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Special Issue 2007: Proceedings from the Workshop on the Future of Biopesticides in Desert Locust Management

Workshop on the Future of Biopesticides in Desert locust Management (Atelier sur l'avenir des Biopesticides en lutte contre le Criquet pèlerin) Saly, Senegal, 12-15 February 2007

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Summaries

Sixty six experts from all over the world took part in a workshop on the future of Biopesticides in Desert locust management at Saly Senegal in mid-February 2007. During both the 1986-89 and 2003-05 plagues of the Desert locust *(Schistocerca gregaria* Forskal), chemicals were applied to millions of hectares of locust bands and swarms, with 13 million hectares treated in 2003-05. During the past 20 years, there were also a number of upsurges, each requiring treatment of hundreds of thousands of hectares. The exclusive reliance on the widespread use of chemical pesticides in Desert locust control programs has led to intensive research efforts to develop alternatives to chemical use. Two of the most promising alternatives are the mycopesticide *Metarhizium anisopliae var. acridum* and the semio-chemical phenyl acetonitrile (PAN). Both products were developed specifically for Desert Locust control and the main focus of the meeting was to discuss how to progress the integration of these biopesticides into Desert locust management.

The workshop was organized under the aegis of the Orthopterists' Society, and was supported financially by the World Bank, l'Organisation Internationale de la Francophonie, the Food and Agriculture Organisation of the United Nations and the International Fund for Agricultural Development. Experts involved in the development, production and use of these products met, along with representatives of donor organisations, to discuss what role the biopesticides *Metarhizium* and PAN should play in the control of the Desert locust, and what key actions were required to ensure that one or both of these products become an integral part of Desert locust management in the near future.

The meeting was conducted under the patronage of the Madame Viviane Wade, First Lady of Senegal and President of the foundation, *Agir pour L'Education et la Santé* (Action for Education and Health). Following the large invasion of the Senegal River Valley by Desert locusts during 2004, during which there was widespread use of chemical pesticides, she has fostered a strong interest in biopesticides as a method of locust control and, through the foundation *Agir pour l'Education et la Santé,* is helping establish the SenBiotech factory in Senegal for the production of *Metarhizium* for locust and grasshopper control. Local production of high quality *Metarhizium* will be an important step in fostering its use for locust and grasshopper control not only in Senegal but elsewhere in Sahelian Africa and beyond.

The opening ceremony and organization of the meeting.

The Mayor of Saly, the host of the workshop, welcomed the Patron, Madame Viviane Wade, sponsors' representatives, and participants to Saly. The opening ceremony chaired by Madame Wade was addressed successively by the Workshop Chairman Mr Amadou Diarra, by M. Amadou Ouattara Representative of FAO to Senegal, by Mr Michel Lecoq President of the Orthopterists' Society, by Madame Mame Fatim Gueye of l'Organisation internationale de la Francophonie and by M. Tall, Directeur de Cabinet du Ministre de l'Environnement et de La Protection de la Nature. Everyone welcomed the initiative to hold the Workshop and wished participants every success in their efforts to define an appropriate strategy for integrating biopesticides into Desert Locust management.

The workshop began with a brief session that endorsed the content and timetable of the workshop. It was agreed that the meeting would begin with oral presentations that would review the lessons learned in field trials and operational use of biopesticides. Further presentations would outline what practical and regulatory steps were required to ensure high quality product was readily available when required.

Participants then worked in three parallel sessions developing draft action plans for the period 2007-2009 that would enable biopesticides to be integrated into operational plague prevention campaigns. Group 1 considered additional research and development priorities, Group 2 examined the supply chain and regulatory processes and Group 3 identified information, coordination, training and awareness programs required at national, regional and international levels.

Oral Presentations: Lessons learned in field and operational use of biopesticides.

To ensure that all participants were fully aware of the most recent developments, Mr Harold van der Valk outlined the results of field applications of *Metarhizium* and PAN. The results of 31 applications of

Green Muscle® (isolate IMI 330189 from Africa), 16 applications of Green Guard® (isolate FI-985 from Australia), and 18 applications of other *Metarhizium* isolates demonstrated that a dose of 50g/ha worked as well as higher doses. In Australia and China, 25g/ha has been shown to work, particularly where secondary pickup from the vegetation has been demonstrated. However, because bands of Desert locust move very quickly and are often in sparse vegetation, there may be less secondary pickup, so it was suggested that initially a dose of 50g/ha be used. Mortality often occurred in 6-14 days in applications with Desert locusts though it took was somewhat longer when it was cool. Initial trials with PAN (phenyl acetonitrile) have shown that it reduces gregarization and feeding and increases susceptibility to predation while seemingly having synergistic effects when applied in combination with *Metarhizium*. It was felt that further field trials are required to clearly demonstrate these effects in a variety of circumstances.

