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Towards the Registration of Microbial Insecticides in Africa: Non-target Arthropod Testing on Green Muscle™, a Grasshopper and Locust Control Product Based on the Fungus *Metarhizium anisopliae* var. *acridum*

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Introduction

For many years, microbial control has been incorporated in pest management strategies in developing countries, enjoying particular success in Asia and South America, although successful approaches for microbial control have evolved worldwide (Fuxa, 1987). Many of these control strategies were developed in response to environmental constraints related to the use of synthetic pesticides. In Africa too, there is a long history of projects that have sought microbial solutions to pest problems, but rarely were these approaches developed into commercial products as in Europe and North America, where development of microbial control agents typically followed the chemical pesticide model, taking a commercial route (Langewald and Cherry, 2000).

In the West African Sahel, since the desert locust outbreak in the late 1980s, there was increasing evidence of negative environmental impact associated with large-scale applications of synthetic insecticides (Everts, 1990; Peveling *et al.*, 1999; Peveling, 2001). Furthermore, there were indications that insecticide applications might even have aggravated grasshopper infestations, due to adverse effects on natural enemies of acridids (van der Valk and Niassy, 1997; van der Valk *et al.*, 1999).

 Biological Control in IPM Systems in Africa (eds P. Neuenschwander, C. Borgemeister and J. Langewald)

In response to these environmental constraints, the LUBILOSA project (Lutte Biologique contre les Locustes et les Sauteriaux), executed by the IITA, Bénin, CABI *Bioscience*, UK, and Centre Régional de Formation et d'Application en Agrométéologie et Hydrologie Opérationelle pour les Pays du Sahel (AGRHYMET), Niger, developed Green Muscle™, an industrially produced fungal insecticide for grasshopper and locust control (Lomer *et al.*, 2001). Green Muscle™ is the first microbial control product specifically developed for the African market. The pathogen on which Green Muscle™ is based is a strain of *Metarhizium anisopliae* var. *acridum* (IMI 330189) Driver and Milner (*Deuteromycotina*, *Hyphomycetes*). Green Muscle™ (Plates 41–44) has been successfully tested against many locust and grasshopper species, particularly *Zonocerus variegatus* (L.) (variegated grasshopper), *Hieroglyphus daganensis* Krauss (rice grasshopper), *Oedaleus senegalensis* (Krauss) (Senegalese grasshopper), *Anacridium melanorhodon melanorhodon* (Walker) (tree locust) and *Schistocerca gregaria* (Forskål) (desert locust) (Lomer *et al.*, 2001).

Registration

The development of any commercial product for plant protection has strong regulatory implications. As a result of growing public concern with respect to side-effects of pesticides and persistent industrial pollutants in the 1960s (Carson, 1962), registration schemes were established for the protection of human health and the environment. The Organisation of Economic Co-operation and Development (OECD) was the first to lay down common Guidelines for Testing of Chemicals (GTC) that aim at harmonizing testing requirements and procedures among member countries and facilitate mutual recognition of results (OECD, 1982 onward). During the late 1970s, international agencies and governments began to examine the need for guidelines and regulations for the evaluation, development and registration of microbial control agents (Greathead and Prior, 1990), and only very recently governments started to develop particular registration regulations for microbial pesticides. In 1983, the US Environmental Protection Agency (EPA) developed the first comprehensive regulatory scheme for such products (EPA Subsection M guidelines), and ecotoxicological studies are now required for microbial control agents, despite them being considered *a priori* environmentally safe (Goettel *et al.*, 1990). It is important to realize that microorganisms used for biological control of pests and diseases have a broad spectrum of properties. At one extreme are the highly specific and virulent pathogens such as the Entomophthoralean fungi (e.g. *Neozygites* sp. on cassava green mite, *Entomophthora muscae* (Chon) Fresenius and *Entomophthora maimaiga* Humber, Shimazu, Soper and Hajek) and some of the nucleopolyhedroviruses (NPV). At the other extreme are microbial control agents such as *Bacillus thuringiensis* (Berliner) that may have quite wide host ranges, but are not expected to multiply, spread or persist in the environment, and in these respects are similar to chemical pesticides. For these agents, which are used only as inundative biopesticides, the issues of mammalian toxicology and residue analysis, etc., are not very different from those for chemical pesti-

cides. Intermediate between these two extremes are species such as *M. anisopliae* var. *acridum* or *Beauveria brongniartii* (Saccardo) Petch or many of the NPVs. They are often applied in industrial quantities characteristic of 'inundative biopesticides', but we expect to see moderate persistence at least within the season during which they are applied, if not for several consecutive seasons. Here both toxicological and environmental issues need to be considered.

The registration of microbial pesticides encompasses a similar range of tests to the registration of chemical pesticides. To date, the most detailed and comprehensive documents available are the Microbial Pesticide Test Guidelines (MPTG) of the EPA (EPA, 1996). Their use has also been recommended by the European Union as long as 'acceptance of specific guidelines at international level is pending' (EU, 2001). In West Africa, the Comité Sahélien des Pesticides (CSP), a subcommittee of CILSS, also requests data equivalent to EPA or EU standards. On the basis of a dossier provided for the registration of Green Muscle™, this organization has now developed their own framework for the registration of microbial pesticides in the Sahel, and also South Africa and Madagascar have developed such legislations.

The MPTG follow a tier-testing approach. This idea was first developed by the US National Academy of Sciences (NAS, 1981). The methodology is characterized by a sequence of tests within a dichotomous decision-making scheme in which evidence of low risk on a lower level of biological and ecological complexity precludes further testing on a higher level. Applied to ecotoxicological studies, this means at low tier-level simple laboratory set-ups, and at high tier-level testing under complex natural conditions in the field. The rationale of tier testing is 'to ensure that only the minimum data sufficient to make scientifically sound regulatory decisions will be required' (EPA, 1996). In the case of non-target testing, the determination of the host range is the main objective of first tier testing. Special attention is given to organisms used for biological control and playing an important role in IPM (EU, 2001). It can also be important to include other pests in host-range screening studies, thereby widening the potential target spectrum and improving commercial prospects. Higher tier laboratory or field tests under environmentally more realistic exposure conditions are only envisaged if first tier tests show adverse effects. In case adverse effects are substantiated, further ecotoxicological tests are carried out under operational conditions in natural environments. The main advantage of tier testing to commercial companies is that it reduces the cost for registration. In this context, the option to waive data requirements on a case-by-case basis is important and makes the process less expensive and fast. While the registration of synthetic pesticides in the USA takes approximately 3 years, the review of microbial pesticide registration dossiers takes on average only 12 months. Requests for waivers have to be based on scientific grounds. A simplified procedure has been developed for the registration of products with limited uses (niche products). In this case, data requirements are less than for extensively used products (EPA-Interregional Research Program no. 4).

