Draft for Review
1 Nov., 1997

PE-1: IPM for a Safer Environment: Integrated Pest Management in Major Agroecosystems in the Americas

ANNUAL REPORT
1997

Centro Internacional de Agricultura Tropical
November 1997

PE-1: IPM for a Safer Environment: Integrated Pest Management in Major Agroecosystems in the Americas

Objective: To develop and transfer improved pest and disease management components for major agricultural production systems and reduce environmental damage due to excessive pesticide use.

Outputs: Improved pest and disease management components and implementation strategy developed on CGIAR commodity crops; improved crop management components relevant to IPM strategies in the Americas developed; NARS capacity to design and execute integrated crop management research and development projects strengthened.

Gains: Increased crop yields and reduced environmental damage; natural enemies of major pests and diseases evaluated. IPM tested and verified on farms. Knowledge on biology, ecology behavior and damage of pests, and diseases. Molecular characterization of major pathogens and diagnostic kits available. Whitefly biodiversity characterized.

Milestones:

1998 Whitefly biodiversity determined and natural enemies identified; cassava mite predators and entomopathogens evaluated and released in LA and Africa; CVMV yield loses determined.

1999 Natural enemies of whiteflies, mites, burrowing bug released in LA and Africa; Molecular markers tagging resistance to CBB and Phytophthora root rot identified; resistance to cassava frog skin virus identified and Elisa diagnostic kit developed.

2000 Biological control implemented for several arthropod pests and root rot pathogens; Cassava geminiviruses characterized. Farmers and extensionists trained in utilization of diagnostic kits.

Users: Biodiversity of agroecosystems determined and available to researchers. NARS scientists, extensionists and farmers trained in IPM methodologies. Crop yields for small producers increased and stable production systems identified.

Collaborators: EMBRAPA, CORPOICA, IITA, ICIPE, CATIE, NARO, NRI, Universities of Florida and Wisconsin, John Innes Center, ETH, OSTROM/CIRAD; and others. NARS, including EMBRAPA, CORPOICA, INIAP, INIVIT, NGO's and biological control industries (COINBIOL).

CG System Linkages: Increasing Productivity 30%; Biodiversity - 20%; Protect Environment - 40%; Strengthening NARS - 10%. Manages Whitefly and Participatory Methods Projects in Systemwide IPM Program.

CIAT Project Linkages: Collaborates with breeding projects (IP1, IP2, IP3, IP4, IP5) in host plant resistance; provides biocontrol agents to project PE5; uses inputs from PE4, SB2, and SN3.

Financing Plan: 20% unrestricted core, 80% special projects.

PE-1. IPM for a Safer Environment: Integrated Pest Management in Major Agroecosystems

Project Objective

To develop and transfer improved pest and disease management components for major agricultural production systems and reduce environmental damage due to excessive pesticide use

Outputs	I **Improved pest and disease management components and implementation strategy developed on CIAT commodity crops**	II **Improved crop management Components relevant to IPM strategies developed in the Americas**	III **NARS capacity to design and execute integrated crop management research and development projects strengthened**	IV **Global whitefly research network to reduce crop losses initiated**
Activities (Subprojects)	• Identification and quantification of major arthropod complexes in selected agroecosystems; development of effective control measures. • Identification and characterization of root rot and superelongation pathogens and CBB races; development of rapid detection methods; development of effective control measures. • Identification and characterization of major viruses; development of rapid detection methods; development of effective control measures.	• Determination of crop management practices that effect pest and diseases populations and dynamics. • Development and evaluation of cultural control practices such as crop rotation, intercropping, etc. for selected pests and diseases. • Evaluation of alternate safer chemical control agents.	• Development of methods for farmer's participatory diagnosis and research in IPM and ISCM. • Ex-post adoption and impact studies on IPM costs and crop productivity. • Integrated management technologies for cassava planting material. • Assessment of impact of IPM measures and policy recommendations. • Dissemination of IPM methodologies through training, workshops, etc. • Assessment of impact of farmer participatory methodologies including participation of women in IPM projects.	• Formation of an international whitefly network. • Diagnosis and characterization of whitefly problem and target areas. • Improvement of understanding of pest and disease dynamics. • Preliminary development and testing of management strategy and tactics through participatory research.

Highlights:

- The first project within the Systemwide Programme on Integrated Pest Management (SP-IPM) to be funded is the project on "Sustainable Integrated Management of Whiteflies as Pests and Vectors of Plant Viruses in the Tropics" (Whitefly IPM Project). The Whitefly IPM Project, which is coordinated by CIAT, was approved and funded by the Danish International Development Agency (Danida) in early 1997.
- The Whitefly IPM Project has successfully brought together 5 International Agricultural Research Centers, 6 basic research institutions in the United Kingdom, Germany and the United States, and 26 national or regional programs in 12 Latin American and 10 African countries to address the whitefly problem.
- The Whitefly IPM Project coordination team has developed a standardized Methodology Guide for collaborative research, which is now in the hands of Project teams in Eastern Africa, the Sub-Saharan cassava belt, the Andean highlands and Central America, Mexico and the Caribbean. Standardized field work on 3 continents is underway.
- A colony of the whitefly parasitoid *Encarsia hispida* was established on *A. socialis* in the greenhouse.
- Laboratory studies show that isolates of pathogens *Verticillium lecanii*, *Beauveria brassiana, Metarhizium anisipliae,* and *Fusarium* sp. cause whitefly nymphal mortality.
- The parasitoids *A. vexans* and *A. diversicornis* responded significantly to the mealybug *P. herreni* induced cassava odours and these volatiles play a major role in their attraction to infested cassava plant.
- Bean plant odours may have a repellent effect on *A. vexans* and when intercropped with cassava the parasitoids could not distinguish infected cassava leaves.
- *A. vexans* parasitism of *P. herreni* was higher in monocropped cassava than when intercropped with beans.
- *A. coccois* parasitoids show no preference for odours of infested over uninfested plants neither as naive nor experienced females.
- All female developmental stages of *P. herreni* feed mainly on phloem sap of cassava with a predominant of extra cellular pathways of stylets before reaching phloem vessels.
- During male development of *P. herreni* only first and second instars feed on cassava phloem sap; older male instars cannot feed due to the absence of mouth parts.
- Biochemical analysis of *P. herreni* shows the presence of a phenolic compound that may play an important role in its development.
- Yield losses due to cassava vein Mosaic virus (CVMV) can be more than 25%.
- A potential pathogen related gene was identified from Cassava Frogskin Disease (CFSD) infected cassava.
- Twenty Two percent of the core collection were determined to be highly susceptible to CFSD and eliminated from further study.
- Phytophtora root rot of cassava found in every important cassava producing region in Colombia and is common in any edaphoclimatic region where cassava is grown.
- Phytophtora identification is best accomplished by hybridizing the region of ribosomal DNA with a Phytophtora-specific DNA probe.

- Pathogenic variation among Phytophthora isolates was determined; the fungus was isolated from Soil, root and sprout tissues.
- No genetic differences were detected bet ween monozoosporic and wild Phythophtora cultures.
- Phytophthora nicotianae were found to have a lower growth rate than Phytophthora drechsleri, and the combination of 50 °C and 20 min (water bath) was lethal to both species in vitro; the fungi were not detected in hot water treated stakes by ELISA test.
- Of 40 Trichoderma isolates tested, three strains inhibited the growth in vitro of P. drechsberi and other Phytophtora spp. the causal agent of root rot in cassava.
- Molecular diversity of Sphaceloma manihoticola (Super-elongation disease-SED) was low, thus facilitating the task of selecting cassava germplasm resistant to SED.
- Cassava varieties resistant to SED identified.
- To address the need critical mass of trained technicians both in research and extension at the state level in the northeast of Brazil, a proposal training is considered a priority by CNPMF.
- Started during the UNDP-IPM project, 25 CIALs are functioning and many are running their third consecutive experiment.
- The training master plan is composed of three modules: diagnosis, planning and experimentation and evaluation. After two cycles, the trainees have the basic skills to facilitate collective problem solving.
- COPALs are making their own decisions using FPR.

- Decisions on the best varieties for the community and the use of fertilizers are examples of the products of the COPALs research.
- The farmer's criteria for selecting the best varieties are more than just yield. Shape of roots, and color of flour are examples of characteristic that were identified as important by farmers.
- Three predatory mites from Brazil are now established in Africa (*Neoseiulus idaeus*, *Typhlodromalus manihoti* and *Typhlodromalus aripo*). The latter two are reducing the pest populations by 30-80%. *T. aripo* has spread 200 km from initial release sites in southern Benin in 2 years. Predator exclusion experiments by IITA indicate that *T. aripo* reduces the pest population by 90% and increases yield by 30%, which produces an additional income of about US$60 per ha.
- Phytoseiid mite species that are candidates for exportation as biological control agents of cassava green mite were evaluated for specificity of prey and food sources that they can consume. *T. aripo* could use a greater variety of food sources, including cassava exudate, immature whiteflies and mealybugs; all of which produce a sugary liquid, while most species depended only on tetranychid mites.
- *Galendromus annectens* from a dry location in coastal Ecuador was exported to Brazil for release by CNPMF/EMBRAPA.
- *Typhlodromalus manihoti* from several high altitude sites in Colombia was sent to IITA, Benin via quarantine at the University of Amsterdam (Mitox) for release in the East African plateau.

- A taxonomic key to 30 phytoseiid species commonly found on cassava in northern South America was further developed in collaboration with Gilberto de Moraes (ESALQ/ Universidade de São Paulo, Piracicaba, Brazil).
- We evaluated 5 strains of the fungal pathogen *Neozygites* cf. *floridana* for host specificity. None of the 3 strains originating from cassava green mite (CGM) infected the two-spotted spidermite (TSM), and one of the strains from TSM failed to infect CGM, indicating a high level of host specificity.
- We improved *in vitro* tissue culture methods for rearing *Neozygites* cf. *floridana* by adding antibiotics to control bacterial contamination, which has permitted us to speed up our DNA characterization work.
- We measured the effect of resistant, tolerant and susceptible cassava varieties on the cassava green mite and a phytoseiid predator, *Neoseiulus californicus*. The variety MEcu-72 provided the best combination of resistance against the pest and compatibility with the predator.

OUTPUT I. IMPROVED PEST AND DISEASE MANAGEMENT COMPONENTS DEVELOPED

Subproject 1. Identification and Quantification of Major Arthropod Complexes: Development of Control Measure

Mealybugs (Phenacoccus herreni)

Numerous species of mealybug attack cassava, but, only *Phenacoccus herreni* and *P. manihoti* (Cox and Williams, 1981) are important economically. Both are of neotropical origin; *P. manihoti* has caused considerable cassava damage in Africa, but is found only in limited areas of Paraguay and Southwestern Brazil where it causes no economic damage. *P. herreni* causes damage similar to *P. manihoti* causes damage to cassava in Colombia, Venezuela, The Guayanas and throughout much of northeast Brazil. These important parasites of *P. herreni, Acerophagus coccois, Apoanagynus diversicornis* (= *Epidinocarsis diversicornis,* and *Aenasius vexans* have been extensively evaluated in the laboratory and field. The release and establishment of these parasites in northeast Brazil has resulted in parasite establishment and reduced mealybug populations. In coordination with these field releases, considerable research has been carried out to determine the effects of plant volatiles on parasitoid behavior.

Subproject 1.1. Chemical Mediated Searching Behavior of Parasitoids

Source of the attraction of *A. vexans* and *A. diversicornis*

In a Y-tube olfactometer *A. vexans* and *A. diversicornis* were tested, to determine the cause of the attraction for infested cassava plants shown in previous work (Bertschy et al., 1997). Plant factors were separated from the mealybug system. In order to isolate the plant factors, the mealybugs and their by-products were removed and the leaves carefully washed. These washed leaves should release common plant odours plus the herbivore induced volatiles. To test the mealybug system, the mealybugs removed from the infested leaves as well as the exuviae were placed on a small wet cotton wool pad, honeydew, sooty mould and all excretion products of the mealybugs were collected with a wet cotton wool.

The washed leaves were offered the two encyrtids against a blank (wet cotton wool), healthy cassava leaves, infested leaves and against the mealybug system. The mealybug system was also tested against the same odour sources, namely : blank, healthy and infested. In order to test the presence of induced volatiles the mealybug system odour source was associated to healthy leaves and opposed to infested leaves.

Results

In previous work (Bertschy et al., 1997), it was reported that 64.5 % of *A. vexans* females preferred healthy plant odour over "Blank", but it was not statistically significant. This experiment was repeated with ninety *A. vexans* females. This time they showed a statistically significant attraction for healthy leaf odours (67.2 %, χ^2=6.89, P<0.01). This discrepancy is attributed to the use of unsterilised cotton wool in the first experiment. It was since found that unsterilised cotton is attractive to the wasps and it may have been used more of it in the blanks than in the sources with plants. A.vexans females responded in a

higher proportion to odours than *A. diversicornis*, but in a similar way **(Fig. 1.1 and 1.2)**. Washed infested leaves ("Washed") were preferred by both species over "Blank" (χ^2=7.78, P<0.01 and χ^2=8.22, P<0.005 respectively for *A. vexans* and *A. diversicornis*) and over "mb" (χ^2=4.05, P<0.05 and χ^2=6.36, P<0.025) **(Fig. 1.1)** which indicates a stronger influence of the plant odours over the mealybug factor odours. Both wasp species could not distinguish "Washed" from "Infested" (χ^2=1.05, P>0.25 and χ^2=0.134, P>0.50), which could confirm the release of induced volatiles by cassava leaves, but they also could not distinguish "Washed" from "Healthy" (χ^2=0.67, P>0.25 and χ^2=1.17, P>0.25), though they can distinguish "Infested" from "Healthy (Bertschy et al., 1997). This may indicate that washed cassava leaves release an intermediate quantity or/and quality of volatiles which stand between the one of healthy and infested leaf odours.

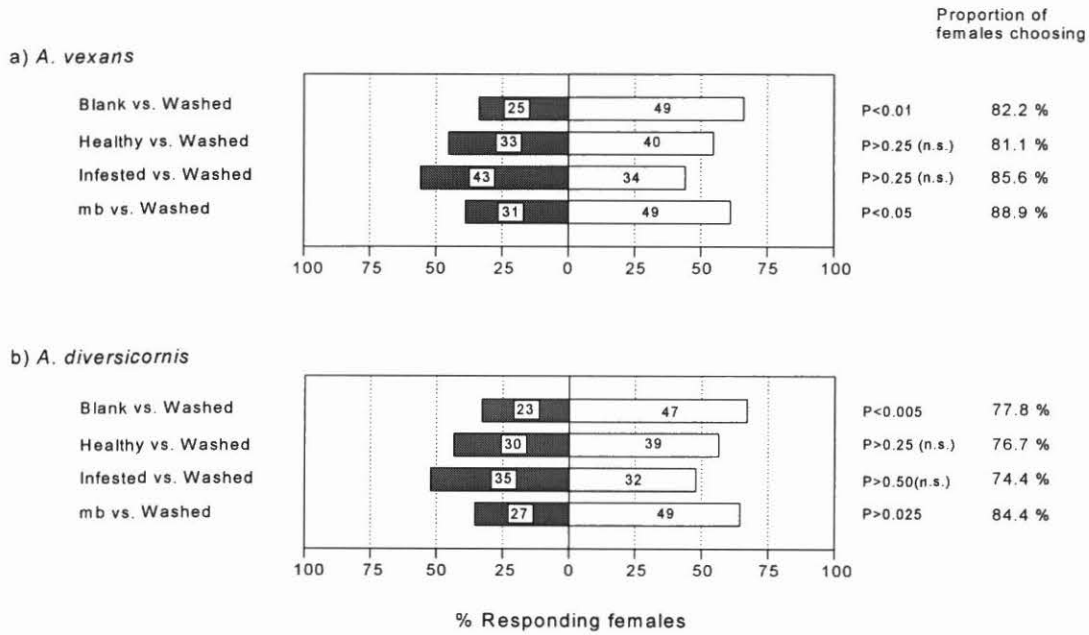


Figure 1.1. Response of *A. vexans* and *A. diversicornis* to washed infested cassava leaves in a Y-tube olfactometer. In each bar the actual number of wasps that made a particular choice is given, while the x-axis indicates the percentages of choosing wasps that the numbers represent. Next to the bars the proportion is given of the females that made a choice for one of the two odours.

In the choice "mb vs. Blank" 60.8 % of *A. vexans* females walked up to the arm leading to the mealybugs and their by-products, which is statistically not significant (χ^2=3.459, 0.1<P>0.05). *A. diversicornis* showed no significant attraction for "mb" over "Blank" **(Fig. 1.2)** as only 54.5 % of the responding females preferred the mealybug system odours (χ^2 =0.545, P>0.5). "Infested" was significantly attractive to both *A. vexans* and *A. diversicornis* over "mb" (χ^2=8.33, P<0.005 and χ^2=13.12, P<0.001 respectively). They also preferred "Infested" over the combination of healthy leaves and the removed mealybug system ("Healthy + mb") (χ^2=20.28, P<<0.001 and χ^2=16.07, P<<0.001 respectively). This indicates the presence of an attractive odour blend in the infested leaves which is not released neither by healthy leaves nor by mealybug and their by-products in a high enough quantity so that the wasps can recognize it. Curiously the wasps could not distinguish between "Healthy" and "mb", though "Healthy" was shown to be attractive to both *A. vexans* and *A. diversicornis* over "Blank". Like the washed leaves, the mealybug system may stand in an intermediate position between "Blank" and "Healthy".

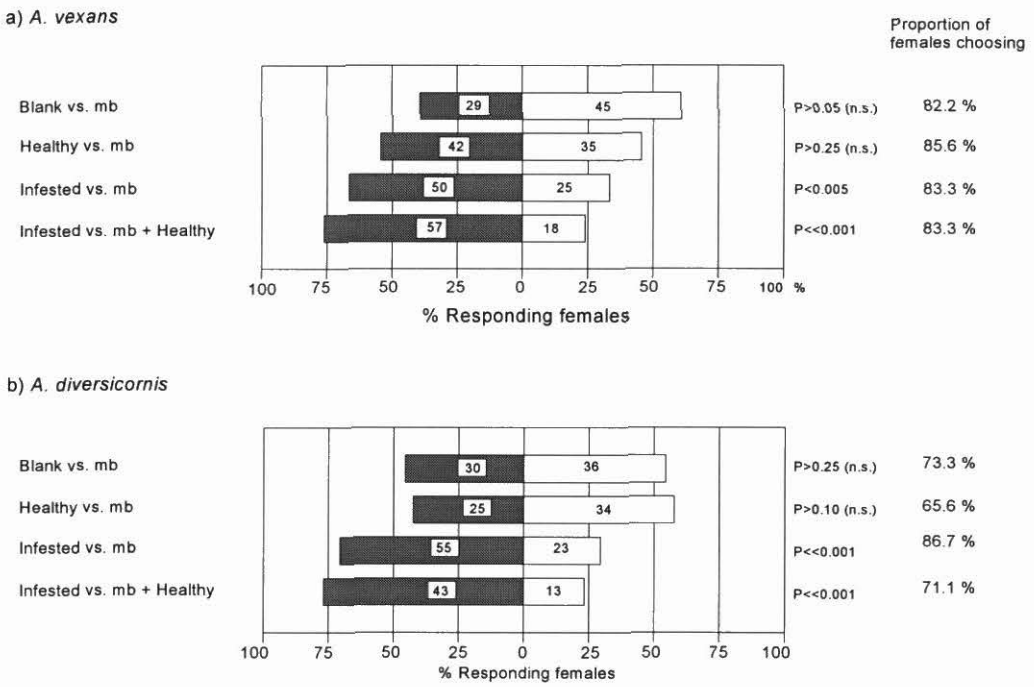


Figure 1.2. Response of *A. vexans* and *A. diversicornis* to mealybugs and their by-products in a Y-tube olfactometer. In each bar the actual number of wasps that made a particular choice is given, while the x-axis indicates the percentages of choosing wasps that the numbers represent. Next to the bars the proportion is given of the females that made a choice for one of the two odours.

A. vexans and *A. diversicornis* responded significantly to *P. herreni*-induced cassava odours. The induced volatiles played the major role in their attraction to infested cassava plant. They preferred them from the mealybug factors and from healthy leaves. The mealybugs may play a more important role in short-range attraction. The difference in volatile quantity seemed to be important for the encyrtids to distinguish between two different odour sources.

Influence of bean plant odours (in laboratory)

Bean leaves were tested in a Y-tube olfactometer to see the influence of their odours on the foraging behaviour of *A. vexans* and *A. diversicornis*. Two bean leaves were used as odour sources and tested against a blank (2 ml-vials filled with water). The test "Infested vs. Blank" was repeated as control of the attraction of the encyrtids for infested cassava plants. The different paired odours were offered to the parasitoid females: "Bean + Infested vs. Bean" (2 bean leaves with 2 infested cassava leaves versus two bean leaves), "Bean + Infested vs. Infested" and "Bean + Infested vs. Bean + Healthy". The five experiments were done the same day.

Bean leaf odours seemed to be repellent to *A. vexans* females, as only 36.4 % of them walked toward this odour source **(Fig. 1.3)**. Females significantly preferred the "Blank" (χ^2 = 4.09, P<0.05), which sustains the low response obtained in this choice. This species was significantly attracted by infested cassava leaves as already shown in previous work (Bertschy et al., 1997) but the presence of bean leaves confused them enough so they did not recognize the cassava leaf odours. 48.3 % of the responding females walked up to "Bean + Infested" side (χ^2 = 0.066, P>0.75). Their capacity to distinguish between infested and healthy cassava leaves (Bertschy et al., 1997) disappeared when they were mixed with bean leaves **(Fig. 1.3)**. Only 56 % of them preferred the infested cassava leaf side, which is not statistically significant (χ^2 = 1.08, P>0.25). 67.1 % of the responding *A. vexans* females preferred infested cassava leaves alone over a mixture with bean leaves (χ^2 = 8.89, P<0.005).

Unlike for *A. vexans*, bean odours seemed to be neutral to *A. diversicornis* **(Fig. 1.4)**. 60.3 % of the responding females went to bean leaf side, which is statistically not significant (χ^2 = 2.68, P>0.10). Presence of bean leaves with cassava did not change their attraction to cassava leaves. 87.3 % of the responding females preferred "Bean + Infested" over the "Blank" (χ^2 = 35.06, P<<0.001), 72.9 % distinguished the infested from the healthy cassava leaves, no matter they were accompanied with bean leaves (χ^2 = 14.628, P<<0.001). Though, like *A. vexans*, *A. diversicornis* females preferred going to infested cassava leaves when they were not with bean leaves (χ^2 = 4.37, P<0.05).

A. vexans seemed to be repelled by bean plant odours and could not distinguish infested cassava leaves when they were associated to bean leaves. Bean plant odours may have a repellent or masking effect on *A. vexans*. *A. diversicornis* was not confused by the presence of bean leaves, it still could distinguish infested cassava leaves from healthy ones. Bean had no effect on this species.

