Evaluation of *Metarhizium anisopliae* mycoinsecticide as an alternative locust control measure in southern Africa

by

Elizabeth Johanna Müller neé Kassimatis

Submitted in partial fulfilment of the requirements for the degree

Magister Scientiae

in the Faculty of Biological and Agricultural Sciences
Department of Zoology and Entomology
University of Pretoria
Pretoria

2000

This work is dedicated to

my parents George and Priscilla Kassimatis

my children Yolandi & Amori

my sister Hannelie

CONTENTS **PAGE NUMBER**

ABSTRACT

Locust plagues always have been, and are likely to remain, a major threat to African agriculture. The repeated application of chemical pesticides has to date been the only proven method of locust control. An entomopathogenic fungus, *Metarhizium anisopliae* var. *acridum* isolate (IMI 330189), was identified as an alternative, potentially safer, non-chemical locust control method. This oil-based mycoinsecticide was imported into South Africa and tested preliminarily at the laboratories against the brown locust, *Locustana pardalina*, the red locust, *Nomadacris septemfasciata* and the African migratory locust, *Locusta migratoria migratorioides*. The fungus was applied topically, by using a micro-applicator and placing individual droplets behind the neck membrane of locusts. All the locust species were highly susceptible to *Metarhizium*, producing >89% final mortality and significantly low control mortality. Treated insects all developed a typical red coloration after death, which is a clear symptom of mycosis, and external sporulation of the fungus subsequently developed when locusts were incubated at high humidity. These laboratory results prompted field trials against gregarious hopper bands of the brown locust in the Karoo and the red locust in Mozambique. Another isolate, the blastospore stage of *M. anisopliae* var. *acridum* isolate Mfl5, has also been successfully evaluated in the laboratory, although the field testing of this aqueous formulation did not seem feasible at the time. The mycoinsecticide acts through direct contact and gives control equal to that of any of the chemical insecticides in current use, although the speed of kill was slow, with most locusts taking 4-21 days to die. This could be a perceived disadvantage during operational implementation, where locust control officers are used to fast acting chemicals. To test the effect of dose rate on increasing the speed of kill, dose rates ranging from 10^3-10^8 conidia per individual, were applied to brown locust hoppers in the laboratory. There was a classic dose response, with higher doses killing insects quicker. Furthermore, locusts are pests largely because of the crop security that is threatened and it is argued that any reduction in food consumption after mycoinsecticide treatment could be valuable regarding future operational application. All laboratory treatments showed the trend of a reduction in food consumption even at a dose rate of 10^4 conidia/hopper. To assess the risk to non-target organisms after release of the mycoinsecticide in the field, the African honeybee, *Apis mellifera scutellata*, was used as a biological indicator. Three free-living colonies were dusted directly with a high dose of dry spores, while two colonies served as a control. No dead bees were found due to mycosis. Colonies maintained their strength and there was no overall significant difference in brood patterns and food collecting activities between treated and untreated colonies, even though temperatures inside hives (25-30°C) were conducive to germination of spores.

Samevatting

Sprinkane is en sal waarskynlik altyd 'n groot probleem in die landboubedryf in Afrika wees. Die herhaaldelike aanwending van chemiese insektisiedes was tot dusver nog die enigste suksesvolle beheermetode vir sprinkane. 'n Entomopatogene swam, *Metarhizium anisopliae* var. *acridum* isolaat (IMI 330189), is geïdentifiseer as 'n alternatiewe, potensieel veiliger, en nie-chemiese sprinkaanbeheermetode. Hierdie mikoinsektisied in 'n olie-basis is na Suid-Afrika ingevoer en voorlopig in die laboratorium teen die bruinsprinkaan, *Locustana pardalina*, die rooisprinkaan, *Nomadacris septemfasciata*, en die Afrikaanse treksprinkaan, *Locusta migratoria migratorioides*, getoets. Die swam is topikaal toegedien, deur indiwiduele druppels met 'n mikro-toediener agter die nekmembraan van sprinkane aan te wend. Al die sprinkaanspesies was hoogs vatbaar vir *Metarhizium*, en het >89% finale mortaliteit getoon, met 'n betekenisvolle lae kontrole mortaliteit. Behandelde insekte het almal 'n tipiese rooi verkleuring na dood ontwikkel, wat 'n duidelike simptome van mikose was. Eksterne sporulering van die swam het ontwikkel toe hierdie dooie sprinkane by 'n hoë humiditeit geïnkubeer is. Laboratorium resultate het tot veldproewe teen migrerende voetgangerswerms van die bruinsprinkaan in die Karoo en die rooisprinkaan in Mosambiek gelei. 'n Ander isolaat, die blastospoor stadium van *M. anisopliae* var. *acridum* isolaat Mfl5, is ook suksesvol in die laboratorium getoets, alhoewel die toets van hierdie water formulasie in die veld, nie op daardie stadium prakties uitvoerbaar was nie. Die mikoinsektisied werk soos 'n direkte kontakdoder, en lewer net soveel beheer as die chemiese insekdoders wat tans in gebruik is, tog is die spoed waarteen insekte doodgaan stadig, en meeste sprinkane neem 4-21 dae om dood te gaan. Dit kan deur sprinkaanbeheerbeamptes, wat gewoond is aan vinnig-dodende chemiese insektisiedes, as 'n nadeel beskou word. Om die uitwerking van verskillende dosisse op die spoed van doodmaak te toets, is bruinsprinkaanvoetgangers met dosisse wat wissel vanaf 10^3-10^8 konidia per indiwidu, behandel. Die resultate dui op 'n klassieke dosisreaksie, met die hoër dosisse wat die insekte vinniger doodmaak. Verder is sprinkane plae as gevolg van die gewassekuriteit wat bedreig word. en daarom kan geredeneer word dat enige afname in hulle voedselinname van groot waarde tydens operasionele toediening kan wees. Alle laboratorium behandelings het die neiging van 'n afname in voeselinname deur die sprinkane getoon, selfs teen 'n dosis van 10^4konidia per sprinkaan Om die risiko te bepaal wat met die vrylating van *M. anisopliae* var. *acridum* mikoinsektisied in die veld gepaard mag gaan, is die Afrika heuningby, *Apis mellifera scutellata*, as 'n biologiese indikator gebruik. Drie vry-lewende kolonies is direk met 'n hoë dosis van die droë spore van *Metarhizium* bestuif, terwyl twee onbehandelde kolonies as kontrole gedien het. Geen dooie bye is as gevolg van mikose gevind nie. Kolonies het hulle sterkte gehandhaaf en daar was geen algehele betekenisvolle verskille in broedpatrone en voedselversamelingsaktiwiteite tussen behandelde en onbehandelde kolonies nie, alhoewel die temperature binne in die kolonies gunstig was vir die ontkieming van spore (25-30°C).

CHAPTER 1

INTRODUCTION

The locust threat - past and present

Locusts are among the most spectacular and notorious of insect pests (Faure 1935; Uvarov 1951) and have plagued man ever since he first began cultivating plants for food, some ten thousand years ago. The earliest known record of the desert locust, *Schistocerca gregaria*, is found at Saqqarah in Lower Egypt, where locusts are depicted on a tomb dating back to the Sixth Dynasty from 2420-2270 BC (Botha 1969b; Meinzingen 1993). The eighth plague of Egypt, recorded in the Book of Exodus (about 1300 BC) in the Bible also reflects the awe with which desert locust swarms were regarded in ancient times (Meinzingen 1993).

Probably the earliest record of locusts in South Africa goes back to before European settlement in the Cape, when visiting sailors reported having seen locusts in Table Bay. The pioneer, Jan Van Riebeeck, documented the suffering of crop loss from locusts in 1653 and again in 1746. Locusts damaged grazing in the Cape to such an extent that many cattle and sheep died of starvation. This resulted in a doubling of the price of meat, and work at Mouille Point had to be abandoned for lack of revenue from the sales of crops to visiting ships (Botha 1969b). According to Lounsbury (1915) the earliest confirmed record of the brown locust in South Africa dates back to 1797, ten years after the founding of the Karoo town, Graaff-Reinet.

Despite modern and powerful new insecticides and novel advances in detection and application technology, locusts still occur in large numbers all over the world and remain a serious threat to agricultural production, especially in many African countries.

Locust species, outbreak dynamics, damage and biology

Of the thousands of grasshopper species (Orthoptera: Acrididae) described world wide, only approximately 12 are classified as true locusts. Eight of these species occur in Africa south of the Sahara (Meinzingen 1993) (Table 1.1). Locusts differ from grasshoppers in that they have the ability to change their behaviour and morphology under crowded conditions. As locusts, they are then able to maintain their cohesion and form gregarious hopper bands and adult swarms, with distinctive synchronised feeding, resting, breeding and migration habits. This biochemical, physiological and behavioural switch from a solitary to a gregarious phase is called phase transformation, and was described by Uvarov in Europe and demonstrated by Faure in South Africa (Botha 1969; Botha 1970; Faure 1932; Lea 1973).

Table 1.1 True locusts that occur in Africa south of the Sahara

Locust species	Scientific name
African migratory locust	*Locusta migratoria migratorioides* (Reiche & Fairmaire 1850)
Brown locust	*Locustana pardalina* (Walker 1870)
Desert locust (Namibia)	*Schistocerca gregaria flaviventris* (Burmeister 1838)
Desert locust (North Africa)	*Schistocerca gregaria gregaria* (Forskål 1775)
Madagascar migratory locust	*Locusta migratoria capito* (Saussure 1884)
Red locust	*Nomadacris septemfasciata* (Serville *1838*)
Tree locust	*Anacridium melanorhodon* (Walker 1870)
Tree locust	*Anacridium wernerellum* (Karny 1907)

Grasshoppers can also occur in numbers sufficient to cause serious economic damage to crops and pastures, but locusts are typical r-strategists, capable of rapid population growth under favorable environmental conditions. Solitary phase locusts of any given species can be found over a very large area. However, not all parts of their natural distribution are suitable for the transformation from solitary to gregarious phase. The parts that are optimal for breeding, and from where plagues originate, are known as outbreak areas. Such areas are much smaller than the geographic range of these species.

Plagues of locusts and grasshoppers have been known to cause significant damage in Australia, parts of Asia, the middle East, North and South America, the Indian subcontinent and, especially, throughout Africa. Because of their high pest status all over the world, locust control is an international problem with intense political ramifications, especially during severe locust plagues when swarms cross international borders.

Locusts in southern Africa generally breed in thinly populated, non-crop producing areas, but their gregarious behaviour enables them to migrate rapidly and cause damage to crops and to pastures. Swarms can cover areas of more than 1000km^2 and can migrate several kilometres per day (Steedman 1990).

Four of the eight African locust plague species are found in southern Africa and pose a regular and significant threat to agriculture. They are:

- The brown locust, *L. pardalina* (Walker)
- The red locust, *N. septemfasciata* (Serville)
- The African migratory locust, *L. migratoria migratorioides* (Reiche & Fairmaire)
- The Southern African desert locust, *S. gregaria flaviventris* (Burmeister).

The brown locust

Economically, the brown locust, *L. pardalina*, (Fig 1.1) is by far the most important locust in southern Africa (Botha 1969b; Lea 1973). Its outbreak region covers an area of about 250 000km^2 of remote desert and semi-desert environments and sparsely populated country in the Karoo region of South Africa and southern Namibia (Fig. 1.2) (Faure & Marais 1936). This remote outbreak area makes the accurate reporting of locust populations unreliable. *L. pardalina* has the highest outbreak frequency of any of the world's plague locusts and intense outbreaks occur almost every year. There have been only 5 years in the past 50 years when no chemical control campaign was undertaken in the Karoo (Bateman *et al.* 1994). Before the first effective insecticides became available around 1945, uncontrolled

plague cycles of brown locust swarms used to regularly escape from the Karoo and threatened food security throughout southern Africa up to the Zambezi river and bred over most of South Africa, Namibia, Lesotho, Botswana, Zambia, Zimbabwe, south-eastern Angola, and the southern half of Mozambique (Faure 1932; Lea 1964; 1973).

L. pardalina lays its eggs in both dry and wet soil and they hatch out approximately 10 days after the first summer rains (Smit 1939). Both diapause and non-diapause eggs can be laid and development is complex (Matthee 1951). Drought resistant eggs are able to lie in the soil in a state of quiescence for up to three years (Botha 1970). If conditions are warm enough, following the first widespread summer rainfall events, mass hatching of eggs that have accumulated during drought periods then cause locust outbreaks to erupt simultaneously over large areas of the Karoo. Locust populations increase rapidly as it is multivoltine, with 2-4 generations per year, short life cycle of ±42 days and a high reproductive potential. *L. pardalina* also has a high fecundity, producing up to five egg pods per female with 40-50 eggs per pod. Hoppers, of which there are five instars, are highly gregarious and march in aggregated column-formation bands. The 4-5th nymphal hoppers are commonly known as the 'rooibaadjie' or 'red jacket stage', derived from their reddish appearance.

Adults are able to swarm over vast areas and can fly over 100km per day. This species has the most pronounced phase polymorphism of any of the world's locusts. The males in the solitary phase are relatively small compared to gregarious phase males (Botha 1969; Botha 1970; Lea 1973; Smit 1960).

Fig.1.1. Fifth instar brown locust hopper nymphs, *Locustana pardalina*.

PHOTO: D.BROWN

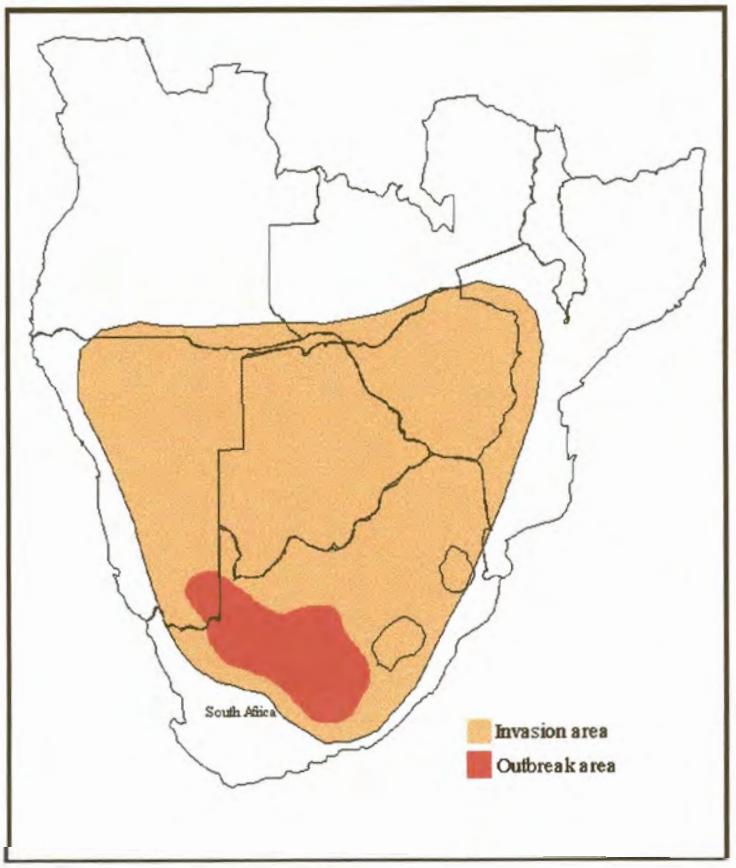

Fig. 1.2 Map of the brown locust outbreak and invasion areas.

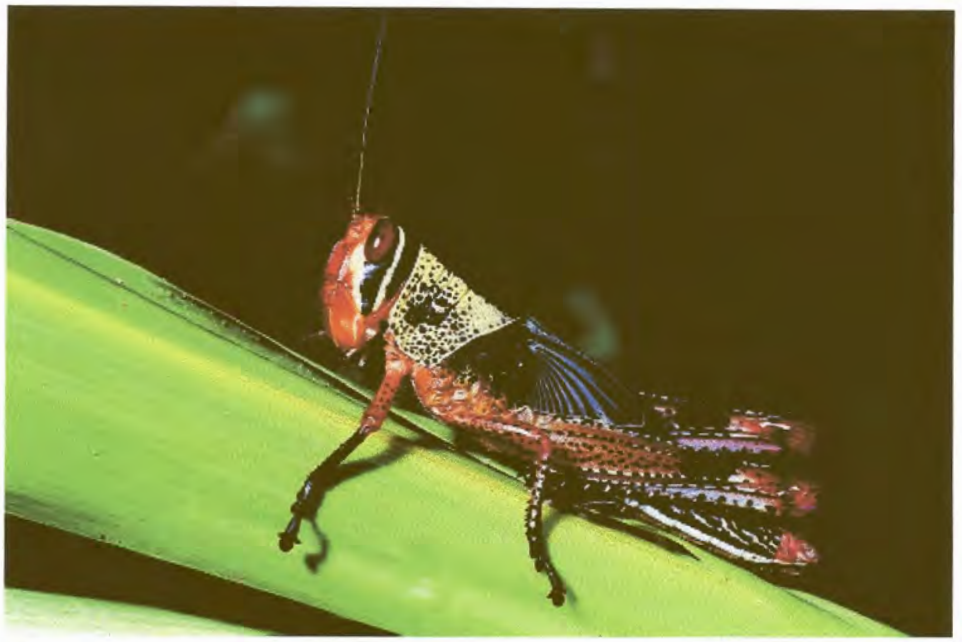

Fig. 1.3 A sixth instar red locust nymph, *Nomadacris septemfasciata*
PHOTO: D.BROWN

The red locust

The red locust, *N. septemfasciata* (Fig.1.3) is the largest of the African plague locusts and earns its name from the bright red colour of the adults in actively migrating swarms (Lea 1973). It is a tropical species with permanent breeding grounds in nine discrete swampland areas scattered across Central and East Africa. The main outbreak areas include those in the vicinity of Lake Rukwa and the Malagarasi and Wemberi areas in Tanzania, the Mweru wa Ntipa and Kafue swamps in Zambia and Lake Chilwa in Malawi (Gunn 1960; Botha 1969) (Fig. 1.4). There have only been three major plagues in the past century, the last one which lasted from 1939-1944. The nearest outbreak area to South Africa lies between the Buzi and the Pungue rivers near Beira in Central Mozambique. It was here, in 1995-1996, in these seasonally flooded grasslands that an intense upsurge of the locust developed for the first time in 20 years. Swarms escaped the Buzi floodplain area and invaded Zimbabwe, Malawi, Botswana, and eventually reached South Africa. Swarms flew over Pretoria for the first time in 50 years in November 1996.

