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Comparison of an Organophosphate Insecticide with a Mycoinsecticide for the Control of *Oedaleus senegalensis* (Orthoptera: Acrididae) and Other Sahelian Grasshoppers at an Operational Scale

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Operational scale field trials were conducted in 1996 and 1997, in the east of the Niger Republic, on 50 and 800 hectare plots, to compare the efficacy of an oil based formulation of the entomopathogenic fungus, Metarhizium anisopliae (flavoviride) *var.* acridum *(Deuteromycotina: Hyphomycetes) with fenitrothion for the control of Sahelian grasshoppers. The Senegalese Grasshopper* Oedaleus senegalensis *Krauss was the most abundant species in the trials.* M. anisopliae *was applied at* 5×10^{12} *spores* ha^{-1} *at volume application rates of 2 and 0.5 l* ha^{-1} *in successive years. Fenitrothion was applied at 220 g* ha^{-1} *at 1.25 and 0.22 l* ha^{-1} *volume application rates. Ultra low volume equipment mounted on a vehicle (1996) or a fixed wing aircraft (1997) was used for application. The* M. anisopliae *treatment reduced the grasshopper population significantly after 7 days and by 93% within 16 days. Fenitrothion caused a population reduction of more than 90% shortly after application, but due to immigration, the grasshopper population recovered to the initial level within 16 days. Grasshoppers treated with the fungus and given the opportunity to thermoregulate in the sun died more slowly than grasshoppers incubated in the shade. The survival of spores in the spray residue of the* M. anisopliae *plots assessed by exposing grasshoppers to the sprayed vegetation at intervals and monitoring disease levels during subsequent laboratory incubation, showed the spray residue to remain highly infective, for three weeks after spraying. At the end of the 1997 season, egg pod density and viability in the plot treated with the fungus was reduced compared with both untreated and the fenitrothion plots.*

Compared with the existing practice of large-scale treatment of grasshopper infestations

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with fenitrothion, use of M. anisopliae *would not only be safer to mammals and less damaging to non-target organisms, but also be more effective in the long-term control of grasshoppers.*

Keywords: *microbial control,* Metarhizium anisopliae, *entomopathogenic fungus,* Oedaleus senegalensis, *grasshoppers, Acrididae, spray residue*

INTRODUCTION

The Senegalese Grasshopper, *Oedaleus senegalensis* Krauss. (Orthoptera: Acrididae) is the most serious grasshopper pest in the Sahel (Cheke, 1990; McDonald, 1988). One of its favoured habitats is the Sahelian grasslands, where the Kram Kram grasses, *Cenchrus* sp are abundant. Since the 1970s, deforestation, overgrazing and droughts have favoured the development of such suitable habitats for *O. senegalensis* (Cheke *et al.*, 1980). At high population densities, *O. senegalensis* undergoes a phase change to a gregarious morph (Ahluwalia *et al.*, 1976). Early *O. senegalensis* instars develop in fallow or grassland. Subsequently, with desiccation of the fallow, or at very high population densities, late instars and adults invade adjacent millet fields which remain green for longer, and can destroy the crops within a few days (Cheke *et al.*, 1980). Between 1986 and 1987 a major outbreak of *O. senegalensis* was observed in the Sahel, during which some five million hectares were treated with insecticides. Major crop damage was avoided, but the overall cost was over US$ 60 million (Brader, 1988). Associated with *O. senegalensis* in the habitat described and also abundant are *Acrotylus blondeli* Saussure and *Pyrgomorpha cognata* Krauss (Orthoptera: Acrididae). Their economic importance, however, is low compared with *O. senegalensis* (Popov, 1989; Lecoq, 1978).

The LUBILOSA (French acronym for lutte biologique contre les locustes et les sauteriaux) programme has carried out trials for grasshopper control using the entomopathogenic fungus *Metarhizium anisopliae* (*flavoviride*) var. *acridum* Driver and Milner in eastern Niger since 1993. Kooyman *et al.* (1997) describe treatments of populations consisting mostly of *O. senegalensis*, which were reduced significantly in comparison with the controls after 9 days, and by 80% after 21 days. The host range of this fungus is very narrow and at field application rates it is safe to non-target Hymenoptera, Coleoptera and Homoptera (Ball *et al.*, 1994; Prior, 1997). This has also been demonstrated, during a very extensive ecotoxicological monitoring programme conducted together with the study presented here (Stolz, unpublished data). The lack of toxicity of *Metarhizium* to mammals (El-Kadi *et al.*, 1983; Zimmermann, 1993) offers an advantage over common chemical pesticides in the Sahel, where knowledge on safe handling and application of pesticides is often lacking.

Insects treated with *Metarhizium* die slowly compared to those treated with synthetic insecticides. Nevertheless, an advantage of the *M. anisopliae* formulation is its long persistence in the field. Thomas *et al.* (1997) reported a half-life of the spray residue of 7.7 days under Sahelian conditions. Healthy grasshoppers migrating into treated plots can still be infected by the fungus up to three weeks after application. The concentration of spores in the environment can be measured using susceptible insects in a bioassay (Müller-Kögler, 1965). Such a method was also developed for the *M. anisopliae* (*flavoviride*) va. *acridium*-grasshopper-system (Thomas *et al.*, 1995; Lomer *et al.*, 1997). For this paper we compared *M. anisopliae* with a standard organic insecticide, fenitrothion. The objective was also to test application techniques using improved *M. anisopliae* formulations and reduced volume application rates in order to make them acceptable for locust control operators (Bateman, 1997). This isolate of *M. anisopliae* had previously been applied by air in South Africa using a micro-light aircraft (Price *et al.*, 1996), but it was important to assess product efficacy when applied in an aerial block spray at a volume application rate of < 1 l ha^{-1}. A high work rate is essential: in order to achieve treatment of substantial infested areas during the narrow 'window of opportunity' early in the morning, when atmospheric conditions are suitable for ultra low volume (ULV) spraying.

Fenitrothion belongs to the group of organophosphate insecticides and has been one of the most widely used insecticides for grasshopper and locust control (MacCuaig, 1983). At operational dose rates fenitrothion provokes considerable noxious effects on aquatic invertebrate fauna (Lahr & Diallo, 1997) and spring tails (Peveling *et al.*, 1997). However, the half-life of fenitrothion is very short, not exceeding 20 h under Sahelian conditions (Gadji, 1997). The fast decomposition of this compound is an advantage in terms of ecotoxicological effects, but often requires repeated treatments (Brader, 1988).