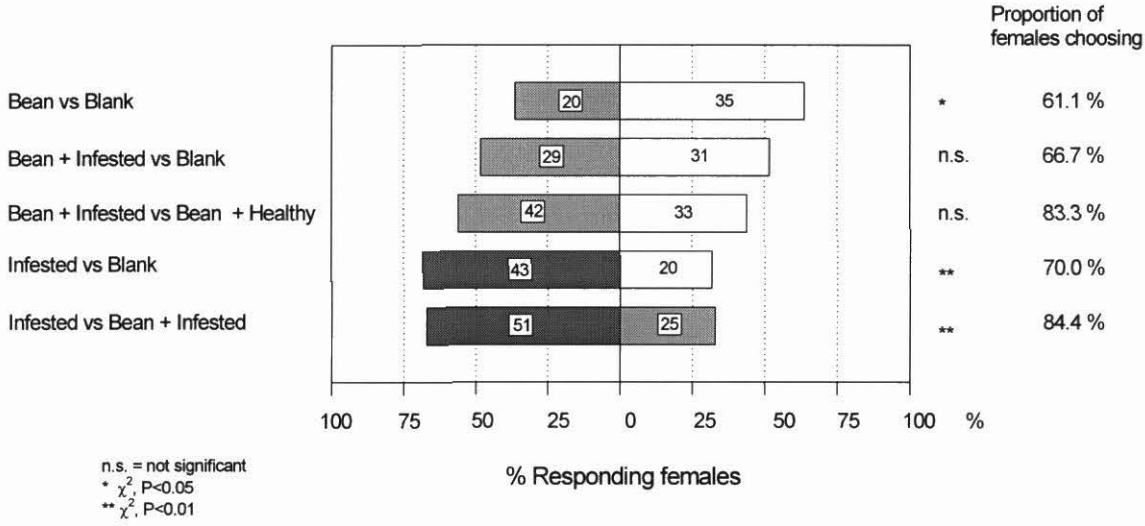


Figure 1.3. Response of *A. vexans* to bush bean plant odours in a Y-tube olfactometer. In each bar the actual number of wasps that made a particular choice is given, while the x-axis indicates the percentages of choosing wasps that the numbers represent. Next to the bars the proportion is given of the females that made a choice for one of the two odours.

Field tests

Three different treatments were arranged in a block of 12 plots of 5mx5m and repeated three times. Between the small plots a row of cassava or bush beans (var. Pijao) was planted. The treatments were: cassava pure stand, cassava intercropped with bush beans and bush beans pure stand. The cassava plot contained 6 rows of 6 cassava plants 1x 1 m. In the four central rows, two plants were missed in each row, leaving a distance of 2 m between two cassava plants. In the mixed culture plots, the same design was used as in cassava pure stand, rows of beans were sown 10 cm from each others between the cassava plants. In the last treatment, bean pure stand, 11 rows of beans were sown. The two outer rows were kept complete, while in each second row of the middle ones two spots of 2 m each were kept free of plants. In all free spots, infested potted cassava plants were brought in an afternoon (2-4 p.m.) and left in the field for parasitism during five days. 20 females and 10 males of *A. vexans* or 20 females of A. *diversicornis*, which is parthenogenetic, were released the next morning (8 a.m.) in the middle of each plot. The two parasitoid species were never released at the same time, but the experimental plots for the different species were contiguous. *A. vexans* seemed to be more aggressive and remain more time in the same place, that is why *A. diversicornis* was always first released and a few weeks later the experiment with *A.*

vexans was started. The parasitism rate of each potted cassava plants was recorded. The three repetitions are significantly different from each other for both *A. vexans* and *A. diversicornis*.

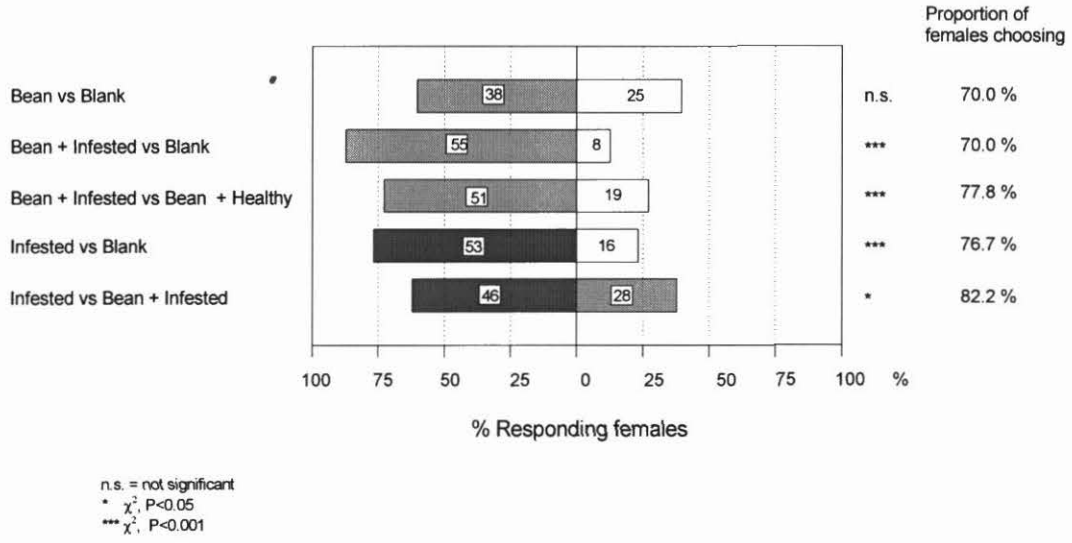


Figure 1.4. Response of *A. diversicornis* to bush bean plant odours in a Y-tube olfactometer. In each bar the actual number of wasps that made a particular choice is given, while the x-axis indicates the percentages of choosing wasps that the numbers represent. Next to the bars the proportion is given of the females that made a choice for one of the two odours.

In the first and second repetitions with *A. vexans* there was no significant difference in the parasitism rate between treatments, though the parasitism doubled in the second repetition, which may be due to the natural *A. vexans* parasitism. In the third repetition, there was a significant difference between cassava pure stand plots and the two other treatments. In this particular repetition bean plants were much higher than in the others. The higher bean mass may have had an influence on the behaviour of *A. vexans*. This result would confirm one of the Y-tube experimental. It may also indicate that *A. vexans* is confused with a high bean plant volatile concentration.

Unlike *A. vexans*, *A. diversicornis* showed a higher parasitism rate in the first repetition. It may be due to the lower natural *A. vexans* parasitism. In the second repetition, there was a significantly higher parasitisation in the mixed plots. In this test some infested plants dried out in the field and the number of mealybugs brought back on the plants was much lower than usual (about 30 % of the infestation against 60 %). The higher parasitisation in the mixed plots may be related to the higher number recovered in these plots.

Conclusion

A. vexans did not react to the presence of bean plants in the field. Though when their volume was important, it means a higher volatile release, the parasitism was significantly higher in the cassava pure stand. But there would be necessary to do more experiments to prove it. *A. diversicornis* did not react to bean plants in the field. There was no significant difference between treatments, but their parasitism rate was very low (ca. 5 %).

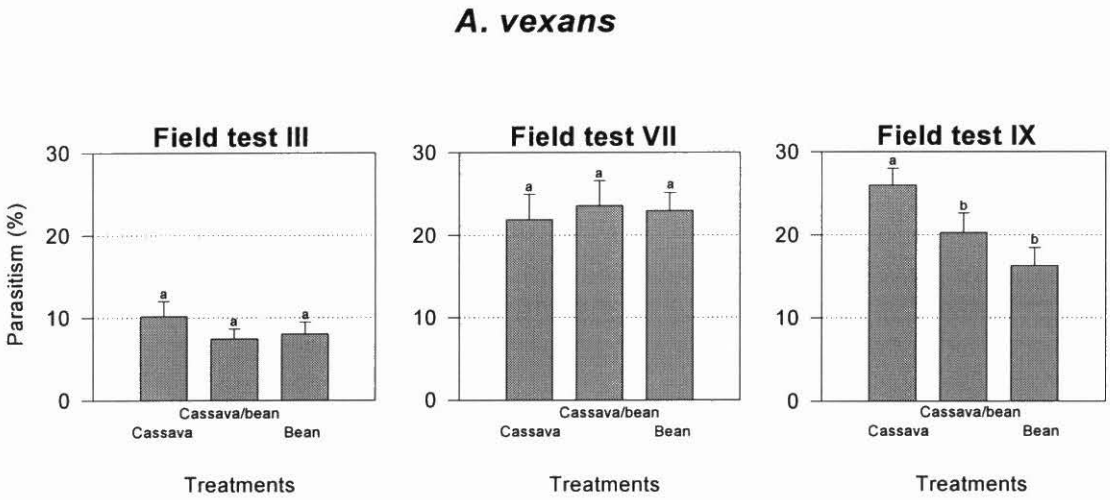


Figure 1.5. **Parasitism rate of *A. vexans* in three repetitions in the field. (+/- S.E.). Differences between plots with the same letter are not statistically significant.**

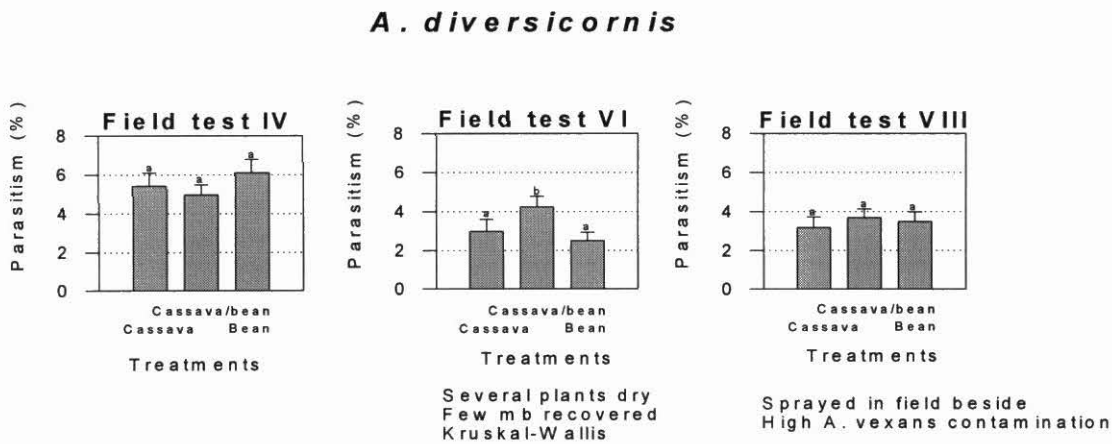


Figure 1.6. **Parasitism rate of *A. diversicornis* in three repetitions in the field. (+/- S.E.). Differences between plots with the same letter are not statistically significant.**

Sex ratio allocation of *A. vexans*

A. vexans were introduced in pairs in petri dishes with live cassava leaves. These leaves were infested with 50 mealybugs of one instar. The parasitism was tested with second, third and fourth mealybug instars and sex ratio recorded. In a second experiment *A. vexans* couples were offered the three different mealybug instars together (20 of each). The sex allocation was recorded as well as the preference and the size of the emerging *A. vexans* (tibia length).

Bigger mealybug instars produced significantly more *A. vexans* females than males **(Fig. 1.7)** in both choice and no-choice experiments. The proportion of males emerging in the choice-experiment was lower for all instars than in no-choice experiments. The parasitoid females preferred parasitising third and fourth mealybug instars **(Fig. 1.8)**. *A. vexans* females were significantly bigger than males. Third mealybug instars produced bigger parasitoids, both males as well as females **(Fig. 1.9)**.

Third mealybug instar seems to be best for *A. vexans* rearing. Fourth instar is also preferred and produces more females than second instar. Third instar produces bigger *A. vexans* than both second and fourth instars and bigger parasitoids are reported to have a better fitness, they lay more eggs in a lifetime.

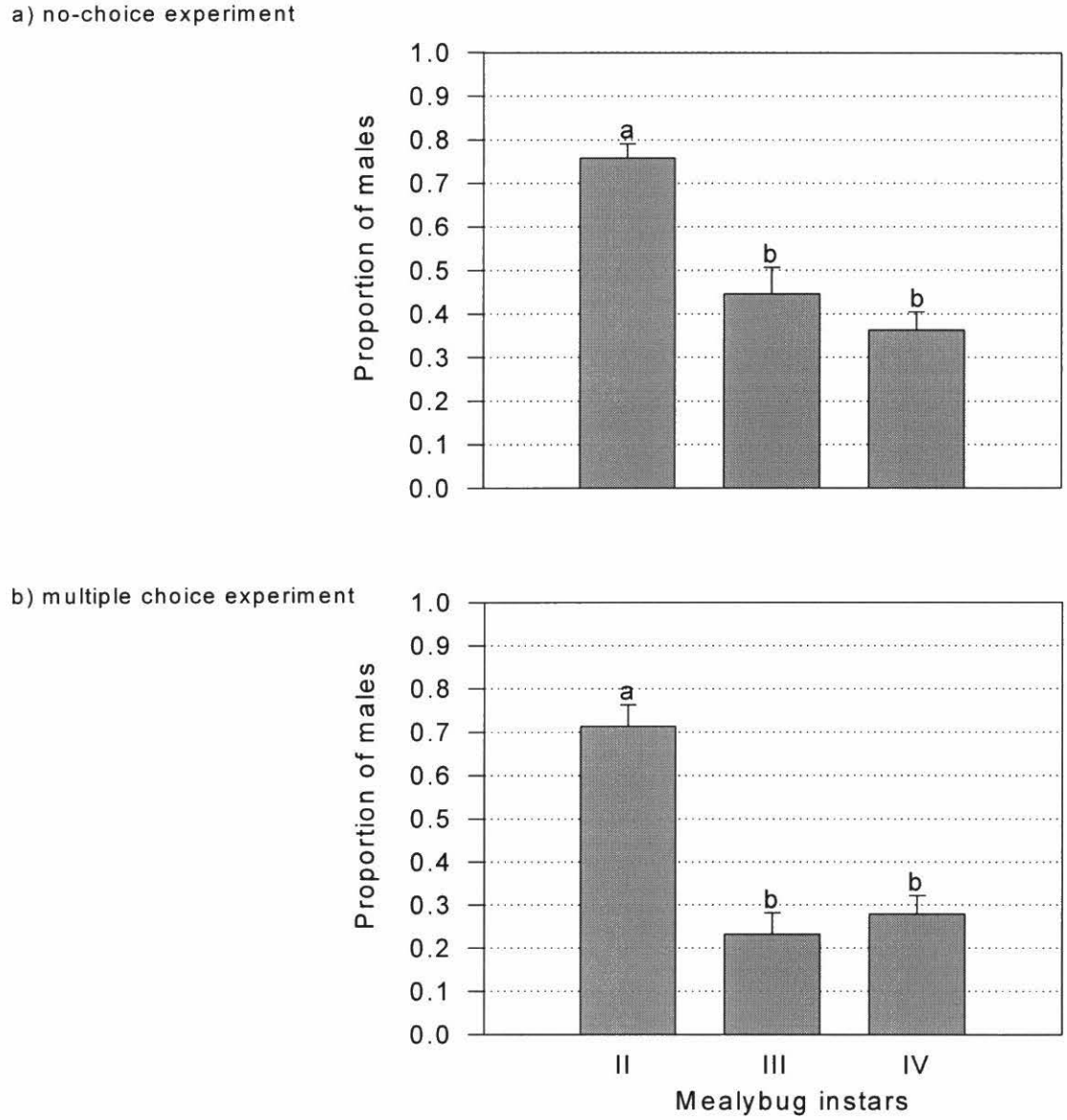


Figure 1.7. **Proportion of *A. vexans* males emerging from different parasitised mealybug instars in (a) no-choice and (b) choice experiments. (+/- S.E.). Differences between plots with the same letter are not statistically significant.**

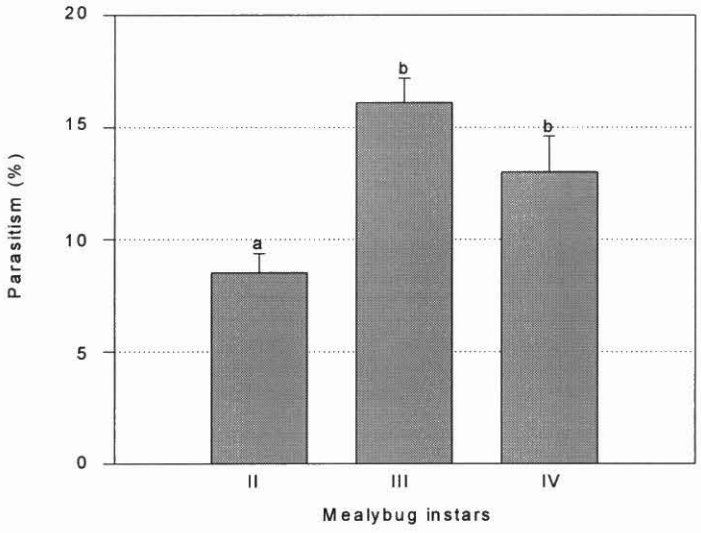


Figure 1.8. **Parasitism rate by *A. vexans* for different mealybug instars in choice experiment. (+/- S.E.). Differences between plots with the same letter are not statistically significant.**

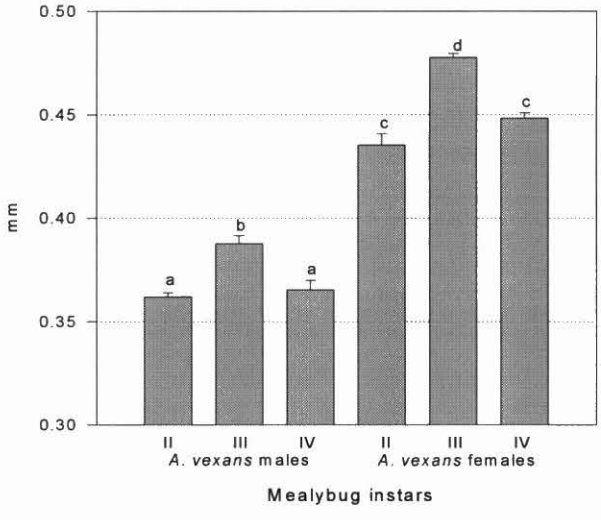


Figure 1.9. **Tibia lengths in mm of *A. vexans* emerging from different parasitised mealybug instars. (+/- S.E.). Differences between plots with the same letter are not statistically significant.**

Subproject 1.2. Innate and learned responses of parasitoids for reliable host finding

Three mealybug (*P. herreni*) parasitoids *A. coccois, A. diversicornis,* and *A. vexans* have been extensively evaluated at CIAT and released in northeast Brazil where they have reduced mealybug populations by nearly 50%. As has been shown, these species, to varying degrees, respond to volatiles released by plant herbivores.

Studies have been initiated to evaluate the learning capacity of two parasitoids *A. coccois* and *A. vexans.* In addition the responsiveness of the two species to different varieties of cassava will also be studied. *A. coccois* is a generalist parasitoid, with numerous hosts. *A. vexans* appears, to have a limited host range and shows high preference for *P. herreni.*

Primarily studies were carried out using the Y tube olfactometer (Y shaped glass-tube, 1.7cm diameter, all three arms 24cm long). Each side arm was connected to a glass chamber (6.0cm diameter) in which odour sources could be placed. Activated charcoal filtered air at a rate of 400ml/min was pushed into each chamber causing a windspread of 0.09 - 0.21 m/sec. At the exit of the common arm. To avoid visual distractions a wooden frame covered with white cloth was placed around the Y-tube. To induce the parasitoids to walk in an upwind direction toward the odour sources an array o five 14-watt fluorescent daylight tubes (producing 1200 lux), were placed at the upwind end of the apparatus.

The mealybug colony is maintained on cassava plants (Var. CMC 40) in the greenhouse. The parasitoids *A. vexans* and *A. coccois* are reared on *P. herreni* on infested cassava (Var. MCol 1505) leaves in screened cages in the greenhouse.

Odour Sources

Two leaves of healthy or infested, same-aged plants were cut and placed into each of the odour chambers, respectively. To avoid withering, the leaf stalks were stuck into small vials with water. The infested leaves carried late 2nd and early 3rd instars of *Phenacoccus herreni* as well as honeydew, sooty mould and exuviate.

Bioassay

Parasitoids 2 - 4 days old were used to avoid pre- and peri-emergence learned odour cues (Bertschy et al. 1997, submitted). From emergence both sexes were held in screen Plexiglas-cages (40 x 40 x 60 cm) and provided with water and honey, isolated from contact and odour of hosts and hosts' plants.

For the trial females were isolated into Plexiglas vials with 2 open ends (2.2 x 5.5 cm, bottom with gauze; the open top fits perfect onto the Y-tube) and provided with abundant honey and water. Thus it is most likely that responders are searching for hosts and not for food or water (Lewis & Takasu 1990).

For experience, females were introduced into a petri dish (14cm diameter, Plexiglas) containing an infested leaf with late 2nd and early 3rd instars of *Phenacoccus herreni* as well as honeydew, sooty mould and exuviate. They were continuously watched until they had completed their very first oviposition or at least stung a mealybug. Right after this experience they were separated into the Plexiglas vials.

All females were left under 1280 - 1680 lux before the trial for at least 45-min.

Anemotactic responses of female parasitoids were assessed in the above-mentioned Y-tube olfactometer between 9:30 am and 8 pm at 26.5 - 28.5 °C and 61 - 92 % RH. A female that penetrated more than 8 cm (= 1/3) into a side arm within 5 min was recorded as a responder. All others were classified nonresponders ("no choice"). In order to obtain sufficient replications the trial was **conducted over several days.**

For statistical analysis chi-square-test was used.

Preexperimental oviposition experience significant affected the behaviour of *Acerophagus coccois* and *Aenasius vexans* females concerning their responsiveness and their choice of odours. Responsiveness decreased when the parasitoids had an oviposition experience **(Table 1.1)**. Experience females are less responsive than naive ones for *A. coccois* (Chi square = 7.92, d.f. = 1, $p < 0.01$, n = 206) and likewise for *A. vexans* (Chi square = 5.39, d.f. = 1, $p < 0.05$, n=198).

Table 1.1. Parasitoid females responding to mealybugs (*Phenacoccus herreni*) infested cassava leaves.

Species and Treatment		Number of Females Responding[1]	Number of Females Making no Choice	Total Number of Females
Acerophagus coccois	naïve	65% (n = 66)	35% (n = 36)	100% (n=102)
Acerophagus coccois	experienced	45% (n = 47)	55% (n = 57)	100% (n = 104)
Aenasius vexans	naïve	56% (n = 62)	44% (n = 48)	100% (n = 110)
Aenasius vexans	experience	40% (n = 36)	60% (n = 53)	100% (n = 88)

1 = Insects that went to either of the odour arms.

Considering the difference in choice of responsive naive and experienced females for either odour of infested leaves or uninfested leaves, there are no differences within *A. coccois* whereas within *A. vexans* there are significant differences (Chi square = 4.84, d.f. = 1, $p < 0.05$, n = 97).

Concerning the choice of responsive females of each of the treatments "naive" and "experienced," only the experienced *A. vexans* exhibit a significant preference to odour of infested over uninfested leaves (Chi square = 6.4, d.f. = 1, $p < 0.05$, n = 35). Whilst neither naïve or experience *A. coccois* nor naive *A. vexans* display any preferences.

Therefore, comparing the naïve females of both species, there is no different pattern of preference behavior to one odour source or the other. But there is a significant difference within the experience females of both species (Chi square = 4.97, d.f. = 1, $p < 0.05$, n = 82):

experience *A. vexans* exhibits a preference for the odour of infested over uninfested leaves, whereas experienced *A. coccois* displays no preference.