The red locust is univoltine, with only one generation per year. Eggs are laid in damp soil at the start of the first rains (November/December) and take about a

month to hatch. There are six hopper instars in the gregarious phase and an additional one in the solitary phase. The adults remain sexually immature throughout the long dry season. Females may lay successive egg pods (3-4) at fortnightly intervals, with each pod containing as many as 100, but usually 65-75 eggs. (Botha 1969; Faure 1935; Jack 1934; Lea 1973; Whellan 1964).

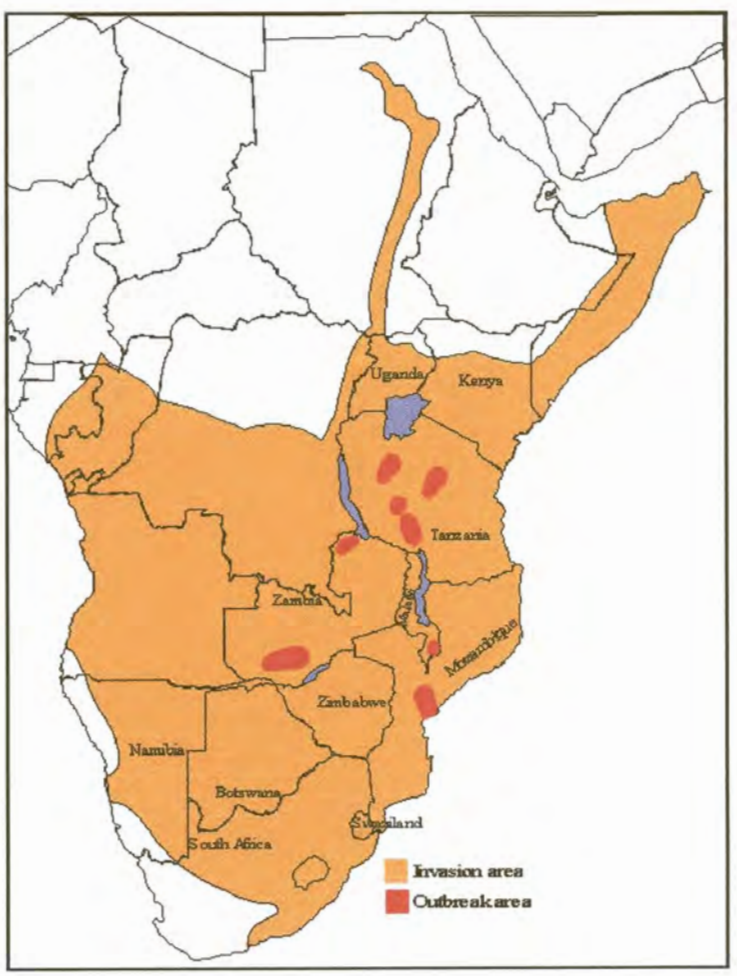

Fig. 1.4. *Map of the red locust outbreak and invasion areas.*

The African migratory locust

The African migratory locust, *L. migratoria migratorioides*, is one of at least nine closely related subspecies occurring in different parts of the world (Lea 1973). Although the main outbreak area lies in the Middle Niger floodplains in Mali, this subspecies occurs throughout Africa south of the Sahara, especially in areas where continuous irrigation and cultivation practices provide favourable breeding conditions (Farrow 1975). In southern Africa, localised outbreak areas are known from South Africa in the Free State, Mpumalanga, the Western Cape, North West and Northern parts of Gauteng Provinces, Botswana, Zimbabwe and the Caprivi Strip of Namibia. The African migratory locust also has five hopper stages and can produce three generations in a season. Eggs are laid in damp soil and hatch in about 12-28 days, depending on the temperature (Botha 1969; Price & Brown 1990, 1992). There has not been a serious outbreak since the late 1980's, with the last small-scale outbreak occurring in 1994.

The Southern African desert locust

This subspecies of desert locust, *S. gregaria flaviventris*, is a permanent inhabitant of the desert parts of Namibia, Western Botswana and the north-west Cape Province in South Africa. It is known mainly only in its solitary phase, but when it does swarm, it is a feeble migrant and as such is not a serious problem. Females lay their eggs only in wet soil, hoppers hatch after 14-27 days and there are five hopper stages (Botha 1969e; Lea 1973).

The history of locust control in South Africa

Traditional methods: 1895-1905

The first organised locust control campaigns started in 1895 (Botha & Lea 1970). Before the use of pesticides became the standard means of locust control in South Africa, traditional and mechanical techniques were applied to control locusts. According to Jack (1934), Lea (1973) and Lounsbury (1915), herding of hoppers into pits (partly filled with molasses) with the aid of "kraals", the harvesting of

hoppers and flyers into bags at night, or ploughing up the locust egg beds and trampling the soil surface with domestic stock to prevent eggs from hatching, were commonly used. Hopper bands were also gathered and killed by slashing them with a whip made of wire strands, or under extreme situations, people even went as far as burning their grazing to drive off locust swarms, and last but not least, tried to 'cast spells' on this pest! Contact sprays using paraffin oil, soap solutions and carbolic sheep dips, were also used as control measures in the early part of this century (Jack 1934; Lea 1973; Lounsbury 1915).

Chemical control

Arsenite of soda

Arsenite of soda was first used in 1895 in Natal to directly douse or drench red locust targets, but was later applied through stirrup pumps when these were introduced in 1899. In 1905, factory prepared arsenic pesticides and baits came into use.

The period 1906 to 1944 marked the general adoption of sodium arsenite poison. Spraying was completely superseded by baiting methods by 1935 (2-3% bait), but no effective alternative was found to arsenite of soda as the active ingredient. This is a Division 1 class poison and extremely hazardous to man and animals. Several stock losses have been recorded (Lea 1973). Wheat bran was the main carrier used in the preparation of these baits. In 1935, aerial spraying with arsenite of soda dusts released into the slipstream of large tri-motor aircraft in Zululand was carried out against red locusts (Lea 1973).

Organochlorine compounds

Between 1944 and 1958, organochlorine based control measures were initiated with a 50:50 dilution of old 3% sodium arsenite maize meal bait together with a 1% gamma Benzeen Hexachloride (BHC) synthetic insecticide. This method was soon superseded by spraying with a wettable powder BHC and dusting directly with the same formulation (also known as *'Double Benhex'*). Mechanised control methods

using power machinery were introduced widely for both spraying and dusting in place of the older hand apparatus. Small experimental campaigns using gamma BHC began in the Karoo with light aircraft for the first time in 1958 against the brown locust (Lea 1973).

Although BHC was effective and cheap (it was regarded as 'seemingly' safe (Botha 1958), public concern was raised over the adverse environmental consequences of gamma BHC in the early 1970's. Its uptake in sheep and resulting high residue levels in human populations in South Africa led to its general banning, except for emergency use (Wiese & Bot 1973). A search for suitable shorter-acting but equally effective replacements was therefore initiated soon afterwards, although BHC was still used until 1986. During the huge 1985/1986 brown locust outbreak, 5 million kg of BHC was used in the Karoo (Brown 1987).

Organophosphorous compounds (OP's)

Within the next twenty years (in the 1970s), the active ingredient diazinon was the first organophosphorous insecticide to be used as an ultra low volume (ULV) spray formulation against locusts, followed by fenitrothion and dichlorvos. It was not as persistent as BHC and did not bio-accumulate in the food chain. OP's were slow acting under cool conditions, which lead to the re-spraying of targets. However, the extremely high toxicity to birds of one of the metabolites (TEPP) rendered diazinon undesirable. There were also incidents of locust control officers admitted to hospital with OP poisoning in 1994 (Price, pers. comm.) This made OP's undesirable for safe, effective, more target-specific locust control and it was consequently replaced by pyrethroids.

Synthetic pyrethroids (PY's)

The initial high volume approach to pesticide application was scaled down to ultra-low volumes of safer formulated pesticides. Synthetic pyrethroids were first used for locusts in South Africa in 1995. These insecticides, e.g. active ingredient deltamethrin, fenvalerate and esfenvalerate, showed greater potency against locusts at substantially lower dosage rates and are relatively safe for mammals, birds and

operators. Dermal toxicity is safe, with LD_{50} of >2000. PY's rapidly knock down hoppers and confine swarms to treated areas (Brown and Kieser 1997). It is less costly, re-application is not needed and it is therefore less harmful to the environment. Current control strategy in the Karoo involves the spot application of synthetic PY's to thousands of individual hopper bands and fledgling swarms. An estimate of about 400 000 litres of chemical insecticides were used in the 1996/1997 brown locust outbreak.

The international search for alternative methods of locust control

The year 1985 will be remembered as the start of the most alarming desert locust campaign since 1963. During the major upsurge of desert locust in Northern Africa during 1986-1989, more than US$ 275 million was committed by donors for locust control and some 13 million litres of insecticides were applied. This caused world-wide concern over the detrimental environmental impact of such extensive chemical control campaigns (OTA 1990). The very high cost of these control operations and the concerns expressed about their safety and effectiveness (less persistent, applied more frequently, more costly) led to unease among the international donor community and locust control methods in the Food and Agricultural Organisation (FAO) Conference were reassessed in 1988. At this conference ideas were put forward by CABI BIOSCIENCE (formerly the International Institute of Biological Control), on the possibility of developing a biopesticide for locust and grasshopper control. As a result of this interest, a total of US$4.5 million was raised in 1989 from donor agencies of Canada (Canadian International Development Agency), Switzerland (Swiss Development Co-operation), the Netherlands (Directorate General for International Co-operation) and the United Kingdom (Department for International Development) for a programme to develop a new biopesticide alternative for locust and grasshopper control.

Within one year a multidisciplinary, multi-institutional and international collaborative programme of research and development was established, involving co-operation between CABI Bioscience, U.K. (who managed the programme); the International Institute for Tropical Agriculture (IITA) in Benin and CILSS in Mali

and Niger (The Centre pour Agronomie, Hydrologie, Meteorologie, an Institute of the Comité Inter-Etats pour la Lutte contre la Secheresse dans le Sahel) and GTZ, Germany (Dent 1998; Moore *et al.* 1995; Prior 1997). The programme was entitled *Lutte Biologique contre les Locutes et Sauteriaux (Biological control of locusts and grasshoppers),* or acronym, LUBILOSA (Bateman 1992).

LUBILOSA was thus set up to develop a biopesticide for the control of locusts and grasshoppers. Hereafter, various international research initiatives into the development of biological pesticides were pioneered, with the aim of providing an effective, environmentally benign alternative to pesticide usage. Biopesticides (entomopathogenic fungi, viruses and other pathogens) are non-polluting, have a low impact on non-target organisms and the environment and were therefore investigated as an alternative control strategy. Prior & Greathead (1989) described Deuteromycete: Hyphomycetes fungi as the most promising agents for biopesticide development against locusts and research based on these naturally occurring fungi was initiated to find a target-specific mycoinsecticide for locusts.

The challenge was that LUBILOSA proposed the use of fungal biopesticides for the control locusts under field conditions in the extremely hot and dry environment of the Sahel. Although a range of entomopathogenic fungi showed potential as control agents of Acrididae, they are highly sensitive to environmental conditions with >95% RH required for effective growth and development (Ullyett & Schonken 1940; Streett & Henry 1990; Bateman 1992).

However, an early mark in the programme was to demonstrate that by formulating fungi in vegetable and mineral oils, high rates of germination of spores and subsequent infection of insects could be obtained even in dry environments (Bateman *et al.* 1993). It was established that the efficacy of fungi as locust killing agents was enhanced by this formulation in oil, which, in practical terms, meant than infection could now occur at low humidities or in a very dry, hot habitats (Bateman *et al.* 1993). Formulating conidia in an oil mixture thus makes it suitable for spraying as a biological pesticide with conventional ultra-low volume (ULV) equipment, independent of environmental factors (Moore *et al.* 1995).

South Africa's involvement in the search for alternative methods of locust control

In the Karoo region of South Africa, local farmers and conservation bodies are increasingly concerned about the repeated spraying of large amounts of chemical insecticides against locusts in this unique semi-arid eco-system, with its rich and endemic fauna and flora. Since locusts are a serious pest problem in South Africa, which requires regular and costly chemical control, there is an urgent need for target-specific, environmentally benign methods of controlling locusts.

South Africa started exploring the use of fungi to control locusts as early as the turn of the century with what has become known as the 'South African White Fungus' debacle (Bateman *et al.* 1994; Butler & Lefroy 1907; Pole Evans 1911; Skaife 1925). This experiment with *Entomophaga grylli* fungi failed. Schaefer (1938) also commented on the 'grey fungus', *Beauveria bassiana,* on the red locust. However, after increased global interest and mostly local pressure, researchers at the Plant Protection Research Institute of the Agricultural Research Council (ARC: PPRI) again started exploring the potential of locust-killing pathogens in the early 1990's. Various pathogens e.g. entomopathogenic fungi, entomopoxvirus and bacteria were tested against southern African locusts species. However, insignificant mortality was achieved in laboratory trials and these agents were therefore considered unsuitable for the control of gregarious local locust species (Price *et al.* 1993; Price & Müller 1993a).

During 1992, visits were made by PPRI to CABI Bioscience in the U.K. to investigate the potential of using entomopathogenic fungi in South Africa and the export of *Metarhizium* isolates to the P.P.R.I. was arranged. The following year this pathogen was imported under strict quarantine conditions into South Africa for further testing. Although the risks associated with releasing exotic isolates (especially biological insecticides) are considered small (Prior 1997), LUBILOSA had to maintain a strict protocol by following the Food and Agriculture Organisation (FAO) code of conduct on the importation of exotic biological control agents. According to South African legislation, the import also involved the issue of a permit from the Directorate of Plant and Quality control of the Department of

Agriculture. Two conidial isolates, in a ready formulated oil mixture, were imported into South Africa in 1993 (import permit no. 14/2/2/1(9/21/172) and the first laboratory research was carried out under strict quarantine conditions prescribed for the basic containment level 1 (UK Advisory committee on dangerous pathogens) at PPRI.

The aims of this study

The aims of this study (1993-1998) were to conduct laboratory tests to evaluate the entomopathogenic fungus *Metarhizium anisopliae* var. *acridum* as a potential biological control agent for the three most important locust species in southern African, namely the brown locust, the red locust and the African migratory locust. Chapter 2 briefly addresses the discovery, biological properties, characterisation, taxonomic status and mode of action of the mitosporic fungus, *Metarhizium*. Chapter 3 describes the first preliminary laboratory bioassay studies conducted in South Africa to investigate effectivity of the conidial stage of *M. anisopliae* var. *acridum* against three locust species, and outlines the various insect pathological techniques used in this study. In Chapter 4, these basic tests are extended to examine the possibility of using the blastospore stage of the same fungus in an aqueous formulation, instead of the oil conidial formulation against the brown locust and the red locust. Chapter 5 then examines the effect of slow speed of kill, by increasing the dose rate, as well as additional qualities of the pathogen such as reduction of food consumption after infection with the mycoinsecticide. Chapter 6 presents the findings of the effect of testing *Metarhizium* on a non-target organism, the African honey bee, *Apis mellifera scutellata*. Finally, Chapter 7 summarizes the implications of the above results in the context of using this mycoinsecticide in an operational management strategy for the control of locusts in southern Africa.

CHAPTER 2

THE FUNGUS *METARHIZIUM ANISOPLIAE* VAR. *ACRIDUM*: BIOLOGICAL PROPERTIES, CHARACTERISATION AND MODE OF ACTION

Discovery of an African strain of the fungus, *Metarhizium*, virulent to acridids

An exploratory programme was mounted in various parts of West Africa, Arabia and the Indian subcontinent to find local and effective fungal pathogens against locusts and grasshoppers (Bateman *et al.* 1996). Following this intensive pathogen survey, CABI BIOSCIENCE isolated and identified about 130 disease-causing (locust-killing) fungal isolates from grasshoppers. These were tested in the laboratory for virulence against locusts (Bateman *et al.* 1996). Potential strains adapted to high temperature conditions were discovered in West Africa and tested, using the oil formulation, for their potential as biopesticides.

The specific isolate *Metarhizium anisopliae* var. *acridum* isolate IMI 330189 was collected in 1988 in Niamey, Niger, from the insect *Ornithacris cavroisi* (Finot) (Orthoptera: Acrididae) and was later selected by LUBILOSA as a 'standard isolate' for laboratory and field research, it being an effective locust-killing disease in various species of African locusts and grasshoppers (Bateman 1992; Bateman *et al.* 1996b).

Characteristics of *Metarhizium*

M. anisopliae var. *acridum* isolate IMI 330189 was chosen because it yields well, it is robust and effective, target-specific to only certain grasshopper and locust species, stores well and is formulated to meet the conditions of its use. It therefore performs well and is reliable under the harshest of field conditions (Bateman *et al.* 1994). Characterisation of isolates is achieved by plating out spores on artificial media, examination of sporogenous structures and DNA and isozyme analysis (Bridge *et al.*, 1997).

Metarhizium is a commonly encountered genus (pan tropical distribution) with powdery conidia and lipophilic cell walls (Bateman *et al.* 1995). The lipophilic cell walls make it easier to prepare oil-based formulations than conventional suspensions in water. On insects, conidia form green crust-like velvet, found on both inner and outer walls of the insect cuticle. On agar, a white mycelial margin is initially formed, with older regions becoming coloured as sporodochial groups of conidiophores develop and become confluent (Bateman 1992). The surface is powdery and finally crustose as chains of conidia are released. Colour varies from pale to yellow/green to pale olivaceous colour. *Metarhizium* conidia are clavate to broadly ellipsoid. They are approximately 6µm long with diameters of 2-3 µm. Assuming that their density is similar to water, each spore weighs approximately 20 picogrammes (Bateman 1992).

