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Efficacy and Environmental Impact for Biological Control of *Nosema locustae* on Grasshoppers in Cape Verde

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A Synthesis Report

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Preface

This report reflects the research undertaken in Cape Verde in 1989, in connection with broader programs seeking to develop improved management methods and strategies against locusts and grasshoppers. The specific purpose of the studies described herein was to evaluate (a) the efficacy of a microbial parasite, *Nosema locustae* Canning, against Sahelian grasshoppers found in Cape Verde, and (b) the possible ecological side effects of the application of this biological control agent.

This report summarizes collaborative research initiated by the Bureau for Africa of USAID, involving several organizations in the Republic of Cape Verde, U.S.A. and Germany. Inputs by these groups were varied in terms of finance, staff and materials. Financial support came from USAID-Washington through its central project "Africa Emergency Locust & Grasshopper Assistance (AELGA)" and the Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH, Eschborn, Germany, through its project "Integrated Biological Control of Grasshoppers and Locusts", financed by the Bundesministerium für wirtschaftliche Zusammenarbeit (BMZ), Germany.

For the sake of simplicity the organisations involved in the work have been listed below including a summary of their involvement and the origin of financial support.

1. Instituto Nacional du Investigacao Agraria (INIA), Ms. M.L. Lobo-Lima, President (INIA) for program liaison and logistics support. INIA provided laboratory and insectary facilities and Mr. M.J. Brito as in country project coordinator participating in project planning and implementation (USAID).

2. Plant Protection Department (DPV) in Cape Verde, Technician Mr. F. Delgado (Chief of DPV) for logistics support and technical assistance (USAID).

3. Montana State University (MSU) as primary institutional counterpart to INIA, provided the Project Coordinator, Dr. J.E. Henry and TDY (Temporary Duty) by three specialists. Mr. J. Evans provided direction and oversight for plot selection, formulations, application, treatment assessment, assemblage of data and preparation of reports. Dr. C. Lange conducted pre- and post-treatment examinations of grasshoppers, initiated development of the grasshopper rearing facility and assisted with formulation of the *Nosema*-wheat bran preparations. Dr. T. Sluss provided assistance in post-treatment assessments, assemblage of data and draft report preparation (USAID).

4. Dr. G. Jansen, team leader of the GTZ Plant Protection Project, Cape Verde, involved in program liaison and logistics support (GTZ).

5. Dr. O. Nasseh, staff member of GTZ Locust Project, participating in field programs (GTZ).

6. University of Saarland, Germany, Institute for Biogeography, Prof. Dr. P. Müller and Dr. V. Guthörl carried out the ecotoxicological studies. The facilities of the Institute were utilised for laboratory studies (GTZ).

7. Ciba-Geigy, Basel, Switzerland.

The overall coordinators of this program, involved in project planning and implemen-

tation, were Stephan Krall for the GTZ and Dr. Walter I. Knausenberger for USAID.

This report, prepared by GTZ Eschborn, is a synthesis based on the reports by Dr. J.E. Henry on the field trials and Prof. Dr. P. Müller covering the ecotoxicological studies. It includes a summary of the most important findings and edited versions of the two reports mentioned including only parts relevant to the effectiveness and environmental impact of *Nosema* application. This report is financed through the GTZ Locust-Project mentioned above.

Efficacy and Environmental Impact of Nosema locustae on Oedaleus senegalensis in Cape Verde

Summary

General Introduction

In the recent past it has been decided by various organisations to improve the control of locusts. Emphasis has been on finding integrated pest management (IPM) methods including biological control agents. These methods should have as little negative impact on the environment as possible and ultimately replace the standard treatments using insecticides only of the past. In order to reach this goal a number of field-oriented research programmes were initiated. Here the results of two studies conducted in the Republic of Cape Verde are presented.

The first study involved large scale field trials applying *N. locustae* by aircraft and the second study looked at the associated effects on non-target organisms.

The insular nature of Cape Verde presents an ideal situation for the introduction and establishment of biological agents, i.e. *N. locustae*, to provide long-term regulation of pest species, here grasshoppers, mainly *Oedaleus senegalensis* and *Diabloca-tantops axillaris*. Earlier studies (Henry *et al*, 1985) indicated that both species can be infected by *N. locustae*.

However experience during the last 200-300 years has shown that introducing new organisms to islands can be met with disastrous consequences. Often islands have endemic fauna, which is very susceptible to being extirpated by man or through introducing new organisms. An example is the giant skink, *Macroscincus coctei*. In addition it should be remembered that parasites are not always host specific. It means they may infect new hosts in the new area. It is therefore essential to make sure that spores of *N. locustae* do not transfer from target to non-target organisms. Hence the second study accompanying the field trials.

To simplify the discussion, the results of the two studies are presented separately and have been edited slightly. The original reports can be obtained from the original authors, the GTZ 'Integrated Biological Control of Grasshoppers and Locusts' Project, or USAID. Below a summary of the most important findings of the two studies has been summarized.

