Program B

Developing Plant Health Management Options

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Project B
Developing plant health management options

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Project rationale

This project aims to improve the food security and well-being of rural and urban Africans by providing farmers with environmentally sound options for pest, disease, and weed control. Its guiding principles are ecological stability and agricultural productivity.

In a make-believe agricultural paradise, farmers would regularly harvest 100% of the genetic potential of the seeds and cuttings they plant. In Africa, bumper crops of cowpea, cassava, maize, banana, yam, and soybean would continuously replenish farm granaries, reliably supply family meals all year round, and generate surpluses that could be sold for cash.

But the real world of day-to-day farming in Africa and other tropical regions is starkly different. A host of insects, aggressive weeds, microscopic pathogens, and other noxious organisms prey on or compete with crops, substantially reducing yields. These "biotic" stresses make agricultural livelihoods uncertain, even precarious, and are a perennial threat to food security. For example, an aggressive form of *African cassava mosaic virus* (ACMV), transmitted by whiteflies, caused total crop failure in parts of Uganda in the early 1990s, resulting in a number of deaths by famine.

Moreover, damage to grains before harvest often leads to storage molds such as the fungus *Aspergillus flavus*, which produces highly toxic and carcinogenic Aflatoxins.

A big challenge for African farmers and IITA researchers, then, is to systematically and continuously dampen the effects of biotic stresses on crops and, ideally, learn to better predict the location and timing of outbreaks. But control measures must be executed without disrupting or destroying the local biodiversity—both friends and foes of food crops that sustain and regulate agroecosystems.

This project builds on IITA's internationally recognized expertise and considerable successes in the management of biotic stresses. It delivers a wide range of plant health management options to farmers throughout sub-Saharan Africa. Research products fall into four major groups:

- Biological control methods, such as the rearing and release of natural enemies to fight mealybugs and cassava green mites.
- Plant protection products, especially biopesticides based on botanical extracts—from papaya or neem, for example or on entomopathogenic fungi, such as *Metarhizium* which is highly effective against grasshoppers and locusts.
- Habitat management techniques, such as eliminating weeds and other plants that serve as disease reservoirs, or mulching crops with plant species shown to be effective against nematodes and other pests.
- Host plant resistance, in the form of improved crop lines developed through cultivar screening and breeding.

Designing and deploying such biologically based solutions is knowledge-intensive. This is due to the vast diversity and complexity of biotic stresses in agriculture and the fact that they are moving targets, constantly adapting to their environment. Project scientists must therefore continually investigate the distribution and biology of pests and their natural enemies, as well as interactions between pests, host plants, natural enemies, and the environment. This requires substantial expertise in plant science, molecular genetics, entomology, mycology, virology, and geographic information systems. The project provides diagnostic and information services for plant protection research by IITA and partner organizations and maintains reference collections and colonies of pests and their natural enemies. All this is done in collaboration with national research programs in Africa, while at the same time building their capacity to develop and apply solutions to pest management problems.

Goal

Improve food security and well-being of rural and urban populations, through enhanced ecological stability and agricultural productivity by developing sustainable plant health management options.

Purpose

Develop effective and ecologically sound options to manage major pest problems and sustain biodiversity in the humid forest, savanna, and midaltitude agroecological zones of sub-Saharan Africa.

Outputs

1 Knowledge base on distribution and biology of pests and their natural enemies improved, and agrobiodiversity characterized

Ongoing and future activities

1.1 Distribution of soybean rust in Nigeria

by R.B. in collaboration with M. Twizeyimana

Soybean rust is a destructive disease and was first reported from Nigeria in 2001. A field survey was conducted across different soybean-growing areas in Nigeria to identify the rust-prevalent areas. During this survey, soybean rust incidence and severity were determined in all locations visited. A total of 150 locations were surveyed and located in 14 states (Oyo, Ogun, Osun, Ekiti, Ondo, Kogi, Benue, Nasarawa Plateau, Kano, Kaduna,

Niger, Kwara, and FCT Abuja). The survey showed that soybean rust infection was very high in Benue State, followed by Osun and Oyo, the rust infection was mild or absent in Ogun, Kwara, and Niger states. We will continue to monitor the disease in Nigeria and elsewhere in the region.

1.2 Monitoring the CMD pandemic

by J.P.L. and M.To. in collaboration with S. Bigirimana, G. Okao-Okuja, R. Obonyo, P. Ntawuruhunga, H. Obiero, S. Ajanga, I. Ndyetabula, S. Jeremiah, and T. Hangy

In partnership with national research teams, an extensive monitoring and diagnostics program was implemented, covering more than 300 sites across East and Central Africa including Kenya, Tanzania, and Burundi. These surveys demonstrated the continued expansion of the CMD pandemic. In Kenya, further southwards spread was recorded in Nyanza Province towards the border with Tanzania. The pandemic-associated virus, *East African cassava mosaic virus – Uganda* (EACMV-UG), has now been recorded virtually throughout the cassava-growing areas of western Kenya. In Tanzania, further southwards spread was recorded in Kigoma region. It appears likely that EACMV-UG will reach Kigoma town on the Lake Tanganyika shoreline in 2005 or 2006. This represents a significant new spread threat since movement of the virus along the shores of this large lake should be relatively easy, a fact that could lead to the rapid spread of the virus to parts of central Tanzania, Democratic Republic of Congo, and Zambia further to the south. On the eastern side of Lake Victoria, within Tanzania, first records were made of the occurrence of severe CMD in areas immediately to the south of the Kenyan border. This represents a disturbing new development and suggests that the "encirclement" of Lake Victoria by EACMV-UG is imminent. Reports of superabundant populations of the whitefly vector of cassava mosaic geminiviruses, *Bemisia tabaci*, were received from various locations in Tanzania, as well as for the first time in western Kenya. The whitefly vector was similarly abundant in parts of Burundi affected by the pandemic. Symptoms of physical damage were often associated with these raised populations, and this seems to be a universal phenomenon in pandemic-affected areas.

In Burundi, in a countrywide survey, EACMV-UG was detected for the first time in Cibitoke, Bubanza, Cankuzo, and Muramvya provinces, leaving only four of the 16 provinces yet to be affected. Severe CMD had a major impact on the first-affected northern provinces, Kirundo and Muyinga, as drought accompanied the severe CMD spread and there were widespread food shortages.

1.3 Characterization of cassava mosaic geminiviruses

by J.P.L. in collaboration with J. Ndunguru, G. Okao-Okuja, R. Obonyo, B. Owor, P. Sseruwagi, P. Ntawuruhunga, H. Obiero, S. Ajanga, I. Ndyetabula, S. Jeremiah, and T. Hangy

More than 1000 CMG diagnoses were completed during the year in the IITA-Uganda laboratory with the primary aim of mapping the spread of the pandemic associated EACMV-UG.

A survey of 94 sites in all 16 provinces of Burundi revealed the occurrence of EACMV-UG in 46% of virus-diseased samples collected, and of these, 51% were in mixed infection with ACMV. In common with similar previous studies, there was a clear association between the occurrence of EACMV-UG with severe symptoms and the most severely-diseased plants had mixed *African cassava mosaic virus* (ACMV) + EACMV-UG infections. In contrast to recent virus data from Tanzania, in Burundi, there was considerable uniformity. EACMV

had been detected from a single sample in 2003, but this was absent from the 2004 survey material, and only two virus species/strains were recorded, EACMV-UG and ACMV.

By contrast, characterization of cassava mosaic geminiviruses in Tanzania revealed a unique level of diversity. The DNA A and B components of seven isolates were fully sequenced. The most striking finding was the occurrence in southwestern Tanzania of two isolates of *East African cassava mosaic Cameroon virus* (EACMCV). These showed a high level of homology with previously described isolates of EACMCV from Cameroon, with 92% sequence identity in DNA A. Isolates collected from other parts of Tanzania, most notably along the eastern coastal zone, shared greatest identity with previously characterized EACMV from Kenya, whilst isolates that were virtually identical to EACMV-UG occurred in the northwestern part of the country, which is the area currently affected by the severe CMD pandemic. This study provided the first full sequence of ACMV from Tanzania. This was found to be 95–97% identical to previously sequenced ACMV isolates from both East and West Africa. The recombined portion of DNA previously reported for EACMCV-CM was also present in EACMCV isolates from Tanzania, but these isolates had additional unique recombinations. This evidence suggests a relatively ancient separation of these isolates and does not support the posit of a more recent introduction. The level of CMG diversity identified in Tanzania suggests that this is an important center of diversity for these viruses within Africa.

1.4 Molecular markers to track CMD epidemic associated *Bemisia tabaci* biotypes in East and Central Africa

by J.P.L. in collaboration with P. Sseruwagi and J. Brown

Bemisia tabaci whiteflies collected in Uganda were characterized through sequencing a c. 850bp portion of the mitochondrial cytochrome oxidase gene (MtCO1), in collaboration with the University of Arizona. In a study examining the host range of "cassava biotypes" of *B. tabaci*, whitefly samples were collected from cassava and 22 other crop and weeds hosts in and around cassava fields. Sequences of MtCO1 were obtained for both adults and nymphs collected on these hosts. Based on the results of the nymphs, the cassava-colonizing Ug1 genotype cluster was found to occur on five non-cassava plant species including: *Manihot glaziovii, Jatropha gossypifolia, Euphorbia heterophylla, Aspilia Africana,* and *Abelmoschus esculentus.* Previous studies have suggested that cassava-colonizing *B. tabaci* populations in Africa are more or less cassava-restricted. These data, in contrast however, suggest that the host range is larger than previously recognized, at least for the environment in central Uganda. Population dynamics data collected before and after the passage of the CMD pandemic have demonstrated the almost 100 fold increase in *B. tabaci* populations on cassava under "post-epidemic" conditions. Whether an increase in host range is a facet of the biology of *B. tabaci* that has contributed to this change remains to be determined.

In a related study, eight genotype clusters of *B. tabaci* were identified from crop and weed hosts in central Uganda, representing an unprecedented level of diversity for a single region. In addition to the two previously recorded cassava-colonizing genotypes, Ug1 and Ug2, a third hitherto undescribed genotype, Ug3, was recorded from a wild mint species, *Ocimum gratissimum*. Other genotypes recorded were B and B-like, Côte d'Ivoire okra-like, and the Uganda sweetpotato type. The finding of the "B biotype" in Uganda is particularly significant. Molecular data were supported by biological assays using squash plants in which B biotype colonized plants produced the characteristic silverleafing symptom elicited by the B biotype. Wherever the B biotype has been recorded elsewhere (USA, Latin America,

Caribbean, Asia, Middle East, Southern Europe), it has been characterized by unusually high populations and a wide host range. In most situations, this has led to it achieving major pest status. Significantly in Uganda, however, populations of the B biotype were low on the small number of host plants on which it was reported. This raises the intriguing possibility that the B biotype may actually be indigenous to East Africa, and held under control here by an existing natural enemy fauna. In 2005, studies are planned first to confirm 100% sequence homology between Ugandan Bs and those from other locations across the globe. Attempts will also be made to determine the factors responsible for sustaining Ugandan B populations at the current low levels. This information could have particular significance for management efforts being undertaken in the parts of the world currently affected by pest populations of the B biotype.

1.5 Surveys for cassava pests and diseases in DRC

by A.K.L., J.L., R.H., M.M., R.B., and M.To. in collaboration with K. Tata-Hangy

During 2004, surveys were carried out in eastern provinces of DRC, i.e. Maniema, Nord Kivu, Sud Kivu, and Province Orientale. Like in the western part of the country, cassava mosaic disease was found to be the most important production constraint for cassava in these provinces. All three viruses (virus strains) of the cassava mosaic disease: African cassava mosaic virus, east African cassava mosaic virus, and the most virulent Ugandan strain (EACMV-Ug) were reported from Nord Kivu, Sud Kivu, and Province Orientale, while most of southern Maniema was still free from EACMV-Ug. The African root and tuber scale was reported damaging cassava in the forest zones of Bénin (Nord Kivu) and Kisangani (P. Orientale). Root-rots are a major constraint to cassava production in all the four provinces. Over 80 % of farmers in Nord Kivu and 95% in Kisangani area have observed root-rots in their fields. Nematodes were also observed as important pests in the eastern part of DRC.

1.6 Diagnostic survey of biophysical constraints to cassava production in southern Cameroon

by C.N., M.M., R.B., D.C., M.Ti., and R.H.

Cassava tuber yields in southern Cameroon are low. The fresh tuber yield in farmers' fields ranges typically from 5 to 10 Mg ha^{-1}. Such a low yield hinders the intensification and commercialization of cassava production. For example, in the Pouma area, halfway between Douala and Yaoundé, UNDP and UNIFEM set up in 1993 a starch-producing factory that could not run at full capacity because of supply problems. Though these were partly due to a wrong pricing policy, the level of production was also far too low. The factory management approached IITA for assistance to raise cassava production in order to revive the factory. Poorly adapted germplasm along with a complex of pest/diseases and soil nutrient constraints was suspected to limit cassava yield. A diagnostic survey was organized to deliver insights into the major biophysical problems of cassava in the region. This would form an excellent basis for subsequently designing, together with farmers, targeted intervention trials. It would also give breeders information about what kinds of plant characteristics are required for intensive production.

Observation plots of 10 x 10 m were established in March/April 2003 in 62 farmers' fields of eight villages (Ngompem, Bihiang, Nkonga, Songsimout, Nkondjock, Sokele II, Pouma center, Sibongo) around Pouma, a village 141 km southwest of Yaoundé. The villages were selected based on discussions with representatives of the farmer organization

AID Cameroun and the *agent de vulgarisation de zone* (AVZ), responsible for the Pouma *arrondissement*. Fields were selected based on planting dates for cassava, in order to minimize variation caused by planting date. Observations for stem, root and tuber rots, anthracnose disease (CAD), bacterial blight (CBB), and cassava mosaic disease (CMD) infections were scheduled at 6, 9, and 12 months after planting (MAP). In each field, five plants were visually examined for presence of different disease symptoms. The severity scores for each of the foliar diseases were taken using a scale of 1–5. For rot assessment, five plants were uprooted and inspected for superficial presence of fungi and actual rotting of the tuberous roots. Volume of roots rotted was estimated for each plant and is termed as rot severity. Samples of rotten tissue were collected for isolation and identification of the fungi in laboratory.

Cassava root rots. At 6 MAP, 41% fields were free from root rot symptoms (Table 1). Low level (less than 10% roots rotted) of root rot was observed in 55% of the fields. About 2% fields had between 11 and 25% of their roots rotted, while more than 50% of root volume was rotted in nearly 2% fields. In such severely infected plants, superficial sign of fungal presence was high on the mother cuttings from where mycelia were seen extending to the base of the stems and to the young tuberous roots.

At 9 MAP, 24% fields were free from root rot symptoms (Table 1). In nearly 64% of the fields the rotted roots had less than 10% of their volume spoilt while in 10% of the fields rotted roots had up to 25% of their volume spoilt. Only about 2% of the fields had an average of up to 50% of the root volume rotted. Complete rotting of tubers in a few plants was observed in only two villages.

At 12 MAP, when 10 plants were evaluated, 44% of the field plots were free of rot symptoms (Table 1). Nearly 46% of the field plots had less than 10% of their total volume rotted, while 11 % of the fields had up to 25% of their total volume rotted. Pathogens most frequently isolated from rot specimens were *Botryodiplodia theobromae, Macrophomina phaseolina, Fusarium* sp., *Sclerotium rolfsii, Armillaria* sp., *Aspergillus* sp. and *Trichoderma* sp.

Table 1. Incidence and severity of cassava root and tuber rots at six and nine months after planting (MAP) in the Pouma area of Cameroon.

	6 MAP		9 MAP		12 MAP	
	Inc	Sev	Inc	Sev	Inc	Sev
Root rot %						
0	41.4	41.4	24	24	43.6	43.6
1–10	34.5	55.2	39.7	63.8	47.3	45.5
11–25	20.7	1.7	31	10.3	9	10.9
26–50	1.7	0	5.2	1.7	0	0
>50	1.7	1.7	0	0	0	0

A total of 58 fields were evaluated. Four fields were not evaluated due to poor access after being abandoned by their owners.

Cassava mosaic disease. At 3 MAP, CMD symptoms were observed in all fields. Plants in 39% fields were moderately infected (level 2.1 to 3) with mild chlorotic to strong mosaic patterns observed on the leaves (Table 2). Plants in 51% fields had severe infection (level 3.1 to 4) with initial signs of leaf distortion and leaf sizes being reduced in some cases. Plants in 10% fields had severe mosaic with many leaflets distorted. At 6 MAP, plants in 72% fields were moderately infected with only mild chlorosis. At this time, plants in 26% fields were severely infected (level 3.1 to 4), which was a reduction when compared to 3 MAP. At 9 MAP, plants in 93% fields were moderately infected (level 2.1 to 3), while plants in the other 7% fields had severe infection. In general, CMD severity decreased as the plant age increased. At 12 MAP, plants in 80% of the fields were moderately infected while only plants in 7% of the fields were severely infected.

Cassava Bacterial blight. At 3 MAP, plants in 93% fields were slightly infected (level 1.1 to 2), showing only angular leaf spots (Table 3). At 6 MAP, infection in 67% fields was severe (level 3.1 to 4) with blighting of leaves, defoliation, and initiation of wilting. Plants in the other 33% fields had moderate infection (level 2.1 to 3). At 9 MAP, plants in 55% fields had severe infection (level 3.1 to 4), while 43% were moderately infected. At 12 MAP, the disease level was about the same as observed at 9 MAP, with plants in 53% fields being severely infected, while plants in 46% fields had moderate infection.

Anthracnose disease. At 3 MAP, plants in 82% were not infected. The other 18% fields had only slight infection with cankers mostly on the lower part of the stem (Table 4). At 6 MAP, anthracnose symptoms were observed in all fields but the severity of infection varied between fields. Plants in 65% fields had moderate infection (level 2.1 to 3) meaning cankers were just beginning to spread from the lower to the middle part of the stem. Plants in about 20% fields had severe infection (above level 3) with cankers having completely

Table 2. Severity level of *Cassava mosaic virus* infection at 3, 6, 9, and 12 months after planting (MAP) in Pouma area of Cameroon.

Mean score	3 MAP	6 MAP	9 MAP	12 MAP
1 (disease-free)	0[a]	0	0	0
–2	0	1.7	0	12.7
2.1–3	39	72	93	80
3.1–4	51	26	7	7.3
4.1–5	10	0	0	0

[a] Figure is the percentage number of fields with the level of infection shown in column 1. At 3 MAP 61 fields were evaluated, 10 plants per field. At 6 and 9 MAP 58 fields were evaluated, 5 plants per field.

Table 3. Severity level of cassava bacterial blight at 3, 6, 9, and 12 months after planting (MAP) in Pouma area, Cameroon.

Mean score	3 MAP	6 MAP	9 MAP	12 MAP
1 (disease-free)	1.6[a]	0	0	0
1.1–2	93	0	3.5	1.8
2.1–3	5	33	43	45.5
3.1–4	0	67	55	52.7
4.1–5	0	0	0	0

[a] Figure is the percentage number of fields with the level of infection shown in column 1.

Table 4. Severity level of cassava anthracnose infection at 3, 6, 9, and 12 months after planting (MAP) in Pouma area, Cameroon.

Mean score	3 MAP	6 MAP	9 MAP	12 MAP
1 (disease-free)	82[a]	0	0	0
1.1–2	18	14	14	54.5
2.1–3	0	65	71	43.6
3.1–4	0	19	15	1.8
4.1–5	0	2	0	0

[a] Figure is the percentage number of fields with the level of infection shown in column 1.

spread to the green part of stem. At 9 MAP, plants in 71% fields had a moderate level of infection (level 2.1 to 3), while 15% fields had severe infection (level 3.1–4). At 12 MAP, anthracnose was observed in all fields but the number of fields with moderately infected plants had reduced to 44%.

The observation of tubers with more than 50% of their volume rotted at six months after planting was significant in that root rots have been assumed to be of importance only in the later stages of cassava plant growth. On the overall, data obtained from this study indicate that root rots could cause substantial loss to cassava production in the Pouma area. However, the extent of loss incurred also depends on how the tubers are utilized. In Pouma, much of the cassava is processed into *baton manioc* for sale or is consumed at home, which means the part of the tuber that is damaged by rot can be chopped off and discarded and the rest utilized. Also, the loss realized could be minimal if cassava is harvested at 12 months. For CBB and CAD, the severity increased significantly between 3 and 6 MAP, which could have been caused by the wet weather prevailing in this period. In conclusion, these two diseases could be of concern in Pouma if wet weather continues for extended periods of time.

1.7 Survey of fungal rot diseases as constraints to cassava production in east DR Congo

by M.M. and R.B. in collaboration with INERA staff

The Democratic Republic of Congo (DRC) is ranked the second highest producer of cassava in Africa and the fifth highest worldwide with almost 15 million tonnes. In 2000, production was estimated to be 15.5 million tonnes, but in the past few years production is known to have reduced significantly especially due to cassava mosaic disease. Tuberous root-rots are known to be a constraint to cassava production in the humid forest and forest transition agroecologies of Central and West Africa. In early 2004, rots were studied as part of a diagnostic survey carried out to determine the occurrence and distribution of major pests and diseases limiting cassava production in the eastern and northern provinces of DR Congo. The survey was carried out in collaboration with NARS partner (INERA). In South Kivu, sites were selected along the Kalehe axis, while in North Kivu, sites were along Masisi and Rutshuru. More sites were surveyed along the 400 km route from Goma to Butembo/ Beni. In Oriental Province, sites were around Kisangani along the routes to Lubutu, Ituri, and Buta. A total of 61 fields were visited. In all cases, minimum intervals of 10 km were allowed between fields. Where fields with mature plants (>10 months) were available, 10 plants were selected randomly, uprooted, and examined for rotting. A questionnaire was used to collect more data by interviewing owners of the selected fields on their perception of the occurrence of cassava tuber rots in their fields.

The survey area could broadly be placed into a forested or nonforest area. Rots were reported to be serious constraint by 53% farmers in the nonforest areas and by 68% farmers in forest areas. About 30–50% of interviewed farmers reported occurrence of rots even before cassava reaches maturity, and that delayed harvesting further enhanced rotting. High rotting appeared to be related to insufficient land for rotation in the highly populated nonforest areas, affecting 84% of farmers. In the forest areas, over 80% of farmers prefer to leave mature plants in the soil for gradual harvesting which is likely to enhance rotting. Farmers in the forest areas also prefer sweet varieties for fresh consumption and because they mature faster. These sweet varieties were reported to be more vulnerable to rots than bitter varieties.

The study concludes that root rots are an important constraint to cassava production in the eastern parts of DRC and can impact negatively on food security of millions inhabiting the region. The study also shows that ecological, socioeconomic, and demographic factors strongly influenced root-rot problem and these factors should be considered while designing intervention strategies for root-rot management. It is recommended that focused research be conducted to search for resistant varieties that can be grown especially in the Kivu areas where opportunities to fallow or rotate are few due to land scarcity. In the forest areas where land is available, farmers could be trained to use varieties of different maturities or stagger planting so that cassava matures at different times in the year rather than all in one season. This will reduce the need to leave mature plants in the soil as a form of storage for prolonged periods thereby making fresh good quality tuberous roots available for extended periods. Training in cultural measures for disease management could contribute substantially.

1.8 Evaluating cassava yield losses due to root-rot and the impact of this disease on rural households and food security in south Cameroon

by M.M., R.B., and J.G.

Various studies have indicated root rots are an important and increasing constraint in cassava production in the humid forest areas. To enable targeting and implementation of intervention measures, it is necessary to identify the causes of the rots and understand the disease dynamics. This study was organized to investigate the extent of cassava yield loss caused by root rots in south Cameroon, study the effect of the rot diseases on the livelihoods of people that are mostly dependent on cassava for food and income, and identify the pathogens responsible. Additionally, the study aims to investigate whether there is a relationship between occurrence of root-rot diseases and the presence of the African root and tuber scale (ARTS).

The trial was designed to take place in four locations, three located within the forest benchmark area (Awae, Akok, Nkometu) and the fourth, Nkolmeyang (nearer Yaoundé), an area noted for its supplies of fresh cassava root to the Yaoundé market. In each village, 15 farmers who planted a cassava crop in August/September 2003 were identified. The 15 farmers were also required to plant a new crop in March/April 2004. Fields were selected in each site so as to have some fields established in long fallow fields converted from bush and secondary forest vegetation and others located in shorter duration fallow lands where *Chromolaena odorata* is the primary vegetation and residual cassava from previous cropping cycles is common.

For each planting season, cassava plants are sampled at 6, 9, and 12 months after planting (MAP). At each sampling time, 5 plants are uprooted for assessment. The number of plants with rot in each field and the number of tuberous roots with rot per plant is recorded as disease incidence. The volume of each tuberous root destroyed by rot is estimated and recorded as disease severity. Plant specimens with rot symptoms are brought to the laboratory for isolation and identification of pathogens. In addition, a questionnaire was used to get information about farmers' perception of root rots relative to other cassava pests and diseases (ARTS, ACMV, green mite, rodents, etc.).

In the first season, rots were observed in all villages on six month-old cassava plants. At 6 MAP, rot incidence was highest at Nkometou with 46.7% of the fields having 1–10% of all tubers rotted, followed by Akok with 20% fields having 1–10% tubers rotted, 7% fields with 11–25% tubers rotted, and 7% fields with 26–50% tubers rotted. In Awae and Nkolmeyang, about 20% fields had 1–10% tubers rotted.

At 9 MAP, rot incidence was highest in Akok with 60% fields having 1–10% tubers rotted and another 13% fields with 11–25% tubers rotted. In Nkometou, rots at 9 MAP affected 40% fields with 1–10% tubers rotted and an additional 7% fields with up to 25% tubers rotted. There was a significant increase in rotting in Nkolmeyang where more than 30% of the fields had over 10% of their tubers rotting.

At the scheduled sampling time at 12 MAP, some farmers had already harvested in Nkomeyang (67%), Akok (40%), and Awae (13%). From the remaining fields, rots were observed to be highest in Awae where 14% fields had 25% or more of the harvested tubers rotted. In Akok, 33% of fields had between 1–10% of all tubers rotted.

In the second season, rotting at 6 MAP was less in the first season in all villages. Incidence was still highest at Nkometou with 20%fields having 1–10% of the tubers rotting. At Akok and Nkolmeyang, about 15% fields had 1–10% of all tubers rotting. The second sampling in the second season was done when plants were 10 months old. At that time, rot incidence was 40% at Akok and Nkometou but severity was higher at Akok where 20% fields had 1–10% tubers rotted and another 20% fields had 11–25% of all tubers rotted. Although incidence was lower at Awae (27% fields), rotting was much more severe with 7% of fields having 11–25% tubers rotted and another 7% of fields having more than 50% of harvested tubers rotted. The last sampling is scheduled for April 2005 when the plants will be 12 months old.

Most of the rotting appeared to be accused by *Fusarium* species pathogens, in many cases occurring together with *Armillaria* infections. Other pathogens isolated were *Botryodiplodia theobriomae,* and *Sclerotium rolfsii,* in addition to several other opportunistic fungi. Although tuber scales in many farms where the trials were carried out heavily attacked cassava tubers, it did not appear to be an implicit relationship between scale injuries and the occurrence of rots. A more conclusive report on this aspect is expected once the final harvest is done. Data on household economics and how tuber diseases affect livelihoods have been collected and are being analyzed by socioeconomists.

1.9 Natural occurrence of *Fusarium* and subsequent fumonisin contamination in preharvest and stored maize in Bénin, West Africa

by K.H. in collaboration with P. Fandohan and B. Gnonlonfin*

The natural occurrence of *Fusarium* and fumonisin contamination was evaluated from 1999 to 2003 in both preharvest and stored maize produced by small-scale farmers in

four agroecological zones of Bénin. Mycological analysis revealed a predominance of both *Fusarium* and *Aspergillus* in maize samples compared to other genera. The two *Fusarium* species most commonly isolated from maize were *Fusarium verticillioides* (68%) and *Fusarium proliferatum* (31%). A typical isolates of *F. verticillioides* with some characteristics of *Fusarium andiyazi* but apparently closer to *F. verticillioides*, because the isolates were all high fumonisin producers, were also found only on preharvest maize. A study of *F. verticillioides* strains showed the presence of extremely high fumonisin producers in Benin with total fumonisin levels ranging from 8240 to 16690 mg/kg. Apart from 2002–2003, *Fusarium* occurrence was not significantly different from one zone to another, although a slight decrease was observed from south, humid, to north, drier. *Fusarium* occurrence varied somewhat from one season to another. It significantly decreased over the six months of storage. Widespread fumonisin occurrence in maize was observed. Most of the maize samples collected were found positive for fumonisin with levels ranging from not detected to 12 mg/kg in 1999–2000, 6.7 mg/kg in 2000–2001, and 6.1 mg/kg in 2002–2003. Fumonisin levels in maize were found to be significantly higher in the two southern zones during all the surveys. The highest mean total fumonisin level was detected in 1999–2000 in maize samples from the southern Guinea savanna (SGS) (12 mg/kg), whereas in both 2000–2001 and 2002–2003, it was in samples from the forest mosaic savanna (FMS) (6.7 and 6.1 mg/kg, respectively). Fumonisin levels varied from one season to another and, throughout the storage time, showed a decreasing trend in each zone. However, this decrease was not significant every season. An increasing trend was observed during some seasons in the SGS and northern Guinea savanna (NGS) zones. The results of this study emphasize that farmers and consumers, not only in Bénin but also in other West African countries, should be alerted to the danger of fumonisin contamination in maize.

1.10 Factors influencing *A. flavus* strains and toxins expression in different agroecozones

by K.H. in collaboration with T. Ekanao, B. Hau, and N. Holst

Soil samples were collected in the four agroecological zones in Bénin to determine *A. flavus* strain distribution. In the four agroecological zones, we collected from 100 farms soil samples. We have collected a total of 484 isolates. The type of strains (S or L) was determined by observation on V8 medium and we have:

- Coastal savanna (Z1): 0 S-strains over 125 isolates
- Southern Guinea savanna (Z2): 6 S-strains over 154 isolates
- Northern Guinea savanna (Z3): 31 S-strains over 97 isolates
- Sudan savanna (Z4): 31 S-strains over 108 isolates

The determination of non-toxinogen strains among L-strains started for Z1 and up to now we have 49 non-toxingenic that have to be confirmed. We have not yet started with Z2, Z3, and Z4.

1.11 Dietary exposure to aflatoxin from maize and groundnut in young children from Bénin and Togo

by K.H. in collaboration with S. Egal, A. Hounsa Y.Y. Gong, P.C. Turner, and C.P. Wild

Aflatoxins are a family of fungal toxins that are carcinogenic to man and cause immunosuppression, cancer, and growth reduction in animals. We conducted a cross-sectional study among 480 children (age nine months to five years) across four agroecological zones (SS,

NGS, SGS, and CS) in Bénin and Togo, to identify the effect of aflatoxin exposure on child growth and assess the pattern of exposure. Prior reports on this study showed that aflatoxin exposure among these children is widespread (99%) and that growth faltering is associated with high blood aflatoxin-albumin adducts (AF-alb adducts), a measure of recent past exposure. The present report demonstrates that consumption of maize is an important source of aflatoxin exposure for the survey population. Higher AF-alb adducts were correlated with higher *A. flavus* (CFU) infestation of maize ($p = 0.006$), higher aflatoxin contamination (ppb) of maize (p b0.0001) and higher consumption frequencies of maize ($p = 0.053$). The likelihood of aflatoxin exposure from maize was particularly high in agroecological zones where the frequency of maize consumption (SGS and CS), the presence of aflatoxin in maize (SGS), or the presence of *A. flavus* on maize (NGS and SGS) was relatively high. Socioeconomic background did not affect the presence of *A. flavus* and aflatoxin in maize, but better maternal education was associated with lower frequencies of maize consumption among children from the northernmost agroecological zone (SS) ($p = 0.001$). The impact of groundnut consumption on aflatoxin exposure was limited in this population. High AF-alb adduct levels were correlated with high prevalence of *A. flavus* and aflatoxin in groundnut, but significance was weak after adjustment for weaning status, agroecological zone, and maternal socioeconomic status (resp. $p = 0.091$ and $p = 0.083$). Ingestion of *A. flavus* and aflatoxin was high in certain agroecological zones (SS and SGS) and among the higher socioeconomic strata due to higher frequencies of groundnut consumption. Contamination of groundnuts was similar across socioeconomic and agroecological boundaries. In conclusion, dietary exposure to aflatoxin from groundnut was less than from maize in young children from Bénin and Togo. Intervention strategies that aim to reduce dietary exposure in this population need to focus on maize consumption in particular, but they should not ignore consumption of groundnuts.

1.12 Cassava and yam chips pests and diseases

by K.H. in collaboration with P. Fandohan, B. Gnonlonfin, and B. Siame

Fungal contamination and insect pests were analyzed on 148 yam chips samples, 76 cassava chip samples, and 20 dried pounded yam samples determined. Moisture content, fungal infection and toxin content was determined. The fungi observed belonged to the *Aspergillus*, *Fusarium*, *Penicillium*, *Phoma*, *Rhyzopus*, *Mucor*, *Emericella*, *Chaetomium*, and *Neosartorya* species. Moisture content for yam chips varied between 10.6 and 15.8% and for cassava chips between 9.7 and 15.0%. About 59.2% of the cassava chip samples have a moisture content of less than $\leq 12\%$, whereas only 10.3% of the yam chips were sufficiently dry not to risk fungal contamination. Again, 30.7% of the analyzed samples were infected with *A. flavus*, with 23.7% of the cassava samples showing *A. flavus* contamination and 36.5% of the yam chips being infected with these fungi. *F. verticilloides* was only found on four samples. This work will provide the baseline data for efforts to control pests and diseases in processed chips to be developed into a project proposal.

1.13 Genetic, morphological, and pathogenic conformity of the yam nematode *Scutellonema bradys* from West Africa established

by D.C. in collaboration with V. Williamson, B. Hughes, A. Tchabi, L. Lalbanna, and N. Labuschagne

In West Africa, the yam nematode, *Scutellonema bradys*, is responsible for causing dry rot of yam (*Dioscorea* spp.). In order to identify resistance to *S. bradys* in yam, it is important that the conformity of the nematode across the yam-growing region is determined. Yam

tubers with symptoms of dry rot and typical nematode damage produced in Nigeria, Togo, Bénin, Burkina Faso, Mali, Ghana, and Côte d'Ivoire were collected. Nematodes were extracted for measurement of genetic and morphological characteristics and assessed for their pathogenicity on yam (*Dioscorea rotundata*). Genetic assessment was undertaken following amplification of internal transcribed spacer (ITS-1, 2) gene by PCR using 10 individuals from each of 26 geographically separate isolates. In addition, PCR-RFLP using Rsa1 digested samples were also performed. Species-specific primer sets were designed using the ITS rDNA alignments of *S. bradys* isolates and other plant parasitic nematodes and amplified ITS-1, 2 rDNA using genomic DNA as a template. Morphological characters (quantitative and qualitative) were measured using a compound microscope. Pathogenicity was assessed by inoculating 250 g seed setts with 1000 *S. bradys* before planting and measuring growth and damage parameters after six months. Nematodes from dry rot infected tubers from all sites across the region were identified as *S. bradys*. Morphologically, characters differed little between sample sites (isolates). In Bénin however, one isolate was different ($P \leq 0.05$) to others, particularly in relation to spear length. Furthermore, there were morphological variations among individuals belonging to the same isolate. Genetically, substantial polymorphism was observed between different individuals within an isolate and between isolates in Bénin. Seven different genetic patterns were identified using Rsa1 digested PCR-RFLP patterns of the ITS gene. Some polymorphism was also observed between samples from sites across West Africa. All isolates reduced ($P \leq 0.05$) yam tuber weights compared with uninoculated, but not compared with each other.

1.14 Distribution and prevalence of nematodes (*Scutellonema bradys* and *Meloidogyne* spp.) on marketed yam (*Dioscorea* spp.) in West Africa established

by D.C. in collaboration with B. Hughes, A. Tchabi, and N. Labuschagne

The distribution, population density, and incidence of plant parasitic nematodes and associated damage to yam (*Dioscorea* spp.) tubers obtained from market stalls in the West African countries of Bénin, Burkina Faso, Côte d'Ivoire, Ghana, Mali, Nigeria, and Togo was determined during the tuber storage periods in 2002 and 2003 (Fig.1). A total of 527 yam tuber samples, exhibiting typical nematode (*Scutellonema bradys*) damage symptoms, were collected and assessed for *S. bradys* densities. In addition, 25 318 tubers on sale in markets were assessed for visual symptoms (except in Nigeria) of nematode damage (*S. bradys* and *Meloidogyne* spp.). *Scutellonema bradys* was present in all countries assessed, with greatest ($P \leq 0.05$) mean population densities occurring in tubers in Bénin (397/g), followed by Nigeria (248/g), and lowest in Togo (28/g). When analyzed by agroecological zone, the greatest ($P \leq 0.05$) mean *S. bradys* density was observed in the midaltitude savanna (890/g), followed by the southern Guinea savanna (488/g). *Scutellonema bradys* occurred in lower ($P \leq 0.05$) densities on *D. alata* (57/g) than other yam species, while *D. rotundata* was the most popular yam species encountered. There was considerable variation in *S. bradys* density between cultivars within country and in some cases between countries. From some cultivars no *S. bradys* were recovered, even though they presented symptoms of damage. Tubers from Ghana had the greatest ($P \leq 0.05$) proportion of tubers visually affected by *S. bradys* (7.53%), when analyzed across yam species and Mali the least (0.28%), while the highest proportion of galled tubers (due to *Meloidogyne* spp.) was observed in Mali (14.4%) on *D. rotundata* (19.6%). *Scutellonema bradys* infestation, based on visible symptoms, was more evident on *D. rotundata* (3.8%) than *D. alata* (0.6%), although 5.18% of yams in the group comprising the unidentified yam species had the greatest mean proportion of visually

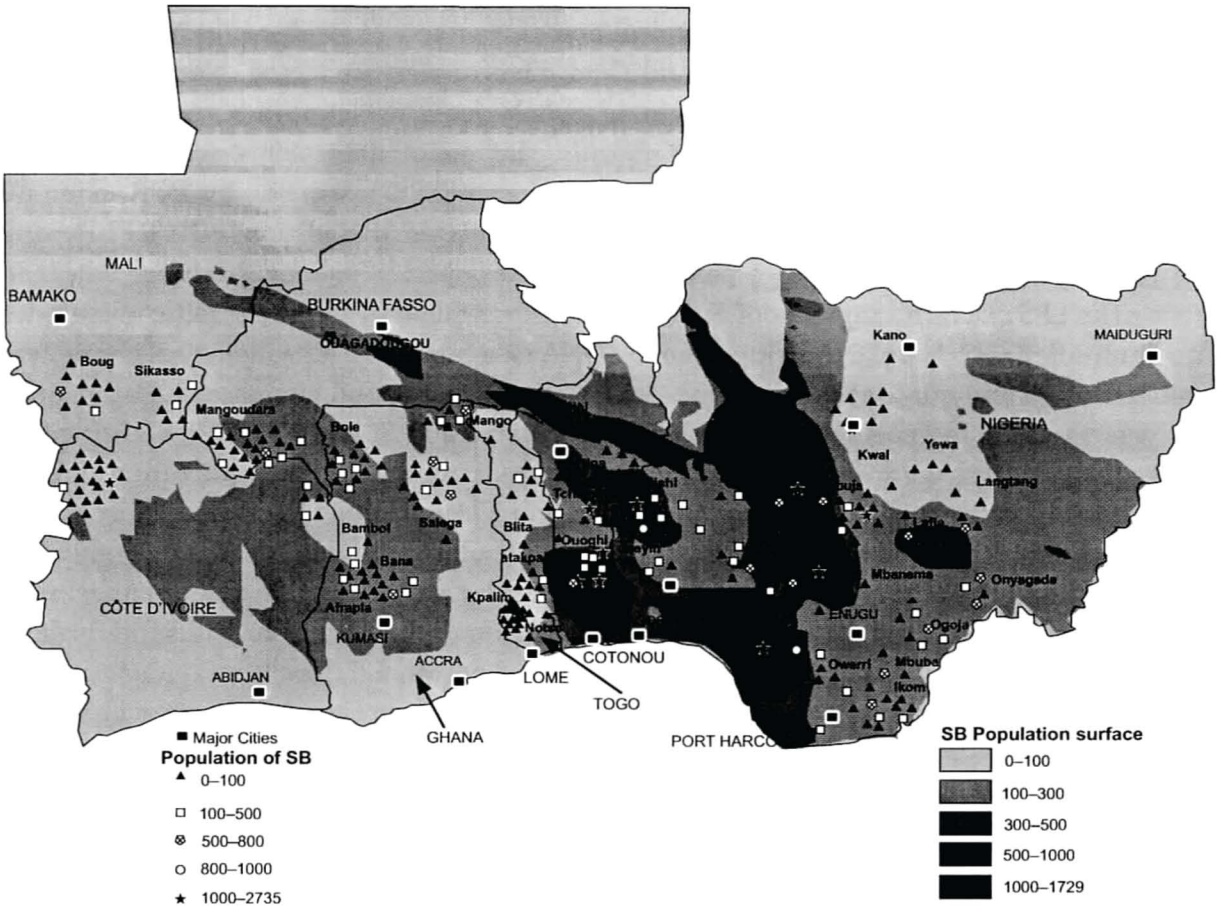


Figure 1. Map of West Africa showing distribution of collection sites of yam tuber samples in relation to *Scutellonema bradys* density and national boundaries.

affected tubers. On market stalls, *D. alata* (4.73%) and *D. rotundata* (3.35%) were most affected by visible galling due to *Meloidogyne* spp.