In this chapter, we focus on non-target arthropod toxicity and pathogenicity testing. Apart from proven beneficial arthropods, we are also reporting on arthropods that are not necessarily beneficial, but still not a target for locust and grasshopper control.

Testing Green Muscle™

Contrary to commercial producers, LUBILOSA did not limit itself to minimum data requirements. The reason is that LUBILOSA was planned and executed as a development assistance project. Development and registration of a mycopesticide against locusts and grasshoppers, i.e. a marketable product, was the principal, but not the only, aim of the LUBILOSA programme. Other goals were to establish collaboration and equitable partnership with African research and plant protection institutions (non-government organizations, as well as government organizations), to build and foster regional biological control capacities, and to conduct participatory on-farm research. Within the scope of this collaborative research, there was a much wider window of opportunity to explore benefits and risks of fungal control than within the scope of commercial pesticide development. Large-scale field applications for efficacy testing of Green Muscle™ were opportunities to carry out large-scale ecotoxicological studies simultaneously – which provided large amounts of additional information on the environmental impact of Green Muscle™ under real field conditions and in comparison with synthetic insecticides.

Most environmental data were generated by the LUBILOSA programme itself, but other partners contributed important data as well. Thus, studies were conducted on various tiers at a time, depending on opportunities in terms of access to research facilities, interest of partners and available financial, technical as well as human resources.

With respect to registration, it is important to note that the genus *Metarhizium* has been reviewed several times. What used to be a distinct group of acridid isolates of *Metarhizium flavoviride* Gams and Rozsypal (Bridge *et al.*, 1993) is now classified as *M. anisopliae* var. *acridum* (Driver *et al.*, 2000). IMI 330189 – the Green Muscle™ isolate – obtained from *Ornithacris cavroisi* Finot (Orthoptera, Acrididae) in 1989, near Niamey, Niger, became the type material for this group. Naturally occurring infections of grasshoppers and locusts with *Metarhizium* are uncommon. A few cases of epizootics, however, have been reported (Lomer *et al.*, 2001). Moreover, observations of LUBILOSA collaborators suggest that a low-level background infection may be common in locusts and grasshoppers in the Sahel and other semiarid biomes.

Green Muscle™ Arthropod Host Range – Laboratory Studies

The arthropod host range and pathogenicity of *M. anisopliae* (reviewed in Goettel *et al.*, 1990; Prior, 1997) varies greatly among genotypes. It is therefore difficult to predict effects of new isolates on non-target arthropods on the basis of susceptibilities to previously tested strains. Previous virulence screening tests using IMI 330189 revealed that 94% of 17 different orthopteran species were susceptible, and 59% – all within Acrididae – highly susceptible (Prior, 1997). In contrast, the corresponding percentages for non-orthopteran insects ($n = 16$) were only 44% and 0%, respectively. The author concluded that high virulence of this isolate seems confined to acridoids. More host-range studies have been

conducted since this first appraisal, partly motivated by findings that some hymenopteran parasitoids were highly susceptible to IMI 330189 and other *M. anisopliae* isolates. An updated and extended list of non-orthopteran arthropods challenged with IMI 330189 (Table 14.1) indicates that while some non-target insects were susceptible under lab conditions, these results were largely due to unrealistic exposure and test conditions. For example, two beneficial

Table 14.1. Susceptibility of non-target arthropods to IMI 330189 (Green Muscle™) (Orthoptera not included; laboratory results).

Taxon	Susceptibility	References	Remarks
Branchiopoda			
Cladocera			
Daphnia magna Straus	–	a	
Anostraca			
Streptocephalus sudanicus Daday	–	b	High acute toxicity but no mycosis
Acari			
Phytoseiidae			
Neoseiulus idaeus Denmark and Muma	–	c	
Isoptera			
Termitidae			
Coptotermes spp.	+	d	
Nasutitermes spp.	+	d	
Psammotermes hybostoma Desneux	+	e	Controlled field test
Blattaria			
Blattidae			
Blatta sp.	–	d	
Neuroptera			
Myrmeleonidae			
Indet.	–	d	
Myrmeleon sp.	+	f	
Heteroptera			
Notonectidae			
Anisops sardeus Herrich-Schäffer	+	b,e	Floating layer of formulation can impede breathing
Lygaeidae			
Cosmopleurus sp.	–	f	Formulation toxic at high volume[m]
Coreidae			
Clavigralla shabadi Dolling	–	d	
Clavigralla tomentosicollis Stal	–	c,d	
Anthocoridae			
Orius albidipennis Reuter	–	c	
Coleoptera			
Cucurlionidae			
Neochetina eichhorniae Warner	–	d	

Continued

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Acari			
Phytoseiidae			
Neoseiulus idaeus Denmark and Muma	–	c	
Isoptera			
Termitidae			
Coptotermes spp.	+	d	
Nasutitermes spp.	+	d	
Psammotermes hybostoma Desneux	+	e	Controlled field test
Blattaria			
Blattidae			
Blatta sp.	–	d	
Neuroptera			
Myrmeleonidae			
Indet.	–	d	
Myrmeleon sp.	+	f	
Heteroptera			
Notonectidae			
Anisops sardeus Herrich-Schäffer	+	b,e	Floating layer of formulation can impede breathing
Lygaeidae			
Cosmopleurus sp.	–	f	Formulation toxic at high volume[m]
Coreidae			
Clavigralla shabadi Dolling	–	d	
Clavigralla tomentosicollis Stal	–	c,d	
Anthocoridae			
Orius albidipennis Reuter	–	c	
Coleoptera			
Cucurlionidae			
Neochetina eichhorniae Warner	–	d	

Continued

Table 14.1. *Continued.*

Taxon	Susceptibility	References	Remarks
Coccinellidae			
Hyperaspis notata (Mulsant)	–	d	
Chilocorus bipustulatus (L.)	–	c	
Scarabaeidae			
Phyllophaga sp.	–	d	
Coleoptera			
Tenebrionidae			
Pimelia senegalensis (L.)	–	g	
Trachyderma hispida (Forskål)	–	g	
Tenebrio molitor L.	+	d	
Hymenoptera			
Encyrtidae			
Anagyrus lopezi (De Santis)	–, +, ++	e,g,h	High susceptibility at high dose and in stressed conditions, but no effect on beneficial capacity (parasitism) at field rate
Braconidae			
Bracon hebetor Say	+, ++	g	Larvae in host also infected
Cotesia flavipes Cameron	–	i	
Phanerotoma sp.		h	
Apidae			
Apis mellifera L.	+	j	Topical exposure of worker bees
Apis mellifera adansonii Latreille	–	k	Hives exposed in treated crop
Apis mellifera scutellata Lepeletier	–	l	Conidia dusted into hives
Formicidae			
Tapinoma sp.	–	d	
Lepidoptera			
Sphingidae			
Hyles livornica (Esper)	–	f	
Noctuidae			
Sesamia calamistis Hampson	+	i	