Field Trials with Nosema locustae against Grasshoppers in Cape Verde

For the field trials paired blocks were selected on the basis of grasshopper density, suitable terrain for aerial application and uniform vegetation. One of each pair was treated with *N. locustae* wheat bran bait, the other served as control. The first three blocks (each 500 ha) were treated with a concentration of 2.5×10^9 spores/ha the last two (200 and 100 ha respectively) with 5.0×10^9 spores/ha by aeroplane between September 9 and 27, 1989.

Effects of treatment were assessed by sampling the number of grasshoppers per square metre in each of the first four block pairs. Assessment was carried out using ten 0.1 sqm aluminium rings along each of four transects in each block, treated and

untreated (Onsager and Henry, 1977). Sampling started 2-3 days before and continued until 41 days after treatment. Block five was assessed visually once a week. In addition 100-150 grasshoppers were collected from each block at weekly intervals and analysed for infection.

It is apparent from the results of this study that applications of 2.5×10^9 or 5.0×10^9 spores/ha of *N. locustae* do not cause significant reductions in densities of *O. senegalensis*, the most important grasshopper in West Africa. One of the major reasons seems to be the rapid development of *O. senegalensis* which does not leave sufficient time for the parasite to cause morbidity. Other reasons for a lack of treatment effect may have been the mobility and rapid migration of this species and the drought conditions prevailing at the time of the experiment.

The results indicate though that in the long-term, *N. locustae* causes subtle population effects that ultimately can alter grasshopper densities, as has been found by Onsager (1985) and Lockwood and Derby (1990).

In the final analysis, the results of this study established that in the short-term *N. locustae* is not a suitable control for grasshopper complexes dominated by *O. senegalensis*. However there is some indication that it may contribute to a subtle long-term regulation of the population, and therefore may still be a useful tactic for the integrated pest management of the Sahelian grasshoppers.

Effects of Nosema locustae on Target and Non-Target Organisms on Cape Verde Islands

The aim of this study was to monitor the environmental impact of *N. locustae* wheat bran bait application. The study looked at the persistence of spores in the soil, infection rates in target and non-target animals and mode of transmission between generations of grasshoppers from one season to the next. In addition the presence of pesticides in consumables and soil was monitored, the results are not presented in this summary.

From September 9 to October 5 1989 and March 3-10 1990 trial blocks were inspected and samples collected. 26,236 insects including locusts, materials for residue analyses, (soil, goat milk, hen eggs) were taken to Saarbrücken alive or frozen for analysis.

In over 2,800 target specimen analysed from the two seasons, *Nosema*-like spores were detected only in 12 grasshopper specimen: *Anacridium melanorhodon* (2 in 1989), *Catantopus* (or *Diabolocatantops*) *axillaris* (1 in 1989 and 2 in 1990), *Oedaleus senegalensis* (5 in 1989 and 1 in 1990) and *Pyrgomorpha cognata* (1 in 1989). However spores were detected in some non-targets: *Labidura* (Dermaptera), in *Ctenosa senegalense* (Carabidae), in one *Chilopoda* specimen, in *Periplaneta americana* (Dictyoptera), in *Gryllus brunneri* (Gryllidae), in *Argiope clarcki* (Arachnida) and in *Butalus occidentalis* (Arachnida), a frequently occurring scorpion.

After three months of a feeding trial in the laboratory *Locusta migratoria* and *Schistocerca gregaria* showed no signs of any *Nosema* infection. During the same period grasshoppers caught in treated areas and kept separately in the laboratory for observations also showed no signs of *Nosema* infection.

Clearly infected *L. migratoria* and *S. gregaria* were examined by scanning microscopy. Eggs of both species were completely free of *Nosema* spores. Microsporidian spores

only reached the outer part of the epithelian layer surrounding the ovarioles, which were not penetrated.

Toxicity trials showed that early instar stages (up to 4th instar) were more susceptible to infection than later stages, though no reason for this fact can be suggested at this stage.

Nosema spores could not be detected from soil samples taken from treated blocks. Bait containing *N. locustae* spores placed on March 6 and collected and frozen on March 8 1990 showed no pathogenicity in subsequent feeding trials. Non-targets (*Periplaneta americana* and *Butalus occidentalis*) fed with *Nosema* infected meal worms showed no signs of infection after four months. *Nosema* could be detected in the fatty tissue of the test insects.

The results of this study show two major findings. As in the field trials the effectiveness of *N. locustae* as a control agent is questionable against the targets under discussion. Apart from the reasons stated above the low pathogenicity may be due to high solar intensity, evapotranspiration and constant winds, as well as the lack of transmission through eggs.

The effect on non-targets was also negligible. No mortality was noted and transmission was rare and not pathogenic. However the findings and interpretation of this study refer only to the species of *Nosema* used here. The effects of other species of *Nosema* would need to be re-evaluated, particularly since transmission of spores to non-targets has been observed.

I. Field trials with Nosema locustae against grasshoppers

Introduction

The Government of the Republic of Cape Verde has expressed interest in the biological control of various insect pests. Rain fed crops, primarily maize and beans are susceptible to the attack by grasshoppers. Conventional control, mainly the manual application of propoxur on wheat bran carrier provides limited protection only. It also presents a health risk to farmers and livestock. Rough terrain, expense and health hazards make aerial application of other insecticides impractical. Therefore *N. locustae* seems an ideal alternative.