The sampling technique described below for the monitoring of grasshopper population densities in treated and untreated plots has been successfully applied to demonstrate the efficacy of *M. anisopliae* against several grasshopper species (Kooyman *et al.*, 1997; Lomer *et al.*, 1993a; Douro-Kpindou *et al.*, 1995; Lomer *et al.*, 1997). A supplementary sampling technique is to collect grasshoppers from treated plots and incubate the samples under controlled laboratory conditions (Lomer *et al.*, 1993a; Johnson & Goettel, 1993). The cause of mortality can be confirmed by incubating cadavers under humid conditions and checking for external sporulation of *M. anisopliae*. In earlier studies it was observed that grasshoppers die faster under laboratory conditions than in the field (Langewald *et al.*, 1997). Blanford *et al.* (1998) demonstrated that *O. senegalensis* after infection with the fungus exposes itself to the sun in order to suppress the development of the fungus by raising its body temperature. This behavioural response increases the incubation time of the fungus. In the 1996 trials grasshoppers were incubated in the sun and the shade to investigate this effect.

In 1997, at the end of the season the impact of the different treatments on the fecundity of the grasshoppers was studied during an egg pod survey. *O. senegalensis* lays eggs in patches, but not in such dense clusters as the Desert Locust. Towards the end of the rainy season *O. senegalensis* females start to lay diapausing eggs, which may hatch at the earliest in March–April of the following year (Fishpool & Cheke, 1983). Egg pods are frequently attacked by predators such as *Systoechus* spp. (Diptera: Bombilidae) (Cheke *et al.*, 1980).

MATERIALS AND METHODS

Field Site

In August 1996 and 1997, sites were chosen in a dry savanna grassland near Maine Soroa, in the south-east of Niger, West Africa (13°22′N 12°06′E; 13°22′N 12°09′E; 13°11′N 12°06′E; 13°11′N 12°09′E). The annual rainfall in this area varies considerably with an average of about 250 mm per year. Two-thirds of the area consisted of range land (Chiffaud-Mestre & Jahiel, 1997); grasses (mainly *Cenchrus* spp.) and herbaceous plants ranged from 5–20 cm in height at the time of spraying. About one-third was sand dunes or planted with pearl millet (*Pennisetum americanum*). The field sites were scattered with trees of *Acacia* spp., *Balanites* spp. and *Pergularia tomentosa* bushes. The grasshopper community can be described as Sahel- to semi-desert-type consisting mainly of the following species: *O. senegalensis*, *Acrotylus blondeli*, *Acorypha clara* (Walker) (Orthoptera: Acrididae), *Poekilocerus bufonius* (Klug) (Orthoptera: Pyrgomorphidae) and *P. cognata* Krauss (Chiffaud-Mestre & Jahiel, 1997), the dominant species being *O. senegalensis* and *A. blondeli* at the time of spraying. The average daily climatic conditions during the observation period of 22 days were in 1996: min. temp. 22.5°C (SE = 0.28), max. temp. 33.1°C (SE = 0.45), min. RH 49.6% (SE = 1.75), max. RH 91.4% (SE = 0.74). In 1997 the daily climatic conditions were: min. temp. 22.4°C (SE = 0.31), max. temp. 43.4°C (SE = 1.09), min. RH 28.4% (SE = 2.7), max. RH 95.5% (SE = 0.94). Temperature and relative humidity were measured using a data logger one metre over the ground.

Application

In 1996, nine 50-ha plots were marked out. Three randomly chosen plots were treated with an oil-based ULV formulation containing dry spores of *Metarhizium anisopliae* (*flavoviride*) var. *acridum* (strain IMI 330189, isolated from *Ornithacris cavroisi* Finot (Orthoptera: Acrididae) in Niger) passed through a 160 μm sieve, and 2% Lumogen, a fluorescent tracer

TABLE 1. Application details and parameters for field trials carried out in 1996 and 1997 comparing *M. anisopliae* (*flavoviride*) var. *acridum* with fenitrothion for grasshopper control

	1996		1997	
Product	*M. anisopliae* (*flavoviride*) var. *acridum*	Fenitrothion	*M. anisopliae* (*flavoviride*) var. *acridum*	Fenitrothion
Plot size (ha) (replication)	50 × 3	50 × 3	800	800
Specification	Dry spores 5×10^{10} spores g^{-1}	200 g a.i. l^{-1}	Oil miscible flowable concentrate 2.5×10^{13} spores	1000 g. a.i. l^{-1}
Formulation oils	Shellsol:Ondina (50:50) plus 2% Lumogen tracer	Non-diluted	Concentrate: diesel (40:60) Plus 2% Lumogen tracer	Non-diluted plus 2% Lumogen tracer
Volume (l ha^{-1})	2	1.25	0.5	0.225
Application rates				
Flow rate (ml s^{-1})	20	15	233	100
Forward speed (km h^{-1})	12	14	167	167
Swath width (m)	30	30	100	100
Sprayer	Micron ULVA mast mark II	Micron ULVA mast mark II	Micronair AU 8000 on Chessna Air truck plane	Micronair AU 8000 on Chessna Air truck plane
Disk speed (rpm)	4700	4700	5500	5500
Volume median diameter of droplets (mm)	85	85	85	85
Emission height (m)	2.5	2.5	10±3	10±3
Cloud cover (%)	80–100	80–100	80–100	80
RH (%)	50–90	50–90	50–90	55–87
Wind speed (ms^{-1})	2–4	2–3.5	1.5–2.5	2.5–3.5

(formulated by Micron Sprayers Ltd, Bromyard, Herefordshire, UK). Inoculum was applied at 5×10^{12} spores ha^{-1}, at a volume application rate of 2 l ha^{-1}, using a vehicle mounted ULVA-Mast Mark II spinning disk sprayer (Micron Sprayers Ltd). The *Metarhizium* plots were treated on three consecutive days. The three fenitrothion plots were treated on the same consecutive days as the *Metarhizium* plots using a separate vehicle and ULVA-Mast sprayer. Fenitrothion 20, containing 200 g a.i. l^{-1}, was applied at 250 g ha^{-1}. Lumogen tracer was only available for the *M. anisopliae* formulation (for application details see Table 1). The remaining three plots (controls) were left untreated, since previous studies have demonstrated no effect of blank formulation treatments on grasshoppers (Shah, 1994).