–, not infected; +, low or moderate susceptibility; ++ high susceptibility.
[a]Confidential registration results.
[b]Lahr *et al.* (2001).
[c]cf. Table 14.3.
[d]Prior (1997).
[e]Everts *et al.* (2001), unpublished results.
[f]J. Tabel (1994) Saarbrücken, unpublished data.
[g]Danfa and van der Valk (1999).
[h]Stolz *et al.* (2002), unpublished results.
[i]V.S. Ayitchehou (1996), unpublished data.
[j]Ball *et al.* (1994).
[k]P. Byrne (1995), unpublished results.
[l]R.E. Price (1997), unpublished results.
[m]8 l ha^{-1}.

insects, *Bracon hebetor* Say (Hymenoptera, Braconidae) and *Anagyrus lopezi* (de Santis) (Hymenoptera, Encyrtidae), were highly susceptible in some assays (Danfa and van der Valk, 1999; J. Everts, 2001, Senegal, unpublished results). It appears that the susceptibility varies depending on the actual exposure regime. For example, laboratory spray-tower treatments (Danfa and van der Valk, 1999) caused higher infectivity and mortality in *A. lopezi* than treatments with spinning-disk sprayers (Stolz *et al.*, 2002). By contrast, assays simulating field-exposure of *A. lopezi* and *Cotesia flavipes* Cameron (Hymenoptera, Braconidae) did not reveal adverse effects on the beneficial capacity (reproduction) of these parasitoids (V.S. Ayitchehou, Bénin, 1996, unpublished results; Stolz *et al.*, 2002). This suggests that the risk under field conditions may be low, despite evidence of high virulence in the laboratory. The first results from the laboratory assays with termites suggest that this group may be more susceptible to IMI 330189 than other non-target insects (J. Langewald, 2000, unpublished data). However, field experiments conducted by the CERES-LOCUSTOX Foundation, near Dakar, Senegal on *Macrotermes subhyalinus* Rambur at recommended field dose for grasshopper control did not reveal any reduction in termite activity (Everts *et al.*, 2001, unpublished data). CERES-LOCUSTOX was particularly interested in studying aquatic non-target organisms. An oil-based formulation of Green Muscle™ had a negative effect on *Anisops sardeus* Herrich-Schäffer (Heteroptera, Notonectidae), as had the blank formulation without *M. anisopliae* spores. *A. sardeus* is a water surface breather and the oil layer on the surface resulting from the application impeded breathing. These effects did, however, not occur under field conditions, due to the breaking-up and dispersal of the oil layer by the wind and the motion of the water.

Until now, *M. anisopliae* var. *acridum* has proved harmful only to non-target organisms in first tier tests, i.e. under maximum exposure conditions. In contrast, higher tier studies under operational conditions (at recommended dose rates) provided no evidence of significant adverse effects of *M. anisopliae* var. *acridum* on non-target organisms (Table 14.2). Significant reductions were either below hazard levels (<25%; Hassan, 1998) or related to overdosing (the tenebrionid *Gonocephalum setulosum*). The host specificity of different strains of entomopathogenic hyphomycete fungi seems to be dose dependent, with the host range increasing with dose. Higher tier test protocols should mainly focus on realistic dose applications, which could in many cases still be done under inexpensive laboratory conditions.

Laboratory case studies

A wide range of insect taxa has been screened for their susceptibility to *M. anisopliae* var. *acridum* strain IMI 330189. Some laboratory case studies are described in the following as examples of the LUBILOSA first tier testing approach. The non-target organisms included in these tests were one potential pest and three beneficial arthropods. All of these species are important elements in natural or newly established food webs of West African agroecosystems, including locust and/or grasshopper habitats. Thus, they are likely to be directly affected by control operations.

Table 14.2. Susceptibility of non-target arthropods to IMI 330189 (Green Muscle™) under field conditions (Orthoptera not included).

Taxon	Max. rate (conidia ha^{-1})	Sign. impact	Remarks	References
Arachnida (except Acari)	5×10^{12}	–	Niger	a
Acari	5×10^{12}	–	Niger	a
Collembola	5×10^{12}	–	Niger	a
Homoptera	2.5×10^{12}	–	Niger	a
Cicadellidae	2×10^{13}	+	Mauritania (22% reduction)[e]	b
Cicadellidae	5×10^{12}	+	Mauritania (18% reduction)	b
Coleoptera				
Carabidae	5×10^{12}	–	Niger	a,c
Tenebrionidae	5×10^{12}	–	Niger	a,c
Pimelia angulata angulosa F.	2×10^{13}	–	Mauritania[e]	b
Zophosis posticalis Deyrolles	2×10^{13}	–	Mauritania[e]	
Gonocephalum setulosum Faldermann	2×10^{13}	+	Mauritania (86% reduction)[e]	b
Scarabaeidae	2.5×10^{12}	–	Niger	a
Anthicidae				
Notoxus sp.	2×10^{13}	–	Mauritania[e]	b
Chrysomelidae				
Euryope rubra F.	2.5×10^{12}	–	Niger	a
Curculionidae				
Dereodus marginellus Boheman	2.5×10^{12}	–	Niger	a
Lepidoptera				
Microlepidoptera	2×10^{13}	–	Mauritania[e]	b
Hymenoptera				
Chalicidoidea	5×10^{12}	–	Niger	d
Encyrtidae	2×10^{13}	–	Mauritania[e]	b
Scelionidae	5×10^{12}	–	Niger	d
Mymaridae	5×10^{12}	–	Niger	d
Trichogrammatidae	5×10^{12}	–	Niger	d
Ichneumonoidea	5×10^{12}	–	Niger	d
Chrysidoidea	5×10^{12}	–	Niger	d
Dryinidae	5×10^{12}	–	Niger	d
Apoidea	5×10^{12}	–	Niger	d
Apoidea	2×10^{13}	–	Mauritania[e]	b
Sphecidae				
Podalonia spp.	2×10^{13}	–	Mauritania[e]	b
Vespoidea	5×10^{12}	–	Niger	d
Formicidae	5×10^{12}	–	Niger	a,c,d
Monomorium areniphilum Santschi	2×10^{13}	–	Mauritania[e]	b
Diptera				
Tachinidae				
Indet.	2×10^{13}	–	Mauritania[e]	b
Ephydridae				
Actocetor margaritatus (Wiedemann)	5×10^{12}	–	Niger	a,c

[a]J. Tabel (1994), Saarbrücken, unpublished results.
[b]This chapter, see Table 14.1.
[c]Peveling *et al.* (1999).
[d]Stolz (1999).
[e]High-volume application rate of 8 l ha^{-1}.

