Plant Production and Protection Division (AGP)
Plant Protection Service (AGPP)
Locusts and other Migratory Pests Group

Guidelines

Field efficacy trials with the entomopathogen
Metarhizium anisopliae var. *acridum* (Green Muscle™)
against the Desert Locust (*Schistocerca gregaria*)

and

Monitoring of its operational use

Version 1.1 – 19 September 2007

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 rate has been established against the Desert Locust (*Schistocerca gregaria*) (FAO, 2004). 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 2.5×10^{12} conidia per hectare.

Recently, the efficacy of *Metarhizium anisopliae* var. *acridum* against the Desert Locust was reassessed (Van der Valk, 2007). The study included new trials that were not yet available at the 9th PRG meeting, but also results of field trials with other strains of the pathogen and other species of locusts and grasshoppers. The review confirmed the dose rate set by the PRG in 2004 as being robust under favourable or moderate temperature conditions. Further field trials were still recommended, though, in particular to assess the environmental limits of the use of *Metarhizium*, especially temperature conditions. Better data were also needed on the relationship between vegetation biomass, hopper displacement and effectiveness of secondary pick-up of spores, and on the effects of the pathogen on the susceptibility of the insects to predation.

The review also concluded that reporting of many previous field efficacy trials had been insufficient and the design and execution of some trials had been inadequate. Furthermore, given the limited opportunities that may occur to organize full-fledged field efficacy trials against the Desert Locust, the importance of adequate monitoring of any operational use of *Metarhizium* against locusts and grasshoppers, and particular the Desert Locust, was underlined.

The objective of these guidelines is to give advice on the design of field efficacy trials with *Metarhizium anisopliae* var. *acridum* against the Desert Locust. This document revises and updates a previous guideline published in 2005 (FAO, 2005) and incorporates recent field experiences with the pathogen and results of the above-mentioned review. Furthermore,

guidance for operational monitoring of the use of *Metarhizium* against the Desert Locust is also provided.

Part 1 of the guidelines describes the design, execution and reporting of medium and large-scale trials of *Metarhizium anisopliae* var. *acridum* against the Desert Locust.

Part 2 of the guidelines provides a checklist for operational monitoring of the efficacy of *Metarhizium anisopliae* var. *acridum* against the Desert Locust.

PART 1

Field efficacy trials with the entomopathogen
Metarhizium anisopliae var. *acridum* (Green Muscle™)
against the Desert Locust (*Schistocerca gregaria*)

1. OUTLINE OF THE TRIAL

The trial concerns one or more medium or large-scale applications of *Metarhizium anisopliae* var. *acridum*[1] against hopper bands of the Desert Locust. Small-scale applications are not included in this guideline because these are not likely to be representative of future operational treatments against the Desert Locust with *Metarhizium*. Also, small-scale treatments have a high probability of underestimating the efficacy of the entomopathogen.

In a few particular situations, small-scale efficacy trials may provide useful new information, for instance to assess the effect of the residence time of locusts in treated areas, and its impact on secondary pick-up of spores and efficacy. Such more specialized trials are not further discussed here.

Given the relative complexity and long duration of a field trial with an entomopathogen, it needs to be carefully planned. However, this does not exclude that the trial can be an integral part of the ongoing Desert Locust control campaign in a country.

2. TRIAL DESIGN

Target type

The spray targets are blocks of land containing one or more hopper bands of the Desert Locust. Even though adult locusts are also susceptible to *Metarhizium*, the high mobility of adults combined with the slow action of the pathogen make the assessment of field efficacy difficult, both in field trials and even more so under operational circumstances. The operational use of *Metarhizium* against adult Desert Locusts is therefore unlikely to be recommended in the future.

The actual targets for the spray droplets are both the individual locusts as well as the vegetation on which they feed or move around in, because secondary pickup is a very 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. Trials against adults are not recommended.

Trial area

Areas with relatively 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.

[1] All reference in the rest of these guidelines to *Metarhizium* refers to *Metarhizium anisopliae* var. *acridum*, isolate IMI 330189. At the time of elaborating the guidelines the only commercially available product against the Desert Locust was Green Muscle™

Trial period

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

Presently available evidence suggests that the performance of *Metarhizium* is likely to be inadequate (i.e. long delay to achieve 90% mortality) under conditions with hot days (> 38°C) and cool nights (< 20°C), or when both the days and nights are cool (< 20°C) (see table below).

Trials should therefore be carried out under favourable or moderate conditions.

Expected performance of *Metarhizium anisopliae* var. *acridum* against locusts and grasshoppers under different ambient temperature conditions (modified after Blanford & Klass, 2004).

Performance category [1]	**Time to achieve 90% mortality**	**Temperature conditions**
Favourable	7 – 14 days	Daytime < 38 ºC and night-time > 20 ºC
	10 – 14 days	Daytime < 38 ºC and night-time < 20 ºC
Moderate	15 – 24 days	Daytime > 38 ºC and night-time > 20 ºC
Unfavourable	> 25 days	Daytime > 38 ºC and night-time < 20 ºC
		Daytime and night-time < 20 ºC

Plot size

The trial plot size should be representative of operational conditions. Furthermore, plot size 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.

