CROP PROTECTION PROGRAMME

Development of biologically based control strategies for environmentally sustainable control of red locust in Central and Southern Africa

R 7818 (ZA0419)

FINAL TECHNICAL REPORT

28 July 2000 - 29 February 2004

Dr Matt Thomas

Imperial College

Date FTR completed: 23 February 2004

"This publication is an output from a research project funded by the United Kingdom Department for International Development for the benefit of developing countries. The views expressed are not necessarily those of DFID." [R7818 Crop Protection Programme]

Executive Summary

The red locust is an important pest in Central and Southern Africa. Current control relies on the use of broad-spectrum chemical insecticides. However, the breeding sites that are targeted are wetland areas that represent rich sources of biodiversity and are of international conservation value. Thus, an alternative to conventional chemical control is urgently needed. This project sought to develop a novel, environmentally benign strategy for control of red locust based on the use of a fungal entomopathogen which is specific to locusts and grasshoppers and which can be formulated and applied as a biopesticide.

Several field trials were conducted with the biopesticide in Zambia and Tanzania. These included treatments against red locust nymphs during the wet season and one trial against adult locusts during the dry season (co-funded by FAO Technical Cooperation Programme funds). Whilst there were some difficulties with the trials (largely logistical difficulties in working on locusts in remote flooded areas, although one trial was also a failure due to use of an inappropriate formulation), the overall results from the trials indicated that red locust nymphs and adults were susceptible to the fungus and that spray applications caused significant population reductions.

The efficacy studies were accompanied by environmental impact studies to determine the effects of the biopesticide on non-target invertebrates relative to a chemical pesticide standard. These studies showed the biopesticide to have significantly less impact on non-target species than the chemical pesticide. Indeed, the only non-target effects recorded were on non-target grasshoppers. This result is not unexpected and whilst it should not be dismissed as irrelevant, its significance needs to be placed in context. First, the direct non-target effects from spray applications of the biopesticide are still far less than with a chemical. Second, long-term effects through establishment and cycling of the pathogen through target and non-target species are likely to be negligible; studies on persistence and sporulation of cadavers in the field indicated high levels of scavenging and predation such that cadavers could rarely be found, making horizontal transmission extremely unlikely.

Supporting ecological studies were conducted to elucidate the effects of temperature and locust thermoregulatory behaviour on performance of the pathogen. These studies provided valuable insights into the mechanisms employed by locusts to combat infection and into the costs of mounting a defence response. The studies of thermal biology also contributed to the development of a GIS-based model that enables us to predict variability in performance of the biopesticide in time and space, based on measures of ambient temperature. The outputs from the model suggest that the biopesticide should be highly effective against red locust nymphs throughout its range during the wet season. It should also be effective against adults in the dry season, although speed of kill is slower and more variable (indicating the need to target adults earlier rather than later in the dry season).

Overall, the assessment of locust control experts who participated in the trials, including those from the organisation with the mandate for locust control in the region (IRLCOCSA), was that the biopesticide provided satisfactory control and, given its limited environmental impact, should be considered for red locust control in the future. Registration for the biopesticide was extended from South Africa to Zambia and Nambia during the study, with registration dossiers submitted for review in Tanzania and Mozambique by a commercial producer in the region. Thus, this project has been extremely successful in setting the scene for a new era of environmentally sustainable red locust control. Further adoption will require locust control donors to now support the technology in place of traditional chemical insecticides.

Background

The red locust (*Nomadacris septemfasciata* Serville) is a serious agricultural pest in central and southern Africa which breeds in seasonally flooded plains. The most extensive and serious red locust plague on record lasted from 1929-1944 and covered all of southern Africa (Morant 1947). Upsurges have since continued to occur in the so-called 'recognised outbreak areas' in eastern-central and southern Africa. The most serious of these near plague upsurges were initiated in outbreak areas in Tanzania, Mozambique, Malawi and Zambia in 1986-89 and 1995-7, and resulted in large numbers of swarms escaping from the outbreak areas. Crops were damaged as a result of these invasions in Burundi, Rwanda, Uganda, Malawi, Zimbabwe, Botswana and South Africa (Anon 1996, 1997). Moreover, the increasing cultivation of crops within outbreak areas makes the red locust a growing problem causing direct crop losses for commercial and subsistence farmers.

The key recognised outbreak areas include the Kafue Flats and Mweru wa Ntipa in Zambia; Wembere Plains, Iku-Katavi, the Malagarasi Plains and Lake Rukwa Valley in Tanzania; Lake Chilwa Plains in Malawi and Buzi-Gorongosa Plains in Mozambique. Subsidiary outbreak areas include the Caprivi plains in Namibia and neighbouring Satau plains along River Chobe in Botswana and the Bahi Valley in Tanzania. These flood plains represent rich sources of biodiversity and many are populated by herds of wild game animals and a wealth of bird life. For example, Kafue Flats is the 4th largest refuge of bird life in the world (Ellenbroek 1987). For this reason, a number of the recession areas have been designated as 'Ramsar Sites' of international conservation importance.

Current control measures are based on aerial application of organophosphate insecticides applied to adults and hopper bands in the outbreak areas. These chemicals are toxic to nontarget organisms, and hazardous to humans and livestock. The pyrethroid, Deltamethrin, which is increasingly used for locust control because of its fast knockdown effect, is less hazardous to mammals but still has a wide spectrum of action. Even the latest chemical insecticide being marketed for locust control, Fipronil, does not seem to be suitable for use in wetlands. Though it is quite safe for humans and livestock, it is highly toxic to all arthropods and fish, and also affects lizards and certain types of birds. Moreover, it is more persistent than the other insecticides in use against locusts.

Thus, the insecticides most commonly used in locust control have direct effects on aquatic and other non-target invertebrates. They also have either direct or indirect effects on birds. Consequently, an alternative to conventional chemical control is desperately needed for the development of new, environmentally sustainable strategies for control of red locust. This demand is endorsed by the international scientific and donor communities. For example, it is implicit in the aims of the FAO EMPRES programme, and in the activities of organisations such as the Australian Plague Locust Commission. Significantly. For Central and Southern Africa in particular, participants at a DFID-funded CPP Migrant Pests Workshop, held in Pretoria in March 1999, identified the testing of pathogens and the development of alternative IPM strategies as the priorities for future locust control.

Throughout the 1990's an international collaborative research programme, called LUBILOSA, worked to develop a mycoinsecticide as a biological alternative to chemicals for locust control (Bateman 1997; Lomer *et al.* 1997). The mycoinsecticide is based upon a naturally occurring pathogen, *Metarhizium anisopliae* var. *acridum*. The mycoinsecticide has proven effective for the operational control of grasshoppers in the Sahel, desert locusts in West and East Africa, and brown locusts in South Africa. Moreover, extensive research into the environmental effects of the mycoinsecticide has indicated that it has minimal impact on non-target species and is harmless to humans, other mammals, reptiles and birds. In 1996 the Desert Locust Pesticide Referee Group of FAO approved the LUBILOSA mycoinsecticide (trade name Green Muscle[™]) for operational locust control in conservation and environmentally sensitive areas. At the end of 1998, Green Muscle was successfully

registered for use in South Africa against brown locust and a commercial producer (Biological Control Products SA (Pty) Ltd.) was licensed to produce the product in the region.

Preliminary trials with the mycoinsecticide were carried out on red locusts by PPRI and Departmento de Sanidade Vegetal in South Africa and Mozambique. These trials showed that the product causes more than 90% mortality in cages within a week (Price *et al.* 1997). In the field, younger instars were completely controlled in 7 days, but flooding prevented a full assessment of mortality in older instars. Nonetheless, these results and research developments indicate a real possibility for using biological control as a substitute for chemicals in red locust control.

