ALTERNATIVE METHODS OF CONTROLLING THE BROWN LOCUST, *Locustana pardalina* (Walker)

by

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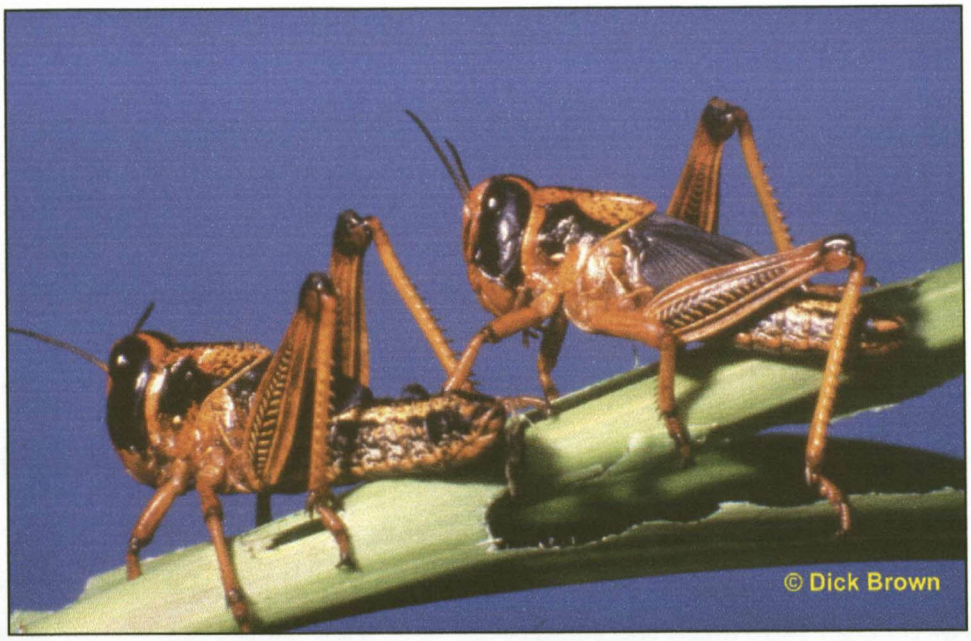
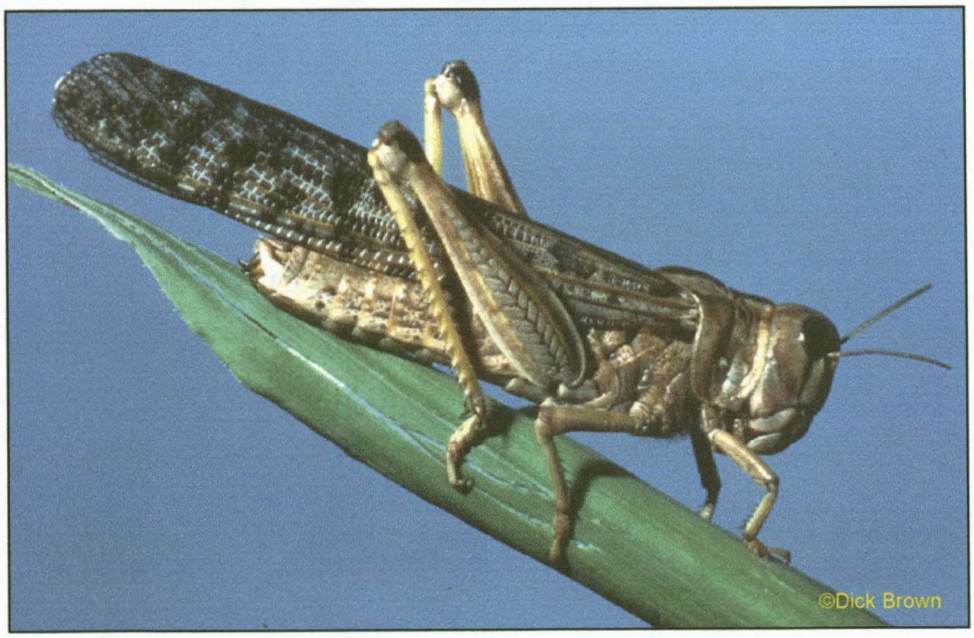


Gregarious 5th instar hoppers of the brown locust, *Locustana pardalina* Walker



Gregarious phase adult of the brown locust, *Locustana pardalina* Walker

DECLARATION

I declare that the thesis, 'Alternative methods of controlling the brown locust, *Locustana pardalina* (Walker)', hereby submitted by me for the Philosophiae Doctor (Entomology) degree at the University of the Orange Free State is my own independent work and has not previously been submitted by me at another university/faculty. I furthermore cede copyright of the thesis in favour of the University of the Free State.

R.E. Price ..... 28 July 2003

R. E. Price

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SUMMARY

Outbreaks of the brown locust, *Locustana pardalina* (Walker), occur almost annually in the semi-arid Karoo region of South Africa and southern Namibia. Current suppressive control strategy relies on the application of fast-acting, synthetic pyrethroid insecticides, applied as ultra low volume drift sprays, to control gregarious brown locust targets at source within the Karoo outbreak region. However, the negative impact that the repeated application of insecticides may have on the rich diversity of endemic invertebrates and reptiles found in the Nama-Karoo biome is of great concern to landholders and conservationists. How to reduce the insecticide load and minimise the environmental impact in the Karoo and yet at the same time control this serious agricultural pest has become a controversial issue. There is thus an urgent need for more environmentally benign methods of locust control, as an alternative to the current spraying of insecticide. As part of a locust research project initiated by the Plant Protection Research Institute, Pretoria, the potential of various alternative methods of controlling the brown locust were evaluated against gregarious hopper populations in the laboratory and in the field.

It was first important to update the available information on the background level of control provided by natural enemies and diseases of the brown locust. Although a range of natural enemies were found to prey upon the various life stages, their impact on brown locust populations in the present study was negligible. Of particular interest was a study of the impact of the sarcophagid fly, *Wohlfarhtia pachytyli*, which is a well-known facultative parasite of late instar brown locust hoppers and fledglings. However, field data suggested that the potential of the fly as a biological control agent may have been over estimated in the past, as the fly failed to cause more than 6% mortality of fledgling swarms in the present study.

Before the first insecticides became available at the turn of the 20th century, farmers had to resort to mechanical methods to protect their crops and pastures from the ravages of locusts. Turning back the clock, the destruction of locust egg beds and the harvesting of locusts were re-examined as control methods. Excavation of locust eggs gave effective control, but the disturbance of the friable soils in the Karoo would damage the vegetation cover and cause severe erosion problems

and is therefore not advocated. Harvesting of live locusts using nets or vacuum machines was not practical due to the avoidance behaviour of locusts. However, the harvesting of locust cadavers lying on the soil surface following insecticide spraying, once they had dried out and insecticide residues had broken down, was possible. With their high protein and fat content, the processing of locust cadavers into animal feed may become economically viable in future.

Before organo-chlorine insecticides became available in the 1940s, bran bait containing sodium arsenite was extensively used for brown locust control. The baiting technique was re-evaluated in the present study using minute dose rates of the phenyl-pyrazol insecticide, fipronil, dissolved in water and mixed into wheat bran as the edible carrier. Bran bait containing 0.02% fipronil 200SC (Regent®) was prepared on site and was broadcast by hand onto the soil surface around bushes occupied by hopper bands as overnight roosting sites.

Excellent control (>95%) of small and medium sized hopper bands was achieved, as long as baiting was undertaken shortly after sunrise, before hoppers scattered from the baited area. Baiting large band targets, or baiting later in the day once hoppers became active, was not effective. Baiting with 0.02% Regent® proved very effective if applied to compact, roosting hopper bands. It was also inexpensive and was easy to prepare and apply, requiring basic equipment and limited training. However, the logistics of the bulk transport, preparation and application of locust baits under operational conditions appear daunting.

Insecticide barrier treatments using fipronil (Adonis® 5UL), applied to 21m-wide strips of Karoo vegetation at a dose rate of 12.5g a.i./ha, were used to intercept gregarious brown locust hopper bands marching through the veld. Barriers of Adonis® proved very effective against mobile L2-L3 bands and against small L4-L5 bands, giving >90% control within 48 hours. However, barriers sometimes failed to adequately control large and mobile L5 bands that had sufficient momentum to march through barriers before the majority of hoppers acquired a lethal dose of Adonis®. Barriers also proved less effective where the vegetation density was sparse or where the vegetation was unacceptable to locusts. The size and density of the hopper bands and the time of day when bands made contact with the barriers also appeared to influence efficacy.

Despite these factors, Adonis® barriers were still considered to have potential for the control of brown locust hopper bands in the more remote areas of the Karoo, especially during the early stages of an outbreak when hopper bands are still young. However, barriers would have to be judiciously applied to restrict the environmental impact of Adonis® against non-target organisms. Large-scale operational trials are recommended.

Insect Growth Regulators (IGRs) have shown promise when applied as barrier treatments against various locust and grasshopper species. However, laboratory experiments with the IGRs, flufenoxuron and teflubenzuron, applied to leaf discs and fed to L5 brown locust hoppers at dose rates of 3-15µg/g, gave variable mortality of 30-70%, with most mortality occurring as the hoppers attempted to moult. In another experiment, diflubenzuron (Dimilin OF6®), was sprayed onto maize plants at volume rates of 1-3ℓ/ha and subsequently fed to L2 brown locust hoppers in the laboratory. Dimilin OF6® produced 100% mortality of L2 hoppers within 11 days at all application rates, as long as hoppers were continuously exposed to treated vegetation. However, irregular exposure to Dimilin® during the inter-moult period produced unsatisfactory mortality, as the product is evidently non-accumulative and is readily excreted.

The fact that brown locust hoppers have to be regularly exposed to IGR-treated vegetation, combined with the sporadic feeding behaviour and high mobility of brown locust hopper bands in the Karoo, would probably make IGR barriers unsuitable for brown locust control operations.

In collaboration with IIBC and the LUBILOSA programme (CABI Bioscience, Ascot, UK), the locust-killing fungus, *Metarhizium anisopliae* var. *acridum*, was imported and evaluated by PPRI locust researchers as a myco-insecticide agent in laboratory and field trials against the brown locust. Under suitable application conditions the myco-insecticide, applied at a standard dose rate of 100g conidia/ha, regularly produced >90% mortality of hoppers maintained in cages, although speed of kill was slow, with median lethal times of 10.3 and 13.4 days for the ground and aerial application trials respectively. In most cases, acceptable >90% mortality was not achieved for at least three weeks after application.

Despite the slow speed of kill, the myco-insecticide agent was considered a significant advance in locust control and the product was subsequently registered as Green Muscle® in South Africa in 1998. However, the lack of a knock-down action and the slow kill currently makes Green Muscle® unsuitable for operational use in the Karoo. The thousands of individual hopper bands treated during control campaigns, and the high mobility of bands, would make the recognition of treated and untreated targets by locust officers impossible. The hot and dry Karoo climate is also usually detrimental for the survival and transmission of fungal conidia, while the thermoregulation behaviour of brown locust hoppers enables them to effectively delay the onset of *Metarhizium* mycosis. An alternative application strategy needs to be developed and tested before Green Muscle® can be recommended for brown locust control.

Other pathogenic micro-organisms evaluated in the laboratory for brown locust control were certain acid-tolerant strains of *Bacillus thuringiensis* and an entomopoxvirus isolated from a West African grasshopper, *Odaleus senegalensis* (De Geer). Unfortunately, none of these micro-organisms proved virulent to the brown locust.

The alternative locust control methods evaluated against the brown locust were all ranked according to various performance criteria and compared with the conventional spraying of ULV insecticides. Of the alternative control methods, only Adonis® barrier treatments and Regent® bait showed sufficient promise for brown locust control. However, none of the alternatives were considered suitable under all locust control situations to entirely replace the spot spraying of conventional ULV insecticides, which will thus remain the backbone of brown locust control strategy. Recommendations on the development of an IPM strategy for brown locust control, to incorporate barrier treatments and baiting in certain areas of the Karoo in order to complement conventional insecticide spraying, are given.

KEYWORDS: Brown locust, *Locustana pardalina*, alternative control methods, natural enemies, mechanical control, baiting, fipronil barriers, insect growth regulators, Green Muscle myco-insecticide, microbial agents, IPM strategy.

CHAPTER 1

Background

1.1 Locusts as pests

Locust plagues have threatened agricultural production in Africa for thousands of years and locusts have become entrenched in fact and folklore as one of the continent's most notorious pests (Steedman, 1990; Meinzingen, 1993). Even today, outbreaks of locusts still have the potential to overwhelm control capacity and to threaten the food security of many nations in Africa, despite the modern application technology and the range of effective insecticides currently available. The dilemma between the political and economic need to control locust outbreaks, while at the same time reducing the environmental damage caused by the application of insecticides and limiting the high financial cost of control campaigns, has become a controversial issue.

Locusts are members of the insect Order Orthoptera, the Suborder Caelifera, the Superfamily Acridoidea and the Family Acrididae, which are commonly known as the 'short-horn' grasshoppers. Grasshoppers occur in many shapes and sizes, although the basic morphology of the Acrididae is that they possess antennae with less than 30 segments and which are shorter in length than the body, tarsi that are 3-segmented, a large hind femur thickened at the base and modified for jumping and a pronotum that does not extend posteriorly over the abdomen (Uvarov, 1966).

There are well over 10 000 described species of grasshoppers and many hundreds of species have been recorded as economically important pests of agriculture and grazing lands throughout the world (COPR, 1982). However, only between 12-15 grasshopper species are regarded as true locusts (Uvarov, 1966; Steedman, 1990; Meinzingen, 1993) due to their ability to occur in different morphological and behavioural states, known as phases.

1.2 Phase theory

The theory of phase change in locusts was proposed by Uvarov (1921) as an explanation for the origin and disappearance of gregarious populations of the Asian migratory locust, *Locusta migratoria*

migratoria L. in Russia. However, evidence for phase change was first demonstrated experimentally in the brown locust, *Locustana pardalina* (Walker), in South Africa by Faure (1932), who showed clear differences between the solitary phase and gregarious phase phenotypes within the same species, depending upon whether the insects were reared in crowded conditions or not. Solitarious and gregarious phases are now considered to constitute the two extremes of a continuous polymorphic series, with most locust populations occurring with intermediate phenotypic characteristics known as the transient phase (Kennedy, 1956; Uvarov, 1966).

Phase polymorphism is a complex phenomenon and the morphological, behavioural and physiological differences between solitarious and gregarious phases can be striking (Faure, 1932; Kennedy, 1956; Uvarov, 1966; Dale and Tobe, 1990). When locusts occur at low densities they are cryptic green or brown in colour and typically feed, bask and roost as individuals. The solitarious hoppers are sedentary in habit, avoiding association, although solitarious adults may undertake long distance nocturnal dispersal flights associated with weather disturbances (Davey, 1959; Farrow, 1990). However, when solitary locust hoppers occur at high density they undergo the process of gregarisation (Uvarov, 1966), which is an endocrine response stimulated by visual and tactile stimulation and pheromone (olfactory) communication factors (Dale and Tobe, 1990; Roessingh, Bouaichi and Simpson, 1998).

As the density of locusts increases they form aggregations characterised by the unidirectional migration of the hopper bands and adult swarms. The bands and swarms maintain cohesion and reassemble if disturbed; the gregarious swarms roost, bask, feed, fly and oviposit as dense aggregations. If aggregation occurs in the early hopper stages there are also changes in the colour, morphology and physiology of the gregarising insects, the degree of which depends upon the intensity of aggregation. However, when gregarious insects are isolated some of these changes are reversible and solitarious traits become dominant. Although phase change has been studied for over 75 years, the complex interaction of factors influencing density-dependent responses and the adaptive function of phase change is not fully understood and has attracted new interest (Roessingh, Simpson and James, 1993; Simpson, McCaffery and Hägele, 1999).

1.3 Southern African locusts

Practically all continents have plague locusts and some species, such as *Locusta migratoria* and *Schistocerca gregaria,* have evolved different subspecies in different continents. Four recognised plague locust species occur in the southern African geographical region, defined as Africa south of the Cunene, Okavango and Zambezi rivers, namely:

African migratory locust, *Locusta migratoria migratorioides* (Reiche & Fairmaire, 1850)
Brown locust, *Locustana pardalina* (Walker, 1870).
Red locust, *Nomadacris septemfasciata* (Serville, 1838).
Southern African desert locust, *Schistocerca gregaria flaviventris* (Burmeister, 1838).

All four of these locust species occur in South Africa and all have produced outbreaks that have required chemical control operations in the southern African region during the past decade. However, the brown locust, *L. pardalina,* is by far the most economically important locust species and is considered to pose a significant threat to agricultural production in South Africa (Botha, 1969; Lea, 1973), with populations periodically reaching plague proportions (Lea, 1953).

1.4 Brown locust

The brown locust is a member of the Oedipodinae subfamily of grasshoppers and is endemic to the semi-arid Karoo regions of South Africa, southern Namibia and southern Botswana (Faure and Marais, 1937). Within this sparsely populated semi-desert country, the brown locust has a recognised outbreak region covering an area of approximately 250 000 km^2 (Faure and Marais, 1937; Lea, 1958; Kieser, Thackrah and Rosenburg, 2002), which mainly lies within the northern Cape Province of South Africa (Fig.1.1). During the plague cycles in the early part of the 20^{th} Century, the invasion area overrun by swarms extended up to the Zambezi river (Fig.1.1), threatening agricultural production throughout the entire southern African region (Lea, 1964; 1970).

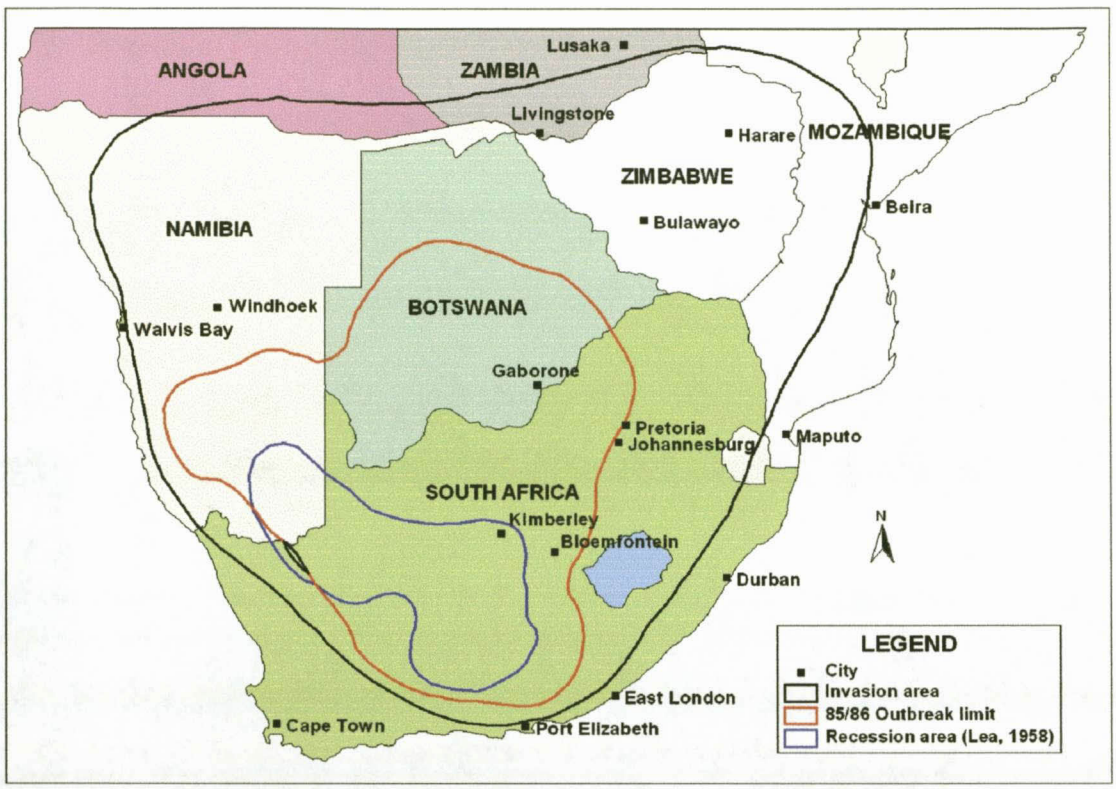

Fig.1.1. Outbreak region and plague invasion area of the brown locust. Limits of the 1985/86 plague are also depicted, courtesy of H.D. Brown and P. L. Napier Bax.

1.5 Karoo outbreak area

1.5.1 Climate

The brown locust outbreak area mainly lies within the Nama Karoo biome (Rutherford and Westfall, 1986) which occupies the central plateau of the western half of South Africa at altitudes between 500 and 2000m, with most of the biome falling between 1000 and 1400m. The climate of the Karoo is dominated by anti-cyclonic weather systems situated over southern Africa (Desmet and Cowling, 1999), causing hot summers (mean maximum air temps of 32-35°C in January) and cool winters (mean maximum air temps of 16-19°C in July). Frost is common in winter at Upper Karoo altitudes. Rainfall varies from approximately 400mm per annum in the eastern Karoo and decreases to <100mm in the western parts. Rainfall is very erratic with wide annual deviation from the long-term means. The northern, central and eastern Karoo is considered a late summer and autumn rainfall area, with rain

generally falling as convection showers under the paths of thunderstorms. Winter rainfall associated with cold frontal systems sometimes occurs in the western (Namaqualand) and southern Great Karoo.

1.5.2 Geology and soils

The complex geology of the Karoo has been the subject of various conflicting interpretations, with an overview of current views given by Meadows and Watkeys (1999). The Great Escarpment is the geologically dominant feature of the Karoo and runs from east to west, dividing the flat plains of the Upper Nama Karoo from the Great Karoo to the south. The Great Escarpment is an ancient geological feature composed of highly deformed and metamorphosed sedimentary and volcanic rocks, with peaks rising to over 2000m in the eastern Karoo.

The geology of the Upper Nama Karoo is dominated by flat-lying sedimentary sandstones, mudstones and shales of the Karoo Supergroup, with the grey 'Dwyka Group glacial tillites' and the black and red 'Ecca shales' found in the north and the grey 'Beaufort Group' shales and sandstones found further south. The Quaternary sands and sedimentary rock formations in Bushmanland and the Central Karoo have produced an extremely flat topography resulting in numerous silty flats (known as 'vloere') associated with internal drainage. In the eastern Karoo the Beaufort sandstones and shales have been intruded by dolerite dykes and sills which have produced flat-topped hills, typical of the Karoo landscape. South of the Great Escarpment the geology of the Great Karoo is dominated by the flat-lying Beaufort Group sediments, while these same sedimentary rocks have become deformed in the Cape fold belt mountains further to the southwest.

The soils of the Karoo have been well mapped and studied, with a recent overview provided by Watkeys (1999). In semi-arid environments, the weathering of the underlying rock, leaching of nutrients and humus production is typically low and results in the very slow production of poor quality soils. Likewise, the Karoo soils vary according to the underlying rock strata and have usually been formed *in situ* by local weathering, which has produced shallow, weakly developed soils with a low organic content. Free-draining red and yellow granitic sandy soils are common in the north and north-western Karoo (Namaqualand), while weakly structured lime-rich clays and sandy loams

derived from the Karoo Sequence sandstones and shales dominate the upper central, eastern and southern Karoo. Low rainfall and limited leaching has resulted in the development of saline soils over calcrete strata in many areas. Although <5% of the annual rainfall reaches the seasonal rivers, the shallow Karoo soils are highly erodible and are easily damaged by flash floods and overgrazing.

1.5.3 Vegetation

Vegetation within the Karoo is dominated by perennial dwarf Karoo bushes and annual desert grasses. Trees are sparse and only occur naturally along drainage lines. Vegetation composition in the Karoo is determined by the topography, soil type and the annual distribution and quantity of 'effective' rainfall (Acocks, 1988; Palmer, Novellie and Lloyd, 1999). The high frequency brown locust outbreak areas fall within three 'veld-types' defined by Low and Rebelo (1998) as 'Eastern Mixed Nama Karoo', 'Upper Nama Karoo' and 'Bushmanland Nama Karoo' (formally described by Acocks (1988) as 'False Upper Karoo', 'False Arid Karoo/Central Upper Karoo' and 'Arid Karoo' respectively).

