Guideline

Operational-scale field trial with Green Muscle®
(*Metarhizium anisopliae* var. *acridum*)

Version 2 – February 18, 2005

1. INTRODUCTION

The Pesticide Referee Group (PRG) in its 9th Meeting in 2004 lists the entomopathogen *Metarhizium anisopliae* var, *acridum* (IMI 330189) as an insecticide for which a verified dose rates has been established for the Desert Locust. The only presently available commercial formulation of this particular strain is Green Muscle®. The recommended field application rate is 50 grams of dry conidia (spores) per ha, which corresponds to about 2.5 x 10^{12} conidia per hectare. This recommendation is primarily based on small and medium-scale field trials on the Desert Locust and on information from large scale use on other locust species.

However, large-scale assessments of this microbial insecticide, during which the application, its efficacy and environmental conditions are well monitored, are lacking. Such information is necessary to confirm the efficacy of the product on a large scale and in varying environmental conditions. The latter is particularly important since the efficacy of the microbial insecticide is more dependent on ambient conditions than is the case for most chemical insecticides.

The objective of this guideline is to give advice on the design of an operational-scale field efficacy trial with *Metarhizium anisopliae* var, *acridum* on the Desert Locust (*Schistocerca gregaria*), formulated as Green Muscle®. Furthermore, logistical needs, a budget and some advice on planning are provided.

The guideline does not include environmental impact assessment of the microbial insecticide.

2. PRINCIPLES OF THE TRIAL

The trial concerns one or more aerial applications of Green Muscle® against hopper bands of the Desert Locust. The size of the trial plots needs to be representative of operational blanket sprays against hopper band targets.

However, because the efficacy of the microbial insecticide is partially dependent on environmental conditions, it is also recommended that as many independent replicate treatments are carried out as logistically feasible. A balance will therefore need to be found between plot size and number replicates.

Only the PRG recommended dose rate will be tested.

Particular attention needs to be given to the collection of meteorological data and information on hopper behaviour during the trial.

In principle, the trial will be an integral part of the ongoing Desert Locust control campaign in the country, and be coordinated by the national locust control unit or plant protection department.

3. TRIAL DESIGN

Target type

The spray targets are blocks of land containing several hopper bands of the Desert Locust[1]. The actual target for the spray droplets are both the individual locusts as well as the vegetation on which they feed or move around in. This is because secondary pickup is an important mode of exposure of the insect to the *Metarhizium* spores.

Target stage

Hopper stages should ideally range from 2nd to 4th instar. First instar hoppers are too susceptible, while 5th instar hoppers may fledge before they die from the microbial insecticide.

Trial area

Areas with sparse and clumpy vegetation are suitable. The vegetation should neither be too dense (where hopper bands are difficult to trace and the microbial insecticide is too much diluted) nor too light (where hopper bands may move too fast out of the spray block and too much pesticide is lost on the soil). Area and vegetation type should in principle be representative of Desert Locust habitat conditions, but a relatively uniform habitat tends to make evaluation easier.

Trial period

The effectiveness of *Metarhizium* is particularly dependent on ambient temperatures, which both influence the growth rate of the pathogen in the insect, as well as development speed and behaviour (especially active thermoregulation) of the hoppers.

Presently available evidence suggests that the performance of Green Muscle® is likely to be insufficient (i.e. >25 days to achieve 90% mortality) under conditions with hot days (> 38°C) and cool nights (< 20°C). Trials should not be carried out under such conditions.

Type of treatment

For operational-scale trials, aerial treatments are recommended.

Plot size

The trial plot size should be representative of operational conditions. Furthermore, it will be dependent on the type of treatment (aerial or vehicle-mounted) and on the speed and direction of displacement of the hopper bands.

The minimum plot size for aerial treatments is about 100 ha, since on smaller plots a uniform cumulative spray deposit cannot be achieved.

The minimum plots size should also be large enough to ensure that hoppers bands do not march out of the sprayed plot before the insects have acquired sufficient spores of the pathogen to cause the required mortality within a reasonable time (an suggested figure would be a mortality of at least 50% of the population within 2 weeks time).

Ideally, hopper should remain within the sprayed plot until they die. However, because of the relatively slow growth of *Metarhizium* in the insect body, a lethal dose will have been acquired some time before the insect dies. The hopper bands may therefore be allowed to move out of a treated plot, as long as a lethal dose of spores has been acquired. This will often have occurred in 2-5 days. Linear displacement of mid instar hopper bands is very variable, and is likely to be several hundreds of

---

[1] Adult locusts are also susceptible to *Metarhizium*, but trials with adults are complicated to assess, and are not recommended.

meters per day[2], which means that in the time needed to acquire a lethal dose, the band may have moved up to 2 km, or sometimes even more. As a result, the minimum needed plot size is likely to be at least 900 ha (i.e. 3 x 3 km block).

The actual sizes of blocks sprayed during a hopper control campaign differ widely. Based on recent experiences operational spray blocks for aerial applications against hopper bands ranged from 400 to 2500 ha[3].

