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## Introduction

IITA's research mission continues to be focused on the ability of sub-Saharan Africa to feed itself on a sustainable basis, while not neglecting related issues of environment, population, and development. It is worth reemphasizing that sub-Saharan Africa is the only region in the world where per capita food production has steadily declined over the last three decades, threatening its marginalized people with malnutrition.

As the contents of this issue reaffirm, IITA's research continues to make significant progress in its attempts to increase the productivity of key food crops, to develop sustainable agricultural systems, and to improve the nutritional status and well-being of low income people in the humid and subhumid tropics of sub-Saharan Africa.

The first article addresses how parasitic flowering plants of the genus *Striga* spread on to farmers' fields. Those parasitic plants can cause extensive damage to cereal and legume crops in Africa, including maize and cowpea. The understanding our scientists have gained of the *Striga* seed dispersal is vital in efforts to control damage.

The second article in this issue addresses another important aspect of the work on controlling damage from weeds. Genetic studies, such as the one reported here involving inheritance of resistance in cowpea to two parasitic weeds, *Striga* and *Alectra*, are important in building resistance in the plant to stress from buildup of the weeds.

Soil fertility is one of the crucial factors governing sustainable agriculture. Soil fertility can be enhanced by alley cropping (growing selected species of trees along food crops) and by the use of rhizobial inoculants in place of commercial fertilizer. One issue that has received inadequate research attention in the past is how long the introduced rhizobia can stay viable and continue to fix nitrogen (and thus enrich the soil). The third article herein addresses that specific question. Combined with other studies on management practices, it provides information that can be useful in developing sustainable systems of agriculture.

Viruses are only one among various causes of plant diseases. They are uniquely difficult to detect, identify, and characterize. This makes control of viral diseases especially difficult. Since 1990, IITA scientists have carried out a collaborative project with scientists in Canada and in African national programs, which has resulted in improved capability within Africa to identify specific viruses and their strains. The fourth article in this issue summarizes the achievements of that collaborative project.

Other items in this issue also document IITA's contributions to its main goal. Theses abstracts offer a glimpse of the results obtained by IITA's research trainees. The information on current literature, recent publications, training courses, and people at IITA is also intended to help better interactions within the scientific community.

Your views continue to be welcome, both on our work and on related issues.

**L. Brader**
*Director General*

## In this issue

### Articles



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For further information about IITA's research and training programs, contact Stephen M. Lawani, Director, Information Services.

# Relative roles of wind, crop seeds, and cattle in the dispersal of *Striga* species*

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**Parasitic flowering plants of the genus *Striga* cause extensive damage to cereal and legume crops in Africa, including maize and cowpea. Yield losses can be greater than 50%. Both the extent and intensity of *Striga* damage appear to be increasing across the region. Yet, factors affecting the dispersal of *Striga* seeds have not been well understood. This study, conducted by IITA scientists at sites of high *Striga* infestation, examined the roles of different potential agents in the dispersal of seeds of the parasites, in an effort to understand and control their spread.**

## Introduction

A diverse number of parasitic seed plants in the savanna zone of Africa pose serious threats to both cereal and legume production (Dodgett 1965, 1984; Aggarwal and Ouedraogo 1989; Anon 1989; Carson 1989). The most devastating of these are species in the genus *Striga*, family Scrophulariaceae (Musselman 1987). The most common and devastating species in the savanna zone is *S. hermonthica* (Del.) Benth., a parasite of millet (*Pennisetum americanum* [L.] K. Schum), sorghum (*Sorghum bicolor* [L.] Moench), maize (*Zea mays* L.), rice (*Oryza sativa* L.), and sugarcane (*Saccharum officinarum* L.) (Pieterse and Pesch 1983; Ogborn 1987; Anon 1989; Lagoke et al. 1991). Cowpea (*Vigna unguiculata* [L.] Walp) is frequently intercropped with cereals in this zone and is a host of *Striga gesnerioides* (Willd.) Vatke, the second most common species of *Striga* (Aggarwal and Ouedraogo 1989; Lagoke et al. 1991).

These species have coevolved with their respective hosts and have been pests in traditional cropping systems for many years (Pieterse and Pesch 1983; Ogborn 1987; Lagoke et al. 1991). Because the traditional cropping systems involved prolonged fallow, crop rotations, and mixed cropping, populations of *Striga* spp. were kept at tolerable levels (Ogborn 1987). With increasing population pressure, however, the demand for increased food production, monocropping, and the intensification of land use, with little or no fallow, populations of these parasites have gradually increased and become threats to food production (Pieterse and Pesch 1983; Ogborn 1987).

Yield losses from these pests are augmented when plants are already in poor health because of drought and low soil fertility. Cost of fertilizers, lack of viable control opportunities, and paucity of resistant cultivars make alleviation of the problem difficult for the African farmer (Bebawi and Abdelaziz 1983; Ogborn 1987; Anon 1989). Estimates in the literature indicate frequent yield losses above 50% attributable to *Striga* spp. on all of the host crops (Pieterse and Pesch 1983; Dodgett 1984; Aggarwal and Ouedraogo 1989; Anon 1989). Arable fields are often abandoned because of prohibitive parasite populations (Anon 1989; Lagoke et al. 1991). Equally alarming are field reports which indicate that the range of these parasitic plants appears to be increasing (Anon 1989; Lagoke et al. 1991).

Species of *Striga* reproduce prodigiously and are capable, in a single crop season, of producing 50,000–500,000 seeds per parasite plant (Pieterse and Pesch 1983). Sorghum fields heavily infested with *S. hermonthica* have been reported to yield more than 900,000 flowering *S. hermonthica* plants per hectare (Dodgett 1965); this would result in $4.5 \times 10^{10}$ parasite seeds per hectare. These seeds are viable for 7–14 years (Saunders 1933; Bebawi et al. 1984).

Despite the seriousness of these parasites and their capability for reproduction, little is known about mechanisms governing their seed dispersal. Because of the small size of these seeds (0.20–0.50 mm long) (Musselman and Parker 1981; Pieterse and Pesch 1983), wind has been assumed to be a major dispersal mechanism (Howe and Smallwood 1982; Pieterse and Pesch 1983). However, *Striga asiatica* (L.) Kuntze has never moved, as wind-dispersed seed would be expected to do, from the original areas of infestation in the United States (Eplee 1981; Sand 1990). There have been no studies on wind dispersal of seeds of *Striga* spp. in Africa.

Contamination of crop seeds with weed seeds is well known, and it is a measure of seed purity (International Seed Testing Association 1976). Although seeds of *Striga* spp. are listed in the United States Federal Noxious Weed Act (Gunn and Ritchie 1988), they have not been documented as being a crop-seed contaminant. The possible importance of this mechanism of dispersal in Africa is unknown.

Incubation of *S. hermonthica* seeds in sheep rumen liquor was shown to generally result in reduced seed viability (Bebawi and El Hag 1983). In another study, however, maize, grown in dung from cattle fed with *Striga* spp. plants, became severely infected (Farquar 1937). Under natural conditions, there are no reports on dispersal of *Striga* spp. seeds through dung or on viability of *Striga* spp. seeds extracted, after prolonged exposure, from dung.

To slow or stop the spread of *Striga* spp., the mechanisms of dispersal need to be better understood. With this knowledge, control strategies could be more effectively structured. The objectives of this study were to examine the roles of wind, host crop seeds, and cattle in the dissemination of *Striga* spp. seed.

* Slightly adapted from an article of the same title originally published by the authors in *Plant Disease* 78(4): 402–406 (1994). 

## Materials and Methods

**Dissemination by wind.** These studies were conducted in the southern Guinea savanna zone of West Africa. In 1991 in Nigeria, a 1-ha field in Mokwa (9° 35' N, 5° 11' E), and a 0.5-ha field in Abuja (9° 12' N, 7° 20' E), were artificially infested with *S. hermonthica* by placing 1,500 germinable parasite seeds in each planting hole 7 days prior to host planting (Ransom et al. 1990). In the Mokwa field, two susceptible maize cultivars (8321–21 and 8425–8) and two susceptible sorghum cultivars (Mokwa local and CK60B) were planted on 3 July in the infested holes in 4-row × 10-m plots. Crop planting density was 53,333 plants $ha^{-1}$, using a 75 × 25 cm spacing. Five plots of each crop or cultivar were planted in a completely randomized design. In the Abuja field, only the susceptible maize cultivars were used. Planting was done on 30 July. Other conditions were the same as for the Mokwa trial. Growth duration of the maize cultivars was 115 days, while that of the sorghum cultivars was 90 days for CK60B and 150 days for Mokwa local. A swath of 40 m around each field was kept free from vegetation that could have impeded the free movement of parasite seeds. Plots were situated so that no trees were in this 40 m swath. Any trees within the fields were left standing, according to local farming practices, and the crops were established around them.

To assay the horizontal extent of wind dispersal of *S. hermonthica* seeds, petrolatum-coated microscope slides were placed in vegetation-free areas outside the fields at intervals of 10, 20, 40, 80, 160, 320, 640, 1280, and 2560 cm away from stands of mature *S. hermonthica* plants on the field borders. Ten radii of slides spaced at these intervals (10–2560 cm) were placed around each field at the time of *S. hermonthica* flowering. Thus there were 10 replications of each distance at each sampling time for each field. The slides were tied to bamboo stakes at 30 cm above the ground. A wooden stick, 1 cm diam, was placed behind the lower edge of the slide, to tilt it upward at an angle of 45°. The entire exposed face of each slide was coated with petrolatum, but only seeds on the center 6.5 $cm^2$ of the slide were counted.

To assay vertical dispersal, slides were hung at 1-, 2-, and 3-m heights from trees within the fields and from trees within a 0.25 km radius of the fields. In each area (within and outside of the field), and in each location, five replications of each height were used. Both faces of the slides hung from trees were coated with petrolatum, and seeds on the center 6.5 $cm^2$ of each face were counted. All slides were changed weekly for 8 weeks, and numbers of captured seeds were counted. Deployment of slides began 116 days after planting (DAP) and continued through 175 DAP. Because *S. hermonthica* seed maturity and release coincide with the onset of the dry season in Nigeria, rain had no effect on the quality of data collected.

In 1992, this dispersal study was repeated at Hadagon (7° 00' N, 2° 10' E), in the Republic of Benin. A 0.5-ha field was infested as described earlier with 1,000 germinable *S. hermonthica* seeds/hill and planted on 21 September during the second rainy season. A 115-day susceptible maize cultivar, 8338–1, was used. Deployment of slides began 63 DAP and continued through 98 DAP. *S. hermonthica* seeds were counted as described earlier. As in 1991, maturity and release of parasite seeds coincided with the onset of the dry season and rain had no effect on data quality.

**Dissemination with crop seeds.** During the postharvest seasons of 1991 and 1992 (December to January) seeds of cowpea, maize, millet, and sorghum were collected from local markets in Abuja, Bida, Kaduna, Kano, Mokwa, and Zaria. These areas are representative sites in the *Striga*-infested savanna of Nigeria. In local markets, crop seeds, whether for consumption or planting, are displayed and sold from large pans. To ensure that small *Striga* seeds could be detected in the grain samples, the upper portion of grain in the pan was removed and only the bottom 1–3 kg of seeds (where small particles settle) were sampled. Sample sizes varied due to seed availability from the sellers. One randomly chosen sample was purchased from each of eight markets in each location.

After collection, seed samples were taken to a laboratory at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria and *Striga* spp. seeds in the samples were isolated. Initially seeds were separated by turbulent, flowing water in a cabinet-top elutriator (Eplee and Norris 1990) and particles the size of the *Striga* seeds were collected on a 90-µm mesh sieve. Particles on the sieve were then backwashed with water into a separatory column containing $K_2CO_3$ of specific gravity 1.4. Particles of approximately the same weight as an intact *Striga* spp. seed ($5 \times 10^{-6}$ g) were suspended around the $H_2O$-$K_2CO_3$ interface, while empty *Striga* spp. seed coats and other lightweight particles were suspended at the surface of the water layer. Particles backwashed into the separatory column were allowed to settle for 20 min. The column was then emptied, without disturbing the suspended layers, through a stopcock at the bottom. Particles were collected on 60-µm mesh sieves, and *Striga* spp. seeds were counted under a dissecting microscope. Seeds were differentiated only at the genus level.

Statistical comparisons were made only for average number of seeds per sample for the 2 years of collection. Statistical comparisons between locations were not made because a general inference was desired only for the *Striga*-endemic area of Nigeria. Comparison of locations, without data on the large number of associated variables that could affect seed lot contamination, would have had little practical value.

**Dissemination by cattle.** After harvest of maize and sorghum in the 1991 study, cattle (*Bos* sp.), managed by local farmers, were allowed to graze in and around the *S. hermonthica*-infested fields. Fields used in these studies were isolated from other *S. hermonthica*-infested fields, so there was no overlap in grazing between infested areas. Two weeks after grazing, 30 samples of 10 dung droppings each were collected from within each infested field, and another 30 samples were collected from a radius of 0.5 km around the fields.

After harvest in 1992, cattle dung was collected from 45 random locations in the savanna zone of northern Nigeria. Within these locations, one sample consisting of 10 droppings was collected from within a *S. hermonthica*-infested field (38 samples from 45 locations) and one was collected from noninfested fields in the same area (50 samples from 45 locations).

In both years, the samples were taken to an IITA laboratory in Ibadan, where *Striga* spp. seeds were isolated. No attempt was made to differentiate species of *Striga* based on seed morphology. To isolate *Striga* spp. seeds, samples were dissolved in water for 1 day, then seeds were separated by elutriation and $K_2CO_3$, as previously described. Particles were collected separately on 60-µm mesh sieves, and intact *Striga* spp. seeds and seed coats were counted. Viability of intact seeds

was determined by the tetrazolium chloride embryo staining technique (Bebawi et al. 1984).