1.15 Identification of constraints to cassava production in Mozambique

by M.To, R.H., M.A., J.L., and D.C. in collaboration with L. Lalbanna, A. Jone, E. Mambo, M. Otema, G. Okao-Ojuja, R. Obonyo, and A. Sitole

A survey in 2003 revealed that CMD, CBSD, and CGM are the most important biotic constraints of cassava in Mozambique. Root-knot nematodes (*Meloidogyne* spp.) were widespread but causing relatively low levels of damage in terms of galling. Further assessment of the data and recent identification of the species involved, reveals that the galling damage was as a result of *Meloidogyne* spp. infection by *M. incognita, M. javanica, M. naasi, M. chitwoodi,* and *M. exigua* as single infections, but more often as mixed infections of two or more species. The data provides the first identification of *M. naasi, M. chitwoodi* and *M. exigua* occurring on cassava and also the first recording of their occurrence in Mozambique. It is also the first record of *M. naasi* and *M. exigua* occurrence in Africa. *M. chitwoodi* has previously been observed in South Africa. *M. incognita* and *M. javanica* are known to inflict severe damage on cassava, depending on conditions and cultivar. The extent to which *M. naasi, M. chitwoodi,* and *M. exigua* affect cassava however, is obviously completely unknown. Identifications were based where possible, on 10 females per root sample, although this was not always possible. Cassava roots can be woody and females

often difficult to remove. Some identifications were therefore based on less than 10 females per sample. Some samples provided no females for identification, although juveniles were recovered from root and soil extractions based on the Baermann technique, confirming their presence. The first record of *M. naasi, M. chitwoodi,* and *M. exigua* on cassava and in Mozambique provides evidence for the need for constructive assessment of *Meloidogyne* spp. occurrence on key crops in Africa, for which only limited information is available. The development and delivery of resistant cultivars is dependant upon such knowledge.

1.16 Interactions between the Senegalese grasshopper, *Oedaleus senegalensis*, its hosts and its natural enemies

by C.K. in collaboration with I. Maiga, J. Axelsen, A. Combari, and J. Bak

Recent work by the PRéLISS Project revealed the importance of natural enemies in the regulation of grasshopper populations. Bombyliid and tenebrionid predators and scelionid parasitoids of egg pods together generally destroy 60–80% of eggs during the dry season. Birds are also effective at reducing grasshopper populations, especially at medium grasshopper densities. All the different components of the millet/grassland-grasshopper-natural enemies ecosystem have been modeled. This SahelEco model incorporates the most important grasshopper species, *Oedaleus senegalensis*, and two other species. It also includes bombyliid and tenebrionid egg predators, *Scelio* egg parasitoids, and about 20 of the most important grasshopper eating bird species. It can make predictions about the effect of grasshopper control using chemical insecticides, Green Muscle, *Nosema,* and combinations. To make it more useful to end users, the model is being integrated into a GIS-based decision support tool. The GIS tool will take into consideration areas that are sensitive to chemical pesticides. Six classes of such areas can be defined: (1) human settlements, (2) wetlands, (3) oases, (4) protected areas, (5) areas with a high or unique biodiversity, and (6) areas with concentrations of (migratory) birds. Most of these can be found on existing maps or in the literature. Class 6 was more problematic. Negotiations are ongoing with Bird Life International to obtain digital maps of important bird areas. Data available at AGRHYMET on weather, soils, water availability, and crop development are currently being fed into the GIS tool.

1.17 Soil survey to map banana nutrient deficiencies and their relation to banana pest and management in four regions across Rwanda

by P.V.A. and C.S.G. in collaboration with S. Gaidashova

Participatory rural appraisals and diagnostic surveys were undertaken in 2001 in the four major banana-growing regions of Rwanda. These activities focused on (1) establishing why Rwandan farmers prefer brewing bananas (ABB) over cooking bananas and annual crops; (2) characterizing banana-based production systems and farmers' perspectives on production constraints; and (3) mapping the distribution of major banana pests and diseases. A subsequent survey was undertaken in 2004 to identify the nutrients that limit banana growth in Rwanda's major production areas. The same 60 farms were visited and soil and plant foliar samples were taken for nutrient analysis. The results showed major K and N deficiencies in all regions, with extremely strong deficiency in Kibuye where nematode populations were also high. Across sites, root necrosis and K foliar concentration were strongly correlated (r2=0.6). Plant zinc concentrations in the sites bordering Lake Kivu were also low.

1.18 Peri-urban vegetable pest biodiversity diagnosed

by B.J., P.N., G.G., M.To., F.B., and D.C. in collaboration with C. Atcha-Ahowé, I. Godonou, H. Baimey, T. Nouhoheflin, E. Adango, J. Boulga, E. Goudégnon, and E. Zannou

The peri-urban vegetable IPM project has generated extensive baseline information on the diversity, distribution, economic importance of pests and diseases, and associated natural enemies in vegetable production in urban and peri-urban areas of southern Bénin. The database covers 11 crops and responses from 46 producers (72% men and 28% women) at 20 sites surveyed in the 2003 dry season, and 34 vegetable producers (88% men and 12% women) at 16 sites surveyed in 2005 rainy season. Across sites, indigenous leafy vegetable "gboma" (*Solanum macrocarpon*) was the most popular vegetable cultivated, followed by amaranth, and lettuce. Gboma accounted for 45, 31, 65, and 42% of vegetables cultivated in Littoral, Ouémé, Atlantique, and Mono regions respectively. Phytophagous root feeding nematodes were the most prevalent on all vegetable crops sampled. In 2004, the prevalence of nematodes across crops was highest for root-knot nematodes (RKN) *Meloidogyne* sp. which was collected from all 15 crops sampled with a mean population density of 1953/g of roots, followed by *Helicotylenchus* sp. collected from 4 crops with a mean population density of 11/g of roots, *Pratylenchus* sp. collected from 5 crops with a mean population density of 7/g of roots and *Ditylenchus* spp. collected from 1 crop with a mean population density of 5/g of roots. The 2005 field diagnoses confirmed RKN as the most prevalent system pest attacking roots of a wide range of vegetables in the UPU areas.

Across sites in 2005, the percentage of plants with moderate to severe damage was approximately 83, 48, 30, and 11% for carrots, cabbage, gboma, and amaranth respectively. The leaf feeding broad mite *Polyphagotarsonemus latus* (Acari: Tarsonemidae) and RKN were the two most economically important pests of gboma across urban and peri-urban sites sampled in southern Bénin. Other economically important pets of the crop were the leaf feeders *Selepa docilis*. (Lepidoptera: Noctuidae), *Helopeltis schoutedeni* (Hemimptera: Niridae), and *Fusarium* disease; and flower and fruit borer *Scrobipalpa ergasima* (lepidoptera: Gelechiidae). The key pests in amaranth were the foliage feeders *Hymenia recurvalis* (Lepidoptera: Pyralidae), *Psara basalis* W. (Lepidoptera: Pyralidae), and *Tetranychus* spp. (Acari: Tetranychidae). The main pests of lettuce were RKN and leaf spot diseases (*Colletotrichum fiscum* and *Fusarium* spp.). RKN were the only economically important pests in carrot. The production of the exotic leafy vegetable cabbage (*Brassica oleracea*) is being abandoned due to heavy damage by caterpillars of the diamond back moth (DBM) *Plutella xylostella* L. (Lepidoptera: Yponomeutidae), *Hellula undalis* L. (Lepidoptera: Pyralidae), and the cost of chemical control of these pests. Amongst arthropods collected on vegetables were diverse natural enemies and harmless species including *Coleoptera: Exochomus troberti* (Coccinellidae), *Cheilomenes vicina* (Coccinellidae); *Serangium* sp. (Coccinellidae); *Scymnus* sp. (Coccinellidae); *Paederus* sp. (Staphylinidae); Hemiptera: *Rhynocoris bicolor* (Het.: Reduviidae); *Rhynocoris albopilosus* (Het.: Reduviidae); *and Hymenoptera: Cotesia plutellae* (Braconidae); *Opius* sp. (Braconidae); and *Oomyzus* sp. (Elophidae).

1.19 Pesticide use patterns diagnosed in urban and peri-urban vegetable production

by B.J. in collaboration with C. Atcha-Ahowé, I. Godonou, H. Baimey, J. Boulga, and T. Nouhoheflin

The peri-urban vegetable IPM project ranked pesticides according to frequency of use on vegetables by 50 farmers at 10 vegetable production sites in UPU areas of southern Benin.

Farmers apply at least 15 different chemical pesticides to control a range of insect pests, nematodes, and diseases on vegetables with little or no training in safe use methods. Cabbage and gboma stood out as indicator crops of harmful pesticide regimes in the vegetable agroecosystems. Cabbage producers apply approximately 46 liters pesticide concentrate on the crop in 19 applications 3 months prior to harvest in attempts to control the diamond back moth (DBM) *Plutella xylostella*. Talstar was applied 12 times and Decis 7 times on each cabbage bed, primarily to control caterpillars of DBM and *H. undalis*. Gboma, received 12 applications amounting to 41.8 liters of pesticide concentrate per hectare within three weeks of crop growth, in farmers attempt to control leaf damage symptoms of the broad mite *P. latus* and root damage by RKN prior to harvest. Lettuce, carrot, amaranth, and cucumber received 26.9l, 17.4l, 10.5l, and 7l pesticide concentrates respectively. Only 27% of the 124 vegetable producers used recommended pesticides, 36% did not adhere to recommended dose for pesticides they applied on the crops.

1.20 Cassava brown streak disease survey in Tanzania, Kenya, Malawi, Mozambique, and DR Congo

by C.H. and J.L. in collaboration with M. Ferguson, E. Kanju, N. Mahungu, M. Andrade, and I. Ingelbrecht

Cassava brown streak disease (CBSD) has been a problem since the 1930s for cassava production in the lowland areas of Eastern Africa where cassava mosaic diseases (CMD) are also endemic. CBSD symptoms are not as distinct in the foliage as CMD but the disease can cause a severe reduction in root yield and produces a dry necrotic rot in the storage roots leading to either complete spoilage or significant reductions in quality. *Cassava brown streak virus* (CBSV) is an ipomovirus (Family: *Potyviridae*, genus: *Ipomovirus*) that has been identified as being associated with CBSD with transmission of the virus also being recently associated with the whitefly *Bremisia tabaci*.

The survey in 2005 will attempt to update the extent of the CDSD problem in East Africa and collect basic data for characterizing the infectious agent. Association of sequence variation to patterns of symptom expression will also be attempted.

1.21 Cassava brown streak disease characterization via molecular means

by C.H. and J.L. in collaboration with M. Ferguson, F. Moonan, and I. Ingelbrecht

Sequence data on at least two areas of the *Cassava brown streak virus* genome (HC-PRO and CP open reading frames) will be collected and analyzed for sequence variation, and phylogenetic analysis, commencing 2005. At least one full length CBSV genome will be fully sequenced, commencing 2005. One output from this will be the data to be used by another project RF-CBSD (Project A) in order to facilitate the best design strategy for a pathogen-mediated approach for CBSV resistance through transgenic means.

1.22 Developing cleaved amplified polymorphic sequence (CAPS) method to delimit specific *Cassava brown streak virus* isolates

by C.H. in collaboration with F. Moonan, M. Ferguson, and I. Ingelbrecht

Cassava brown streak virus genome and other sequence data will be compared to that of known potyviruses from the public databases, and analyzed using different techniques for areas of low, medium, and high of predicted nucleotide changes. The outcoming predictions will be tested using *Cassava brown streak virus* isolates collected and RT-PCR products from the different areas of the genome. If the method can be verified, that is robust enough

to distinguish isolates from each other it could be a useful tool to track temporal and spacial changes of the pathogen for the future, commencing 2006.

1.23 Cassava brown streak disease characterization via biological means

by C.H. and J.L.

CBSV indexing experiments are to be set up using herbaceous indicator species for the biological characterization, commencing 2005. Screenhouse experiments with different cassava phenotypes-will be set up in order to test the types of virus reaction, mechanical and whitefly virus inoculation methods, and the distribution of the virus through vegetative propagation, commencing 2005.

1.24 Epidemiological trials for cassava brown streak disease in Tanzania

by C.H. and J.L. in collaboration with E. Kanju

Multilocational field trials with the most popular landraces grown in Tanzania will be initiated in 2006. The aim is to study the temporal and spacial movement of CBSD in the field setting, study the *Bremisia tabaci* populations and the variable reactions of the phenotypes in the different locations to natural CBSD infection.

1.25 Faunistic surveys and agrobiodiversity characterization

by G.G., R.H., and M.Ti. in collaboration with D. Gnanvossou

The biodiversity center at the IITA/BCCA Station, Cotonou carries out regular surveys in different agroecological zones of the subregion to assemble and complement a representative reference collection of West African arthropods. This activity constitutes the basis for the identification service offered to IITA scientists and NARES collaborators. Building on the scientific knowledge base accumulated over the years, the unit provides extensive assistance when specific projects need to study whole insect guilds or in the case of newly emerging pest problems which occasionally requires external taxonomic expertise.

Following the initial discovery in Bénin of a new invasive fruit fly pest for West Africa, the biodiversity center started to develop a capacity in Tephritidae taxonomy. Diagnostic surveys using parapheromone traps were set up in various ecological zones in Ghana, Togo, Bénin, Nigeria, and Cameroon. This enabled within 12 months, the collection of more than 50% of all known frugivorous fruit fly species of economical importance in tropical Africa. Additional material and information was obtained from the laboratory, rearing a wide range of horticultural and wild fruits. The reference collection has increased to 49 tephritid species distributed in 14 genera. Curatorial work revealed that the most frequently recovered species belong to the genera *Dacus*, *Ceratitis*, and *Bactrocera* spp. Efforts deployed in parapheromone trapping and fruit rearing were used to monitor in parallel the presence, distribution, and host range of the new discovered species, recently described as *Bactrocera invadens*. The pest that has incidentally been recorded in East and Central Africa appears to be highly invasive and polyphagous. Data presently gained suggest that *B. invadens* has widely spread over most of tropical Africa and develops on a wide range of crops including mango, citrus, guava, papaya, cashew, pepper, and several wild host plants.

In collaboration with the University of Lomé, faunistic surveys were initiated to study the diversity and importance of white grub insects of the scarabeid subfamilies Melolonthinae and Rutelinae in agricultural and natural environments. The research, conducted by two MSc students in their thesis, was aimed at exploring the native white grub biodiversity in

the midaltitude region of Togo and at gathering base line information on larval and adult host plant range of these traditionally cryptic and thus neglected insect groups. Insects' samples collected from direct observation in the field, larval rearing, and light trapping were identified at IITA's biodiversity center. The first assessment showed that the recovered Rutelinae are more abundant in species but less diverse at generic level than the closely related group Melolonthinae. Whilst the identification work has only been partially achieved, the study has led to the discovery and description of a melolonthid species previously unknown to science.

In Cameroon, studies that started three years ago on the bioecology, distribution, and importance of the African root and tuber scale (ARTS) *Stictococcus vayssierei* Richard (Homoptera: Stictococcidae) were jointly pursued with IITA scientists working on cassava IPM. Following the successful development of a rearing method which avoids the steady transfer onto fresh host plants, the life cycle of the root scale could be studied with better accuracy. Continuous laboratory observations showed that females undergo three developmental stages, two larval instars and an adult stage. A key based on the size and specific morphological features was developed to discriminate between the different developmental stages. In further studies, the field ecology of *Anoplolepis tenella* (Hym.: Formicidae), an ant associated with ARTS was examined to gather information for the development of control options against the pest. Field results showed that nests of this species that multiplies by budding were generally found with ARTS infested plants. Moreover cassava field housed significantly higher numbers of nests than fallows, secondary, and primary forest.

For the first time, some root scale insects of the family Stictococcidae were discovered in the Niger delta on cassava and cocoyam but yet their true identity and the possible attendance by ants have not been verified. In this respect, original material from all presently know genera and species that was previously obtained on loan from the natural history museum of Paris will be particularly useful for comparative studies.

1.26 Mite fauna on leafy vegetables in southern Bénin

by A.O., R.H., and B.J. in collaboration with E. Adango and P. Atachi

A survey of the mite fauna associated with *Amaranthus cruentus* L. (amaranth), *Solanum macrocarpon* L. (gboma), and *S. aethiopicum* L. (African eggplant), three major vegetable crops produced in Bénin, was carried out from July to October 2004 at IITA-Bénin Station, and on a vegetable farm at Togba in Abomey-Calavi area. Thirty-three species of mites belonging to 12 mite families were found on the three vegetables. The mites included phytophagous, predatory (of the family Phytoseiidae), and a group, whose feeding habits were not clearly determined. The following phytophagous mites were found: *Tetranychus urticae* (Koch) and *T. ludeni* (Zacher), in the family Tetranychidae; *Polyphagotarsonemus latus* (Banks) and another unidentified mite, in the family Tarsonemidae. The two *Tetranychus* species were present on all the vegetable whereas *tarsonemids* were found only on the two solanaceous plants. *Polyphagotarsonemus latus* was the most dominant pest during the course of the experiments. It was also the major mite pest on solanaceous plants, especially on gboma in vegetable farms where large spectrum pesticides are used. Damage caused by *P. latus* is most severe on young terminal leaves that curl downwards as the mite feeds on the lower side of the leaves that become rigid or bronzed and present a shriveled and scorched aspect. Flower buds become malformed, blooms abort, and plant growth slows as apical leaves die. The phytoseiids group was the most diversified with 16 species out of the 33 identified and more predominant in on-station plots where

no pesticides were applied compared with plots on commercial farms where pesticides were regularly applied on nearby plots. The other mites identified on the three vegetables belong to the following families: Acaridae, Tydeidae, Erythraeidae, Cunaxidae, Anystidae, Ascidae, Bdellidae, Uropodidae, and Oribatidae. Additional surveys and yield/quality loss trials are underway.

1.27 Baseline surveys of coconut mite and assoicated fauna in Bénin and Brazil

by R.H. in collaboration with M. Gondim, E. Lawson-Balagbo, K. Negloh, G.M. de Moraes, and P. Schausberger

Several surveys were conducted in Bénin to determine the abundance and distribution of the coconut mite *Aceria guerrerons* Keifer and its associated natural enemies on coconut palm *Cocos nucifera* L. Similar surveys were conducted in several states in the north and northeastern Brazil in search of effective natural enemies of the coconut mite for introduction into Africa and elsewhere where the coconut mite is a problem. Initial results from Brazil showed that faunal diversity under the coconut bracts (where the coconut mite feeds and multiplies) was greater than what was found in Bénin (and in Tanzania according to preliminary analysis of data from a survey conducted in 2005).

In Bénin, nut infestations ranged from 54 to 100% with up to 72% of the nuts with severe surface scarring. Coconut mites were found in large numbers underneath the perianth and on the meristematic part of the nuts, mostly at the base of scares. Coconut mite densities varied greatly among fields and reached at times over 30 000 mites per nut. Mite densities were generally highest on the young nuts and showed a decreasing trend with increasing nut age. In contrast to the findings from Bénin, both coconut scarring and coconut mite densities were considerably less in Brazil than in Bénin. In addition to coconut mite, a new species of tarsonemid mite was also found to be causing damage to coconut in parts of northeastern Brazil. The following predatory mites were found on coconut in Brazil: *Proctolaelaps bickleyi*, *Proctolaelaps* n. sp., *Neoseiulus baraki*, *Neoseiulus aff. paspalivorus*, *Lasioseius* sp., *Amblyseius largoensis*, and *Euseius alatus*. In addition to *A. guerreronis*, *Tyrophagus* sp. *Steneotarsonemus* n. sp., and *Lorryia* sp. were among the phytophagus mites found on coconut in Brazil. Additional surveys are planned for Bénin and Brazil, and a survey in Tanzania. We are presently still in the preliminary stages in identifying natural enemies for introduction into Africa, but there is mounting evidence that at least one of the predatory mites *N. aff. paspalivorus* and/or a Brazilian population of *N. baraki* would be likely candidates for introduction into Africa. Detailed comparative biological studies are underway to determine which of the predators would be eventually selected for introduction.

1.28 Biology and ecology of *Anoplolepis tenella*, an ant associated with the African root and tuber scale

by M.Ti., R.H., and G.G. in collaboration with A. Fotso Kuate and M. Kenne

The ant *Anoplolepis tenella* Santchi is closely associated with the African root and tuber scale (ARTS) *Stictococcus vayssierei* Richard and is considered essential for ARTS survival, dispersal, and proliferation. A large component of the work on the biology and management of ARTS consists therefore of understanding the biology and ecology of *A. tenella*. To investigate *A. tenella* colony boundaries, nests of this ant were collected from three localities (Awaé II, Atin-odzoé, and Mbalmayo) in Cameroon and reared in the laboratory on honey

and grasshoppers. After five weeks, aggressive bioassays were conducted in Petri dish (Ø = 10 cm), by means of one-to-one confrontations between workers belonging to (1) the same nest, (2) different nests from the same locality and (3) different nest from different locality. During a 5-minute confrontation, we recorded the occurrence of predescribed and coded behaviors on a scale of increasing aggressiveness with four levels: (1) lightly touch, brief antennation, mutual antennation, stay motionless at the contact of a nestmate, trophallaxis; (2) prolonged antennation, pursuit, avoidance, raising of gaster; (3) pulling of leg or antennae; (4) fighting; grapple. The results showed a low intraspecific aggressiveness on a local scale indicating that *A. tenella* can form supercolonies covering large areas. Our studies of *A. tenella* biology and ecology provide a rare opportunity to strengthen IITA's research capacity in social insects (such as ants and termites) that play a significant role both as direct and indirect pests of agricultural crops, and as highly significant components of biodiversity in tropical biomes.

1.29 Biodiversity of the African root and tuber scale in Central Africa

by M.Ti, G.G, and R.H. in collaboration with A. Fotso Kuate, A. Lema, A. Ndoumtsop, A. Nguenkam, and K. Tatahangy

The African root and tuber scale (ARTS), *Stictococcus vayssierei* Richard is the only known underground species of the family Stictococcidae. The wide range of attacked plants together with concurrently observed differences in host preference suggest that ARTS populations are more diverse than previously thought. To establish baseline information for future taxonomic studies, newly emerged ARTS larvae were collected from infested cassava in various sites in southern Cameroon and maintained in IITA's Entomology Laboratory in Nkolbisson. Morphological changes were observed with head lenses (10×) during rearing and with microscopic observations of slide-mounted individuals of all developmental stages. ARTS females go through three developmental stages, two larval instars and an adult stage. First and second instars are purple-red while the third instar is brown. In addition to increasing body size and sclerotization of the dorsal line with each developmental stage, white waxy secretions on the basal periphery of the second instar and both dorsum and periphery of the adults distinguish them from the first instar, which lacks white waxy secretions. On slide-mounted specimens, however, certain aspects of the anal complex—number of opercula plates and opercula setae have been found to be the most discriminating features among life stages of ARTS. These results are included in the first step (pest identification) of an integrated pest management campaign against ARTS. Additional studies are underway to develop molecular markers and host specificity studies to separate geographic and host populations of ARTS.

1.30 Identification of constraints to cassava production in Mozambique

By M.To., R.H, M.A., J.L., and D.C. in collaboration with A. Jone, E. Mambo, M. Otema, G. Okao-Ojuja, R. Obonyo, and A. Sitole

Three countrywide surveys were conducted in April–May and September–October 2003, and in May–June 2004 throughout the cassava-growing areas of Mozambique including, Gaza, Inhambane, Sofala, Manica, Zambezia, Nampula, and Cabo Delgado. The April–May 2003 and May–June 2004 surveys were planned to assess the distribution, incidence, and damage severity of diseases and pests that could affect cassava production. Although the September–October survey also included the evaluation of pests and diseases, it's main purpose was to determine the impact of the main pests and diseases identified in April–May 2003 on cassava production. Using the methodology developed by IITA, 202 and 175 cassava

fields between 4 and 8 months old were sampled in April–May 2003 and May–June 2004, respectively. The results indicated that cassava whiteflies (WF) appeared to be the second most important pest in Mozambique after cassava green mite. Generally known as vectors of the cassava mosaic disease, the populations of whiteflies were so high that they caused heavy black sooty mould covering the whole canopy. This was particularly severe in the northern Zambezia province. The incidence and severity of other known common pests of cassava such as cassava mealybug, termites, and grasshoppers were insignificant. The cassava mosaic virus disease (CMD) is the most common cassava disease in Mozambique. However, except in Gaza, Inhambane, Sofala, and Nampula, where severe damage symptoms (> 3 on a 1–5 scale) were encountered, the disease was either absent or present with only slight to moderate (2–3) damage severity.

Of the CMD-causing viruses, the African cassava mosaic virus (ACMV) was the most common particularly in Inhambane, Zambezia, and Nampula, but was absent in Cabo Delgado and Gaza. Mixed infections of African cassava mosaic virus (ACMV) and East African cassava mosaic virus (EACMV) were found in about 34% of the fields, while 17% of the fields were found harboring the East African cassava mosaic virus (EACMV). The devastating EACMV-Ugandan variant (EACMV-UG2) was not found in any of the fields during the April–May survey, but 4.8% of the fields sampled in September–October 2003 showed the presense of EACMV-UG2, but only from Nampula province. The incidence of cassava brown streak disease (CBSD) was high but only limited to the Zambezia, Nampula, and Cabo Delgado provinces. Damage severity was highest in Zambezia and in one district in Nampula. Other foliar diseases, e.g., cassava bacterial blight and cassava anthracnose were insignificant. Nematodes were present throughout but their severity varied from low to moderate. Root rots were common particularly in the CBSD infected provinces. Although we did not find a clear relationship between damage severity of pests and diseases in the April–May survey and cassava yield parameters at harvest in September–October, we found increasing dry root-rot incidence with increasing CBSD incidence and severity, but only in Nampula and Cabo Delgato provinces. The results of the two surveys are used to target interventions for the management of lingering and emerging pests and diseases of cassava in Mozambique.

1.31 The distribution and abundance of cassava pests in Tanzania with a note on spiralling whitefly

by D.G., R.H., and M.To. in collaboration with O. Mfugali, E. Nsami, and B. Pallangyo

Two surveys were conducted during May–June 2003, and June–July 2004 to assess the incidence and severity of cassava pests in Tanzania. The surveys covered four agroecological zones, including the Lake (Mara, Mwanza, and Kagera regions), Western (Kigoma and Shinyanga areas), Southern Highlands (Mbeya and Ruvuma regions), and Eastern (Tanga and central and southern coast regions) zones. A total of 193 and 94 fields were surveyed in 2003 and 2004 respectively. Six arthropod pest species (mites and insects) were recorded, including cassava green mite *Mononychellus tanajoa* (Bondar), cassava mealybug *Phenacoccus manihoti* (Matile-Ferrero), spiralling whitefly *Aleurodicus dispersus* (Russels), the whitefly *Bemisia tabaci* (Gennadius), the white scale *Anidiomyticus albus* (Ckll), the red spider mite, and several species of termites. The elegant grasshopper *Zonocerus elegans* (Thunberg) was not found in any of the surveyed fields. Low infestations of cassava green mite were found in all regions except in the Lake zone (Mwanza region), where high infestations (> 100 mites/leaf) were recorded. The exotic predatory mite *Typhlodromalus aripo*

DeLeon was found in all regions, with the highest infestations (>80%) occurring in the Eastern zone (central and southern coast regions) and the lowest (<18%) occurring in the Lake zone (Mwanza region). Low to moderate infestations (9–72%) of cassava mealybug were recorded in the Lake (Mara, Kagera and Mwanza regions), Western (Kigoma region), and Eastern (coast region) zones.

High infestations (80–100%) of spiraling whitefly, a first record (found only in 2004) of this species on mainland Tanzania, were recorded in the Eastern zone (Tanga and central and southern coast regions). *Bemicia tabaci* (probably in mixed infestations with *Bemicia afer*) were found in all regions, with the highest infestation occurring in the Lake and Western (Kigoma) zones. Low termites infestations were found in all regions, while white scale and red spider mites were found only in few fields in the Lake (Mara area) and Eastern (Tanga area) zones. The results of the two surveys are used to highlight regions where interventions are needed. This is to address continuing and emerging important biotic constraints of cassava and the recent invasion by the spiralling whitefly.

1.32 Tephritid fruit flies of economic importance in West and Central Africa

by *R.H., G.G., J-F.V., D.G., M.Ti., - in collaboration with F.X.N. Abanda, M. de Meyer, N. Famah, J. Gwinner, D. Haymer, S. Mengomo, A. Ojetola, K. Tatahangy, L. Traore, J. van Alphen, F.G. Zalom*

Surveys using male lures (cue lure and methyl eugenol) and fruit collections in nine sites across a latitudinal gradient in Cameroon were undertaken to determine species diversity, host range, and fruit infestation rates of tephritid fruit flies in Cameroon. To date, we have obtained data on 24 fruit fly species belonging to five genera: *Bactrocera* (two species), *Ceratitis* (six species), *Dacus* (12 species), *Perilampsis* (one species), and *Thririthrium* (one species) occurring in Cameroon. Depending on locality, *Bactrocera invadens* (Drew, White, and Tsuruta) was most common in methyl eugenol traps and *Dacus punctatifrons* Karsch or *D. bivittatus* (Bigot) were most common in cue lure traps. With a record number of 30281 individuals trapped in one month in May 2005 in one trap in one site, *B. invadens* was by far the most abundant fly in traps. But, the abundance of this species was found to vary greatly among locations and probably followed vegetation and elevation gradients, with lowest occurrences in highlands and in heavily forested regions. Fourteen species of fruit flies emerged from fruits of 39 out of 46 cultivated and wild plant species from which fruits were collected during surveys. Generally, *Dacus* spp. were restricted to vegetables while tree fruits were mostly attacked by *Bactrocera* and *Ceratitis* spp., with notable exception of *C. capitata* infesting vegetables and *D. bivittatus* emerging from some tree fruits. In decreasing order, *D. bivittatus*, *B. invadens*, *C. annonae*, *C. capitata*, and *D. punctatifrons* were the most polyphagous species. *Braconids* and *Chacidoids parasitoïds* emerged from fruits attacked by *D. bivittatus*, *D. vertebratus*, *C. annonae*, *C. capitata*, and *C. cosyra*. Latitudinal gradient trapping and host range studies are continuing, and will be augmented with additional traps containing trimedlure, vertlure, and torula yeast.

Similar surveys are underway in Bénin, where fruit fly trapping across a south-north gradient was initiated using cue lure and methyl eugenol to determine the species composition and seasonal dynamics of fruit flies attracted to the two lure types. Host surveys were conducted on several occasions in all provinces in Bénin and generally in the proximity of the trapping sites to establish a host inventory and rates of infestations of host plants of tephritid fruit flies. Similar to the findings in Cameroon, *B. invadens* was almost always the only species found in methyl eugenol, while depending on location, either *D. punctatifrons*,

D. bivittatus, or *B. cucurbitae* were most common in cue lure traps. It should be noted that several fruit flies e.g., *D. ciliatus*, *D. africanus*, and *D. vertebrates* - that infest fruits and vegetables in Bénin are not attracted by cue lure or methyl eugenol. The latter is attracted to vertlure while the other two do not have known attractants. Overall, trap catches of *B. invadens* were generally lower in Bénin than in Cameroon, with the highest trap count in Bénin of ca. 4000 flies. During the surveys, fruits from 52 plant species belonging to 22 plant families (Anacardiaceae, Annonaceae, Arecaceae, Asteraceae, Averrhoaceae, Bombacaceae, Caricaceae, Combretaceae, Cucurbitaceae, Euphorbiaceae, Irvingiaceae, Lauraceae, Moraceae, Musaceae, Myrtaceae, Ochnaceae, Passifloraceae, Rutaceae, Sapindaceae, Sapotaceae, Solanaceae, and Verbenaceae) were selected to determine their infestation by tephritid fruit flies. Of the 52 plant species, at least 30 were attacked by fruit flies. Of special interest, *D. punctatifrons* was found infesting only *Passiflora foetida* and at much less frequency *Momordica charantia* in Bénin. In Cameroon, *D. punctatifrons* was found attacking *Capsicum annuum*, *Cucumus sativus*, *Cucurbita pepo*, *Luffa cylindrica*, *Lycopersicum esculentum*, and *Zehneria scabra*. In ongoing studies in Cameroon and Bénin, we are comparing in on-station and on-farm trials susceptibility of various gourds and solonaceous plants to fruit flies, with special emphasis on *D. punctatifrons*, *D. ciliatus*, and *B. cucurbitae*. These results are especially important for the on-going efforts to understand the biology and ecology of *D. punctatifrons* in West and Central Africa.

In May 2002, IITA responded to concerns expressed by the government of Equatorial Guinea to the sudden appearance of an insect that was devastating tomato production on Bioko Island, about 50 km off the western coast of Cameroon. A survey of several farms in the vicinity of the putative site where the pest was first detected showed that nearly 100% of tomato, bell pepper, and zucchini fruits were infested by a fruit fly identified as *D. punctatifrons*. This apparent new infestation and subsequent spread on Bioko Island is now thought to be an introduction from Cameroon where *D. punctatifrons* was first recorded as a pest. A subsequent survey in 2003 and 2004 using cuelure traps showed that *D. punctatifrons* was present throughout the island. But, tomato losses were greatest in Moco, a large vegetable-producing area situated on the eastern side of the island at about 1300 masl. The new occurrence of *D. punctatifrons* on Bioko Island shows that *D. punctatifrons* is an invasive species, and is an ominous sign of a developing and potentially serious fruit fly pest problem on tomato in Central Africa. The same species which is widely distributed in Africa, does not attack tomato and cucurbits outside of Cameroon and Equatorial Guinea. Present and future research efforts include (1) molecular and morphological studies to study the biodiversity of geographic populations of *D. punctatifrons*; (2) garden plot studies to determine the phenology, seasonality, development of infestations, and crop losses due to *D. punctatifrons* and other tephritids attacking solonaceous and cucurbit crops; (3) country-wide surveys initially in Bénin, Cameroon, Equatorial Guinea, Ghana, Nigeria, and later in other countries, to determine the distribution and level of fruit infestations by *D. punctatifrons* and other tephritids and their associated natural enemies; and (4) develop management programs to reduce crop losses due to infestations by *D. punctatifrons* and other tephritids on solanaceaous and cucurbit crops.

We have recently initiated collaborations with the University of Hawaii-Manoa USA, to use molecular genetics studies to determine the relationship among the various tephritid fruit fly taxa found in sub-Saharan Africa and elsewhere. Of particular interest is the need to sort out the variation in geographic populations of *D. punctatifrons* from several countries in sub-Saharan Africa, as well as the diversity of the exotic species *B. cucurbitae*

and *B. invadens* and their relationship to other populations occurring outside Africa; and in the longer term, the relationship within and among several other *Dacus* spp. native to Africa.

2 New knowledge on the interactions between pests, host plants, natural enemies, and the environment generated

Ongoing and future activities

2.1 Diversity in *Fusarium* species causing stalk and ear rot of maize and sorghum in West Africa

by R.B. in collaboration with J. Leslie

Fungi in the genus *Fusarium* are ubiquitous in the soil, and as endophytes or pathogens of native and commercial plants worldwide. *Fusarium* spp. can cause serious production losses due to stalk rots, ear rots, and grain mold in both maize and sorghum, which are sometimes grown in the same agroecosystem. These fungi cause significant economic damage each year through direct yield losses and from the contamination of otherwise apparently sound food and feedstuffs with mycotoxins (produced by these fungi) that are detrimental to both humans and domesticated animals. Risks associated with *Fusarium* toxins are usually assessed based on the *Fusarium* species present, as not all species produce all toxins. Important toxins produced by these fungi include the fumonisins and moniliformin. *Fusarium* species occurring on maize and sorghum are diverse, and may have been commonly confounded in the past, probably all identified as *F. moniliforme* from sorghum and maize, resulting in the confusingly different toxicological and phytopathogenic properties that are simultaneously attributed to *F. moniliforme* in different agroecologies. Knowledge of the identity of the pathogen is a prerequisite to develop strategies for the management of crop damage due to *Fusarium* species. We studied the diversity *Fusarium* species infecting maize and sorghum to understand the distribution of these species in different agroecologies in West Africa. This will allow specific targeting of technologies to these pathogens.

Samples collected in Ghana in 2003 were purified through micromanipulation, and DNA extracted (~600 of ~700 samples). All samples are now in KSU culture collections. There appears to be no more than three species present on maize, with *F. verticillioides* present at 95%+ frequency. Sorghum spectrum appears to be different species from maize and more diverse. No evidence was found for extensive clonality in the *F. verticillioides* population. Analysis for relatedness of samples with fields and agroecozones not yet complete, but populations do not appear to be significantly differentiable on the basis of neutral genetic markers. Given the species present, the potential for fumonisin contamination in subsistence maize in Ghana is very high.