Table 1.2. Parasitoid females responding to mealybug (*Phenacoccus herreni*) infested cassava leaves.

Species and Treatment		Number of Females Responding to Odour of Infested Leaves	Number of Females Responding to Odour of Infested Leaves	Total
Acerophagus coccois	naive	45.5% (n = 30)	54.5% (n = 36)	100% (n = 66)
Acerophagus coccois	experienced	46.8% (n = 22)	53.2% (n = 25)	100% (n = 47)
Aenasius vexans	naive	48.4% (n = 30)	51.6% (n = 32)	100% (n = 62)
Aenasius vexans	experience	71.4% (n = 25)	28.6% (n = 10)	100% (n = 35)

As demonstrated for other species it was expected that previous oviposition experienced will increase parasitoids' general responsiveness through sensitization or priming. But our data show just the opposite. Therefore it is likely that the handling involved in the experienced had an effect and makes them respond less. It is unlikely that reduced eggload is responsible for the lack in responsiveness because dissection showed that the females of both species contain at least one dozen eggs and, -if allowed - females go on parasitizing several mealybugs.

Furthermore the usually strong stimulus of an oviposition experience was expected to result in an increased preference for odour of infested plants over uninfested. We also expected the assumed less specialist parasitoid *Acerophagus coccois* to respond more strongly through associative learning to the previously experienced odour than the more specialist parasitoid *Aenasius vexans*. But in these results the more generalist species does not exhibit any preference but the more specialist species displays a strong preference after being experienced.

As *Acerophagus coccois* females show no preference for odour of infested over uninfested plants neither as naïve nor as experience females, it was not possible to see the effect of learning. The bioassay may need to be charged in order to evaluate learning capacity.

Subproject 1.3. Biological Control of Cassava Whiteflies

Whiteflies (Homoptera: Aleyrodidae) are one of the most damaging arthropod pests of agricultural crops throughout the tropical and subtropical regions of the world. They damage crops through their direct feeding and are vectors of numerous plant viruses. Several species of whiteflies feed on cassava. In Colombia *Aleurotrachelus socialis* is the dominant species; *Bemisia tuberculata* and *Trialeurodes variabilis* occur in lower populations although they may be important in virus transmission.

In recent years the whitefly problem has become more serious, especially in the neotropics. The silverleaf whitefly, *Bemisia tabaci,* B biotype (=*Bemisia argentifolli*) has now disseminated throughout much of the region. Its presence in Colombia was confirmed during the last year and was recorded at CIAT in recent months. This species has been found feeding on cassava in several countries, giving additional justification to our current

emphasis on whitefly research. This research on cassava has traditionally emphasized host plant resistance (see Project IP3). However in recent years research on biological control have been initiated.

Establishment of whitefly parasite colonies and evaluation of parasites

For the past three years considerable survey work has identified numerous natural enemy parasitoid species associated with the cassava whitefly complex. Two species *Encarsia hispida* and *Eretmocerus* sp. "b" were the most widely distributed throughout the regions surveyed and were also the most abundant species record.

Based on these observations, research was initiated to evaluate the effect of parasitoid species on whitefly populations. These studies are being done in the greenhouse on two whitefly species *A. socialis* and *B. tuberculata.* Colonies of both whitefly species are being maintained in greenhouse cultures.

After considerable effort we were able to establish a colony of *Encarsia hispida* on *A. socialis* in the greenhouse. Parasite adults are released in cages (1x1x1 m) containing cassava plants infested with whitefly nymphs. After 2 to 3 days of exposure to the parasitoids, plants are removed and stored in cages until parasitoids emerge. Using this procedure a constant supply of parasitoids can be maintained.

Studies have been initiated to identify the whitefly instar most susceptible to the parasitoid and to determine the biological cycle of *E. hispida.* Two whitefly species are being studied as hosts, *A. socialis* and *B. tuberculata.*

Evaluation of entomopathogens for whitefly control:

Field surveys were carried out in several cassava growing regions of Colombia, including Valle del Cauca, Cauca, Chocó, Caldas, Santander and Tolima. Cassava leaves infested with whiteflies were collected at numerous sties and placed in humidity chambers for 48 hours. In the laboratory, with a stereomicroscope, whitefly nymphs with symptoms of fungal infection or unusual discolorations, were placed on PDA and SDA. Fungal pathogens observed were purified and isolated on microcultures. *Penicillium* sp. and *Fusarium* sp. were isolated from whitefly nymphs.

Bioassays were carried out with these pathogens as well as with isolates of *Beauveria brassiana* and *Metarhizium anisipliae* and *Verticillium lecanii.* The bioassay used the Lunda method; nymphs of *Trialeurodes vaporarium* with a suspension of conidia of each entomopathogen. The slide was placed in a petri dish with moist filter paper. A second slide with nymphs was inoculated with water. Observations were made over a 7-day period.

Results show that the entomopathogens *V. lecanii* (20%), *B. brassianaI* (15%), *M. anisopliae* (10%), and *Fusarium* sp. (10%) caused whitefly nymphal mortality. *Penicillium* sp. did not cause any mortality and was eliminated from further evaluation. From those pathogens that caused mortality, pure cultures were obtained. In a second evaluation whitefly nymphs of *A. socialis* were inoculated with conidia as described above.

Results from this experiment show higher levels of mortality for all entomopathogens **(Table 1.3).** *B. bassiana* and *M. anisopliae* resulted in 95% and 90% mortality respectively. We are not aware of additional report of *M. anisopliae* causing whitefly mortality.

Table 1.3. Whitefly (*A. socialis*) nymphal mortality caused by several fungal entomopathogens.

Pathogen	% Mortality Original Isolate	Reactivated Isolate
Verticillium lecanii	20%	60%
Beauveria bassiana	15%	95%
Metarhizium anisopliae	10%	90%
Fusarium sp.	10%	50%
Control	35%*	13%*

* No whitefly adult emergence.

Preliminary studies, based on the above results, were designed to evaluate the effects of *B. bassiana* on *A. socialis* under greenhouse conditions. The *B. bassiana* strain was originally isolated from *Hypotenemus hampei* Ferrari, cultivated on Saborand Dextrose Agar, and reactivated on *A. socialis,* using the Landa et al (1994) methods. The reactivated pathogen was multiplied on precook rice and conidia collected with domestic kerosene. The collected conidia were mixed with sunflower seed oil, resulting in a $1 \times 10^9 / ml^{-1}$ formulation.

Applications were directed to leaf undersurfaces of potted cassava plants in the greenhouse. Cassava leaves contained populations of I, II, and III instar *A. socialis* nymphs. The control applications of the sunflower oil without conidia, primary purpose was to confirm the absence of the entomopathogen under "natural" conditions. Evaluations were done 5, 7, and 9 days after application.

Results show that *B. bassiana* caused mortalities of 28,55 and 39 percent for I, II, and II instar nymphs respectively. It was established that the second instar is most susceptible to *B. bassiana* under the experimental conditions. The position of the leaf (apical, middle or lower) on the plant had no effect on mortality. It was also established that the seventh day after application is the most appropriate for evaluation.

The entomopathogens *M. anisopliae* and *V. lecaniiare* also being evaluated as described above for *B. bassiana.*

Subproject 2. Identification and Characterization of Root Rot and Superenlongation Pathogen: Development of Rapid Detection Methods and Effective Control Measures

Cassava Germplasm Identified for Resistance to Phytophthora Root Rot Disease

Introduction

Phytophthora root rot cause heavy economic losses in the cassava crops of Latin America and Africa. We first developed methods for isolating *Phytophthora* spp. from cassava sprouts and roots and new inoculation methods for detecting pathogenic variation. We also adapted molecular techniques to determine the genetic variability of *Phytophthora* spp. The pathogens of root rots are disseminated through infected vegetative seed, and the need to have seed free of disease is high. The objective of our second study was to design an efficient method of eradicating *Phytophthora* spp. from planting material. With the idea of controlling different *Phytophthora* species, we set up in vitro tests to select efficient isolates of *Trichoderma* spp.

Collection and characterization of *Phytophthora* spp.

Phytophthora strains were obtained from 38 cassava fields in the Colombian departments of Valle, Quindío, Cauca, Bolívar and Putumayo, where losses can be as high as 80% of the total cassava production **(Fig. 1.10)**. We collected 170 samples of infected tissues, from which 80 isolates were obtained by directly plating the infected tissue on media selective to *Phytophthora* spp. and by baiting with stem fragments of cassava plantlets propagated in vitro or in the greenhouse.

An excellent way of identifying *Phytophthora* is to hybridize the region of ribosomal DNA with a *Phytophthora*-specific DNA probe (Lee et al. 1993). We used a selection of 13 isolates from our cassava *Phytophthora* collection and we found that most of the strains belong to *Phytophthora* spp. **(Table 1.4)**.

Lee SB; White TJ; Taylor JW. 1993. Detection of *Phytophthora* species by oligonucleotide hybridization to amplified ribosomal DNA spacers. Phytopathology 83:177-181.

All the strains were characterized using restriction analysis of rDNA fragments amplified by the PCR and RAPD. Seven strains identified in earlier works by morphological characteristics as *Phytophthora drechsleri* (P12), *Phytophthora nicotianae* (P4), *Phytophthora cryptogea* (P7), *Phytophthora erythroseptica* (P13), *Pythium* spp., *Thielaviopsis paradoxa* and *Sphaceloma manihoticola* were used as references. Amplification of the ITS regions and the gene 5.8 S of the rDNA was obtained using the universal primers ITS1 and ITS4 from extracted total DNA. *Alu*I, *Msp*I and *Taq*I restriction enzymes were used to digest the PCR products. Ten random 10-mer primers (Operon Technologies, Inc) were tested and five (OPA-01, OPA-02, OPA-03, OPA-04, and OPH-05) gave reproducible bands. A similarity matrix and dendrogram were made using the UPGMA from the NTSYS-pc.

Figure 1.10. Distribution of *Phytophthora* spp. in sampled regions of Colombia.

Table 1.4. Grouping of some *Phytophthora* isolates of Colombia according to morphology and DNA hybridization.

Isolate	Source	Origin	Morphology Group[a]	Hybridization[b]
P4	-	-	1	+++
P7	-	-	2	++
70	Root	Caicedonia (Valle)	2	+
59	Root	María La Baja (Bolívar)	3	++
74	Root	Palmira (Valle)	3	+++
62	Root	La Encinada (Quindío)	3	+
35	Sprout	Barcelona (Quindío)	4	+
37	Root	Sevilla (Valle)	5	+
78	Root	Barranquilla (Atlántico)	6	++
24	Rhizosphere	Barcelona (Quindío)	7	-
45	Rhizosphere	Palmira (Valle)	8	-
81	Rhizosphere	Córdoba (Quindío)	9	-
P12	-	-		+++

[a]Mycelium type; shape of culture; daily growth rate; presence of oospores, chlamydospores, zoospores, oogonia, sporangia, and antheridia; and form of sporangia.
[b]Hybridization is strong (+++) to negative (-).

The size of the amplified product for the ITS regions was approximately 900 bp for all the Colombian isolates and the *Phytophthora* reference strains, different to other fungi species. Restriction digestion of the amplified products showed different patterns. Isolates from the same geographical region presented several restriction patterns. The cluster analysis separated the isolates into several groups at a similarity index between 0.3 and 0.5 **(Fig. 1.11).** Several subgroups are observed at a similarity index of 0.7. The strains obtained from different plant tissues or soil, clustered into different subgroups. RAPD banding patterns showed polymorphism between the isolates clustered in the same restriction group.

The *Phytophthora* reference strains demonstrates that the isolates belong to the same genus with no distinction between the species tested. Based on the restriction patterns, several molecular groups are present in the Colombian fields. Not only different groups of isolates are observed in the same region, but also different regions share the same genetic groups.

Some isolates group with the identified *Phytophthora* species but some other cluster independently, suggesting the possible existence of other *Phytophthora* species affecting cassava in Colombia. This hypothesis could be evaluated by sequencing analysis of representative isolates and comparison with known sequences of *Phytophthora* species.

The majority of isolates from the southern departments Valle and Quindío, cluster together, indicating that a molecular group predominates in these neighbor regions. On the other hand, the remaining isolates from these regions are closer to all that from North of Colombia (Atlántico and Bolívar departments) suggesting a possible exchange of plant material between these places. In spite of the reduced number of strains from Cauca department, they cluster in a different molecular group. This result suggests that collection

efforts would be worthy in order to verify the distinct genetic nature of the pathogen populations predominating in Cauca.

According to the dendrogram, there is no evidence of the presence of *P. drechsleri* and *P. nicotianae* respectively in the Colombian sampled regions, contrary to earlier reports. An increased sampling could corroborate this discrepancy.

According to the existence of several groups of *Phytophthora* in Colombia and to the high level of polymorphism showed by RAPD method, further analysis and an accurate identification of species is suggested.

Eighty strains were compared in pathogenicity tests; sprouts of young plants were inoculated in two cassava cultivars (M Bra 12 and M Col 1505) under greenhouse conditions. Symptoms caused by individual strains were very similar, although the severity of symptoms differed among strains.

We could therefore determine that the *Phytophthora* fungus causes wilting and root rots in the different regions we visited; and that the inoculation method we developed, permits to estimate the pathogenic variability in isolates. Through PCR and RAPDs, we could also determine the wide genetic variability of the isolates. These results will help develop diagnostic techniques and adapt efficient control measures, like durable host resistance, to the different genotypes of this pathogen.

We evaluated 429 varieties from the core cassava collection held at CIAT for resistance to Phytophthora root rot, inoculating stems from 20-cm height cassava plants. Thirteen varieties were found to tolerate the aggressive strain P12, identified in earlier works as *Phytophthora drechsleri*. The most promising varieties will be evaluated in several root rot endemic regions.

Figure 1.11 **Phenogram of *Phytophthora* spp. from Colombia based on restriction patterns of rDNA using the UPGMA method. Development of resistant cassava varieties to Phytophthora root rot**

Subproject 2.1 Thermotherapy of Asexual Cassava Seed to Control *Phytophthora* spp. and the Use of ELISA Diagnostic Kit

Three methods of thermotherapy were evaluated for cleaning cassava stakes: hot water, water vapor, and dry heat. We selected hot water as being the most promising method, because it affects stake germination less than the other two techniques **(Fig. 1.12).**

To know the temperature and time for inactivating *Phytophthora* spp., we first inoculated 5-mm discs of cassava tissue with *P. nicotianae* and *P. drechsleri* cultures that had been isolated from cassava. The inoculated discs were placed in glass tubes, which were filled with liquid V-8 medium and placed in a hot water bath. We submitted the whole to different combinations of temperature and time. The tubes were then placed in the dark for incubation for 48 h at 27° C.

We discovered that *P. nicotianae* had a lower growth rate than *P. drechsleri*, and that the combination of 50° C and 20 min was lethal to both species **(Fig. 1.13).** Encouraged by these results, we determined how long the temperature took to reach 50° C in the center of the stakes: 5 min for stakes with a diameter of 1.6 cm and 15 min for 2.5 cm. The combination of 50° C and 48 min did not show significant differences in the germination rate of 20 cultivars susceptible to *Phytophthora* spp. **(Fig. 1.14).** Although stakes can be treated at 51° C, care needs to be taken in controlling the temperature exactly.

The control of the fungus in stem cuttings was evaluated with the ELISA technique, made specific for detecting *Phytophthora* spp. (*Phytophthora* ELISA multiwell kit of E. Neogen, Inc., East Lansing, MI). The test did not find the fungus in stakes that we cleaned by the hot water method **(Table 1.5).**

Because thermotherapy is the only system that eliminates, in a fast and environmentally friendly way, *Phytophthora* present in cassava stakes, and the pathogen is unlikely to develop resistance to heat treatment, this technology should be implemented, as a matter of urgency, at research centers and programs for germplasm interchange. However, treatments will need to differ in terms of temperature and timing from region to region, according to which *Phytophthora* species are causing the rots. Ongoing research includes pre-treatment of the cassava stakes. We hope, eventually, to extend this methodology to the control of other cassava diseases.

Figure 1.12 Effect of thermotherapy on the germination rate of cassava.

Figure 1.13 Effect of temperature on the growth of *Phytophthora spp.*

Figure 1.14. Reaction of commercial cassava varieties susceptible to Phytophthora rot, to hot water treatment.

Figure 1.15. Inhibition of *Phytophthora* spp. by *Trichoderma* isolates.

Table 1.5. Presence of *Phytophthora* spp. in cassava stakes after treatment with hot water. Results are from an ELISA test, specific to *Phytophthora* spp.

Samples	Detection
Phytophthora spp., pure in vitro cultures	+
Phytophthora spp., treated at 60° C	+
Stems of inoculated plants	+
Hair roots of greenhouse plants naturally infected by *Phytophthora* spp.	+
Hair roots of field plants naturally infected by *Phytophthora* spp.	+
Stakes of field plants naturally infected by *Phytophthora* spp.	+
Treated stakes from field plants naturally infected by *Phytophthora* spp.	-

Subproject 2.2. Development of Biological Control of Rot Diseases.

Cassava

We selected 50 *Trichoderma* spp. strains from a collection of 350 isolates that originated from the rhizosphere of cassava grown in different edaphoclimatic zones of Latin America. The isolates were evaluated for their virulence against the *Phytophthora* fungus. We used two methods; in the first, we placed the antagonist directly with *P. nicotianae* in culture plates. Thirteen of the *Trichoderma* isolates constrained *P. nicotianae* to a zone no larger than ten mm in diameter, and colonized more than 75% of the culture.

The same 50 strains were also evaluated in the greenhouse for their control of *P. nicotianae*. Rooted stem cuttings were inoculated with both *P. nicotianae* and *Trichoderma* spp. by application to the soil. Four *Trichoderma* isolates showed significant differences in incidence, but high variability of results occurred in replications.

We did not find a correlation between the in vitro and greenhouse results. We therefore developed a second in vitro method, using *Trichoderma* spp. filtrates that were free of fungal structures. We evaluated the effect of 40 of the same strains on *P. drechsleri*, *P. nicotianae,* and other *Phytophthora* spp. Suspensions of *Trichoderma* isolates, grown in liquid Richard medium, were prepared and mixed with potato-dextrose agar to obtain a medium carrying toxins of the biocontrol agent. The *Phytophthora* pathogens were placed in this medium. Of the 40 *Trichoderma* isolates tested, the strains 12PDA-4, 14PDA-4, and 41PDA-3 inhibited the growth of *P. drechsleri* and other *Phytophthora* spp. in 80% **(Fig. 1.15)**. The strain 14PDA-4 was effective in greenhouse trials.

Subproject 2.3 Development of Biological Control of the Causal Agent of Bud Rot of Oil Palm

Collection of antagonistic microorganisms

Fifty-four *Bacillus* spp., 30 *Pseudomonas* spp. and 14 *Trichoderma* spp. strains were isolated from three oil palm plantations located at Los Llanos Orientales in April 1997. Six bacterial strains of the University of Auburn (Alabama) were included in the experiments.

In vitro selection of antagonists

Ten *Trichoderma* isolates were used to identify their capacity to produce metabolites that inhibit the growth of four isolates of *Thielaviopsis* spp. from four different regions infected with the disease. According earlier works *Thielaviopsis paradoxa* is the causal agent of oil palm bud rot. The pathogens were transferred to PDA that contained the filtrate of the *Trichoderma* isolates cultured in Richard medium. The growth -colony diameter- of the pathogen was measured daily. Highly significant differences were observed between the isolates and the control without *Trichoderma* filtrates. In a preliminary test two isolates, were highly effective in the control of the four *Thielaviopsis* isolates. Other strains do not inhibit the pathogen at all. Experiments will be continued using the developed methodology.

Eleven *Trichoderma* isolates were tested for their capacity to control the pathogen by using the dual plating technique. The culture medium used was prepared with palm tissue, distilled water and agar. Some *Trichoderma*-pathogen combinations showed inhibition zones. In some cases *Trichoderma* was able to grow on the colony of the pathogen. Strongest inhibition was observed by the strain 14PDA-4, a promissory strain to control cassava Phytophthora root rot and isolated from cassava. The applied methodology is promising to be effective to differentiate *Trichoderma* strains on their capacity to control *Thielaviopsis* in vitro; by greenhouse and field experiments the most efficient strains will be evaluated.

Ten isolates of *Bacillus*, *Pseudomonas*, *Serratia* and others were used to evaluate their effect on the growth of the same four pathogenic strains. Isolates were transferred to a culture medium prepared from a soil extract (soil, calcium carbonate, agar and distilled water) and nutrient agar medium between two colonies of the pathogen. Growth of the pathogen was recorded daily. Two *Bacillus subtilis* isolates and one *Bacillus pumilus* inhibited E 103B, a pathogenic strain of *Thielaviopsis*. Not one isolate was able to inhibit the growth of all four pathogenic strains. Because of the physical properties of the soil extract medium it was difficult to observe the inhibition of the growth caused by the bacterial isolates. New experiments are ongoing using sterile filter paper discs saturated by the bacterial suspensions placed on PDA and other isolates will be tested.

Growth room trials to induce resistance to bud rot

Ten bacterial strains were tested for their ability to control *T. paradoxa* strain E 103 B in young oil palm seedlings. Three inoculation methods were used for the introduction of the bacteria in the plant. The pathogen was inoculated by perforation of the leaves one day after the inoculation of the plants with the bacteria. Significant differences in control of the pathogen with several bacterial strains were observed. Differences were observed between inoculation methods, bacterial isolates, and type of leaf inoculated. Greenhouse trials will be continued to evaluate the consistency of the results.

Subproject 2.4. Cassava Germplasm Identified for Resistance to SED

Introduction

Super-elongation disease (SED) in cassava, caused by the fungus *Sphaceloma manihoticola*, is considered a major disease because it significantly reduces harvests and disperses easily by wind, water, and planting material. Earlier studies demonstrated variability in the morphology of the pathogen in culture, but little is known about its genetic structure. The objective of this study was to characterize isolates originating from two edaphoclimatic zones in Colombia. The methods used were colony morphology, pathogenicity, and molecular techniques.

Thirty-three strains of *Sphaceloma manihoticola* were obtained from infected leaves, stems, and petioles of cassava from different regions of Colombia. They were then characterized by their morphology, growth and pathogenicity, and by site-directed (rDNA) PCR and RAPD. Growth area and color of colony were not stable parameters over time, and thus unsuitable for distinguishing strains. Spore size showed no significant differences, averaging at 5.8 x 3.0 μm. Amplification of the ITS region of the rDNA was obtained with template DNA from all strains. The product amplified for the ITS region was 650 bp, and the amplified product showed one single restriction pattern for all strains tested when digested with *Msp*I enzyme. Thus, the strains were all from one specie. Twenty-five, random, 10-mer primers (Operon Technologies, Inc.) were tested, and OPA-03, OPA-04, OPA-09, OPA-10, OPA-18, and N18 gave reproducible bands, indicating genetic variability among strains. Polymorphism with OPA-03, OPA-18, and N18 single primers differentiated the strains. Despite the morphological and pathogenic variability of the isolates, molecular diversity is low, thus facilitating the task of selecting cassava germplasm resistant to SED.

Pathogenicity

Inoculations were conducted in a growth room with 31 strains of *S. manihoticola* isolated from different cassava fields in Colombia affected by SED, inoculated on varieties M COL 22 y M VEN 77. Evaluations were made 15 days after inoculation, using a scale from 1 to 5. Varieties had intermediate reaction to most of the isolates, although high variability between strains and high significant differences between isolates were observed. Host pathogen interaction was found. Using these strains, no correlation was found between molecular groups and pathogenicity.