Mr Peter Spurgin of the Australian Plague Locust Commission outlined the lessons learned from treating more than 70,000 ha of locusts with *Metarhizium* in Australia. When it is warm to hot, an efficacy of 80% and higher is generally achieved in 10-14 days after application of a dose of 25 g/ha. The main problem with applying Green Guard® is that the spores settle into a sludge after vibration caused by transportation. The problem has been solved by field mixing as outlined in a poster below. The APLC uses Green Guard® as part of an early intervention strategy and a study recently completed by the Australian Bureau of Agricultural and Resource Economics has demonstrated that early intervention results in a 1:20 return in cost benefit. The early intervention strategy involves the integrated use of three products. Fipronil is applied against nymphal bands as widely spaced barriers placed 300-500m apart. Only a part of the area is treated, so less pesticide and less aircraft time is used so that the cost of application (aircraft + pesticide) is only US$2.60/ha. Fenitrothion is mainly used to treat adult swarms and is applied as a blanket treatment with aircraft + pesticide costs being higher at US$4.60/ha. While the cost of aircraft + *Metarhizium* is substantially higher at US$11.50/ha, it has proven to be a valuable in environmentally sensitive areas and overall forms about 10% of the locust spraying program.

Oral Presentations: Ensuring production and use of biopesticides

An important limitation on use of biopesticides is their higher cost relative to chemical pesticides. It was clear at the meeting that most people compare costs of product alone, but the cost of the product is only a small part of the total actual cost of treatments. Dr Adrian Leach of Imperial College, London, described an approach that estimated the total direct and indirect costs of using biopesticides as opposed to chemicals for locust control campaigns. Direct costs include not only the cost of the product, but also the generally much higher additional costs of finding the locusts, delineating infestations suitable for spraying and then treating them. To these costs must be added the Environmental Impact Quotients (EIQs) of the product used, and with chemical pesticides these costs can be significant. Including all of the real costs in using a given product substantially reduces the difference in the cost of using biopesticides or chemicals.

Mr Ken Neethling of Biological Control Products (BCP) that produces Green Muscle® gave an industry perspective on the ongoing initiatives to ensure that *Metarhizium* is readily available when required. BCP has advanced production and testing facilities to ensure its products are of high quality but these facilities are generally used for other products that have more consistent markets. Often there are few locusts but when, from time to time, populations increase dramatically, the requirements for control products are substantial. Green Muscle® is specific to locusts and grasshoppers and some can be produced and stored for when locusts appear. From the industry's perspective, payment for product needs to be received soon after manufacturing costs have been incurred. When locusts are increasing, warning must be given of an impending upsurge, and when product is required, there must be streamlined processes that allow for rapid purchasing, formulation and transport of *Metarhizium* to where it is required. The current price is US$18 per 50g of spores formulated in oil as Green Muscle® which is enough to treat one ha at current recommended doses. Costs could be a major constraint but use of lower doses, as has been demonstrated in trials, and purchase of higher volumes would reduce the price. Substantial costs are involved in registration and standardisation of registration procedures over a number of countries would also mean a lower price in the end.

Joan Kelley from CABI then outlined licensing procedures. Agreements were signed in 2001 that provided a single entity that could license Green Muscle®. CABI's obligations were to establish the LUBILOSA Trust; to use the IPR (i.e. licenses), to "underpin" the quality of the product. Monies from licenses go to the LUBILOSA Trust "to promote and support biopesticide research development and use in Africa". Licenses are restricted to specific countries and territories with BCP currently licensed to supply in eastern and southern Africa and negotiations are in progress for SenBiotech in Senegal to have the license for west Africa. Mr Amadou Diarra of Mali then outlined the procedures required for registration in the countries of Sahelian Africa. Eight dossiers and a sample of the formulated product are required in the submission. Registration authorities are recommending the increased use of biopesticides but when asked if exceptions could be made to registration procedures to help overcome the problems companies are facing trying to register Green Muscle® in Sahelian countries, it was stated that there is no flexibility in the document signed by the nine Member Countries to grant exceptions. Consequently, Green Muscle® must fulfill all current requirements but efforts are being made to standardize registration requirements across the Sahelian countries.

