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DESERT LOCUST TECHNICAL SERIES

Review of the efficacy of *Metarhizium anisopliae* var. *acridum* against the Desert Locust

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Review of the efficacy of *Metarhizium anisopliae* var. *acridum* against the Desert Locust

by

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

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1 Introduction

The management of the Desert Locust is still mainly based on control of gregarious adults and hoppers with chemical synthetic insecticides. Concern about the environmental and human health effects of the large quantities of these insecticides used in the late 1980s sparked renewed interest in the development of biological control options. One of the approaches that were researched was the use of entomopathogenic fungi, the most promising of which appeared to be belonging to the genus *Metarhizium* (Deuteromycotina: Hyphomycetes) (Lomer *et al.*, 2001).

Based on screening of large number of isolates, the isolate IMI 330189 of *Metarhizium anisopliae* var. *acridum* Driver & Milner (Driver *et al.*, 2000) was selected for development as a biopesticide against the Desert Locust (*Schistocerca gregaria*), the Red Locust (*Nomadacris septemfasciata*) and various species of African grasshoppers. This isolate is now commercialized under the name Green Muscle™. Additional isolates were developed against other species of locusts and grasshoppers, such as the isolate FI-985 (Green Guard™) against Australian Plague Locust (*Chortoicetes terminifera*), Migratory Locust (*Locusta migratoria*) and various other species, isolate I91-609 against Variegated Grasshopper (*Zonocerus variegatus*), or isolate CG 423 against Mato Grosso Grasshopper (*Rhammatocerus schistocercoides*).

The efficacy and use of *Metarhizium*[1] for locust and grasshopper control has been reviewed in the past for Africa (e.g. Douthwaite *et al*., 2001; Lomer *et al.*, 2001), Australia (e.g. Milner & Hunter, 2001; Hunter, 2005) and Brazil (Magalhães *et al*., 2001). The underlying study is different in the sense that it attempts to bring together all the field efficacy trials that were carried out with *Metarhizium anisopliae* var. *acridum* on all species of locusts and grasshoppers. The review can be seen as a meta-analysis of these trials, which breaks down the reports of the individual studies to the lowest feasible experimental units (i.e. an individual sprayed plot, or a set of plots if only averaged results were reported).

The review concentrates on the efficacy of isolate IMI 330189 against the Desert Locust. Whenever possible, results from trials against other species, and with different isolates, will be extrapolated to the Desert Locust.

The review focuses mainly on direct mortality due to *Metarhizium*. Potential effects of the pathogen on reproduction, feeding reduction or higher mortality due to increased predation are not explicitly evaluated. Furthermore, the efficacy of *Metarhizium* is reviewed in its isolation, presuming that the product will be used alone, and not in combination with other pest management techniques. Obviously, this does not exclude that *Metarhizium* will be used as part of integrated pest management against the Desert Locust, or against other species of locusts and grasshoppers (e.g. Lomer *et al.*, 1999; Hunter, 2005).

Chapter 2 of this report describes the data collection process for the review, and discusses data quality. A review of all Desert Locust field efficacy trials is provided in Chapter 3. In Chapter 4, field trials with other species of locusts and grasshoppers and other isolates of *Metarhizium* are discussed, as far as they provide information relevant for Desert Locust control. A brief discussion of the findings can be found in Chapter 5, while recommendations for future work are summarized in Chapter 6.

An initial version of this review was presented at the *Workshop on the future of biopesticides in Desert Locust control*, which was held from 12 – 15 February 2007 in Saly, Senegal (FAO, 2007).

[1] In the rest of this report, any sole mention of *Metarhizium* always refers to *Metarhizium anisopliae* var. *acridum*

2 Data collection and analysis

2.1 Data collection

Published reports and journal publications of field efficacy trials of *Metarhizium anisopliae* var. *acridum*[2] against locusts and grasshoppers were compiled, irrespective of the isolate of the pathogen or the species of locust or grasshopper involved. Only studies with oil-based formulations were assessed; water-based or water-emulsion formulations, or baits, were not reviewed since these are unlikely to be used for Desert Locust control. Trials in which locusts were sprayed and contained in large field cages (or "bomas") were not included in the review either.

A first list of 38 reports was prepared, based on a detailed literature search, and sent to experts in the field of biological control of locusts and grasshoppers for comments, amendments and additions (Annex 1). Bibliographic references in the collected reports and journal articles were also checked for possible omissions. This process yielded a total of 45 reports, covering 61 different field efficacy trials (Annex 2).

Field trials with nine different *Metarhizium* isolates were reported. More than half of the field trials were carried out with the African isolate IMI 330189, followed by the Australian isolate FI-985 and the African isolate I91-609 (Fig. 2.1).

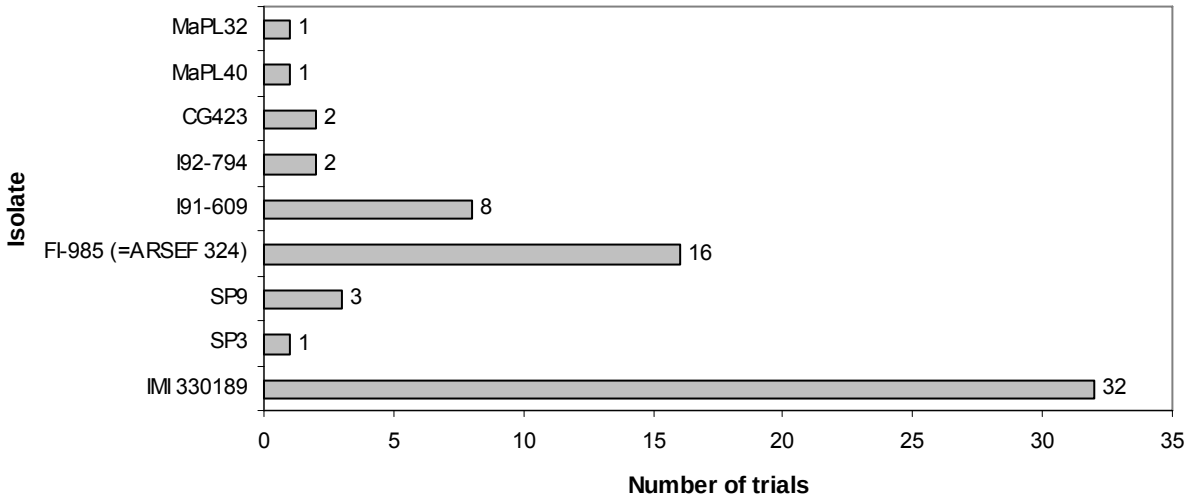

Figure 2.1 Isolates of *Metarhizium anisopliae* var. *acridum* used in the field efficacy trials carried out on locusts and grasshoppers.[3]

About 40% of the field trials were carried out on African grasshoppers, such as *Oedaleus senegalensis*, *Hieroglyphus dagenensis* and *Zonocerus variegatus*. Approximately half of the trials were done on locusts, nine of which on the Desert Locust (Fig. 2.2). Roughly 70% of the field efficacy trials were carried out in Africa, about a quarter in Australia/Asia and three trials were reviewed from South America (Fig. 2.3).

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[2] This includes all reports referring to *Metarhizium flavoviride*, the name that was used for the fungus before the taxonomic revision by Driver *et al.* (2000).

[3] The total number of trials in the graph (66) is more than the 61 reviewed because in certain trials more than one isolate was tested.

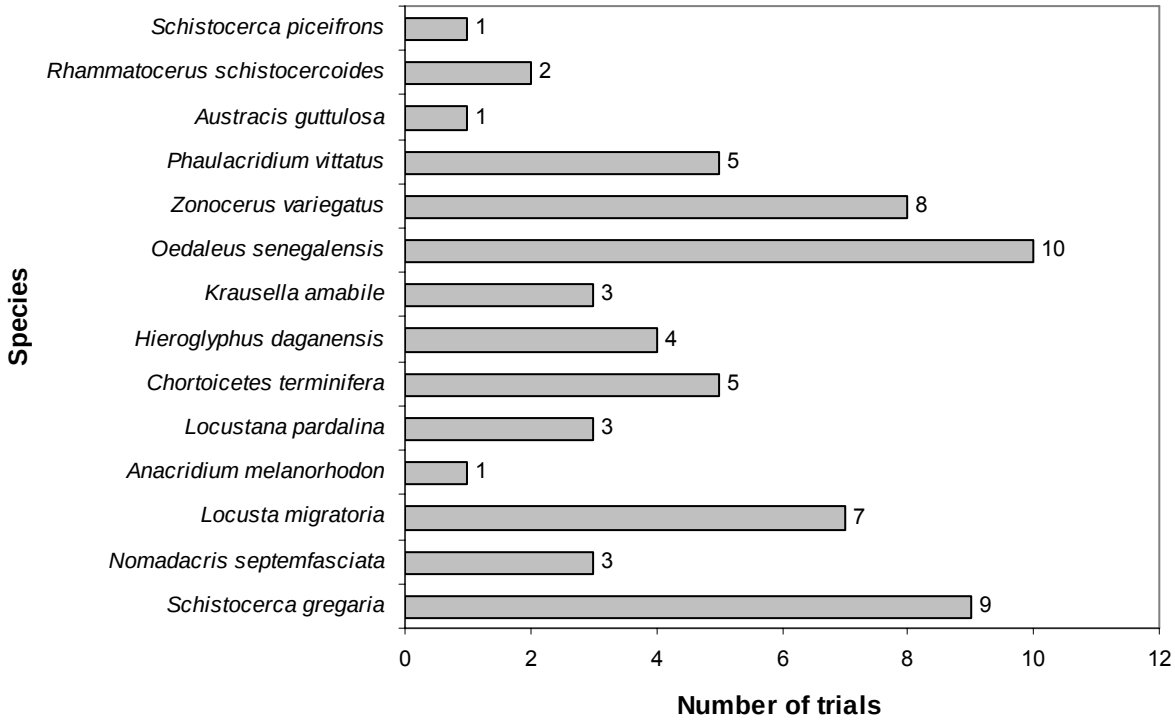

Figure 2.2 Locust and grasshopper species covered by the field efficacy trials carried out with *Metarhizium anisopliae* var. *acridum*.[4]

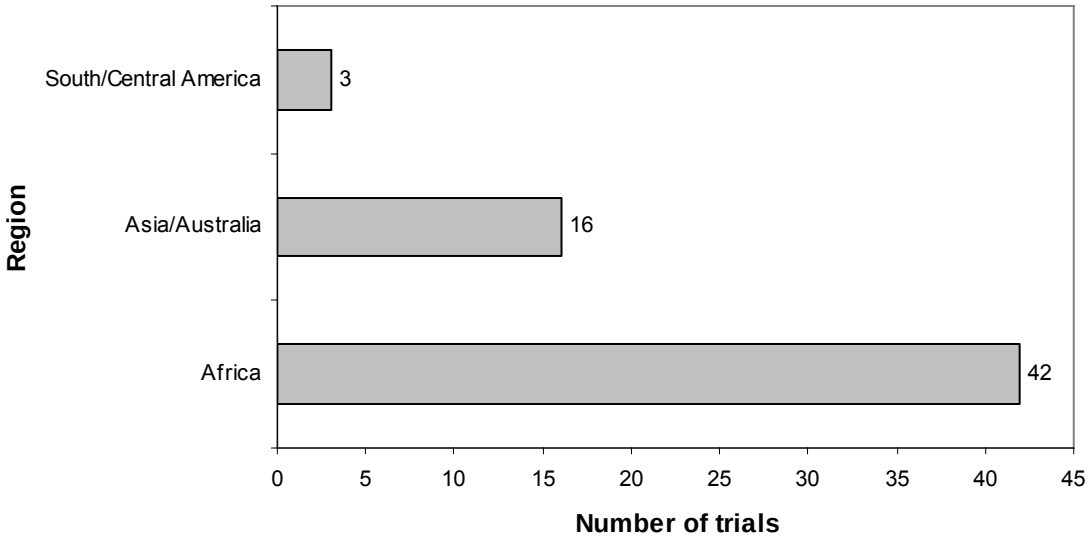

Figure 2.3 Regional distribution of field efficacy trials carried out with *Metarhizium anisopliae* var. *acridum* against locusts and grasshoppers.

[4] The total number of trials in the graph (62) is more than the 61 reviewed because in certain trials the effect of *Metarhizium* on more than one species was evaluated.

Most of the efficacy trials were done on small spray plots (< 4 ha); only 16% of the trials were relatively large scale applications (> 100 ha) of the entomopathogen (Fig. 2.4)

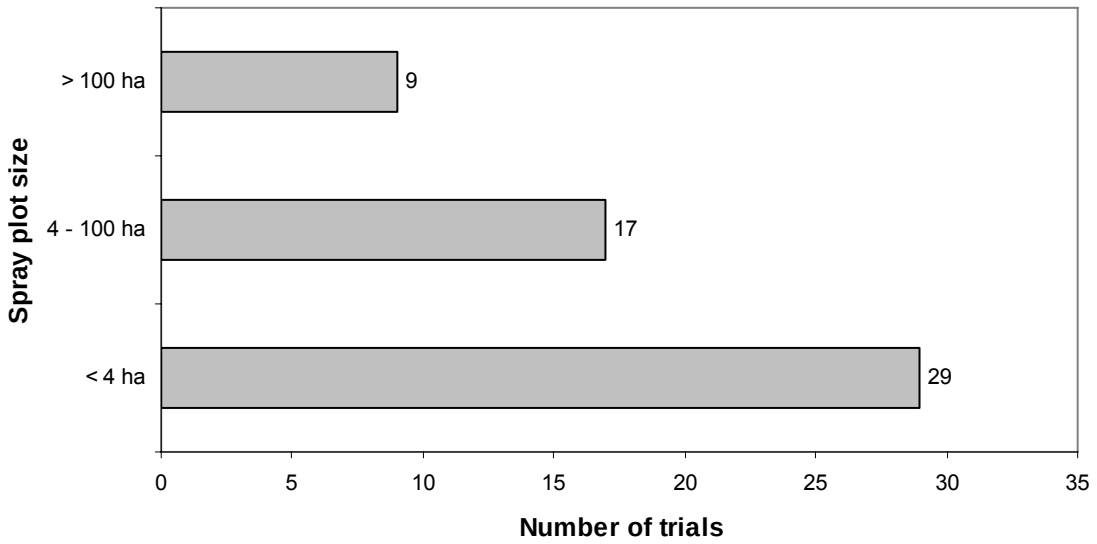

Figure 2.4 Sizes of the spray plots used in the field efficacy trials carried out with *Metarhizium anisopliae* var. *acridum* against locusts and grasshoppers.[5]

2.2 Evaluation of the quality of the studies and reports

All field trial reports were reviewed to assess the quality of the experimental design, pesticide application procedures, mortality assessment methods and reporting. A summary of the principal parameters and results of all trials is provided in a spreadsheet, which is available on request.

A number of criteria were used to evaluate trial quality. They are based on the *FAO Guidelines for pesticide trials on Desert Locust hoppers* (FAO, 1991), meeting reports of the FAO Pesticide Referee Group, and the Lubilosa Project *Insect pathology manual* (Lomer & Lomer, undated). A more recent guideline for *Metarhizium* trials on the Desert Locust (FAO, 2005) was not used since most studies were carried out before its publication. However, since this latest guideline was to a large extent based on the previous documents, trial design criteria are very similar.

Since it became rapidly clear that none of the trial reports would comply with the full set of recommendations for field efficacy trials made in these sources, a more limited selection of criteria was applied for the review. These minimum requirements for field efficacy trials with microbial insecticides, and data requirements for the subsequent reporting, are summarized in Table 2.1.

The criteria were used in a relatively flexible manner, and studies that did not fulfil all criteria were not automatically excluded from the review. It was attempted to assess the effect that non-fulfilment of a criterion would have on the results of the trial. For instance, the minimum plot sizes in Table 2.1 have been defined to ensure that an even pesticide deposition plateau would be reached on most of the trial plot following ULV drift-spraying. Application on smaller plots will likely lead to under-dosing, which can be taken into account when interpreting the results of a trial.

[5] The total number of trials in the graph (55) is less than the 61 reviewed because spray block sizes were nota always reported.

Table 2.1 Quality criteria for the evaluation of the field efficacy trials with *Metarhizium anisopliae* var. *acridum* against locusts and grasshoppers, applied in the review.

Criteria	Requirement for:		Comment	Ref. [1]
	Trial setup	Reporting		
Trial design				
Untreated control plot(s) used	Conditional		if field population assessments are carried out	i.
Untreated control cage(s) used	Conditional		if cage mortality assessments are carried out	i.
Minimum plot sizes:				
Hand held sprayer: 1 ha	Always			i., [iii.]
Vehicle mounted sprayer: 4 ha	Always			i., iii.
Aircraft: 100 ha	Always			i., iii.
Minimum plot separation:				
Hand held sprayer: 100 m	Always			i., iii.
Vehicle mounted sprayer: 200 m	Always			i., iii.
Aircraft: 500 m	Always			i., iii.
Environmental conditions				
Vegetation cover & height reported		Conditional	For review of vegetation effect	i.
Daily max. and min. temperatures over observation period reported		Always		i.
Rainfall reported		Conditional	For review of persistence	i.
Insects				
Species reported		Always		i.
Stage(s) reported		Always		i.
Insecticide				
Isolate reported		Always		iii.
Germination rate just before/after application reported		Always		iii.
Formulation composition reported		Always		iii.
Application				
Sprayer with rotary atomisers	Always			i.
Calibration details reported		Always		i.
Nominal droplet size (VMD) 50 – 100 µm	Always			ii.
Volume application rate 1 – 3 L/ha (or down to 0.5 L/ha if low vegetation density)	Conditional		Higher rates acceptable for trial, but not practical for operational use.	ii.
Wind speed > 1m/s	Always			i.
Area dosage measured		Conditional	Unless area dosage can be calculated from the reported application parameters	i.
Detailed application parameters reported		Conditional	If area dosage not measured	i.
Assessment				
Method of field assessment reported		Conditional	if field population assessments are carried out	i.
Method of caging reported		Conditional	if cage mortality assessments are carried out	i.
Timing of sampling for caging reported		Conditional	if cage mortality assessments are carried out	iii.

Criteria	Requirement for:		Comment	Ref. [1]
	Trial setup	Reporting		
Type of vegetation (sprayed/ unsprayed) provided in cages reported		Conditional	if cage mortality assessments are carried out	iii.
Cage control mortality <40 % at time of reported treatment mortality.	Always		Treatment mortality to be corrected by control mortality if latter >10%	

[1] Sources of the criteria: i. FAO (1991); ii. Pesticide Referee Group (various sessions); iii. Lomer &Lomer (undated)

Similarly, if an untreated control plot was not present in the study, the data on field population densities were not included in the review; however, cage assessments would still be considered as long as control cages with unsprayed locusts/vegetation were used in the study. In such a manner, the maximum of information was extracted from the trials, even if not all criteria were fulfilled.

A summary of the quality assessment of all trials is provided in Annex 3. None of the trials/reports satisfied the minimum criteria listed in Table 2.1. Only five out of the total 61 trials satisfied, and an additional seven partially satisfied, 80% or more of the criteria. Five studies did not satisfy, or did not report on, 75% or more of the criteria that were required for the respective trials, and they were excluded from the review. One additional study was excluded from the review because the formulating oil was found to be toxic to *Metarhizium*. A further 12 studies did not satisfy, or did not report on, 50% – 75% of the criteria that were required for the respective trials; they were evaluated with particular scrutiny.

It should be underlined that failure to satisfy the quality criteria was often due to insufficient reporting but not necessarily to inappropriate trial design. Thus the quality of the trial may have been acceptable, but this could not be confirmed from the trial report.

A partial explanation why the minimum criteria were not satisfied may lie in the publication policy of certain scientific journals, which do not always accept descriptions of study methodology, experimental conditions or trial results at the level of detail needed for a proper evaluation. However, insufficient reporting was also regularly encountered in trial reports that were not published in the scientific literature, and should not have encountered such publication restrictions.

Information that was in particular lacking in many reports includes the environmental conditions (e.g. vegetation cover and height in the plot, ambient temperature throughout the study), sprayer calibration and pesticide application details, and the caging methodology and conditions used in the cage mortality assessments. Regular shortcomings in the trial design include small spray plots, the lack of verification of spore viability at the time of the treatment, and the absence of the measurement of the actual application rate. Another problem for the optimal use of the data was that sometimes average mortalities or population densities were reported of all plots in a given treatment, rather than for individual plots. This reduces the number of available data sets and limits the possibility to reduce variance in the results or evaluate correlations.

Overall, the reporting of the efficacy trials was unsatisfactory. As a result, it was not possible to assess the quality of many studies in sufficient detail. This should be taken into account when evaluating the results of the studies.

2.3 Data analysis

For the field efficacy trials, control corrections for population fluctuations in the untreated plots were made using Henderson and Tilton's (1955) formula. For the cage incubation assessments, percentage maximum mortality was corrected for control cage mortality using either Abbott's formula (Abbott, 1925) or Schneider-Orelli's (1947) formula, depending on the format of the data. The *LdP Line* online control correction web page was used for these calculations (http://www.ehabsoft.com/ldpline/onlinecontrol.htm).

Median lethal times (MLT) reported in this review were either calculated by the author of the trial report or, in the absence of such a calculation, estimated by the reviewer through linear extrapolation of the data in the graph or table presented in the trial reports. Time to 90% mortality (LT_{90}) was always estimated by the reviewer. Whenever the reviewer estimated the MLT or LT_{90}, no statistical models were used to determine the precision of these estimates.

Average survival times (AST) reported in this review were always calculated by the author of the trial report, generally by survival analysis such as the Kaplan-Meier method.

In all laboratory studies where a median lethal dose (LD_{50}) could be calculated, but was not reported by the author of the trial, the LD_{50} values were estimated by the reviewer with the non-parametric Trimmed Spearman-Karber method (Hamilton *et al.,* 1977), using the *TSK – version 1.5* computer programme (US Environmental Protection Agency – http://www.epa.gov/EERD/stat2.htm)

All other statistical assessments were carried with the *XLSTAT* package, versions 2007.3 & 2007.4 (Addinsoft, New York, USA).

3. Desert Locust trials

3.1 Study quality

In total, nine field efficacy trials on the Desert Locust (*Schistocerca gregaria*) were obtained for the review, described in eight different reports (Table 3.1). The studies were carried out in the period from 1995 to 2005. A study done in Mauritania in 2006 was not yet reported at the time of the review, and was therefore not included.

Table 3.1 Field efficacy trials carried out against the Desert Locust (*Schistocerca gregaria*) with *Metarhizium anisopliae* var. *acridum* (isolate IMI 330189) that were reviewed.

Country	Locality	Year of trial	Type of target	Inclusion in review	Trial no. [1]
Mauritania	Oued El-Kharob	1995	Hopper bands & fledglings	yes	1
Mauritania	Boumdeid	n.r. [2]	Hopper bands	yes	2
Mauritania	Tin-Ouich	1995	Hopper bands	yes	3
Mauritania	Tijirit	2003	Hopper bands	yes	65
Niger	Agagala	2003	Hopper bands & fledglings	yes	4
Sudan	Aeit1	n.r.	Hopper bands	no	5
Sudan	Aeit2	n.r.	Hopper bands	no	6
Algeria	Oum et Thiour	2005	Hopper bands	yes	7
Niger	Aghéliough	2005	Adults	yes	8

[1] Trial number as listed in Annex 2 (note that more than one trial may have been described in a study report, and they have been numbered separately).

[3] n.r. = not reported.

The quality assessment of the studies and their reports, in line with the criteria listed in Table 2.1, showed that none of the trial reports fulfilled all these criteria (Table 3.2 and Annex 3). The application of the pesticide was often not described in sufficient detail to make a complete assessment of the quality of the treatment. The pesticide application rate was often not measured, but based on the sprayer calibration without subsequent confirmation. Germination rates of the pathogen just before or after the applications were sometimes not verified, in spite of transport and storage of the product in (apparently) hot conditions which may have reduced spore viability. Furthermore, the description of the cage assessment methods that were used in the trials was often lacking detail, in particular whether the treated locusts were caged with treated or untreated vegetation.

Based on this quality assessment, the two trials in Sudan (trials no. 5 & 6) were excluded from the review, because reporting on the whole was insufficient. The seven other trials were included, in spite of certain omissions in reporting and problems with the trial setup or execution. However, it was attempted to take these shortfalls into account in the interpretation of the results of the trials.

Table 3.2 Quality assessment of the Desert Locust field efficacy trials and their reporting.