Based on the above observations, combining application requirements, biological information and the reality from the field, appropriate plot sizes would range from about 900 to 2500 ha.

And finally, the plot size will be determined by the spray aircraft type that is available and the pesticide load it is able to carry. In principle, the trial plot should be sprayed on one day, and depending on the distance between spray plot and air strip, this may mean that only one sortie is possible.

Given the likely available amount of Green Muscle (400 L), it is suggested that a plot size of about 1200 – 1300 ha is used for this trial, if it is possible to spray this in one day. This will allow 3 replicates to be sprayed. Otherwise, plot size may be reduced to a minimum of 900 ha. Smaller plots are not recommended for this large scale trial.

Plot number (replicates)

Since the objective of the operational-scale trial is to confirm the field dose rate rather than to set a new rate, there is less of need to treat several replicates with similar hopper populations and similar environmental conditions. However, different plots need to be treated, preferably under different environmental and meteorological conditions that can be encountered in Desert Locust control, to assess the robustness of the recommended dose rate.

Therefore, it is strongly recommended that at least 2 and preferably 3 plots, are treated independently one from the other. Treatments need to be independently carried out to ensure that potential errors made in the execution of one treatment are not "carried over" to the next one. For all practical purposes for this type of trial, treatments can be considered independent if:

(i.) the tank formulations used in each treatment are prepared individually and are not be part of a single batch (the formulation concentrate may be from one bath, though),

(ii.) plots are treated during different aircraft sorties, and

(iii.) sprayer/atomiser settings are (re-)calibrated before each treatment[4].

Unsprayed control plots

One unsprayed control plot should be included in the trial. For slow acting microbial insecticides, like *Metarhizium*, an untreated control plot gives an indication, although not a completely certain one, of what would have happened to the locust population within the sprayed plots had they not been sprayed. Untreated control plots are particularly useful to check on major changes in background population, such as mass

---

2 The distances that Desert Locust hopper band can march vary enormously, depending of hopper stage, ambient temperature, the size of the band, the density, structure and composition of the vegetation, among others. Wilps (2004) recently evaluated the literature on this subject and cites daily displacement of 10 to 1600 m, for mid-instar hopper bands.

3 Based on an assessment of aerial treatments against hopper bands done in Mauritania in 2004, 10-percentile and 90-percentile spray block sizes were about 400 and 2500 ha respectively, while the median value was about 1100 ha. Note that spray block sizes were estimates based on volumes of insecticides applied, and real block sizes are likely to have been somewhat smaller.

4 Ideally, hopper populations in each plot should also be genetically/ecologically distinct, but this can with the highly mobile Desert Locust hardly ever be ensured.

exodus after fledging, or mass hatching if several events of egg laying occurred in the same area.

Since the function of the control plot is primarily to assess general changes in untreated hopper populations, it is more important that the age of the hoppers is similar between treated and control plots, rather then that the vegetation is homogeneous among plots.

The national locust control organization may want to ensure that no locusts will fledge from the control plot. But since there is no real need to monitor the control plot anymore when fledging starts, an agreement can be made that the control plot will be sprayed with a convention contact insecticide at that moment. No budgetary reservation is made for this in the trial protocol, as it is assumed that spraying of such a plot would have occurred anyway using the available national capacity. Clear arrangements need to be made with the locust control unit about this, however.

Plot layout

Trial plots should be well separated to prevent spray drift from one to another. Furthermore, hopper bands should not be able to move from one trial (or control) plot into another. Distances between plots should therefore be at least 3 km. Untreated control plots are preferably positioned upwind from the treated plot.

Test Product

Green Muscle® OF, an oil miscible flowable concentrate containing 500 g of spores per litre (equivalent to 2.5×10^{13} spores/L) is the test product. It will be diluted with diesel oil, to a tank concentration of 2.5×10^{12} spores/L (i.e. a dilution ratio for Green Muscle:diesel of 1:9)

Area dosage

The tank mixture mentioned above will be applied at 1 L/ha.

Product quality assessment

A germination test must be carried out on the batch of formulation concentrate 24 – 48 hours before the first treatment[5]. If certain treatments are done more than 1 week after this first test, a second germination test needs to be done, especially if the product has been stored under hot conditions in the field.

Germination tests are best done in a laboratory (need to sterilize agar plates and use a microscope). Alternatively, sterilized and properly packed plates could be taken to the field, as well as a microscope.

Reference product

No reference product is required[6].

Aircraft

Due to the plot size required (preferably 1600 ha, but a minimum of 900 ha) the spray aircraft should have sufficient hopper capacity to allow the plot to be sprayed in one day. Assuming some ferry time between airstrip and trial plot, often only one sortie will be feasible.

Spray aircraft with a hopper volume that is sufficiently large to take at least 900 L of insecticide are for example the Turbo Thrush 510 or the Air Tractor AT-401 or AT-402.