The neotropical mite *Neoseiulus idaeus* (Denmark and Muma) (Acari, Phytoseiidae) has been introduced to West Africa to control the cassava green mite (Yaninek *et al.*, 1993; Yaninek and Hanna, this volume). The pod bug *Clavigralla tomentosicollis* Stal (Heteroptera, Coreidae) is a major pest of cowpea in West Africa (Singh and Allen, 1979). Both *Beauveria bassiana* (Bals.) Vuill (Deuteromycotina, Hyphomycetes) and *M. anisopliae* showed promise as fungal control agents in virulence screening assays (Ekesi, 1999). Flower or minute pirate bugs are among the most abundant beneficials in African cropping systems (Hernández and Stonedahl, 1999). The species *Orius albidipennis* Reuter (Heteroptera, Anthocoridae) is particularly important in the natural control of lepidopteran eggs (e.g. *Helicoverpa armigera* (Hübner)), mites and thrips (*Frankliniella* spp.). The coccinellid *Chilocorus bipustulatus* L. var. *iranensis* (Coleoptera, Coccinellidae), a predator of scale insects, is widely distributed in the South Palearctic. In Mauritania, *C. bipustulatus* var. *iranensis* was released in the late 1960s to control *Parlatoria blanchardi* Targioni-Tozzetti, a scale of date palm, *Phoenix dactylifera*.

Details on the experimental procedures and design are summarized in Table 14.3. In all tests, desert locust nymphs (Bénin) or immature adults (Mauritania) were used as positive controls, and subjected to the same treatments as the non-target organisms (Table 14.4).

In all tests compared with the controls, *M. anisopliae* did not increase mortality in any of the non-target organisms; it also had no effect on reproduction

Table 14.3. Effects (mortality and reproduction) of *Metarhizium anisopliae* var. *acridum* (IMI 330189) on non-target arthropods.

	Test 1 *Neoseiulus idaeus* (Phytoseiidae)[a]	Test 2 *Clavigralla tomentosicollis* (Coreidae)[b]	Test 3 *Orius albidipennis* (Anthocoridae)[c]	Test 4 *Chilocorus bipustulatus* (Coccinellidae)[d]
Test organisms				
Stage (age)	Nymph	Adult (variable)	Adult (2 days)	Adult (7 days)
No. of tests (series)	2	1	2	1
Treatments	2	3	3	2
Replicates per test	4	5	4	3
No. per replicate	25	10	10	30
Total no. tested	400	150	240	180
Test conditions				
Application	Conidia mixed with 25 host eggs[e]	Micro-Ulva field treatment[f]	Micro-Ulva field treatment[f]	Burkard microapplicator
Exposure	Contact	Residual[g]	Residual[g]	Topical
Dose	6×10^8 conidia g^{-1} (fresh weight)[h]	5×10^{12} conidia ha^{-1}	5×10^{12} conidia ha^{-1}	2.5×10^3 conidia per beetle[i]
Duration (days)	8	14	14	14

Continued

Table 14.3. *Continued.*

	Test 1 *Neoseiulus idaeus* (Phytoseiidae)[a]	Test 2 *Clavigralla tomentosicollis* (Coreidae)[b]	Test 3 *Orius albidipennis* (Anthocoridae)[c]	Test 4 *Chilocorus bipustulatus* (Coccinellidae)[d]
Mortality (% ± SE)				
(1) Untreated control	3.0 (1.0)[1]	28.0 (8.6)[1]	50.0 (7.6)[1]	–
(2) Carrier control	–	18.0 (5.8)[1]	81.2 (5.8)[2]	5.6 (3.0)[1]
(3) *M. anisopliae*	3.0 (1.5)[1]	36.0 (7.5)[1]	77.5 (4.5)[2]	7.8 (4.0)[1]
Statistics (ANOVA)	$F_{1,14} = 0.0$[j] $P = 1.0$	$F_{2,12} = 1.49$[k] $P = 0.265$	$F_{2,21} = 7.84$[j] $P = 0.003$	$F_{1,4} = 0.18$[k] $P = 0.690$
Efficacy (%)[l]				
(1) Carrier control	–	−13.9	62.5	–
(2) *M. anisopliae*	0.0	11.1	55.0	2.3
Reproduction (±SE)[m]				
(1) Untreated control	68.6 (8.4)[a]	–	–	–
(2) *M. anisopliae*	75.5 (11.3)[a]	–	–	–
Statistics (ANOVA)	$F_{1,14} = 0.24$ $P = 0.633$	–	–	–
Efficacy (%)[l]	−22.0	–	–	–

Means within columns in successive lines with different superscript numbers are significantly different at $P < 0.05$.

[a]Phytoseiids reared according to protocols of Friese *et al.* (1987) and Mégevand *et al.* (1993).

[b]*C. tomentosicollis* was reared using the protocol of Ekesi (1999). The general outline of the bioassay was similar to the one with *O. albidipennis*.

[c]*O. albidipennis* were collected in the field and reared according to Fritsche and Tamò (2000).

[d]Field-collected *C. bipustulatus* var. *iranensis* were reared under conditions similar to those described for the coccinellid *Pharoscymnus anchorago* F. (Peveling and Demba, 1997).

[e]Alternative host eggs (*Tetranychus urticae* Koch).

[f]Recommended field dose formulation and application according to protocols by Bateman *et al.* (1992).

[g]Permanently exposed to spray residues as described by Stolz *et al.* (2002).

[h]Maximum challenge dose.

[i]Approximate maximum field exposure (equal volumes of Shellsol T® and Ondina®).

[j]Differences among test series were not significant. Therefore, replicates from different series were pooled and analysed with one-way ANOVA.

[k]One-way analysis of variance (ANOVA).

[l]Abbott's formula.

[m]Combined number of viable eggs and protonymphs.

of *N. idaeus*. Furthermore, no sporulation occurred on cadavers. Hence, *M. anisopliae* was not pathogenic to any of the species tested. The carrier oils caused some increased mortality in *O. albidipennis*, indicating toxic and/or physical (e.g. clogging of tracheae) effects of the oil formulation (Table 14.3), but the average longevity of females (controls) is only about 14 days anyway (Fritsche and Tamò, 2000) (controls, Table 14.3). Nevertheless, a medium risk is assumed according the IOBC risk classification scheme

Table 14.4. Mortality of desert locust, *Schistocerca gregaria*, and percentage mycosis tested as positive control under the same conditions as the non-target test organisms listed in Table 14.3.

	Test 1[a]	Tests 2 and 3[a]	Test 4[a]
Test locusts			
Stage (age of imago)	5th instar	5th instar	Adult (variable)
Group size	20	4–10	12
Replicates	4	1	3
Treatments	2	3	2
No. of tests (series)	2	2	1
Total number in test	320	42	72
Test conditions			
Application	Conidia mixed with wheat bran	See Table 14.3	See Table 14.3
Exposure	See Table 14.3	See Table 14.3	See Table 14.3
Dose	5×10^6 conidia g^{-1} (dry weight)	See Table 14.3	See Table 14.3
Duration (days)	8	7–14	14
Mortality (% ± SE)			
(1) Untreated control	0.6 (0.6)[1]	14.3[1]	–
(2) Carrier control	–	14.3[1]	33.3 (0.0)[1]
(3) *M. anisopliae*	97.5 (1.6)[2]	71.4[2]	100.0 (0.0)[2]
Statistics (ANOVA or χ^2 test)	$F_{1,14} = 3057.7$[c] ($P < 0.001$)	$\chi^2 = 13.7$ ($P = 0.001$)	No variance
Efficacy (%)[b]			
(1) Carrier control	–	0.0	–
(2) *M. anisopliae*	97.5	66.6	100.0
Sporulation (mycosis) (%)			
Untreated control	0	0	–
Carrier control	–	0	11.1
M. anisopliae	95.6	71.4[c]	77.8

Means within columns in successive lines with different superscript numbers are significantly different at $P < 0.05$.