In 1997, three plots of 800 ha were chosen, each with millet fields in the centre. Both products were applied in the early morning (07:30–09:00) by air on separate days (4th and 6th August), using a Cessna Ag. Truck 188 spray plane belonging to the Plant Protection Service of Niger. It was fitted with four Micronair AU5000 nozzles. One plot was treated with a mixture of an oil miscible flowable concentrate (OF) formulation of *M. anisopliae* (*flavoviride*) var. *acridum* (strain IMI 330189), diluted with diesel and 2% Lumogen. Inoculum was applied at 5×10^{12} spores ha^{-1} at a volume application rate of 0.5 l ha^{-1}. The second plot was treated with technical Fenitrothion 1000 as a chemical standard, containing 1000 g a.i. l^{-1}, at 220 g ha^{-1} and a volume application rate of 0.22 l ha^{-1} (for application details see Table 1). The remaining plots (control) were left untreated.

Population Density Monitoring

In 1996, a double nested repeated measure experimental design with three replicates was used to monitor population density. In the centre of each of the three different treatment

plots, four 100 m long diagonal transects were laid out, starting 10 m from the central point and extending towards the four corner points of each plot. The grasshopper population density was monitored by counting the number of grasshoppers within 25 imaginary one-square-metre-quadrates along these lines (Douro-Kpindou *et al.*, 1995). The same observer always counted the same line to decrease variability.

In 1997 a pseudo-replicated triple nested set up was used. Each of the three plots contained three sampling zones in the non crop area, which were designed in the same way as the main repeated plot in 1996. Both data sets were analysed using mixed model repeated measure analysis of covariance designs (Sokal & Rohlf, 1997).

Cage Incubation Samples

Samples of 50 grasshoppers were collected in the *M. anisopliae* plot using a sweep net immediately after spraying. In 1997, samples were collected also in the fenitrothion treated plots. By examining the insects under UV light the droplets of fluorescent tracer were counted, allowing an estimate of the proportion of insects initially hit by the spray. After the assessments the grasshoppers were discarded.

For cage incubation a second set of mixed samples of 50 late instar nymph and adult grasshoppers were caught in the *M. anisopliae* plot around the edges of each sampling zone. In 1996 this was done by walking. It was observed that the proportion between adults and nymphs in the catches varied according to the forward speed of the person collecting the insects. Thus in 1997, sampling was standardized by dragging a sweep net at a constant speed of 20 km h^{-1} around the sampling zone twice, using a vehicle. The samples were introduced into portable wooden cages with metal wire mesh sides (25 × 30 × 25 cm). In the treated and the control plots, the first sample was taken 3 days before treatment. Further samples were taken in the treated plots one day after application (day 1), and subsequently on day 4, 6, 10, 13, 16, 19 and 22 after application. The caged samples were stored under a shaded roof (22–43°C; 28–95% RH) and mortality was observed over 21 days. In 1996 an identical set of grasshopper samples was held in cages exposed to the sun (22–55°C; 15–95% RH) to allow the insects to thermoregulate.

Monitoring of the Spore Residue

The persistence of the spores from the spray residue in the *M. anisopliae* plot was estimated using a field bioassay, in which healthy insects were caged and exposed to the vegetation in the treated plots for 3-day periods after spraying. Two foldable field cages with polyester mesh sides (50 × 50 cm surface, 50 cm height, with a zip in the top and no floor) were each placed over some vegetation in each of the three *M. anisopliae* plots or sampling sites immediately after the treatment. Twenty healthy non-treated late instar nymph and adult grasshoppers were collected at least 300 m from the treated plots and introduced into each field cage. Three days later, the insects were retrieved from the field cages and incubated in containers, made out of plastic mineral water bottles with the bottom replaced by mosquito screen, in the shade until the 21st day after collection. At the same time, the position of the field cages was changed and new grasshoppers were introduced. In this way, secondary pick-up was monitored over a period of 19 days.

Cadavers from the cage samples were transferred onto Petri dishes containing wet filter paper. The number of cadavers with external formation of *M. anisopliae* spores (sporulation) were noted.

No samples for cage incubation were collected in the fenitrothion plots because most grasshoppers died in a few hours. The short persistence of the product made sampling for the decline of the spray residue obsolete.

Contamination During Sample Collection

In 1997 the importance of the contamination of grasshoppers during sampling with sweep nets was studied in the plot treated with *M. anisopliae*. Six sweep nets were disinfected by

leaving them for 10 min in 12% sodium hypochloride solution. One net was dragged for 3 min over the contaminated vegetation around one sampling zone. Grasshoppers from a non-treated area were then introduced into the contaminated sweep net by shaking it for 3 min. The same treatment was repeated with 6-min intervals. These evaluations were carried out in all three sampling zones and repeated at three-day intervals. The grasshopper samples were incubated in the shade as described above.

Population Structure

The population structure was monitored by sampling grasshoppers using sweep nets around each of the sampling zones. In 1996 this was done by walking. In order to decrease the sampling error, in 1997 the samples were collected using a vehicle at constant speed, as described above. Between 25 and 50 grasshopper nymphs and adults were collected in cages; instar and species were recorded. After the assessments the grasshoppers were discarded.

Egg Pod Survey

At the end of the rainy season in 1997, four months after the aerial applications of *M. anisopliae* and fenitrothion, an egg pod survey was carried out. Ten squares (10 m^2) were marked out, one in the centre, the others along the edges of each of the three sampling sites in the three plots. The upper 10 cm of soil at the surface was removed and the egg pods dug out. The number of viable and non-viable egg pods was noted. The data were arcsine $\sqrt{x}$ transformed where necessary and compared using two-way-ANOVA.

RESULTS

Application

In 1996, mixing of the dry spores with the formulation oils was more time consuming, but did not cause major problems, as in the years before, when sieves clogged regularly, while in 1997 the preparation of the formulation was much easier since the flowable miscible concentrate had only to be poured into the aircraft tank together with diesel. All plots sprayed received an evenly distributed spray, despite the reduction of the volume application rate of *M. anisopliae* in 1997, to one-quarter of the previous standard rate of 2 l ha^{-1}.

In 1996 examination of insects marked with tracer the evening after the application using UV light revealed an average hit rate of 87% (SE = 4.2%) for all grasshopper instars combined in the three *M. anisopliae* plots. In 1997, the percentage of grasshoppers covered with droplets of tracer was 79.4% (SE = 4.1%) for nymphs and 81% (SE = 1.9%) for the adults after the application of fenitrothion and 83% (SE = 3.5%) and 82% (SE = 2.4%) for nymphs and adults, respectively, in the *M. anisopliae* plot.