---

5 The germination test is described in Lubilosa (undated-a).

6 A reference product is often included in the trial to detect if there are any general problems with the trial, such as a defective atomiser or unfavourable meteorological conditions. Its mode of action should ideally be similar to the test product. However, no such product exists for *Metarhizium* in locust control. The requirement for independency of treatments should reduce the risk of a general problem going undetected.

The Turbo Thrush 660 or the Air Tractor AT-502 or AT-802, for example, will take enough insecticide to treat a 1600 ha plot. Small spray planes, such as the Cessna Ag Truck 188 or the Piper PA-25 Pawnee are quite certainly not appropriate for this large scale trial.

Sprayer

Rotary atomisers give the narrowest drop spectra and should always be used in trial work.

The pesticide pump system should preferably be electrical (or otherwise independent) rather than propeller-driven, to allow calibration on the ground. However, since this may often not be available, the aircraft should always be equipped with an onboard automatic flow control linked to the track guidance system.

Before treatment, the aircraft pesticide hopper, tubing system and atomisers should be rinsed well with diesel or kerosene, to wash out as much of leftover chemical insecticides as possible. This is best done by having the aircraft fly and spray out (at least) 200 L of diesel or kerosene 3 times.

A sample of the final rinsate should be taken and properly stored. This can be sent for residue analysis to exclude residues as a confounding factor, e.g. in case a sudden drop in populations is observed shortly after spraying the pathogen.

Aircraft navigation equipment

The aircraft should be equipped with GPS-based agricultural navigation equipment, permitting spray track guidance for the pilot, and an output showing exact location of the treatment, delimitation of spray blocks and plotting of spray tracks.

An automatic flow control unit should also be fitted. This should be linked to the track guidance system to give an output of the total volume of pesticide been applied (e.g. systems such as Satloc® or Ag-Nav® will give a detailed treatment map showing the volume of liquid applied per hectare.

4. TRIAL PROCEDURES

Calibration of equipment

Before the trials start, the spray equipment should be calibrated to apply the required area dosage. The spray equipment should be recalibrated, or calibration checked, before each individual treatment. Note that atomiser flow rates may vary from day to day, or even during the day, but this will be controlled by the onboard flow control system.

If the aircraft is equipped with well-know rotary atomisers, there is no need to carry out a swath width estimate before the trial. The blade angle of the atomisers should be set to achieve a VMD of 75 µm based on the operating handbook.

Laying out of the plot

Spraying must be carried out as close to crosswind as possible. A rough plot layout can be delimitated the day(s) before treatment, based on prevailing wind direction in the area. This will allow pre-spray sampling of the hopper bands in the central area of the plot, with a reasonable certainty that these populations will indeed be sprayed. The actual spray plot will be delimitated on the day of the treatment, by ground crews marking the four plot corners with GPS. These are the co-ordinates passed to the pilot for use in the aircraft track guidance system.

Application conditions

Spraying should start early in the morning and finish before the onset of heat convective turbulence, characterised by the wind beginning to vary considerably in strength and direction. The time that this occurs will depend on factors such as cloud cover and temperature, so no absolute time can be given. Further spraying can be carried out in the hour or so before sunset. It is by far the best to spray the entire plot on one day.

Wind speed should be greater than 2 m/s, to ensure that the spray is carried over a reasonable swath. The stronger the wind, the better, up to a wind speed of around 5 m/s. Strong wind will carry spray droplets horizontally, increasing their likelihood of impaction on locust and vegetation (the intended targets) and reducing wastage on the bare ground.

Wind speed and direction (measured at 2 m above ground level), temperature, relative humidity, estimated cloud cover (in octas), possible (temporary) onset of convection and rainfall must all be measured at the start, during (at about half-hour intervals) and at the end of the application.

Spray technique

Applications should be made on tracks at right angles to the wind. To obtain a reasonably even deposit, a track spacing of 100 m should be used and a flying height of 10 m. This corresponds with operational aerial spray practice against the Desert Locust.

Area dosage measurement

The exact volume of pesticide actually applied per unit area of plot will never be precisely what is intended, so every effort should be made to accurately determine it. The use of a spray aircraft equipped with a GPS-based agricultural navigation system, coupled to an onboard (computerised) flow meter, will allow easy calculation of the area dosage. GPS data for the application should be downloaded to a computer for calculation of the actual spray block. The flow meter should provide total volume of pesticide applied. If the latter is not available, the volume of pesticide loaded before and left over after treatment should be measured, taking into account the "dead volume" of the sprayer plumbing system.

Droplet deposition assessment

An assessment of droplet deposition on vegetation or on droplet samplers after treatment can give a useful indication of application quality (though it is indicative only). However, diesel and kerosene do not stain on oil-sensitive papers, and magnesium oxide ribbons or slides are easily damaged, so their use is not practical.

Fluorescent dyes, can be used instead, such UVITEX OB. It is mixed in the insecticide at a concentration of 1 g per 10 L of formulation. The additional advantage of using a fluorescent dye is that it will also give an indication of deposit on locusts.