Desert Locust hoppers will be exposed to spray droplets during the application, but will subsequently also collect a major part of the total spore load through secondary pick-up from the vegetation. The minimum plot size should therefore 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 adequate mortality within a reasonable time.

Ideally, hoppers 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 should remain in the treated plot for a period long enough to accumulate a sufficient number of viable spores through secondary pick-up to ensure a reasonably short time to death. It has been shown that this period is at least 2 days, but the longer the better.

Linear displacement of mid instar hopper bands is very variable, and is likely to be several hundreds of meters per day[2], which means that in the time needed to acquire

[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.

a lethal dose, the band may have moved as much as 0.5 to 1 km, or sometimes even more.

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.

Taking into account the above considerations, the minimum needed plot size is likely to be at least 400 ha (i.e. 2 x 2 km block). Smaller spray plots can be considered if it is clear that hopper displacement is limited.

Plot number (replicates)

Since the objective of the medium to large-scale trials is to confirm the recommended field dose rate rather than to set a new rate, there is less of a need to treat several replicates with similar hopper populations and similar environmental conditions. However, various 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 recommended that at least 2, and preferably 3 plots, are treated independently from each 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 batch, though);

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

(iii.) sprayer/atomiser settings are (re-)calibrated, or the calibration verified, before each treatment[3].

If no replicates are feasible, a trial with single sprayed plot may still provide very useful data and should not be excluded. Condition is, however, that the trial is reported in sufficient detail so that its results can be compared with other trials.

Unsprayed control plots

At least 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 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 structure of the hopper population 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 after fledging starts, an agreement can be made that the control plot will be sprayed with a conventional contact insecticide at that moment.

---

[3] 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.

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 x 10^{13} spores/L) is the test product. It needs to be diluted with diesel oil to a tank concentration of 2.5 x 10^{12} spores/L (i.e. a dilution ratio for Green Muscle:diesel of 1:9), to obtain the recommended dose rate of 50 g spores/ha applied in 1 L of formulation/ha.

Metarhizium spores may settle at the bottom of the containers, in particular after road transport over rough terrain, and form a solid deposit. It is very important that any deposit is re-suspended before (or in the course) of dilution, to ensure that the nominal dose rate is indeed applied. Electrical mixers are best used for re-suspension and mixing of the concentrate.

Type of treatment

For large-scale trials, aerial treatments are recommended.

If plots smaller than about 100 ha can be validly treated (see **plot size**), vehicle-mounted sprayers could also be used.

In the sections below, it will be presumed that aerial treatments are carried out.

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[4], to check spore viability levels. If at all possible, a germination test should also be done on the tank mix, to confirm that the diluent did not have any adverse effect on spore viability.

If treatments are spread over a prolonged period (e.g. more than about a week), 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, because of the 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[5].

Aircraft

Due to the plot size required (a minimum of 400 ha, but ideally more) 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.

---

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

5 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.

Sprayer/atomisers

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 must be thoroughly rinsed 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 always 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 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 trial starts, the spray equipment should be calibrated to apply the required area dosage. The spray equipment should be recalibrated, or the calibration checked, before each individual treatment. Note that atomiser flow rates may vary from day to day, or even during the day, but this can be verified by the onboard flow control system.

If the aircraft is equipped with well-know rotary atomisers, such as Micronairs™, 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 about 80 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 the ground crew marking the four plot corners with GPS. These are the coordinates 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 locusts 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 or %), possible (temporary) onset of convection and rainfall must all be measured at the start and at the end of the application, and if possible during the operation (at about half-hour intervals).

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 approximately 10 m. This corresponds with operational aerial spray practice against the Desert Locust.

Area dosage measurement

The volume of pesticide actually applied per unit area of plot will never be exactly what is intended, so every effort should be made to accurately determine it. The use of a spray aircraft equipped with a GPS-based track guidance system, coupled to an onboard (computerised) flow meter, will allow easy estimation of the average area dosage. GPS data for the application should be downloaded to a computer for calculation of the actual spray block size. 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 does not stain well on oil-sensitive papers, while 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 with 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 droplet deposition on the locusts.

At least two lines of droplet collection cards should 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 insects die. Both emigration 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 all the answers needed to assess efficacy in a sufficient manner. If possible, one of the two first methods (or both) should be combined with caging.

Monitoring of individual hopper bands

Individual hopper bands can be monitored to assess the impact of the entomopathogen on the insects. This method is relatively precise but also very labour intensive. It is particularly useful if the spray plot is relatively small 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 recommended. A number of scouts are recruited to follow one (or sometimes two) hopper band(s) each during the entire day, until the band stops to roost. The band location is then marked (both with a flag and 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 two sprayed plots plus one control plot are monitored in one trial, up to 15 scouts may be needed for such a task.