Poster presentations

Additional information on *Metarhizium* was provided in posters including one which outlined the key steps that resulted in the LUBILOSA collaborative research program to successfully develop Green Muscle® as an effective biopesticide for locust and grasshopper control. LUBILOSA scientists established a number of quality control criteria for the spore product and it is essential that these are strictly adhered to if we are to have a product with a long storage life and consistently high efficacy. With improved formulation, the recommended dosage is now 50 g/ha, diluted to 0.5-2.5 L with oil (e.g. diesel fuel) depending on the type of ULV application. Field trails during the past few years have demonstrated that a lower dose of 25 g/ha is adequate in many circumstances.

Several posters reported detailed results of field trials using Green Muscle® including increased predation of treated locusts being an important contribution to mortality, particularly in the second week after treatment when the locusts became sluggish. A second report of a trial against field populations of the Senegalese grasshopper found that a low dose of 25g/ha and the standard 50g/ha dose both resulted in a 50% decline in grasshopper numbers within 9 days and a 90% decline within 2 weeks.

The results of 4 years of ground and aerial applications of Green Guard® against the oriental migratory locust, *Locusta migratoria manilensis* in China were reported. There was good efficacy (81-97% decline in 8-14 days) at doses of 25-75g/ha, the lower dose being similar to that regularly used in Australia. A poster outlined the mixing procedure used during operational applications of Green Guard® in Australia: a large mixing tank and engine driven impeller pump is mounted on a rugged trailer suitable for remote area use. This system allows spray payloads to be prepared easily so that they can be rapidly loaded into the spray aircraft. Results were also reported of laboratory tests of Green Guard® and Green Muscle® on North American grasshoppers that showed that both caused high mortality of grasshoppers though when temperatures were low at night, mortality was delayed substantially. Outdoor cage trials with are planned for 2007, provided exotic isolate permits can be obtained.

The effects of phenylacetonitrile (PAN) in reducing feeding of nymphs was reported and, provided PAN was applied three days before fenitrothion, low doses of PAN were shown to enhance the activity of fenitrothion in allowing the dose of the latter to be reduced by up to a third. In another study, PAN enhanced the efficacy of a sub-lethal dose (1/4 of normal lethal dose) of Neem Oil in causing increased mortality as well as deformations in the fledglings that made them unable to feed fly and mate. The effects of various natural pesticides was also reported: Neem which inhibited development and caused up to 70% mortality; the bacterium *Bacillus subtilis* with a dose of 7.3x10 8 spores/mL giving 100 % mortality of nymphs in 8 days; and *Metarhizium* which caused a substantial decline in respiration and heart rate 6th days after treatment. Examination of the haemolymph of locusts treated with *Metarhizium* revealed that the number of haemocytes had decreased after 6 days, and the haemocytes that were present were retracted, their cytoplasmic contents had deteriorated, with clusters of spores observed within the blood cells. When the fungus inoculum was applied to the sand in which the

females laid their eggs, the rate of egg hatchings was 40% instead of the 90% observed in untreated controls.

Development of action plans.

For the main part of the meeting, participants met in parallel sessions for two and a half days to develop work plans aimed at overcoming scientific, regulatory, supply, training and promotional constraints to integrating biopesticides into locust control operations. Each group developed a two year plan of action, which was then discussed in a final plenary session on the last day.

Participants then worked in three parallel sessions based on their expertise: Group 1 examined research & development priorities required to further biopesticide use; Group 2 examined the supply chain and regulatory processes; and Group 3 identified information, coordination, training and awareness programs required nationally, regionally and internationally.

In each group, discussions began with the current status, including factors limiting biopesticide use, but the main part of proceedings then focused on finding *solutions* to current limitations and to provide ways to ensure integration of biopesticides into Desert locust control programs. The discussions proved to be very fruitful under the leadership of a professional facilitator and of two group members appointed as recorders and reporters of items discussed. Critical to the success of the group meetings was that most of the proceedings were conducted in French, though Group 1 met in the main meeting room where there were translation facilities. The use of French meant that local end users were then able to articulate clearly what was required for them to integrate the use of biopesticides into Desert locust management. The proposed key actions required to ensure integration and use were those appropriate to *their* circumstances. Requirements and solutions were discussed intensively with appropriate key actions agreed upon either by consensus or majority vote. When it was felt that there might be several different ways forward appropriate to different regions, several alternatives were proposed.

Summary of recommendations.