Country	Trial no. [1]	Degree of fulfilment quality criteria of the trial and the report [2]					Important omissions
		Fulfilled	Fulfilled in part	Not fulfilled	Not reported	Not applicable	
Mauritania	1	75%	13%	0%	13%	0%	Spray plot size not reported; application details incomplete; application rate not measured; description of cage assessment method incomplete
Mauritania	2	54%	17%	13%	13%	4%	Certain spray plots too small; application details incomplete; application rate not measured; description of cage assessment method incomplete.
Mauritania	3	71%	21%	0%	8%	0%	Spray plots too small; application details incomplete; application rate not measured.
Mauritania	65	75%	13%	13%	0%	0%	Application details incomplete; description of cage assessment method incomplete.
Niger	4	71%	17%	13%	0%	0%	Germination rate not verified; description of cage assessment method incomplete.
Sudan	5	17%	50%	4%	17%	13%	Germination rate not verified; no application details; no description of cage assessment method; no description of environmental conditions.
Sudan	6	17%	50%	4%	17%	13%	Germination rate not verified; no application details; no description of cage assessment method; no description of environmental conditions.
Algeria	7	67%	21%	8%	4%	0%	Locust stage not reported; VMD too large for the aerial applications; application rate not measured; description of environmental conditions incomplete.
Niger	8	83%	8%	4%	0%	4%	No control plot used.

[1] Trial number as listed in Annex 2

[2] Criteria as listed in Table 2.1

3.2 Summary of the studies

A short narrative summary of the six Desert Locust efficacy trials that were retained for the review is provided below. More detailed data tables of all the trials can be found in Annex 4.

All studies below were carried out with oil-based formulations of *Metarhizium anisopliae* var. *acridum*, isolate IMI 330189.

3.2.1 Trial no. 1 – Mauritania, 1995 (Oued-El Kharob)

The study by Langewald (1995) concerned the treatment of three late instar hopper bands and one group of immature adults in north-eastern Mauritania. Treatments were carried out with hand-held ULV sprayers at a nominal dose rate of 5×10^{12} conidia/ha. Spray plot sizes were not reported. Temperatures ranged from 17 to 34 ºC during the observation period.
Mortality was assessed through incubation of treated locusts in cages that were placed in the field. It was not reported whether this vegetation had been treated with the pathogen. Samples of hoppers taken a few hours after treatment reached a maximum control-corrected cumulative mortality of 95% at 17 days after treatment; this was 100% for the immature adults at 11 days

after treatment. Average survival times calculated by the author of the study were 8.8 days for the hoppers and 8.7 days for the immature adults. The median lethal times obtained from the survival curves were 8 days for both locust stages. Samples taken 3 or 7 days after treatment showed considerably less mortality than the day-0 sample.

The development of hopper bands was also monitored in the field. The median number of insects per hopper band showed a control-corrected reduction of 88% after 9 days of observation, after which monitoring was stopped because the locusts started fledging in both treated and control bands.

3.2.2 Trial no. 2 – Mauritania (Boumdeid)

Kooyman and Godonou (1997) report the treatment of four mid-instar hopper bands in south-eastern Mauritania. Treatments were carried out with hand-held ULV sprayers at a nominal dose rate of 5×10^{12} conidia/ha. Treated hopper bands varied in size from 0.01 to 2.5 ha and the sprayed plot sizes were slightly larger. Temperatures ranged from 20 to 40 ºC during the observation period.

Mortality was assessed through incubation of treated locusts in cages that were placed in the full sun. It was not reported if the vegetation that was fed to the insects had been treated or was unexposed to *Metarhizium*. Samples of hoppers taken after treatment reached a maximum cumulative control-corrected mortality of 100% at 15 – 22 days after the applications for all hopper bands. The time after treatment when hoppers were sampled was not reported. Average survival times calculated by the author of the study ranged from 7.2 to 9.8 days; they were 18.5 days for the untreated controls. The median lethal times obtained from the survival curves ranged from 7.4 to 10 days.

3.2.3 Trial no. 3 – Mauritania, 1995 (Tin-Ouich)

The study reported by Langewald *et al.* (1997a) refers to the treatment of three early instar hopper bands in south-western Mauritania. *Metarhizium* was applied using hand-held ULV sprayers at a nominal dose rate of 5×10^{12} conidia/ha. Spray plot sizes varied from 0.3 to 0.65 ha. Temperatures ranged from 16 to 40 ºC during the observation period.

Locusts were sampled from treated and control bands one hour after application and incubated in cages that were placed in the full sun. It was not reported if the vegetation that was fed to the insects had been treated or was unexposed to *Metarhizium*. The insects showed an average maximum control-corrected cumulative mortality of 99%, 18 days after treatment. The average survival time calculated by the author was 7.5 days. The median lethal time obtained from the survival curves was 6 days. Samples that were taken 3, 6, 9 or 12 days after treatment showed considerably less mortality than the day-0 sample.

The development of one hopper band could be followed in the field. It showed a control-corrected reduction in total population size of 68% at 7 days after treatment.

3.2.4 Trial no. 4 – Niger, 2003 (Agagala)

Aston (2005) reports on a trial carried out in central Niger on a mid- and late instar hopper population. Treatments were carried out with a vehicle-mounted ULV sprayer at a measured dose rate of 3×10^{12} conidia/ha. One plot of 245 ha was sprayed. Temperatures in the trial area ranged from 9 to 26 ºC during the observation period.

Mortality was assessed through incubation of treated locusts in cages that were placed in the full sun in the field. It was not reported if the vegetation that was fed to the insects had been treated or was unexposed to *Metarhizium*. Samples of hoppers taken two hours after treatment reached a

maximum control-corrected cumulative mortality of 74% at 22 days after treatment. Locusts sampled either soon after treatment (day-0 and day-1) or later (up to 12 days after treatment) showed less cumulative mortality than the sample taken two days after the *Metarhizium* application. The median lethal time estimated from the survival curve was 16 days.

Assessments of the density of hoppers, and later of fledglings appearing in the plot, were also carried out. The hopper population in the sprayed plot showed a relative population increase compared to the control of 470% by the end of the observations at 20-22 days after treatment. The density of fledglings, however, was reduced by 24% when compared to the control.

3.2.5 Trial no. 5 – Sudan (Aeit 1)

Trial not included in the review because of incomplete reporting

3.2.6 Trial no. 6 – Sudan (Aeit 2)

Trial not included in the review because of incomplete reporting

3.2.7 Trial no. 7 – Algeria, 2005 (Oum et Thiour)

A trial carried out on hopper bands (stages not reported) in north-east Algeria was reported by Kooyman *et al.* (2005). Two 700 ha plots were treated by air at a nominal dose rate of 2.5×10^{12} conidia/ha. An additional 4 plots of 25 ha were sprayed with a vehicle-mounted ULV sprayer at the same nominal dose rate. Temperature conditions during the observation period ranged from 19 to 37 ºC.

Locusts were sampled from treated and control bands three days after application and incubated on untreated vegetation in cages that were placed in the shade. The insects showed maximum control-corrected cumulative mortalities ranging from 59% to 94%, 7 days after treatment. The median lethal time estimated from the survival curves ranged from 4.8 to 5.9 days.

A number of hopper bands were followed in the field and the effect of treatments on total population sizes assessed. Four of the five monitored bands showed 100% population reduction within 5-6 days after treatment; the fifth had a control-corrected reduction of 74%, 13 days after treatment.

3.2.8 Trial no. 8 – Niger, 2005 (Aghéliough)

Ouambama *et al.* (2006) carried out a trial in west-central Niger on an adult Desert Locust population. A plot of 492 ha was aerially treated at a measured dose rate of 5.9×10^{12} conidia/ha. Temperature conditions during the observation period ranged from 16 to 34 ºC.

Locusts were sampled 1 day after treatment and incubated in cages that were placed outside the field base in the shade. They were fed unsprayed vegetation. These insects showed a maximum control-corrected cumulative mortality of 100%, 10 days after treatment. The median lethal time obtained from the survival curve was 7 days. Samples that were taken 4, 8, 12, 16 or 20 days after treatment showed similar or less mortality than the day-1 sample.

Field population density was also followed in the treated plot, but since no untreated control plot was available for monitoring, these data were not included in the review.

3.2.9 Trial no. 65 – Mauritania, 2003 (Tijirit)

Ould Taleb (2004) reports a field trial carried out in central Mauritania on mid-instar hopper bands. A plot of 400 ha was treated with an UlvaMast sprayer at a measured dose rate of 1.1 x 10^{12} conidia/ha. Temperature conditions during the trial ranged from 15 to 29 ºC.

Locusts were sampled from treated and control bands two hours after treatment, and subsequently on a daily basis for 23 days. Insects were incubated in cages that were placed in the shade under a tent in the field. It was not reported if the vegetation that was fed to the insects had been treated with *Metarhizium*. The insects caught on the days of treatment showed maximum control-corrected cumulative mortality of 100%, observed at 11 days after treatment. Samples that were taken at later dates showed less mortality than the day-0 sample. The median lethal time estimated from the survival curves was 9.5 days.

Five hopper bands were followed in the field and the effect of treatments on population densities assessed. However, Desert Locust hopper bands may maintain fairly similar densities, even if mortality occurs. Since no measures of hopper band sizes were made, changes in population densities in isolation therefore do not provide a valid indication of the effect of the pathogen. The field density measurements were thus not further used in the review.

3.3 Assessment of field efficacy

The efficacy of a microbial pesticide can be assessed by monitoring the size of field populations of Desert Locust hoppers or adults. However, such assessments are generally cumbersome due to the mobility of the insects and the difficulty of estimating population sizes in a reliable manner. Therefore, insects are generally also captured after treatment and placed in cages for observation of mortality. But cage incubations of treated locusts may not represent field mortality properly. Insects may be stressed in cages, which can increase their susceptibility to pathogens. Infection rates in cages may be different from the situation in the field because the duration and intensity of contact with treated vegetation is not similar. Furthermore, disease development in infected locusts in cages may be either slower or faster than in the field, depending on whether the cages are exposed to field (temperature) conditions or kept in the laboratory.

Combined assessments of field densities and cage mortality are therefore preferred when assessing the efficacy of entomopathogens against the Desert Locust. Table 3.3 shows the results of the seven trials selected in Chapter 3.1, which comprise of a total of 16 data sets. For seven data sets, efficacy was determined in parallel by caging and by field population assessments. In the other cases, only one of these two techniques was applied.

It is generally considered that more than 95% efficacy is considered very good, and less than 90% is inadequate, for Desert Locust field trials with synthetic chemical pesticides (FAO, 1991). If the same criteria are applied to these trials, one treatment showed adequate efficacy in both field and cage assessments. Twelve other treatments resulted in adequate efficacy in one of the two assessments.

Only one trial, carried out in Niger in 2003 (see Chapter 3.2.4), did not show an operationally significant effect of the pathogen on the locust population. No clear reductions in field populations were observed, and the cage mortality did not surpass 74% after 22 days. The reason why efficacy in this trial was relatively low is not clear. The author of the study suggests that the fairly low ambient temperatures during the trial may have slowed down mortality. However, the temperatures still were in the range which is considered favourable for performance of the fungus (see Chapter 3.3). Another possibility is that spore viability was low at the time of treatment in Niger, since it was only 78% when shipped from the manufacturer in South Africa. Reporting of this study was not sufficiently detailed to further interpret the lack of efficacy.

Dose rates in the field trials ranged from 1.1 x 10^{12} to 5.9 x 10^{12} conidia/ha. All these dose rates resulted in adequate control. However, the lowest dose rate tested, of 1.1 x 10^{12} conidia/ha, was applied against early instar hoppers, and only cage incubation efficacy results were available for this trial. Therefore, the minimum effective dose rate that provided "robust" adequate control will be considered 2.5 x 10^{12} conidia/ha.

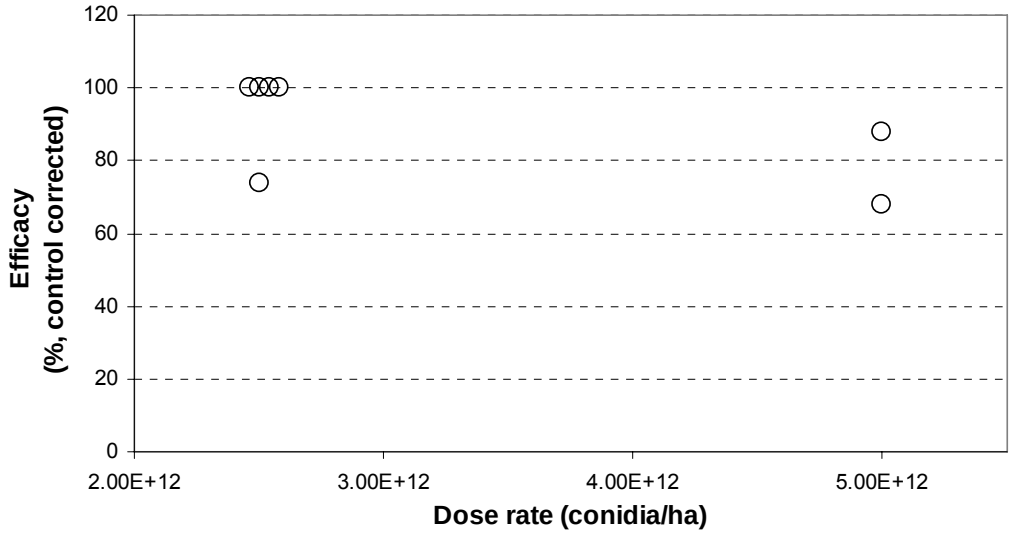

Figure 3.1 Efficacy of *Metarhizium anisopliae* var. *acridum* against the Desert Locust, as assessed through field population reductions (nymphs and adults). Efficacy is the final population reduction observed in a study, corrected for fluctuations in untreated controls (see Table 3.3). Trial no. 4 was excluded from the graph. There was no significant linear correlation between dose rate and arcsin(%-field efficacy) (n = 7; R^2 = 0.42; *P* = 0.11).

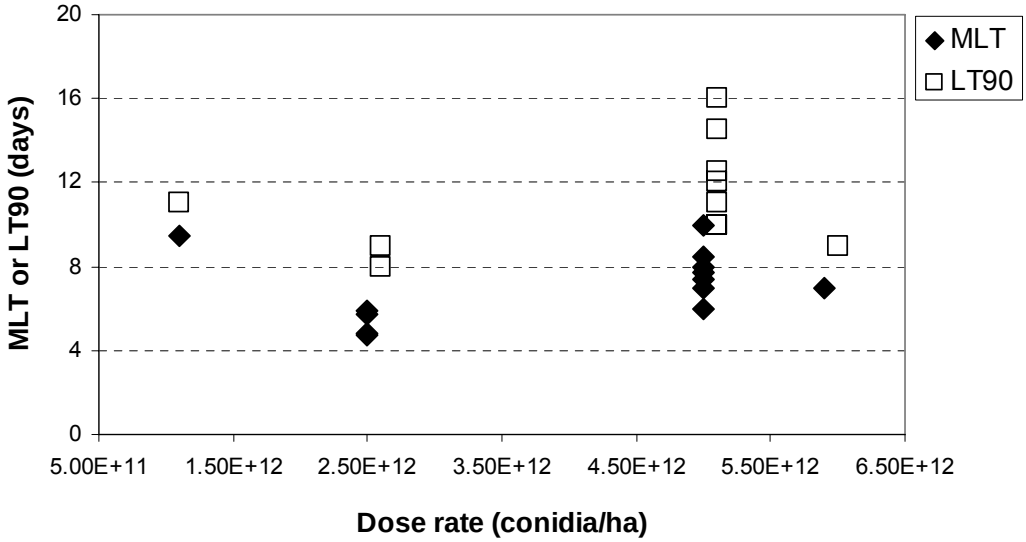

Figure 3.2 Efficacy of *Metarhizium anisopliae* var. *acridum* against the Desert Locust, assessed through cage incubation after treatments. Efficacy is expressed as median lethal time (MLT) or 90% mortality levels (LT_{90}) (see Table 3.3). Trial no. 4 was excluded from the graph. There was no significant linear correlation between dose rate and logMLT (n = 13; R^2 = 0.12; *P* = 0.24), nor between dose rate and logLT_{90} (n = 11; R^2 = 0.12; *P* = 0.30).

Table 3.3 Field efficacy of *Metarhizium anisopliae* var. *acridum* against the Desert Locust monitored through the reduction of field populations and mortality after cage incubations. Each row represents one treated plot, unless stated otherwise.

Dose rate conidia/ha	Locust stage [1]	Type of treatment	Spray plot size ha	Field assessments Efficacy [2]		Cage assessments Maximum mortality [4]			MLT [5]	LT_{90} [6]	Factors that may have reduced efficacy	Trial no. [10]
				% reduction	days AT [3]	%	reached at days AT	sample taken days AT	days	days		
1.1×10^{12}	L2 – L3	ground	400	--	--	100	11	0	9.5	11	Uneven spray deposit	65
2.5×10^{12}	L?	air	700	74	13 *	89	7	3	5.9	9	Large VMD	7
2.5×10^{12}	L?	air	700	100	6	76	7	3	4.7		Large VMD	7
2.5×10^{12}	L?	ground	25	100	5	94	7	3	5.7	8		7
2.5×10^{12}	L?	ground	25	100	5	59	7	3	4.8	--		7
2.5×10^{12}	L?	ground	25	100	5	--	--	--	--	--		7
3.0×10^{12}	L3 - L5	ground	245	-470 [7]	22 *	74	22	2	16		Spore viability possibly low (not verified); possibly unfavourable temperature conditions	4
	Fl			24 [8]	22 *	--	--	--	--	--		
5.0×10^{12}	L4 - L5	ground	?	88 [9]	9 *	95 [9]	17	0	8	16	Possibly small plots	1
	Fl			--	--	100	11	0	7.7	10		
5.0×10^{12}	L3 - L4	ground	0.01-2.5	--	--	100	22	0?	7.4	14.5	Possibly small plot	2
5.0×10^{12}	L4	ground	0.01-2.5	--	--	100	20	0?	7	10	Possibly small plot	2
5.0×10^{12}	L4 - L5	ground	0.01-2.5	--	--	100	17	0?	8.5	11	Possibly small plot	2
5.0×10^{12}	L5	ground	0.01-2.5	--	--	100	15	0?	10	12.5	Possibly small plot	2
5.0×10^{12}	L2 - L3	ground	0.3 – 0.65	68 [9]	7 *	99 [9]	18	0	6	12	Small plots; possibly unfavourable temperature conditions	3
5.9×10^{12}	Ad	air	492	--	--	100	10	1	7	9		8

[1] Locust stages: L = nymphs; Fl = fledglings; Ad = adults. [2] Efficacy is the % population reduction corrected for fluctuations in the untreated control plots according to Henderson and Tilton (1955).

[3] Days AT = number of days after treatment when the efficacy level was attained. Values with an asterisk (*) were the end of the monitoring period of the trial.

[4] Maximum mortality is corrected for control mortality using Schneider-Orelli's correction (Schneider-Orelli, 1947). Specified is the day after treatment at which maximum morality was reached; values with an asterisk are also the last day of the cage incubation period. Also noted is the day after treatment (AT) when the cage incubation sample was taken in the field.

[5] MLT = median lethal time; or number of days after treatment until 50% cumulative mortality. [6] LT_{90} = number of days after treatment until 90% cumulative mortality.

[7] Relative population increase compared to the control plot. [8] Relative reduction of fledglings is compared to the average hopper densities before treatment.

[9] Average value of 3 treatments. [10] Trial number as listed in Annex 2.

There was no positive correlation between dose rate and efficacy, either in the field population assessments or in the cage mortality evaluations (Figures 3.1 and 3.2). The most appealing explanation for the absence of a clear dose response relationship is that the lowest dose that was tested (1.1×10^{12} conidia/ha) is just as effective against the Desert Locust as the higher rates. However, given the large variability in assessment methods, environmental conditions and observation periods between the various trials, any dose-response relationship may have been concealed. Some of these variables will be further assessed in the next chapter.

3.4 Variables influencing field efficacy

In addition to the product dose rate, various factors may have influenced the efficacy of *Metarhizium* on the Desert Locust. They are briefly discussed here.

3.4.1 Product composition and quality

Since all trials against the Desert Locust were carried out with the same isolate, product quality is mostly influenced by the final tank mix used in the sprayer, the spore viability and possible physical formulation problems (e.g. clogging of atomisers).

All treatments were carried out with a tank mix composed of a vegetable oil or oil-flowable formulation combined with either diesel or kerosene (Annex 4). This is a relatively non-volatile formulation and problems with rapid evaporation of spray droplets are not expected.

Spore viability was measured in all reviewed trials just before or after the treatments. Only in trial no. 4 was the germination rate after manufacturing less than 80%; the exact germination rate at the time of spraying was not measured. Spore viability was satisfactory in all other cases (i.e. > 80%).

In trial no. 8 difficulties were encountered in preparing the tank mix since the spores had coagulated in the shipping drums. However, after vigorous mixing and sieving, the final spray formulation did not clog the atomisers. In trial no. 65 spores did block the atomisers resulting in lower flow rates, in particular the last two days of treatments. This may have been due to coagulation of spores in the sprayer. Since the spray volume was measured after application, the applied dose rate could be corrected for this anomaly.

Overall, in spite of some problems of spore coagulation, product composition and quality were comparable between the different studies. It is unlikely that these will have influenced efficacy to a large extent. However, it cannot be excluded that low spore viability in trial no. 4 may have reduced efficacy, but this could not be confirmed.

3.4.2 Application parameters

Details about the principal application parameters of the different trials are listed in Annex 4.

All the efficacy trials against the Desert Locust retained for the review were carried out with rotary atomisers. Spray drop sizes were in the recommended range of 50-100 µm VMD in five out of seven trials. In trial no. 3 and the ground treatments of trial 7, drop sizes were not reported nor could they be deduced from the sprayer settings. In the aerial treatments of trial no. 7, a VMD of 140-200 µm was deduced from the sprayer settings, which is larger than recommended for Desert Locust control (FAO, 2001).

Track spacing should be sufficiently narrow to ensure that an even spray deposit is achieved. FAO (2001) recommends maximum track spacing of 10 m for hand-held sprayers, 30 m for

vehicle-mounted equipment and 100 m for aerial applications. Track spacing was reported in six out of the seven reviewed trials and in all cases satisfied these recommendations (Annex 4).

Wind speed during the application is an essential parameter for the success of ULV drift spraying. A minimum wind speed of 1 m/s is recommended (Table 2.1). Higher wind speeds (up to a maximum of about 10 m/s) tend to result in better deposition of the pesticide, especially if the spray plot is large enough. Wind speed during spraying was reported in five out of seven trials, and generally ranged from 2-3 m/s (Annex 4). In all cases, the average wind speed was more than 1 m/s. It is unlikely that wind speed would have resulted in substantial variability in efficacy, because differences were small among the trials.

High temperature becomes only a determining factor during ULV spraying when, combined with low wind speed, it causes upward convection. This may lead to a significant reduction of pesticide deposition on the plot. Heat convection was only reported during one aerial treatment in trial no. 7. This did not appear to have reduced efficacy in a significant way, however.

Overall, application parameters for the seven trials were fairly similar. No further assessment of the impact of application parameters on efficacy was therefore carried out.

3.4.3 Vegetation

Vegetation density can influence the efficacy of the biopesticide in various ways. A higher vegetation density may reduce direct impaction of the spray droplets on the insects because they are shielded from the spray cloud. Secondary pick-up of spores from the vegetation may also be reduced because the product is "diluted" over a higher vegetation volume. On the other hand, locusts may stay and feed longer in dense than in sparse vegetation, thus increasing the probability of secondary pick-up of spores from the plants.

Vegetation density was quantified only in two out of seven trials, through estimation of vegetation cover and height. The other studies did not, or incompletely, describe vegetation density. It was therefore not possible to assess the potential impact of vegetation density on the efficacy of the entomopathogen against the Desert Locust.

3.4.4 Environmental conditions

Temperature

In a recent review of environment effects on the performance of *Metarhizium anisopliae* var. *acridum*, Blanford & Klass (2004) conclude that temperature is the key environmental determinant of control efficacy.

Elliott *et al.* (2002) and Blanford & Klass (2004) describe three processes that contribute to the influence of ambient temperature on interactions between locusts and fungal pathogens. First, temperature affects the ability of the pathogen to infect and grow within the insect. Low ambient temperature thresholds for the growth of most isolates of *M. anisopliae* var. *acridum* are around 8-11 ºC. Most rapid growth occurs at temperatures between 25 and 30 ºC, while upper temperature limits for pathogen growth are around 37-39 ºC.

Second, many orthopterans, including the Desert Locust, are active behavioural thermoregulators and will select environments close to their desired body temperature, where they can further fine-tune internal temperature through adjustments in body posture. Optimal temperatures for development of all hopper stages of the Desert Locust are between 40 and 42 ºC, with lower development thresholds of 13-15 ºC and an upper development threshold at 49ºC. Desert Locusts will, whenever possible, actively try to raise (or lower) body temperatures to approach optimal

values. Since the upper threshold of pathogen growth is about 37-39 ºC, maintenance of optimum body temperatures through thermoregulation will delay fungus growth and thus mortality.