Taxonomic status of *M. anisopliae* var. *acridum* isolate IMI 330189

The genus *Metarhizium* is placed in the Deuteromycotina: Moniliales and contains two species virulent to Acridoidea (Orthoptera), namely *M.anisopliae* var. *anisopliae* and *M. flavoviride*. This genus has been regarded by some workers as a single species, *M. anisopliae*, containing numerous varieties (var. *album*, var. *flavoviride* etc.) (Prior 1997).

Before 1996, many scientific papers referred to isolate IMI 330189 and similar isolates as *Metarhizium flavoviride*. CABI Bioscience first assessed IMI 330189 as *M. flavoviride* based on spore morphology, the accepted taxonomic criterion for species definition at the time (Bridge *et al.* 1993). As more similar isolates became available it became clear that these isolates formed a distinct and genetically homogenous group with a unique combination of morphological and molecular characteristics which did not conform to the then-accepted *Metarhizium* taxa based on conventional morphology (Bridge *et al.* 1996). Recent taxonomic studies using molecular methods have indicated that they are genetically more similar to *M. anisopliae*. In the latest revision these appear to belong to a genetically homogenous group which is distinct from other isolates of *M. flavoviride* virulent to acridids and

are now tentatively referred to as "var. *acridum*" (Milner *et al.* 1994; Bridge *et al* 1997; Prior 1997). The group virulent to acridids includes IMI 330189 which has been the LUBILOSA "standard isolate" for testing, and is now thus named *M. anisopliae* var. *acridum* isolate IMI 330189.

Mode of action of *Metarhizium*

When a spore (conidium) of *M. anisopliae* var. *acridum* lands on a grasshopper or locust, it attaches to the insect's external cuticle via a germ tube (Bateman 1992). It germinates after 24-48h and then penetrates, using enzymes and mechanical force, through the insect cuticle (which is lipophilic) to the inside of the body cavity. Here it develops and reproduces in the insect, progressively digesting the body cavity. Approximately four to 10 days after infection, the insect dies. Death from this isolate appears to occur without the production of toxins by the fungus. This is an advantage over other mycoinsecticides, since toxins would complicate the introduction of the technique by making the registration requirements more strict (Bateman 1992).

Metarhizium thus acts through direct contact, and field trials with oil-formulated spores have demonstrated effective control of a number of target species under a range of natural field conditions (Scherer *et al.* 1992; Milner *et al.* 1994; Douro-Kpindou *et al.* 1995; Thomas *et al.* 1996; Kooyman *et al.* 1997 and Kooyman & Godonou 1997).

CHAPTER 3

LABORATORY BIOASSAYS WITH AERIAL CONIDIA

Introduction

There are four economically important locust species in southern Africa, namely the brown locust, *Locustana pardalina*, the African migratory locust, *Locust migratoria migratorioides*, the red locust, *Nomadacris septemfasciata* and the southern African desert locust, *Schistocerca gregaria flaviventris*. Since locusts are a serious pest problem in South Africa, which require regular and costly chemical control, there is an urgent need for more target-specific, environmentally benign methods of controlling locusts. In this regard, researchers at the Plant Protection Research Institute of the Agricultural Research Council (ARC - PPRI) explored the potential of controlling locusts with alternative, more environmentally acceptable methods. They collaborated with scientists at CABI Bioscience, U.K., who discovered that various entomopathogenic fungi, especially certain strains of *Metarhizium anisopliae* var. *acridum* (Deuteromycotina: Hyphomycetes), showed great promise as locust killing agents. The formulation of the aerial conidia of these fungi in oil greatly enhanced their infectivity in semi-arid environments and has overcome the reliance of high humidity for the mycoinsecticide to be effective. *M. anisopliae* var. *acridum* conidia were thus imported into South Africa in 1993 (import permit no. 14/2/2/1(9/21/172) and initially kept under strict quarantine conditions prescribed for the basic containment level 1 (UK Advisory committee on dangerous pathogens). Here the results of preliminary laboratory bioassay studies, that were conducted to investigate the efficacy of *M. anisopliae* var. *acridum* conidia against three local locust species, are presented.

Materials and methods

Spore suspension

Growing spores in the laboratory

Initially, two *M. anisopliae* var. *acridum* isolates, IMI 330189 and IMI 324673, were imported in a ready formulated oil mixture. Thereafter test samples of the standard isolate, IMI 330189, obtained from the production plant at IITA, Benin, West Africa, were sent to PPRI in a dry spore formulation. These spores were mixed in an aqueous formulation and plated out on potato dextrose agar and Sabauraud dextrose agar by streaking evenly and flaming the needle between microbial transfers. Cultures were incubated at 22-24°C in 12 hours artificial light and 12 hours darkness until ready for harvesting, following CABI protocol.

Harvesting

Conidia were harvested between 10 and 30 days after inoculation. Carriers used were good quality oils (5-10ml of pure groundnut oil or a 50:50 Ondina oil/kerosene mixture) for the conidial stage. The conidia were brushed into the oil with a small paintbrush, or gently detached from the substrate with a needle. The suspension was sieved to remove hyphal fragments. After sieving, the suspension was agitated in a shaker for 3-5 minutes, using glass beads, and the formulation used within one day (Bateman *et al.* 1996a).

Determining spore concentration

Conidial concentration was obtained using a haemacytometer (giving concentration per ml) and the formulation adjusted by dilution with oil to give approximately 10^7 conidia/ml (Bateman *et al.* 1996a). When topically applied at a rate of 1µl per insect, this gave a dose rate of 10^4 conidia per locust.

Spore viability

To check spore viability, a small quantity (±0.1µl) of the oil formulation was spread as thinly as possible onto water agar. After 24h incubation, between 100 and 300 spores were examined to determine percentage germination.

Inoculation

Laboratory reared locusts

The three locust species, the progeny of field collected parents, were reared and kept in the laboratory in standard wooden or metal cages at a constant temperature of 30°C. Locusts were fed daily on a diet of either fresh green wheat, maize or kikuyu grass (*Pennisetum clandestinum*) and dry bran. Dry grass and sticks or mesh provided perching sites in the cages. Locusts were collected by hand and untreated (control) samples were set up separately to avoid contamination before applying the fungus. The number of locusts used per bioassay depended on the availability of laboratory reared insects. Prior *et al.* 1995 confirmed that sex of the locust did not significantly influence the pathogen-induced mortality, therefore no effort was made to divide the groups up according to sex.

Topical application

The first laboratory test samples of the standard isolate, IMI 330189 was applied to the hopper and adult stages of both brown locust and African migratory locust species, while the isolate IMI 324673 was only applied to brown locust adults (Price & Müller 1993a). Inoculation took place by applying the spore suspension topically with either a Hamilton micro-syringe, by means of an electrically driven micro-applicator, or with a hand-held micropipette. Individual 1µl droplets, containing a 10^4 conidial suspension, were placed behind the dorsal pronotal shield, on the neck membrane of individual locust hoppers (Fig. 3.1). Batches of locusts were also inoculated with a higher dose rate of 6 X 10^4 spores. Two replicates of each treatment and controls were carried out (n = 16-32). Mortality in all the treatments was compared with control hoppers and adults treated with a similar dose of pure peanut oil, although initial experiments by Prior *et al.* (1995) showed that

inoculation with the blank oil had no ill effect on the insects, nor did the oil affect conidial viability.

For red locust treatments, dry conidia (harvested from agar plates) of the standard isolate IMI 330189 was mixed in an oil formulation (Ondina oil + kerosene) and the spore concentration measured. In one experiment, droplets containing doses of 2 X 10^4, and 7 X 10^5 conidia/individual insect were topically applied under the pronotum of second instar red locust hoppers. A sample of similarly aged, but untreated nymphs served as a control. In another experiment, doses of 2 X 10^5, 2 X 10^6, 3.74 X 10^6 were applied to third instar hoppers. Since the red locust is regarded as an insect that is difficult to kill with insecticides (R.Price pers.comm.), a high dose rate of 3.75 X 10^7 was also tested against sixth instar red locust hoppers. Controls were untreated third and sixth instar hoppers (n = 18-56 hoppers per dose for both experiments).

Maintaining locusts

After inoculation, brown and African migratory locusts were kept in groups of 10-15 in wire-gauze cages, measuring 40 X 25 X 25 cm and maintained in a temperature and humidity controlled quarantine glasshouse. Treated and control locusts were fed fresh green wheat seedlings and dry wheat bran. No attempt was made to increase the relative humidity artificially because high humidity is detrimental to brown and African migratory locusts, causing stress and secondary infection of bacterial diseases.

Following inoculation, red locust hoppers were placed in clean, autoclaved glass jars with mesh screw-top lids and maintained at 30°C (±2°C) and 85% rh (±5%) in the laboratory and were fed on freshly cut green maize leaves. In contrast to the brown locust experiment, here relative humidity was increased by spraying insects regularly with water to create a high humidity (>80%) similar to that occurring in the natural habitat of red locust hoppers.

Daily cumulative percentage mortality of treated hoppers was compared with that of untreated hoppers.

Fig. 3.1 Application of a 1µl droplet of *Metarhizium anisopliae* var. *acridum* mycoinsecticide behind the pronotal shield of the brown locust, *Locustana pardalina*.

Mortality assessment

Symptoms of fungal infection, daily activity and behaviour (such as moulting) and mortality of locusts were recorded daily for 21 days after treatment. Dead insects were removed from their cages and were incubated in containers inducing high humidity e.g. inside plastic tubes, plastic sandwich bags, or an air tight glass container, to encourage external growth or sporulation of the fungus.

Statistical analysis

Survival analysis was used to compare the different isolates and application rates following the LIFETEST procedure (SAS Institute Inc. 1986). The Log-Rank test was used to test for significant differences at a 5% level. Combined figures of replicates were used and comparisons between treated and control groups within

species were made. No attempt was made here to compare dose responses between species as the main aim was to check for efficacy of the isolate to the individual locust species.

Results

General fungal infection symptoms

The first deaths of infected locusts occurred 3-4 days following treatment, where after mortality rates increased dramatically. Apart from loss of appetite and somewhat sluggish behaviour if compared with controls, hoppers and adults of all three species that were treated with the mycoinsecticide showed few outward signs of infection, making early diagnosis of *M. anisopliae* var. *acridum* infection difficult. However, shortly before death they all assumed the conspicuous red coloration (mycosis) characteristic and symptomatic of *Metarhizium* infection (Fig. 3.2). In the majority of cases, the whole body turned pink to bright red, while in other insects the red coloration was restricted to particular parts of the body such as around the mouth parts or on the legs. There were also signs of late mycosis (a normal coloured cadaver turning red sometimes only hours after death), and cadavers were therefore subsequently checked until two days after death for the red coloration and/or sporulation to develop. The position of the locust at death was also characteristic. Death was sudden, with individuals dying *in situ*, still clinging to the sides of the cage or to the vegetation. Mycosed locusts were rigid compared to bacterial infection (e.g. *Serratia* spp.), where insects appear limp and bodies are soft with a black coloration under the thorax or abdomen.

At high humidity, external fungal sporulation was observed as early as 24 or 48h after death. There was sometimes rapid growth of contaminating non-locust killing fungi such as *Aspergillus* or *Mucor* spp. (Prinsloo 1960; Prinsloo 1962), but the green *Metarhizium* fungus could usually clearly be seen on the intersegmental parts of the locusts, the antennae or on the tarsi, spreading rapidly to the rest of the cadaver over the following few hours (Fig.3.3). In cases where the pathogen did not

develop upon incubation, *Metarhizium* may still have been the cause of death, as not all infected individuals go on to show full mycosis (Thomas pers.comm). In such a case, comparison with a healthy control batch showed whether the pathogen was the cause of mortality. If the controls were healthy then all mortality was assumed to be a result of infection.

Another symptom of fungal infection observed was that many fifth instar brown locust hoppers died during the final moult. In contrast, in the red locust, normal moulting occurred after treatment. Skins (exuvae) were shed in the normal way during the moult. Although most behavioural activities appeared to be normal, feeding appeared to decrease in all the treatments and also ceased at the time of moult in both treatments and control for all locust species.

Fig. 3.2 A brown locust adult, *Locustana pardalina*, treated with *Metarhizium anisopliae* var. *acridum*. The locust died *in situ*, still clinging to the grass stalk, while the typical red coloration further provides a clear symptom of mycosis.

Fig. 3.3. External fungal sporulation on brown locust hoppers, *Locustana pardalina*, treated with *Metarhizium anisopliae* var. *acridum*.

Mortality of brown locusts and African migratory locusts treated with the IMI 330189 isolate

Hoppers

Doses of 3 X 10^4 and 6 X 10^4 spores produced mortality of 90% and 93% in fifth instar brown locusts hoppers after 21d with median lethal times (LT_{50}) of 9.8d and 7.5d respectively (Fig. 3. 4). Control mortality was 7%. Mean air temperature during this time was 27.2°C (range 24-34°C) and rh 19% (range 13-23%). Against the African migratory locust hoppers, the same dose rates produced mortalities of 97 and 100% with LT_{50} periods of 10.7 and 10.3d respectively. Control mortality was 13%. Air temperature here averaged 20°C (range 17-23°C) with 40% rh (35-55%).

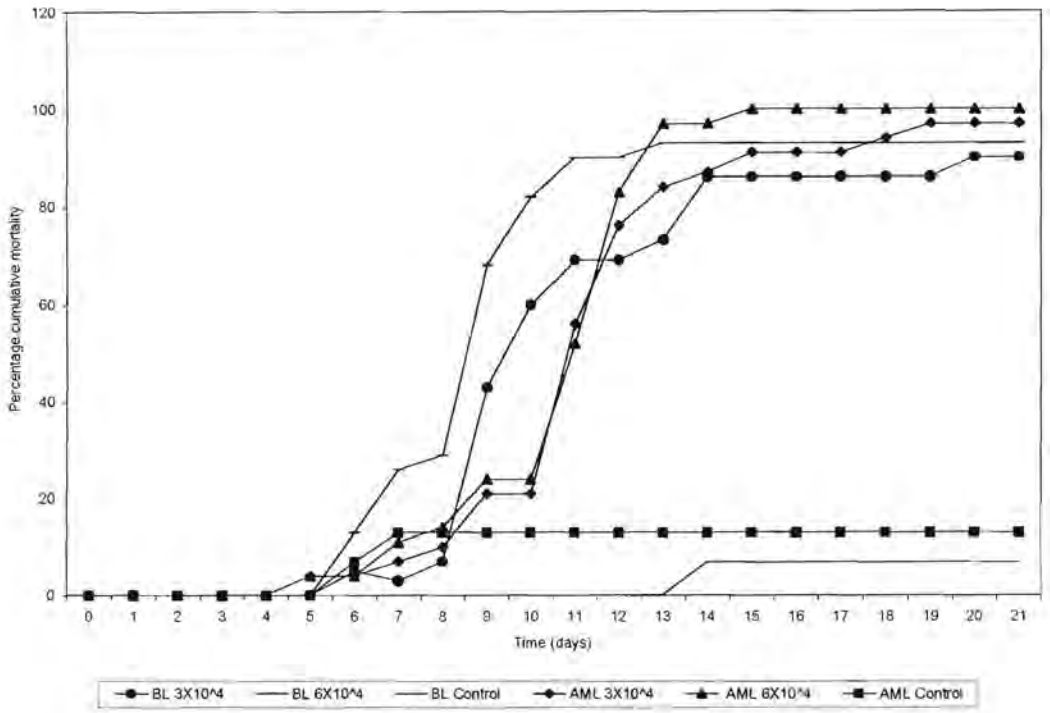

Fig. 3.4 Percentage cumulative mortality of brown locust and African migratory locust hoppers treated topically with *Metarhizium anisopliae* var. *acridum* mycoinsecticide.

Adults

Both the dose rates above gave 100% control of brown locust adults after 12d (LT_{50} = 10.1d) and 9d (LT_{50} = 4.4d) respectively (Fig. 3.5). Control mortalities were 8 and 14%. Mean air temperature was 28°C (range 24-34°C) and rh 40% (range 28-52%). Against African migratory adults, kept caged under similar conditions, 100% mortality was achieved after 18d (LT_{50} = 10.8d) and 15d (LT_{50} = 8.5d) respectively.

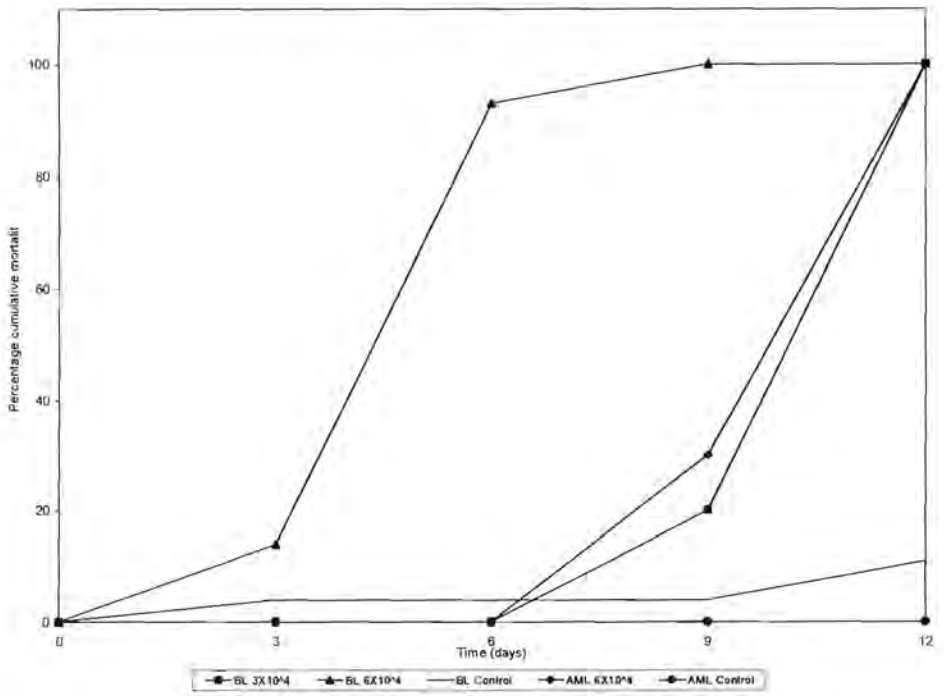

Fig. 3.5. Percentage cumulative mortality of brown locust and African migratory locust adults treated topically with *Metarhizium anisopliae* var. *aridum* mycoinsecticide.