Group 1. Research and Development

To facilitate the use of *Metarhizium* in Desert locust management, it was recommended that the FAO, in collaboration with Desert locust control organisations, define the role of *Metarhizium* in both preventive and curative control and to map, in each region, the ecologically sensitive areas where *Metarhizium* should be used as a priority. It was felt that a critical next step is to verify efficacy against Desert locusts on an operational scale, and such operations should be conducted by multi-organisational teams. Recognizing that a lower dose would lead to lower costs and higher uptake, models of the effect of environmental parameters on *Metarhizium* development should be used to provide maps of areas and periods of optimal speed of action and efficacy and where the use of lower doses might be appropriate.

As part of decreasing the time to mortality and minimizing costs, low doses of *Metarhizium* should be tested in combination with PAN (phenylacetonitrile). The environmental risks from using *Metarhizium* and PAN both individually and in combination need to be more accurately assessed so that any effects can be accurately compared to those of chemical pesticides. FAO, in collaboration with producers, should act to improve the formulation with respect to reduced sedimentation, increased shelf life during storage and persistence in the field, and in having packaging adapted to easier operational use. It was felt that there needed to be improved exchange of information among stakeholders both in research and in end use to maximise progress of integrating biopesticides into Desert Locust management strategies.

Group 2. Ensuring availability and quality of biopesticides

For *Metarhizium* to be readily available early in upsurges as part of a program of preventive control, the FAO was urged to create a biopesticide bank, where biopesticides are purchased and stored in anticipation of an upsurge. There should be special funds set aside for their purchase, with an initial target of at least 15% of the pesticide budget earmarked for biopesticides. Improved forecasting of when an upsurge is likely is needed so that biopesticides can be accessed in a timely manner: an alert needs to be issued and then orders for biopesticides must be placed rapidly so that supplies from the pre-existing biopesticide bank can be formulated and delivered to storage facilities in the country or countries under threat. Streamlined procedures for ordering need to be in place, along with

logistical, transport and customs procedures at both regional and national levels. At times, product will be required within a month: often a problem is first recognised in a specific area when there are reports of adult swarms ovipositing in an environmentally sensitive area and product needs to be in place a month later when the nymphs have hatched out and are forming bands.

Recognizing that costs are an important limitation to the uptake of biopesticides, FAO should promote the harmonisation and simplification of registration and CABI should streamline licensing procedures to overcome existing constraints. CABI, with the help of FAO, should ensure contractual requirements of licenses are met and end product is tested so that consistent high quality product is delivered to the end user. Stewardship of best practices for the handling, use and disposal of biopesticides should be documented and be readily available in the form of an electronic User Manual.

Group 3. Information, Coordination, Training and Promotion

To ensure proper use integrated with control programs, all stakeholders, whether local national or international, need to be made aware of the value of biopesticides and what is required to ensure their efficient use. *Metarhizium* is very specific and isolates virulent against locusts and grasshoppers can only be used to control these pests, so that when locusts are in recession, there will be limited stocks available. A biopesticide bank will serve to supply requirements early in an upsurge, but once an upsurge is underway, FAO should ensure that donors commit to biopesticides by rapidly supplying the finance for substantial additional purchases so that companies will rapidly switch production to these products.

To ensure best practise in biopesticide use, all stakeholders need to have rapid access to the latest information. Research and development programs need to be well coordinated on a national and international scale with information disseminated through bulletins, research reports and informal and formal contacts. National policy makers need to be well informed of the value of biopesticides and permanent contacts will need to encourage national policy makers to use biopesticides and monitor the integration of biopesticides into their Desert locust control programs. Critically important is the training of operational "Green Teams": officers specially trained in the use and monitoring of biopesticides, ensuring that control operations are successful. And when successes do

Figure 1: General Session preparations and networking

occur, all stakeholders including scientists, national policy makers, and locust officers both locally and in other regions, need to be made fully aware of the success and of the processes involved in ensuring success. Trained locust officers need to keep up to date through readily available web-based training manuals that contain the latest information to ensure best practise in biopesticide use. And when *Metarhizium* is part of a control program, villagers farmers and the media need to understand and accept its slower action and fully recognize the environmental benefits so that they will support its widespread use.

Final summary

The meeting of these sixty-six experts began with an outline of the current level of knowledge and use of biopesticides that demonstrated that they can control locusts effectively and that in Australia, the biopesticide *Metarhizium* has been integrated into the locust control program. There then followed group discussions of specific aspects of biopesticide use, which focused on finding solutions to overcome current impediments that have limited the use of biopesticides against the Desert locust. The official sessions were complemented by discussions and networking among the participants after hours which together resulted in a consensus reflected in the overall conclusions agreed to on the final afternoon. The detailed official report to the FAO on the workshop is entitled "The Future of Biopesticides in Desert Locust management (2007)" and can be accessed at: **http://www.fao.org/ag/locusts/en/publicat/meeting/topic/misc/documents_1117.html.**

At the meeting, it was realized that while many of the solutions will be implemented through the processes outlined in the report, continuing less formal cooperation between the participants will be important in encouraging the increased use of biopesticides so that they become fully integrated into Desert locust management.