## Results

**Dissemination by wind.** In all locations, seed catches declined sharply with increasing distance from the seed source. Seed catches at distances greater than 80 cm ($\log_{10} = 1.9$) declined to less than a half of those at 10 cm. In all locations, maximum extent of dispersal was 1280 cm ($\log_{10} = 3.10$) at a height of 30 cm.

In Mokwa, the greatest number of seeds caught per slide at 1280 cm was 1.5 at 160 DAP. At 640 cm ($\log_{10} = 2.8$), the greatest number of seeds caught per slide was 3.5 at 160 DAP (Fig. 1). The largest seed catch, averaged over all slides, was at 145 DAP. A few seeds were caught as early as 116 DAP, but the bulk of seed dispersal appeared to be between 145 and 160 DAP.

In Abuja, the greatest number of seeds caught per slide at 1280 cm was 1.3 at 160 DAP (Fig. 1). At 640 cm, the greatest number caught per slide was 3.3 at 147 DAP. The largest seed catch was also at 147 DAP. Only small amounts of seeds were caught at any distance before 130 DAP and after 160 DAP, and the period of maximum dispersal was between 138 and 147 DAP.

In Hadagon in 1992, the greatest number of seeds caught per slide at 1280 cm was 1.2 at 91 DAP (Fig. 1). At 640 cm, the greatest number caught per slide was 1.3 at 84 DAP. The period of substantial seed catches was between 77 and 91 DAP. Outside this range, few seeds were caught at any distance.

The period of maximum seed dispersal in all locations was from 25 November through 6 January, which is the middle of the annual harmattan season.

The maximum vertical distance at which *S. hermonthica* seeds were caught was 2 m. A maximum of 10 seeds was caught at this height and only from traps in the 15 trees within infested fields. No seeds were caught on any of the 45 tree-hung traps outside the infested areas.

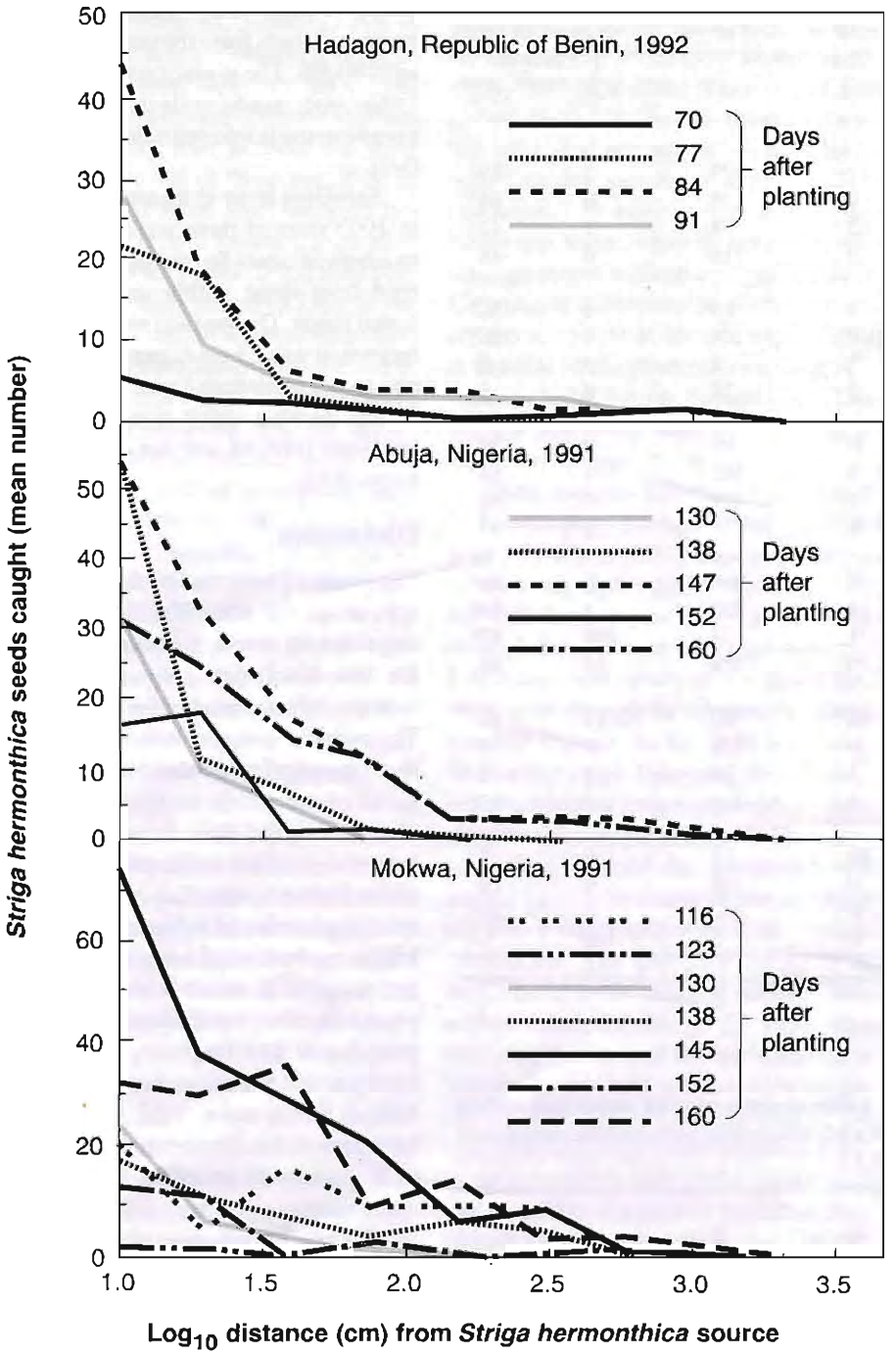


**Figure 1. Average number of *Striga hermonthica* seeds caught on coated microscope slides placed at 10, 20, 40, 80, 160, 320, 640, 1280, and 2560 cm from mature *S. hermonthica* plants. Data were collected from a mix-planted sorghum and maize field in Mokwa, Nigeria and a maize field in Abuja, Nigeria in the early dry season of 1991, and from a maize field in Hadagon, Republic of Benin in the early dry season of 1992. Data points are averages of 10 samples taken at the indicated times after planting.**

**Dissemination with crop seeds.** *Striga* spp. seeds were found in all grain samples. The amounts found in the different crop seed samples varied each year. The overall contamination of crop seeds was 19.4% (42/216 samples) in 1991. An average of 16.6% samples (9/54) of cowpea, 33.3% (18/54) of millet, 14.8% (8/54) of sorghum, and 13.0% (7/54) of maize (Table 1) were contaminated with *Striga* seeds. In 1991, the largest amounts of *Striga* seeds in a single sample were found in millet (over 300) and sorghum (over 200) from the Kano market (Table 1). The greatest frequency of contamination in 1991 was also found in Kano in all of the crop seeds, except for cowpea. Contamination of maize seeds was found only in Kano and Kaduna, the latter being the principal maize-growing region of Nigeria.

In 1992, seed samples of all crops from all sites contained *Striga* seeds. Relatively few market samples did not contain parasite seeds. The overall *Striga* seed contamination of crop seeds was 63.9% (138/216) of all the samples collected. An average of 61.1% samples (33/54) of cowpea, 55.5% (30/54) of millet, 62.9% (34/54) of

sorghum, and 75.9% (41/54) of maize were contaminated with *Striga* seeds (Table 1). These numbers were much higher in 1992 than in 1991, particularly for cowpea and maize. Maximum and average numbers of *Striga* seeds in the samples were also much greater for all crops (Table 1), but particularly so for cowpea and maize.

**Dissemination by cattle.** Few *Striga* spp. seeds were found in dung samples from areas outside infested fields in the 1991 study (Table 2). Approximately twice the number of *Striga* spp. seed coats as intact seeds—638 vs 387—were found in the dung samples. Considering total number ingested, this results in 37.7% intact seeds after passage through the cow gut. The average number of intact *Striga* spp. seeds per sample from within infested fields was 7 (range 0–98). As 30 samples were taken from each field, this would yield a reinfestation of 210 intact seeds per preinfested field. Viability of intact seeds was 22.0%, leaving a reinfestation of 46 viable seeds per infested field, or, overall, 8.3% of the total ingested (22% viability of 37.7% intact seeds). Viability of freshly harvested seeds from the same fields averaged 80.5%. The average number of intact *Striga* spp. seeds collected from dung samples outside infested fields was 0 (range 0–2).

Sampling from 45 locations (88 fields) in 1992 showed there were nearly equal numbers of intact *Striga* spp. seeds recovered from dung within and outside infested fields. The average number of seeds recovered was 23 seeds per infested field, and 15 per noninfested area. Viability was 21.6%, leaving infestations of 5 and 3 seeds per infested and noninfested areas, respectively.

**Table 1. Presence of *Striga* spp. seeds in samples[1] of cowpea, maize, millet, and sorghum seeds collected from eight markets in each of six locations in Nigeria during 1991 and 1992 postharvest seasons.**

| Location | No. of samples with seeds | | Maximum no. of seeds in any sample | | Mean no. of seeds per sample[2] | |
|---|---|---|---|---|---|---|
| Crop | 1991 | 1992 | 1991 | 1992 | 1991 | 1992 |
| Abuja | | | | | | |
| Cowpea | 1 | 8 | 12 | 227 | 2 | 88* |
| Maize | 0 | 8 | 0 | 148 | 0 | 84* |
| Millet | 3 | 7 | 2 | 88 | 1 | 42* |
| Sorghum | 0 | 7 | 0 | 116 | 0 | 48 |
| Bida | | | | | | |
| Cowpea | 1 | 6 | 4 | 89 | 1 | 36* |
| Maize | 0 | 8 | 0 | 222 | 0 | 108* |
| Millet | 4 | 1 | 1 | 1 | 0 | 0 |
| Sorghum | 2 | 3 | 2 | 25 | 0 | 5 |
| Kaduna | | | | | | |
| Cowpea | 0 | 6 | 0 | 98 | 0 | 29 |
| Maize | 3 | 8 | 9 | 162 | 1 | 54* |
| Millet | 0 | 6 | 0 | 101 | 0 | 30 |
| Sorghum | 0 | 7 | 0 | 71 | 0 | 31 |
| Kano | | | | | | |
| Cowpea | 0 | 6 | 0 | 200 | 0 | 45* |
| Maize | 4 | 6 | 14 | 250 | 2 | 56* |
| Millet | 8 | 5 | 321 | 74 | 109 | 19* |
| Sorghum | 5 | 7 | 230 | 388 | 64 | 86 |
| Mokwa | | | | | | |
| Cowpea | 4 | 5 | 36 | 90 | 11 | 30 |
| Maize | 0 | 6 | 0 | 178 | 0 | 74* |
| Millet | 3 | 3 | 3 | 38 | 1 | 8 |
| Sorghum | 1 | 8 | 3 | 345 | 0 | 92* |
| Zaria | | | | | | |
| Cowpea | 3 | 2 | 30 | 15 | 6 | 3 |
| Maize | 0 | 5 | 0 | 36 | 0 | 10 |
| Millet | 0 | 8 | 0 | 202 | 0 | 80* |
| Sorghum | 0 | 3 | 0 | 2 | 0 | 1 |

1. Samples were cleaned, market-quality seed. Sample sizes ranged from 1 to 3 kg, depending on quantity available from the seller.
2. All averages have been rounded to nearest integer.

* = Significant difference between years ($P \leq .05$).

**Table 2. Presence of *Striga* spp. seeds in cattle dung samples collected within *Striga hermonthica*-infested fields and from a 0.5 km radius outside infested fields during December 1991 and December 1992.[1]**

| Sample sites | No. of samples | | No. of intact seeds | | No. of seed coats | | Mean no. of intact seeds/ sample/ field | |
|---|---|---|---|---|---|---|---|---|
| | 1991 | 1992 | 1991 | 1992 | 1991 | 1992 | 1991 | 1992 |
| Within infested fields | 60 | 38 | 387 | 857 | 638 | 24 | 7 | 23 |
| Within 0.5 km radius of infested fields | 60 | 50 | 5 | 730 | 10 | 35 | 0 | 15 |

1. During 1991, 30 samples of 10 droppings each were taken from each site in two fields. During 1992, individual samples of 10 droppings were taken from each field in 45 randomly selected locations in northern Nigeria.

## Discussion

The results of these studies show that *Striga* spp. seeds are not efficiently or widely dispersed by wind. Supporting evidence for this conclusion comes from the *S. asiatica*-infested areas of the United States. The parasite was accidentally introduced to maize-growing areas of North and South Carolina sometime in the 1950s (Sand 1990). Since that time, the parasite has not appeared in other maize-producing areas of the United States. This has been due to strict quarantine of infested areas (Eplee 1981), but had wind been a primary dispersal agent, *S. asiatica* would have appeared in other areas despite quarantine procedures. The frequency and force of hurricanes along the eastern coast of the United States since 1950 would surely have provided sufficient wind for dispersal of *S. asiatica* to areas far from those in quarantine.

The relative unimportance of wind as a dispersal agent is particularly relevant when control options are being considered. Had this study shown widespread wind dispersal of *Striga* spp. seeds, the option of localized eradication would not be feasible and control efforts would be best directed to limiting host damage by host plant resistance or crop protection. As it is, localized eradication should be

possible by a combination of exclusion of new influxes of the parasite, crop rotations to reduce soil levels of parasite seeds, and methods to stop parasite reproduction.