Two lines droplet collection cards can be set out perpendicular to the flight direction before treatment on each plot. Cards are positioned vertically on a stick at the height of the grassy vegetation and facing the wind. Sticks can be placed at 50 m intervals, and the length of the sampling line can be about 1000 m in the centre of the plot.

5. ASSESSMENT OF MORTALITY & ENVIRONMENTAL CONDITIONS

Methods

Metarhizium is a slow acting agent, with 90% mortality typically occurring between 7 and 20 days after treatment. This means that hopper bands can move considerable distances before the last hoppers die. Both emigrations of treated hopper bands out of the plot, and immigration of untreated bands into the plot, may perturb the assessment. Three assessment methods can be used to assess mortality under such circumstances:

1. Monitoring of individual hopper bands
2. Presence / absence sampling along transects
3. Caging

Each of the three methods has advantages and inconveniences, and none is likely on its own to provide the answers needed to assess efficacy in a sufficient manner. If possible, all three methods should be applied.

Monitoring of individual hopper bands

Individual hopper bands can be monitored to assess the impact of the microbial insecticide on the insects. This method is relatively precise but also very labour intensive. It is particularly useful if the spray plot is relatively small when compared to hopper band movement, and it is likely that sprayed hopper bands may move out of the plot. Monitoring individual bands will then ensure that such bands are not lost for the efficacy evaluation.

Because individual hopper bands may be very difficult to find again if not continuously observed (especially in denser vegetation or in dense band infestations), a scouting system is often used. A number of scouts are recruited to physically follow one (or sometimes two) hopper band(s) each during the entire day, till the band stops to roost. The band location is then marked (both with a flag and with GPS) and the scout returns to the spot to continue his/her work the following morning, before the band starts to march again. Shepherds or other local people with good knowledge of the surroundings have been used for this task. If we assume that at least 5 hopper bands need to be followed in each plot, and three prayed plots plus one control plot may need to be monitored for one trial, at least 20 scouts are needed for such a task.

An assessment team will then visit each hopper band several times during the trial period and estimate hopper populations. Insects can also be sampled for caging (see below). Precise estimates of hopper population sizes in bands are notoriously difficult to obtain. During each visit the following information should be collected: size estimate of the hopper band (m^2), hopper density estimate (number/m^2), hopper stage(s), band location (GPS reading), type of hopper activity (marching, roosting), abnormalities in behaviour, development or colour of the hoppers.

Langewald et al. (1997) describe a more precise method, based on digital photography, but it is quite labour intensive and may not be feasible for operational-scale dose confirmation trials.

Presence/absence sampling along transects

A method to determine the efficacy of slow acting pesticides in large plots with a large number of hopper bands is to compare the "percentage band infestation" before and at intervals after spraying. This is done by driving parallel transects through the plot and noting at regular intervals whether one is in a band or not.

The proportion of points in a band is a valid measure of the proportion of the area covered by bands. The change in percentage band infestation can then be used as a measure of efficacy, as long as there is only limited immigration or emigration of bands from the plot.

Density estimates can be improved by assigning density categories to each point and calculating the percentage of points in each density category before and at intervals after spraying (see FAO, 1991, for indicative categories).

Caging

Collecting samples of hoppers in the field after treatment and caging them can provide useful supplementary information to the field assessments. However, various factors may complicate interpretation of such data, such as cross-contamination of the insects during capture or in the cages, and increased mortality due to stress.

To minimise cross-contamination, the first sample should be taken on day 3 after treatment. This also maximises secondary spore pick-up. In most Desert Locust habitats, secondary pick-up becomes negligible after day 3. Stress mortality can be estimated from the control samples.

Insects can be collected from each band that is followed by a scout, several times after treatment, and caged on unsprayed vegetation to assess mortality. Dead insects are subsequently incubated in Petri dishes containing wet filter paper to assess sporulation. Unsprayed hoppers are also caged on unsprayed vegetation, to quantify control mortality.

Secondary pickup of spores can be assessed by caging unsprayed hoppers onto sprayed vegetation, using field cages.

More details on methods and pitfalls of caging can be found in FAO (1991) and Lubilosa (undated-b).

Behaviour

Basic information should be collected on the behaviour of the insects, especially when this is likely to influence the action of the pathogen, or is a result of the action of the pathogen.

Important observations are presence and duration of active thermoregulation (basking in the sun, at unusual times of the day), reduction in speed and coordination of marching, reduction in feeding, increased predation, etc. Insects could also be sampled for gut analysis, and link this information to feeding behaviour.

Cadaver counts

Counts of dead locusts in the field are not necessary since they cannot be linked quantitatively to efficacy. Furthermore, they tend to disappear rapidly due to scavengers.

However, incubation of a sample of the cadavers, if they are found, to check for sporulation should always be done as it provides a qualitative confirmation of the likely cause of death of the insect.

Place

Populations assessments are best started in a central area in the upwind part of the block. Since hopper bands will likely move downwind, this will result in the highest likelihood of hopper bands remaining in the sprayed plot as long as possible. However, marching direction is also strongly affected by topography, and this should be taken into account.