Dwarf, perennial Karoo bushes are the dominant vegetation on the rocky, loamy and silty soils over most of the Karoo, but the Karoo bushes become progressively replaced by grass in areas receiving >400mm rainfall in the Eastern Mixed Karoo. Annual desert grasses are the dominant vegetation on the sandy soils in the arid north and northwest (Bushmanland Nama Karoo). The grasses found in the summer rainfall areas are generally of the C4 type that respond rapidly to early summer rainfall and high temperatures, while grass cover is usually sparse in the winter rainfall areas of the western and southern Karoo (Milton and Dean, 1996). The perennial Karoo bushes mainly grow following rainfall in autumn and winter as long as temperatures remain above 5°C. Drought resistant 'succulent' vegetation ('vygies') assumes more importance in the drier western and south-western areas.

Vegetation density can vary significantly between years according to the distribution and quantity of rainfall, with local rainfall events triggering the lush growth of grasses and other annual plants, such as *Tribulus* spp. and *Mesembryanthemum* spp. ('opslag'). However, the percentage vegetation canopy cover of perennial plants that live for two years or more is generally between 20-40% in the eastern Karoo and between 10-20% in the more arid central, western and southern Karoo (Milton and

Dean, 1996). Large herds of antelope, eg. springbuck (*Antidorcas marsupialis*), used to roam the Karoo in search of seasonal grazing, but by the end of the 19th Century the herds had been largely exterminated. The more remote areas of the Bushmanland Nama Karoo were finally fenced off into privately owned sheep farms during the 1930s.

The Eastern Mixed Nama Karoo is considered prime sheep ranching country and has been intensively farmed for mutton and wool since the late 1800s. The Bushmanland Nama Karoo can be productive during good rainfall seasons, but long-term droughts make stock farming on small farms marginal in these areas. According to Acocks (1988), the selective grazing of the annual grasses and the palatable Karoo bushes by sheep, combined with over optimistic stocking rates by the early settlers, has caused the Great Karoo, False Upper Karoo and Upper Central Karoo vegetation types to have become degraded to near desert or poor quality 'Arid Karoo' in many areas. This has resulted in a decline in the stock carrying capacity of the veld during the past century (Dean and Macdonald, 1994).

Although the diet selection by sheep varies in different areas of the Karoo and also between seasons (Du Toit, Bloem and Immelman, 1995), the long-term stocking rates recommended by the National Department of Agriculture are currently 1 small stock unit per 2-3ha/year in the Eastern Mixed Karoo and 1 unit per 5-8ha/year in the Arid Karoo. However, better veld-management practices, driven by a realisation of the long-term limitations of the Karoo for animal production, have enabled some veld recovery to occur in certain areas (Dean and Macdonald, 1994).

1.6 Biology of the brown locust

The brown locust displays extreme phase polymorphism and sexual dimorphism (Du Plessis, 1939), with the differences between the solitaria and gregaria phase being more extreme than in any other locust species (Uvarov, 1966). The biology and population dynamics of the brown locust have been well studied in the laboratory and the field (Faure, 1923; Potgieter, 1929; Du Plessis, 1938; Smit, 1939, 1960; Lea, 1968, 1969). The brown locust is considered a typical r-strategist, capable of rapid population growth and is multivoltine, producing 2-3 generations between the months September-May (Smit, 1939), with 4 generations recorded under exceptional circumstances (Lea, 1958).

The solitarious phase of the brown locust lays eggs that have an obligate diapause, which is usually broken after 9-45 days under dry soil conditions, while the gregarious phase lays only non-diapause eggs that develop continuously to hatching under warm and wet conditions (Matthee, 1951). Transient phase locusts lay eggpods containing differing proportions of diapause and non-diapause eggs, depending upon factors such as day length, phase, age and nutrition of the female (Matthee, 1951). Under dry conditions the non-diapause eggs, as well as diapause eggs that have broken out of diapause, stop developing further and enter a state of quiescence. This only occurs at a single embryological state just before katatrepsis takes place (Matthee, 1951). Quiescent eggs are very drought tolerant and have been known to survive for 2-3 years under dry conditions in the field, (Potgieter, 1929; Botha, 1967; Price, *pers.obs.*). However, Botha (1967) dismisses any eggs surviving for more than 12 months as irrelevant to the population dynamics of the brown locust.

An effective rainfall of 20-25mm is considered necessary for the widespread hatching of eggs (Smit, 1939; Price, 1988a) with non-diapause eggs hatching after 10-14 days under warm and moist conditions. There are five hopper instars, with solitarious hoppers developing within 21-30 days (Smit, 1939). Gregarious phase hoppers are larger and develop more slowly, with each instar taking 7-12 days to develop under summer field conditions; gregarious hopper bands thus typically develop in 42-45 days (Smit, 1939; De Wet and Webb, 1951; Price, 1988a). Under field conditions the fledgling adults mature within 2-3 weeks and gregarious females will lay 3-4 eggpods containing a mean of 45 eggs/pod at weekly intervals between pods (Price, 1988a). Under laboratory conditions the females mature faster and may lay 10-15 egg pods (Faure, 1923). Gregarious adults live for 2-3 months in summer and for a longer period under cooler conditions (Faure, 1923; Coetzee, 1994).

1.7 Outbreak frequency

Outbreaks of the brown locust develop almost annually somewhere or other in the Karoo, although the intensity of outbreaks varies greatly between years. The high outbreak frequency, measured by the number of Magisterial Districts in South Africa, southern Namibia and Botswana where brown locusts were chemically controlled each year is shown in Fig.1.2, which confirms that there have only been 5 years in the past 55 when no chemical control was used by the Department of Agriculture

(Price and Brown, 2000). The brown locust has the highest outbreak frequency of any African plague locust (COPR, 1982). Cycles of swarming activity over the past 50 years have generally lasted 10-12 years with short, drought induced, recession periods in between (Fig.1.2).

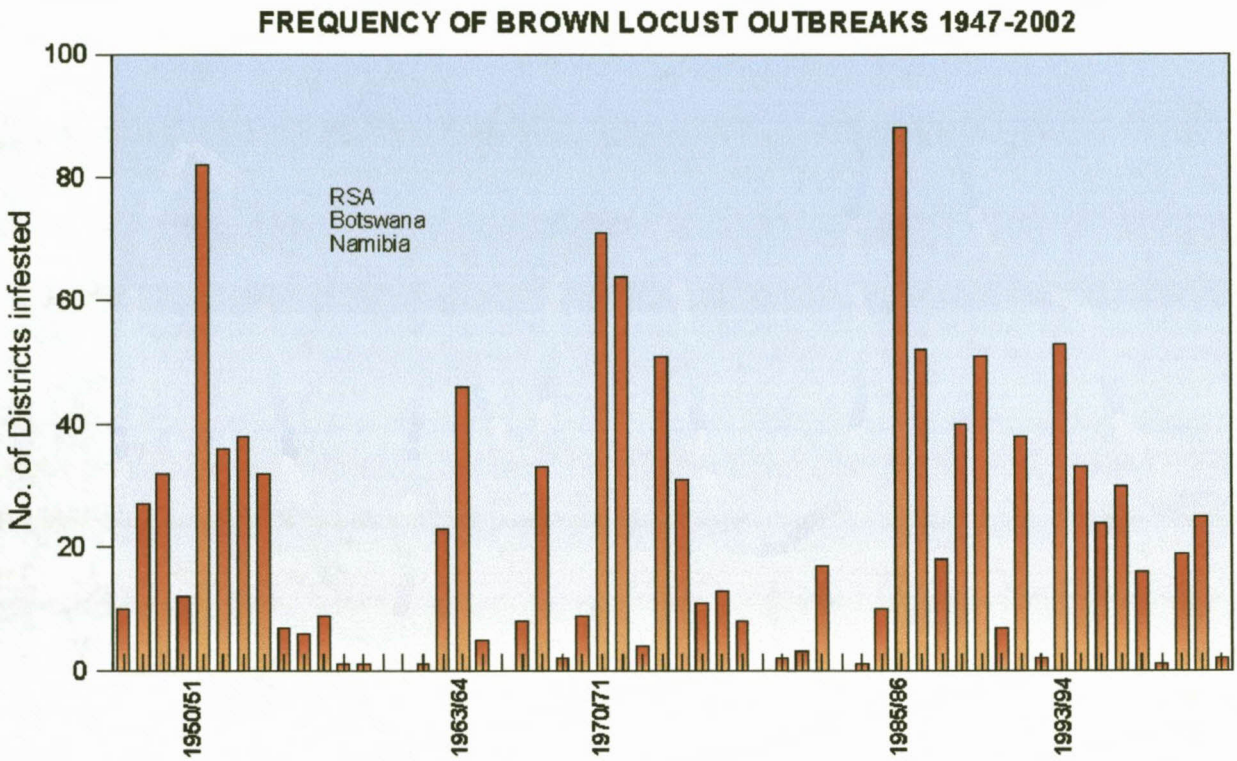

Fig. 1.2. Frequency of outbreaks of the brown locust 1947 – 2002. The dates of notable plague seasons are shown. Data courtesy of H.D. Brown and M. Kieser.

The Magisterial Districts in South Africa that have recorded the highest incidence of gregarious outbreaks, where chemical control has been used in >25 of the past 55 years, are the Kenhardt, Carnarvon, Britstown, Hopetown, De Aar and Hanover districts in the Northern Cape Province. Large-scale outbreaks have tended to occur once or twice per decade, following widespread drought-breaking rains, and are characterised by the dramatic increase in the density of the solitary phase adult population over wide areas of the Karoo leading to crowding of locusts and phase transformation (Du Plessis, 1938; Smit, 1941; Lea, 1969). Favourable rainfall and temperature conditions then allow the synchronised development of widespread, incipient outbreaks. Following this initial upsurge the gregarious swarming populations may then persist for a few years by exploiting the different summer

and winter rainfall areas within the Karoo. The gregarious populations are the focus of intense chemical control campaigns, but the last of the swarms usually only die out due to unfavourable climatic conditions, such as drought or the onset of winter, which prevents further breeding.

1.8 History of brown locust control

Before the first arsenical insecticides became available at the turn of the 20th century, farmers were at their wits end trying to save their crops and pastures from the ravages of locusts (Anon., 1907; Brown, 1988). Previous efforts to control hopper bands by spraying soap solution, paraffin oil and cattle dip formulations, or by setting fire to the grassland, were largely ineffective. In response to the locust threat to agricultural production, the South African Government first sponsored chemical control campaigns against the brown locust in 1906 when, in terms of Government aid to farmers (defined in Government Notice 1036 of 1905), arsenite of soda solution and spray pumps were issued to farmers free of charge by the Resident Magistrate in each District (Lounsbury, 1906; Anon., 1907). The history of brown locust control is summarised in Table 1.1.

Table 1.1. History of brown locust control in South Africa.

Before 1906	Mechanical and cultural control methods, such as harvesting locusts, digging up eggbeds, trampling and beating hopper bands to death, burning pastures, soap, paraffin sprays, sheep dip and arsenic baiting.
1906-34	Sodium arsenite (liquid spray and dusting powder)
1934-45	Sodium arsenite bait (bran bait broadcast by hand to hopper bands)
1945-87	Organochlorine insecticide: Benzene hexachloride (BHC & gamma-BHC) as bait and EC formulations, mainly as a wettable powder (dry or in aqueous solution) from vehicle-mounted dusting and spray machines.
1975-94	Organophosphate insecticides: Diazinon + dichlorvos (DDVP) and fenitrothion applied as UL formulations.
1990-to date	Synthetic pyrethroids: (fenvalerate, esfenvalerate and deltamethrin UL formulations)
Future	New products recently registered for brown locust control include fipronil (Adonis®), alpha-cypermethrin (Fastac®) and *Metarhizium anisopliae* var. *acridum* mycoinsecticide (Green Muscle®).

1.9 Current control strategy

The pro-active control of sub-swarming brown locust concentrations has always been considered

uneconomic and impractical (Du Plessis, 1939; Lea, 1968) and brown locust control has always relied on an emergency response to upsurges of swarming populations. Brown locust outbreaks have been traditionally combated at source within the Karoo outbreak region, in a suppressive control strategy that can be described as upsurge elimination (Lecoq, 2001), before the migrating swarms can escape and threaten cereal crop producing areas outside the Karoo. The current control strategy relies on the application of broad-spectrum, fast-acting contact insecticides, applied as ultra low volume (ULV) drift sprays to individual hopper band or fledgling swarm targets.

Landholders are legally required to report the presence of locusts on their land while locust control is the responsibility of the Directorate of Land and Resource Management, National Department of Agriculture (NDA). There are a number of policies and legislation relating to the management of the locust problem in South Africa, the most important of which are 'Article 6 of the Agricultural Pests Act of 1983 (Act No.36 of 1983)' and the 'Fertilizer, Farm Feeds, Agricultural Remedies and Stock Remedies Act (Act No.36 of 1947)'. The revised National Policy on locust control was complied by a Ministerial Policy Committee in 1998 following consultation with a wide range of stakeholders.

1.10 Locust control tactics

Locust control is undertaken using the 'commando system' whereby an army of temporary employed locust officers track down and control individual locust targets. The main target of control are the L4-L5 hopper bands as they are easier to locate and spray than the mobile adult swarms. Locust control operations are coordinated from three regional centres, situated at De Aar, Upington and Kimberley, where large stocks of formulated insecticide and spraying equipment are stored. The regions are subdivided into locust control 'districts' that are usually based on Magisterial boundaries. A District Locust Officer is appointed by the NDA and supervises control actions in each 'locust district'. Residents from the local community are appointed on a temporary basis during outbreaks as supervisor-drivers and pest control operators (assistants) to form the 'commando system'. The District locust officers, supervisors and assistants claim day-wages and all travel costs using private vehicles are remunerated (Peters, Lindeman and Van der Westhuizen, 2000). A training manual for locust control operators is available (Heyns, Greyvenstein and Van der Westhuizen, 1995).

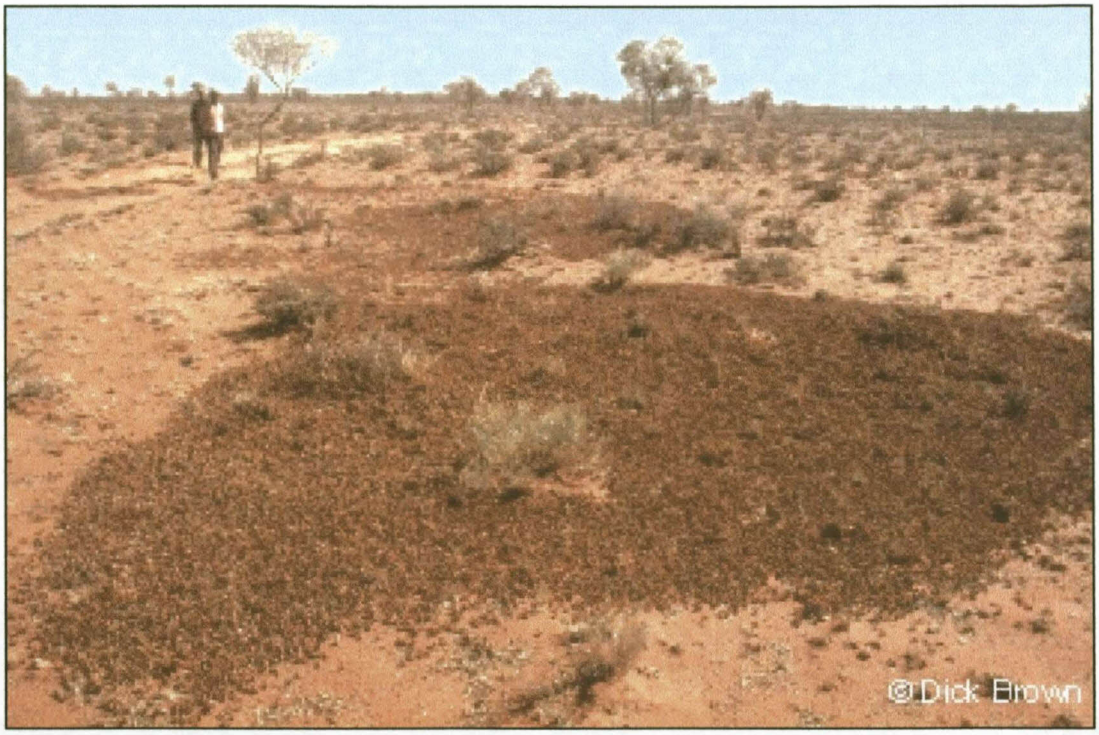

Fig.1.3. Gregarious brown locust hopper bands basking and feeding around their roosts, form ideal targets for the spot application of insecticide.

Fig.1.4. Roosting brown locust swarms are generally 10-14ha in extent and are usually sprayed at night using vehicle-mounted spray equipment.

As briefly mentioned, brown locusts are controlled by the spot application of fast-acting synthetic pyrethroid insecticides, applied as ULV drift sprays to individual hopper bands and fledgling swarm targets early in the morning while still occupying their overnight roosts (Heyns, Greyvenstein and Van der Westhuizen, 1995). Roosting hopper bands are generally small in size (0.1-1ha) but are very densely aggregated (Fig.1.3), with densities of over 5000 hoppers/m^2 recorded. Roosting adult swarms average 10-14ha in size (Fig. 1.4), although large swarms may be hundreds of hectares in extent. Once the hopper bands descend from their overnight roosts and begin to march during mid-morning the control operations are usually abandoned for the day. Large adult swarms are usually sprayed throughout the night by a fleet of vehicles before the swarms start to fly in the morning.

Insecticide is mainly applied using motorised 'Solo Port' (Model 423) knapsack and 'Power Solo' vehicle-mounted spray machines, which are calibrated to deliver a volume application rate of 2.5ℓ/ha. In general, the smaller band targets are controlled with the knapsack Solo Port mistblower, fitted with an ultra low volume orifice device, while larger bands and swarms are controlled with the Power Solo (Heyns, Greyvenstein and Van der Westhuizen, 1995). Spray aircraft, both fixed-wing and helicopters, are occasionally employed during large-scale outbreaks. During a typical outbreak season some tens of thousands of locust targets are controlled (Table 1.2), requiring the application of hundreds of thousands of litres of formulated insecticide. Seasons where low numbers of control actions were undertaken, e.g. 1992-93 and 1998-99 (Table 1.2), were drought years. In these years the egg population remained quiescent in the soil until good widespread rains fell the following season and triggered mass hatching from the dormant eggbank.

During the past 50 years the commando system of locust control has worked well and swarming populations of the brown locust have largely been confined to the Karoo, with only short-term invasions of neighbouring countries reported, such as Botswana in 1986 and Lesotho in 1994. Virtually no crop damage outside the Karoo has been reported and the threat to food security within the SADC region has been averted. Although large-scale locust control campaigns have been regularly conducted in the Karoo, the prevention of plagues has been a success (Price and Brown, 2000).

Table 1.2. The number of locust targets controlled each season in the Karoo between 1985 and 2002 by officers of the National Department of Agriculture.

Season	Hopper bands	Adult swarms
1985-86	175 500	38 600
86-87	68 902	14
87-88	5 618	1 123
88-89	85 935	12 642
89-90	36 553	1 392
90-91	1 142	357
91-92	18 131	1 603
92-93	72	0
93-94	34 581	9 565
94-95	20 895	663
95-96	24 489	6 577
96-97	75 890	8 081
97-98	1 018	80
98-99	2	0
1999-2000	40 115	9 021
2000-2001	28 642	1 135
2001-2002	1 905	137

During the period 1985-2002 an average of 36 435 hopper bands and 5 352 adult swarms were controlled per season in the Karoo by the NDA.

1.11 Recurring outbreaks

Before large-scale chemical control intervention became possible, the period of major brown locust swarming activity (plagues) generally lasted 7-11 years with similar periods of low activity (recessions) in between (Lea, 1968). Despite the intense locust control campaigns undertaken over the past 50 years however, the frequency and intensity of outbreaks has actually become greater in the recent past, with fewer years of recession between major outbreaks (Fig.1.2) (De Villiers, 1988; Price and Brown, 2000). Lea (1968) came to the conclusion that even though the chemical control

campaigns significantly lowered the locusts' biomass during plagues and prevented large-scale emigration of swarms, the widespread application of insecticide failed to prevent the initiation or curtail the duration of outbreaks. Lea (1958b) postulated that chemical control campaigns actually broke-up the swarming population, preventing the outbreaks from fully gregarising and burning themselves out. Chemical control may actually be perpetuating a locust problem in the Karoo by preventing the 'locust pot from boiling over'.

1.12 Locust control insecticides

Following the massive 1985-86 control campaign, the chlorinated hydrocarbons BHC and gamma-BHC (as WP and EC formulations) were finally withdrawn and were replaced by the ULV organophosphate insecticides, fenitrothion and diazinon + dichlorvos, as the mainstay of brown locust control (Pretorius, 1976; Brown, 1988). However, numerous bird poisonings resulting from organophoshate (OP) overdosing, and alarm expressed by the National Department of Health over the high number of locust control officers requiring hospital treatment due to cholinesterase inhibition during autumn 1994 (T. Heyns, *pers. comm.*, former Deputy Director, Directorate of Resource Conservation), caused the Government to ban the use of OPs for locust control. This opened the door for the adoption of safer synthetic pyrethroid insecticides as alternatives to the OP sprays.

1.13 Synthetic pyrethroid insecticides

In search of new and promising insecticides for brown locust control, the Locust Research Division of the ARC-PPRI evaluated various UL formulated insecticides, including the synthetic pyrethroid insecticide, deltamethrin (Decis®). These were tested under a range of climatic conditions and veld types in the Karoo between 1986 and 1991. Hundreds of field trials of Decis®, mainly applied from Micron Micro-Ulva® spinning-cup, hand-held, sprayers were undertaken against gregarious L4 and L5 hopper bands – which are the main control targets. Decis® produced a rapid knockdown action and was effective under a wide range of environmental conditions (Brown, 1988; Brown and Kieser, 1997). The rapid knockdown of treated locusts served to keep them within the treated area where further dose uptake was maximized. This also served as a vital marker of successful control for the

locust officers and largely eliminated the wasteful and environmentally damaging re-spraying of locust targets that was such a problem with the slower acting OPs (Brown and Kieser, 1997). To overcome the problem of the recovery of treated locusts following application of sub-lethal dose rates of Decis®, which is typical of the mode of action of synthetic pyrethroids against a range of insects, a relatively high dose rate of 15-17.5g a.i./ha Decis® was found to be the minimum effective dose rate against the brown locust, producing >90% mortality within 72 hours.

Decis® was also evaluated under operational locust control conditions (Brown and Kriel, 1994) and was commercially registered for locust control with the South African NDA in compliance with Act 36 of 1947 (Vermueulen, Krause, Nel, Hollings and Greyling, 1992). Other synthetic pyrethroid insecticides, namely esfenvalerate (Sumi-alpha®) and alpha-cypermethrin (Fastac®) have since also been registered for brown locust control. Sumi-alpha® has been applied operationally as a ULV spray and as a dust formulation. Typical speed of knockdown and mortality of L5 brown locust hoppers (n=5 trials) following application of Decis® 7 UL, applied at the registered area dose rate of 17.5g a.i./ha using a hand-held Micron Ulva+ sprayer under summer conditions, are depicted in Fig.1.5.