The prevalence of *Striga* spp. seeds in market samples of crop seeds indicates the importance of this mechanism of dispersal when seeds are used as planting materials. Although differences in crop seed contamination were observed both years, it is not clear whether these differences reflect increasing amounts of contamination or increased proficiency of sampling and seed isolation processes. However, the overall levels of contamination indicate that this mechanism may well account for most new establishments of these parasites. In this study, crop seeds were sampled between December and January, soon after crops had been harvested. Local seed purchases for planting materials later in the year (May–July) might be expected to contain even greater amounts of parasite seeds, as these small seeds settle near the bottom of crop seed containers, and the uppermost contents of the containers would have been gradually sold or consumed since harvest the previous season.

Because *S. hermonthica* is obligately allogamous (Safa et al. 1984), at least two viable plants would have to survive to cross and establish a new infestation focus. Many samples contained over 50 seeds. However, with autogamous *Striga* species such as *S. gesnerioides* and *S. asiatica* (Musselman 1987), only one seed is sufficient to establish a new focus of infestation. Contaminated imported seed stocks may have been the source of the initial *S. asiatica* infestation in the United States, which subsequently developed into a serious long-term agricultural problem.

Of significance is the source of the samples in these studies. All were market-quality seeds that had been thoroughly winnowed and contained little, if any, visible field trash, such as leaves, husks, and panicle or pod fragments. Seeds saved by farmers for planting in the following season are not of this market quality, and they probably contain even greater amounts of *Striga* spp. seeds. The reason for this amount of contamination is probably the method of harvest of these crops. Stalks of cereal crops are cut at the base and laid in rows in the field to dry. After drying, the grains are threshed and stalks are used as building materials. In *Striga*-infested fields, *Striga* plants are frequently intermixed with the drying grain crop and parasite seeds become intermixed with crop seeds upon threshing. Since cowpea is often intercropped with cereals, harvest of cowpea incorporates not only the cowpea parasite (*S. gesnerioides*) but also the cereal parasite (*S. hermonthica*).

The best solution to contamination of crop seeds with lightweight *Striga* spp. seeds is field sanitation. Farmers need to be made aware of the ease with which their planting materials can become contaminated, and they must learn to avoid laying crops in the vicinity of these parasites. Plant quarantine services and seed industries also need to be aware of potential crop seed contamination with *Striga* spp. Unaided sieving of crops seeds is inadequate to remove the lightweight *Striga* spp. seeds, which do not pass readily through sieves without external pressure. Cleaning of contaminated seeds by washing or vacuum (D. K. Berner, unpublished) is feasible at the plant quarantine or seed industry level, but the current procedures involve too much time and labor to be viable on farmers' fields.

Dung samples collected from outside *S. hermonthica*-infested fields contained relatively few parasite seeds in either year of the study. After the 1991 study, it was felt that because passage of green matter through the cattle gut may take more than 1 or 2 days, our survey area may not have been wide enough to adequately sample material ingested in the field and deposited farther away. However, the relatively high numbers of *Striga* spp. seeds found in dung samples within infested fields in 1991 seemed to indicate that greater amounts should have been found in the surrounding area if this was an important dispersal mechanism. The low percentages of intact, viable seeds in dung samples from within infested fields in the 1991 study indicated that animal ingestion and deposition may be only a short-distance and relatively minor dispersal mechanism. The results of the 1992 study confirmed this, as an average of only three viable seeds per noninfested area was found. If these were *S. hermonthica* seeds, the probability of only three seeds establishing a new focus of parasite infestation is low, since this species is obligately allogamous.

The importance of dispersal of seeds on animal hooves and fur, however, was not addressed in this study. Because animal herds roam widely across the savanna zone of Africa, this possible mechanism needs to be examined in more detail. There have been no reports of *Striga* spp. seed dispersal by birds.

A mechanism that may account for widespread parasite seed dispersal is the transportation (and often sale) of cowpea fodder from infested fields to areas deficient in animal feed during the dry season. Depending on location, inspection of the contents of any bundle of fodder could reveal the presence of seed bearing *S. hermonthica*, *S. aspera* (Willd) Benth., *S. gesnerioides*, and *Alectra vogelii* Benth., either individually or collectively, since all of these parasites can be found within a single field. Control of this means of dispersal in Africa will be very difficult during times of critical need for animal feed. Spread of parasite seeds by fodder can be arrested only by the localized reduction of parasite populations from fodder-producing areas.

The overall results of these studies indicate that man, through agricultural produce and animal movement, is the primary factor in dispersal of *Striga* spp. This spread can be controlled through farmer education and better awareness among staff of plant quarantine services. Because annual influxes of *Striga* spp. seed by wind do not appear to occur in farmers' fields, localized eradication could be made effective by preventing recontamination of fields by man and through appropriate control measures aimed at existing *Striga* spp. populations.

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# Independent inheritance of *Striga* and *Alectra* resistance in cowpea genotype B301*

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**The production of cowpea, a major food legume in the semi-arid regions of sub-Saharan Africa, is threatened by two parasitic weeds, *Striga* and *Alectra*. Yield losses can be as high as 80%. IITA scientists have been attempting to incorporate host plant resistance into improved cowpea genotypes, as well as studying cultural practices to contain the weeds. Genetic studies, such as the one reported here, help to better understand and build resistance in the cowpea plant.**

## Introduction

Cowpea is a major food legume in the semi-arid region of sub-Saharan Africa. Recently, two parasitic weeds, *Striga gesnerioides* (Willd.) Vatke and *Alectra vogelii* (Benth.), have become major threats to its cultivation in this region. Yield losses can exceed 80%. Host plant resistance is accepted as the most practical and economical strategy to control these weeds (Aggarwal 1985).

Several sources of resistance have been identified (IITA 1982; Riches 1987; Bailey and Terry 1990; Singh and Emechebe 1990b), of which B301, a landrace from Botswana, has shown complete resistance to both weeds (Bailey and Terry 1990). Genetic studies have revealed in B301 a single dominant gene for *Striga* resistance and duplicate dominant genes for *Alectra* resistance (Singh and Emechebe 1990a,b; Singh et al. 1993). This line has thus been used as a donor parent (Singh and Emechebe 1991), and many improved, resistant breeding lines are currently undergoing adaptability trials across Central and West Africa. They are being used also as parents in a crossing program involving local cultivars, to develop a range of improved varieties differing in plant type, maturity, and seed characteristics to suit different cropping systems and regional preferences.

The present study was undertaken to ascertain whether the genes controlling resistance to these parasites are independent of each other.

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**Table 1. Segregation for *Striga* and *Alectra* resistance in the different cowpea populations.**

| Population | Generation | Resistant to both *Striga* and *Alectra* | Resistant to *Alectra*, susceptible to *Striga* | Resistant to *Striga*, susceptible to *Alectra* | Susceptible to both *Striga* and *Alectra* | Genetic ratio | $X^2$ | *P*-range |
|---|---|---|---|---|---|---|---|---|
| | | ----------Number of plants---------- | | | | | | |
| B301 | $P_1$ | 68 | 0 | 0 | 0 | | | |
| IT84S–2246–4 | $P_2$ | 0 | 0 | 0 | 39 | | | |
| IT84S–2246–4 × B301 | $F_1$ | 17 | 0 | 0 | 0 | | | |
| B301 × (B301 IT84S–2245–4) | $BC_1F_1$ | 28 | 0 | 0 | 0 | | | |
| B301 × IT84S–2246–4 | $F_2$ | 349 | 118 | 15 | 8 | 45:15:3:1 | 2.92 | 0.30–0.50 |
| IT84S–2246–4 × (B301 × IT84S–2246–4) | $BC_1F_1$ | 10 | 8 | 1 | 4 | 3:3:1:1 | 1.91 | 0.50–0.70 |

## Materials and Methods

This study was conducted at the Kano Station of the International Institute of Tropical Agriculture (IITA) in Nigeria, and at the Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria, Nigeria. Materials were derived from the cowpea germplasm line B301, resistant to both *Striga* and *Alectra*, and a susceptible cultivar IT84S–2246–4, which is resistant to several diseases and insect pests and has high yield potential. Adequate $F_1$, $F_2$, and backcross seed from crosses involving these parents were developed in the screenhouse in 1989–1990 and then screened for combined resistance to *Striga* and *Alectra* in 1991 by a pot culture technique.

Plastic pots (13 cm diam, 13 cm deep) were used for screening. Each pot contained 1 L (1:1 vol/vol) of unsterilized sieved sand : topsoil mixture, previously inoculated uniformly with about 800 seeds each of *Striga* and *Alectra*. Two cowpea seeds were planted per pot. Pots were kept on benches in the screenhouse and watered daily. Weeds other than *Striga* and *Alectra* were removed. The experiment was terminated 10 weeks after planting (WAP) by submerging each pot in a 20 L bucket of water for about 5 min, and washing the soil off the plant roots. The roots of each plant were separated gently, and the number of *Striga* and *Alectra* attached to each plant were counted. Data on the number of resistant and susceptible plants were subjected to the chi-square test for goodness of fit to different genetic ratios.

## Results and Discussion

Emergence of *Striga* and *Alectra* seedlings on susceptible plants began about 6 WAP, and the differences between resistant and susceptible plants had become clear by 8 WAP. The infected plants showed leaf chlorosis, stunted growth, and partial defoliation, all of which were visible on some plants even before the parasitic weeds emerged. However, we classified the plants as resistant or susceptible only after washing the roots and observing attachment of the parasites.

All plants of B301, $F_1$, and backcross $F_1$ involving B301 were free of infections from both *Striga* and *Alectra* (Table 1), indicating that dominant genes govern their resistance. All plants of IT884S–2246–4 were susceptible to both *Striga* and *Alectra*.

**Table 2. Contingency table showing segregation for resistance to *Striga* and *Alectra* in $F_2$ population of cowpea.**

| Segregation for *Alectra* | Segregation for *Striga* Resistant | Susceptible | Total |
|---|---|---|---|
| Resistant | 349 | 118 | 467 |
| Susceptible | 15 | 8 | 23 |
| Total | 364 | 126 | 490 |

$X^2$ for 3:1 *Striga* resistance segregation = 0.13 ($P$ = 0.75–0.95)
$X^2$ for 15:1 *Alectra* resistance segregation = 2.03 ($P$= 0.10–0.20)
$X^2$ for ratio for independence of *Striga* and *Alectra* resistances = 0.76 ($P$ = 0.75–0.90)

**Table 3. Contingency table showing segregation for resistance to *Striga* and *Alectra* in $F_1$ backcross involving the parent, IT84S–2246–4.**

| Segregation for *Alectra* | Segregation for *Striga* Resistant | Susceptible | Total |
|---|---|---|---|
| Resistant | 10 | 8 | 18 |
| Susceptible | 1 | 4 | 5 |
| Total | 11 | 12 | 23 |

$X^2$ for 1:1 *Striga* resistance segregation = 0.04 ($P$ = 0.80–0.90)
$X^2$ for 3:1 *Alectra* resistance segregation = 0.23 ($P$ = 0.50–0.70)
$X^2$ for ratio for independence of *Striga* and *Alectra* resistances = 1.75 ($P$ = 0.10–0.20)

When resistance to the two parasitic weeds was considered separately, the $F_2$ plants segregated into 364 resistant : 126 susceptible for *Striga* and 467 resistant : 23 susceptible for *Alectra*, giving close fits to 3:1 and 15:1 ratios, respectively (Table 2).

These results were consistent with the data from the backcross population involving IT84S–2246–4, which segregated in a ratio of 11 resistant : 12 susceptible for *Striga*, and 18 resistant : 5 susceptible for *Alectra*, as expected for monogenic (1:1) and digenic (3:1) inheritance, respectively (Table 3). The data confirmed previous results on the inheritance of resistance to *Striga* and *Alectra* (Singh and Emechebe 1990a,b; Singh et al. 1993).

When the combined reactions to both *Striga* and *Alectra* were considered, the $F_2$ segregated into 349 plants resistant to both *Striga* and *Alectra*, 118 susceptible to *Striga* and resistant to *Alectra*, 15 susceptible to *Alectra* and resistant to *Striga*, and 8 susceptible to both *Striga* and *Alectra* (Table 1). These results fit closely to a 45:15:3:1 ratio expected for trigenic independent inheritance, involving one dominant gene for one character and duplicate dominant genes for the other.

The backcross involving IT84S–2246–4 segregated into a ratio of 10 resistant to both *Striga* and *Alectra* : 8 susceptible to *Striga* and resistant to *Alectra* : 1 susceptible to *Alectra* and resistant to *Striga* : 4 susceptible to both *Striga* and *Alectra* (Table 1). This gave a close fit to the expected 3:3:1:1 ratio and confirmed the $F_2$ segregation. The independent chi-square analysis of $F_2$ and backcross data with contingency tables further confirmed independent inheritance of resistance to *Striga* and *Alectra* (Tables 2 and 3). The $F_2$ as well as backcross data had fewer plants susceptible to *Alectra* than expected, but the differences were not significant. This may be due to occasional escapes.

We concluded from this study that the single dominant gene for *Striga* resistance and the duplicate dominant genes for *Alectra* resistance are nonallelic and independent of each other. Thus, breeding for resistance will require testing individual plants or lines for resistance to both parasites. If $F_2$ plants are selected initially for *Striga* resistance, most of them (15/16) will still be resistant to *Alectra*, which greatly facilitates selection for combined resistance in subsequent generations. Good progress has already been made in incorporating *Striga* and *Alectra* resistance into acceptable improved cultivars using B301 as the resistant source (Singh and Emechebe 1991). A set of isogenic lines is being developed to facilitate future studies on the biochemical nature of resistance to *Striga* and *Alectra*.