A third factor which reinforces locust thermoregulation is behavioural fever, whereby the insect can increase its body temperature to 42-44 ºC in response to a disease infection. These fever temperatures are further above the pathogen's upper growth threshold. For *Metarhizium* these temperatures are not lethal and there is no evidence that the locust can cure itself from the infection. Behavioural fever will, however, further slow down fungus growth and delay mortality.

These three temperature-related processes are summarized in Figure 3.3.

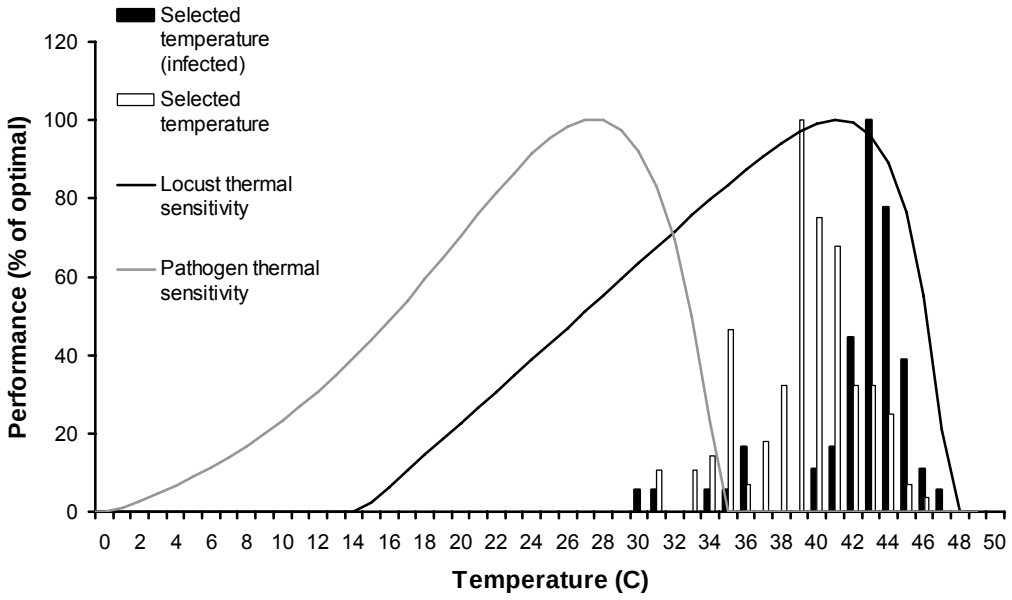

Figure 3.3 Comparison of the thermal growth profile *of Metarhizium anisopliae* var. *acridum*, thermal development rate profile of *Schistocerca gregaria* hoppers, and thermal preferences of healthy and *Metarhizium*-infected *S. gregaria* (graph from Blanford & Klass, 2004, modified after Elliott *et al.*, 2002)

Based on the processes described above, Blanford and Klass (2004) identified three performance categories for the efficacy of treatments against locusts and grasshoppers with *M. anisopliae* var. *acridum* (Table 3.4). Favourable temperature conditions include warm nights (> 20 ºC) and not overly hot days (< 38 ºC), which allow rapid development of the pathogen without the locusts being able to develop behavioural fever for a long time. Cooler nights would still be favourable, but would slow down mortality. Moderate temperature conditions are represented by hot days (> 38 ºC), which allow locusts to raise their body temperatures to levels that stop pathogen growth for a considerable part of the day. The warm nights in this scenario, however, would still result in mortality, though delayed.

Table 3.4 Expected performance of *Metarhizium anisopliae* var. *acridum* against locusts and grasshoppers under different ambient temperature conditions (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

[1] Classification based on both laboratory and field studies

Unfavourable conditions are hot days combined with cool nights (< 20 ºC), when development of *Metarhizium* is stopped or much delayed because of behavioural fever of the host during the day, and low development rate of the fungus at night.

The results of the field trials against the Desert Locust are classified according to these performance categories in Table 3.5. There are no significant differences in median lethal times (MLT) or in times to 90% mortality (LT_{90}) among the three classes (Figure 3.4a).

Table 3.5 Efficacy of *Metarhizium anisopliae* var. *acridum* against the Desert Locust, grouped according to ambient temperature conditions.

Average temperature		Temperature conditions for pathogen performance [1]	Cage location (all in field)	Cage incubation results [2]				Field Efficacy [3]	Trial no. [4]
Max.	Min.			MLT (days)	Average MLT [±SD] [8]	LT_{90} (days)	Average LT_{90} [9] [±SD]		
26 ºC	9 ºC	favourable	Full sun	16	7.7 [±3.5]	-- [5]	10.5 [±2.9]	24 % [6]	4
29 ºC	15 ºC		Shade	9.5		11		n.d. [7]	65
34 ºC	16 ºC		Shade	7.0		9.0		n.d.	8
34 ºC	17 ºC		?	8.0		16		88 %	1
34 ºC	17 ºC		?	7.7		10		n.d.	1
37 ºC	19 ºC		Shade	5.9		9.0		74 %	7
37 ºC	19 ºC		Shade	4.7		--		100 %	7
37 ºC	19 ºC		Shade	5.7		8.0		100 %	7
37 ºC	19 ºC		Shade	4.8		--		100 %	7
40 ºC	20 ºC	moderate	Full sun	7.4	8.2 [±1.3]	14.5	12 [±2.0]	n.d.	2
40 ºC	20 ºC		Full sun	7.0		10		n.d.	2
40 ºC	20 ºC		Full sun	8.5		11		n.d.	2
40 ºC	20 ºC		Full sun	10		12.5		n.d.	2
40 ºC	16 ºC	unfavourable	Full sun	6.0	6.0	12	12	68 %	3

[1] See Table 3.4. [2] See Table 3.3. [3] See Table 3.3.

[4] Number of the trial as listed in Annex 2.

[5] Mortality did not reach 90%.

[6] Field efficacy for fledglings; efficacy for hoppers was worse.

[7] n.d. = No field population data available.

[8] The difference in MLTs among the three temperature classess were not significant [ANOVA logMLT; n=14, P=0.68]

[9] The difference in LT_{90}s among the three temperature classess were not significant [ANOVA logLT_{90}; n=11, P=0.54]

Various explanations can be put forward for this apparent lack of impact of the temperature conditions on field efficacy of *Metarhizium*. First, the data set is limited, in particular for the moderate and unfavourable classes. This reduces the overall power of detecting an effect of temperature.

Second, the temperature data provided in the reports were not sufficiently detailed or precise. This was definitely the case for some studies which only reported a maximum and minimum temperature over the entire study. Only in a few cases were daily readings reported. The average maximum and minimum temperatures in Table 3.5 may thus not correctly represent the ambient temperature ranges experienced by the locusts.

Third, the performance classification in Table 3.4 is not correct, even though it was based on the most extensive (laboratory) data set available. It is unlikely, however, that minor changes (i.e. ± 1-3 ºC) in the temperature ranges of the classification would make much difference in the outcome of the assessment in Table 3.5.

The results of the Desert Locust efficacy trials show one clear "outlier" with respect to the performance classification, which is trial no. 4. It was already indicated that spore viability may have been too low in this study, explaining the low efficacy. But temperatures were also the lowest of all studies, with averages ranging from 9 to 26 ºC. The lower end of this range would basically halt pathogen development. However, average ambient temperature during the entire observation period, based on hourly measurements, was 17 ºC (Annex 4) which would result in 40-60% pathogen performance (Figure 3.3). One could argue, however, that situations where minimum temperatures fall below 10-15 ºC, and maximum temperatures allow only limited pathogen development, should be classified as unfavourable performance conditions, rather than favourable as is the case in Table 3.4. A reclassification of trial no. 4 from the favourable to the unfavourable performance class clearly improves the results, but variability is still high (Figure 3.4b).

In conclusion, no clear-cut effects of temperature on the performance of *Metarhizium* on Desert Locust were observed, when based on the analysis of the field efficacy trials that were available.

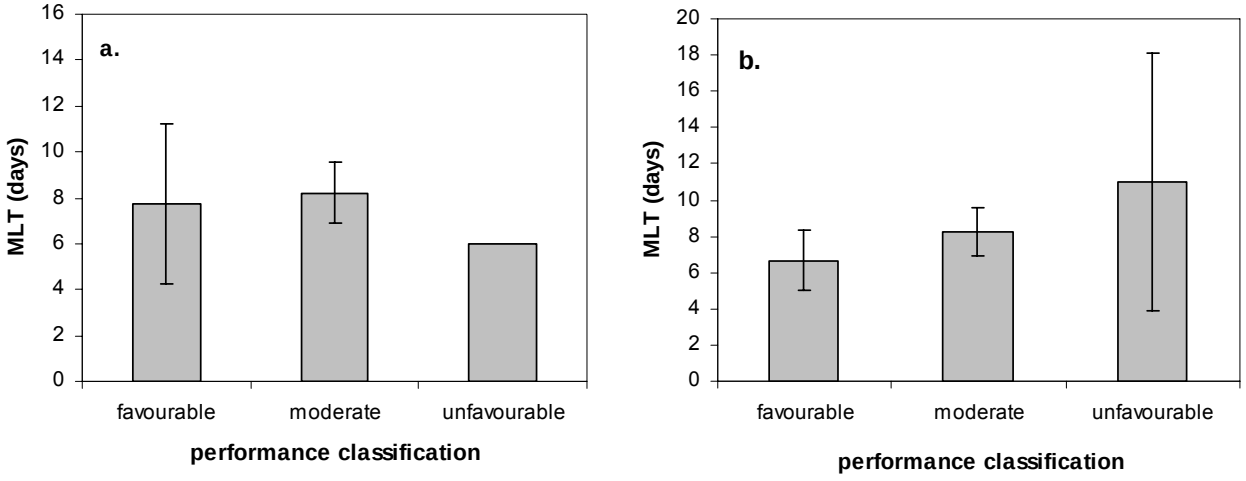

Figure 3.4 Average Median Lethal Times (MLT) (± 1 SD) observed in efficacy trials with the Desert Locust, for each temperature performance category of *Metarhizium anisopliae* var. *acridum*. **a.:** trials ordered as in Table 3.5; **b.:** trial no. 4 reclassified from the favourable to unfavourable category. Differences among performance categories were not significant (ANOVA logLMT, n=14; **a:** $P = 0.68$, **b:** $P = 0.21$).

Solar radiation

Exposure to high radiation can rapidly reduce survival of *Metarhizium* spores. However, Blanford and Klass (2004) conclude that the impact of radiation on spore survival in the field does not seem to be an important constraint to the successful use of the pathogen. They suggest two reasons for this: First, the dose rates generally applied provide a sufficient amount of spores to infect and kill, in spite of losses due to radiation. And second, radiation will only affect directly exposed spores. Those deposited in shaded conditions (e.g. the underside or other shaded parts of leaves, insect inter-segmental membranes) will be protected.

No quantification of solar radiation was provided in any of the Desert Locust trials, and the above assertions could not be confirmed with field data.

Relative humidity

Ambient relative humidity does not influence mortality of locusts due to *Metarhizium* (Fargues *et al.* 1997). It has been shown that infection with *M. anisopliae* var. *acridum* can proceed at relative humidity levels as low as 35%, but that formulation of the spores in oil greatly improves infectivity, in particular at such low relative humidity levels (Bateman *et al.* 1993). It is therefore generally considered that low ambient relative humidity, as may be encountered in Desert Locust control, is not a major impediment to successful control by the pathogen (Blanford and Klass, 2004).

Relative humidity was reported in 4 out of the 6 reviewed efficacy trials on the Desert Locust. Ranges were generally very wide between day and night (Annex 4). Only trial no. 4 had consistently low ambient relative humidity throughout the observation period. No further assessments were carried out.

4 Other species of locusts and grasshoppers

4.1 Introduction

Only a limited number of field efficacy trials on the Desert Locust are presently available (Chapter 3). Therefore, uncertainties remain about the effect that in particular environmental parameters may have on the efficacy of the *Metarhizium*.

However, many more field efficacy trials have been carried out on other species of locusts and grasshoppers, either with the isolate IMI 330189 or with other isolates (Annex 2). Because there appears to be no fundamental difference in the mode of action of these isolates on the different species of locusts and grasshoppers, this much larger database can be used to try to answer questions relevant for Desert Locust control. In this chapter, an attempt will be made to extrapolate efficacy data obtained with other species of locusts and grasshoppers, and with other isolates of *M. anisopliae* var. *acridum*.

4.2 Comparison of intrinsic susceptibility among species

Locust and grasshopper species may have different intrinsic susceptibilities to *Metarhizium*, with certain species being more affected by specific isolates of the pathogen than others. As a first step in the comparison of efficacy among species, an assessment is therefore made of the variation in intrinsic susceptibility. This is normally assessed in laboratory-based toxicity or susceptibility tests. Only tests with topically applied *Metarhizium* were assessed in this review, because they mirror the direct impingement or secondary pick-up of spores in the field, which is the main route of exposure of the locusts to the pathogen.

Various parameters have been used to quantify the susceptibility to *Metarhizium* in the laboratory. In some studies, the median lethal dose (LD_{50}) (dose killing 50% of the test population) was determined. The limitation of the LD_{50} is that it is time-dependent; i.e. it is established for a given incubation (or observation) time. Generally, an increase in the incubation time results in a reduction of the LD_{50}.

Other studies have determined the median lethal time (LT_{50} or MLT) (time required to kill 50% of the test population). The limitation of the MLT is that it is dose-dependent; i.e. it is established for a given dose of the pathogen. An increase in the test dose often means a reduction in the MLT. Sometimes, the average survival time (AST) was established. The AST is calculated in a different manner from the MLT, but the outcome is often similar. Significant differences between AST and MLT may occur when the time-response curve is very asymmetrical and/or final mortality at the end of the experiment is well below 100%. Similar to the MLT, the AST is dose-dependent.

No single value therefore covers the susceptibility of a locust or grasshopper species to *Metarhizium*, although attempts to model time-dose-response parameters have been made in the past (Nowierski *et al.*, 1996). Therefore, in the assessment below, time-response data (MLTs) are always presented as a function of dose, and dose-response data (LD_{50}s) as a function of time.

Figure 4.1 shows MLTs of laboratory susceptibility studies with species of locusts and grasshoppers for which a minimum number of field efficacy trials was also available (Table 4.1). Only those species which had two or more MLTs were included. The full data set, and its sources, is provided in Annex 5A.

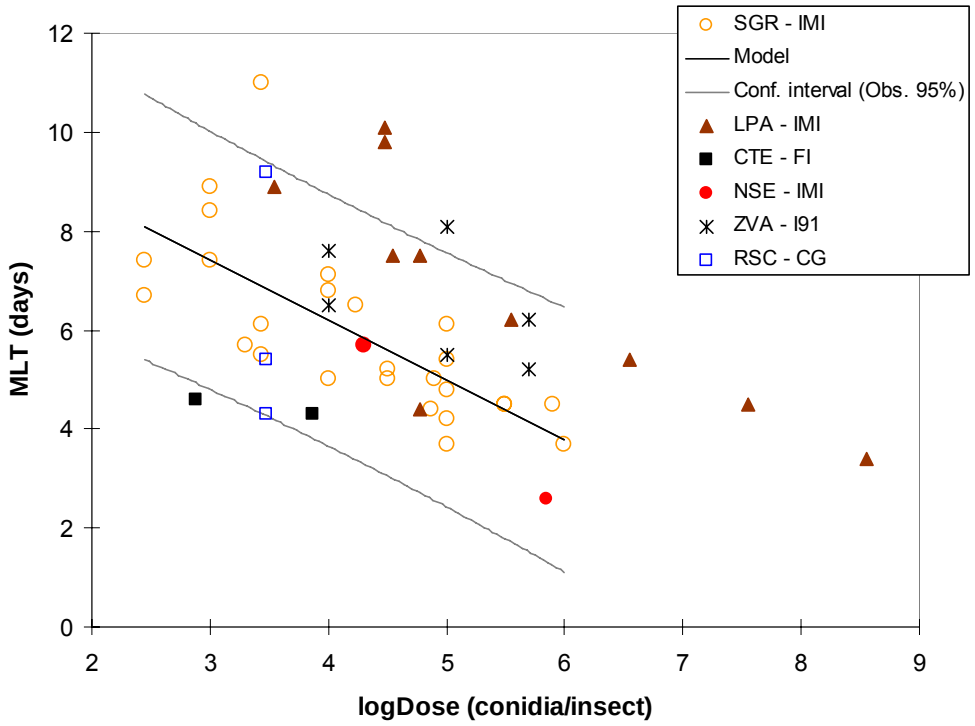

Figure 4.1 Median lethal times (MLT) of *Metarhizium anisopliae* var. *acridum* as a function of topically applied dose for various species of locusts and grasshoppers. Shown are combinations of locust species and *Metarhizium* isolates which are also applied in the field: Desert Locust, Brown Locust and Red Locust treated with IMI 330189 (SGR-IMI, LPA-IMI and NSE-IMI respectively), Australian Plague Locust with FI-985 (CTE-FI), Variegated Grasshopper with I91-609 (ZVA-I91) and Mato Grosso Grasshopper with CG-423 (RSC-CG). Linear regression *("Model & Conf. interval")* is for Desert Locust data only (adjusted R^2 = 0.51, n = 26, P < 0.001). Data sources are provided in Annex 5A.

MLTs of the Desert Locust decrease as a linear function of the logDose. Although the regression is highly significant, the 95% confidence intervals are rather wide, averaging about 2.5 days. Figure 4.1 shows that the large majority of MLTs of other species than the Desert Locust fall within the 95% confidence intervals of the Desert Locust data set, indicating that the intrinsic susceptibility of these species to the respective *Metarhizium* isolates applied against them in the field is similar. Australian Plague Locust is slightly more susceptible to FI-985 than the Desert Locust to IMI 330189, with its MLTs roughly on the lower 95% confidence intervals of the Desert Locust regression. Brown Locust appears to be slightly less susceptible to IMI 330189 than the Desert Locust.

In a similar manner, LD_{50} values can be plotted against incubation times (Figure 4.2). This data set is considerably smaller (Annex 5B). LD_{50} values were determined only in a few studies, and more than half of the values included in the assessment were calculated by the reviewer based on the data presented in the reports of the laboratory studies.

The variation in LD_{50} values was very much larger than in the MLTs (note that Figure 4.2 shows $LogLD_{50}$ values on the Y-axis). The regression of $logLD_{50}$s of the Desert Locust against incubation time was not significant. However, the indication that Brown Locust may be slightly less susceptible to IMI 330189 than the Desert Locust, and Australian Plague Locust slightly more susceptible to FI-985, is not contradicted by the data in Figure 4.2.

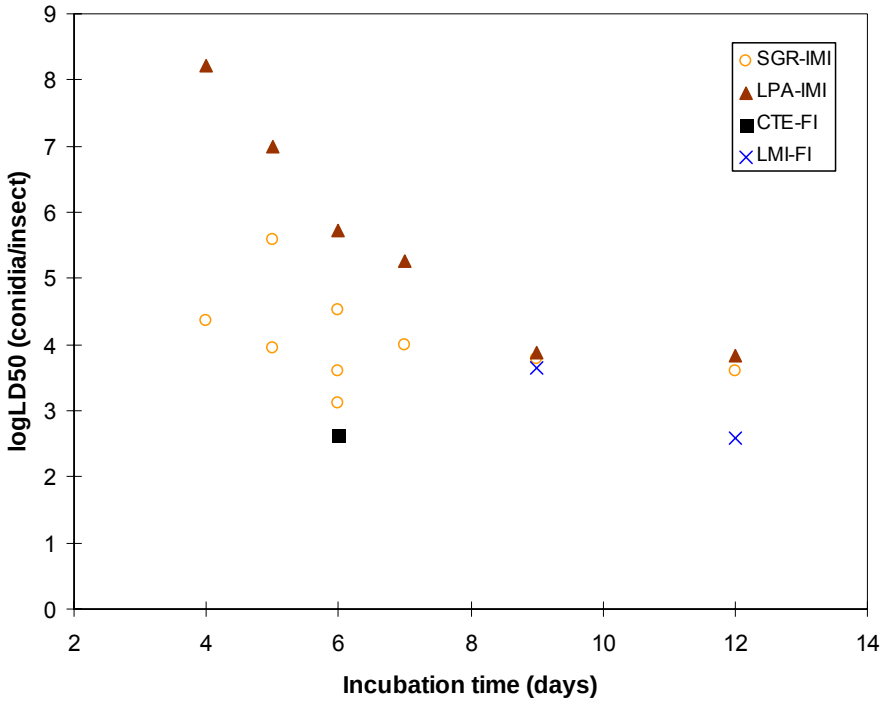

Figure 4.2 Median lethal doses (LD_{50}) of *Metarhizium anisopliae* var. *acridum* as a function of incubation time for various species of locusts and grasshoppers. Shown are combinations of locust species and *Metarhizium* isolates which are also applied in the field: Desert Locust and Brown Locust treated with IMI 330189 (SGR-IMI and LPA-IMI respectively), and Australian Plague Locust and Migratory Locust with FI-985 (CTE-FI and LMI-FI respectively). Linear regression for Desert Locust data is not significant (adjusted R^2 = 0.05, n = 9, P = 0.28). Data sources in Annex 5B

On the basis of this assessment, the intrinsic susceptibilities of the different locust and grasshopper species shown in Figures 4.1 and 4.2 to the *Metarhizium* isolates applied against them in the field can be considered similar. There are indications, however, that Brown Locust may be less susceptible than the Desert Locust and the Australian Plague Locust more susceptible.

4.3 Comparison of field efficacy

With the information on the intrinsic susceptibility of different locust and grasshopper species obtained in Chapter 4.1, the relative efficacy of *Metarhizium* isolates can now also be assessed in the field. The results of field trials against various species of locusts and grasshoppers other than the Desert Locust have been summarized in Table 4.1.

Only those species are listed for which a minimum of 5 data sets were available at dose rates that ranged over a factor of at least 2. In total, 7 species of locusts and grasshoppers could be evaluated, treated with 4 different isolates of *Metarhizium*. Efficacy is described either based on cage incubation assessments of treated locusts, or on population reductions observed in the field. Only in a few cases were both types of assessment available for the same trial. More details of each of the trials are provided in Annex 6.

Table 4.1 Efficacy of *Metarhizium anisopliae* var. *acridum* for selected species of locusts and grasshoppers other than the Desert Locust. Included are trials for which at least 5 data sets were available at dose rates that ranged at least over a factor 2.

Species	Dose (range)	Efficacy											Trial no. [8]
		Cage incubation assessment [1]							Field population assessment				
		No. of data sets [2]	Max mort. [3]		MLT		LT_{90} [4]		No. of data sets	Field efficacy [5]			
			[range] %	[avg] %	[range] days	[avg] days	[range] days	[avg] days		[range] %	[avg] %	@ days AT	
M. anisopliae var. *acridum:* **isolate IMI 330189**													
Red Locust	1.25 – 1.5 x 10^{12}	2	72 – 87	80	11	11			--				10, 11
	2.5 – 5 x 10^{12}	5	90 – 100	94	3.6 – 11	6.9	8 – 21	15	--				9, 10
Brown Locust	1.8 – 2 x 10^{12}	8	61 – 100	93	5 – 12	9.2			--				23
	3 – 5 x 10^{12}	3	72 – 100	90	6.5 – 12	8.8			--				22, 23
	7.4 – 9.4 x 10^{12}	5	39 – 100	88	8 – 17	11	12 – 19	13	--				21
Senegalese Grasshopper	2 – 2.5 x 10^{12}	2	53 – 87	70	7 [6]		13 [6]		1		79	24	34, 42
	4.2 – 5 x 10^{12}	6	72 – 100	90	8 – 9	8.6	13 – 15	14	5	43 - 96	74	18 – 22	36, 37, 38, 39, 40, 42
M. anisopliae var. *acridum:* **isolate FI-985**													
Australian Plague Locust	1 – 2 x 10^{12}	2	60 – 90	79					3	90 – 99	94	13 – 21	25, 26, 27, 63
	3 – 5 x 10^{12}	--							3	85 – 100	93	13 – 21	25, 26
Migratory Locust	1 – 2.1 x 10^{12}	--							5	-13 – 97	53	8 – 18	16, 17, 18, 19
	3 – 5 x 10^{12}	--							12	65 – 99	91	8 – 18	15, 16, 17, 18, 19
M. anisopliae var. *acridum:* **isolate CG 423**													
Mato Grasso Grasshopper	5 x 10^{12}	3	81 – 91	85					--				59
	1 – 2.1 x 10^{13}	4	58 – 82	73					3	69 – 86	80	11 – 13	58, 59
M. anisopliae var. *acridum:* **isolate I91-609**													
Variegated Grasshopper	1 – 7 x 10^{11}	5		69 [6]	12 – 17	15			2	82 – 90	86	21	46, 50, 51, 52
	1 – 5 x 10^{12}	10	89 – 100	94 [7]	5 – 12	8.6	8 – 25 [7]	16	4	87 – 98	91	14 – 21	45, 46, 47, 48, 49, 50, 51, 52
	1 x 10^{13}	1				9.7			1		98	21	50

[1] All data from cages placed outside in the sun, when available. [2] A data set represents a spray plot or a set of spray plots for which individual application data and efficacy results were available. [3] Maximum mortality % is corrected for control mortality. [4] Only LT_{90}s are listed for those cases which reached 90% mortality during the observation period. [5] Field efficacy is corrected for control population fluctuations. [6] Only 1 data set. [7] Only 5 data sets. [8] Trial number as listed in Annex 2.