Population Reduction

In 1996 the population counts in the untreated plots remained constant over the whole observation period of 22 days. The counts in the plots treated with *M. anisopliae* declined with time and differed significantly from the control plot from day seven after application and counts were significantly lower ($P < 0.05$) compared with the fenitrothion plots from day 16 onwards. Correction of changes in the treated populations densities for changes in the control populations and differences in initial density using the Henderson and Tilton (1955) formula revealed the efficacy of the biopesticide application to be 86% after 22 days. Fast kill was observed in the plots treated with fenitrothion. Many dead grasshoppers were found. First counts after application demonstrated a treatment efficacy of more than 90% using the Henderson and Tilton formula. These counts differed significantly from the counts in the control plots until day ten after application ($P < 0.05$). With time, however, the population counts in the fenitrothion plots increased again, presumably due to immigration

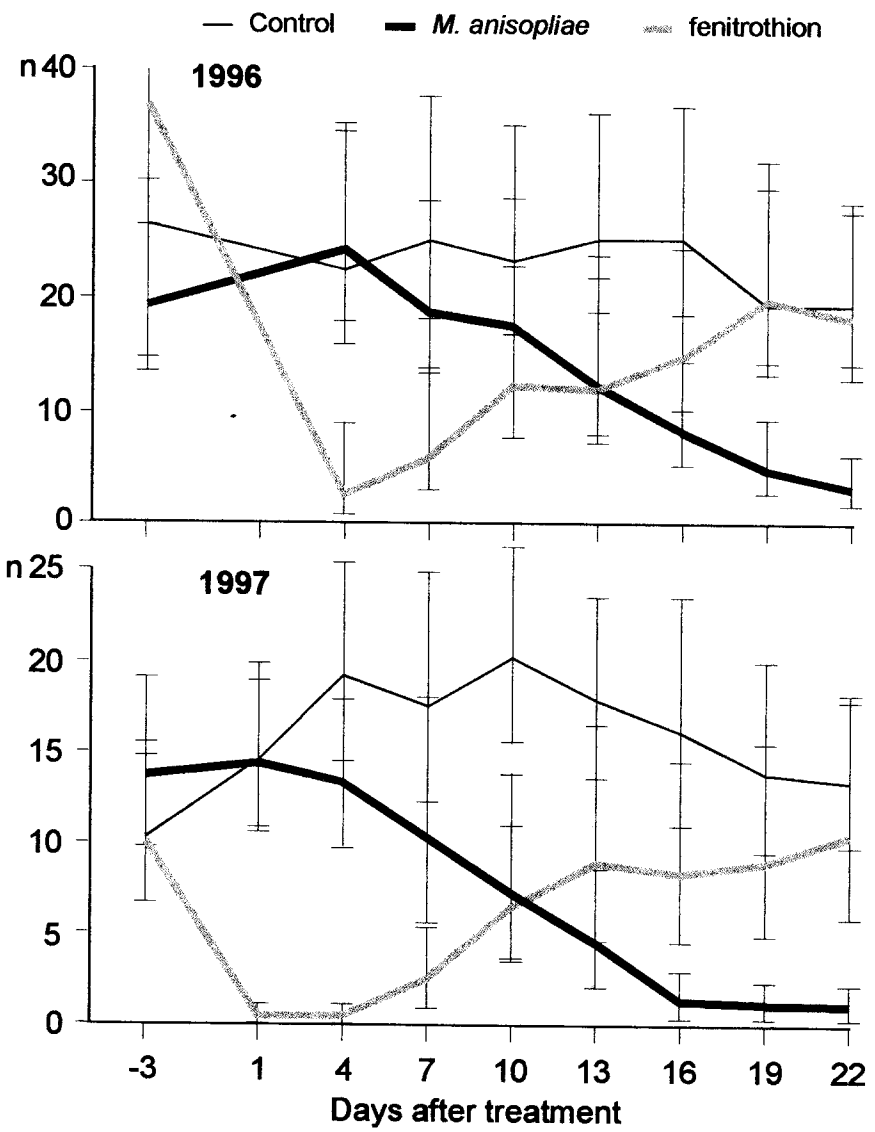

FIGURE 1. Mean counts (±SE) of grasshoppers per square metre over a period of 22 days in untreated plots, plots treated with an oil based formulation of *Metarhizium anisopliae* (*flavoviride*) var. *acridum* and with fenitrothion during two rainy seasons in east Niger.

of adults. The grasshopper counts in the plots treated with fenitrothion were significantly lower compared with the counts in the *M. anisopliae* plots until day 10 ($P < 0.05$) (Figure 1).

In the repeated measurement analysis of covariance over all three treatments, 82% of the total variability between the population density counts was due to variability between the replicates and the four counting transects, which were always evaluated by the same four technicians. The time–treatment interaction was highly significant ($P < 0.05$).

In 1997, the population counts in the untreated plots first increased and later declined again to the initial level over an observation period of 22 days. The counts in the plots treated with *M. anisopliae* differed significantly from the controls starting from day 16 after application.

Using the Henderson and Tilton formula, the corrected population counts demonstrated 93% treatment efficacy for the *M. anisopliae* application after 22 days (Figure 1). As in 1996, fast kill with many cadavers was observed in the plots treated with fenitrothion. In the first counts after application, the population counts had declined by more than 90% (Figure 1). The day 1 and day 4 counts differed significantly ($P < 0.05$) from the counts in the control and the *M. anisopliae* plots. The population recovered subsequently, as observed in 1996. On day seven after application there was no significant difference between the fenitrothion treatment and the control.

In the repeated measurement analysis of covariance over all three plots and time, the variability between the four counting transects, which were always evaluated by the same four technicians, explained 28% of the total variance. Variability between the counting sites within each plot accounted for 49% of the total variance. The time–treatment interaction was highly significant ($P < 0.01$).

Cage Sample Mortality

The cumulative mortality curves from the cage incubations in the shade of the samples collected in the *M. anisopliae* plots are shown in Figure 2. In 1996, mortality levels in the caged samples were very high for all sampling dates and reached 100% after 12 days and, in 1997, 100% after 16 days for those insects collected one day after spraying (Figure 2). The fungus sporulated in 1996 on a mean of 85% (SE = 1.65%) and in 1997 on a mean of 53% (SE = 1.6%) of the cadavers from all field samples. Mortality in untreated samples from the control plots reached 19.8% (SE = 6.7%) at the end of the cage incubation period of 21 days in 1996. No sporulation was observed on these cadavers. In 1997, day one and control sample data were lost.