We collected 28 maize (25) and sorghum (3) samples from subsistence farms in five agroecozones in Cameroon with NARS scientists. About one-half of the samples were sent to KSU where isolation of *Fusarium* species from grains using micromanipulation techniques is continuing. The other half will be dispatched to KSU in 2005. We also collected six maize samples from the northern Guinea savanna zone of Nigeria. *Fusarium* spp. from all Nigerian samples collected during 2003 and 2004 were purified using dilution-plating technique. More than 1600 single-spore isolates of *Fusarium* were obtained and multiplied for DNA extraction. A large-scale DNA extraction and AFLP analysis protocol is being developed

at IITA in conjunction with the Biotechnology Lab of IITA. All Nigerian *Fusarium* isolates collected during 2003 and 2004 are now in a back-up collection in KSU.

2.2 Nutrient omission × pest trials in central and southwestern Uganda

by P.V.A., C.S.G., and A.A. in collaboration with B. van Lauwe, J. Jefwa, and W.K. Tushemereirwe

Poor management and low productivity has been characteristic of banana production in central Uganda for decades. In southwestern Uganda, where management and yield levels are much higher, banana has gained increasing importance as a commercial crop. Increased marketing of banana has resulted in the export of plant nutrients from farms to the urban centers and has accelerated soil nutrient decline. Unlike commercial banana farmers elsewhere in the world, Ugandan farmers do not use chemical fertilizers, but rely on organic materials to restore their nutrient stocks. However, with increasing land pressure, the availability of organic matter becomes limited. Many farmers have thus abandoned or reduced their input of organic amendments. Increased nutrient export through bunch sale and reduced availability of organic fertilizers leads to the depletion of soil nutrient stocks, eventually followed by yield decline. Several studies have already revealed that nutrient deficiencies of K, N, and Mg are common, but little information is available about the importance of micronutrients.

Banana weevil and nematodes further aggravate the decline of banana cropping systems. These pests attack the root and vascular system, interfering with nutrient uptake and preventing the plant from converting applied nutrients into increased yields. Thus, the positive effects of soil fertility interventions (e.g., use of mineral fertilizers) can largely be eliminated by the banana weevil and nematodes. Poor plant growth reduces the nutrient stocks in the living biomass, and the opening of the canopy can lead to increased losses of nutrients through leaching and erosion. A better understanding of these processes is essential if we want to develop integrated soil and pest management interventions that improve the system productivity and sustainability.

Although mulch is generally considered as beneficial for nutrient addition and water conservation, it is likely to exacerbate banana weevil problems. Adult banana weevils are strongly hydrophilic and factors that promote soil moisture conservation encourage this pest. Research results have shown that banana weevils cause higher yield loss in mulched systems. Though the evidence is less clear, there are several indications that show that stressed plants are also more susceptible to pest attack. In other words, mulching increases weevil numbers, while stress reduces plant tolerance to a given level of attack.

Earlier studies have shown that it is not sufficient to address soil fertility and pest problems independently to improve banana production in existing plantations. In this project, we wish to look at the dynamics of both new banana plantations and established banana stands to address the issues of soil nutrient status and moisture conservation and the related issues of weevil and nematode damage. There are clear indications that bananas in new plantations respond better to improved management than existing plantations, which has a large impact on the cost-benefit ratio of the proposed interventions.

In order to develop integrated soil and pest management options for banana growers in eastern Africa, it is critical to gain a better understanding of key nutrient deficiencies and how they relate to pest attack. In 2004, we have planted nutrient omission × pest trials in central and southwestern Uganda. The objectives of these trials are to:

- Identify what nutrients are limiting highland banana production on the trial sites.
- Determine what the nutrient requirements are of highland bananas.
- Identify/confirm what the critical and optimal nutrient concentrations are in different plant parts of highland bananas.
- Estimate potential production of highland cooking banana.
- Determine recovery rates of fertilizers in highland cooking bananas, in order to allow the calculation of cost-benefits of different fertilizer recommendations.
- Determine the effect of banana pests (nematodes and weevils) on plant nutrient uptake and fertilizer recovery.

Although much soil fertility related studies in EA highland banana systems have been conducted, there is a lack of basic knowledge on how much nutrients the banana plant requires, what its potential production is under well fertilized conditions, what nutrients are limiting plant growth in different areas, and what would be the fertilizer doses needed for optimum yields and optimum returns to inputs used when pests and diseases are no major cropping constraint. Treatments consist of N, P, K, and Mg+B+Mo+S fertilizer doses combined and with a series of treatments in which each time one of the fertilizers is omitted. Pests are controlled using insecticide (furadan), except for in an additional control and full fertilizer treatments in which pests are allowed to come in. The trials were installed in the second rainy season of 2004 and the first harvest will occur in late 2005 or early 2006.

2.3 On-farm monitoring of banana plant growth and banana pests

by P.V.A., C.S.G., and A.A. in collaboration with B. van Lauwe, J. Jefwa, and W.K. Tushemereirwe

This activity is being carried out on farmers' fields in Ntungamo district, Uganda. The objective of the study is to determine which environmental factors (water, light, nutrients, and pests/diseases) that cause variations in bunch weights within and between farms. Most banana surveys to date have concentrated on only a few elements of banana cropping (either pests, diseases, or soils), but few have tried to capture all environmental factors that determine banana growth and bunch weight. Moreover, most survey work is done at one point in time, whereas bunch weights are the result of variable growing conditions during the plant's growth cycle. We try to capture all environmental factors that can directly influence the plant growth and will try to monitor growth during one full cropping cycle. Ten farms were selected in SW Uganda after an initial characterization survey of 50 farms. The 10 farms represent a range of landscape positions (from crest to valley bottom) and wealth classes (from poor to rich). Within each farm, 15 mats are selected in both good and poor growing parts of the field. This survey work should help us to explain why bunches are often small and why large variation exists in bunch sizes within and between farms.

2.4 Understanding the relationship between nematode damage and plant nutrient uptake

by P.V.A. in collaboration with C. Dochez and S. Gaidashova

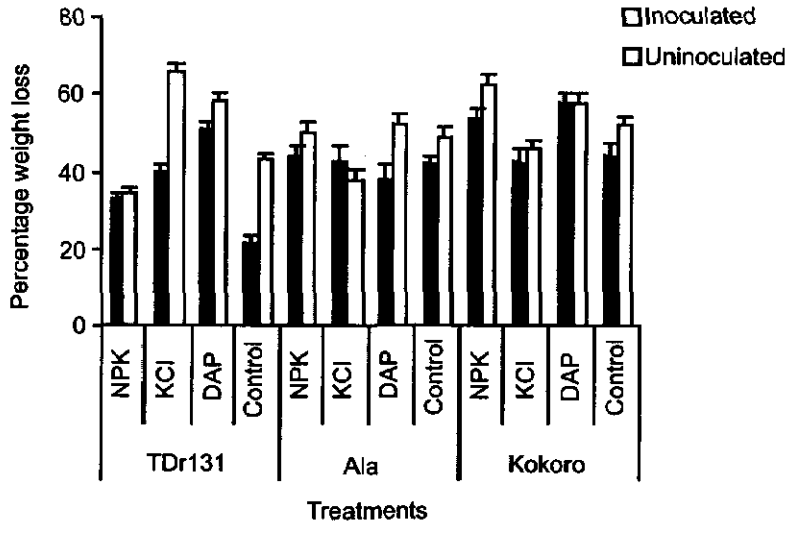
This research was commenced in 2004 and will continue through 2005. The objective of this experiment was to understand the effects of nematode damage on nutrient uptake. Existing nematode-infested trials were used in Uganda and Rwanda. The treatments consisted of three highland banana cultivars (AAA) displaying a range in susceptibility to

nematodes; nematode treatment (i.e., with and without nematicide); and mulch (with and without mulch). The hypothesis to be tested is that nematodes will affect water and nutrient uptake of the susceptible cultivars. Foliar samples are being taken to identify which nutrient deficiencies are induced by nematode damage.

2.5 Relationship between inorganic fertilizer application and nematode damage on yam in West Africa investigated

by D.C. in collaboration with H. Baimey

Three yam (*Dioscorea* spp.) cvs: two *D. rotundata* (cvs TDr 131 and Ala), and one *D. cayenensis* (cv Kokoro) were evaluated for the effect of NPK fertilizer application on nematode reproduction and damage in the field and during storage at IITA-Cotonou for the effect of NPK, KCl, and DAP fertilizers on yam. Plants were inoculated or not with 1000 *Scutellonema bradys* and fertilized or not with NPK (300 kg ha^{-1}), KCl (155 kg ha^{-1}) and DAP (60 kg ha^{-1}) (Fig. 2). Studies were carried out at IITA-Cotonou and at Save in Bénin. Nematode inoculation and fertilizer application were compared to uninoculated and no fertilizer control in field plots. At harvest, yield and the number of tubers were not affected ($P \leq 0.05$) by fertilizer application or nematode inoculation. However, the fertilizers suppressed nematode multiplication in tubers, especially DAP ($P \leq 0.05$). In storage, tuber weight loss was recorded in all treatments. Weight loss was more pronounced in tubers from plots receiving fertilizer application than not and when infected with *S. bradys* than not, and mostly in tubers that were infected and had received fertilizer. Nematode multiplication rate was greater ($P \leq 0.05$) in tubers during the first three months of storage, reducing in the fourth and fifth months. However, the effect of fertilizer and nematodes was both cultivar and fertilizer type dependant.



Error bars represent Standard Errors.

Figure 2. Effect of fertilizer treatments and *Scutellonema bradys* inoculation on post-harvest weight loss of yam cvs TDr131, Ala, and Kokoro tubers at five months after harvest.

2.6 Assessment of the importance of nematodes to cassava production

by D.C. in collaboration with A. Dixon, T. Munga, and M. Ogunlolu

Cassava is associated with numerous species of plant parasitic nematodes, among which *Meloidogyne* spp. is the most damaging. Evidence supporting the level of damage observed in controlled studies is limited under field conditions and quantification of severe damage is largely lacking. Field experiments were conducted at four sites in Uganda (Serere and Sendusu) and Kenya (Mtwapa and Msabaha) between mid 2002 to late 2003 to establish the effect of *Meloidogyne* spp. on cassava production in Kenya and Uganda under field conditions. The experiments were 2 × 2 factorial, laid out in a randomized block design with six replicates (except for Serere with five replicates). Cultivars (factor 1) comprised SS4 (Nase4) and Migyera (TMS 30572) for Uganda sites, Kibandameno (KIB) and Mtwapa1 (Brown root) for Kenya sites. Nematicide treatment (factor 2) were none (control) and Nemacur® (Fenamiphos) applied at 20 g/ m^2 (10 Kg a.i/ha) at planting and repeated at three months after planting to create a low and high nematode density.

Data collected included nematode population density, crop growth characters and yield, and yield components. *Meloidogyne* spp. occurred and resulted in galling damage at all sites with Serere having the highest incidence of the nematode recovered from roots and Mtwapa recording the highest galling damage and intensity. Other nematodes frequently recovered from the sites especially at harvest included *Scutellonema* spp. and *Pratylenchus* spp. Nematicide treatment reduced ($P \leq 0.05$) number of galls per 50 cm of feeder roots. Densities of the most commonly occurring nematodes and galling damage were lower in nematicide-treated plots but not significantly ($P > 0.05$). The majority of crop growth and yield parameters measured at harvest, across sites, and cultivars were not affected by nematicide treatment, except number of rot-affected tubers. Factor analysis of crop growth and nematode damage parameters across sites, cultivars, and nematicide treatments revealed strong negative interaction of *Meloidogyne* spp., number of root-knot galls per 50 cm of feeder root and *Pratylenchus* spp. with yield (tonnes ha^{-1}) and marketable tuber number. Regression analysis of the relationship between *Meloidogyne* spp. density at harvest and marketable tuber numbers revealed nonsignificant ($P > 0.05$) negative linear correlations at Serere and Sendusu sites, but highly significant correlations between marketable tuber number and *Meloidogyne* spp. density for cv Migyera ($P = 0.0024$, $R^2 = 37.5\%$). Yield (t/ha^{-1}) was also negatively correlated with *Meloidogyne* spp. density for cv Migyera ($P = 0.019$, $R^2 = 24.6\%$) and also cv SS4 ($P = 0.0072$, $R^2 = 14.8\%$).

2.7 Importance of root-knot nematode attack and incidence of fungal rots established on cassava

by D.C. and R.B. in collaboration with A. Rotimi and D. Fawole

Pot experiments undertaken at Ibadan to further establish the relationship between the root-rot fungi *Botryodiplodia theobromae* and the root-knot nematode *Meloidogyne* spp. were conducted over a four-month period on cv TME1 and cv TMS30572. Fungi or nematodes were inoculated separately or in combination, within two-weeks interval between fungus and nematode inoculations, or at the same time. Although galling damage on cassava roots was greater in some combined treatments than in nematode only at two months, the damage score was less severe by four months after planting. Root weights were lower, however, in combined inolculations than nematode alone, and it is speculated that the galled roots had decomposed, with nematodes unable to multiply to cause further damage.

This is also speculated on the grounds that combined treatments recoded higher root-rot scores than in fungus or nematode only inoculations—3 and 4 compared with 2 for fungus only (on a scale of 1–5). The presence of nematodes increased the rot score in roots. Shoot fresh weight was affected by *B. theobromae* to a greater extent than *Meloidogyne* spp. but in combination, generally resulted in further weight reduction. *Meloidogyne* spp. densities at four months were lower in inoculum combinations than alone, which was possibly due to the poor health of roots providing poor conditions for multiplication. Experiments and data collection continues.

2.8 Importance of root-knot nematode attack and incidence on yam in East Africa

by D.C. and R. A. in collaboration with J. Mudiope and A. Ekwamu

Pot and field experiments were undertaken at IITA-Sendusu to establish the effect of *Meloidogyne* spp. nematodes on yam using varying levels of inoculum, inoculated as suspensions in pots and as infected tomato root material in the field. Representative cvs of three yam species were used in the study: cv Ndaggu Nziba (*D. alata*), cv Kyetutumula (*D. cayenensis*), and cv DRC 97/00725 (*D. rotundata*). The results indicated that the minimum initial nematode population level required to significantly reduce the tuber quality appears similar in pots as in the field, but varies depending on the yam species (or cultivar) used. The minimum inoculum density (*Pi*) to affect the quality of DRC 97/00725 tubers was 100 *Meloidogyne* spp. nematodes per plant, whereas for *D. cayenensis* and *D. alata*, the tuber quality was reduced at *Pi* 1000 from field experiments harvested at seven months after planting. Similar results were obtained for the root galling damage in the pot experiment harvested at three months (Table 5). Tubers of *D. alata* and *D. cayenensis* developed fewer galls than those of *D. rotundata*. The study further revealed that *Pi* 3000 was the minimum *Meloidogyne* spp. population density that suppressed tuber weights of *D. rotundata* and *D. cayenensis* but not *D. alata*, in the field. This indicated that *D. alata* tolerated higher *Pi* providing higher tuber yields than *D. rotundata* and *D. cayenensis* under nematode pressure. The current findings show that *Meloidogyne* spp. can affect tuber yield on *D. rotundata* and *D. cayenensis*, which contradicts other studies from West Africa and Central America. Furthermore, galled *D. rotundata* tubers caused by *Meloidogyne* spp., were found to have reduced sprouting ability compared with non-galled, which reduced with severity of galling damage. Increasing levels of galling on *D. rotundata* tubers also resulted in increasing levels of weight loss and desiccation during storage.

Table 5. Mean percentages of *D. rotundata* tubers with varying severity of galling damage that sprouted in storage at monthly intervals.

Damage level	A			B			
	[1]1	2	3	1	2	3	4
No galls	87.5	100	100	60.0	80.0	100	100
Moderate	57.0	64.0	93.0	50.0	80.0	100	100
Severe	38.0	62.0	69.0	20.0	60.0	70.0	70.0

[1]1, 2, 3, and 4 indicate one, two, three and four months of storage (after harvest), respectively; A and B are experimental repeats.

2.9 Investigation into *Cassava brown streak virus* movement within cassava phenotypes

by C.H. and J.L. in collaboration with E. Kanju

Inoculated screenhouse experiments with different cassava phenotypes will be set up and assessed by RT-PCR in order to determine the plant tissues where the virus is present, commencing 2005.

2.10 Characterization and epidemiology of *Musa* viruses

by J.d'A.H. in collaboration with T. Oben and G.I. Atiri

Leaf samples from plants showing typical symptoms of *Banana die-back virus* (BDBV) were collected monthly between January–September 2004 from the same *Musa* plots at IITA, Ibadan that was used for identifying and monitoring the virus 2003. The samples were indexed by enzyme-linked immunosorbent assay (PAS/TAS-ELISA) for previously characterized banana viruses.

April, two plants out of 16 were indexed, and tested positive for only *Banana die-back virus* (BDBV) with absorbance values A_{405nm} ×2 of the healthy control. An attempt to move these plants to an insect-proof screenhouse was not successful as the plants died in the screenhouse some days after. A BDBV-infected *Musa* plant is presently in a screened cage in the screenhouse, though this plant has a low concentration of *Banana streak virus (BSV)*, genus Badnavirus.

Further work on the biochemical properties of BDBV in 2004 revealed that the 29.175Kda coat protein molecular weight reported for BDBV in 2003 is not consistent with recent findings. Several discontinuous sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) were done using the protocol described by Laemmli (1970) with slight modifications to determine the protein molecular weight of the virus. Mini preparations were made from plants that tested positive for only BDBV. Mean molecular weight of bands from four different gels was 52 KDa as band weights ranged between 51 and 54 KDa. The band size reported in 2003 is most likely for the coat protein of BSV.

Based on symptoms observed, eight banana leaf samples were collected from the Western Province of Cameron and indexed for known banana viruses. Two out of the eight samples indexed were positive (with absorbance values A_{405}nm greater than x 2 of the healthy control) for *Banana bunchy top virus* BBTV by TAS-ELISA. Antibodies for BBTV were a gift from Dr J.E. Thomas, Queensland Department of Primary Industries, Australia. Total nucleic acids from BBTV-infected leaves were used for polymerase chain reaction (PCR) using the published BBTV primer pair BT1F2.30/BT1R13.30 (Hafner 1995). A PCR band size slightly higher than the expected (1.1bp) was generated. This is the first time *Banana bunchy top virus* (BBTV), genus *Potyvirus* is identified and detected in Cameroon.

Subsequent indexing of 40 banana plant leaves from the Western and Littoral Provinces in Cameroon, noted that nine plants tested positive for BDBV by PAS-ELISA (3 of them in mixed infection with BBTV), 4 for BBTV-10, and 2 for BBTV-1. Antibodies for BDBV were got from IITA. This is the first time BDBV is detected in Cameroon.

2.11 Characterization of yam viruses

by J.d'A.H., R.A., D.C., and S.O. in collaboration with A. Eni, C. Rey, and O. Adeniji

With a view to understanding the epidemiology of yam viruses and isolation of uncharacterized yam viruses occurring in West Africa, a survey of the major yam-producing zone of the Republic of Bénin was done. A total of 29 villages were visited and 204 yam leaf samples collected from farmer's field in the locations visited. These leaf samples and 79 others collected from symptomatic yam plants from IITA's fields in Nigeria were indexed for virus infection. Twenty-three leaf samples collected from the IITA screenhouse in the Republic of Bénin were also indexed.

The leaf samples were tested by enzyme-linked immunosorbent assay (ELISA) for *Yam mosaic virus* (YMV), genus *Potyvirus*, *Dioscorea alata virus* (DaBV), genus *Badnavirus*, *Dioscorea mottle virus* (DMoV), genus (*?Comovirus*), *Dioscorea alata virus* (DAV), genus *Potyvirus* and *Cucumber mosaic virus* (CMV), genus *Cucumovirus*. Triple antibody-sandwich (TAS) ELISA was used for the detection of YMV and DMoV, while protein-A sandwich (PAS) ELISA was used for the detection of DaBV, DAV, and CMV.

ELISA results summary in Table 6 show that DaBV was the most prevalent virus infection in the Republic of Bénin while YMV was the most prevalent in Nigeria. DMoV was not encountered in any of the two countries. Over 65% of DAV infections from the Republic of Bénin occurred as mixed infections with DaBV, CMV, or YMV. Thirty seven percent DaBV infections were mixed with DAV, CMV, or YMV. All the CMV infections occurred as mixed infections with DaBV or DAV. Only a single unmixed infection of YMV was recorded in Bénin. Although mixed infections were also present in the samples from Nigeria, it was not as frequent as in Bénin and it was mostly mixtures of YMV and DaBV.

IC-PCR and IC-RT-PCR to validate the ELISA results is on. Symptomatic samples that tested negative to all of the viruses indexed for will be mechanically inoculated to test plants in a bid to isolate uncharacterized viruses.

YMV is the causal agent of a viral mosaic disease of yams that occurs throughout the yam-growing belt of West Africa. However, the effects of YMV on yield of yams (*D. rotundata*) in the region have not been adequately quantified. A study was conducted to investigate the influence of YMV infection on the growth, development, and yield of *D. rotundata*, the most popular yam in West Africa. Two genotypes of *D. rotundata* (TDr 93-31 and TDr 95-127) identified based on their performance in previous trials as moderately resistant to YMV were used for this study. It was found that YMV infection caused 65.39% yield loss in TDr 93-31 and 52.62% yield loss in TDr 95-127. It was also found that tubers with higher severity of virus scores had low tuber yield.

Table 6. Results from screening of yam samples from Nigeria and the Republic of Bénin for yam viruses.

Virus	Number of positive samples		Percentage of positive samples	
	Nigeria N = 79	Bénin N = 227	Nigeria (%)	Bénin (%)
CMV	0	10	0	4
DaBV	27	138	34	61
DAV	1	64	1	28
DMoV	0	0	0	0
YMV	46	6	58	3

Minitubers of these genotypes, produced in a screenhouse from certified virus-free *in vitro* plantlets, were obtained from the Tissue Culture Unit at IITA. Minitubers weighing between 5 g and 50 g were buried in burnt rice husk in baskets until they sprouted in 2002. Yam tubers cut into sett size of 100–150 g were used in 2004. They were transplanted into a screenhouse built on an experimental plot at IITA on 26 March 2002 and 2 April 2004 on mounds, at a spacing of 1 m between rows and 0.75 m within rows. Each plot was 5.25 m long and consisted of 7 rows 0.75 m apart, and a width of 5 m per plot. The experiment was 2 × 2 factorial laid out in a randomized complete block design (RCBD) with three replications. The four treatments were:

- TDr 93-31 uninoculated (Ro)
- TDr 95-127 uninoculated (So)
- TDr 93-31 inoculated (R_1)
- TDr 95-127 inoculated (S_1)

Serological indexing of plants was done using TAS-ELISA, as described by Njukeng (1998) six weeks after sprouting of the minitubers to ensure that the plants were free from YMV before inoculation. Mechanical inoculation of the young leaves was done 8 and 11 weeks after sprouting using sap obtained by grinding *D. rotundata* leaves infected with YMV in inoculation buffer (0.01M phosphate buffer pH 7.7 containing 0.001M ethylene diamine-tetraacetic acid (EDTA) and 0.0001M cysteine). A second laboratory screening was done two weeks after inoculation that is, at 10 and 13 weeks after sprouting to detect the presence of YMV.

Scoring of the plants started four weeks after inoculation and this was repeated six weeks after. Each of 20 plants in a plot was assessed for symptoms of virus infection, leaf scorch (anthracnose), leaf spot, and leaf blight on a scale of 1–5 for no observable symptoms to 100% symptoms on leaves.

At four weeks after inoculation (WAI) in 2002 and 2004, the mean virus symptom score was higher for inoculated compared to uninoculated plants of TDr 93-31 and TDr 95-127. At 10 WAI in 2002, no difference was detected between inoculated and uninoculated of both genotypes. However, at 10 WAI in 2004, inoculated plants showed higher leaf virus mean scores and maximum virus scores than the uninoculated ones for both genotypes. A positive correlation ($r = 0.7$) was obtained between the virus visual estimation scores at 10 WAI and virus concentration at $P < 0.0001$. In 2004, these diseases and pests symptoms were negligible.

There were no significant differences in the yield with regards to the treatment at $P \leq 0.05$ for the two genotypes, but there was higher yield depression in TDr 95-127 which had maximum virus score of four compared to three of TDr 93-31 (Table 7). In 2004, tuber yield was twice what was obtained in 2002. Analysis of variance showed significantly higher yields in uninoculated plants (27.6 compared to 9.55 kg/plot for TDr 93-31 and 30.2 compared to 14.31 for TDr 95-127). The genotype (TDr 93-31) with highest virus maximum score also had the highest percent yield loss (Table 7).

Virus symptom scores at 4 and 10 WAI were positively correlated with ELISA results ($r = 0.6$ and 0.7 respectively at $P< 0.0001$) this indicated that as the virus score increased, the virus concentration was also increased. However, the yield was negatively correlated with the virus scores and ELISA readings at $P < 0.0001$ ($r = -0.9$ for virus scores and $r = -0.6$ for ELISA absorbance) the implication of this was that, as the yield increased, the virus score and virus concentration decreased.

Table 7. Yield loss due to YMV of two genotypes of *D. rotundata*.

Genotype	Treatment	2002		2004	
		Yield/plot (kg)	% Yield loss*	Yield/plot (kg)	% Yield loss*
TDr 93-31	Inoculated	10.50	0.94	9.55	65.39
	Uninoculated	10.60		27.60	
TDr 95-127	Inoculated	9.40	18.30	14.31	52.62
	Uninoculated	11.25		30.20	
	Mean	10.5		20.42	
	LSD	8.95		4.38	

* difference in yield values between uninoculated and inoculated multiplied by 100.

Analysis of variance showed no significant effect of the treatment on dry matter percentage of the tubers at $P \leq 0.05$ (ranges of 27–30% in 2002 and 35–36% in 2004).

The percentage of sugar content of the tuber parenchyma values were, 2.76–3.12 in 2002 and 2.11–2.99 in 2004. Analysis of variance showed that the treatment had no effect on the percentage of sugar (db) at $P \leq 0.05$.

The percentage of starch content of the tuber parenchyma values for the two genotypes were 64 and 65 in 2002, while in 2004, values were 68–70. There was no significant difference ($P \leq 0.05$) in the treatment. The result of analysis of variance indicated that inoculation of the plant with virus does not affect the dry matter percentage of the tubers, percentage of sugar content and starch of the two genotypes.

2.12 Population dynamics of coconut mite in Bénin and Brazil

R.H. in collaboration with, M. Gondim, E. Lawson-Balagbo, K. Negloh, G.M. de Moraes, and P. Schausberger

Detailed population dynamics studies were initiated in several locations in southern Bénin and northeastern Brazil. The aim was to trace the process of coconut mite population development and nut infestation and damage from inflorescence to nut harvest. The Brazilian study focuses on mite dynamics within selected nut ages, particularly in relation to the distribution of associated natural enemies. The Bénin study focuses on the distribution and dynamics of coconut mite and its associated fauna among nuts. The data from these studies will be used to obtain insight into the biotic and abiotic factors affecting coconut mite populations in Brazil and Bénin. Bénin is to serve as baseline population data for comparison with similar data in the event that promising natural enemies of coconut mite are introduced from Brazil into Africa.

2.13 Biology of mites associated with the coconut mite in Brazil and Bénin

R.H. in collaboration with M. Gondim, E. Lawson-Balagbo, K. Negloh, G.M. de Moraes, and P. Schausberger

Several camparative life-history studies are underway to determine the rates of development, reproduction, and survivorship of predatory mites associated with coconut mite in Brazil and Bénin. This information will be used along with additional laboratory studies on intraguild interactions and field observations and eventually population modeling. This is to understand individual population and community processess that affect the dynamics of the mite fauna on coconut, and to determine the best suitable candidate natural enemies for

introduction into Africa. Initial results indicate that the Ascid mite *Proctolaelaps bickleyi* associated with coconut mite in Brazil is highly voracious, polyphagous (feeding on pollen, fungal spores, as well as mites), and develops rapidly on several diets. Cultures of this predator can be maintained on fungal spores as well as combinations of spores and mites. Studies with the Beninese strains of *Neoseiulus baraki* indicate that this predator feeds and can be maintained on coconut mite as well as on two spotted mite, but attempts to maintain colonies on various pollens (e.g., coconut and maize pollen) showed that pollens are not suitable for maintenance of *N. baraki* cultures. Comparative studies are underway to determine the effects of various food combinations on selected life-history traits of Beninese populations of *N. baraki*. A permit has already been obtained to import a Brazilian population of this predator for side-by-side comparisons with a Beninese population. While the two populations belong to the same species, we do not know if they have the same life-history characteristics in relation to diet breadth that could affect their relative potential impact on coconut mite populations. Comparative studies are also planned for Brazilian populations of *P. bickleyi* and *N. aff. paspalivorus* (a promising natural enemy for introduction into Africa) and Beninese populations of *N. paspalivorus*.

2.14 Interactions between ARTS and its associated ant *Anoplolepis tenella*

by R.H., M.Ti., G.G., and L.W. in collaboration with A. Fotso Kuate, A. Lema, S. Nanga Nanga, J. Ngeve, A. Nguenkam, and K. Tatahangy

We have completed several studies with the broad objective of understanding the relationship between ARTS and *A. tenella*. In field surveys, we determined that among 18 ant species encountered in cassava fields, *A. tenella* was most closely associated with ARTS (81% of all cases of ant-ARTS associations). This ant nests at the base of plants harboring ARTS, and is frequently seen tending the scale and removing its honeydew secretion. We demonstrated in laboratory studies that ARTS would "drown" in its own secretion if the honeydew was not removed. In a two-factor field experiments (ant exclusion and itial scale infestation), we found that when ants are not given access to cassava infested with ARTS, the scale disappeared from the plants within a three-month period. But, increased to two folds of initial infestations (109 ± 38.2; mean ± SE of two sampling dates at three and six months after initiation of the experiment) on initially infested plants from which *A. tenella* was not excluded. If, however, ants were given access to plants that were not previously infested by ARTS, densities of the scale reached a level of 37 ± 11.7 scales per plant within a six-months period. But remained absent on initially non-infested plants from which ants were excluded. Taken together, the results of the laboratory and field experiments showed that *A. tenella* is essential for ARTS survival. This is implicated in active dispersal of the scale as workers have often been observed carrying scale crawlers and placing them on uninfested cassava roots or other suitable underground cassava plant parts. We are looking into the development of means of disrupting ant-scale association as a sustainable scale control measure.

2.15 Cassava root yield losses due to ARTS infestations in Cameroon and DR Congo

by R.H., M.To., and A.L. in collaboration with A. Dixon, A. Fotso Kuate, J. Negeve, A. Nguenkam, and K. Tatahangy

In previous experiments in DR Congo, ARTS exclusion experiments (with chemicals) provided the first insight into the impact of ARTS infestations on cassava productivity. In those experiments, heavy ARTS infestations caused up to 67% losses in cassava root

yield. Additional information was needed to determine the level of cassava losses under various conditions. Two trials: one in Cameroon and another in DR Congo were concluded in 2004, and one trial in Cameroon was to be concluded in August 2005. In the ongoing Cameroon trial, we are using a three-factor experiment fertilizer, scale exclusion, and cassava cultivar, to determine the relative effects of scale infestations on cassava growth and yield under different soil fertility regimes. Protecting cassava from ARTS infestations resulted in an average of 71 and 63% increase in cassava yield in the Cameroon and DR Congo trials respectively. Most importantly, the trials showed that protecting the plant from scale infestations during the first six months of its growth is most important in protecting storage root yield. Data from the two completed trials and from the ongoing trial in Cameroon will be used to develop a functional relationship for ARTS infestations and their impact on cassava yield loss.

2.16 Interactions among introduced and indigenous phytoseiids and impact on CGM biocontrol

by R.H. and A.O. in collaboration with P. Nagel, K. Negloh, N. Ntonifor, and C. Zundel

We conducted various experiments on intraguild interactions in the phytoseiid guild that occurs on cassava in Africa. In one recently concluded experiment, we were interested in determining the relative impact on cassava green mite by midaltitude and lowaltitude strains of *Typhlodromalus aripo*. In previous experiments, we showed that a midaltitude strain from the state of Minas Gerais in Brazil has a much greater intrinsic rate of increase than a lowland strain at constant temperatures between 20 and 25 °C. The midaltitude strain was therefore targeted for the midaltitudes of tropical and subtropical Africa where the lowland strains had not succeeded in establishing and persisting. In subsequent comparative studies in northwestern Cameroon, we showed that the lowland and midaltitude strains were equally likely to establish in the midaltitudes. However, neither showed a strong negative impact on cassava green mite populations, largely because of asynchronous development of predator and prey populations. We then tested the two strains in screehnouse conditions in Fonta, Cameroon (midaltitude) and at IITA's station in Bénin (lowland). The results of the two experiments showed that, contrary to the initial study that compared life-history traits of the two strains, both lowland and midaltitude strains, alone and together, were capable of suppressing cassava green mite populations in the Cotonou experiment. The Fonta experiment, however, showed that the lowland strain had a slightly greater capacity than the midaltitude strain in suppressing cassava green mite densities. Subsequent functional response experiments supported the finding of equal predation capacity by the two strains on cassava green mite. That the initial studies favoring the selection of the midaltitude strains was not supported by field evaluations, highlights again the pitfalls of relying on life-table data for determining eventual success of a predator in the field.

2.17 Cassava varietal suitability to the exotic predatory mite *Typhlodromalus aripo*

by R.H., M.To., and A.O. in collaboration with A. Dixon, A. Ojetola, S. Olabowale, and C. Zundel

In previous studies, we showed that *T. aripo* preferred to inhabit cassava varieties with large hairy apices. On-farm (in Bénin, Togo, and Uganda) and on-station (Bénin, Nigeria, and Uganda) variety evaluations for *T. aripo* suitability and impact on CGM were completed in 2002. In addition, over 360 clones maintained in the yield gain germplasm collection in Ibadan (Nigeria) were characterized over a two-year period with respect to characteristics

affecting cassava green mite and its predators. Preliminary analysis of the data in relation to clone pedigrees showed that apex hairiness (a favorable trait for *T. aripo*) is heritable, meaning it could very well be incorporated into cassava improvement programs. We are continuing to evaluate germplasm collections in Ibadan to identify germplasm suitable for *T. aripo* and to develop deeper understanding of the genetic basis of heritability of *T. aripo*-suitable traits.

2.18 Biological and molecular characterization of indigenous and exotic isolates of the acaropathogen *Neozygites tanajoae*

by R.H. and A.C. in collaboration with I. Delalibera, M. Egas, A. Hajek, F. Hountondj, and M. Sabelis

Biological studies were conducted in the laboratory to determine the nature of interactions between CGM and each of three *N. tanajoae* isolates (two Brazilian and one Beninese). Also, to enhance our understanding of the underlying mechanisms for differences in pathogenicity of the exotic and indigenous isolates of *N. tanajoae*, and to develop a rational approach for new introductions of the exotic isolates.

Response of *N. tanajoae* to host-induced plant volatiles. Blends of volatile chemicals emanating from cassava leaves infested by the cassava green mite were found to promote conidiation in *Neozygites tanajoae*. One compound frequently present in blends of herbivore-induced plant volatiles (HIPV) as well as that of mite-infested cassava is methyl salicylate (MeSA). We investigated the effect of methyl salicylate in pure form on the production of pre-infective spores (conidia) by a Brazilian isolate and a Beninese isolate of *N. tanajoae*. Mummified mites previously infected by the fungal isolates were screened under optimal abiotic conditions for sporulation inside tightly closed boxes with or without methyl salicylate diffusing from a capillary tube. Production of conidia was consistently higher when the Beninese isolate was exposed to MeSA than when not exposed to it (37%; 305.5 ± 52.62 vs 223.2 ± 38.13 conidia per mummy) whereas no consistent difference was observed for the Brazilian isolate (-7%; 387.4 ± 44.74 vs 415.8 ± 57.95 conidia per mummy, respectively). These effects of MeSA were strikingly similar to those obtained under exposure to the complete blends of HIPV for the case of the Beninese isolate, but dissimilar (no promoting effect of MeSA) for the case of the Brazilian isolate. This shows that MeSA, being one compound out of many HIPV, can be a factor promoting sporulation of *N. tanajoae*, but it may not be the only factor as its effect varies with the fungal isolate under study. Additional studies are planned in which the actual amount of MeSA is measured, and if time permits, to use additional levels of MeSA to measure the fungus response across a gradient of MeSA concentrations.

Population level virulence. Two experiments were conducted in climate-controlled walk-in rooms with two large plastic cages inside each room designed to produce the high relative humidity needed to induce sporulation by *N. tanajoae*. One Brazilian isolate (Colal.brz) and one Bénin isolate (Coton.ben) were evaluated for virulence on CGM. The experiments consisted of introducing either 50 or 25 live mites (high and low inoculum) previously exposed to spores of *N. tanajoae* to each of 24 mite-infested plants and monitoring the performances of the isolates along with a control without fungus. All experiments (except the controls) showed establishment of the fungus and an associated decrease in the mite populations relative to the control, but prevalence of the fungus (measured by the proportion of infected mites) increased with inoculum density. At high inoculum density, the Beninese isolate performed better than the Brazilian isolate, whereas the Brazilian

isolate performed better at low inoculum density. We also measured dispersal in the cage to assess its role in the differential performance of the isolates in the field. Spore dispersal appeared to mainly depend on the infection level whereas more mites (healthy and infected) dispersed when infested plants were inoculated with the Brazilian isolate than with the Beninese isolate. These results suggest that population-level virulence tests under controlled climatic conditions are better predictors of the performance of fungal isolates in the field, than individual-level tests. As the experiments consisted of only one replicate of each prevelance level, we plan to conduct two additional replications under the same conditions.

Modeling host-pathogen dynamics. Previous models of *N. tanajoae* dynamics predicted that the fungus alone would not be able to control cassava green mite. We used the same Kermack-McKendrick model but with a new per capit rate of halo loss derived from laboratory observations. Model results suggest a reconsideration of how transmission rate of *N. tanajaoe* has been previously estimated. We suspect that the previous method does not take into account that live infected mites act as Trojan horse to carry the fungus to dense mite patches on the plant where it dies and sheds spores. This parasite-induced behavior may well promote the efficacy of microbial control of cassava green mite. Further development and validation of the model prediction await the results of ongoing pathogen transmission experiments.

Molecular characterization and development of molecular markers. The molecular probe developed in collaboration with Cornell University USA is now available. We will start using it latest, early 2006 in evaluating the spread of the Brazilian isolates in Bénin and neighboring countries and in ongoing and future releases of *N. tanajoae* in eastern and southern Africa.