Laboratory susceptibility data were available for 6 of the 7 species of locusts and grasshoppers listed in Table 4.1 (Figures 4.1 and 4.2). No laboratory susceptibility tests with the relevant isolate of *Metarhizium* (IMI 330189) were available for the Senegalese Grasshopper.

Mortality levels or population reductions at or exceeding 90% were again considered to represent adequate efficacy[6]. Figure 4.3 shows dose ranges for which adequate efficacy was observed for the 7 species listed in Table 4.1 and the Desert Locust. In Table 4.1 and Figure 4.3, different isolates of the pathogen are compared, depending on the locust or grasshopper species against which they are applied. The isolates reviewed can be considered "standard" for each of the species. Since the same species–isolate combinations were also reviewed in Chapter 4.1, direct comparisons between laboratory and field data can be made.

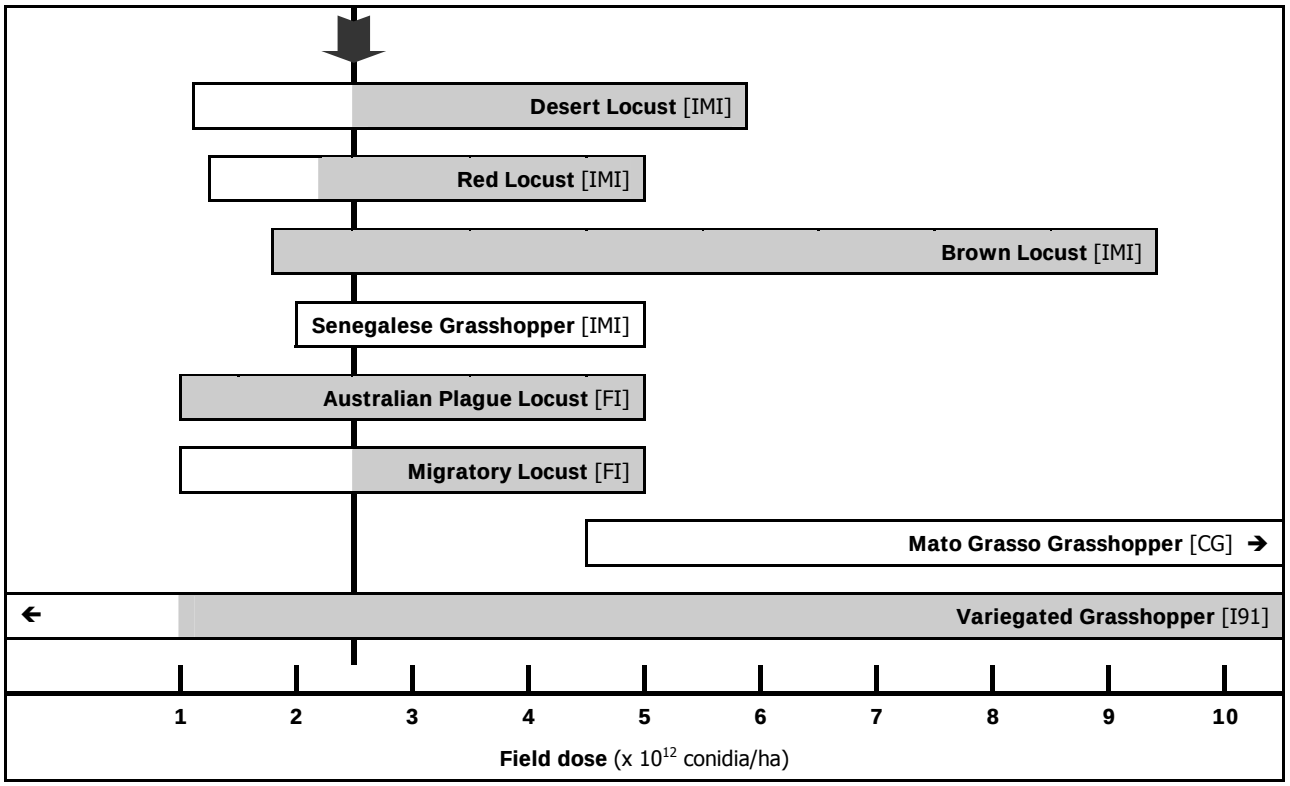

Figure 4.3 Relative field efficacy of *Metarhizium anisopliae* var. *acridum* for various species of locusts and grasshoppers. The dose range tested for each species is represented by the horizontal bars; the grey-hatched part of each bar covers the dose range that showed average efficacy of ≥90%. The minimum effective dose rate against the Desert Locust is shown by the vertical line with arrow. Dose ranges tested for Mato Grosso Grasshopper and Variegated Grasshopper extended outside the scale (indicated by small arrows). [IMI] = isolate IMI 330189, [FI] = isolate FI-985, [CG] = isolate CG423, [I91] = isolate I91-609. All data are from Tables 3.3 and 4.1.

The minimum field dose rates of *Metarhizium* which resulted in adequate efficacy are similar for the Desert Locust, the Red Locust, the Brown Locust and the Migratory Locust, at around 2.5 x 10^{12} conidia/ha. This is supported by the laboratory susceptibility of the four species, which was also similar (Figures 4.2 and 4.2).

[6] It should be stressed that the 90% efficacy level considered adequate refers in particular to the Desert Locust. For other species of locusts or grasshoppers, required minimum efficacy may well be different, depending on population dynamics of the species, migratory behaviour and damage potential.

Australian Plague Locust and Variegated Grasshopper have been controlled adequately at dose rates as low as 1 x 10^{12} conidia/ha. The laboratory data for the Australian Plague Locust indicate that it is indeed slightly more susceptible to its "standard" isolate than the Desert Locust. The Variegated Grasshopper appears more susceptible in the field than is expected from the laboratory data presented in Figure 4.1.

Field efficacy trials against the Mato Grosso Grasshopper were carried out at dose rates ranging from 5 x 10^{12} to 2.1 x 10^{13} conidia/ha, but did not result in adequate efficacy. This apparent lack of efficacy in the field was not expected from the laboratory data presented in Figure 4.1, but the number of laboratory tests was only limited. The Senegalese Grasshopper could not be controlled adequately with isolate IMI 330189 at dose rates up to 5 x 10^{12} conidia/ha. However, no laboratory susceptibility tests were available to assess if its intrinsic susceptibility to this isolate was relatively low.

On the basis of these data, one may conclude that the lower field dose of IMI 330189 providing adequate control of the Desert Locust, 2.5 x 10^{12} conidia/ha, appears robust when one takes into account efficacy trials with other species of locust and grasshoppers. The majority of other species with similar relative susceptibilities are adequately controlled at this dose rate. Only the Mato Gross Grasshopper is an exception to this observation.

The data also show that there may be limited room for a further reduction of the field application rate of IMI 330189 against the Desert Locust. Both the Brown Locust and the Variegated Grasshopper show adequate control at slightly lower dose rates than those tested against the Desert Locust, while there relative intrinsic susceptibilities are similar or lower. This is supported by partial trial results against early instars of the Desert Locust (see Table 3.3). However, the data do not suggest that a major reduction in dose rate (i.e. more than a factor 2) is likely to provide robust and adequate control for the Desert Locust with the present commercial formulation.

4.4 Environmental factors influencing field efficacy

4.4.1 Temperature and thermoregulation

In Chapter 3.4.4., ambient temperature and thermoregulation by the insects, were identified as key factors influencing the field efficacy of *Metarhizium*. These parameters are therefore assessed in more detail below, incorporating data sets of other species of locusts and grasshoppers.

In a number of field trials, locusts or grasshoppers were collected after the treatments and were incubated simultaneously in cages that were placed both in the sun and in the shade (the latter either in or outside the laboratory). Data for 4 species for which that was the case were combined in Figure 4.4 (Red Locust, Brown Locust, Senegalese Grasshopper and Variegated Grasshopper – see Annex 7).

The average median lethal time increased from 8.7 (±2.4) days in the shade to 12.5 (±2.8) days in the sun; the average LT_{90} increased from 9.5 (±3.1) days in the shade to 15 (±5.5) days in the sun (Figure 4.4). A paired comparison showed these differences to be highly significant.

The reduction in the speed of mortality observed in the sun may either be due to overall higher temperatures in the cages and to (additional) active thermoregulation by the insects. The effects of these two factors cannot be clearly dissociated because temperature data were not available for all trials. The four species are known to thermoregulate, and it is expected that this will have contributed to the reduced speed of kill.

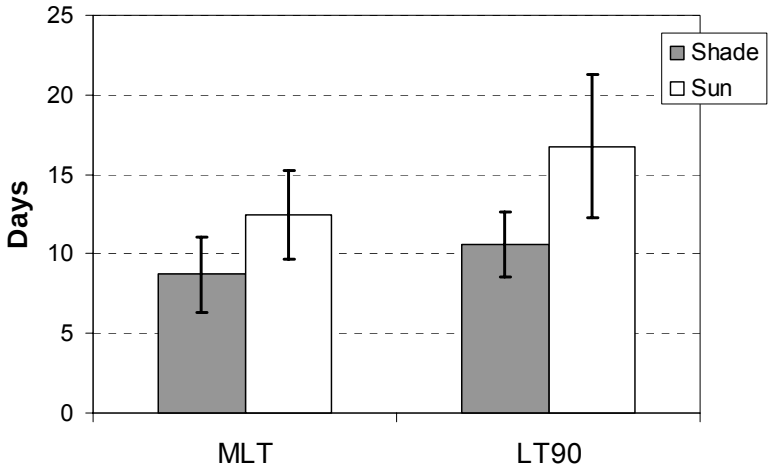

Figure 4.4 Average median lethal times (MLT) and times to 90% mortality (LT90) for locusts and grasshoppers incubated in cages that were placed simultaneously in the sun (thermoregulation possible) and in the shade (thermoregulation limited or impossible). Combined data for 4 species (see Annex 7). Error bars are one standard deviation. Differences between sun and shade incubations were statistically significant (paired Student t-test – MLT: n = 17, $P < 0.001$; LT_{90}: n = 5, $P = 0.029$).

A second analysis was carried out to assess the effect of thermoregulation on efficacy of *Metarhizium*. All field efficacy trials for which MLTs were available or could be calculated, and which reported if incubation cages were placed in the shade or in the sun were compiled. Median lethal times were then plotted as a function of dose rate for species with similar susceptibility to the entomopathogen (Figure 4.1).

The results, shown in Figure 4.5, indicate a clearly lower speed of kill for insects incubated in the sun than in the shade, over most of the dose range tested. The MLT declines in a, statistically significant, linear manner as a function of ln(dose), for insects incubated in the shade. However, the MLTs for insects incubated in the sun are more variable and no significant regression can be fitted against the dose rate of *Metarhizium* (either linear or logarithmic). This suggests that when locusts are able to thermoregulate, the dose-response relationship is weaker, probably because thermoregulation partly masks the effect of the dose of the pathogen.

The results of these field efficacy studies therefore confirm the effects of temperature and thermoregulation on the performance of *Metarhizium* that were observed for the Desert Locust in the laboratory (Chapter 3.4.4). In addition, the conclusion of Chapter 4.2 that there may only be limited room for reduction of the dose rate to be used against the Desert Locust is supported by Figure 4.5. It shows that the median lethal time starts to steeply increase at dose rates below about 1.5×10^{12} conidia/ha.

4.4.2 Other environmental factors

Other environmental factors that may have influenced efficacy of *Metarhizium*, such as vegetation biomass and radiation intensity, were not described in a sufficient number of trials to carry out any further assessments.

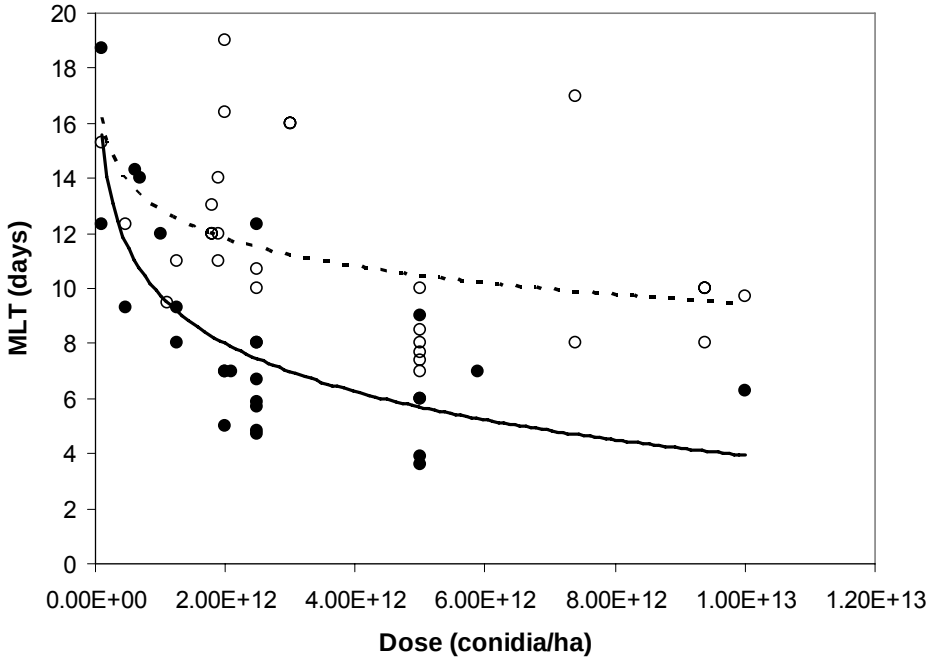

Figure 4.5 Median lethal times as a function of field dose rates of *M. anisopliae* var. *acridum,* for locusts and grasshoppers incubated in cages placed in the shade (solid circles) or in the sun (open circles). Combined data for the Desert Locust, Red Locust, Brown Locust (all sprayed with IMI 330189) and the Variegated Grasshopper (sprayed with I91 609), which are considered of similar susceptibility (see Figure 4.1).

Regression sunny incubations (dotted line): MLT = -1.47ln(dose) + 53.5 [n=29, R^2 = 0.19, $P > 0.05$]
Regression shaded incubations (solid line): MLT = -2.52ln(dose) + 79.5 [n=26, R^2 = 0.58, $P < 0.01$]

4.5 Modes of exposure

4.5.1 Introduction

Locusts and grasshoppers will in principal be exposed to spores of *Metarhizium* in three ways:

- Direct exposure during and immediately after the application of the product, when spray droplets directly impinge on the insects. For this exposure pathway to be effective, the treatment should be carried out when the locusts are exposed, i.e. roosting or marching over relatively open spaces. The window of opportunity during the day for this type of exposure is limited, and high vegetation density may shield the insects from the droplets.
- Secondary pickup of spores from the treated vegetation, either when the insects feed or when they move through the vegetation. For this way of exposure to be effective, the spores need to remain viable for some time on the vegetation and a minimum residence time of the insects in the vegetation is likely to be needed for sufficient spores to be picked up by the insects.
- Horizontal transmission from infected cadavers, when these sporulate again. This transmission pathway occurs with a considerable delay after the treatment. For it to be effective, only limited scavenging of cadavers or predation on debilitated insects must take place. Also, locusts need to be present in the area where sporulation occurs.

Horizontal transmission will not be further discussed in this review. It is unlikely to be very important for Desert Locust control, since hopper populations will often have moved or fledged from the areas where mortality took place, and thus sporulation could occur.

Direct exposure to the spray droplets to *Metarhizium* is mainly influenced by the quality of the application, the biomass of the vegetation and the behaviour of the insects. This is not different from a synthetic chemical insecticide, and general recommendations for the optimization of control efficacy are therefore also applicable to *Metarhizium* (e.g. FAO, 2001).

The effectiveness of secondary pick-up of *Metarhizium* spores from the treated vegetation will to a large extent be determined by the biological persistence of the pathogen, combined with the time that the locusts spend within the treated vegetation. These two factors will be assessed in the rest of this chapter.

4.5.2 Persistence of biological activity

The persistence of *Metarhizium* has been assessed in several field efficacy trials. This was generally done through bioassays, by caging untreated insects onto treated vegetation at different times after the application of the pathogen. All field efficacy trials in which such evaluations of persistence of biological activity were carried are listed in Annex 8. Regressions (either linear or exponential) of mortality against age of the spray deposit were carried out by the reviewer for those data sets containing at least four values. Table 4.2 shows the data sets that yielded statistically significant "decay curves".

No biological half-lives of *Metarhizium* following Desert Locust control could be determined, because statistically significant decay curves could not be fitted to the available data. The other data sets show fairly consistent biological half-lives ranging from 3.5 to 8.4 days, irrespective of locust species or pathogen isolate. Only one trial with Senegalese Grasshopper resulted in a very long half-live of 34 days (a second trial with the same species also showed high persistence, though did not yield a significant decay curve – Annex 8). It is not exactly known why *Metarhizium* persisted so long in these two trials, but likely the cloudy conditions during the study (reducing spore mortality caused by irradiation) or possibly horizontal transmission of spores, may have played a role.

Only for the Rice Grasshopper were infectivity half-lives available at different dose rates (Table 4.2). An increase in dose rate resulted in corresponding increases in half-lives.

It should be noted that to be able to fit a statistically significant decay curve of the biological activity of *Metarhizium* spores, generally at least 7-10 observations were required over time. Several field studies did assess the infectivity of spores at various times after treatment, but the number of observations was too limited to yield statistically significant half-lives. Such data sets could therefore not be used.

A number of studies specifically evaluated biological persistence of spores, but the calculations used mortality values corrected to give risk of infection per day, and were based on positive mycosis rather than just mortality. However, in spite of the difference in methodology, the half-life of infectivity is expected to be fairly similar to the ones calculated in this review. Indeed, half-lives for instantaneous risk of infection ranged from 5.0 days for Variegated Grasshopper treated with isolate I91 609 (Thomas *et al.* 1996), to 6.8 days for Rice Grasshopper treated with IMI 330189 (Thomas *et al.*, 1997), and to 7.7 days for Senegalese Grasshopper treated with isolate IMI 330189 (Kooyman *et al.,* 1997).

In conclusion, the half-lives of biological activity of *Metarhizium anisopliae* var. *acridum* (isolates IMI 330189, FI-985 and I91 609) range from about 4 to 8 days, under semi-arid and sub-tropical environmental conditions.

Table 4.2 Biological half-lives of *Metarhizium anisopliae* var. *acridum* assessed by caging untreated insects onto treated vegetation at different times after spraying. Shown are trials that resulted in statistically significant curves (Annex 8).

Species	Isolate	Dose rate (conidia/ha)	Half-life[1] (days)	Type of curve	Trial no.[2]
Rice Grasshopper	IMI 330189	1×10^{11}	3.3	Exponential	
		4.65×10^{11}	5.0	Exponential	50
		2.15×10^{12}	5.8	Exponential	
		5×10^{12}	8.4	Linear	49
Senegalese Grasshopper	IMI 330189	5×10^{12}	34	Linear	40
Migratory Locust	FI-985	3.5×10^{12}	5.0	Exponential	15
Australian Plague Locust	FI-985	various	5.3	Exponential	25

[1] Half-life based on the percentage mortality.

[2] Trial numbers as listed in Annex 2.

4.5.3 Importance of secondary pick-up

A measure of the importance of secondary pick-up of *Metarhizium* spores that can be obtained from field efficacy trials is the comparison of the maximum cumulative mortality of locusts that were exposed only to the spray residue with mortality of insects that were sprayed and subsequently incubated in cages. However, a prerequisite for this assessment is that the exposure duration to the treated vegetation should be similar for both cases.

Annex 9 lists all the field efficacy studies in which both the above assessments were done. The average mortality following exposure of untreated locusts on treated vegetation on the day of treatment was 90 ± 11%. The average percentage mortality of insects that were exposed to the spray droplets and subsequently incubated in cages was 96 ± 7%. This difference was not significantly different.

However, the results of this comparison should be interpreted with caution. The data set in Annex 9 is rather variable: exposure times of untreated insects on treated vegetation ranged from 2 days to continuously. Similarly, timing of sampling of the field-treated insects ranged from "immediately" to 2 days after treatment. Also, in many cases it was not reported whether the vegetation that was fed to the insects in the cages had been treated. Thus, the exposure duration of the field-treated locusts after the application was likely quite variable.

As a result, the only valid conclusion that one can draw from this assessment is that mortality due to secondary pick-up only is high. Furthermore, if one assumes that at least limited secondary pick-up would likely also have occurred in the cases of incubation of field-treated locusts, direct impingement of spray droplets is at most just as effective as secondary pick-up, but likely to be less so.

A few studies have explicitly addressed the relative importance of secondary pick-up of spores compared to direct exposure. Bateman *et al.* (1998) studied adult Desert Locust in large semi-permanent field cages and found that there was no significant difference in the average survival time between locusts exposed directly and those picking up spores exclusively from the vegetation. Thomas *et al.* (1997), combining a field trial on the Rice Grasshopper with a population dynamics model, estimated that exposure to residual spores accounted for 40-50% of the total infection measured. Thomas *et al.* (1998), in a field study carried out on the Rice Grasshopper in short mixed-grass vegetation, concluded that the dose of spores acquired through

contact with the spray residue exceeds that from direct contact with the airborne spray droplets alone. The same conclusion was drawn by Kassa *et al.* (2004) following a field trial carried out on the same species of grasshopper in high density sorghum and millet plots. Finally Scanlan *et al.* (2001), modelling the effect of *Metarhizium* on Migratory Locust in Australia, conclude that secondary pick-up from vegetation is important at low to moderate dose rates of the pathogen and at moderate vegetation cover.

Based on the above assessments, one may conclude that secondary pick-up of *Metarhizium* spores from treated vegetation is a major, if not the major mode of exposure of locusts and grasshoppers. Its relative importance to direct hits by airborne spray droplets is likely to become more important at lower dose rates and higher vegetation densities.

4.5.4 Residence time of locusts in treated areas

With secondary pick-up of *Metarhizium* from the vegetation being recognized a vital exposure pathway, it is important to assess how long locusts should remain in a treated area to accumulate the maximum amount of viable spores, resulting in the highest level of mortality.

To get an indication of these minimum required residence times, an evaluation was carried out of the mortality curves of locusts and grasshoppers that were sampled from treated plots at different times following the treatments. Such cage mortality assessments have been carried out as part of many field trials.

Most mortality curves look like the (theoretical) one shown in Figure 4.6. Maximum cumulative mortality levels tend to decrease as sampling dates progress. This decrease in mortality is to a large extent due to the reduction of infectivity over time of the spore residue on the vegetation (see Chapter 4.5.2). However, other factors also play a role. Since locusts of grasshoppers within a given population will have variable susceptibilities to the pathogen, as time progresses after a treatment the more resistant ones will survive, and are increasingly less likely to die. Furthermore, sampling efficiency may be different between healthy and infected insects: On the one hand, diseased insects may thermoregulate in the canopy of the vegetation and be captured easier; on the other hand they may loose motility and remain low in the canopy or on the soil, and thus be captured less easily.

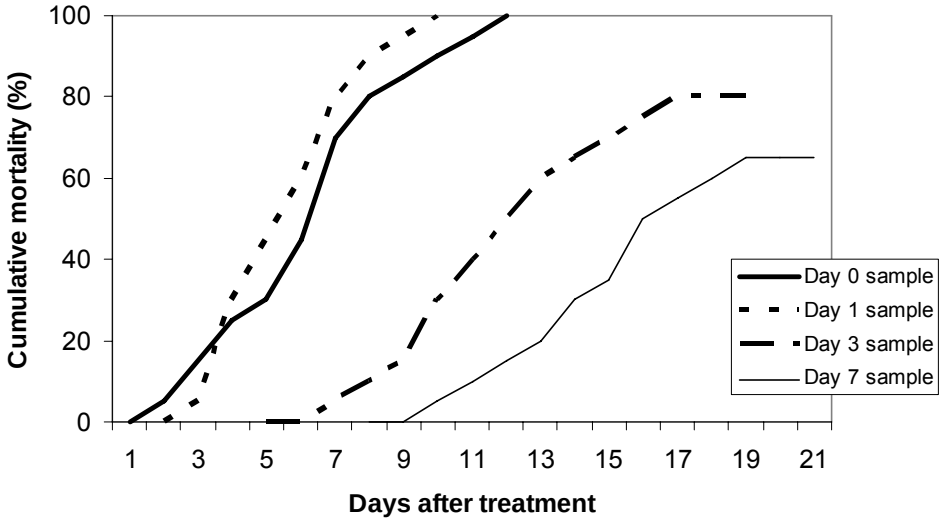

Figure 4.6 Theoretical cumulative mortality of samples of locusts or grasshoppers that have been treated with *Metarhizium*, collected at different times after spraying and subsequently incubated in cages. Note that maximum cumulative mortality levels tend to taper off as sampling time progresses.