N. locustae is a microsporidium that infects the fat tissue of grasshoppers and therefore competes with the host for energy reserves. Pathogenicity is expressed by increased mortality and reduced fecundity. Field trials in North America have resulted in a reduction of grasshopper density by 30-70% between six and nine weeks after application (Henry, 1971; Henry *et al*, 1974; Ewen and Mukerji, 1979; Johnson 1987). Disease prevalence among survivors varied between 20 and 50%.

N. locustae has been registered in the U.S. against grasshoppers and has been marketed under several trade names. As a microbial pesticide *N. locustae* has not met with user satisfaction, because of insufficient short-term mortality. It has not been used to regulate grasshopper populations long-term. It has been applied mainly in situations where the use of chemical pesticides is not permissible.

Methods

Bait Formulation

Spores of *N. locustae* were purchased from a commercial producer (Evans BioControl) through Ciba-Geigy. A small sample was used in a pre-treatment bioassay to insure infectivity. The remainder was maintained frozen till 24 hours before formulation. The bait carrier, wheat bran, had been stored for one year by the CV Crop Protection Service. It was screened through a 1/4-mesh hardware cloth to remove clumps. Prior to formulation the spores were re-suspended at a concentration of 1.0×10^{11} spores/1.5 l distiled water. A thixotroph (hydroxymethyl cellulose) was added to the suspension at the rate of 0.2 w/w.

The spore suspensions and wheat bran were batch-mixed in diesel powered cement mixer. To each batch (20 kg) 750 ml of suspension containing 5.0×10^{10} spores were applied as a spray over a 2.2 minute interval. The spray apparatus was pressurized with CO_2 at 20lbs/sqin. The resulting bait preparations were packed in polyethylene bags at 15 kg each and stored on pallets at about 15°C until applied, 3-5 days later. Moisture content of the bran was 8% and 11% before and after formulation respectively. Finished bait was screened through 1/4-mesh hardware cloth before aerial application.

Application

The formulated bran was applied by a turbine Air Tractor equipped with an SSL spreader. Prior to treatment the equipment was calibrated using screened untreated wheat bran as follows:

Swath width	36 m
Airspeed	192 km/h (3200 m/min)
Altitude	30 m
Hectares/min	11.52
KG bait/min	11.52 (1 kg/ha or 23.04 (2 kg/ha)

Bran flake deposition was assessed using deposit card on a grid. Depositions were 316 and 630 flakes/sqm for 1 and 2 kg bran/ha applications respectively.

Guidance was provided by two flaggers at each end of the plot, who were also in radio contact with the pilot.

Plot Design

Plots (Blocks) were selected on the basis of grasshopper densities, suitable terrain for aerial application and uniform vegetation. Treated and untreated plots were paired and a total of 5 pairs were chosen, the first three treated differently from the last two pairs:

Block Designation	Treated Plot	Control Plot	Size ha	Treatment spores/ha
1	San Martinho	Trinidad	500	2.5×10^9
2	Calabaceira	Joao Varela	500	" "
3	Val da Custa	Monte Bode	500	" "
4	Achada Barnelo	Joao Valente Gouveia	200	5.0×10^9
5	Tarrafal (Achada Tomaz)	Tarrafal (Achada Boi)	100	" "

While block 4 was close to the first three blocks, block 5 was located at the opposite end (north) of the island (Fig. 1).