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# Persistence and recovery of introduced *Rhizobium* ten years after inoculation on *Leucaena leucocephala* grown on an Alfisol in southwestern Nigeria*


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**Sustainable agriculture depends on many factors. Among them is soil fertility, which can be enhanced by growing trees along food crops, as in alley cropping, and by the use of rhizobial inoculants in place of commercial fertilizer. One issue that has received little attention in this context is how long the introduced rhizobia can stay viable and continue to fix nitrogen (and thus enrich the soil). This article discusses that specific issue. Combined with other studies on management practices that will maximize returns from alley cropping, it provides useful information for developing sustainable systems of agriculture.**


## Introduction

Because of its $N_2$-fixing ability and exceptional capacity to produce biomass, forage, and wood, and to improve soil fertility, *Leucaena leucocephala* (Lam) de Wit., a fast-growing, tropical leguminous tree species, is used as a hedgerow tree in alley farming systems (Kang et al. 1981). In many soils, however, *L. leucocephala* requires inoculation with the appropriate and specific *Rhizobium* to provide $N_2$ fixation, particularly where *L. leucocephala* has not previously been cultivated (Halliday and Somasegaran 1983; Sanginga et al. 1985, 1988).

In 1982, field experiments were undertaken at Ibadan (transition forest-savanna zone) and at Fasbola (savanna zone, 70 km north of Ibadan), in southwestern Nigeria to examine the $N_2$-fixing efficiency of various strains of *Rhizobium* and select those that proved most efficient. These studies indicated that in 6 months, inoculated *L. leucocephala* fixed 196–264 kg N $ha^{-1}$ $yr^{-1}$, equivalent to about 40% of the total N in the plant (Sanginga et al. 1989). Inoculated plants produced as much biomass as those fertilized with 150 kg N $ha^{-1}$, and more biomass than noninoculated plants.


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Inoculation with an effective and persistent *Rhizobium* strain has several advantages, and it is preferable to the repeated application of N fertilizers. This is particularly true for alley farming systems where hedgerow trees are left in the field for a long period (more than 10 years) without additional input of N fertilizer. Despite its importance, there is as yet little information concerning the fate of introduced rhizobia in alley cropping fields.

To become established in the field, introduced rhizobia must coexist with competitors and predators and maintain themselves during periods of low nutrient availability (Alexander 1986). Fluctuating environmental conditions and management practices can also influence the survival of inoculants in the field. An understanding of the changes occurring in *Rhizobium* populations and the factors influencing such changes may lead to improved use of *L. leucocephala* inoculants, and to the selection of adapted inoculant strains.

The present study evaluated whether *Rhizobium* strains IRc 1045 and IRc 1050, introduced in 1982 as *L. leucocephala* seed inoculants, persisted when the field was left fallow for 10 years, and how much they then sustained *L. leucocephala* growth and biomass production.

## Materials and Methods

The experiment was conducted in a field at the International Institute of Tropical Agriculture (IITA), Ibadan (Sanginga et al. 1988). The soil, an Alfisol of the Iwo series, has the following physicochemical characteristics: 0.14% total N; 9.2 mg kg$^{-1}$ P (Bray 1); 0.92% organic C; pH of 6.0; CEC 4.6 Cmol kg$^{-1}$; 9% clay; 85% sand; and 6% silt.

Single strains of *Rhizobium* IRc 1045 (isolated from *L. leucocephala* grown in Fashola soil) and IRc 1050 (isolated from *L. leucocephala* grown in Ibadan) were used separately to inoculate *L. leucocephala* in the 1982 inoculation trial (Sanginga et al. 1988). These were applied to the seed as peat-based inoculants, using Nitracoat adhesive (Nitragin Co.), and inoculated seeds were planted in moist soil. The noninoculated and fertilizer N treatments had also been used in this trial, with 150 kg N ha$^{-1}$ of urea applied once, at planting. Two years after planting, *L. leucocephala* was uprooted and assessed for its response to inoculation (Sanginga et al. 1988). The field was used for a soybean multiplication trial in 1985, and then left fallow for about 8 years.

In 1992, the field was cleared and noninoculated *L. leucocephala, Senna siamea,* and *S. spectabilis* were sown in the previous *L. leucocephala* plots.

The experiment was laid out as a split plot design, with 3 replicates. The main treatments were (1) previously noninoculated, (2) inoculated with *Rhizobium* IRc 1050 or 1045, and (3) noninoculated plus N fertilizer; the three woody legumes were the subtreatments: the $N_2$-fixing *L. leucocephala* and two non-$N_2$-fixing plants *(S. siamea* and *S. spectabilis)*, giving a total of 36 plots. Each plot measured 96 m$^2$ (8 × 12 m), with 3 rows spaced 2 m apart and a planting distance of 1 m within rows. One month after planting, 20 kg N ha$^{-1}$, as ammonium sulfate labeled with 10 atom % $^{15}$N excess, was applied onto isotope subplots (6 m$^2$) containing the 4 central trees, after the seedlings had been thinned. An adjacent field, which had never been planted with *L. leucocephala,* was used as a control.

Soil samples were collected three times in 1992: (1) at planting; (2) a few months after the onset of the rainy season; and (3) during the dry season, about two months before the first rains. A soil auger of 3 cm diam was used to collect soil at depths of 0–15, 15–30, and 30–60 cm. Ten cores were collected at random in each of the previous plots in the alleys and beneath trees of *L. leucocephala, S. siamea*, and *S. spectabilis*. They were mixed into composite samples and refrigerated.

Rhizobia were enumerated—either immediately or within 1 month of collection—by the most probable number (MPN) method (Alexander 1965), using plastic pouches (Weaver and Frederick 1972). A 10-fold dilution series, with five replications per dilution, was used, with *L. leucocephala* as the legume host. Plants received Jensen's solution (Vincent 1970) as required. The pouches were incubated at 28°C under fluorescent lighting tubes, and nodulation was assessed 35–42 days after inoculation.

Plants from the experimental plots were sampled twice a year, 6 months after planting (at the end of the rainy season) and 6 months still later (at the end of the dry season). At each sampling, the above-ground and below-ground plant materials were harvested. The above-ground plant parts were separated into leaves, stems, and branches, chopped into 10–20 cm pieces; and then oven-dried, weighed, ground, and analyzed for total N and atom % $^{15}$N (Fiedler and Proksch 1975). The isotope dilution method (Fried and Middleboe 1977) was used to calculate $N_2$ fixation.

Roots were carefully removed and examined for nodulation. Fresh nodules were counted, cleaned of soil particles, weighed, and then used for strain identification. From each treatment, 40 nodules were chosen at random and typed, using the ELISA technique for IRc 1050 (Clark and Adams 1977), and the intrinsic antibiotic resistance to 500 mg streptomycin ml$^{-1}$ for IRc 1045 (Schwinghamer and Dudman 1973).

## Statistical analysis

Analysis of variance was performed on the log-transformed numbers of rhizobia per gram of oven-dry (100°C, 24 h) soil. ANOVA was also performed on data of all plant parameters measured, using the SAS software (SAS Institute 1986). LSD (at $P = 0.05$) was calculated to determine statistical differences between treatments.

## Results

Prior to sowing in 1982, rhizobia able to nodulate *L. leucocephala* were less than 300 cells per g of soil. The rhizobia numbers increased at 24 weeks after planting (WAP) due to seed inoculation, and no substantial change occurred thereafter in the two inoculation treatments at 48 WAP, nor after years of fallow in 1992 (Table 1). In general, inoculated plots contained more rhizobia than the noninoculated plots in 1982, but in 1992 rhizobial numbers were the same in both inoculated and noninoculated plots. Plots in the adjacent field and in the noninoculated plus N fertilizer treatments contained the fewest rhizobia.

Soil collected beneath *L. leucocephala* plants had more rhizobia than that collected under *S. siamea* and *S. spectabilis*. Mean rhizobial counts were 26, 5.3, and 4.6 × 10$^3$ for *L. leucocephala, S. siamea,* and *S. spectabilis,* respectively. These numbers were not affected by sampling depths.

In 1982, only inoculated plants were nodulated, and all nodules were produced by the inoculant strains IRc 1045 and IRc 1050 (Table 2). Shoot dry weight and total N were statistically equal in inoculated and N fertilized plants, and they were higher here than in noninoculated and adjacent plots. In 1992, nodules were present in all treatments except in the adjacent field. Assays for the identity of nodule isolates (Table 2) showed that nearly all the nodules from the inoculated

**Table 1. $Log_{10}$ of the numbers of *L. leucocephala* rhizobia $g^{-1}$ of soil in the field before planting and at 24 and 48 weeks after planting (WAP) *L. leucocephala* in 1982 and after planting noninoculated *L. leucocephala* in 1992.**

| Treatments in 1983 | Year of *L. leucocephala* establishment | | | | | |
|---|---|---|---|---|---|---|
| | 1982 | | | 1992 | | |
| | 0 WAP | 24 WAP | 48 WAP | 0 WAP | 24 WAP | 48 WAP |
| Noninoculated | 2.3 | 3.2 | 3.0 | 4.3 | 4.0 | 4.5 |
| Inoculated + IRc 1045 | 2.3 | 5.5 | 5.4 | 5.1 | 5.0 | 4.9 |
| Inoculated + IRc 1050 | 2.3 | 4.8 | 4.9 | 5.2 | 4.9 | 4.7 |
| Noninoculated + N fertilizer | 2.3 | 2.9 | 2.6 | 3.0 | 2.9 | 2.6 |
| Adjacent field | 2.3 | 2.2 | 2.3 | 1.7 | 1.3 | 1.1 |
| LSD 5% within years | NS | 1.8 | 1.5 | 2.0 | 1.8 | 2.1 |
| between years | 1.3 | | | 1.1 | | |

**Table 2. Percent nodules formed by inoculant strains IRc 1045 or IRc 1050 on *L. leucocephala* in 1982 and after 10 years of fallow.**

| Inoculation | Percent nodules formed | | | |
|---|---|---|---|---|
| | IRc 1045 | | IRc 1050 | |
| | 1982 | 1992 | 1982 | 1992 |
| Noninoculated | 0 | 78 | 0 | 18 |
| Inoculated + IRc 1045 | 100 | 98 | 0 | 2 |
| Inoculated + IRc 1050 | 0 | 0 | 100 | 95 |
| Noninoculated + N fertilizer | 0 | 75 | 0 | 15 |
| Adjacent field | 0 | 0 | 0 | 0 |

**Table 3. Total N, proportion, and amount of $N_2$ derived from atmospheric $N_2$ by *L. leucocephala* grown in the field in 1982 and following 10 years of fallow at 24 WAP after planting.**

| Previous treatments | Total N (mg $plant^{-1}$) | | $N_2$ fixed (%) | | $N_2$ fixed (mg $plant^{-1}$) | |
|---|---|---|---|---|---|---|
| | 1982 | 1992 | 1982 | 1992 | 1982 | 1992 |
| Noninoculated | 17 | 22 | 0 | 35 | 0 | 7 |
| Inoculated + IRc 1045 | 44 | 39 | 45 | 43 | 20 | 17 |
| Inoculated + IRc 1050 | 39 | 33 | 40 | 39 | 15 | 13 |
| Noninoculated + N fertilizer | 44 | 37 | 0 | 16 | 0 | 6 |
| Adjacent field | 16 | 17 | 0 | 0 | 0 | 0 |
| LSD (5%) within years | 6 | 3 | 4 | 6 | 5 | 4 |
| between years | 11 | | NS | | NS | |

plants were produced by the introduced rhizobia IRc 1045 (98%) and IRc 1050 (95%). For the noninoculated and N treatments, nodules were due mainly to IRc 1045 (76% as against only 16% for IRc 1050). About 8% of the rhizobia in nodules could not be identified, and they were classified as indigenous rhizobia.

In further analysis, the proportion and amount of $N_2$ fixed was estimated by the isotope dilution technique, with the noninoculated *L. leucocephala* serving as the nonfixing control in 1982, while *S. siamea* and *S. spectabilis* were used as controls in 1992. Nodulated *L. leucocephala* in 1982 and noninoculated plants in 1992 fixed about the same amounts of $N_2$ (approximately 170 kg N $ha^{-1}$), equivalent to about 42% of the total N in the plants (Table 3).

## Discussion

Competitive ability and persistence of rhizobia are among important criteria for the selection of strains to be used as inoculants for legumes, especially in developing countries (Vincent 1970). However, these attributes are among the least studied, especially when rhizobial strains are used as inoculants for perennial legumes (Danso et al. 1992). Information is easily found on the response of some $N_2$-fixing trees, such as *L. leucocephala*, to inoculation with specific strains of rhizobia (Halliday and Somasegaran 1983; Sanginga et al. 1988), but very little information is available on the persistence or the competitive ability of these rhizobial strains in the field.

It is clear from the results presented here that *Rhizobium* strains IRc 1045 and IRc 1050, introduced with *L. leucocephala* seeds, were able to colonize the soil. They were detectable in the soil in high numbers, and able to effectively nodulate *L. leucocephala* 10 years later. The high percentage of nodule occupancy by the introduced rhizobial strains IRc 1050 and IRc 1045 indicates that these strains survived well and outperformed the indigenous strains.

In most reported cases for grain legumes, inoculant rhizobia numbers decrease soon after introduction. For example, when the persistence of *Bradyrhizobium japonicum* in a field soil was monitored for 56 weeks following inoculation (Ellis et al. 1984), significantly more of the inoculant strain than of the indigenous rhizobia was detected within the first 7 weeks. Thereafter, the inoculant rhizobial population decreased to the background level. However, some *Rhizobium* strains, including those used in commercial inoculants, have been successfully established in soils for at least 4 years, after which reinoculation may be necessary (Eaglesham 1989).