2.19 The Asian fruit fly *Bactrocera invadens* in West and Central Africa: infestation rates and seaonsonal dynamics in West and Central Africa

By *R.H., G.G., J-F.V., M.Ti., D.G. in collaboration with F.X.N. Abanda, S. Ekesi, N. Famah, S. Lux, A. Ojetola, S. Olabowale, K. Tatahangy, and L. Traoré*

The tephritid fruit fly *Bactrocera invadens*, described as a new species in March 2005, was first recorded in Kenya in February 2003, and was subsequently found in Tanzania in July 2003. Present evidence indicates that Sri Lanka is the aboriginal home of *B. invadens*, but it is not known if Sri Lanka is the source of *B. invadens* now present in Africa. Within the span of one year of its discovery in Africa, *B. invadens* was recorded from several countries in West and Central Africa, extending from DR Congo to Senegal. The sequential finding of *B. invadens* does not necessarily represent, however, a point of introduction and subsequent spread in continental Africa. Farmer surveys in Bénin suggest that *B. invadens* has been present in Bénin since 2003, as mango, cashew and possibly citrus infestations by fruit flies have increased sharply since that year, particularly in the southern and central provinces of Bénin. Our present knowledge of the ecology and biology of *B. invadens* indicates that this is a highly invasive and polyphagous species with high reproductive potential. Known hosts in West and Central Africa include citrus, mango, cashew, papaya, guava, sheanut, pepper, and several wild host plants, with high infestation rates of fruits of some of the host plant species.

In Bénin on mango, *B. invadens* infestations were highest in the southern and central provinces, with infestations reaching upward of 72% during peak infestations, while in

the northern provinces, *B. invadens* was absent during the beginning of the mango season (*C. cosyra* being the primary fruit fly species on mango at that time). But proportion of fruits infested by *B. invadens* increased sharply in May (although overall fruit fly infestation remained relatively unchanged) and decreased again in June and the last part of the mango season in July, when *B. invadens* was the only fruit fly infesting mango. In sharp contrast to the northern provinces, *C. cosyra* was present in low frequency in the central provinces while *B. invadens* was the only species infesting mango in the southern provinces. If these trends continue, *B. invadens* will likely displace *C. cosyra* in the central provinces. Complete displacement is unlikely in the northern provinces, where *B. invadens* disappears during the dry season, and may need to reinvade (by natural dispersal and/or movement of infested fruits from the southern provinces) at the start of the rains and as populations of this fly develop in central and southern provinces.

Of the host plants so far known to be infested by *B. invadens*, mango, *Irvingnia gabonensis*, sheanut, guava, *Terminalia catappa*, and *Annona muricata* were most infested (30–78% infestation), while infestations of citrus, cashew and other hosts ranged between 12 and 23%. Improved mango varieties (particularly in the southern and central provinces of Bénin), *I. gabonensis*, and sheanut produced between 23 and 45 adult flies per fruit, while the rest of the hosts produced 2–12 adults per fruit. In Bénin and Cameroon, where continuous male trapping has been in place for nearly a year, male abundance in methyl eugenol traps shows at least one peak and one trough corresponding respectively to peak rain and peak dry seasons. While *B. invadens* is already widely distributed in Africa, this species appears to be most adapted to lowland humid regions, as supported by the Bénin host infestations data. Taken together, all available information on its rapid continental spread, host range, and reproductive potential indicate that *B. invadens* is a highly invasive species with substantial destructive potential. The international scope of this species requires an equally international effort to contain its spread and develop and implement integrated options for its control.

3 Efficient biological control options against important pests in farming and aquatic systems developed

Ongoing and future activities

3.1 Biological control of aflatoxin

by R.B. in collaboration with J. Atehnkeng, S. Kiewnick, R. Sikora, and P.J. Cotty

Biological control of the aflatoxin-producing *Aspergillus* fungi can be considered a potentially promising strategy to reduce dietary aflatoxin exposure. There is a great diversity of *A. flavus* phenotypes in agricultural fields. Some strains are toxic and some are atoxic. Toxigenicity is apparently unrelated to a strain's ability to colonize and/or infect living or dead plant tissues. It is possible to displace toxigenic strains of *A. flavus* with strains of *A. flavus* that do not produce aflatoxins (atoxigenic). This method is currently used on cotton and maize in USA, and tested on groundnut in India. As part of a GTZ/BMZ funded project on aflatoxin risk assessment, biological control and other interventions, we conducted research on biological control of aflatoxin. Highlights of the results are presented.

At IITA, we completed the isolation of *Aspergillus* spp. from maize grains collected in 2003 from farmers' stores in 52 locations (in southern Guinea savanna and derived savanna). Isolations from these Nigerian samples yielded a total of 4296 single spore isolates of the

A. flavus type. In 2004, grain and soil samples were also collected from another six locations in the northern Guinea savanna ecological zone. Thus, our *Aspergillus* populations are from 56 locations covering the main maize-growing areas in Nigeria. The large number of locations sampled and isolates obtained offer confidence that screening and selection of atoxigenic strains will be robust.

A two-step protocol was developed at Bonn and IITA to screen the large number of *Aspergillus* isolates to identify atoxigenic strains. The first step involved small-scale fermentation, extraction of aflatoxin, and qualitative toxin estimation using TLC to identify putative atoxigenics. The second step consisted of large-scale fermentation of putative atoxigenics followed by extraction and estimation of the toxin. Isolates that did not produce aflatoxin in the second step are considered as confirmed atoxigenics. Due to problems associated with handling of *Aspergillus* spp. in Bonn, all fermentations and extractions were carried out at IITA by the PhD student Joseph Atehnkeng, and extracts shipped to Bonn where the PhD student Matthias Donner performed aflatoxin analysis.

A total of 1550 isolates from four districts (Ogbomosho, Mokwa, Makurdi, and Lafia) were screened for toxin production and 503 isolates were selected as putative atoxigenics. Subsequently, 86 isolates were confirmed as atoxigenics.

A field trial was inoculated to test the competitive ability of 24 atoxigenic isolates from six locations. Treatments consisted of the 24 atoxigenics inoculated alone and together with a strong toxin producer. The trial will be harvested during the first week of March 2005.

Tester development work for some of the atoxigenics from Nigeria was started at IITA. Similar tester development work for Benin isolates is in progress in Bonn.

It is expected that by mid-2006, candidate strains for biocontrol of aflatoxin in maize would be available in Nigeria.

3.2 Evaluating the efficiency of aphelinid parasitoids as biocontrol agents of whitefly vectors of cassava mosaic geminiviruses

by J.P.L. in collaboration with M. Otim, P. Asiimwe, S. Kyamanywa, and D. Gerling

Surveys of parasitoids of the cassava whitefly, *Bemisia tabaci* were conducted in major cassava-producing areas of Uganda. The two principal species encountered were *Encarsia sophia* Dodd and Girault and *Eretmocerus mundus* Mercet. In contrast to earlier similar studies, *Er. mundus* was the more abundant of the two, but neither were sufficient to adequately control whitefly populations in the field.

Laboratory studies were undertaken to describe some of the life history parameters of these two principal parasitoids species. Pre-imaginal development duration times were 19.7 days and 18.0 days respectively for *Er. mundus* and *E. sophia* respectively. These were comparable with durations recorded elsewhere for related species. Mean adult longevities were 5 days and 6 days for *Er. mundus* and *E. sophia* respectively when reared on honey. These values increased to 5.5 days and 11.3 days for *Er. mundus* and *E. sophia* respectively when provided with whitefly nymph hosts. The increased longevity of *E. sophia* when fed on live hosts was typical of results obtained with other aphelinids elsewhere, but by contrast, the absence of a similar increase for *Er. mundus* is unusual. Reproductive rate parameters were estimated for the two species. Values calculated for *E. mundus* (R_0 = 13.1; T_C = 5; r_m = 0.2) were lower than those for *E. sophia* (R_0 = 15.1; T_C = 8; r_m = 0.342), suggesting that the latter is likely to be a more efficient parasitoid than the former.

Studies of the searching behavior of *Er. mundus* and *E. sophia* adults on leaves of hairy and glabrous cassava varieties revealed important differences. Both species were more inclined to leave hairy leaves than glabrous ones. However, for the adults that remained on the hairy-leaved variety, searching behavior was as efficient for *E. sophia* as on glabrous leaves. By contrast, *Er. mundus* was less efficient in its searching activity on hairy leaves in comparison with those of the glabrous variety. Overall searching efficiencies were 26% (glabrous) and 25% (hirsute) for *E. sophia* and 47% (glabrous) and 38% (hirsute) for *Er. mundus*. *E. sophia* seemed to spend more time resting in comparison with *Er. mundus* females. This helps to explain the different host encounter rates. Observations of egg loads following emergence revealed that whilst *Er. mundus* females on average emerged with 35 mature eggs, *E. sophia* females had only three. It is thought that this major difference between these two species is behind the differences in searching behavior observed. Previous studies have demonstrated that the level of host searching activity is closely related to the mature egg load following emergence. Based on these behavioral studies, *Er. mundus* seems to be the best candidate for augmentation in any biologically-based whitefly management program.

Since earlier field-based survey and population dynamics studies have shown that neither species, nor the two together, are able to hold superabundant *B. tabaci* populations in check, additional natural enemy activity is clearly required if an effective biologically-based control approach is to be developed for *B. tabaci* on cassava in East Africa. The data developed through these and allied studies, however, are providing essential baseline information for future work on developing such an approach.

3.3 Cataloging and evaluating the efficiency of predators as biocontrol agents of whiteflies on cassava in East Africa

by J.P.L. in collaboration with P. Asiimwe, M. Otim, S. Kyamanywa, and D. Gerling

Plants of the cassava mosaic disease-resistant variety, Nase 4, were observed over the cropping cycle in order to assess the occurrence and relative abundance of predators feeding on the whitefly, *Bemisia tabaci*. The main predator groups observed included: adults and nymphs of the coccinelid species—*Serangium*, ants, spiders, and syrphid larvae. Predator numbers declined following a hailstorm but increased rapidly after this as fresh new cassava growth sprouted. Preliminary direct observations of feeding behavior have suggested that *Serangium* spp. are likely to be the most efficient predator. Future studies will be attempting to characterize tritrophic interactions involving the cassava host, whitefly, and nymph and adult stages of *Serangium*.

Life table studies were used to assess mortality factors affecting whitefly populations in south-central Uganda. Sucking predation by predators accounted for 4% and 23% in egg and nymph mortality respectively. However, 46% of eggs and 58% of nymphs went "missing" during record taking and it is likely also that a significant proportion of this loss is a result of predation. Based on these results, it seems apparent that predators are an important and hitherto underestimated source of whitefly mortality. Future studies will aim to identify the factors responsible for "missing" mortality and to identify approaches to incorporating the augmentation of predator activity into biologically-based integrated pest management approaches for whiteflies.

3.4 Determining an appropriate sampling unit for banana weevil predators with respect to their distribution in banana plantations

by A.A. and C.S.G. in collaboration with R. van Driesche

In this study, we measured abundance and spatial distribution within banana fields of previously described predators of banana weevil and used the information to identify the best sampling unit for work with such predators in future studies. We sampled crop residues and live plants by destructive sampling and used visual observations to look for predators on the ground surrounding mats and in the soil. Experiments were conducted in a trial at the Kawanda Agricultural Research Institute (KARI) in Kampala, Uganda and in farmers' fields in Ntungamo District, Uganda.

In plots at KARI, 32% of all the predators were in prostrate residues, 44% in standing residues on mats, and 24% on living plants on mats. Together (prostrate and standing), residues accounted for 76% of all the predators in plots. On mats, flowered plants had 2.5 times more predators than maiden suckers and five times more predators than preflowered plants. Most predators in live plants, equivalent to 71–100% were in pseudostems rather than corms for plants older than six months. Predators per m^2 were 3–4 times more in residues than in live plants on mats, and several hundred times more than were in the soil. On farmers' fields, the number of predators per m^2 was 35 times higher in residues than on living plants. Absence of predators on living plants was a result of leaf sheath removal by farmers to deny weevils' oviposition sites.

These data demonstrated that banana weevil predators are most abundant in residues. Residues are the most appropriate sampling unit for estimating abundance of banana weevil predators because (1) they were the only sampling unit in which all important species of predators were present, (2) they had the greatest number of predators per m^2 of any sampling unit, and (3) they are the most important breeding site of the banana weevil and can be sampled without harm to the banana crop, which is not true for live plants.

3.5 The composition and abundance of indigenous non-formicid natural enemies in banana farming systems in Uganda

by A.A. and C.S.G. in collaboration with R. van Driesche

The abundance of previously identified natural enemies of the banana weevil was studied in major banana zones in Uganda during the rainy and one dry season in 2001. Work was done in farms using four key banana management practices: pure banana stands mulched with banana trash, pure banana mulched with grass, banana stands intercropped with beans, and banana stands intercropped with coffee. Our goal was to understand abundance of banana weevil predators in banana fields and how these management practices affected predator numbers found in crop residues.

Hydrophylidae were the most abundant group, comprising 47–62% of total predators per residue, dermaptera comprised 23–35%, staphylinids 2–24%, and histerids 1–7% of total predators. Management practices affected predators by affecting level of residue removal at the beginning of rains. Removal of 47% of residues in banana/banana trash led to 35% decline in predators and 35% removal of residues from banana/bean intercrop to 49% decline in predators. In contrast, predators increased by 13% in banana/grass mulch system despite 47% residue removal. Data from this study suggest that residue removal practices interfered with predator abundance while mulching banana with grass favored increased numbers.

Of the four farming practices studied, mulching banana with grass was the practice most likely to enhance biological control of the banana weevil.

3.6 The impact of crop residue removal on the abundance and efficacy of natural enemies of the banana weevil in Uganda

by A.A. and C.S.G. in collaboration with R. van Driesche

Indigenous predators of the banana weevil have been reported in a number of banana growing areas; however, their effectiveness may have been hampered in some cases, by management practices that are unfavorable for natural enemy activity. In this paper, we report studies conducted in Uganda to determine how crop management practices affect natural enemy abundance and their efficacy as biological control agents. Field experiments were conducted at the Kawanda Agricultural Research Station to examine the effect on predators of mulching and different levels of crop residue removal.

Destruction of banana plant residues by chopping to promote rapid desiccation reduced predator abundance by 59% per residue and 77% per hectare at high residue removal levels and by 28% per residue and 65% per hectare at moderate residue removal. Conversely, mulching with grass in plots with low residue removal increased predator abundance by 67% per residue and 82% per hectare. Residue removal in high sanitation plots reduced trap catch of adult banana weevils by 31–38% per trap and weevil population per hectare by 46–48% relative to other sanitation practices, but did not significantly reduce damage. Moreover, predator: prey ratios in residues were three times higher per hectare in plots with low residue removal compared to those with high residue removal.

On mats (clusters of standing banana stems from a common rhizome), residue removal at high sanitation reduced predator abundance by 82% and adult banana weevils by 47% per mat compared to low sanitation plots. However, adult banana weevil numbers on living plants on mats (excluding postharvest stumps) were not significantly different among treatments. Predator: prey ratios on mats were also three times higher in plots with low vs high residue removal. In general, natural enemy abundance was favored by residue presence, and better predator: prey ratios in plots with low residue removal offset higher absolute banana weevil numbers such that lowest damage occurred in plots with low residue removal.

3.7 Composition, distribution and relative abundance of ants in banana farming systems in Uganda

by A.A. and C.S.G. in collaboration with R. van Driesche

We present results of a survey of ant species' composition and relative abundance in farmers' plots in the banana growing areas of Uganda. Through sampling at 39 farms in four regions, we found 55 species of ants using pitfall traps and 24 species using fish and honey food baits. When banana weevil larvae were exposed as baits, we encountered 11 species, of which *Pheidole* sp. 1, *Pheidole* sp. 2, *Pheidole* sp. 3, *Paratrechina* sp. 1, and *Lepisiota* sp. 1 were the most abundant. These five species were also the major species found as colonies or forager inside harvested postharvest pseudostem and corm residues. In such crop residues, we recovered 17 species in pseudostems and 34 in corms, either as colonies or foragers. High species richness and abundance especially inside plants and residues suggests that ants are important foragers in banana plantations.

Seven of the ant species found in plants demonstrated significant ability to remove banana weevil eggs artificially inserted in corms. Two species, *Odontomachus troglodytes* Santschi and *Pheidole* sp. 2, were able to remove eggs from naturally infested corms. *Odontomachus troglodytes* removed 33–68% of the eggs in naturally infested corms, while *Pheidole* sp. 2 removed 38–60% of such eggs. The attraction of these species to banana weevil-infested plant pieces, their close association with the banana plants, and their ability to extract artificially and naturally introduced banana weevil eggs from plant tissue. This suggests that these two ant species may have significant potential to consume weevil immature stages in plants and residues.

3.8 The effect of selected ant species on banana weevil immature density in plants, and damage to banana plants in Uganda

by A.A. and C.S.G. in collaboration with R. van Driesche

Studies were conducted in both microcosm and field experiments using ant exclusion in control plots and ant enhancement in treatment plots. The study was to determine if the ant species *Pheidole* sp. 2 and *Odontomachus troglodytes* Santschi both common in banana farming systems in Uganda, had potential to affect banana weevil population dynamics and damage to plants. Both species caused significant mortality to banana weevil eggs in live plants in microcosm shade house experiments and in the field, and to banana weevil larvae in residues. In potted plants allowed to grow for three months, ants reduced banana weevil immature stages when starting density of banana weevils were low (2 females per plant) but failed to cause a reduction in larvae and pupae numbers at higher banana weevil densities (3 or 5 females per plant). In potted plants that were allowed to grow for six months before sampling, *Pheidole* sp.2 reduced banana weevil larvae by 42% compared to controls and in general. Plants from plots with ants were more vigorous, grew taller, had more leaves, and lower damage than control plants.

In a field trial, *Pheidole* sp. 2 and *O. troglodytes* reduced egg numbers by 64% and 38% respectively in suckers planted as sample units and by 82 and 76% in naturally growing suckers. In banana plantations, ants appear to affect banana weevil population dynamics and plant damage through predation on banana weevil eggs and larvae in plants and, predation on banana weevil larvae in residues. Data for this trial will be collected through 2005.

3.9 Efficacy and persistence studies of the entomopathogen *Beauveria bassiana* under different banana management practices

by C.N., C.S.G., and T.D. in collaboration with W.K. Tushemereirwe, E. Magara, V. Tumuhaise*, and S. Kyamanywa*

The banana weevil has long been a major constraint for banana production in Uganda. Several control options have been developed and implemented to address the banana weevil problem, but with varying levels of success. IITA in collaboration with UNBRP, CABI Biosciences and the University of Reading has implemented an integrated pest management (IPM) approach with emphasis on using the entomopathogen *Beauveria bassiana* as an important component of the IPM strategy.

Development of microbial control of the banana weevil has gone through a series of stages involving, isolation and characterization, screening and pathogenicity testing, and evaluation of mass production and delivery systems. Various strains of *B. bassiana* have been isolated from soil and insects hosts in Uganda, which can cause 50–100% mortality in two

weeks. Some of the *B. bassiana* isolates have shown good growth and spore production on locally available substrates such as cracked maize and maize bran. Field evaluation of possible delivery systems of *B. bassiana* showed that application of the entomopathogenic fungi with planting material, pseudostem traps or soil around the banana plants can be used to infect banana weevil in the field and also reduce the damage caused to the plant. These studies have demonstrated that good potential exists for use of *B. bassiana* as microbial control agent and would fit well with the broad IPM context being developed for the banana weevil.

It was necessary to further investigate the applicability of this entomopathogenic fungus to the banana farming system in more ecological and complex farming conditions.

This weevil project has two trials running concurrently with on-station and on-farm experiments. The on-station trial was initiated in October 2002, while that of on-farm in July 2003.

On-station experiment. This trial aims at (i) evaluating the effect of banana spacing on the efficacy and persistence of *B. bassiana* and (ii) evaluating the effect of banana spacing on the agronomic performance of the crop.

Efficacy and persistence of *B. bassiana*. To assess the efficacy and persistence of the fungus, *B. bassiana* is applied as solid maize-based formulation; applied at 2 doses and 2 application frequencies (every 2 and 3 months). The following 2 doses have been applied and are being compared with the control (untreated plots). Dose 1 (high dose) = 200g of maize-based formulation; measuring to approximately $10^{15\text{-}16}$ conidia/hectare. Dose 2(low dose) = 100g of maize-based formulation, measuring to approximately 10^{11} conidia/hectare. Due to limited mass production space, it was not possible to have all the *B. bassiana* inoculum for the whole field produced at the same time. It was decided to stagger the application time (at approximately 2–3 weeks intervals) among the three replicates depending on the prevailing production capacity. We continued with fungus application the course of this period. Six applications have been made in the fields where the fungus is applied on two months interval (i.e. twice on all the replicates) and for the three months interval, fungus has been applied three times (i.e., once on every replicate). The efficacy and persistence of the fungus is being assessed by monitoring corm damage of the harvested plants and weevil population: Data for this trial are being collected into 2005.

Effects of spacing/plant density on agronomic performance of the crop. Banana plants were planted at spacing of 2 × 2 m, 2.5 × 2.5 m, and 3 × 3 m. These spacing were chosen to simulate possible plant densities at farmers' fields. The hypothesis being tested; different plant densities will affect the soil environment and growth characteristics of the plant that will directly or indirectly influence the performance of the fungus in the field. To investigate the influence of plant spacing on agronomic and yield parameters, data has been continually collected on: plant girth, plant height, number of suckers per mat, number of leaves per plant (mother and daughter), flowering and harvesting dates, bunch weight, and number of clusters per bunch. In addition to the above, data on soil temperature and soil moisture has also been collected. Meteorological data are also being collected. To enhance growth and vigor of the plants, artificial fertilizers was applied in both the blocks i.e., block 10 and extension G. Fertilizers were applied per mat at the recommended rates; 69g of NPK, 28g of urea, 31g of KNO_3, and 3g of Mg SO_4. The plantation was mainly mulched using dry banana leaves cut from the plants.

On-farm experiments. Preliminary on-farm studies investigating *Beauveria bassiana* persistence and infectivity under farmers' management conditions are being conducted at Kisekka benchmark sites in Masaka district. The trial was established in July 2003, and so far implemented on 10 farmers' field. Maize-formulated *B. bassiana* at a rate of 200g per mat was applied in two different ways: 1) applied around the banana mats without mulched and 2) applied 30–45 cm away from banana mats and then mulched. Farmers when putting mulch, some mulch away from the banana mats while others just put blanket mulch in the field covering the banana stools. This trial was intended to investigate how *B. bassiana* would perform under these farmers' practices. In each farmer's field, 20 mats were used for each treatment and control. Assessment of the efficacy and persistence of *B. bassiana*, data was collected on Weevil abundance and monitoring the extent of the corm damage. Agronomic parameters were collected on plant girth, plant height, number of suckers per mat, number of leaves per plant (mother and daughter), flowering and harvesting dates, bunch weight, and number of clusters per bunch. Data will be collected through 2005. Farmers have been impressed with the whole trail because it has enabled them to reduce the number of weevils in their farms especially in the areas where the study is being undertaken. Some farmers said that the fungus applied has enabled their plants to grow much taller and healthier. More farmers need to be included in this study.

The way forward will include the following activities:

- Development of an improved technique of producing consistent good quality inoculum of *Beauveria bassiana* in an appropriate formulation developed and optimum conditions for deployment described.
- Development of a method of producing, harvesting, and packaging of *B. bassiana.*
- Further testing of *B. bassiana* delivery systems under farmer's conditions.
- Evaluation of a protocol of integrating different banana weevil control options with *B. bassiana.*

3.10 Biological control of mango mealybug

by P.N. in collaboration with O. Ajuonu

In Bénin, local infestations on single trees in the big northern towns of Parakou and Natitingou continue to be reported; but in all cases these orchards suffer from insecticide treatments or drift, which interferes with the biological control agents.

3.11 Biological control of spiraling whiteflies

by P.N. in collaboration with O. Ajuonu

In 2001, spiralling whitefly was reported for the first time from Cape Verde Islands and no parasitoids could be detected. In 2002, the two parasitoids *Encarsia haitiensis* and *E. guadeloupae* from IITA Benin were released by the Instituto Nacional de Investigação e Desenvolvimento Agrário (INIDA) and by 2003, both parasitoids were found to be established. Following the poor field recovery of *E. guadeloupae* by the end of 2003, an additional shipment of both parasitoids was sent from IITA Bénin to Cape Verde Islands in 2004. In Bénin, populations were not followed up in 2004.

3.12 Biological control of water weeds

by P.N. in collaboration with O. Ajuonu and M. Hill (PPRI)

A starter colony of the moth *Niphograpta albiguttalis* was produced in 2004 and shipped to the Kenya Agricultural Research Institute.

In Bénin, water hyacinth infestations are no longer monitored regularly. In a collaborative project between IITA and the Ministry of Forest Economy of the Congo (Brazzaville), four weevil species had been introduced in 1999/2000 against three floating water weeds that threatened local fishing activities and transport in rivers, flooded forests and lakes of the Congo: *Neochetina eichhorniae* and *N. bruchi, Neohydronomus affinis*, and *Cyrtobagous salviniae*. In a vast survey in 2003 by the Congolese national program and IITA Bénin, with some follow-up activities in 2004, it was found that as a result of this biological control project, water fern (*Salvinia molesta*) and water lettuce (*Pistia stratiotes*) were under total control wherever the two specific weevil species had reached. Long closed waterways were opened up again and people returned to previously abandoned villages/parts of villages. Control of water hyacinth (*Eichhornia crassipes*) was found to be slower, but showed good progress. In interviews, documented for national television, villagers (Bantu as well as pygmies) acknowledged this successful implementation of biological control.

In the framework of an MSc by O.A., a study on the interactions between three biocontrol agents released against water hyacinth was undertaken. Under laboratory conditions, naturally occurring intensities of old and fresh feeding scars from *Neochetina eichhorniae* and *N. bruchi* were simulated and offered to the mirid *Eccritotarsus catarinensis* for feeding and oviposion. The study investigated the interactions between the two weevil species and the mirid first released in African in 1996, several years after the weevils have established. Adult mirid *E. catarinensis* had high mortality on plants with old feeding scars. They survived and produced better on undamaged plants and on plants were adult *Neochetina* spp. were actively making fresh feeding scars. This result underscores the compatibility of the two types of control agents.

3.13 Biological control and habitat management of *M. vitrata*

by M.T. in collaboration with C. Agboton, N.S. Talekar, R. Srinivasan, and B.B. Singh

Collaborative research activities at AVRDC, funded within the scoping study sponsored by the Gatsby Charitable Foundation, continued in 2004 and have led to the discovery of another natural enemy of *M. vitrata* with high potential for control, this time it is a entomopathogenic virus *Mv*NPV (*Maruca vitrata* Nuclear Polyhedrosys virus). Preliminary laboratory experiments revealed very high mortality rates at commonly used concentrations of occlusion bodies (Fig. 3).

The rearing of the first parasitoid identified in Taiwan, *Apanteles taragamae*, continued at AVRDC, where a visit is planned for 12–18 June, 2005, to assess the level of parasitism in the field, and particularly to bring back to our laboratory in Cotonou the first shipment of pupae.

Concerning the tachinid parasitoids, no import permit into Bénin could be obtained prior to a final and indisputable identification of the organism, which has been tentatively identified as *Nemorilla maculosa*.

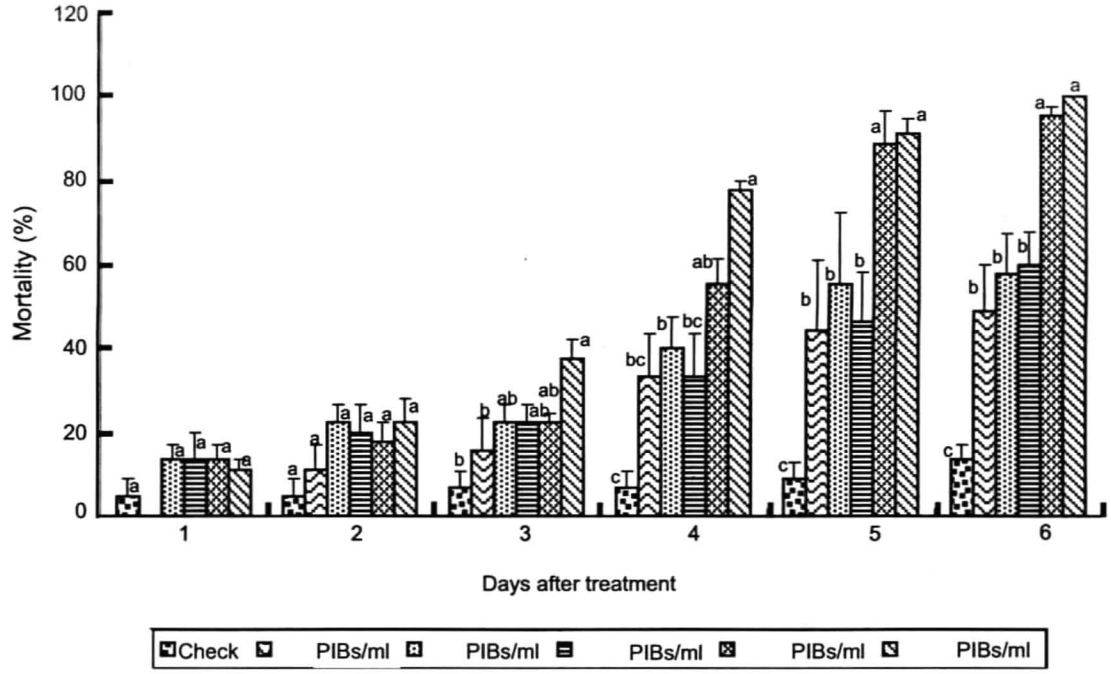


Figure 3. Mortality (%) inflicted by *Maruca vitrata nuclear polyhedrosis virus* (MvNPV) on second instar *M. vitrata* larvae under laboratory condition at AVRDC.

3.14 Biological control of cowpea flower thrips

by M.T. in collaboration with C. Agboton, J. Sagbohan, and T. Cudjoe

Surveys to monitor the establishment of the exotic thrips parasitoid *Ceranisus femoratus* (Hymenoptera, Eulophidae) introduced in 1999 from Cameroon continued in southern and central Bénin in 2004. As in the previous year, *C. femoratus* showed seasonally fluctuating population dynamics, mostly depending on thrips host plant. Parasitims levels as well as populatin dynamics of *M. sjostedti* are being recorded, in order to start preliminary assessment of its impact. The laboratory production at BCCA could be maintained at about 2000 parasitoids per 3 weeks cycle.

In Ghana, plots of *Tephrosia candida* were planted at three different locations, Pokuase, Kumasi, and Techiman, in order to increase field populations of *C. femoratus*.

Experimental releases were also carried out on flowering *T. candida* plots at the IITA Ibadan campus late 2004. Monitoring to assess establishment is currently ongoing.

3.15 Ecofriendly alternatives to harmful pesticide regimes in cabbage production

by, B.J. and C.K in collaboration with I. Godonou, C. Atcha-Ahowé, S. Vodounhè, F. Onikpo, and G. Heviefo

The peri-urban IPM project has screened eight local isolates of entomopathogenic fungi and selected *Beauveria bassiana* 5653 as a promising candidate biopesticide agent against DBM in UPU areas of Bénin. The biopesticides agents screened were the fungi *Beauveria bassiana* isolates 5648, 5653, and 5654 and *Metarhizium anisopliae* isolates 180 and 182. All isolates were previously collected on Lepidoptera species in Bénin: *B. bassiana* 5648 on

Acigona sp., *B. bassiana* 5653, 5654, and *M. anisopliae* 180 on *Sesamia calamistis*; and *M. anisopliae* 182 on *Eldana saccharina*. Laboratory trials have determined the pathogenicity, virulence, and persistence of *B. bassiana* 5653, *B. bassiana* 5654, and *Metarhizium anisopliae* 180 compared to the virulence of the granulosis virus *Plxy*GV-Nya01. Amongst the eight isolates, *B. bassiana* 5653 caused the highest sporulation and mortality (94%) of DBM larvae three days after inoculation (a fast killing ability) followed by *M. anisopliae* 182 which caused 74 % mortality. All other pathogens caused less than 65% mortality nine days after inoculation. Practically, no mortality and sporulation were obtained with *M. anisopliae* 180, *B. bassiana* 5648, and the control. The results underlined the project's findings that *B. bassiana* 5653 is a good biopesticide candidate against DBM; the isolate was therefore selected for further tests on its formulation and persistence against DBM larvae and for large scale participatory field trials.

3.16 Ecofriendly alternatives to harmful pesticides against root nematodes in vegetables

by, B.J. and D.C. in collaboration with H. Baimey and S. Loumedjinon

In potted experiments, the effect of four doses of each of the two botanicals on RKN incidence and damage severity was compared to four doses of fungal nematicide *Paecilomyces lilacinus*. The doses were 5, 20, 35, and 50 g dried epidermal peels of cassava or orange and 1, 2, 3, and 4 mg of *P. lilacinus*. The nematicide Rugbi 10 was applied as a check treatment. The botanicals and *P. lilacinus* were mulched into sterilized potted soil. The test crops were gboma and carrot. Each pot was inoculated with 1500 juvenile RKN. Nematode abundance in gboma was inversely related to dosage of botanicals and *P. lilacinus*. The effect of cassava skin peels and of the fungus on RKN abundance was similar at each of dosage tested. RKN abundance was zero for Rugbi 10, as well as for cassava peels applied at 50 g per pot and *P. lilacinus* applied at 4 mg per pot. The effect of orange skin peel treatment on number of RKN was less than for other treatments. The percentage of roots with galls was inversely related to dosage of botanicals and fungus applied. In treatments with the chemical nematicide Rugbi 10, roots of gboma plants remained free of galls. Carrot responded more favorable to the botanicals and other treatments than gboma. In carrot treated with cassava peels at 5 and 20 mg per pot, the percentage of plants with galls was 22 and 14% respectively. In potted plants with treated with *P. lilacinus* at 1 and 2 mg per pots, the percentage of plants with galls was 14 and 12% respectively. All treatments resulted in 100% of the plants without galls at the highest doses. The botanicals hold particular promise in nursery management and crop-based approaches control RKN in vegetables. Subsequent activities focus on participatory development of appropriate formulations for small-scale production by the farmers.

3.17 Introduction and establishment of Brazilian isolates of the acaropathogenic fungus *Neozygites tanajoae* and impact on population dynamics of cassava green mite; and prevelance of *N. tanajoae* infections in eastern and southern Africa

by R.H. and M.To. in collaboration with B. Agboton, A. Cherry, F. Hountondji, R. Irungu, A. Jone, C. Kariuki, J. Maniania, B. Pallangyo, and M. Sabelis

Regular monthly monitoring and/or countrywide surveys were conducted in 2003 and 2004 to determine the incidence of indigenous strains of *N. tanajoae* in Kenya, Tanzania, Zambia, and Mozambique. The fungus was present but in very low incidence in Tanzania and

Zambia, and completely absent from Kenya and Mozambique. The Tanzania and Zambia samples were identified as indigenous *N. tanajoae* using the molecular probe developed to distinguish exotic and indigenous isolates. The virulence of the Tanzania strains are presently being compared to the Bénin and Brazil strains. Preliminary results indicate that rates of mite mortality due to infections by the Tanzanian strains are much lower than those of the Beninese or Brazilian strains.

In a follow-up to findings from previous surveys after the failure of *T. aripo* to establish and persist in some parts of Bunda and Geita districts in Tanzania, a Brazilian strain was recently released in the two districts. Follow-up evaluations are planned for October/ November 2005. Additional releases are planned in Malindi in Kenya and Inhambane province in Mozambique.

3.18 Experimental releases of exotic phytoseiids and evaluations of their impact on cassava green mite and cassava productivity in collaboration with NARS partners

by R.H., M.To., A.O., M.Ti., D.G., and A.L. in collaboration with B. Agboton, J. Anania, M. Andrade, C. Asanzi, O.S. Bah, W. Bwana, T. Cudjoe, A. Dixon, F. Hountondji, A. Jone, C. Kariuki, N. Mahungu, E. Mambo, C. Malambo, V. Mgoo, K. Negloh, P. Nagel, J. Ngeve, E. Neukenine, N. Ntonifor, J. Ogwang, M. Otema, B. Pallangyo, G. Paraiso, G. Phiri, M. Pivi, U. Scheideger, K. Tatahangy, L. Traore, I. Zannou, and C. Zundel

The biological control campaign for cassava green mite continued at several levels in all participating countries. One survey was conducted in 2004 in the central and eastern provinces of DR Congo, one survey in Cameroon (in conjunction of the ARTS survey in 2004), and one survey was conducted in Tanzania in 2005.

The exotic predatory mite *Typhlodromalus aripo* has shown excellent persistence and impact on CGM in most regions with a few exceptions—western Zambia, limited areas in central Mozambique and along the shores of Lake Victoria in Tanzania, parts of Salima district in Malawi, and most of Katanga province in DR Congo. In Cameroon, the predator is widely distributed, but has not persisted well in the midaltitudes of the northwestern province.

Case study: the biological control campaign of cassava green mite in Tanzania. CGM biological control in Tanzania was initiated in 1998 collaboration with the Tanzania National Biological Control Program (NBCP). The campaign included new introductions and redistributions of *T. aripo*, follow-up surveys on its establishment and spread, impact assessment, and farmer training on pest and natural enemy recognition and means of enhancing predator efficacy. *Typhlodromalus aripo* was first found in March of 1998 in the Tanga region, most likely invading from the southern Kenya coast where it was released in cassava fields adjacent to the Tanzania border in 1996. Subsequently, numerous introductions and redistributions of the predator were carried out by Tanzania NBCP and IITA. Surveys conducted in the following 6 years revealed successful establishment, persistence, and spread in four agroecological zones including the Lake (Mara and Kagera regions, except Mwanza region), western (Kigoma region, except Shinyanga region), southern highlands (Mbeya and Ruvuma regions), and eastern (Tanga and Coast regions) zones. Cassava green mite mean densities have declined to low levels (less than 20 actives per leaf) in all regions where *T. aripo* was present, while they remain 5–10 fold higher in certain localities in some areas of Bunda and Geita districts, where the predators is established on in limited areas. In on-farm impact assessment trial, *T. aripo* was capable of reducing the

population density of cassava green mite densities by 62.5% and increasing total and marketable cassava root weights by 61.3 and 24.4%, respectively. There was also a significant increase in total number of roots (25.4%), number of marketable roots (20.2%), and stem weights (15.8%) where *T. aripo* was not eliminated. Present efforts in Tanzania are aimed at release and follow-up on establishment of a Brazilian isolate of *N. tanajoae* into Bunda and Geita districts where *T. aripo* has not done well.

4 Effective host-plant resistance against pests identified and developed

Ongoing and future activities

4.1 Early screening of banana and plantain for resistance to black leaf streak

by R.B. in collaboration with M. Twizeyiman, F. Moonan, and A. Tenkouano

Black leaf streak (BLS), caused by *Mycosphaerella fijiensis*, is the most important foliar disease of *Musa* worldwide. The pathogen infects leaves and causes severe and premature defoliation. This results in reduction in yield and premature ripening of fruits. Although fungicides remain the mainstay of BLS management in commercial plantations, host-plant resistance is the control option of choice for resource-poor, small-scale banana farmers who produce the crop for domestic consumption and sale.