Note that sampling should not be done within roughly one swath width of the upwind plot boundary, since this area will be underdosed.

Environmental conditions

Because the efficacy of *Metarhizium* is influenced by the ambient conditions, especially temperature, it is essential that a number of meteorological measurements are carried out on a regular basis during the entire trial. They include ambient temperature and relative humidity at "locust heights". These are best taken on a regular basis using a simple data logger.

Light intensity measurements, using a light meter that measures UV light, may be useful to get an idea about the potential exposure of spores to UV irradiation.

Furthermore, rainfall and an indication of cloud cover should be noted daily. Wind speed and direction are particularly important during the treatments. However, if measured on daily basis, it may provide useful information with respect to its influence on the direction of hopper band movement (important for future trials).

6. SPECIALIZED OBSERVATIONS

To get a better understanding of the efficacy of *Metarhizium* under varying environmental conditions, more in-depth observations on locust behaviour, physiology and pathology and environmental conditions after exposure to the spores in the field would be very useful (Blanford & Klass, 2004). It is therefore recommended to link that a more fundamental research group is invited to participate in the operational trial, to collect such data on Desert Locust while this is possible in the field.

7. REPORTING

The report should be concise, but should contain all information necessary to understand and independently evaluate the quality of the treatment, the quality and results of the biological monitoring exercises and the environmental and meteorological conditions during the trial. The original, not analysed or otherwise transformed data should be annexed to the report. Statistical analyses should be used, where appropriate, by clearly explained and referenced methods.

8. LOGISTICS & PERSONNEL

Organization

The trial will be part of the ongoing control campaign. The national coordinator of the trial will be a staff member of the locust control unit or plant protection department, but will be specifically assigned to the trial during its preparation and execution (a budget line for possible reimbursement of his time as a national consultant is included in the budget. In principle, the national coordinator will participate as efficacy monitoring staff during the entire trial. In addition, a specialist (international) consultant with intimate knowledge of all aspects of trials with *Metarhizium* needs to monitor the entire trial.

The amount of flight hours that has been reserved for the trial presumes that an aircraft company is carrying out locust control in the country, and that extra flying hours can be purchased, or reserved, on a local basis. No aircraft positioning costs have been included for planes to be flown in from outside the trial country.

Aerial spraying

Below are a number of scenarios regarding the required flying hours for aerial application. They are indicative only since the aircraft type is not yet known. The number of plots is based on the available amount of Green Muscle concentrate® (400 L). For details on the calculations, see Annex 1.

Plot size	# Replicates	Total flying hours	
		large spray plane e.g. Turbo Thrush/Air Tractor	**small spray plane** e.g. Ag Truck
900 ha	3	9	20
1200 ha	3	10	24
2000 ha	2	10	25

Ground support for spraying

Ground support for spraying consists of:

- team at the airstrip for mixing loading (presumed to be arranged by the company that carries out the treatment, as part of the contract)
- transport of pesticides to the airstrip
- 1 project staff to supervise mixing and loading of Green Muscle (3 – 4 days at the airstrip) and check on calibration. This staff will also compile AgNav data and check leftover pesticide after each treatment.
- 1 project staff on the ground to ensure ground to air communication at the plot sites (3 – 4 days on plots), independent from the efficacy monitoring staff.

Mortality assessments

Various mortality assessments have to be carried out. Staff and vehicle requirements (for 2 or 3 treated plots) are listed below, based on the tentative sampling schemes provided in Annex 2

Activity	Needs	Number of plots	
		2 treated & 1 control	**3 treated & 1 control**
hopper band observations	scouts	15	20
	monitoring staff	1 (+1 during treatments)	2
	vehicles (4x4)	1 (+1 during treatments)	2
Hopper band transects	monitoring staff	1 (+1 during treatments)	2
	vehicles (4x4)	1 (+1 during treatments)	2
Sampling for mortality in cages & Caging for persistence/secondary pick-up	staff	no extra	no extra
	vehicles	no extra	no extra
	camp staff (supervision of cages)	1	1

9. BUDGET

All estimates in this indicative budget are based on treatment and monitoring of three 1200 ha plots and 1 control plot.

Cost reductions could be obtained by spraying only 2 plots instead of 3 (but they are limited, amounting to a total of 168 000 US$ instead of 189 000 US$). A shorter monitoring period (i.e. if full efficacy has been achieved after 3 weeks rather than 4) would lead to a total budget of about 177 000 US$.