Field experiments

345 genotypes were evaluated at Villavicencio and 387 at Carimagua, corresponding to various crop cycles. 181 were evaluated during five years. In 1997, 187 varieties (crop cycle five) were evaluated in field experiments at Carimagua and Villavicencio. Three evaluations were performed to determine disease severity, which was measured on a scale of 1-5. Each variety was planted in a randomized complete block design: three replications with five plants per plot. 135 varieties were resistant to SED during five growth cycles. Of this group 51 varieties were also resistant to CBB.

Subproject 2.5. Genetic Stocks

750 cassava varieties have been established in the field for Phytophthora root rot, CBB and SED projects.

Collection of microbial strains assembled and made available to CIAT partners: 100 strains of *Phytophthora* spp. and *Pythium* spp., 320 strains of *Trichoderma* spp., 85 strains of *Bacillus* spp. and *Pseudomonas* spp., 86 strains of *Fusarium* spp., 31 strains of *Sphaceloma manihoticola* and 55 strains of *Diplodia manihotis*.

Subproject 3. dentification and Characterization of Major Viruses: Development of Rapid Detection Methods

Subproject 3.1. CVMV yield loss field trials

One of the important questions regarding Cassava Vein Mosaic Virus (CVMV) is the effect on the yield. Since the absence of symptoms is not a reliable indicator that the cassava clone is free of CVMV, *in vitro* plants were used for this yield trial experiment. The plants were grown in the EMBRAPA station near Petrolina Pernanbuco. A randomized block design was used. The yield was determined and the roots were analyzed for starch content. The yields of the infected plants averaged 10% less than the healthy controls. The starch content of the roots from infected plants was 20% less than that of the healthy controls. By combining the decrease in yield and starch content, the losses attributed to CVMV are more than 25%. This disease is endemic over most of the northeast of Brazil and the incidence in the field is often very high, therefore it appears that the economic importance is much higher than previous estimates.

Subproject 3.2 Molecular marker and characterization of cassava frogskin disease (CFSD)

The objective was to isolate a part of the genome of CFSV and to develop a molecular marker for CFSD. First total RNA was isolated and converted into DNA using random primers and amplified using specific primers of ten oligonucleotides. A total of 220 combinations of oligonucleotides from Operon Inc. were used to amplify the DNA products of the reverse transcriptase reaction. The PCR products from healthy secundina and CFSD cassava were compared and those bands that were only in the diseased plants were selected for further study.

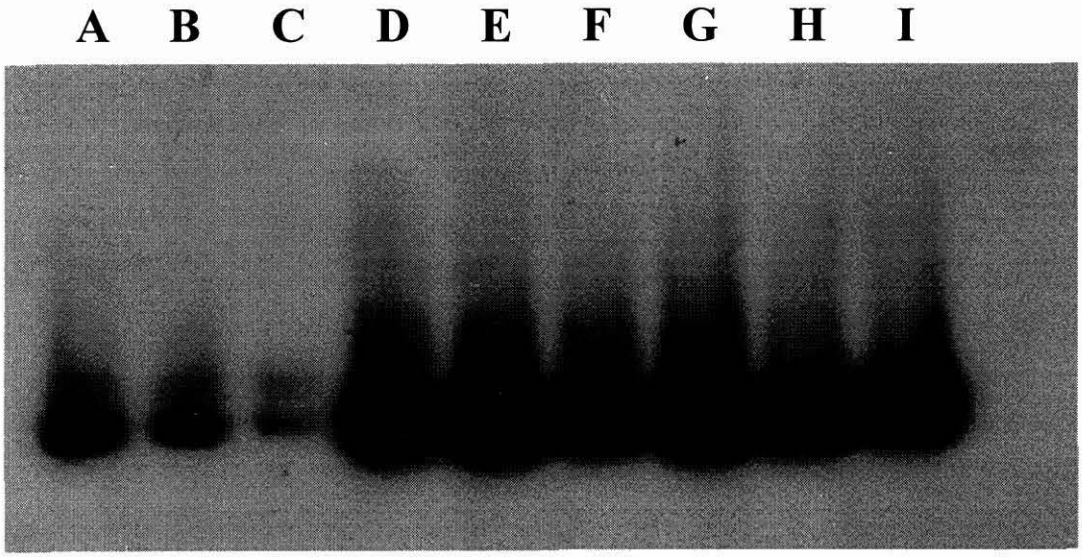
The amplified product from one set of primers was cloned and sequenced. It was a complex clone and it contained a region with a moderated degree of similarity with rice ragged stunt reovirus (RRSV) RNA 5 and the encoded minor structural protein (MSP). This clone has a 332 nucleotide region with 57% nucleic acid identity and 62% amino acid similarity compared to the RRSV RNA5 and the MSP. Adjacent to this region is the sequence of a CTTT/GAAA micro-satellite. The clone also has a small region of DNA homology with a heat shock (Hsp70) protein. A deletion was made using the Hinc II-Cla I sites. This clone

will be referred to as the FSD-Cla. Oligonucleotide primers were designed that would amplify a 258 base fragment within the CFSD-Cla clone.

The clone was analyzed for specificity hybridization with three isolates of CFSD. The controls were secundina, M Peru 83, and a passionfruit plant infected with a potyvirus. Total RNA was purified and amplified using the specific oligonucleotide primers. The PCR products were run on agarose gels, transferred to nitrocellulose filters, and hybridized with the CFSD-Cla insert at high strigency. The probe reacted with all of the samples but at very different levels of intensity **(Fig. 1.16)**. The intensity of the band is related to level of PCR product amplified and to the degree of homology with the CFSD-Cla hybridization probe. The most surprising result was that the passion fruit infected with a potyvirus reacted with the cDNA probe. There is slightly a stronger reaction than the healthy cassava controls. The reaction in all the cassava samples infected with CFSD are much more intense.

Since there were specific PCR products in the healthy controls and even in the passion fruit, it was suspected that the clone CFSD-Cla represented a plant gene. To confirm this, *in vitro* plants of Arg 2, Nig 9 and Secundina were used in the following cloning experiment. The temperature of annealing was lower to 42C to optimize the production of PCR products. The predicted size PCR products were amplified from Arg2, Nig 9 and Secundina. The PCR products from Nig 9 were cloned and sequenced. The sequence of the cDNA clone Nig-PR was identical to the clone CFSD-Cla. This confirmed that the cDNA clone CFSD-Cla represents a plant gene and that it is highly conserved.

It appears that the partially cloned gene has higher expression levels in FSD infected cassava as compared to healthy plants. The reaction in passion fruit suggests that this gene is common in plants and may indicate that it is a pathogen related protein. Further studies are ongoing to test if the differential level of expression is a practical detection method.



A: Passion fruit infected with a potyvirus, All other are cassava B-F samples from leaves, G-I samples from green stems. B: MPer 83, C: Secundina, D: CFSD 5, E: CFSD 80, F: CFSD 86, G: CFSD 5, H: CFSD 80, I: CFSD 86.

Figure 1.16. Autoradiograph of total RNA that was amplified by reverse transcriptase-PCR using specific primers designed from the sequence of the clone CFSD-Cla. The clone CFSD-Cla was used as the hybridization probe.

Subproject 3.3. Analysis the of the signs of CFSD

Cytological studies of CFSD affected roots and leaves were made using both light and electron microscope. The characteristic fissures on the surface of the roots are caused by a hyperplasia of the cells in the root cortex. The root cortex beomes thicker and starch granules are fewer than in normal roots. This pattern of root symptoms is typical of CFSD. Less common are the leaf symptoms. In some varieties, there are severe mosaic symptoms on the leaves. Since CFSD does not cause leaf symptoms in most varieties, it was at first thought that the leaf mosaic was caused by another disease. Graph transmission studies have demonstrated that the mosaic symptom is associated with the variety but not the source of inoculum. This demonstrates that leaf and root symptoms are caused by the same disease. In all cases, the root symptom expression is affected by temperature. Higher temperatures tend to inhibit the roots symptoms, but it is not known if the soil or air temperature is more important. The inhibition of symptoms causes problems in the selection of clean planting material. In developing practical control strategies, understanding the differential expression of symptoms is essential. The vector transmission of CFSD is somewhat inefficient and the primary spread is caused by propagation of infected stem cuttings. One challenge is to develop a system of rapid propagation using CFSD-free cassava and linking this with the

farmer's intervention to rogue out infected plants. Previous epidemiological trials suggest this can minimize losses caused by CFSD for 4-8 years. In those areas with endemic CFSD, these activities can be part of an IPM and transfer by farmers' participatory methods.

Subproject 3.4. Identification of sources of resistance or tolerance to CFSD

During 1995, a field experiment was begun to analyze the 630 accession of the cassava core collection for tolerance or resistance to CFSD. A single source of CFSD was used to inoculate the most accessions of the core collection. The screening of root symptoms is done in the field using a simple classification of severe, light and no symptoms. To date 459 of the core collection accession are in the field trial. A total of 139 of these accessions were identified as having severe symptoms and were eliminated from further evaluation. 124 accession were determined to have mild or no symptoms and were replanted for further evaluation. Another 196 plants are in the first year of evaluation and these will be analyzed in the first semester of 1998. There are 171 accession that need to be entered into this resistance trial. Because of the nature of CFSD and the long growth cycle of cassava, this is a long-term experiment that will take several more years to complete. The elimination of 22% of the accessions is progress in selecting the most tolerant cassava accessions. As the sample number becomes smaller, more intensive characteristics including yield and starch accumulation will be analyzed and compared with CFSD free accessions.

Subproject: 3.5. Identification and quantification of major arthropod complexes in selected agro ecosystems

Acarology

The principal objectives of the acarology unit are to find natural enemies of the cassava green mite (CGM, *Mononychellus tanajoa*), evaluate them for safety and suitability as biological control agents, and send them to EMBRAPA in Brazil and IITA in Benin for release. This pest is native to South America; however, it was accidentally introduced into Africa in the early 1970s, and it quickly spread across the continent causing root yield losses of 30-80%. Since there are few natural enemies that attack the pest in Africa, we have been searching for predators and pathogens to release there to reestablish the natural equilibrium that controls the pest throughout most of Latin America. Mites are also an important pest in dry regions of Latin America such as in semiarid region of Northeast Brazil, where it causes losses of about 25%.

Subproject 4.1. Selection of phytoseiids for control of cassava green mite in dry and high altitude regions

Three predatory mites from Brazil are now established in Africa (*Neoseiulus idaeus*, *Typhlodromalus manihoti* and *Typhlodromalus aripo*). The latter two are reducing the pest population by 30-80%. *T. aripo* has spread 200 km from initial release sites in southern Benin in 2 years. Predator exclusion experiments by IITA indicate that *T. aripo* reduces the pest population by 90% and increases yield by 30%, which produces an additional income of about US$60 per ha. *T. aripo* is now established in 11 countries in Africa; however, it does not appear to be very successful in drier regions, such as the sahel, and in higher altitude/subtropical regions, such as Zambia. Because of these developments we are now focusing our explorations on two climatic regions: 1) hot semiarid (corresponding to the sahel and interior Northeast Brazil) and 2) high altitude/subtropical (corresponding to the East African plateau). Target release sites in Zambia were analyzed using principal component analysis to map regions in Latin America with similar climatic patterns **(Fig. 1.17)**. The climatic data included long-term monthly averages for each of the 12 months for daily mean temperature, daily temperature range, and precipitation. This identified target regions to explore in Minas Gerais, Brazil. Collaborators from Brazil and Zambia began to make collections in this region. The other target region is in southwestern Mexico, but we have not been able to collect in this region for lack of financial resources.

Figure 1.17. Regions in Latin America that have similar climatic patterns to target sites in Zambia that were identified using geographical information systems (GIS).

We continued to establish fresh phytoseiid cultures and maintain them for experimental evaluation. Those maintained during the year are listed in **Table 1.6**

Table 1.6. Phytoseiid cultures established and maintained during the year

Species	Country	Dept./State	Municipality	Location	Collection
T. manihoti	Venezuela	Yaracuy	Marín	San Felipe	Mar-95
	Brazil	Bahia	Cruz das Almas		Feb-93
	Colombia	Cauca	Cajibio		Jun-95
		Antioquia	Barbosa	El Hoyo	Jan-96
		Antioquia	Copacabana	Montañita	Jan-96
		Magdalena	Medialuna		Feb-96
		Guajira	Villanueva		Jan-96
		Caldas	Chinchiná	San Gregorio	Ago-96
		Santander	Bucaramanga	Bijagual	Ago-96
		Santander	Bucaramanga	Los Colorados	Ago-96
		Risaralda	Sta. Rosa de Cabal	UNISARC	Ago-96
		Quindío	Armenia	Armenia	Ago-96
		Antioquia	Barbosa		Fe-97
		Antioquia	Barbosa		Fe-97
		Magdalena	Pivijay	La Colorada	Jun-97
		Valle	Palmira	CIAT	Oct-97
T aripo	Colombia	Valle	Palmira	CIAT	Sep-96
		Magdalena	Pivijay	La Colorada	Jun-97
	Brazil	Bahia	Cruz das Almas		Mar-97
T. tenuiscutus	Ecuador	Manabí	Portoviejo		Nov-94
		Manabí	Puerto Cayo	Cantagallo	Dec-95
T. limonicus	Brazil	Sao Paulo	Jaguariuna		Jun-90
N. idaeus	Ecuador	Manabí	Rocafuerte	Entrada a Danzarín	Dec-95
	Colombia	Guajira	Carretalito		Mar-97
		Cesar	La Paz		Mar-97
		Guajira	Fonseca		Feb-97
N. californicus	Ecuador	Manabí	Portoviejo		Nov-94
G. annectens	Ecuador	Manabí	Portoviejo	Crucita/La sequita	Dec-95
	Colombia	Guajira	Fonseca		Feb-97
G. helveolus	Ecuador	Manabí	Puerto Cayo	Cantagallo	Dec-95
Euseius ho	Ecuador	Manabí	Rocafuerte	Entrada a Danzarín	Dec-95
N. anonymus	Colombia	Quindio	Armenia		Aug-96

Phytoseiid species that are candidates for exportation as biological control agents were evaluated for the specificity of prey and food sources that they can consume. This is to assure that we select species that will not harm non-target hosts after being imported to Africa or Northeast Brazil. The ability to use pollen or sugary exudates of cassava, mealybugs or whiteflies is an advantage in helping to maintain predator populations when cassava green mites are scarce. *Galendromus annectens* and *G. helveolus* showed much higher longevity in the presence of tetranychid prey (*Mononychellus caribbeanae* or *Tetranychus urticae*) than for the other food sources tested (**Fig. 1.18**). *Neoseiulus idaeus* was similar except that it could also utilize castor bean pollen. *Typhlodromalus tenuiscutus*, *Euseius ho* and *T. aripo* could use a greater variety of food sources, including cassava exudate (a sugary liquid), immature whiteflies (*Aleurotrachelus socialis*) and mealybugs (*Phenacoccus herreni*) -- both of which produce sugary exudate.

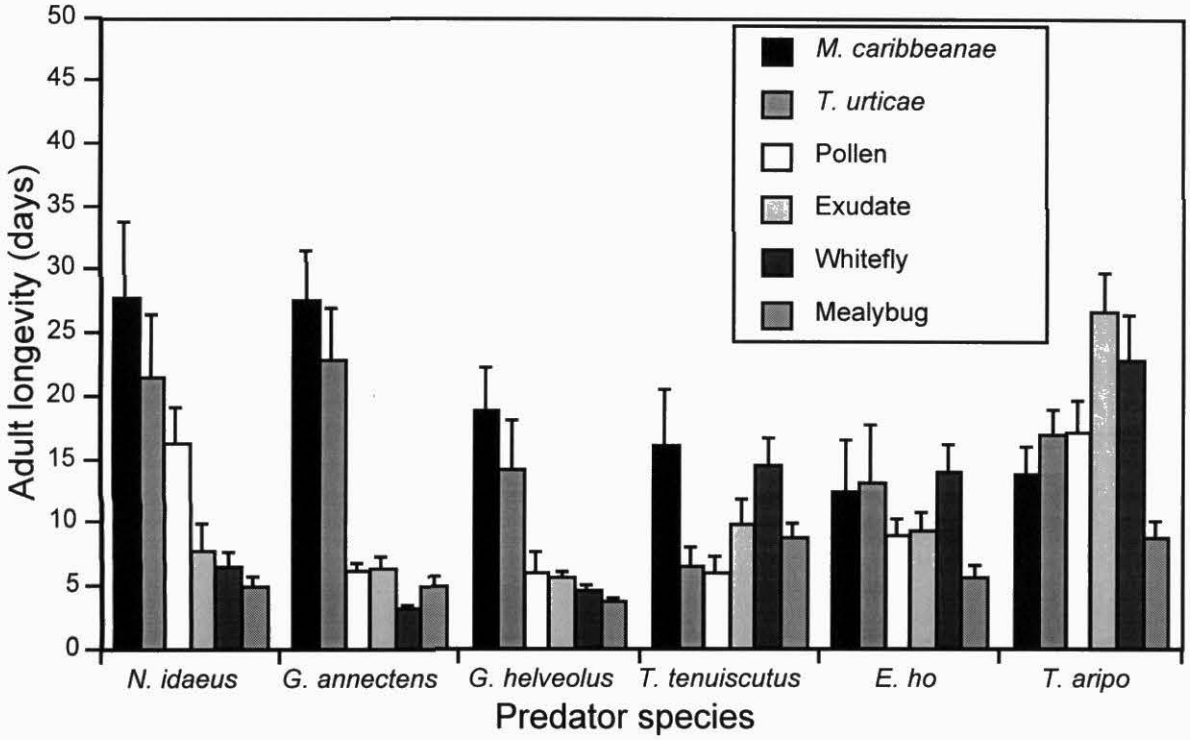


Figure 1.18. Effect of different food sources on female adult longevity of several species of predatory phytoseiid mites.

Predatory mites must to consume protein in order to produce eggs, so fecundity is an important measure of the suitability of different food sources to maintain predator populations. *Typhlodromalus tenuiscutus* showed the highest specificity laying substantial numbers of eggs only when fed *Mononychellus caribbeanae* (which is equal to the cassava green mite in preference). *Neoseiulus idaeus*, *Galendromus annectens* and *G. helveolus* also attacked the two-spotted spider mite, *Tetranychus urticae* **(Fig. 1.19)**. *Euseius ho* was the only species of this group that could produce many eggs when fed only castor bean pollen. *Typhlodromalus tenuiscutus* and *Euseius ho* could also reproduce at low rates when held on cassava leaves infested with various developmental stages of immature whiteflies (*Aleurotrachelus socialis*).

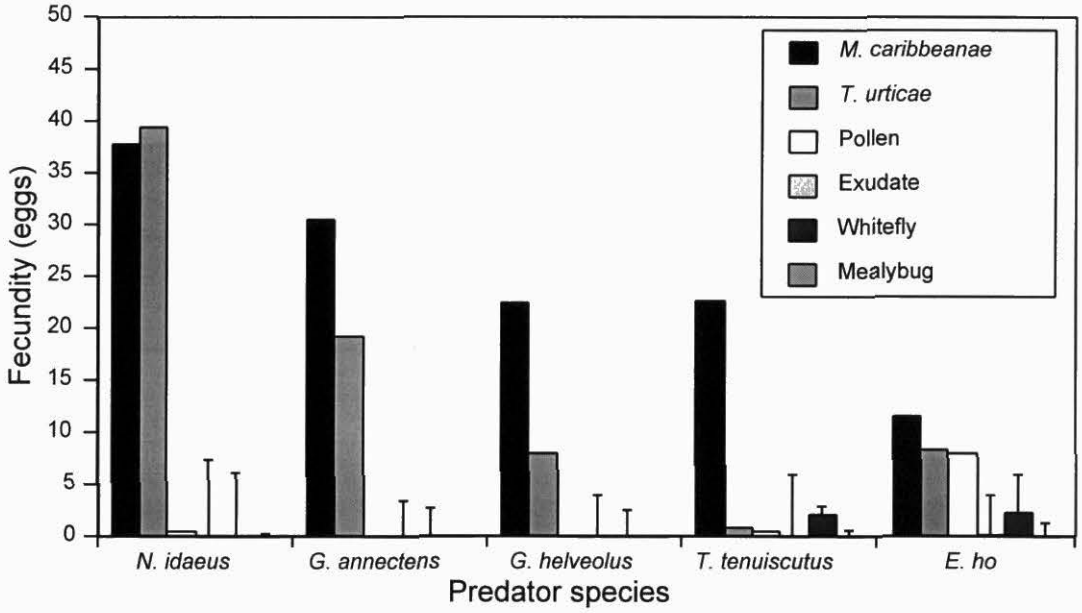


Figure 1.19. Effect of different food sources on fecundity of several species of predatory phytoseiid mites.

Exportations of natural enemies during 1997 included *Galendromus annectens* from a dry location in coastal Ecuador (Portoviejo, Manabí) which was sent to CNPMF/EMBRAPA, Cruz das Almas, Bahia via quarantine at CNPMA, Jaguariúna, São Paulo Brazil **(Table 1.7)**. *Typhlodromalus manihoti* from several high altitude sites in Colombia (Bucaramanga, Armenia & Santa Rosa de Cabal) was sent to IITA, Benin via quarantine at the University of Amsterdam (Mitox). *Neoseiulus idaeus* from Fonseca, Colombia was sent to Amsterdam for evaluation as a predator of *T. urticae* in greenhouses because of its known association with hot dry regions.

Table 1.7. Recent natural enemy exportations.

Date shipped	Species	Source	Start of colony	Number sent	Number received	Destination
12/6/96	*T. manihoti*	Los Colorados, Bucaramanga, Colombia	8/96	480	ca. 60%	Amsterdam/ Benin
"	"	Armenia, Colombia	8/96	300	ca. 60%	Amsterdam/ Benin
"	"	Chinchina, Colombia	8/96	120	ca. 60%	Amsterdam/ Benin
"	"	Santa Rosa de Cabal, Colombia	8/96	180	ca. 60%	Amsterdam/ Benin
"	"	Bijagual, Bucaramanga, Colombia	8/96	180	ca. 60%	Amsterdam/ Benin
3/11/97	*G. annectens*	Portoviejo, Ecuador	12/95	1,995	800?/ 357	CNPMA/ CNPMF
4/17/97	*N. idaeus*	Fonseca, Colombia	2/97	863	512+eggs	Amsterdam
	Neozygites cf. *floridana*	*M. tanajoa*, CIAT, *M. tanajoa*, Benin				CNPMA/ CNPMF

We analysed our database of phytoseiids collected on cassava using a subset of sites in Colombia, Venezuela and Ecuador, representing 1728 sample sites, to determine if any of the more common species had strong associations with either high elevation or dry climate. Both these criteria are important in regard to our two priority ecological zones for release in Africa and Northeast Brazil. Geographic coordinates associated with each collection site were used to obtain approximate values for number of dry months per year (a month with <60 mm precipatation) from a long-term climate GIS database. Elevation was classified in four levels and dryness in three levels **(Table 1.8)**. The data are reported as incidence (proportion of samples from a given classification cell in which the species is present) to adjust for uneven number of samples in each cell. The results indicate that *Typhlodromalus manihoti* is by far the most cosmopolitan species and is commonly found at all elevations and all durations of dry months. Species such as *Neoseiulus anonymus* are more restricted to moderate levels of elevation (800-1600 m) and dry months (<5). *Galendromus annectens* and *Euseius ho* showed a relatively high association with dry low elevation, in relation to its incidence at the other climatic classifications.