Figure 2: Writing of group reports

Poster Summaries

The LUBILOSA Legacy: A Mycoinsecticide Success Story

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Introduction.
The International LUBILOSA Programme was a collaborative research initiative, which brought to the market an effective biopesticide for control of locusts and other grasshopper pests. A crucial ancillary benefit was the development of a number of "enabling technologies" (e.g. fungal mass production, storage, formulation, product packaging and application) both for this product and other mycoinsecticides. In bioassays, isolates belonging to *Metarhizium anisopliae var. acridum* were found to be most infectious to *Schistocerca gregaria* and isolate IMI 330189 was commercialized under the name 'Green Muscle®'. The first company to be granted a license was Biological Control Products (BCP) of South Africa, which launched the product in 1998. The foundation *Agir pour l'Education et la Santé* in Dakar has completed a production plant for the product and intends to obtain a license this year. An Australian isolate belonging to the same clade is marketed as 'Green Guard' using technology similar to that developed by LUBILOSA.

Production and delivery systems.
Spores (aerial conidia) are prepared in a 2-phase system and separated from solid substrates using specialized equipment. The product is packed in sealed, laminated bags, and experimental models suggest it can be stored for up to 10 years under suitable conditions. An oil flowable concentrate (OF formulation) that contains 500 g spores/L in an oil base, was developed for ease of use in the field with standard ultra-low volume (ULV) sprayers.

Field rates and validation.
'Green Muscle' has been registered in a number of eastern and southern African countries and was granted a temporary sales license in the CILSS countries. Early application rates were at 100 g. spores/ha, including a successful series of wide-area trials against *Oedaleus senegalensis*. With improved formulation, the recommended dosage is now 50 g/ha, diluted to 0.5-2.5 L with oil (e.g. diesel fuel) depending on the type of ULV application. However, it has been demonstrated over the past few years that 25 g/ha is adequate in many circumstances, with only dense vegetation requiring higher dosages (of up to 75 g/ha). The efficacy of entomopathogenic fungi is subject to abiotic factors such as high temperature; nevertheless trials have shown that 'Green Muscle' can be effective against *S. gregaria*.

Necessities for implementation.
LUBILOSA scientists established a number of quality control criteria for the spore product and it is **essential** that these are strictly adhered-to. A product that conforms to high particle size specifications enables stable formulation and prevents blockages in application equipment. A very high proportion of individual, viable, virulent spores is required for achieving the low (and thus economically viable) application rates. Other specifications include: absence of contaminating microorganisms and low moisture content to facilitate storage.

The specificity of *M. anisopliae* confers immediately practical as well as proven environmental benefits. Both vertebrate and invertebrate natural enemies are known to contribute to regulation of pest populations and there have been repeated observations of treated, sluggish, hopper bands being eliminated by predators within a week of treatment. Besides achieving its scientific objectives, LUBILOSA undertook very substantial participatory training in order to inform farmers and extension workers of the benefits and issues entailed in acridid biological control.

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Field Trials of Low Dose Rate of Green Muscle Against Grasshoppers In Senegal

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Summary.
The cost of using biopesticides for locust/grasshopper management is proportional to the dose. A field trial in Khelcom, Senegal followed the fate of mixed-species adult grasshopper populations after the application of Green Muscle (*Metarhizium anisopliae var. acridum*, isolate IMI330189), at 25g spores/ha and 50g spores/ha. The 25g/ha dose was as effective as the 50g/ha dose. Populations in unsprayed parcels remained stable over a month. Populations in both high and low dose parcels dropped to 50% within 9 days. Within two weeks, the low and high dose populations were 10% and 6% of their original density, respectively, further falling to 6 and 3% by 21 posttreatment.

Conclusions.
1. Both 25g/ha and 50g/ha of Green Muscle were effective at reducing the population of adult grasshoppers.
2. Despite significant differences in the treatment means, both doses were equally effective from the perspective of grasshopper management.
3. Given the 50% lower cost of a 25g/ha dose and the negligible decrease in performance compared to a 50g/ha dose, these results support a 25g/ha option on the registration label.

Cross-Stage Effects of the Desert Locust, *Schistocerca gregaria*, Aggregation Pheromones on Their Immune Factors, Behaviour And Susceptibility To a Sub-Lethal Neem Oil, *Azadirachta indica.*

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Objectives.
• To determine the cross-stage effects of *S. gregaria* aggregation pheromones on their immune factors and

behaviour (communal oviposition).