Both hopper and adult stages of the brown locust and the African migratory locust gave final mortalities of >90%. All treated locusts showed significant reductions in survival times compared with their respective controls. There was a significant difference between the speed of kill achieved with the standard dose (1µl) compared to a double dose (2 µl) i.e., the higher the dose, the quicker the kill. A statistical analysis of comparisons is provided in Table 3.1. The best results with the mycoinsecticide were obtained with the double dose treatment against brown locust adults (Table 3.2).

Table 3.1 Survival analysis, using the Log-Rank test, of brown locust and African migratory locust hoppers and adults treated topically with the standard isolate IMI 330189 of the mycoinsecticide, *Metarhizium anisopliae* var. *acridum*.

Test of equality over time			
Comparison	Chi-square	d.f	p<
Hoppers			
BL 1 µl vs. Control	28.4	1	0.001
BL 2 µl vs. Control	31.24	1	0.001
AML 1 µl vs. Control	24.6	1	0.001
AML 2 µl vs. Control	25.9	1	0.001
Adults			
BL 1 µl vs. Control	19.8	1	0.001
BL 2 µl vs. Control	25.7	1	0.001
AML 2 µl vs. Control	22.72	1	0.001

BL = brown locust, AML = African migratory locust, d.f = degrees of freedom

Table 3.2. Statistical comparison between topical application of 1 µl vs. 2 µl of the standard isolate IMI 330189 on all brown locusts, *Locustana pardalina*.

Test of equality over time			
Comparison	Chi-square	d.f	p<
BL 1 µl vs. 2 µl	28.15	1	0.001

BL = brown locust, d.f = degrees of freedom

The Log-Rank test indicated that all comparisons between treated insects and controls made in Tables 3.1 and 3.2 were highly significant ($p < 0.001$).

Mortality with the IMI 324673 isolate

This isolate achieved 100% morality within 12d with LT_{50} values of 10.25 and 4.5d with the 3 X 10^7 and 6 X 10^7 dose rates respectively. Control mortalities measureed 17% and 14%. Results of the survival analysis test carried out on the two isolates of *M. anisopliae* var. *acridum* against the brown locust and the African migratory locust are given in Table 3.3.

During the course of the laboratory bioassays, CABI Bioscience decided to use isolate IMI 330189 as their standard isolate and isolate IMI 324673 was discontinued in future laboratory bioassays in South Africa. Preliminary laboratory results against the brown locust, however, showed that there was no significant difference between the two isolates. It was found though, although not quantified, that isolate IMI 324673 did not grow and yield as well as the standard isolate.

Table 3.3. A statistical comparison between topical application of isolate IMI 330189 and isolate IMI 324673 against the brown locust, *Locustana pardalina*.

Test of equality over time			
Comparison	Chi-square	d.f	p<
IMI 330189 1 µl vs. IMI 324673 1 µl	0.4	1	0.5
IMI 330189 2 µl vs. IMI 324673 2 µl	3.1	1	0.1

d.f = degrees of freedom

Mortality of the red locust treated with the standard IMI 330189 isolate

In the first treatment with the mycoinsecticide, applied at dosage rates of 2 X 10^4 and 7 X 10^5 to second instar red locust hoppers, final mortalities of 89% and 92% were obtained, with mortality of 18% in the control (Fig.3.6).

In the second treatment, dosage rates of 2 X 10^5 and 2 X 10^6 again gave mortalities of 89% and 92% after 6 –9 days in second instar red locust hoppers. A dosage rate of 3.7 X 10^6 was applied to third instars and gave 96% mortality after 9 days and 100% mortality after 7 days. Again, there was 18% mortality in the controls (second and third instar). The sixth instar, which is the final red locust instar, is a large, 'tough' insect to kill, and although speed of kill was slower, a 100% mortality was obtained after two weeks (11d) with a high dosage rate of 3 X 10^7 conidia/insect. No control mortality occurred. (Fig. 3.7).

The results of a survival analysis, comparing the dosage rates 10^5, 10^6 and 10^7 to second, third and sixth instar red locust hoppers respectively, are shown in Table 3.4. These revealed a significant reduction in survival time due to infection in all treatments relative to controls.

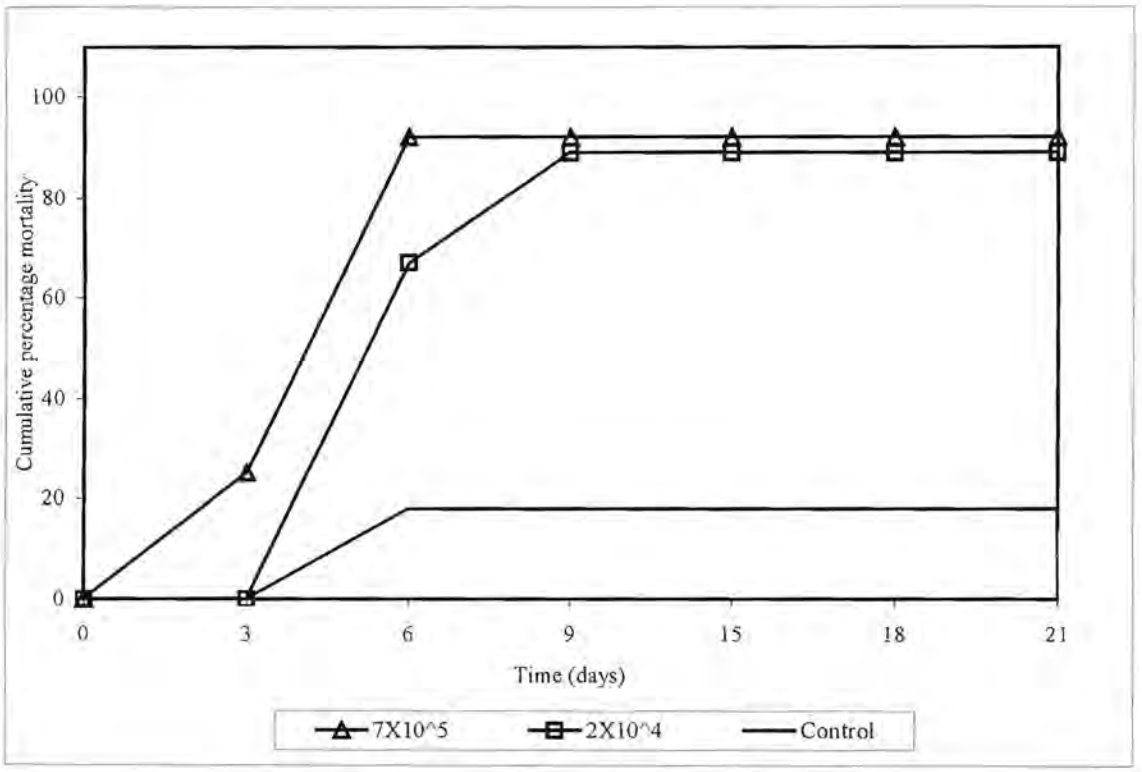

Fig. 3.6. Mean cumulative percentage mortality of second instar red locust hoppers treated with dosages of 2 X 10^4 and 7 X 10^5 conidia of the isolate IMI 330189.

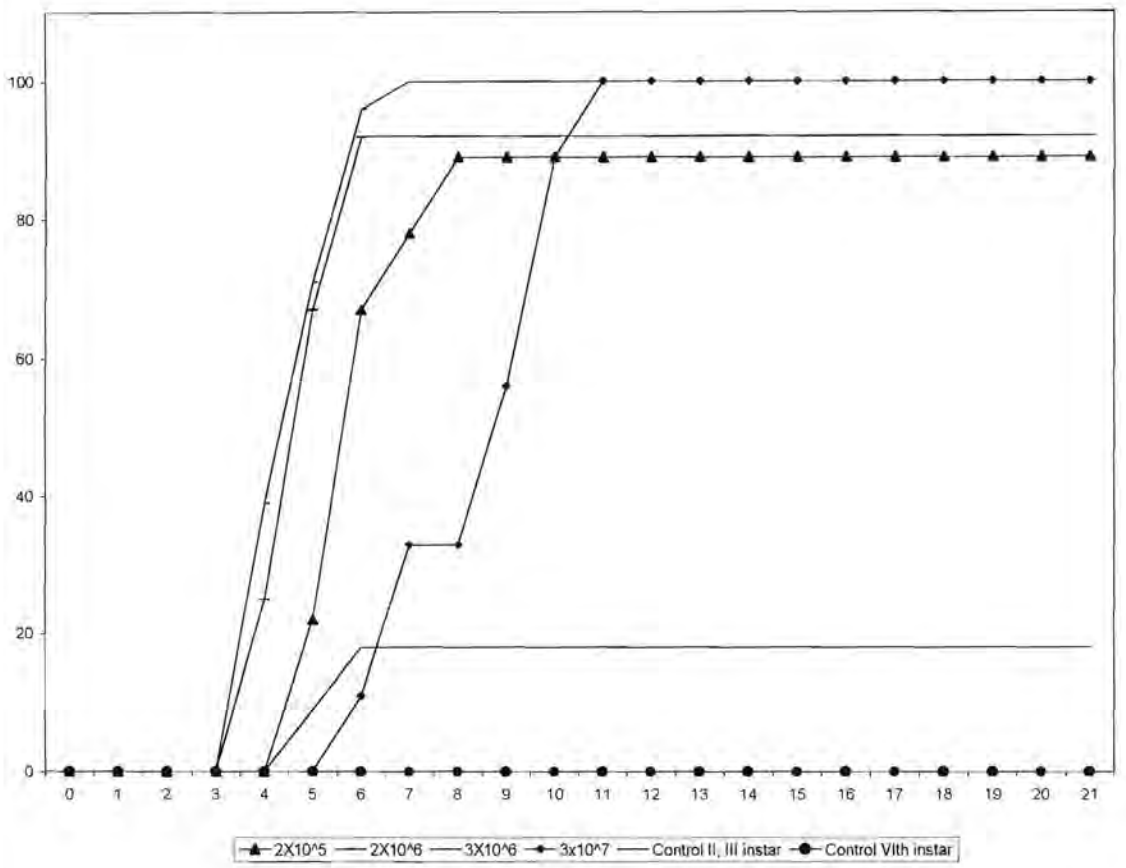

Fig. 3.7 Mean cumulative percentage mortality of second (2 X 10^5 and 2 X 10^6), third (3.7 X 10^6) and sixth (3.7 X 10^7) instar red locust hoppers treated with conidia of the isolate IMI 330189.

From the graphs it can be seen that the control shows the lowest mortality and Table 3.4 indicates that all differences from the control over time is significant at $p<0.001$.

Table 3.4 Survival analysis, using the Log-Rank test, of three red locust hopper instars, *Nomadacris septemfasciata*, treated topically with three dosage rates of the standard isolate IMI 330189.

Test of equality over time			
Comparison	Chi-square	d.f	p<
Second instar 10^5 vs. control	27.3	1	0.001
Third instar 10^6 vs. control	69.73	1	0.001
Sixth instar 10^7 vs. control	41.47	1	0.001

d.f = degrees of freedom

Discussion

The above results showed that hoppers and adults of the brown locust, the African migratory locust and hoppers of the red locust were highly susceptible to topical application of an oil-based mycoinsecticide formulation containing *M. anisopliae* var. *acridum* conidia.

With the brown locust and African migratory locust hoppers, both dosage rates applied produced excellent final mortality levels, but there was a significant difference in speed of kill between the standard dose (1µl) and a double dose (2 µl). The double dose produced a more rapid rate of mortality in both species. Therefore, the higher the dose, the quicker the kill. Also, adults of both locust species proved more susceptible than hopper stages to isolate IMI 330189 when applied at both dose rates.

Previous laboratory bioassays with chemical insecticides against the red locust have shown that this species is more resistant to a range of insecticides, requiring very high doses to obtain successful control. In contrast, excellent mortality of red locust with the mycoinsecticide was achieved, with none to very low mortality in the controls.

These preliminary laboratory bioassays showed that the mycoinsecticide was effective against three southern African locust species. The promising results provided motivations for undertaking field trials against gregarious hopper bands in the field.

CHAPTER 4

LABORATORY BIOASSAYS WITH BLASTOSPORES

Introduction

The mycoinsecticide, *Metarhizium anisopliae* var. *acridum* isolate IMI 330189 has shown promise for use as an alternative, target-specific and benign locust control method in southern Africa. Aerial conidia of this Deuteromycete are easy to produce, show good storage characteristics, and can be produced in large quantities on solid culture. In submerged culture, most fungal strains produce mycelium, mycelial pellets and hydrophilic blastospores and are often produced in liquid cultures (Stephan *et al.* 1995). Although blastospores have a shorter shelf-life than conidia and conidia are often thus most likely to be employed for the reason of viability, blastospores are at least as pathogenic as conidia of the same strain (Burges 1970-1980). Veen (1968) reported that blastospores are able to cause high mortalities in the desert locust, *Schistocerca gregaria*, while Kleespies (1993) has proved its effectiveness against the African migratory locust, *Locusta migratoria migratorioides*.

Metarhizium conidia have lipophilic cell walls and can be suspended in an oil formulation to enhance their efficacy under dry environmental conditions. Blastospores on the other hand, are used with water-based formulations because they have hydrophilic cell walls and generally can not be suspended in oil. Since oil-based formulations and the ULV spray equipment can be expensive, the use of water as a carrier for the control of certain acridids in the humid tropics and cooler climatic zones may be viable. Recent research has demonstrated the feasibility of preparing blastospores and submerged hydrophilic conidia of *Beauveria* and *Metarhizium* spp. and aqueous suspensions of both types of spores have shown to be infective (Jenkins & Goettel 1995; Jenkins *et al.*, 1998).

Within the framework of the GTZ project, investigations by the Institute of Biological Control (BBA), Darmstadt, Germany found a suitable ULV formulation for blastospores and successfully carried out semi-field trials by using spray-dried

Metarhizium blastospores against the desert locust (Stephan *et al.* 1995). It was then decided to undertake initial laboratory bioassays of the same Mfl5 isolate against the brown locust and red locust.

Materials and methods

Brown locust

A bioassay with a sample of the *Metarhizium* isolate Mfl5 blastospores was undertaken using laboratory reared fifth instar brown locust, *Locustana pardalina*, hoppers. This fungal isolate 5 has its origin in Madagascar, and its host insect was *Locusta migratoria* (Stephan *et al.* 1995). Blastospores were suspended in an aqueous formulation (sterile, distilled water) and the concentration measured with a haemacytometer. Before application, viability of these blastospores was determined by streaking out a small quantity of the formulation on Potato Dextrose Agar (PDA) plates and counting the number of germinating spores after 24h.

Two doses (n = 20±5 locusts per dose) were applied. Droplets containing doses of 6.8×10^4 and 6.8×10^5 blastospores per insect were topically applied behind the dorsal neck membrane of the hoppers using an electrically driven micro-applicator. A sample of untreated, but similarly aged, insects served as a control (20±5 individuals).

Viscosity of the Mfl5 aqueous suspension was low and this facilitated droplet extrusion from the Hamilton syringe, which made application easier than with the conidial/oil mixture (isolate IMI 330189).

After treatment, each batch of hoppers was placed separately in the wire-gauze cages and maintained at room temperature (25°C (±2°C) and 70% RH (±5%) in the laboratory. The days were cool and hoppers were taken out each morning to bask in direct sunlight for ±4h. Hoppers were fed green kikuyu grass and dry bran daily and mortality was assessed over the following 21 days. Behaviour, such as feeding and moulting, was observed and compared with the controls. Cumulative percentage

mortality was compared with the untreated controls. The cause of locust death was, as before, verified by the presence of mycosis and incubating all cadavers under high humidity conditions and checking for sporulation of *Metarhizium*.

A survival analysis was undertaken between the control and the two doses, using the Log-Rank test (SAS Institute Inc. 1986).

Red locust

Blastospores were again suspended with an aqueous formulation (sterile, distilled water) and the concentration and viability measured.

Droplets containing doses of 7×10^3, 7×10^4, and 3×10^4 blastospores/individual insect were topically applied to the dorsal neck membrane of laboratory reared second instar red locust nymphs, *Nomadacris septemfasciata* (n = 21-27 individuals/treatment). A dose of 3.4×10^4 and 2×10^5 blastospores/insect was assayed against third instar insects (n =16-17 individuals/treatment). Mortality and speed of kill were compared with the untreated control (n = 20±5 individuals).

After treatment, each batch of nymphs was placed in a glass jar with a mesh screw-top lid and maintained under controlled conditions of 30°C (±2°C) and 85% RH (±5%) in the laboratory. Hoppers were sprayed with water to simulate the humidity of >80% commonly found in their grassland outbreak regions in central Africa. Hoppers were fed green maize leaves daily and mortality was assessed over the following 21 days. Moulting behaviour was especially monitored, because of earlier results obtained with brown locust nymphs, suggesting high mortality during their moulting period. Cumulative percentage mortality was compared with similarly aged, but untreated, red locust nymphs and a survival analysis conducted using the Log-Rank test (SAS Institute Inc. 1986).

Results

Brown locust

Blastospore viability measured before treatment was excellent, with 95-98% germination recorded after 24h. The 6.8 X 10^4 dose gave 80% control after 21 days, while the 6.8 X 10^5 concentration gave 100% control after 21 days (LT_{50} = 12.5 & 10.3d respectively) (Fig. 4.1). Control of fifth instar brown locust nymphs was found to be dose dependent. Mortality of 10% was recorded in the control batch after 21days. Although some individuals moulted into adults, most of the nymphs died in the final moult, a similar observation to that recorded with the conidial isolate IMI 330189 treatment against the brown locust (Chapter 3). A cessation in feeding was also observed in batches of treated locusts, though it was not quantified. Basking behaviour appeared to be normal. Immediately before death, all infected nymphs developed a characteristic red coloration which provided clear evidence of mycosis. When such cadavers were incubated under high humidities, external *Metarhizium* sporulation always occurred after 24-48h. The statistical analysis showed that there were highly significant differences between the two blastopore dose rates versus their respective controls ($p<0.001$) (Table 4.1).