Hopper bands are best selected for monitoring 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 that they remain in the sprayed plot as long as possible. However, marching direction is also strongly affected by topography, and this should be taken into account.

An assessment team will then visit each hopper band several times during the trial period and estimate hopper population sizes. 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, indication of predation and/or scavenging, and 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 regular 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 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.

Insects are collected from the treated plots several times after treatment and caged with unsprayed vegetation to assess mortality. The hoppers should be collected from different parts of the sprayed plot, but can subsequently be pooled in one large sample for each treated plot. Insects can then be drawn from this sample to be caged in the various replicate cages. Note that sampling should not be done within roughly one swath width of the upwind plot boundary, since this area will be underdosed.

The first sample is best taken about 2 days after treatment, and a second sample 4 or 5 days after spraying. It is not very useful to take samples after this period, since maximum cumulative mortality will generally not increase anymore.

If resources are limited, and only one sample can be taken for caging, this is best done 2 days after spraying, when the effect of secondary pick-up on mortality is expected to be highest.

Control cages containing unsprayed hoppers placed on unsprayed vegetation should always be prepared. This should be done for each sampling round, to quantify control mortality.

At least two, and preferably more, replicate cages should be incubated for each sample that is taken from the field. This will minimize the loss of efficacy data if one or more of the cages are lost for unforeseen reasons (e.g. contamination, predation). Cages should best be placed outside, in a location that is only partly shaded. This will allow the locusts to thermoregulate. If cages are placed in the full shade or in a building, the observed mortality is likely to be an overestimate of the field situation.

All locusts that die in the cages, both the unsprayed control insects and the treated ones, are incubated in Petri dishes containing humid filter paper to assess sporulation.

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.

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 be done whenever possible as it provides a qualitative confirmation of the cause of death of the insects.

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" (i.e. generally at average vegetation height). These are best taken on a regular basis throughout the trial, preferably every 2 hours, or even more regularly. Simple and relatively cheap data loggers exist for ambient temperature and relative humidity.

The level of exposure to ultraviolet (UV) radiation influences spore viability. The most relevant for *Metarhizium* spores deposited in the field is radiation in the UVB spectrum (290 – 320 nm), and a UV light (radiation) meter that can measure in this range should be used. Radiation in the UVA range (320 – 400 nm) does not have much adverse effect on spore viability.

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).

Persistence of spore viability

If resources permit, it is very useful to assess the persistence of spore viability using field bioassays. Untreated locusts are caged onto treated vegetation, preferably in the field. Insects are kept in these cages for 3 days and then transferred to cages with unsprayed vegetation, where mortality is assessed.

The first persistence bioassay is set up just after treatment, and subsequent bioassays should be done at (approximately) 3-day intervals. In most cases, this needs to be repeated until about 15 days following treatment, after which mortality will have returned to control levels. In some cases spore persistence may be longer, and the exact duration of the bioassays needs to be determined during the trial.

Sporulation should be assessed of all insects that die in the cages and that can be recovered.

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 may be useful (Blanford & Klass, 2004). However, it is recommended that a more specialized research group is invited to participate in the trial to collect such data.

7. REPORTING

Reporting is an essential phase of the trial and without a good report the trial is basically useless because its results cannot be exploited by others! Many past reports of field efficacy trials with *Metarhizium* against locusts and grasshoppers have been found to be incomplete (Van der Valk, 2007).

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. In principle, the report should provide sufficient information for the reader to exactly repeat the trial.

The original, not analyzed or otherwise transformed data should be annexed to the report. Statistical analyses should be used, where appropriate, by clearly explained and referenced methods.

An outline of elements for a trial report with *Metarhizium* is provided in Annex 1

8. LOGISTICS & PERSONNEL

Organization

Because of its relative complexity and duration, a medium or large-scale efficacy trial with *Metarhizium* requires an independent team of field staff with its proper logistics. However, the trial can be part of an ongoing control campaign.

The trial is best coordinated by a national staff member who is based at the national locust control organization, or who has very close operational links with the locust control organization. This is important to ensure that trial logistics, in particular the location of targets and the execution of spraying operations, can be organized smoothly. The national trial coordinator needs to be specifically assigned to the trial during its preparation and execution. The national coordinator should also participate in the entire trial itself.

It is recommended that a specialist (international) consultant with intimate knowledge of all aspects of trials with *Metarhizium* is responsible for the execution of the trial. This may be the national coordinator, but could also be someone else.

Planning of the trial needs to start about three months before the *Metarhizium* application. At this stage, it is often not clear yet if and where suitable Desert Locust targets will be present. The first stage of this process is therefore contingency planning, which may need to be done in several countries, while the actual trial will only be carried out in one of them.

An indicative timeline for the various actions that have to be taken is provided in Annex 2.

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. For more details on the calculations, see Annex 3.