- To assess the mortality and growth disruptive effects of PAN combined with a sub-lethal dose of neem oil on nymphal desert locusts.

Conclusions.

- There are cross-stage pheromonal effects that affect immune factors in *S. gregaria* as measured by hemocyte counts, probably resulting from inhibition of the pheromonal communication of the insects.
- PAN enhanced the efficacy of a sub-lethal dose of neem oil (1/4 of the dose used by Wilps and Nasseh (1994)) with regard to mortality and growth disruption properties.
- PAN and neem caused deformations of the wings, antenna, and hind legs of fledglings. The deformed insects are unable to feed, fly, and mate.
- Both PAN and Nymphal aggregation pheromone (NAP) have no toxic or growth regulation properties on nymphal and adult desert locusts and represent environment-friendly indirect control agents.
- NAP caused a random distribution off egg-pods by gregarious gravid females similar to that of solitarious females. This effect results from the inability of treated gravid females to detect their own oviposition pheromones. By disturbing the communal oviposition which is a key factor in promoting gregarisation of the hatching nymphs, NAP may increase the tendency of them becoming solitarious.

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Effects Of Phenylacetonitrile On the Food Uptake of Desert Locust Nymphs, *Schistocerca gregaria,* And Its Combination To Sub-Lethal Doses Of Fenitrothion.

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Objectives.

- To determine the effect of PAN on the feeding behaviour of *S. gregaria* nymphs.
- To assess the efficacy of the combination of PAN and fenitrothion on nymphs and the most suitable time of the application.

Conclusions.

- PAN enhanced the efficacy of all doses of fenitrothion used either on fourth or fifth instars nymphs, but not significantly (Pr>F >0.05) when the two compounds were applied on the same day.
- On third instar nymphs, only the higher dose of fenitrothion (0.5 x 10-2 mg a.i / nymph) was significantly enhanced by PAN (Pr>F < 0.0001) when the former was applied three days after the onset of PAN treatment.
- The efficacy of all doses of fenitrothion used alone on third and fifth instars nymphs significantly (Pr>F < 0.0001) decreased with the age of the nymphs, the younger ones being more susceptible than the older ones.
- In contrast, for the PAN-treated nymphs, age of the test insect had very little effect on the efficacy of fenitrothion.
- Both dose of PAN (3,90 and 0.93 mg / day) similarly enhanced (Pr>F > 0.05) the efficacy of fenitrothion (1.8 x 10-2 mg a.i / nymph) when the latter was applied three days after the onset of PAN treatment with a mortality rate of 66,19% and 66,16%, respectively.
- The best combination for this study is: the lower dose PAN (0.93 mg / day) and medium dose of fenitrothion (1.8 x 10-2 mg a.i / nymph, that is three quarter of the recommended dose) when the latter was applied three days (“Day 3”) after start of PAN treatment on fifth instar nymphs.
- PAN reduced the food uptake of fourth instar nymphs from day 2 after application, this effect was significant on days 4 (Pr > t = 0.020) and 5 (Pr > t = 0.030).

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Mixing Green Guard® ULV & Spray Adjuvant Oil In the Field For Aerial Application – the APLC Method

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Mixing Green Guard® ULV in the field prior to aerial application.

APLC Field Officers have found that one of the most difficult aspects of working with the biopesticide Green Guard® ULV during aerial control operations in remote areas is mixing the concentrated spore suspension with spray adjuvant oil prior to loading into spray aircraft. Problems during this process can lead to critical blockages of aircraft application equipment with poor results on targets due to reduced dose and poor coverage. The APLC has developed a relatively low cost mixing system to make this task simpler and more efficient. A rugged vehicle trailer (suitable for travel on bush tracks) incorporating a large mixing tank, an engine driven impeller pump, a system of valves, hoses and a filter, is currently used. With this equipment and a simple mixing procedure, spray payloads can be prepared easily and loaded into the spray aircraft.

The mixing method.

1. The spray adjuvant oil (Caltex Summer Spray oil) is transferred into the large mixing tank using the pump. All pumping operations are controlled using a series of on/off valves. Current mixing ratio is one 205 L drum to 42 L of Green Guard® ULV concentrate (3 buckets). When applied at 0.5 L/ha this results in a dose of 25 g of *Metarhizium anisopliae* spores/ha.

2. The Green Guard® ULV concentrate is stirred by hand using a suitable implement until the spore material is thoroughly resuspended. The open "bucket" 20 L container allows easy access. Once mixed the concentrate is added to the spray oil in the large mixing tank and agitated.