Project Purpose

To develop a novel, environmentally benign strategy for control of red locust based on the use of a fungal entomopathogen, formulated and applied as a mycoinsecticide.

Research Activities

Activity 1. Field testing to quantify performance of a mycoinsecticide based on M. anisopliae var acridum (isolate IMI 330189) at a range of sites under different environmental conditions

Four main field trials were conducted during the course of the project:

February 2001 in Kafue Flats, Zambia

This study was conducted in collaboration with the International Red locust Control Organization for Central and Southern Africa (IRLCO-CSA, Ndola, Zambia), CABI African Regional Centre (CABI-ARC, Nairobi, Kenya), the Plant Protection Research Institute (Pretoria, RSA) and national programme staff of Zambia (Mt. Makulu Research Station), Tanzania (PPD, Tanzania) and trainees from Namibia (Ministry of Agirculture).

The field site consisted of a mosaic of long grasses (*Echinochloa* sp. and *Spoloborus pyramidalis*) and short grasses (*Cyanadon* sp. and *Panicum-Setaria*). Locust populations were low, generally $<1 \text{ m}^{-2}$ from sweep net sampling, but were heterogeneously distributed within the mosaic, principally in the shorter grasses along the borders with the taller. In these areas populations reached $3m^{-2}$. Meanwhile, co-occurring grasshoppers, principally *Orthochtha* spp., were very abundant.

A treatment area of 50ha was selected for treatment and within this, four square plots were delimited with borders of 350m. Three similar control plots were delimited upwind of the treated plots. The Green Muscle formulation of *Metarhizium* was mixed with Jet A1 fuel to give a spore dose of 50gha⁻¹ at 2 Lha⁻¹ with germination counts prior to application indicating spore viability of 60%.

Attempts to monitor locust and grasshopper populations in the treated and control plots were hindered by time limitations due to flying restrictions, low populations and flooding of the site. Instead, the day following application, four samples of approximately 5 red locusts and 20 *Orthochtha* sp. were collected from each of the treated and control areas and placed in incubation bottles, subsequently taken to the laboratory were they were daily fed fresh maize leaves. Mortality was assessed daily and all cadavers were removed. The cumulative mortality was analysed using Kaplan-Meier survival analysis.

February 2002, Iku-Katavi, Tanzania

The 2002 trial was on a larger scale than in the previous year, in an environmentallysensitive area in Tanzania, the Katavi National Park, one of the major recession areas for the red locust. The study was conducted in collaboration with the International Red Locust Control Organization for Central and Southern Africa (IRLCO-CSA, Ndola, Zambia), CABI African Regional Centre (CABI-ARC, Nairobi, Kenya), the Plant Protection Research Institute (PPRI, Pretoria, RSA) and Plant Health Services of the Ministry of Agriculture and Food Security, Tanzania. Following initial surveying in Iku-Katavi, three target areas were identified around concentrations of 4th to 5th instar locust hoppers, beginning to coalesce into high density bands (e.g. at least 23 locusts m⁻², range 11-37).

The size of locust bands and the practicalities of aerial application meant that each area was assigned as a treatment rather than subdividing them into replicated blocks. The three treatments were: *Metarhizium* treatment (i.e. biopesticide), Fenitrothion treatment (i.e. conventional chemical pesticide) and an untreated control. Each area was a square or rectangle of 100 ha.

The Green Muscle formulation of *Metarhizium* was mixed with Jet A1 fuel to give a spore concentration of 50 g/ha. Fenitrothion was sprayed at a volume rate of 0.5 l/ha with 450 g a.i./ha.

Locust populations were assessed by two workers walking transects of 100 m through concentrations of hoppers. Every 5m the workers stopped and counted the number of locusts visible in front of them in an 'imaginary' 1m quadrat. This was repeated at day 1 in the fenitrothion plot and thereafter every 2-3 days until day 16. On day 15 an additional assessment of parts of dead locusts was made in the *Metarhizium* and control plots to obtain an indication of locust mortality.

February/March 2003, Wembere Plains, Tanzania

On the strength of promising results from the trials above, and in the face of a growing locust problem in the region, FAO provided funds to the Tanzanian Government through the Technical Cooperation Programme to support a large-scale application of the Green Muscle. By combining expertise and resources of the FAO project with the DFID-funded project, 150Kg of fungal spores were purchased and transported to the field. This large-scale trial was implemented by representatives from CABI Bioscience, IRLCOCSA, The Australian Plague Locust Commission (as consultants for FAO), EMPRES and the Plant Health Services of the Ministry of Agriculture and Food Security, Tanzania.

The trial was carried out in the Wembere Plains, about 150 km by road north-east of Tabora in central Tanzania. The product used was an oil miscible flowable concentrate (OF formulation) of conidia (spores) of *Metarhizium anisopliae* var. *acridum* (Green Muscle), obtained from Biological Control Products (BCP) of Pinetown, South Africa. It had been air-freighted to IRLCOCSA HQ in Ndola, Zambia, and subsequently transported by road to Masenge. The viability of the conidia had been about 93-95% on leaving the factory. Samples taken from 4 drums just before spraying indicated viability between 84-94%.

Two blocks were sprayed between 15 and 21 February 2003. 1776 ha was sprayed at a dose of 50 g a.i. per ha and 1770 ha at 25 g/ha. An area of about 600 ha was used as control block. The OF formulation, which contained 400 g of spores per litre, was mixed with Jet-A1 fuel at the rate of 1:7 to give a spore concentration of 50g/l and 1:15 to obtain 25g/l. Volume application rate was 1.2 l/ha.

Locust samples were taken the day after the last spray sortie. The hoppers were held in 1.51 empty plastic water bottles in numbers not exceeding 10 per bottle. Fresh feed was provided on a daily basis and mortality checked. Dead insects were collected and air-dried for 24 hours to check for the characteristic red colouration caused by *Metarhizium*. They were then surface sterilised using ethanol 70%, rinsed with distilled water and placed on moist tissue paper in Petri dishes to observe the external growth of the fungus.

Field locust populations were monitored in areas of tall grass consisting mostly of *Sorghum sudanicum*. Numbers of hoppers and fledglings were counted along transects of 100 steps. Two or three transects were counted in each of three sampling areas in each block including an unsprayed one. Assessments started as soon as the grass was dry and most locusts had moved into the upper half of the vegetation, sometime between 8.00 and 9.00 am. By midday, the locusts would usually descend because of rising temperatures, making them

more difficult to observe. Assessments therefore had to be completed well before that time. Blocks were visited on consecutive days, so that each block was monitored every three days.

August/September 2003, Iku Plains, Tanzania

The initial end date of the project was July 2003. However, the previous trial conducted in February-March 2003 did not produce the expected result, apparently due to the wrong choice of diluting oil (see results section). It was therefore necessary to conduct another trial using a more suitable diluent. Furthermore, it was considered worthwhile to try the product on adult red locusts instead of just nymphs. An extension to the project was therefore requested to conduct a final trial. As with the previous trial, this was co-funded by FAO and was implemented by representatives from IRLCOCSA, CABI, APLC and the Plant Health Services of the Ministry of Agriculture and Food Security, Tanzania.

The trial was carried out in the Iku Plains, which are part of the Katavi National Park, about 50 km south of Mpanda in south-western Tanzania. Two products were used in this trial. One was Fenitrothion 96% ULV, which has been the standard insecticide for red locust control in recent years. The other was an oil miscible flowable concentrate (OF formulation) of conidia (spores) of *Metarhizium anisopliae* var. *acridum* (Green Muscle), obtained from Biological Control Products (BCP).