One of the requirements for breeding of resistant cultivars is the availability of screening methods to identify sources of resistance. Such methods should be simple, efficient, repeatable and economical so that large numbers of breeding populations can be evaluated in a cost-effective manner both in time and space. Most often, BLS resistance evaluation is carried out in the field. For field evaluation, plants are grown in the field until bunch formation after which resistance is evaluated. As a result, field evaluation requires more than one year and large area per unit plant making such evaluation time consuming and expensive. Development of a resistance screening technique for rapid evaluation of resistance at an early stage that overcomes the need to grow large number plants in the field and wait until bunch formation would considerably reduce time and other resources thereby speeding up breeding for BLS resistance.

The use of tissue culture techniques offers valuable means of generating and selecting variants of *Musa* resistant to BLS. In vitro selection of BLS-resistant clones can take place at several tissue levels such as callus, cell suspension, stem apices, in vitro plantlets, and pieces of plants. Such techniques allow screening of not only newly generated clones but also of plants obtained from collections or prospected from the wild. Evaluation of BLS resistance has been carried out experimentally by exposing cell suspension of *Musa* to the toxin produced by *M. fijiensis*. However, to our knowledge, this technique has not been used for routine evaluation of BLS resistance. Resistance evaluation has been also performed on detached leaves using agar plugs containing mycelial growth of the pathogen.

In vitro plantlets in tissue culture tubes and detached leaf technique appear to be promising plant material for use in early screening method. However, there is a need to understand several aspects of inoculation methods before a screening method can be devised with these plant materials. The purpose of this study was to develop resistance-screening method using in vitro plantlets in tissue culture tubes and detached leaves. We also reported the use of these techniques to evaluate *Musa* clones for resistance to BLS.

An isolate of *M. fijiensis* from IITA-Onne Station was used for this study. The fungus was grown on V8 juice agar for 26 days under near-UV light at 20 °C to encourage the growth of the fungus and conidial production. Plants in the field provided leaves for the tests on detached leaves. In vitro tissue culture plantlets were obtained from IITA's Tissue Culture Laboratory. Plantlets with at least 4–5 leaves and with a well-formed leaf-whorl were selected.

Development of tissue culture plant inoculation technique for resistance evaluation. Several inoculum types were tested to determine the best infective propagules for infection. Mycelial fragments (10^6 conidia ml^{-1}) and two conidial concentrations (5×10^5 conidia ml^{-1} and 10^4 conidia ml^{-1}) were evaluated as inoculum source. A drop of inoculum was carefully placed in the leaf whorl ensuring that the inoculum drop did not fall off the plants onto the tissue culture growth medium. This step was crucial to avoid contamination of the growth medium with the inoculated fungus. A non-inoculated control with sterile water, and another non-inoculated control without water was also maintained. All inoculations were done under aseptic conditions in a laminar flow hood. Tubes containing the tissue culture plants were incubated at 25 °C with a 12-hour diurnal cycle following treatment. Four clones were used for this experiment: Agbagba (susceptible), FHIA-23 (moderately susceptible), TMBx 4479-1 (resistant), and Calcutta 4 (highly resistant). Each treatment consisted of three plants and was replicated thrice in a split plot design with clones as main plots and inoculation treatments as subplots. Plants were observed on every fourth day to record the percentage leaf area damaged due to BLS.

Mycelial inoculum induced maximum leaf area damage on all clones followed by conidial suspension at higher (5×10^5 conidia ml^{-1}) and lower concentration (Fig. 1). Mycelial inoculum also contained toxins since the inoculum had the agar medium on which the fungus grew and produced the toxin. Conidial suspensions were devoid of agar and the disease incidence was purely due to infection induced by conidia. Since mycelia are not the normal propagules causing infection in nature, we selected 5×10^5 conidia ml^{-1} inoculum suspension for further experimentation.

Use of tissue culture plant inoculation technique for resistance evaluation. We selected 10 clones of *Musa* with different reaction to BLS under field conditions to determine the appropriateness of in vitro tissue culture plantlet inoculation for resistance evaluation. Conidial suspension (5×10^5 conidia ml^{-1}) was used to inoculate plantlets as described above. Two plants were inoculated (with an additional non-inoculated plant) for each clone. The experiment was conducted in randomized block design with three replications. Incubation period, symptom evolution time and leaf area damaged of plants were recorded on every fourth day. The experiment was repeated two times.

The highly susceptible clone Agbagba was most susceptible whereas the resistant clone Calcutta 4 was most resistant in the in vitro test (Table 8). The clone TMBx 4479-1, which has Calcutta 4 in its pedigree, was also resistant (Fig. 4). The FHIA clones, though susceptible to BLS, were less so than Agbagba (Fig. 4). The reaction of the 10 clones in in vitro plantlet evaluation was similar to known field reaction of these clones.

Development of detached leaf technique for resistance evaluation. Detached leaves are prone to degradation and yellowing after they are excised from mother plants. We initially conducted experiments to determine methods to keep detached leaves green for a long duration by reducing degradation and yellowing. The leaves had to be kept green for a long time since *M. fijiensis* requires several weeks to express symptoms on leaves. We tested several hormones at different concentrations and combinations in water agar

(1%) to identify a suitable hormone that can keep leaves green. The hormones tested were gibberellic acid (5, 8, and 15 ppm), kinetin (10, 30, and 50 ppm), and benzimidazole (20, 40, and 60 ppm) (Table 9). A hormone-less water agar (1%) treatment was maintained as control. Water agar with or without hormones was poured into 9 cm diameter petri dishes under aseptic conditions. Newly unfolded, symptom free leaf pieces (3 × 4 cm) were excised

Table 8. Evaluation of *Musa* clones for resistance to black leaf streak using in vitro tissue culture plantlets and detached leaf techniques.

	In vitro plantlets in tube			Detached leaves		
Musa clones	IP (d)[1]	SET (d)[2]	LAWS (%)[3]	IP (d)	Time to stage 4 (d)[4]	LAWS (%)
Agbagba	5.3 c[5]	16.7 e	80.9 a	7.8 d	20.3 d	31.2 a
FHIA-23	6.2 c	24.7 d	36.2 b	12.0 c	29.3 c	15.8 bc
FHIA-25	6.5 c	29.3 cd	28.4 bc	15.3 c	29.8 c	16.6 b
PITA-21	7.3 c	30.6 c	27.9 bc	13.3 c	28.7 c	10.0 c
TMPx25291-S44	7.0 c	31.5 c	23.2 cd	-[6]	-	-
TMPx26388-1	8.0 c	28.7 cd	22.3 cd	15.3 c	29.9 c	15.2 cb
TMPx24203-14	7.3 c	31.3 c	19.8 cd	-	-	-
TMPx25291-S26	8.8 bc	30.0 c	16.5 de	11.6 cd	27.8 c	11.6 cb
TMBx 4479-1	12.3 b	*[7]	6.4 ef	22.0 b	**[8]	2.1 d
Calcutta 4	27.3 a	*[7]	1.1 f	29.3 a	**[8]	0.8 d

[1] Incubation period (number of days from inoculation to first appearance of symptoms, (i.e., Fouré Stage 1)
[2] Symptom evolution time (number of days from inoculation to occurrence of mature lesions, (i.e., Fouré Stage 6)
[3] Leaf area with symptoms (%) 32 d after inoculation
[4] Number of days from inoculation to occurrence of elliptical spot, i.e., (Fouré Stage 4)
[5] Means followed by same alphabet in columns are not significantly different from each other in Duncan Multiple range Test ($P \leq 0.05$)
[6] Data not available
[7] Mature lesions (or Fouré Stage 6) did not occur
[8] Elliptical spots (or Fouré Stage 4) did not occur

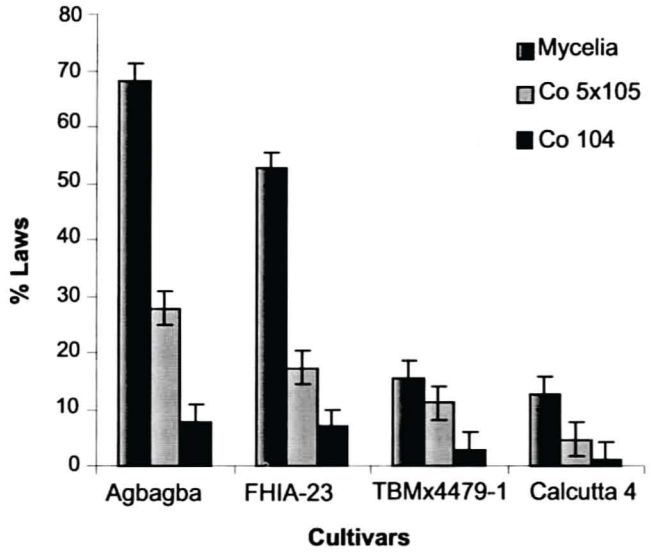


Figure 4. Percent leaf area with symptoms (LAWS) of black leaf streak on tissue culture plantlets of four banana clones 14 days after inoculation with mycelial fragments (mycelia) and conidia (5 × 10^5 spores ml^{-1} and 1 × 10^4 spores ml^{-1}) of *Mycosphaerella fijiensis*. Control (sterile water) plantlets did not show any symptoms.

Table 9. Green leaf area of detached *Musa* leaves maintained on different hormone amended water agar plates at 28 and 52 days after incubation.

Hormones and concentration	Green leaf area score[1]	
	28 days	52 days
Gibberellic acid 5 ppm	1.0 c[2]	1.6 d
Gibberellic acid 8 ppm	1.2 c	1.9 cd
Gibberellic acid 15 ppm	1.3 c	2.6 c
Benzimidazole 60 ppm	4.3 b	7.5 b
Benzimidazole 40 ppm	7.8 a	9.0 a
Benzimidazole 20 ppm	8.7 a	9.0 a
Kinetin 10 ppm	9.0 a	9.0 a
Kinetin 30 ppm	9.0 a	9.0 a
Kinetin 60 ppm	9.0 a	9.0 a
None	9.0 a	9.0 a

[1] Scored on a 1 to 9 scale where 1 = >90% green leaf area and 9 = <20% green leaf area

[2] Means followed by same alphabet in columns are not significantly different from each other in Duncan Multiple range Test ($P \leq 0.05$)

from field grown plants of Agbagba. It was washed several times in sterile water, surface sterilized in sodium hypochlorite solution (1.5%) for a minute, washed three times in water, and placed abaxial side up on agar plates (2 pieces per plate) ensuring that the leaf pieces lay flat on agar surface without curling. Plates were incubated at 25 °C with a 12-hour diurnal cycle. Leaf pieces were rated for green leaf area on every fourth day on a 1–9 scale where 1 = > 95% green leaf area, 2 = 80–95% green leaf area, 3 = 70–80% green leaf area, 4 = 60–70% green leaf area, 5 = 50–60% green leaf area, 6 = 40–50% green leaf area, 7 = 30–40% green leaf area, 8 = 20–30% green leaf area, and 9 = <20% green leaf area. Each treatment consisted of three replications, each with three plates (or 6 leaf pieces). The experiment was repeated twice.

Gibberellic acid maintained detached leaves green for more than 52 days. Even 5 ppm of gibberellic acid was sufficient to retard degradation of leaves. Surprisingly, both kinetin and benizimidazole were not effective in spite of their proven capacity to reduce chlorophyll degradation in other plant species. Source of agar was an important consideration. Agar powder with impurities led to contamination of water agar plates, Technical grade agar powder (Agar Technical from Difco or Unipath Ltd., UK) was used since it did not support contaminating microbes on agar plates.

Use of detached leaf inoculation technique for resistance evaluation. To determine the effectiveness of detached leaf technique for resistance evaluation, we selected eight clones of *Musa*. Leaf pieces were prepared as mentioned above and placed on 1% water agar amended with 5 ppm gibberellic acid. After two days of stabilization of leaf pieces on the medium, 50 µl of conidial suspension (5×10^5 conidia ml^{-1}) was placed at six spots on each detached leaf piece. For each clone, two plates (each with 2 leaf pieces) were inoculated. The plates were incubated at 25 °C with a 12-hour diurnal cycle. Incubation period, symptom expression and leaf area damaged were recorded on every fourth day. The experiment was repeated two times.

As in in vitro tissue culture plantlet inoculation, Agbagba was the most susceptible clone on which incubation period was shortest and leaf area damage highest (Table 8). Elliptical lesions or mature spots did not develop on the resistant clones TMBx 4479-1 and Calcutta 4. The other clones were intermediate in reaction and had similar reaction pattern normally observed under field conditions.

Statistical genetic validations tests. Current BLS disease field evaluations primarily focus on a relative indexing method based upon the youngest leaf spotted (YLS) at flowering, though a crop cycle and ratoon cycle occurring over up to a 3-year planting period. The impact of YLS to yield in the field has been well studied and characterized, validating it as a screening method for BLS. The TMB, TMP, and Calcutta 4, plantlets as illustrated in Figure 4, and Table 8 represent a partial sib-related series of plantlets comprising well studied YLS field indexed plants across multiple environments in Nigeria. In addition, the sensitive reference landrace accession of Agbagba also has substantive field data on YLS also collected. While data from the three methods (two in vitro, and one field) cannot be directly compared to each other for analysis of variance (ANOVA), the relative nature of the expression of the measured genetic components across this germplasm base can be analyzed by stepwise linear regression models of the trends. This is to identify the consistency of the in vitro data in relation to the field data. As shown in Table 8, measured phenotypic components for the host-pathogen interaction include, incubation period (IP), symptom evolution time (SET) to stages 4 and 6, and percent leaf area with symptoms (LAWS), which may be drawn to expression of possible separate genetic contributions in regards to host response time to infection as well as cell to cell movement of the pathogen within infected tissues. Within a partial sib series, statistical genetic models cannot only be developed that validate the use of the particular in vitro methods presented here, but to develop mathematical formulas of greater predictive value by utilizing the data of each of these in vitro measured subcomponents, and the development of such predictive statistical genetic models is ongoing.

Conclusions. Evaluation of BLS resistance in the field requires a substantive investment in land and labor resources over individual timeframes which may approach as high as three years each through a plant crop and ratoon cycle, depending on the particular environment. Results suggest that both in vitro tissue culture plantlet inoculation method and detached leaf method can be used as early screening methods for BLS resistance evaluation, for a substantive cost savings in both resource and time investment. The detached leaf method is easier of the two to use since production of in vitro tissue culture plantlets requires sophisticated facilities, synchronization of plantlet growth in tissue culture, and is expensive. However, resistance evaluation can be performed more rapidly with in vitro tissue culture plantlets once they are synchronized for growth and micropropagated in tissue culture, compared to detached leaf technique. These two methods should prove valuable to breed *Musa* accessions with BLS resistance.

4.2 Methods to evaluate soybean for rust resistance

by R.B. in collaboration with M. Twizeyimana

A field and laboratory-based method to evaluate soybean for rust resistance was developed. The filed screening method is based on infector row system and the lab method is based on detached leaves.

Infector rows (test line rows surrounded by highly susceptible line rows). A trial was established in 2003 and repeated in 2004 at IITA to study the screening method using infector rows. Many combinations were used which include, one, three, five, and seven rows of test line rows surrounded by two infector rows on either sides of the test rows. Data from trials revealed that the combination of three rows of test line surrounded by infector rows was the best because the rust inoculum from infector rows spread equally in all three rows of test line, which was not the same case in other combinations where inoculum spread unequally among test lines rows. This system was subsequently followed for resistance evaluation in the field.

Detached leaf technique. As an obligate parasite, the soybean rust fungus requires living tissue to develop and multiply. Detached leaf technique was used to provide living and healthy tissue for the fungus to develop. It consisted in putting a piece of soybean leaf on a kinetin-amended agar medium in plastic petri dish. The piece of leaf had to stay healthy at least three weeks to allow the fungus grow, develop, and multiply. Several growth hormones at various concentrations were tested to identify the best hormone for use in maintaining the detached leaves green longest on agar medium. We found that 1% agar technical amended with 10 ppm Kinetin was the best-amended medium; it could maintain pieces of soybean leaves healthy for more than three weeks. This technique will be used in screening for rust resistance in the laboratory and in rust variability study.

4.3 Identification of soybean lines with rust resistance

by R.B. in collaboration with M. Twizeyimana

During the last two years, we have been evaluating soybean-breeding lines to identify resistance to rust. Until now, we have screened more than 150 lines and 36 of these lines were further evaluated in 2004 in a replicated trial. Infector rows were used to enhance the source of inoculum, and three rows of test lines were sown between two adjacent infector rows. From this evaluation, 11 lines were identified as resistant (<15% leaf area damaged by rust at R6 growth stage). These were: TG× 1903-3F, TG× 1871-12E, TG× 1835-10E, TG× 1895-50F, 1897-17F, TG× 1891-3F, TG× 1740-2F, TG× 1895-9F, TG× 1864-17F, TG× 1895-6F, and TG× 1869-13E. We plan to evaluate these lines in 2005 to determine the mechanism of rate-reducing resistance that these lines appear to possess.

4.4 Identification of sources of resistance to fumonisin contamination in maize

by R.B. in collaboration with C. Afolabi and A. Menkir

Fusarium ear rot caused by *Fusarium verticillioides* has become a significant concern of the maize (*Zea mays* L.) producers, consumers, and the processing industries all over the world, because of the significant losses in both grain yield and quality. Host plant resistance is one of the best strategies to manage fumonisin contamination in maize. The purpose of this study was to evaluate a wide range of maize inbred lines for resistance to *Fusarium* ear rot, fumonisin contamination, and kernel infection under natural and artificial infections. One hundred and three maize inbred lines and four maize hybrids used as checks were evaluated for resistance to *Fusarium* ear rot, fumonisin accumulation, and discolored kernel. The maize inbred lines and hybrids were evaluated under natural and artificial infections in Ikenne and Ibadan respectively, during the maize growing season of 2003. Fifty inbred lines with ear rot severity rating of ≤ 3, and four inbred lines with higher severity ratings in both trials were selected for fumonisin analysis and reevaluation in 2004. Inoculation was done

through the silk channel by injection method at blister (R2) stage. Eight inbred lines were identified with fumonisin concentration ≤ 10µg g^{-1} in the inoculated trials across the two years, out of which two (02C14585 and 02C14624) inbreds had fumonisin concentration ≤ 4µg g^{-1}, which is below the level of concern for human consumption. In the non-inoculated trials, 26 inbred lines were identified having fumonisin concentration ≤ 10µg g^{-1}, out of which, nine inbred lines had fumonisin concentration ≤ 4µg g^{-1}. Pearson correlation coefficients between fumonisin concentration and percent-discolored kernel gave indication of high levels of fumonisin in the grain. There was no significant interaction between inbred lines and year, suggesting that the inbred lines behaved similarly in both years under inoculation. Further research work needs to be done on the identified inbred lines with low fumonisin concentration under different agroecological conditions. The inbred lines are useful sources of resistance for breeding programs.

4.5 Studies on expression of host plant resistance

by C.S.G. in collaboration with G. Night and S. Power

Three modes of resistance are recognized in plants: non-preference, antibiosis and tolerance. Screening trials and field surveys have shown that many banana cultivars are highly to moderately resistant to banana weevil. Non-preference is not an important factor. The female weevil is attracted to and lays eggs on all cultivars in both the laboratory and field. Studies on plant tolerance to banana weevil attack require multiple cycle trials and are not well understood. All evidence to date suggests that resistance is antibiotic in nature.

Treatment of eggs with sap (latex) from resistant cultivars has been reported to reduce hatchability. However, it is not clear whether the negative effects of sap are due to its physical properties, chemical elements, or both. Furthermore, an antibiotic mechanism in some cultivars was suggested based on high performance liquid chromatography (HPLC) analysis of extracts from various cultivars. Increased developmental time, reduced vigor and increased mortality of larvae were observed when larvae were reared on corm tissue of resistant cultivars. Antibiosis may be expressed either as a chemical toxin or antifeedant.

In addition to chemical composition of plants, physical factors such as corm hardness may confer antibiotic resistance to banana weevil. Increased corm hardness may lead to failure of larvae to tunnel and feed. Tissue toughness may lead to wearing of larval mandibles and reduced feeding.

In banana, patterns of corm damage by weevils suggest uneven distribution of plant defenses. Distribution of damage in the plant may also influence yield loss. Additionally, differences in oviposition and larval performance on different host phenological stages have been reported. By evaluating resistance at different growth stages, useful patterns can be identified that have applications for breeding programs. An understanding of these phenological patterns is also important for purposes of pest management and timing of control measures.

Expression of resistance is a result of interplay between genetic traits and environmental factors. One of the environmental factors that may influence resistance of banana plants to the weevil is plant nutrition. Given a limited set of resources, plants might change their allocation pattern in response to such factors as herbivore attack. Also, succulence may be influenced by such nutrients as nitrogen.

The objectives of this research activity were to: (1) characterize the chemical and physical factors responsible for banana resistance to weevil; (2) study the distribution of resistance

factors within the plant and variation of resistance with plant phenology for selected cultivars; and (3) determine the influence of plant nutrition on expression of resistance.

Bioassays carried out on feeding larvae on corm pieces in the laboratory showed delayed development of larvae feeding on corms of resistant cultivars. For instance, larval developmental period on resistant Kisubi (AB) was 37d, while that on susceptible Atwalira (AAA-EA) was 25 d. Adult weight was similar for individuals feeding on different cultivars. However, survivorship in laboratory studies was not consistent with resistance patterns observed in the field, that is, we were able to rear weevils from egg to adult in highly resistant cultivars (e.g., Yangambi-Km 5, AAA) that show virtually no damage under field conditions. It was hypothesized that resistance breaks down quickly in cut plants.

A pot experiment was therefore set up to study development rates and survivorship in growing plants. The data from potted plants did agree with cultivar resistance patterns observed in the field. Development rates and survivorship were lower on resistant cultivars. For instance, survivorship on Yangambi Km5 (resistant) was 5% after 45 days compared to 30% on Atwalira (susceptible). Survivorship correlated well with corm damage observed in the field. These observations suggest that laboratory studies may not be suitable for evaluating all the facets of resistance mechanisms although they can provide useful insights.

Laboratory observations showed that larvae generally took longer to tunnel into corms of resistant cultivars compared to susceptible cultivars. It is not clear whether the basis of this resistance is physical or biochemical. Data that will be taken on corm hardness will be used to determine the mechanism.

Feeding deterrence is one way in which antibiosis may be manifested. An experiment was set up to observe settling and tunneling time of newly hatched larvae offered corms of different cultivars (Atwalira, Kabula, Mbwazirume, Calcutta, Kisubi, Kayinja, and Yangambi Km 5). Settling time was increased on resistant cultivars. While on susceptible cultivars (Atwalira, Kabula, Mbwazirume) all larvae penetrated the corm completely in 1–1.5 hours, up to 40% of the larvae had not penetrated the corms of resistant cultivars (Kisubi, Kayinja and Yangambi Km 5) in the same time. In another study, newly hatched larvae took more than twice as long to tunnel into corms of a resistant cultivar (Kayinja, 693 minutes) compared to those of a susceptible one (Atwalira, 275 minutes). In this experiment, entry holes were made for the larvae. In another study where entry holes were not made, larvae took an average of 126 minutes to penetrate corms of Atwalira compared to 231 minutes on corms of Kayinja.

Laboratory bioassays indicated that the food consumption rate on resistant cultivars was lower than that on susceptible cultivars. However, total food consumption was similar over the duration of the larval stage, the larval period being longer on resistant cultivars. This explains why adult weights do not differ for individuals feeding on cultivars of varying resistance. Based on these results, it can be concluded that the differences in corm damage observed in the field on different cultivars are more a function of survivorship than of differences in total tissue consumption of individual larvae. Also, the susceptible cultivars would suffer higher damage as they support more weevil generations per unit time.

Studies were also taken on expression of resistance in different parts of the banana plant and for different plant phonological stages. Larvae feeding on pseudostem had longer developmental periods (59 d) than those feeding on the corm (31 d). Also, survivorship of individuals feeding on pseudostem was low (59% for early instars; 29% at adult stage)

compared to that of larvae feeding on corm tissue (77% for early instars; 53% at adult stage). Adult weight of weevils raised on corm tissue was 0.054g compared to 0.033g for those on pseudostem tissue.

Banana weevils preferentially feed on the corm but will occasionally attack the pseudostem. However, oviposition is often in the leaf sheaths, indicating that the first instar larvae must tunnel through pseudostem tissue to reach the corm. Failure of larvae to tunnel into the corm (for instance if corms are deep) should reduce survivorship. Studies are underway to examine the migration of larvae from the corm to the pseudostem in different cultivars.

A field experiment was planted in October 2003 to determine the earliest plant age at which differences in resistance of cultivars become apparent. Due to delayed establishment of plants (attributed to weevils), the first sample for damage assessment was taken nine months after planting. At this age, cultivars could be distinguished as resistant, susceptible, or intermediate. While in the past evaluation of resistance has been carried out on harvested plants, this study suggests that resistance screening data can be obtained earlier. While sampling was delayed in this experiment due to poor plant establishment, there is potential for further reduction in time resistance data can be obtained in the field. Data collected from younger plants (due to repeated filling of gaps left by dead plants) suggests that resistance differences are observable at six months of age.

The next set of activities concerned the effects of plant nutrition on the expression of weevil resistance. A field experiment was planted in December 2002 and consisted of two treatments. The nutrition treatment had two levels: a fertilizer treatment and a control. There were six cultivars with a gradation of resistance levels. Banana plants displayed only limited response to fertilizer in the plant crop, but differences are becoming evident in the first ratoon cycle. Data will be taken for ratoon cycles through 2005.

A follow-up pot experiment is underway to determine the influence of nutrient rates and balance on weevils. The study will examine the effects of N, K, Mg, Ca, P, and the micronutrients Zn, B, and Mn. The experiment consists of eight treatments. In one treatment all the nutrients are omitted from the sand growth medium while in another, all nutrients are applied. In the other treatments, we omit one nutrient (or group of nutrients for the micronutrients) at a time. Nakyetengu, an East African Highland cooking cultivar, was used. Newly hatched larvae will be inserted into the plants and their development rates and survivorship observed.

4.6 Varietal differences in fumonisin contamination levels in maize varieties in Bénin

by K.H. in collaboration with P. Fandohan and P. Dewaminou*

The present research carried out from June to October 2004 at IITA-Benin station aimed to study the varietal behavior of maize to *Fusarium verticillioides* infection. Twelve maize varieties out of which one local and eleven improved were sown in a split-plot device of two treatments (inoculated and uninoculated) with four repetitions. The plants were inoculated at the 49th day after sowing (JAS) above the collar of the first internod, with toothpicks previously infected with a pure suspension of a toxinogenic strain of *F. verticillioides*. Samples of stems were collected at the 48th (before inoculation), 62th, 76th, 90th, and 104th JAS and cultivated on PDA to appreciate on the one hand, the incidence of the microflora in general and particularly that of *F. verticillioides* in the stems, and on the other hand, the systemic evolution of *F. verticillioides* in the internod. Parameters such as ear slope,

husk covering, and color of the grains were measured to evaluate their influences on the fungic infection of grains at the harvesting time.

The microflora identified on stems and grains were mainly constituted of genus *Fusarium*, *Aspergillus*, and *Penicillium*. Other genus found were *Mucor*, *Alternaria*, and *Rhyzospus*. This microflora was dominated by *Fusarium* and in particular by the species *F. verticillioides*. The results of the culture of peaces of stems showed a strong incidence of *F. verticilliodes* on the inoculated plants (37.99 ± 1.06) and it evolved in time, dropping in intensity from the lower internods to the upper ones. The behavior of various varieties to stems infection did not vary significantly from one variety to another. However, the incidence of *F. verticillioides* was higher on the improved variety DMRESRY (24.53 ± 1.62%) and weaker on DMRESW (17.99 ± 1.62%). Furthermore, the inoculation of the stems by *F. verticillioides* had had a significant effect on the infection of the grains by an increase of 34.88% of its incidence. The improved variety DMRESRW was the less infected (20.84 ± 6.71 %), while the local variety Gbogboué and the improved HPG97 were most vulnerable, with respectively 37.79 ± 6.71 and 37.36 ± 6.71 percentage of infection. The color did not have a significant effect on the grains infection by *F. verticillioides*. The infection by *F. verticillioides* was negatively correlated with the slope and the ears husk covering.

4.7 Screening of *Musa* hybrids for resistance against nematode species

by D.C. in collaboration with F. Moonan and A. Tenkuoano

Screening of *Musa* hybrids and cultivars has been established in Ibadan, Nigeria against the main *Musa* nematode parasites in West Africa: *Radopholus similis, Helicotylenchus multicinctus, Pratylenchus coffeae, Hoplolaimus pararobustus,* and *Meloidogyne* spp. for identification of resistance to nematodes and also to map the heritability of nematode resistance. Diploid and polyploid hybrids derived from crosses using cvs Calcutta 4, Yangambi Km5, Heva, and Padri are being screened against all four species. Differential responses are being observed by some hybrids to some of the species, but not all, while observed resistance against one species does not necessarily apply for all nematode species. Screening is being repeated and continuing to provide reliable results.

4.8 Cassava cultivars for resistance to nematode pests routinely screened

by D.C. in collaboration with P. Ntawuruhunga and A. Dixon

Cassava cultivars were assessed for their reaction to two root-knot nematode species commonly associated with cassava: *Meloidogyne incognita* and *Meloidogyne javanica* and also a mixture of *M. incognita* and *M. javanica* in sterile sawdust in IITA-Sendusu to establish the reaction of cvs against the two common root-knot species (Table 10). The experiment consisted of five replications in a randomized complete block design, using 10 cassava lines planted in 30 × 40 cm polythene bags. The 10 cassava lines included TME12, 00067, 2324, 0414, 0427, 0063, 2327, 00057, TME14, and 00087 obtained from IITA cassava breeding program. Uninoculated controls followed each treatment combination.

Plants were inoculated at three weeks after planting with a suspension of 1000 eggs of the three *Meloidogyne* spp. treatments. Data was collected at two months after inoculation: plant height, root fresh weight, galling index, number of galls, and nematode population density g^{-1} root fresh weight. Cultivars reacted differently to the different species of nematode depending on species and/or cultivar. Some cultivars were more affected by *M. incognita* than *M. javanica* in some cases and the reverse in others. Also, inoculation of

Table 10. Effect of the different *Meloidogyne* spp. treatments on growth parameters and damage indices across cultivar.

	Plant ht (cm)	Fresh root wt	Galling index (1–5)	No. of galls	*Meloidogyne* spp.
Control	11.2a	5.7a	1.0b	0.0c	0.0b
M. javanica	9.3b	3.8b	2.5a	3.5a	213a
M. incognita	8.7b	3.6b	2.5a	3.3ab	216a
Mixture	9.9ab	4.2b	2.2a	1.7b	132ab
Sign. level	**	***	***	***	***

mixed species resulted in greater galling for some cultivars and less for others than single inoculations. Galling index was higher for *M. incognita* and mixture in variety 2324. Cultivar 0063 also had higher galling index for *M. javanica* than other treatments. The results suggest complex inter-species reactions with sources of resistance different for the different species. The trial will be repeated at least once to help confirm results, but suggests the need for screening against the separate species, and possibly in combination also.

4.9 Development of technique for screening cassava genotypes for resistance to root-knot nematodes

by D.C.

Screening cassava for resistance has been undertaken in a relatively ad hoc manner by various workers, usually using a variety of related methods. They tend to be time and space consuming, however, and often reliant on a reasonable level of nematological expertise, a commodity in short supply in many national programs. Efforts have been undertaken therefore to try and identify a relatively simple method for rapid screening of genotypes, that is transferable to most conditions existing in national programs. The study took the example of hanging plastic tubes from a frame, as developed by the cassava green mite project at IITA-Cotonou, with the aims of identifying an appropriate irrigation and inoculation method/rate in combination with appropriate plant (stem) spacing and position in the tubes. Studies are being conducted at IITA-Sendusu. Four node cuttings of *Meloidogyne* spp. susceptible cv Nase 4 (SS4) were planted in 20 cm diameter polythene tubings filled with sawdust and hung on a wooden frame. The tubings (1 m length each) were arranged in a split-split plot design with 4 factors: inoculation method, irrigation method, plant spacing and cutting (stem) position using three replications.

Three weeks after planting, tubes were inoculated with a suspension of ~ 1000 *Meloidogyne* spp. The plants were harvested after two months. Data collected at harvest included plant height, fresh root weight, galling index and *Meloidogyne* spp. root density. Data on the volume of water required was also taken. Inoculation method influenced ($P \leq 0.05$) root fresh weight, galling index and *Meloidogyne* spp. density (Table 11). Irrigation method influenced plant height, galling index, *Meloidogyne* spp. density and tuber weight. Spacing influenced plant height and fresh root weight. Cutting position affected only plant height. Notable interactions of inoculation method × irrigation method and inoculation method × cutting position significantly influenced *Meloidogyne* spp. density and galling index respectively. The results are being used to modify the arrangements towards developing a robust design with consistent results.

Table 11. Analysis of variance for effect of irrigation method, inoculation method, plant spacing, and sett position on growth and nematode incidence of cassava under screen.

Source of variation	F values					
	d.f	Plant height (cm)	Fresh root weight	Galling index	*Meloidogyne*[1] 1g	Tuber weight
Inoculation method (A)	1	0.54ns	5.58*	148.5***	254.01***	0.86ns
Irrigation method (B)	1	39.88***	1.52ns	17.13***	5.46**	46.49***
Spacing (C)	2	5.93**	12.48***	2.22ns	0.56ns	2.65ns
Sett position (D)	2	6.61**	0.56ns	2.00ns	0.13ns	2.89ns
A*B	1	0.37ns	0.70ns	1.57ns	8.67**	0.37ns
A*D	2	1.97ns	0.86ns	4.14*	1.44ns	0.10ns
A*C	2	5.57**	0.48ns	1.23ns	1.29ns	4.99**
B*D	2	1.90ns	0.68ns	0.69ns	0.70ns	2.43ns

[1] square root transformed values used; ns, *, **, and *** represent not significant (P > 0.05), significant at 0.05, 0.01 and 0.001 respectively.

4.10 Development and identification of root-rot resistant cassava germplasm

by M.M., R.B., and A.T. in collaboration with A. Dixon

The deployment of resistant cassava varieties is seen as the most appropriate and cost-effective approach to manage root and tuber pathogens that occur widely in the humid forest ecological zone. Various activities have been undertaken towards developing and identifying resistant cassava germplasm. These include:

Development of germplasm screening techniques. To enable efficient screening of improved cassava varieties for resistance to rot pathogens, it is important to have reliable methods for inoculating plants with the pathogen. Starting 2003, experiments were designed and carried out to determine the most suitable plant growth stage to inoculate test plants and the most appropriate method to deliver inoculum as well as the best place to deliver the inoculum for optimal infection. Different inoculation methods evaluated included (1) placing inoculum on soil surface; (2) exposing tuber and pouring inoculum on top, (3) exposing tuber and creating injuries to facilitate pathogen entry and (4) placing inoculum onto the tubers borne on toothpicks. Inoculating periods evaluated were three, six, and nine months after planting. After the first year of evaluation results indicated there was no benefit when plants were inoculated at three months because tuber formation had not started and infection was not optimal. Infecting at later growth stages, which appeared to be most suitable, is being evaluated in a repeat trial. Delivering inoculum by toothpick method resulted in the highest infection levels. In addition, the toothpick method enabled easier identification of the rot occurring specifically due to the applied organism, which can be easily confused with rots resulting from other pathogens naturally present in the soil.

Evaluation of improved cassava varieties. In 2003–2004 10 elite cassava lines recommended from the Cassava Breeding Unit were planted in a diseased-evaluation block at IITA research farm Ibadan. The main soilborne pathogens in the evaluation plots are *Botryodiplodia theobromae, Fusarium solani, and Sclerotium rolfsii* among others. High inoculum levels of these pathogens have been established over time, encouraged by growing cassava in the same block for two or more consecutive seasons, and each time, the infected

cassava tubers are chopped and incorporated into the soil-using tractor. At 12 months after planting, the 10 clones were harvested and evaluated for rotting. In addition to data from the field, healthy tubers of the same clones were brought to the laboratory and artificially inoculated with the different pathogens using two different methods i.e., (1) root slices cut from the tubers and asceptically inoculated with the pathogen and (2) holes drilled into whole tubers and inoculum placed inside. Data obtained from these different methods were considered in selecting the best five lines for further evaluation. The clones evaluated and their reaction to different pathogens is summarized in Table 12. All the 10 clones were observed to be highly susceptible to *B. theobromae*. However, five clones showed tolerance to either *F. solani* or *S. rolfsii* or both. These five were selected and planted, and are being evaluated for a second season to select some that can be used as breeding material. Another 495 improved cassava varieties recommended from the breeding unit were planted in 2004 at IITA-Ibadan and are being screened for resistance to *B. theobromae* and *F. solani* using artificial inoculation techniques. Results are expected in 2005.

Evaluation of cassava germplasm in Cameroon. Nineteen improved cassava varieties were screened for resistance to rot pathogens at IITA research farm in Mbalmayo, Cameroon. This was a preliminary screen with each variety evaluated in two replicate plots. Results (Table 13) indicated most of the varieties were highly susceptible to rot pathogens but cultivars W94/0009, 96/0867, 81/0010, 91/02325, and I89/02831 were considered suitable for further evaluation.

Evaluating the role of selection criteria on occurrence of rots. Increasingly ,breeders and farmers are selecting cassava varieties that have specific traits such as low cyanide content, high ß-carotene content, thin peels, etc. These choices are made mostly considering processing methods and the eventual end use. It is thought that preference for some traits such as reduced cyanide in tubers could lower the capacity of cassava plants to defend them against attack by soilborne fungal pathogens. Other traits such as ß-carotene content are thought to affect the balance of reactive oxygen and other radicals in the tubers, which could affect how a particular cultivar reacts to tuber pathogens. To determine how differ-

Table 12. Reaction of 10 cassava clones to three root and tuber rot pathogens at Ibadan.

Clone	Whole tuber test			Root slice test			Rot intensity in field	
	Bt	Fs	Sr	Bt	Fs	Sr		Remark
TME 163	S	R	R	S	R	S	Low	Selected
188/00188	S	S	R	S?	S	R	Moderate	Selected
181/01610	S	R	R	S?	S	R	Low	Selected
92/0211	S+	S+	R-	S	S	S	High	–
187/01004	S	R -	R-	S	S	S	High	–
TME 139	S	S	R-	S	S	S	Moderate	–
TME 514	S	R	R-	S	S	S	Moderate	–
92/0057	S+	R	R-	S	R	S	Low	Selected
0082/01438	S	S?	S	S	R	R	Moderate	Selected
184/00460	S	R	S	S	R	S	High	–

Bt = *Botryodiplodia throbromae*; Fs = *Fusarium solani*; Sr = *Sclrotium rolfsii*, S = susceptible; R = resistant (no symptoms developed).

ent selection criteria may impact on the occurrence of rots, 30 improved cassava varieties with wide ranging variations in cyanide and ß-carotene content, as well as other aspects, were obtained from breeding unit and planted in diseased plots. The study is expected to end in 2006.

Table 13. Incidence and severity of fungal tuber rots on 19 IITA improved cassava varieties evaluated at Mbalmayo Farm, Cameroon.