[a]Locusts were kept as previously described (Peveling and Demba, 1997; Stolz *et al.*, 2002). All tests were conducted under ambient laboratory conditions at the IITA (Bénin) or Akjoujt field station (Mauritania).

[b]Abbott's formula.

[c]Test series pooled.

[d]Pairwise comparisons with Fisher's exact test of pooled series.

(Hassan, 1998). In the target organism (desert locust), *M. anisopliae* treatments caused high mortality, and sporulation of the fungus on cadavers confirmed its pathogenicity for treated locust in all tests (Table 14.4). In conclusion, the isolate IMI 330189 proved not pathogenic to the non-target organisms, but showed high virulence to one of the principal target acridids, desert locust.

Green Muscle™ Arthropod Host Range – Field Tests

Studies on the impact of Green Muscle™ on non-target arthropods were usually conducted within the scope of efficacy testing (e.g. Langewald *et al.*, 1999; Fig. 14.1). The spatial scale varied from ≈ 0.1 ha in Mauritania (Table, 1994, unpublished data) to 800 ha in Niger (Stolz, 1999), and the temporal scale from 5 weeks (Table, 1994, unpublished data) to 1 year post-treatment (Peveling *et al.*, 1999). Most studies used the standard dose rate of 5×10^{12} (100 g) conidia ha^{-1}, but one also tested four times this dose (Table, 1994, unpublished data) and another one only half of it (Stolz, 1999; field case study, see below). In medium- to large-scale field trials (Langewald *et al.*, 1999), synthetic insecticides were included as toxic standards (fenitrothion and fipronil) (Fig. 14.1). This not only allowed comparison of product efficacy but also provided a means to validate monitoring methods. Fenitrothion was applied according to label rates. However, rates of fipronil changed several times since its registration for locust and grasshopper control. In Dogo (Niger) 1998, LUBILOSA used a rate of 2 g a.i. ha^{-1}, which was lower than the former label rate but higher than specifications for grasshopper control today. In 1996, Peveling *et al.* (1999) studied non-target effects of Green Muscle™ and the organophosphate fenitrothion at Maine Soroa in Niger. While both products were highly efficient in reducing grasshopper populations (Langewald *et al.*, 1999) (Fig. 14.1), only fenitrothion caused reductions in non-target ground-dwelling species. Overall, 75% of the observed taxa, including Carabidae, Tenebrionidae, Formicidae and Ephydridae, were significantly reduced by fenitrothion. Most of the non-target fauna recovered

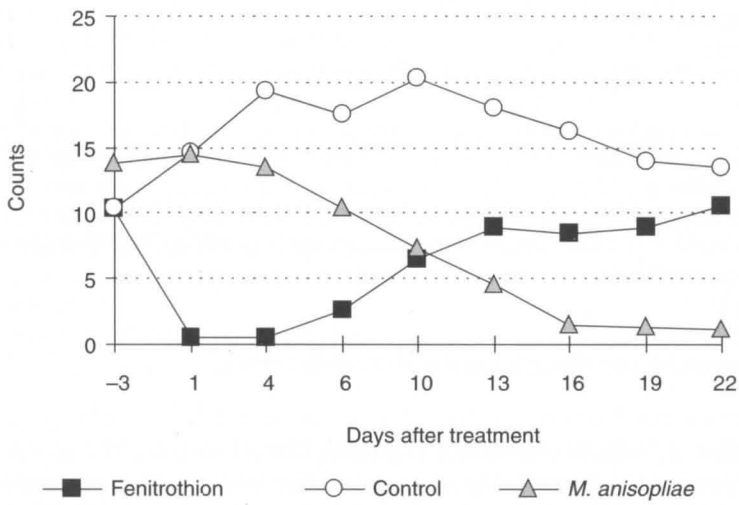

Fig. 14.1. Mean counts of grasshoppers per square metre over a period of 22 days in untreated plots, plots treated with an oil based formulation of *Metarhizium anisopliae* var. *acridum* (Green Muscle™) and with fenitrothion during the 1997 rainy season in east Niger (Langewald *et al.*, 1999).

after 51 days, except one ant species, which had still not recovered 1 year after application (Peveling *et al.*, 1999). During the 1997 and 1998 seasons in Niger, Stolz (1999) studied the impact of Green Muscle™ and fenitrothion compared with untreated sites on parasitoid Hymenoptera in 50 and 800 ha plots. Hymenoptera were monitored using yellow mini sticky traps (160 per study) and malaise traps (32 per study). Treatment effects were compared with the pre-treatment situation and the untreated control plots. She did not find any negative impact of any of the products on this non-target group. Non-target effects on arthropods of IMI 330189 under field conditions are summarized in Table 14.2. The existing evidence clearly confirms the impression from laboratory bioassays that the host range of this genotype of *Metarhizium* among non-orthopteran arthropods is very narrow. However, non-target orthopterans are *a priori* highly at risk, and use restrictions may be necessary in areas where particular grasshopper species or overall grasshopper diversity warrant protection.

Field case study

As an example for ecotoxicological work in the field, the following study is presented in more detail. The study was conducted during the rainy season in the most important millet producing zone in Niger. After conducting field experiments in the dryer parts of the millet belt, where millet is grown under marginal conditions (see above), this site was selected because of its importance for the millet production in Niger. Grasshoppers, in particular *O. senegalensis* Krauss, are a major problem in most years in this area and may cause severe damage to both crops and pastures (Cheke, 1990; Nwanze and Harris, 1992; Jago, 1993). In 1998, the grasshopper fauna consisted of *Acrotylus* spp. and *O. senegalensis*, but densities remained exceptionally low, despite good breeding conditions. Thus, experimental conditions were not ideal for efficacy testing. The effect of *M. anisopliae* var. *acridum* IMI 330189 (Green Muscle™) on non-target arthropods was compared with fipronil (Adonis® 4 UL) and an unsprayed control (for details see Table 14.5). Different taxa of arthropods were studied in cropland (millet) and pastures. In millet, the emphasis was on beneficial organisms, in particular hymenopteran parasitoids. A detailed account of this study was given by Stolz (1999). In pastures, the focus was on ground-dwelling arthropods – including grasshoppers – and two arboreal, herbivorous beetles, the chrysomelid *Euryope rubra* Fabricius, and the curculionid *Dereodus marginellus* Boheman. Here we present results from the study of non-target arthropods in pastures.