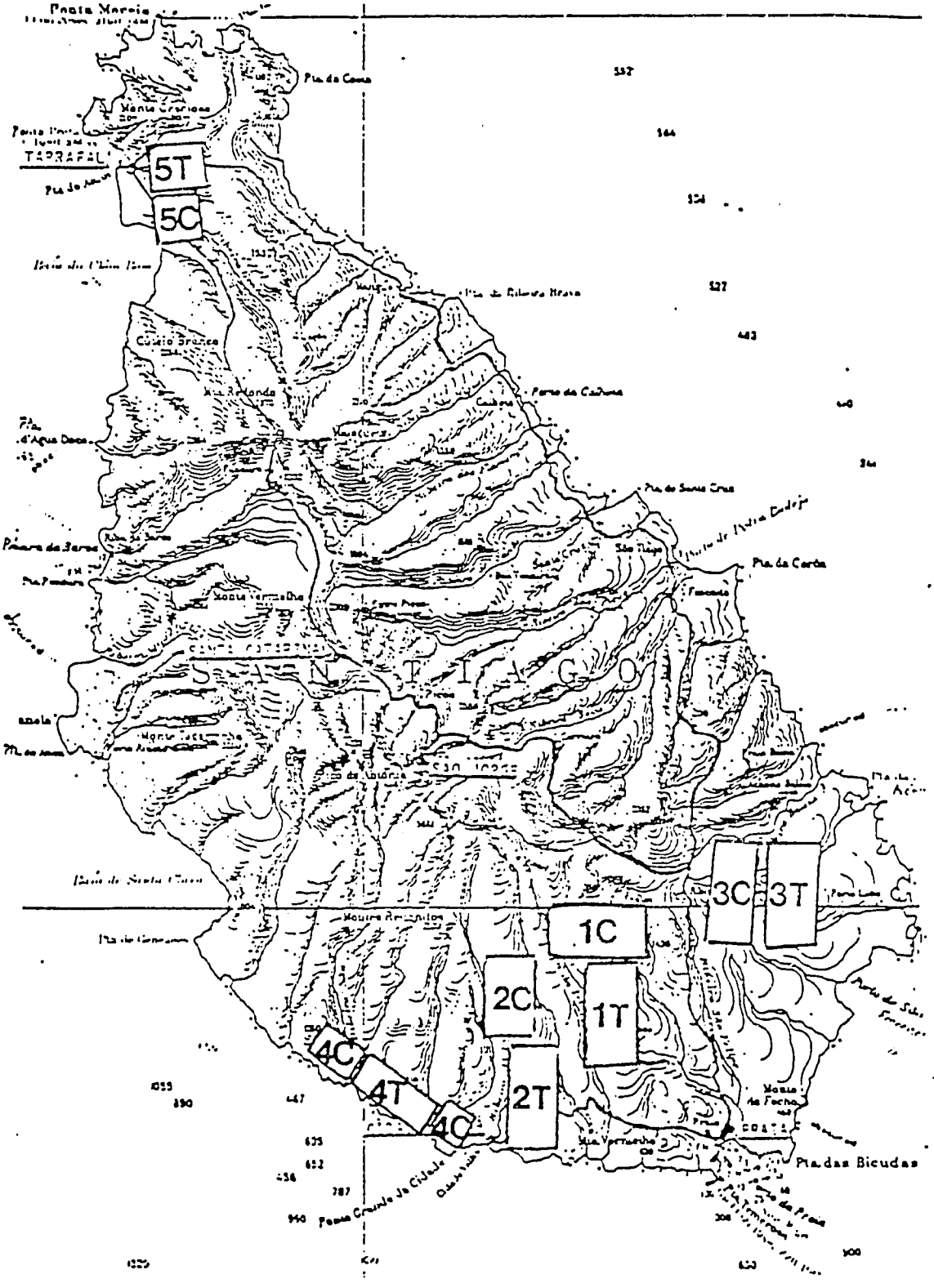
Treatment Design

The first three blocks were treated at 1 kg/ha, blocks 4 and 5 with 2 kg/ha. For application conditions see table 1.

Table 1: Conditions, timing and volumes of bait applied

Block No.	Actual Date	Julian Date	Volume kg	Time of Day	Mean Temp C^0	Wind Velocity Knots
1	Sept 9	252	500	0740-1047	27	7-8
2	Sept 10	253	500	0736-1047	28	0-2
3	Sept 14	257	500	0628-0842	26	2-3
4	Sept 15	258	500	0630-0730	28	3-4
5	Sept 27	270	200	0730-0830	26	8-10

Figure 1: Relative disposition of treated and check plots, by block on Santiago Island, Cape Verde.



Treatment Assessment 1989

Two permanent sampling sites were established in each of the first 4 block pairs. Grasshopper densities were determined using 0.1 sqm aluminium rings along four transects each with 10 rings (Onsager and Henry, 1977). Distances between rings and between transects were 5-7 metres. Sampling started 2-3 days before application and continued at 3-4 day intervals until 41 days after application. Grasshopper density per square metre in block 5 was estimated visually along a transect 100 times at 10 m intervals once a week.

In addition 100-150 grasshoppers specimen were collected from the centre of each plot at weekly intervals starting 2-3 days before and continuing until about 50 days after treatment. The pre-treatment samples were used to assess species composition and stage of development. Post-treatment specimen were also assessed for disease prevalence.

Specimens collected were taken to the laboratory and frozen. Specimens were individually homogenized in distiled water from which samples were drawn and examined under 500 diameters phase microscope for spores of *N. locustae* and for other microbial organisms. Examinations for disease prevalence were limited to *O. senegalensis* and *D. axillaris*.

Treatment Assessment 1990

Population density and infection assessment for the 1990 season were initiated August 15 and continued till November 21. Density estimates were conducted according to the PRIFAS method of counting animals/sqm along a multidirectional transect within each block. Disease prevalence estimates were based on up to 100 grasshoppers from each subplot and analysed by microscope. Only *O. senegalensis* and *D. axillaris* were examined for spores of *N. locustae*.

Conditions of Study

The blocks of the southern part of the island were between 65 and 266 meters altitude. All blocks were characterised by irregular terrain consisting of volcanic solid and rocks with intermittent vegetation. Most of the area served as pasture for goats and cattle with little or no cultivation. All blocks were included in reforestation programmes. The type of vegetation was similar between blocks and in particular between paired blocks.

Maximum daily temperatures varied between 28 and 32°C and the average temperature ranged from 19-24°C. Rainfall predominated in August varying from 185.2 mm at Tarrafal to 61.3 mm at Trinidad. In August the rains concentrated between the 12th and 18th, and moisture was sufficient to initiate the hatching of eggs of *O. senegalensis*. Rainfall during September, October and November was very sporadic and not sufficient to maintain rain-fed crops.