Experimental blocks were selected based on the density of present locust populations. Four blocks were marked, one of 1400 ha (block D) for Green Muscle at 50 g/ha, two of 800 and 400 ha (blocks C and X) for Green Muscle at 25 g/ha and one of 600 ha (block F) for Fenitrothion 96% ULV. An area of about 400 ha (block B) was used as an untreated control. The north-western part of block D was mostly covered by tall grasses (>1.5 m) with many tall trees (>5 m). The south-eastern part of the block was burnt, but two large islands of unburnt grass remained. The burnt area had sparse vegetation with most of the grass tussocks sprouting. The other blocks were covered with grass of <1 m. About 15% of block X was burnt.

The experimental blocks were sprayed between 15 and 22 August 2003. Block D was sprayed with Green Muscle at 50 g/ha over a three-day period. It also took three days to spray blocks C and X with Green Muscle at 25 g/ha. Block F was sprayed with Fenitrothion in a single day. The OF formulation of Green Muscle, which contained 500 g of spores per litre, was mixed with diesel fuel at the rate of 1:9 to give a spore concentration of 50g/l and 1:19 to obtain 25g/l. Volume application rate was 1 l/ha. The Fenitrothion 96% ULV was sprayed undiluted at 0.5 l/ha.

The spraying of block D was technically difficult. The block was long and narrow (2x7 km) and had to be sprayed along its length, otherwise the spray plane would have had to make too many turns thereby substantially increasing the time needed for spraving. As the window available for spraying each day was limited to around 2 hours, with inversion conditions early in the morning and thermals from mid-day onwards, too many days would then have been spent on this block. Unfortunately, this meant that the angle between the flight path and the prevailing wind was less than 45°. The wind speed was also quite variable and often less than ideal. In some places, the presence of trees forced the pilot to fly higher than was desirable. Finally, the spray plane that had been made available had not been fitted with differential GPS. A helicopter was therefore used to mark individual spray runs at one end of the block, but because of the smoke haze conditions present on the plains, it was still extremely difficult for the pilot to stay on the correct track during the long spray runs. This most likely resulted in overdosing and under dosing of certain parts of the block. Another problem was that contrary to our expectations, most locusts that were in tall grass areas did not fly up when the plane passed overhead, which will have severely limited direct hit. Conditions during spraying of the other blocks were, however, good and they most likely received a better and more uniform coverage.

Field populations were monitored by helicopter flying slowly at low level to get an idea of the extent and density of the swarms and to check whether the swarms had moved out of the blocks or joined with other swarms or broken up into smaller ones. The blocks were visited on alternate days. Estimating the populations by counting the locusts along transects was not possible because of the constant movements within the blocks. Additionally, observations were made on the behaviour of the locusts and attempts were made to find cadavers. Forty freshly killed locusts were placed in two different spots to check the activity of scavengers.

Activity 2. Studies of host-pathogen interactions and thermal ecology

Research on host-pathogen interactions and thermal ecology followed two paths. The first was to collect data and gain sufficient insight into the thermal biology of red locust to enable us to develop a temperature-based pathogen performance model. Further details of this work are provided in Activity 5 and supporting appendices.

The second theme to the host-pathogen research was to better understand specific aspects of the biology of the host-pathogen interaction and the influence of temperature on these. Because of difficulties in culturing red locusts we used the Desert locust, *Schistocerca gregaria*, as model locust species for these studies (and Desert locust is arguably the most important pest species in its own right).

The specific studies we conducted were:

- 1. A study of the consequences of behavioural fever for survival of locusts and performance of the fungal pathogen.
- 2. A study on the effects of the fever response on locust phase state and its potential implications for control.
- 3. A study further exploring the costs of fever and mechanisms through which fever affects locust life history.

Summaries of this work are present in the Outputs section below with full details provided in the manuscripts appended to this report.

Activity 3. Studies on pathogen persistence and horizontal transmission

As part of the initial field trial, a study was conducted to determine persistence of spores in the spray residue after treatment. The technique used a bottomless field cage of 2m height and 50cm sides, which was placed over grass in each of the four treated plots. The day after application, this was filled with ca. 20 red locusts from an untreated area. These were left for three days and then removed and taken to the laboratory to assess mortality as above. Upon locust removal, the cages were moved to a new site close by and restocked for another three days. This was only possible twice due to the extent of flooding of the field site. In addition, an effort was made to assess the effect of height in the vegetation upon initial dose and spore persistence. On days 1, 4 and 7 following application, vegetation was cut from the top (67cm+), middle (33-67cm) and bottom (0-33) strata, and soil was taken. One such sample set was taken from each treatment and placed individually in incubation bottles. These were then stocked with ca. 20 *Orthochtha* and taken to the laboratory to assess mortality as described above.

Horizontal transmission of the fungus requires that locusts infected from the original spray application die from the disease, persist as cadavers and produce new spores to infect further individuals. Studies to evaluate the potential for horizontal transmission of the pathogen after treatment centred around observational studies on the frequency and persistence of cadavers in the various field trials and also the fate of cadavers actively placed in the field.

Activity 4. Evaluating environmental impact of the mycoinsecticide

The most comprehensive study on the environmental impact of the mycoinsecticide relative to chemical alternatives was conducted as part of the field trial undertaken in the Iku-Katavi

grasslands in SW Tanzania during the wet season in 2002. Methodology involved sampling of a range of non-target taxa from treated and control plots with one pre-treatment sample and 5 post treatment samples up to 15 days after application. Sampling methods included sweep netting, visual counts and activity assay techniques. Full details of the methods and approaches are given in Appendix 4.

Activity 5. Development of host-pathogen models to define optimum use strategies

Although the biopesticides being developed and tested for locust and grasshopper control in various parts of the world hold considerable promise, their use is complicated by a high degree of variability in performance, determine largely by host thermal biology and diurnal temperature range. The practical significance of this variability is that standard control operations in which chemical products are sprayed and efficacy is assumed may not be appropriate for *Metarhizium*-based biopesticides. Rather, successful deployment of the biopesticide requires the development of novel use strategies where expected performance parameters are defined in advance and decisions on where and when to spray adjusted accordingly (Thomas *et al.*, 2000; Thomas, 2000; Lomer *et al.*, 2001). In the absence of such use strategies, the conventional 'spray and pray' tactics will likely compound the inherent variability and critically undermine the adoption of the biopesticide technology into new integrated control practices (this argument is supported, in part, by the current situation in South Africa where, although a locust biopesticide has been registered since 1997, adoption of the technology has been minimal (Thomas *et al.*, 2000)).

Our approach to addressing this issue was to develop a model which captures the effects of environmental temperature and host thermal biology on the growth of *M. anisopliae* var. acridum, and enables us to predict speed of kill of the pathogen following infection. Our aim is to use this model to predict likely efficacy of biopesticide applications against different orthopteran targets in different environments, and to use this information to define effective strategies for implementing biological control of locusts and grasshoppers. The model is developed in three key steps. First, we characterise the thermal behaviour of locusts/grasshoppers and develop a suite of models to describe the relationship between body temperature and ambient temperature for a range of species. We then build a basic model which enables us to link the effects of ambient temperature, through host body temperature, to the rate of development of the fungal pathogen in an infected host (i.e. allows us to predict speed of kill). We then extend this model into a GIS framework to enable us to explore variability in expected pathogen performance in time and space (and hence inform use strategy). Although the primary interest of the modelling work for the current project was the Red Locust, in developing the model we consider a range of locust and grasshopper species from different countries/systems, both to act as points for validation of the modelling approach and to extend the impact of the approach to other target systems.

These modelling studies were conducted as part of a PhD thesis of Ms Justine Klass at Imperial College, supervised by Dr Matt Thomas. Full details of the work are presented in three chapters from the thesis that are annexed to this report.

Activity 6. Technical support to the producer and authorities to provide the necessary guidance and information on regulatory matters to initiate product registration in the target countries

A limited amount of support was provided to the producers (BCP) by scientists associated with the project (particularly from CABI Bioscience) to help in issues associated with registration. However, given the existing registration dossier from South Africa together with the new data from the collaborative field trials, it was relatively straightforward to move ahead with registration procedures.