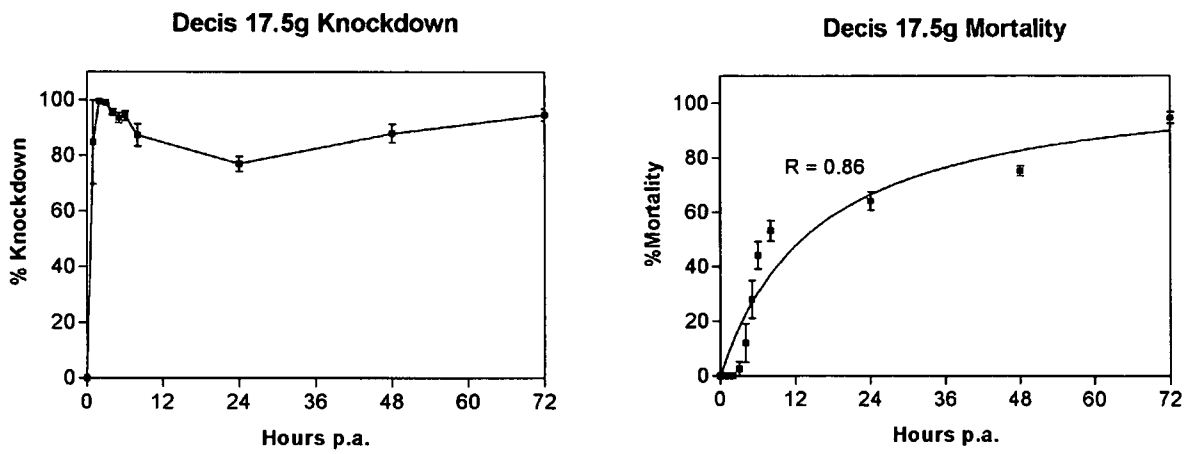

Fig.1.5. Typical speed of knockdown and rate of mortality produced by Decis® 7 UL, applied at a standard dose rate of 17.5g a.i./ha, against gregarious L5 brown locust hoppers.

Decis® 7 UL applied at a dose rate of 17.5g a.i./ha produced a rapid knockdown of L5 hoppers with first toxic effects evident within 20 minutes and >90% recorded as moribund within 1 hour. The initial

heavy knockdown effect was followed by the partial recovery of some hoppers (Fig.1.5), but most of these hoppers relapsed again and were recorded as moribund after 48-72h post-application. Mortality generally started 4-5 hours after application and reached >90% after 72h (Fig.1.5).

1.14 Environmental impact

The high priority given to acridid control in many countries has often provided the driving force for the development of new insecticides in agriculture, such as the introduction of the arsenite compounds in the early 1900s and the organochlorines and organophosphates in the 1940s (Riegert, Ewen and Lockwood, 1997). These insecticides were initially hailed as miracle compounds that gave man the upper hand in the war against locusts, even though most of the early insecticides were very toxic. However, the vast amount of development work undertaken during the past 50 years on the evaluation of new insecticides for acridid control has also resulted in the steady introduction of environmentally safer insecticides, as evident with the phasing out of the organochlorines and some of the organophosphate products (Riegert, Ewen and Lockwood, 1997). Likewise in the Karoo, there has been a long history of research into more effective and safer insecticides for use against the brown locust (Brown, 1988; Price and Brown, 2000). Despite these developments, the environmental impact of acridicides has become a contentious issue and serious questions are being raised regarding the environmental sustainability of conventional acridid control in many countries (Peveling, 2001). There is no doubt that even the modern, so called 'environmentally softer' and non-persistent synthetic pyrethroids, carbamates and organophosphate insecticides currently used for locust control still cause high mortality of non-target invertebrates within sprayed areas (Everts, 1990; Peveling, 2001).

Large-scale aerial spraying tactics, employed against the desert locust during the mid 1980s, are known to produce serious environmental damage as there is a slow recruitment of non-target organisms back into block sprayed areas (Everts, 1990). In contrast, the small-sized spray plots (usually <1ha) typically treated with Decis® during brown locust control operations produced less medium and long-term impact on the non-target fauna as there was a fairly rapid recolonisation of the spray plots by invertebrates from the surrounding untreated areas (Stewart, 1998; Roux, 1998).

1.15 Current problems with brown locust control

1.15.1 Operational problems

Although the commando system has been effective in suppressing brown locust plagues over the past 50 years, the intense locust control campaigns waged almost annually in the Karoo have become more difficult to sustain. Operational costs have escalated rapidly due to high transport and insecticide costs, while changing demographics and the ongoing depopulation of many areas of the Karoo, due to depressed farming conditions, has reduced the efficiency of the outbreak reporting and control network that formed the basis of the commando system. The high number of absentee farmers and locked farm gates also has serious consequences for locust control operations as outbreaks on the unoccupied farms often now go unreported and the control capacity can be overwhelmed by swarm escapes. More effective methods of controlling locusts in the remote areas of the Karoo are urgently required as an alternative to the location and spot treatment of individual locust targets. In response to these problems the Locust Research Division of PPRI activated a project titled 'Alternative Control Strategies' with the author as project leader.

1.15.2 Environmental concerns

Although the spot application of synthetic pyrethroid insecticides to roosting brown locust targets is very effective and has a relatively transient environmental impact, the high frequency of brown locust outbreaks has meant that chemical control is undertaken on some farms almost every season. The repeated application of broad-spectrum insecticides in the unique Nama Karoo biome, with its high diversity of endemic invertebrates and reptiles, is thus a serious cause for concern and there is increasing demand for more environmentally acceptable and sustainable methods of controlling the brown locust. This is especially important for National Parks and designated conservation areas, for 'organic' farming areas relying on residue-free animal products for export and on farms where landholders currently object to the application of ULV insecticides. South Africa also has obligations under Agenda 21 to reduce the environmental risks associated with pesticide application and to develop environmentally sound alternatives (UNCED, 1992).

CHAPTER 2

Natural Enemies and Diseases

2.1 INTRODUCTION

Despite regular locust outbreaks in the Karoo and the intensive research undertaken over the past 100 years into the population dynamics and chemical control of the brown locust, there is surprisingly little recent information available on the impact that predators, parasites and diseases have upon brown locust populations (Erasmus, 1988). Although the role that natural enemies and disease organisms play in regulating the population dynamics of the brown locust is not clear, the local impact of insect natural enemies can sometimes be high, with isolated records of >90% mortality of eggpods caused by various beetle and fly larvae and 50-90% mortality of hopper populations caused by larvae of the sarcophagid fly, *Wohlfahrtia pachytyli* Townsend (Potgieter, 1929). A wide range of opportunistic predators such as mammals, birds and reptiles together with scorpions, spiders, ants and robber flies also take their toll (Botha and Lea, 1970). Our knowledge of all the known insect parasitoids and predators of Acridoidea has been reviewed by Greathead (1963; 1992).

There has been a remarkably low incidence of fungal or bacterial disease recorded amongst brown locust populations in the field (Smit, 1939; Lea, 1964). Fungal epizootics, caused by the grey enotomopathogenic fungus, *Entomophaga grylli* (Fresenius), were occasionally observed amongst brown locust swarms that invaded the humid areas of northern Namibia and Zimbabwe during the locust plagues of the 1920-30s (Skaife, 1925; Jack, 1931), although the literature does not state whether any *E. grylli* epizootics were recorded in the semi-arid Karoo. However, fungal epizootics probably occur on rare occasions in the Karoo as certain locust officers recall observing adult brown locust cadavers covered with a grey fungus (probably *E.grylli*) during the unusually wet summer of 1974/75 in the eastern Karoo (John Watermeyer, Aberdeen District, *pers. comm.*).

Despite the low incidence of disease recorded in the field, there is often high and dramatic mortality amongst brown locust colonies maintained in the laboratory due to infections by the entomopathogenic fungus, *Beauveria bassiana* (Bals) Vuill. (Prinsloo, 1962). Mortality due to bacteria infection by *Serratia marcessens* (Bizo) has been reported from brown locust populations in the Karoo (Prinsloo, 1960; Bateman, Price, Müller and Brown, 1994), while it can regularly decimate laboratory colonies (Prinsloo, 1960; Price, *pers.obs.*). According to Venter (1966), the parasitic protozoa, *Malamoeba locustidae* King and Taylor, is also a major cause of mortality in laboratory cultures of the brown locust, while heavy infestations in the field are believed to reduce the fecundity and cause the early demise of field populations.

A long list of bird species have been observed feeding on the various locust stages, including birds which are normally seed eaters, such as certain lark species (Botha and Lea, 1970; Erasmus, 1988). The local decimation of hopper bands and fledgling swarms by flocks of White Storks (*Ciconia ciconia)*, White-bellied storks (*Sphenorhynchus abdimii),* Cattle egrets (*Bubulcus ibis),* Lesser Kestrels (*Falco naumanni*) and Wattled starlings (*Creotophora cinerea)*, has regularly been reported by locust officers (Potgieter, 1929; Botha and Lea, 1970). The potential of White storks as important predators of brown locusts has been described by Lea (1964). Small mammals, such as bat-eared foxes and various mongoose species, have been observed feeding on brown locusts (Botha and Lea, 1970; Price, *pers. obs.*) and a number of lizard species, especially the lacertid, *Pedioplanis lineo-ocellata*, regularly prey on young instar hoppers (Price, Seesink and Brown, 1996).

Of the principal invertebrate natural enemies of the brown locust, Van Schalkwijk (1939) considered the sarcophagid fly, *W. pachytyli*, to be the most promising candidate as a biological control agent and discussed the possible mass rearing and augmentative release of flies. Although *W. pachytyli* is often associated with carrion in the Karoo and southern Namibia, the fly is also a well-known facultative parasite of late instar hoppers and fledglings of the brown locust (Van Schalkwijk, 1939; Greathead, 1963). Locusts dying as a result of parasitism by fly maggots can be commonly found at the base of Karoo bushes occupied as overnight roosting sites by hopper bands or fledging swarms. The first report of *W. pachytyli* parasitising the brown locust dates back to Bairstow (1894), while

high infestation rates of 50-90% were recorded in hopper bands and fledgling swarms by Potgieter (1929), who considered the fly to be an important natural enemy contributing to the decline of brown locust outbreaks. However, apart from anecdotal reports from locust control officers, little recent information on the level of *W. pachytyli* parasitism within brown locust populations is available.

Field and laboratory observations on the impact of invertebrate natural enemies of the brown locust, especially the locust fly, *W. pachytyli,* are now described. The work on the locust fly also incorporates observations made by former colleagues, J. Arbuthnot, F. McLaren and L. Seesink. The development of a myco-insecticide for locust control, incorporating spores of a locust-killing fungus, will be described in a later chapter.

2.2 MATERIALS and METHODS

2.2.1 Invertebrate enemies of the egg stage

Surveys were made of ten brown locust eggbeds in the Namaqualand and Britstown districts in autumn 1991 and the Aberdeen district in winter 1994. Eggbeds were usually difficult to find and we relied on information from locust officers where gravid locust swarms had been recently sprayed or where farmers had observed oviposition taking place. Eggbeds were located by searching for areas of disturbed soil, caused by the diggings of small mammals within the eggbeds. Eggpods were found by brushing away the soil surface along ten line transects, measuring 30x1m, with a yard-broom or by scraping the top 1cm of soil with a spade to reveal the white froth plugs of the eggpods *in situ*. Transects were selected where the soil surface could be readily brushed and scraped within different areas of the eggbed. Eggpods found along transects were marked with matchsticks and samples of intact eggpods (n=50-70) were excavated to determine the incidence of parasites and predators.

2.2.2 Distribution of the locust fly, *Wohlfahrtia pachytyli*

The distribution of *W. pachytyli* was investigated by capturing adult flies in 12 house-hold fly traps (Red Top®), erected for 72h periods at various localities in the Karoo during 1992-96. Most traps

were erected during the summer and autumn months when flies were most numerous. Further records were also obtained by collecting parasitised locusts from the base of bushes after the hopper band or swarm had departed from their overnight roost sites. Such stragglers were collected by hand and placed in a jar of damp soil where the maggots were allowed to emerge and pupate. Additional records of *W. pachytyli* were determined from specimens in the Locust Research Division's natural enemy collection held at Rietondale and the National Collection of Insects, ARC-PPRI, Pretoria.

2.2.3 Impact of *W. pachytyli*

The incidence of fly parasitism in L5 brown locust hopper bands, fledgling swarms and migrating adult swarms was determined by collecting samples (Table 2.1) directly after they had been sprayed with Decis® 7 UL. The insecticide produced a paralysis lasting 3-5 days and which conveniently spanned the 2-5 day larval development period of the parasite at 20-35°C (Price, Seesink and Brown, 1996). Such paralysed locusts were collected by hand and placed in plastic buckets, measuring 40x30x25cm, containing a 5cm layer of damp sand in which the emerging parasite larvae pupated over the next few days. Buckets were sealed with nylon netting and were maintained at room temperature for 5-7 days until fly larvae had all emerged from the locust cadavers. The number of locust cadavers and fly puparia sieved from the sand were used to calculate percentage parasitism.

2.3 RESULTS

2.3.1 Invertebrate enemies of brown locust eggs

Surveys of four eggbeds near Aggenys in Bushmanland found an average of 12% (range 9-15%) infestation by larvae of the woolly bee fly, *Systoechus* sp. (Diptera: Bombyliidae), in samples of eggpods (n=60-70) excavated for examination. Generally, only one yellow coloured *Systoechus* larva was found per eggpod, with two larvae per pod found on only two occasions. At the time of survey, damage within individual eggpods was low and undamaged eggs hatched in the laboratory. A follow-up survey to two of the above eggbeds in winter, approximately four months after the eggs had hatched, found *Systoechus* larvae (n=15) overwintering in the remnants of eggpods. However, at least

some hoppers had hatched from eight of these pods, which proved that *Systoechus* predation at these low levels of infestation did not destroy entire eggpods.

Systoechus was rare in six other eggbeds sampled in the Britstown and Aberdeen districts with an average of one larva (range 0-2) recorded in samples of intact eggpods (n = 50) excavated from each eggbed. Adult *S. albidus* flies were commonly observed in Bushmanland during the summer months, although attempts to rear larvae in the present study under a range of laboratory conditions failed, with only two *S. albidus* adults eventually emerging over 15 months later. Greathead (1963) also had limited success in rearing *Systoechus* larvae in the laboratory.

Various beetle larvae (Tenebrionidae and Elateridae spp.) were found feeding within overwintering brown locust eggpods in the Aberdeen district, but levels of infestation in five samples were <2% (n=250 eggpods). Two *W. pachytyli* larvae were also found overwintering in these eggpod samples. The only beetle larvae successfully reared and identified were *Zophosis sp.* (Tenebrionidae), which are generalist scavengers in the soil. A *Scelio* sp. (Hymenoptera: Proctotropidea) was recorded on rare occasions from solitarious brown locust eggpods found in maize fields in the Free State Province (Price, 1988b), but was not found in any of the eggbeds sampled in the Karoo. According to Greathead (1963), *Scelio* spp. only attack pods laid by solitary locusts and grasshoppers and are not, for example, recorded from desert locust eggbeds in North Africa

2.3.2 Mammal and bird predation of eggs

The conspicuous diggings of small mammals and fragments of partially eaten eggpods scattered on the soil were observed at seven of the ten eggbeds examined. Although there were no direct observations on the small mammals responsible for digging up eggbeds, it is likely that suricates ("stokstertmeerkat") and porcupines were mainly responsible. A collection of mammal scats found on the eggbeds were forwarded to the Mammal Research Unit, Pretoria University, who also confirmed that droppings from black-backed jackal, bat-eared fox and various field rodents were present. There were also indications from bird tracks on some of the eggbeds that eggs had been scratched out by guinea fowl, kori bustards and blue cranes.

The intensity of the diggings on the eggbeds gradually increased during the winter and by the date of hatching in mid October an estimated 10% of the area of some eggbeds, measured by pacing out affected areas, showed evidence of diggings. In addition, the soil disturbed by the diggings effectively covered other pods with a soil overburden which was observed to prevent hatching from buried pods, although no estimates of this indirect mortality were obtained.

2.3.3 Invertebrate enemies of hoppers

Predation of brown locust hatchlings was quantified on only one occasion, following the mass hatching from an eggbed that had overwintered on the farm Mountain View, Aberdeen district. The eggbed measured 3.5ha in area with a mean eggpod density of 59.4 pods/m^2 (range 10-72 pods/m^2, n=10 quadrats) containing a mean clutch of 44.7 (22-67) eggs per pod (n=50 pods). Hatching was first observed at 10h30 on 10 October 1994 at an air temp. of 24°C and a soil surface temp. of 44°C. By 12h00 thousands of eggpods were hatching and the soil was covered with pale-coloured hatchlings engaged in discarding their vitelline membranes. The hatchlings later assumed the black appearance characteristic of gregarious phase hoppers. Under the overcast conditions occurring at the time, the hoppers formed conspicuous dense basking parties on the bare patches of soil.

During the peak period of hatching a series of ten transects, measuring 100x2m, were walked across the eggbed and the incidence of predation of hatchlings by *Camponotus fulvopilosus* ants was recorded. A total of 13 ant colonies were observed actively seizing hoppers along transects (mean of 1.3 ant colonies per 100m transect). Other predators observed were two lizards *(Lacertidae* sp.), two hunting spiders (Lycosidae), one robber fly (Asilidae), one preying mantis and one bird (Ant-eating Chat). Mass hatching of hoppers evidently stimulated *C. fulvopilosus* ants into great activity with thousands of ants running around searching for prey. Ants usually attacked hoppers shortly after they emerged from the eggpod and while still trying to shed their white vitelline membranes. Hoppers were seized in the mandibles and carried aloft back to the nests. Rate of predation, measured by recording the number of hoppers carried back to individual ant nests (n=5 nests) during 15 minute observation periods, averaged about one hopper per minute (range 11-21 hoppers/ 15 min). Although ants were initially very active predators their level of activity soon declined. The 'pugnacious ant',

Anoplolepis custodiens, was also observed as a predator of brown locust hoppers in the eastern Karoo, confirming observations by Lea (1964).

The locust hunting wasps, *Priononyx viduatus* (Critz.) and *P.subfuscatus* (Dahlbom) (*Sphegidae*), were sometimes found associated with L5 hopper bands and fledgling swarms where they attacked and paralysed individual locusts before dragging them into their burrows. Wasps deposited a single egg on the hind femur of the locust host, thus provisioning the wasp larvae with food. Although previously unrecorded as predators of the brown locust (Fred Guess, Albany Museum, pers.com.), *Priononyx* wasps were widely distributed in the Northern Cape Province during the summer months.

Robber flies (Asilidae spp.) were occasionally observed hawking early instar hoppers in the present study and were considered to be effective predators of solitarious brown locust hoppers by Smit (1939). Isolated records of larvae of the sarcophagid fly, *Blaesoxipha filipjevi*, parasitising brown locust adults, were obtained from near Hopetown and Van Wyksvlei, complimenting the record of *B. filipjevi* from Graaff-Reinet (Van Someren Greve, 1965). Other occasional predators of the brown locust observed in the current study included various scorpions, spiders, solifugids and centipedes. These are all opportunistic predators and prey on locusts when available.

2.3.4 The locust fly, *W. pachytyli*

2.3.4.1 Distribution

Localities from which *W. pachytyli* flies were collected by various locust researchers during 1992-1996 are plotted in Fig.2.1. Since observations were made at different times, the localities in Fig 2.1 indicate the presence of the fly and not its relative abundance. The fly was widely recorded across the Northern Cape Province, Eastern Province and southern Free State in a distribution that closely coincides with the recognised outbreak region of the brown locust, as described by Lea (1958).

Adult *W. pachytyli* flies were recorded during every month of the year, but were most numerous during late summer and autumn (February-April). Flies were most common in the Namaqualand, Kenhardt, Carnarvon and Prieska districts of the Northern Cape Province, where they sometimes

reached nuisance status. Although usually associated with brown locust populations, fly maggots were also found on carrion, such as sheep, bat-eared fox and mongoose

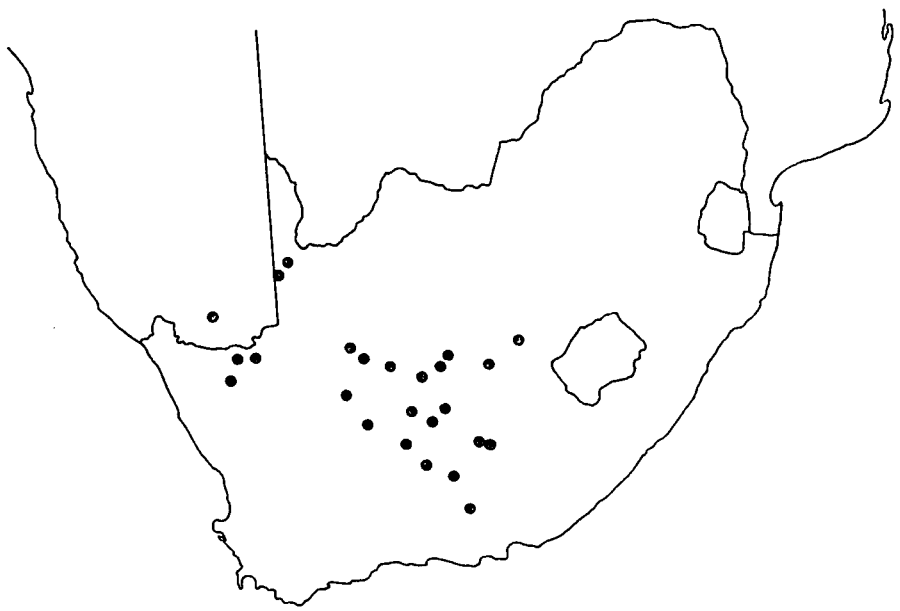

Fig.2.1. The distribution of the locust fly, *W. pachytyli,* in the Karoo region.

2.3.4.2 Impact

Incidence of parasitism by *W. pachytyli* flies of different brown locust stages is shown in table 2.1.

Table 2.1. The incidence of *W. pachytyli* parasitism in gregarious brown locust populations.

No. locusts sampled	Locust stage	% parasitism	Range (%)
13 000	L5 hoppers	0.1	0 - 0.3
2 000	Fledglings	5.7	0 - 6.0
2 000	Migrating adults	0	0

Levels of parasitism in L5 hoppers, sampled from 30 hopper bands in the Britstown, Herbert, Middelburg and Prieska and Carnarvon districts were negligible (0.1%), while highest parasitism levels were found in fledgling adults in the Carnarvon district (Table 2.1). No parasitism was detected

in any samples taken from migrating swarms (post teneral adults >10 days old), indicating that parasitised fledglings had already dropped out of the swarm before migration occurred.

Observations on moulting brown locust hoppers, undertaken by Ms L.D. Seesink at the ARC-PPRI laboratory, showed that the moult from L4 to L5 hoppers averaged ten minutes at 34°C (n=25 hoppers). However, fledging, i.e. the final moult into the adult stage, averaged 31 minutes at 34°C (n=25), which provided more time for the fly to parasitise the immobilised locusts. The locust was thus more vulnerable to attack from flies at this stage and this observation may possibly explain the higher rate of parasitism found in field collected fledglings compared with hoppers (Table 2.1).

2.4 DISCUSSION and CONCLUSION

The possibility of using classical biological control to regulate locust and grasshopper populations, using indigenous or introduced natural enemies, has long fascinated locust control workers. However, almost all such introductions have failed (Prior and Greathead, 1989); some success against the rice grasshopper, *Oxya chinensis*, in Hawaii has been claimed, while the only sustainable biological control record followed the introduction of the Indian Myna bird (*Acridotheres tristis)*, in 1762 to control the red locust, *N. septemfasciata*, in Mauritius (Prior and Greathead, 1989). Greathead (1992) concluded that migratory locust species are poor targets for classical biological control and that natural enemies have little impact on gregarious locust populations. According to Prior and Greathead, (1989) and Greathead (1992), the mass rearing and augmentative release of insect enemies for locust control is not viable.

Natural enemies with a classic "k" reproductive strategy can rarely produce effective control a "r" strategist having a chaotic or irruptive nature, such as the brown locust (Lea, 1964; Chapman, 1976; Wallner, 1987). With its multivoltine life cycle and high reproductive rate, brown locust populations are able to increase rapidly during favourable climatic conditions and overwhelm the more static populations of their natural enemies. Such rapid fluctuations in locust populations are also not

conducive for the continuous maintenance of high numbers of obligate predators and parasites, especially in a harsh environment like the Karoo. The strong migratory behaviour of the brown locust also enables swarms to vacate outbreak areas before the local natural enemy complex can build-up sufficiently to exert appreciable biological control.