Analysis of Data

To date data has been analysed by ANOVA and regression analyses. Further analyses are planned.

Results

Species Composition and Density

O. senegalensis was the predominant species in all blocks before and shortly after application; it made up over 90% of all grasshopper species (Fig. 2). The other two species were *Acorypha clara* (7%) and *Pseudosphingonotus savignyi* (3%). Other species collected during the study, but not present in the samples were:

Diablocatantops axillaris
Acrotylus patruelis
Aiolopus simulatrix
Sphingonotus rubescens
Pyrgomorpha cognata
Stenohippus epacromioides
Acrotylus longipes
Aiolopus thalassinus
Anacridium melanorhodon
Sphingonotus canariensis
Stenohippus bonneti

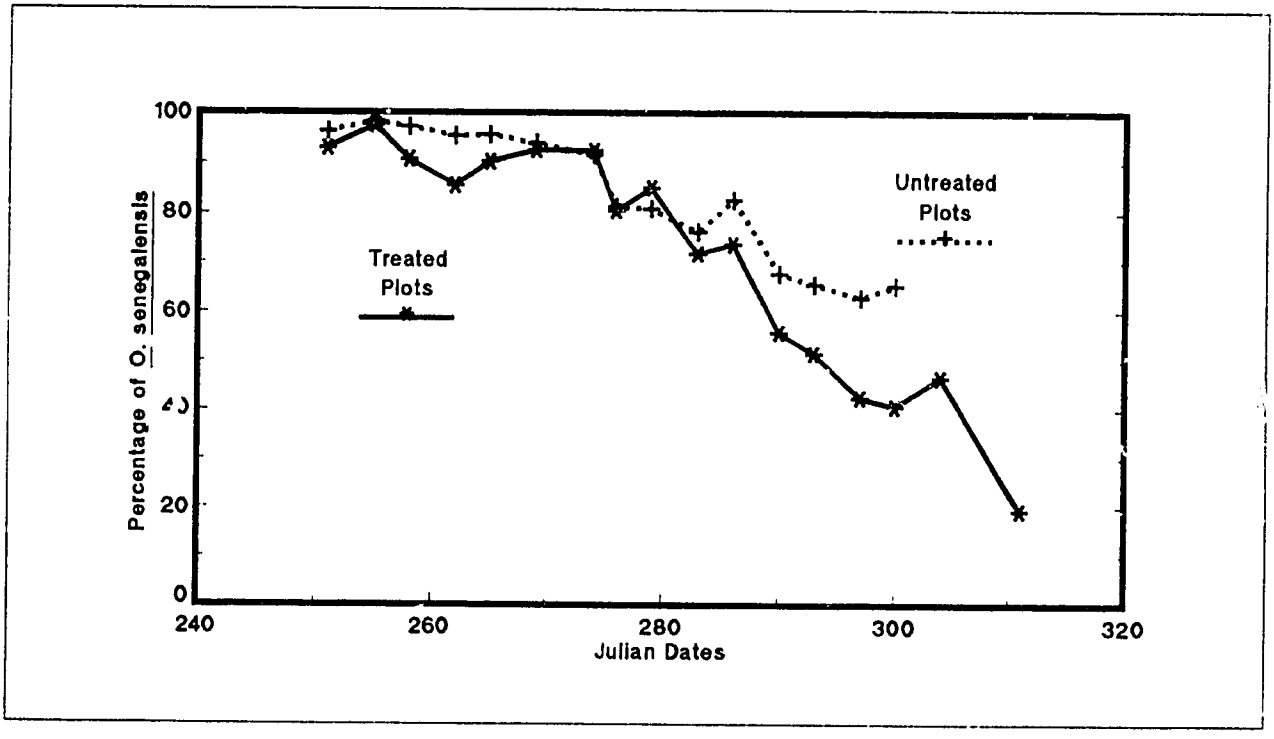


Fig. 2: Average proportion of O. senegalensis in blocks 1, 2, 3 and 4 grasshopper complex.

Analysis of the post-treatment sampling showed no difference in composition either between treated and untreated block pairs or between blocks (Fig. 2). Prevalence of *O. senegalensis* remained fairly constant till about 27 days after treatment.

The composition of grasshoppers in block 5 at the north end of the island was quite different from the remaining blocks. Here the proportion of *D. axillaris* was higher with a large proportion of the first instar (50%). Treatment resulted in a reduction in the proportion of *D. axillaris*.

Results in blocks 1-3 demonstrated the rapid development of *O. senegalensis* in Cape Verde. Hatching began on August 25 and by September 9 some grasshoppers had reached the fifth instar.

Initial densities of *O. senegalensis* was much higher in treated blocks. About 14 days after treatment this difference was not any more apparent suggesting some treatment effect. This difference was not apparent when data from all three blocks was combined (Fig. 3).