Activity 7. Examination of costs and benefits of implementing biologically-based locust control

Our intention at the outset of the project was to conduct an analysis of the costs and benefits of using the biopesticide technology for control of red locust. A formal cost:benefit analysis was not conducted, in part because funds were diverted in the last year of the project to enable the last field trial to proceed (approval was given for a no-cost extension with the trial costs met through partial reallocation of funds within the project), but also because the relevance of a cost:benefit analysis was unclear given the political and economic complexities of locust control and the current level of experience in using the biopesticide in the region. However, progress was made towards defining essential parameters including efficacy, dose, environmental impact, ease of use, costs of production and end-user perception. In addition there has been an active dialogue between project scientist, BCP and FAO on cost of product and issues of supply and demand.

Activity 8. Training

The field trials were conducted in collaboration with scientists from IRLCOCSA and local plant health services. This participatory approach provided 'on the job' training for key stakeholders in the region. In addition, two training courses have been organised by BCP to train plant protection officers in the properties and use of Green Muscle for locust and grasshopper control. The first of these is to be held in Tanzania in Dodoma which is about 6 hours drive from Dar es Salaam on 24th - 26th February. The outline of the programme is as follows:

- 23rd p.m. : arrival of delegates
- 24th a.m. : Official opening by the Government officials
 - p.m. : Introduction, (BCP and Green Muscle)
 - : Green Muscle trials in Tanzania 1; Locust control operation in Wembere
 - : Open discussions, questions etc
- 25th a.m. : Green Muscle trials in Tanzania 2; Locust control Iku
 - : Green Muscle trials; Grass hopper control Dodoma
 - p.m. : Green Muscle application; practicals (Hand-held sprayers and mist blowers)
 - : Open discussion and conclusion
 - : Official closing and departure of delegates.

The delegates will mostly be technicians (Field officers) in the plant health department from all the regions. They will be 35 - 40 in number.

The second training course will take place in Namibia in March/April (exact dates have not yet been finalized). The programme will more or less be the same as the Tanzanian course with some changes in the Trials sessions. Emphasis will be on the Brown and Red locusts as well as in application (mixing and spraying).

Outputs

Output 1. Effectiveness of the mycoinsecticide against red locust quantified and sufficient data obtained from outbreak areas to demonstrate efficacy of the product for registration

The key results from the field trials were as follows:

February 2001, Kafue Flats, Zambia

The results of the mortality assessments of field-collected locusts and grasshoppers are shown in Figure 1. It is evident that the animals from the treated plots had a more rapid mortality than those from the control plots (Kaplan-Meier survival analysis gave mean survival times \pm C.I. of: 15 ± 2 versus 26 ± 2 days for red locusts and 10 ± 1 versus 22 ± 1 days for *Orthochtha*), indicating that the application of the biopesticide was successful. Logrank comparisons gave significant differences between all survival curves at $P \le 0.0025$. Approximately 80% of the red locusts from the treated plots had died by 23 days post-

application, while nearly 100% of the *Orthochtha* had died (probably explicable by the differential mortality of control insects).



Figure 1. Proportional survival of red locust nymphs and Orthochtha sp. nymphs and adults following a field application of an oil-based formulation of Metarhizium anisopliae var. acridum. Insects were collected one day after application and maintained in a field laboratory

February 2002, Iku-Katavi, Tanzania

Hopper bands of red locusts are generally not very cohesive, but are made up of dense clusters sitting near to each other in the vegetation. In the control plot, the locusts stayed more or less together and it was fairly easy to follow them and assess the same clusters every time. Populations in the control area remained fairly constant through the trial (Fig. 2). However, in the *Metarhizium*-treated plot, the initially very dense clusters spread out and the main band fell into several smaller bands. Three of these bands were followed and assessed. A decline in density of ca. 50% was observed plots by day 12, falling to <10% by day 16 (Fig. 2). This could be explained partly by locusts moving away from these bands, but their sizes remained fairly constant during the first assessments. It was only when mortality became apparent from the number of body parts found on the ground, that the areas covered by the bands became clearly smaller. In the fenitrothion plot, locusts were eliminated the day after application and there was no recovery observed for the remaining period (Fig. 2).



Figure 2. Visual assessments of red locust populations in the field following treatment with Metarhizium or fenitrothion. Day 0 is day of application.

February/March 2003, Wembere Plains, Tanzania

The mortality in the first set of locust samples collected after spraying is shown in Fig. 3. This figure suggests some effect of the fungus, which is supported by up to 32.5% of cadavers also showing the red colouration characteristic of fungal infection. No control locusts showed signs of infection.

Overall, however, the effects of the fungus appeared rather week. This is supported by the field counts shown in Figure 4. Initially, there was an increase in populations, though this was only slight in the 25 g block. This was apparently caused by a steady influx of younger instars from the shorter grass. Towards the end, all populations declined, but there was no indication that this was due to *Metarhizium* induced mortality. The most obvious explanation is that the fledglings started dispersing. Intensive searches yielded only a few pieces of cadavers and none had the characteristic red colouration. More mortality could have taken place, however, because fresh cadavers placed on the ground all disappeared in less than a day except for some wings and pieces of leg. The same happened to cadavers fixed to the grass at various heights above the ground.

Since the viability of the product was good and that we know red locust to be susceptible to the fungus, these relatively poor results indicate that there was unsatisfactory dose transfer. In some parts of the blocks, this may have been caused by spraying at relatively high temperatures, which may have prevented the droplets from drifting down to the intended area. Another reason may have been that the very tall grass of up to 3.5 m prevented penetration to a level where the hoppers would have easily picked up the spores from the vegetation. The spores trapped in the higher levels of the grass would have been more exposed to UV light and been disabled. However, the assessment of the field team was that the high proportion of Jet-A1 in the spray mixtures, especially the one of 25 g/l, probably reduced impact of the spray. Though Jet-A1 is perfectly acceptable as a diluent, having no adverse effect on the spores, it is highly volatile and when spraying from an aircraft at relatively high temperatures, a high proportion of the spray mixture can be expected to evaporate leaving very small droplets. Such droplets would be carried away before drifting

down to the vegetation and would not impact well on locusts or grass stems. Thus, in spite of considerable effort and a very large-scale application, this trial was generally considered a failure due to inappropriate selection of diluent (the guidance notes on the Green Muscle product label, which were prescribed by CABI Bioscience and which indicated Jet-A1 to be a suitable diluent, have since been altered).



Figure 3. Mean percent survival per dose rate in red locust samples collected from field after spraying



Figure 4. Mean population counts of red locusts per dose rate in the field

August/September 2003, Iku Plains, Tanzania

Locust numbers in the fenitrothion block declined rapidly following treatment. Three hours after spraying it proved impossible to catch sufficient healthy locusts for caging. By 24 hours, few live locusts were observed in the target area and mortality was estimated at more than 90 %.

Mortality in the *Metarhizium* blocks was difficult to detect. However, about two weeks after spraying, it became evident that many locusts had been infected. Some could be caught by hand and many were seen basking on the ground at midday indicating that the locusts were inducing fever to slow down disease development. Uninfected locusts do not display such behaviour. Subsequently, the infected locusts became more and more reluctant to fly. After about three weeks, population reduction became evident in all blocks. This was most obvious in the 25 g/ha blocks, where at the end of the trial, the medium to high densities (3-10 locusts/m²) found before spraying had reduced to low densities (ca. 1 locust/m²) with few medium density pockets. An estimated 70% of the locusts had died by day 27.