Clone name	% number of rotted tubers	% rotted volume	Clone name	% number of rotted tubers	% rotted volume
W94/0009	5.5	4.0	97/4013	54.9	98.2
96/0867	30.0	27.0	95/0967	55.0	45.0
81/00110	33.3	30.8	95/0061	60.7	60.7
91/02325	33.5	34.3	98/0506	63.1	63.1
I89/02831	34.8	34.8	I82/00058	66.7	64.1
96/0963	39.4	39.4	GBAZEKOUT	70.0	68.5
M94/0121	39.5	38.8	92/0342	72.1	65.3
MS20	44.7	43.8	30040	73.7	73.7
84/0031(4X)	48.5	48.5	89/00003-1(3X)	75.3	75.3
97/0270	53.2	47	Local check	75.5	92

4.11 Population dynamics of ARTS on local and improved cassava germplasm and identification of sources of cassava resistance to ARTS

by R.H., M.Ti., K.L., A.T., C.N., and A.L. in collaboration with A. Dixon, A. Fotso Kuate, J. Ngeve, A. Ndoumptsop, A. Nguenkam, and K. Tatahangy

This is a broad activity that encompasses long-term studies on population dynamics of ARTS and the identification of sources of cassava resistance to ARTS and the subsequent development of improved varieties that can be integrated into other tactics for ARTS management. Over a period of two years, population abundance of ARTS and its associated ants were followed on a monthly basis for the length of the crop cycle in six fields in Cameroon and four fields in DR Congo and under different fallow history. Taken together, the data from the 10 fields indicated similar scale dynamics. Regardless of variety, scale populations increase rapidly during the first half of a normal 12 month crop cycle, followed by a peak between six and eight months after planting, and a sharp decline until crop harvest.

A follow-up of ARTS population dynamics was conducted on two cassava cultivars, a sweet variety Lueki and a bitter variety Mvuama, in a highly infested site of secondary forest of Bas-Fleuve in DR Congo. Three planting dates (early, intermediate and late planting) were used to follow the changes in the development of populations of ARTS on two cassava varieties. ARTS population followed similar temporal trends on both varieties and for the three planting dates. Peak ARTS populations occurred at the onset of the main dry season, in May in the plots of the early planting and in June in the intermediate and late planted plots. The late planting date showed the highest peaks and higher infestations throughout. Dramatic drops in ARTS populations occurred just after the peaks, from 700 individuals to < 100 individuals for the highest peak. The drop was likely due to dispersal,

reduced fecundity, and natural mortality. No evidence of predation or parasitism was observed. These observations show that cassava varieties, cultivation season, dry season effects, natural mortality, and dispersal are major factors influencing ARTS population abundance on cassava. Several years of population dynamics data from Cameroon and DR Congo are presently being compiled for a global analysis and publication.

In addition to population dynamics studies, we are conducting several germplasm evaluations to identify sources of cassava resistance to ARTS. The varieties used were either land races in use by farmers, improved varieties available for use by farmers, improved varieties recently selected for release to farmers, or improved varieties undergoing selection for multiple desirable traits.

In Cameroon, we proceeded with both short- and long-term goals for developing cassava germplasm resistant to ARTS. For the short-term goal, the program evaluated 10 varieties selected from IITA and IRAD's cassava improvement programs for resistance/tolerance to ARTS. Two productive varieties (TMS 96/0023 and TMS 92/0326) supported acceptable ARTS levels. They were selected (along with one NARS variety) as a component of an IPM package combining host plant resistance, vegetation management, and planting method for ARTS management in 16 fields in Central Province of Cameroon. For the long-term goal, we collected representative cassava land races (a total of 189 accessions) throughout the distribution range of ARTS in Cameroon. These land races were planted in two locations (Atin-Odzoe and Mbalmayo) in Central Province of Cameroon where they are presently being characterized and multiplied for further on-farm studies to identify sources of resistance to ARTS for incorporating them into programs developing ARTS-resistant cassava germplasm.

In DR Congo, the cassava IPM project has been working closely with the USAID-financed cassava project, which has as one of its main activities the development of disease-/pest-resistant cassava germplasm with emphasis on CMD. Several clones are presently being evaluated on-farm in Bas-Fleuve and Kisangani where ARTS infestations are quite severe. Preliminary data indicates that the two varieties Mvuama and Sadissa have considerable resistance to ARTS and could well serve as sources of resistance for incorporation into the cassava improvement program in DR Congo and Cameroon. In the other Bas-Fleuve trial, over 60 CMD-resistant clones (originating from preliminary yield trials) are being evaluated for their resistance to ARTS.

5 Effective habitat management options against pests developed

Ongoing and future activities

5.1 Use of clean planting material for nematode management promoted in Nigeria through demonstration plots for improved *Musa* production

by D.C. in collaboration with S. Hauser and A. Tenkuouano

Improved *Musa* production was promoted at 15 farm sites in Nigeria using clean, healthy planting material in combination with organic mulching. On-farm demonstration plots were established in 2003 to demonstrate alternatives to farmer practice towards increased longevity of plantain plantations and increased production per crop cycle through nematode management and increased nutrient availability. Demonstrations included a small plot each of: (a) farmers' own traditional planting style (involving the use of suckers without

removal of roots or treatment, and without mulching); (b) use of suckers with roots removed from the corm and then the corm dipped in boiling water for 30 s; and (c) treated suckers (roots removed and dipped in boiling water) plus *Tithonia diversifolia* mulch added at a recommended rate of one handful per plant per month when possible.

The use of three treatments provides farmers with the ability to assess the separate use of healthy material and then additionally with mulch added, in comparison with their own practice. The use of boiling water to heat-treat suckers has been developed at IITA to disinfest suckers of debilitating nematode pests. It is a modification, which is proving more convenient and suitable for smallholder farmers, than conventional hot water treatment recommendations. The addition of even small amounts of mulch was also promoted. Plant height and girth was greater in treated+mulch treatments at 6, 9, and 12 months after planting compared with the other two treatments; nematode damage was reduced in both treated sucker treatments compared with farmer practice. Yield data is being collected, but to date, the treatments with treated suckers are flowering and bunching ahead of the farmers practice. Farmers initially proved skeptical to using pared suckers and also to heat-treating them.

Initial preliminary demonstrations were therefore necessary to gain farmer confidence that suckers had not been killed by the procedure, before commencing on demonstration plots. Even then, farmers remained apprehensive until visual differences became obvious to the farmers between the various treatments. During 2004, approximately 12–18 months after planting, farmer field days were held in conjunction with national agricultural programs, farmer groups, and individual farmers at participating farms to discuss the merits of the various treatments by the farmers to other farmers. Demonstrations of "cleaning" suckers by removing roots (paring) and boiling water treatment was organized at the field days. There has been an overwhelming response by farmers to use both healthy material and to add *T. diversifolia* mulch, which is readily available in most locations. An increasing demand for healthy suckers is being witnessed in the pilot study areas, with some farmers establishing multiplication plots for sale of healthy (premium) suckers. Support for expanding the program wider and across *Musa* growing areas is currently being sought, to promote this simple, yet effective method.

5.2 Farmer awareness campaign for banana bacterial wilt in Uganda

by C.N., C.S.G., A.A., and R.B. in collaboration with W. Tushemereirwe

In early 2001, a serious outbreak of banana *Xanthomonas wilt* (BXW), caused by the bacterium *Xanthomonas campestris* pv. *musacearum* (×*cm*), was reported in Mukono district, Uganda. This devastating disease has quickly become the most serious threat to the banana production in the country. BXW can spread rapidly and is now present in eastern, central, and northern Uganda. Recent reports of BXW in Kabarole and Bushenyi districts place the disease on the edge of the country's primary banana growing regions in southwestern Uganda. If unchecked, the disease would cause massive losses in Uganda's western districts, an area of intensive banana cultivation. The livelihoods of millions of Ugandan farmers are now at risk. BXW has also been confirmed in the Democratic Republic of Congo and it seems only a matter of time that it will enter Rwanda and Burundi.

Bacterial wilt was initially reported in Ethiopia on *Ensete*. ×cm infection can result in severe losses in banana production due to early ripening and rotting of fruits (even in the absence of apparent external signs of the disease), and to wilting and death of banana

plants. Ratoon crops arising from infected mats are severely diseased and often wilt before producing bunches or produce bunches with rotted fruits. All types of bananas appear susceptible, although certain cultivars (e.g., ABB beer bananas) probably play a critical role in facilitating the spread of BXW.

Field observations suggest that the primary means of disease spread is by insect transmission through the male flowers. Secondary infection may occur through the roots or the use of contaminated tools. Wilting of floral bracts and the male bud, premature yellowing and rotting of fruits, and bacterial exudation from the peduncle are symptoms of infection through the inflorescence. Yellowing of leaves, yellow to pink bacterial exudates in the leaf sheaths and plant wilting are symptoms of infection from soilborne inoculum or from a previously-infected pseudostem. BXW appears to be similar to Moko disease (*Ralstonia solanacearum*) of banana with respect to disease development, transmission and damage. Stingless bees, wasps and flies are believed to be important vectors of Moko disease with infection commonly occurring through the moist cushions or scars of recently dehisced male flowers and floral bracts. However, virtually no information is available about the vectors, infection courts, transmission epidemiology and biology of BXW since the disease is new. A good understanding of these factors is required for developing and targeting BXW management practices.

The Uganda National Banana Research Program (UNBRP), in collaboration with IITA and other national and international partners, have undertaken an intensive BXW mitigation program and countrywide awareness campaign. The primary objective of this campaign is to contain the disease and prevent its spread. The principal recommendation has been to protect banana plants by removal of the male flowers to eliminate the most likely infection court. This recommendation has been drawn from the research and management of Moko disease in the Americas. However, the presence of similar groups of vectors and disease pathways for BXW remains to be confirmed.

Since the late 1980s, IPM technology development, evaluation, promotion, and dissemination efforts in Uganda have been focused on a range of priority constraints. Banana weevil, nematodes, black sigatoka, *Fusarium wilt*, and *Banana streak virus*, have been identified by UNBRP, IITA, and other partners. However, the recent emergence and rapid spread of BBW, first recognized in Mukono district in September 2001, is already having a devastating effect on banana cultivation in central Uganda in particular and is threatening the highly productive regions of south and southwest Uganda. It also has the potential to significantly counteract progress made with respect to IPM development and implementation, including the uptake of improved banana varieties. The disease is now known to be present in 28 districts. Of major concern is the realization that all banana varieties in Uganda, including improved varieties possessing resistance to other pests and diseases and currently being promoted, appear to be susceptible to the disease. BBW has now been recognized as the primary threat to banana production in Uganda and the potential implications with regard to future food security in the country realized at the highest level. Steps to halt further spread of the disease and to eradicate it from affected areas are currently being developed and implemented as a matter of urgency.

Although intensive research is still required to fully understand BBW, measures that will help to contain the disease such as destruction of affected plants, removal of the male flower bud, and use of clean, unaffected planting materials are recognized. Many stakeholders within and beyond BBW affected areas are already aware of such practices, symptoms

associated with the disease, and mechanisms of BBW introduction and on-farm spread as part of IPM sensitization. Recommended practices are now being applied in many areas but with variable degrees of success.

In close collaboration with NARO, IITA engages in farmer awareness campaigns, delivery of best bet technologies, identification of vectors, and gaining a better understanding of disease dissemination Various methods have been used to make the Ugandan public aware of the disease and also to enforce the implementation of the control measures at various levels. The approaches employed included:

- Formation of national BBW task force, steering committee, and BBW working groups to draw a national action plan on BBW in Uganda.
- The National Action Plan recommended integrating BBW control activities into ongoing food security programs in the National Agricultural Research Organization (NARO), The Uganda Ministry of Agriculture Animal Industries and Fisheries (MAAIF), National Agricultural Advisory Services (NAADS), local government programs, NGOs, and international research partners on bananas.
- Training of trainers on BBW in public agricultural extension and NGOs.
- Mass media publicity.
- Going public such as in market places, sociocultural, and religious functions.
- Reaching people through participatory development communication (PDC).

UNBRP in collaboration with IITA and other partners worked together in using a PDC approach to foster farmers' participation in BBW control and application of other banana integrated pest management (IPM) technologies. The research team was convinced that PDC, as opposed to top down technology dissemination, is a methodology that promises sustainable technology adoption by farmers. However, PDC is a process that takes time, yet BBW control demanded quick action during which farmers and researchers had to learn and share information about the symptoms, mode of spread and control of the new disease.

The PDC approach was largely adhered to in order to identify and bring together a broad range of banana stakeholders to discuss and share their knowledge, perceptions and opinions of banana pest and diseases and their management. By doing so, the stakeholders sought to find ways in which they may become better farmers by becoming more informed of prevailing constraints and related management approaches, and thereby become better placed to implement improved management strategies that would help to alleviate the yield and revenue losses experienced on a day to day basis. Primarily through activities held within the stakeholders' communities, including a series of workshops and other meetings, on-farm visits, training, and hands-on exposure to pest and disease problems and management technologies, constraints were identified and prioritized as were management options perceived as being appropriate to stakeholders' needs. A variety of communication materials were specifically developed based on needs identified by stakeholders, to convey knowledge of the available technologies in an appropriate format. The strengths and weaknesses of potential partners in the communication process were identified and partnerships established to facilitate provision of technologies, by intermediaries to end-users. Community action plans were developed for implementing improved pest and disease management, including BBW, from district to village level. Improved technologies have been taken up and applied by farmers and, based on the results of limited monitoring and evaluation undertaken to date, are proving successful. Where implementation is limited, key obstacles

to uptake and adoption have already been highlighted and will inform future communication efforts. In-depth evaluation of the success of PDC process in promoting awareness of BBW control activities is being planned in future banana research activities.

The research team worked with farming communities starting by testing the PDC approach in three BBW affected districts (Mukono, Kayunga, and Luwero). Through this interaction, an extensive and comprehensive range of dissemination outputs and communication materials on BBW have been produced and being disseminated to various BBW frontline districts and unaffected districts. A video was developed highlighting: (1) PDC procedure and protocols followed when working with Mukono and Kayunga district communities to identify IPM and BBW communication needs and related technologies for solving them; (2) Training and sensitization on BBW symptoms, transmission, and control; and (3) processes used in BBW sensitization workshops, highlighting the implementation of BBW control. In addition, fact sheets and posters were produced in English and five local languages.

Through the various stakeholder workshops and meetings, extensive feedback has also been obtained with regard to how they perceive the communication process and promotional activities, factors that continue to limit uptake of existing information and implementation of recommended practices and, of major importance, how these may be addressed. All of this information is critical in ensuring that future development, provision and adoption of banana management technologies is undertaken with the needs of farmers as end users in mind, and that farm management is a practical and attractive proposition.

5.3 Adoption of improved clay store for maize storage in central and northern Bénin

by K.H. and O.C. in collaboration with M. Guirguissou and H. Skovgaard*

Storage structures are key components of postharvest systems in developing countries and particularly in West Africa. Research and extension services are challenged to improve those structures for the reduction of postharvest losses in maize storage. The study was carried out in central and northern Bénin to identify the factors that affect the adoption of improved mud granary for maize storage. The univariate probit analysis model was used to access the combined effect of three kinds of variables: farmers' socioeconomic factors, technology characteristic, and the farm specific factors on the adoption of improved clay store. The results showed that, factors related to market and farm specific factors are negatively correlated with adoption of the improved clay store while the years of farming experience, access to extension service, the extent of maize production and the clay store possibility to reduce maize losses are positively correlated to the adoption.

5.4 Mycotoxin occurrence in *Adoyo*, a traditional maize based beverage in West Africa

by K.H. in collaboration with P. Fandohan, D. Baguilima, and W.F.O. Marasas*

The objective of this study was to find out the importance of the traditional beverage *adoyo* among the maize-based beverages traded and consumed in Bénin, West Africa, and to evaluate aflatoxin and fumonisin occurrence in this product. The importance of *Adoyo* was evaluated by conducting an appraisal survey in two cities of Bénin, during which women sellers of beverages were visited. Aflatoxins and fumonisins were quantified in collected *adoyo* samples, following the VICAM method. Of the beverages encountered, *adoyo* was the most important, traded by 74% of the women visited. Mycotoxins were present in most of the samples, but at low levels (aflatoxin levels < 2 µg kg-1 and fumonisin level < 2 mg

kg-1). A reduction of mycotoxin levels was observed during the preparation of *adoyo* (86% of aflatoxins and 65% of fumonisins). The presence of mycotoxins in *adoyo* is dangerous and calls for more attention from consumers as this product is widely consumed in West Africa.

5.5 Fate of aflatoxins and fumonisins during the processing of maize into food products in Bénin

by K.H. in collaboration with P. Fandohan, J. Hounhouigan, and W.F.O. Marasas*

The fate of aflatoxins and fumonisins, two mycotoxins that cooccur in maize, was studied through the traditional processing of naturally-contaminated maize in *mawe*, *makume*, *ogi*, *akassa*, and *owo*, maize-based foods common in Bénin, West Africa. Levels of total aflatoxin and fumonisin were measured at the main unit operations of processing, and the unit operations that induce significant reduction of mycotoxin level were identified. Overall reduction of mycotoxin level was more significant during the preparation of *makume* (93% reduction of aflatoxins, 87% reduction of fumonisins) and *akassa* (92% reduction of aflatoxins, 50% reduction of fumonisins) than that of *owo* (40% reduction of aflatoxins, 48% reduction of fumonisins). Sorting, winnowing, washing, crushing, combined with dehulling of maize grains were the unit operations that appeared effective in achieving significant mycotoxin removal. Aflatoxins and fumonisins were significantly recovered in discarded moldy and damaged grains and in washing water. Fermentation and cooking showed little effect. During the preparation of *ogi* and *akassa*, reduction of fumonisin levels measured in food matrix was lower (50%) compared to *mawe* and *makume*, probably due to significant fumonisin release in *ogi* supernatant. Consequently, the use of *ogi* supernatant for preparing beverages or traditional herbal medicines could be harmful as it is likely to be contaminated with mycotoxin from the raw maize.

5.6 Participatory testing of aflatoxin management package for maize production in Bénin and Togo

by K.H. in collaboration with T. Houndekon

Trials were established in two villages in northern Bénin/Togo and in two villages in southern Bénin/Togo. In each village, 10 farmers participated in the evaluation. Management package encompassed variety, use of fertilizer, drying, sorting, insect control, and storage in stores with less risk for aflatoxin development. This was compared to the conventional maize production practices. Maize samples were taken at harvest and at two-monthly intervals during storage up to six months. Fungal contamination, aflatoxin levels, and pest level and damages were evaluated.

In the north, losses rose to up to 2% in maize that was stored with the husk. When maize was stored on the husk and neem leaves were added to protect against insects the farmers traditional method, losses were even higher (14.55%). *Prostephanus truncatus* was found on stored maize from the fourth month. When maize was stored without the husk, 1% loss was observed. In the northern village, no aflatoxin was detected on the maize samples. It seems that farmers were able to better manage their maize and store for longer periods with the intervention of the project.

In southern Bénin, there was a high prevalence of samples contaminated with *A. flavus*. In one village, 9% of the samples were contaminated with aflatoxins, with levels rising up to 104.17 ppb, whereas in the other villages, no aflatoxin was found even though up to 25% of the samples showed growth of *A. flavus*. Of the contaminated samples, 71% of the

farmers were storing maize in bags, a storage form that previously was associated with high aflatoxin contamination. Maize stored as grain was heavily attacked by insects, most prevalent were *Sitophilus* spp., *Tribolium* spp., and *Carthatus* spp. Insects prevalence were higher on the local maize variety. Losses of 3.29% were observed in bags, and 6.45% when stored on the platform. When *P. truncates* was present, losses rose up to 8.8%.

5.7 Impact of indigenous storage systems and insect infestation on the contamination of maize with fumonisins

by K.H. in collaboration with P. Fandohan, W.F.O. Marasas, and B. Gnonlonfin*

Four storage systems of maize commonly used by farmers in Bénin, West Africa, were tested to determine infection of maize with *Fusarium* fungi and subsequent contamination with fumonisins. The study showed that *F. verticillioides* was the predominant *Fusarium* species found in all maize samples. *Fusarium* incidence was significantly higher when maize was stored on a cemented floor in a house (40.3 ± 17.4%) than in the other systems. The lowest *Fusarium* incidence was recorded when maize was stored in a bamboo granary (25.5 ± 13.5%) ($p = 0.04$). This suggests that storage systems used by farmers may affect *Fusarium* infection on maize, if these systems create conditions favorable to fungal growth. The storage systems did not seem to have a significant effect on fumonisin contamination, total fumonisin level being not significantly different from one system to another ($p > 0.05$). This indicates that a high *Fusarium* infection level in maize in a granary during the storage period may not necessarily result in a high level of fumonisin contamination. A significant decrease of fumonisin content in maize during storage was observed. This is likely to depend on the storage systems. A 35% decrease was observed in maize stored on the cemented floor in a house, 41% in that stored on a platform, 57% in maize stored in a mud silo, and 76% in maize stored in a bamboo granary. Damage by lepidopterous pests was significantly and positively correlated with both *Fusarium* infection ($r = 0.802$, $p < 0.01$) and fumonisin contamination ($r = 0.852$, $p < 0.01$). The number and damage of coleopterous insects were significantly and negatively correlated with *Fusarium* infection and fumonisin contamination. *Sitophilus* spp. was found positively but not significantly related to *Fusarium* during the first month of storage ($r = 0.229$, $p > 0.05$).

5.8 Impact of mechanical shelling and dehulling on *Fusarium* infection and fumonisin contamination in maize

by K.H. in collaboration with P. Fandohan, P. Houssou*, and R. Ahouansou**

Mechanical shelling and dehulling methods were tested to evaluate their impact on *Fusarium* infection and fumonisin contamination in maize. The mechanical shelling methods tested were found to damage the grains. The motorized sheller type of IITA caused the highest level (up to 3.5%) of damage ($p < 0.01$). This could be due to the operation mode of that machine. Fusarium populations were higher on damaged grains. The highest number of colonies was recorded from grains damaged by the IITA sheller (2533.3 cfu/g) ($p < 0.05$).

Total fumonisin levels were also higher in damaged grains, the highest being in maize shelled by the IITA sheller (2.2 mg/g) ($p < 0.01$). Fumonisin levels correlated positively and significantly with the percentage of damage caused by the shelling methods ($r = + 0.6$; $p < 0.01$), and also with the number of *Fusarium* colonies from maize ($r = + 0.7$; $p < 0.01$). In contrast to the other shelling methods, an increase of the fumonisin level was observed during the first month of storage in maize shelled with the IITA sheller. On the other hand, the mechanical dehulling methods significantly reduced fumonisin levels in maize

($p < 0.01$). The use of dehullers resulted in a reduction of 64–68% for Mini-PRL, 62–67% for Engelberg, and 56–62% for the attrition disc mill.

This study has clearly shown the effects of shelling and dehulling methods on fungal infection and mycotoxin contamination of maize. It is important for farmers to choose appropriate shelling methods to reduce mycotoxin contamination. Also, dehulling, which is an important step in the processing of maize in Africa should be widely promoted for the reduction of mycotoxins in maize. This is a major challenge for all agricultural institutions in Africa.

5.9. Clean seed yam production practices identified and promoted with farmers in Nigeria for improved yam production

by D.C., R.A., and M.A. in collaboration with A. Claudius-Cole, L. Kenyon, S. Morse, and N. McNamara

Various studies have shown that scarcity and expense of clean planting material (seed yams) is a major constraint to increasing yam production and productivity in West Africa. Nematode pests and diseases of yam are perpetuated from season to season through the planting of infested seed yams or setts. Traditional methods of seed yam production under low-input systems supply inadequate quantities required by farmers. The minisett technique developed the by the National Root Crops Research Institute (NRCRI) and IITA in the 1970s as a rapid means of multiplying yam germplasm, is relatively familiar to farmers in Nigeria. But, few actually practice it due to a number of reasons including socioeconomic reasons in addition to scarcity of complementary inputs and the tedious and technical nature of the procedures involved.

A study through a DFID-funded project was established to evaluate methods for producing healthy seed yams in Nigeria and assess the reasons behind the lack of adoption of methods and continued scarcity of healthy material. The yield advantage of using good seed yams over poor ones was consistently reported as being between 2 and 4 times greater, with 3 times being the most usual response among farmers questioned in Ekiti and Kogi states during a survey in 2003. Seed yams of the most popular cultivars are scarce. This is perhaps because these cultivars are "sweet" and more susceptible to nematodes and other diseases. To overcome the problems of seed yams, it has been proposed that seed companies and interested NGOs should handle the production of yam planting material (specifically minitubers) and sell to the farmers as is done by hybrid seed companies. The current study is undertaking an on-farm, livelihoods/cost-benefit analysis in Ekwuloko (Kogi State) and Ado Ekiti (Ekiti State) to produce their own clean seed yams in an economically sustainable manner using simple methods identified from on-station experiments in 2003. The methods are also being promoted through NGO's and commercial seed farmers across a wider area of Nigeria. In the on-station experiments at IITA-Ibadan and with at the Diocesan Development Service Farm in Kogi, setts were prepared by cutting yam tubers of locally popular cultivars into 80–100 g pieces before treating with hot water (20 min @ 53 ^{0}C), neem leaf slurry, wood ash or mild pesticides (fungicide + insecticide) and comparing with untreated control.

The experiments were laid out in a randomized complete block design with five replicates. At eight weeks after planting, plants treated with the mild pesticide had a higher ($P \leq 0.05$) percentage sprouting than those of other treatments, which varied with location and cultivar. At harvest, setts treated with pesticide also produced heavier seed tubers than those from the other treatments, except hot water-treated setts for some cultivars. Seed tuber production from hot water-treated setts was highly variable depending on cultivar.

Pesticide-treated and hot water-treated setts provided healthier seed tubers than either the wood ash, neem treatment or untreated controls, which were less affected by tuber rots, nematodes and associated deterioration in storage, following three months storage. In 2004, on-farm participatory studies in Ekiti and Kogi states showed that pesticide-treated setts provided substantially greater yield of yam tubers, which were larger and of better quality than using the farmers traditional practice of wood ash.

5.10 Clean seed yam production practices identified and promoted with farmers in Nigeria for improved yam production

by D.C. in collaboration with E. Oyekanmi and D. Fawole

A series of experiments were undertaken during 2004 to assess the potential of fungal antagonists against nematodes on maize in both pots and the field. Application of *Glomus mosseae*, an arbuscular mycorrhizal fungus (AMF) was assessed alone, in the various combinations with or without *Pratylenchus* spp. inoculation and NPK fertilizer under different levels of water availability. In field plots, grain yield was greater under favorable water availability as opposed to drought-stressed. Under drought-stressed conditions, plots that received fertilizer had higher grain yields than those not receiving. Application of AMF made no difference to yield as neither did nematode inoculation compared with untreated control. Under favorable water availability however, nematode inoculation reduced grain yield compared to the control, but not when combined with AMF or carbofuran (nematicide).

Under favorable water availability, root densities of *Pratylenchus* spp. were between 3 and 5 times greater than under drought-stressed conditions. Application of AMF suppressed ($P \leq 0.05$) nematode root densities under high as opposed low water availability, while carbofuran was more effective at reducing nematode populations under the dryer conditions. Mycorrhization was however, poor, with only low percentage root colonization: lower than 10% for all but the AMF+fertilizer+*Pratylenchus* spp. treatment, with lower colonization under the dryer conditions. Untreated plots also had relatively high levels of natural *Pratylenchus* spp. infection. Results form the pot experiments generally reflected field results, but AMF application in combination with fertilizer and with *Pratylenchus* spp. more than doubled cob weight per plant compared with all other treatments. This treatment was also associated with high levels of percent AMF colonization, particularly under high water availability (32%). Dry conditions again resulted in low nematode root population densities in most treatments compared to wetter conditions. In an additional, but similar microplot experiment, application of an unidentified *Paecilomyces* sp. appeared to compete with *G. mosseae*, but suppressed nematode densities and led to higher grain yield in the presence of *Pratylenchus* spp. than carbofuran or AMF. However, AMF colonization was again poor (7.5% for AMF alone). Studies are continuing, including the identification of AMF species with ability to provide higher levels of colonization and/or maize cultivars, which are more compatible with AMF.

5.11 Maize–legumes–cassava intercropping in the control of maize cob borers with special reference to *Mussidia nigrivenella*

by K.A. and M.T. in collaboration with S. Gounou

Effects of intercropping maize with cowpea, lima bean, soybean, three leguminous cover crops (*Tephrosia vogelii*, *Canavalia ensiformis*, *Sesbania rostrata*), and cassava on the infestation of *Mussidia nigrivenella* and other cob borers were studied. Field experiments were conducted in four different locations in Bénin using 4 × 2 pattern of maize/legumes

or cassava planting. Intercrops reduced the number of eggs (>25%) and larvae of *M. nigrivenella* (17.9–53%) compared with the monocrop. Maize/*C. ensiformis* and maize/*T. vogelii* proved to be the most effective combinations for reducing *M. nigrivenella* populations in the different locations. Yield loss and cob damage were significantly affected by the intercrops, which reduced losses by 0.9–46.8%, and they were significantly correlated with the number of insects in the cob. No parasitized larvae were found in any of the locations.

5.12 Impact of cassava variety combinations on population densities of the cassava green mite and its predator *T. aripo*, and on cassava yield in Bénin and Nigeria

By A.O., R.H., and M.To. in collaboration with A. Dixon, D. Gnanvossou, B. Ojo, and I. Zannou

There is an increasing awareness that biological diversity can promote community stability, and that plants can play a major role in their defence against herbivores. Using data from cassava fields, we tested whether combining different cultivars of the same plant species can increase predator load on the naturally unfavorable cultivars, thereby reducing herbivore densities and increasing plant productivity. The study builds on evidence that the predatory mite *T. aripo* prefers cassava cultivars with hairy apices. The study was conducted in two cassava fields in West Africa, one in Bénin and the other in Nigeria. The objectives were to:

- Confirm the capacity of *T. aripo* to control cassava green mite in a chemical exclusion trial.
- Determine, based on the differential preference by *T. aripo* to cassava cultivars, how combinations of two morphologically different cassava cultivars with differential suitability to the predator can enhance population densities of the predators on the unsuitable cultivar, and suppress *M. tanajoa* densities and increase in cassava yield.

The experiments confirmed that *T. aripo* effectively suppresses *M. tanajoa* populations, and showed that at both experimental sites and on both cassava varieties (Agric with hairy apices, and Gbézékouté with glabrous apices), densities of *M. tanajoa* and *T. aripo* are significantly affected by cultivar combinations. Although trends in the sprayed plots were not clear. In the unsprayed plots and at both sites, regression of the proportion of Agric (*T. aripo* preferred variety) on *M. tanajoa* and *T. aripo* densities showed generally positive relationships between *T. aripo* densities on Gbézékouté (non-preferred cultivar) and the proportion of Agric in the plots. In contrast, and as expected, the relationship for *M. tanajoa* was generally negative. Our results have shown that targeted crop cultivar diversity can effectively improve biological control of a crop pest. This provides a precious tool for compensating morphological and physiological features undesirable for natural enemies observed in some cassava cultivars to increase their productivity and increase chances of their adoption by farmers. Current efforts are aimed at integrating these findings into cassava improvement programs through the promotion of the use of cassava cultivars with hairy apices for the enhancement of cassava green mite biological control. These findings are also disseminated where feasible in farmer field school and field days.

5.13 Vegetation management, modes of land preparation, and soil fertility improvement to reduce ARTS infestations in cassava fields in Cameroon and DR Congo

by R.H., M.Ti., A.L., L.W., M.To., and C.N. in collaboration with A. Dixon, A. Lema, A. Nguenkam, J. Ngeve, and K. Tatahangy

In previous surveys in Cameroon and DR Congo, we determined that fallow vegetation consisting of various wild host plants and leftover cassava served as significant reservoir of ARTS. These findings provided an opportunity to use vegetation management to reduce ARTS reservoir and therefore ARTS infestations in cassava fields. In addition, there were various indications that prevailing modes of land preparation and depth of tillage in the Congo Basin may affect ARTS infestations on cassava, but experimental data on these practices were lacking.

Vegetation management–Cameroon. We conducted an experiment in nine farmer fields in each of two villages in a forest margins benchmark area in southern Cameroon. The experiment was to determine the effect of removal of host plant residues prior to field establishment on subsequent ARTS infestations of cassava. Two plots, removal or nonremoval of host plant residues, were set up in each of the 18 farmer fields. Vegetation cover, composition, and densities of known ARTS host plants were established prior to field preparation in the selected planting sites and in the surrounding vegetation three months later. In addition, ARTS infestations on the known host plants in the fallow were categorized according to severity. After planting, the fields were managed by the farmers according to common practices.

ARTS populations were censured on a sample of 10 plants in each plot at three, six, and nine months after planting (MAP). Overall mean scale densities per plant were not significantly different between the two villages at three MAP. But, there were more scales per plant at six MAP in Akok compared with Awae, and more in Awae compared with Akok at nine MAP. Mean scale densities per plant varied considerably between fields within village. Some fields were free of scale. The highest mean scale densities of 588±179 per plant were recorded in Akok at six MAP. One field remained free of scale throughout the experiment. Mean scale densities per plant in plots with host-plant removal ('treated') were not significantly different from "untreated" plots during any of the census periods. In addition, ARTS densities on the planted cassava were not correlated with the densities of potential hosts in the preceding fallow and in the surrounding vegetation, contrary to expectations. It appears from this one-year experiment that removal of host plant residues prior to planting cassava after fallow does not affect ARTS populations in the cassava crop.

However, the outcome of this study was probably affected by various factors such as a significant interaction between plot and farmer field within village, indicating a considerable heterogeneity in treatment effect. Indeed, comparisons of ARTS densities in all three pooled censuses showed that ARTS population densities in "untreated" and "treated" plots were similar, higher, and lower, respectively in 12, 21, and 21 censuses. The heterogeneity in treatment effects could be due to such factors as (among others) variations in initial scale population densities, proximity of the plots to the surrounding vegetation, and ant abundance. A farmer-participatory trial was recently initiated in two villages in Cameroon to further test the effects of host removal from surrounding vegetation on ARTS populations in cassava field. The trial also uses two land preparation methods and a minimum of two

varieties, one of which was identified as tolerant to ARTS infestations in previous trials and the other a susceptible local variety. A similar trial is planned for DR Congo.

Soil fertility–Cameroon and DR Congo One of the lingering issues with rising ARTS infestations in short fallows is the possible role of declining soil fertility and resultant nutrient stress on the cassava plant, which could in turn exacerbate already existing scale problems. A fertilizer trial conducted in the Bas-Fleuve district resulted in greater scale densities on fertilized compared to unfertilized plants. While this result was unexpected, it highlights the need for further detailed studies on soil fertility and ARTS infestations. A collaboration is underway with IITA and NARS agronomists and plant protectionists (Cameroon and DR Congo) to determine the response of cassava to inputs of various soil nutrient elements and abundance/incidence of several cassava pests and diseases (e.g., ARTS, whiteflies, mites, cassava anthracnose disease, cassava bacterial blight, cassava mosaic virus disease, and root rots). Preliminary analysis of the results of recently concluded trials indicate that cassava can benefit considerably from various fertility management inputs, which can in turn mitigate some of the negative effects of severe ARTS infestations on cassava yield.

6 Crop protection products based on entomopathogens, botanicals, semiochemicals, and elicitors developed towards commercialization

Ongoing and future activities

6.1 Optimization of Green Muscle to operational grasshopper control

by C.K. in collaboration with Z. Ouambama and D. Kpindou

Though Green Muscle has been commercialized, certain issues remain to be resolved. One of those is the dose rate. The currently recommended dose of 50 g/ha leads to high control costs compared with chemical pesticides and should therefore be reduced if possible. Lower dose rates of Green Muscle have consequently been tested during three seasons. Unfortunately, heavy grasshopper migration occurred through at least some of the trial plots in each season, which made it difficult to interpret the data. However, the results consistently showed similar control efficiency between 50 and 25 g/ha, which means that the recommended dose for grasshopper control can be reduced leading to lower control costs. It is still unclear whether further dose reduction is possible. The problem of the slow mode of action of Green Muscle has been addressed by testing mixtures with a pyrethroid pesticide, Cyhalothrin, in Senegal. The trials first of all demonstrated that the recommended dose of Cyhalothrin can easily be reduced on its own. The dose can be further reduced in combination with a reduced dose of Green Muscle. A quarter dose of Cyhalothrin mixed with a quarter dose of Green Muscle provides better control than each at recommended dose and at lower cost.

6.2 Field testing of *Nosema locustae* against Sahelian grasshoppers

by C.K. in collaboration with J.P. Tounou

A field trial was carried out with *Nosema locustae* in Maine-Soroa, Niger. *Nosema* was applied on 25 ha plots (3 replicates per treatment) as $2.5*10^9$ spores per ha in wheat bran with and without 4% sugar and with and without Green Muscle (50 g/ha) and compared with an application of Green Muscle (50 g/ha) alone. The treatments did not have any effect on the adult populations, but larval populations were reduced in all treatments, while those in the control plots increased slightly. The combined *Nosema*/GM treatment

produced the highest reduction (90%) followed by GM (70%), *Nosema* (60%) and *Nosema* with sugar (55%).

6.3 Chemical ecology and integrated management of the banana weevil in Uganda

by C.S.G. and C.N. in collaboration with W. Tinzaara, A. van Huis, and M. Dicke

Infochemicals play an important role in the biology of many insect species. An understanding of their role in plant-herbivore-carnivore interactions can be used in the development of tools for the enhancement of environmentally-benign pest control options. Infochemicals, especially pheromones, can be used in insect monitoring and in direct control by mating disruption, mass trapping, and as a means of aggregating herbivores at delivery sites for biological control agents. Pheromones and kairomones may potentially be used for control of the banana weevil. The males produce an aggregation pheromone that attracts both males and females. The attractive *Isomer sordidin* has been identified and synthesized, and is commercially available. The objective of the research project described in this thesis was to investigate whether an effective infochemical-based trapping system can be used to control banana weevil under Ugandan conditions.

Different bioassays for investigating orientation responses of the banana weevil to host plant volatiles and the synthetic pheromone (cosmolure+) were compared using a locomotion compensator, double pitfall olfactometer and double port olfactometer. The weevil responded in additive way to the combination of the fermented plant tissue and the aggregation pheromone in the different set ups. The locomotion compensator set-up was recommended as a sensitive device for evaluating weevil response to infochemicals in a no-choice situation, while both the dual port and pitfall olfactometer can be used in a dual choice situation. Further detailed laboratory and field experiments, confirmed that host plant volatiles had an additive effect on attraction of weevils to the pheromone. The effect was more pronounced in laboratory than field experiments. The results suggest that pheromones can be used alone instead of combining with the pseudostem tissue.