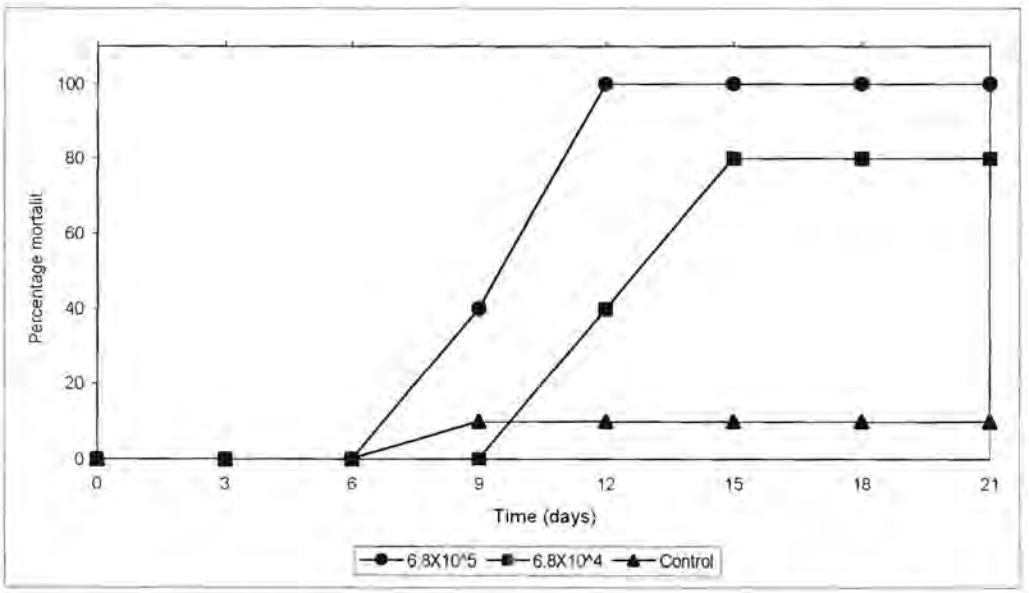

Fig.4.1 Mean cumulative percentage mortality of brown locust hoppers, *Locustana pardalina*, treated with *M. anisopliae* var. *acridum* blastospores, isolate Mfl5.

Table 4.1 Statistical comparison between the topical application of two blastospore dose rates of *Metarhizium anisopliae* var. *acridum*, isolate Mfl5, and a control treatment against fifth instar brown locust hoppers, *Locustana pardalina*.

Test of equality over time			
Comparison	Chi-square	d.f	p<
6.8×10^4 vs. control	11.0	1	0.001
6.8×10^5 vs. control	22.23	1	0.001

d.f = degrees of freedom

Red locust

Measured blastospore viability before treatment was similar to that recorded during the brown locust bioassay, between 95 - 98%. Here, interestingly enough, normal moulting occurred, regardless of the dose rate administered and no insects died during the moulting process. Both treated and control second instar hopper batches moulted into third instar from days 7 to 11 post application (mean = 9d for all treatments and controls), and then subsequently moulted into fourth instar hoppers, 14 to 15 days after exposure. Third instar hopper batches moulted into fourth instars from days 5 to 8 post application (mean = 6.5 days) and into fifths from day 14. Infected locusts all died after completion of the moult and skins were shed in the normal way. The intermoult period did not vary between treated insects and untreated controls. See Figure 4.2. Surviving locusts completed their final moult (sixth instar), contra to the brown locust and eventually became adults.

Feeding appeared to be normal initially, then moderately low to zero after a few days and ceased for all insects during the moulting period. Other behavioural

activities (e.g. jumping, grooming) appeared to be normal and were similar in comparison with the controls. No abnormalities were observed. Just before death, all infected nymphs developed the red coloration characteristic of *M. anisopliae* var. *acridum* infection. This provided clear evidence of mycosis and when such insects were incubated under high humidities, they developed external fungal growth on their bodies after 24-48h.

The results of doses 3.4 X 10^4, 7 X 10^4, 2 X 10^5 were significantly different to control batches ($p = 0.01$), while doses of 7 X 10^3, 3.2 X 10^4 were apparently too low and showed no significant difference compared to the control (Table 4.2).

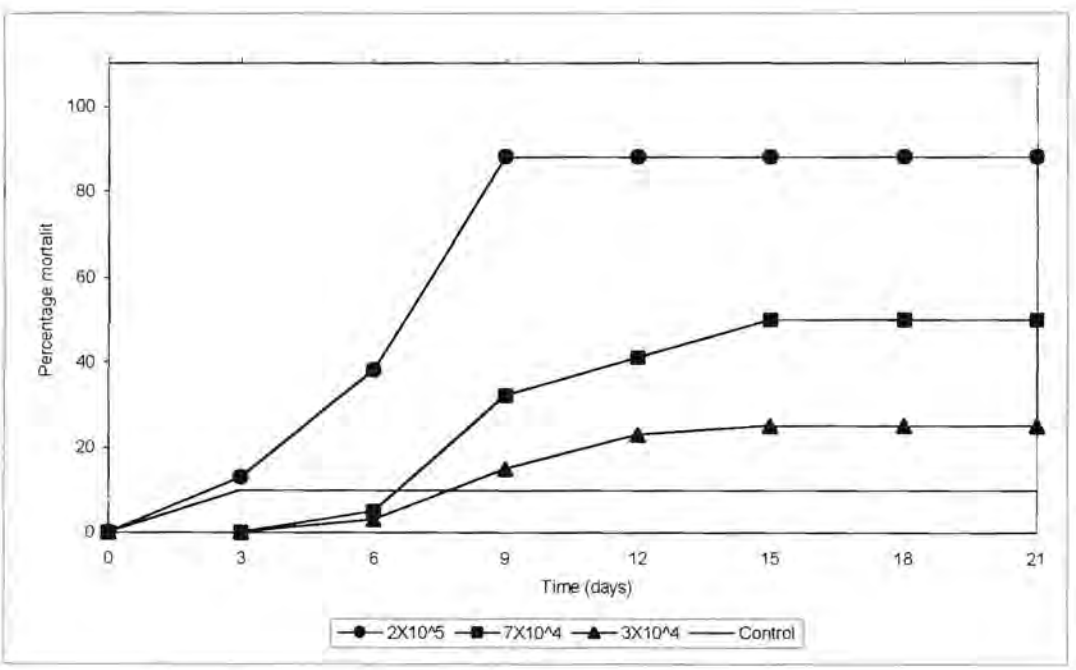

Fig. 4.2. Mean cumulative percentage mortality of red locust hoppers, *Nomadacris septemfasciata*, treated with *M. anisopliae* var. *acridum* blastospores, isolate Mfl5.

Table 4.2 Statistical comparison between the topical application of different dose rates of blastospores of *Metarhizium anisopliae* var. *acridum* (isolate Mfl5) and controls against the red locust, *N. septemfasciata*.

Test of equality over time			
Comparison	Chi-square	d.f	p<
7 X 10^3 vs. control	0.17	1	0.7
7 X 10^4 vs. control	7.21	1	0.01
3.2 X 10^4 vs. control	0.22	1	0.64
3.4 X 10^4 vs. control	6.0	1	0.02
2 X 10^5 vs. control	22.27	1	0.001

d.f = degrees of freedom

Discussion

The results found with blastospores compare with the 93% mortality of brown locust hoppers (Lt_{50} = 7.5d) achieved with a $6X10^4$ oil-based *M. anisopliae* var. *acridum* conidia suspension at 27°C (Chapter 3). Good control of red locust hoppers was achieved with five different dose rates of Mfl5 blastospores against second and third instars, although the standard dose of 10^4 conidia/insect (10^7/ml) did not produce good results. The red locust is generally regarded as a 'tougher' species to kill and when a higher dose was administered, good mortality was achieved. This data opened yet another door for mycoinsecticide application.

Although not quantified, the cessation in feeding in both locust species was apparent. Interestingly, while some of the treated brown locust hoppers died in the

final moult, red locust hoppers all succeeded in completing their moulting stages and survivors all reached the adult stage.

Topical application of an aqueous blastospore mixture thus gave excellent control of brown locust and red locust hoppers and highlighted the need for field experimentation with this formulation, although field experiments did not not seem feasible at the time.

CHAPTER 5

ADDRESSING THE PROBLEM OF SPEED OF KILL: DOSE-RATE MORTALITY AND REDUCTION IN FOOD CONSUMPTION

Introduction

An oil-based formulation of the entomopathogenic fungus, *Metarhizium anisopliae* var. *acridum* (Deuteromycotina: Hyphomycetes), LUBILOSA isolate IMI 330189, has been developed and successfully tested for the control of various locusts and grasshopper species.

One of the perceived disadvantages of the operational implementation of the mycoinsecticide, however, is the length of time it takes for the target insect to die after application (Bateman 1996; 1997). This disadvantage may have important ramifications, especially in the Karoo in South Africa, where locust control officers and farmers are used to fast-acting insecticides (Bateman *et al.* 1994).

The mycoinsecticide acts through direct contact and although *M. anisopliae* var. *acridum* germinates and invades the hemocoel within 24h (Gunnersson 1988) following application of conidia to the locust cuticle, the speed of kill achieved against brown locust hoppers is slow, with most insects taking 4-21 days to die (Bateman 1994).

The speed of kill can be related to the numbers of conidia received by each insect, which is theoretically determined by the number and size of droplets deposited on the locust, the conidial concentration and germination success or viability. Locust targets are sprayed in the field at ultra low volume (ULV) rates of 1 or 2 litres per ha at a standard dose rate of 100g dry conidia/ha, which is about equivalent to a dose rate of 5×10^{12} conidia/hectare (Bateman 1992).

Examining higher dose rates thus appeared to be the first and most obvious potential solution to the problem of increasing the speed of kill.

While the relatively long duration between mycoinsecticide application and death of the locusts is undesirable, successful control may not only be determined by direct mortality alone. Infection may also be considered to bring about a level of control by modification of the treated insect's behaviour, such as by reducing food consumption (Thomas *et al.* 1997; Moore *et al.* 1992). These authors argue that if the impact of a locust that has ceased to feed due to infection is reduced, then this could also constitute effective control. Seymoun *et al.* (1994) also showed reduced flying capability in desert locust adults after mycoinsecticide infection, which would also affect the pest status of the locust.

Reduction in feeding following treatment with pathogens has been shown with many grasshopper and locust species (Johnson & Pavlikova 1986; Moore *et al.* 1992; Thomas *et al.* 1997). Food consumption of brown locusts infected with *Metarhizium* was therefore investigated to determine if this species showed a similar reduction in food consumption.

Materials and methods

Six dose rates of *M. anisopliae* var. *acridum* isolate IMI 330189 conidia (10^3-10^8 conidia/insect) were assayed against laboratory reared fifth instar brown locust hoppers, the progeny of field collected parents from the Karoo. No attempt was made to divide hoppers into equal numbers of each sex, because previous experiments have shown that both sexes are equally vulnerable to *Metarhizium* infection (Moore *et al.* 1992; Prior *et al.* 1995).

M. anisopliae var. *acridum* conidia were formulated in groundnut oil and placed in a shaker to break up the conidial chains. Conidial counts were made with a haemacytometer. Spore concentrations in the formulations were adjusted by serial dilution in oil to give dose rates of 3.52×10^3 - 3.52×10^8 per insect.

Each spore dose rate, suspended in 1 µl droplets of oil, was topically applied behind the dorsal pronotal shield on the neck membrane of individual insects (n=35 per batch for each dose). Control locusts (n=25) were untreated because previous studies by proved that the blank oil formulation had a negligible effect on locust mortality in the laboratory and field (Chapter 3; Bateman et al. 1994, Shah 1994). Two replicated assays were undertaken with a total of 70 locusts per treatment.

Batches of hoppers were placed in metal observation cages (measuring 40 X 40 X 25cm) at a constant temperature of 30°C, 24h prior treatment, to allow acclimatization. They were also provided with perching sites. Green kikuyu grass, *Pennisetum clandestinum* (n = 10 samples) was oven dried for 24h at 90°C to determine the average percentage dry weight of the grass so that the weight of food supplied could be calculated (Moore *et al.* 1992).

Feeding was assessed by calculating the amount of food eaten daily. Each day a known/weighed amount of freshly cut kikuyu grass was given to batches of hoppers and after 24hrs all the uneaten grass and faeces in cages was removed, oven dried at 90°C for 24hrs and weighed.

The total dry mass of grass consumed was divided by the number of individuals still alive in the batch to give daily food consumption per individual as dry mass/insect. This figure was then used to calculate the quantity of fresh food consumed per individual locust per day.

Mortality was recorded daily and all dead locusts were removed from cages and placed in a humid environment to encourage *Metarhizium* sporulation. The results of the two experiments were combined and presented as a mean.

For dose-rate mortality, a survival analysis was used to test for significant differences between different doses and controls. The LIFETEST procedure in the SAS system and the Log-Rank test were used to test for differences at a 1% level of significance (SAS Institute Inc. 1986).

With food consumption, a one-way analysis of variance was used to compare the controls and different dose levels. Tests for normality and homogeneity of variances were undertaken and data were transformed using a square root. Dunnett's t-test comparisons were made between the different dose levels and control.

Results

The average percentage dry weight for replicated batches of kikuyu grass was 18.6% of the fresh green mass.

Dose-rate mortality

The locusts treated with the highest dosage rates began to die as early as 1d after treatment, with full symptoms of mycosis following from day three. Doses of 10^5-10^8 spores/insect produced 100% final mortality with the rate of mortality directly related to spore dose applied. With the highest dose (10^8), all insects were dead on days 3-4, whereas with the 10^7 and 10^6 dosage rates, complete kill was achieved after 5 and 6 days respectively.

The 10^5 dose took 6-10d to achieve 100% mortality while a dose of 10^4 spores took 12-14d to achieve mortality of 75-87%. The lowest dose rate (10^3) gave no significant mortality until 9-10d after inoculation, with final mortality at 21 days in both replicates reaching only 60%. Average control mortality was 32% after 21 days with no symptoms of mycosis recorded.

The mean cumulative percentage mortality of brown locust hoppers in the two treatments is shown in Fig. 1.1. There is a clear dose response with the mean dose-rate mortality (in days after treatment) decreasing as the dose rate increases. Mortality achieved by the 10^3 dose was not significantly different from the control, whereas mortality achieved from doses 10^4 and higher were highly significant ($p = 0.001$) (Tables 1.1 & 1.2).

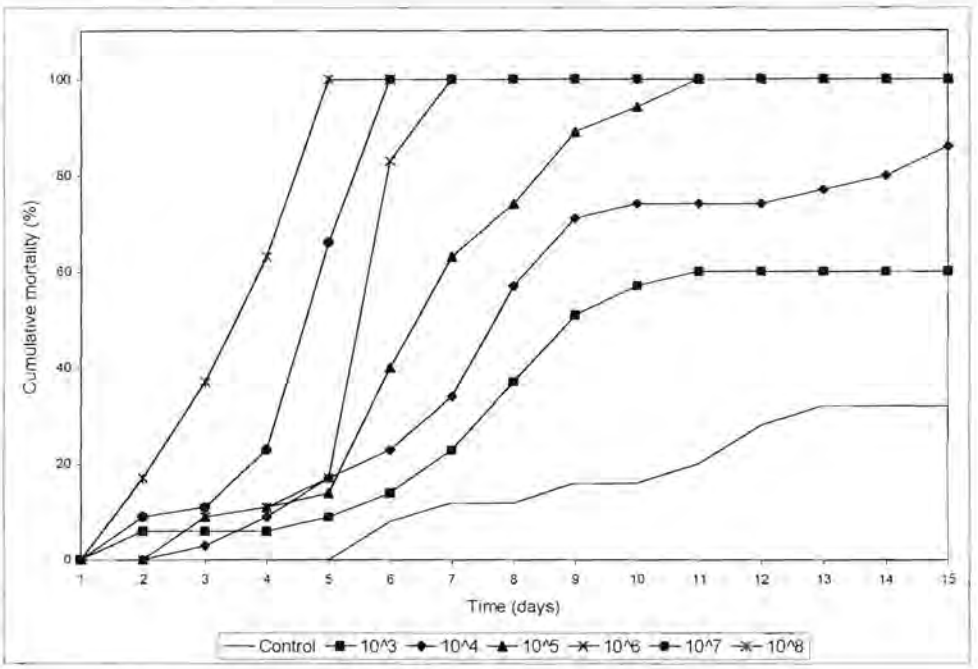

Fig. 5.1 The mean cumulative percentage dose-rate mortality of fifth instar brown locust hoppers treated with different dose rates of *M. anisopliae* var. *acridum*.

Table 5.1 Summary statistics using the Log-Rank test at a significance at 1% of dose-rate mortality of fifth instar brown locust hoppers, *Locustana pardalina*, treated with different dose rates of *M. anisopliae* var. *acridum*

Dose	Mean	Standard error (SEM)
Control	10.9	0.48
10^3	7.9	0.42
10^4	8.1	0.64
10^5	6.1	0.35
10^6	4.6	0.20
10^7	3.9	0.20
10^8	2.8	0.20

Table 5.2 Dose-rate mortality of fifth instar brown locust hoppers treated with different dose rates of *M. anisopliae* var. *acridum* using the Log-Rank test.

Test of equality over time			
Comparison	Chi-square	d.f	P<
10^3 vs. control	5.38	1	0.02
10^4 vs. control	15.21	1	0.001
10^5 vs. control	42.76	1	0.001
10^6 vs. control	49.12	1	0.001
10^7 vs. control	50.12	1	0.001
10^8 vs. control	54.23	1	0.001

Food consumption

During the acclimation period, the first 24h before the insects were inoculated, and immediately after treatment, food intake was not noticeably different between treated and control locusts, except for the highest dose (10^8 conidia per insect). On the first day after treatment, a marked reduction in food consumption had already occurred in all hoppers treated with doses 10^4 spores/insect and higher.

There were, however, no significant differences in food consumption of hoppers in the 10^3 treatment, which behaved much like the untreated controls even until the last day of the experiment. With the 10^6 dose rate, food consumption decreased from day three and the hoppers treated with 10^7 dose rates reduced their feeding from day two. The highest dose, 10^8, showed a rapid drop in food consumption.