In the sun, the samples collected in 1996 in the *M. anisopliae* plots died more slowly than insects incubated in the shade (Figure 2). Mortality levels in the caged samples reached 100% after 20 days for those insects collected one day after spraying (Figure 2). A mean of 81% (SE = 1.3%) of the cadavers from the field samples sporulated with the fungus. Mortality in non-treated samples from the control plots reached 11.8% (SE = 0.6%) at the end of the cage incubation period of 21 days. No sporulation was observed on these cadavers.

Spore Residue

In 1996, the cumulative mortality in the secondary pick-up samples from the plots treated with *M. anisopliae* reached 100% after 18 days of incubation in the laboratory, for the sample exposed to the treated vegetation on the day of application (Figure 3). Total mortality in consecutive samples remained high, still reaching 91% (SE = 11%) in the sample taken 19 days after application. Sporulation occurred on 81% (SE = 1.1%) of the cadavers incubated under moist conditions. Mortality in grasshopper samples exposed to non-treated vegetation reached 20% (SE = 1.9%). No sporulation was observed on these cadavers.

In 1997, in the day four secondary pick-up samples all grasshoppers died by the 12th day of incubation. The total mortality was 100% in all samples taken until day 22 after treatment. Later, total mortality declined and reached a level similar to the control mortality 46 days after treatment. Sporulation occurred on 59% (SE = 1.2%) of the cadavers. Mortality in non-treated samples from the control plots reached 10.6% (SE = 0.6%) at the end of the cage incubation period of 21 days. No sporulation was observed on these cadavers (Figure 3).

Contamination During Sampling

Samples of grasshoppers exposed to sweep nets which were dragged over the sprayed vegetation were contaminated with spores of *M. anisopliae*. The percentage mortality in the first samples after spraying was 78% (SE = 8.7%) in those exposed over 6 min and 85% (SE = 2.6%) of the hoppers exposed over 3 min. At the end of the observation period 19 days after application mortality due to contamination remained high at 85% (SE = 1.1%) after 6 min of exposure and 45% (SE = 11.4%) after 3 min of exposure (Figure 4). The

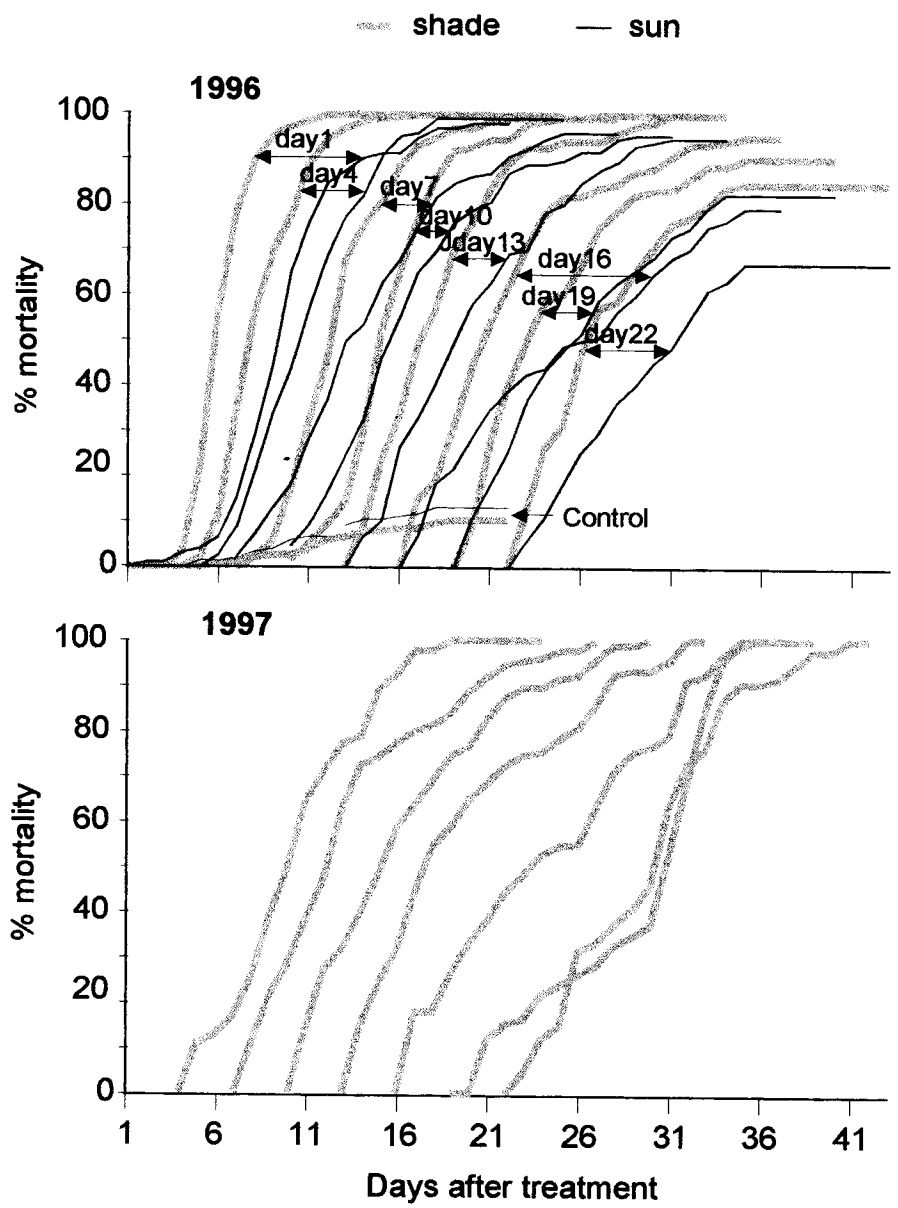

FIGURE 2. Cumulative mortality curves of caged grasshopper samples collected in plots treated with an oil based formulation of *Metarhizium anisopliae* (*flavoviride*) var. *acridum*, at three-day intervals and incubated in the shade over 22 days, during two rainy seasons in east Niger. In 1996 subsamples were also exposed to the sun.

percentage sporulation in the contamination samples was 28% (SE = 1%) after 6 min of exposure and 17.3% (SE = 4.8%) after 3 min of exposure to the treated vegetation, one day after spraying. At the end of the observation period 1.9% (SE = 1.1%) of the insects sporulated after 6 min of exposure and 1.3% (SE = 1.3%) after 3 min of exposure (Figure 4). Thus it seems likely that handling mortality rather than actual contamination is a more important factor in the increased mortality in these samples.