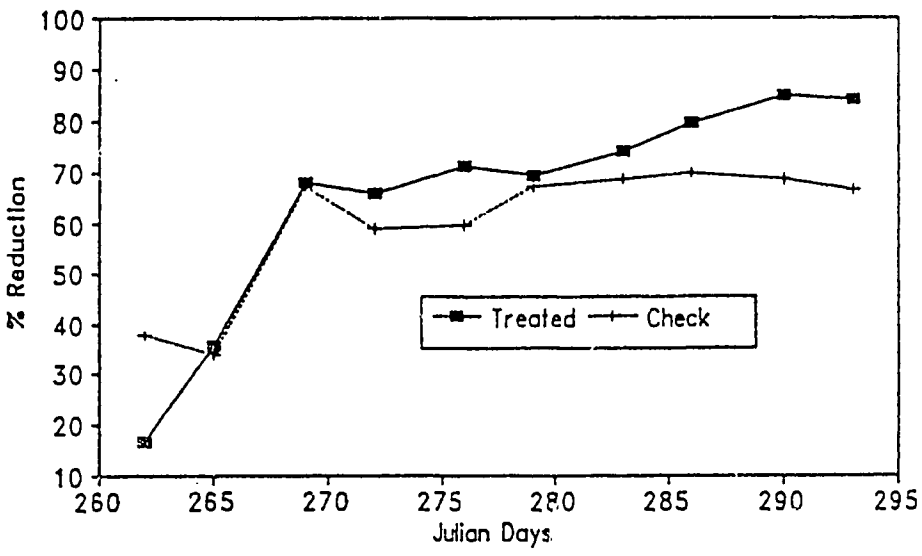


Fig. 3: Overall densities of O. senegalensis in blocks 1, 2 and 3.

The results do not conclusively demonstrate that the variations within blocks and between block pairs (treated and untreated) were due to treatment. These variations could have been brought about by the movement of grasshoppers, inexperience of samplers or the results of moulting which increases mortality rates naturally.

Infection by Nosema locustae

Examinations for infection were restricted the dominant species, *O. senegalensis*. About 10,000 specimens were examined between 1-52 days following treatment. First infection was observed on day 27 and the maximum infection in the first three blocks was about 18% (Fig 4). In blocks 4 and 5, sprayed at higher concentrations, infection rate was somewhat higher (Fig. 5). In none of the untreated blocks was infection noted, indicating that infections were solely due to the treatment.

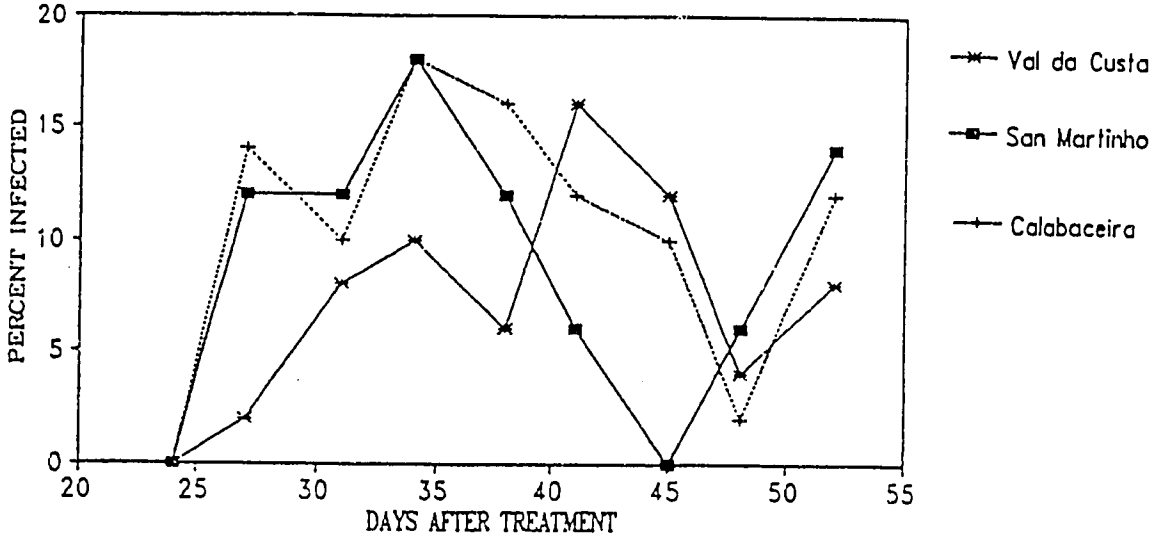


Fig. 4: Infection of O. senegalensis in treated blocks 1, 2 and 3

Grasshopper Density and Disease Prevalence in 1990

To check long-term effects the plots were sampled again in the 1990 season.

Table 2 shows that the overall densities were lower than in 1989 but no differences were found between treated and untreated plots. Table 3 indicates that the infection in treated plots was extremely low and declining. Almost no infections were noted in the untreated blocks.

Table 2. Grasshoppers per square metre.