Epigeal arthropods were sampled with pitfall traps (Table 14.5). The two herbivorous beetles live on the succulent shrub *Leptadenia pyrotechnica* (*Asclepiadaceae*), which was by far the most abundant woody species. *D. marginellus* feeds on the flowers and *E. rubra* on young shoots. The beetles are easily visible due to their large size and conspicuous colour, and due to the peculiar morphology of the shrub. *L. pyrotechnica* has only tiny leaves and is

Table 14.5. Application and monitoring details for a field study on non-target effects of the application of *Metarhizium anisopliae* var. *acridum* IMI 330189 (Green Muscle™) and fipronil on ground dwelling arthropods and arboreal beetles.

Location (year)	Dogo, 45 km south of Zinder, Niger (1998)	
Crop	Millet (heads developed, milky grain)	
Product	*M. anisopliae* var. *acridum* (Strain IMI 330189)	Fipronil (Adonis® 4 UL)
Plot size × (replication)	50 ha × (3)	50 ha × (3)
Specification	2.5×10^{12} cfu ha^{-1}	2 g a.i. l^{-1}
Volume application rates	0.5 l ha^{-1}	0.5 l ha^{-1}
Swath width	30 m	30 m
Sprayer	Micron ULVA mast mark II	Micron ULVA mast mark II
Wind speed	3–5 m s^{-1}	3–5 m s^{-1}
Non-targets	Ground-dwelling arthropods including grasshoppers; arboreal beetles (*E. rubra* and *D. marginellus*)	
Monitoring[a,b]	Pitfall traps[c] (ground-dwellers); random counts on 50 shrubs of *L. pyrotechnica* (arboreal beetles)	

[a]Arthropods were monitored for about 8 weeks, starting 1 week before treatment.
[b]Only the main effects were significant.
[c]Sampling scheme described by Peveling *et al.* (1999).

generally seen in leafless condition, with narrow, green and juicy branches for assimilation. Another consequence of its morphology is that spray drift is unhampered by stems or foliage and leads to homogenous deposits all over. For example, 10 days post-treatment, tracer-marked droplets were found in >50% of all shoots collected in plots treated with Green Muscle™. Thus, the insecticide–milkweed–herbivore system appeared to be an ideal model to assess effects in a maximum exposure scenario (Table 14.5).

The grasshopper abundance decreased in all treatment groups as the season progressed, including the control (Fig. 14.2). Nevertheless, the decline was highest in plots treated with Green Muscle™. The efficacy of IMI 330189 against Sahelian grasshoppers at standard dose rates was found to be lower than in previous studies (Kooyman *et al.*, 1997; Langewald *et al.*, 1999; Fig. 14.1). The low efficacy in the present study resulted from low grasshopper densities rather than insufficient dose rates. This can be inferred from an equally 'poor performance' of fipronil, an agent that has shown nearly 100% efficacy even at lower dose rates than those tested here (Balança and de Visscher, 1997).

More than 40 different epigeal non-target arthropods, in addition to the two beetles mentioned before, were monitored on species level. For conciseness, we focus on higher taxonomic levels (Arachnida (except Acari), Acari, Collembola, Homoptera, Carabidae, Scarabaeidae, Tenebrionidae, Formicidae, Ephydridae). The study revealed no adverse effects of Green Muscle™ on any of these non-targets. With the exception of Formicidae

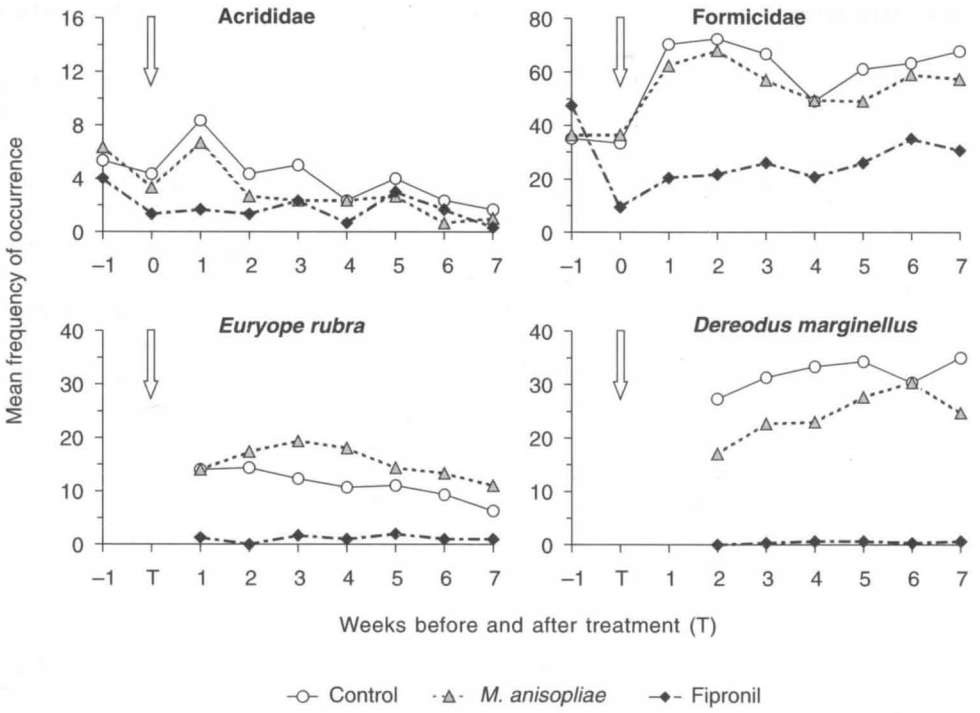

Fig. 14.2. Effects of Green Muscle™ (*Metarhizium anisopliae* var. *acridum*) and Adonis® (fipronil) on target grasshoppers and non-target epigeal (Formicidae) and arboreal (*Euryope rubra* and *Dereodus marginellus*) insects. The frequency of occurrence (presence/absence sampling) was monitored at different points in time as a measure of relative abundance. Repeated measures analyses revealed significant effects of the main factor treatment in all cases. Sidak multiple comparison of means shows that both target and non-target taxa were significantly reduced by fipronil, whereas *M. anisopliae* only affected target grasshoppers. The arrow indicates the time of treatment; error bars are not shown to avoid the cluttering of graphs.

(Fig. 14.2), fipronil had no detectable effects either. Similar results had been obtained by Balança and de Visscher (1997). Further detailed studies on the environmental impact of fipronil for locust control have been carried out by Tingle and McWilliam (2001) and Peveling *et al.* (2001) in Madagascar.