Table 1.8. Distribution of phytoseiid species with respect to elevation and number of dry months based on long-term climatic data associated with collection sites in Colombia, Venezuela and Ecuador.

No. of dry months	Elevation above sea level (meters)			
Typhlodromalus manihoti				
	< 800	800-1200	1200-1600	>1600
< 3	0.54	0.25	0.25	0.29
3-5	0.35	0.25	0.25	0.23
> 5	0.24	.	.	.
Neoseiulus anonymus				
	< 800	800-1200	1200-1600	>1600
< 3	0.05	0.15	0.15	0.07
3-5	0.06	0.13	0.13	0.06
> 5	0.07	.	.	.
Galendromus annectens				
	< 800	800-1200	1200-1600	>1600
< 3	0.02	0.10	0.10	0.03
3-5	0.04	0.05	0.05	.
> 5	0.09	.	.	.
Typhlodromalus aripo				
	< 800	800-1200	1200-1600	>1600
< 3	0.03	0.06	0.06	0.08
3-5	0.05	0.05	0.05	0.06
> 5	0.05	.	.	.
Galendromus helveolus				
	< 800	800-1200	1200-1600	>1600
< 3	.	0.10	0.10	0.08
3-5	0.03	0.08	0.08	0.03
> 5	0.05	.	.	.
Neoseiulus idaeus				
	< 800	800-1200	1200-1600	>1600
< 3	.	.	.	.
3-5	0.04	.	.	.
> 5	0.01	.	.	.
Euseius ho				
	< 800	800-1200	1200-1600	>1600
< 3	0.04	0.01	0.01	0.05
3-5	0.01	0.03	0.03	0.03
> 5	0.16	.	.	.

TOTAL SAMPLES	Number of sites in each classification cell				
	< 800	800-1200	1200-1600	>1600	Total
< 3	111	410	86	32	639
3-5	728	75	35	4	842
> 5	245	0	1	1	247
Total	1084	485	122	37	1728

Subproject 4.2. Improved methods for mass-rearing and release

Investigations were conducted to develop a stage-structured computer simulation model **(Fig. 1.20)** to help optimize and facilitate the management of the mass-production of phytoseiids. Data on life history, rate of stage-specific prey consumption, and intraspecific competition were collected using the phytoseiid predator *T. tenuiscutus* to provide inputs for the model. Data collected from mass-rearing cultures were used to "validate" the model to see if it would correctly predict changes in the phytoseiid population **(Fig. 1.21).** Discrepancies between predicted population sizes and the observed sizes could be corrected by adding cannibalism to the model. This indicates that additional experimental information is needed on the rate of cannibalism.

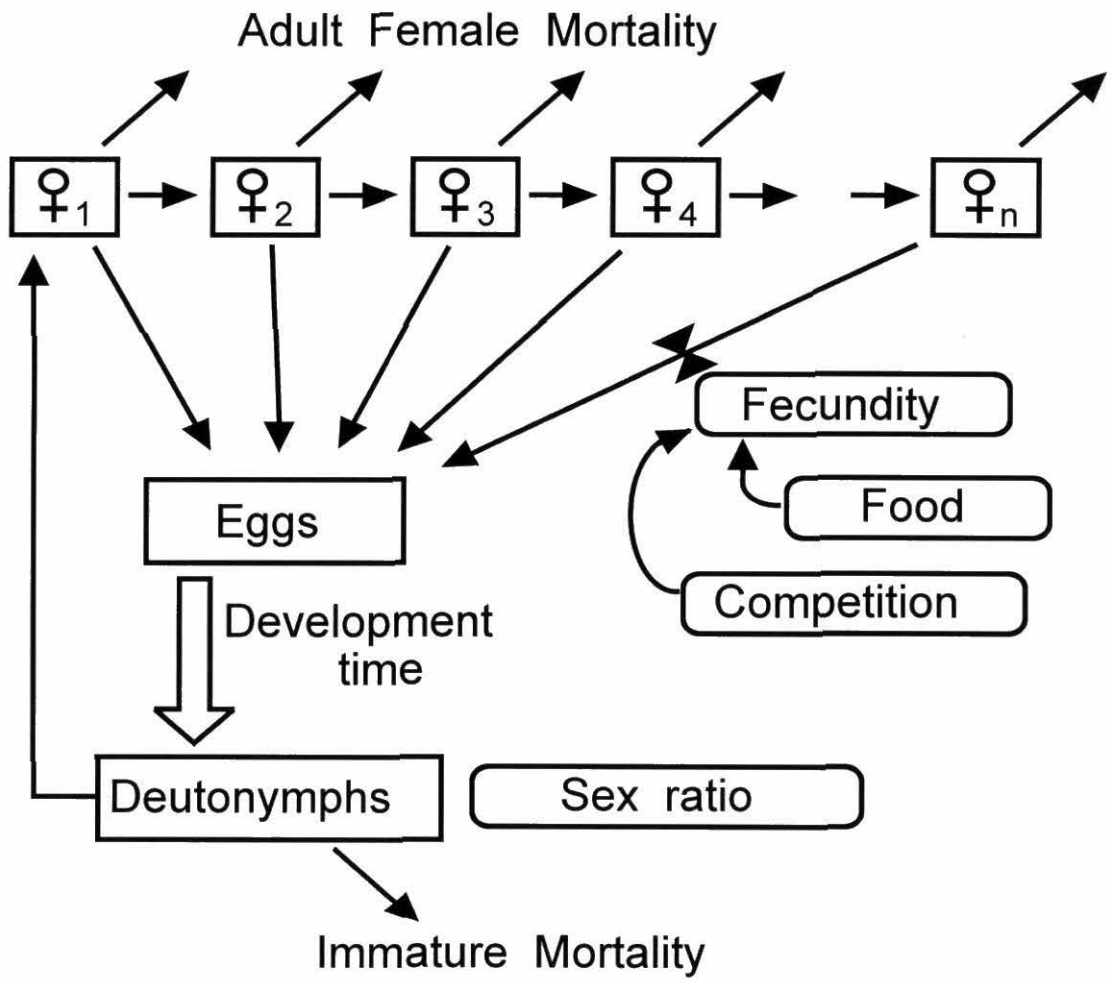


Figure 1.20. Structure of the computer model to simulate population growth and prey consumption of a phytoseiid predator.

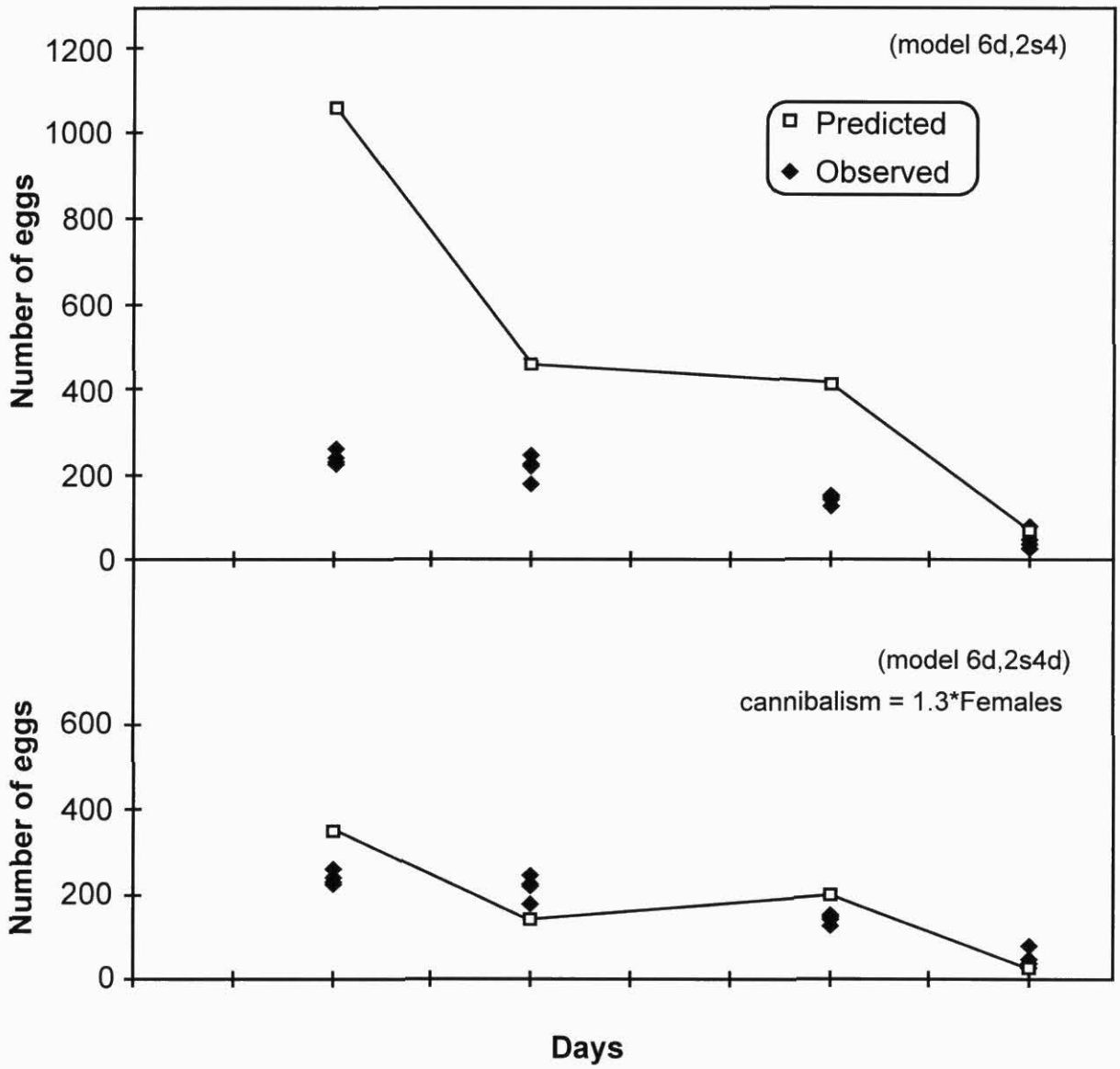


Figure 1.21. Effect of including cannibalism of eggs by phytoseiid adults to impove the fit of the simulation model to experimental data.

Subproject 4.3. Taxonomic documentation of phytoseiids and tetranychids

A taxonomic key to 30 phytoseiid species commonly found on cassava in northern South America was further developed in collaboration with Gilberto de Moraes (ESALQ/ Universidade de São Paulo, Piracicaba, Brazil). Work on description of new species also continued under the same collaboration. Phytoseiids collected in the field during explorations and experiments were identified and curated. The taxonomic collection of tetranychid and phytoseiid mites has been reviewed to verify old identifications and correct the computer database. AFLP analysis of genetic variation showed that geographic strains of *N. idaeus* were very homogeneous whereas those of *T. manihoti* differed greatly. Genetic crosses are being made between field strains of *T. manihoti* to determine if there is any evidence of reproductive incompatibility which may reveal the presence of subspecies.

Subproject 4.4. Biological control by the fungus *Neozygites*

Neozygites

Neozygites cf. *floridana* is a fungal pathogen (Zygomycetes: Entomophthorales) that causes periodic outbreaks in Colombia and Northeast Brazil that kill up to 100% of cassava green mites in one-to-two weeks. This pathogen has also been found in Africa, but it has never been observed to cause such dramatic control of cassava green mite populations. Therefore, we are collaborating with CNPMF, and IITA to find and characterize isolates to introduce into Africa.

We evaluated 5 strains of this pathogen to test them for host specificity and virulence on 3 species of tetranychid mites: *Mononychellus tanajoa*, *M. caribbeanae* and *Tetranychus urticae* **(Fig. 1.22).** The experiment was conducted on cassava leaf disks (variety CMC-40) 1.7 cm in diameter held on a saturated sponge. A mummified mite placed in the center of each disk was held in darkness for 16 hours at 24°C and 90% RH to stimulate sporulation. 25 healthy newly emerged female mites were placed on each leaf disk to be exposed to the secondary (infective) conidia (24°C, 65% RH, 12 hour photophase). The mites were examined daily for signs of morbidity and mortality. None of the 3 strains originating from *M. tanajoa* infected *T. urticae*, and one of the strains from *T. urticae* failed to infect *M. tanajoa* indicating a high level of host specificity. All 5 strains infected *M. caribbeanae*, and only the African strain isolated from *T. urticae* failed to infect *M. tanajoa*. Specimens were sent to Siegfried Keller (Swiss Federal Res. Station for Agronomy, Zurich, Switzerland) a taxonomic specialist for this group of fungi, who considered them to belong to the same species,*Neozygites* cf. *floridana* .

Because the taxonomy of this genus is not well known, and because we would like to be able to distinguish between the African strain and the strain we would like to release, we are conducting DNA analysis. It has been necessary to rear the fungus in *in vitro* tissue culture to obtain uncontaminated DNA for analysis. We improved *in vitro* methods previously developed by Luis Leite and Donald Roberts (Boyce Thompson Institute, Ithaca, NY) by adding antibiotics to control bacterial contamination **(Fig. 1.23)**. We have also tested some modifications of the tissue culture media to improve multiplication of the fungal hyphal bodies. Adding fetal bovine serum to the standard tissue culture media (TNMFH + lactalbumin + yeastolate) generally improved fungus growth. However, growth of strains isolated from *Mononychellus* still grow very slowly **(Fig. 1.24)**, indicating the need to continue improving culturing conditions.

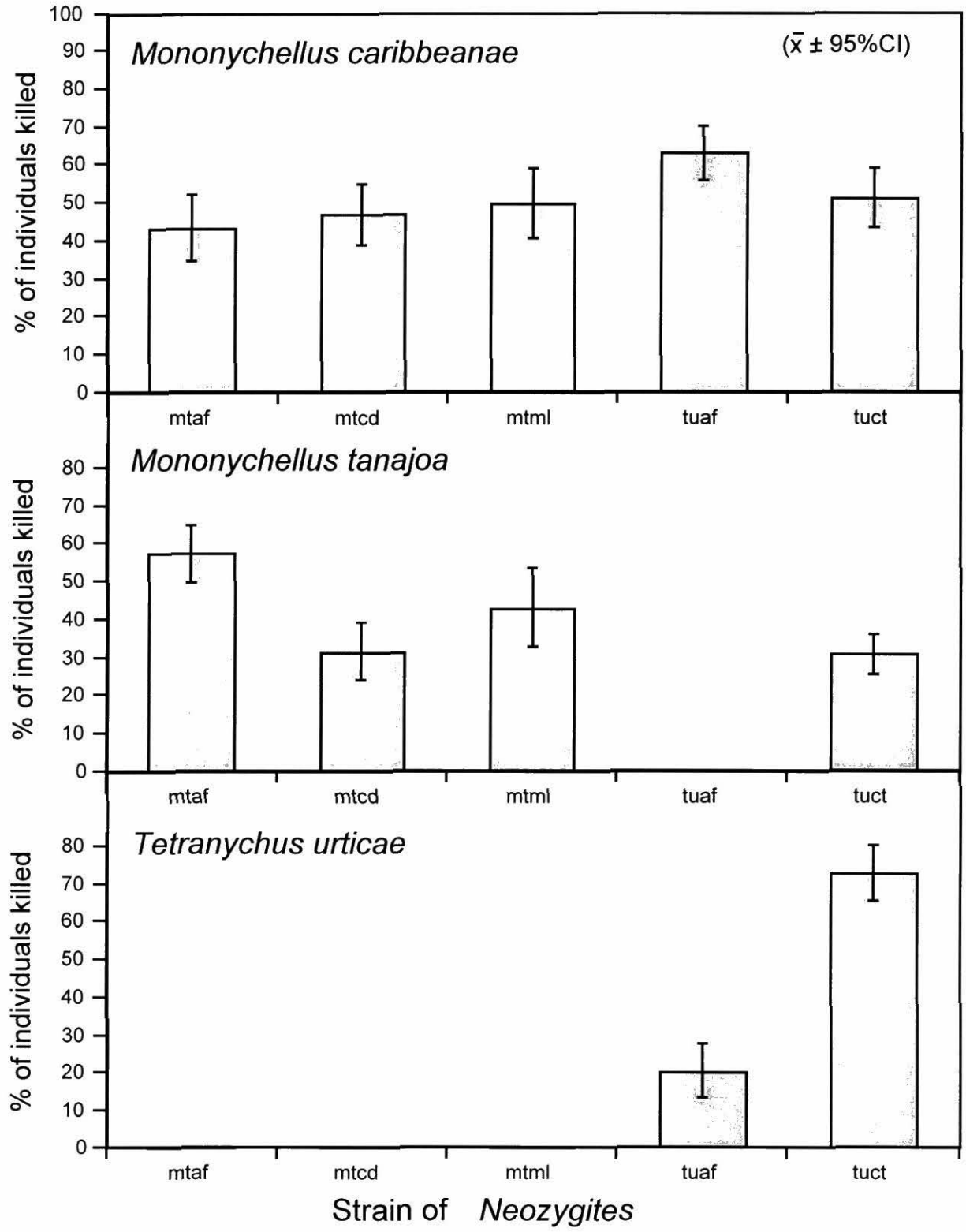


Figure 1.22. Specificity of five strains of *Neozygites* cf. *floridana* on three species of tetranychid mites (mt - isolated from *M. tanajo*, af - isolated from *T. urticae*, .

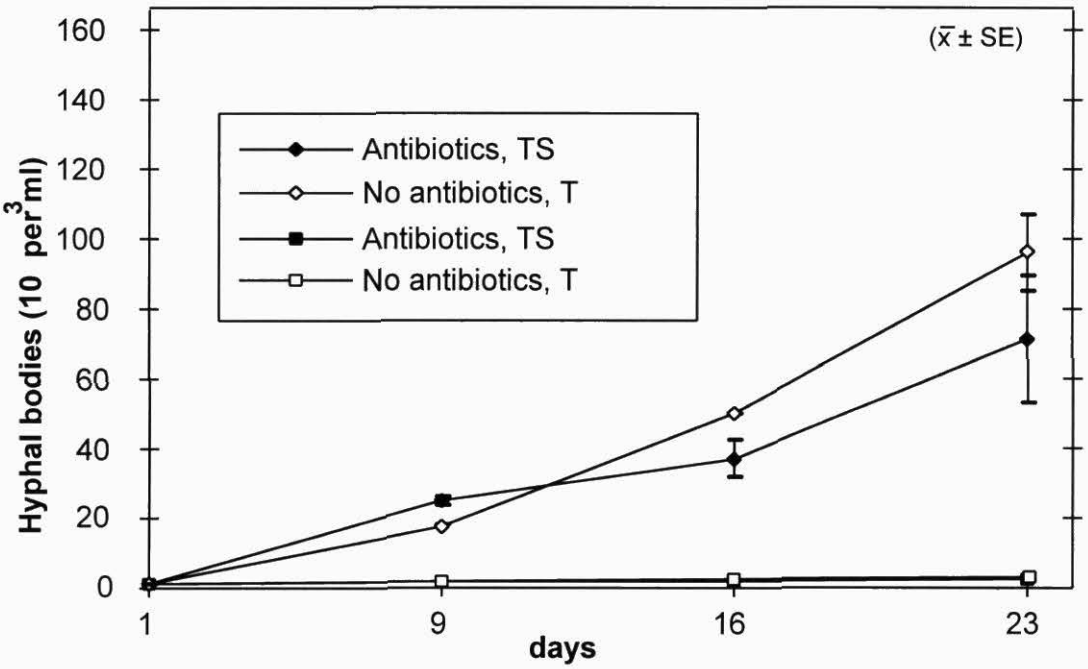


Figure 1.23. **In vitro multiplication of a *Neozygites* cf. *floridana* strain isolated from *T. urticae* (TuCIAT1) with and without antibiotics (streptomycine + tetracycline) in different tissue culture media TNMFH + lactalbumin + yeastolate (T) and TNMFH + lactalbumine + yeastolate + fetal bovine serum (TS).**

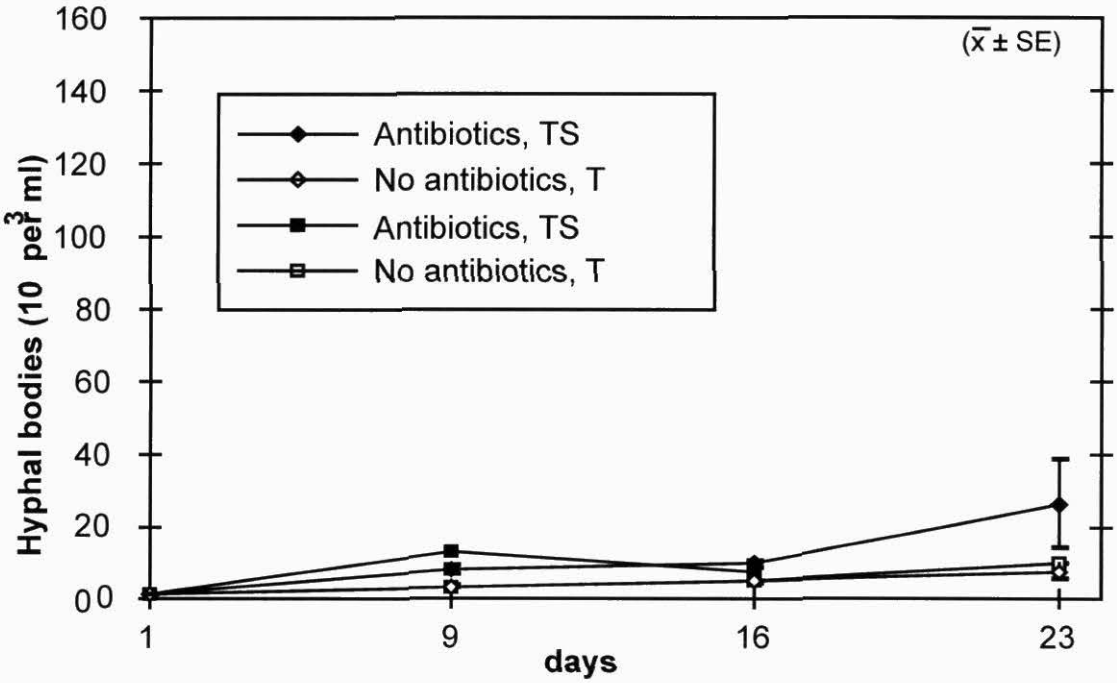


Figure 1.24. **In vitro multiplication of a *Neozygites* cf. *floridana* strain isolated from *M. tanajoa* (MtCIAT1) with and without antibiotics (streptomycine + tetracycline) in different tissue culture media TNMFH + lactalbumin + yeastolate (T) and TNMFH + lactalbumine + yeastolate + fetal bovine serum (TS).**

Methods for extraction of DNA from in vitro cultures of Neozygites have been satisfactorily developed. Despite the slow production of Neozygites strains isolated from M. tanajoa, we have been able to make some initial AFLP analyses, which indicate that the strains can be distinguished, but the technique still needs further deveolopment before genetic distances can be measured.

Subproject 4.5. Integration of Biological Control of cassava green mite with cassava varietal resistance.