There was an overall drop in food consumption in both control and treated batches, around day 10, when hoppers were moulting into fledglings (Fig. 1.2).

When food consumption is presented as cumulative food eaten, there was a marked reduction in the total amount of food eaten per treatment in all the doses of 10^4 and higher (Fig 1.3). The p-value was <0.001 which indicated significant differences between dose-levels.

Dunnet's t-test showed significant differences between the controls and all the dose levels of 10^4 and higher at the 5% significance level (Table 1.3). It is also clear from Table 1.3 that the mean food consumption (mg/hopper) decreased as the dose level increased.

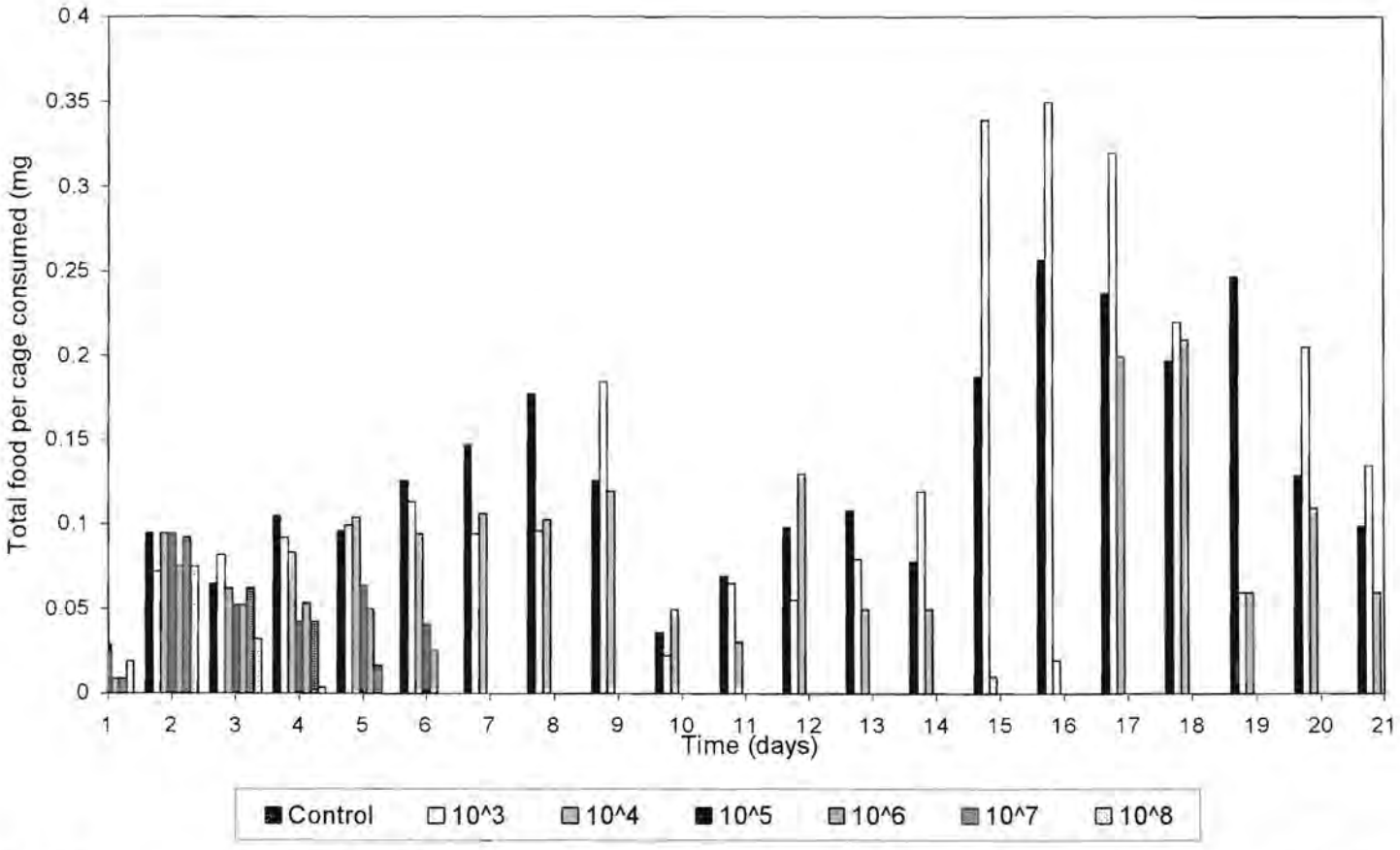

Fig. 5.2 Daily food consumption (mg/h) of fifth instar brown locust hoppers treated topically with different doses of *Metarhizium anisopliae* var. *acridum* mycoinsecticide

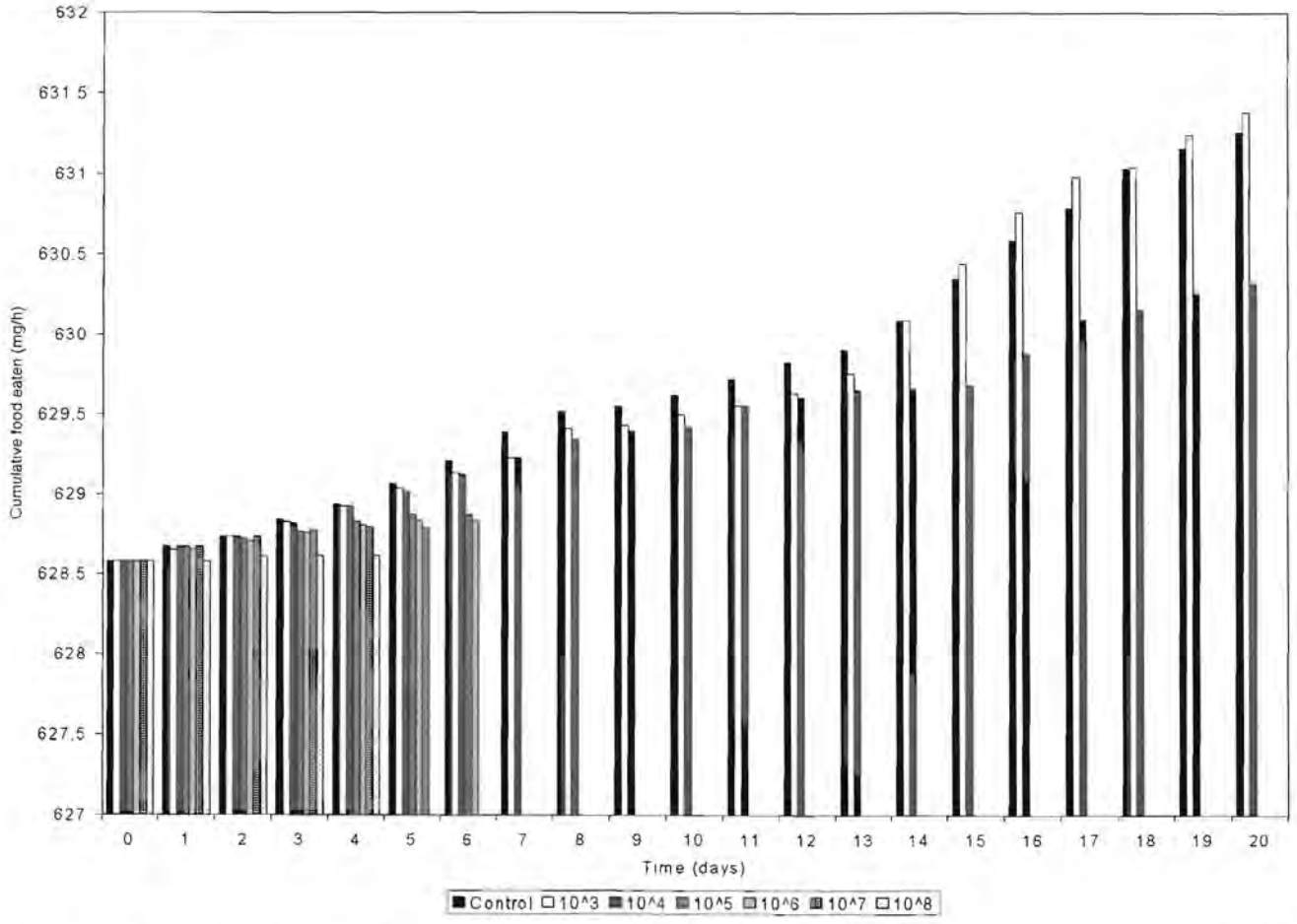

Fig. 5.3 Cumulative food consumption (mg/h) of fifth instar brown locust hoppers treated topically with different doses of *Metarhizium anisopliae* var. *acridum* mycoinsecticide.

Table 5.3 Dunnett's t-test comparisons at a 5% level of fifth instar brown locust hoppers treated with different dose rates of *M. anisopliae* var. *acridum*.

Dose level	Transformed mean (square root) food consumption	Mean food consumption (mg/hopper)
Control	0.3799	0.1443
10^3	0.3616	0.1308
10^4	0.3056	0.0934
10^5	0.2740	0.0751
10^6	0.2606	0.0677
10^7	0.2263	0.0512
10^8	0.1960	0.0384

Discussion

The *M. anisopliae* var. *acridum* isolate IMI 330189 mycoinsecticide produced excellent control (>90%) of brown hopper bands in the field, a level of control equal to that of any of the conventional insecticides currently in use, although speed of kill is slow (Bateman *et al.* 1994). Although the prolonged time taken to produce high rates of mortality may not be so important during control campaigns against small scale incipient locust outbreaks or when undertaken within conservation areas, it would create serious problems during large-scale operational control campaigns in the Karoo, where a quick knockdown of the pest is required.

Laboratory bioassays using different doses of *Metarhizium* showed that speed of kill was directly proportional to dose rate of spores applied. The higher the dose rate, the quicker the speed of kill. This dose response effect has important consequences for field operations. Current field experimentation on other field insects, leads to an

estimation that 10,000 conidia (10^4/individual) per locust applied by ultra-low-volume spinning disc applicators will lead to death by 9 days post application (Moore *et al.* 1992). The 4-21 days taken to kill brown locust hoppers under field conditions is similar to the rate achieved with the 10^{4-5} bioassay dose, where locusts take. Thus, the foremost solution to improve the speed of action of the mycoinsecticide in the field, would be to increase the dosage rate. However, the logistics, economics and technical problems of vastly altering the existing formulation and application rates do not allow this direct strategy. Instead, the treated area around a hopper band could be increased, thus increasing the dosage by secondary pick up of spores.

The average survival times of the treated insects, especially the higher doses, were much shorter than those typically observed in field trials (>4 days). Significant colonization and full mycosis would not be expected to occur until 48h post-inoculation (Gunnarsson 1988), although, in this present study, high doses died early and showed a rapid drop in food consumption as early as one day after treatment.

Thomas *et al.* (1997) mention that reduction in feeding may be attributed to degradation of tissues in combination with the production of secondary metabolites, but also suggest that a separate contributory mechanism is involved. Gunnarsson (1988) indicates that an immune response in insects 12h after inoculation of the pathogen is possible, alerting the insect to infection at an early stage. Therefore, a further field study into the mechanisms involved and reduction in food consumption after insects have been treated, should be investigated for the brown locust.

Locusts are pests largely because of the crops that they eat and therefore any reduction in food consumption is of great value. All laboratory treatments showed the trend of a reduction in food consumption even at a dose rate as low as 10^4. These and other supported field results could be valuable to convince farmers that, although gregarious hopper bands treated with *Metarhizium* mycoinsecticide may

still be able to march across their veld and still appear to be healthy for a few days, they are indeed sick and will not consume large amounts of food.

Lastly, the pest status of locusts depend on their migratory ability as well, especially the highly mobile brown locust. Although the target of control is the fifth instar hopper stage in the Karoo, it is still suggested that adults be treated with the mycoinsecticide, to further investigate whether reduction in food consumption is accompanied by a poorer flight performance.

CHAPTER 6

RISK ASSESSMENT USING THE AFRICAN HONEY BEE

Introduction

According to Greathead & Prior (1989), fungal control agents pose a minimal risk to non-target organisms when compared with chemicals, and offer a method of control that has a very narrow target host range. It is, however, still important to establish the environmental impact of each new isolate on non-target organisms before releasing it and to evaluate the mycoinsecticide for registration purposes.

Prior (1997) reviewed the susceptibility of target acridoids and non-target organisms to *Metarhizium anisopliae* var. *acridum* isolate IMI 330189. Of all the species and groups tested, only the Orthopteran families Acrididae and Pyrgomorphidae were shown to be highly to moderately susceptible to this mycoinsecticide (Table 6.1). The risks associated with releasing this isolate are thus considered to be small and are discussed by Prior (1992).

The Department of Plant and Quality Control in South Africa required a risk assessment of the final *M. anisopliae* var. *acridum* product, 'Green Muscle', against the brown locust, *Locustana pardalina*. For assessing the risk associated with the release of the mycoinsecticide, it was decided to use the honeybee, *Apis mellifera* Linnaeus, (Hymenoptera: Apidae), as a biological indicator.

There are two honey bee races in South Africa, and the African honeybee, *A. mellifera scutellata*, found in southern Africa from the Karoo region northwards, was selected for this risk assessment. This bee is found over most of the unique semi-arid brown locust outbreak biome, which includes considerable commercial honeybee farming activity, incorporating both honey production and commercial

crop pollination. For example, a single beekeeping operation in Douglas, a town in the Karoo Region, comprises more than 2 000 honeybee colonies (Allsopp, pers.comm.). Honey bees are important for the pollination of indigenous flora are significant – at least 50% (Richard Cowling pers. comm.) and hence critical for floral conservation and biodiversity. If this mycoinsecticide were to pose any threat to these bees, its application could severely affect beekeeping and agricultural activity in the region, and bring into question suitability of mycoinsecticides as an environmentally safer locust control measure.

In previous studies, spore applications of *Metarhizium* to bees at realistic field doses result in low mortality under laboratory conditions. For example, the European honeybee, *Apis mellifera mellifera*, has been reported to show low infectivity following application of massive doses of *Metarhizium* (Ball *et al.* 1994 & Prior 1997). Assessments done by Ball *et al.* (1994), involved briefly anaesthetising young bees with carbon dioxide and transferring them to cages containing 25 bees each. This procedure exerts unusual, artificial stress on the test animals. Laboratory testing of entomopathogenic fungi against social insects such as the honeybee thus pose unique problems and laboratory results on non-target susceptibility to fungal agents must, at best, be considered as being over conservative. However, laboratory stress creates artificial stress for any pesticide evaluation but remain a fundamental part of any pesticide evaluation.

In this specific non-target assessment, the caging of individual African honeybees for two to three weeks would be detrimental to the bees, prohibiting natural daily beehive activities. Thus, as far as establishing degree of safety and assessing risk factors, natural field conditions where unrestricted daily activities should rather be allowed. Therefore, the impact or susceptibility assessment of applying the *M. anisopliae* var. *acridum* mycoinsecticide against the African honeybee, was conducted on normal free-living colonies, permitting unrestricted activities approximating natural field conditions, such as foraging for nectar, pollen and

water, as well as other hive activities such as the ripening of nectar, rearing of brood and hive maintenance.

Table 6.1. Arthropod host range data for Metarhizium anisopliae var. acridum (Prior 1997).

Host		Susceptibility
Orthoptera: Acrididae		
	Schistocerca gregaria	High
	Locusta migratoria	High
	Locustana pardalina	High
	Chortoicetes terminifera	High
	Oedaleus senegalensis and *O. nigeriensis*	High
	Kraussaria angulifera	High
	Diabolocatantops axillaris	High
	Acorypha glaucopsis	High
	Aiolopus simulatrix and *A. thalassinus*	High
	Phaulacridium bivittatum	High
	Hieroglyphus daganensis	Moderate
	Cataloipus cymbiferus and *C. fuscocoerulipes*	Moderate
	Homoxyrrhepes punctipennis	Moderate
Orthoptera: Pyrgomorphidae		
	Zonocerus variegatus	Moderate
	Pyrgomorpha cognata	Moderate
Orthoptera: Gryllidae		
	Teleogryllus commodus	Very low
Orthoptera: Tettigonidae		
	Unidentified sp.	None
Coleoptera: Curculionidae		
	Neochetina eichhorniae	None
Coleoptera: Tenebrionidae		
	Pimelia sinensis	None
	Trachyderma hispida	None
	Tenebrio molitor	Low
Coleoptera: Coccinellidae		
	Hyperaspis notata	None
Coleoptera: Scarabaeidae		
	Phyllophaga spp.	Low
Heteroptera: Coreidae		
	Clavigrella shabadi and *C. Tomentosicollis*	None
Hymenoptera: Formicidae		
	Tapinoma sp.	None
Hymenoptera: Encyrtidae		
	Epidinocarsis lopezi	None
Hymenoptera: Braconidae		
	Bracon hebetor	Moderate
Hymenoptera: Apiidae		
	Apis mellifera	Low
Isoptera: Termitidae		
	Coptotermes and *Nasutitermes* spp.	Moderate
Dictyoptera: Blattidae		
	Blatta sp.	None
Neuroptera: Myrmeleonidae		
	Unidentified spp.	Low

Materials and methods

Five viable, healthy African honeybee colonies (in Langstroth-type hives), regarded as very strong and aggressive colonies (having foraged on *Aloe* sp. the whole winter) were obtained from the Plant Protection Research Institute (PPRI) Bee Research Division. The hives were placed randomly at 20m intervals in a field at Rietondale Research Centre, Pretoria, during spring (September to October) 1995. The hives were situated near *Acacia galpinii* trees, which provided an excellent pollen source for foraging bees.

As a potential worst case scenario of exposure of colonies to the mycoinsecticide, one gram dry weight *M. anisopliae var. acridum* dry spore powder (TC formulation; concentration 5 X 10^{10} conidia/g) was dusted into three of the hives. This was done using a hand operated air blower connected to a plastic tube long enough to reach the centre of the hive so that spores could contaminate bees and frames. This massive application represents a dose rate approximately 100 times higher than the recommended field dose rate. Two hives served as controls and were not treated.