Plot size	# Replicates	Total flying hours	
		large spray plane e.g. Turbo Thrush/Air Tractor	small spray plane e.g. Ag Truck
400 ha	3	6	10
900 ha	3	9	20
1200 ha	3	10	24

Ground support for spraying

Ground support for spraying consists of:

- A team at the airstrip for mixing and 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 and check on calibration (3 – 4 days at the airstrip). This staff will also compile data from the track guidance system and check leftover pesticide after each treatment.
- At least 1 project staff on the ground to ensure ground to air communication at the spray sites (3 – 4 days on the plots), independent from the efficacy monitoring staff.

Mortality assessments

Various mortality assessments have to be carried out. Minimum staff and vehicle requirements (for 2 or 3 treated plots) are listed below, based on the tentative sampling schemes provided in Annex 5. It is recommended that, if resources are available, the number of monitoring staff listed below is doubled, so that field teams of two staff can be constituted and work speeded up.

Activity	Needs	Number of plots	
		2 treated & 1 control	3 treated & 1 control
Hopper band observations	scouts	up to 15	up to 20
	monitoring staff	1 (+1 extra during and shortly after treatments)	2
	vehicles (4x4)	1 (+1 extra during and shortly after treatments)	2
Hopper band transects	monitoring staff	1 (+1 extra during and shortly after treatments)	2
	vehicles (4x4)	1 (+1 extra during and shortly after treatments)	2
Sampling for mortality in cages & Caging for persistence/secondary pick-up	staff	1	1
	vehicles	1	1
	camp staff (supervision of cages)	1	1

Equipment and supplies

A tentative list of equipment and supplies is provided in Annex 4. Some of this can be purchased or manufactured locally; other items will need to be imported or temporarily be brought into the country. Equipment requirements and availability need to be carefully determined well before the trial starts (also see Annex 2).

PART 2

Monitoring of the operational use of the entomopathogen
Metarhizium anisopliae var. *acridum* (Green Muscle™)
against the Desert Locust (*Schistocerca gregaria*)

1. OUTLINE OF THE MONITORING OPERATION

At present, a dose rate of 2.5 x 10^{12} conidia/ha or 50 g conidia/ha of *Metarhizium anisopliae* var. *acridum* (IMI 330189) is being recommended against the Desert Locust. However, only limited operational experience has yet been gained with this entomopathogen. It has therefore been recommended that as many operational treatments as possible be monitored in sufficient detail to assess efficacy.

In principle, any treatment of *Metarhizium* against the Desert Locust can be monitored, but data collection and interpretation is considerably easier for hopper populations. The minimum data requirements have been listed in the form of a checklist in the next section.

It is strongly recommended to first read the full trial guideline in Part 1 before carrying out any monitoring exercises, as it provides details on the type of data that need to be collected, and the approaches and methods required.

This checklist should be considered as a model, not as a fixed form. It can be modified or added to, depending on the time and resources available for monitoring. In particular the number of replicates in the various efficacy assessments may be increased. Issues that could also be added are additional sampling dates to assess efficacy through caging, assessments of persistence of spore viability, or more specific behavioural observations. However, the data listed in the checklist are a minimum requirement and should in principle always be collected.

2. CHECKLIST

Operational monitoring of *Metarhizium* applications against the Desert Locust

One checklist needs to be completed for each plot that is treated. The observations on untreated control plots are incorporated into the forms of the treated plots.

General information		
Country:		Name of organization carrying out the treatment:
Date of treatment:		Name of person filling out this form:
Plot location		
GPS coordinates of corners of plot that was actually sprayed	1:	Nearest town or village:
	2:	
	3:	Distance and direction to nearest town or village:
	4:	
Habitat		
Vegetation type (e.g. grassland, shrubs, woodland, crop):		State of vegetation (e.g. greening up, green, drying out, dry):
Average height of grass/herb layer: (cm)		% vegetation cover: (%)
Locust population		
Species: *Schistocerca gregaria*		Dominant stage(s):
Average hopper band size: (m^2)		Average hopper band density: (insects/m^2)
Behaviour (e.g. speed and direction of displacement):		
Biopesticide		
Entomopathogen: *Metarhizium anisopliae* var. *acridum* (IMI 330189)		Brand name:
Batch number:		Formulation concentration: (g spores/L)
Expiry date:		Colour of temperature indicator on container (if present):
Diluent:		Dilution ratio:
Germination rate: (%)		Date sampled:
		Product sampled (concentrated formulation or diluted tank mix):
Observations about or problems with the product:		