Julian Day	229	235	342
Treated			
San Martinho (1)	5.1	5.7	2.7
Calabaceira (2)	2.2	1.4	4.2
Val de Custa (3)	2.8	5.5	2.4
Average	3.4	4.2	3.1
Untreated			
Trinidad (1)	8.7	6.1	3.8
Joao Varela	5.5.	1.9	4.2
Monte Bode	0.9	1.8	1.6
Average	5.0	3.3	3.2

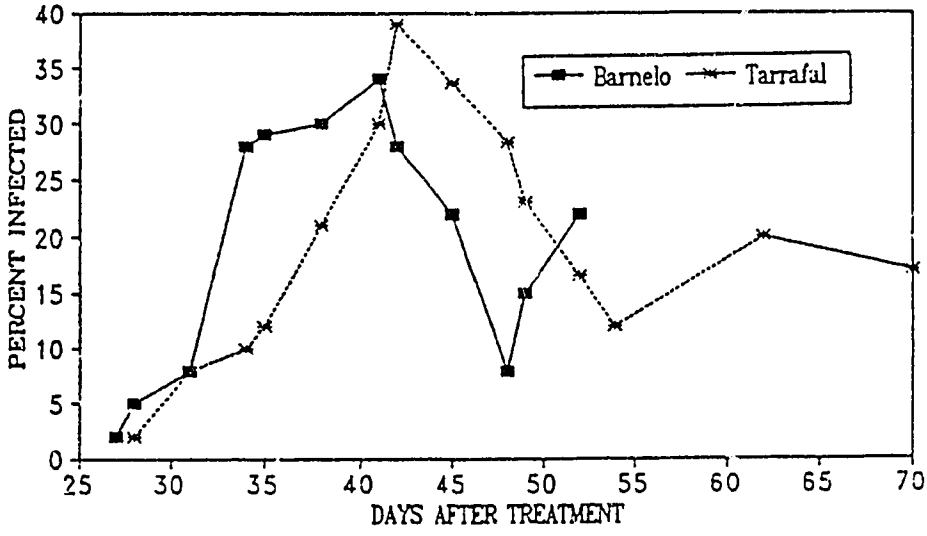


Fig. 5: Infection of O. senegalensis in treated blocks 4 and 5

Table 3. Percentage of O. senegalensis infected by N. locustae in treated and untreated blocks one year after treatment (Figures in brackets represent sample size).

Julian day	2.5×10^9 spores/ha Treated	Untreated	$5.9\ 10^9$ spores/ha Treated	Untreated
227	3.8(599)	0.0(588)	4.2(190)	1.4(139)
242	2.2(600)	0.0(600)	1.8(163)	0.0(200)
256	5.1(603)	0.0(568)	0.5(190)	0.0(190)
269	3.0(369)	0.0(460)	2.2(139)	0.0(140)
283	1.9(105)	0.0(144)	0.9(111)	0.0(72)
298	0.0(34)	0.0(92)	0.0(21)	0.0(23)
311	0.0(159)	0.0(239)	0.0(34)	0.0(44)
325	0.0(60)	1.7(116)	0.0(68)	0.0(71)

Discussion

It is apparent from the results of this study that applications of 2.5×10^9 or 5.0×10^9 spores/ha of *N. locustae* do not cause significant reductions in densities of *O. senegalensis*, the most important grasshopper in West Africa. One of the major reasons seems to be the rapid development of *O. senegalensis* which does not leave sufficient time for the parasite to cause morbidity.

While the populations of grasshoppers in treated blocks were larger than in untreated plots in 1989 they were very similar and low in 1990. These results indicate an effect over the period of two seasons. As pointed out by Onsager (1985) and Lockwood and Derby (1990), *N. locustae* can cause subtle population effects that ultimately could alter grasshopper densities.

Surprising was the virtual absence of infection in *D. axillaris*, since Henry *et al* (1985) reported the susceptibility of this species. It is possible that *D. axillaris* did not take the bait. The carrier, wheat bran, had been stored near bran formulated with the insecticide propoxur. There may have been some airborne contamination acting as a feeding deterrent. An unpublished report in the US (USDA/ARS Rangeland Insect Laboratory, Bozeman) describes a situation in which at least one species of grasshopper would not accept wheat bran-insecticide baits.

In the final analysis, the results of this study established that in the short-term *N. locustae* is not a suitable control for grasshopper complexes dominated by *O. senegalensis*. However there is some indication that it may contribute to a subtle long-term regulation of the population, and therefore may still be a useful tactic for the integrated pest management of the Sahelian grasshoppers.

II. Effects of Nosema locustae on Target and Non-Target Organisms on Cape Verde Islands

Introduction

N. locustae was isolated from the fat tissue of *Locusta migratoria migratorioides* and described in 1953 (Canning 1953, 1962). Since then has been used with limited success against grasshoppers in North America. However its pathogenicity varied considerably between different species of grasshoppers and its spores can survive in other biological matrices. The question regarding the host specificity and host changes of *Nosema* has not been studied in detail. For these reasons and those mentioned in the introduction of the summary it was decided to run ecotoxicological studies with the *Nosema* field trials. The results are presented below.