Population reduction was more difficult to assess in the 50g/ha block, because the initial density was high throughout the block and there was a lot of movement between the tall grass and adjacent burnt areas. A significant decline was, however, noticeable in the eastern half of the block, where densities went down from 3-20 locusts/m² to $1-2/m^2$, though high density groups remained along the north-eastern edge of the tall grass. In the western half, the locusts moved into the tall grass in the north-western quarter and concentrated there forming numerous medium to high density swarmlets (10->50/m²). It is difficult to put a percentage on the overall reduction in this block, but over 50% was definitely achieved by day 32.

Cadavers were difficult to find, most likely because the activity of scavengers was high. The 40 locusts that had been killed and placed in the 50g/ha block, had almost all completely disappeared after three days. Pieces of only three of the cadavers were found. The most common scavengers observed were ants and certain beetles. Another reason could have been predation of sick locusts by birds. Some mammals are also likely to have taken lots of locusts. Finally, finding cadavers among the dense grass was an almost impossible task because of the locusts' good camouflage colours.

Unlike the previous trial on nymphs, there were clearly no problems with spray deposition. Diesel as a diluent worked very well. However, the droplets did not penetrate well into the tall grass, a problem that has also been experienced in other trials elsewhere in Africa and in Australia. Since the locusts that were settled in the tall grass did not fly up when the plane flew over, they would have experienced little direct hit. Infection in those locusts therefore had to rely mostly on secondary pickup. In contrast, the locusts in the short grass were well exposed and most of them are expected to have experienced direct hit.

At the end of the observation period, there was a clear reduction in locust numbers in all blocks sprayed with *Metarhizium*. Because of the way the assessments were done, it was difficult to come up with reliable percentages. In the experience of the Australian Plague Locust Commission, it is hard to notice a reduction of less than 50-70% in sprayed areas containing adult locust swarms. It seems reasonable to assume, therefore, that more than 50% of the locusts died in all blocks, with probably in excess of 75% in the 25g/ha blocks (mortality in the 50 g/ha block may indeed have been higher but the dense concentrations of locusts in several parts of the block made it difficult to ascertain). Since the 25g/ha treatment gave a result that was at least as good as the 50g/ha treatment, a good case can be made for the former to become the recommended dose rate.

In conclusion, the trial team (which included representatives from IRLCOCSA, the Tanzanian PPD and FAO – i.e. the key stakeholders) considered that the level of mortality achieved would probably be sufficient for effective red locust control in most years, especially as efficacy is likely to increase under actual operational use rather than the trial conditions of

the current study (where the requirement for separate blocks, together with a limited amount of Green Muscle product, meant that application efficiency was less than optimum).

Output 2. Thermal ecology of the host-pathogen interaction determined and models to predict speed of kill under different environmental conditions developed

Details of the modelling studies are presented in Output 5 and the accompanying appendices as these feed into use strategy.

Summaries of the research on the effects of temperature and behavioural fever are presented below with full details in appendices 1-3.

The consequences of behavioural fever for survival of locusts and performance of the fungal pathogen

We demonstrated how variable temperatures, mediated by host thermoregulation and behavioural fever, critically affect the interaction between a host (the desert locust, Schistocerca gregaria) and a pathogen (the fungus Metarhizium anisopliae var. acridum). By means of behavioural thermoregulation, infected locusts can raise their body temperatures to fever levels. The adaptive value of this behaviour was examined using three thermal regimes wherein maximum body temperatures achievable were: (i) below, or (ii) at normally preferred temperatures, or were (iii) unrestricted, allowing heightened fever temperatures. All infected locusts ultimately succumbed to disease, with median survival times of 8, 15 and 21 days post-infection, respectively. Crucially, only those locusts able to fever produced viable offspring. This represents, to our knowledge, the first demonstration of the adaptive value of behavioural fever following infection with a naturally occurring pathogen. By contrast, although normal host thermoregulation moderately reduced pathogen reproduction (by 35%), there was no additional negative effect of fever, resulting in an asymmetry in the fitness consequences of fever for the host and the pathogen. The dependency of the host-pathogen interaction upon external abiotic conditions has implications for how virulence and resistance are treated both theoretically and in the management of pests and diseases. (See appendix 1).

The effects of the fever response on locust phase state and its potential implications for control

Natural enemy attack can cause transgenerational shifts in phenotype such that offspring are less vulnerable to future attack. Desert locusts (Schistocerca gregaria) show density dependent variation in their resistance to pathogens, such that they are less vulnerable to pathogens when in the high-density gregarious phase state (when they would probably be more exposed to pathogens) than when in the solitarious phase state. We therefore hypothesized that infected gregarious parents would maintain this phenotype in their offspring. We infected gregarious desert locust nymphs with the fungal pathogen Metarhizium anisopliae var. acridum, and allowed them to survive to reproduction by means of behavioural fever. The phase state of the locust offspring was assessed by their colouration and behavioural assays. Contrary to our hypothesis, we found an increase in solitarization in the infected population (14.6% solitarious offspring from infected parents, vs. <2% from uninfected counterparts at equivalent density). In a second experiment, we simulated behavioural fever temperatures and obtained a similar result (13.6% solitarious offspring vs. 4.4% from controls), implying that the phenomenon is probably a side-effect of the hosts' fever response. Identification of this novel environmental factor affecting locust phase state could have important implications for the biological control of these major pests. (See appendix 2).

Exploring the costs of fever and mechanisms through which fever affects locust life history

Fever is one mechanism by which animals may defend themselves against pathogens but, as with other defenses, may have consequences for the host. In ectotherms such as the desert locust (*Schistocerca gregaria*), fever temperatures are attained through modified behavioural thermoregulation. We previously demonstrated that the fitness benefits of behavioural fever in *S. gregaria* can be substantial: a fever temperature just 2-4°C higher

than the normal can make the difference between zero and near-normal reproduction when infected with a fungal pathogen. Here we examined whether there are costs associated with these elevated temperatures by holding adult, gregarious S. gregaria at elevated temperatures for one or five hours per day and for ten or twenty days. We found that, while fever temperatures did affect locust life history traits, this was expressed through the ability of locusts to sustain flight and to compete for mates, rather than the primary fitness correlates of survival and fecundity. In addition, there was no relation between time spent at fever temperatures and magnitude of the response. While these effects could result from production of molecular chaperones, for example, indicating a direct cost of fever, they are also consistent with an effect of fever on the locust hormonal system and a shift towards the solitaria phase state. Although this can still be interpreted as a cost of fever, in the field context, a shift towards solitary behaviour could also be viewed as adaptive life history response to limit the impact of disease. These conflicting interpretations highlight the need for careful consideration in identifying response traits and the importance of considering complex defence mechanisms and trade-offs in an appropriate ecological context. (See appendix 3).

Output 3. Fate of pathogen in the environment determined and the impact of secondary cycling on host populations following spray applications evaluated

Investigations into persistence of spores in the spray residue revealed that insects held in a sprayed area from days 1 to 4 after treatment survived for less time (mean 10 ± 2 days by survival analysis) than those held from days 4 to 7 (mean 18 ± 2 days, log-rank comparison of two survival times *P* < 0.00005), suggesting decay of the applied spores on the vegetation. Quantifying the decay pattern further would require data from later than day 7 but the site was then too heavily flooded.

Studies on the survival of *Orthochtha* sp. exposed to sprayed vegetation collected from four strata revealed that one day after application, insects appeared to die more rapidly when exposed to the top stratum, probably due to limited penetration of the spray down the canopy. Subsequent exposures led to reduced mortality in the top two strata but mortality was similar in the bottom of the canopy, suggesting that decay was faster higher in the canopy. These data are summarised in Figure 5 where mean survival times are converted into estimates of infectivity relative to the top stratum one day after application. It is apparent that the top stratum initially received the highest dose of *Metarhizium* but the spores in the top and middle strata decayed over the six days such that the bottom stratum was the more infective by the end of the assays. No decay is apparent in the bottom stratum and washdown from above may even have supplemented inoculum (although the upward trend is not significant).