The net effect of these processes on capture efficiency is not clear, but there are indications that, even if infectivity of the spores remains stable over time, apparent cumulative mortality as measured through sampling and caging will decrease. This is supported by the trials carried out by Langewald *et al.* (1999), where infectivity of spore residues remained virtually unchanged for the first 15 days of the trial, but apparent cumulative mortality decreased over the same period. It is for this reason that evaluations of the persistence of biological activity are always done by caging untreated insects on treated vegetation, and not by assessing the mortality curves of the insects that were treated and subsequently sampled.

However, mortality curves as shown in Figure 4.6 contain other information relevant for operational locust control. This is the fact that very often the maximum cumulative mortality is not reached in the samples of insects that were caught immediately after spraying, but one or more days later. So in spite of a general decrease in mortality over time due to the factors mentioned in the paragraphs above, initially cumulative mortality due to *Metarhizium* may increase (or be attained more rapidly). This mortality increase is caused by the secondary pick-up of spores in addition to any spores obtained by direct hits of spray droplets during treatment.

The sampling day resulting in the highest maximum cumulative mortality can therefore be seen as a measure of the minimum residence time that a locust or grasshopper is required to spend in treated vegetation to ensure maximum secondary pick-up of viable spores.

In total, 36 data sets were identified in which samples of locusts or grasshoppers were taken on more than one occasion after the treatment (Annex 10). They concern eight species of locusts and grasshoppers. The large majority (86%) of first samples was taken on the day of treatment, while the rest was taken the day after (Fig. 4.7). On average, the first sample was taken at 0.14 days after treatment. However, maximum cumulative mortality was observed in 72% of the cases in samples taken later than the day of spraying (on average at 2.0 days after treatment).

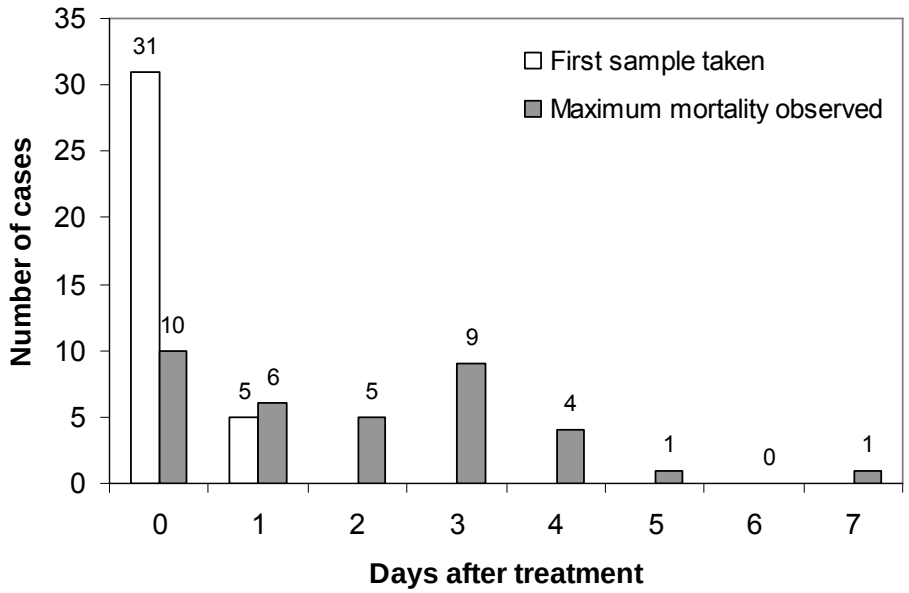

Figure 4.7 Timing at which maximum cumulative mortality was observed in samples of locusts or grasshoppers taken from treated plots at different times after spraying, when compared with the timing of the first sampling. The average timing of the first sample was at 0.14 (±0.35) days after treatment, while the average timing of maximum mortality was at 2.0 (±1.7) days.

A Kruskal-Wallis test showed an overall significant difference between the various species/isolate combinations (df = 7; $P = 0.04$), but no individual contrast between species was significant. However, the average timing of maximum cumulative mortality of both Desert Locust and Brown Locust was at the lower range of the spectrum (at ~0.8 days after treatment), while the Rice Grasshopper was at the high end (at 4.2 days after treatment). This may have been because treatments against the former two species are generally carried out in relatively sparse vegetation, where the relative importance of direct impingement of spray droplets is greater than in denser vegetation. A certain observation bias may also come into play, because in sparse, aggregated, vegetation hopper bands may altogether leave sprayed vegetation patches fairly rapidly and thus cannot continue to pick up spores anymore. However, due to the lack of data on vegetation cover, this could not be further ascertained.

In conclusion, the results of this assessment reaffirm the importance of secondary pick-up for effective control of locusts and grasshoppers. The data suggest that, on average, insects should remain in the treated area for at least 2 two days after the treatment, for maximum efficacy. With a half-life of spore infectivity ranging from 4 to 8 days (see above), the infection risk at 2 days after treatment would still be 70 – 85% of the initial levels.

The operational implication of these observations is that ideally spray plots should be large enough so that locusts will remain in the treated area for about 2 days, in particular if vegetation density is relatively high. If, on the other hand, spot-applications of moving hopper bands have to be carried out, higher dose rates are likely to be required for the insects to obtain sufficient spores for adequate mortality.

5. Discussion

5.1 Study quality

The underlying review showed that the quality of the field efficacy studies with *Metarhizium* was in a number of cases inadequate. More often, however, reporting of the trials was unsatisfactory. This limited the possibilities to assess the quality of the trials, but also to draw conclusions about the efficacy of the pathogen, in particular with respect to the effects of environmental factors. Furthermore, it restricted opportunities to extrapolate results from trials carried out with other species of locusts and grasshoppers to the Desert Locust.

One of the reasons for the incomplete reporting is probably the limitation imposed by certain scientific journals on the amount of detail accepted for publication. However, in many other cases where this was possible, study authors did not fully report the trial methodology and results either. Since opportunities to carry out field efficacy trials on migratory locusts tend to be scarce, it is of the utmost importance those studies which are carried out are reported in sufficient detail. For instance, it is essential that application parameters and results are reported on a plot-by-plot basis, as this will make effective meta-analysis over a series of trials possible, and allows better extrapolation of results between species, isolates and experimental conditions.

It is strongly recommended that an internationally accepted guidance document is elaborated on the design, execution and reporting of field efficacy trials of *Metarhizium* against locusts and grasshoppers. The document should provide guidance for fully-fledged replicated field trials, but also for monitoring of operational use of *Metarhizium* against migratory locusts. The elaboration of such a guidance document should be relatively easy since several good partial guidelines are already available (FAO, 2001, 2005; Lomer & Lomer, undated).

5.2 Efficacy of *Metarhizium* against the Desert Locust

The results of this review indicate that a dose rate of 2.5×10^{12} conidia/ha of *M. anisopliae* var. *acridum* (isolate IMI 330189), or 50 g conidia/ha, is efficacious against the Desert Locust. This dose rate is relatively robust and is expected to provide adequate control under favourable and moderate environmental conditions (see below), and against all stages of the insect.

Comparison with field trial results of other species of locusts and grasshoppers, for which the relative susceptibility to *Metarhizium* could be estimated from laboratory tests, supports the robustness of this dose rate.

Both limited trial results on the Desert Locust, and data from other species of locusts and grasshoppers, indicate that some reduction of the dose rate may be possible. However, there is no data yet to assess the robustness of a lower dose rate against the Desert Locust.

The persistence of biological activity of *Metarhizium* spores on vegetation was assessed through bioassays in several field studies. They showed fairly consistent biological half-lives ranging from 4 to 8 days, under semi-arid and sub-tropical conditions.

The analysis of the field efficacy trials supported the observations from specialized studies that secondary pick-up of spores from vegetation is a major, if not the major, exposure route of locusts and grasshoppers. The data suggest that insects should remain at least two days in the treated areas, for maximum efficacy. The operational implication is that it may not be effective to use *Metarhizium* against fast-moving hopper bands of the Desert Locust, since spray block sizes would need to cover several days marching of the insects, or the dose rate would need to be increased so that direct impingement by spray droplets yields a lethal dose of the pathogen. Slow-moving hopper populations (often observed in higher density vegetation) would be better targets

for treatments with the pathogen. The available data on vegetation biomass in Desert Locust trials were insufficient, both in number and in detail, to either confirm or contradict this hypothesis.

It should be stressed that the definition of adequate efficacy applied in this review focuses entirely on mortality. However, *Metarhizium* may have other effects on locusts that contribute to population reductions or damage limitation. These include a reduction in feeding (Moore *et al.*, 1992; de Faria *et al.* 1999; Müller, 2000; Arthurs & Thomas, 2001b) and increased susceptibility to predation (Kooyman & Godonou, 1997; Arthurs & Thomas, 2001b; Kooyman *et al.*, 2005; Mullié, *pers. comm.*). Such effects have not been taken into account, mainly because they have not been described in a consistent manner in field trial reports. However, while sublethal effects may contribute to the overall efficacy of *Metarhizium*, it is unlikely that they will be used as a tool on its own for Desert Locust management.

5.3 Effects of environmental factors on efficacy

Of the various environmental factors that may influence the efficacy of *Metarhizium*, only the effect of temperature could be assessed to a limited extent. A clear reduction in the speed of mortality was observed in the field trials whenever locusts or grasshoppers were allowed to thermoregulate. This supports laboratory-based observations on the importance of temperature on the efficacy of the pathogen.

The performance classification of *Metarhizium* under different ambient temperature conditions, as defined by Blanford & Klass (2004) (Table 3.4) could not be confirmed by the results of the field trials on the Desert Locust. No significant differences were observed in MLTs for the three performance classes. This may have been due to the limited data set that was available (especially for unfavourable conditions) or the lack of detailed temperature information collected during the field trials. It is also possibly that the classification is not entirely correct. In particular, situations where both day-time and night-time temperatures are low may need to be classified as unfavourable, while in Table 3.4 these fall under the favourable performance class. Such situations may be rare in the semi-arid and (sub-)tropical climate zones, but can be encountered in the Sahara Desert or in Mediterranean parts of North-Africa. How exactly to define these unfavourable conditions is beyond this review, but likely average day-time temperatures below ~20 ºC and average night-time temperatures below ~15 ºC would qualify.

There clearly is a need to get better data on the efficacy of *Metarhizium* under different temperature conditions, as temperature seems to set the limits to the use of the pathogen for Desert Locust control. The collection of more frequent temperature data during the entire trial is conditional for any further field testing. Also, a better description of caging conditions (temperature, shading) when assessing efficacy is absolutely necessary if the results of the trials are to be interpreted more precisely.

Of the other environmental factors that may influence efficacy, better data on vegetation biomass (both height and density) and on irradiation intensity seem most urgent. The former is needed to get more information on minimum residence time that locusts need to spend in the treated areas for effective secondary pick-up of spores; the latter to be able to estimate persistence of biological activity of the spores more precisely.

5.4 Effects of predation

From the very first field trials with *Metarhizium* on locusts and grasshoppers, there have been reports that the pathogen increased the susceptibility of the insectics to predation (e.g. Kooyman and Godonou, 1997; Kooyman *et al.*, 2005). Arthurs and Thomas (2001b) suggest that this increased susceptibility may be due to behavioural changes. Mullié (2007), after reviewing evidence from field work with several biopesticides, implies that entomopathogens and predation may work in a synergistic manner, and result in overall increased efficacy of *Metarhizium* in the

field. However, both the quantity and the quality of comprehensive field data that show this effect are scant. It is therefore important that in future field studies, the effect of predation on efficacy is assessed in as detailed a manner as feasible, and that attempts are made to separate out the mortality due to the pathogen in itself from the additional increased predation.

5.5 Future field trials

The review shows that there is a continued need to collect data on the efficacy of *Metarhizium* on locusts and grasshoppers in general, and on Desert Locust in particular. However, this will only contribute to better operational advice on the use of the pathogen if the quality of these data is improved (see above).

Based on the results of this review, the priority for additional field trials would be to further assess the robustness of the use of a dose of about 2.5×10^{12} conidia/ha against the Desert Locust. Trials would therefore need to be carried out in areas or environmental conditions that are expected to be moderately favourable or unfavourable to *Metarhizium*. Such trials would help define the limits of effective use of *Metarhizium* against the Desert Locust. At the present stage, it is considered less of a priority to carry out further trials to reduce the dose rate, in spite if the fact that it is recognized that lower dose rates would reduce the costs of control.

It should be strongly stressed that trials which show inadequate efficacy should not be considered wasted time and resources. As long as the study is properly reported, data on the lack of efficacy are just as important in setting operational limits to the use of the *Metarhizium* as so-called "successful" trials.

It was found during this review that many trial reports were either relatively difficult to obtain because they were unpublished documents stored at research institutions (so-called "grey" literature), or they were published in the scientific literature but did not contain sufficient detail. To ensure that all results of trials with *Metarhizium* on locusts and grasshoppers can be optimally accessed for technical review and further use, it is suggested that an electronic repository is set up of all trial reports, as well as possible subsequent scientific publications, that is freely accessible to the scientific community and to pesticide registration authorities.

5.6 Operational use and monitoring of *Metarhizium* against the Desert Locust

Based on the underlying review, the use of *Metarhizium anisopliae* var. *acridum* against the Desert Locust should provide adequate control at a rate of about 2.5×10^{12} conidia/ha. Until more field data are available on the environmental limits of the pathogen, it is recommended that operational use is restricted to favourable or moderate temperature conditions.

The use of *Metarhizium* against the Desert Locust should initially be limited to control of hopper bands, and should not include the treatment of adult groups or settled swarms. There is no doubt that the pathogen will also be effective against adult locusts (Ouambama *et al.,* 2006), but monitoring of its efficacy is relatively complicated and results are difficult to interpret. Operational treatments of adults are therefore, at this stage, not recommended.

To facilitate the use of *Metarhizium* for operational Desert Locust control, it is recommended that locust control organizations are provided with guidance on the areas and the seasons in their countries where and when the pathogen can be used with a high likelihood of success. Initially, such guidance should be robust, avoiding situations that might limit the effectiveness of the product. At a later stage detailed GIS-based models could be developed, particularly when more field data become available to allow validation (e.g. Klass *et al*., 2007a,b).

All operational use of *Metarhizium* against migratory locusts in general, and the Desert Locust in particular, should be monitored at a reasonable level of detail. Such monitoring should include a

description of the application parameters, an assessment of efficacy, and of vegetation and temperature conditions. While these assessments are not as detailed as in a fully-fledged field efficacy trial, they would definitely be more elaborate than the usual efficacy monitoring which is carried out following control operations with chemical insecticides. However, it is strongly recommended that operational monitoring of *Metarhizium* use is carried out on a standard basis, even if this would require limited additional funding for control operations, because it likely provides the best opportunity to obtain further data on efficacy of the pathogen relatively rapidly.

6. Recommendations

Study quality

1. To improve the quality of trial setup and reporting, it is recommended that:
   - FAO should elaborate a guideline on the design, execution and reporting of efficacy trials with *Metarhizium* against migratory locusts, based on already existing guidance documents and the insights gained through this review;
   - future field efficacy trials of *Metarhizium* on the Desert Locust should follow this FAO guideline;
   - a specific sub-set of reporting requirements should be defined for the monitoring of the operational use of *Metarhizium* against migratory locusts.

Field trials against the Desert Locust

2. Further field efficacy trials of *Metarhizium anisopliae* var. *acridum* (IMI 330189) against the Desert Locust should be carried out when appropriate targets present themselves, at a dose rate of about 2.5 x 10^{12} conidia/ha, and should in particular focus on:
   - the environmental limits of the use of *Metarhizium*, in particular temperature conditions;
   - the relationship between vegetation biomass, hopper displacement and effectiveness of secondary pick-up of spores;
   - the effect of the pathogen on the susceptibility of the insects to predation.

   Field efficacy trials aiming at further reduction of the dose rate do, at this stage, not have immediate priority.

3. All field trials should at least be reported in a full technical report, even if efficacy was not adequate, before they are eventually submitted for publication to a scientific journal.

4. An internet-based repository should be set up where all trial reports of *Metarhizium* against locusts and grasshoppers are collected and published, so that they become available for use by scientists and pesticide registration authorities.

Operational use of *Metarhizium* against the Desert Locust

5. There is sufficient field-based evidence to allow the immediate operational use of *Metarhizium anisopliae* var. *acridum* (IMI 330189) against the Desert Locust at a dose rate of about 2.5 x 10^{12} conidia/ha. However, to ensure that locust control units gain further confidence in applying the pathogen, it is recommended that its initial use is limited to:
   - favourable or moderate temperature conditions;
   - nymphal stages of the insects.

6. All operational use of *Metarhizium* against migratory locusts should be monitored for efficacy, on the basis of the monitoring guidance referred to under point 1, even if this requires limited additional funding.

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Vaughan L, Maniania J & Rajaonarison J (2004) Large-scale field trial on the efficacy of *Metarhizium anisopliae* var. *acridum* isolate SP9 against the migratory locust, *Locusta migratoria capito*, in Saodona, Madagascar. 28 April – 19 May 2003. Field trial report 1. Virginia Polytechnic Institute and State University, Blacksburg, USA. **[46]**

Zhang L & Hunter DM (2005) Laboratory and field trials of Green Guard® (*Metarhizium anisopliae* var. *acridum*) (Deuteromycotina: Hyphomycetes) against the oriental migratory locust (*Locusta migratoria manilensis*) (Orthoptera: Acrididae) in China. *Journal of Orthoptera Research* 14(1): 27-30. **[14]**

Annex 1 – Data collection

Data collection for the review followed the stages below

1. Searches for reports on field efficacy trials were carried out, using the following sources:
   a. Documentation available with the reviewer.
   b. Documentation available at FAO Migratory Pests Group.
   c. Reports listed by the FAO Pesticide Referee Group.
   d. Bibliographic searches were carried out using scientific publishers databases Ingenta Connect (www.ingentaconnect.com), BioOne (www.bioone.org), as well as through Google Scholar (http://scholar.google.com) and the ISPI – Pest Directory (CD-ROM of April 2006; also see www.pestinfo.org).

      Search terms that were used:

      Metarhizium AND locust OR grasshopper OR Orthoptera

      Metarhizium anisopliae AND locust OR grasshopper

      M. anisopliae AND locust OR grasshopper

      Metarhizium flavoviride AND locust OR grasshopper

      M. flavoviride AND locust OR grasshopper
   e. In addition, the archives of a number of specific scientific journals were screened: Journal of Orthoptera Research, Biocontrol Science and Technology and Crop Protection.
   f. Various review articles on the use of *Metarhizium* for locust control were screened for additional field trial information.
   g. Publication lists were reviewed of the former Lubilosa Project, the Australian Plague Locust Commission, and the Locust Literature 2003 CD-ROM (ISPI)

2. This yielded a first list of publications which was sent to various experts on locust biocontrol with the request to complete it. Feedback was received from:

   Christiaan Kooyman (International Institute of Tropical Agriculture), David Hunter (pest management consultant, Australia), Peter Spurgin (Australian Plague Locust Commission, Australia), Michel Lecoq (CIRAD, France), Larry Vaughan (Virginia Tech, USA), Bonifácio Magalhães (Embrapa, Brazil), Roger Price (ARC – Plant Protection Research Institute, South Africa) and Jürgen Langewald (BASF, Germany).

3. A final list of field efficacy reports was then prepared which was used as a basis for the review.

4. One additional report was obtained after the *Workshop on the future of biopesticides for Desert Locust control* (February 2007, Saly, Senegal), and incorporated in the review.

Annex 2 – Field efficacy trials included in the review

The following 45 reports of 61 different field efficacy trials with *Metarhizium anisopliae* var. *acridum* were reviewed.

Report no.	Trial no.	Country	Locality	Year of trial	*Metarhizium anisopliae* var. *acridum* isolate	Reference
DESERT LOCUST (*Schistocerca gregaria*)						
1	1	Mauritania	Oued El-Kharob	1995	IMI 330189	Langewald (1995)
2	2	Mauritania	Boumdeid	?	IMI 330189	Kooyman & Godonou (1997
3	3	Mauritania	Tin-Ouich	1995	IMI 330189	Langewald *et al.* (1997a)
4	4	Niger	Agagala	2003	IMI 330189	Aston (2004)
5	5	Sudan	Aeit1	??	IMI 330189	Bashir (2004)
5	6	Sudan	Aeit2	??	IMI 330189	
6	7	Algeria	Oum et Thiour	2005	IMI 330189	Kooyman *et al.* (2005)
7	8	Niger	Aghéliough	2005	IMI 330189	Ouambama *et al.* (2006)
49	65	Mauritania	Tijirit	2003	IMI 330189	Ould Taleb (2004)
RED LOCUST (*Nomadacris septemfasciata*)						
8	9	Mozambique	Buzi	1997	IMI 330189	Price *et al.* (1999)
9	10	Tanzania	Wembere plains	2003	IMI 330189	Kooyman *et al.* (2003a)
10	11	Tanzania	Iku plains	2003	IMI 330189	Kooyman *et al.* (2003b)
MIGRATORY LOCUST (*Locusta migratoria*) [note: LMI *capito* in Madagascar; LMI *manilensis* in China]						
12 [a,b]	13	Madagascar	?	1994	SP3, SP9	Delgado *et al.* (1997a,b)
13	15	Australia	Clermont	1998	FI-985	Hunter *et al.* (1999)
13	16	Australia	Emerald	1999	FI-985	
13	17	Australia	Meandarra	1999	FI-985	
14	18	China	Tianjin	2002	FI-985	Zhang & Hunter (2005)
14	19	China	Henan	2003	FI-985	
46	62	Madagascar	Saodona	2003	SP9	Vaughan *et al.* (2004)
TREE LOCUST (*Anacridium melanorhodon*)						
15	20	Sudan	Tendelti	?	IMI 330189	Kooyman & Abdalla (1998)
BROWN LOCUST (*Locustana pardalina*)						
16	21	South Africa	Nuwefontein	1994	IMI 330189	Bateman *et al.* (1994)
17	22	South Africa	Deelfontein	?	IMI 330189	Price *et al.* (1997)
18	23	South Africa	Britstown	1998	IMI 330189	Arthurs & Thomas (2000)
AUSTRALIAN PLAGUE LOCUST (*Chortoicetes terminifera)*						
19	24	Australia	Coleambally	1993	FI-985	Hooper *et al.* (1995)
20	25	Australia	Griffith	1999	FI-985	Hunter *et al.* (2001)
20	26	Australia	Windorah	2000	FI-985	
20	27	Australia	Gammon Ranges	2000	FI-985	
47	63	Australia	New South Wales	2005	FI-985	Spurgin (unpublished)
RICE GRASSHOPPER (*Hieroglyphus daganensis*)						
21	28	Benin	Malanville	1993	IMI 330189	Lomer *et al.* (1997)

Report no.	Trial no.	Country	Locality	Year of trial	*Metarhizium anisopliae* var. *acridum* isolate	Reference
21	29	Benin	Malanville	1993	IMI 330189	
23	31	Benin	Malanville	1996	IMI 330189	Thomas *et al.* (1998)
24	32	Niger	Tahoua	1999	IMI 330189	Kassa *et al.* (2004)
SENEGALESE GRASSHOPPER (*Oedaleus senegalensis*) [note: often dominant species in mix with other grasshoppers]						
26	34	Mali	Mourdiah	1992	IMI 330189	Douro Kpindou *et al.* (1997)
26	36	Mali	Mourdiah	1994	IMI 330189, I92-794	
28	37	Niger	Gouré	1995	IMI 330189	Maïga *et al.* (1998)
29	38	Niger	Maine Soroa	1995	IMI 330189	Kooyman *et al.* (1997)
30, 31	39	Niger	Maine Soroa	1996	IMI 330189	Langewald *et al.* (1999); Peveling *et al.* (1999)
30	40	Niger	Maine Soroa	1997	IMI 330189	
32	41	Senegal	Nioro du Rip	2002	IMI 330189	Douro Kpindou *et al.* (submitted)
32	42	Senegal	Nioro du Rip & Kaffrine	2003	IMI 330189	
32	43	Senegal	Khelcom	2004	IMI 330189	
12 [a,b]	14	Cape Verde	?	1994	SP9	Delgado *et al.* (1997a,b)
OTHER SAHELIAN GRASSHOPPERs (*Kraussella amabile*) [note: often dominant species in mix with other grasshoppers]						
25	33	Mali	Bandiagara	1996	IMI 330189	Douro Kpindou *et al.* (2001)
26	35	Mali	Mourdiah	1993	IMI 330189, I92-794	Douro Kpindou *et al.* (1997)
26	36	Mali	Mourdiah	1994	IMI 330189, I92-794	
VARIEGATED GRASSHOPPER (*Zonocerus variegatus*)						
34	45	Benin	Lama Forest	1991	I91-609	Lomer *et al.* (1993)
34	46	Benin	Lama Forest	1991	I91-609	
35	47	Benin	Mono	1993	I91-609	Douro Kpindou *et al.* (1995); Thomas *et al.* (1996)
35	48	Benin	Quémé	1993	I91-609	
36, 37	49	Benin	Ketou	1994	I91-609	Langewald *et al.* (1997b)
38	50	Benin	Azové	1996	I91-609	Douro-Kpindou *et al.* (2005)
38	51	Benin	Azové	1997	I91-609	
38	52	Ghana	Nyakrom	1997	I91-609	
WINGLESS GRASSHOPPER (*Phaulacridium vittatum)*						
39	53	Australia	Dalgety	1993	FI-985	Baker *et al.* (1994)
40	54	Australia	Tarago	1994	FI-985	Milner *et al.* (1997)
41	55	Australia	Carabost	1994	FI-985	Milner (1997)
41	56	Australia	Amaroo	1994	FI-985	
48	64	Australia	Dalgety	1999	FI-985	Anonymous (undated)
SPUR-THROOATED LOCUST (*Austracis guttulosa)*						
41	57	Australia	Clermont	1995	FI-985	Milner (1997)
MATO GROSS GRASSHOPPER (*Rhammatocerus schistocercoides)*						
42	58	Brazil	Chapada dos Parecis	1998	CG423	Magalhães *et al.* (2000)
43	59	Brazil	Campos de Julio county	1999	CG423	de Faria *et al.* (2002)
CENTRAL AMERICAN LOCUST (*Schistocerca piceifrons)*						
44	*60*	Mexico	Tizimín	?	MAPL32, MAPL40	Hernández-Velásquez *et al.* (2003)

Annex 3 – Assessment of the quality of the trial setup and its reporting

Quality assessment of each of the reviewed trials, following the criteria set out in Table 2.1. Trials blocked out in grey were excluded from the review because of insufficient quality of the trial or the report, or for reasons clarified in the footnotes.