Laboratory and field experiments were conducted to investigate the effect of pest biology, pheromone efficacy, trap parameters, cropping system, and environmental factors on response of banana weevil to aggregation pheromone. The studies aimed at providing information that can be used in making decisions on pheromone trap deployment for the control of banana weevil. Weevil age did not influence response to pheromones in the laboratory but differences were observed in field experiments. Equal numbers of males and females responded to the pheromones in the laboratory while significantly more females than males were captured in pheromone traps in the field. Weevil response to the pheromone was not significantly influenced by weevil density. Trap efficiency decreased with distance from the pheromone trap. There was no relationship between temperature or wind speed and catches of banana weevil in pheromone traps. Relative humidity but not rainfall showed a strong positive relationship with banana weevil catches in pheromone-baited traps. Pheromone-baited traps with banana leaves covering around the trap captured higher numbers of banana weevil compared to uncovered traps. Weevil catches in pheromone-baited traps from both mulched and unmulched plots were generally similar. We conclude that mulching is compatible with pheromone trapping.

The effects of pheromone trap densities on banana weevil population and plant damage was evaluated in an on-farm experiment. Changes in banana weevil populations and corm

damage due to pheromone trapping were negligible. Doubling the number of traps caused a negligible increase of the number of weevils caught per ha per month from 0.4 to 0.6% and did not reduce damage to the plants. The pheromone-trapping system on farmers' fields was not effective at the trap density recommended by supplier and doubling the density did not have a significant effect.

Responses of banana weevil predators *Dactylosternum abdominale* (Coleoptera: Hydrophilidae) and *Pheidole megacephala* (Hymenoptera: Formicidae) towards volatiles from banana pseudostem tissue (kairomones) and the synthetic banana weevil pheromone Cosmolure+ were evaluated in a two choice olfactometer. *Dactylosternum abdominale* was attracted to fermenting banana pseudostem tissue and Cosmolure+, while *P. megacephala* was attracted only by fermented pseudostem tissue. Fermented plant volatiles appeared to be more important to the predators of the banana weevil than volatiles from the prey itself during habitat and prey location. There was no evidence that the pheromone influences predator distribution around the trap in the field.

Use of pheromone-baited traps to enhance dissemination of the entomopathogenic fungi, *Beauveria bassiana* to control banana weevil was investigated. Previously, candidate strains of the fungal pathogen, *Beauveria bassiana* have been identified for use in integrated pest management of the banana weevil. However, the lack of an economic and effective delivery system to maximize field effects has been an important limiting factor to their application. Integration of pheromone trapping and application of *B. bassiana* may provide a cost-effective strategy for the control of the pest.

We conducted field studies to determine the potential for pheromone-baited traps to aggregate the banana weevil around the trap mat. Field transmission and use of different delivery systems of *B. bassiana* using pheromones were also investigated. We observed that weevils could be aggregated on banana mats on which pheromone-baited traps are placed and on adjacent mats. We further observed that infected weevils could transmit the fungal pathogen to healthy individuals. Most of the dead weevils due to *B. bassiana* infection were found at the base of the plant in the leaf sheath and from soil near the mat. There were significantly more weevils that died after incubation due to pathogen infection from plots where pheromones were used in combination with *B. bassiana* applied on the trap mat and four adjacent mats than when the pathogen was applied without the pheromones. Our data demonstrate that the banana weevil aggregation pheromone Cosmolure+ could be used to enhance the dissemination of *B. bassiana* for the control of banana weevil.

This project provides ample experimental evidence to further develop the application of the synthetic aggregation pheromone to control the banana weevil in Ugandan banana production by small-scale farmers. The aggregation pheromone should be considered to be a good component of an IPM system in which it may not be effective by itself but stimulate several mortality factors for the control of the banana weevil. The next major strategy for use of pheromones is to further exploit the potential to integrate entomopathogenic fungi (*B. bassiana)* and nematodes in the trapping system.

6.4 Fungal endophytes for the microbial control of the banana weevil, nematodes, and *Fusarium wilt*

by T.D., C.S.G., D.C., and C.N. in collaboration with B. Niere, R. Sikora, E. Adipala, A. Viljoen, S. Kapindu, N. Labauschagne, S. Athman*, P. Paparu*, P. Kilama*, and E. Mukwaba**

Banana weevils (*Cosmopolites sordidus*) and nematodes (*Radopholus similis*, *Helicotylenchus multicinctus*, and others) are soilborne pests which attack the roots, corm and vascular system weakening plant support and impeding nutrient uptake. Clean planting propagules, like tissue culture derived plants, can be used to establish new fields, although pest reinfestation remains a vital concern. Microbial control offers excellent possibilities for controlling these pests. The primary focus of this project is the use of endophytes for the management of banana pests and diseases. Fungal endophytes are microorganisms that colonize plant tissue internally for at least part of their life cycle without causing symptoms of disease. Many endophytes have formed mutualistic relationships with their host plants and serve as antagonists to pests and diseases. These endophytes can be inoculated into tissue culture plants and reduce pest and disease pressure. In 2004, we undertook the following activities:

Screening of endophytes against banana weevils and nematodes. Using 12 *F. oxysporum* strains that had been isolated at the beginning of the project, 8 bioassays were conducted against banana weevil eggs and 5 bioassays were conducted against *R. similis* motile stages (males, females, and juveniles). Banana weevil egg mortality was 32.8–50.3 %. In all *R. similis* bioassays, fungal filtrates caused 84.2–100.0% mortality. Some strains were equally effective against both pests, creating the possibility of using a single endophyte strain to target the two pests simultaneously as they usually occur together in the field. During the reporting period, various new *F. oxysporum* strains have been isolated and identified from Masaka, Uganda. Five of these newly isolated endophytic *F. oxysporum* strains have been tested in various in vitro screening experiments for their pathogenicity against banana weevil eggs. All isolates induced mycosis and reduced eclosion compared to untreated eggs.

Twenty-six newly isolated *F. oxysporum* strains were tested for their effect against *R. similis* motile stages in three bioassays. Whereas all strains originating from banana roots displayed toxicity against *R. similis*, only the minority of strains originally isolated from banana corms were effective against *R. similis* motile stages. In addition, a rapid and consistent protocol, using banana stem discs that are infiltrated with fungal broth or filtrate and on which a larva is placed, has been developed for in vitro testing of endophytic *F. oxysporum* against banana weevil larvae. This protocol was used to test the newly isolated *F. oxysporum* strains and demonstrated that the latter strains reduced larval feeding and activity. Two endophytic *F. oxysporum* strains (*V5w2* and *III4w1*) were screened in two in vivo experiments for *R. similis* control in five banana cultivars (Enyeru AAA-EA, Kibuzi AAA-EA, Gros Michel AAA, Valery AAA, and Kayinja ABB). The number of *R. similis* juveniles was significantly lower among endophyte-treated plants compared to control plants in only one experiment. Endophyte-treated plants did not exhibit less nematode damage than control plants. The number of juvenile *R. similis* was independent of cultivar in both experiments. Relatively low sample size and a too short nematode challenge period were probably responsible for these inconclusive results. In another set of three in vivo experiments, results were more promising.

Each experiment comprised of plants belonging to three cultivars (Enyeru, Kibuzi, and Kayinja). Plants were inoculated with *F. oxysporum* strain *V5w2* and challenged with two different *R. similis* population densities. Across all experiments, the total number of nematodes was much lower in control plants than in endophyte-treated plants. The three banana cultivars showed major differences in the densities of *R. similis* extracted from their root tissues. These results suggested that the East African highland banana cultivars were more susceptible to nematodes than the exotic *Musa* cultivars such as Kayinja. In none of the experiments was there any difference in the number of *R. similis* between plants that had received a high nematode dose and plants that had received a low nematode dose. Therefore, the activity of strain *V5w2* against *R. similis* reproduction seems not to be influenced by initial nematode density. Strain V5w2 is currently being tested in a large field trial for its effect against *R. similis*.

Studies on endophyte colonization, persistence, and interactions. Two in vivo experiments were carried out to investigate the effect of combinations of different endophyte strains on *R. similis* reproduction. After 63 days, endophyte-free plants and plants that had been inoculated with a single endophytic strain supported higher *R. similis* densities than plants that were simultaneously inoculated with two strains. Lowest *R. similis* densities were recorded when plants were inoculated with a combination of all three endophytic strains. We therefore conclude that combining two or three endophytes appears to enhance their potential to lower *R. similis* densities in a synergistic way. High colonization and subsequent persistence of *F. oxysporum* endophytes is necessary for effective biological control of banana weevils and *R. similis*. Three inoculation methods were investigated: inoculation through dipping the roots and corms in a spore suspension, inoculation through dipping the roots and corms in a spore suspension after the plants were grown in an additional nutrient solution to enhance root growth, and inoculation using a solid substrate. Corms were colonized to a higher extend (91 %) than roots (63 %). Increased root development prior to endophyte inoculation also maximized root colonization by fungal endophytes. Persistence was investigated by inoculating plants of two cultivars (Kibuzi and Nabusa) with either strain *V2w2* or *III4w1* using root and corm dip and reisolating the endophytes at weekly intervals. Root and corm tissue colonization was not influenced by fungal endophyte strain or cultivar.

Though root tissue colonization by both strains was always much lower than corm tissue colonization at four weeks after inoculation, the persistence of the strains in the roots of inoculated plants was sustained for up to 25 weeks. Even after 33 weeks, both endophytic strains had not entirely disappeared from the roots. In contrast, corm tissue colonization decreased rapidly. As a result, 21 weeks after colonization, *F. oxypsorum* endophytes had disappeared from the corm. The pattern of root and corm colonization by these *F. oxysporum* strains was further investigated in both banana cultivars using light microscopy. Percentage colonization in the corms (93%) was higher than in the roots (56%), but hyphal density in the roots (1.73/mm^2) was higher than in the corms (0.21/mm^2). The latter result might explain the difference in persistence between roots and corms.

Biodiversity and isolation of fungal endophytes in highland banana. Plant samples from banana plants (Kibuzi) were collected from Masaka district in Western Uganda. Fungal endophytes were isolated from roots, inner corm, and pseudostem of symptomless flowering and sucker plants. A total of 675 strains were recovered from the analyzed plant samples. One hundred and fifty-four *Fusarium* strains were used for in depth morphological characterization to species level. The most predominant endophytic *Fusarium*

species recovered from the analyzed banana plant samples were *F. oxysporum* (57 strains), followed by *F. solani* (18 strains), *F. subglutinans* (15 strains), and *F. anthophilum* (9 strains). *F. oxysporum* and *F. solani* were predominantly isolated from the root tissues while *F. subglutinans* and *F. anthophilum* were mainly isolated from pseudostem base tissues. *F. solani* was most frequently recovered from suckers. *Fusarium oxysporum* was equally isolated from both flowering and sucker plants. The results clearly indicate that colonization of banana plants by different endophytic *Fusarium* spp. in Uganda is tissue specific and affected by plant age.

Characterization of ***Fusarium wilt*** in Uganda. To distinguish endophytic *F. oxysporum* strains from its nonspecific *Fusarium wilt* strains, 115 *Fusarium wilt* strains in Uganda are being characterized using detailed morphological, molecular (AFLP), biochemical (VCG), and biological (pathogenicity tests) traits. The isolates were obtained from diseased-banana plants in the major banana-growing areas in central and southwestern Uganda and as far north as Arua district. A total of 23 screenhouse pathogenicity tests conducted, comprised of 385 Bluggoe, 215 Gros Michel, and 341 Valery plants. Pathogenicity was determined by assessing both external (yellowing of the leaves and splitting of the pseudostem) and internal (discoloration of the corms) symptoms. Almost all isolates were pathogenic to Gros Michael, the race 1 differential host. Over 25% of isolates caused internal *Fusarium wilt* symptoms in Bluggoe, the race 2 differential host, although external disease symptoms were less noticed. None of the isolates was pathogenic to Valery, the race 4 differential host. Race 2 was up until now thought to be absent in Uganda. Our data suggest that both race 1 and race 2 of the pathogen are present in Uganda, a finding that has profound implications in banana breeding programs. We were able to create at least two *nit* mutants for 107 out of the 115 isolates. All *nit* mutants have been found to be self-complementary and are ready to be grouped in VCG groups based on complementation with tester strains. In addition, mycelia have been stored for all 115 isolates and await RFLP analysis.

Field-testing of different banana planting materials. Although tissue culture technology is already accepted and used in many Central and East African countries, this technology is currently not available to Ugandan farmers. Tissue-cultured banana is however an excellent source of pest- and disease-free planting material. High costs of the tissue culture-derived planting material is seen as one of the reasons for slow dissemination and adoption of this technology in Uganda. To further in the promotion and acceptance of tissue culture technology in Uganda, a field trial comparing different types of planting material of banana (tissue culture, hot-water treated suckers, and untreated suckers) of the widely grown East African highland banana cultivar Enyeru was planted in 2002. Harvest of the mother plants is finished but data recording is ongoing for ratoon crops. In the ongoing field trial, each type of planting material is tested under high nematode pressure and under two different management practices, with and without mulch and manure. Economic analysis of the obtained data will help to determine the best type of planting material for farmers in Central Uganda. Preliminary results show that tissue culture banana plants produce heavier bunches and grow faster than sucker-derived plants. Since the present project depends on the promotion and acceptance of tissue culture plants, this finding is exciting because it highlights the potential of using tissue culture plants during the first crop cycle, even without endophytes, and will without doubt aid in the promotion of tissue culture in Uganda.

Use of *Paecilomyces* spp. against banana nematodes. *Paecilomyces lilacinus* is a facultative parasite of nematode females and eggs and is the most extensively field-tested

biological control agent for nematodes, but control has generally been variable. *P. lilacinus* is known to control nematodes using two important mechanisms: production of toxic secondary metabolites and penetrative growth within nematodes. *P. lilacinus* was isolated among other soil-inhabiting fungi from rhizosphere soil and the root surface of banana plants from two fields using a modified dilution plate technique. One of the fields was infested with *R. similis*. The frequency of *P. lilacinus* among strains isolated indicated its high abundance in soil. A total of 23 *Paecilomyces* spp. strains were isolated, of which 13 were *P. lilacinus* and 7 were *P. marquandii*. Total abundance of *P. lilacinus* in the fields with and without *R. similis* was similar, indicating no density relationship between the fungus and nematode. A possible explanation is that the fungus can survive saprophytically in soil. However, the distribution of *P. lilacinus* across niches was different in both fields. In the *R. similis*-infested field, *P. lilacinus* was more abundant on the root surface than in the rhizosphere soil, whereas the opposite was detected in the *R. similis*-free field.

Seven in vitro bioassays were conducted, incorporating various modifications, to develop an effective protocol for testing the effect of filtrates of the isolated *P. lilacinus* against *R. similis*. These modifications include pH adjustment, alterations of fungal filtrate concentration and manipulation of nematodes while counting. A successful protocol was established whereby the effect of pH was standardized and whereby nematodes were rinsed and probed before assessment. Subsequently, four in vitro bioassays assessed the potential of 12 *P. lilacinus* strains in controlling *R. similis* using the effective protocol. Two of the *P. marquandii* strains were included to compare their nematode control potential. *P. lilacinus* strain *23N5-2* caused the highest percentage of *R. similis* paralysis (38.4–56.4 %). Strains isolated from *R. similis*-infested and non-infested fields were not different, nor was there a difference between strains from the rhizosphere and the banana root surface. Interestingly, nematode paralysis decreased after 24 hours, suggesting that the toxic paecilomycins produced by *P. lilacinus* are sublethal and subsequently only caused temporary paralysis. There was no difference in percentage paralysis between *P. lilacinus* and *P. marquandii* strains. *P. marquandii* has been shown to produce paecilotoxins but was previously less known as a nematode parasite.

The mode of action of two *P. lilacinus* strains (*23N5-2* and *22R5-1)* against *R. similis* was further studied *in planta* under screenhouse conditions. Individual roots within plants were cupped and treated with either broth containing spores and mycelia or treated with spore- and mycelia-free filtrate treatments. *P. lilacinus* applied within broth controlled *R. similes* better than the fungal filtrates at four weeks. Probably, fungal filtrates were degraded quickly whereas fungal broth treatments allowed spores and mycelia colonized the soil. The effect of the 12 *P. lilacinus* strains against *R. similis*-infested banana plants was tested in two screenhouse experiments, using maize bran as a fungal substrate. Unexpectedly, plants treated with *P. lilacinus* stimulated plant growth and subsequently *R. similis* reproduction. This may suggest that the fungus switched to its saprophytic mode due to absence of nutritional stress and depended entirely on the maize bran for its nutrition without producing its paecilotoxins.

Tissue culture facility. A tissue culture lab is fully operational and routinely producing plants of six banana cultivars for all in vivo screenhouse and field experiments conducted at Sendusu, Uganda. In addition, small numbers of other banana cultivars are produced for the breeding program. Compared to the previous reporting period, monthly and yearly output were increased from 312 to 473 plants and from 3750 to 5680 plants, respectively. Output is currently maximized due to space limitations.

Mode of action of endophytes against banana weevils and *R. similes*. Four in vitro choice bioassays were conducted to investigate feeding preference of banana weevil larvae. Banana pseudostem disks were partitioned in two halves: one side was treated with a fungal spore suspension or fungal filtrate whereas the other half was left untreated. Reduction in number of tunnels was consistently observed in tissues treated with two (*Eny 7-11o* and *M-87*) endophytic *Fusarium* strains, indicating that these strains produce some substance with a deterrent effect. An in vivo choice experiment was carried out to investigate the effect of endophyte-enhanced plants on oviposition preference of adult banana weevils. Results indicate that the number of eggs extracted from endophyte-treated and non-treated plants was the same. The effect of endophytic *F. oxysporum* strains on nematode penetration was studied both in vitro (using single excised banana roots) and in vivo. (Using banana plants in buckets). In the laboratory, strain, *Eny1.31i* caused the highest reduction (37%) in root penetration by *R. similis* compared to the control plants. The other strains also reduced root penetration though by a lesser percentage.

In the screenhouse, sampling of *R. similis* density at three-day intervals demonstrated that penetration was reduced by some strains. Both in vitro and in vivo host choice tests were conducted to investigate whether migration of *R. similis* mobile stages towards roots was influenced by endophyte colonization. No clear attraction or repulsion towards untreated or endophyte-enhanced banana plants was evident. To investigate the induction of systemic resistance against *R. similis* following endophytic colonization, split-root experiments were conducted. Half of the roots was challenged with *R. similis* and physically separated from the other half that was inoculated with *F. oxypsorum* endophytes. Nematode damage was lower in the split roots treated with *Eny7.11o* and *Eny1.31i* than in control plants. This is an indication that prior inoculation of banana plants with the fungal strains may be inducing systemic resistance against *R. similis*.

Capacity building. Graduate training and capacity building of national staff is a major aspect of this project. Three MSc students (registered at Makerere University, Uganda) finished their research during the reporting period, and two have submitted their thesis. Two PhD students (registered at the University of Pretoria, South Africa) are currently continuing their research at Sendusu, Uganda. As part of their academic training, the PhD students isolated 176 endophytes from South African banana plants and, among those, identified 60 endophytic *Fusarium* spp. that were characterized using genetic techniques (AFLP and RFLP). Results of these experiments are being published. In Uganda, the PhD students are looking into mechanisms of action of endophytes against nematodes and banana weevils. Research conducted by the MSc students has focused on the use of *Paecilomyces* spp. against *R. similis*, the use of combinations of endophytic isolates, the response of endophytes against different pest levels, and the distribution, spread, and persistence over time of endophytes within the banana plant.

6.5 Efficacy of four indigenous plant extracts in the control of maize cob borer *Mussidia nigrivenella*

by K.A. and M.T. in collaboration with K.M. Agbodzavu and S. Gounou

Aqueous extracts of *Tephrosia vogelii*, *Hyptis suaveolens* (15 and 20%), and oils of *Azadirachta indica* and *Jatropha curcas* (2.5 and 5%) and Furadan 5G were evaluated for their efficacy in reducing *Mussidia nigrivenella* infestation and its damages under practical field conditions and their oviposition deterrent effect in cages in Bénin. The results showed that *M. nigrivenella* preferred to lay eggs on non-treated plants. Approximately, all eggs

were found on untreated ears showing the oviposition deterrent of all extracts and oils. Under field conditions, Furadan, Neem, and *Jatropha* oils (both concentrations) reduced significantly the number of *Mussidia* larvae (by 16–49.2%) compared to *T. vogelii* and *H. suaveolens*, which were similar to the control. These results show that oil emulsions of *A. indica* and *J. curcas* oils act not only as more persistent oviposition deterrent but also as insecticides. Thus they could be used in integrated control of *Mussidia* in maize cropping system.

6.6 Effect of essential oils on the growth of *Fusarium verticillioides* and fumonisin contamination in corn

by K.H. in collaboration with P. Fandohan, J.D. Gbenou*, and B. Gnonlonfin*

Essential oils extracted by hydrodistillation from local plants in Bénin, West Africa, and oil from seeds of the neem tree (*Azadirachta indica*) were evaluated in vitro and in vivo for their efficacy against *Fusarium verticillioides* infection and fumonisin contamination. Fumonisin in corn was quantified using a fluorometer and the Vicam method. Oils from *Cymbopogon citratus*, *Ocimum basilicum*, and *Ocimum gratissimum* were the most effective in vitro, completely inhibiting the growth of *F. verticillioides* at lower concentrations over 21 days of incubation. These oils reduced the incidence of *F. verticillioides* in corn and totally inhibited fungal growth at concentrations 8, 6.4, and 4.8 íL/g, respectively, over 21 days. At the concentration 4.8 íL/g, these oils did not affect significantly fumonisin production. However, a marked reduction of fumonisin level was observed in corn stored in closed conditions. The oils adversely affected kernel germination at 4.8 íL/g and therefore cannot be recommended for controling *F. verticillioides* on stored corn used as seeds, when used at this concentration. The oil of neem seeds showed no inhibitory effect but rather accelerated the growth of *F. verticillioides*.

6.7 Efficacy of teak seeds for the control of *P. truncatus* in stored maize

by K.H. in collaboration with D. Agbidinoukoun and A. Agbaka*

A series of experiments was conducted from November 2004 to June 2005 at IITA-Bénin with an aim of testing the effectiveness of teak seeds (*Tectona grandis* L.) in the reduction of the damage and weight losses caused on the shelled maize (*Zea mays* L.) by *Prostephanus truncates* (Horn).

In a first trial, bags of jute containing 10 kg of corn mixed with various quantities of teak seeds (0; 0.5; 1 and 1.5 kg) and artificially infested by various numbers of *P. truncatus* (5,10, and 20 *P. truncatus*) were stored for five months during which samples of 500 g are monthly taken on all the bags.

To study the influence of the odors of the different hosts substrates on the behavior of *P. truncatus*, 160 sexed beetles insects were individually subjected to the odors of teak and maize seeds using four arms olfactometer.

At the end of the storage, it showed that the amount of 1.5 kg ensures a maximum reduction as well damage and losses as highly significant differences appeared between this treatment and the controls treatment all samplings. The various initial infestations did not have a decisive effect on the evolution of the different parameter measured. However, proportionality was noted between the level of the initial infestation and the damage and losses, the total number of insects on the maize.

The olfactometric study as for it showed a highly significant difference ($P < 0.0001$) between time average passed by *P. truncatus* in the teak seeds odor compartment and the one passed in the maize and the control odor compartments.

6.8 Potential for local entrepreneurship in IPM

by, B.J. and C.K. in collaboration with I. Godonou, C. Atcha-Ahowé, S. Vodounhè, F. Onikpo, and G. Heviefo

New biopesticide candidates such as the *B. bassiana* isolate 5653 which seem to hold good promise for DBM control need to be screened properly and undergo biosafety and ecotoxicology tests before they can be moved to large scale production and use. IITA's ability to produce large quantities of the potential product in response to any high demand for such a product is limited to the capacity of the "Green Muscle" facility. Private sector partners will be required to move the product from experimental to industrial level, especially to focus on promoting local entrepreneurship to produce such products. When such products become available in large quantities one expects significant changes both in terms of reduced pesticide load on vegetables and in increased revenue for the producers.

6.9 Development of pheromone traps for *Maruca vitrata*

by M.T., A.T., and O.C. in collaboration with M. Downham, B. Datinon, S. Adetonah, and D. Rurema

Replicated, on-station trials for control of cowpea insect pests in Bénin and Ghana built upon earlier results to show that a variety of botanical pesticides, with or without pheromone traps for the legume podborer, *Maruca vitrata*, yields and infestations were generally intermediate in effectiveness between conventional pesticides and untreated controls. Neem seed oil was the best botanical for controlling flower thrips and *M. vitrata*. Farmer field school trials (FFS) in the first year (18 villages) largely confirmed these findings and recommendations were developed for the optimal use of traps.

On-farm trials of these recommendations were carried out in the second year with PRONAF, OBEPAB, CRI, and GOAN in 15 villages across Bénin and in central Ghana. Feedback from farmers and researchers indicated that the use of traps with a flexible approach to choice of control agent enabled better integration with existing farmer practice and better understanding and interest among farmers than previously. A survey of several villages carried out to assess the potential for farmer-to-farmer transmission of information about the technologies showed that such informal transmission occurs among the majority of farmers. Farmers also wanted more practical information regarding the use of pheromone traps and this influenced the production of two posters on the traps intended for distribution to FFS, as well as a leaflet for FFS facilitators and extension staff.

Progress to develop pilot-scale systems of manufacture and distribution of pheromone traps and lures, and of botanical insecticides was mixed. It has not yet been possible to identify local commercial companies to either manufacture or supply pheromone traps or lures for *M. vitrata* (partly due to unavailability of the project leader to undertake travel on medical grounds). As a result, planned activities intended to sensitize regulatory authorities to the novel characteristics of pheromone and botanical products could not be covered. Studies of the social and economic feasibility of technologies showed that a substantial proportion of farmers were willing to pay the estimated economic cost of traps and lures. In the longer-term, farmers wished to make purchases of traps, lures and botanical pesticides through existing providers, but farmer production of traps was successfully carried out and a short-term supply route for lures (through PRONAF from the UK supplier) has been identified.

Replicated trials with a putative *M. vitrata* new pheromone blend component were carried out at five sites across West Africa. These provided no evidence that the new component produced any improvement in catches. In Burkina Faso, the greatest catches were with the single (already known), major component alone, not with the standard 3-component blend. At Savè (central Bénin) all the blends did equally well. On the basis of these results, expansion of on-farm work to Burkina Faso (in the final project phase), but not to northern Nigeria, is appropriate.

6.10 Entomopathogenic fungi associated with the African root and tuber scale in Cameroon

by R.H., M.Ti., L.W., and C.K. in collaboration with, A. Fotso Kuate, S. Nanga Nanga, J. Ngeve, A. Nguenkam, and P. Tondje

The cassava IPM program has invested considerable time and resources in searching for natural enemies of the scale. We have succeeded in isolating and identifying the entomopathogens *Metarhizium anisopliae* (Metschnikoff) Sorokin, *Metarhizium* sp., and *Beauvaria* sp. from several locations in Cameroon. Natural infections were generally low, which is generally expected from these fungi; but in one of the locations, infection levels reached 23.5%. The fungi were cultured and their virulence was tested in the laboratory where they showed a capacity to produce up to 80% infection levels. Other laboratory tests showed that high does of *M. anisopliae* could also kill all individuals in nests of *A. tenella*. Additional surveys are planned in DR Congo and Cameroon to look for other isolates of *M. anisopliae*. We are also making plans to work with a company in USA that has identified isolates of *M. anisopliae* for ant control. The isolates are reportedly effective because of their "late sporulating" biology i.e., they do not sporulate and infect the ants until the spores have been introduced into the ant colony. If successful in killing *A. tenella*, we plan to multiply and release the fungus in several key locations in Cameroon (initially) and then in other countries affected by ARTS.

7 Information and diagnostics support for plant protection provided; reference collections and colonies of pests and natural enemies maintained

Ongoing and future activities

7.1 Reference collection and database development for enhanced biosystematics capacity

by G.G.

For the 2004 inventory activities, emphasis on pest and beneficial insects of IITA's mandated crops were pursued in following different ecological sites:

- Bénin: daily catches from a permanent light trap device installed on BCCA's ground have provided throughout the year, an abundant and diverse insect material. The quality of these samples is particularly valuable as freshly captured insects can immediately be handled and integrated in the reference collection. Parallely, periodic surveys carried out in the frame of the IPM project in peri-urban gardens of Cotonou lead to the collection of numerous insect samples obtained in 19 different production sites on common horticultural crops such as lettuce, cabbage, cucumber, amaranth, carrot, tomato, fresh beans, okra, sweet/hot pepper, eggplant, etc.
- Nigeria: this year's collection activities consisted mainly in the assessment and monitoring of the local fruit fly diversity by means of parapheromone baited traps.

In addition, numerous samples were obtained from a light trap running continuously IITA's campus at Ibadan. Recently, the equipment, which is daily operated by a technician, has entirely been renovated. This resulted in a noticeable increase of the trapping efficiency.

- Ghana, Togo, and the northern parts of Bénin: as in the previous years, faunistic surveys were punctually conducted in remote but typically biodiverse sites such as the Volta delta area, the humid forest zones of Ghana, the midaltitude area of Togo, and the dry savanna in the northern part of Bénin. Accessions in these distant geographical areas were cost effectively achieved through the assistance of local collectors who are permanently present in these sites.

The selection of adequate specimens within the newly acquired material, the subsequent pin/slide mounting or alcohol preservation, the identification, integration, and indexing process remain ongoing activities that contribute to a steady increase of the reference collection. In the course of this year, another 18600 specimens, representing an average processing rate of 84 specimens per working day, were added to the existing collection. With presently roughly 210 000 specimens (Fig. 5), IITA's reference collection is consistently consolidating its position of most active and important biosystematics center within West Africa.

Identification work of the newly acquired arthropod material has permitted to increase this year the existing collection to a total of 4200 species adding thus representatives of 17 new insect families to the already existing 305 ones. This expansion in taxonomic coverage for West African arthropods has undoubtedly improved the ability to provide reliable identifications of agriculturally important pest and beneficial insects and of other representative organisms of general relevance. At its present state, IITA's reference collection encompasses at least one representative from 54% of all recognized families and 17% of all currently known arthropod biodiversity from the whole subregion.

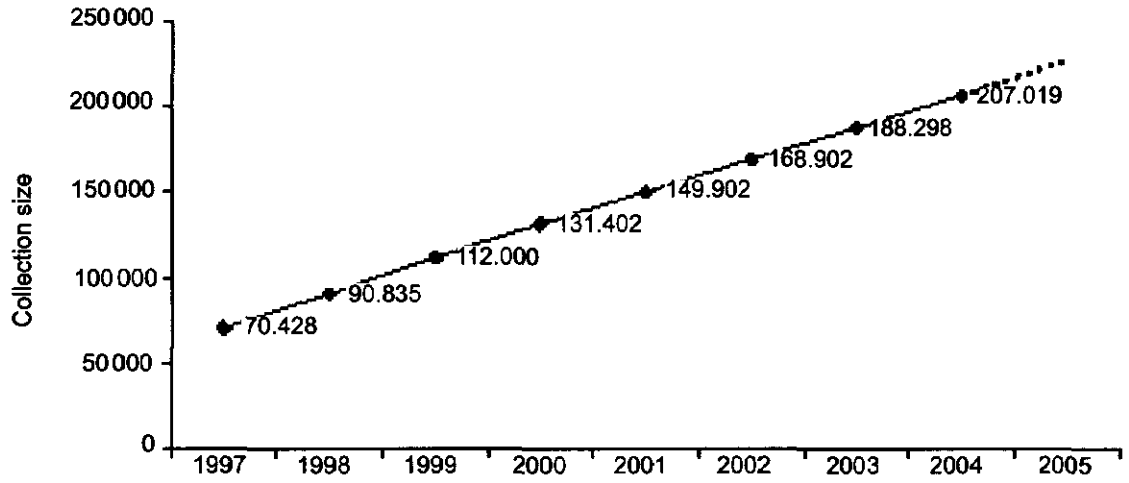


Figure 5. Increase of the reference collection for the period 1997–2004.

7.2 Arthropod identification support service

by G.G.

As taxonomic services in the region are scarce and biosystematics expertise by long-established institutions barely affordable for NARES, IITA's biodiversity center is committed to the provision of an effective and free regional identification service. Continued demand for such taxonomic assistance and supply of concomitant biodiversity information is illustrated in Table 14.

For 2004, the demand for free taxonomic assistance markedly increased as compared to the previous years. Queries originating from NARES in Bénin outnumbered those from IITA scientists. The two latter accounted together for 84% of the 626 submitted samples. During the period under review, support was provided to collaborators from nine countries in West and Eastern Africa.

As underpinned in Table 14, about 95% of the 2116 invoiced insect specimens were identified at least to genus level. Samples containing Dipteran insects were the most abundant. While majority of submitted insects were identified to a satisfactory level, partially resolved requests were forwarded to group specialists when desired. The numerous identification requests invoiced in the course of this year consistently contributed to the collection augmentation. The identification work partly derives from the taxonomic support rendered to in-house studies. The assistance provided to IITA scientists and NARES collaborators is being substantiated in joint publications.

7.3 Reference collection of plant parasitic nematodes

by D.C. and G.G.

Plant parasitic nematode cultures are continuously being cultured and maintained in vitro and in vivo for use in nematode pathogenicity and biology studies at IITA and also for access by national programs. A reference collection of plant parasitic nematode microscope slides is continually gathered and stored for training purposes from samples from diagnostic surveys. Photographic images of nematodes on microscope slides are continuously added for electronic storage at the Biodiversity Museum in Cotonou, and elsewhere.

Table 14. Identification requests handled for 2004.

	Samples	Insects	Col.[1]	Dip.	Hem.	Hym.	Lep.	Others	Level identified:		
									sp.	gen.	fam.
Bénin[2]	314	823	152	138	136	340	13	44	449	303	71
Burkina Faso[2]	1	10	0	0	10	0	0	0	10	0	0
Congo[2]	5	15	7	0	8	0	0	0	15	0	0
Ghana[2]	4	25	6	0	0	10	9	0	25	0	0
IITA scientists	218	962	112	748	2	12	71	17	877	49	36
Niger[2]	12	108	0	0	0	0	0	108	58	50	0
Nigeria[2]	31	105	17	59	14	0	0	15	99	6	0
Sudan[2]	1	10	0	0	10	0	0	0	10	0	0
Tanzania[2]	2	20	0	0	20	0	0	0	20	0	0
Togo[2]	38	38	38	0	0	0	0	0	33	5	0
Total	626	2116	332	945	200	362	93	184	1596	413	107

[1]Col.= Coleoptera; Dip.= Diptera; Hem.= Hemiptera; Hym.= Hymenoptera; Lep.= Lepidoptera; sp.= species; gen.= genus; fam.= family.

[1]Requests from NARES.

7.4 Development and screening of monoclonal antibodies to *Cassava brown streak virus*

by J.d'H. and C.H.

Proteins specific to Cassava brown streak virus-infected plants are to be purified (Tanzania) and monoclonal antibodies (MCA) raised in mouse cell lines (Ibadan). Potential MCA will be initially screened, then tested in a field situation (Tanzania) commencing 2005.

7.5 Mass rearing of insect pests with special emphasis on pests of cowpea and maize

by J.d'A.H. and M.T.

The Insect Rearing Unit (IRU) is responsible for the production of all life stages of insect pests for use by the scientists to carry out various research activities, which include;

- Screening for resistance of crop types and lines to insect pests infesting the Institute's mandate crops, including field and postharvest infestation and damage.
- Conducting field and screenhouse infestation studies.
- Biological studies: Producing insects used for the evaluation of the efficiency of indigenous and exotic natural enemies species and strains, and for mass rearing of natural enemies including entomophagous organisms.
- Laboratory studies to identify the feeding habits of the insect pests damaging newly bred lines of the mandate crops.
- The IRU also collaborates with IITA staff in Bénin, by participating in the monitoring of insect pests and their antagonists on wild hosts.
- Conducting field inspection, collection, and identification of new pests found on the experimental and multiplication sites.
- Providing entomological assistance for research on insect pests of other IITA mandate crops.

Millions of each of the following insects were produced in 2004: maize stem borers (*Sesamia calamistis* and *Eldana saccharina*), legume pod borer (*Maruca vitrata*), Cicadulina leafhopper (*Cicadulina storeyi*), cowpea aphids (*Aphis craccivora*), pod sucking bug (*Clavigralla tomentosicollis*), bruchids (*Callosobruchus maculatus*), and thrips (*Megalurothrips sjostedti*). Though the production fluctuated with time and according to need, a peak production of several thousands of individuals was being reared weekly as shown below:

Insect species	Eggs/neonates per week
Sesamia calamistis	500000
Eldana saccharina	500000
Maruca vitrata	400000
Cicadulina storeyi	200000
Callosobruchus maculatus	>20000
Clavigralla tomentoscollis	>16000
Aphis craccivora	80000
Megalurothrips sjostedti	>70000

The IRU collected large numbers of eggs, larvae, pupae, nymphs, and adults of insect pests of cowpea and maize, and from other alternate host plants, in various parts of Nigeria. The healthy ones were used to establish new colonies or integrate into the existing laboratory colonies in order to ensure that the insects mass-reared exhibit genetic diversity, aggressiveness and vitality as manifested by each pest population in nature.

Scientists working at IITA did mass rearing of the following species for use:

Species	Total number reared in 2004
Sesamia calamistis	408073 pupae
Eldana saccharina	378502 pupae
Maruca vitrata	401336 pupae
Cicadulina storeyi	>800000 adults
Clavigralla tomentosicollis	>400000 adults
Megalurothrips sjostedti	>200000 adults
Aphis craccivora	>150000 adults
Callosobruchus maculatus	> 400000 adults

The Unit was able to establish and standardize special media using imported and local materials for the rearing of each insect type. In the laboratory, the maize stem borers (*S. calamistis* and *E. sacharina*) are mass-reared on artificial diet (soyflour and wheat germ-based diet), legume pod borer (*M. vitrata*) on artificial diet made up of cowpea flour and wheat germ, bruchids (*C. maculatus*) on susceptible cowpea seeds, and pod sucking bugs (*C. tomentosicollis*) were reared on pods of susceptible pigeon peas and some susceptible cowpea lines.

Cicadulina leafhoppers (*C. storeyi*) are mass-reared on pearl millet plants in the glasshouse and the cowpea aphid (*A. craccivora*) is reared on susceptible cowpea plants in the screenhouse. Pigeon peas (*Cajanus cajan*), lablab and some selected potted cowpea plants where pod sucking bugs and thrips are mass-reared.

7.6 Production and maintenance of diagnostics capacity for viruses infecting IITA's mandate crops and viruses occurring in the farming systems

by J.d'A.H. and S.O. in collaboration with A. Eni, C. Rey, and G. Pietersen

In a bid to isolate and reestablish clean cowpea viruses, monitor strain mutation in the existing viruses and possibly isolate new cowpea viruses, a survey of six cowpea-producing states in northern Nigeria was done. The states surveyed were Kaduna, Kano, Kebbi, Kogi, Niger, and Sokoto states. A total of 103 diseased cowpea samples were collected and indexed for *Cowpea Moroccan aphid-borne mosaic virus* (CABMV), *Bean common mosaic virus* (BCMV), Blackeye strain, *Cowpea mosaic virus* (CPMV), genus *Comovirus*, *Cowpea mottle virus* (CPMoV), genus *?Carmovirus*, *Southern bean mosaic virus* (SBMV), and *Cucumber mosaic virus* (CMV) by ELISA using antisera from the IITA antisera bank.