Surprisingly few invertebrate natural enemies of gregarious brown locusts were found during numerous surveys in the Karoo. Although all locust life stages are prone to attack and suffer variable mortality, there was no evidence that any of the natural enemies gave effective control of brown locust populations examined in the present study. This supports the view of Lea (1964; 1968) and Botha and Lea (1970) that natural enemies have minimal impact on the outbreak dynamics of gregarious populations of the brown locust.

The facultative locust fly, *W. pachytyli,* was found to be widespread in the Karoo, although the rates of parasitism within brown locust populations under natural conditions were usually negligible. Hoppers were only vulnerable to attack from flies during their moulting periods, especially while fledging into the adult stage. Maximum parasitism rates of 6% in fledgling swarms were recorded during the present study. However, due to the large numbers of locusts contained in a swarm, even these low rates of parasitism resulted in numerous locusts being left behind at fledgling sites. This has possibly led to over estimates of the degree of parasitism of brown locusts by *W. pachytyli* in the past.

Predation of overwintering eggs was mainly due to the diggings of small mammals and birds whose excavations caused up to 10% mortality. Further mortality was also caused by eggpods being buried with soil overburden from these diggings which prevented the hatchlings from escaping from their pods. The digging-up of brown locust eggbeds in the Karoo by small mammals and birds has been reported by Potgieter (1929), while burying of eggpods during early spring ploughing of maize fields was an important mortality factor of overwintering eggs of the African migratory locust, *L. m. migratorioides*, in the Free State Province (Price, 1988b).

The low level of mortality of eggs due to insect predators was probably due to the fact that most of

the eggbeds examined were laid at the start of winter and hatched following the first good rains and rising temperatures, giving little opportunity for insect predation to take place. The low impact of natural enemies on brown locust populations in the present study contrasts sharply with the high levels of predation and parasitism of eggs and hoppers reported in the 1920s by Potgieter (1929). For example, larvae of the locust egg fly, *Stomorhina lunata* (Calliphoridae) and *W. pachytyli* (Sarcophagidae), were regarded as the most important predators of brown locust eggs in the past, with each of these flies occasionally causing up to 90% destruction of eggpods (Potgieter, 1929). However, during the current surveys no *S. lunata* and only two *W. pachytyli* larvae were recorded. A larger eggbed sample thus needs to be investigated in the Karoo, including a study of eggbeds that lie dormant through a summer season, before conclusions can be drawn.

It is well known that the most vulnerable stage of the life cycle of locusts is during the first few days after hatching. For example, post-natal mortality of hatchlings of *L. m. migratoriodes* can be >90% within the first 24h due to unfavourable environmental factors and high levels of predation (Farrow, 1975; Price, 1988b). However, apart from isolated observations on predation, the factors affecting the mortality of brown locust hoppers in the field are virtually unknown. Brown locust hoppers are relatively difficult to rear in the laboratory and only thrive under hot (30-35°C) and relatively dry (RH<50%) conditions. High mortality of hatchlings occurs under cool and damp conditions, especially when confined in cages with too much green food. It has also been observed that hatchlings can survive for 2-3 days on their residual yolk reserves and then tend to initially feed on dry grass or dry wheat bran before later consuming green grass.

The Karoo ecosystem has been subjected to regular chemical control interventions for nearly 100 years with a range of insecticides, from arsenic to synthetic pyrethroids, being repeatedly applied for locust control. It is possible that the repeated and sometimes extensive application of insecticide has depleted the background levels of natural enemies, but further investigation was beyond the scope of this study. In future it is important to undertake locust control in the most environmentally benign methods possible in order to conserve the available natural enemy complex in the Karoo.

During the incidental observations undertaken during the present study, the impact of recorded natural enemies and diseases on brown locust populations appeared negligible and was clearly unable to prevent locust upsurges from occurring.

2.5 BENEFITS and CONSTRAINTS

2.5.1 Potential benefits of natural enemies

- Background levels of natural enemies will theoretically increase if no insecticides are applied, which will lead to increased levels of biological control.
- Huge cost savings and environmental benefits from not having to spray insecticides.

2.5.2 Potential constraints

- Natural enemies are unreliable and unpredictable control agents.
- Natural enemies are slow to act and will not provide direct crop protection.
- Natural enemies are unable to suppress large-scale locust eruptions.

CHAPTER 3

Mechanical control of the brown locust

3.1 INTRODUCTION

Before arsenite of soda insecticide became available for brown locust control in 1906, farmers had to rely on a range of home remedies and mechanical control methods to save their crops and pastures from locusts, which included beating hopper bands with sticks and wire whips, trampling hoppers under the hooves of stock animals or by digging trenches to trap marching bands (Anon., 1907). The collection of locusts into sacks or the physical digging up of locust eggbeds was another popular method of locust control, especially as the Governments of the Cape Colony, Orange River Colony and Natal offered a bonus of two shillings for every sack (muid bag) of hoppers collected and one shilling per bag of fliers. However, this scheme was soon abandoned as the hundreds of thousands of bags collected threatened to bankrupt these Colonies (Anon., 1907).

The age-old physical methods of protecting crops from the ravages of locusts are still practised in various parts of the world. Rural communities in Madagascar use the smoke from fires and actively disturb the locusts to prevent swarms settling in their crops, while the digging up of eggpods for the control of the pest grasshopper, *Zonocerus variegatus* L., in cassava fields in West Africa has been undertaken on a local scale (Page, 1978). The mechanical disturbance of soils in crop areas by the early ploughing or by harrowing the soil between rows of maize plants was an effective method of destroying eggpods of the African migratory locust (*L. m. migratorioides)*, in the north-west Free State Province, South Africa (Price, 1988b).

Following a report to the South African NDA on the damage caused to brown locust eggbeds by small mammals and birds (Price, Seesink and Brown, 1996), the NDA requested additional information on the possible mechanical destruction of locust eggbeds in the Karoo. Since the harvesting of swarms has been advocated as an alternative to the use of insecticides for locust control in the Karoo, a preliminary study of harvesting of brown locusts was also undertaken.

3.2 MATERIALS and METHODS

3.2.1 Mechanical destruction of eggs

Three overwintering eggbeds of the brown locust were located on the farm Chelmsford in the Aberdeen District, Eastern Province, during May 1994. The soil surface was brushed away using a stiff yard-broom or was scraped away with a spade along transects through the eggbeds to reveal the froth plugs of the eggpods *in situ*. Areas with a high density of eggpods (>80 eggpods/m^2) were identified within each eggbed (n=3) and a series of $1m^2$ quadrats (n=7) were marked off and dug over with garden forks to expose the eggpods. Visits were made to the excavations after 24 and 48 hours and then 35 days later to check on the fate of the exposed eggpods. Control quadrats (n=3 in each eggbed), containing similar high densities of unexcavated eggpods, were then covered with a thin layer of soil and marked for later examination. In a separate experiment at one of the above eggbeds, three batches of 500 individual locust eggs that had been broken open from excavated pods and placed out on the soil surface were examined 24 and 48 hours later.

3.2.2 Harvesting of locusts

Harvesting live adult locusts by sweep netting was briefly investigated on the farm Gelukspan in the Prieska District during April 2000 where a large swarm roosted overnight. The mass of locusts harvested by individual collectors (n=3), using 50cm diameter sweep nets during a 20 minute collection time, was weighed using a spring balance.

Attempts were also made to collect live L5 hoppers and fledglings from their overnight roosts by means of a hand-held, commercially available, garden leaf collector (Wolf Garden Master®). The collector acted like a vacuum cleaner powered by a 2-stroke motor. The investigation was undertaken near Griquatown under cool autumn conditions in 1996 when the locusts roosted as dense aggregations on tufts of grass and within spiny Karoo bushes. In a comparative trial undertaken near by, the vacuum leaf collector was used to suck up locust cadavers that had been sprayed one week previously with Decis® 7UL during normal control operations.

3.3 RESULTS

3.3.1 Mechanical destruction of eggs

The digging up and exposure of brown locust eggpods caused 100% mortality. Eggpods exposed by the digging began to disappear within 24-48h and after 35 days no trace of the eggs, apart from a few remnants of the froth plugs, could be found. Although the tracks of various small mammals and birds were observed on site, it could not be established whether eggpods had been removed by these potential scavengers. Likewise, batches of 500 eggs scattered on the soil surface all disappeared with 48h. Numerous ants, especially *A. custodiens* (the pugnacious ant), were observed carrying away individual eggs within minutes of exposure. *Zophosis* sp. beetles were also observed feeding on the exposed eggs. No similar loss of intact eggpods was observed in the control quadrats, with the pods eventually hatching in mid October. Mortality in the controls (n=150 pods) was low with 4.5-6.1% loss of eggs due to desiccation and <1% loss due to damage by insect larvae; a total of five *Systoechus* sp. (Diptera: Bombylidae) larvae and two larvae of a Tenebrionidae beetle were found. The diggings of small mammals and birds were observed in some other areas of the eggbeds.

3.3.2 Harvesting of locusts

Although brown locust swarms typically roost as a dense mass on low Karoo bushes, the harvesting of adult locusts in nets was impractical. Under the cool morning conditions the sample swarm remained immobilized on its overnight roost for at least an hour after sunrise, but as temperatures rose the locusts were easily disturbed and could avoid capture. The densely packed locusts were either swept directly from the top of the bushes or were physically shaken from the bushes into nets placed on the ground. Individual collectors (n=3) each captured 25-30kg of locusts within the initial 20 minute collection time. However, as temperatures rose the locusts were scattered by the disturbance or retreated into the interior of the thorny Karoo bushes, where the nets readily snagged and made the further collection impossible. Collecting roosting locusts at night would greatly extend the harvesting period, although this would require additional lighting.

The hand-held vacuum collector proved inefficient for collecting live locusts as they soon became

disturbed by the noise of the machine and the movements of the operator. Locusts either dropped to the ground and scattered, or retreated into the interior of the bushes and formed a huddled mass of bodies that were virtually impossible to extract. Maneuvering the bulky vacuum collector into the depth of the spiny bushes was very difficult, while chasing the scattering hoppers was ineffective and only succeeded in sucking up a detritus of sand, dry leaves, sticks and sheep droppings.

The vacuum collector, however, proved an effective method of collecting dry cadavers of hoppers and adult locusts lying on the open soil surface. In some areas the cadavers lay in drifts under Karoo bushes and had accumulated in hollows in the ground where they were easy to collect. However, the cadavers were light and bulky and the collection bag of the machine soon became full. Dry plant detritus was also readily sucked up and the process of regularly having to empty and restart the machine proved laborious and time consuming.

3.4 DISCUSSION and CONCLUSION

The mechanical destruction of brown locust eggbeds was an effective method of locust control. However, the physical disturbance or ploughing of the soil in semi-arid areas such as the Karoo, where most of the soils were found to be shallow and easily eroded, is not advocated as it would likely cause serious environmental damage.

The physical collection of live locusts using nets or a vacuum collector was ineffective as the disturbance caused the locusts to scatter or to retreat deep into the interior of the vegetation. Harvesting of live locusts in the current trials did not provide a viable alternative to the application of insecticides for locust control. However, the collection of insecticide-treated locust cadavers lying on the soil surface, using vacuum machines or by hand, is certainly possible. Insecticide residues are known to break down comparatively rapidly under Karoo conditions of high temperatures and strong sunlight. Following application of the standard dose rate of 17.5g a.i./ha Decis®, residues on Karoo vegetation dropped to <0.5ppm within 17d post-application (Stewart, Seesink and Du Preez, 1997).

The utilization of harvested brown locusts for animal feed or as a fertilizer was investigated nearly a 100 years ago (Anon., 1907) and dried locust meal mixed with hay was commonly sold as food for live stock animals and poultry in the Northern Cape Province during the 1920s (Jack, 1924). With the spiraling costs of imported animal feeds, it may soon become economically viable to re-examine harvesting of locusts for processing into animal feeds. During large-scale brown locust outbreaks thousands of swarms are controlled in the Karoo, which represent an enormous quantity of insect protein. For example, some exceptionally large brown locust swarms have been estimated to contain up to 50 billion insects weighing 50 000 tonnes (Cilliers, van Someren-Greve and Lea, 1964). However, more practical methods of collecting locusts need to be developed before the question of mechanical methods of control can be considered.

3.5 BENEFITS and CONSTRAINTS

3.5.1 Potential benefits

- Harvesting locusts would be labour intensive, creating jobs and business opportunities in previously disadvantaged Karoo communities.
- Commercial utilisation of harvested locusts for processing into animal feeds.
- If harvesting of live locusts became practical it would benefit the environment.

3.5.2 Potential constraints

- Disturbance of soil for control of locust eggs would cause environmental damage.
- Little labour is available in remote areas for harvesting locusts. There are also huge logistical problems involved in the bulk collecting, transportation and storage of locusts.
- Commercial companies processing locusts into animal feeds require a regular supply of harvested locusts – supplies will be unavailable during years with no outbreaks.

CHAPTER 4

Brown locust control by means of selective baiting

4.1 INTRODUCTION

The semi-arid Nama and succulent Karoo eco-region is considered one of the world's biodiversity 'hotspots', rich in endemic invertebrate and reptile species, and is considered a priority area for conservation (Olson and Dinerstein, 1998; Peveling, 2001). As mentioned earlier, the repeated application of broad-spectrum ULV insecticides for locust control in the environmentally sensitive Karoo is being increasingly questioned by landholders and conservationists (Price and Brown, 1997). How to reduce the insecticide load in the Karoo and yet at the same time control locust outbreaks has become a controversial issue. There is therefore an urgent need for more cost effective and environmentally benign methods of locust control.

One way of reducing the environmental risk associated with locust control is to modify the mode of application and to deliver the insecticide in a more target-specific manner. Baiting offers one method of presenting a toxic dose to the pest, while at the same time reducing the chances of non-target organisms coming into contact with the insecticide.

In South Africa, bran bait containing 2-3% arsenite of soda was extensively used against brown locust and red locust hopper bands during the 1930s, applied by hand at rates of 70-135kg/ha (Coaton, 1939; Du Plessis and Botha, 1939). Extensive field testing of various bait carriers such as wheat bran, maize meal, oats, sawdust and even horse dung, showed that moistened wheat bran was the most acceptable carrier (Du Plessis and Botha, 1939; De Wet, 1941). The addition of sugar, salt or vegetable oil made no difference to the palatability of bait to hoppers. Arsenite bait was only phased out for brown locust control when the organochlorine insecticide, benzene hexachloride (BHC), became available in 1945. Insecticide baits, using wheat or maize bran as an edible carrier, were successfully used for locust control in different parts of the world for many years (Steedman, 1990). Baits have now largely been replaced by oil-based ULV sprays because of the logistical problems

involved with bulk transport and storage of baits. Until recently, however, bran bait containing carbaryl, applied from aircraft or vehicle mounted motorised spreaders, was used for the control of rangeland grasshoppers in the western USA (Onsager, Henry, Foster and Staten, 1980).

In the search of more cost effective and environmentally acceptable methods of controlling the brown locust, the baiting technique was re-evaluated as an alternative to the cover spraying of insecticide. Bran bait containing the non-ester pyrethroid insecticide, silafluofen (Neophan®), was evaluated against L5 brown locust hoppers in the laboratory and in small-scale field trials (Price and Brown, 1997). Bait containing 0.2% silafluofen 80 EC was considered optimal for the control of the brown locust, but unfortunately the product was not presented by the manufacturer for commercial registration in South Africa under Act 36 of 1947.

In search of other promising insecticides for incorporation into locust bait, laboratory bioassay and field trials of bait containing the phenyl pyrazol insecticide, fipronil, are now described. The potent stomach action of technical-grade (96%) fipronil against brown locust hoppers was demonstrated by Butler and Du Preez (1994) and highlighted the potential of fipronil as a bait preparation. The current chapter describes laboratory bioassays and field trials with fipronil bait.

4.2 PART 1 : LABORATORY BIOASSAY

4.2.1 MATERIALS and METHODS

4.2.1.1 Bait preparation

Fipronil 200 SC (Regent®) was supplied by Rhône-Poulenc (Pty) Ltd, Pretoria. Serial dilutions of this formulation were prepared with distilled water and thoroughly mixed volume/mass with dry wheat bran to produce bait containing 5, 15, 25, 50, 100, 200 and 400 parts per million fipronil. Bait was then allowed to air dry. Bran mixed with distilled water only was prepared as a control.

4.2.1.2 Test insects

Bioassays were undertaken against laboratory reared L5 hoppers, the progeny of parents collected from hopper bands in the Karoo. Hoppers were reared in metal cages, measuring 40x25x25cm, and were fed a diet of green maize leaves, green kikuyu grass and dry wheat bran at a temperature range of 26-30°C and a 12:12h photoperiod. Hoppers were used for bioassay 4-5 days after the moult to the L5 stage. In order to standardise the dose response of insects, only hoppers weighing between 0.5-0.6g, of both sexes, were used in the experiments. Hoppers were starved for 24h prior to exposure to bait to standardise feeding response.

4.2.1.3 Exposure periods and bait acceptability

Previous work has shown that starved hoppers became satiated within ten minutes of continuous feeding and that there were no significant differences between the quantity of bran consumed by individual hoppers during a 10, 30 or 60 minute exposure period (Kieck and Price, 1992). A ten minute exposure time was therefore subsequently used as standard throughout the bioassay work.

Choice experiments were first undertaken to determine whether Regent® bait, at the two highest concentrations, was acceptable to hoppers. Small heaps (0.5g) of moistened 200ppm and 400ppm Regent® bait and untreated wheat bran were placed at random on the floor of metal-gauze locust cages (n=3), and batches of ten hoppers, starved for 24h, were randomly introduced into the cages. The feeding preferences of the hoppers were recorded over a ten minute observation period.

4.2.1.4 Toxicity of bait

Hoppers (3 replicates of 10 insects) were exposed to each of the seven concentrations (5-400ppm) of Regent® bait in plastic containers measuring 18x25x30cm. A rate of 180mg dry weight of bait per container, equivalent to an extrapolated field application rate of 40kg/ha, was used for each dose trial. The dry bait was moistened with 10mℓ distilled water to make it more acceptable to hoppers before it was scattered over the base of the containers. Batches of control hoppers were confined over clean bran in the same manner. After ten minutes exposure to bait the batches of hoppers were removed, immersed in distilled water to remove any bait adhering to their bodies, and placed in clean cages.

Hoppers were provided with green maize leaves and kept at 30°C and a 12h day. Hoppers were closely monitored and efficacy was recorded at hourly intervals for the first 6h and then at 24, 48 and 72h after treatment. Hoppers were classified as moribund when they were lying on their sides and were unable to right themselves, while death was recorded when all visible movements had ceased.

4.2.1.5 Mode of action of bait

Toxic effects resulting from exposure to insecticide bait could either be due to the direct contact action on the insect cuticle or from the actual ingestion of bait. The toxicology of contact action and stomach action of the different baits were therefore examined separately.

Contact action: Mouthparts of L5 hoppers were waxed closed with low melting point beeswax to prevent feeding. Outwardly this did not appear to affect their mobility; hoppers walked and hopped in a manner similar to those that did not have their mouthparts waxed. Hoppers (3 batches of 10) were then confined over each of the seven dose rates of Regent® bait scattered over the floor of the containers, as described above. Hoppers were observed to walk over the bait during the ten minute exposure period but could not feed. Batches of control hoppers also had their mouthparts sealed with wax for ten minutes and were confined over untreated bran. After the exposure the wax masks were peeled off and hoppers were immersed in distilled water to remove any bait adhering to their bodies. Each batch of hoppers were then transferred to a clean cage at 30°C and provided with green maize leaves. Rates of knockdown and mortality were recorded over the following 72h.

Stomach action: Individual hoppers, starved overnight, were fed 2, 4 or 6mg of 50ppm Regent® bait (n=20 hoppers per treatment) by means of metal tweezers. Although laborious, this method of feeding individual hoppers with an exact mass of bait proved effective with starved hoppers that were not so easily disturbed. This feeding method also ensured that there was minimal body contact with the bait. Only hoppers that consumed the entire test dose were used in the experiment while controls were fed clean bran only. Other hoppers (n=10) were fed excess 50ppm bait until they became satiated, and the quantity of bait consumed recorded Treated hoppers were kept isolated in glass jars with green maize leaves and their fate monitored over the following 72h.

4.3 BIOASSAY RESULTS

4.3.1 Bait acceptability

Starved hoppers were observed to feed on the heaps of Regent® bait and no obvious differences were noticed between the acceptability of the 200 and 400ppm baits compared with clean bran. All candidate Regent® bait concentrations were thus considered suitable for further evaluation.

4.3.2 Efficacy of bait

Rate of knockdown (% moribund plus dead hoppers combined) and mortality (% dead only) of brown locust hoppers following exposure to seven concentrations of Regent® bait are given in Table 4.1.

Table 4.1. Percentage knockdown and mortality of L5 brown locust hoppers (n=30) following exposure to different concentrations of Regent® bait in the laboratory.

% Knockdown							
Hrs p.a.	5ppm	15ppm	25ppm	50ppm	100ppm	200ppm	400ppm
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	0	10	12
4	0	0	7	0	10	43	80
5	0	0	20	13	57	83	100
6	0	0	23	67	80	90	100
24	43	83	90	100	93	100	100
48	83	93	93	100	93	100	100
72	93	93	97	100	97	100	100
% Mortality							
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	0	0	0	0	0	0	10
6	0	0	0	0	0	7	17
24	0	7	23	30	33	47	97
48	33	70	87	100	90	100	100
72	93	93	97	100	97	100	100

Rate of knockdown (KD) produced by Regent® bait was related to the concentration, with higher rates producing a more rapid toxic effect (Table 4.1). Once hoppers became moribund there was no recovery at all rates. Excellent mortality rates of 93-100% were achieved with all concentrations, although mortality was slow under laboratory conditions (Table 4.1).

Knockdown and mortality data for each bait concentration (Table 4.1) were subjected to curve fitting statistics (GENSTAT 5 program). The time to produce 50% knockdown (KDT_{50}) and 90% knockdown (KDT_{90}), as well as the median lethal time (LT_{50}) and LT_{90} values, were calculated from these curves (Table 4.2). The KD and mortality data clearly demonstrated the strong dose response produced by Regent® bait against L5 brown locust hoppers.

Table 4.2. Calculated time required to produce 50% and 90% levels of knockdown and mortality of L5 brown locust hoppers following a ten minute exposure to bran bait containing different concentrations of Regent®.

Knockdown		
Bait dose (ppm)	**KDT_{50} (hours)**	**KDT_{90} (hours)**
5	27.5	62.4
15	15.7	43.5
25	11.2	33.0
50	8.1	20.6
100	5.6	15.8
200	3.9	9.1
400	3.4	4.7
Mortality		
Bait dose (ppm)	**LT_{50} (hours)**	**LT_{90} (hours)**
5	50.7	64.9
15	39.8	65.1
25	31.4	51.2
50	25.7	31.3
100	28.5	28.0
200	24.2	29.7
400	19.5	24.5

4.3.3 Mode of action

Direct contact: Although a few hoppers initially exhibited some signs of toxicity, none of the treated hoppers that had been confined over the different bait concentrations for a ten minute exposure period died during the 72h post-exposure period. The direct contact action produced by Regent® bait at all test concentrations was therefore weak.

Stomach action: The 50ppm Regent® bait proved lethal to hoppers. The 2mg quantity of bait produced 90% mortality within 72h, rising to 100% after 120h, while the 4 and 6mg of 50ppm bait produced 100% mortality within 48h. However, individual hoppers (n=10) provided with excess 50ppm bait consumed an average of 11.4±4.9mg (range 7.4-14.0mg), indicating that hoppers could consume more toxic bait than was actually required to kill them. The potent stomach action of Regent® bait confirmed previous results by Butler and Du Preez (1994), who showed that technical grade fipronil was highly toxic to brown locust hoppers when ingested on maize leaves with a calculated LD_{90} at 72h of 0.199µg/g as compared with 1.184µg/g for direct topical application.

4.4 CONCLUSION

Bran bait containing a range of concentrations of Regent® 200 SC (from 5 to 400ppm) gave 93-100% mortality of L5 brown locust hoppers within 72 hours. At these low dose rates, toxicity against hoppers was almost entirely due to stomach action of the product. Although all bait dose rates killed hoppers, the quantity of Regent® bait that could be potentially consumed by individual hoppers was of concern, which is very important considering the logistics of bait preparation, bulk transport and application. It was thus decided to undertake preliminary trials with two of the higher bait concentrations (100 and 200ppm) against brown locust hopper bands in the field. These concentrations were thereafter referred to as 0.01 and 0.02% Regent® 200 SC bait.