Figure 5. Risk of infection through time in the three vegetation strata. *Orthochtha* sp. were exposed to vegetation 1 to 7 days after application with *Metarhizium anisopliae* var. *acridum* (IMI 330189). Inverses of mean survival times as estimated by Kaplan-Meier survival analysis give an indication of infectivity. These data are expressed as proportions of the infectivity on the higher vegetation stratum one day after application (see large point). Trends in mean survival times were tested by log-rank comparisons for height at each exposure time (*P* values shown at top of graph; n.s. is P > 0.05) and for exposure time at each height (*P* values adjoining lines).

Monitoring of cadavers in each of the field trials indicated high activity of predators and scavengers with very few cadavers (if any) persisting for sufficient time to allow fungal sporulation and subsequent infection of further locusts. Thus, the potential for secondary cycling to contribute to the overall impact of the biopesticide is considered negligible. This result is consistent with some other studies on fate of the fungal entomopathogen conducted in West Africa (see Thomas *et al.* 1998; Arthurs *et al.* 2003).

Output 4. Environmental impact of the mycoinsecticide relative to chemical alternatives determined

This trial demonstrated that an aerial application of Green Muscle could successfully control red locust hopper populations, while at the same time producing far less environmental impact than a conventional application of fenitrothion UL. With the exception of the grasshopper fauna, which proved susceptible to Green Muscle, no direct mortality or other negative effects on non-target organisms were detected during the 15d post-application observation period. Similarly, non-target beetles and bugs collected in the Green Muscle spray plot and maintained in plastic bottles, showed no signs of mycosis during a 4 week observation period. Unfortunately, no studies on long-term sub-lethal effects were possible during the trial period. However, the results confirmed previous environmental impact studies undertaken in the Sahel, which showed that apart from the susceptible grasshopper fauna, there was minimal impact of Green Muscle on non-target organisms (Peveling, 2001).

In contrast, the toxic standard insecticide, fenitrothion 500g a.i./ha, had a devastating direct effect on virtually all non-target invertebrates inhabiting the middle to upper grass canopy. Almost all invertebrates were completely eliminated from the grass canopy within 24-72h post-application. However, there was less direct toxic effect on some of the geophile ant and beetle fauna, which probably survived by being shielded by the dense grass from the direct impact of the fenitrothion.

Although fenitrothion is known to be an effective broad-spectrum insecticide, with a high direct impact on non-target invertebrates (Peveling, 2001), it is also known to have a short

toxic half-life under tropical conditions. In the 2002 study the more mobile grasshoppers, flies, wasps and dragonflies had started to re-colonise the fenitrothion spray plot within 9 days post-application.

The minimal impact of Green Muscle against non-target organisms in the red locust grasslands, compared with conventional insecticides, supports the findings of a number of previous impact studies (Peveling, 2001). The FAO pesticide referee group has already recommended *Metarhizium* myco-insecticides for acridid control in environmentally sensitive areas (FAO, 2000). The operational adoption of the Green Muscle® technology by IRLO-CSA is therefore advocated as a more environmentally benign alternative to fenitrothion spraying, especially during the wet season when biodiversity is greatest.

The negative impact of Green Muscle® on non-target grasshoppers is, however, a cause for concern. Grasshoppers are a vital component of the ecology of tropical grassland ecosystems and play important roles as herbivores and in the re-cycling of nutrients and energy flow systems (Gandar, 1982; Samways, 1997). The decimation of grasshopper populations from large areas of grassland following broad-acre application of most conventional insecticides, as well as Green Muscle®, is therefore very detrimental to the functioning of the grassland ecosystem. However, the targeted application of Green Muscle® to high-density red locust populations, allowing refuge areas for grasshopper populations between the treated areas, would likely have no long-term impact on grasshopper populations or the ecology of the grasslands. (See appendix 4 for full details of this study).

Output 5. Optimum use strategies for the mycoinsecticide developed and built into an overall strategy for sustainable control of red locust

The detailed results from the body temperature and pathogen performance modelling studies are presented in appendices 5-7. An illustration of the GIS outputs indicating spatial variation in expected pathogen performance for red locust nymphs and adults is given in Figure 6a,b below. The key findings with respect to red locust were that the environmental conditions characteristic of the wet season, when control operations would be targeted at nymphs, are highly suitable for the biopesticide. Even though red locusts can actively thermoregulate the dense grass vegetation, often over standing water, combined with long periods of cloud cover, create a very stable thermal environment. As such, we predict that the biopesticide should cause high levels of mortality (i.e. 90% mortality of infected individuals) within about 2 weeks of application. This rapid mortality is highly stable across space and time during the wet season period (a result which contrasts considerably with certain other species in different environments - see appendices). Thus we conclude that the biopesticide should be highly effective in controlling red locust nymphs; a particularly valuable result given concerns over using chemical pesticides in the environmentally sensitive wetland habitats. The main constraint to implementing the biopesticide is actually in locating populations in remote and inaccessible areas (identifying a need for good surveying and monitoring infrastructures) and possibly in targeting populations effectively in the dense grass vegetation (identifying a need for good application techniques and equipment to provide effective spray coverage).

The hotter and drier conditions that coincide with the adult populations reduce the speed of kill of the biopesticide and increase variability over time and space. Nonetheless, we still predict high levels of mortality (again time until 90% mortality of infected individuals) with slowest speed of kill in the range of 25-35 days. Whilst this is much slower than during the wet season, the red locust is rather unusual amongst pest locust species in that it has a very long sexual maturation period across the dry season. This creates a large window for control before adults reproduce and undergo long-range dispersal. Thus, whilst a period of 25-35 days to achieve 90% mortality would likely be too slow against adults of species such as

Desert or Moroccan locust, it is considered perfectly acceptable for control of red locust adults as long as applications take place before the very end of the season (i.e. any time from June to late August should be appropriate). Again, the key constraint, as evidenced by the trial results reported above, is effective application such that spores contact a high proportion of locusts.

Overall, we consider that the thermal conditions and life history characteristics of red locust make it an ideal target for using the biopesticide with very little modification to current control operations. Both nymphs and adults can be targeted with minimal environmental impact.



Figure 6. GIS map of predicted rates of mortality of red locusts following application of the *Metarhizium*-based biopesticide. The different colours indicate number of days to achieve 90% mortality in the treated population (LT90) following application. (a) Indicates expected performance against nymphs assuming treatment in January 2000; (b) indicates expected performance against adults assuming treatment in August 2000.

Output 6. Draft registration dossiers compiled in a number of locust affected countries

Based on the positive field trial results from the project together with the existing registration dossier from South Africa, BCP (the licensed commercial producer) has prepared a dossier to register Green Muscle for red locust control. So far, registration has been achieved for Namibia and Zambia. The Plant Health Services (PHS) officials in Tanzania are currently reviewing the dossier and initial feedback indicates registration is likely to be granted. BCP have also translated the dossier into Portuguese and have lodged the documentation with the appropriate authorities in Mozambique. Thus registration is well underway throughout Southern Africa representing a substantial success for the project.

Output 7. Costs and benefits of developing a biologically-based locust control strategy determined

The efficacy and environmental benefits of the mycoinsecticide have been determined. The results indicate that the mycoinsecticide can provide effective control and has substantially less environmental impact than chemical insecticide alternatives. A remaining issue is cost of the product.