We measured the responses of several life history parameters of the cassava green mite, *Mononychellus tanajoa* (Bondar), on three varieties of cassava, previously classified as resistant (MEcu-72), tolerant (MBra-12) and susceptible (CM3306-4). Mites initially isolated from a cassava variety even more conducive to rearing *M. tanajoa* (CMC-40) were reared for two generations on excised lobes of the three cassava varieties. In the parental generation, intrinsic rate of increase (r_m) was lowest on MBra-12 and MEcu-72 **(Fig. 1.25).** In the next generation, r_m decreased relative to the parents on both CM3306-4 and MBra-12, suggesting that some resistant mechanisms may have a residual action, affecting the second generation more strongly. The mites had longer development times and lower F1 immature survival on MBra-12 and MEcu-72 than on CM3306-4 **(Table 1.9)**. Sex ratio was lowest on MBra-12, and MEcu-72 and CM3306-4 did not differ significantly. Fecundity of the parental generation was lower on both MBra-12 and MEcu-72, but it rebounded in the F1 generation of MEcu-72, whereas it continued to stay low on MBra-12. This suggests that after one generation the mites were able to adapt to the resistance mechanism in MEcu-72 that caused reduced fecundity in the parental generation. The fact that MEcu-72 has the highest trichome density, while MBra-12 has the highest overall resistance, suggests that trichomes are not the only resistance mechanism of importance. The differential responses of the mite life history characters suggest the existence of least two resistance mechanisms.

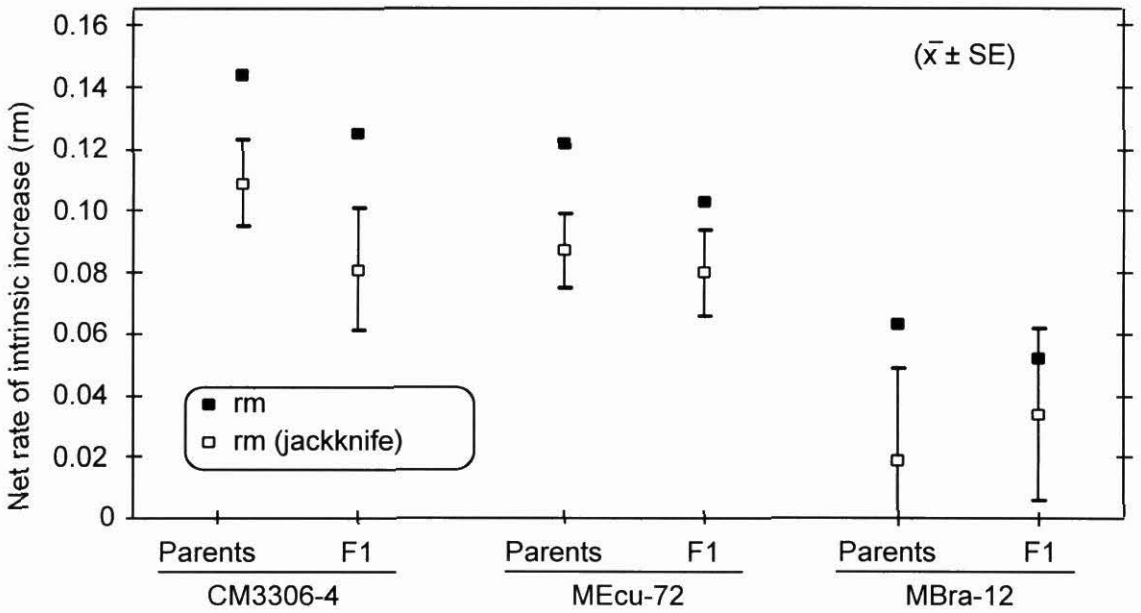


Figure 1.25. Intrinsic rate of natural increase (r_m) of *M. tanajoa* on three varieties of cassava. Jackknife estimates of r_m are low because of skewness in frequency distribution of fecundity, but 95% CI error bars indicate level of precision.

Table 1.9. Population parameters of *M. tanajoa* on three varieties of cassava (CM3306-4, "susceptible"; MBra-12, "tolerant"; MEcu-72, "resistant").

Population parameters	Cassava varieties					
	CM3306-4		MBra-12		MEcu-72	
	Parents	Progeny	Parents	Progeny	Parents	Progeny
Lamda[1]	1.18	1.16	1.12	1.08	1.11	1.12
T[2]	17.2	17.2	18.8	18.1	18.0	18.8
R_o[3]	16.8	11.3	8.6	4.2	6.8	10.7
DT[4]	4.2	4.9	6.1	8.8	6.5	5.5
Development time[5]	12.4	11.5	13.2	12.3	13.6	12.7
SE	0.09	0.13	0.09	0.17	0.18	0.12
Fecundity[6]	22.6	17.4	16.8	13.9	13.5	21.8
SE	1.8	2.1	2.3	2.1	1.3	2.8
Immature survival (%)	90	96	81	59	83	64
Longevity[7]	9.3	9.1	9.9	8.9	8.8	9.6
SE	0.4	0.6	0.7	0.6	0.6	0.6
Sex ratio[8] (%)	82.2	68.8	63.0	50.8	63.9	76.6

1 finite rate of increase (day^{-1}), i.e. 1.18 means that the population grows at 18% per day.
2 generation time (days).
3 net reproductive rate (females/female/generation).
4 doubling time (days).
5 egg to adult emergence (days).
6 eggs per female per lifetime.
7 females, from adult emergence.
8 Percent females.

We measured the responses of several life history parameters of a phytoseiid predator, *Neoseiulus californicus*, of the cassava green mite, *Mononychellus tanajoa*, on three varieties of cassava, previously classified as resistant (MEcu-72), tolerant (MBra-12) and susceptible (CM3306-4). Development time was significantly longer for the parental than for the F1 generation as a main effect (ANOVA; $F(1, 244) = 17.8$, $P = 0.0001$; **Table 1.10).** The reduction in development time suggested that the mites were adapting to each of the three cassava varieties after the first generation. Development time was longest on MEcu-72 (7.6 ± 0.1 [SE] d), intermediate on MBra-12 (7.1 ± 0.1 d) and shortest on CM3306-4 (6.72 ± 0.08 d) (ANOVA; $F(2, 244) = 20.6$, $P = 0.0001$; Fisher's protected LSD, alpha = 0.05). Immature survival was higher on CM3306-4 (98.0%) and MEcu-72 (81.6%) than on MBra-12 (44.8%) in the parental generation (Chi-square tests, alpha = 0.05). However there was no difference among the three varieties in the F1 generation, suggesting a possible delayed effect of CM3306-4 and MEcu-72 on reducing immature survival. Low survival rates for the second generation could plausibly have been caused by some change in experimental conditions, but we do not know what this could have been. There was no difference in sex ratio between generations or between cassava varieties (Chi-square tests, alpha = 0.05). Longevity of adult females was not affected by generation (ANOVA; $F(1, 144) = 0.9$, $P > 0.3$) nor cassava variety (ANOVA; $F(2, 144) = 1.5$, $P > 0.2$), nor was there a significant generation x variety interaction (ANOVA; $F(2, 144) = 0.1$, $P > 0.8$. Lifetime fecundity did not differ between the two generations on any of the three cassava

varieties (ANOVA; $F(1, 144) = 0.2$, $P > 0.6$), suggesting no adaptation to any of these varieties between the two generations. Fecundity on MBra-12 was significantly lower (7.4 ± 0.9 [SE] eggs) than on CM3306-4 (16.1 ± 1.1) or MEcu-72 (15.1 ± 1.4) (ANOVA; $F(2, 144) = 12.1$, $P = 0.0001$; Fisher's protected LSD, alpha = 0.05). Peak daily fecundity was similar on all three cassava varieties, but oviposition rate decreased rapidly with increasing maternal age on MBra-12. Although lifetime fecundity did not differ between CM3306-4 and MEcu-72, peak fecundity was delayed by 2-to-3 days on the latter. Preoviposition period was shortest on MBra-12 for both the parental and F1 generations (2-way ANOVA; variety: $F(2, 144) = 22.7$, $P = 0.0001$; variety x generation: $F(2, 144) = 1.19$, $P = 0.0066$). Preoviposition period did not differ between the two generations on MBra-12 or on CM3306-4; however, it increased in the F1 generation on MEcu-72, suggesting a possible delayed effect of a resistance factor in the latter variety. Neither the oviposition period nor postoviposition period were affected by cassava variety or phytoseiid generation.

The finite rate of increase (lambda; = $\exp(r_m)$, where r_m is the intrinsic rate of natural increase) comprises development time, immature and adult survivorship, sex ratio and age-specific fecundity. It indicates the proportional rate of increase of a population with a stable age distribution (i.e., lambda = 1.16 means the maximum population growth rate is 16% per day). Jackknife calculations permit the estimation of the standard error **(Fig. 1.26)**. Normally jackknife estimations of r_m are unbiased; however, there is clearly bias for our data. This was probably caused by the presence of relatively few individuals with high fecundity, making the frequency distribution asymmetrical. The estimate of r_m is shifted downward when each of these extreme values is sequentially omitted during the jackknife calculations. Although the jackknife estimates were biased, we believe that the standard error estimates still provide a good indication of the level of significance of the differences in r_m observed. Thus Fig. 1.26 should be read by relying on the actual r_m values but using the Jackknife 95% confidence intervals as an indication of precision. Population growth rate of *N. californicus* was highest on CM3306-4 and MEcu-72, and lowest on MBra-12. In contrast, population growth of the prey, *M. tanajoa*, is highest on CM3306-4 and lowest on MEcu-72 and MBra-12. This suggests that cassava variety MEcu-72 has the advantage of both higher resistance to the pest and higher suitability for the predator.

The effects of MBra-12 on *N. californicus* included reduced fecundity, immature survival and net reproductive rate (R_o) and longer development time than CM3306-4, but with no significant effect on adult female longevity. MEcu-72 was associated with longer development time and generation time than CM3306-4 or MBra-12, but with no other significant differences relative to CM3306-4. The pattern of daily fecundity of the phytoseiid differed on the two resistant cassava varieties, suggesting that there may be different mechanisms of resistance. MEcu-72 has the highest density of leaf trichomes (16,830 ± 807 SE per cm^2) of the three varieties (CM3306-4 1,681 ± 367, MBra-12 4,231 ± 608). Impairment of mobility of immature mites caused by trichomes may help explain the longer development time on MEcu-72, and the prolonged preoviposition period. But it does not appear to have much effect on *N. californicus*'s other life history traits. The negative impacts of MBra-12 are more likely to be caused by chemical resistance factors because this variety has fewer trichomes.

Table 1.10. Population parameters of *Neoseiulus californicus* on three varieties of cassava.

	Cassava varieties					
	CM3306-4		MBra-12		MEcu-72	
Population parameters	**Parents**	**Progeny**	**Parents**	**Progeny**	**Parents**	**Progeny**
Lamda[1]	1.16a	1.13b	1.06c	1.05c	1.13b	1.11b
T[2]	14.2	13.0	12.3	11.7	16.9	17.2
R_o[3]	7.7	5.1	2.2	1.8	7.9	5.9
DT[4]	4.8	5.6	11.0	13.3	5.7	6.7
Development time[5,6]	6.8	6.3	7.5	6.7	7.7	7.4
SE	0.1	0.2	0.1	0.1	0.2	0.2
Fecundity[6]	15.8	17.0	9.0	6.5	13.8	17.0
SE	1.3	1.9	1.2	1.2	1.7	2.4
Immature survival (%)[7]	98a	47c	45c	46c	82b	45c
Longevity[8]	20.7	19.8	16.8	12.7	17.9	15.1
SE	2.9	3.8	3.4	2.1	2.2	2.3
Sex ratio (%)[7,9]	50.0a	64.3a	53.8a	61.5a	70.0a	76.9a

[1] finite rate of increase (day^{-1}); values with the same letter have overlapping Jackknife 95% CIs
[2] generation time (days)
[3] net reproductive rate (females/female/generation)
[4] doubling time (days)
[5] egg to adult emergence (days)
[6] eggs per female per lifetime
[7] horizontal multiple comparisons (2x2 X^2 tests, alpha = 0.05)
[8] females, from adult emergence (days)
[9] percent females

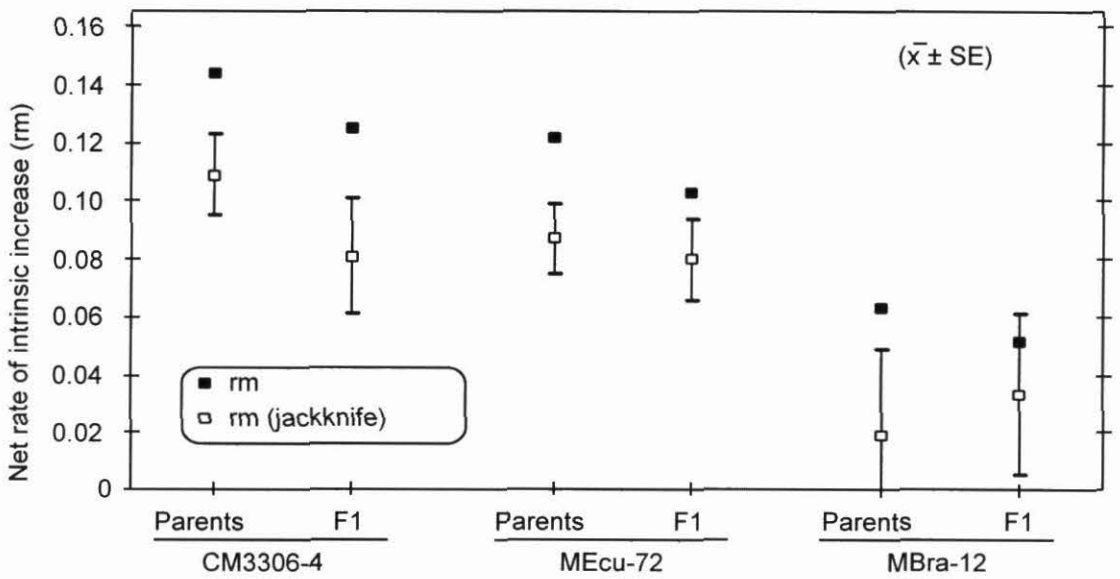


Figure 1.26. r_m of two generations of *Neoseiulus californicus* on three varieties of cassava (CM3306-4, "susceptible"; MBra-12, "tolerant"; MEcu-72, "resistant").

OUTPUT II. IMPROVED CROP MANAGEMENT COMPONENTS RELEVANT TO IPM STRATEGIES.

Subproject 1. Determine the Influence of Drought in the Relationships Between the Cassava Mealybug *Phenacoccus herreni* and its Host Plant

Introduction:

Several pests (mealybugs, whiteflies and mites) affect cassava yield, causing severe crop damage. It is well known that the population dynamics of these pests depend on environmental factors, including drought. In fact, the long dry periods frequently induce a strong outbreak in the number of these pests on cassava. This phenomenon partly depends on the changes of the trophic quality of cassava during the drought.

Our purpose is to determine the effect of drought tolerance in cassava on pest population dynamics. The mealybug is of primary interest because it has already been studied in this research area, and because it is one of the major pests in both the Americas and Africa. For the present, our research is focused on the importance of drought tolerance mechanisms, and the changes they might trigger in plant physiology or biochemistry, and what changes this might trigger in mealybug behavior and development. This research could serve as a model for future studies with other pests such as whiteflies.

Subproject 1.1. Identification of plant physiological changes induced by drought associated with the nutritional needs of the mealybug *Phenacoccus herreni*

Determination of the feeding behavior of male and female of *Phenacoccus herreni* on cassava

The feeding behavior of adult female was analyzed by the electrical penetration graph technique (EPG, DC-system). This study confirmed a typical phloem-feeding behavior with a predominance of extracellular pathways of stylets **(Fig 2.1)**. Similarities of EPGs from *P. herreni* with those of *P. manihoti*, of aphids and whiteflies allowed adoption of standard pattern labeling.

Comparison of the feeding behavior of insects on two locations on the leaf, insects near to or far from a primary leaf vein, allowed differentiation of penetration profiles. The phloem cell **52**punctures with sustained phloem sap feeding (noted E2) were markedly higher on insects located near to a leaf vein. The EPG profiles of mealybugs far from a primary leaf vein frequently showed an absence of phloem vessel puncture. This result explains the observations in natural conditions that the insects are mainly located near a primary leaf vein on cassava, the best location to reach phloem sap.

It is well known that adult male *P. herreni* does not possess mouth parts for feeding, and they do not feed. Nevertheless, little is known about the feeding behavior of immature male developmental stages. Using scanning electron microscopy (SEM), the second and third stage (note that the third stage is in a cocoon) showed a labium similar to that of females and to most other phloem-feeding insects. Nevertheless, when male third instars were tested by

EPG (n=18), none showed plant penetration, indicating that their mouth parts are not functional. The feeding behavior of male second instars is presently being studied by EPG. Furthermore, the microscopic observations of fourth instar and adult male confirmed the absence of mouth parts, indicating that they can't feed.

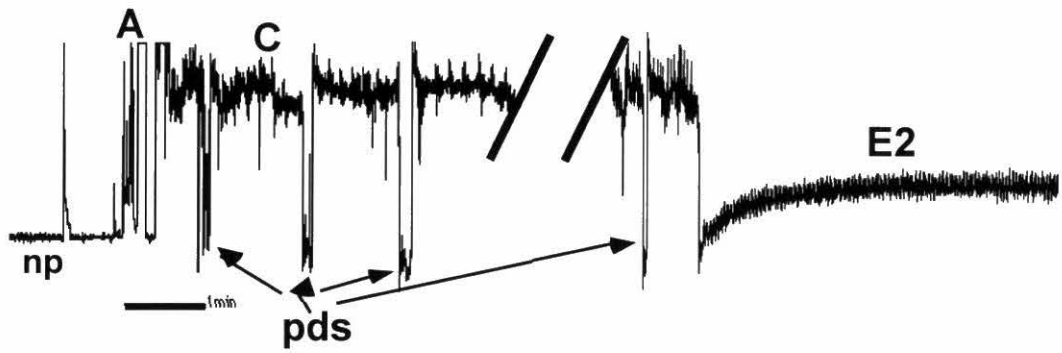


Figure 2.1. Cassava mealybug EPG patterns (adult female).
np: non penetration
A: beginning of stylet penetration in plant tissues
C: extracellular pathways of the stylets
pds: potential drops due to cell penetrations by the stylets
E2: penetration in a phloem cell with sustained phloem sap feeding

Subproject 1.2. Identification of secondary compounds in the cassava mealybug *P. herreni* and their role in insect's development

In previous studies, we detected in an other mealybug species *P. manihoti* the presence of a phenolic compound potentially synthetized by the insect. The concentration of this compound depends on the developmental stage. In fact, it is abundant in eggs and first instars, decreases in second and third instars, and increases in adults. This result suggests that this compound may play a role in the development of *P. manihoti*. Furthermore, in laboratory conditions we observed that plant water deficit induced a significant increase of the level of phenylalanine and tyrosine, amino acids known to be precursors of phenolic compounds in plants, in cassava leaves. These preliminary experiments give us a potential relationships between the plant physiological changes induced by drought and some nutritional needs of the mealybug, by the fact that the insects rearing on cassava in drought stress could synthetize a level more important of their phenolic compound mentioned above and potentially implied in their development because their alimentation are more

phenylalanine-rich and tyrosine-rich. This phenomenon suppose that these amino acids are also precursors of the phenolic compound found in mealybugs.

The first biochemical analysis obtained on *P. herreni* showed the presence of a phenolic compound apparently similar to the one found in *P. manihoti*. The role of this compound and the identification of its precursors will be investigated.

Subproject 1.3. Influence of cassava in drought stress conditions on the development and the fecundity of *P. herreni*

The cassava varieties used are CMC 40 and CM 507-37 known to be less and more drought tolerant, respectively. In glasshouse conditions, one-month-old plants were used. Drought stress was imposed by decreasing the irrigation volume from 500 ml (three times a week per plant, control) to 50 ml (two times a week per plant, stress). After 30 days of water deficiency, shoot development was markedly affected. The stems stopped growing from the onset of the period of water limitation. The number of leaves per plant decreased because of a dramatic acceleration of leaf senescence and abscission and a substantial decrease in leaf emergence. In addition, the area of the mature leaves was about half that of the control, and the stomatal conductance was very low under water limitation, whatever the leaf on the same plant for the both varieties. These modifications of plant growth and morphology demonstrate that our experimental conditions mimic water stress conditions well and thus allow study of the development of *P. herreni* on drought-stressed cassavas.

In order to limit the influence of parental trophic feeding, four distinct populations of mealybugs that were reared for 4 generations on the studied host plants that are well-watered or unwell-watered are in process and will be used for studying the development and the fecundity of *P. herreni* on CMC 40 and CM 507-37 under the two water conditions.

OUTPUT III. NARS CAPACITY IN INTEGRATED PEST AND CROP MANANGEMENT STRENTHENED

3. Training and Farmer Participatory Research Activities

3.1 Training

During 1997, training events organised by PROFISMA were limited to a Seminar held at CNPMF, Cruz das Almas, Bahia, with participation of 20 researchers and extension workers from the state-level agencies that have been collaborating with CIAT and EMBRAPA in the implementation of the project. The objectives of this seminar were to evaluate the training program, discuss results obtained by the COPALs and formulate action plans for the rest of 1997 and for the period starting in January 1998. Two principal points emerged from the discussions during the seminar: first, the need to increase the critical mass of trained technicians at the state level, for both research and extension agencies, and second, the importance of establishing strategies and special mechanisms aimed at raising consciousness and commitment among directors and managers of these agencies so that technicians could get more support from them to implement the FPR-based research and extension activities that PROFISMA has been promoting around the cassava crop. In relation to increasing in the region the number of technicians trained and consequently improving local agencies' capacity to disseminate the use of these methods, the agencies from the States of Bahia, Ceará and Pernambuco submitted to EMBRAPA/CNPMF concrete proposals requesting support (in the form of trainers from CIAT, training materials and the participation of trainees from other states) to organize state-level courses along the same training strategy used by PROFISMA.

Responding to these requests, and as part of a global joint planning exercise involving CIAT and EMBRAPA, a proposal was elaborated and submitted to a World Bank-financed program (PRODETAB) with the specific objective of implementing in Northeast Brazil a training program in FPR (Farmer Participatory Research) methods. This proposal is based in three components: a) internalization among a group of researchers from CNPMF of the basic principles of FPR methods, b) formation of a regional team of trainers in FPR for Northeast Brazil and c) state-level courses on FPR-methods taught by the regional trainers team. This proposal has been considered a priority by CNPMF, and efforts are currently being made to identify financial support for its implementation.