A total of 68 samples tested positive while 35 were negative. Again, 29.41% of the positive samples had single infections while 70.58% had mixed infection. Of the six viruses tested for, five (CABMV, BCMV, CPMoV, SBMV, and CMV) were isolated as single stains.

CPMV was not present in any of the samples indexed. The need for cheap, accessible, sensitive, and user-friendly virus diagnostics necessitated the lateral flow test kit. Work on the development of the kit is based on the principles and existing protocols for production of pregnancy test kits. The finished test kit will consist of the sample pad, the conjugate release pad, capture line, control line and an absorbent pad. The principle is to detect the target virus(es) using antibodies bound to a support phase such as colloidal gold or dyed microspheres which can then be detected visually.

Assembly of literature and information on lateral flow tests (immuno-chromatographic assays) has been completed. Leading test product manufacturers have donated samples of membranes and conjugate release sheets as well as acrylic pressure sensitive adhesive. Different protocols for the conjugation of colloidal gold and polystyrene latex to antibodies are currently being assessed. Antisera with high specificity and sensitivity as well as absence of antibodies against contaminating proteins are needed for this work. Cross reactivity observed with some of the antisera in the IITA antiserum bank means that new, high quality antisera must be produced, in addition to highly specific monoclonal antibodies. Based on ELISA results, the available *Yam mosaic virus* (YMV), genus *Potyvirus* polyclonal and monoclonal antisera and *Cucumber mosaic virus* polyclonal antibodies have been found to be of high specificity and sensitivity, so these will be used to produce prototype lateral flow test strips.

Due to problems encountered in purifying antiviral immunoglobulin (IgG) from YMV monoclonal antibodies (tissue culture supernatant) available at IITA for the lateral flow tests (LFT), YMV antibody producing cell lines were rejuvenated, isolated from solution, and injected into primed mice for ascetic fluid production. High quality monoclonal antibodies (in the form ascetic fluid) with an ELISA optimum working dilution of 1: 80000 have been produced from these mice. Antiviral IgG purified from the YMV monoclonal antibodies produced and from *Cucumber mosaic virus* polyclonal antibodies from the IITA antiserum bank, are currently being used for the development of the LFT.

The optimal concentrations of CMV antiviral IgG required for gold conjugation and for the capture line were determined by titration and by spotting various concentrations of the purified IgG on nitrocellulose membrane respectively. An immunogold conjugate of CMV has been produced using 40nm gold colloids.

Three buffers were investigated for use as capture line antibody application buffer on five available membranes; PRIMA 40, PRIMA 60, PRIMA 85, CHEM A, and CHEM K. Three of the membranes are backed while two are not. Five membrane-blocking solutions were also investigated. CHEM K, one of the unbaked membranes was found to have better wettability and protein binding properties and thus will be used for the rest of the developmental work for the LFT.

The desired single straight lines for the membrane reagents (capture and control lines) can only be achieved by mechanization. Pipettes were used for the application of the capture and control reagents at this developmental stages, results are seen as circular spots instead of lines but each spot is distinctive. This will by adjusted as soon as the developmental stages are over and all parameters optimized

As at the time of this report, CMV infected leaf sap will migrate laterally along the membrane and produce a circular test spot as positive and a circular control spot to show that the test was completed. Further work to completely get rid of membrane background color, to optimize and to assemble the test kit is in progress.

7.7 Germplasm Health Unit ensuring safe international movement of IITA's improved germplasm

by J.d'A.H. and M.A. in collaboration with A. Eni, C. Rey, A. Ala, Okorie, and S. Winter

Production of polyclonal antibodies. The Antiserum bank at IITA is in continuous demand and it is imperative that stocks of antibodies are maintained or improved.

Diagnostics for CMV are used for detecting of the virus in banana and plantain, yam, cowpea, soybean, and other crops. Demand is high. CMV was purified using a method described by Roossinck and White (1998). After purification, the virus was emulsified with equal volume of Freund's complete adjuvant and injected intramuscularly into a healthy rabbit. Subsequent intramuscular injections (three injections at two-week intervals) were done with Freund's incomplete adjuvant. Test bleeding was done after the third injection, and from the ELISA test results, the serum contained antibodies against CMV. Further tests after a booster injection showed the antibody titer in the serum of >1: 25600. Over 20 ml of this polyclonal antiserum are now available for experimental use and distribution, on request, to collaborators.

To isolate BDBV from other proteins, an SDS-PAGE was done and four bands (4ul of antigen in each band) corresponding to BDBV was cut from the gel slab and washed several times in deionized water. The gels were crushed in 0.5 ml of Phosphate buffered, Saline (PBS), and injected subcutaneously into a rabbit. After two sets of injections (one every fortnight), the first test bleed was taken for titration to monitor antibody production.

Initial titration of the serum revealed the antibody titer to be low, positive reactions were observed only at 1:8 dilutions. Titration for the second test bleed, which was taken after the third injection, is in progress.

Production of monoclonal antibodies. To boost monoclonal antibody production at IITA, the monoclonal antibody technician was trained in monoclonal antibody production at Deutche Sammlung von Mikroorganismen Zellkulturen GmbH (DSMZ), Braunschweig, Germany. A total of 20 balb-c mice—10 males and 10 females were imported from the Noguchi Memorial Medical Research Institute, Accra, Ghana.

Low concentration of desired anti-viral IgG and interference of IgG from calf serum in the YMV monoclonal antibody (tissue culture supernatant) available at IITA antibody bank for LFT necessitated the production of ascetic fluid. YMV monoclonal antibody secreting hybridoma cells from the IITA antibody bank were used.

For ascetic fluid production, six weeks-old balb-c mice were primed by injecting 0.3 ml of incomplete Freund's adjuvant into the peritoneum of each mouse. Rapidly growing hybridoma cells were centrifuged at 1000 rpm for 10 minutes and washed once by mixing gently in PBS in a falcon tube and centrifuging for 1000 rpm for 10 minutes. Two mice were injected with 5×10^6 washed hybridoma cells in 0.3 ml or 0.5 ml volume intraperitoneally. The mouse were monitored daily after the injection for tumor formation. Soft tumors were tapped for acetic fluid collection two week after injection of hybridoma, by inserting a 22-gauge syringe needle into the lower abdomen of the mouse. The ascites fluid was incubated at 37 °C for 1 hour and transferred to 4°C overnight. The fluid was then centrifuged at 1000 rpm for 10 minutes and the supernatant was carefully removed from the cell pellet and stored at 4 °C.

High quality monoclonal antibodies in the form of ascetic fluid have been produced with an ELISA optimum working dilution of 1:80000. Anti-viral IgG for LFT has been purified from these antibodies.

7.8 Isolation, detection, and identification of viruses, bacteria and fungal pathogens in seeds and plant propagules of IITA mandate crops

by M.A. and J.d'A.H. in collaboration with O. Afolabi and O. Oguntade

A total of 29 (fields, 1 screenhouse, and containment) active growth health inspections and certification were carried out. These crop species were multiplied in the field and screenhouses at various locations to produce disease-free seeds/plant propagative material. Inspections and certifications were conducted on the following accessions/clones:

Ibadan	110	cowpea lines
	35	herbaceous legumes
	256	cassava (plantlets)
	2853	yams (plants, tubers)
	191	yam (plantlets)
	340	maize lines
	28	soybean lines
	2491	rice lines
Ikenne	25	cowpea lines
	28	soybean lines
Abuja	209	yam clones (TDr)
Ubiaja	98	yam clones (TDr)
Ado Ekiti	05	seed yam clones (TDr 2 local and 3 IITA genotypes)
Kogi State	05	seed yam clones (TDr: 2 local and 3 IITA genotypes)
Kano	210	cowpea lines
Zaria	210	cowpea lines
	35	herbaceous legumes
Saminaka	692	maize entries
Wamco	905	maize entries

Some of the fields were also inspected by the Plant Quarantine Service to issue phytosanitary certificates to cover exchanged germplasm material.

7.9 Assessment of seedborne pathogens in germplasm intended for international distribution

by M.A. and J.d'A.H.

As part of the Germplasm Health Unit (GHU) activities, cowpea, soybean, and herbaceous legume seeds were assessed both visually and by enzyme-linked immunosorbent assay (ELISA) for virus infection. In addition, cassava and yam plants were tested by ELISA and polymerase chain reaction (PCR) for virus infection.

A total of 141 cowpea lines were assessed by visual inspection and by ELISA for virus infection in 2004. These comprised material from the genebank (6 lines) and from seed

multiplication plots (135) .The germination rate of seeds from the genebank ranged from 37 to 64%, and none showed virus symptom. The germination rate of seeds from the seed multiplication plot ranged from 5.2 to 99%, and of the 135 lines indexed, seven lines (IT98K-506-1, IT97K-818-35, IT98K-206-9, IT98K-503-1, IT98K-131-2, IT99K-491-7, and IT97K-569-9) were rejected on the basis of virus symptom expression in the seedlings.

A total of 98 lines of herbaceous legumes from Ibadan were assessed visually for symptom expression. Before planting, the seeds were scarified and treated with fungicide (Benlate at 10g/l) to encourage early germination and prevent fungal infection. Some accessions had 95% germination rates while for some accessions, the germination rate was only 7%. None of the accession showed virus symptoms by visual inspection.

Again, 28 soybeans lines from the IITA demonstration plot showing severe leaf symptoms of virus infection were tested to determine the causal agent. A total of 25 out of 28 lines were severely virus infected. The lines were tested by ELISA with antisera raised against four viruses: *Bean pod mosaic virus* (BPMV) genus *Comovirus*, *Tobacco streak virus* (TSV) genus *Ilavirus*, *Southern bean mosaic virus* (SBMV) genus *Sobemovirus*, and *Cucumber mosaic virus* (CMV) genus *Cucumovirus*. Sixteen lines tested positive to CMV while none was positive to the other three antibodies.

As part of the process for the production of virus-tested plantlets for international distribution, 55 yam plantlets from the Tissue Culture Unit were inspected and indexed by ELISA for five viruses known to infect yams in West Africa. The following yam viruses were tested for: *Yam mosaic virus* (YMV) genus *Potyvirus*, *Dioscorea alata virus* (DaBV), genus *Badnavirus*, *Dioscorea mottle virus* (DMoV), genus (*?Comovirus*), *Dioscorea alata virus* (DAV), genus *Potyvirus*, and *Cucumber mosaic virus* (CMV), genus *Cucumovirus*. Of the 55 yams that were indexed, 23.6% were infected with DaBV and 12.7% with YMV. About 9.6% of the samples were weakly positive to DAV and 5.4% had mixed infection of two or three of the viruses tested for. None was positive for CMV and DmoV. A total of 53 cassava plantlets from the Tissue Culture Unit also meant for international distribution were indexed by ELISA for *African cassava mosaic virus* (ACMV) and *East African cassava mosaic virus* (EACMV), both genus Begomovirus. All the plants tested negative for both viruses.

7.10 Eliminate viruses and other pathogens in germplasm

by M.A. and J.d'A.H.

Cowpea. One hundred and ten cowpea lines were inspected during active growth, and the harvested seeds were submitted for laboratory health assessment. Although 39 lines showed virus symptoms on the field, only six lines were found to be infected with viruses by ELISA results. *F. oxysporum* was isolated from 43 lines, *F. solani* from lines lines and *Macrophomina phaseolicola* from six lines. Four lines IT00K-835-45, IT99K-7-14, IT00K-835-4, and IT99K-216-24-2 were highly infected with rust. *Septoria vignae* was isolated from IT99K-7-14, IT99K-1122, IT99K-529-1, IT97K-494-3, and IT98K-506-1. One line IT98K-205-9 was infected with *Entyloma vignae* (smut). Sixteen lines were infected with *X. axanopodis vignicola*, and seven lines with *P. syringae* pv *Phaseolicola*.

Maize. Sixty-seven maize lines, which had been field-inspected during active growth by PQS and GHU, were harvested and the seeds submitted to GHU for seed health testing. Forty nine lines were found to be heavily-infected with *Fusarium verticillioides, (F. moniliforme)* (causal agent of kernel rot) cosmopolitan, and worldwide in distribution, has been constantly isolated from both imported and exported germplasm maize seeds, *Xanthomonas zea maydis* from three lines and *Clavibacter* sp. from one line.

Herbaceous legumes. Seeds from the 85 species of herbaceous legumes inspected during active growth were health-tested. Ten species were found to be infected with *F. oxysporum* while 23 with *P. syringae* pv *phaseolicola* and 21 with *X. axanopodis phaseoli.* While *Aeschynomene histrix* CT 8911, CT8902, CT 8539, CT 9690, and CT 7884 had a germination percentage ranging from 0.0 to 10% were severely infected with *X. phaseoli* and *Stylosanthes verano. S. capitata* I 9052 was infected with *P. syringae* pv *Phaseolicola* and *X. axanopodis pv phaseolicola.*

Yam. Two thousand, eight hundred and fifty-three yam genotypes inspected during active growth in all the locations, were found to be infected with viruses and anthracnose. In Abuja, 138 TDr genotypes were found to be infected with anthracnose, and 132 TDr genotypes. In Ubiaja, 67 genotypes with viruses and 90 infected with anthracnose. The pathogenicity testing and the evaluation of biopesticides for the control of yam anthracnose are still ongoing. Another set of genotypes were selected to evaluate the various *C. gloeosporioides* isolates causing different foliar symptoms (blights and spots) in *D. rotundata,* and also testing the efficacy of a range of available fungicide and biopesticide for the control of the disease and eradication of the pathogen.

Soybean. Twenty-four soybean lines health-tested were found to be infected with *F. oxysporum* on 12 lines, *F. solani* on four lines, while *P. glycinea* was isolated from one line.

7.11 Interceptions on imported germplasm

by M.A. and J.d'A.H.

Maize from Zimbabwe. Three hundred and eighty two lines of maize imported from Zimbabwe, health-tested were infected with *Fusarium verticilloides, Fusarium oxysporum, and Penicillium* spp.

Rice from Côte d'Ivoire. Two thousand, four hundred and ninety-one rice lines imported by WARDA Ibadan from WARDA, Côte d'Ivoire were health-tested .All the varieties were found to be infected with one or all of the following pathogens: *Xanthomonas oryzae, Pseudomonas oryzae, Fusarium oxysporum, Bipolaris oryzae, Tilletia baclayana Microdochium oryzae, Phoma* spp. and *Curvularia lunata.* The infected lines were given a fungicidal seed treatment of Mancozeb. The treated seeds, after 72 hours exposure, were plated on both agar and blotter and incubated for four and seven days respectively. *Xanthomonas oryzae, Pseudomonas, Fusarium oxysporum, Fusarium solani,* and *Fusarium verticilloides* were isolated from the lines.

Sweetpotatoes from Kenya. Vines of 27 sweetpotato genotypes imported from CIP, Kenya were planted under containment and indexed by enzyme-linked immunosorbent assay (ELISA) for virus infection. Twenty-five genotypes tested negative for both *Sweetpotato feathery mottle virus* and *Cucumber mosaic virus.* Two genotypes 440037 IMBY 3102 was positive to Sweetpotato potyvirus and 440001 Resisto was found to be infected with *Sweetpotato chlorotic stunt virus.* These materials are still under containment processing and have not yet been cleaned. A group from CIP Kenya visited the containment facility in February to assess the performances of the material.

Pigeon pea were imported from India. Two hundred lines of pigeon pea imported from India health-tested were found to be infected with *Pseudomonas syringae* and *Curvularia lunata.* The seeds were further planted under containment for virus testing by ELISA (ongoing).

Cassava plantlets from South Africa and Colombia. Forty-three genotypes of cassava in vitro plantlets imported from South Africa and 370 genotypes from Colombia were health tested and released to the Tissue Culture Unit for further culturing.

7.12 Import permits

by M.A. and J.d'A.H. in collaboration with O. Afolabi and O. Oguntade

A total of 2038 lines, clones, and/or varieties of IITA mandate crops, herbaceous legumes, rice, and dried soil and plant samples were imported by various scientists from 15 collaborating countries. Thirty-two permits were obtained during the year. The import consisted of 382 lines of maize, 43 accessions of cassava in vitro plantlets, 822 of rice, 27 clones of sweetpotatoes, 200 lines of pigeon pea and dried plant parts (maize, cassava, soybean), and soil samples. The imports were released to scientists after having undergone postentry quarantine processing and GHU health tests. Interceptions on the different crop types were recorded.

7.13 Phytosanitary certificates (export)

by M.A. and J.d'A.H. in collaboration with O. Afolabi and O. Oguntade

Twenty-eight phytosanitary certificates were obtained from the Nigerian Plant Quarantine Services to cover seven crop types for germplasm exchange to 26 collaborating countries worldwide. Crop types accompanied by phytosanitary certificates were cassava (417 genotypes plantlets), cowpea (83 lines), herbaceous legumes (11 species), maize (140 lines), yams (280 genotypes, minitubers, and plantlets), soybean (20), *Musa* (30), and miscellaneous (2).

7.14 Collections of plant pathogenic organisms

by M.A. and J.d'A.H. in collaboration with O. Oguntade

The pathogen collection held under different crop type or plant host is kept under refrigerated conditions in the Unit. The collection is continuously upgraded with new microorganisms isolated and identified. These microorganisms are released on request to university lecturers, NARES, and students for research purposes and records kept on the releases made.

7.15 Culture of exotic phytoseiids and mite pathogens

by R.H., M.To., A.O., and F.B. in collaboration with F. Hountondji, K. Negloh, and N. Famah

The maintenance of mother cultures continued at an acceptable level for six phtyoseiid species and a total of 12 colonies. The exotic species still in culture include *Neoseiulus idaeus* (one Brazilian populations including one from a release field in Bénin), *T. aripo* (three Brazilian populations including one from a release field in Bénin and two established in the highlands of northwestern Cameroon) *T. manihoti* (one Brazilian populations from release fields in Bénin), *Iphiseius degenerans* (one population from Malawi), *Euseius fustis* (one population from Malawi), *N. baraki* from coconut groves in Bénin. Four isolates of the pathogenic fungus *N. tanajoae* from Brazil and four indigenous isolates (Bénin, Ghana, Tanzana, and Zambia) are also maintained with IITA's microbial collection. No new exotic phytoseiids or acaropathogens were received in 2004.

8 NARES capacity to develop and apply biologically-based pest management components enhanced

Ongoing and future activities

8.1 Farmer training in vegetable IPM

by, B.J. in collaboration with C. Atcha-Ahowé and I. Godonou

The peri-urban vegetable IPM project completed two training and trainers (ToT)/farmer field school (FFS) sessions at Houéyiho the biggest vegetable-growing site in Cotonou. At Houéyiho, 289 farmers (21% women) cultivate gboma, amaranth, carrot, lettuce, cabbage, bell pepper, tomato, aubergine, and cucumber as the most common crops on the 15ha site in Cotonou. The host farmer organization *"Union Communale des Producteurs de Cotonou"* (UCP-Cotonou) established criteria for selecting farmer participants. This was to ensure that each nomiated participant represented and reported to a wider community of farmers at 14 vegetable production sites in UPU areas of southern Bénin. The 41 ToT participants with 25% being women in each of the first training sessions represented 194 (13% of them women). The FFS/ToT curriculum comprised six interrelated learning modules: Features of vegetable production in the localities; identification of pests, and diseases in vegetable production; crop-based IPM practices in vegetable production; biologically-based alternatives to chemical pesticides in vegetable production; safe use of pesticides; planning/budgeting vegetable plant production and plant protection.

Training has empowered farmers to correctly identify and understand biodiversity associated with vegetables, distinguish between economically important pests from beneficial and harmless species, and to understand the need for correct and timely crop and pest observations coupled with field plot experiments as the bases for informed decision-making for plant protection interventions. Participants' evaluation of the training shows some changes in production practices. For example, in cabbage production, a water-based formulation of *B. bassiana,* 5653 out-competed chemical pesticides and is being followed up as a key recommendation in the management of DBM. Participating farmers were convinced that use of Dipel plus Decis on the crop can be replaced with the biopesticide. The farmers are eager to establish large scale trials on *B. bassiana* isolate 5653 for DBM control. The farmers also discovered the most suitable dose of fowl droppings (which they routinely apply to gboma and carrot against perceived pest and diseases).

In gboma, farmers traditionally apply 5kg of fowl drops/bed (7.2 m2) + 140g of NPK; + 92g of urea and chemical insecticide Talstar at the rate of 3l/bed to promote plant health and prevent leaf tapering, and bronzing. In the IPM plot, farmers discovered the superior benefits of fowl droppings applied in two splits of 14 kg of fowl drop/bed plus urea at 54 g of urea/bed, and no NPK or pesticide. Trained farmers are reducing the use of chemical pesticide and increase the dosage of the fowl drops to 40t/ha to enhance vegetable health and limit pest damage on their vegetable production plots. Trained farmers discovered that lettuce plants treated only with Manèbe in IPM plots were healthier (free of necrosis) than lettuces treated with farmers' practice of Talstar/Manèbe application to control pests and diseases. The use of Talstar is therefore superfluous in lettuce in that location. A public awareness campaign has also been initiated on radio and TV for the trained farmers to present and discuss their new experiences.

Farmer training is backed by IPM research capacity building through academic training of postgraduate students and collaborative research to identify and strengthen the national

capacity, test and adapt taxa for use in vegetable IPM. The degree-related training has been carried out in collaboration with national universities in Bénin and neighboring Togo. The studies include inventory of mite pests of gboma, analysis of pesticide use patterns in vegetable production, feasibility of fungal and virus pathogens to control DBM on cabbage; and the efficacy of botanicals against root-knot nematodes on gboma and carrot.

8.2 Specialized training of NARES scientists in identification of *Fusarium* species

by R.B. in collaboration with J. Leslie

Taxonomy of the genus *Fusarium* has undergone tremendous changes during the past decade. New research and diagnostic tools are used for *Fusarium* identification. We sponsored a training workshop dedicated to identification of *Fusarium*. The workshop was held in the University of Pretoria, South Africa. It provided hands-on training in microscopy, microbiological, and molecular methods to handle and identify *Fusarium* species. We plan to sponsor the same workshop in 2005 in Kansas State University, USA.

8.3 Rehabilitation of plant pathology in DR Congo

by M.M., R.B., A.D., and K.L.

Through the USAID-supported project on cassava in DR Congo, funds were obtained to purchase some equipment that were required to rehabilitate the Plant Pathology Laboratory at INERA, Mvuazi Research Center. The equipments purchased were a laminar flow hood, a microscope, and water distiller. A training program was organized for a newly recruited INERA pathologist who would be working in the rehabilitated laboratory. The training, which took place in January 2005, exposed the staff on methods to identify and score disease infections in the field, collect diseased samples, and isolate pathogens in the lab for identification. The staff also learnt various techniques to evaluate germplasm for tolerance to various biotic stresses.

8.4 Capacity building for quality control of root and tuber products

by K.H. in collaboration with P. Fandohan, M. Elegbede, and T. Houndekon*

National training held from 8 to 12 March 2004 at IITA-Cotonou involved technicians working on quality control of cassava and yam chips. This training was funded by a national project « Programme de Développement des Racines et Tubercules » (PDRT). The objective of the Bénin Government is to increase root and tuber production and improve the quality of chips for local consumption and exports.

Agents from PDRT and «Direction de la promotion de la qualité et du conditionnement des produits agricoles » (DPQC) attended this training organized by IITA with the collaboration of INRAB. The main objective of the training was to empower participants on necessary tools to strengthen and guide processors in enhancing cassava and yam chip safety and quality.

The content of the course was:

- Origin and postharvest problem development
- Moldy: nature, contamination mode, and mycotoxins
- Inventory of traditional techniques of chip production
- Improved technologies of cassava and yam chip production

- Pests of chips
- Other root and tuber-based products
- Management strategy of stored product pests
- Estimation methods of field and postharvest losses
- Mycotoxins and public health

Some training materials were also distributed to participants. From general discussions, some hypothesis were elaborated:

- Non-fermented chips would be less attacked than fermented ones
- Cooked chips would be less attacked by pests than simple chips
- Pest infestation would affect the texture of chip pastry

The main emphasis was to train and sensitize farmers and consumers on the importance to produce and consume safe and quality chips. Improve safety and quality of chips will increase farmer incomes and then reduce the risks of public health linked to the consumption of mycotoxins.

8.5 NARES capacity building to maintain virus-free propagative material of cassava

by C.H. and J.L. in collaboration with E. Kanju

With national and international partners, we will establish cassava tissue culture, virus indexing, and thermotherapy at the Mikocheni Agricultural Research Institute, Tanzaniza, and train the relevant national staff in virus indexing, thermotherapy, and culture maintenance methods, commencing 2005.

8.6 Capacity building for the regional quarantine station for East Africa (KEPHIS-PQS Muguga)

by C.H. in collaboration with E. Kanju

With national scientists, we will hold appropriate training sessions on the use of equipment and molecular techniques (PCR, ELISA) for virus detection, commencing 2005.

8.7 NARES training in virology

by J.d'A.H.

Messrs Michel Michel, Koumandian Camara, Jidiane Diallo, Mamadou Diallo, and Ansoumane Syila from the Institut de recherche agronomique de Guinée (IRAG), Conakry, Guinea were trained on symptom recognition and field scoring for the *African cassava mosaic virus* (ACMV) and *East African cassava mosaic virus* (EACMV). The training included theoretical discussions on the principles of TAS-ELISA and polymerase chain reaction (PCR) for the detection of these viruses. Both detection techniques were also demonstrated. Mmes Carla Torre do Vale and Nurbibi Saifodine Cossa from the Agriculture Research Institute, Maputo, Mozambique were also trained on cassava virus diagnostics.

Dr Danladi Balarebe Dangora of the Ahmadu Bellow University (ABU), Zaria, Nigeria was trained on various protein and nucleic acid-based virus diagnostic techniques. His training was for six months and funded through the USDA/USAID-project on strengthening collaboration between Ghanaian, Nigerian, and IITA scientists in biotechnology research. The training consisted of initial theoretical discussions of plant virology principles and

techniques, and practical work covering protein-based monoclonal and polyclonal antibody diagnostics using several ELISA formats, nucleic acid-based diagnostics, use of herbaceous indicator plants, virus purification, and antibody production. He was also trained on cell line rejuvenation and monoclonal antibody production.

8.8 Strengthening NARS capacity for collection, and conservation, an enhancement of plant genetic resources and seed health assessment

by M.A., J.d'A.H., and S.O. in collaboration with G. Konate and B. Ronellenfitch

Dissemination of virus diagnostics to NARES. Requests for virus diagnostics were received from within and outside sub-Saharan Africa. Virus diagnostics were sent to: Drs J. Lamptey (Ghana), M.L. Jeeva (India), and M.D. Alegbejo (Nigeria).

When a first request is made for antibodies, a 20µl or 40µl aliquot is sent. When the recipient has successfully used the reagents, larger volumes may be sent on a repeat request. The following antibodies and volumes were distributed in 2004 (Table 15).

Laboratory manual on virus diagnostics. The manual on virus diagnostics has been published. It deals extensively with basic nucleic acid isolation techniques, RNA/DNA based detection protocols, and protein-based detection protocols. It also contains characteristics of most of the viruses infecting crops in sub-Saharan Africa, modes of transmission and procedures for their detection, as well as addresses of agents and supplies of laboratories equipment and reagents. Copies have been sent to some laboratories on request.

Table. 15. Antibodies and volumes distributed in 2004.

Virus	Antibody type	Volume
Yam mosaic virus (YMV), genus *Potyvirus*	Polyclonal	40µl
Yam mosaic virus (YMV), genus *Potyvirus*	Monoclonal	80µl
Cucumber mosaic virus (CMV), genus *Cucumovirus*	Polyclonal	1520µl
Dioscorea alata virus (DaV), genus *Badnavirus*	Polyclonal	40µl
Dioscorea alata virus (DAV), genus *Potyvirus*	Polyclonal	40µl
Rice yellow mottle virus (RYMV)	Monoclona	50ul

8.9 IITA visit by the Nigerian Plant Quarantine officers

by M.A.

Twenty-eight plant quarantine staff from different stations in Nigeria who were on a three-week training course at the Plant Quarantine Service (PQS) Headquarters, Moor Plantation, Ibadan, visited IITA's plant health facilities as part of their course requirements.

The participants were given an overview on the IITA Germplasm Unit, its mandate, responsibilities, compliance to the International Plant Protection Convention (IPPC) requirements and IITA's policy on germplasm exchange and international.

GHU's activities in the context of the safe international movement of germplasm, the diagnostic procedures and tools used in the GHU. Emphasis was made on the importance for close collaboration and mutual trust between IITA and the NPQS whose main goal is the prevention of the spread of pest accompanying plant consignment across borders. The participants were shown around the germplasm import and export, virology, nematology, and entomology laboratories as well as the genebank, a major user of the NPQS.

The participants were impressed on the descriptive information found in the IITA Germplasm Health statement, as well as the quality and wide scope diagnostic activities involved in ensuring the health of germplasm for international distribution.

8.10 PRONAF project

by O.C. and M.T. in collaboration with T. Adam, M. Touré, C. Dabiré, C. Agli, and M. Dike

The PRONAF (Projet Niébé pour l'Afrique) project has focused during 2004–2005 on assessing the impact of technologies and institutional innovations on the beneficiaries, continued diffusion of improved technologies including high yielding varieties, cropping practices, IPM technologies and FFS (farmer field school) approach to address the constraints of poor farmers with special emphasis on women.

Participatory technology development and diffusion. Attention was focused on the diffusion of improved cowpea technologies. New varieties are being successfully adopted and diffused among farmers and this process has been going on since the inception of PEDUNE and PRONAF. Plant based pesticides and storage techniques are also being used at large scale in West and Central African cowpea-growing agroecological zones. The participatory development and diffusion of cowpea technologies is being implemented through the collaboration in IFAD-supported rural development project sites in Bénin, Burkina Faso, Mali, and Niger.

Capacity building. PRONAF made a significant contribution to capacity building and empowerment of its stakeholders and partners through group and individual training and open door site visits and observations. About 22 NARS scientists of "Programme de développement des plantes à racines et tubercules" (PDRT), an IFAD-investment project in Bénin were trained during 2004–2005. Technical backstopping has been to PDR-MS, and another IFAD-investment project from Mali in February 2004 for evaluating the impact of the project's actions on beneficiaries. The evaluation results showed that PDR-MS has been making substantial progress in community-based organizations and empowerment in land, credit, and human resource management. PDR-MS has created facilitation to diversify rural community revenues including agricultural inputs distribution facilities, credit availability, healthy community centers, potable water, and rural community empowerment through various trainings in cropping practices, resource management and animal fattening techniques. In terms of farmer's capacity building, PRONAF continued to empower farmers on safe and efficient cowpea production in Bénin, Burkina Faso, Mali, Niger, and Nigeria in collaboration with partners. PRONAF on the other hand, conducted significant number of trainings towards farmers, farmers' organizations, private sector, NGOs, and extension agents, using a novel participatory extension approach called farmers' field fora (FFF) approach (ex FFS), and contributing to technological changes in beneficiary countries.

During the 2004–2005 season, PRONAF collaborated with many other partners and IITA Projects (APPUI, VECO, and INRAB) to develop training curricula and train vegetable and rice farmers through FFF.

PRONAF initiated collaboration with Bénin national programs against HIV/AIDS, the creation of a focal unit to sensitize farmers, IITA, ADRAO, and IPGRI staffs on HIV/AIDS.

Socioeconomics. Socioeconomic studies have been carried out through the assessment of cowpea technologies impact on food security and poverty reduction, the distribution analysis of incomes from improved cowpea technologies through computing Gini coefficient. This study showed that cowpea technologies like improved varieties and neem/papaya leaf-based

extracts, contribute to reduce inequity between adopters of cowpea technologies of their scale neutrality. Seed protection cost studies in Niger showed that the major constraint to a sustainable seed production system is the high cost of input (pesticides and fertilizers). Farmer field school impact studies also have been done and the analysis is in progress.

Collaboration with partners. Strong collaboration with Bean Cowpea CRSP of Purdue University through joint projects, African Development Bank (AfDB) and WECARD has been established. PRONAF got funds from WECARD and IFS for students and many proposals are in the pipeline of Global Fund against HIV/AIDS, malaria and tuberculosis through the Bénin Government, and UNU/INRA. Five graduate students (two PhD and three MSc) will complete their degree by 2006.

Conclusion. PRONAF has been making substantial progress in impacting cowpea technologies in various countries of PRONAF. These efforts are contributing to food security, poverty reduction, and environmental protection. Linkages have been strengthened between PRONAF and IFAD-supported rural development projects through capacity building and impact evaluation of their actions on beneficiaries. Efforts will be concentrated in collaboration with private sectors for improved seed and neem extracts production.

8.11 NARES training in insect identification and curation

by G.G.

The focus of the biodiversity center is on the valorization of the scientific information contained with the arthropod collection through knowledge transfer. Thus short-term bench- or group training for IPM or quarantine practitioners, and hands-on tuition to individual NARES scientists and collaborators from universities in the subregion interested in systematics. Identification of pests and beneficials, curatorial techniques is provided on demand. In response to the recent introduction of a new devastative fruit fly pest into tropical Africa, the biodiversity center has developed a new capacity in the taxonomy of Tephritidae. Plans include degree related training courses in monitoring techniques and reliable identification of frugivorous species of economical importance. Further capacity building in biosystematics for NARS is being provided in the supervision of MSc thesis by students of national universities. In 2004, two students from the University of Lomé are given the opportunity to start their MSc thesis at the biodiversity center focusing on the diversity and biosystematics of Rutelinae and Melolonthinae, two white grub families occurring on diverse crop and noncrop host plants. In addition, we appreciate the assistance provided by the biodiversity center. One guest student at the University of Lomé completed his MSc thesis on the diversity of termites occurring in various ecological conditions in the republic of Niger.

With educational exhibitions of insect material from IITA projects and running scientific studies at the fourth agricultural fair at Cotonou/Benin, efforts were deployed to raise public awareness of IITA research and development activities in the field of biological control of pests, faunistic surveys, and biodiversity.

8.12 Acarology support to national programs

by R.H., M.To., A.O., and D.G. in collaboration with B. Agboton, F. Hountondji, K. Negloh, and I. Zannou

IITA's support to national biological control programs continues in the form of specific training in acarology, including mite identification; technical assistance with field surveys; preparation of appropriate work plans and proposals for bilateral donor support;

data analysis, training courses on impact assessment; and supplying natural enemies for experimental releases. Support activities were carried out in Bénin, Cameroon, Democratic Republic of Congo, Ghana, Guinea-Conakry, Kenya, Malawi, Mozambique, Republic of Congo, Tanzania, Togo, Uganda, and Zambia.

8.13 Backstopping of Bénin Root and Tuber Crop Development Program (PDRT)

by R.H., M.To, O.C., and K.H. in collaboration with R. Asiedu, A. Dixon, J. Hughes, D. Coyne, and R. Bandyopdhyay

In September 2003, IITA signed an agreement with the Ministry of Agriculture, Bénin, to provide technical backstopping to their root and tuber crop development program. The agreement covered the following six activities: (1) Introduction of improved and multiple disease- and pest-resistant cassava and yam varieties; (2) leadership of the efforts on morphological and molecular characterization of local and improved cultivars of cassava and yam in use in Bénin; (3) training NARS staff in tissue culture techniques; (4) training NARS staff in diagnostic techniques of cassava and yam pests and diseases, and conduct countrywide survey on cassava and yam pests and diseases; (5) assessing the level of aflatoxin contamination and prevelance of insect pest infestation in stored cassava and yam products; and (6) enhancing NARS capacity in impact assessment. Most of these activities have been initiated and are proceeding on a continuous basis. In addition, IITA staff participate regularly in workshops and scientifique meetings organized by PDRT, and one IITA scientist is a member of PDRT's Steering and Scientifique Committees. We are presently making plans for similar backstopping of the IFAD-financed PNDRT in Cameroon.

8.14 The training program of the cassava green mite project in Eastern and Southern Africa

by R.H., M.To, M.Ti. and G.G. in collaboration with A. Fotso Kuate, R. Irungu, C.W. Kariuki, M. Kenne, K. Kruger, J. Legg, A. Lema, J. Maniania, N. Mahungu, P. Nagel, A. Ndoumtsop, J. Ngeve, B. Pallangyo, M. Sabelis, U. Scheideger, K. Tatahangy, and C. Zundel

Training of farmers and technicians of partners working on cassava has been and continues to be an important activity for the cassava IPM and coconut mite projects. Training of trainers (Bas Congo, Kinshasa, and Plateau de Bateke) in the framework of farmer field schools (FFS) and training of technicians and extension agents were the main training activities in DR Congo. This training was essentially practical, revolving around the unique theme of identification of cassava pests and diseases and means for controlling them. For example, the trainers learned to identify and monitor populations of cassava green mite and its exotic predator *T. aripo* and means to enhance predator activity with the use of cassava varieties with hairy apices. They were also trained on methods of collecting predators from one field and releasing them in other fields to augment predator abundance.

Twenty-seven trainers were trained in each of the four locations. Training of technicians and extension agents took place in Kasai Oriental and Kasai Occidental. Technicians were trained in the principles and practices of cassava IPM, including elements of the training of trainers in Western DR Congo. Emphasis was also placed on the production of healthy planting materials, as many of the trainees were from organizations involved in the production of healthy cassava planting materials. Respectively, 14 and 16 technicians received training in Kasaï Occidental and Kasaï Oriental. A weeklong training course on cassava IPM was carried out in Nampula province in Mozambique in October 2004. Thirty

participants from NGO (Save the Children and World Vision) and government extension agents received training in diagnosis and management of cassava pests and diseases. The course was cosponsored by the cassava IPM program, the USAID project on multiplication and distribution of improved and disease-free cassava in Mozambique, Save the Children, and World Vision.

One MSc student from Malawi completed her studies on predation by the native phytoseiid *Iphiseius degenerans* on cassava green mite and whiteflies, and one DEA student from Cameroon completed his studies on the biology of *A. tenella* and ARTS. One PhD student from Bénin will complete his studies on microbial control of cassava green mite with *N. tanajaoe* in November 2005, and one PhD student has completed her studies on the midaltitude strains of *T. aripo* in northwestern Cameroon and will defend her thesis in April 2006. One MSc student from Kenya will complete her studies in December 2005 on climate matching models for identifying appropriate sites for the introductions of *N. tanajaoe* into Eastern Africa. Two students from Cameroon will continue their studies on the biology of *A. tenella* and ARTS and on the interactions between the two insects with the aim of developing options that could be successfully deployed for interfering in *A. tenella* foraging and for controlling ARTS. Two PhD students will complete their degrees in June 2007 on classical biological control of the coconut mite.

IITA's support to national biological control programs continues in the form of specific training in acarology and entomology, including mite and insect identification; technical assistance with field surveys; preparation of appropriate work plans and proposals for bilateral donor support; data analysis, training courses on impact assessment; and supplying natural enemies for experimental releases. Support activities were carried out in Bénin, Cameroon, Democratic Republic of Congo, Kenya, Malawi, Mozambique, Tanzania, and Zambia.

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