Abbreviations used: Y = yes, criteria fulfilled; N = no, criteria not fulfilled; P = partial fulfillment of criteria; u = unclear if criteria are fulfilled (incomplete reporting); n.a. = criteria not applicable to the trial.

			Trial and report quality criteria																							**Totals**				
Report number	***Trial number***	**Species**	Control plots used	Control cages used	Plot size OK	Plot separation OK	Vegetation reported	Temperature reported	Rainfall reported	Species reported	Stage reported	Isolate reported	Germination rate reported	Formulation reported	Rotary atomisers used	calibration details reported	VMD OK	Volume application rate OK	Windspeed OK	Dosage measured	Application details reported	Method field assessment reported	Method cage assessment reported	Timing of sampling reported	Type vegetation in cage reported	Cage control mortality OK	**Y**	**N**	**P**	**u**	**n.a.**
1	***1***	*SGR*	Y	Y	u	u	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	u	N	Y	Y	Y	Y	N	Y	18	3	0	3	0
2	***2***	*SGR*	Y	Y	u	u	P	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	u	N	P	na	P	N	N	Y	13	4	3	3	1
3	***3***	*SGR*	Y	Y	N	u	N	Y	N	Y	Y	Y	Y	Y	Y	Y	u	Y	Y	N	N	Y	Y	Y	Y	Y	17	5	0	2	0
4	***4***	*SGR*	Y	Y	Y	N	P	Y	N	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	P	Y	P	Y	N	Y	17	4	3	0	0
5	***5***	*SGR*	na	N	Y	na	N	N	N	Y	Y	Y	N	N	u	N	u	u	u	N	N	na	N	P	N	N	4	12	1	4	3
5	***6***	*SGR*	na	N	Y	na	P	N	N	Y	Y	Y	N	N	u	N	u	u	u	N	N	na	N	N	N	N	4	12	1	4	3
6	***7***	*SGR*	Y	Y	Y	u	N	P	N	Y	N	Y	Y	Y	Y	Y	N	Y	Y	N	Y	Y	P	Y	Y	Y	16	5	2	1	0
7	***8***	*SGR*	N	Y	Y	na	P	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	20	2	1	0	1
49	***65***	*SGR*	Y	Y	Y	Y	Y	P	N	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	P	Y	P	Y	N	Y	18	3	3	0	0
8	***9***	*NSE*	Y	Y	Y	u	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	u	Y	Y	N	P	P	P	Y	N	Y	17	2	3	2	0
9	***10***	*NSE*	Y	Y	Y	u	P	P	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	P	N	Y	18	2	3	1	0
10	***11***	*NSE*	Y	Y	Y	u	P	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	19	3	1	1	0
12	***13***	*LMI*	Y	Y	N	u	N	N	N	Y	Y	Y	N	Y	Y	N	u	P	N	N	N	N	Y	Y	Y	Y	11	10	1	2	0

Report number	Trial number	Species	Trial and report quality criteria																								Totals				
			Control plots used	Control cages used	Plot size OK	Plot separation OK	Vegetation reported	Temperature reported	Rainfall reported	Species reported	Stage reported	Isolate reported	Germination rate reported	Formulation reported	Rotary atomisers used	calibration details reported	VMD OK	Volume application rate OK	Windspeed OK	Dosage measured	Application details reported	Method field assessment reported	Method cage assessment reported	Timing of sampling reported	Type vegetation in cage reported	Cage control mortality OK	Y	N	P	u	n.a.
12	***14***	*OSE*	Y	Y	N	u	N	N	N	Y	Y	Y	N	Y	Y	N	u	P	N	N	N	N	Y	Y	Y	Y	11	10	1	2	0
13	***15***	*LMI*	Y	Y	Y	u	Y	P	N	Y	Y	Y	Y	Y	Y	Y	u	Y	Y	N	Y	Y	Y	Y	Y	u	18	2	1	3	0
13	***16***	*LMI*	Y	Y	Y	u	Y	P	N	Y	Y	Y	Y	Y	Y	Y	u	Y	Y	N	Y	Y	Y	Y	Y	u	18	2	1	3	0
13	***17***	*LMI*	Y	Y	Y	u	Y	P	N	Y	Y	Y	Y	Y	Y	Y	u	Y	Y	N	Y	Y	Y	Y	Y	u	18	2	1	3	0
14	***18***	*LMI*	Y	na	Y	u	P	Y	N	Y	Y	Y	N	Y	u	N	u	Y	u	N	N	Y	na	na	na	na	9	5	1	4	5
14	***19***	*LMI*	N	na	Y	u	P	N	N	Y	Y	Y	N	Y	u	N	u	Y	u	N	N	Y	na	na	na	na	7	7	1	4	5
15	***20***	*AME*	Y	N	Y	Y	N	P	P	Y	Y	Y	Y	Y	u	N	u	P	u	N	N	Y	Y	Y	N	u	11	6	3	4	0
16	***21***	*LPA*	Y	Y	N	u	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	P	N	Y	na	Y	Y	N	Y	17	4	1	1	1
17	***22***	*LPA*	na	Y	N	u	P	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	na	Y	Y	N	Y	16	4	1	1	2
18	***23***	*LPA*	na	Y	u	u	N	P	N	Y	Y	Y	Y	Y	Y	N	u	Y	Y	N	N	na	Y	P	N	Y	11	6	2	3	2
19 [7]	***24***	*CTE*	Y	Y	u	Y	N	Y	N	Y	Y	Y	Y	P	Y	N	Y	Y	u	N	N	N	N	Y	Y	Y	14	7	1	2	0
20	***25***	*CTE*	Y	Y	u	u	N	P	N	Y	N	Y	N	Y	Y	N	u	Y	Y	N	P	Y	N	N	N	u	9	9	2	4	0
20	***26***	*CTE*	Y	Y	u	u	N	P	N	Y	N	Y	N	Y	Y	N	u	Y	Y	N	P	Y	N	N	N	u	9	9	2	4	0
20	***27***	*CTE*	na	Y	u	u	N	P	N	Y	N	Y	N	Y	Y	N	u	Y	Y	N	P	Y	N	Y	N	u	9	8	2	4	1
21	***28***	*HDA*	Y	Y	Y	u	N	N	P	Y	N	Y	N	Y	Y	Y	Y	Y	u	N	N	Y	N	Y	N	u	12	8	1	3	0
21	***29***	*HDA*	na	Y	N	u	N	N	P	Y	N	Y	N	Y	Y	Y	Y	Y	u	N	N	na	N	Y	N	u	9	9	1	3	2
23	***31***	*HDA*	na	Y	u	na	N	P	N	Y	N	Y	N	Y	Y	N	u	Y	u	N	N	na	Y	Y	Y	Y	10	7	1	3	3
24	***32***	*HDA*	na	Y	Y	na	N	Y	N	Y	Y	Y	Y	Y	Y	Y	u	Y	Y	N	N	na	Y	Y	N	P	14	5	1	1	3
25	***33***	*KAM*	Y	Y	N	u	N	N	N	Y	N	Y	N	Y	Y	N	u	Y	Y	N	N	Y	N	Y	N	Y	11	11	0	2	0

[7] Trial excluded from the review because the oil used for the formulation was found to be toxic to *Metarhizium*.

Report number	Trial number	Species	Trial and report quality criteria																									Totals				
			Control plots used	Control cages used	Plot size OK	Plot separation OK	Vegetation reported	Temperature reported	Rainfall reported	Species reported	Stage reported	Isolate reported	Germination rate reported	Formulation reported	Rotary atomisers used	calibration details reported	VMD OK	Volume application rate OK	Windspeed OK	Dosage measured	Application details reported	Method field assessment reported	Method cage assessment reported	Timing of sampling reported	Type vegetation in cage reported	Cage control mortality OK	Y	N	P	u	n.a.
26	***34***	*OSE*	na	Y	N	u	N	N	N	Y	Y	Y	Y	Y	Y	Y	u	Y	Y	N	Y	na	N	Y	N	Y	13	7	0	2	2
26	***35***	*KAM*	na	Y	N	u	N	N	N	Y	Y	Y	Y	Y	Y	Y	u	Y	Y	N	Y	na	N	Y	N	Y	13	7	0	2	2
26	***36***	*OSE*	Y	Y	Y	u	N	N	N	Y	Y	Y	Y	Y	Y	Y	u	Y	Y	N	Y	Y	N	Y	N	Y	16	6	0	2	0
28	***37***	*OSE*	Y	Y	Y	u	P	N	N	Y	Y	Y	N	Y	Y	Y	u	Y	u	N	N	Y	N	Y	N	P	12	7	2	3	0
29	***38***	*OSE*	Y	N	Y	Y	P	N	N	Y	N	Y	N	Y	Y	N	u	Y	Y	N	P	Y	N	Y	N	N	11	10	2	1	0
30/31	***39***	*OSE*	Y	Y	Y	u	Y	Y	Y	Y	N	Y	N	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	N	Y	19	4	0	1	0
30/31	***40***	*OSE*	Y	N	Y	u	P	Y	N	Y	N	Y	N	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	N	u	15	6	1	2	0
32	***41***	*OSE*	Y	Y	Y	u	P	N	N	Y	Y	Y	N	N	N	N	N	Y	u	N	N	Y	N	Y	N	Y	10	11	1	2	0
32	***42***	*OSE*	Y	Y	Y	u	N	N	N	Y	Y	Y	N	N	Y	N	u	Y	u	N	N	Y	N	Y	N	Y	11	10	0	3	0
32	***43***	*OSE*	Y	na	Y	u	N	N	N	Y	Y	Y	N	N	Y	N	u	Y	u	N	N	Y	na	na	na	na	8	8	0	3	5
34	***45***	*ZVA*	Y	Y	N	N	N	Y	N	Y	Y	Y	Y	Y	Y	N	u	Y	Y	N	N	P	P	Y	N	Y	13	8	2	1	0
34	***46***	*ZVA*	Y	Y	N	N	N	Y	N	Y	Y	Y	Y	Y	Y	N	u	Y	N	N	N	P	P	Y	N	Y	12	9	2	1	0
35	***47***	*ZVA*	Y	Y	Y	u	N	N	N	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	N	N	Y	N	Y	N	Y	15	8	0	1	0
35	***48***	*ZVA*	Y	Y	Y	u	N	N	N	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	N	N	Y	N	Y	N	Y	15	8	0	1	0
36/37	***49***	*ZVA*	Y	Y	Y	Y	N	Y	N	Y	Y	Y	N	Y	Y	N	u	Y	u	N	N	Y	Y	Y	N	Y	15	7	0	2	0
38	***50***	*ZVA*	Y	Y	Y	Y	P	N	N	Y	Y	Y	N	Y	Y	N	u	Y	N	N	N	Y	N	Y	N	Y	13	9	1	1	0
38	***51***	*ZVA*	na	Y	Y	Y	P	N	N	Y	Y	Y	N	Y	Y	N	u	Y	N	N	N	na	N	Y	N	Y	11	9	1	1	2
38	***52***	*ZVA*	na	Y	Y	u	P	N	N	Y	Y	Y	N	Y	Y	N	u	Y	N	N	N	na	N	Y	N	Y	10	9	1	2	2
39	***53***	*PVI*	Y	Y	N	N	Y	Y	Y	Y	Y	Y	N	Y	P	N	P	P	P	N	P	Y	P	Y	N	Y	12	6	6	0	0
40	***54***	*PVI*	Y	u	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	N	Y	P	u	N	N	P	N	N	N	u	11	8	2	3	0
41	***55***	PVI	Y	u	u	u	N	N	N	Y	N	Y	N	N	N	N	N	u	u	u	N	N	N	Y	N	u	4	13	0	7	0

Report number	Trial number	Species	Trial and report quality criteria																							Totals				
			Control plots used	Control cages used	Plot size OK	Plot separation OK	Vegetation reported	Temperature reported	Rainfall reported	Species reported	Stage reported	Isolate reported	Germination rate reported	Formulation reported	Rotary atomisers used	calibration details reported	VMD OK	Volume application rate OK	Windspeed OK	Dosage measured	Application details reported	Method field assessment reported	Method cage assessment reported	Timing of sampling reported	Type vegetation in cage reported	Cage control mortality OK	Y	N	P	u	n.a.
41	***56***	PVI	Y	u	N	u	N	N	N	Y	N	Y	N	N	Y	N	u	u	u	u	N	N	N	Y	N	u	5	12	0	7	0
41	***57***	AGU	N	u	u	u	P	N	N	Y	N	Y	N	N	u	N	u	u	u	u	N	N	N	Y	N	u	3	11	1	9	0
42	***58***	*RSC*	Y	Y	N	u	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	u	Y	Y	Y	Y	Y	Y	Y	N	Y	20	2	0	2	0
43	***59***	*RSC*	Y	Y	N	u	Y	N	N	Y	Y	Y	Y	Y	Y	Y	u	Y	Y	N	P	Y	Y	Y	N	Y	16	5	1	2	0
44	***60***	*SPI*	Y	na	Y	u	N	P	P	Y	Y	Y	Y	Y	u	N	u	Y	Y	N	N	Y	na	na	na	na	10	4	2	3	5
46	***62***	*LMI*	Y	Y	Y	P	N	Y	N	Y	N	Y	Y	Y	u	N	u	Y	u	N	N	Y	Y	Y	Y	u	13	6	1	4	0
47	***63***	*CTE*	u	u	Y	u	Y	Y	N	Y	Y	Y	N	P	Y	N	u	Y	u	u	N	na	N	Y	N	u	9	6	1	7	1
48	***64***	*PVI*	Y	Y	Y	na	N	P	N	Y	N	Y	N	P	Y	N	u	Y	u	u	N	P	N	N	N	u	7	9	3	4	1
		Totals																													
		Y	45	47	36	8	12	21	6	61	45	61	29	49	50	25	17	51	30	5	16	36	22	48	12	35					
		N	3	5	15	4	32	26	51	0	16	0	32	9	2	36	3	0	6	51	35	6	27	6	45	3					
		P	0	0	0	1	17	14	4	0	0	0	0	3	1	0	1	5	2	0	10	5	8	3	0	2					
		u	12	4	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	4	4	4	4					
		n.a.	1	5	10	42	0	0	0	0	0	0	0	0	8	0	40	5	23	5	0	0	0	0	0	17					

Annex 4 – Field efficacy trials of *Metarhizium anisopliae* var. *acridum* (isolate IMI 330189) against populations of the Desert Locust (*Schistocerca gregaria*)

The tables below summarize the details of the field efficacy trials against the Desert Locust that were reviewed. Note that report no. 5 was not further used in the assessment (see Chapter 3.1).

A: Trial identification, locust stages and product details.

Trial identification				Trial location & year			Control target								Product (*Metarhizium*) details		
								Stage[3]									
Report no.[1]	Trial no.[1]	Data set no.[2]	Number of plots per data set	Country	Locality	Year	Type	L1	L2	L3	L4	L5	L6	Fl	Ad	Isolate	Germination rate[4] (%)	Formulation[5]
1	**1**	1	3	Mauritania	Oued El-Kharob	1995	band				x	x				IMI 330189	>90	3 groundnut oil : 7 kerosene
1	**1**	2	1	Mauritania	Oued El-Kharob	1995								x		IMI 330189	>90	3 groundnut oil : 7 kerosene
1	**1**	3	3	Mauritania	Oued El-Kharob	1995	band				x	x				control (form.)[6]	--	3 groundnut oil : 7 kerosene
1	**1**	4	1	Mauritania	Oued El-Kharob	1995								x		control (form.)	--	3 groundnut oil : 7 kerosene
2	**2**	1	1	Mauritania	Boumdeid	?	band			x	x					IMI 330189	>80	3 groundnut oil : 7 kerosene
2	**2**	2	1	Mauritania	Boumdeid	?	band				x					IMI 330189	>80	3 groundnut oil : 7 kerosene
2	**2**	3	1	Mauritania	Boumdeid	?	band				x	x				IMI 330189	>80	3 groundnut oil : 7 kerosene
2	**2**	4	1	Mauritania	Boumdeid	?	band					x				IMI 330189	>80	3 groundnut oil : 7 kerosene
2	**2**	5	1	Mauritania	Boumdeid	?	band			x	x					control	--	--
3	**3**	1	3	Mauritania	Tin-Ouich	1995	band		x	x						IMI 330189	>90	3 groundnut oil : 7 kerosene
3	**3**	2	3	Mauritania	Tin-Ouich	1995	band		x	x						control (unspr.)[6]	--	--
4	**4**	1a	1	Niger	Agagala	2003	band			x	x	x				IMI 330189	<78	1 OF : 9 diesel
4	**4**	1b	1	Niger	Agagala	2003								x		IMI 330189	<78	1 OF : 9 diesel
4	**4**	2a	1	Niger	Agagala	2003	band		x	x	x	x				control (unspr.)	--	--
4	**4**	2b	1	Niger	Agagala	2003								x		control (unspr.)	--	--

Trial identification				Trial location & year			Control target								Product (*Metarhizium*) details		
								Stage[3]									
Report no.[1]	Trial no.[1]	Data set no.[2]	Number of plots per data set	Country	Locality	Year	Type	L1	L2	L3	L4	L5	L6	Fl	Ad	Isolate	Germination rate[4] (%)	Formulation[5]
5	**5**	1	3 (?)	Sudan	Aeit1	?	band			x	x	x				IMI 330189		OF : ?
5	**5**	2	3 (?)	Sudan	Aeit1	?	band			x	x	x				IMI 330189		OF : ?
5	**5**	3	3 (?)	Sudan	Aeit1	?	band			x	x	x				IMI 330189		OF : ?
5	**6**	1	3 (?)	Sudan	Aeit2	?	band			x	x	x				IMI 330189		OF : ?
5	**6**	2	3 (?)	Sudan	Aeit2	?	band			x	x	x				IMI 330189		OF : ?
5	**6**	3	3 (?)	Sudan	Aeit2	?	band			x	x	x				IMI 330189		OF : ?
6	**7**	1	1	Algeria	Oum et Thiour	2005	band									IMI 330189	82	1 OF : 9 diesel
6	**7**	2	1	Algeria	Oum et Thiour	2005	band									IMI 330189	89	1 OF : 9 diesel
6	**7**	3	1	Algeria	Oum et Thiour	2005	band									IMI 330189	90	1 OF : 19 diesel
6	**7**	4	1	Algeria	Oum et Thiour	2005	band									IMI 330189	90	1 OF : 19 diesel
6	**7**	5	1	Algeria	Oum et Thiour	2005	band									IMI 330189	89	1 OF : 19 diesel
6	**7**	6	1	Algeria	Oum et Thiour	2005	band									IMI 330189	89	1 OF : 19 diesel
6	**7**	7	1	Algeria	Oum et Thiour	2005	band									control (unspr.)	--	--
7	**8**	1	1	Niger	Aghéliough	2005									x	IMI 330189	91	1 OF : 4 diesel
7	**8**	2	1	Niger	Aghéliough	2005									x	control (unspr.)[7]		
49	**65**	1	1	Mauritania	Tijirit	2003	and		x	x						IMI 330189	84-94	1 OF : 19 diesel
49	**65**	2	1	Mauritania	Tijirit	2003	band		x	x						control (unspr.)	--	--

[1] report no. and trial no. as listed in Annex 2

[2] a data set is a spray plot or a set of plots for which complete application and efficacy details were available

[3] larval stages L1 – L5, fledgelines (fl) and adults (ad)

[4] germination rate of *Metarhizium* in the spray formulation, just before or after the treatment

[5] composition of diluents in the formulation. OF = the oil flowable commercial formulation of Green Muscle

[6] control (form.) = control plots treated with the blank formulation; control (unspr.) = unsprayed control plots

[7] only control cages available, but no control plots.