Application					
Type of application (aerial, ground):			Brand and type of atomizer:		
Name of last used pesticide in the application equipment:			Cleaning procedures used for application equipment:		
Atomiser settings (e.g. blade angle, VRU setting):			Rotational speed of atomiser		(rpm)
Speed of sprayer/aircraft:		(km/h)	Track spacing:		(m)
Total flow rate:		(L/min)	Emission height:		(m)
Total volume applied:		(L)	Actual plot size:		(ha)
Planned application rate:		(g spores/ha)	Actual application rate:		(g spores/ha)
Observations or problems encountered during the application:					
Meteorological conditions during the application					
Temperature: (at 2 m height)	at start:	(ºC)	Wind speed: (at 2 m height)	at start:	(m/s)
	at finish:	(ºC)		at finish:	(m/s)
Wind direction (relative to spray tracks):			Estimated cloud cover:		(%)
Rainfall (on day of application, or days immediately after):		(mm)	on date:		

Effect assessment – monitoring of individual hopper bands (if applicable)

Carry out this assessment for two or more hopper bands in each plot

Band #		**Before treatment**	**After treatment**			
Treated plot						
T1	Date:					
	Location (GPS coordinates):					
	Band size (m^2)					
	Band density (insects/m^2)					
T2	Date:					
	Location (GPS coordinates):					
	Band size (m^2)					
	Band density (insects/m^2)					
T3	Date:					
	Location (GPS coordinates):					
	Band size (m^2)					
	Band density (insects/m^2)					
T…	(replicates can be added)					
Control plot						
C1	Date:					
	Location (GPS coordinates):					
	Band size (m^2)					
	Band density (insects/m^2)					
C2	Date:					
	Location (GPS coordinates):					
	Band size (m^2)					
	Band density (insects/m^2)					
C3	Date:					
	Location (GPS coordinates):					
	Band size (m^2)					
	Band density (insects/m^2)					
C…	(replicates can be added)					

Remarks (e.g. merging or splitting up of bands, behaviour, predation) :

Effect assessment – presence/absence sampling (if applicable)

Carry out this assessment along two or more parallel transects traversing most of the plot

Transect #		Before treatment		After treatment							
Treated plot											
T1	Date:										
	Length of transect (m):										
	Surface area sampled at each stop (m^2):										
	Number of sampling stops:	with locusts:	without locusts:	with locusts:	without locusts:	with locusts:	without locusts:	with locusts:	without locusts:	with locusts:	without locusts:
T2	Date:										
	Length of transect (m):										
	Surface area sampled at each stop (m^2):										
	Number of sampling stops:	with locusts:	without locusts:	with locusts:	without locusts:	with locusts:	without locusts:	with locusts:	without locusts:	with locusts:	without locusts:
T3	Date:										
	Length of transect (m):										
	Surface area sampled at each stop (m^2):										
	Number of sampling stops:	with locusts:	without locusts:	with locusts:	without locusts:	with locusts:	without locusts:	with locusts:	without locusts:	with locusts:	without locusts:
Control plot											
C1	Date:										
	Length of transect (m):										
	Surface area sampled at each stop (m^2):										
	Number of sampling stops:	with locusts:	without locusts:	with locusts:	without locusts:	with locusts:	without locusts:	with locusts:	without locusts:	with locusts:	without locusts:
C2	Date:										
	Length of transect (m):										
	Surface area sampled at each stop (m^2):										
	Number of sampling stops:	with locusts:	without locusts:	with locusts:	without locusts:	with locusts:	without locusts:	with locusts:	without locusts:	with locusts:	without locusts:
C3	Date:										
	Length of transect (m):										
	Surface area sampled at each stop (m^2):										
	Number of sampling stops:	with locusts:	without locusts:	with locusts:	without locusts:	with locusts:	without locusts:	with locusts:	without locusts:	with locusts:	without locusts:

Remarks:

Effect assessment – Caging of treated locusts on untreated vegetation (if applicable)

Carry out this assessment 2 days after treatment. Collect at least 100 locusts from the treated plot and cage in at least 2 (but preferably more) separate cages. Also collect at least 100 locusts from a non-treated area and cage in at least 2 (but preferably more) separate cages. Only provide untreated vegetation as food.

Date of collection: (=2 days after treatment)	Location of cages: (e.g. field/inside; sun/shade)

Mortality (at days after introduction in cages)

Cage #	Number of insects introduced in cage	1 day			3 days			5 days			7 days			9 days			11 days			13 days			15 days			17 days			19 days		
		alive	dead	missing	alive	dead	missing	alive	dead	missing	alive	dead	missing	alive	dead	missing	alive	dead	missing	alive	dead	missing	alive	dead	missing	alive	dead	missing	alive	dead	missing
Treated plot																															
T1																															
T2																															
T3																															
T4																															
…																															
Control plot																															
C1																															
C2																															
C3																															
C4																															
…																															

Observations (e.g. if insects lost through predation; if sporulation incubations have been carried out, and results):

Meteorological conditions during the efficacy assessment(s)

Take measurements several times per day, until the end of the efficacy assessments. The use a data logger is highly recommended.

	Days after treatment																				
Temperature (at locust height)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Max.																					ºC
Min.																					ºC
Average																					ºC
Number of hours during day between 20°C and 35°C																					
Relative humidity (at locust height)																					
Max.																					%
Min.																					%
Rainfall																					mm
Cloud cover																					%

Remarks:

(e.g. add records from data logger or the complete list of measured temperatures)

Other observations

Increased predation on the locusts observed?