Green Muscle production costs are between US\$10 and 20 per ha. There is still scope for reducing these costs, particularly through economies of scale (see 'Contribution of Outputs' section below for more detailed breakdown). However, this price does not allow for distribution costs, or spraying costs. In comparison, some of the organophosphate or pyrethroid insecticides used against locusts and grasshoppers cost as little as US\$5-12 per ha. Whilst there is some overlap between these basic unit costs, Green Muscle will generally be more expensive. However, this simple assessment based on unit costs fails to take account of differences in product specification. In particular, the environmental, animal and human health externalities of the biopesticide are much less than for the chemical insecticide alternatives. If these were factored in, then it is likely that the products would be more equitable. In reality this might mean that environmentally benign biopesticides will have to be subsidised by the public sector, or chemical pesticides will need to be taxed for their externalities, if a product like Green Muscle is to compete with chemical pesticides (Douthwaite *et al.* 2001).

Output 8. Key stakeholders informed as to the principles and practice of mycoinsecticide use for locust control

Many of the most important stakeholders have gained experience of the mycoinsecticide through active participation in the field operations in Zambia and Tanzania. These include representatives from IRLCOCSA and the Tanzanian and Zambian Plant Health Services. Moreover, the large-scale field operations were conducted in collaboration with FAO who represent the most significant stakeholder for locust and grasshopper control globally. In addition, BCP have arranged for two training workshops to engage a wider range of stakeholders in the region (approximately 70 trainees in total).

Contribution of Outputs to developmental impact

The main objectives of this project were to test the *Metarhizium*-based biopesticide, Green Muscle, against red locust and demonstrate its potential for environmentally sustainable locust control. The results of the field trials and supporting research were generally considered good (and where control was poor, explanations could be found which were not, on the whole, the fault of the product *per se*). Registration has been achieved, or is underway, in the target countries. In addition, the IRLCOCSA scientists, who participated in the project and who represent a key stakeholder in the region, have indicated that they are sufficiently convinced with the results that they see no reason why Green Muscle shouldn't be the product of choice for standard control operations in the future. More broadly, FAO also continue to show their support for the product with, for example, 20Kg of spores purchased from BCP for trials against desert locust in Niger this winter. FAO are also discussing

possibilities for using *Metarhizium* in Iran and Afghanistan. Thus, there is considerable interest in the product and this is now being translated into actually use through the likes of FAO, who are the key implementing agency for locust control worldwide. All these outputs together represent an extremely significant developmental impact for this project.

However, whilst we believe the project was a substantial success, challenges still remain to the widespread adoption and implementation of Green Muscle in Africa. Experience in South Africa, for example, reveals that demonstrating efficacy, achieving registration and establishing production capacity are not sufficient for technology acceptance. From the current project we have encouraging support from the likes of FAO and the regional locust control organisation, together with an effective and motivated SME (the producer) who wish to see the product a commercial success. Nonetheless, given the complexities of locust and grasshopper control [i.e. donor-funded programmes with generally preventive control actions taken in areas/countries far away from the ultimate beneficiaries] and the fact that the benefits of the technology are linked to 'non-market' environmental values, we believe that there is still a need for further support to ensure adoption of this promising and innovative technology.

Further funding could support a number of activities aimed at championing the new technology including:

- Further development of the GIS-based efficacy model to produce a decision support tool available to donors, policy makers and end users globally - A key constraint with any new technology is uncertainty and limited track record compared with established technologies (these are major factors contributing to 'path dependency'). Understanding where and when it is appropriate to use the biopesticide and predicting how quickly it will work once applied could be a considerable aid to effective deployment, particularly in taking the product to new targets/areas. Outputs from the model have already informed operators in the current programme and also locust control officers in Spain and Australia.
- 2. Further demonstration and extension trials As indicated above, the much more limited body of research data and practical experience with biological control adds to the degree of uncertainty surrounding the efficacy and economic viability of the biopesticide and acts as a barrier to adoption. Conducting further evaluation and demonstration trials with the product against different targets and in different regions would help considerably to define the confidence limits on product performance and reduce risk aversion. Even if the product turns out to be less reliable and cause less direct mortality of locust populations compared with chemicals, it is far from clear whether this is actually a problem in terms of preventing locust damage (i.e. for the majority of locusts and grasshoppers we have very little understanding of how effective control needs to be to prevent damage or what the value of current control measures actually is). Possibilities for an SME, such as BCP, to undertake extensive trials (which include product, trained personnel and operational expenses) are very limited without some kind of assistance.
- 3. Creating a stable demand The price of the product remains an issue, as does supply and demand. These problems could be resolved, in part, through improvements in production efficiency and economies of scale. To give an indication of this, using a full costing which takes into account factory overheads for the current facility, depreciation on equipment, a contribution to administration charges, licence fee, royalties, Rest of Africa Sales Manager's salary and travel costs, registration trial fees and marketing expenses (brochures, training symposia), BCP makes approximately 29% net profit at the current wholesale price of R3,811.50 per kg or R38,115 per 25litre drum. With this scenario the production capacity would be 1500 kgs per annum sufficient to treat 30,000 hectares at 25g per hectare at a cost of R95 (approximately \$13) per hectare.

However, it would be relatively straightforward to scale up to an output of 2,300kg per annum – sufficient to treat 92,000 hectares. With the same percentage net profit, BCP could reduce the wholesale price to R2,600 per kg or approximately \$9 per hectare at an

application rate of 25g per hectare. For areas greater than these, further economies of scale would be applicable.

If there were some way of guaranteeing the annual purchase of volumes of this order, BCP would be prepared to commit to the lower price. Given this might be a tall order due to the unpredictable nature of outbreaks, a refund, payable annually in arrears and based on the quantities purchased for the year could be negotiated. As a further suggestion, a letter of intent stating that should locust control be required in Africa, a specified percentage of the area treated would be treated with BCP's Green Muscle, would allow the investment in the increased capacity

At present then, there is something of a 'chicken and egg' situation where the likes of FAO say they might be interested in purchasing large quantities of spores but are uncertain whether BCP can do it, whilst BCP say they could produce large quantities of spores if FAO would guarantee they would buy it. This problem derives in part from the public sector nature of biopesticide development (Langewald & Cherry 2000) and is confounded by the nature of the products themselves whose main advantage is savings in externalities (i.e. environmental values); savings that by definition the market cannot capture without policy changes. One additional requirement, therefore, is continued support for 'product championing' activities to lobby for policy changes and so change the environment in favour of the technology (Douthwaite *et al.* 2001). Ultimately, widespread adoption rests with donor commitment to purchase product and pay for protection of the environment as well as controlling locusts.

Sources of funding for such activities are unclear. However, given their investment to date we would hope DFID would be interested in some continued role in, what is arguably, one of the most significant developments in locust control in the last 20 years.

The main dissemination outputs of the project are summarised below:

Scientific publications

ELLIOT, S.L., BLANFORD, S. and THOMAS, M.B. (2002). Host-pathogen interactions in a varying environment: temperature, behavioural fever and fitness. *Proceedings of the Royal Society of London* B **269**, 1599-1607.

GARDNER, S.N. and THOMAS, M.B. (2002). Costs and benefits of fighting infection in locusts. *Evolutionary Ecology Research* **4**, 109-131.

ELLIOT, S.L., BLANFORD, S., HORTON, C. and THOMAS, M.B. (2003). Fever and phenotype: Transgenerational effect of disease on desert locust phase state. *Ecology Letters* **6**, 830-836.

THOMAS, M.B. and BLANFORD, S. (2003). Thermal biology in insect-pathogen interactions. *Trends in Ecology and Evolution* **18**, 344-350.

ELLIOT, S.L., BLANFORD, S., HORTON, C. and THOMAS, M.B. (submitted). Fever impacts on host life history traits but is this a cost? *Proceedings of the National Academy of Sciences.*

ELLIOT, S.L., BLANFORD, S., THOMAS, M.B., KLASS, J.I., BAHANA, J., KATHERU, J.N., PRICE, R. and KOOYMAN, C. (in prep). Potential of the biopesticide *Metarhizium anisopliae* var. *acridum* for control of the red locust, *Nomadacris septemfasciata* in Africa.