4.5 PART 2 : FIELD TRIALS

4.5.1 MATERIALS and METHODS

4.5.1.1 Trial sites

Initial range-finding trials (n=9) of two dose rates of Regent® SC bait (0.01 and 0.02% concentrations) were undertaken on the farm Middledeurvlei (29°31'S, 19°27'E) near Pofadder in the Kenhardt district of the Northern Cape Province during March 1996. The aim of these preliminary trials was to establish the correct application rate of Regent® bait under field conditions and to determine the factors unique to the success or failure of the baiting technique against the brown locust. Subsequent trials comprised replicated treatments (n=7) with the 0.02% Regent® bran bait under autumn conditions near Augrabies (28°47'S, 20°21'E) in the Kenhardt district during April 1997. Both the above areas supported a semi-desert vegetation, classified as Arid Karoo (Acocks, 1988), comprising lush stands of bushman grass, *S. ciliata*, and scattered 1-1.5m high *Rhigozum trichotomum* and *Lycium* sp. bushes. Soils were deep red quartz sands.

In order to obtain product registration in South Africa under the Agricultural Remedies Act (Act 36 of 1947), additional field trials are required under different climatic and vegetation conditions in an alternative area of the Karoo. Additional trial work with 0.02% Regent® bait (n=8 replicates) was thus undertaken under hot summer conditions on the farm Gelukspan (29°39'S; 21°58'E) 40km south west of Marydale in the Prieska District, Northern Cape Province, during March 2000. A total of five bands were treated with clean bran bait (no Regent®) and served as controls. The vegetation on the farm was classified as 'Arid Karoo' (Acocks, 1988) with Bushman grasses, *S. obtusa* and *S. ciliata*, predominating. A variety of succulent herbs ('Brakveld') and 1-1.5m high *R. trichotomum* bushes were also common. Following the good summer rain season the vegetation in this desert area was unusually lush and green. Vegetation basal cover, estimated from 500 point records made along line transects, was 36%. The area had a low undulating topography and soils comprised shallow red quartz sands overlying calcareous tufa. Farms in the trial areas are used for sheep and game ranching, although many farms are currently unoccupied due to the depressed farming conditions in the Karoo.

4.5.1.2 Locust targets

Targets selected for baiting trials comprised individual bands of gregarious L4-L5 hoppers. These marched throughout most of the day, strung out in characteristic column formation, covering a kilometre or more per day, before concentrating to roost at sunset in discrete, tightly packed aggregations on prominent bushes. Roosting bands appeared as red colored masses on the canopies of bushes in the morning sunshine and made highly conspicuous targets for baiting.

4.5.1.3 Bait preparation and application

Regent® 200g/ℓ SC (batch No. 96400504) was received from Aventis Environmental Science, Centurion. Bait was prepared in the field shortly before application as bait that was allowed to stand for more than 24h started to ferment and became less acceptable to hoppers. The 0.01% and 0.02% bran baits were prepared using 2.5mℓ or 5mℓ of Regent® 200g/ℓ SC dissolved into 5ℓ water respectively, which was then thoroughly mixed into 5kg of dry wheat bran in a plastic basin. Bait was mixed using a wooden paddle and gloved hand until all the bran became moist and assumed a crumbly consistency. Bait that was too dry tended to blow away in the wind, while sodden bait tended to clump and could not be distributed evenly (Price and Brown, 1997).

Previous trial work had shown that time of baiting was crucial for successful control and was best undertaken early in the morning while hoppers were still densely aggregated on their overnight roosts (Price and Brown, 1997). Baiting roosting bands before they descended to the basking sites caused the least possible disturbance to hoppers and resulted in the most economical and effective use of bait. Baiting of basking or marching bands proved unsuccessful, as the hoppers were readily disturbed and rapidly dispersed when bait was broadcast amongst them.

Two methods of applying bait were tested, either by broadcasting it by hand or placing it out in small heaps, applied from a 250mℓ scoop. Access to heaped bait was restricted by the first arrivals, which crowded out subsequent hoppers attempting to feed. The most effective method was to broadcast bait in a 1-2m wide strip on the bare soil around the bases of bushes containing roosting hoppers (Fig.4.1). The hoppers descended from the bushes to bask on the ground in the morning sun and

Fig.4.1. 0.02% Regent® bran bait was applied by gloved hand around the bases of Karoo bushes containing roosting brown locust hoppers.

Fig.4.2. Following bait application the hoppers descend from their overnight roosts to bask in the morning sun and to feed on the bait.

crawled around and fed on the bait. More bait was applied around the east-side of the bushes which was where the hoppers tended to bask in the sun (Fig.4.2).

Application of dry or moistened bait through manually operated knapsack seed dispensers or through motorised 'Solo' spray machines proved ineffective, as bait rapidly clogged the apparatus. Specialist bait applicators, used in the USA in the past for baiting rangeland grasshoppers, were not evaluated.

Previous trial work on the quantity of insecticide bait required to produce acceptable control (>90% mortality) of roosting brown locust bands of various sizes has been described by Price and Brown (1997). In the current trials a 5kg quantity of fipronil bait was applied as a standard rate against small and medium-sized bands roosting over an area of 10-50m^2 and 50-100m^2, respectively, and containing up to 100 000 hoppers (Price and Brown, 1997). A 10kg quantity was applied to larger bands roosting over an area of 100-250m^2. Larger or more dispersed targets were considered unsuitable for baiting, due to the logistics of baiting large areas in the limited time available before bands became unduly disturbed and scattered.

4.5.1.4 Mortality assessment

Following bait application each band was closely observed from approximately 5m away with the aid of Pentax 7x35 wide-field binoculars, so as not to disturb the hoppers. Observations on hopper behaviour, toxic symptoms, speed of knockdown and mortality were recorded. Air and soil surface temperatures during the trial period were continually recorded with a data logger.

The distribution of cadavers radiating out from the bait site 24h post-application was demarcated with flags and their area calculated. Density of cadavers was determined by means of 25 quadrats (1m^2) thrown at random within the demarcated area. The mean density of cadavers per m^2 multiplied by the area gave an estimate of the total number of hoppers killed by the bait.

Surviving hoppers were followed throughout the day as they marched from the baiting site. Their subsequent roosts were marked and visited early the next morning when the aggregated hoppers

could be closely observed without disturbance. Hoppers that had died overnight were added to the mortality total. Numbers of unaffected hoppers at 24h post-application were either visually estimated or the 'survivors' roosts were sprayed with Decis® 7UL to produce a fast KD of hoppers *in situ,* after which the number of moribund hoppers were counted. The number of these 'survivors' was then added to the bait mortality counts and the percentage mortality caused by baiting calculated. Levels of control below 90% were considered as failures.

4.6 FIELD TRIAL RESULTS

4.6.1 Preliminary trials

Range-finding trials undertaken near Pofadder showed that a 5kg application of 0.01% bait to small-medium sized bands (roosting over an area of 30-50m^2) produced unacceptable levels of mortality (estimated at 50-75%). Although band cohesion broke down following baiting with this bait concentration, some groups of surviving hoppers were large enough to warrant further treatment at 24h post-application. However, an increased application rate of 10kg per band of 0.01% bait produced excellent levels of control (estimated at >90%) in two similarly sized bands. However, it was concluded that the additional quantity of bait required for each target would produce additional logistical problems and this rate was therefore considered impractical for operational use.

However, application of the higher concentration 0.02% Regent® bait, applied at 5kg/band, gave excellent (>95%) control of small to medium sized bands (n=3 bands) within 24h. The 0.02% Regent® bait was therefore considered suitable for further trial work.

4.6.2 Efficacy of bait during autumn

Trials with 0.02% Regent® bait under autumn conditions (n=7 treatments) were undertaken near Augrabies. Climatic conditions were calm and cloudless, with mean maxima and minima air temperatures measuring 25.4°C (range 23.2-27.6°C) and 16.1°C (15.5-16.8°C) respectively. Maxima

and minima soil surface temps measured 38.5°C (36-41°C) and 18.2° (17-19°C) respectively. No rain fell during the trial period, although there was heavy dew in the mornings.

Under autumn conditions at Augrabies hoppers remained on their roosts until comparatively late in the morning and only began to descend to the ground after 09h00. By 09h30 most hoppers were observed basking in the morning sunshine and by 09h45 most were observed feeding on bait within the basking areas. First toxic effects became evident at approximately 10h15 when individual hoppers were observed trembling and exhibiting uncoordinated movements before collapsing on their sides. By 10h30 (+1.5h) the level of knockdown had increased substantially (estimated at >30%KD) and the ground was strewn with moribund hoppers. Once hoppers became moribund there was no recovery. Affected hoppers showed no aggregated shade seeking or climbing behaviour. Hoppers not yet adversely affected continued to feed on bait, while others began to walk or march away from the roost site and out of the baited area.

By 11h00 band cohesion had broken down completely (>50%KD) and moribund hoppers were scattered over a wide area around the original baited site. The remaining hoppers, as yet unaffected by the bait, marched in small columns in random directions among the carpet of dying hoppers. Control bands were meanwhile marching strongly in cohesive column formation, typical of gregarious bands, and were at least 30-50m from their overnight roost sites at this time.

An interesting observation noted with Regent® bait was that marching columns of hoppers leaving the bait site often circled back to rejoin the moribund hoppers within the baited area, which thus again exposed these hoppers to the bait and added to the final mortality. Small numbers of affected hoppers could still be observed milling around the bait site 5-6h after the majority of the band was already moribund. First mortality was observed after 5h and >95% of hoppers were estimated dead after 24h.

4.6.2.1 Mortality assessment

Percentage mortality in seven bands baited with 5kg 0.02% Regent® bran bait under autumn conditions was estimated from 25 quadrat counts of cadavers (Table 4.3).

Table 4.3. Estimated mortality of L5 brown locust hopper bands following application of 0.02% Regent® bait under autumn conditions.

Band No.	Area of cadavers (m^2)	Mean density of cadavers per m^2	Estimated No. hoppers killed by bait	Estimated No. of survivors	Total No. hoppers in band	Estimated % mortality by baiting
1	137	99.3	13 604	252	13 856	98.2
2	373	74.9	27 953	30	27 983	99.9
3	180	243.6	43 852	75	43 927	99.8
4	280	51.9	14 552	12	14 564	99.9
5	92	394.5	36 294	550	36 844	98.5
6	160	371.0	59 360	2 950	62 310	95.2
7	187	262.5	49 087	2 140	51 227	95.8

Application of 5kg of 0.02% Regent® bran bait gave 95.2-99.9% control of small and medium sized roosting bands of the L5 brown locust under autumn climatic conditions.

4.6.2.2 Control bands

Two roosting hopper bands, treated with bran carrier (no Regent®), fed avidly on the moistened bran before marching away from the bait site. No mortality was evident.

4.6.3 Efficacy of bait during Summer

During these trials sunrise occurred at 06h40, which gave very little time to locate and bait the experimental bands before they descended from their roosts. However, five bands marked the previous evening were baited early the next morning (07h00-07h30) while still densely aggregated on the canopies of *Rhigozum* and *Lycium* bushes. Four of these bands roosted over an area of $<100m^2$ and were treated with 5kg 0.02% Regent® bait. The fifth band roosted over an area of $180m^2$ and was baited with 10kg. Baiting took 10-20 minutes to undertake and minimal disturbance of the roosting hoppers occurred during bait application. Air temperatures at the time of baiting

ranged between 17.5-20°C. Due to time constraints another two bands could only be baited after hoppers had already descended to the basking sites, while a third band was baited while marching.

Hoppers began descending from their roosts at 07h15. Within 30 minutes over 50% of hoppers were observed basking in the sun at the base of the bushes. Basking hoppers spread out to carpet the strip of bait surrounding the bushes and feeding was first observed after 08h00. Hoppers fed avidly on the moist bait and most of it was consumed, especially in areas where basking hoppers had concentrated. Some hoppers were observed feeding on grass as well as bait. First symptoms of toxicity were observed after 08h30 (+1.5h post-application) when individual hoppers became agitated and displayed uncoordinated movements. Individual columns of outwardly unaffected hoppers started to assemble and marched from the basking areas after 09h30, when air temperatures were >24°C. However, as increasing numbers of hoppers became uncoordinated, the cohesion and momentum of the bands started to break down and the advance marching columns circled back to rejoin the mass of affected hoppers at the bait site, as previously reported during the autumn trials.

First moribund hoppers were observed between 08h45-09h00 and by 09h30 (+2.5h) over 50% of hoppers were clearly affected and milled around in scattered formation near the bait site. By 10h30 (+3h), >50% of hoppers were recorded as moribund (lying flat on their sides with their legs trembling and mouthparts twitching). Once hoppers became moribund and lay on their sides there was no recovery. Under these summer conditions the moribund hoppers lying on the bare soil in full sun rapidly died on the hot soil surface. First mortality was recorded at 11h00 and mortality increased rapidly as soil surface temperatures rose to 53-58°C during the heat of the day. Maximum air temperatures of 30-33°C were recorded at 14h00-15h00.

Some columns of hoppers marched away from the bait sites, but left a trail of dead and dying hoppers in their wake for a distance of 100-150m before the columns eventually disintegrated. After 24h post-application a small number of affected hoppers were still milling on the bait sites, but these did not survive for long and after 48h the mortality achieved within each of the five bait treatments was considered excellent and was estimated at >95%. Small groups of survivors milled around bushes

outside the bait plots but these did not form into cohesive marching bands and did not pose an economic threat.

4.6.3.1 Efficacy of bait against basking bands

Two bands that had descended from their roosts and were basking on the ground were baited with 5kg 0.02% Regent® bait between 08h00-08h15. Such basking hoppers were more active and scattered as the bait was broadcast amongst them. This resulted in some columns of hoppers marching away without feeding on the bait. Some of these columns circled back to rejoin the main band within the baited area, as described previously, while others continued to march away. Although the baiting of basking bands killed large numbers of hoppers, many escaped control because of the disturbance factor. Mortality was estimated at less than 75% after 48h,which was considered unsatisfactory as most of the survivors regrouped to form small bands.

4.6.3.2 Efficacy of bait against marching bands

A large-size band was baited with 10kg of 0.02% Regent® bait while actively marching through the veld during mid-morning (10h30) when the air temperature was 26°C. Bait had to be again widely broadcast in order to intercept the marching columns and hoppers were easily disturbed by the bait as it landed amongst them. Although numerous hoppers stopped to feed on bait, the momentum of the marching columns soon took most of the hoppers out of the baited area. Widely scattered corpses later indicated that some hoppers had consumed bait, but the majority of the band remained unaffected and control was clearly a failure.

4.6.3.3 Control bands

Three roosting L5 bands, baited only with moist bran, descended to the basking sites where they fed on the bait and basked in the sun until approximately 09h00. Groups of hoppers started to walk and then marched away from the roosting site in typical column formation. All hoppers had left the baited area by 09h30. No evidence of mortality was observed.

4.7 DISCUSSION and CONCLUSION

Application of 0.02% Regent® bran bait consistently produced good mortality of gregarious L5 brown locust hopper bands under both summer and autumn conditions, with efficacy comparable to the ULV spraying in current use for locust control. Toxicity was almost exclusively due to the potent stomach action of Regent®. Contact action at these low concentrations was apparently negligible.

Regent® bait was easy to mix and apply on a small scale, requiring no special application equipment. Bait was simply broadcast by gloved hand in a 1-2m wide barrier around the base of bushes containing roosting hoppers. Baiting was most effective if undertaken early in the morning while hoppers were still densely aggregated on their overnight roosts and were less readily disturbed. Baiting later in the morning while hoppers were basking or actively marching was less effective as hoppers became scattered during bait application.

Baiting was effective against small and medium sized bands roosting over areas of $<100m^2$. Baiting of larger or more diffuse targets was impractical. However, the characteristic high-density nature of gregarious brown locust bands, which roost as discrete and highly visible aggregations, generally provide ideal targets for spot treatment with bait. Baiting appears to provide a highly target-specific method of insecticide dose transfer to hoppers with reduced environmental contamination, as compared with the usual ULV cover spraying, with its associated downwind drift of insecticide. Furthermore, the fact that hoppers consumed practically all the bait and subsequently scattered to die over a wide area effectively dispersed the insecticide load.

Typical diurnal behaviour of brown locust hopper bands in the Karoo mainly consists of periods spent roosting, basking, feeding, walking and marching. The proportion of time spent in these different activities varied between seasons and was mainly dependent upon light and temperature conditions (Price and Brown, 1997). Thus, baiting was very effective during autumn due to the slower descent of hoppers from the roosts and the extended time spent basking and feeding under the cool morning conditions. This behaviour therefore extended the period of exposure to bait and enhanced dose

uptake. There was also more time during the early morning in which to locate the band targets. However, during summer the hoppers vacated their roosts more rapidly and there was thus a shorter window of opportunity for locating and baiting targets. However, this could be overcome in some instances by the marking of target bands the previous evening for bait treatment in the morning.

Bait preparation, transport and application is very labour intensive and could produce huge logistic problems during locust campaigns, especially in the more remote areas. However, a baiting strategy would additionally involve local disadvantaged communities in the Karoo, thereby creating temporary job opportunities during locust outbreaks. The baiting technique was safer for the operator to undertake, requiring minimal application equipment and training.

Regent® 0.02% bait has the potential to provide an effective, low cost, method of controlling brown locust hopper bands in certain areas of the Karoo and could be incorporated into the National locust control strategy as a complement to mainstream ULV ground spray operations. The reduced environmental impact associated with the more target-specific baiting technique may make it a more acceptable method of locust control for use in conservation areas, where there is current concern over the negative impact caused by the drift spraying of ULV insecticide. Operational trials with Regent® bait are now recommended.

4.8 BENEFITS and CONSTRAINTS

4.8.1 Potential benefits

- When applied under optimal conditions, baits consistently produced excellent control of brown locust hopper bands within 24h.
- Regent®has a very potent stomach action against the brown locust. Baiting is thus an ideal method of delivering minimum lethal dose rates of Regent® to locusts without unnecessary environmental contamination.

- Baits are relatively target-specific and have a reduced environmental impact against non-target organisms compared with ULV drift application. The scattering of hoppers following ingestion of bait also effectively dispersed the dose rate of Regent® applied.
- Regent® locust bait was easy to prepare and apply, requiring minimal application equipment and training of operators.
- Baiting has the potential to provide numerous temporary jobs in disadvantaged Karoo communities during locust outbreaks.

4.8.2 Potential Constraints

- Baiting was only effective if undertaken early in the morning before hoppers descended from their roost. Baiting against basking or marching bands was not effective.
- Baiting was less effective against large-size bands or against widely dispersed targets.
- Under summer conditions in the Karoo, the window of opportunity in the morning for the location and effective baiting of bands is very limited.
- Labour is not always available in the remote areas of the Karoo. The labour will also have to be collected early in the morning and transported to the bait sites.
- Huge logistical problems involved in bait preparation, transport and application.

CHAPTER 5

Barrier treatments

5.1 INTRODUCTION

The technique of 'barrier or strip spraying' was extensively used to control marching hopper bands of the desert locust in North Africa during the 1950-1960s and was based on the application of the organochlorine insecticide, dieldrin, to narrow strips of vegetation (Bennett and Symmons, 1972; Bennett, 1976; Steedman, 1990). Dieldrin was active both by contact and by ingestion and barriers remained persistent for months after application, relying on the mobility of hopper bands which marched into the treated vegetation and consumed or accumulated lethal doses of insecticide. Although only a small percentage of the infested area required treatment with barriers to provide effective locust control, dieldrin was withdrawn from use in desert locust control during the 1970s owing to its excessive persistence and the adverse effects of bio-accumulation in the environment (Brader, 1988).

The fact that most modern synthetic insecticides used for locust control have short persistence has meant that, for the past 25 years, few suitable residual compounds were available for use in barriers. The block spraying of broad-spectrum ULV insecticides, applied from aircraft, thus became the main focus for locust control around the world, although many of the products now in use are expensive and are environmentally damaging when sprayed over large areas (Everts, 1990; Murphy, Jepson and Croft, 1994). There is thus a need to find a suitable replacement for dieldrin for use in barrier treatments which may allow for more sustainable locust control.

One of the most promising candidates to have recently emerged as a possible replacement for dieldrin in barrier treatment is the phenyl pyrazole insecticide, fipronil. This compound proved effective when applied in a UL formulation (Adonis®) in barrier trials against the desert locust in Mauritania (Rachadi and Foucart, 1996) and has been applied as barrier treatments and as cover sprays in large-scale control operations against the Malagasy migratory locust (Lecoq, 2001).

As mentioned in Chapter 4, laboratory bioassays in the PPRI laboratories have demonstrated the potent stomach action of fipronil technical grade against L5 brown locust hoppers (Butler and Du Preez, 1994): the LD_{90} dose at 72h was 0.199µg/g as compared with 1.184µg/g for direct topical application. Adonis® UL, applied as a full cover spray, was registered for brown locust hopper and adult control in 1994 at a rate of 7.5g a.i./ha (Price, Butler and Brown, 1994). However, the product had not yet been tested in barrier treatments against the brown locust.

The efficacy of 21m wide barriers sprayed with a dose of 12.5g a.i./ha Adonis® UL were therefore evaluated as a barrier treatment against migrating brown locust hopper bands under summer conditions in the Karoo. This relatively narrow barrier strip was used in order to simulate application from vehicle-mounted sprayers in current use for locust control in South Africa.

5.2 MATERIALS & METHODS

5.2.1 Trial sites

Trial work was undertaken during March-April 2000 on the farms Stompoor, Sononder and Gelukspan, lying 40-60km south west of Marydale town (29°25'S, 22°07'E) in the Prieska district of Bushmanland in the Northern Cape Province. This area is known locally as 'Die Bult' due to its low undulating, treeless topography. Soils generally comprise shallow red quartz sands overlying calcareous tufa with stony ridges. The vegetation was classified as 'Arid Karoo' (Acocks, 1988) with Bushman grasses, *S. obtusa* and *S. ciliata*, predominating. Green tufts of *Schmidtia kalihariensis* grass ('suurgras') and *R. trichotomum* ('driedoring') bushes, 1-1.5m high, were also common in the sandy areas. Vegetation on the stony ridges and slopes was dominated by low *Salsola tuberculata* ('blomkoolganna') bushes and mats of green *Enneapogon* sp. grass ('agtdaegras'). A variety of succulent herbs ('Brakveld') were also common in some of the more overgrazed areas.

Although the majority of farms in the study area were currently unoccupied, this semi-desert scrub-grassland is exclusively used for sheep and game ranching. The nearby farm, Jagbult, is a

well-studied outbreak centre for the brown locust (Smit, 1939). The present study area receives a mean annual rainfall of 150-200mm, with most rain falling as erratic convectional thunder storms during late summer and autumn (February-April). Although long-term droughts are common in Bushmanland, the good early rains (>100mm) that fell between December 1999 and February 2000 stimulated a lush growth of annual grasses which were green at the time of the trial work. The good rains also triggered an intense outbreak of brown locust in the Northern Cape Province which achieved incipient plague status during March-June 2000.

5.2.2 Fipronil formulation

Fipronil formulated as Adonis® 5UL (5g a.i./litre, Batch No. OP 970967) was supplied in 10ℓ containers by Aventis Environmental Science, Centurion, South Africa. The prescribed volume application rate for the barrier treatments was 2.5ℓ/ha, which is the standard volume application rate currently used for locust control in South Africa and Namibia. This gave an area dosage rate of 12.5g a.i./ha within the treated barrier.

5.2.3 Spray apparatus

Adonis® barriers were applied using a 'Solo 423' motorised knapsack, mistblower, powered by a two-stroke engine, which is one of the standard sprayers in use in the Karoo. This is a robust and reliable air blast machine, which when fitted with the ULV restrictor plate, generates small spray droplets by means of air-shear. Its use in these trials was dictated by logistical and calibration constraints. The vehicle-mounted 'Solo Trac 419' mistblower in routine use in the Karoo is known to be difficult to calibrate and therefore was not suitable for accurate experimental work.