3.2 Farmer participatory research

Implementation of technology development and transfer activities based on the adaptation of the CIAL (farmer research committee) methodology to Northeast Brazil conditions has continued during 1997. The number of CIALs organised during the project remained the same (25), and six of these CIALs, after three years of existence, continued to present problems related to both lack of internal organization and poor technical assistance and support. The other 19 farmer researchers groups continue to function normally, some of them already running their third consecutive experiment and most of them already getting prepared to harvest their second participatory experiment for technology adaptation at the community level. The dissemination in the region of the use of FPR-methods for

Centro Internacional de Agricultura Tropical
Apdo. Aéreo 6713, Cali, Colombia
Internet: http://www.ciat.cgiar.org/
Correo electrónico: ciat@cgnet.com
Fax: (57-2)445-0073 (directo), (1-415)833-6626 (vía USA)
Teléfono: (57-2)445-0000 (directo), (1-415)833-6625 (vía USA)

technology development and transfer has been based on the formation of a group of researchers and extensionists from state-level agencies and the implementation with this group of a training master plan aimed at preparing them to use these methods efficiently and help farmers adapt them into their cassava-based systems. The training master plan was composed of three modules: a) diagnoses, b) planning and experimentation and c) evaluation. These three modules were to be connected at field level through the establishment of CIALs. Good results with modules a) and b) were rapidly and easily obtained. Trainees learned basic skills and developed abilities to facilitate collective problem identifying exercises at the community level with 75 groups of farmers in the States of Bahia, Ceara, Paraiba and Pernambuco. Later on, with a smaller group of 25 communities, trainees supported farmers' efforts to establish CIALs; select high priority topics and problems; and plan, install and administrate technology testing trials based on these topics. Experiences obtained by PROFISMA'S trainees with the use of these two modules indicate that two consecutive cycles is enough time for them to master the basic skills and knowledge required to use the modules efficiently.

Results with the third module, the evaluation of the trials, is demanding a longer term approach. The first batch of experiments evaluated (corresponding to the 1995-96 growing cycle), showed trainees giving emphasis to agronomic evaluation of trials, probably due to the fact that these methods are already known and used by the researchers and most of the extensionists. The only participatory evaluation method used by trainees during this period was the preference ranking methodology which was used mainly to select winning treatments (a one-shot farmer's opinion about the performance of a given technology). This method does not allow the capture of information about the criteria that farmers have used to select these treatments as their best choices and as a consequence, limits the feedback of valuable information, especially for researchers and breeders,

In 1997, during the harvest of the second trials (growing cycle 1996-97), an effort was made to stimulate trainees to start gaining experience with the use of other participatory methods to evaluate trials so that valuable information on farmers' criteria about their technological choices could be recorded and fed back to researchers and extensionists. To illustrate this process, the next section presents an analysis of the results obtained by the CIAL Colonia Agricola Roberto Santos, during their first two years running participatory experiments.

3.3 COPAL: Colonia Agrícola Roberto Santos, Inhambupe, Bahia-Experiment 1995-96

The objective of the experiment was to evaluate six cassava varieties for its resistance/tolerance to Cassava Green Mites (CGM). This pest had been prioritized by farmers as the main constraint for cassava production in the region. The six varieties were chosen locally by farmers from genetic materials they have planted and selected for many years. This first experiment did not included any variety from EMBRAPA/CNPMF. Farmers in this community represent an atypical example of cassava growers in most of Northeast Brazil since they are used to put heavy amounts of fertilizers on their crops (cassava, citrus and passion fruit) and have been doing it over many years. The excellent yields obtained in this experiment confirm the beneficial effect of fertilizer on yield. **Figure 3.1** presents the results obtained for

yields (ton/ha), fresh root dry matter content (ton/ha) and the preference ranking that farmers made of the treatments included in the experiment. Two of the varieties gave yields higher than 35 tons, four of them higher than 30 tons, figures that are well over state, regional and national average yields for cassava (12.0 ton/ha in Bahia, 10.6 in Northeast Brazil and 12.8 for the whole country. The local variety, used as check in this experiment (Platina) gave the lowest yield (28,2 ton/ha) although farmers ranked it as 2nd best in their preferences. The two varieties that gave higher yields were ranked by farmers in 3rd and 4th place. The main lesson learned from this COPAL and their collaborating farmers was the discovery of the excellent performance of other varieties different from the local ones that the farmers had been planting.

3.4 COPAL: Colonia Agrícola Roberto Santos, Inhambupe, Bahia-Experiment 1996-97

Based on the results obtained during their first experiment, the CIAL decided to continue testing varieties for resistance/tolerance to cassava green mite damage. This time, four varieties recommended by researchers at CNPMF were included in the experiment, using the best two local varieties as checks. The results of the experiment are presented in **Figure 3.2.** Two varieties, Voadeira (local) and 189/11 (introduced), gave the best yields and were also ranked by farmers as their favorite varieties during the preference ranking exercise. The additional activity conducted this year was to conduct evaluation interviews with the members of the COPAL and other farmers of the community with the purpose of identifying the principal criteria that farmers use to select the best varieties. These interviews were based on the open-ended evaluation methodology during which a total of 16 farmers evaluated and ranked each one of the six varieties and gave their opinions about what they liked and disliked about each one of them. Box 1 presents an example of the type of information that was obtained using this evaluation methodology. From this results it can be seen that farmers give a lot of importance to criteria such us a) the size and form of the roots because these means facility of using the knife when they peel the roots before the processing them into cassava flour, b) the colour of the flour is very important for them, if they see that the pulp is not 100% white they get worried because dark colour cassava flours are difficult to sell, c) the yield (amount of roots) is another important criteria and d) the flour yield which they call as "rende bem na massa" meaning that the variety has a good conversion factor from roots into farinha. Another criteria mentioned by farmers such as granulated, good flavour flour, good starch content, good dry matter, are all related to the cassava flour which gives an idea of how much these farmers are market-oriented, their main objective is to produce flour of good quality and good acceptance in the market.

Another form of interpreting this information is presented in Figure 3 using the concept of accumulated frequency that has been proposed recently by Luis Alfredo Hernandez (CIAT). In this methodology, the ranking made by farmers of the six varieties is analyzed using the number of times that each variety receives a given ranking position to calculate the proportion of farmers choosing that rank **(Tables 3.1, 3.2, 3.3)**. With this information, an accumulated frequency is calculated and plotted against the ranking preference **(Fig. 3.3).** This type of graph expresses the probability that a given variety has of being selected by farmers as 1st, 2nd, etc, in preference. This information could be very important for breeding programs and

Source: IBGE (1996). Instituto Brasileiro de Geografia e Estatistica.

especially in cases when a relatively large number of varieties have to be evaluated in short periods of time.

Table 3.1. Evaluation of six cassava varieties at the CIAL Colonia Agricola Roberto Santos by collaborating farmers.

	Farmers[1]															
Varieties	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Platina	3	3	3	4	4	4	4	4	4	4	4	5	5	6	6	6
47-19	2	2	3	3	3	3	3	3	3	3	3	4	4	4	5	5
194-16	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2
Voadeira	1	1	1	1	1	1	1	1	1	1	1	1	2	2	3	5
128-08	4	5	5	6	6	6	6	6	6	6	6	6	6	6	6	6
189-11	3	3	3	4	4	4	5	5	5	5	5	5	5	5	5	5

1 1 = Preferred Variety
2, 3, 4, 5, 6 = Less Preferred Variety

Table 3.2. Proportion for each preference ranking.

	Farmers' preferences					
Varieties	**1**	**2**	**3**	**4**	**5**	**6**
Platina			0.1875	0.5	0.125	0.1875
47-19		0.125	0.5625	0.1875	0.125	
194-16	0.25	0.75				
Voadeira	0.75	0.125	0.0625	0.0625		
128-08				0.0625	0.125	0.8125
189-11			0.1875	0.1875	0.625	

Table 3.3. Accumulated frequency for each preference ranking.

	Farmers' preferences					
Varieties	**1**	**2**	**3**	**4**	**5**	**6**
Platina	0.00	0.00	0.19	0.69	0.81	1.00
47-19	0.00	0.13	0.69	0.88	1.00	1.00
194-16	0.25	1.00	1.00	1.00	1.00	1.00
Voadeira	0.75	0.88	0.94	1.00	1.00	1.00
128-08	0.00	0.00	0.00	0.06	0.19	1.00
189-11	0.00	0.00	0.19	0.38	1.00	1.00

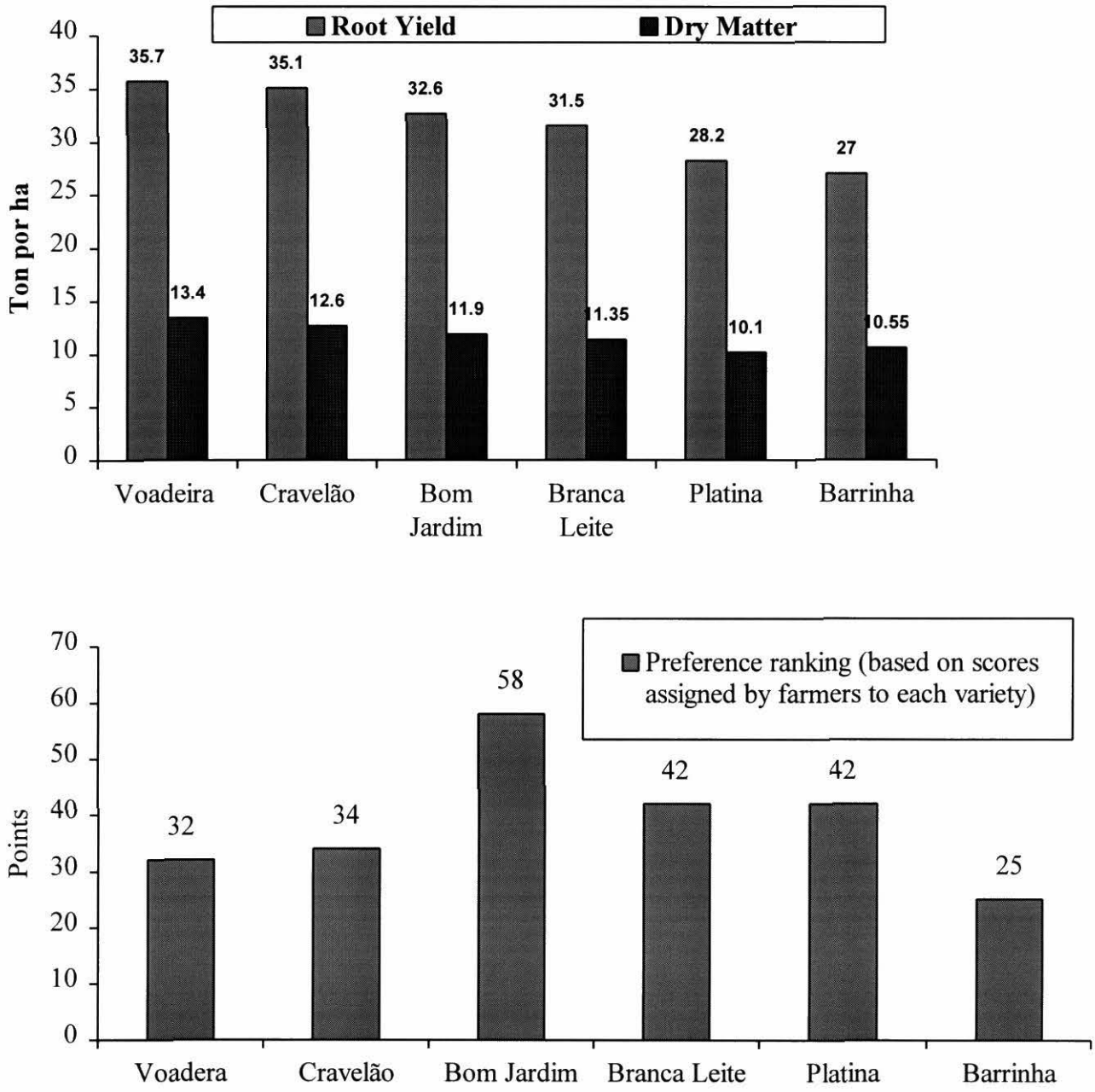


Figure 3.1. **Evaluation of six cassava varieties for resistance/tolerance to CGM Agronomic and Preference Ranking Evaluation (growing cycle 1995-96).**

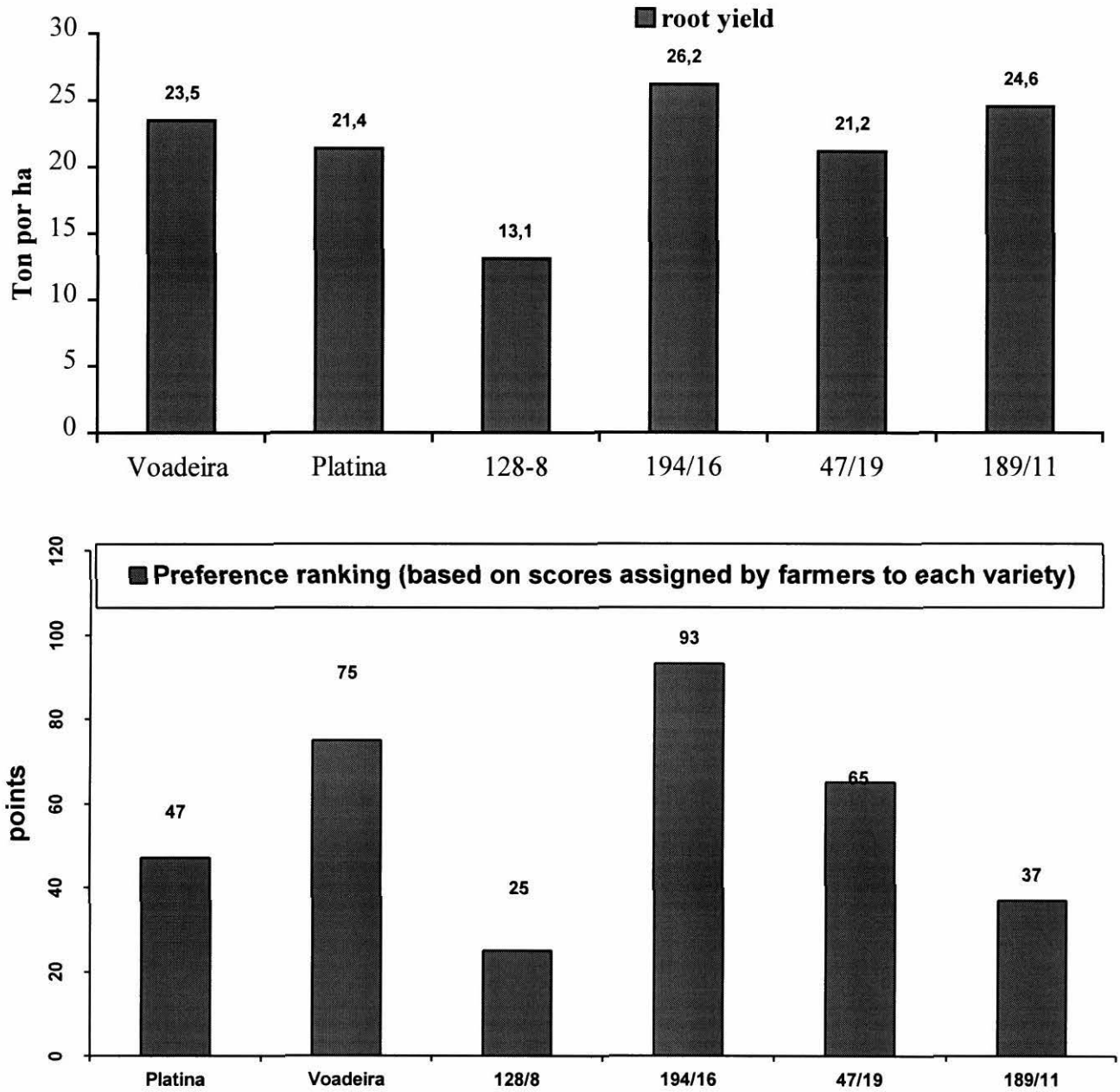


Figure 3.2 **Evaluation of six cassava varieties for resistance/tolerance to CGM Agronomic and Preference Ranking Evaluation (growing cycle 1996-97).**

Box 1.
CIAL: COLONIA AGRICOLA ROBERTO SANTOS
EXPERIMENTAL CYCLE 1996-97
OPEN-ENDED EVALUATION OF SIX CASSAVA VARIETIES

Variety: Platina

Farmers comments:[2]	**No. of Farmers**
• Susceptible to diseases in the leaves	(3)
• Low ramification	(3)
• Good roots yield	(12)
• Good dry matter	(5)
• Good starch content	(8)
• Good flour yield	(10)
• Beautiful colour of the flour	(11)
• Is regular for weed control because the ramification is very low	(4)
• The form of the roots is very good for processing	(12)
• Good top part production	(2)
• Easy to harvest	(3)
• Flour is easy to commercialise	(1)
• Flour has good granulation and good flavour	(6)
• Good size of the roots	(4)
• Top part is good to use feeding animals	(6)
• Too much water in roots but this is because the experiment was weeded out before harvest	(1)
• Not very good yield	(1)

Codes for comments:

Researchers 's criteria	**Positive aspects**	**Negative aspects**
a) Yields	+ (good roots yields)	
b) Height of the plant		
c) Height of the ramification		-(difficult to control weeds)
d) resistance (disease, insects)		- (leaves are attacked)
e) size of the roots		
f) form of the roots	+ (easy to peel out the roots)	
g) root roting		
h) dry matter of the roots	+ (good flour yield)	
i) colour of the pulp	+(flour colour is beautiful)	
j) colour of the epiderm		
k) number of roots per plant		
l) flour yield	+ (good starch content)	
m) easy to manage (manual weed control)		-(difficult to weed out because of the low ramification)
n) commercialisation	+(flour with good granulation and good flavour)	

[2] Numbers in brackets refer to the No. of farmers that mentioned the same criteria is good for this variety. Total number of farmers was 16.

Figure 3.3 **Accumulated preference ranking of six cassava varieties in Bahia, Brazil based on farmers´s evaluations.**

OUTPUT IV. GLOBAL WHITEFLY RESEARCH NETWORK TO REDUCE CROP LOSSES INITIATED

Subproject 1. Development of Whitefly IPM Project and Formation of an International Whitefly Network

Historically, the 18 centers of the CGIAR System have worked in a relatively independent fashion. However, it became increasingly clear that many benefits would be derived by pooling human capital, infrastructure and economic resources to jointly tackle research problems that numerous Centers were working on independently. As a result, Inter-Center Initiatives were born in the areas of: Participatory Research; Soil, Water, Nutrient Management; Livestock; Genetic Resources; and Integrated Pest Management.

The Systemwide Programme on Integrated Pest Management (SP-IPM) was established by the CGIAR in recognition of the crucial importance of integrated pest management to sustainable agricultural development. In February of 1995, representatives often International Agricultural Research Centers (IARCs) and the Chairman of the IPM Working Group, met at Den Hague in The Netherlands. The participants of this meeting recommended that the CG System form a System-wide Program on Integrated Pest Management, with an Inter-Center Working Group on IPM to function as the steering committee. To-date, 12 Inter-Center IPM projects have been proposed as part of SP-IPM:

Whitefly IPM Project
Cereal stem borers
Grain legume pests
Parasitic flowering plants
Weeds in rice
Farmer participatory research in IPM
Functional agrobiodiversity
Soil-borne pathogens
IPM implementation and impact assessment methodologies
Integrated weed management
Crop loss assessment
White grubs

CIAT was designated as the convening center to formulate a system-wide proposal on Integrated Management of Whiteflies as Pests and Vectors in the Tropics.

Whitefly IPM Project

To that end, a Task Force Meeting for the CGIAR Whitefly IPM Project was held at CIAT from February 13-15, 1996. The objectives of the meeting were: a) to discuss the Outputs and Activities that should be proposed for the Project; b) to discuss a structure for the global Whitefly IPM Project, as well as how to link and coordinate the institutions that would be involved in the Project; and c) to discuss how each of the institutions represented on the Task Force might contribute to the proposed Project Activities.

The Task Force Meeting included 24 participants, whitefly experts, representing CGIAR Centers, national and regional agricultural programs, and basic research institutions in the United Kingdom and the United States. After three days of discussion, the Task Force reached consensus on the nature of the whitefly problem and what action was needed.

The Task Force agreed that it was possible to define three whitefly problems that should be prioritized:
1) whiteflies as pests in Tropical highlands;
2) whiteflies as vectors in mixed cropping systems in low to mid altitudes of the Tropics; and
3) whiteflies as vectors and direct pests in cassava.

And, as a result, within the framework of an eco-regional problem approach, it was further agreed that the Whitefly Project should be structured into a series of Sub-projects, reflecting those different problems **(Fig. 4.1)**:
1) Whiteflies as direct pests in the tropical highlands of Latin America;
2) Whiteflies as vectors in legumes and mixed cropping systems in the tropical lowlands of the Caribbean, Mexico and Central America;
3) Whiteflies as vectors in vegetables and mixed cropping systems in eastern Africa; and
4) Whiteflies as vectors of virus in cassava and sweet potato in Africa

The Task Force also agreed upon the Goal and Purpose of the Project, was able to reach consensus on the Outputs of the Project, and defined which activities should be undertaken to achieve each Output. With this information, a work breakdown for the Whitefly IPM Project was constructed **(Fig. 4.2)**. The Whitefly Project is one of the most complex and most advanced of the SP-IPM Projects proposed to-date. Both the product and the process of Whitefly Task Force Meeting were well received by the Inter-Center Working Group on IPM, and are being used as a model and prototype for other SP-IPM projects.

Whitefly IPM Project - Phase 1

After the Task Force meeting, in March of 1996 the Danish International Development Agency (Danida) invited CIAT to submit a proposal for the start-up phase (Phase 1) of the Whitefly IPM Project. That proposal was submitted in September of 1996, and was approved by Danida in February of 1997.

Phase 1 focuses on Outputs 1 and 2, as defined in the work breakdown **(Fig, 4.2)**, that is the formation of a network of whitefly professionals working in the tropics, and the establishment of a collaborative research agenda by which we can better characterize whitefly problems in the tropics. The strength and innovation of this new project lies in its collaborative and integrative nature.

Figure 4.1

Figure 4.2

Within this project structure, we have already been able to bring together an impressive number of partners. At this initial phase the project includes 5 International Agricultural Research Centers, 6 basic research institutions in the United Kingdom, Germany and the United States, and 29 national program or regional research institutions in 22 Latin America and Africa countries **(Table 4.1)**.

One of the obstacles to research progress has been the diversity of methodologies employed, to the extent that even similar data sets cannot be compared. For that reason, and taking advantage of this incredible group of collaborators, significant attention has been given to standardizing the methodologies and protocols for the data that we collect and generate. In that way, we hope to compare and analyze data on the whitefly problem from very different contexts.

The objective of the CGIAR Whitefly IPM Project is not only to establish a collaborative research project, but also to integrate research across disciplines, across eco-regions, and across national and international boundaries. This project is transdisciplinary and global in its nature. We believe that by learning to work together in new ways and integrating previously compartmentalized disciplines and geographical zones, we can strengthen the products that we produce, and generate knowledge and solutions for whitefly problems in a more efficient manner.

Progress to date

Project funding was transferred to CIAT in March of 1997. All initial administrative tasks have been completed, and funding has been transferred to project partners.

Related to networking, efforts have begun on development of a WWW InterNet site, a directory of whitefly professionals in the tropics, and the annotated bibliography of whitefly grey literature. The whitefly network in Latin America is relatively advanced due to the existence of the Latin American Whitefly and Geminivirus Network, established in 1993. In July of this year, ICIPE took the initiative of organizing what we expect to become the African Whitefly and Geminivirus Network. Their initial meeting, a satellite meeting of the African Entomology Congress, included 32 scientists from 7 African countries.