Our study indicates that, in addition to high competitiveness and persistence, the introduced strains maintained the same $N_2$-fixing capacities after 10 years of fallow. Thus we can assume that if adequate strains of rhizobia are introduced into a soil, the populations will survive. In this case, inoculation with reasonable populations of persistent rhizobial strains constitutes an evident advantage over N fertilization, which has to be applied frequently for consistent high yields.

Survival of inoculants in the soil has been related to the rate of plant debris decomposition (Schroth et al. 1979). Nutrient deposition and acquisition from decomposing litterfall, and prunings from *L. leucocephala* might explain why rhizobia persist longer in alley farming systems than in annual grain legumes.

Management practices, such as cutting or pruning of hedgerows, also affect populations of rhizobia in the tree rhizosphere and its subsequent nodulation (Sanginga et al. 1990). It was shown that 2–3 weeks after cutting, nodules were decaying at the same time new ones were being formed. The new nodules sustained $N_2$ fixation in the alley farming system, while the decomposing nodules probably released large numbers of rhizobia into the rhizosphere.

Plant species affect the number of rhizobia in the soil. Rhizobial population was greater in the soil collected under *L. leucocephala* than in the soil collected under *S. siamea* and *S. spectabilis*. Marked increases have been observed in native and introduced rhizobia in the rhizosphere of various legumes (Bushby 1984). This phenomenon has been interpreted as being due to (a) selective growth stimulation of the symbiont by its specific host legume; (b) a nonspecific rhizosphere effect, indicating that plant roots provide a more favorable environment than soil for rhizobia and other microorganisms; or (c) as a result of rhizobial release following nodule decays.

Our data substantiate the selective stimulation of rhizobial growth in the root zone of *L. leucocephala*. The results presented here suggest that effectively nodulated plants provide an enriched environment and sustain high populations of root-nodule bacteria. Thus, after 10 years the effective strains still dominate the nodule-forming rhizobial population. Further studies are needed to define the management practices that will ensure high numbers of rhizobia in soils for effective nodulation and $N_2$ fixation of hedgerow trees in long-term alley farming systems.

## Acknowledgement

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# Identification of cowpea viruses and their strains in tropical Africa—an international pilot project*

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**Viruses are only one among various causes of plant diseases. They are uniquely difficult to detect, identify, and characterize. This makes control of viral diseases especially difficult. Since 1990, IITA scientists have carried out a collaborative project with scientists in Canada and in African national programs, which has resulted in improved capability within Africa to identify specific viruses and their strains—a prerequisite for designing suitable control measures. This article summarizes the achievement of that project.**

## Introduction

Characterization and identification of viruses is a prerequisite for developing effective management of viral diseases (Hamilton et al. 1981). In Africa, a large number of economically important virus diseases is yet to be fully characterized and their relationships to similar viruses occurring in other countries are still to be determined. Several plant viruses produce very similar symptoms but are unrelated, and strains of the same virus can produce very different symptoms (Reddy 1990). Moreover, mixed infections of unrelated viruses may occur.

More than 20 viruses are reported from various cowpea-growing areas worldwide (Thottappilly and Rossel 1985; Mali and Thottappilly 1986); however, only 8 viruses are reported from cowpea in tropical Africa (Taiwo and Shoyinka 1988; Thottappilly and Rossel 1992). It is more difficult to identify viruses than other plant pathogens; elaborate and expensive equipment is required (Bos 1976). Because of inadequate facilities and lack of support, most national program scientists find it difficult to accurately identify viruses. Serology is by far the most reliable method currently available for virus identification in African facilities.

In addition to being important for virus identification, serological diagnostic techniques are reliable and easy to perform in a variety of laboratories. In order to make serological diagnostic methods available to national programs in Africa, the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, hosted a meeting in March 1988 with a few key scientists from these programs to initiate a collaborative network.

The group decided to seek financial support from the International Development Research Centre (IDRC), Ottawa, Canada, and complementary scientific support from the Vancouver Research Station (VRS) of Agriculture Canada. Fourteen scientists from national programs in eastern, western, and southern Africa were involved in this pilot project.

As the results from the IDRC-sponsored research were to be applied by the national programs, the methodology was jointly developed and implemented. The chosen strategy for virus identification and geographical distribution studies in this pilot project was to produce either virus-specific or strain-specific monoclonal antibodies, and to optimize diagnostic tests usable under field conditions.

The first phase (three years) of the collaborative project was successful in the development and application of serological methods for the identification of several cowpea viruses: cowpea aphid-borne mosaic virus (CAMV); blackeye cowpea mosaic virus (BlCMV); cowpea mosaic virus (CpMV); cowpea mottle virus (CMeV); and southern bean mosaic virus (SBMV). This phase was extended to other national programs to cover all cowpea-growing regions in Africa, and further extended to cover other food crops (cassava, maize, pepper) infected by economically important viruses.

## Project strategy

IDRC funded this pilot project from 1990 to 1992. The plan involved:

- Establishment by IITA of a collection of cowpea viruses, followed by preliminary characterization and production of polyclonal antisera for some of them;
- Selection by IITA of scientists from national programs in Africa to be involved in a collaborative project on the detection of cowpea viruses;
- Propagation and purification at VRS of cowpea virus isolates recognized as potyviruses, for comparison with reference isolates for identification purposes, and for preparation of specific antibodies

* Slightly adapted from an article of the same title originally published by the authors in *FAO Plant Protection Bulletin* 41(2): 65–71 (1993).

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(polyclonal antisera and monoclonal antibodies);

- Development at VRS of diagnostic tests using monoclonal antibodies for detection of BICMV and CAMV;
- Participation of selected national program scientists in annual workshops at IITA on the application of monoclonal antibodies in plant virology, with specific sessions and laboratory practice on the use of standardized serological diagnostic tests for detection of BICMV and CAMV, and roundtable discussions on modalities of surveying in selected countries, various applications of diagnostic tests, and interpretation of results;
- Completion of surveys for cowpea viruses by national program scientists in their own countries, testing of collected samples for the presence of BICMV or CAMV with the diagnostic kit provided (Fig. 1), and establishment of the incidence of infection and geographical distribution for each country;
- Collation of incoming data from the national programs, to provide a general overview of the incidence of these two viruses in the surveyed regions, and to prepare recommendations for cowpea breeding programs;
- Discussion among collaborators about possible improvements of the diagnostic tests, before releasing them to the other African national programs;
- Application by the network collaborators of a similar strategy to deal with other virus diseases in major African food crops.

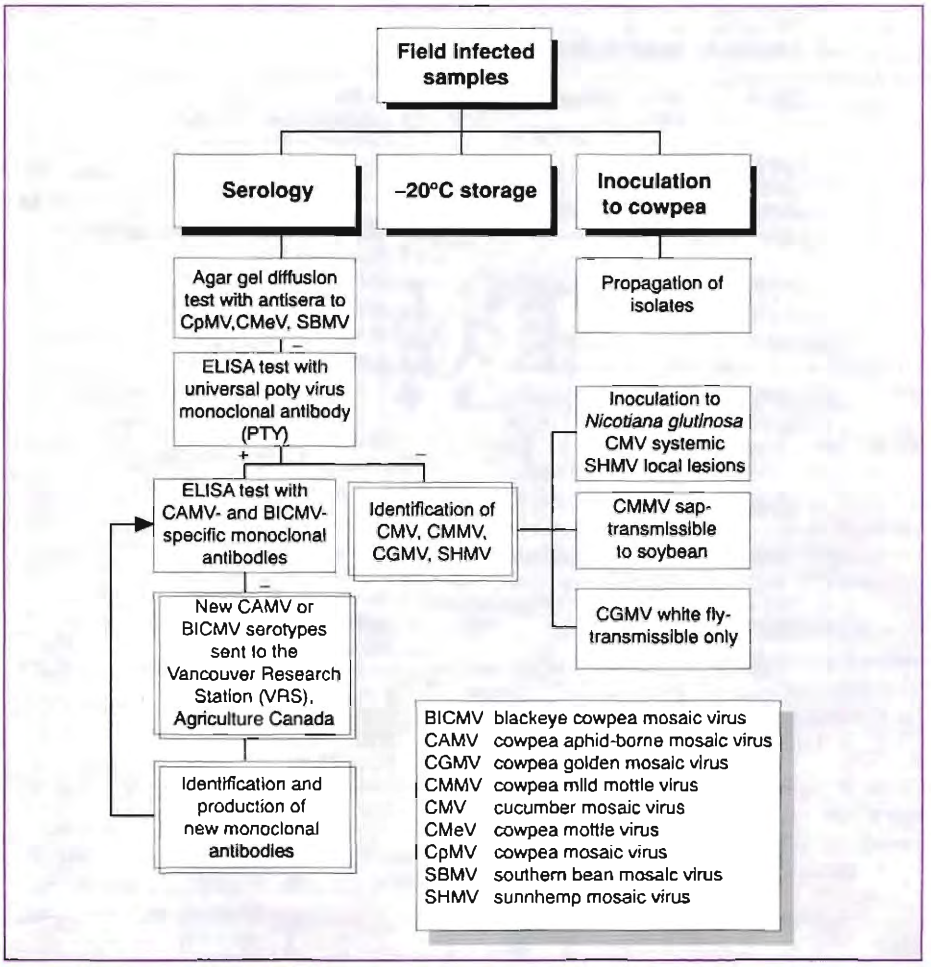



**Figure 1. Strategy for cowpea virus identification.**

## Results

**Network development.** A functional and dynamic network of scientists from IITA, VRS, and national programs in 19 countries (Burkina Faso, Cameroon, Côte d'Ivoire, Ethiopia, Ghana, Kenya, Madagascar, Malawi, Mozambique, Niger, Nigeria, Rwanda, Senegal, Sierra Leone, Tanzania, Togo, Uganda, Zambia, and Zimbabwe) collaborated in the identification and detection of cowpea viruses. National program scientists participated in week-long workshops at IITA in April 1991, November 1992, and January 1994 on the use of monoclonal antibodies in plant virology. Participants gained hands-on experience, and they took home with them virus-detection kits, to enable them to carry out tests in their locations.

A large-scale study on cowpea viruses, especially BICMV and CAMV, was evaluated at IITA in November 1992.

**Development of diagnostic tools.** Polyclonal antisera were raised at IITA against CpMV, CMeV, and SBMV for use in the agar gel diffusion test in the first screening of infected cowpea samples. The monoclonal antibody PTY (Agdia Inc., Indiana, USA)[2] for universal detection of potyviruses was used, with the supplier's instructions to detect the presence of potyvirus in cowpea samples. Polyclonal antisera and monoclonal antibodies were raised at VRS against various isolates of viruses collected in Nigeria. The potyviruses in cowpea were identified as BICMV and CAMV, and further classified into serogroups (Huguenot et al. 1993).

**Table 1. DAS-ELISA[1]: basic procedure.**

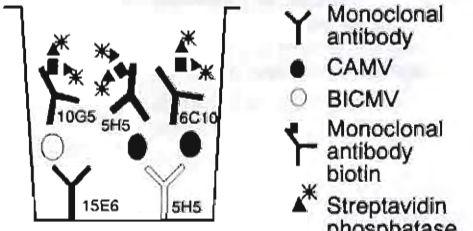
| Step | Reagent | Incubation | Rinse |
|---|---|---|---|
| Coating | Trapping mAb[2] at suitable dilution in coating buffer | 2 h at 37°C | With PBS-T[3] |
| Blocking | 1% BSA[4] in PBS-T | 30 min at 37°C | None |
| Antigen | Plant sap at suitable dilution in PBS-T | 2 h at 37°C | With PBS-T |
| Antibody | Second antibody (mAb. Biot)[5] at suitable dilution in PBS-T | Overnight at 4°C | With PBS-T |
| Conjugate | Conjugate (Str. AP)[6] at suitable dilution in PBS-T | 2 h at 37°C | With PBS-T |
| Substrate | Substrate (pNPP)[7] 1 mg/ml in substrate buffer | 1 h at 37°C | |

1. Double antibody sandwich, enzyme-linked immunosorbent assays.
2. Monoclonal antibody.
3. Phosphate-buffered saline containing Tween 20.
4. Bovine serum albumin.
5. Monoclonal antibody conjugated with biotin.
6. Streptavidin conjugated with alkaline phosphatase.
7. Para nitrophenylphosphate.

2. Use of the trade or company names is for reader information only. It does not imply endorsement of any product or service.