The knapsack mistblower was regularly calibrated using a measuring cylinder and stopwatch, to ensure that the emission rate remained constant. The same person, whose walking speed was monitored with a stopwatch, was used throughout in order to standardise application. Track intervals were marked by flagmen situated at either end of the barrier. The correct emission rate of 131mℓ/min was obtained by using the second smallest orifice (0.8mm diameter) on the restrictor plate while the machine ran at full throttle. Barrier treatments were made at a constant walking speed of 4.5km/hr and a track interval of 7m to give the required volume application rate

of 2.5ℓ/ha and achieve a dosage rate of 12.5g a.i./ha. A single barrier line thus consisted of three spray passes, which produced a 21m wide strip of treated vegetation.

5.2.4 Barrier application

Brown locust hopper bands characteristically march in narrow column formation rather than in a broad front, as occurs with other locusts. Barriers were usually applied in the early morning (07h00-08h30), while air and soil surface temperatures were still cool (<20°C), across the line of march of bands coming off their roosting sites. Each barrier was applied relatively close to the target band so that observations on basking, feeding, marching and roosting behaviour, before and after the band crossed the fipronil barrier were made. All barrier spraying took place downwind of band targets in order to avoid any accidental drift onto the band. Only a single barrier line was applied for each band to cross. Spray passes were made at right angles to the prevailing wind with the air stream directed downwind. Barriers varied in length from 110-270m.

Wind speed was monitored during spray application using a hand-held anemometer, while air temperature and relative humidity were recorded with a whirling hygrometer. Spray droplets emitted during application were collected on oil-sensitive papers and magnesium oxide coated glass slides set out at 2m intervals across one of the barriers. Slides were later analysed with a 'Vidas Kontron' Image Analyser at the PPRI laboratories and the mean droplet size and density determined. Percentage vegetation cover at ground level was determined within all barriers, using a point count method consisting of 500 points per barrier site.

5.2.5 Mortality assessment

After bands entered the spray barrier the speed of knockdown and mortality of hoppers were recorded. Efficacy was estimated by comparing the size of bands measured at the end of each day. In addition, samples of hoppers (n = 100-200) that had crossed spray barriers were netted shortly after exposure and placed in open topped field enclosures, measuring 1.5x1.5x0.4m, erected over nearby unsprayed vegetation. These samples were monitored daily over four days and the mortality compared with the residual band. The size, displacement and direction covered each day by these residual sub-bands were recorded. Air and soil surface temperatures were continually

logged throughout the assessment period.

5.2.6 Barrier persistence

Hundreds of gregarious marching bands were present in the surrounding areas and during the weeks following barrier treatments, successive waves of unsprayed bands also marched through some of the existing barriers. These provided an ideal opportunity for monitoring barrier persistence. In addition, the persistence of barriers against L5 hoppers was investigated in detail by confining them within open-topped field enclosures, erected over sprayed vegetation within selected barriers, at 12 and 25 days post-application. Untreated L5 hoppers (n=100–200) collected elsewhere were released into these treated enclosures (n=3 replicates per barrier) during mid-morning. After a 2h exposure to treated vegetation (the average time that marching bands took to cross a barrier), during which feeding was noted, the hoppers were transferred to other enclosures erected over untreated vegetation where their subsequent mortality was recorded over the following four days. Three control enclosures of untreated hoppers were similarly erected over untreated vegetation to monitor any stress effects from handling and confinement.

5.3 RESULTS

5.3.1 Application parameters

Application parameters and meteorological conditions recorded during each barrier treatment are given in Table 5.1. Although wind speed and direction were often low and variable during treatment, as observed visually, a good spray cloud was produced which was confirmed on oil sensitive papers. Droplet sizing and deposition, as shown on the Magnesium oxide slides (n=10), gave a volume mean diameter (vmd) of 118μm (range 86-158) and a numerical mean diameter (nmd) of 64μm (51-80) with a mean density of 33.2 droplets per cm^2 (range 11-80).

Table 5.1. Application parameters and meteorological conditions for the different Adonis® barriers treatments used against brown locust target bands in Bushmanland.

Barrier No.	Date	Farm	Hopper instar (dominant)	Band size (m) (roosting)	Barrier length (m)	Barrier orientation	Time of application	windspeed (m/sec) + direction	Air temp. (°C)	% veg. ground cover
1	10/03/00	Stomp Oor	L2	80 x 50	220	80°E	07h10	3 - 4, NW	25	35
2	10/03/00	Son Onder	L3	12 x 10	110	16° N/NE	08h00	2 -3, NW	26	28
3	10/03/00	Son Onder	L3	50 x 33	180	50°NE	08h20	2, NW	27	27
4	11/03/00	Son Onder	L5	75 x 50	240	10°N/NE	07h20	0 -1, E	20	22
5	11/03/00	Son Onder	L3	70 x 40	130	58°NE	07h50	0 , --	21	15
6	11/03/00	Son Onder	L4	25 x 20	135	170°S/SE	08h15	0, --	24	24
7	12/03/00	Stomp Oor	L3	100 x 60	270	10°N/NE	07h10	0 - 0.5, E	19	51
8	12/03/00	Stomp Oor	L3	54x14, 16x12	150	70°NE/E	07h50	0 - 0.5, N	22	57
9	12/03/00	Driehoek	L3	47x10, 42x18, 33x20	240	40°N/NE	08h40	1.5 - 2, N	24	44
10	13/03/00	Son Onder	L3	30x5	150	70°E	18h15	0.5 - 1,N	25	40
11	28/03/00	Gelukspan	L5	20x10, 12x8, 10x5, 4x3	200	100°E	07h00	0, --	20	48
12	28/03/00	Gelukspan	L5	8x6, 4x3	158	100°E	08h00	0 – 0.5, .SW	23	37

5.3.2 Vegetation cover

Vegetation cover, estimated for each barrier in Table 5.2, was expressed as percentage grass, succulent herbs, Karoo bush and dead vegetation together with bare ground. Barrier sites were generally well vegetated as a result of the good summer rains this season.

Table 5.2. Percentage bare soil and vegetation cover for each Adonis barrier site.

Barrier No.	Site description	% Bare soil	% Grass	% Herbs	% Karoo bush	% Dead vegetation	Total % ground cover
1	Stony ridge	65.0	28.4	2.2	3.0	1.4	35.0
2	Sandy flats	72.1	10.2	7.1	6.7	3.9	27.9
3	Sandy slope	72.8	10.4	8.9	5.1	2.8	27.2
4	Sandy slope	78.1	7.9	10.6	2.1	1.3	21.9
5	Sandy flats	85.0	5.8	4.5	4.1	0.6	15.0
6	Sandy slope	75.9	10.5	6.6	4.9	2.1	24.1
7	Eroded slope	49.0	7.0	39.5	3.3	1.2	51.0
8	Sandy flats	43.0	34.3	2.9	19.8	0	57.0
9	Stoney slope	55.8	34.6	2.1	5.8	1.7	44.2
10	Gravel flats	58.3	21.1	1.1	18.2	1.3	41.7
11	Gravel flats	52.0	29.8	1.6	14.6	2.0	48.0
12	Pan veld	63.4	13.4	3.4	16.8	3.0	36.6
Means		64.2	17.8	7.5	8.7	1.8	35.8

Vegetation cover ranged from 15-57%. Flowering Bushman grass was common on the sandy sites (e.g. Barrier 2, 3 & 6), while 'agtdaegras' was plentiful in the more stony areas (e.g. Barriers 1, 9 & 11). A dense growth of 'suurgras' was present at Barrier 8. Grass, which is the main food of brown locust hoppers, varied from 6-35% cover. Various succulent herbs were common in degraded areas where grass cover was sparse (e.g. Barrier 7).

5.3.3 Meteorological conditions during barrier trials

Air and soil surface temperatures and percentage relative humidity, logged over ten days during the barrier trial, are given in Table 5.3. Field conditions were hot and dry and were considered typical for Bushmanland during early autumn. Sunrise occurred at 06h40 and sunset at 18h50.

Table 5.3. Mean maximum and minimum air and soil surface temperatures (°C) and % relative humidity recorded during the trial and mortality assessment period.

	Mean maximum	Range	Mean minimum	Range
Air temperature °C	32.1	29.5 - 37.2	18.2	13.5 – 23.5
Soil surface temp.°C	53.2	50.6 - 57.7	20.5	16.0 – 24.0
Relative humidity %	68	65 - 90	30	25 – 40

5.3.4 Behaviour of control bands

The behaviour of L5 untreated bands all showed a similar pattern: bands roosted at dusk on prominent Karoo bushes where they formed conspicuous, red coloured aggregations, visible from afar. At sunrise (06h40), when temperatures were usually below 20°C, hoppers were relatively insensitive to disturbance, but they gradually became more active and then moved up the vegetation to bask in the sunshine. By 07h45-08h30 they descended from the bushes and dropped to the ground and spread out to bask in the sunlit areas. They mainly concentrated on the eastern side of the bushes, where they packed together in dense groups, aligning themselves parallel to the sun. As their body temperatures rose, they became increasingly active and started to crawl about on the ground and began to feed. Spontaneous group behaviour developed, which led to the formation of hopping columns of hoppers which gradually marched off the basking sites.

Displacement of bands progressively increased throughout the morning and was punctuated by short feeding stops. The numerous tributary-like streams of marching hoppers, coalescing and snaking their way in between the vegetation and maintaining their cohesion and common direction, are characteristic of the gregarious phase (Fig.5.1). Brown locust marching columns usually moved in an easterly or north-easterly direction, which is typical for brown locust hopper bands in the Karoo (De Wet and Webb, 1951). During the heat of the day (12h30-14h30), when air temperatures rose to >30°C and soil surface temperatures were 50-57°C, bands typically halted. The hoppers then all climbed up to perch in the vegetation, so as to escape the heat and to regulate their body temperatures.

Fig. 5.1. A typical L3 brown locust target band, comprising numerous tributary-like columns strung out behind, in its daily march through the trial area. © D.Brown

Fig. 5.2. Final instar brown locust hoppers feeding on a tuft of Bushman grass growing within an Adonis® treated barrier. © D.Brown

After the mid-day roost, bands descended to the ground and resumed their marching activity, which intensified during the afternoon and when little to no feeding was observed. Five such control bands in these trials covered distances of 1.2-2.5km per day. Towards dusk, the marching columns slowed down and the band then contracted to form static basking groups. From 17h45-18h30 hoppers began to gradually aggregate and then roost in the larger bushes. At this time air and soil surface temperatures ranged from 30-33°C and 32-36°C respectively.

5.3.5 Behaviour of bands entering Adonis® barriers

By 09h00, target bands began to gradually stream off the basking sites in columns and enter the Adonis® barriers, which had been laid across their path. All target bands, irrespective of size and age structure, marched directly into the Adonis® barriers without any evidence of repellency. Hoppers displayed normal marching, basking and feeding behaviour within barriers.

Feeding behaviour was as follows: Individual hoppers stopped at regular intervals to feed on the available tufts of grass. Such hoppers turned aside from the marching columns and climbed into the nearest grass tufts and fed for short periods while the column continued its advance. After a short period, feeding would cease and these hoppers then rejoined the nearest marching column. Hoppers fed on the seed heads and leaves of the grass and sometimes grazed individual tufts down to the ground. Most feeding took place throughout the mid-morning period (Fig.5.2). Within barriers, hoppers fed avidly on grass; incidental observations suggest that they fed longer within the Adonis® treated zones. Feeding continued for 2-3 hours after initial exposure to barriers. Although brown locust hoppers feed mainly on grass (Smit, 1939), they will also sometimes chew on other vegetation. However, the 'Brakveld' succulent areas were rapidly vacated by hoppers, suggesting that these herbs were less acceptable.

5.3.6 Efficacy of Adonis® barriers

Observations on the behaviour, estimated mortality and the distance and direction travelled by bands after crossing the Adonis® barriers at various times after application are presented in Table 5.4 and are discussed below.

5.3.6.1 Immediate impact on L2-L3 bands

Barriers 1, 2, 3, 7, 8, 9 and 10 (T+ 1 day)

Observations made on ten individual L2-L3 bands that entered barriers within the first day post-application, showed that first toxicity was observed 45-60 minutes following contact with barriers. Affected hoppers broke ranks, making increasing uncoordinated jumping movements and then lost their equilibrium, falling on their sides. First moribund hoppers were observed 75 minutes after contact with a barrier and by 90 minutes, the ground was strewn with moribund hoppers. These hoppers lay prostrate on the bare soil between the plants, violently kicking and twitching their mouth-parts and were unable to crawl or right themselves. There was no recovery of moribund hoppers and band cohesion started to break down after 2h. Survivors milling about amongst an ever expanding zone of dying hoppers which accumulated in drifts under the bushes and in depressions. Other hoppers lay exposed out in the open.

First hoppers died between 11h00-11h30, at which time soil surface temperatures measured 48-50°C. The first columns of survivors, still maintaining their cohesion, proceeded to exit the barrier lines after 60-90 minutes. However, some marching columns that had crossed the Adonis® barriers, typically circled back to re-unite with the dead and dying. This abnormal back-tracking behaviour served to retain the band within or near the barrier zone.

Mortality in the majority of L2-L3 bands was estimated at >90% by the end of the first day (*see* Table 5.4). Survivors from some of the larger bands, nevertheless regrouped to form small sub-bands which still roosted at night in cohesive groups. Although control appeared initially incomplete, subsequent observations on enclosure samples showed that most survivors died over the following 2-4 days. Some groups marched up to 190m from the barriers before dying. With an estimated 99% mortality after four days, final control of L2-L3 bands was thus excellent.

After these initial observations the majority of early instar bands in the trial area moulted, so that only L4-L5 targets were now available for study.

Table 5.4. Immediate impact of 12.5g a.i./ha Adonis® barriers against L2-L5 brown locust bands that entered barriers, within the first day following application.

Barrier No	Nymph instar and initial band size	End of first day (6-10 h) after band crossed barrier	End of 2nd day (+36h)	End of 3nd day (+60h)	End of 4th day (+84h)
1	L2 (90x60m) = 5,400m2	Majority now dead and moribund. Survivors in cohesive marching group 100m 130° SE from barrier.	More died overnight. Band cohesion breaking down. Starting to moult to L3. Disorientated groups 150mE	Dwindling group of L3 survivors milling 25m2, 170m SE. No marching in columns. Still dying.	Band still shrinking. Now only 10m2 of milling nymphs 190m SE. Control virtually complete.
2	L3 (15x10m) = 150m2	Band fed on treated grass - heavy mortality. Survivors milling near barrier line.	Small group of survivors milling 30m, 150°SE. Affected nymphs still dying.	Low density band 12m2 now 55m 130°SE. Marching slowly. Moulting to L4.	Survivors now static and dwindling. Random milling. No target left.
3	L3 (70x40m) = 2,800m2	Most nymphs dead within or near barrier line. Good (>90%) of control. Survivors milling around.	Survivors still milling 20-50m E from barrier line. Moulting to L4. Forming small milling groups.	Trail of affected nymphs but survivors formed a dense marching band (25m2). Still a sprayable target 120m SE.	All survivors healthy. Small band 20m2 still marching 300m SE. Survivors escaped.
4	L5 (90x60m) = 5,400m2	Band reached barrier late in day and most roosted on edge of barrier. Little effect so far (<30% moribund).	Most (>50%) now affected and roosting in bushes. 40% lying dead at base of roosts. Rest milling within barrier.	Most dead at base of roost (>99%). Good control. Scattered nymphs crossed barrier up to 25m 140°SE.	Scattered nymphs static 30-40m from barrier. No target left for control.
5	L5 (30x30m) = 900m2	Most dead and moribund. Band cohesion broken down. Remaining nymphs milling.	Most nymphs now lying dead 20m 120°SE of barrier.	A few scattered affected nymphs remain. Good >99% control. No target left.	Control complete.
6	L5 (80x50) = 4,000m2	Majority of band roosted on edge of barrier. First nymphs now moribund.	Many dead at base of roost. Others still roosting within barrier. Band broken down.	Most now dead. A few small groups crossed barrier but still affected 30m 100°E.	Scattered nymphs static 30-40m 100°E. Control virtually complete.
7	L3 (120x50m) = 6,000m2	Band crossed barrier in 'brakveld' but many now moribund. Band now static – milling. A few columns left.	Most dead (>95%) . Band broken down 0-50m 150°SE of barrier. A few milling nymphs remain. No columns	>99% dead. Trail of dead nymphs now up to 70m from barrier line 150°SE. No target left.	
8	L3 (2 bands) 54x14m and 16x12m	Bands roosting in barrier. Devoured much 'suugras'. Nymphs dying at leading edge of band.	Most dead and moribund within barrier. Small group of survivors (5m2) crossed barrier roosting 10m 120°SE	Trail of scattered moribund nymphs up to 85m 140°SE from barrier. Survivors band only 3m2. Moulted to L4.	Scattered trail of dying nymphs now 170m 180°S. Band dispersed. No target left
9	L3 (3 bands) 47x10m, 42x18m and 33x20m.	Bands only entered barrier late in day and partially roost along barrier. Some now moribund.	Many died within barrier. A few small columns crossed barrier – trail of moribund up to 30m 120°E.	Bands broken down (>99% control). Isolated nymphs milling up to 50m 130°E.. No target left.	Scattered nymphs dispersed but still dying.
10	L3 (30x10m) = 300m2	Band milling,,many dead and dying. No columns crossed through barrier.	Most now dead within barrier. Only isolated survivors milling about.	Minimal survivors. Excellent control achieved.	
11	L4-L5 (4 bands) 20x10, 12x8, 10x5, 4x3m	Bands crossed barrier. Trail of moribund lying up to 50m 25°NE. Bands joined up to roost over area of 180m2.	Many died overnight, but survivors still marching in columns. Poor control.	Band still marching. Only a few still affected.	Poor control. Target controlled by locust officer.
12	L5 (2 bands) 8x6m, 4x3m.	Bands stopped by barrier. Most nymphs moribund. Band milling and static.	Some scattered nymphs crossed barrier. Trail of moribund up to 25m 35°NE	Only isolated affected nymphs remain in static groups. No target left	

5.3.6.2 Immediate impact on L4-L5 bands

Barriers 4, 5, 6, 11 and 12 (T+1 day)

Observations on nine L4-L5 bands that entered barriers within the first day after application, summarised in Table 5.4, showed that they initially behaved normally after encountering barriers. Again, it appeared that these bands spent more time basking and feeding within the barrier sites. First toxic effects became evident ±60 minutes after the L4-L5 bands entered the Adonis® barriers and the first moribund hoppers were observed after 90 minutes, which was longer than for L2-3 bands. The first dead L5 hoppers were similarly observed during late morning when soil temperatures were high. Some columns of hoppers also exhibited the circling and back-tracking behaviour described earlier, causing high mortality in the vicinity of the barrier. However, less successful control was achieved with the four bands that crossed Barrier 11 (Table 5.4), which continued marching and had to be controlled with Decis® by the local locust control officer.

5.3.7 Persistence of Adonis® barriers

The persistence of the Adonis® barriers was monitored for up to 25 days after treatment. The behaviour and mortality of these additional L4-L5 bands that crossed barriers at various time intervals post-application, are briefly discussed below.

5.3.7.1 Persistence 2-7 days post treatment

Barrier 11 (T+ 2 days)

A dense L5 band, measuring approximately 0.4ha when roosting, marched north-east through Barrier 11 after T+2days along virtually the same route taken by the initial targets. Advance columns of this L5 band entered the barrier at 16h00 and the first affected and moribund hoppers were recorded before dusk. Part of the band roosted overnight within the barrier and by the next morning many of the hoppers basked and fed within the treated zone of Adonis® vegetation, resulting in high mortality over the following 48h. Columns that had crossed the barrier turned around to rejoin the mass of dead and dying hoppers from the original target band.

Barrier 7 (T+2 days)

During the late afternoon a large, marching L4 band crossed Barrier 7 along the same route taken

by the initial target band, 48h earlier. Vegetation within the barrier mainly consisted of succulent 'brakveld' on which little feeding occurred. This band continued in a SE direction to its overnight roosting site, 120m distant, leaving a sparse trail of moribund hoppers in its wake. Although there was evidence of control, with scattered moribund hoppers carpeting an area of 2-3ha the next morning, the majority of hoppers had marched away. They were tracked and were found to have split up into two cohesive sub-bands, which measured 80x50m and 20x15m, when roosting.

A sparse debris trail of affected and dying L4 hoppers was followed over the next two days, as these two residual bands continued their march. By now they were 3km distant from the barrier site. Mortality was unsatisfactory and the locust control officer later treated them with Decis®.

5.3.7.2 Persistence 8-14 days post treatment

Barrier 9 (T+10 days)

During the late morning, a large L5 band (70x30m) marched rapidly through Barrier 9 in an easterly direction. Little feeding on treated vegetation within the barrier was observed and only a few affected and moribund hoppers were found after the band had marched away. This band continued to march strongly during the remainder of the day and roosted that evening >1km away. Although some moribund hoppers were found around the roost the following morning, poor control had evidently occurred. The band was subsequently traced and treated with Decis®.

Barrier 1(T+11 days)

A small (<10m^2) L4-L5 band marched south east through Barrier 1 and fed on treated vegetation during the morning, leaving a 120m long trail of moribund hoppers. Survivors stopped marching and broke up into small groups during the second day. Good mortality (>90%) was achieved.

Barrier 2 (T+11 days)

A large (80x10m), fast marching L5 band crossed the barrier strip during the mid-afternoon. Only scattered moribund and dead hoppers were found along its route over the next 2 days. Control was poor (<10%) and this band escaped.

5.3.7.3 Persistence 15-25 days post treatment

Barriers 4 & 5 (T+15-25 days)

A trail of thinly scattered corpses indicated that other L5 bands had crossed Barriers 4 and 5, sometime between 15-25 days after treatment. No other evidence of control of these bands was found and they were presumed to have escaped.

5.3.8 Persistence of barriers against enclosed hoppers

Persistence of Adonis® barriers was further examined using the field enclosure technique by exposing batches of untreated L5 hoppers for two hours to selected barriers laid down 12 and 25 days earlier. Mortality data at 120h post-exposure is given in Tables 5.5 and 5.6. Vegetation in the trial site was still green after 12 days, but became senescent after 25 days. Heavy rain (>50mm) also fell in the trial area from 21-22 days after barrier application, which might have adversely influenced persistence.

Table 5.5. Cumulative percentage mortality of L5 brown locust hoppers, following a two hour exposure to Adonis® barriers, 12 days after treatment.

Barrier site/ replicate No.	No. Hoppers	Mortality Hours post-exposure					Mean @ 120h
		24h	48h	72h	96h	120h	
B2/1	n = 225	0	0	0	0	0	15.5
B2/2	n = 126	0	0	1.6	3.2	3.2	
B2/3	n = 173	14.4	30.0	39.9	41.0	43.4	
B3/1	n = 190	4.7	10.0	12.1	13.7	13.7	47.6
B3/2	n = 175	3.4	12.6	24.0	32.6	34.3	
B3/3	n = 192	75.0	89.6	93.8	94.8	94.8	
B8/1	n = 113	0	0	0	0	0	2.3
B8/2	n = 126	0	0.8	1.6	1.6	1.6	
B8/3	n = 132	3.0	3.8	4.5	5.3	5.3	
Control	n = 97	0	0	0	0	0	0

Despite the enclosures being erected on spray barriers with suitable vegetation for feeding, the rate of mortality of hoppers following a 2h exposure period was generally poor and varied greatly, both within and between sites. The best control achieved was only 47.6% (n=557 hoppers) after 12 days in Barrier 3 (Table 5.5).

Table 5.6. Cumulative percentage mortality of L5 brown locust hoppers, following a two hour exposure to Adonis® treated barriers, 25 days after treatment.