B: Entomopathogen application details

Trial identification			Application details													
Report no.[1]	Trial no.[1]	Data set no.[2]	Date of treatment	Type of applic.[3]	Plot size (ha)	Sprayer/ atomiser	Volume applic. Rate[4] (L/ha)	Dose rate[5] (conidia/ha)	Dose rate[5] (g/ha)	Dose Type[6]	Emiss. height[7] (m)	Track spacing (m)	Drop Size[8] (µm)	Deposit. meas.[9]	Wind speed (m/s)	Temp. (°C)
1	**1**	1	Mar 96	G	?	ULVA-plus	2	5×10^{12}	100	calib.	{1}	5	{50-100}	lumogen	?	27-34
1	**1**	2	Mar 96	G	?	ULVA-plus	2	5×10^{12}	100	calib.	{1}	5	{50-100}	lumogen	?	27-34
1	**1**	3	Mar 96	G	?	ULVA-plus	2	0	0	calib.	{1}	5	{50-100}	lumogen	?	25-31
1	**1**	4	Mar 96	G	?	ULVA-plus	2	0	0	calib.	{1}	5	{50-100}	lumogen	?	25-31
2	**2**	1	?	G	0.01-2.5	Micro-ULVA	2	5×10^{12}	100	theor.	{1}	5	{50-100}	lumogen	?	?
2	**2**	2	?	G	0.01-2.5	Micro-ULVA	2	5×10^{12}	100	theor.	{1}	5	{50-100}	lumogen	?	?
2	**2**	3	?	G	0.01-2.5	Micro-ULVA	2	5×10^{12}	100	theor.	{1}	5	{50-100}	lumogen	?	?
2	**2**	4	?	G	0.01-2.5	Micro-ULVA	2	5×10^{12}	100	theor.	{1}	5	{50-100}	lumogen	?	?
2	**2**	5	?	--			0	0	0							
3	**3**	1	Oct 95	G	0.3-0.65	Ulva plus	2	5×10^{12}	100	theor.	{1}	?	?	lumogen	2.5-3.5	27-32
3	**3**	2	Oct 95	--			0	0	0							
4	**4**	1a	10-14 Dec 03	G	245	Ulvamast V3E	1	3×10^{12}	49	meas.	{2.5}	30	100	no	1.5-7	?
4	**4**	1b	10-14 Dec 03	G	245	Ulvamast V3E	1	3×10^{12}	49	meas.	{2.5}	30	100	no	1.5-7	?
4	**4**	2a	10-14 Dec 03	--	153		0	0	0							
4	**4**	2b	10-14 Dec 03	--	153		0	0	0							
5	**5**	1	?	G	1	mistblower	?	1.25×10^{12}	?	theor.	{1}	?	?	?	?	?
5	**5**	2	?	G	1	mistblower	?	2.5×10^{12}	?	theor.	{1}	?	?	?	?	?
5	**5**	3	?	G	1	mistblower	?	5×10^{12}	?	theor.	{1}	?	?	?	?	?
5	**6**	1	?	G	1	mistblower	?	1.25×10^{12}	?	theor.	{1}	?	?	?	?	?
5	**6**	2	?	G	1	mistblower	?	2.5×10^{12}	?	theor.	{1}	?	?	?	?	?
5	**6**	3	?	G	1	mistblower	?	5×10^{12}	?	theor.	{1}	?	?	?	?	?
6	**7**	1	3 May 05	A	700	AU5000	1	2.5×10^{12}	50	calib.	6	50	{140-200}	OSP	2-3	23-28
6	**7**	2	4 May 05	A	700	AU5000	1	2.5×10^{12}	50	calib.	6	50	{140-200}	OSP	0.8-1.2	28-31

Trial identification			Application details													
Report no.[1]	Trial no.[1]	Data set no.[2]	Date of treatment	Type of applic.[3]	Plot size (ha)	Sprayer / atomiser	Volume applic. Rate[4] (L/ha)	Dose rate[5] (conidia/ha)	Dose rate[5] (g/ha)	Dose Type[6]	Emiss. height[7] (m)	Track spacing (m)	Drop Size[8] (µm)	Deposit. meas.[9]	Wind speed (m/s)	Temp. (°C)
6	**7**	3	2 May 05	G	25	AU8100	2	2.5×10^{12}	50	calib.	2.5	30	?	no	2-2.5	28-30
6	**7**	4	2 May 05	G	25	AU8100	2	2.5×10^{12}	50	calib.	2.5	30	?	no	3-3.5	24-26
6	**7**	5	4 May 05	G	25	AU8100	2	2.5×10^{12}	50	calib.	2.5	30	?	no	2	26-28
6	**7**	6	5 May 05	G	25	AU8100	2	2.5×10^{12}	50	calib.	2.5	30	?	no	2-2.5	26-29
6	**7**	7	--	--			0	0	0							
7	**8**	1	5 Nov 05	A	492	AU5000	1.18	5.9×10^{12}	118	meas.	5	50	80-110	OSP	2-3.5	23-27
7	**8**	2	--	--			0	0	0							
49	**65**	1	14-18 Dec 03	G	400	UlvaMast	1.08	1.1×10^{12}	27	meas.	2.5	16	~50	lumogen	4.6-4.9	26-27
49	**65**	2	--	--				0	0							

[1] report no. and trial no. as listed in Annex 2

[2] a data set is a spray plot or a set of plots for which complete application and efficacy details were available

[3] G = ground application, A = aerial application

[4] Volume application rate in litres/ha

[5] Dose rate in number of conidia/ha and in grammes/ha. If one of the two was not reported, it was calculated on the basis of 5×10^{12} conidia = 100 g (for IMI 330189)

[6] meas. = dose rate applied actually measured, calib. = dose rate based on calibration only; theor. = dose rate theoretical, i.e. not justified

[7] emission height between brackets {…} were not reported but set by the reviewer: hand-held sprayer = 1 m; vehicle mounted sprayer = 2.5 m

[8] drop sizes are VMD (ranges); values between brackets were set by the reviewer based on atomiser settings and using the atomiser handbooks (if details available).

[9] Type of depositions measurement (if applicable): lumogen fluorescent tracer mixed into the formulation or use of oil sensitive paper (OSP) without tracer

C: Environmental conditions and efficacy assessment methods

Identification			*Vegetation*			Meteorology							Efficacy assessments[5]			
						Temperature[3]				Relative humidity[4]						
Report no.[1]	Trial no.[1]	Data set no.[2]	Height (cm)	Cover (%)	Stage	T_{max} (°C)	T_{min} (°C)	$_{avg}T$ (°C)	Conditions	H_{max} %	H_{min} %	$_{avg}H$ %	Rainfall (mm)	Cage Mortality	Sporulation	Field density	Persistence
1	**1**	**1**	33	50	dry	34	17		favourable	80	7		?	yes	yes	yes	no
1	**1**	**2**	33	50	dry	34	17		favourable	80	7		?	yes	yes	no	no
1	**1**	**3**	33	50	dry	34	17		--	80	7		?	yes	yes	yes	--
1	**1**	**4**	33	50	dry	34	17		--	80	7		?	yes	yes	no	--
2	**2**	**1**	?	10-50	?	40	20		moderate	60	20		?	yes	no	yes	no
2	**2**	**2**	?	10-50	?	40	20		moderate	60	20		?	yes	no	yes	no
2	**2**	**3**	?	10-50	?	40	20		moderate	60	20		?	yes	no	yes	no
2	**2**	**4**	?	10-50	?	40	20		moderate	60	20		?	yes	no	yes	no
2	**2**	**5**	?	10-50	?	40	20		--	60	20		?	yes	no	yes	--
3	**3**	**1**	?	?	?	40	16		unfavourable	85	2		?	yes	yes	yes	yes
3	**3**	**2**	?	?	?	40	16		--	85	2		?	yes	yes	yes	--
4	**4**	**1a**	70	?	drying	26	9	17.3	favourable	34	3		?	yes	yes	yes	yes
4	**4**	**1b**	70	?	drying	26	9	17.3	favourable	34	3		?	yes	yes	yes	yes
4	**4**	**2a**	70	?	drying	26	9	17.3	--	34	3		?	yes	yes	yes	--
4	**4**	**2b**	70	?	drying	26	9	17.3	--	34	3		?	yes	yes	yes	--
5	**5**	**1**	?	?	?	?	?		?	?	?		?	yes	no	no	no
5	**5**	**2**	?	?	?	?	?		?	?	?		?	yes	no	no	no
5	**5**	**3**	?	?	?	?	?		?	?	?		?	yes	no	no	no
5	**6**	**1**	?	45?	?	?	?		?	?	?		?	yes	yes	no	no
5	**6**	**2**	?	45?	?	?	?		?	?	?		?	yes	yes	no	no
5	**6**	**3**	?	45?	?	?	?		?	?	?		?	yes	yes	no	no
6	**7**	**1**	?	?	?	37	19		favourable	?	?		?	yes	yes	partial	no
6	**7**	**2**	?	?	?	37	19		favourable	?	?		?	yes	yes	partial	no
6	**7**	**3**	?	?	?	37	19		favourable	?	?		?	yes	yes	partial	no

Identification			*Vegetation*			Meteorology								Efficacy assessments[5]			
						Temperature[3]				Relative humidity[4]			Rainfall (mm)				
Report no.[1]	Trial no.[1]	Data set no.[2]	Height (cm)	Cover (%)	Stage	T_{max} (°C)	T_{min} (°C)	$_{avg}T$ (°C)	Conditions	H_{max} %	H_{min} %	$_{avg}H$ %		Cage Mortality	Sporulation	Field density	Persistence
6	**7**	**4**	?	?	?	37	19		favourable	?	?		?	yes	yes	partial	no
6	**7**	**5**	?	?	?	37	19		favourable	?	?		?	yes	yes	partial	no
6	**7**	**6**	?	?	?	37	19		favourable	?	?		?	yes	yes	partial	no
6	**7**	**7**	?	?	?	37	19		--	?	?		?	yes	yes	partial	no
7	**8**	**1**	100	?	drying	34	16	26	favourable	?	?		0	yes	yes	yes	yes
7	**8**	**2**	--	--	--	34	16	26	--	?	?		0	yes	yes	no	yes
49	**65**	1	20	75	?	29	15		favourable	?	?		?	yes	yes	no	yes
49	**65**	2	20	80	?	29	15		--	?	?		?	yes	yes	no	yes

[1] report no. and trial no. as listed in Annex 2

[2] a data set is a spray plot or a set of plots for which complete application and efficacy details were available

[3] minimum and maximum temperatures during the observation period, and the period average temperature, if available. Temperature conditions for *Metarhizium* induced mortality according to Table 3.4

[4] minimum and maximum relative humidity during the observation period, and the period average relative humidity, if available

5 Various efficacy assessments hat were carried out: cage mortality after cage incubation of field-treated insects; assessment of sporulation rate in field-treated insects; assessment of fluctuations in field densities after treatment; and assessment of persistence on vegetation of the entomopathogen after treatment, through bioassays with untreated locusts.

Annex 5 – Laboratory susceptibility of locusts and grasshoppers to *Metarhizium*

A: Time reponse data

Median lethal times (MLT) or average survival times (AST) for various isolates of *Metarhizium anisopliae* var, *acridum*.

Selected were locust and grasshopper species which are listed in Table 4.1 and for which at least 2 values were determined. Only tests carried out at constant temperatures within the range of 25 – 30 °C were included. All treatments were topical, with aerial conidia in an oil-based formulation.

Insect stage tested [1]	**Temp.** [2] °C	**R.H.** [2] %	**Dose** conidia/insect	**Time response**			**Reference {report number}**
				MLT [4]		**AST** [5]	
				days	type	days	
Desert Locust treated with IMI 330189							
Ad	30	~30	280	7.4	A	7.8	Bateman *et al.* (1996) {L1}
			280	6.7		7.0	
			2 700	6.1		6.1	
			2 700	5.5		5.8	
			32 000	5.0		5.0	
			32 000	5.2		5.2	
			75 000	4.4		4.4	
			310 000	4.5		4.5	
			310 000	4.5		4.5	
Ad	30	~35	2 000	5.7	R		Bateman *et al.* (1993) {L4}
			10 000	5.0			
			100 000	4.2			
			1 000 000	3.7			
Ad	25		1 000	n.a.		8.4	Arthurs & Thomas (2001a) {L8}
	30		1 000			8.9	
	25		10 000			7.1	
	30		10 000			6.8	
	25		100 000			6.1	
	30		100 000			5.4	
Ad	25		100 000	4.8	A		Fargues *et al.* (1997) {L9}
	30		100 000	3.7			
Ad	~30		1000	7.4	R	8.5	Blanford & Thomas (2001) {L12}
Ad	30	30-40	800000	4.5	R		Moore *et al.* (1992) {L27}
			80000	5.0			
			17000	6.5			
			2750	11.0			
Red Locust treated with IMI 30189							
L3	30	65-90	20 000	5.7	R		Price *et al.* (1999) {L13}
			700 000	2.6			

Insect stage tested [1]	Temp. [2] °C	R.H. [2] %	Dose conidia/insect	Time response			Reference {report number}
				MLT [4]		AST [5]	
				days	type	days	
Brown Locust treated with IMI 330189							
L5	30		1 000	8.9	R	7.9	Müller (2000) {L22}
			10 000	7.5		8.1	
			100 000	6.2		6.1	
			1 000 000	5.4		4.6	
			10 000 000	4.5		3.9	
			100 000 000	3.4		2.8	
L5	24-34 (avg. 27)	13-23	30000	9.8	A		Bateman *et al.* (1994) {L14}
			60000	7.5			
Ad	24-34 (avg. 27)	28-52	30000	10.1			
			60000	4.4			
Australian Plague Locust treated with FI-985							
L4	29	~100	7500	4.3	A		Milner & Prior (1994) {L2}
L5	29	~100	750	4.6			
Mato Grosso Grasshopper treated with CG 423							
L3	27	55	3000	4.3	R	4.5	Magalhães *et al.* (1997) {L3}
L3	25-27		3000	5.4	G		Magalhães *et al.* (2003) {L6}
L5	25-27		3000	9.2			
Variegated Grasshopper treated with I91-609							
Ad	25		5.00E+05			6.2	Thomas & Jenkins (1997) {L25}
	30		5.00E+05			5.2	
	25		1.00E+05			8.1	
	30		1.00E+05			5.5	
	25		1.00E+04			7.6	
	30		1.00E+04			6.5	

[1] Ad = adults, L = nymphs. [2] Temp. = temperature (range) during the test and subsequent incubation; R.H. = relative humidity. [3] Median lethal times (MLT) were either reported by the author of the study (type "A"), or estimated by the reviewer by linear interpolation of data presented in a graph or table in the publication (type "R"). [4] Average survival times (AST) were always reported by the author of the study.

B: Dose reponse data

Median lethal doses (LD_{50}s) for various isolates of *Metarhizium anisopliae* var, *acridum*.

Selected were locust and grasshopper species which are listed in Table 4.1 and for which at least 2 values were determined. Only tests carried out at constant temperatures within the range of 25 – 30 °C were included. All treatments were topical, with aerial conidia in an oil-based formulation.

Insect stage tested [1]	Temp. [2] °C	R.H. [2] %	Dose response [4]				Reference {report number}
			Incubation time [3] days	LD_{50} conidia/insect	95% C.I. [5] conidia/insect	type	
Desert Locust treated with IMI 330189							
Ad	30	~30	6	1 297	403 – 4 059	R	Bateman *et al.* (1996) {L1}
			6	4 057	2 298 – 7 160		
Ad	30	~35	4	23 000	n.a. [6]	A	Bateman *et al.* (1993) {L4}
			5	8 900	6 300 – 11 800		
Ad	30	30-40	5	387 950	180 570 – 833 490	R	Moore *et al.* (1992) {L27}
			6	33 750	28 520 – 39 930		
			7	9 750	7 170 – 13 260		
			9	6 000	4 700 – 7 670		
			12	3 960	2 230 – 7 040		
Brown Locust treated with IMI 330189							
L5	30		4	167×10^6	$84 \times 10^6 – 333 \times 10^6$	R	Müller (2000) {L22}
			5	9.7×10^6	$4.8 \times 10^6 – 19.5 \times 10^6$		
			6	529 700	278 200 – 1 008 600		
			7	179 200	91 500 – 250 800		
			9	7 500	2 300 – 24 700		
			12	6 700	1 500 – 29 900		
Australian Plague Locust treated with FI-985							
L5	29		6	417	220 – 721	A	Milner & Prior (1994) {L2}
Migratory Locust treated with FI-985							
Ad	28-29		9	4363	987 – 16 460	A	Milner *et al.* (1996) {L20}
			12	387	68 – 1 602		

[1] Ad = adults, L = nymphs. [2] Temp. = temperature (range) during the test and subsequent incubation; R.H. = relative humidity.
[3] Median lethal doses (LD_{50}) were either reported by the author of the study (type "A"), calculated by the reviewer with the trimmed Spearman-Karber method, using data presented in a graph or table in the publication (type "R"). [5] C.I. = confidence interval.
[6] n.a. = not available.

Annex 6 – Field trials on other species of locusts and grasshoppers which were retained for the review

Summary of the field efficacy trials carried out against other spcies of locusts and grasshoppers which were included in the review. A more detailed data set is available as a spreadsheet. If no value is listed in the table, data were not available.

Trial identification					**Insect stage** [3]	**Metarhizium isolate** [4]	**Pesticide application**			**Assessments** [6]				**Effect**					
														Cage mortality [7]				**Field population assessment** [8]	
Report no. [1]	Trial no. [1]	Data set no. [2]	Country	Year			Type [5]	Plot size (ha)	Dose (conidia/ha)	Cage mortality	Sporulation	Field efficacy	Persistence	Location	MLT (days)	LT_{90} (days)	Max. mortality (%)	Max. efficacy (%)	Attained at (days AT)
Red Locust (*Nomadacris septemfasciata*)																			
8	9	1	Mozambique	1997	L3	IMI 330189	G	1	5×10^{12}	Y	N	N	P	shade	3.6	8	100	--	--
8	9	4			L3	control (unspr.)		1	0						--	--	23		
8	9	2			L3	IMI 330189	G	1	5×10^{12}						3.9	9	98		
8	9	5			L3	control (unspr.)		1	0						--	--	13		
8	9	3			L4	IMI 330189	G	1	5×10^{12}						6	21	90		
8	9	6			L4	control (unspr.)		1	0						--	--	17		
9	10	1	Tanzania	2003	L4 – Fl	IMI 330189	A	1776	3×10^{12}	Y	N	Y	N	?	11	20	90	-44	24
9	10	2			L4 – Fl	IMI 330189	A	1770	1.5×10^{12}						11		72	43	21
9	10	3			L4 – Fl	control (unspr.)		600	0						18		52		
10	11	1a	Tanzania	2003	Ad	IMI 330189	A	1400	2.5×10^{12}	Y	Y	P	N	sun	10	17	90	--	--
10	11	1b			Ad	IMI 330189	A	1400						shade	8	10	98		
10	11	2a			Ad	IMI 330189	A	400 & 800	1.25×10^{12}					sun	11		87		
10	11	2b			Ad	IMI 330189	A	400 & 800						shade	8	12	98		
10	11	3			Ad	control (unspr.)		400	0					?			10		
Migratory Locust (*Locusta migratoria*)																			
12	13	1	Madagascar	1994	L3-4	SP3	G	0.005	2.5×10^{13}	Y	Y	N	N	shade	5.4		89	--	--
12	13	2			L3-4	SP9	G	0.005	2.5×10^{13}					shade	5	6.6	100		
12	13	3			L3-4	control (unspr.)			0					shade			4		
13	15	1	Australia	1998	L3-5	FI-985	A	50	3.5×10^{12}	Y	Y	Y	Y	n.a	n.a	n.a.	n.a	89	15-18
13	15	2			L3-5	FI-985	A	50	3.5×10^{12}									98	15-18

Trial identification					Insect stage [3]	Metarhizium isolate [4]	Pesticide application			Assessments [6]				Effect					
														Cage mortality [7]				Field population assessment [8]	
Report no. [1]	Trial no. [1]	Data set no. [2]	Country	Year			Type [5]	Plot size (ha)	Dose (conidia/ha)	Cage mortality	Sporulation	Field efficacy	Persistence	Location	MLT (days)	LT_{90} (days)	Max. mortality (%)	Max. efficacy (%)	Attained at (days AT)
13	15	3			L3-5	FI-985	A	77	3.5×10^{12}									99	15-18
13	15	4			L4-5	FI-985	A	49	3.5×10^{12}									89	15-18
13	16	5			L3-5	FI-985	A	58	3.5×10^{12}									98	15-18
13	16	6	Australia	1999	L2-4	FI-985	A	65	3.5×10^{12}	Y	Y	Y	Y	n.a	n.a	n.a.	n.a	90	15-18
13	16	7			L3-4	FI-985	A	80	1×10^{12}									30	15-18
13	17	8			L2-4	FI-985	A	56	3.8×10^{12}									99	15-18
13	17	9			L3-5	FI-985	A	25	3.8×10^{12}									99	15-18
13	17	10			L3-5	FI-985	A	21	3.8×10^{12}									26	15-18
13	17	11	Australia	1999	L4-5	FI-985	A	89	3×10^{12}	Y	Y	Y	Y	n.a	n.a	n.a.	n.a	98	15-18
13	17	12			L4-5	FI-985	A	106	3×10^{12}									92	15-18
13	17	13			L4-5	FI-985	A	133	2.1×10^{12}									73	15-18
13	17	14			L4-5	FI-985	A	25	2.1×10^{12}									-13	15-18
13	15-17	15	Australia	1999		control (untr)			0	Y	Y	Y	Y	n.a	n.a	n.a.	n.a	-18	15-18
14	18	1			L3-5	FI-985	G	20	3×10^{12}									72	8
14	18	2	China	2002	L3-5	FI-985	G	20	2×10^{12}	N	N	Y	N	n.a	n.a	n.a.	n.a	97	8
14	18	3			L3-5	control (formul.)	G	10	0									-19	8
14	18	4			L3-5	control (untr.)		20	0									12	8
14	19	5	China	2003	L3-5	FI-985	G	80	5×10^{12}	N	N	Y	N	n.a	n.a	n.a.	n.a	65	11
14	19	6			L3-5	FI-985	G	80	2×10^{12}									76	11
46	62	1				SP9	G	9.3	2.1×10^{12}										
46	62	2				SP9	G	9.8	2.1×10^{12}									56	14
46	62	3				SP9	G	12.6	2.1×10^{12}										
46	62	4	Madagascar	2003	n.a	control (untr.)		29	0	Y	N	Y	Y	n.a	n.a	n.a.	n.a		
46	62	5				control (untr.)		17	0										
46	62	6				control (untr.)		27.6	0										

Trial identification					Insect stage [3]	Metarhizium isolate [4]	Pesticide application			Assessments [6]				Effect					
														Cage mortality [7]				Field population assessment [8]	
Report no. [1]	Trial no. [1]	Data set no. [2]	Country	Year			Type [5]	Plot size (ha)	Dose (conidia/ha)	Cage mortality	Sporulation	Field efficacy	Persistence	Location	MLT (days)	LT_{90} (days)	Max. mortality (%)	Max. efficacy (%)	Attained at (days AT)
Tree Locust (*Anacridium melanorhodon*)																			
15	20	1	Sudan	n.a	L4-5	IMI 330189	G	17	5×10^{12}	Y	Y	Y	N	shade	n.a.	n.a.	97	66	18
15	20	2			L4-5	control (untr)		18	0										
Brown Locust (*Locustana pardalina*)																			
16	21	1			L4-5	control (formul.)	G	0.1	0								22		
16	21	2			L4-5	IMI 330189	G	0.1	7.4×10^{12}						17		55		
16	21	3			L4-5	IMI 330189	G	0.1	7.4×10^{12}						8	11	100		
16	21	4	South Africa	1994	L4-5	IMI 330189	G	0.1	9.4×10^{12}	Y	N	N	N	n.a	10	12	100	n.a	n.a.
16	21	5			L4-5	IMI 330189	G	0.1	9.4×10^{12}						10	14	100		
16	21	6			L4-5	control (formul.)	G	0.1	0								30		
16	21	7			L4-5	IMI 330189	G	0.1	9.4×10^{12}						8	13	100		
17	22	1	South Africa	n.a	L5	IMI 330189	A	0.1-1	3×10^{12}	Y	N	N	N	n.a	12		76	n.a	n.a.
17	22	2a			L5	IMI 330189	A	0.1-1	3×10^{12}						8		98		
17	22	2b			L5	IMI 330189	A	0.1-1	3×10^{12}						16		85		
17	22	3a			L5	IMI 330189	A	0.1-1	1.9×10^{12}						8		95		
17	22	3b			L5	IMI 330189	A	0.1-1	1.9×10^{12}						11		98		
17	22	4a			L5	IMI 330189	A	0.1-1	1.9×10^{12}						5		100		
17	22	4b			L5	IMI 330189	A	0.1-1	1.9×10^{12}						14		88		
17	22	5a			L5	IMI 330189	A	0.1-1	1.9×10^{12}						5		100		
17	22	5b			L5	IMI 330189	A	0.1-1	1.9×10^{12}						12		85		
17	22	6a			L5	IMI 330189	A	0.1-1	2×10^{12}						12		67		
17	22	6b			L5	IMI 330189	A	0.1-1	2×10^{12}								38		
17	22	7a			L5	IMI 330189	A	0.1-1	2×10^{12}						11		96		
17	22	7b			L5	IMI 330189	A	0.1-1	2×10^{12}						19		62		
17	22	8a			L5	IMI 330189	A	0.1-1	1.8×10^{12}						10		98		
17	22	8b			L5	IMI 330189	A	0.1-1	1.8×10^{12}						12		98		
17	22	9a			L5	IMI 330189	A	0.1-1	1.8×10^{12}						11		98		

Trial identification					Insect stage [3]	Metarhizium isolate [4]	Pesticide application			Assessments [6]				Effect					
														Cage mortality [7]				Field population assessment [8]	
Report no. [1]	Trial no. [1]	Data set no. [2]	Country	Year			Type [5]	Plot size (ha)	Dose (conidia/ha)	Cage mortality	Sporulation	Field efficacy	Persistence	Location	MLT (days)	LT_{90} (days)	Max. mortality (%)	Max. efficacy (%)	Attained at (days AT)
17	22	9b			L5	IMI 330189	A	0.1-1	1.8×10^{12}						13		98		
17	22	10a			L5	IMI 330189	A	0.1-1	$1.8 \times 10^{12}12$						12		98		
17	22	10b			L5	IMI 330189	A	0.1-1	1.8×10^{12}						12		96		
17	22	11			L5	control (untr.)			0								16		
17	22	12			L5	control (untr.)			0								14		
18	23	1a	South Africa	1998	Fl	IMI 330189	G	n.a	5×10^{12}	Y	N	N	N	shade	6.5	9	100	n.a.	n.a.
18	23	2a			Fl	control (untr.)			0					shade			30		
18	23	1b			Fl	IMI 330189	G	n.a.	5×10^{12}					sun	38		89		
18	23	2b			Fl	control (untr.)			0					sun			42		
Australian Plague Locust (*Chortoicetes terminifera*)																			
20	25	1	Australia	1999	L?	FI-985	A	n.a.	$3\text{-}4 \times 10^{12}$	Y	N	Y	N	n.a.	n.a.	n.a.	n.a.	100	21
20	25	2			L?	FI-985	A	n.a.	$1\text{-}2 \times 10^{12}$									99	21
20	25	3			L?	control(untr.)	A	n.a.	0									0	20
20	26	4	Australia	2000	L?	FI-985	A	n.a.	5×10^{12}	Y	N	Y	N	n.a.	n.a.	n.a.	n.a.	85	15
20	26	5			L?	FI-985	A	n.a.	3×10^{12}									95	13
20	26	6			L?	FI-985	A	n.a.	1×10^{12}									94	13
20	26	7			L?	control(untr.)	A	n.a.	0									94	13
20	27	8	Australia	2000	L?	FI-985	A	n.a.	1×10^{12}	Y	N	Y	N	n.a.	n.a.	n.a.	90	90	16
47	63	1	Australia	2005	L3-4	FI-985	A	200	1×10^{12}	Y	N	N	N	n.a.	n.a.	n.a.	60-75	n.a.	n.a.
Rice Grasshopper (*Hieroglyphus daganensis*)																			
21	28	1	Benin	1993	n.a.	IMI 330189	G	4	5×10^{12}	Y	Y	Y	N	shade	9		78	62	28
21	28	2				control (untr.)		4	0					shade			7		
21	29	1	Benin	1993	n.a.	IMI 330189	G	0.25	5×10^{12}	Y	Y	N	Y	shade	11	12	100	n.a	n.a.
21	29	2				IMI 330189	G	0.25	5×10^{12}					shade	8	21	90		
21	29	3				IMI 330189	G	0.25	5×10^{12}					shade	8	21	92		
21	29	4				control (formul.)	G	0.25	0					shade			11		