Item	Number / quantity	Number of days	Cost per unit ($US)	Total cost ($US)		Comments / assumptions
				intern'l	local	
Local trial preparation						
international consultant	1	7	350	2450		salary & field per diem ($300+$50)
national consultant/staff	1	10	100		1000	salary & field per diem ($70+$30)
4x4 vehicle	1	6	100		600	
fuel (litres)	200		0.5		100	
Pesticide application						
Flying hours - (incl. fuel & logistics)	12		2500	30000		large spray aircraft
Ground support (transport of fuel & insecticide loading)	--		--	0		part of aircraft contract
Green Muscle OF (litres)	400		180	72000		
Diesel fuel for mixing insecticide (litres)	3500		0.5		1750	
Small truck for transport of pesticides	1	3	200		600	rent & fuel & driver
Extra ground support staff at airstrip	1	7	100		700	salary & field per diem ($70+$30)
Extra ground support staff at spray plots	1	7	100		700	salary & field per diem ($70+$30)
droplet deposition equipment	1			100	50	UV dye, sticks, etc.
anemometer	1			50		
germination tests				500		to be done by laboratory with proven experience
Efficacy monitoring						
scouts (local)	20	28	10		5600	salary ($10)
international consultant	1	30	350	10500		salary & field per diem ($300+$50)
national consultants/staff	4	30	100		12000	salary & field per diem ($70+$30)
4x4 vehicles	4	30	100		12000	all commercially rented
HF/UHF radios (in vehicles)	4			0		available nationally?
walkie talkies (possibly integrated with GPS)	8		175		1400	
drivers	4	30	50		6000	salary & field per diem ($35+$15)

Item	Number / quantity	Number of days	Cost per unit ($US)	Total cost ($US)		Comments / assumptions
				intern'l	local	
fuel for 4 vehicles (litres)	4800		0.5		2400	40 L/vehicle/day
cages	200		5		1000	160 cages for mortality & 24 cages for persistence (local production)
insect nets	20		25	500		
bleach water to sterilize nets					10	
Petri dishes etc.	500			100		for incubation
General equipment						
GPS	4			0		available nationally or from FAO.
portable computer, printer & mapping software	1			0		available from FAO or intern. consultant?
electronic data logger (temperature/RH)	2		150	300		
infrared thermometer	1			100		
digital camera	1			0		available from FAO or intern. consultant?
small portable generator	1				250	available nationally or rent?
Satellite telephone + calling costs				1200		
various small equipment				500	500	
Camping equipment						
large tents	4		1000		4000	available nationally?
camping beds	15		100		1500	available nationally?
cooking material					500	available nationally?
water jerrycans/drums	10		25		250	available nationally?
gas lamps + bottles	4		50		200	available nationally?
folding tables & chairs	15		25		375	available nationally?
various camping equipment					1000	
General personnel						
Cook	1	30	50		1500	salary & field per diem ($35+$15)
Guard (camp)	1	30	20		600	salary ($20)
Report writing						
international consultant	1	5	300	1500		work at home
national consultant	1	5	70		350	work at home
International travel						
international consultant	1			3000		

Subtotals	*intern'l*	*local*	
	122800	**56935**	
Unforeseen (5%)	6140	2847	
Grand total	**188722**		

10. TIMELINE

Below is an indicative timeline for the various actions that have to be taken before the trial. This timeline will certainly be modified as the trial is being organized. Rather than a fixed planning, it should be seen as a checklist of actions to be dealt with before the trial.

When?	What?	Who?
D – 3 months	Preparatory meeting with national locust control organization or PPD – to be done in all countries where trials may likely be carried out	FAO HQ
D – 3 months	Purchase of Green Muscle (keep at supplier until potential targets and thus country of trial has been confirmed)	FAO HQ
D – 3 months	Establishment of short-list of possible international / national consultants and their periods of availability	FAO HQ
D – 3 months	Purchase of equipment (as far as it is unlikely to be available in the country) and store at FAO HQ, or Discuss the purchase/supply of equipment by the consultants/groups that may carry out the trial.	FAO HQ Consultants
D – 1 month	**Decision on trial country**	FAO HQ
D – 1 month	Obtain experimental permit (if needed)	National PPD
D – 1 month	Raise Field Authorisation for FAOR	FAO HQ
D – 1 month	Dispatch of Green Muscle from supplier to country	FAO HQ
D – 1 month	Establish aircraft contract or reserve flying hours	FAO HQ
D – 1 month	Dispatch of equipment to country	FAO HQ
D – 1 month	Recruitment of national coordinator	Government & FAOR
D – 1 month	Recruitment international consultant	FAO HQ
D – 1 month	Arrange appropriate storage of Green Muscle	National coordinator
D – 1 month	Initiate customs clearance Green Muscle & equipment	FAO HQ & FAOR
D – 20 days	Recruitment other national staff	National coordinator & FAOR
D – 20 days	Rent of vehicles	National coordinator & FAOR
D – 16 days	Arrival insecticides and other equipment in country	--
D – 15 days	Pesticides and other equipment out of customs	National coordinator & FAOR
D – 15 days	Reception of experimental permit	National coordinator
D – 15 days	Initiate local purchase of equipment	National coordinator
D – 10	Identification of potential treatment locations	National coordinator
D – 7	Arrival international consultant	--
D – 7	Organize logistics discussions with PPD and aircraft company	National coordinator & international consultant
D – 6 to 4	Filed visits / identification definitive plot locations	National coordinator & international consultant
D – 4	Travel team and equipment to trial location	all technical staff involved
D – 3	Methodology session with entire team	all technical staff involved
D – 2	Work session with pilot / calibration aircraft (if needed)	National coordinator & international consultant & national application expert
D – 1	Collection pre-spray data	all field monitoring staff
D	Treatments	all staff
D + 28	Monitoring of plots	all field monitoring staff