Barrier site/ replicate No.	No. Hoppers	Mortality Hours post-exposure					Mean @ 120h
		24h	48h	72h	96h	120h	
B2/1	n = 144	0	0.7	0.7	1.4	1.4	3.6
B2/2	n = 99	0	3	6.1	7.1	7.1	
B2/3	n = 133	0.7	2.3	2.3	2.3	2.3	
B3/1	n = 121	0	4.1	21.5	28.1	30.6	19.9
B3/2	n = 75	2.7	6.7	17.3	25.3	28.0	
B3/3	n = 92	0	0	1.1	1.1	1.1	
B4/1	n = 116	4.3	5.2	5.2	6.9	6.9	11.0
B4/2	n = 88	0	6.8	9.1	10.2	15.9	
B4/3	n = 78	0	5.1	7.7	10.3	10.3	
B6/1	n = 123	0	0	2.4	4.1	4.9	3.6
B6/2	n = 91	0	0	0	1.1	1.1	
B6/3	n = 62	1.6	1.6	4.8	4.8	4.8	
Control/1	n = 95	0	1.1	1.1	2.1	2.1	1.3
Control/2	n = 81	0	0	0	0	0	
Control/3	n = 106	0.9	1.9	1.9	1.9	1.9	

However, residual toxicity from 12.5g a.i./ha Adonis® barriers to L5 hoppers after 25 days attained a maximum of only 20% (n=288) (Table 5.6). The indications are that at these 2h exposure periods these barriers would not effectively control L4-L5 brown locust bands. Hoppers placed in the untreated control enclosures were largely unaffected by the experimental procedure.

5.3.9 Non-target effects

Incidental observations with Adonis® 12.5g a.i./ha barriers showed that there was a heavy initial impact on non-target insects, especially if they were exposed to spray during application. Cadavers of various Caelifera species, (e.g. *Acrotylus patruelis*, *Sphingonotus scabriculus*), Ensifera (e.g. *Acanthoplus* sp. and Gryllidae), Formicidae, Diptera, Neuroptera, Lepidoptera, Tenebrionidae and Pentatomidae were found lying on the soil surface within barrier sites during follow-up inspections. However, after 24h post-application, a few ants and ground beetles were found on the soil surface in some barriers. It was unfortunately not possible to undertake residue studies, or carry out an environmental impact study because of limited resources.

Generally, insects were absent within barrier strips for at least a week after treatment. After this period, ants, beetles and armoured crickets were regularly encountered within the barriers.

Few birds were observed feeding on hopper bands in the trial area. However, a few finch larks, flew into Barrier 1 and fed on L2 moribund hoppers, but the birds were not followed further. In the same barrier, sub-adult lacertid lizards were also noted feeding on affected hoppers. Lizards which appeared outwardly unaffected were regularly encountered on the spray sites for up to 28 days post-application. A small tortoise found in Barrier 3, was tracked for several days and also appeared unaffected. No dead reptiles or birds were found in the trial plots.

5.4 DISCUSSION and CONCLUSION

The gregarious brown locust hopper bands found in the trial area were generally large and highly mobile and severely tested the 21m wide 12.5g a.i./ha Adonis® barriers. The numerous hopper bands generally marched in the same direction and tended to follow similar routes across the veld. This resulted in successive bands crossing the same barriers at various post-application intervals and indicated that barrier tactics were a viable control option for brown locust management.

All target bands entered and fed freely on treated grass within barriers, without any repellency being observed. Indeed, some observations suggested that the hoppers preferred the Adonis-treated grass within the barriers, probably due to the attractiveness of the canola (rape seed) oil carrier used in the UL formulation (Aventis Crop Science, 2000).

Single barriers of 12.5g a.i./ha Adonis® generally produced excellent control of L2-L5 marching bands during the first day following application. Mortality of bands that crossed the barriers continued for at least four days, giving final estimated control results of 99%, except for the L4-L5 bands which crossed Barrier 11. However after the first day, the residual control of older L4-L5 bands by the Adonis® barriers became much more variable. Under current field conditions, the efficiency of barriers against brown locust hopper bands appeared to be influenced by a

combination of behavioural responses by the hoppers and environmental factors, from which the following assumptions could be made:-

1. *The time of day* when a brown locust band entered a barrier appeared important. Bands which encountered barriers during the early part of the morning were less mobile and were observed to spend more time feeding, resulting in excellent control. In contrast, bands crossing barriers during the afternoon moved more rapidly in column formation and spent less time feeding, which gave poor control.

2. The *instar of the target band* also influenced the level of control produced by the Adonis® barriers. These proved more effective against the younger L2 and L3 hopper bands. Barrier treatments should consequently be implemented as early as possible after the start of locust control campaigns.

3. The *size of the target band* might have influenced dose uptake in barriers. For example, Adonis® barriers appeared more effective against small-sized bands of different age structure, but the level of control diminished when large-sized bands crossed the barrier. The momentum generated by these large gregarious bands enabled them to cross barriers with little evidence of control.

4. P*lant density and composition of the flora* also appeared important under Karoo conditions. Plant cover in the study area ranged from 15-51%, while plant composition ranged from succulents, dwarf shrubs to grasses. Hoppers selectively fed on the available vegetation within barriers and where grass cover was sparse little feeding occurred and poor control resulted. It is well known that brown locust hoppers feed preferentially on grasses (Smit, 1939) and the observations on the unacceptable 'brakveld' vegetation found in Barrier 7 have already been described. It should be noted that in many parts of the Arid Karoo and Namaqualand, succulent veld-types predominate, and it is likely that hoppers encountering brakveld barriers would not feed. Under these circumstances control will therefore be much reduced since Adonis® has been shown to work mainly by stomach action.

Furthermore, LD_{90} bioassay results have demonstrated that Adonis® has a potent stomach action against brown locust hoppers and is 6X stronger than by contact action (Butler and Du Preez, 1994).

5. The *time after the barrier was applied* also determined the level of control achieved. Despite a high application rate of 12.5g a.i./ha Adonis® UL, the level of persistence was generally of short duration and only gave up to 48% control after 12 days post-treatment (Table 5.5). This is far less than the 21 days persistence reported for desert locust hoppers in Mauritania (Aventis Crop Science, 2000). Without examining residue data on the Adonis® treated vegetation in these trial plots, which was beyond the scope of the present study, it is impossible to comment further.

5.5 RECOMMENDATIONS

The 12.5g a.i./ha Adonis® dose applied in a single 21m wide barrier in the current study was high in comparison with the 7.5g a.i./ha rate applied by air in 100m wide multiple barriers, used for control of migratory locust bands in Madagascar. According to reports from Kazakstan, a 40m wide barrier gave good results against the Asiatic migratory locust (Aventis Crop Science, 2000). In the present case, the use of wider Adonis® barriers might give more effective control of L5 brown locust hoppers. Further trial work with wider barriers, tested in various areas with different semi-desert vegetation types, is required before final recommendations can be made. Multiple barrier strips should also be investigated.

Brown locust bands often follow the path of least resistance during their progress through the veld and prefer the bare, open, areas as they hop along in their respective columns. Bands are thus naturally funnelled into open areas, such as along sheep tracks and roads (*see* Fig.5.1). Adonis® barriers deployed along roadside verges, where the grass vegetation is usually more lush due to runoff of rain from the road surface, may provide optimum sites for barrier application. In this case, access to unoccupied farms would hence be unnecessary.

5.6 BENEFITS and CONSTRAINTS

5.6.1 Potential benefits

- Only a fraction of the area infested with locusts requires chemical treatment with barriers.
- Adonis® UL has been commercially registered for brown locust control.
- Barriers would be ideal for use in remote areas using 'spray and forget' tactics.
- Barrier treatments offer a greatly extended 'window' for spraying as compared to current
- seek and spray tactics.
- High efficacy against hopper bands, especially younger instars, under most circumstances.

5.6.2 Potential constraints

- Barriers are only effective against hopper bands and are not suitable for adult swarm control.
- The environmental impact of Adonis barriers requires further elucidation before they can be recommended for operational implementation.
- Some areas of the Karoo have sparse or unpalatable vegetation cover, which may be unsuitable for effective barrier application.
- Control failures may sometimes occur, especially against larger, late instar bands.

CHAPTER 6
Insect Growth Regulators

6.1 INTRODUCTION

The benzoylphenyl urea compounds, which are also known as insect growth regulators (IGRs), interfere with the process of chitin synthethsis and deposition in the cuticle (Reynolds, 1987), causing serious malformations and death of juvenile insects undergoing the moult. The IGRs are persistent compounds that primarily act as potent stomach poisons against a range of insects (Grosscurt and Jongsma, 1987), although they also produce some contact activity against locusts and grasshoppers (Coppen, 1994).

The IGRs only affect the juvenile stages of insects and are thus relatively target specific with a limited impact on non-target invertebrates (Everts, 1990) and have no known toxicity against vertebrates at field dose rates. These compounds may thus be potentially suitable candidates for application in barrier spraying against hopper bands, having suitable persistence on vegetation and low environmental impact in semi-desert areas (Symmons, 1992). A total cover application of the IGR, diflubenzuron, produced negligible environmental impact on Karoo invertebrates, as compared to a standard Decis® application (Roux, 1998).

Field trials of diflubenzuron, applied to strips of vegetation as a barrier treatment, have been undertaken against various locust and pest grasshopper species (Hoffman, 1988; Bouaichi, Coppen & Jepson, 1994; Cooper, Coppen, Dobson, Rakotonandrasana and Scherer, 1995). Significant reduction in population densities were achieved in the above trials, although speed of kill was slow. Factors affecting the rate of mortality produced by IGR barriers included the track interval between the barriers, the number of barriers traversed by the hoppers, density of vegetation, development stage of the hoppers and the time interval before moulting took place.

A preliminary laboratory investigation of the IGR products, flufenoxuron and teflubenzuron, using technical grade material, was undertaken to evaluate their toxicity against hoppers of the

brown locust with the intention of comparing their efficacy with Adonis®. Work was also undertaken on a diflubenzuron EC formulation (Dimilin®) in a simulated field trial in order to evaluate its potential for use in barrier treatments against the brown locust. This IGR compound was selected because the supplier of the product (Uniroyal Chemical BV, South Africa) was interested in obtaining registration of its Dimilin OF6 formulation for brown locust control.

6.2 MATERIALS and METHODS

A laboratory culture of brown locust hoppers, derived from parents collected in the field, was established at the PPRI research station, Rietondale, Pretoria. Locusts were reared in metal-gauze cages, measuring 40x25x25cm, and fed a diet of green maize leaves and dry wheat bran. Insects were maintained at a temperature range of 26-30°C and kept under constant 24h photoperiod.

6.2.1 Experiments with flufenoxuron and teflubenzuron

The acute stomach toxicity of technical grade flufenoxuron and teflubenzuron was evaluated against gregarious L5 brown locust hoppers, of known age and weight class, in order to establish dose responses. Flufenoxuron and teflubenzuron technical powder was supplied by Cyanamid South Africa (Pty) Ltd. Specific concentrations of the above IGRs were prepared by dissolving technical powder in pure 99% alcohol, which was then thoroughly mixed using a 'Vortex-Genie 2' shaker. The dissolved IGR was applied as 0.1µℓ droplets by means of a 10µℓ Hamilton syringe, driven by an electrically powered micro-applicator, to 1cm diameter leaf discs cut from potted maize plants grown in the glasshouse.

In order to standardise the age class, only mid-instar L5 hoppers were used in the experiments. Hoppers were first starved for 24 hours in order to stabilise their mass and standardise their feeding response, before being individually weighed and assigned to different weight classes. The concentration of IGR required to give precisely the same dose rate (µg/g) to individual hoppers within each of the different weight classes was then calculated (Tables 6.1 and 6.2), so that the dose rate remained constant regardless of the body weight of the hopper. Three dose

rates of flufenoxuron, viz. 3, 6 and 15µg/g, and three rates of teflubenzuron, viz, 6, 9 and 15µg/g, were examined.

6.2.1.1 Feeding experiments

The 0.1µℓ droplets of IGRs were applied to the centre of the maize discs and allowed to air dry. Each dose rate of IGR was tested against batches of 30 hoppers (of mixed sex) individually placed in glass tubes and fed their specific maize discs. Only insects that consumed the entire maize disc within 2h were included in the experiment and substitutes replaced those failing to consume their discs. Hoppers that had ingested the same IGR dose were then grouped together in wire-gauze observation cages, maintained on a diet of green maize leaves and dry wheat bran and kept at 26-30°C under continuous lighting. Controls (n=30) received similar treatment and were fed maize discs treated with alcohol alone. All sub-lethal effects and mortality of hoppers following the single exposure to the IGRs were recorded over the following 21 days.

6.2.2 Experiments with diflubenzuron

The toxicity of Dimilin OF6 (60g a.i./ℓ) was evaluated against L2 brown locust hoppers following a simulated field application using volume rates of 1, 1.5 and 3ℓ/ha sprayed onto young maize plants. Dimilin OF6 was applied by means of a hand-held Micron 'Ulva +' spinning-cup sprayer (Micron Ltd, Bromyard, UK) to potted maize plants (30cm high) placed outdoors at Rietondale. The sprayer was selected because it provided greater precision during calibration and was powered by 6 alkaline batteries producing ±7000rpm, which according to the manufacturer's handbook, produced droplets in the 70-100µm size range.

Flow rate per minute from the sprayer was varied by selecting different colour-code nozzles and was checked using a measuring cylinder and a stopwatch. The same operator, whose walking speed was monitored by means of a stopwatch, was used to standardize application speed. Track interval was measured with a measuring tape. Spraying started downwind, moving upwind to cover the maize plants (n=10) with five parallel swaths, which ensured good incremental spray coverage. Replicated spray applications (n=3) were undertaken with each of the designated volume application rates.

Following spray application the maize plants were allowed to dry off before being placed in a glasshouse. Maize plants sprayed with the same volume application rate of Dimilin OF6 were labeled and pooled together. Treated leaves were cut from the plants when required.

6.2.2.1 Exposure to diflubenzuron

Batches of newly moulted L2 gregarious hoppers (n=10) were placed in glass jars (1ℓ capacity) fitted with wire-gauze lids. Hoppers were provided with sticks on which to perch and were maintained at 30±2°C and under 24h lighting. Batches of hoppers were provided *ad lib.* with maize leaves, sprayed with their specific Dimilin OF6 volume rate, for a 2h period in the morning and again 4h later. All vegetation was removed from the jars between the exposure periods, which ensured that hoppers only fed on freshly cut vegetation. A total of three replicates (n=30 hoppers) were used for each experiment. Three replicated batches of control hoppers (n=10/batch) were fed unsprayed maize leaves under the same experimental conditions as above.

The efficacy of the three volume application rates of Dimilin® OF6 were examined under two different exposure periods as follows:

Exposure A

Hoppers were provided with Dimilin® treated vegetation for a 2h period, twice per day, over the entire inter-moult period of 7 days (time taken for L2 hoppers to moult to the L3 stage).

Exposure B

Hoppers were provided with Dimilin® treated vegetation for a 2h period, twice per day, but only over the first half of the inter-moult period (4 days). Hoppers were fed untreated maize leaves during the remainder of the inter-moult period.

Following the above exposure periods the hoppers were provided with an unlimited diet of unsprayed maize leaves and wheat bran. Mortality of hoppers, or morphological defects following ecdysis, were recorded for up to 21 days post-treatment. All mortality data were smoothed using Abbot's formula.

6.3 RESULTS

6.3.1 Experiments with flufenoxuron and teflubenzuron

Concentrations of the above IGRs required to produce specific dose rates to be applied against the different L5 hopper weight classes, are given in (Table 6.1 and 6.2).

Table 6.1. Concentrations of flufenoxuron (%m/v) required to produce specific dose rates (µg/g) against different weight classes of L5 brown locust hoppers.

Dose	Weight class of hopper (g)							
µg/g	0.2-0.29	0.3-0.39	0.4-0.49	0.5-0.59	0.6-0.69	0.7-0.79	0.8-0.98	0.9-0.99
3	7.5	10.5	13.5	16.5	19.5	22.5	25.5	28.5
6	15.0	21.0	27.0	33.0	39.0	45.0	51.0	57.0
9	22.5	31.5	40.5	49.5	58.5	67.5	76.5	85.5
15	37.5	52.5	67.5	82.5	97.5	112.5	127.5	142.5

Table 6.2. Concentrations of teflubenzuron (%m/v) required to produce specific dose rates (µg/g) against different weight classes of L5 brown locust hoppers.

Dose	Weight class of hopper (g)							
µg/g	0.2-0.29	0.3-0.39	0.4-0.49	0.5-0.59	0.6-0.69	0.7-0.79	0.8-0.89	0.9-
3	5.0	7.0	9.0	11.0	13.0	15.0	17.0	19.0
6	10.0	14.0	18.0	22.0	26.0	30.0	34.0	38.0
9	15.0	21.0	27.0	33.0	39.0	45.0	51.0	57.0
15	25.0	35.0	45.0	55.0	65.0	75.0	85.0	95.0

The toxic effects produced by the above IGRs against L5 brown locust hoppers were complex and were therefore categorized as described in Table 6.3.

Table 6.3. Types of morphological defects occurring in L5 brown locust hoppers following exposure to flufenoxuron and teflubenzuron.

Defect category	Description of morphological defect
1	Ecdysis was successful and insect survived with no obvious ill-effects
2	Ecdysis was completed but insect suffered non-lethal deformities
3	Ecdysis completed but insect was seriously deformed and later died
4	Insect died while undergoing ecdysis.
5	Insect died before ecdysis was attempted (during the pre-ecdysis stage)

The morphological defects and mortality of L5 brown locust hoppers produced by a single ingestion of three different dose rates of flufenoxuron and teflubenzuron varied considerably (Table 6.4). Both products sometimes killed the hoppers before or during the moult (defect category 4 and 5), or conversely, produced no visible effect (category 1). Very few "walking wounded" were produced (category 2 and 3). Mortality in the controls reached 10% after 21d.

Table 6.4. Number of L5 brown locust hoppers (n=30) showing different toxic effects produced by a single ingestion of various dose rates of flufenoxuron and teflubenzuron. Final percentage mortality was corrected using Abbot's formula.

Dose µg/g		No. hoppers assigned to morphology defect category					% mortality (Class 3+4+5)
		1	2	3	4	5	
Flu	3	18	0	0	3	9	33.4
	6	11	2	0	11	6	51.8
	15	9	0	3	6	12	66.7
Tef	6	26	2	0	0	2	0
	9	15	0	0	4	11	44.4
	15	10	0	3	4	13	62.9
Con	0	0	0	0	0	3	10.0

Maximum mortality achieved with the highest dose rate (15ug/g) of both these IGRs examined, was 67% and 63% respectively (Table 6.4), which was considered unsatisfactory for brown locust control. Some of the locusts that survived the lowest dose rates (flufenoxuron 3µg/g and

teflubenzuron 6μg/g) laid viable eggs after the end of the experiment. However, none of the surviving fledglings exposed to the higher dose rates of these IGRs laid eggs.

6.3.2 Experiments with diflubenzuron

Application and meteorological data for the simulated field application of three volume rates of Dimilin OF6 are given in Table 6.5.

Table 6.5. Application parameters for three volume application rates of Dimilin OF6, applied by means of a Micron Ulva+ spinning-cup sprayer, to maize plants.

Dose (ℓ/ha)	Restrictor colour	Emission rate (mℓ/min)	Application speed (km/h)	Track interval (m)	Air temp. (°C)
1	Pink	28	5.7	3	14
1	Red	23	5.7	2.5	15
1	Red	23	5.7	2.5	15
1.5	Pink	28	5.7	2	14
1.5	Pink	28	5.7	2	15
1.5	Pink	29	5.7	2	16
3	Black	52	5.2	2	15
3	Black	52	5.2	2	16
3	Black	52	5.2	2	17

The L2 hoppers were observed to feed on maize leaves sprayed with the three different Dimilin OF6 rates, proving its acceptability. Toxic effects of Dimilin OF6 manifested themselves in a range of morphological defects, both during and after ecdysis, which were classified according to the criteria established in Table 6.3. Mortality data for hoppers following exposure 'A' and for exposure 'B' are given in Tables 6.6 and 6.7 respectively.

Complete (100%) mortality of L2 hoppers was achieved with all three application rates of Dimilin OF6 following exposure 'A' (Table 6.6). Treated hoppers took an average of 10.3±0.2 days to die at all three dose rates (range 10-11 days). All control hoppers moulted to the L3 stage within 6-8 days and no mortality was recorded during the 21 day observation period.

Table 6.6. Exposure A. Brown locust L2 hoppers (n=30) fed maize leaves treated with three different rates of Dimilin OF6 twice per day for the entire inter-moult period.

Dose	No. hoppers assigned to morphology defect category					Total % mortality
	1	2	3	4	5	
Control	30	0	0	0	0	0
1ℓ/ha	0	0	0	4	26	100
1.5ℓ/ha	0	0	0	0	30	100
3ℓ/ha	0	0	0	1	29	100

Mortality mainly occurred during the pre-ecdysal period (defect category 5) with the remaining hoppers dying while attempting ecdysis (category 4) (Table 6.6). Little evidence of a dose response was observed with the three dose rates examined. Treated hoppers were observed to cease feeding 12-24 hours before ecdysis was attempted, while untreated controls continued feeding up until a few hours before the moult.

Hoppers that died during pre-ecdysis (category 5) became bloated with the cuticle becoming loose and the abdomen elongated. Haemolymph was often observed extruding from behind the pronotum and from neck and leg membranes. Hoppers only made feeble movements at this stage. The few hoppers that were able to attempt ecdysis were then unable to fully shed the exuviae (category 4). The head and legs became trapped in the exuviae making further movements impossible. All these trapped hoppers died within 24 hours.

When hoppers had a reduced exposure to Dimilin OF6 (exposure B), the results were confusing, with a range of morphological defects produced. No dose response was evident and control was incomplete with many survivors at all dose rates (Table 6.7).

Table 6.7. Exposure B. Brown locust L2 hoppers (n=30) fed maize leaves treated with three rates of Dimilin OF6 twice per day for the first half (4d) of the inter-moult period.

Dose	No. hoppers assigned to morphology defect category					Total % mortality
	1	2	3	4	5	
Control	30	0	0	0	0	0
1ℓ/ha	5	2	1	15	7	77
1.5ℓ/ha	29	0	0	1	0	3
3ℓ/ha	13	1	0	6	10	53

Final mortality rates varied greatly between treatments with highest mortality of 77% achieved with the 1ℓ/ha application. Most of the mortality at the 1ℓ and 3ℓ/ha rates occurred between 8-12d post-application, but was extended up to 15-17d on two occasions. The three hoppers that survived the moult suffered deformities to the legs or lost the hind legs completely (category 2). These hoppers later failed to fledge properly and suffered crumpled wings. However, all survivors placed into category 1 successfully fledged into adults with no wing deformities evident. Untreated hoppers all successfully moulted to the L3 stage within 5-7 days and no mortality was recorded in the controls during the 21 day observation period.

It was evident that final mortality of L2 hoppers following a discontinuous exposure to Dimilin® OF6, applied at three different dose rates to maize plants, was generally unsatisfactory.

6.4 DISCUSSION and CONCLUSION

A single ingestion of 3 dose rates of flufenoxuron or teflubenzuron by L5 brown locust hoppers gave unsatisfactory control, with 30-100% of treated hoppers surviving for at least 21d following treatment at all dose rates. These IGRs produced variable mortality and the effects were difficult to interpret; hoppers either died before or during the moult or were entirely unaffected. Very few intermediate stages of deformed survivors were produced. A single ingestion of relatively high dose rates of three different IGRs eventually produced good control of L2 hoppers of the desert locust (Coppen and Jepson, 1996).

The duration of exposure to Dimilin®OF6 by L2 brown locust hoppers was found to be a critical factor influencing efficacy. Exposure to treated vegetation twice per day during the entire inter moult period gave 100% control with all three volume application rates examined, while control was unsatisfactory when hoppers were only exposed to treated vegetation during the first half of the inter-moult period (4 days). Some of these survivors showed no morphological defects at all. Duration of exposure is evidently very important and the longer the exposure the greater the toxicity. Diflubenzuron is non-accumulative and is readily excreted in the feces (Neuman and Guyer, 1987), which means that insects can recovery from sub-lethal exposure.