3. This is carried out by recirculating the contents of the mixing tank through the pumping system for a minimum of 15 minutes.

4. After mixing, the valve settings are changed and the spray load is transferred into the spray aircraft. At this final stage an in-line filter stops any aggregated material reaching the aircraft's spraying system (the mesh size for this filter is 30 to 40). The 500 L mixing tank can accommodate a load of two 205 L drums of Summer Spray oil and six buckets of Green Guard® ULV concentrate, enough to treat an area of approx. 990 ha.

Synergy of Predation And Entomopathogens: An Essential Element Of Biocontrol.

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Birds as predators of locusts and grasshoppers.

Birds are well known to be efficient locust predators. Unfortunately, most of the observations on locust-feeding birds were done during locust outbreaks, and came to the conclusion that the efficacy of the bird predation on adult swarms is very limited, only between 0.25 and 5% (Elliott, 1962). However, the Desert locust hopper bands may face much higher losses due to bird predation. Observations by Ashall and Ellis (1962) showed that birds can destroy about 1/2 of hopper population. According to Wilps (1997), birds were able to destroy 95-99.5% of Desert locust nymphs. Currently over 500 bird species of 55 families are known to prey upon locusts in Africa; several bird families, such as Coraciidae, are specialized acridophages.

Birds increase mortality rates of targets in plots treated with entomopathogens.

Desert locust hopper bands treated with *Metarhizium anisopliae var. acridum* (Green Muscle, GM) appeared to be attacked much more seriously by birds. In various trials over several years, bird predation rates in treated plots were significantly higher than in controls. Two operational size trials with GM in 2005 and 2006 showed that synergy of predation and the action of the entomopathogen is a natural phenomenon. Up to 26% of 1 million hoppers were eliminated by birds in one month time. After the locusts started to become affected by GM, bird impact increased rapidly, and the birds took up to 4% of the hopper population daily.

Why does biocontrol enhance predator activity and chemical control does not?

An essential difference between chemical and biological control agents is their speed of action on locusts. The relatively slow speed of action of GM actually help building up bird and other predator populations. Affected by GM, hoppers become sluggish, extend their basking time to induce fever, and thus become easier capture. In the case of chemical control, huge numbers of intoxicated prey become available in a matter of hours. Some debilitated insects may attract birds which, in turn, may become intoxicated as well.

Predation as an element of an integrated preventive control strategy.

Predator effectiveness will be enhanced by use of biological control agents, but reduced by chemical control. Predator potential of birds should be assessed as part of regular scouting activities. Several bird species, such as Golden Sparrow, are considered as pests of cereal crops. However, their role as key predators of the Desert locust nymphs is very important, and their status should be reappraised.

Potential Of Several Alternative Control Agents For Use Against Locusts: A Case Of *Metarhizium anisopliae var. acridum* and *Schistocerca gregaria.*

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Summary

Within the framework of biological control of locusts, we tested the following entomopathogenic organisms and insecticides on *Schistocerca gregaria* (Forskal, 1775):

- A bacterium *Bacillus subtilis* with the amount of 7.3x10 8 spores/ml, gives 100 % of mortality out of the first 4 larval stages at the end of 8 days;

- *Azadirachta indica* (the neem) and *Melia azedarach* (the melia), exhibited both repellent and acridicide properties. The oil of neem inhibits the growth and the development of the locust. Rates of malformations and mortality going up to 70 % are observed.

- Teflubenzuron, the Insect Growth Regulator, tested on L5 by ingestion, showed significant reductions of all the metabolites studied: cuticular chitin and proteins as well as proteins, hemolymphatic lipids and glucides and ovaries.

- The fungus *Metarhizium anisopliae var. acridum* was obtained in May 2005 of the National Institute of Plant Protection of Algiers in the form of biospesticide "Green Muscle" (GM) formulated as oil concentrate of spores. Following the treatment of *S. gregaria* with this product with the amount of 14x10 8 spores/ml, we noted on the 6th day a significant reduction in the rate of respiration (88 to 19 and 78 to 18 openings/min in the females and the males). Similarly, the rate of heartbeat went down (73 to 22 and 81 to 24 beats/min in the females and the males). On the level of the haemolymph, a strong toxic activity of cytoparasites was detected. The affected haemocytes retracted, their cytoplasmic contents were deteriorated and the clusters of spores were observed within the blood cells. The numbers of hemocytes decreased. When the fungus inoculum was applied to0 the sand in which the females laid their eggs, the rate of egg hatchings was 40% whereas it reached 90% in the untreated controls. This rate went as low as 13.33% when the treatment was realised directly on eggs the same day of the oviposition.