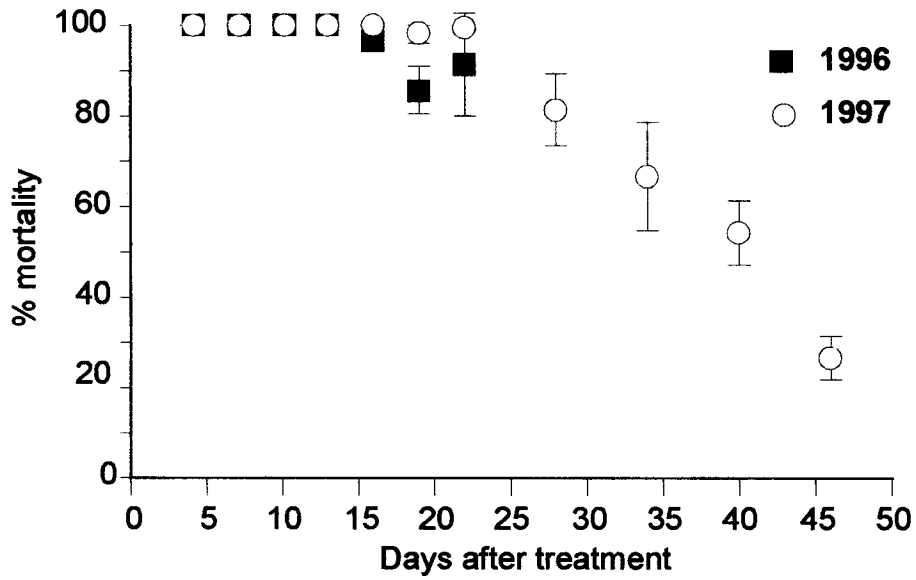

FIGURE 3. Cumulative total mortality (±SE) of caged grasshopper samples, which had been exposed for three days on vegetation treated with an oil based formulation of *Metarhizium anisopliae* (*flavoviride*) var. *acridum*, at three-day intervals and incubated in the shade over 21 days, during two rainy seasons in east Niger.

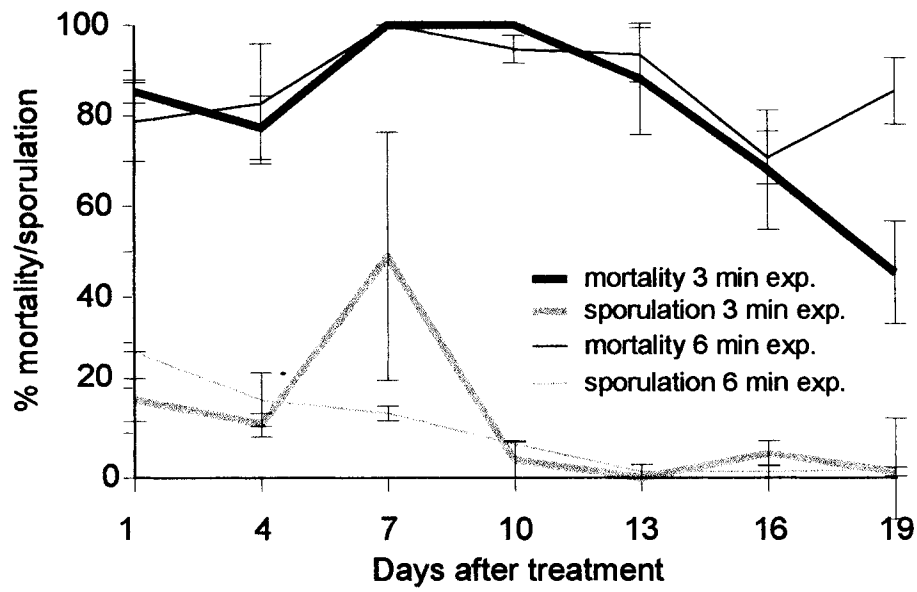

FIGURE 4. Cumulative total mortality and sporulation (±SE) of caged grasshopper samples, which had been exposed for 3 or 6 min to sweep nets which had been dragged for 3 or 6 min over a vegetation treated with an oil based formulation of *Metarhizium anisopliae* (*flavoviride*) var. *acridum* and at three-day intervals and incubated in the shade over 21 days, during the 1997 rainy seasons in east Niger.