**GENERAL DIAGNOSIS**

| Virus | Detection of serotype | ELISA procedure *(mAbs - Ag - mAbs.Biot - Str.AP)* | |
|---|---|---|---|
| BICMV and CAMV | A+B<br>C+D+E+F+G | 15E6+5H5 | (10G5+5H5+6C10).Biot |







**SEROTYPING**

| Virus | Detection of serotype | ELISA procedure *(mAbs - Ag - mAbs.Biot - Str.AP)* | |
|---|---|---|---|
| BICMV | A | 16G5 | 10G5.Biot |
| | A+B | 15E6 | 10G5.Biot |
| CAMV | C+D+E+F+G | 5H5 | 5H5.Biot |
| | C | 1F5 | 1F5.Biot |
| | D+E | 5H5 | 7D9.Biot |
| | E | 12F9 | 6C10.Biot |

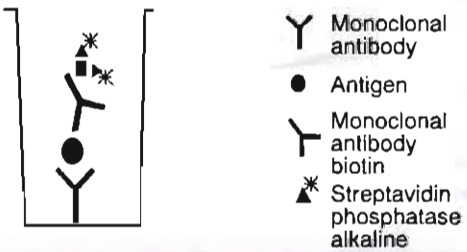



**Figure 2. Serological detection of cowpea aphid-borne mosaic virus (CAMV) and blackeye cowpea mosaic virus (BICMV) by double antibody sandwich, enzyme-linked immunosorbent assays (DAS-ELISA).**

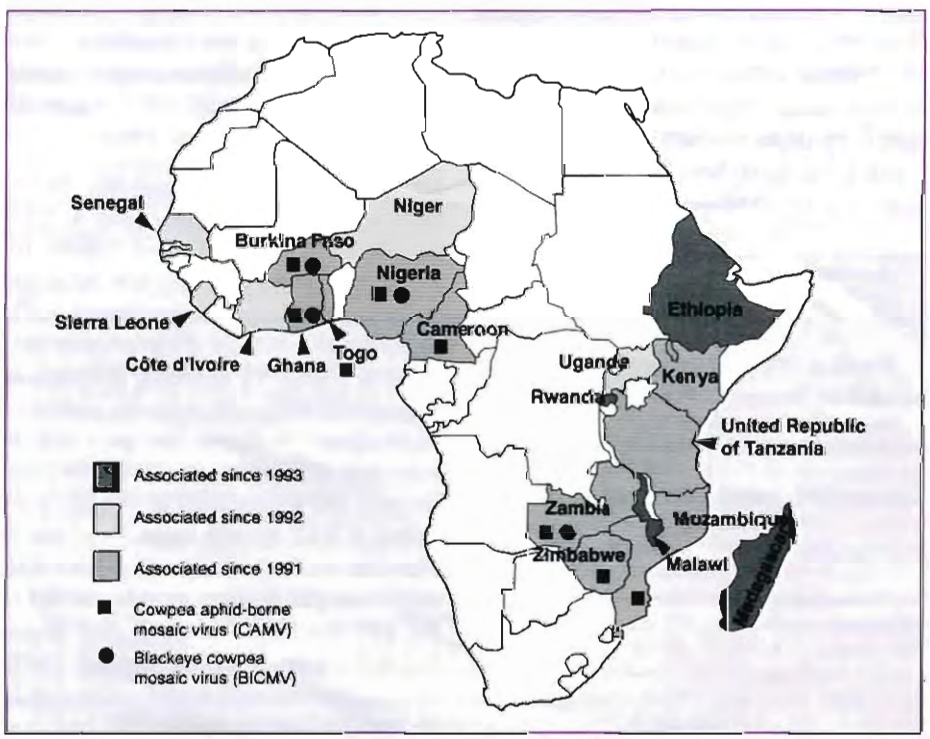



**Figure 3. Associated countries in which cowpea fields are being surveyed for detection of BICMV and CAMV.**

Double antibody sandwich, enzyme-linked immunosorbent assays (DAS-ELISA) (see Table 1), using the VRS monoclonal antibodies, were designed to be either virus-specific (CAMV or BICMV) or strain-specific (Fig. 2). The general diagnostic test was achieved by mixing several monoclonal antibodies for both coating the plate and detecting the antigen. The serotype was determined by using a specific monoclonal antibody for coating, and either the same or a different specific antibody, conjugated with biotin, for detecting the antigen (Huguenot et al. 1993).

Different diagnostic tests that do not require an incubator or ELISA reader were designed for use in any laboratory facility in Africa, and the different reagents were produced in sufficient quantities to supply all collaborators.

**Large-scale survey by national program scientists.** After surveying over one or two cowpea seasons and testing samples for the presence of CAMV and BICMV, preliminary results from eight countries indicated that the viruses occurred in each participating country (Fig. 3), suggesting a very important economic impact. Moreover, the serological diagnostic test has proved to be efficient in detecting the viruses in seeds (single seed, portion of cotyledon, or embryo), and it can be used by national programs for seed transmission studies and for checking seed stocks.

## Discussion

After completion of the first phase of the project, three major points were obvious. First, either CAMV or BICMV can cause a similar mosaic disease in many African countries. Until now, CAMV was reported as the only causal agent of the mosaic disease on cowpea. Both BICMV and CAMV are potyviruses, but they are serologically distinct (Huguenot et al. 1993), distinguishable on differential hosts (Taiwo et al. 1982; Dijkstra et al. 1987; Huguenot et al. 1993), and different resistance genes are required to prevent infection (Providenti et al. 1983). Cowpea breeding programs, therefore, have to consider both viruses.

Second, for several of the countries investigated, a low incidence of the viruses was found on landraces and a very high incidence on imported lines, suggesting that these seed-transmissible viruses were probably imported in selected cowpea lines.

Third, in the countries surveyed so far in Africa, both viruses have been found to comprise a number of strains with different serological properties and to occur on crops other than cowpea (e.g., African yam bean in Nigeria, and bambara groundnut in Nigeria and Burkina Faso), thus stressing the need to develop a reliable diagnostic test. The present network of collaborators has the potential to develop a general test of this sort. The large-scale survey operating in the 19 countries offers the greatest opportunity to identify new strains of CAMV and BICMV, and thus to improve the efficacy of the present diagnostic tests through preparation of new antibodies against specific strains.

In the second phase of this pilot project, which was extended for an additional 2 years, 12 virologists from other African countries have joined the network. The success of the project will depend on the free exchange of materials, ideas, and experiences. Collaborators will publish the results of their surveys and identification work independently.

With more support from donors, this collaborative approach has the potential to accelerate the pace of identifying economically important viruses in Africa, and to facilitate epidemiological studies.

## Acknowledgement

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# Thesis abstracts

## results of research by IITA trainees*

**Bruce-Oliver, S.J. 1993. Evaluation of the indigenous African Phytoseiid, *Euseius fustis* (Pritchard and Baker) (Acari: Phytoseiidae), as a potential biological control agent of the cassava green mite *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae), on cassava (*Manihot esculenta* Crantz.) in West Africa.** PhD thesis, University of California at Berkeley, USA. **Supervisors:** M.A. Hoy and J.S. Yaninek†.

Experiments were conducted to establish which foods most favor the development of *Euseius fustis* (Pritchard and Baker) (Acari: Phytoseiidae) a predator of the cassava green mite, *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae). The results were in descending order (1) maize + castor bean pollen and maize pollen + *M. tanajoa*; (2) maize pollen alone, castor bean pollen alone, and castor bean pollen + *M. tanajoa;* (3) *M. tanajoa* and *Oligonychus gossypii* (Zacher) as prey; and (4) cassava pollen, cassava exudate, and cassava mealybug honeydew. Rearing *E. fustis* for multiple generations on castor bean pollen did not adversely affect its life history attributes.

Estimates of intrinsic rates of increase at 18, 20, 25, 30, and 32°C were higher on a diet of maize pollen (0.059 to 0.202) than on *M. tanajoa* (0.006 to 0.157). *E. fustis* was sensitive to low relative humidity, with no egg hatching at 43%.

Two generations of females reared from egg to adult and held under potential diapause-inducing conditions showed no indication that they were in diapause. Empirical observations of low densities of *E. fustis* during the dry season are thus unlikely to be caused by diapause.

Repeated sampling of cassava for 13 months showed no correlation in phenology between *E. fustis* and populations of *M. tanajoa* and *O. gossypii*. The abundance of *E. fustis* during the wet season when *M. tanajoa* is absent was related to the availability of pollen from plants such as maize, which is usually intercropped with cassava, and which depends on the rainfall pattern. The phenology of *E. fustis*, *O. gossypii*, and *M. tanajoa* was strongly, but differently, influenced by the alternation of wet and dry seasons.

Laboratory studies showed that *E. fustis* consistently fed, developed, and reproduced better on maize and castor bean pollens than on *M. tanajoa*. This ability to feed on various foods makes *E. fustis* a "generalist" predator. Based on the phenology studies, the potential of *E. fustis* to control *M. tanajoa* is considered limited because these two mite populations are abundant on cassava at different times of the year.

**Njock, T.E. 1994. Epidemiology and disease recovery phenomenon of African cassava mosaic virus in resistant and susceptible cassava clones.** PhD thesis, University of Ibadan, Nigeria. **Supervisors:** G.I. Atiri and G. Thottappilly†.

Laboratory and field experiments were conducted to investigate three factors related to the occurrence and spread of African cassava mosaic disease (ACMV): (1) virus distribution within cassava stems; (2) disease recovery; and (3) general epidemiology, including studies of vector (whitefly) populations. Resistant (TMS 30001), moderately resistant (TMS 4(2)1425), and susceptible (60506) cassava clones were used.

In field evaluation, as expected, disease incidence and severity were significantly highest in clone 60506, and lowest in TMS 30001. Whitefly population was significantly higher on TMS 4(2)1425 than on either 60506 or TMS 30001. Vector transmission of the virus was significantly high in 60506, and low in TMS 30001.

Within clones, there were significant differences in disease incidence and severity on plants from cuttings of different stem sections of clone 60506. Among clones, however, disease incidence and severity were significantly high on plants from cuttings from the base section of stems of clone 60506. The top, middle, and base sections of the stems of TMS 4(2)1425 and TMS 30001 were not significantly different from each other in disease incidence and severity. Most nodes were diseased in the base section of clone 60506, and least in the top section of TMS 30001. For each clone, the frequency was significantly higher in the base than in the top section. The enzyme-linked immunosorbent assay (ELISA) was used to detect ACMV among different nodes of the three clones.

Disease incidence, severity, and whitefly population were not significantly different between two sites at IITA, but they were significantly different among the planting times of June, August, October, and December. Significantly higher disease incidence, severity, and whitefly population occurred on cassava intercropped with groundnut than on cassava intercropped with maize. In multilocational trials, disease incidence and severity were highest on cassava planted in the transition savanna/forest zone (Ibadan), followed by that in the mangrove/humid forest zone (Onne), but least in the Sudan savanna zone (Kano).

Components of ACMV resistance in cassava to be considered for better control should include restricted upward movement of ACMV, especially in resistant cassava clones, the tendency for basal localization, and incomplete systemic invasion of the plant system.

**Ntonifor, N. N. 1993. The potential of host shifts of some insect pests from cowpea (*Vigna unguiculata* (L) Walp) to soybean (*Glycine max* (L) Merrill).** PhD thesis, University of Ibadan, Nigeria. **Supervisors:** F.K. Ewete and L.E.N. Jackai†.

With the expansion of soybean as a crop in tropical Africa, the possibility of some known cowpea pests, *Clavigralla tomentosicollis* (Stal), *Riptortus dentipes* (Fabricius), and *Maruca testulalis* (Geyer), shifting to soybean was investigated. Two pests of soybean, *Nezara viridula* (Linnaeus) and *Spodoptera littoralis* (Boisduval), were also included in the study.

Soybean pods were used as a food to rear the various pod sucking bugs. Nymphal *C. tomentosicollis* did not survive on soybean beyond 8 days, and adults beyond 12 days.

The nymphal duration of *R. dentipes* was 20.7 days on cowpea (VITA 3) and 21.3 days on soybean (TGX 536–02D), despite a significantly higher relative food

* A full list of topics on which IITA trainees complete their graduate thesis research each year is carried in the *IITA Annual Report.*

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consumption rate (RCR) during the 4th and 5th instars on soybean than on cowpea. The efficiency of conversion of ingested food (ECI) and that of digested food (ECD) were remarkably lower on soybean than on cowpea. The 2nd and 3rd instars preferred feeding on cowpea pods, while the 4th and 5th instars and adults showed no clear preference. Adults preferred soybean over cowpea for egg laying, but a substantially higher number of eggs were laid on the mesh walls of the cages.

*N. viridula* had a nymphal period of 24.4 days on cowpea and 25.4 days on soybean. Nutritional indices of the 4th and 5th instars (with the exception of the ECD and ECI) were similar on both crops. Regardless of the initial host plant and the duration of preconditioning, *N. viridula* always preferred cowpea pods in all dual choice tests. Soybean leaves were, however, preferred for oviposition.

Third to 5th instars of *M. testulalis* were unable to bore into soybean pods to consume the seeds. They fed on flowers, young tender shoots, and leaves. Total developmental period was 16.1 days on 2–5 day old cowpea, and 15.7 days on soybean leaves of the same age. The RCR of 4th and 5th instar larvae on cowpea leaves was significantly higher than that on soybean, while the approximate digestibility (AD), ECD, and ECI were higher on soybean. Larvae preferred soybean leaves in all dual choice tests, regardless of previous exposure on cowpea leaves; however, adults preferred cowpea in all oviposition preference tests.

*S. littoralis* had a mean larval duration of 18.2 days on cowpea leaves and 19.4 days on soybean leaves. Higher relative growth rates (RGRs), RCRs, ECDs. and ECIs were obtained from larvae fed on cowpea leaves, while higher ADs were obtained from larvae fed with soybean leaves during the 4th and 5th instars. The 4th and 5th instars of *S. littoralis* were also less discriminatory in their feeding preferences.

In screenhouse host selection studies conducted with *M. testulalis* adults obtained from larvae reared on soybean leaves, cowpea pods, or artificial diet, only cowpea plants were utilized for oviposition by these adults and subsequently by the larvae as trophic niches. *R. dentipes* adults obtained from nymphs reared separately on cowpea and soybean pods showed that adult distribution on different cowpea : soybean ratios was a function of the proportion of cowpea in that treatment only when podding occurred earlier in this crop; but when podding was synchronized in both crops, there was no obvious preference.

Field infestation of cowpea by *M. testulalis* was uniform. Temporal occurrence and spatial distribution of flowers on the field apparently played a role in larval distribution. There was no field infestation of soybean by *M. testulalis*.

Field population assessment of pod sucking bugs during the two cropping seasons of 1991 at Ibadan in different treatments with varying proportions of cowpea to soybean showed *Clavigralla* spp. to be the dominant bug species on cowpea during both seasons. *R. dentipes* was dominant on soybean during the first season, while *N. viridula* was dominant during the second season.