Green Muscle™ had no significant effect on *E. rubra* and *D. marginellus* (Fig. 14.2). In contrast, fipronil had a highly significant adverse effect, with 90 and 99% population reduction, respectively. Neither of the species re-colonized the treatment plots during the observation period. This appeared to be related to their limited dispersal capacity and the high persistence of fipronil. Results confirm the hypothesis that insecticide exposure of insects feeding on the milkweed was particularly high. Sokolov (2000) also noted a strong effect of fipronil on Chrysomelidae and other, in particular herbivorous, insects. The results confirm that Green Muscle™ poses a lower risk than the widely used chemical control agents.

Conclusions

While a vast majority of pest control strategies using microbials such as *Metarhizium* and similar products in developing countries is conducted with non-registered products, showing large differences in quality and efficacy, there is a worldwide tendency towards improving product quality and towards reducing use of non-registered products. In terms of ecological impact under Sahelian conditions, Green Muscle™ is probably the most thoroughly studied insecticide. For the development of registration frameworks for microbial pesticides in Africa, Green Muscle™ played a major role, too. From a purely academic point of view, the amount of knowledge achieved is remarkable, and hopefully, these studies will help to support the development of similar products. On the other hand, this immense scientific work could only be carried out through donor support. A small private producer of microbial pesticides serving a small niche market will hardly be able to finance ecotoxicological and ecopathological studies to such an extend. Therefore, Green Muscle™ is probably a rather untypical example with respect to the generation of environmental impact data. Registration authorities cannot expect small companies producing microbial pesticides to develop registration dossiers with a similar amount of data.

Apart from environmental safety, mammalian toxicity is a major concern of pesticide regulations. Deuteromycete fungi, at least *in vitro*, can metabolize a wide range of compounds. As many fungi, *Metarhizium* produces mycotoxins, too. Mycotoxins enable the fungus to combat the immune reaction of its host. It is important to test mammalian safety for each single *Metarhizium* strain to be registered as a product. However, destruxins, the common mycotoxins in *M. anisopliae*, appear to be mainly produced by isolates of the *M. anisopliae* var. *anisopliae* variety. Apart from ARSEF 324, Kershaw *et al.* (1999) found no destruxins produced by *M. anisopliae* var. *acridum* isolates, including the Green Muscle™ isolate IMI 330189. These findings are supported by tests with IMI 330189 on ring-necked pheasant, which revealed no pathological responses (Smits *et al.*, 1999). Likewise, *Metarhizium* spp. have only rarely been associated with fungal pathologies in mammals (C.J. Lomer, J.E. Eilenberg, J. Langewald, C. Nielsen, S. Vestergaard and H.H. Strasser, Swansea, UK, April 2001, unpublished results). For the future of microbial control, it is essential that registration costs remain substantially lower than the ones for synthetic pesticides, a request supported by EPA regulations. Decision-making schemes developed for microbial control products might be complex, but the key to fast and less expensive registration consists in waiving required tests, if scientifically justifiable. In Africa, registration authorities need to develop a knowledge base to make case-by-case decisions, and here the Green Muscle™ data can be very helpful. To facilitate the process, institutions like the CERES-LOCUSTOX Foundation, CABI *Bioscience*, IITA or NLU, which have a long-standing experience in microbial control and ecotoxicology, can provide scientific support.

References

Balança, G. and de Visscher, M.N. (1997) Effects of very low doses of fipronil on grasshoppers and non-target insects following field trials for grasshopper control. *Crop Protection* 16, 553–564.

Ball, B.V., Pye, B.J., Carreck, N.L., Moore, D. and Bateman, R.P. (1994) Laboratory testing of a mycopesticide on non-target organisms: the effects of an oil formulation of *Metarhizium flavoviride* applied to *Apis mellifera*. *Biocontrol Science and Technology* 4, 289–296.

Bateman, R.P., Godonou, I., Kpindou, D., Lomer, C.J. and Paraiso, A. (1992) Development of a novel field bioassay technique for assessing mycoinsecticide ULV formulations. In: Lomer, C.J. and Prior, C. (eds) *Biological Control of Locusts and Grasshoppers*. CAB International, Wallingford, UK, pp. 255–263.

Bridge, P.D., Williams, M.A.J., Prior, C. and Paterson, R.R.M. (1993) Morphological, biochemical and molecular characteristics of *Metarhizium anisopliae* and *M. flavoviride*. *Journal of General Microbiology* 139, 1163–1169.

Carson, R. (1962) *Silent Spring*. Houghton Mifflin Company, Boston, Massachusetts, USA.

Cheke, R.A. (1990) A migrant pest in the Sahel: the Senegalese grasshopper *Oedaleus senegalensis*. *Philosophical Transactions of the Royal Society London B* 328, 539–553.

Danfa, A. and van der Valk, H. (1999) Laboratory testing of *Metarhizium* spp. and *Beauveria bassiana* on Sahelian non-target arthropods. *Biocontrol Science and Technology* 9, 187–198.

Driver, F., Milner, R.J. and Trueman, J.H.W. (2000) A taxonomic revision of *Metarhizium* based on a phylogenetic analysis of ribosomal DNA sequence data. *Mycological Research* 104, 135–151.

Ekesi, S. (1999) Selection of virulent isolates of entomopathogenic Hyphomycetes against *Clavigralla tomentosicollis* Stal. and evaluation in cage experiment using three cowpea varieties. *Mycopathologia* 148, 131–139.

EPA (1996) *Microbial Pesticide Test Guidelines* – Series 885. United States Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances (OPPTS), Washington, DC, USA (continuously updated).

EU (2001) Commission Directive 2001/36/EC. *European Union, Official Journal* L 164, 20/06/2001, 1–38.

Everts, J. (1990) *Environmental Effects of Chemical Locust and Grasshopper Control – A Pilot Study*. Project ECLO/SEN/003/NET, Food and Agriculture Organization of the United Nations, Rome, Italy.

Friese, D.D., Mégevand, B. and Yaninek, J.S. (1987) Culture maintenance and mass production of exotic phytoseiids. *Insect Science and its Application* 8, 875–878.

Fritsche, M.E. and Tamò, M. (2000) Influence of thrips prey species on the life-history and behaviour of *Orius albidipennis*. *Entomologia Experimentalis et Applicata* 96, 111–118.

Fuxa, J.R. (1987) Ecological considerations for the use of entomopathogens in IPM. *Annual Review of Entomology* 32, 225–251.

Goettel, M.S., Poprawski, T.J., Vandenberg, J.D., Li, Z. and Roberts, D.W. (1990) Safety to nontarget invertebrates of fungal biocontrol agents. In: Laird, M., Lacy, L.A. and Davidson, E.W. (eds) *Safety of Microbial Insecticides*. CRC Press, Boca Raton, Florida, USA, pp. 209–231.

Greathead, D.J. and Prior, C. (1990) The regulation of pathogens for biological control with special reference to locust control. In: Somme, L. and Bie, S. (eds) *Proceedings of the Workshop on Health and Environmental Impact of Alternative*

Control Agents for Desert Locust Control. NORAGRIC Occasional Papers Series C: Development and Environment 5, 65–80.