(provide details)

Behavioural abnormalities of the locusts observed?

(provide details)

Dead insects from cage assessment incubated for sporulation?

(describe method and results)

Dead insects collected from field and incubated for sporulation?

(describe method and results)

Other remarks of observations?

REFERENCES

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.

FAO (2004) Evaluation of field trial data of the efficacy and selectivity of insecticides on locusts and grasshoppers. Report to FAO by the Pesticide Referee Group. Ninth meeting, Rome, 18 – 21 October 2004. Food and Agriculture Organization of the United Nations, Rome, Italy.

FAO (2005) Guideline – Operational-scale field trial with Green Muscle® (*Metarhizium anisopliae* var. *acridum*). Version 3 – February 28, 2005. Migratory Pests Group, Food and Agriculture Organization of the United Nations, Rome, Italy.

Langewald J, Kooyman C, Douro-Kpindou O, Lomer CJ, Dahmoud AO & Mohamed HO (1997a) 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.

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.

Van der Valk H (2007) Review of the efficacy of *Metarhizium anisopliae* var. *acridum* against the Desert Locust. Report to FAO, July 2007. Food and Agriculture Organization of the United Nations, Rome, Italy.

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

ANNEX 1 – REPORTING

The efficacy trial 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. In principle, the report should provide sufficient information for the reader to exactly repeat the trial.

An indicative list of elements of a *Metarhizium* efficacy trial report is provided below. It will need to be structured and adapted to fit the exact conditions of each trial.

Report element	Minimum information that needs to be described or provided
Introduction	
	Brief introduction about the objectives and the organization of the trial
Site description	
Location	Description of trial location, in particular: ▪ GPS and/or map coordinates ▪ Name of the site, or of the nearest town or village and distance/direction in relation to this town or village
Habitat	Description of terrain and vegetation, in particular: ▪ Dominant species of herbs, shrubs and trees ▪ Average height of the grass/herb layer, shrub layer and tree layer ▪ Patchiness of vegetation ▪ % vegetation cover (overall, within patches, between patches) ▪ State of vegetation (green , drying out, green, etc.) ▪ Degree/form of isolation of the study plots from surrounding habitats (if relevant) *Annex* : (Aerial) photographs of representative parts of the study plots
Locust population	
	Description of the locust population in the study area, in particular: ▪ Species (*Schistocerca gregaria*) ▪ Type of population (hopper bands, diffuse population, etc.) ▪ Population structure and stages of the insects (just before the application, and at various intervals after this during the observations) ▪ Hopper band sizes and approximate densities ▪ Details on band behaviour (e.g. speed of band displacement) *Annex* : Photographs of representative hopper bands
Pesticide	
	Description of the composition and quality of the pesticide, in particular: ▪ Species and isolate (*Metarhizium anisopliae* var. *acridum* – isolate IMI330189 ▪ Trade name ▪ Manufacturer ▪ Batch number and formulation or expiry date ▪ Formulation concentration (g conidia/litre and number of conidia/litre) ▪ Physical state of commercial product before dilution (e.g. presence of caked deposit in container) ▪ Short description of itinerary of the product from the manufacturer to the field site (mode of transport, duration). ▪ Status of temperature indicator on the container (if present) ▪ Diluent ▪ Dilution ratio ▪ Germination rate (commercial formulation – just before treatment) ▪ Germination rate (diluted tank mix – just before treatment) *Annex* : Report of germination test(s)

Report element	Minimum information that needs to be described or provided
Application	
Equipment	Description of the application equipment, in particular: ▪ Brand and type of aircraft or vehicle ▪ Brand and type of atomizer ▪ Name of spray aircraft company ▪ Name of last used pesticide in the equipment ▪ Cleaning procedures of pesticide hopper and spray system
Plot details	Description of the spray plots and control plot(s), in particular: ▪ Plot location (GPS coordinates of all plot corners), of each plot, as defined before the treatments ▪ Distances between plots ▪ Differences in habitat, vegetation or locust populations (if relevant, provide data as listed under *Habitat* and *Locust population* on a plot-by-plot basis) *Annex*: Map, at scale, of study location and plot layout
Application	Description of the application, on a plot-by-plot basis, in particular: ▪ Atomizer settings (variable restrictor unit settings, blade angle, etc.) ▪ Rotational speed of the atomizer (rpm), and how measured or estimated ▪ Forward spraying speed of the sprayer/aircraft (m/sec or km/h) ▪ Track spacing (m) ▪ Emission rate (litres/min), and how measured ▪ Emission height (m) ▪ Number of spray runs ▪ Area actually sprayed (ha) ▪ Coordinates of spray plot corners, or drawing of spray runs and spray plot from the aircraft onboard computer ▪ Total volume sprayed ▪ Nominal area dosage (litres/ha and g conidia/ha) ▪ Measured area dosage (litres/ha and g conidia/ha), and how measured ▪ Problems encountered or other relevant remarks about the application *Annex*: Printouts from onboard GPS-based track guidance system and flow meter
Droplet deposition	Description of droplet deposition estimates (if applicable), in particular: ▪ Size and position of oil-sensitive cards ▪ Number and location of cards ▪ Concentration of fluorescent dye ▪ Method of droplet density estimation *Annex*: Photographs of oil sensitive papers
Meteorological conditions	Description of the meteorological conditions during the application, in particular: ▪ Temperature (at start and at end of application) ▪ Relative humidity (at start and at end of application) ▪ Wind direction (relative to spray tracks) ▪ Wind speed (range and average) at start, during and at end of application, at standard height (~ 2 m) ▪ Cloud cover (estimated %) ▪ UVB radiation intensity ▪ Rainfall (mm) on day of application
Effect assessments	
Monitoring of hopper bands (if applicable)	Description of the monitoring of the individual hopper bands, in particular: ▪ Number of bands monitored per plot ▪ Frequency and type of observations carried out ▪ Estimates of size (m^2) and density (#/m^2) of the bands, and their location (GPS coordinates), just before spraying and at subsequent visits after spraying ▪ Population composition and overall band population estimates (#/band) for each