Reports

ELLIOT, S.L. (2001). Report on first field trial of *Metarhizium anisopliae var. acridum* against the red locust, *Nomadacris septemfasciata*, in Central and Southern Africa. (Internal report).

THOMAS, M.B. (2001). Summary and action points from partners meeting, CABI Bioscience, Ascot, 3-4 September 2001. (Internal Report).

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (2002). Expert Consultation and Risk Assessment on the Importation and Large-Scale Use of Mycopesticides against Locusts. Rome, 2-7 December 2001. 35pp.

ELLIOT, S.L. (2002). Report on second field trial of Metarhizium anisopliae var. acridum against the red locust, Nomadacris septemfasciata, in Tanzania. 12pp. NERC Centre for Population Biology (Internal report).

THOMAS, M.B. (2002). Report on "Planning meeting for joint locust control operations with a biopesticide in Tanzania", FAO, Rome, October 4, 2002. 7pp. NERC Centre for Population Biology (Internal report).

KOOYMAN, K., BAHANA, J., GODONOU, I., KATHERU, J., MAGOMA, R. and SEYOUM, E. (2003). Operational trial of Green Muscle[®] against red locust nymphs in the Wembere plains, Tanzania. Scientific report for the combined projects DFID R7818 and TCP/URT/201/NOR, 7pp. (Internal Report).

KOOYMAN, K., BAHANA, J., KATHERU, J., MUTAHIWA, S. and SPURGIN, P. (2003). Operational trial of Green Muscle® against red locust adults in the Iku, Tanzania. Scientific report for the combined projects DFID R7818 and TCP/URT/2802, 10pp. (Internal Report). PRICE, R.E. and MITCHELL, J.D. (2003). The environmental impact of biological and chemical intervention for locust control against non-target arthropods in a red locust recession area in Tanzania. Scientific report for the project DFID R7818, 26pp. (Internal Report, ARC-PPRI).

Oral presentations

ELLIOT, S.L., BLANFORD, S. and THOMAS, M.B. (2001). Behavioural fever in an ectotherm increases host fitness and reduces pathogen fitness. British Ecological Society Annual Winter Meeting, 18 – 20 December 2001, University of Warwick. (Scientific presentation / abstract).

THOMAS, M.B., LYNCH, L. and KLASS, J.I. (2001). Use of models in evaluating impact of insect pathogens against target and non-target species. Annual meeting of the Royal Entomological Society, 10-12 September, Aberdeen, UK, 2001. (Scientific presentation / abstract).

THOMAS, M.B. (2001). Use of *Metarhizium anisopliae* var *acridum* for biocontrol of locusts and grasshoppers: understanding the ecological benefits and constraints. FAO Desert Locust Control Committee 36th Session, September 24-28, FAO Rome, 2001. (Oral presentation).

THOMAS, M.B. (2001). Use of *Metarhizium anisopliae* var *acridum* for biocontrol of locusts and grasshoppers : safety, registration and market issues. FAO Expert Consultation and Risk Assessment on the Importation and Large-Scale Use of Mycopesticides against Locusts. December 3-7, FAO Rome, 2001. (Oral presentation).

Training workshops

BANDA, B., MAGOMA, R. (2004). Use of Green Muscle for Locust and Grasshopper Control. Dodoma, Tanzania, 24-26 February 2004. [Two-Day Training Workshop for 35 Field Officers].

Cited References

Anon (1996). Annual Report of the Director for 1996. International Red Locust Control Organisation for Central and Southern Africa. Mimeograph. IRLCO-CSA, Ndola, Zambia. 44pp.

Anon (1997). Annual Report of the Director for 1997. International Red Locust Control Organisation for Central and Southern Africa. Mimeograph. IRLCO-CSA, Ndola, Zambia. 36pp.

Arthurs, S., M.B. Thomas & J. Langewald (2003) Field observations of the effects of fenitrothion and *Metarhizium anisopliae* var. *acridum* on non-target ground dwelling arthropods in the Sahel. *Biological Control.* **26**:333-340.

Bateman, R. P. (1997). The development of a mycoinsecticide for the control of locusts and grasshoppers. *Outlook on Agriculture* **26**: 13-18.

Douthwaite, B., Langewald, J. & Harris, J. (2001). *IMPACT: Development and Commercialization of Green Muscle Biopesticide*. *IITA publication 1, 22 pp*

Ellenbroek, G. A. (1987). *Ecology and productivity of an African wetland system. The Kafue Flats, Zambia*. Dr W Junk Publishers, Lancaster.

FAO (2000) Evaluations of field data on the efficacy and selectivity of insecticides on locusts and grasshoppers. *Report to FAO by the Pesticide Referee Group*, 8th meeting, Rome, 11-14 October 1999.

Gandar, M.V. (1982) The dynamics and tropic ecology of grasshoppers (Acridoidea) in a South African savanna. *Oecologia* **54**: 370-378.

Langewald, J. and A. Cherry. 2000. Prospects for microbial pest control in West Africa. Biocontrol News and Information 21, 51N–56N

Lomer, C.J., Prior, C. and Kooyman, C. (1997) Development of *Metarhizium* spp. for the control of grasshoppers and locusts. *Memoirs of the Entomological Society of Canada* **171**: 265-286.

Lomer, C.J., Bateman, R.P., Johnson, D.L., Langewald, J., & Thomas, M. (2001) Biological control of locusts and grasshoppers. *Annual Review of Entomology*, **46**, 667-702.

Morant, V. (1947). Migrations and breeding of the Red Locust (*Nomadacris septemfasciata* Serville) in Africa, 1927-1945. Anti-Locust memoir, London **2**, 60 pp.

Peveling, R. (2001) Environmental conservation and locust control – possible conflicts and solutions. *Journal of Orthoptera Research*, **10**: 171-187.

Price, R.E., Müller, E.J., Brown, H.D., D'Uamba, P. and Jones, A.A. (1997) First use of a *Metarhizium flavoviride* myco-insecticide for the control of the red locust in a recognised outbreak area. Research Report for the Departamento de Sanidade Vegetal (DSV) and DANIDA, Mozambique. 17 pp.

Samways, M.J. (1997) Conservation Biology of Orthoptera. In: Gangwere, S.K., Muralirangan, M.C. and Muralirangan, M. (eds) The Bionomics of Grasshoppers, Katydids and their kin. CAB International, Wallingford, UK.

Thomas, M.B., Blanford, S., Gbongboui, C. & Lomer, C.J. (1988). Experimental studies to evaluate spray applications of a mycoinsecticide against the rice grasshopper, *Hieroglyphus daganensis*, in northern Benin. *Entomologia Experimentalis et Applicata* **87**, 93-102.

Thomas, M.B. (2000) Development of a Myco-insecticide for Biological Control of Locusts in Southern Africa. pp173-182. In Cheke, R.A., Rosenberg, L.J and M.E. Kieser (eds.) Workshop on Research Priorities for Migrant Pests of Agriculture in Southern Africa, PPRI, Pretoria, South Africa, 24-26 March 1999. Natural Resources Institute, Chatham. pp220+

Thomas, M.B., J. Klass & S. Blanford (2000) The Year of the Locust. *Pesticide Outlook*, pp.192-195.

Biometricians Signature

The projects named biometrician must sign off the Final Technical Report before it is submitted to CPP. This can either be done by the projects named biometrician signing in the space provided below, or by a letter or email from the named biometrician accompanying the Final Technical Report submitted to CPP. (Please note that NR International reserves the right to retain the final quarter's payment pending NR International's receipt and approval of the Final Technical Report, duly signed by the project's biometrician)

I confirm that the biometric issues have been adequately addressed in the Final Technical Report:

Signature: Name (typed): Position: Date:

Dr Sam Elliot Research Ecologist 23 Feb 2004 Dr Matt Thomas PI 23 Feb 2004