Hive activities such as the ripening of nectar, rearing of brood and hive maintenance were monitored and assessed weekly by inspecting the brood patterns on frames of five randomly selected frames from each hive. The frames were assessed by the same person to assure consistency. Scale maps of each frame were drawn in the field during the weekly inspections. These maps were later superimposed onto a 100-square grid (size of grid cells = $1cm^2$) and the percentage area of the following categories accurately determined: eggs, larvae, capped brood, drone cells, queen cells, nectar, pollen, honey and empty cells. Before smoking the hives during the weekly inspection, foraging flights, collecting of pollen and bee behaviour were monitored for 10min/hive for any signs of behavioural and foraging changes. The presence of the queen and new queen cells were also monitored during weekly inspections. The continuation of these activities after spore application was monitored for six weeks post application.

The percentages obtained from the results represented frequencies out of 100, therefore a 2X2 chi-square test was used to test for significant differences of food gathering activity and the reproductive performance between treated and control colonies.

A data logger recorded hourly ambient air temperature, while probes inside the hive recorded temperature and relative humidity.

Results

Beehives

Bee behaviour

Immediately after treatment, bees began to beat their wings rapidly in an attempt to ventilate their hives. Fanning behaviour is not a defence mechanism; rather a temperature regulating and orientation behaviour (M.Allsopp pers. comm.). Weekly inspections and observations indicated that foraging, collecting of pollen and the production of honey continued apparently undisturbed after the Metarhizium application. Pollen was carried into the hives; pollen sacks on the hind legs of bees were clearly visible. No dead bees were found due to mycosis over the six weeks post application period, no disease or pest problems were detected in the hives and none of the colonies swarmed. Colonies maintained their strength, and there was no need for supplement feeding. The only shortcoming of the experiment was that the frames of one of the control hives carried too much honey and heavy honey combs broke off approximately three weeks post treatment, leaving a gap in the final results. However, after termination of the experiment, all five hives were returned to the Bee Research Division and no ill effects were reported on that specific control beehive.

Temperature

The temperature and relative humidity in the hives over a typical 11-day period are shown in Fig. 6.1. The temperature inside the hives was regulated by the bees and constant temperatures of between 25-30°C were maintained.

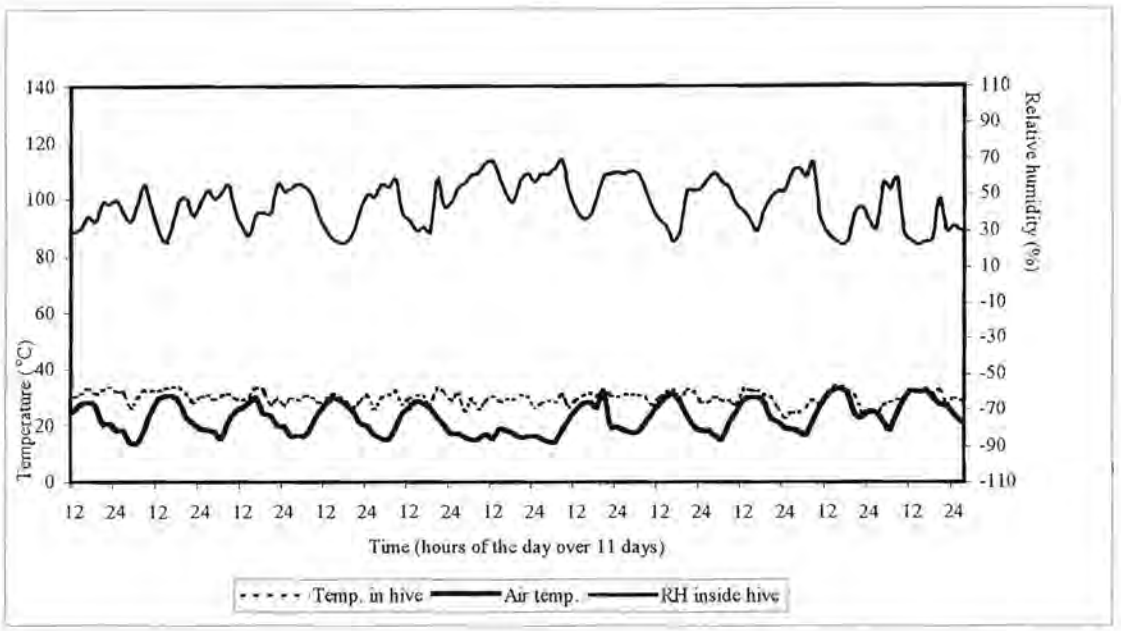

Fig. 6.1. Ambient temperature, and temperature and relative humidity inside an *Apis mellifera scutellata* hive treated with *Metarhizium anisopliae* var. *acridum* isolate IMI 330189. 12 = 12h00, 24 = 24h00, temp. = temperature, RH = relative humidity

Food collecting activity and reproduction

There were no observed differences in brood patterns between treated and untreated hives. New eggs and larvae were regularly produced in all hives, suggesting no ill affects on brood production. The summary of data (mean of five frames per hive) of each hive over six weeks were grouped as food collecting activity (% area of the frame covered by pollen, nectar and honey) (Table 6.2) and reproduction (% area of the frame covered by capped and uncapped brood) (Table 6.3). Drone, queen and

empty cells were included in the "other" category. Mean results per hive over six weeks of these three groups are shown in Fig. 6.2.

Table 6.2 Percentage food collecting activity (pollen, nectar and honey) data (mean of five frames per hive) from four African honey bee hives over six weeks.

Hive	Week						Mean
	1	2	3	4	5	6	
1	44.70	28.50	19.50	21.00	33.30	41.40	31.40
2	16.20	12.10	10.30	8.40	25.70	24.90	16.27
3	35.90	33.60	30.10	42.90	54.60	49.60	41.12
Control	22.00	17.20	29.50	21.60	30.40	33.20	25.65

Table 6.3 Percentage reproduction data (capped and uncapped brood) (mean of five frames per hive) from four African honey bee hives over six weeks.

Hive	Week						Mean
	1	2	3	4	5	6	
1	25.20	28.10	48.10	36.50	38.00	44.10	36.67
2	56.00	57.30	54.30	58.60	38.80	56.00	53.50
3	23.50	37.20	38.30	27.30	25.40	36.30	31.33
Control	48.30	36.30	40.00	51.60	52.10	31.10	43.23

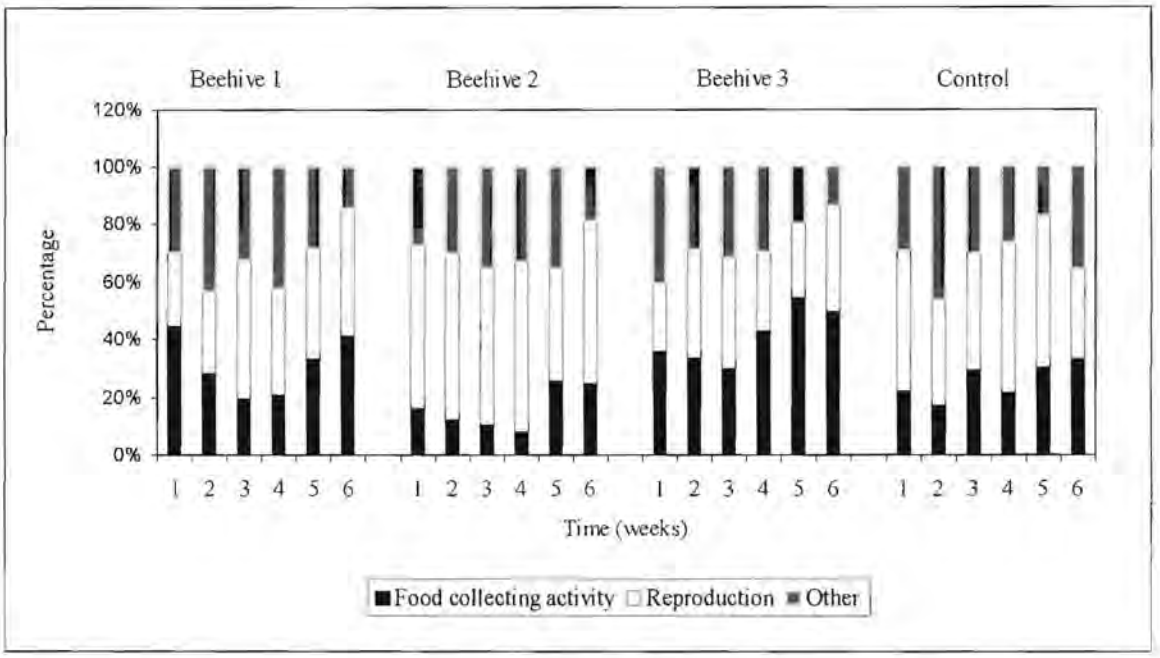

Fig. 6.2 Food collecting activity and reproduction of four *Apis mellifera scutellata* hives over a six-week period after treatment with *Metarhizium anisopliae* var. *acridum* isolate IMI 330189.

Statistical analysis of data

The 2 x 2 chi-square test results of weekly differences in hives between the food gathering activity data and the reproductive performance data are summarised in Table 6.4. There was overall no clear pattern of difference between control and treated colonies at the 1% level even if taking into account that temperature and humidity inside the hives were conducive for fungal development.

Over the six-week period no significant differences occurred in proportions of honey to brood in the treated and control hives and no trends of ill effects were observed. Such a trend would have been indicative of an ailing colony (see Tables 6.2 and 6.3). New queen cells were produced and the presence of the queen in every hive was confirmed during the weekly assessments.

Table 6.4 Two by two chi-square tests to detect differences between hives treated with *M. anisopliae* var. *acridum* and a control (d.f. = 1, test level = 0.01) and tabled chi-squared value = 3.841).

Week	x^2 value	P<
1	5.16	0.02
2	0.33	0.57
3	3.34	0.06
4	0.98	0.32
5	5.61	0.02
6	0.42	0.52

Discussion

Large doses of *M. anisopliae* var. *acridum* isolate IMI 330189 dry conidia dusted directly into African honeybee hives had no apparent harmful effect during a six-week field trial, even though warm, dark and humid conditions in the hives were ideal for germination of spores. Colonies were still strong, healthy and reproducing, even after a year. It could be agreed and recognised that results are limiting and that there are gaps in the trial, e.g. there was a lack of replication in the controls. Conducting a field trial using the oil based formulation rather than dry spores could also have been more detrimental to bees. However, at the time it was decided to use this experimental design as a worst case scenario, which showed most importantly that brood pattern and honey production proportions showed no significant differences between treated and control and no mortality due to mycosis was found in this specific field application. Although it is likely that diseased bees (if any),

could have been removed from hives as routine hive hygiene before external conidiation would have occurred, the obvious absence of any signs of mycosis in the beehives (mycosed corpses) or any other ill effects of such massive dose rates administered suggests that this isolate is not pathogenic to the African honeybee. All five hives (including the discarded control) were healthy and viable even after a year (D.Swart pers.comm), and the above results of the risk assessment against the African honeybee were reported to the Directorate of Plant and Quality Control (National Department of Agriculture), accepted in LUBILOSA's Ecotoxicology Profile (1998) and was regarded as suffiecient value to grant mycoinsecticide registration.

Although preliminary trials failed to indicate any negative effect on the African honeybee, these trials are not regarded as fully conclusive and results suggest that more comprehensive trials of bees as biological indicators should be performed. Although no natural epizootic caused by potential fungal control agents have been reported on bees or any other pollinator, there could nevertheless be a slight risk to insects if large-scale field application of fungi is pursued and Goettel *et al.* (1990) recommend caution if the application included direct exposure to bee colonies or foragers. In any risk assessment, there should be careful consideration of how the applied product might come into contact with the non-target organism. *Metarhizium* application against the brown locust takes place by spraying gregarious hopper bands early in the morning when temperatures are still cool, and while gregarious roosting locust band densities are high. As locust hopper bands are treated by spot application, there is no immediate risk of spray drift onto nearby honeybee colonies, although spores could come in contact with bees via secondary pick up from vegetation.

Subsequent, preliminary and similar trials were performed against the Cape honeybee in South Africa, and although these results were damaging (unpublished), it could not be confirmed that *Metarhizium* was the direct cause of ailing and dying treated and control hives. Overall, it is also believed that more comprehensive

impact assessments have to be done to support the use of the 'Green Muscle' product, for example the effect on other Orthopteran families in the Karoo Region, e.g.many indigenous grasshopper species.

CHAPTER 7

CONCLUSION

The aim of the work was to find out if it was possible to kill southern African locust species with the entomopathogenic fungus, *Metarhizium anisopliae* var. *acridum*.

Although two isolates of this mycoinsecticide, namely isolates IMI 330189 and IMI 324673, were imported and tested initially, LUBILOSA decided to use the first isolate as their standard isolate. Doses of 3 X 10^4 and 6 X 10^4 spores per individual locust, of the standard isolate, produced mortality of 90 and 93% in fifth instar brown locust hoppers after 21 days, with median lethal times of 9.8 and 7.5 days respectively. This indicated from the start that: this fungus is able to infect and kill locusts at a rate equal to that to any of the chemical insecticides in current use; there was dose dependence, but speed of kill was slower compared to fast acting insecticides. Control mortality was 7%, significantly lower than the treated batches.

Against African migratory locust hoppers, the same dose rates produced mortalities of 97 and 100% with median lethal times of 10.7 and 10.3 days respectively. Control mortality was 13%. Final mortality rates were higher with the latter hopper species, but LT_{50} periods were longer, probably due to the lower air temperature that locusts were maintained at. A fascinating symptom of infection, only observed in the brown locust hopper, was that many insects died during the final moult.

The same dose rates of 3 X 10^4 and 6 X 10^4 spores per individual gave 100% control of the brown locust adults after 12 and 9 days respectively, with a median lethal time of the higher dose rate at only 4.4 days. Against African migratory locust adults, 100% mortality was achieved after 18 and 15d. Control mortalities were 8 and 14%. Adults of both locust species clearly showed a higher mortality rate,

although median air temperature and relative humidity were higher than for the hopper stages.

It was decided to increasingly use higher dosage rates with the red locust, because, when comparing previous laboratory experience using chemical insecticides, this locust species is a large, tough insect to kill. Dosage rates of 2×10^4, 2×10^5, 7×10^5 and 2×10^6 spores per individual were applied to second instar locust hoppers, which gave final mortalities of 89 and 92%. Initial results indicated promising, effective control.

A dosage rate of 3.7×10^6 killed 100% of the sample after 9 days, and a high dosage rate of 3.7×10^7 gave a 100% mortality after 11 days. It is possible that the daily spraying of water to increase humidity in the containers could have had a slight effect on speed of kill. Control mortality was 18% for second and third instar hoppers, and no control mortality occurred in the final instar.

Overall, insects were susceptible to all topical applications of the oil formulation. Treated locusts developed the typical red coloration after death, which is a clear symptom of mycosis, and external sporulation subsequently developed when insects were incubated under high humidities. All locusts were compared with untreated controls; a blank treatment with oil had initially no mortality effect.

As opposed to the oil-based conidial formulation of *M. anisopliae* var. *acridum*, an aqueous blastospore formulation was also tested in the laboratory against brown and red locusts. These topical applications were much easier to conduct, because viscosity of the suspension was lower and thus facilitated droplet extrusion better than the oil formulation. Dosage rates of 6.8×10^4 and 6.8×10^5 gave 80 and 100% control of fifth instar brown locust hoppers after 21 days, with median lethal times of 12.5 and 10.3 days respectively. Control mortality was 10%. Again, most of the hoppers died in the final moult and it was now clearly seen that this phenomenon could have immense potential consequences in the understanding of the overall

population dynamics of this mycoinsecticide and certainly requires additional research.

Five doses, ranging from 3.4 X 10^4 to 2 X 10^5 were applied to third and fourth instar red locust hoppers, but only three of the doses were significantly different to the control batches; clearly the lower doses were not effective enough to kill these red locust hopper stages. However, the main aim was to see if hoppers also died in the final moult as the brown locust did in previous laboratory tests. Fascinating enough, all insects completed the final moult, the intermoult period did not even vary between treated insects and control, and all eventually became adults.

Contrary to the fast acting chemicals that locust control officers and farmers are used to in South Africa, it is clearly shown that, although carefully developed and successfully tested, this mycoinsecticide is slower acting. Some locusts started dying as early as one day after treatment, but full symptoms of mycosis usually started on the third day after application.

To test the effect of dose-dependence, two treatment were carried out under strict controlled environment, where six doses of *M. anisopliae* var. *acridum* isolate IMI 330189 spores (10^3-10^8 spores per insect) were assayed against fifth instar brown locust hoppers. With the highest dose (10^8), all insects were dead between 3-4 days while complete kill was achieved after 5 and 6 days respectively with the 10^7 and 10^6 dose rates. The 10^5 dose took 6-10 days to achieve 100% mortality and the lower dose of 10^4 took 12 to 14 days to achieve mortality of 75-87%. The lowest dose rate (10^3) gave no significant mortality until 9-10d after inoculation, with final mortality only reaching 60% after 21 days. The speed of kill showed a classic dose related response, with higher dose rates killing locusts more quickly, confirming earlier results.

All preliminary laboratory treatments in the feeding experiment showed the trend of a marked cumulative reduction in food consumption even at a dose rate of 10^4.

There was also an overall cessation in feeding while locusts were moulting. These results are highly significant regarding future application of the mycoinsecticide in the field. Locusts are pests largely because of the crops that they consume and therefore any reduction in food consumption is of great value. It could convince farmers that, although bands treated with *Metarhizium* may still be able to march across their veld and appear to be healthy for a few days, they are indeed sick and will not threaten crop security. As such, this chapter has given insight in the operational implementation of the brown locust.

Results by other authors showed that reduction in food consumption is also accompanied by poorer flight performance and this clearly should be investigated in the highly mobile brown locust in future.

The experimental design for risk assessment of the African honey bee was at that stage regarded as a worst case scenario. Large doses of *M. anisopliae* var. *acridum* isolate IMI 330189 dry conidia dusted directly into African honeybee hives had no apparent harmful effect during the six-week field trial. Unrestricted activities such as foraging for nectar, pollen and water, as well as other hive activities, such as the ripening of nectar, rearing of brood and hive maintenance were carried out. Pollen was observed being carried into the hives and no dead bees were found in or around hives due to mycosis over the assessment period. The fungus did not develop inside any of the treated hives although warm, dark and humid conditions in the hives were ideal for spore germination, where constant temperatures of between 25-30°C were maintained.