Report element	Minimum information that needs to be described or provided
	band, over time ▪ Merging or splitting up of bands (if applicable) ▪ Timing of immigration of untreated bands and emigration of treated bands, from the Sprayed plots ▪ Behaviour and activity of the insects ▪ Indications of predation ▪ Statistical methods applied (if applicable) *Annex*: Maps of band locations; photographs of bands (for density estimates); raw count data
Presence/absence sampling (if applicable)	Description of the presence absence sampling of hoppers along transects, in particular: ▪ Frequency of sampling (before and after spraying) ▪ Sampling method (length and number of transects per plot; number of sampling points in the transect; surface are of the sampling point; distance between sampling points) ▪ Density categories of hoppers ▪ Overall percentage band infestation, over time ▪ Weighted percentage band infestation (if density categories are applied), over time ▪ Statistical methods applied (if applicable) *Annex*: Maps of transect locations and sampling points; photographs of density categories; raw count data
Caging	Description of mortality assessments by caging hoppers, in particular: ▪ Method, location and timing of capture of the insects ▪ Procedures for cleaning sweep nets ▪ Number and stages of insects placed in each cage ▪ Number of cages prepared per sampling date ▪ Type of vegetation placed in cage (sprayed/unsprayed, origin of vegetation) ▪ Location of cages (field/under roof; full sun/shade; possibility to thermoregulate) ▪ Temperature and RH during the caging period ▪ Duration of the caging period ▪ Daily counts of number of dead/alive insects ▪ Number of insects incubated for sporulation assessments ▪ Method of sporulation incubation (humidity " regulation" ; storage conditions of Petri dishes; maximum number of days of incubation) ▪ Behavioural abnormalities ▪ Observations of predation/scavenging *Annex*: Raw count data; photographs of cage location
Persistence bioassay	Description of the bioassay to asses persistence of spore viability, in particular: ▪ Method, location and timing of capture of the untreated insects ▪ Location and model of cages in the treated and control plots ▪ Method used to limit predation (e.g. by ants) inside cages ▪ Number and stages of insects placed in each cage ▪ Timing of the start of the bioassays (days after treatment) ▪ Number of cages prepared per bioassay date ▪ Temperature, RH and rainfall during bioassay period ▪ UVB-radiation readings during bioassay period ▪ Daily counts of number of number of dead/alive insects ▪ Number of insects incubated for sporulation assessments ▪ Method of sporulation incubation (humidity " regulation" ; storage conditions of Petri dishes; maximum number of days of incubation) ▪ Behavioural abnormalities ▪ Observations of predation/scavenging *Annex*: Raw count data; photographs of cages

Report element	Minimum information that needs to be described or provided
Meteorological conditions	Description of the meteorological conditions during the various mortality assessments, in particular: ▪ Method, frequency and readings for: ambient temperature, relative humidity, rainfall, UVB-radiation; cloud cover, wind direction and speed. *Annex*: Raw data
Other observations	Description of any other observations relevant for the efficacy assessment, in particular: ▪ Cadaver observations in the field and incubation for sporulation assessment ▪ Behavioural abnormalities of the insects ▪ Increased predation on the insects *Annex*: Photographs, raw data
Discussion	
	Discussion of the results of the trial, in particular the observed efficacy at the applied dose rates. Evaluation of the methodology that was used and its possible implication on the conclusions of the trial, in particular if the observed efficacy is likely to be an underestimate or overestimate of "real" operational conditions. Assessment of any problems or limitations that were encountered and their possible consequences for the conclusions of the trial. Recommendations for modifications in the future trial setup or execution.