Hassan, S.A. (1998) Pesticides and beneficial organisms. International Organization for Biological and Integrated Control of Noxious Animals and Plants – West Palearctic Regional Section (IOBC/WPRS). *IOBC/WPRS Bulletin* 21.

Hernández, L.M. and Stonedahl, G.M. (1999) A review of the economically important species of the genus *Orius* (Heteroptera: Anthocoridae) in East Africa. *Journal of Natural History* 33, 543–568.

Jago, N.D. (1993) *Millet Pests of the Sahel: Biology, Monitoring and Control.* Natural Resources Institute, Overseas Development Administration, Chatham, UK.

Kershaw, M.J., Moorhouse, E.R., Bateman, R., Reynolds, S.E. and Charnley, A.K. (1999) The role of destruxins in the pathogenicity of *Metarhizium anisopliae* for three species of insects. *Journal of Invertebrate Pathology* 74, 218–223.

Kooyman, C., Bateman, R.P., Langewald, J., Lomer, C.J., Ouambama, Z. and Thomas, M. (1997) Operational-scale application of entomopathogenic fungi for control of Sahelian grasshoppers. *Proceedings of the Royal Society of London* B 264, 541–546.

Lahr, J., Badji, A., Marquenie, S., Schuiling, E., Ndour, K.B., Diallo, A.O. and Everts, J.W. (2001) Acute toxicity of locust insecticides to two indigenous invertebrates from Sahelian temporary ponds. *Ecotoxicology and Environmental Safety* 48, 66–75.

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

Langewald, J., Ouambama, Z., Mamadou, A., Peveling, R., Stolz, I., Bateman, R., Attignon, S., Blanford, S., Arthurs, S. and Lomer, C. (1999) Comparison of an organophosphate insecticide with a mycoinsecticide for the control of *Oedaleus senegalensis* Krauss (Orthoptera: Acrididae) and other Sahelian grasshoppers in the field at operational scale. *Biocontrol Science and Technology* 9, 199–214.

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

Mégevand, B., Kläy, A., Gnanvossou, D. and Paraiso, G. (1993) Maintenance and mass rearing of phytoseiid predators of the cassava green mite. *Experimental and Applied Acarology* 17, 115–128.

NAS (1981) *Testing for the Effects on Ecosystems.* National Academy Press, Washington, DC, USA.

Nwanze, K.F. and Harris, K.M. (1992) Insect pests of pearl millet in West Africa. *Review of Agricultural Entomology* 80, 1133–1155.

OECD (1982 onward) *Guidelines for Testing of Chemicals.* OECD, Paris, France.

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

Peveling, R. and Demba, S.A. (1997) Virulence of the entomopathogenic fungus *Metarhizium flavoviride* Gams and Rozsypal and toxicity of diflubenzuron, fenitrothion-esfenvalerate and profenofos-cypermethrin to non-target arthropods in Mauritania. *Archives of Environmental Contamination and Toxicology* 32, 69–79.

Peveling, R., Attignon, S., Langewald, J. and Ouambama, Z. (1999) An assessment of the impact of biological and chemical grasshopper control agents on ground-dwelling arthropods in Niger, based on presence/absence sampling. *Crop Protection* 18, 323–39.

Peveling, R., Rasolomanana, H., Raholijaona, Rakotomianina, L., Ravoninjatovo, A., Randimbison, L., Rakotondravelo, M., Raveloson, A., Rakotoarivony, H., Bezaka,

S., Ranaivoson, N. and Rafanomezantsoa, J.-J. (2001) Effets des traitements aériens de fipronil et de deltaméthrine en couverture totale sur la chaîne alimentaire. In: Zehrer, W. (ed.) *Lutte Antiacridienne à Madagascar – Tome III – Ecotoxicologie*. DPV/GTZ, Ministère de l'Agriculture, Antananarivo, pp. 525–572.

Prior, C. (1997) Susceptibility of target acridoids and non-target organisms to *Metarhizium anisopliae* and *M. flavoviride*. In: Krall, S., Peveling, R. and Ba Diallo, D. (eds) *New Strategies in Locust Control*. Birkhäuser Verlag, Basel, Switzerland, pp. 369–375.

Singh, S.R. and Allen, D.J. (1979) Cowpea pests and diseases. Ibadan. International Institute of Tropical Agriculture (IITA). *IITA Manual Series 2*.

Smits, J.E., Johnson, D.L. and Lomer, C. (1999) Pathological and physiological responses of ring-necked pheasant chicks following dietary exposure to the fungus *Metarhizium flavoviride*, a biocontrol agent for locusts in Africa. *Journal of Wildlife Diseases* 35, 194–203.

Sokolov, I.M. (2000) How does insecticidal control of grasshoppers affect non-target arthropods? In: Lockwood, J.A., Latchininsky, A.V. and Sergeev, M.G. (eds) *Grasshoppers and Grassland Health*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 181–192.

Stolz, I. (1999) The effect of *Metarhizium anisopliae* (Metsch.) Sorokin (=*flavoviride*) Gams and Rozsypal var. *acridum* (Deuteromycotina: Hyphomycetes) on non-target Hymenoptera. PhD thesis, University of Basel.

Stolz, I., Nagel P., Lomer, C. and Peveling, R. (2002) Susceptibility of the hymenopteran parasitoid *Apoanagyrus* (=*Epidinocarsis*) *lopezi* (Encyrtidae) and *Phanerotoma* sp. (Braconidae) to the entomopathogenic fungus *Metarhizium anisopliae* var. *acridum* (Deuteromycotina: Hyphomycetes). *Biocontrol Science and Technology* 12, 349–360.

Tingle, C.C.D. and McWilliam, A.N. (2001) Evaluation de l'effet à court terme sur les organismes non cibles des traitements en barrières à grande échelle dans le cadre de la lutte antiacridienne d'urgence. In: Zehrer, W. (ed.) *Lutte Antiacridienne à Madagascar – Tome III – Ecotoxicologie*. DPV/GTZ, Ministère de l'Agriculture, Antananarivo, pp. 115–182.

van der Valk, H. and Niassy, A. (1997) Side effects of locust control on beneficial arthropods: research approaches used by the LOCUSTOX project in Senegal. In: Krall, S., Peveling, R. and Ba-Diallo, D. (eds) *New Strategies in Locust Control*. Birkhäuser, Basel, Switzerland, pp. 337–344.

van der Valk, H., Niassy, A. and Beye, A.B. (1999) Does grasshopper control create grasshopper problems? Monitoring side effects of fenitrothion application in the western Sahel. *Crop Protection* 18, 139–149.

Yaninek, J.S., Onzo, A. and Ojo, B. (1993) Continent-wide releases of neotropical phytoseiids against the exotic cassava green mite in Africa. *Experimental and Applied Acarology* 17, 145–160.