The preparatory meeting with national locust control organization or PPD and with FAOR should deal with the following issues:

- Agreement on trial
- Legal requirements (experimental permit; customs formalities)
- Aerial contract possibilities
- Identification national coordinator
- Short list for national staff (recruitment/reimbursement modalities)
- Needs for outside recruitment
- Discussion equipment list (available for use; local purchase; international purchase)
- Vehicle rent possibilities
- Communication links between FAO HQ and the national coordination/PPD

References

General

Blanford S & Klass J (2004) Review of environment effects on the performance of *Metarhizium anisopliae* var. *acridum* against locusts under field conditions. Unpublished report. FAO EMPRES Programme. Food and Agriculture Organization of the United Nations, Rome, Italy.

EPPO (2004) Hopper bands of *Schistocerca gregaria* under natural conditions. EPPO Standard for the efficacy evaluation of plant protection products, 2nd edition, Volume 3. Standard no. PP 1/191(2). European and Mediterranean Plant Protection Organization, Paris, France.

FAO (1991) Guidelines for pesticide trials on Desert Locust hoppers. June 1991. Food and Agriculture Organization of the United Nations, Rome, Italy.

LUBILOSA (1999) Green Muscle user handbook. Version 4. LUBILOSA Programme, International Institute of Tropical Agriculture, Cotonou, Benin.

LUBILOSA (undated-a) Insect pathology manual – Section III.: Laboratory techniques. LUBILOSA Programme, International Institute of Tropical Agriculture, Cotonou, Benin.

LUBILOSA (undated-b) Insect pathology manual – Section V.: Field experimentation. LUBILOSA Programme, International Institute of Tropical Agriculture, Cotonou, Benin.

Wilps H (2004) Study on barrier treatments as a means of controlling migratory locusts. Unpublished report.

Previous field trials with *Metarhizium* against the Desert Locust

Aston R (2004) Medium scale field trials with the insect pathogenic fungus *Metarhizium anisopliae* ("Green Muscle") against Desert Locust (*Schistocerca gregaria*) in Niger. Draft project report OSRA/RAF/801/USA. February 2004. Food and Agriculture Organization of the United Nations. [PRG report code: not yet submitted]

Bashir MO (2004) Use of the fungus *Metarhizium anisopliae* var. *acridum* in the control of locusts with reference to *Schistocerca gregaria* (Forskal) and *Locusta migratoria migratoroides* (R&F). Report submitted to the 70th Meeting of the Pests and Disease Committee. June 2004. International Centre of Insect Physiology and Ecology, Nairobi, Kenya & Plant Protection Directorate, Khartoum, Sudan. [trial country: Sudan – PRG report code: 04-26]

Kooyman C & Godonou I (1997) Infection of *Schistocerca gregaria* (Orthoptera: Acrididae) hoppers by *Metarhizium flavoviride* (Deuteromycotina: Hyphomycetes) conidia in a oil formulation applied under desert conditions. *Bulletin of Entomological Research* **87:**105-107 [trial country: Mauritania – PRG report code: 93-11]

Langewald J (1995) Control of hopper bands of *Schistocerca gregaria* with the entomopathogenic fungus *Metarhizium flavoviride* in the North-East of Mauritania in April 1995. LUBILOSA report, IITA, Cotonou, Benin. [PRG report code: 95-11]

Langewald J, Kooyman C, Douro-Kpindou O, Lomer CJ, Dahmoud AO & Mohamed HO (1997) Field treatment of Desert Locust (*Schistocerca gregaria* Forskål) hoppers in Mauritania using an oil formulation of the entomopathogenic fungus *Metarhizium flavoviride*. *Biocontrol Science and Technology* **7:** 603-611. [PRG report code: 95-12]

Other relevant medium to large-scale field trials with *Metarhizium* (particularly with respect to field trial methodology)

Hunter DM, Milner RJ, Scanlan JC & Spurgin PA (1999) Aerial treatment of the migratory locust, *Locusta migratoria* (L.) (Orthoptera: Acrididae) with *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) in Australia. *Crop Protection* **18:** 699-704

Hunter DM, Milner RJ & Spurgin PA (2001) Aerial treatments of the Australian plague locust, *Chortoicetes terminifera* (Orthoptera: Acrididae) with *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes). *Bulletin of Entomological Research* **91:** 93-99

Kooyman C, Bahana J, Godonou I, Katheru JN, Magoma RN, Seyoum E & Spurgin P (2003) Operational trial of Green Muscle® against red locust nymphs in the Wembere plains, Tanzania. Internal report projects DFID R7818 and FAO TCP/URT/201/NOR.