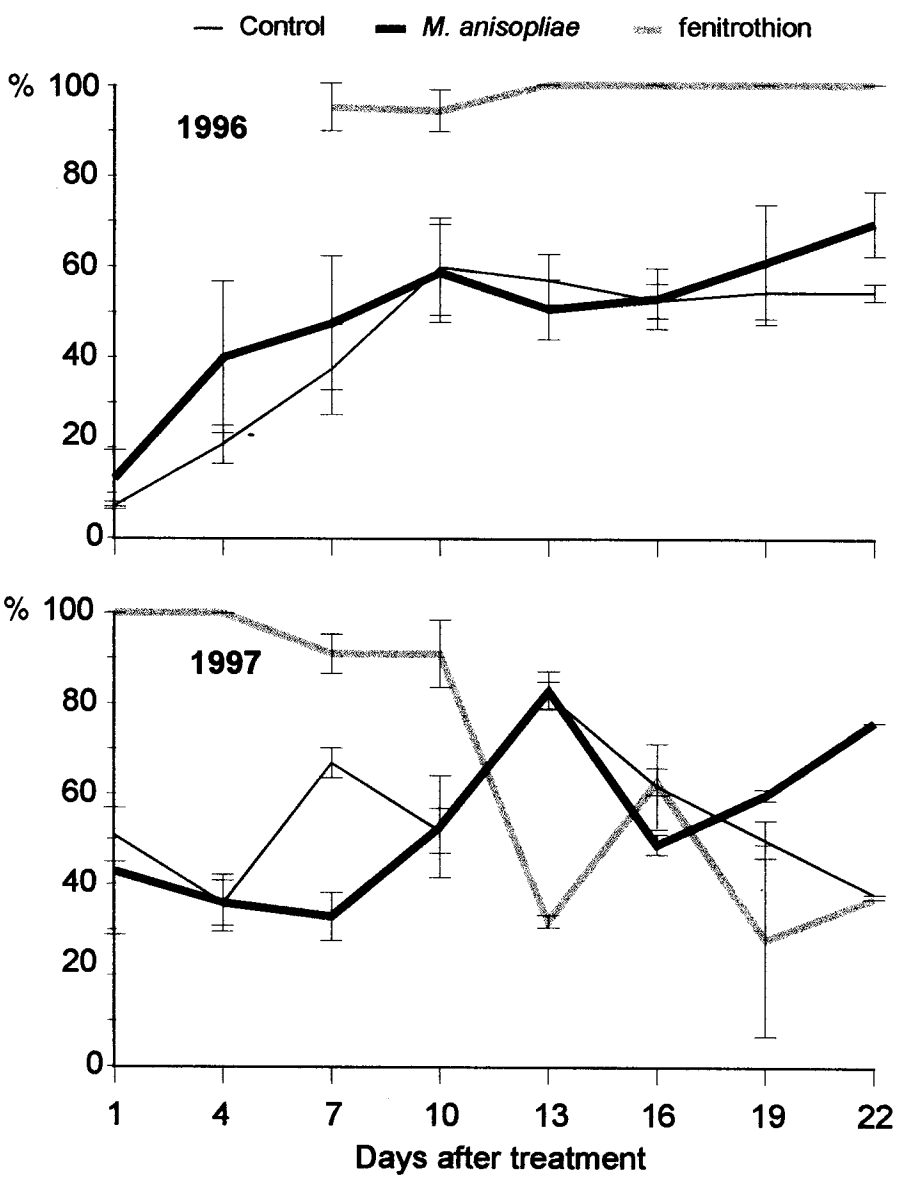

FIGURE 5. Proportion of adult grasshoppers (±SE) in sweep net samples over a period of 22 days in untreated plots, plots treated with an oil based formulation of *Metarhizium anisopliae* (*flavoviride*) var. *acridum* or with fenitrothion at three-day intervals, during two rainy seasons in east Niger.

Population Structure

The population structure of the trial area varied between 1996 and 1997. In the first year, *O. senegalensis* was strongly dominant (95%, SE = 1.5%). In the second year, the dominant grasshopper species were *O. senegalensis* (39%, SE = 2.3%), *A. blondeli* (35%, SE = 1.2%) and *P. cognata* (26%, SE = 2.1%). The proportion of adult grasshoppers in the 1996 plots treated with *M. anisopliae* and the non-treated plots increased from less than 10% to about 50% at the end of the observation period (Figure 5). In the plot sprayed with fenitrothion,

however, the proportion of adults increased rapidly after application, and reached nearly 100% in the day seven sample. No grasshoppers were collected on days one and four after application, because the population density was too low (Figure 5). In 1997, the situation was similar. While the initial proportion of adults was similar in all plots, it increased drastically after application in the fenitrothion plot. Starting on the tenth day after application, the proportion of adults decreased again due to hatching. This occurred in all plots (Figure 5).

Egg Pod Survey

Four months after the treatment when all vegetation was dried out, the density of viable and non-viable egg pods was examined in the three different plots. The treatment effect on the average egg pod density was significant ($P < 0.001$). In the *M. anisopliae* plot, the density was low (1.9 egg pods m^{-2}, SE = 0.46) compared with the non-treated (5.3 egg pods m^{-2}, SE = 0.74) and fenitrothion treated (6.7 egg pods m^{-2}, SE = 1.18) plots. Also the proportion of viable egg pods varied significantly between the differently treated plots ($P < 0.05$). In the plot treated with the fungus only 14% (SE = 7%) of the egg pods were viable compared with 70% (SE = 6.6%) in the fenitrothion plot and 63% (SE = 7%) in the non-treated plot. The average egg pod density in the field was 3.84 egg pods m^{-2} (SE = 0.8), compared with 5.49 egg pods m^{-2} (SE = 0.7) in the grass land, calculated across all plots.

DISCUSSION

In Europe and North America the organochlorine insecticide dieldrin was banned at the beginning of the 1970s due to growing public concern over environmental effects. However, in Africa even in 1988, 13% of the locust control campaigns were still carried out using this noxious product (Crick, 1990). A long persistence in the environment was, in terms of locust and grasshopper control, one of the big advantages of dieldrin. This insecticide used to be applied at an overall application rate of 10 g a.i. ha^{-1}, i.e. one-fiftieth of the quantity used for contact spraying (Courshee, 1990). Thus dieldrin was not only cheap, but its persistence also allowed the development of barrier spraying where large areas could be treated economically with little effort. Locusts crossing such a barrier and feeding on the contaminated vegetation were still killed after a couple of days, even several weeks after the application (Courshee, 1983).

Later environmentally less noxious, non-persistent contact insecticides proved to be very useful for direct air to air swarm control (Courshee, 1990). For ground operation, fenitrothion became the most widely used insecticide for locust and grasshopper control (MacCuaig, 1983). However, its short persistence often makes repeated applications necessary, when treated areas become re-invaded by migrating grasshoppers or locusts (Brader, 1988).

The current study is the first where the efficacy of the novel LUBILOSA mycopesticide is compared with a chemical insecticide under operational conditions. An innovative product such as the *M. anisopliae* formulation, however, is unlikely to be accepted unless it is easy to handle. The OF formulation proved to be easy to handle, involving a minimal amount of preparation before spraying, and thus helped to achieve an acceptably high work rate. A volume application rate of 0.5 l ha^{-1} enables the treatment of approximately 1000 ha in a single sortie, with an aircraft that has a tank capacity of 500 l. Although one-quarter of the 2 l ha^{-1} applied previously, this volume application rate provided an adequate droplet coverage, and insect hit rate only slightly lower than in 1996. In order to improve the formulation, a new method for the separation of conidia from its solid substrate resulted in more homogeneous particle size and a much higher purity of the spore powder (N. E. Jenkins *et al.*, unpublished observations). Compared with previous trials (Kooyman *et al.*, 1997), the mixing of spores became much easier in 1996. When formulations have to be prepared very quickly and in large quantities, the mixing of dry spores would still be too time consuming.