The distribution and abundance of cowpea plants with pods were crucial in the dynamics of *C. tomentosicollis* and *C. shadabi* (which preferred cowpea), while *N. viridula, R. dentipes, Acrosternum acuta, Aspavia armigera, Mirperus jaculus* and, to a lesser extent, *Anoplocnemis curvipes* did not show a definite preference for either crop. Highest bug count on cowpea was observed during the stage of fully formed green cowpea pods, while that on soybean was at the R3 stage of pod development and at podfill (R6).

The results suggest that a number of the pests encountered could become important pests on soybean, given the right conditions.

**Simwambana, M.S.C. 1993. Environmental factors modifying the growth and flowering behavior of four cassava cultivars in Nigeria.** PhD thesis, University of the West Indies, St. Augustine, Trinidad. **Supervisors:** T.V. Ferguson and I.J. Ekanayake†.

Field experiments were conducted at IITA, Ibadan, in Oyo state and Ubiaja in Edo state to assess the effects of time of planting, soil type, and climatic factors on the growth and flowering behavior of four cassava cultivars, TME1, TME2, TMS 30555, and TMS 91934. Three planting periods were assessed: March, May, and July.

In all experiments, the period of peak flowering occurred between August and October of each year, during conditions of high rainfall, high relative humidity, high available moisture, moderate temperature, and high solar radiation. Cassava planted in March produced more flowers during the first season while cassava planted in July had more flowers in the following season. Moisture deficits at IITA seemed to inhibit flowering of cultivars TME1 and TME2, compared with Ubiaja. Shading of cassava (40–60% light) delayed the first flowering and reduced the number of flowers per plant. High relative humidity (59–90%) promoted lower branching height and induced cultivar TMS 91934 to flower.

No single environmental factor promoted flowering in these four cassava cultivars. Effects of temperature, relative humidity, soil moisture, and daylength on flowering in cassava should be evaluated under simulated environmental conditions. Soil samples from IITA, Ibadan and Ubiaja should be studied for their moisture content, moisture retention characteristics, hydraulic conductivity, and effects on the flowering of cassava.

**Adejuyigbe, C.O. 1994. Soil microarthropods and litter decomposition under different cropping systems and fallow management in the humid tropics.** MSc thesis, University of Ibadan, Nigeria. **Supervisors:** G.O. Adeoye and G. Tian†.

The study attempted to quantify the effect of bush fallow and cropping systems (traditional, *Pueraria phaseoloides* relay cropping, and *Leucaena leucocephala* alley cropping) on soil microarthropods and their roles in litter decomposition in the humid tropics.

Population dynamics showed an increase generally in September at the second rainfall peak. The soil microarthropod population was lower under crops than under secondary forest. *Leucaena* alley cropping had the highest mean population over the study period among the cropped plots; however, the cropping systems studied seemed to have no significant influence on soil microarthropod populations. After 1 year of fallow, the soil microarthropod population was restored to the same level as in the secondary forest.

Compared to the secondary forest, cropping decreased the rate of leaf decomposition. Alley cropping showed the highest decomposition rate within the cropped plots. Fallowing improved litter decomposition. Decomposition rates, in decreasing order, were bush fallow (3rd year) > secondary forest (15 years) > bush fallow (2nd year) > bush fallow (1 year) > *Leucaena* alley cropping > *Pueraria* relay cropping > traditional cropping. The

decomposition rate constant, however, did not respond to fallow length after 1 year of fallow. The presence of soil microarthropods increased decomposition rates, particularly in traditional cropping.

The results suggest that improved cropping systems, such as alley cropping and fallow management (one year bush fallow), could restore soil microarthropod activities and thus improve litter decomposition.

**Chikere, A.C. 1994. Preservation of soymilk with extracts from *Aframomum danielli* and *Allium sativum*.** MSc thesis, University of Ibadan, Nigeria. **Supervisors:** O.C. Aworh and S.M. Osho†.

*Aframomum danielli* and *Allium sativum* (garlic) were screened in vitro for their ability to inhibit the growth of seven spoilage microorganisms isolated from soymilk. *Aframomum danielli* successfully inhibited *Escherichia coli* and *Aspergillus niger* at 1% concentration, and *Bacillus* spp., *Aspergillus flavus*, and *Penicillin* spp. at 10% concentration. *Allium sativum* inhibited *Aspergillus flavus* and *Penicillin* spp. at 0.1%, and *Staphylococcus epidermis Bacillus* spp., *Lactobacillus* spp., *Escherichia coli*, and *Aspergillus flavus* at 1% concentration.

The antimicrobial activity of *Aframomum danielli* was significantly reduced when it was applied directly to soymilk at 1%, 1.5%, and 2% concentrations. Changes in acceptability, pH, titratable acidity, and microbial load were measured during a 3-day storage period in ambient and refrigerated conditions. All treated samples were rated inferior to a reference sample with no additives during the first 2 days of storage in all five attributes measured. Spoilage set in after 2 days storage under ambient conditions.

**Ezeji, T.C. 1994. Production and preservation of tofu with *Aframomum danielli*.** MSc thesis, University of Ibadan, Nigeria. **Supervisors:** O.C. Aworh and S.M. Osho†.

Tofu was produced using as coagulants extracts from the leaves of sodom apple *(Calotropis procera)* with lime juice and calcium sulfate. The sensory quality of the tofu was tested by a 10-member panel for color, flavor, firmness, mouthfeel, and general acceptability. Yield, fat, and protein recoveries in the tofu were evaluated. The effects of different percentage levels of *Aframomum danielli* on shelf life and sensory characteristics were investigated.

Tofu treated with no spice and 0.5% spice had a shorter shelf life than that treated with either 1.0% or 1.5% of *A. danielli*, but the latter percentages resulted in lower scores for sensory qualities. Tofu treated with 0.8% of *A. danielli* performed intermediately in all investigations.

The shelf life of tofu under three different storage systems was examined. Packaged tofu had a shelf life of 2 days, tofu stored in tap water lasted 3 days, and salted tofu stored in 0.8% brine lasted 7 days.

**Manga, G.A. 1994. Sprouting and establishment of cassava.** MSc thesis, University of Ibadan, Nigeria. **Supervisors:** C.A. Fatokun and R. Asiedu†.

During 1993, two studies were conducted at IITA, Ibadan to investigate (a) genotypic variability in sprouting and establishment capabilities of 17 cassava genotypes under three soil moisture regimes (no watering, 117 ml/week per plant, and 117 ml/day per plant) in the glasshouse and on nursery beds; and (b) emergence and establishment of progenies from interspecific crosses between cultivated cassava *(Manihot esculenta)* and some of its wild relatives, up to 8 weeks after planting.

Low to moderate (10–40%) variability was observed for sprouting, growth characteristics, and establishment of the 17 cassava genotypes. Moisture deficit affected sprouting, growth, and establishment in both glasshouse and nursery beds. Sprouting and establishment traits had a low genetic coefficient of variability (less than 30%), and they were greatly influenced by environment.

High heritability estimates were found for weight, volume, number of nodes per 25 cm cutting length, and shoot height 7 days after planting. Number of nodes per 25 cm length accounted for the greatest variability observed in sprouting and establishment of the different genotypes. TMS 71173 had high percentages of sprouting and establishment in both glasshouse and nursery beds. TMS 58308 had the lowest values for these traits.

Progenies from interspecific crosses emerged faster than the wild parents, but more slowly than the cultivated cassava. Hybrids from *M. glaziovii* performed best in emergence and establishment. Performance of reciprocal crosses between *M. tristis* and TMS 42025 suggested the presence of cytoplasmic influences on the progenies. Previous results on the difficulty of establishing wild *Manihot* species outside their natural environment were confirmed.

To clarify the relationship between sprouting and yield, evaluation of different genotypes from planting to harvest is suggested. Using TMS 71173 as a parent for interspecific crosses would help in understanding the basis of sprouting and establishment characteristics, and in providing additional information about the mode of inheritance of these traits.

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***This list updates our previous issue (September 1994). Readers who need reprints of articles here may request them from the authors or from Publications Unit, IITA.***


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# Recent IITA publications

**Tropical Root Crops in a Developing Economy.** Edited by F. Ofori and S.K. Hahn. 551 pp. ISBN 978 131 094 4. ISTRC/Government of Ghana/IITA copublication. (Copies available from IITA to cooperating scientists, upon request.)

Tropical root and tuber crops, such as cassava, cocoyam, Irish potato, sweet potato, and yam, have been recognized as the greatest source of dietary food energy for many developing countries. They also contribute significantly to national economies and rural income, and provide employment for most rural economies of sub-Saharan Africa.

This volume, resulting from the ninth symposium of the International Society for Tropical Root Crops (ISTRC) held at Accra, Ghana during 20–26 Oct 1991, brings together 79 papers that discuss various aspects of root crop production.

The papers are organized by crop. Also included are abstracts of papers presented at the symposium, but not published in full here.

**Cassava Safety.** Edited by M. Bokanga, A.J.A. Essers, N. Poulter, H. Rosling, and O. Tewe. 416 pp. ISBN 90 6605 326 7. Acta Horticulturae No. 375. International Society for Horticultural Science (ISHS), Waginengen, the Netherlands (copublication with ISTRC and IITA). ISHS Price: 130 Dutch guilders. (Copies available from IITA to cooperating scientists, upon request.)

While cassava is an important staple crop, which ranks fourth in the tropical world (after rice, wheat, and maize), reports of the presence in it of cyanogenic compounds have been a matter of concern.

A Working Group on Cassava Safety (WOCAS) was formed in 1992, and it convened an international workshop at IITA, Ibadan, Nigeria during 1–4 Mar 1994. This volume brings together the 42 papers presented at that workshop.

The papers are grouped as follows: biology of cyanogenesis; analytical methods; agronomic research; processing and cyanogen removal; cassava in livestock feeds; human health and nutrition; socioeconomic aspects.

## Also available from IITA

**Dealing with the Issues of our Times.** Text by S.M. Lawani. IITA, Ibadan, Nigeria. 15 pp. (Available free of charge upon request.)

This brochure discusses IITA's activities in the context of contemporary developmental challenges: hunger and poverty, environment, sustainability, economic growth, private sector development, women and equity, and self-reliance.

**IITA Annual Report 1993.** 64 pp. (Available free of charge to agricultural institutions, media or media-related agencies, and scientists.)

Provides an illustrative summary of IITA's research activities and achievements during 1993, with special reports on selected themes.

Smith, J., and G.K. Weber. 1994. Strategic research in heterogenous mandate areas: An example from the West African savanna. Pages 545–565 *in* Agricultural technology: Policy issues for the international community, edited by J.R. Anderson. CAB International (in association with the World Bank), Wallingford, Oxon, UK.

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# A third King Baudouin Award for IITA: promise of more fruits to follow

IITA's work in developing plantain hybrids resistant to the fungal disease, black sigatoka (and on some aspects of *Musa* genetics), won for it the 1994 King Baudouin Award. The award, given once every two years by the Technical Advisory Committee (TAC) of the Consultative Group on International Agricultural Research (CGIAR), is intended to recognize excellence in research among the centers of the CGIAR, as well as to reward research that has the most potential impact.

Ismail Serageldin, Chairman of the CGIAR, who presented the award to IITA Director General Lukas Brader at a ceremony in Washington, DC, on 24 October 1994, said: "millions of small African farmers and their families, for whom plantains are a nutritious and tasty basic food, will benefit from IITA's scientific advances. This is truly a milestone contribution toward stemming the long-term decline of per capita food production in sub-Saharan Africa."

***Lukas Brader, IITA Director General (right), receives the King Baudouin Award for 1994 from Ismail Serageldin, Chairman, CGIAR.***

Black sigatoka, a leaf spot disease caused by the fungus *Mycosphaerella fijiensis* Morelet, is generally considered to be the most serious constraint to plantain and banana production in sub-Saharan Africa. First identified in Fiji, the disease was accidentally introduced into southern Africa in the 1970s and spread rapidly, first in Central and West Africa, and later in East Africa. Since the fungal spores are dispersed by wind and water, disease spread cannot be slowed by plant quarantine measures alone. Once established, the pathogen causes severe leaf necrosis, reducing yields by 30–50% and seriously affecting smallholder farmers. All traditional plantain cultivars are susceptible to black sigatoka, as are at least some of the most widely grown banana cultivars in East Africa.

As black sigatoka became a serious problem across the African continent in the 1980s, several African governments encouraged IITA to launch an urgent research campaign to control the disease. IITA scientists were impelled by the need to save plantains for the millions of smallholders who depend on them for subsistence and livelihood. In 1987, *Musa* genetic improvement was initiated at the Institute, aimed mainly at the incorporation of durable host plant resistance to black sigatoka.

IITA scientists expected that it would take at least 10 years to develop improved plantain germplasm resistant to black sigatoka, given the complexity of the task and given the fact that plantain was generally considered a difficult crop to improve genetically. But within 5 years from the beginning of their concerted efforts, by using a combination of conventional and novel approaches, including interspecific hybridization, ploidy manipulations, in vitro culture, and field testing and selection, they were able to develop plantain germplasm resistant to black sigatoka.

In 1993, they registered 14 such improved hybrids and placed them in the public domain through publication in *HortScience* [28(9): 957–959]. Twenty-six national programs in 11 countries of sub-Saharan Africa and tropical America are already evaluating these lines for release to farmers.

The value of annual plantain production in Africa, currently estimated at US$ 2.8 billion, could potentially increase to US$ 9 billion if prices stay constant and all of the crops' current area is planted with improved hybrids. Postharvest assessments have indicated a high potential for adoption of the improved hybrids.

In the process of developing resistance to black sigatoka, IITA scientists have gained new insights into the Musa genome, as well as in plantain agronomy, which can now provide the basis for new strategies to address other constraints in the production of plantain and banana.

