (Buckley, 1987).

Boric acid solution was more promising in killing ant workers than Amdro and Borax, especially when dissolved into sucrose solution. Sugar baits exploits the natural feeding habits of sweet-eating ants that collect honeydew or nectar (Klotz and Williams 1996). This was in line with the feeding habit of *A. tenella* given that honeydew collected from hemipteran insect especially *Stictococcus vayssierei* constitute the main component of *A. tenella* diet (Fotso Kuate et al 2008). Results from this study showed that control of *A. tenella* colonies in the field can be achieved through boric acid sucrose bait solution. It also demonstrated that ant control consequently reduced scale infestation on cassava, resulting in cassava yield increase. In a similar experiment using Lorsban 4E as a 6% solution, control of Argentine ant in vineyard caused 92% decrease in mealybug cluster and 7.1% yield increase (Phillips and Sherk, 1991). Similarly, 0.5% boric acid caused significant reduction in Argentine ant with reduction in mealybug density on vineyards and less crop damage (Daane et al., 2006). Some of the benefits of scales from *A. tenella* attendance (dispersal, survival) have been demonstrated in this study and confirm the hypothesis that control of *S. vayssierei* in crop fields is contingent on the reduction of the density of associated ant *A. Tenella*.

References

Ambe, J.T., Ntonifor, N.N., Awah, E.T. and Yaninek, J.S. 1999. The effect of planting

dates on the incidence and population dynamics of the cassava root scale, *Stictococcus vayssierei* in Cameroon. Int J Pest Manage **45**: 125-130.

Bani, G., Mapangu Divassa, S., Nzemba, M.D. and Maguem, J.-M. 2003. Presence au Congo-Brazzaville de *Stictococcus vayssierei* Richard, ravageur du manioc (*Manihot esculenta* Crantz) (Hem., Stictococcidae). Bull Soc Entomol Fr. **108** (5): 529-530.

Buckley, R.C. 1987. Interactions involving plants, homoptera, and ants. Annu Rev Ecol Sys 18:111-135

Cushman, J.H., Addicott, J.F. 1991. Conditional interactions in ant-plant-herbivore mutualisms. In: Huxley CR, Cutler DF (eds) Ant-plant interactions. Oxford University Press, Oxford, pp 92-103

Daane, K.M., Sime, K.R., Hogg, B.N., Bianchi, M.L., Cooper, M.L., Rust, M.K., Klotz, J.H., 2006. Effects of liquid insecticide baits on Argentine ants in California's coastal vineyards. Crop Prot 25: 592-603.

Degen, A.A., Gersani, M., Avivi, Y. and Weishrot, N. 1986. Honeydew intake of the weaver ant *Polyrhachis simplex* (Hymenoptera: Formicidae) attending the aphid *Chaitophorus populialbae* (Hymenoptera: Aphididae). Insectes Soc 33: 211-215.

Delabie, J.H.C. 2001. Trophobiosis between Formicidae and Hemiptera (Sternorrhyncha and Auchenorrhyncha): an overview. Neotropical Entomol **30**(4): 501-516.

El-Ziady, C. 1960 : Further effect of *Lasius niger* L. on *Aphis fabae* Scropoli. Pro Roy Entomol Soc London (A) 35:30-38

Fotso Kuate, A., Tindo, M., Hanna, R., Kenne, M. and Goergen, G. 2006. Field abundance and nest structure of the ant *Anoplolepis tenella* associated with the African root and tuber scale in the Congo Basin. 1st international symposium on crops integrated pest management in the CEMAC zone. (Mouliom, P.A., Tindo, M., Ngoko, Z. and Dibog, L., Eds) University of Dschang, 6-9 December 2005. pp 203-209.

Fotso Kuate, A., Tindo, M., Hanna, R., Kenne, M. and Goergen, G. 2008. Foraging activity and diet of the ant, *Anoplolepis tenella* Santschi (Hymenoptera: Formicidae), in southern Cameroon. African Entomol **16**(1): 107-114.

Hanna, R., Tindo, M., Wijnans, L., Goergen, G., Tata Hangy, K., Lema, K., Toko, M., Ngeve, J.M., Dixon, A. and Gockowski, J. 2004. The African root and tuber scale problem in Central Africa: the nature of the problem and the search for control options. In: Book of Abstracts of the 9th Triennial Symposium of the International Society for Tropical Root Crops - Africa Branch, 31 October-5 November 2004. Mombasa, Kenya. pp 57.

Hölldobler, B. and Wilson, E.O. 1990. *The Ants*. The Belknap Press of Harvard University Press, Cambridge, Mass. 732 pp.

Klotz, J.H. and D. F. Williams. 1996. New approach to Boric acid ant bait. The IPM Pract 18:1-4

Lutete, D., Tata-Hangy, K. and Kau, T. 1997. Présence au Zaire de *Stictococcus vayssierei* (Homoptera: Stictococcidae), un ravageur du manioc (*Manihot esculenta*). J Afr Zool y **111**: 71-73.

Ngeve, J.M. 2003. The cassava root mealybug (*Stictococcus vayssierei*) (Homoptera: Stictococcidae): a threat to cassava production and utilization in Cameroon. Int J of Pest Manage **49**: 327-333.

Phillips, P.A., Sherk, C.J., 1991. To stop mealybugs, stop honeydew-seeking ants. Calif Agric 45: 26-28.

Pierce, N.E., Braby, M.F., Heath A., Lohman, D.J., Mathew, J.R., Rand, D.B. and Travassos, M.A. 2002. The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). Annu Rev Entomol 47: 73371.

Tata-Hangy, K., Legg, J., Hanna, R., Toko, M., Lema, K.M., Dixon, A. and Mahungu, N.M. 2006. Incidence and distribution of cassava diseases and pests in the Democratic Republic of Congo. Proceedings of 9th ISTRC-AB symposium: Advances in Root and Tuber Crops Technologies for Sustainable Food Security, Improved Nutrition, Wealth and Environmental Conservation in Africa. (Mahungu, N.M. and Manyong, V.M., Eds) Mombasa, Kenya, pp 614-622

Tindo, M., Hanna, R., Georgen, G., Zapfack, L., Tata-Hangy, K., and Attey, A. 2009. Host plants of *Stictococcus vayssierei* Richard (Stictococcidae) in non-crop vegetation in the Congo Basin and implications for developing scale management options Int J of Pest Manage **55**(4): 339-345.

Way M.J., 1954. Studies on the association of the ant *Oecophylla longinoda* Latr (Formicidae) with the scale insect *Saissetia zanzibarensis* Williams (Coccidae). *Bull Ent Res* 45: 113-134