The effect of grasshopper invasion into plots treated with fenitrothion became obvious during this study. This could not only be demonstrated with the population count data which showed the effects of re-immigration after only one week, but also with the population structure data. After the application of fenitrothion, the empty niches were occupied first by adults, which can fly and migrate much faster than nymphs. In 1996, in the fenitrothion plots, after application only adults could be observed up to the end of the observation period. In 1997, the high proportion of adults in the plots treated with fenitrothion declined towards the end of the observation period, probably due to hatching of young grasshoppers after heavy rainfall. The experimental design used in 1996 resulted in clearer differences between the different treatments compared with 1997 data. This is not surprising since a fully replicated set-up was used in the first year. The set-up using pseudo-replicates is less powerful. When comparing the variability components of the different levels of nesting, the large variability between the four counting transects became apparent. The perception of grasshopper densities varied between the four technicians, and restricting them always to the same transects proved to be valuable.

The population count results demonstrate that, compared with fenitrothion, *M. anisopliae* provides much better long term control. Previous studies on *Z. variegatus* (Langewald *et al.*, 1997) and *Schistocerca gregaria* (Bateman *et al.*, 1998) suggested a strong effect of the spray residue in the treated plots, infecting both immigrating grasshoppers and those that escaped infection during the initial application. In the case of the present study on *O. senegalensis*, mortality in the secondary pick-up samples from the *M. anisopliae* plots remained high for a long period, indicating good persistence of the spores in the treated vegetation. In 1997, spores in the vegetation were still virulent after more than six weeks. This is surprising, since Sahelian climatic conditions and especially solar radiation are supposed to be less favourable for spore survival than the conditions in cassava fields in the southern humid forest zone. In the Sahel during the rainy season, however, temperatures and solar radiation can be significantly reduced during cloudy and rainy days and the conditions for spore persistence may be better than commonly supposed. Thomas *et al.* (1997) fitted the total cumulative mortality of grasshopper samples that had been exposed to treated vegetation to negative exponential curves. In the present study this was not possible simply because the spore residue remained extremely infective over such a long period. Cycling of the fungus through grasshopper cadavers making new spores available after some time might also be a plausible explanation. However, a special experimental set-up would be needed to separate the effect of spore survival from the effect of cycling. A possible approach could be to spray a plot with a non-persistent organic knock-down insecticide first, and apply the *Metarhizium* formulation afterwards. Cycling through cadavers could be avoided, and assuming little immigration, spore survival could be monitored as described for this study.

A long-term consequence for the grasshopper population in the *M. anisopliae* plots is its reduced potential hatching, which can have an important impact on the next season's population density. The 1997 late season egg pod survey revealed not only that the egg pod density in the plots treated with the fungus was reduced compared with the control and fenitrothion treated plot, but also that a higher proportion of egg pods was destroyed by natural enemies. Many natural enemies like *Systoechus* spp. (Diptera, Bombylidae) or *Trox* spp. (Coleoptera, Trogidae) attack egg pods after wandering around in search of their prey (Greathead, 1992). There might be a defence mechanism in egg pods which is weakened in grasshopper populations infested with the fungus, but a more detailed study is needed to support this hypothesis.

Compared with organophosphate and pyrethroid insecticides, mortality due to *M. anisopliae* is slow, and this may lead to a slow uptake of the product by users. Efficacy in terms of reduced damage on the crops after the application of the fungus is, however, higher than expected from the figures of mortality, because the feeding of the infected grasshoppers or locusts is significantly reduced (Moore *et al.*, 1992; Thomas *et al.*, 1997). In a preventative strategy, as recommended by FAO, where grasshopper nymphs are treated before they

migrate into the millet crop (Cheke, 1990), slow speed of kill would not be a major disadvantage. Grasshopper populations could be monitored from the beginning of the rainy season during their development outside the fields, and preventative treatments applied when high densities of nymphs are encountered.

Blanford *et al.* (1998) demonstrated the importance of behavioural response of grasshoppers to the fungal infection. As soon as *O. senegalensis* has the opportunity to thermoregulate it will increase its body temperature above ambient by basking and thus slow down the development of the fungus. In Canada, Inglis *et al.* (1996) demonstrated a suppression of *Beauveria bassiana* in the grasshopper *Melanoplus sanguinipes* due to thermoregulation resulting in highly reduced mortality. During this study, results from 1996 grasshopper samples incubated in shaded cages and cages which were exposed to the sun support these observations. Grasshoppers in the cages exposed to the sun died more slowly. The proportion of cadavers sporulating under moist conditions in Petri dishes was similar between the grasshoppers which were incubated in the sun and those which were incubated in the shade. This shows that only some cloudy days are sufficient for the fungus to develop, whereas with grasshoppers having the permanent opportunity to thermoregulate the fungus would sporulate on fewer cadavers (Blanford *et al.*, 1998).

In 1997, the percentage of cadavers from the plot sprayed with the fungus, sporulating in moist Petri dishes was lower compared with the 1996 season. This can be explained with the average maximum daily temperature being 5°C higher compared with 1996, and temperature affects sporulation of *Metarhizium* (Ouedraogo, 1996). The initial mortality of the cumulative curves for samples of grasshoppers taken from the treated plots at days 6 and 9 was higher than at day 0. This indicates that the samples were taken from a diseased population dying in the field and that the grasshoppers were not predisposed to the disease by cage incubation.

An additional factor explaining the difference in the speed of kill between grasshoppers incubating in the field and those kept in cages in the laboratory might be the effect of enhanced contamination due to the sampling process. The mortality in the samples taken during the sweep net contamination study was high, but only a low proportion of the cadavers sporulated with the fungus. Mortality and the proportion of sporulating cadavers did not differ between the 3- and the 6-min treatments. A high proportion of the mortality in the samples taken for the sweep net contamination study was probably caused by handling and not by mycosis. Handling of the insects during the contamination study took much longer compared with sweep net sampling for other purposes.

The standardized sampling technique was developed principally to decrease the variability in the population structure data. However, samples of sweep nets dragged at a constant speed did not have an advantage over less cautious sampling. In 1997, the variability between the sampling dates in the population structure data was even higher than in 1996. A less homogeneous species composition in 1997, however, makes difficult a direct comparison with the 1996 data.

There is no doubt that *M. anisopliae* can be successfully applied against Sahelian grasshoppers. Furthers studies are planned for the development of a general integrated grasshopper management strategy involving plant protection services, non governmental organizations and farmers to optimize the usefulness of this novel product. In the near future *M. anisopliae* will be produced on an industrial scale and made available in West Africa.

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