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Application Of Green Guard® *(Metarhizium anisopliae var. acridum)* Against the Oriental Migratory Locust *(Locusta migratoria manilensis)* In China.

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The *Metharhizium* insecticide Green Guard® has been used as part of preventive control operations against locusts in Australia since 2000. Between 2003 and 2005, there was a major outbreak of Australian plague locust and nearly 50,000 ha of locust infestations were treated with Green Guard®.

However, locust outbreaks in Australia are sporadic and to ensure more continuous use of Green Guard®, and consequent ready availability wherever and whenever it is needed, tests have been conducted in China that has more regular outbreaks.

As part of ensuing food security for its large population, 300,000 to 1,000,000 ha of locust and grasshopper outbreaks are treated every year.

The target species for Green Guard® in China was the oriental migratory locust, *Locusta migratoria manilensis*.

The application of Green Guard® was done by back pack sprayers and by airplane. In the first case, Green Guard® was mixed with soybean oil and applied at 1.1 – 1.5 L/ha to give good coverage and a high number of lethal doses/m^2. Applied by teams of people walking 5 m apart. Tests with Green Guard were done in 2002-2004 at dose rates of 25-75g/ha. High level of locust mortality (81-97%) was registered 8-14 d posttreatment. The dose rate of 50 g/ha produced consistent results. Even the lower dose rate (25 g/ha) yielded 81% mortality 14 d posttreatment.

Aerial ULV applications were done at 1.1 – 1.5 L/ha after mixing Green Guard® with soybean oil. The mixture was sprayed from a 10 m height at 50-75 m track spacing in a crosswind of 2-5 m/s. The dose rates tested were 25 and 37 g/ha. The locust mortality 20 d posttreatment was 94 and 91%, respectively. Despite the fact that locusts continued hatching after the treatment, Green Guard® was effective in killing the new hatchlings.

Conclusions: Green Guard® provided excellent locust control under varying environmental conditions and applied from different spraying platforms. The tested ULV rate of 1.1 – 1.5 L/ha appears adequate to ensure sufficient control.

Assessing Green Guard and Green Muscle for a "Red/Blue" America: Susceptibility of North American Orthoptera and Importance of "Behavioral Fever"

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Introduction

As a preliminary to field trials of either the IMI330189 (GM, Green Muscle) or FI-985 (GG, Green Guard) strains of the fungus *Metarhizium anisopliae* var. *acridum,* I have assessed their infectivity and virulence against a range of North American acridids and the Mormon Cricket *A. simplex* (a katydid).

Results – Bioassays.

Schistocerca americana: Based on the mean LD50s *Schistocerca* was 66.5-fold more susceptible to GG than GHA and 34-fold more susceptible to GM than

GHA. *Anabrus simplex*: Both GG and GM were more efficacious than GHA, yet much less so than for acridids. Adults were equally susceptible as 5th/6th instar nymphs to both *Metarhizium* strains. 5th Instar Mormon Crickets were more susceptible to FI985 relative to GHA. Adults were much more susceptible to FI985 than to GHA. This differential effect seems to be caused by a large decrease in the susceptibility of adults to GHA but not FI985. A similar situation exists for GM. Both *Metarhizium* isolates killed all target acridids faster than GHA, usually with an abrupt mortality 5-7 days after exposure (see above. Cumulative mortality of Mormon Cricket from GHA and GM was similar; that from GG was slower.

Results – Low-Volume Spray Assay.
At the common rate of 1x1013 conidia/ha, GG was significantly more efficacious than GHA for all species except *A. deorum* and *E. costalis*, and was especially so for many of the *Melanoplus* spp. Similar tests with GM have not yet been conducted.

Body Temperatures.
The effect of body temperatures among actively thermoregulating grasshoppers could be a serious constraint to acceptable field efficacy. Behavioral fever occurs in many if not most N. American acridids. In addition, low nighttime body temperatures further slow progression of mycosis. Observed death from *Beauveria* and *Metarhizium* mycosis did not occur until Day 29 of the trial. Field-treated crickets incubated indoors at 28° C suffered 100% mortality from both fungi within 10 days.

Prognosis for FI985 and GM?
Both strains have higher upper thermal than B. bassiana GHA. But both are also more sensitive to the lower temperatures than GHA. Outdoor cage trials with *A. simplex* and *Melanoplus differentialis* are planned for 2007, if exotic isolate permits can be obtained.

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