Methods

During the period from 9 September to 5 October 1989 and 3 to 10 March 1990 staff of the Institute for Biogeography and of the University of Saarland inspected the trial and control blocks and collected 26,236 samples (grasshoppers and other arthropods, soil, goat milk, hen eggs) for residue analysis and examination for *Nosema* infection. Insects collected were shipped to Saarbrücken (Saarland) in PEG, alive or frozen in liquid nitrogen. The results from the residue analysis will not be presented, because they have no direct relevance to the discussion here. They can be taken from the original report.

Results and Discussion

It should be noted that there is some uncertainty about the taxonomic status of some Acrididae. *Diabolocatantops axillaris* could be *Catantopus axillaris* and *Caloptenopsis insignis*, though found listed in Cape Verde, may have a different name in other listings.

Persistence of *Nosema*

The 'persistence' of the *Nosema* application is used as a criterion of the effectiveness of the pathogen. Therefore 800 and 2,062 target insects (*Caloptenopsis insignis, Diabolocatantops axillaris, Oedaleus senegalensis, Anacridium melanorhodon, Pyrgomorpha cognata*) were collected in September 1989 and March 1990 respectively, stored in PEG and examined for spores in the laboratory.

Nosema-like spores were detected only in *D. axillaris* (one specimen from Tarrafal, 1989 and two from Vale da Custa, 1990) and one specimen of *O. senegalensis* (Tarrafal), in *A. melanorhodon* (two specimens from Tarrafal, 1989). in *O. senegalensis* (two specimens from Tarrafal, 1989, one from Tarrafal, 1990, three from Vale de Custa, 1989) and in *P. cognata* (one specimen from Vale de Custa, 1989). No differences could be found between the results from treated and those from untreated blocks, suggesting that there was little 'persistence' of *Nosema* following application.

Possible Transmission of Nosema Spores via the Eggs

The non-persistence of *N. locustae* spores six months after baiting was surprising. To investigate possible causes further laboratory tests were conducted with freshly supplied spores (by CIBA-GEIGY) in 40% PEG solution of *N. locustae* and *Nosema cuneatum*. Infected specimens were dissected and examined by scanning

electron microscopy.

The eggs of clearly infected *L. migratoria* and *S. gregaria* were found to be completely free of *Nosema* spores. The same applied to the nymphs, which hatched from the eggs. *O. senegalensis* managed to oviposit in two cases only, and again the eggs were not infected.

The above findings are in line with observations made by Darwish on *L. migratoria* (1985). That is, infected locusts, and in particular *L. migratoria*, lacked detectable quantities of ovarian tissues or egg debris, making transmission from one generation to another impossible.

Susceptibility of Different Hopper Stages to Infection by Nosema

When specimens of *L. migratoria* were subjected to toxicity tests, the fourth and fifth nymph stages showed only a minimal reaction to *N. locustae* in feeding trials. Up to the fourth nymphal stage the results are satisfactory. To date it could not established why there is a drastic drop in the rate of infection once the fourth stage is reached.

Effectiveness of Nosema Spores in the Field

It has been shown in the past that *Nosema* spores succumb to heat or UV light and only a small proportion of its spores are able to survive in the soil (Canning, 1980).

Nosema spores could not be detected from soil samples taken from baited blocks. In addition bait containing *N. locustae* spores were placed near Tarrafal in March 6 and collected on March 8, stored in PEG and shipped to Saarbrücken. No pathogenicity could be observed in subsequent feeding trials.

It appears that the combined effects of a high rate of evaporation, continual winds and high solar intensity reduces the pathogenicity of *Nosema* outside its host organism.

Susceptibility of Non-Target Organisms to Nosema

At least 40 species of non-target arthropods were collected from treated and untreated blocks and examined for *Nosema* infection. In seven species *Nosema spp.* spores (not necessarily *N. locustae)* could be detected (*Scolopendra morsitans* (Chilopoda), *Argiope clarcki*, *Butalus occidentalis* (Arachnida), *Peripianeta americana* (Dictyoptera), *Labidura riparia* (Dermaptera), *Gryllus brunneri* (Gryllidae) and *Ctenosa senegalense* (Carabidae).

Feeding trials with non-target organisms showed that after four months of feeding *P. americana* and scorpions (*B. occidentalis*) with *N. locustae* in meal worms, no signs of infections could be observed. *Nosema* could however be detected in the fatty tissues of the test insects.

Conclusion

The *Nosema* used in the Cape Verde trials had a low target efficacy in terms of rate of infection and causing mortality. The effect on non-targets was negligible as well, with a few possible nonpathogenic transmissions only. Ecotoxicologically no impact is expected. However these conclusions refer to the species used here only. Other species would have to be re-evaluated, particularly since possible transmission of *Nosema* to non-target organisms appeared to be indicated by this study.

Abbreviations used

ANOVA	Analysis of Variance
ARS	Agricultural Research Service
DPV	Plant Protection Service
GTZ	Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH
INIA	Instituto du Investigacao Agraria
IPM	Integrated Pest Management
MSU	Montana State University
PEG	Polyethyleneglycol
PRIFAS	Programme Interdisciplinaire Francais sur les Acridiens ou Sahel
TDY	Temporary Duty
USAID	United States Agency for International Development
USDA	United States Department of Agriculture

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