Kooyman C, Bahana J, Katheru JN, Mutahiwa S & Spurgin P (2003) Operational trial of Green Muscle® against red locust adults in the Iku plains, Tanzania. August – September 2003. Internal report. Projects DFID R7818 and FAO TCP/URT/2802. [PRG report code: 04-25]

Price RE. Müller EJ, Brown HD, D'Uamba P & Jone AA (1999) The first trial of a *Metarhizium anisopliae* var. *acridum* mycoinsecticide for the control of the red locust in a recognized outbreak area. *Insect Science and its Application* **19(4):** 323-332

Kooyman C, Bateman RP, Langewald J, Lomer CJ, Ouambama Z & Thomas MB (1997) Operational-scale application of entomopathogenic fungi for control of Sahelian grasshoppers. *Proceedings of the Royal Society London B* **264:** 541-546

Annex 1 – Indicative calculations for flying hours

Plot size (ha)	**Volume tank mix needed**	**Volume GM concentrate needed**	**# replicates possible with 400 L GM**		
900 (3x3 km)	900 L	90 L	4		
Indicative flying hours per plot *[airstrip 100 km from plot; treatment and ferry speed 160 km/h]*					
Aircraft type	**max. hopper capacity***	**# sorties**	**spray time of plot** (hours)	**ferry time** (hours)	**total flying hours** (hours)
Turbo Thrush 510	1900	1	1.5	1.2	2.7
Air Tractor 401B	1500	1	1.5	1.2	2.7
Ag Truck 188	280	4	1.5	5	6.5

Plot size (ha)	**Volume tank mix needed**	**Volume GM concentrate needed**	**# replicates possible with 400 L GM**		
1200 (3x4 km)	1200 L	120 L	3		
Indicative flying hours per plot *[airstrip 100 km from plot; treatment and ferry speed 160 km/h]*					
Aircraft type	**max. hopper capacity**	**# sorties**	**spray time of plot** (hours)	**ferry time** (hours)	**total flying hours** (hours)
Turbo Thrush 510	1900	1	2	1.2	3.2
Air Tractor 401B	1500	1	2	1.2	3.2
Ag Truck 188	280	5	2	6	8

Plot size (ha)	**Volume tank mix needed**	**Volume GM concentrate needed**	**# replicates possible with 500 L GM**		
2000 (4x5 km)	2000 L	200 L	slight less than 2		
Indicative flying hours per plot *[airstrip 100 km from plot; treatment and ferry speed 160 km/h]*					
Aircraft type	**max. hopper capacity**	**# sorties**	**spray time of plot** (hours)	**ferry time** (hours)	**total flying hours** (hours)
Turbo Thrush 510	1900	2	2.5	2.4	4.9
Air Tractor 401B	1500	2	2.5	2.4	4.9
Ag Truck 188	280	8	2.5	9.6	12.1

* actual pesticide loads are generally lower, depending on the needed ferry time between airstrip and plot, and the length and condition of the airstrip.

Annex 2 – Indicative sampling regime

Presuming 3 replicate plots; underlined plots are pre-spray samples; unsprayed control plot is D

Type of sampling	**Day**																											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
Treatment (plots A, B & C)		**A**	**B**	**C**																								
Hopper band observations	<u>A</u>	<u>B</u> D	<u>C</u> A	B D	A C	B D	C	A	B D	C	A	B D	C	A	B D	C	A	B D	C	A	B D	C	A	B D	C	A	B D	C
Hopper band transects	<u>A</u>	<u>B</u> D	<u>C</u> A	B D	A C	B D	C	A	B D	C	A	B D	C	A	B D	C	A	B D	C	A	B D	C	A	B D	C	A	B D	C
Sampling for mortality in cages					A	B D	C	A	B D	C	A	B D	C															
Caging for persistence/secondary pick-up		A	B D	C	A	B D	C	A	B D	C	A	B D	C															
Number of vehicles in the field	2	4	4	4	4	4	2	2	4	2	2	4	2	2	4	2	2	4	2	2	4	2	2	4	2	2	4	2
Number of monitoring staff in the field	2	4	4	4	4	4	2	2	4	2	2	4	2	2	4	2	2	4	2	2	4	2	2	4	2	2	4	2
Number of spray staff in field/airstrip	2	2	2	2																								
Number of staff at camp (cages)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Presuming 2 replicate plots; underlined plots are pre-spray samples; unsprayed control plot is D

Type of sampling	**Day**																											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
Treatment (plots A & B)		**A**	**B**																									
Hopper band observations	A	B D	A	B	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D
Hopper band transects	A	B D	A	B	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D
Sampling for mortality in cages					A	B	D	A	B	D	A	B	D															
Caging for persistence/secondary pick-up		A	B	D	A	B	D	A	B	D	A	B	D															
number of vehicles in the field	2	4	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
number of monitoring staff in the field	2	4	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Number of spray staff in field/airstrip	2	2	2																									
Number of staff at camp (cages)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1