Trial identification					Insect stage [3]	Metarhizium isolate [4]	Pesticide application			Assessments [6]				Effect					
														Cage mortality [7]				Field population assessment [8]	
Report no. [1]	Trial no. [1]	Data set no. [2]	Country	Year			Type [5]	Plot size (ha)	Dose (conidia/ha)	Cage mortality	Sporulation	Field efficacy	Persistence	Location	MLT (days)	LT_{90} (days)	Max. mortality (%)	Max. efficacy (%)	Attained at (days AT)
23	31	1a	Benin	1996	L5	IMI 330189	G	n.a.	5×10^{12}	Y	N	N	N	n.a	7.1	12.6	99	n.a.	n.a.
23	31	1b			L5	IMI 330189	G	n.a.	5×10^{12}					n.a	10	18.7	98		
23	31	1c			L5	IMI 330189	G	n.a.	5×10^{12}					n.a	7.7	12.5	100		
23	31	2			L5	IMI 330189	G	1	5×10^{12}					shade	3		73		
23	31	3			L5	control(untr)		n.a.	0					shade			25		
24	32	1	Niger	1999	Ad	IMI 330189	G	1	5×10^{12}	Y	N	N	Y	shade	3	8	100		
24	32	1			Ad	control(untr)			0					shade	17		33		
Krausella amabile																			
25	33	1	Mali	1996	n.a.	IMI 330189?	G	0.25	5×10^{12}	Y	N	Y	N	n.a.	5	11	93	60	4
25	33	2				control(untr)		0.25	0					n.a.			22		
26	35	3	Mali	1993	L1-3	IMI 330189	G	0.25	2×10^{12}	Y	N	N	N	shade	9		74	n.a.	n.a.
26	35	4			L1-3	I92-794	G	0.25	2×10^{12}					shade	7	11	94		
26	35	5			L1-3	control (formul)	G	0.25	0					shade			9		
26	36	6b	Mali	1994	L3-5	IMI 330189	G	1	5×10^{12}	Y	N	N	N	shade	7		85	n.a.	n.a.
26	36	7b			L3-5	I92-794	G	1	5×10^{12}					shade	6		80		
26	36	8b			L3-5	control (formul)	G	1	0					shade			6		
Senegalese Grasshopper (*Oedaleus senegalensis*)																			
12	14	1a	Cape Verde	1994	L3	SP9	G	0.5	2.5×10^{13}	Y	Y	N	N	shade	4.1	5	100	n.a.	n.a.
12	14	1b			L3	SP9	G	0.5	2.5×10^{13}					n.a.	5.9	8	90		
12	14	2a			L3	control (unspr.)			0					shade			11		
12	14	2b			L3	control (unspr.)			0					n.a.			35		
26	34	1a	Mali	1992	L3-5	IMI 330189	G	0.25	2×10^{12}	Y	N	N	N	n.a.	7	13	90	n.a.	n.a.
26	34	1b			L3-5	IMI 330189	G	0.25	2×10^{12}					shade	7		82		
26	34	2a			L3-5	control (formul)	G	0.25	0					n.a.			25		
26	34	2b			L3-5	control (formul)	G	0.25	0					shade			48		

Trial identification					Insect stage [3]	Metarhizium isolate [4]	Pesticide application			Assessments [6]				Effect						
														Cage mortality [7]					Field population assessment [8]	
Report no. [1]	Trial no. [1]	Data set no. [2]	Country	Year			Type [5]	Plot size (ha)	Dose (conidia/ha)	Cage mortality	Sporulation	Field efficacy	Persistence	Location	MLT (days)	LT_{90} (days)	Max. mortality (%)	Max. efficacy (%)	Attained at (days AT)
26	36	6a	Mali	1994	L3-5	IMI 330189	G	1	5×10^{12}					shade	8		76	72	22
26	36	7a			L3-5	I92-794	G	1	5×10^{12}	Y	N	Y	N	shade	8	13	94	73	22
26	36	8a			L3-5	control (formul)	G	1	0					shade			13	96	22
28	37	1a	Niger	1995	L3-4	IMI 330189	G	5	5×10^{12}			Y		shade	8.5	11	96	43	11
28	37	1b			L3-4	IMI 330189	G	5	5×10^{12}	Y	Y	N	N	n.a.	9	12	93	n.a.	n.a.
28	37	2a			L3-4	control (untr)		5	0			Y		shade			30		
28	37	2b			L3-4	control (untr)		5	0			N		n.a.			39		
29	38	1	Niger	1995	n.a.	IMI 330189	G	50	4.2×10^{12}	Y	N	Y	Y	shade	n.a.	n.a.	85	80	21
29	38	2			n.a.	control (untr)			0	N	N	Y	N						
32	41	1	Senegal	2002	L3-Ad	IMI 330189	G	50	2.5×10^{12}	Y	N	Y	Y	n.a			70	55	21
32	41	2			L3-Ad	control (untr)	G	50						n.a			22		
32	42	1	Senegal	2003	L2-4	IMI 330189	G	25	2.5×10^{12}	Y	N	Y	N	n.a			65	79	24
32	42	2			L2-4	control (untr)	G	25						n.a			25		
32	43	1	Senegal	2004	L2-4	IMI 330189	G	25	2.5×10^{12}	N	N	Y	N	n.a			n.a	93	22
32	43	2			L2-4	control (untr)	G	25						n.a			n.a		
Variegated Grasshopper (*Zonocerus variegatus*)																			
34	45	1	Benin	1991	L3-6	I91-609	G	0.05-0.17	2×10^{12}	Y	N	Y	N	shade	7	19	90		
34	45	2			L3-6	control (formul)	G	0.025-0.1	0					shade			10		
34	46	1	Benin	1991	L5-Ad	I91-609	G	0.05-0.06	7×10^{11}					shade	14		75		
34	46	2			L5-Ad	I91-609	G	0.05-0.06	2.1×10^{12}	Y	N	Y	N	shade	7	14	98		
34	46	3			L5-Ad	control (formul)	G	0.05-0.06	0					shade			20		
35	47	1	Benin	1993	L5-Fl	I91-609	G	1	2×10^{12}	Y	N	Y	N	shade	7	14	96	87	15
35	47	2			L5-Fl	control (untr)			0					shade			5		
35	48	3	Benin	1993	Ad	I91-609	G	1	2×10^{12}	Y	N	Y	N	shade	5	8	100	90	18
35	48	4			Ad	control (untr)			0					shade			25		
36	49	1	Benin	1994	L5-Ad	I91-609	G	1	5×10^{12}	Y	N	Y	Y	shade	9	25	90	89	14
36	49	2			L5-Ad	control (formul)	G	1	0					shade			13		

Trial identification					Insect stage [3]	Metarhizium isolate [4]	Pesticide application			Assessments [6]				Effect					
														Cage mortality [7]				Field population assessment [8]	
Report no. [1]	Trial no. [1]	Data set no. [2]	Country	Year			Type [5]	Plot size (ha)	Dose (conidia/ha)	Cage mortality	Sporulation	Field efficacy	Persistence	Location	MLT (days)	LT_{90} (days)	Max. mortality (%)	Max. efficacy (%)	Attained at (days AT)
38	50	1a	Benin	1996	L4-Ad	I91-609	G	1	1×10^{11}	Y	Y	Y	Y	shade	12.3*			90	21
38	50	1b			L4-Ad	I91-609	G	1	1×10^{11}		N	N	N		15.3*				
38	50	2a			L4-Ad	I91-609	G	1	4.65×10^{11}		Y	Y	Y	shade	9.3*			82	21
38	50	2b			L4-Ad	I91-609	G	1	4.65×10^{11}		N	N	N		12.3*				
38	50	3a			L4-Ad	I91-609	G	1	2.5×10^{12}	Y	Y	Y	Y	shade	8*			98	21
38	50	3b			L4-Ad	I91-609	G	1	2.5×10^{12}		N	N	N		10.7*				
38	50	4a			L4-Ad	I91-609	G	1	1×10^{13}		Y	Y	Y	shade	6.3*			98	21
38	50	4b			L4-Ad	I91-609	G	1	1×10^{13}		N	N	N		9.7*				
38	50	5a			L4-Ad	control (untr)		1	0		Y	Y	Y	shade	18.3*				
38	50	5b			L4-Ad	control (untr)		1	0		N	N	N		20.3*				
38	51	1	Benin	1997	L4-6	I91-609	G	1	1×10^{11}	Y	Y	N	N	shade	18.7*				
38	51	2			L4-6	I91-609	G	1	1×10^{12}					shade	12*				
38	51	3			L4-6	I91-609	G	1	2.5×10^{12}					shade	12.3*				
38	51	4			L4-6	control (untr)			0					shade	20.7*				
38	52	1	Ghana	1997		I91-609	G	1	2.5×10^{12}	Y	N	Y	N	shade	6.7*				
38	52	2				I91-609	G	1	1.25×10^{12}					shade	9.3*				
38	52	3				I91-609	G	1	6.25×10^{11}					shade	14.3*				
38	52	4				control (untr)		1	0					shade	18.7*				
Wingless Grasshopper (*Phaulacridium vittatum*)																			
39	53	1	Australia	1993	L4	FI-985	G	0.25	3.56×10^{12}	Y	Y	Y	N	shade	6	8	95	56	32
39	53	2			L4	FI-985	G	0.25	3.56×10^{12}					shade	6	8	98	56	32
39	53	3			L4	control (oil)	G	0.25	0					shade			24		
39	53	4			L4	control (water)	G	0.25	0					shade			24		
40	54	1	Australia	1994	Ad	FI-985	G	5	3.1×10^{12}										
40	54	2			Ad	control (untr)		5	0										
48	64	1	Australia	1999		FI-985	A	300	3×10^{12}	Y	Y	Y	Y				95	76	28
48	64	2				control (untr)			0										

Trial identification					Insect stage [3]	Metarhizium isolate [4]	Pesticide application			Assessments [6]				Effect					
														Cage mortality [7]				Field population assessment [8]	
Report no. [1]	Trial no. [1]	Data set no. [2]	Country	Year			Type [5]	Plot size (ha)	Dose (conidia/ha)	Cage mortality	Sporulation	Field efficacy	Persistence	Location	MLT (days)	LT_{90} (days)	Max. mortality (%)	Max. efficacy (%)	Attained at (days AT)
Mato Grasso Grasshopper (*Rhammatocerus schistocercoides*)																			
42	58	1	Brazil	1998	L3	CG 423	G	0.21	2.14×10^{13}	Y	N	Y	N	shade			77	84	14
42	58	2			L3	CG 423	G	0.22	2.08×10^{13}					shade			77	69	12
42	58	3			L3	CG 423	G	0.3	2.06×10^{13}					shade			77	86	16
42	58	4			L3	control (untr)			0					shade			11		
42	58	5			L3	control (untr.)			0					shade			11		
42	58	6			L3	control (untr.)			0					shade			11		
43	59	1	Brazil	1999	L2	CG 423	G	0.01-0.03	5×10^{12}	Y	N	Y	Y				85	82	15
43	59	2			L2	CG 423	G	0.01-0.03	5×10^{12}								93		
43	59	3			L2	CG 423	G	0.01-0.03	5×10^{12}								86	84	15
43	59	4			L2	CG 423	G	0.01-0.03	1×10^{13}								81	62	16
43	59	5			L2	CG 423	G	0.01-0.03	1×10^{13}								67	62	16
43	59	6			L2	CG 423	G	0.01-0.03	1×10^{13}								86	80	16
43	59	7			L2	control (untr)			0								21		
43	59	8			L2	control (untr)			0								21		
43	59	9			L2	control (untr)			0								21		
Central American Locust (Schistocerca piceifrons)																			
44	60	1	Mexico		L1-2		G	5	2.5×10^{12}	N	N	Y	N					84	13
44	60	2			L1-2		G	12	2.5×10^{12}									95	13
44	60	3			L1-2				0										

[1] Report no. and trial no. as listed in Annex 2. [2] A data set is a spray plot or a set of plots for which complete application and efficacy details were available. [3] larval stages L1 thtough L6, fledgelings (Fl) and adults (Ad). [4] Control (untr.) = uintreated control, control (formul.) = formulation control. [5] Type of application: G = ground, A = air. [6] various types of efficacy assessments that are reported for the trial. [7] cage incubation assessments of mortality: Location of the cages (in shade or sun), MLT = median lethal time, LT_{90} = time to 90% mortality, Maximum cumulative mortality observed in the cages. [8] Field population assessments: max. control-corrected efficacy, and the day after treatment that this was reached.

Annex 7 – Comparison of speed of mortality in sunny or shady conditions

MLT and LT_{90} values for locusts and grasshoppers treated with *M. anisopliae var. acridum* and subsequently incubated in the sun or in the shade. Locusts were either reported or presumed to have been caged on untreated vegetation.

Dose (spores/ha)	**Caging conditions**				**MLT** [1] (day)		**LT_{90}** 1 (day)		**Trial no.** [2]
	Sun		Shade						
	location	temp.	location	temp.	Sun	Shade	Sun	Shade	
Red Locust treated with IMI 330189									
1.25×10^{12}	field	--	field	--	11	8	23	12	11
2.5×10^{12}					10	8	17	10	
Brown Locust treated with IMI 330189									
1.8×10^{12}	field	--	field?	--	12	10			22
1.8×10^{12}					13	11			
1.8×10^{12}					12	12			
1.9×10^{12}					11	8			
1.9×10^{12}					14	5			
1.9×10^{12}					12	5			
2×10^{12}					16	12			
2×10^{12}					19	11			
3×10^{12}					16	8			
Senegalese Grasshopper treated with IMI 330189									
5×10^{12}	field	--	lab.	--	9	8.5	14	12.5	37
5×10^{12}	field	22-55 °C	field	22-43 °C	9	6	13	8	39
Variegated Grasshopper treated with I91 609									
1×10^{11}	field	--	lab.	--	15.3	12.3			38
4.65×10^{11}					12.3	9.3			
2.15×10^{12}					10.7	8			
1×10^{13}					9.7	6.3			
Average (all species)					*12.5*	*8.7*	*15.0*	*9.5*	
Standard deviation					*2.8*	*2.4*	*5.5*	*3.1*	

[1] MLT = median lethal time, or time to 50% mortality; LT_{90} = time to 90% mortality

[2] Trial no. as listed in Annex 2

Annex 8 – Biological assessments of the persistence of *Metarhizium anisopliae* var. *acridum* in the field

Studies in which untreated locusts or grasshoppers were caged onto treated vegetation at different times after the spraying. Only studies where mortality was reported are included. Curves were fitted for data sets containing ≥ 4 observations.

Trial no.[1]	**Dose** (conidia/ha)	**Exp. Dur.**[2] (days)	**Maximum cumulative mortality** (% control corrected) (number of days after treatment when insects were caged onto treated vegetation)																			**Fitted decay curve**[3]		
			0	**1**	**2**	**3**	**4**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**14**	**15**	**19**	**22**	**28**	**34**	**40**	**46**	**Equation**	**R^2**	**$t_{1/2}$** (days)
Desert Locust treated with IMI 330189																								
3	5×10^{12}	3	91			75		85			0												n.s.	
8	5.9×10^{12}	19	100																				--	
65	1.1×10^{12}	3	72			60		18				11				25	6						n.s.	
Migratory Locust treated with FI-985																								
15	3.5×10^{12}	2	80	78	75	83	45		25			26											$Y = 90.5e^{-0.14t}$	0.84	5.0
17	various	2		85			35		45														--	
Australian Plague Locust treated with FI-985																								
25	various	cont.	95		78	54	38		50			20			16								$Y = 87.3e^{-0.13t}$	0.89	5.3
26	various	cont.	80																				--	
Mato Grosso Grasshopper treated with CG423																								
59	1×10^{13}	14	61		79		42																--	
Rice Grashopper treated with IMI 330189																								
31	5×10^{12}	cont.	100																				--	
32	5×10^{12}	3	98				100																--	
Senegalese Grasshopper treated with IMI 330189																								
38	4.2×10^{12}	3	74			74																	--	
39	5×10^{12}	3	100				100		100				100		100		81	88					n.s.	
40	5×10^{12}	3	100				100		100				100		100		96	100	78	64	47	18	$Y = -1.66t+116$	0.8	34

Trial no.[1]	Dose (conidia/ha)	Exp. Dur.[2] (days)	Maximum cumulative mortality (% control corrected) (number of days after treatment when insects were caged onto treated vegetation)																		Fitted decay curve[3]		
			0	1	2	3	4	6	7	8	9	10	11	12	14	15	19	22	28	34	40	46	Equation	R^2	$t_{1/2}$ (days)
Variegated Grasshopper treated with I91 609																									
49	5×10^{12}	2			83		77	56		54		37											Y = -5.75t+96	0.95	8.4
50	1×10^{11}	3				81		31			31					10	2						$Y = 153e^{-0.21t}$	0.94	3.3
50	4.65×10^{11}	3				86		44			35			39		18	7						$Y = 130e^{-0.14t}$	0.9	5.0
50	2.15×10^{12}	3				100		93			57			35		27	15						$Y = 167e^{-0.12t}$	0.98	5.8
50	1×10^{13}	3				26		90			85			95		38	22							n.s.	

[1] Trial no. as listed in Annex 2. [2] Exp. dur. = duration of exposure of the insects onto the treated vegetation. cont. = continuous exposure. [3] Either an exponential or linear curve was fitted to the data, whichever had a larger correlation coefficient. Equations and half-lives are only given for statistically significant curves (P<0.05; n.s. = not significant)

Annex 9 – Comparison between secondary pick-up of and direct exposure to *Metarhizium* spores

Maximum cumulative mortality of locusts or grasshoppers due to *Metarhizium anisopliae* var. *acridum*. Compared are unsprayed insects that were exposed only to the spray residue on the day of application with insects that were sprayed in the field and subsequently incubated in cages. Only trials for which results from both assessment methods were reported are included.

Locust species	**Metarhizium isolate**	**Application rate** (conidia/ha)	**Exposure to spray residue only, on day of treatment**		**Exposure to spray droplets and sprayed vegetation**			**Trial no.** [4]
			Exposure duration [1] (days)	Maximum cumulative mortality (%)	Substrate [2]	Timing of sampling, after treatment [3]	Maximum cumulative mortality (%)	
Desert Locust	IMI 330189	5×10^{12}	3	91	untreated vegetation	1 hour	99	3
Desert Locust	IMI 330189	5.9×10^{12}	19	100	untreated vegetation	not reported	100	8
Desert Locust	IMI 330189	1.1×10^{12}	3	72	not reported	2 hours	100	65
Migratroy Locust	FI-985	3.5×10^{12}	2	80	untreated vegetation	1 day	90	15
Australian Plague Locust	FI-985	various	continuous	95	not reported	2 days	100	25
Australian Plague Locust	FI-985	various	continuous	80	not reported	2 days	95	26
Rice Grasshopper	IMI 330189	5×10^{12}	continuous	100	treated vegetation	"immediately"	98	31
Rice Grasshopper	IMI 330189	5×10^{12}	3	98	not reported	"immediately"	98	32
Senegalese Grasshopper	IMI 330189	4.2×10^{12}	3	74	not reported	"shortly"	77	38
Senegalese Grasshopper	IMI 330189	5×10^{12}	3	100	not reported	"immediately"	100	39
Senegalese Grasshopper	IMI 330189	5×10^{12}	3	100	not reported	"immediately"	100	40
Average ± SD [5]				*90 ± 11*			*96 ± 7*	

[1] Number of days that the insects were caged onto sprayed vegetation, before they were transferred to unsprayed vegetation. [2] Type of vegetation to which the insects were exposed in the cages. [3] The time after treatments when the insects were sampled from the sprayed plot. [4] Trial no. as listed in Annex 2.

[5] There is no significant difference between the two exposure scenarios: Paired t-test on arcsine transformed percentages: n=11, $P = 0.072$

Annex 10 – Residence time for secondary pick-up of *Metarhzium* spores

Timing of maximum cumulative mortality of locusts and grasshoppers sampled from the sprayed plot at various days after treatment, and subsequently incubated in cages to assess mortality.

Trial no.[1]	Species[2]	Isolate	Dose (conidia/ha)	Cage incubations[3]			
				Day at which maximum mortality (days AT)	First sample taken (days AT)	Last sample taken (days AT)	Total no. of samples taken
53	FVI	FI-985	3.6×10^{12}	1	1	32	4
28	HDA	IMI 330189	5×10^{12}	3	0	37	7
29	HDA	IMI 330189	5×10^{12}	3	0	14	4
29	HDA	IMI 330189	5×10^{12}	7	0	14	4
29	HDA	IMI 330189	5×10^{12}	3	0	14	4
32	HDA	IMI 330189	5×10^{12}	5	0	20	5
35	KAM	IMI 330189	2×10^{12}	3	0	7	3
36	KAM	IMI 330189	5×10^{12}	3	0	7	3
21	LPA	IMI 330189	7.4×10^{12}	1	0	3	3
21	LPA	IMI 330189	7.4×10^{12}	0	0	3	3
21	LPA	IMI 330189	9.4×10^{12}	0	0	3	3
21	LPA	IMI 330189	9.4×10^{12}	0	0	3	3
21	LPA	IMI 330189	9.4×10^{12}	3	0	3	3
34	OSE	IMI 330189	2.0×10^{12}	3	0	7	3
36	OSE	IMI 330189	5×10^{12}	3	0	7	3
37	OSE	IMI 330189	5×10^{12}	0	0	3	2
38	OSE	IMI 330189	4.2×10^{12}	3	1	21	6
39	OSE	IMI 330189	5×10^{12}	4	1	22	8
58	RSC	CG 423	2.1×10^{13}	1	1	7	3
59	RSC	CG 423	5×10^{12}	2	0	4	3
59	RSC	CG 423	5×10^{12}	2	0	4	3
59	RSC	CG 423	5×10^{12}	1	0	4	3
59	RSC	CG 423	1×10^{13}	2	0	4	3
59	RSC	CG 423	1×10^{13}	1	0	4	3
59	RSC	CG 423	1×10^{13}	2	0	4	3
1	SGR	IMI 330189	5×10^{12}	0	0	7	3
3	SGR	IMI 330189	5×10^{12}	0	0	12	5
4	SGR	IMI 330189	3×10^{12}	2	0	12	9
8	SGR	IMI 330189	5.9×10^{12}	1	1	20	6
65	SGR	IMI 330189	1.1×10^{12}	0	0	20	6
45	ZVA	I91 609	2×10^{12}	0	0	21	5
46	ZVA	I91 609	7×10^{11}	4	0	16	4
46	ZVA	I91 609	2.1×10^{12}	4	0	16	4
47	ZVA	I91 609	2×10^{12}	0	0	15	3
48	ZVA	I91 609	2×10^{12}	0	0	14	3
49	ZVA	I91-609	5×10^{12}	4	0	10	6
			Average	*2.03*	*0.14*		
			Standard deviation	*1.72*	*0.36*		

1 Trial number as listed in Annex 2. [2] Species: FVI = Wingless Grasshopper, HAD = Rice Grasshopper, KAM = Krausella amabile, LPA = Brown Locust, OSE = Senegalese Grasshopper, RSC = Mato Gross Grasshopper, SGR = Desert Locust, ZVA = Variegated Grasshopper. [3] For cage incubations: Day after treatment (AT) at which maximum cumulative mortality was observed; Days that first and last samples were taken, and number of samples taken in this period.