Studies to explore newer techniques in crop improvement, such as those involving plant biotechnology, as well as to further consolidate genetic stability, are in progress with advanced laboratories in Belgium, UK, and USA.

IITA had already won the King Baudouin Award twice in the past: in 1986 for its work in developing and incorporating resistance to the maize streak virus, and in 1990 jointly with CIAT (Centro Internacional de Agricultura Tropical, with headquarters in Cali, Colombia) for the immensely successful campaign for the biological control of the cassava mealybug.

All three of IITA's awards have thus been for work that exemplifies an ecologically sound pest management strategy. Such work minimizes the need for pesticide interventions and promises greater sustainability in food crop production in an area of the world that requires it urgently.

# IITA training continues to strengthen national programs across Africa

*IITA's group training efforts over the past four years have steadily moved away from a dominant orientation toward headquarters (as explained in our past issues). Those moves have aimed at strengthening the institutional capability of national systems in sub-Saharan Africa to conduct the research and training required for agricultural development. They have also attempted to spread training opportunities to many countries of sub-Saharan Africa.*

## Training courses (Apr–Dec 1995): proposed schedule

| Dates | Course | Venue |
|---|---|---|
| **International courses** | | |
| 22 May to 9 Jun | Sustainable food production systems: integration of crops and livestock* | Ibadan, Nigeria |
| 3–28 Jul | Breeding of root crops | Ibadan, Nigeria |
| 3–5 Sep | Technology development, testing, and dissemination: working with nongovernmental organizations (NGOs) | Ibadan, Nigeria |
| 16–27 Oct | Implementing classical biological control of cassava green mite (CGM)* | Cotonou, Benin |
| 23 Oct to 10 Nov | Characterization and conservation of yam biodiversity | Ibadan, Nigeria |
| **Regional and national courses** | | |
| 10–29 Apr | Application of mycoinsecticide for control of grasshoppers and locusts | Niamey, Niger |
| 1–5 May | Workshop for trainers and training coordinators (with CRUDAN) | Jos, Nigeria (E) |
| 1–12 May | Alternatives to slash and burn: agricultural policy and sustainability issues* | Yaoundé, Cameroon |
| 22 May to 16 Jun | Crop management research on root crops | Bouaké, Côte d'Ivoire (F) |
| 19–30 Jun | Biological control techniques* (with ESCaPP) | Cotonou, Benin |
| 19 Jun to14 Jul | Crop management research on banana | Kampala, Uganda (E) |
| 11–22 Sep | Agricultural research management for senior scientists (with ICRISAT-SC, WARDA, ISNAR) | Niamey, Niger (F) |
| 11–22 Sep | Extension/training materials preparation and communication skills* (with ESCaPP) | Kumasi, Ghana (E) |
| 11 Sep to 6 Oct | Crop management research on cowpea and soybean | Kamboinse, Burkina Faso (F) |
| 11 Sep to 6 Oct | Postharvest research on selected food crops: banana, cassava | Kampala, Uganda |
| 30 Oct to 17 Nov | Integrated development of cassava (with FAO/EMBRAPA) | Venue to be determined (P) |
| 6–10 Nov | Cassava plant protection field plot techniques* (with ESCaPP) | Umudike, Nigeria |
| 6 Nov to 1 Dec | Crop management research on plantain | Buea, Cameroon (F) |
| 20 Nov to 15 Dec | Crop management research on root crops | Kumasi, Ghana (E) |

* Participation limited to project-related personnel.
All courses are bilingual (English + French) unless otherwise indicated: E = English; F = French; P = Portuguese.
ESCaPP = Ecologically sustainable cassava plant protection in Africa; NGOs = nongovernmental organizations; CRUDAN = Christian Rural Development Association of Nigeria; ICRISAT-SC = ICRISAT Sahelian Center, Niamey, Niger.; WARDA = West African Rice Development Association; ISNAR = International Service for National Agricultural Research; FAO = Food and Agriculture Organization of the United Nations; EMBRAPA = Empresa Brasileira de Pesquisa Agropecuária (Brazilian Agricultural Research Enterprise).

The schedule in progress for 1995 reinforces that trend toward augmenting regional capability (see box on courses planned for Apr–Dec 1995). While IITA intends to make these courses as widely available as possible, some courses are restricted to project-related personnel.

Courses already completed in the first two months of 1995 include a 2-week workshop on "The use of geographical information systems (GIS)" held at Ibadan, Nigeria, 16–27 Jan. The second such course in as many years, it focused on the same 14 countries each year to build up institutional capability in GIS techniques.

As we go to press, two 2-week workshops on "Ecological plant protection in cassava" (in English at Ekona, Cameroon and in French at Cotonou, Benin Republic, both to be held 27 Feb to 10 Mar) are planned, as is a 4-week course on "Crop management research on cowpea and soybean," to be held in English 13 Mar to 7 Apr at Lusaka, Zambia.

The two cassava plant protection courses are part of a special project on ecologically sustainable cassava plant protection (ESCaPP), based at IITA in Cotonou, and they aim to train projected-related staff from Benin, Cameroon, Ghana, and Nigeria.

Yet another direction in which IITA's training has made considerable progress is in courses organized jointly with research networks in the region (see box on page 23).

## FAO training workshop

Also being planned currently with FAO is an international workshop at IITA headquarters, Ibadan, during 27–31 Mar on "Major food crops production." The workshop is a follow-up of two previous training courses conducted by the FAO in Bangkok during 1992 and 1994, which included participants from both Asia and Africa. It will identify constraints and problems in the production of major food crops in sub-Saharan Africa, and explore possible solutions to them, including technologies used in southeast Asia.

## Is IITA on the right track?

In an effort to find out if IITA's efforts are targeted to the needs of our cooperators and national programs in the region, IITA has invited national program and network partners (about 35 people in all) to take part in a workshop that will assess its approach, compare alternative approaches, and chart the future course of partnership for research training in West and Central Africa. The workshop will be held at IITA headquarters, Ibadan, during 4–6 April 1995. We expect to carry a fuller report of the workshop in the next issue of *IITA Research*.

## Research Training Guides

To facilitate training, IITA's Training Materials Unit produces research guides on various subjects, determined in collaboration with scientists who initiate the training. About 50 research guides have so far been produced in English and French (there are plans for translating some into Portuguese). Another 100 are in different stages of production. A maize poster has also been produced in English, French, and Portuguese. Unlabelled copies are available to national programs or interested agencies for translation into local languages. Posters of other IITA mandate crops are also being produced.

Most research guides include information on how to plan, conduct, and analyze experiments, as well as suggestions for experimental design, data recording, and analysis. Publications are mailed selectively to a mailing list of 200 addresses in 45 African countries. Individuals can receive copies on request. *For more information, please contact the Training Materials Unit, IITA (see inside cover for addresses).*

# Training through research networks

***IITA collaborates actively with research networks in sub-Saharan Africa, thus achieving a synergistic effect in its training. By virtue of their activities, research networks are well placed to identify research and training needs, and to disseminate the knowledge that can help propel and sustain agricultural development. Thus collaboration in training is a mutually beneficial mode for IITA as well as the parties involved to achieve their common objectives.***

As we go to press, a course in English on "Equipment maintenance for personnel of African laboratories" is being planned for 25 Mar to 8 Apr at Marondera, Zimbabwe, in cooperation with the Soil and Plant Analytical Laboratories Network of Africa (SPALNA), and a similar one in French (3–14 April) will be held at Ouagadougou in Burkina Faso. Similar courses with SPALNA have been held in Nigeria during recent years, while several more are planned for later this year.

A schedule of other network-based courses follows.

## Network-based training courses (May–Dec 1995)

| Dates | Course* | Venue† |
|---|---|---|
| 1–5 May | Rapid multiplication of root crops (EARRNET + local and international NGOs) | Bujumbura, Burundi (F) |
| 24 Jun to 8 Jul | Good laboratory practices and information management (SPALNA, University of Ibadan) | Ibadan, Nigeria (E) |
| 7 Aug to 1 Sep | Crop management research on root crop-based systems** (SARRNET, EARRNET) | Venue to be determined |
| 14–25 Aug | Seed production techniques (WECAMAN) | Kumasi, Ghana |
| 3–8 Sep | Soil and plant analysis (SPALNA, University of Nigeria, Nsukka) | Nsukka, Nigeria (E) |
| 16–30 Sep | Soil and plant analysis (SPALNA, several other institutions) | Ouagadougou, Burkina Faso (F) |
| 30 Sep to 14 Oct | Equipment maintenance for personnel of African laboratories (SPALNA, University of Nigeria, Nsukka) | Nsukka, Nigeria (E) |
| 16–20 Oct | Rapid multiplication of root crops (EARRNET + local and international NGOs) | Kigali, Rwanda (F) |
| 11–25 Nov | Equipment maintenance for personnel of African laboratories (SPALNA, several other institutions) | Niamey, Niger (F) |
| 3–8 Dec | Equipment maintenance for lab technologists from southwestern Nigeria (SPALNA, University of Ibadan) | Ibadan, Nigeria (E) |
| To be scheduled | Integration of livestock into alley farming research (AFNETA) | Addis Ababa, Ethiopia |
| " | Participatory rapid appraisal for alley farming research (AFNETA) | Kumasi, Ghana |
| " | Participatory rapid appraisal for alley farming research (AFNETA) | Yaoundé, Cameroon (F) |

* Cooperators in parentheses.
** Participation limited to network-related personnel.
† Language of the course: E = English; F = French. Where no letter code is given, courses are bilingual (English and French).
EARRNET = East Africa Root Crops Research Network; NGOs = nongovernmental organizations; SPALNA = Soil and Plant Analytical Laboratories Network of Africa; SARRNET = Southern Africa Root Crops Research Network; WECAMAN = West and Central African Maize Network; AFNETA = Alley Farming Network for Tropical Africa.

# People

Two of IITA's three major research divisions came under new directors in September 1994. **Doyle C. Baker** became Director of the Resource and Crop Management Division. He had been an economist with the USAID/IITA project at the National Cereals Research and Extension (NCRE) project, Cameroon since 1988. **Peter Neuenschwander,** entomologist with IITA's Biological Control Program since 1983, assumed charge as Director of the Plant Health Management Division.

**Jan Diels** joined IITA's Resource and Crop Management Division in January 1995 as a postdoctoral fellow. Dr Diels received his MSc in agricultural engineering (1986) and PhD in biological sciences (1994), both from the Catholic University of Leuven, Belgium. He is based at Ibadan, Nigeria and will work on modeling of soil organic matter.

**Jacqueline d'Arros Hughes** joined IITA's Plant Health Management Division in December 1994 as a virologist. Dr Hughes obtained her PhD from the University of Reading, UK in 1987. More recently (1990–1993), she was a technical cooperation officer (for the control of cocoa swollen shoot virus, funded by the Overseas Development Administration, UK) with the Cocoa Research Institute in Ghana. Dr Hughes is based in Ibadan.

**Christopher J. Lomer** joined IITA's Plant Health Management Division in January 1995 as an entomopathologist. He received his PhD in entomopathology from the Imperial College, London in 1986. Most recently (1991–1994), he was with the International Institute of Biological Control (IIBC) as the leader of a collaborative research project on biological control of locusts based in Cotonou. Dr Lomer continues to be based in Cotonou.

**Kaku Sagary Nokoe** joined IITA in October 1994 as biometrician. Dr Nokoe obtained his PhD (resources inventory/biometrics) in 1976 from the University of British Columbia, Canada. Most recently, he was head of the Computer and Biometrics Unit at the International Livestock Centre for Africa (ILCA), Addis Ababa, Ethiopia. Dr Nokoe is based in the Resource and Crop Management Division at Ibadan.

**Louise Haly Ouraga-Djoussou** joined IITA's Crop Improvement Division at Ibadan in November 1994 as a visiting scientist, to work on aspects of cassava utilization. Dr Ouraga-Djoussou obtained her PhD in agricultural economics (1990) from Purdue University, Indiana, USA. From 1991 on, she has been an assistant professor at the University of Abidjan in Côte d'Ivoire, where she also led two research projects, one on women in the cassava economy and the other on integrated development.

## Institute of Biology honors IITA scientist

**George Thottappilly,** Head, Biotechnology Research Unit in IITA's Crop Improvement Division, was elected a Fellow of the Institute of Biology, with headquarters in London, in September 1994. This honor indicates that his knowledge and achievements are held in high esteem by professional peers.

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## About IITA

The goal of the International Institute of Tropical Agriculture (IITA) is to increase the productivity of key food crops and to develop sustainable agricultural systems that can replace bush fallow, or slash-and-burn, cultivation in the humid and subhumid tropics, thus helping especially to improve the nutritional status and well-being of low-income people. Crop improvement programs focus on cassava, maize, plantain, cowpea, soybean, and yam. Research findings are shared through international cooperation programs, which include training, information, and germplasm exchange activities, in a concerted effort to strengthen national agricultural research systems in sub-Saharan Africa.

IITA was founded in 1967. The Federal Government of Nigeria provided a land grant of 1,000 hectares at Ibadan, for a headquarters and experimental farm site, and the Rockefeller and Ford foundations provided financial support. IITA is governed by an international Board of Trustees. The staff includes around 180 scientists and professionals from about 40 countries, who work at the Ibadan campus and at selected locations in many countries of sub-Saharan Africa.

IITA is one of the nonprofit, international agricultural research centers currently supported by the Consultative Group on International Agricultural Research (CGIAR). Established in 1971, CGIAR is an association of about 40 countries, international and regional organizations, and private foundations. The World Bank, the Food and Agriculture Organization of the United Nations (FAO), and the United Nations Development Programme (UNDP) are cosponsors of this effort.


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