ANNEX 2 – TIMELINE

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

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

The preparatory meeting with national locust control organization 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 Trial organizer and the national coordination/NLCO

ANNEX 3 – INDICATIVE CALCULATIONS FOR FLYING HOURS

Plot size (ha)	**Volume tank mix needed**	**Volume GM concentrate needed**
400 (2x2 km)	400 L	40 L

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	0.8	1.2	2.0
Air Tractor 401B	1500	1	0.8	1.2	2.0
Ag Truck 188	280	2	0.8	2.5	3.3

Plot size (ha)	**Volume tank mix needed**	**Volume GM concentrate needed**
900 (3x3 km)	900 L	90 L

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**
1200 (3x4 km)	1200 L	120 L

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

* 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 4 – RESOURCES AND EQUIPMENT FOR AN EFFICACY TRIAL WITH *METARHIZIUM* AGAINST THE DESERT LOCUST

The list below is based on a trial with three treated plots and one unsprayed plot.

Item	Number / quantity	Remarks
Local trial preparation		
4x4 vehicle & fuel	1	
Maps	PM	
GPS	1	
Digital camera	1	
Pesticide application		
Flying hours - (incl. fuel & logistics)	PM	
Green Muscle OF	PM	
Diesel fuel or kerosene for triple rinsing aircraft hopper and spray system	PM	
Diesel fuel for mixing insecticide	PM	
Small truck for transport of pesticides	1	
4x4 vehicle & fuel	2	Minimum; for the larger plots 4 vehicles may be required
Clean mixing drum(s)	2	
Electrical mixer	1	
Calibration equipment	1	Buckets, funnels, measuring cylinders, stopwatch, tachometer
Droplet deposition equipment	1	150 sticks; 3 packages of 50 sheets of oil sensitive paper; double sided tape; 500 g UVITEX OB; "Bateman" droplet gauge; UV lamp
Meteorological equipment	1	Anemometer; thermo-hygrometer; rain gauge; UVB-meter
Maps	PM	
GPS	2	
Compass	2	
Short range VHF or UHF walkie talkies	5	Communication with aircraft and among ground staff
Long range HF radio	1	Communication with aircraft
Flagging material & poles	1	Track marking (optional; depending on aircraft navigation equipment and level of air-ground communications)
Digital camera	1	
Germination tests	PM	10 glass wide-mouth sample bottles (100 ml); sampling device Tests to be done by laboratory with proven experience.
Personal protective equipment		
Overalls (cotton)	10	
Disposable coveralls (Tyvek)	6	
Nitrile gloves (pair)	12	
Protective glasses	2	

Item	Number / quantity	Remarks
Boot covers (pair)	12	
Emergency eye wash bottle	1	
Half-mask respirator with cartridges	2	
Washing materials	PM	Soap, jerry cans, clean water
Efficacy monitoring		
4x4 vehicles & fuel	4	
HF/UHF radios (in vehicles)	4	
Walkie-talkies VHF	8	
Cages	~160	~100 cages for mortality & ~64 cages for persistence (local production)
Sweep nets & replacement nets	8 & 24	
Bleach water to sterilize nets	PM	
Data loggers (temperature, RH)	2	Including software & cables to link to notebook computer
UVB-meter	1	
Infrared thermometer	1	Optional: measurement of surface temperature
Rain gauge, anemometer	1	
GPS	4	
Binoculars	4	
Digital cameras	4	
Tally counters	4	
Tape measure (50 m)	4	
Dissection set	1	
Fluorescent marking ribbons	20 rolls	Different colors
Petri dishes for sporulation assessment & filter paper & adhesive tape	500	
General equipment		
Notebook computer, printer & mapping software	1	
Small portable generator	1	
Mobile telephone or satellite telephone	1	Satellite telephone if local network does not provide coverage
Transport cases for equipment	PM	
Spare batteries for various equipment	PM	
Duck tape, cords	PM	
Tool set	1	
Camping equipment		
Large tents	4	
Camping beds	15	
Cooking material	PM	If required
Water jerry cans/drums	10	
Gas lamps + bottles	4	
Folding tables & chairs	15	

ANNEX 5 – INDICATIVE SAMPLING REGIME

Presuming 3 replicate treated plots (A, B & C); 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	A	B D	C 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	A	B D	C 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 assessment in cages				A D	B	C D	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	A	B D	C													
Number of vehicles in the field	2	4	4	4	4	4	3	3	3	2	2	3	2	2	3	2	2	3	2	2	3	2	2	3	2	2	3	2
Number of monitoring staff in the field	2	4	4	4	4	4	3	3	3	2	2	3	2	2	3	2	2	3	2	2	3	2	2	3	2	2	3	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

Presuming 2 replicate treated plots (A & B); 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 assessment in cages				A D	B		A D	B																				
Caging for persistence/secondary pick-up		A	B	D	A	B	D	A	B	D	A	B	D	A	B	D												
number of vehicles in the field	2	3	2	3	2	3	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	3	2	3	2	3	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