Colonies were still strong, healthy and reproducing, even after a year. Results of the risk assessment against the African honeybee were reported to the Directorate of Plant and Quality Control (National Department of Agriculture) and accepted in LUBILOSA's Ecotoxicology Profile (1998).

The major findings of all the laboratory experiment implicated that it was possible to kill southern African locust species with the entomopathogenic fungus, *M. anisopliae* var. *acridum* without harming a non target organism such as the African honeybee. These evaluations prompted field trials with the mycoinsecticide against the brown locust in the Karoo, and the red locust in Mozambique. (The African migratory locust was not a serious problem at the stage), which again confirmed the laboratory findings.

Product registration was permitted against the brown locust, (commercial trade name Green Muscle™), under Act 36 of 1947 and recommended by the FAO for locust control in environmentally sensitive areas. The *M. anisopliae* var. *acridum* mycoinsecticide will thus be known as the first alternative, more environmentally benign and non-chemical method of locust control in southern Africa and as such, this work is regarded as a milestone.

REFERENCES

BALL, B.V., PYE, B.J., CARRECK, N.L., MOORE, D. & BATEMAN, R.P. 1994. Laboratory testing of a myco-pesticide 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. 1992. Controlled droplet application by myco-insectides: an environmentally friendly way to control insects. *Antenna* **16**: 6-13.

BATEMAN, R.P. 1996. The use of ULV formulations of *Metarhizium flavoviride* for the biological control of locusts. A submission to the desert locust pesticide referee group of the Food and Agriculture Organisation of the United Nations.

BATEMAN, R.P. 1997. Methods of application of microbial pesticide formulations for the control of grasshoppers and locusts. *Memoirs of the Entomological Society of Canada* **171**: 67-79.

BATEMAN, R.P., CAREY, M., BATT, D., PRIOR, C., ABRAHAM, Y., MOORE, D., JENKINS, N. & FENLON, J. 1996b. Screening for virulent isolates of entomopathogenic fungi against the desert locust, *Schistocerca gregaria* (Forskål). *Biocontrol Science and Technology* **6**: 549-560.

BATEMAN, R.P., CAREY, M., DOURO-KPINDOU, O.-K., GODONOU, I, GOETTEL, M., JENKINS, N., KOOYMAN, C., LOMER, C.J., MOORE, D., PARAÏSO, PRIOR, C. & SHAH, P.A. 1995b. Collection of insect pathogens. *LUBILOSA Technical bulletin* no. 2.

BATEMAN, R.P., CAREY, M., DOURO-KPINDOU, O.-K., GODONOU, I., GOETTEL, M., HEVIEFO, G., JENKINS, N.E., KOOYMAN, C., LANGEWALD, J., LOMER, C.J., MOORE, D., PARAÏSO, A. & PRIOR, C 1996a. Laboratory techniques in insect pathology. *LUBILOSA technical bulletin* no.3.

BATEMAN, R.P., CAREY, M., MOORE, D. & PRIOR, C. 1993. The enhanced infectivity of *Metarhizium flavoviride* in oil formulations to desert locusts at low humidities. *Annals of Applied Biology* **122**: 145-152.

BATEMAN, R.P., PRICE, R.E., MÜLLER, E.J. & BROWN, H.D. 1994. Controlling brown locust hopper bands in South Africa with a myco-pesticide spray. *Proceedings of the Brighton Crop Protection Conference – Pests and Diseases* **6A-3**: 609-616.

BOTHA, D.H. & LEA, A. 1970. Do brown periods come in cycles? *Farming in South Africa* (February).

BOTHA, D.H. 1958. Is BHC-behandelde weiding nadelig vir skape. *SA Tydskrif vir Landbouwetenskappe* No. 4.

BOTHA, D.H. 1969. Locusts and their control in SA. *Farming in SA*. August. 45-55.

BOTHA, D.H. 1970. Locusts and their control in South Africa. Part 6. Life cycle of the brown locust. *Farming in South Africa*.

BRIDGE, P.D., 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.

BRIDGE, P.D., PRIOR, C., SAGBOHAN, J., LOMER, C.J., CAREY, M. & BUDDIE, A. 1997. Molecular characterisation of isolates of Metarhizium from locusts and grasshoppers. *Biodiversity and Conservation* **6**: 177-189.

BROWN, H.D. & KIESER, M.E. 1997. Locust control with deltamethrin. In: (Eds.) *New strategies in locust control*. Birkauser Verlag Basel: 249-250.

BROWN, H.D. 1987. Current pesticide application: effectiveness and persistence. *Proceedings of the Locust Symposium. MacGregor Museum, Kimberley.*

BURGES, H.D. (ed) 1970-1980. Microbial control of pests and Plant diseases. New York: Academic Press.

DENT, D. 1998. Locust control with *Metarhizium*. *British Mycological Society's International Symposium on "The future of fungi in the control of pests, weeds and diseases", University of Southampton, U.K.*, 5-9 September.

DOURO-KPINDOU, O.K., GODONOU, I., HOUSSOU, A., LOMER, C.J. & SHAH, P.A. 1995. Control of *Zonocerus variegatus* with ULV formulations of *Metarhizium flavoviride* condia. *Biocontrol Science and Technology* **5**: 131-139.

FARROW, R.A.1975. The African migratory locust in its main outbreak area of the Middle Niger.: Quantative studies of solitary populations in relation to environmental factors. *Locusta Bulletin* No. 11.

FAURE, J.C & MARAIS, S.J.S 1936. The control of *Locustana pardalina* in its outbreak centre. *Proceedings of the IVth International Locust Conference Cairo Application* 37.

FAURE, J.C. 1935. The life history of the red locust (*Nomadacris septemfasciata*) Servillle. *Department of Agriculture And Forestry. Bulletin* No. 144.

FAURE, J.C.1932. The phases of locusts in South Africa. *Bulletin of Entomological Research* **23**: 298-405.

GREATHEAD, D.J. & PRIOR, C. 1990. The regulation of pathogens for biological control with special reference to locust control. In *Proceedings of the workshop on health and environmental impact of alternative control agents for desert locust control,* ed. L. Somme and S.Bie. NORAGRIC Occasional Papers series C: Development and Environment **5**: 67-80.

GREEN MUSCLE SYMPOSIUM. 1998. *Biological Control Products (BCP), South Africa.*

GUNN, D.L. 1960. The biological background of locust control. *Annual Review of Entomology* **5**: 279-300.

GUNNARSSON, S.G.S. 1988. Infection of *Schistocerca gregaria* by the fungus *Metarhizium anisopliae*: cellular reactions in the integument studied by scanning electron and light microscopy. *Journal of Invertebrate Pathology* **52**: 9-17.

JACK, R.W. 1934. The destruction and control of locust hoppers. *Rhodesian Agricultural Journal. Bulletin* 938.

JENKINS, N. & GOETTEL, M.S. 1997. Methods for mass production of microbial agents of grasshoppers and locusts. *Memoirs of the Entomological Society of Canada* **171**: 37-48.

JENKINS, N.E., HEFIEVO, G., LANGEWALD, J., CHERRY, A.J. AND LOMER, C.J. 1998. Development of mass production technology for aerial conidia for use as mycopesticides. *Biocontrol News and Information* **19**: 21-31.

JOHNSON, D.L. & PAVLOKOVA, E. 1986. Reduction of consumption by grasshoppers (Orhtoptera: Acrididae) infected with *Nosema locustae* Canning (Microsporida: Nosematidae). *Journal of Invertebrate Pathology* **48**: 232-238.

KLEESPIES, R.G. 1993. Untersuchungen zur Biologie und Anwendung des entomopathogenen Pilzes *Metarhizium anisopliae* (Metsch.) Sorokin zur biologischen Bekämpfung afrikanischer Wanderheuschrecken. PhD. Thesis. Technische Hochschule Darmstadt. 348 pp.

KOOYMAN, C., BATEMAN, R.P., LANGEWALD, J., LOMER, C.J., OUAMBAMA, Z. & THOMAS, M.B. 1997. Operational-scale application of entomopathogenic fungi for control of Sahelian grasshoppers. *Proceedings of the Royal Society of London* **264**: 541-546.

KOOYMAN, C. & GODONOU, I. 1997. Infection of *Schistocerca gregaria* (Orthoptera: Acrididae) hoppers by *Metarhizium flavoviridae* (Deuteromycotina: Hyphomycetes) conidia in an oil formulation applied under desert conditions. *Bulletin of Entomological Research* **87**: 105-107.

LEA, A. 1964. Some major factors in the population dynamics of the brown locust, *Locustana pardalina* (Walker). *Monographiae biologicae* **14**: 269-283.

LEA, A.1973. Locust control and Research in southern African. *Entomological.Memoir* no 31.

LOMER, C.J., PRIOR, C. AND KOOYMAN, C. 1997. Development of *Metarhizium* spp. for the control of grasshoppers and locusts. *Memoirs of the Entomological Society of Canada* **171**: 265-286.

LOUNSBURY, C.P. 1915. Some phases of the locust problem. *South African Journal of Scienc.* **11**: 33-45.

LUBILOSA. 1998. Ecotoxicological Profile. *Metarhizium* isolate IMI 330189.

MATTHEE, J.J. 1951. The structure and physiology of the egg of *Locustana pardalina* (Walker). *Scientific Bulletin of the Department of Agriculture, Union of South Africa* no. 316.

MEINZINGEN, W.F. 1993. A guide to migrant pest management in Africa. FAO, Rome, Italy.

MILNER, R.J.; DRIVER, F.; CURRAN, J.; GLARE, T.R.; PRIOR, C.; BRIDGE, P.D. & ZIMMERMAN, G. 1994. Recent problems with the taxonomy within the genus *Metarhizium*, and a possible solution. *Proceedings of the VIth International Colloquium on Invertebrate Pathology and Microbial Control, Montpellier, Society of Invertebrate Pathology.* 109-110.

MILNER, R.J. & PRIOR, C. 1994. Susceptibility of the Australian plague locust, *Chortoicetes terminifera*, and the wingless grasshopper, *Phaulacridium vittatum*, to the fungi *Metarhizium* spp. *Biological Control* **4**: 132-137.

MOORE, D., BATEMAN, R.P., CAREY, M. & PRIOR, C. 1995. Long-term storage of *Metarhizium flavoviride* conidia in oil formulations for the control of locusts and grasshoppers. *Biocontrol Science and Technology* **5**: 193-199.

MOORE, D., REED, M., LE PATOUREL, G., ABRAHAM, Y.J. & PRIOR, C. 1992. Reduction of feeding by the desert locust, *Schistocerca gregaria*, after infection with *Metarhizium flavoviride*. *Journal of Invertebrate Pathology* **60**:304-307.

OTA. 1990. A plague of locusts - special report. OTA-F-450, Washington, D.C., U.S. pp129. Washington D.C. Government Printing Office.

POLE EVANS, I.B. 1911. The South African locust fungus. *Farmers Bulletin, Department of Agriciculture, Union of South Africa* No 8.

PRICE, R.E. & BROWN, H.D. 1990. Reproductive performance of the African migratory locust, *Locusta migratoria migratorioides* (Orthoptera: Acrididae) in a cereal crop environment in South Africa. *Bulletin of Entomological Research* **80**: 465-472.

PRICE, R.E. & BROWN, H.D. 1992. Incubation and overwintering in the egg stage of the African migratory locust, *Locusta migratoria migratorioides* (Orthoptera: Acrididae), on the highveld of South Africa. Bulletin of Entomological Research **82**: 235-241.

PRICE, R.E. & MÜLLER, E.J. 1993. Laboratory bioassay of *Metarhizium flavoviride* against the brown locust and the African migratory locust. *Plant Protection Research Institute Report 05: 7/93 for IIBC., South Africa.*

PRICE, R.E. & MÜLLER, E.J. 1993a. Preliminary screening of *Oedaleus senegalensis* entomopoxvirus (OsEPV) against african locusts. *Plant Protection Research Institute Report 05:8b/93 for Rangeland Insect Laboratory, Montana State University, USA.*

PRICE, R.E., STEWART, D.A.B. & MÜLLER, E.J. 1993. Bioassay of 5 strains of *Bacillus thuringiensis* against brown locust nymphs. *Plant Protection Research Institute Report (05:8/93) for CSIR, South Africa.*

PRINSLOO, H.E. 1960. Parasitiese mikro-organismes by die bruinsprinkaan *Locustana pardalina* (Walk.). *Suid Afrika Tydskrif vir Landbouwetenskap* **3**: 551-560.

PRINSLOO, H.E. 1962. The incidence of *Beauveria bassiana* (Bals.) Vuill. In laboratory populations of the brown locust. *South African Journal of Agricultural Science* **5**: 331-332.

PRIOR, C. & GREATHEAD, D.J. 1989. Biological control of locusts: the potential for the exploitation of pathogens. *FAO Plant Protection Bulletin* **37**: 37-48.

PRIOR, C. 1992. Discovery and characterisation of fungal pathogens for locust and grasshopper control. In: Lomer, C.J. and Prior, C (eds) 1992. *Biological control of locusts and grasshoppers*. CAB International, Wallingford, U.K: 159-180.

PRIOR, C. 1997. Susceptibility of target acridoids and non-target organisms to *Metarhizium anisopliae* and *M. flavoviride*. In *New strategies in Locust Control*: Eds: S.Krall, R.Peveling & D. Ba Diallo: 369-375.

PRIOR, C., CAREY, M. ABRAHAM, Y.J., MOORE, D. & BATEMAN, R.P. 1995. Development of a bioassay method for the selection of entomopathogenic fungi virulent to the desert locust, *Schistocerca gregaria* (Forskal). *Journal of Applied Entomology* **119**: 567-573.

SAS INSTITUTE INC. 1986. SAS/STAT User's Guide for personal computers. Version 6.

SCHAEFER, E.E. 1938. Die witswamsiekte (*Beauveria bassiana*) onder rooisprinkane in Suid-Afrika en 'n paar opmerkings oor die grysswamsiekte (*Empusa grylli*). *Wetenskaplike pamflet* no.160.

SCHERER, R., BATEMAN, R.P., MOORE, D. & MCCLATCHIE, G.V. 1992. Control of the migratory locust, *Locusta migratoria capito*, in Madagascar: the potential for the use of a myco-pesticide. *Brighton Crop Protection Conference - Pest and Diseases*: 357-362.

SEYOUM, E., MOORE, D. & CHARNLEY, A.K. 1994. Reduction in flight activity and food consumption by the desert locust, Schistocerca gregaria Forskål (Orthoptera, Cyrtacanthacrinae), after infection with *Metarhizium flavoviridae*. Journal of Applied Entomology **118**: 310-315.

SHAH, P.A. 1994. Field studies on the development of *Metarhizium flavoviride* Gams & Rozsypal as a microbial insecticide for locust and grasshopper control. PhD thesis, University of London.

SKAIFE, S.H. 1925. The locust fungus, *Empusa grylli*, and its effects on its host. *South African Journal of Science* **22**: 298-308.

SMIT, 1960. The behaviour of the brown locust in its solitary phase. *Technical Communication, Department of Agriculture and Technical Services, Republic of South Africa* **1**: 1-132.

SMIT, C.J.B. 1939. Field observations on the brown locust in an outbreak centre. *Scientific Bulletin of the Department of Agriculture South Africa,* no. 190.

STEEDMAN, A. 1990. Locust handbook, 3rd edition. Natural resources Institute, London.

STEPHAN, D., WELLING, M. & ZIMMERMANN, G. 1995. Locust control with *Metarhizium flavoviride*: formulation and application of blastospores.

STREETT, D.A. & HENRY, J.E. 1990. Microbial control of locusts and grasshoppers in the semi-arid tropics. *Bol. San. Veg. Plagas (Fuera de serie)* **20**: 21-27.

THOMAS, M.B, BLANFORD, S. & LOMER, C.J. 1997. Reduction of feeding by the variegated grasshopper, *Zonocerus variegatus*, following infection of the fungal pathogen, *Metarhizium flavoviride*. *Biocontrol Science and Technology* **7**: 327-334.

THOMAS, M.B., LANGEWALD, J. & WOOD, S.N. 1996. Evaluating the effects of a biopesticide op populations of the variegated grasshopper, *Zonocerus variegatus*. *Journal of Applied Ecology* **33**: 1509-1516.

ULLYETT, G.C. & SCHONKEN, D.B. 1940. A fungus disease of *Plutella maculipennis* Curt. In South Africa, with notes on the use of entomogenous fungi in insect control. *Science Bulletin* **218**: 1-24.

UVAROV, C.M.G.1951. Locust Research and Control. 1929-1959. *Colonial Research Publication*. No 10.

VAN DEN BERG, J.D., STREETT, D.A. & HERBERT JR, E.W. 1990. Safety of grasshopper entomopoxviruses for caged adult honey bees (Hymenoptera: Apidae). *Journal of Economical Entomology* **83**: 784-787.

VEEN, K.H. 1968. Recherches sur la maladie, due à *Metarhizium anisopliae* chez le criquet péerin. Mededeling en Landbouwhogeschool: Wageningen 77 pp.

WHELLAN, J.A.1964. The red locust - a forgotten menace. *Rhodesia Agricultural Journal. Bulletin* No 2257.

WIESE, I.H. & BOT, J. 1973. Insecticides, their hazards and control. *Entomology Memoir* **30**: 1- 13.

WELLING, M.; NACHTIGALL, G. & ZIMMERMAN, G. *Metarhizium* spp. Isolates from Madagascar. Morphology and effect of high temperatures on growth and infectivity to the migratory locust, *Locusta migratoria*. *Entomophaga* (in press).

ACKNOWLEDGEMENTS

The author wishes to thank the following people and/or organisations without whose support this work would not have been done:

ARC - Plant Protection Research Institute for funding, time and opportunity

Melodie McGeoch and Matt Thomas for contribution as supervisors

Roger Price and Mike Allsopp for reviews and comments

Everyone at Locust Research for support

The LUBILOSA team

Les Oates and Dick Brown for photos

Fanie Schönfeldt for scanning

My children for constant motivation

God for strength and endurance