The Whitefly IPM Project Coordination Team held its first meeting at CIAT in February of 1997, and has overseen the development of a Methodology Guide which lays out the standardized procedures and protocols for the project field work. Research teams in all four sub-projects have begun field survey work. The GIS unit at CIAT is setting up the geographical information system for the whitefly-related project data. And, junior staff members at the Latin American (CIAT) and African (ICIPE) coordinating centers have begun receiving international training related to their project responsibilities.

In conclusion, while project work is well underway, due to the incipient nature of the Whitefly IPM Project, we do not anticipate being able to report on Project results until 1998.

Table 4.1. Partners in Phase 1 of the Whitefly IPM Project.

INTERNATIONAL AGRICULTURAL RESEARCH CENTERS

Centro Internacional de Agricultura Tropical (CIAT)
International Center for Insect Physiology and Ecology (ICIPE)
Asian Vegetable Research and Development Center (AVRDC)
International Institute of Tropical Agriculture (IITA)
International Potato Center (CIP)

NATIONAL PROGRAMS AND REGIONAL RESEARCH INSTITUIONS

Agricultural Research Corporation (ARC), Sudan
Agricultural Research Institute (IRA), Cameroon
Bvumbwe Agricultural Research Station (BARS), Malawi
Centro Agronómico Tropical de Investigación y Enseñanza (CATIE), Costa Rica
Centro de Investigación y de Estudios Avanzados (CINESTAV), México
Centro Nacional de Tecnología Agropecuaria (CENTA), El Salvador
Corporación Colombiana de Investigación Agropecuaria (CORPOICA), Colombia
Escuela Agrícola Pan-American (EAP), Honduras
FOFIFA, Madagascar
Horticultural Research and Training Institute (HORTI), Tanzania
Instituto de Ciencia y Tecnología Agrícola (ICTA), Guatemala
Instituto Nacional de Investigaciones Forestales y Agropecuarias (INIFAP), Mexico
Instituto de Investigaciones de Sanidad Vegetal (IISV), Cuba
Instituto Nacional Autónomo de Investigaciones Agropecuarias (INIAP), Ecuador
Instituto Superior de Agricultura (ISA), Dominican Republic
Kampala Agricultural Research Institute (KARI), Uganda
Lunyangwa Agricultural Research Station (LARS), Malawi
Mt. Makulu Research Station Plant Protection Section (MMRSPPS), Zambia
Kenya Agricultural Research Institute (KARI), Kenya
National Agricultural Research Institute of Benin (INRAB), Benin
National Agricultural Research Organization (NARO), Uganda
National Horticultural Research Station (NHRS), Kenya
National Plant Protection Service (NPPS), Guatemala
National Root Crop Research Institute (NRCRI), Nigeria
Plant Protection and Regulatory Services Division (PPRSD), Ghana
Programa de Frijol Centro Americano (PROFRIJOL), Guatemala
Programa de Frijol de Haiti (PRONATHAR), Haiti
Tanzania Agricultural Research Organization (TARO), Tanzania
Universidad Nacional Agraria (UNA), Nicaragua

BASIC RESEARCH INSTITUTIONS

Biologische Bundesanstalt fur land und Fortwirtschaft (BBA), Germany
John Innes Centre (JIC), UK

Natural Resources Institute (NRI), UK
University of Arizona-Tucson (UA), USA
University of Florida-Gainesville (UFL), USA
University of Wisconsin-Madison (UW), USA

COORDINATION TEAM

Dr. Pamela K. Anderson (CIAT)	Project Coordinator
Dr. Anthony C. Bellotti (CIAT)	Project Leader
Dr. César Cardona (CIAT)	Coordinator Sub-Project 1
Dr. Francisco Morales (CIAT)	Coordinator Sub-Project 2
Dr. S. Sithanantham (ICIPE)	Coordinator Sub-Project 3
Dr. James Legg (IITA)	Coordinator Sub-Project 4
Dr. Richard Markham (IITA)	Secretary, SP-IPM
Dr. Peter Neuenschwander (IITA)	Representative, SP-IPM
Dr. Peter Markham (John Innes)	Project Scientist
Dr. Lee Calvert (CIAT)	Project Scientist

Subproject 2. Diagnosis and Characterization of Whitefly Problem and Target Area

Develop reliable method for identification of "B"biotype of *B. tabaci*

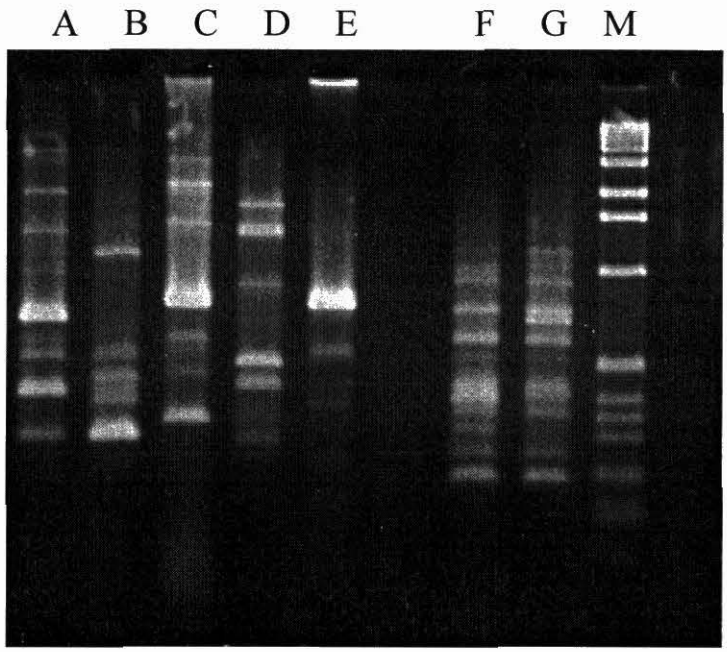
This activity was expanded to the development of molecular detection methods to identify whitefly species and biotypes that are important in the Americas. This complements the African initiative in the whitefly IPM project. One of the major emphasis of this activity is to standardize the methodology between the African and American groups. This will make the data collected in different areas of the world more comparable.

For a regional survey to function without quarantine problems, it is essential to develop methods that utilize dead insects. Methods to extract DNA where analyzed using pupa stored in 70-95% alcohol. One method has been used routinely in our laboratory for several years. This method works for single pupa or adult insects but is somewhat time consuming and it is difficult to extract from more that 32 individuals in a day. The DNA isolated by this method is very stable even when stored for a several years. A second method used in the laboratory of Peter Markham is simpler than our standard method. The method is highly repeatable and moderately rapid. With this method, it is possible for one person to extract DNA from 80 individual insects per day. This is currently the method of choice in the survey of whiteflies.

Four different oligonucleotide primers have been selected to biotype *B. tabaci.* These primers were used to develop baseline data not only for *B. tabaci* but also other whitefly species. The results of PCR product analysis (primer H9) of four species of whiteflies compared with the *B. tabaci* "B" biotype are shown in **Figure 4.3.** Each species is distinct and the "B" biotype is readily identified. A Colombian isolate suspected to be the "B" biotype as analyzed using this primers and was very similar to the Egyptian and Israel

isolates (see report of César Cardona). Further testing needs to be done using more sources of whiteflies to determine if these primers are appropriate for distinguishing biotypes within the four species. The group of primers were selected to rapidly separate the "A" and "B" biotypes of *B. tabaci* and in the preliminary testing differences are being found with the *B. tabaci* complex of tropical America.

A more detailed analysis of variation is being done that allows a comparison of evolutionary relatedness. This involves cDNA cloning and sequencing of a region that is commonly used in studies of biodiversity. A region of the 16S ribosomal RNA gene was selected for this study. A representative of *B. tabaci, B. tuberculata, T. variabilis* and *T. vaporariorum* were cloned and sequenced. We need to determine if this is the most appropriate region and if there is sufficient level of polymorphism between species and biotypes. The preliminary analysis is that there is sufficient polymorphism between species. The next step is to determine the variation within species.



Line A: B. tabaci-CIAT, B: B. tuberculata-CIAT, C: T. vaproariorum, D: A. Socialis, E: T. variabilis, F: B. tabaci "B"-Israel, G: B. tabaci "B"-Egypt, and M:1 kb-BRL markers. The primer was Operon Technologies Inc. oligonuclotide H9 (5'TGTAGCTGGG).

Figure 4.3. A comparison of the pattern of PCR products of five whitefly species and B. tabaci biotype "B".

Subproject 2.1. Monitor "B" biotype of *Bemisia tabaci* spread throughout Americas

This activity is part of the survey of whiteflies and this has been expanded to identifying biotypes of the different species of whiteflies found in the Americas. The original objective was to use molecular markers to rapidly identify the "B" biotype of *B. tabaci* which is considered by some to be a new species named *B. argentifolii*. Using oligonucleotide primers described above, the survey of biotypes within the whitefly species has begun. For example, *B. tuberculata* was collected from cassava in three different regions of Colombia and all appear to be quite similar. Regarding the "B" biotype, it has been expanding its range and is now a problem in most countries in tropical America. With the limiting sampling to date and using the oligonucleotide primers, this biotype looks very similar to the "B" biotype found in Israel, Egypt and Florida. This activity is supplements the survey of whiteflies by allowing resolution of biotypes.

In addition to the survey, there are reports in many different countries of the "B" biotype. It is a major problem in several regions of Brazil. It is also in Ecuador, Colombia, most of Central America, Mexico and the Caribbean region. The expansion of the range of this biotype has been extremely rapid. In the areas of the United States, this biotype was very aggressive and apparently replaced the formerly predominate "A" biotype. With molecular methods, we can determine if there is genetic mixing or simple replacement. This data will complement biological data.

Project Staff - CIAT

Entomology

A. Bellotti
B. Arias
D. Sánchez
G. Trujillo
G. Pérez
C. Ñañes
A. Ortega (student)

ORSTOM

P. Calatayud
M.A. Polanía (student)
C.D. Seligmann (student)

ETH/ZIL

C. Bertschy (Ph.D. student)
V. Feichtinger (Ph.D. student)
H. Morales

Whitefly IPM/Danida

P. Anderson
C. Cardona
L. Calvert
F. Morales
A. Bellotti
M.P. Hernández
M. Cuervo
O. Villareal

Pathology

E. Alvarez
J.B. Loke
G.A. Llano
M.I. Chacón (Colciencias)
D.M. Castañeda (Colciencias)
G.G. Castañeda (UNDP)
S.L. Reyes (student)
M.L. Molina (student)
N.J. Sánchez (student)
F.A. Bedoya (student)
J.A. Ramírez (student)

Virology

L. Calvert
N. Balcázar
J. Arroyave
T. Rodríguez

PROFISMA/UNDP

L. Smith
B. Ospina
J.M. Guerrero
M.E. Cuéllar
E.L. Melo
A. Bohórquez
R. Zúñiga
T.C. Agrono
L. F. Larrahondo
A. Muñoz
R.B. Riascos
J.R. Escobar
J.Martinez

Students:

E.G. Burbano

Publications

Caicedo, V., A.M. and A.C. Bellotti. 1996. Reconocimiento de Nematodos Entomopatógenos Nativos Asociados con *Cyrtomenus bergi* Froeschner (Hemiptera: Cydnidae) en Ocho Localidades de Colombia. Rev. Col. Entomol. Vol. 22. No. 1. Pp. 19-24.

De Moraes, G.J., E.L. Melo and M.G.C. Gonclim, Jr. 1997. Description of a New Species of Phytoseiid Mite from Northeastern Brazil and Redescription of *Neoseiulus gracilis* (Acari: Phytoseiidae) Florida Entomologist 80(3); pp 319-314

Bertschy, C., T.C.J. Turlings, A.C. Bellotti and S. Dorn. 1997. Chemically-mediated attraction of three parasitoid species to mealybug - infested cassava leaves. Florida Entomologist. 80(3), pp. 383-395.

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

Calvert, L.A., Cuervo, M., Ospina, M.D., Fauquet, C., & Ramirez, B.C., 1996. Characterization of cassava common mosaic virus and a defective RNA species. Journal of General Virology 77: 525-530.

Calatayud, P.A., Le Ru, B., 1997. Control of the cassava mealybug in Africa. Les cashiers de la Rechererche et Développement. 43:59-66.

Burbano-M., E. G. and L. Smith. submitted. Effect of Resistant Cassava Varieties on Life History Characteristics of Cassava Green Mite *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae). Experimental and Applied Acarology.

Osorio-Rios, L., M. E. Cuellar -Jímenez, L. Smith. submitted. Estudios Básicos Tendientes a la Optimización de un Sistema de Cría Masiva de *Typhlodromalus tenuiscutus* McMurtry & Moraes (Acari: Phytoseiidae) un Depredador de Acaros de Yuca. Revista Colombiana de Entomología.

Noronha, A. C. S., G. J. de Moraes & L. Smith. In press. Introdução de ácaros predadores da família Phytoseiidae para o controle biológico do ácaro verde da mandioca, *Mononychellus tanajoa* (Bondar), no Nordeste do Brasil. Pesquisa em Andamento, CNPMA, EMBRAPA. [Introduction of mite predators of the family Phytoseiidae for biological control of the cassava green mite*Mononychellus tanajoa* (Bondar), in Northeast Brazil].

Edwards, E., E. L. Melo, L. Smith & M. A. Hoy. In press. Discrimination of Three *Typhlodromalus* species (Acari: Phytoseiidae) Using Random Amplified Polymorphic DNA (RAPD) Markers. Exp. Appl. Acarol. 21: __-__.

de Moraes, G. J., E. L. Melo & M. G. C. Gondim Jr. 1997. Description of a new species of phytoseiid mite from northeastern Brazil and redescription of *Neoseiulus gracilis* (Acari: Phytoseiidae). Florida Entomologist 80(3): 319-324.

Book Chapter

Bellotti, A. 1996. Las plagas de la yuca y su manejo. In. La Yuca Fuente al Hambre del Mundo Tropical. Ed. A. Montaldo. Universidad Central de Venezuela. Talleres de Anauco Ediciones, C.A. Caracas, Venezuela.

Conferences and Posters

Anonymous. 1997. Control Biológico de los Agentes Causales de la Pudrición de Cogollo en Palma de Aceite. CENIAVANCE No. 40. CENIPALMA and CIAT. 6 p.

Alvarez, E. 1997. Characterization of *Phytophthora* spp. Hand out presented at symposium at CIAT. April 23th.

Alvarez, E., Chacón, M.I., Sánchez, J. and J.B. Loke. 1997. Molecular Characterization of Phytophthora species from Cassava in Colombia. Poster presented during two training courses titled *Training in Molecular Biology with Emphasis on the Characterization of Phytopathogenic Fungus*. October 5-10 and 13-18, 1997, at the International Center for Tropical Agriculture (CIAT), Cali, Colombia.

Alvarez, E., J. Loke and R. Mayer. 1996. Progress towards biological control of Cassava Stem and Root Rots. Poster presented at the Workshop Sobre Podridões Radiculares no Cultivo da Mandioca. Cruz das Almas, Bahía Brazil, 1996.

Alvarez, E., Chacón, M.I., Loke, J.B., N. J. Sánchez. 1997. Genetic variation in strains of *Phytophthora* spp. affecting cassava. Phytopathology Vol. 87, No. 6, p. S3 - S4. Abstract presented at APS-Annual Meeting, 9-13 August, Rochester, N.Y., USA.

Alvarez, E. and M. L. Molina. 1997. Caracterización Morfológica y Molecular de Aislamientos de Sphaceloma manihoticola (Bitancourt & Jenkins) en Colombia. ASCOLFI Congress at the International Center for Tropical Agriculture (CIAT), Cali, Colombia, August 1.t

Alvarez, E., Ramirez, J.A. and J.B. Loke. 1997. Termoterapia en Semilla Asexual de Yuca para el Control de *Phytophthora* spp. ASCOLFI Congress at the International Center for Tropical Agriculture (CIAT), Cali, Colombia, August 1.

Alvarez, E., M. Valencia, J.B. Loke and E. Rosero. 1995. A new approach to characterize cassava root rot pathogens. CIAT Internal Review (Plant Breeding for Sustainable Agriculture).

Bedoya, F.A., Alvarez, E. and J.B. Loke. 1997. Control Biológico de la Pudrición Radical en Yuca: Selección *In Vitro* de Aislamientos de *Trichoderma* spp. ASCOLFI Congress at the International Center for Tropical Agriculture (CIAT), Cali, Colombia, August 1.

Sánchez, J., Alvarez, E., Chacón, M.I. and J.B. Loke. 1997. Avances en la Caracterización de Aislamientos de *Phytophthora* spp. el Agente Causal de Pudriciones de Raíces en Yuca. ASCOLFI Congress at the International Center for Tropical Agriculture (CIAT), Cali, Colombia, August 1.

Anderson, P. K., 1997. Emerging Plant Diseases: The Case of Geminiviruses in Beans and Tomatoes in the Americas, case study workshop, Havard Conference on Emerging Infectious Diseases, June 11 & 12.

Anderson, P. K., 1997. Food Security and Emerging Plant Diseases, keynote presentation, Harvard Conference on Emerging Infectious Diseases, June 13.

Anderson, P. K. , 1997. The Epidemiology of Geminiviruses, keynote presentation at the VI Latin American Workshop on Whiteflies and Geminiviruses, Santo Domingo, Dominican Republic, August 18.

Anderson, P. K., 1997. 1997The CGIAR Whitefly IPM Project: A New Form of Collaboration, presentation at the National Whitefly Workshop, Guatemala City, November 11.

Anderson, P. K., 1997. The CGIAR Whitefly IPM Project: A New Form of Collaboration, presentation at the International Workshop on Geminivirus in the Caribbean, Havana, Cuba, November 28.

Bellotti, A.C., C. Cardona, and L. Riis. 1997. Burrowing Bugs and Whitegrubs, Major Soil Pests of Food Crops in Colombia. Third Brisbane Workshop on Soil Invertebrates. University of Queensland. 15-17, July. (Proceeding papers submitted).

Bellotti, A.C. 1997. Introduction to the CGIAR System-Wide IPM Program. Third Brisbane Workshop on Soil Invertebrates. University of Queensland. 15-17, July.

Anderson, P.K., and A.C. Bellotti. 1997. Sustainable Integrated Management of Whiteflies as Pests and Vectors in the Tropics. International Workshop on the Silverleaf Whitefly, *Bemisia argentifolli*. EMBRAPA/CENARGEN. Brasilia, Brazil. 4-6, November.

9/29/97Establishment and Success of Phytoseiid Mites Introduced from South America to Africa for Biological Control of Cassava Green Mite. L. Smith, J. S. Yaninek, G. J. de Moraes. USDA/ARS/NPA, Sidney, Montana.

7/16/97Distribución Geográfical del Hongo *Neozygites* sp. (Zygomycetes: Entomophthorales) en Acaros de la Familia Tetranychidae, Especialmente Colectados Sobre Yuca, *Manihot esculenta* Crantz. J. M. Guerrero, L. Smith, A. C. Bellotti. 24° Congresso de SOCOLEN, Sociedad Colombiana de Entomología, 16-18 July, 1997, Pereira, Colombia.

7/17/97Relación Tritrófica entre *Neoseiulus californicus* (McGregor), y su Presa *Mononychellus tanajoa* (Bondar), Sobre Variedades Resistentes de Yuca. E. G. Burbano, P. Chacón de U., L. Smith. 24° Congresso de SOCOLEN, Sociedad Colombiana de Entomología, 16-18 July, 1997, Pereira, Colombia.

7/17/97Establecimiento y Exito de Acaros Fitoseidos Introducidos de Sur América a Africa para el Control Biológico del Acaro Verde de la Yuca. L. Smith, J. S. Yaninek, G. J. de

Moraes. 24° Congresso de SOCOLEN, Sociedad Colombiana de Entomología, 16-18 July, 1997, Pereira, Colombia.

7/18/97Desarrollo de una Clave Taxonómica de Fitoseidos Encontrados en Yuca para un Programa de Control Biológico. E. L. Melo, G. de Moraes, L. Smith. 24° Congresso de SOCOLEN, Sociedad Colombiana de Entomología, 16-18 July, 1997, Pereira, Colombia.

3/2/97 Geographical Distribution and Climatic Associations of Phytoseiid Mites Associated with Cassava in Colombia, Venezuela and Ecuador. L. Smith, J. R. Escobar and E. L. Melo. 16° Congresso Brasileiro de Entomología, 2-7 March, 1997, Salvador, Brazil.

3/2/97 Suitability of Different Types of Food for Several Phytoseiid Predators Being Evaluated for Biological Control of *Mononychellus tanajoa* (Acari: Tetranychidae) in Cassava. L. Smith, M. E. Cuellar J. and E. L. Melo. 16° Congresso Brasileiro de Entomología, 2-7 March, 1997, Salvador, Brazil.

12/15/96 Foreign exploration for phytoseiid mites in northern South America for classical biological control of the cassava green mite in Africa. L. Smith, E. L. Melo, J. R. Escobar, M. E. Cuellar & J. M. Guerrero. Entomological Society of America National Conference, Louisville, Ky.

Donor Institutions

USAID
UNDP Ecologically Sustainable Cassava Plant Protection: A Global Strategy (Preparatory Assistance), GLO/97/119/A111/31. $447,272 for one year, 1997.
Danida
ETH/ZIL
French Ministry of Education
Colciencias "Caracterización de poblaciones del hongo entomopatógeno *Neozygites* sp. (Zygomycetes: Entomophthorales) sobre el ácaro verde *Mononychellus tanajoa* (Bondar), por medio de técnicas moleculares." Adriana Bohórquez Chaux scholarship for one year.

Research Collaborators: Other Institutions

Chigeru Fukuda; CNPMF, Brazil
Paulo Meissner; CNPMF, Brazil
Peter Markham; John Innes Center
Gina Bank; John Innes Center
Sylvia Dorn; Swiss Federal Institute of Technology (ETH)
Letizia Mattiacci; ETH
Aristóteles Pires de Mattos; CNPMF, Brazil
Armando Takatsu; Universidad de Brasilia, Brazil
Aristóbulo López; CORPOICA, Colombia
Michael Coffey; University of California, Riverside
Alber(io Lima; Universidad Federal do Ceara, Brazil
B. Delobel; INSA-INRA, France
T. Lamaze; Université de Tailouse, France

Collaborating Institutions

INRA - INSA Laboratory of Applied Biology, Villeurbanne, France.
CNPMF - Brazil
Universidad de Brasilia, Brazil
Universidad Federal do Ceara, Fortaleza, Brazil
Biologische Bundesanstalt fur land und Fortwirstschaft (BBA), Germany
John Innes Center (JIC) UK
University of California, Riverside, USA
Auburn University, Alabama, USA
University of Arizona - Tucson (UA), USA
University of Florida (UFL), USA
University of Wisconsin - Madison (UW), USA
CORPOICA (Colombia)
CATIE (Costa Rica)
Escuela Agrícola Panamericana (EAP), Honduras

ICIPE, Kenya
IITA, Nigeria/Benin
CIP, Perú
AVRDC, Taiwan
Universidad Nacional de Colombia, Santafé de Bogotá, Colombia
Universidad Nacional de Colombia, Palmira, Colombia
Universidad del Valle, Cali, Colombia
NARS (See Output IV, Global Whitefly Project)
Natural Resources Institute (NRI), United Kingdom
Université Paul Sabatier, Tailouse, France
Swiss Federal Institute of Technology (ETH)
Royal Veterinary and Agricultural University, Inst. of Ecology and Molecular Biology