In field trials with diflubenzuron against migratory locust bands in Madagascar, one spray barrier, applied in dense grass vegetation, was generally sufficient to significantly reduce band size (Cooper *et al.*, 1995). This was ascribed to the pre-moult effects of diflubenzuron leading to reduced mobility and thus greater predation pressure on the hoppers. Morphological deformities produced by sub-lethal exposure in the bioassays against brown locust hoppers would also undoubtedly contribute to increased mortality from predation in the field.

Brown locust hopper bands potentially present a difficult target for the effective use of IGRs in the Karoo, as the hoppers typically march in narrow columns rather than on a broad front as with other locust species, such as the African migratory locust. They also march rapidly over the sparsely vegetated habitat found in the Karoo, with L5 bands typically covering 1-2km per day under summer conditions. The brown locust is also a geophile and marches on bare soil, rather than climbing through dense vegetation, as do the migratory locust and the red locust. These factors will minimize the time that marching brown locust hopper bands would spend in contact with IGR-treated vegetation and would probably lead to the failure of IGRs when applied as a barrier spray.

In order to maximise efficacy and to increase the possibility of hoppers encountering barriers and ingesting IGR-treated vegetation on a regular basis, large areas of Karoo veld would have to be cover sprayed by aircraft. However, this application strategy is considered both uneconomic and environmentally unacceptable in the Karoo.

One possible method of increasing exposure of hopper bands to IGRs could be to spray the vegetation along road verges in the Karoo. Hopper bands often congregate along the side of roads where the grass is more plentiful due to the additional run-off of rain from the road and due to the exclusion of grazing sheep. The application of IGRs to these road verges would act as a 'trap-crop' and would probably increase the exposure of bands to treated vegetation, although it is doubtful whether this increased exposure would be sufficient to enhance mortality.

In conclusion, the extended period of ingestion of IGRs required by brown locust hoppers, combined with the slow and variable mortality produced by intermittent exposure, suggests that barriers of IGRs would not produce the same efficacy as recorded for the narrow, 21m wide, Adonis® barriers against brown locust hopper bands.

6.5 BENEFITS and CONSTRAINTS

6.5.1 Potential benefits

- IGRs produce a reduced environmental impact on non-target organisms compared to conventional insecticides.
- Good persistence of IGRs on vegetation makes them suitable for barrier application.
- Spray and leave tactics. Barrier spraying with IGRs does not require the time, manpower and expense of searching for individual brown locust targets.

6.5.2 Potential constraints

- IGRs produce no knockdown of treated hoppers and are slow acting.
- Variable mortality according to hopper age and duration of exposure to IGR.
- IGRs only work against the juvenile hopper stages. Other insecticides must be used against the adult stage.
- The sparsely vegetated Karoo and the marching behaviour of the brown locust bands would not favour the efficacy of IGR barriers.

CHAPTER 7

Metarhizium anisopliae var. *acridum* myco-insecticide

7.1 INTRODUCTION

The biological control of locusts and economically important grasshoppers using introduced natural enemies, or the augmentive release of pathogenic organisms, has been considered for over a century (Prior and Greathead, 1989). However, after evaluation of the wide range of parasitoids, predators and diseases known to affect acridids, and considering the mass production technology currently available, scientists concluded that the most promising candidates for development as biological control agents were the deuteromycete entomopathogenic fungi, *Beauvaria* spp. and *Metarhizium* spp. (Prior and Greathead, 1989), as these fungi work by direct contact through the insect cuticle and can be mass produced on artificial media.

A large number of entomopathogenic fungal isolates have since been collected from locusts and grasshoppers in the field (Kooyman & Shar, 1992; Bateman, Carey, Batt, Prior, Abraham, Moore, Jenkins and Fenlon, 1996) and many more are likely to be discovered if more intensive surveys are undertaken (Prior, 1992). However, entomopathogenic fungi usually require high humidity conditions in order to germinate successfully under field conditions and natural fungal epizootics within locust populations in the field are usually rare and, as such, are considered an unreliable mortality factor (Prior and Greathead, 1989; Greathead, 1992).

The only recorded fungal epizootics reported in locust populations in southern Africa occurred during the 1920s and 1930s amongst red locust, *N. septemfasciata*, swarms in the humid areas of northern Botswana and Namibia and in Natal Province of South Africa and were caused by the entomopthoralean fungus, *Entomophaga grylli* (Fresenius) Batko (= *Empusa grylli*), (Skaife, 1925). Records of brown locust swarms also being infected by the *E.grylli* fungus in northern Botswana were reported by Jack (1931; 1933).

The first attempt at manipulating entomopathogenic fungi as a biological control agent against locusts was the South African 'Locust Fungus' debacle during 1896-89, when scientists at the Cape of Good Hope Bacteriological Institute in Grahamstown claimed to have successfully cultured *E. grylli* on agar plates. Spore cultures were commercially distributed to farmers for locust control, but the cultures were ineffective and were later proved to comprise only a common mucor, pin-mould (Pole Evans, 1911; Skaife, 1925). Even today it is still not feasible to commercially mass produce *E. grylli* on artificial media (Goettel & Roberts, 1992).

The first field trials with entomopathogenic fungi as a potential myco-insecticide against locusts date back to the 1930s in Natal, South Africa, when red locust hoppers were sprayed with an aqueous suspension of spores of *Beauvaria bassiana* Balsamo (Vuillemin) (Schaefer, 1936). However, once the age of synthetic insecticides dawned and organochlorine insecticides became available in the 1940s, research into the biological control of locusts was largely abandoned. Research into the potential of fungi for locust control was only resumed following concern about the adverse environmental effects of insecticides during the 1986-89 desert locust plague in north Africa, when over 13 million litres of broad-spectrum insecticides were applied from aircraft over vast areas, at a cost of USD 275million (Everts, 1990; Greathead, 1992).

The most important breakthrough in the development of myco-insecticides was the discovery that aerial conidia of the deuteromycete fungi, *Metarhizium* and *Beauvaria* spp., showed greatly increased virulence against insects, including locusts, when formulated in vegetable or mineral oils instead of water (Prior, Jollands and le Patourel, 1988; Bateman, Carey, Moore and Prior, 1993). Formulation of the lipophilic conidia in oil eliminates the reliance on conditions of high humidity for germination and this opened the door for the development of myco-insecticides for locust control in semi-arid environments (Lomer, Prior and Kooyman, 1997).

An international research programme, implemented by CABI Bioscience (formerly the International Institute of Biological Control (IIBC)) at Silwood Park, Ascot, UK, and funded by international donors, was established in 1989 to develop strains of the locust-killing deuteromycete fungus, *Metarhizium anisopliae* var. *acridum* (Deuteromycotina: Hyphomycetes),

(previously known as *M. flavoviride* (Driver, Milner and Trueman, 2000)), as commercially viable myco-insecticides for use against various locust and grasshopper species. The main UK-based research thrust was later renamed 'Lutte Biologique contre Locustes et Sateriaux' (LUBILOSA).

After intensive field surveys for fungal isolates and following the laboratory evaluation of the virulence and growth characteristics of the large number of *Metarhizium* isolates collected, LUBILOSA selected the isolate IMI 331089, which originated from a grasshopper, *Ornithacris cavroisi* (Finot) collected in Niger, as their 'standard isolate' for further development due to is high virulence against African locusts and due to its favourable culture, viability and storage characteristics. By formulating the aerial conidia in oils and applying the myco-insecticide as a ULV spray, effective control of a number of pest acridid species has been achieved under a range of field conditions (Bateman, 1997; Kooyman and Godonou, 1997; Lomer, *et al.*, 1997).

In search of alternative and more environmentally acceptable methods of brown locust control, PPRI locust researchers visited IIBC in the UK and the Montana State University, Bozeman, USA, in 1992 in search of promising isolates of *Metarhizium* and *Beauvaria* spp. The standard IIBC *Metarhizium* isolate IMI 330189 was imported under quarantine into South Africa in 1993 (import permit No.14/2/2/199/21/172, issued by the Directorate of Plant and Quality Control: NDA) and cultured in the laboratory. Bioassays were undertaken at the PPRI laboratories in Pretoria, to determine its virulence against the brown locust and the African migratory locust. Topical application of oil droplets containing $3.0x10^4$ and $6.0x10^4$ conidia, applied to the neck membrane of L5 brown locust hoppers, gave 90-93% mortality within 21 days with median lethal times of 9.8 and 7.5 days respectively (Price and Müller, 1993, Müller, 2000a).

Following these encouraging results the NDA issued a release permit for the IMI 330189 isolate to allow small-scale field trials to be undertaken. A number of field efficacy trials and associated ecological studies with the myco-insecticide were undertaken in the Karoo between 1994-1997 (Bateman *et al.*, 1994; Price, Bateman, Brown, Butler and Müller, 1997), culminating in the commercial registration of the isolate as Green Muscle® in 1998, under the Agricultural

Remedies Act (Act No. 36 of 1947). The most important field trials with Green Muscle® against the brown locust are summarised below and the prospects for its possible adoption for brown locust control in the Karoo are discussed.

7.2 MATERIALS and METHODS

7.2.1 Preliminary ground trials

7.2.1.1 Field site

Field trials of the IMI 330189 myco-insecticide were undertaken against gregarious L4-L5 brown locust hopper bands on the farm Nuwefontein (30°56'S, 24°11'E) in the Hanover District of the Northern Cape Province during February 1994. Good summer rains had triggered a widespread brown locust outbreak in the central Karoo and numerous hopper bands were being controlled throughout the area by the South African NDA. The topography of the trial area was undulating and vegetation was classified as 'False Upper Karoo' (Acocks, 1988) with low Karoo bushes, *Pentzia*, *Chrysocoma* and *Lycium* sp. and knee-high grasses, *Eragrostis* and *Stipagrostis* spp., present. Vegetation was green and plant cover was estimated at 30%.

7.2.1.2 Spray formulation and application

Batches of spray formulation were made up on site before application by suspending 100g dried *M. anisopliae* var. *acridum* conidia in a 2ℓ mixture of 30% groundnut oil and 70% refined paraffin (Jet A1). Samples of each formulation were retained and spore counts were made using a haemocytometer in the laboratory. Germination viability was examined by streaking out samples of myco-insecticide formulation onto potato dextrose agar plates within 12 hours of application. Plates were incubated at room temperature for 24 hours and the percentage of germinating conidia counted under a binocular microscope.

Spore formulation was applied using a hand-held Micron 'Ulva +' sprayer, powered by six alkaline batteries, producing a rotational speed of 7000 RPM. A similar spray configuration with a blank oil formulation produced droplets with a volume mean diameter (vmd) of 68μm at

emission, as measured with a Malvern 2600 particle size analyser in the laboratory.

Under field conditions the Micron 'Ulva +' sprayer produced a constant emission rate of 50mℓ/min when fitted with an orange restrictor nozzle. The required volume application rate of 2ℓ/ha (giving the standard dose of 100g spores/ha) was produced using a track interval of 3m and a walking speed of 1.4km/hr. Track interval was marked by flagmen at either side of the spray plots and walking speed was calibrated with a stopwatch.

Gregarious L5 hopper bands, identified for treatment, were sprayed early in the morning (06h45-09h00) while still densely aggregated on their overnight roosts. Individual hopper band targets were generally small in size (0.05-0.1ha) and were covered with 10-15 spray swaths. Spray plots varied in size according to the size of the band, but the plot layout ensured that the band and the surrounding vegetation for 5-10m were covered incrementally with spray drift, as is standard practice in all insecticide trial work in the Karoo. Five bands were treated with the myco-insecticide and two control bands were sprayed with blank oil formulation only. Air temperature, relative humidity and wind speed and direction were monitored during application.

7.2.1.3 Mortality analysis

Immediately after spray application, a portion of the treated bands were corralled in open-topped field enclosures, as described earlier. After being placed over the hoppers, the bottom of the enclosures was quickly sealed with soil in order to prevent escapes. Samples of hoppers (n=±50) were subsequently removed from each enclosure after 0, 24 and 72 hours and placed into metal-gauze cages, measuring 40x25x25cm. Samples of untreated hoppers (n=±50), collected from two bands located upwind of the trial site were used as controls for all treatments. Cages of hoppers were transported back to the laboratory at Rietondale and maintained outdoors, where daily inspections of mortality were undertaken. Mortality data were analysed using the MELTIMOR (v.3.31) programme (CABI Bioscience), which calculated the median lethal time and average survival time for treated locust samples. Final mortality figure for each treatment was adjusted according to the final mortality in the two untreated controls, using Abbot's formula.

7.2.2 Aerial spray trials

7.2.2.1 Field site

Following good rains in January 1995, an intense brown locust outbreak developed in the central and western Karoo during February-March. A series of ten ULV aerial spray trials of the *Metarhizium* myco-insecticide were conducted against gregarious L5 hopper bands on the farm Wonderboom (31°02'S, 23°46'E) near Deelfontein in the Richmond District, Northern Cape Province, during mid February 1995. The veld type was 'False Upper Karoo' (Acocks, 1988), with dwarf Karoo bushes, *Pentzia* sp. and *Chrysocoma* sp., predominating. Veld conditions were dry and vegetation canopy cover, measured using the point counts method (n=500 points), averaged 26%, with very little grass (<2%) present.

7.2.2.2 Calibration and application

Fresh batches of myco-insecticide were prepared in the field each day prior to application. Dry spore powder was first mixed into a paste with pure grade paraffin in a plastic bucket and then diluted down to the required volume with a 50:50 mixture of Jet A1 and Ondina oil (Shell Oils, UK). The spore mixture was repeatedly strained through a household kitchen sieve before being poured into the aircraft droptank. The standard dose rate of 100g/ha conidia ($5x10^{12}$ conidia/ha) was applied at two different volume rates of 1ℓ and 2.5ℓ/ha from a "Thunderbird" microlight aircraft (Davis Engineering, Midrand), fitted with six Micronair AU7000 atomisers mounted on a boom under the wings. Atomiser blade settings varied from 25-45°, in order to vary the droplet sizes during different applications. Calibrations were made on the landing strip, using plastic bags and measuring buckets, to obtain a constant flow rate over one minute intervals.

The six Micronair atomisers fitted to the aircraft could accommodate the required flow rate of 6ℓ/min for the 2.5ℓ/ha application rate. However, difficulties were experienced with the lower volume rate (1ℓ/ha) and five of the atomisers had to be closed off to produce the required emission rate of 2ℓ/mim from a single atomiser. Both rates were applied at an aircraft speed of 100km/h and at an emission height of 2-4m. Track spacing was marked with flagmen and measured 12m for the 1ℓ/ha and 14m for the 2.5ℓ/ha volume rates. The 2.5ℓ/ha applications (n=5) were made by the aircraft flying swaths in opposite directions over the target, while the

1ℓ/ha applications (n=5) were made with the aircraft flying only in a single direction, so that the single spray atomiser was always on the downwind wing.

Myco-insecticide was applied to hopper band targets in the morning (06h30-08h00) while still densely aggregated on their overnight roosts. Spray targets were 0.1-0.5ha in extent, typical of medium to large-sized brown locust hopper bands. Application was made at right angles to the wind, starting downwind and covering the target with 8-12 parallel swaths, producing downwind incremental drift of myco-insecticide over the hopper bands. Spray plots measured 2-3ha in extent and meteorological conditions during application were recorded with a data logger. Spore concentrations from spray samples were later estimated using haemocytometer counts in the laboratory. The viability of spore samples was determined on agar plates, which were incubated at 20°C for 48h and the percentage germination determined under a binocular microscope.

7.2.2.3 Mortality analysis

After aerial application, a portion of each sprayed band (n=10) was corralled within an open-topped enclosure, described earlier. After 24h a sample of hoppers (n=±50) was removed from each enclosure and transferred to metal cages which were then kept in the shade at ambient temperature. One untreated control sample was also caged. The remaining hopper samples were maintained for a further ten days under field conditions in the enclosures in the spray plots, to compare the effects of caging. Two unsprayed control samples were placed in field enclosures over unsprayed vegetation, situated upwind of the above trials, for the same period. All samples were fed untreated grass each day. Hoppers in enclosures had to be protected from bird predation with nylon netting, which produced approximately 10% shade cover. Rate of hopper mortality in the enclosures and cage samples was monitored daily. Air temperature, soil surface temperature and relative humidity in the field were monitored with a data logger. After ten days all hopper samples in the field enclosures were removed to metal observation cages and transported back to Pretoria, where their mortality was monitored for up to 28 days post-application. Mortality data were analysed using the updated MELTIMORE programme (CABI Bioscience, UK), which corrected mortality in the treated samples according to mortality in the untreated controls. Average survival times were compared by pair-wise log rank tests.

7.2.2.4 Persistence of the myco-insecticide

Preliminary investigations of the persistence of the myco-insecticide on treated vegetation at Wonderboom farm were undertaken by confining untreated L5 brown locust hoppers for 48h within wire-mesh implant cages, measuring 1x0.5x0.5m, erected over sprayed vegetation within three selected aerial trial spray plots. Three replicates, with ten hoppers per cage, were set up within the three spay plots. Additional hoppers (n=3 cages) were set up over untreated vegetation as a control. The persistence trial was undertaken seven days post-application and was repeated after 28d and 50d post-application. Hoppers were removed from the field cages to metal observation cages after 48h exposure and were transported back to Pretoria where mortality was monitored for 28d. Cadavers were incubated in glass tubes at high humidity to induce fungal sporulation in order to confirm any *M. anisopliae* var. *acridum* mycosis.

7.3 RESULTS

7.3.1 Preliminary ground trials

7.3.1.1 Application conditions

Meteorological conditions recorded during five applications of the myco-insecticide agent and two blank oil controls are shown in Table 7.1.

Table 7.1. Meteorological conditions recorded during the ground-based myco-insecticide trials.

Band No.	Time of application	Air temp. °C	R.H. %	Av. wind speed (m/s)	Wind direction
Myco 1	07h35	21	80	0	E
Myco 2	07h50	22	57	0.8	E
Myco 3	08h10	22.5	80	1.7	E
Myco 4	08h30	22	80	1.2	E
Myco 5	07h30	19	80	2.2	E
Control 1	06h50	18.5	92	0.8	S
Control 2	07h15	19	80	2.1	E

Wind speed during application was variable and in some cases produced poor spray drift of myco-insecticide over the band target (e.g. Myco 1). Hoppers also scattered from their roosts following disturbance during application, which made the collection of adequate hopper samples from the small-size target bands difficult.

Mean maximum air and soil surface temperatures during the field trial period were 26.5°C and 42.5°C respectively. Relative humidity was high at night (80-90%) but was low in the afternoons, falling to a minimum of 25%. Caged samples were subsequently maintained outdoors in Pretoria where they experienced temperatures ranging from 14-26°C and 60-90% R.H.

7.3.1.2 Efficacy

Haemocytometer counts of the concentration of *Metarhizium* conidia in spray samples collected after application, were $3.7\text{-}4.7 \times 10^{12}$ conidia per two litre volume rate. Samples subsequently streaked onto agar plates produced 65-70% germination, which was considered satisfactory. Time to produce 50% mortality (median lethal time (MLT)) and 90% mortality (LT_{90}) following exposure of L5 hoppers to treated vegetation in field enclosures for 0h, 24h and 72h post-application are given in Table 7.2.

Table 7.2. Efficacy data for caged L5 brown locust hoppers treated with myco-insecticide agent applied by Micron Ulva + sprayer. Samples were removed after 0, 24 and 72 hours post-application. Percentage mortality @ 21d were corrected using Abbot's formula.

	MLT in days & sample (n=)			LT_{90} (days)			% mortality @ 21d		
	0h	24h	72h	0h	24h	72h	0h	24h	72h
Myco 1	- (85)	- (66)	- (81)	-	-	-	19.6	24.5	14.7
Myco 2	8.5 (76)	9.3 (42)	10.5 (64)	10.7	11.6	-	99.3	96.5	80.5
Myco 3	10.4 (84)	9.9 (61)	10.1 (72)	12.7	13.1	-	96.4	100	88.1
Myco 4	11.0 (53)	9.8 (44)	13.1 (46)	16.8	12.7	-	96.9	100	77.1
Myco 5	- (89)	10.3(39)	8.6 (61)	-	13.8	15.5	35.0	96.7	94.4
Cont 1	- (63)	- (70)	- (42)	-	-	-	11.1	22.9	11.9
Cont 2	- (75)	- (67)	- (59)	-	-	-	21.0	7.5	23.8

MLT and LT_{90} values not assigned (-) where mortality was <50% and <90% respectively.

Small numbers of hoppers in some of the treated samples and the controls died within a few days due to bacterial infection by *S. marcescens*, which could be attributed to the stress of handling and confinement of hoppers. However, all cadavers from treated samples that died between 7-21 days turned a red colour, indicative of *Metarhizium* mycosis.

The Myco 1 trial with a maximum mortality of 30-36% after 21 days was a failure (Table 7.2), and was almost certainly due to the poor spraying conditions of zero wind recorded during application (Table 7.1). In the four other myco-insecticide treatments, higher mortalities were achieved compared with blank oil controls (Table 7.2). However, the speed of mortality of different hopper sub-samples enclosed within the spray plots for 0, 24 or 72h failed to produce any clear trends. Highest mortality was achieved in the sub-samples removed from the enclosures after 24 hours (Table 7.2). Mortality in some of the control samples was also comparatively high at 7-24%. A mean cumulative mortality curve, using Graphpad PRISM software, for the four 24h sub-samples is depicted in Fig.7.1.

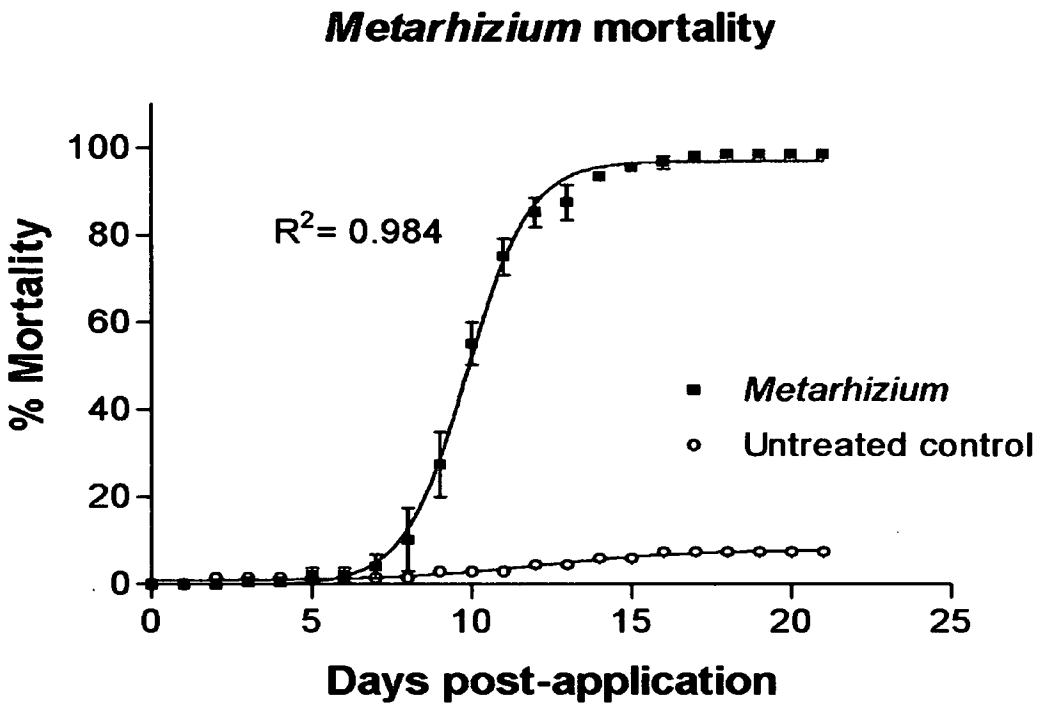

Fig.7.1. Rate of mortality of L5 brown locust hoppers following treatment with *Metarhizium anisopliae* var. *acridum* myco-insecticide.

7.3.2 Aerial spray trials

7.3.2.1 Application conditions

Spray application data for the ten aerial treatments of the myco-insecticide agent made on Wonderboom farm are given in Table 7.3.

Table 7.3. Spray application data during aerial spray trials with the myco-insecticide agent applied against L5 brown locust hopper bands (n=10).

Trial no.	Flow rate (ℓ/min)	Volume rate (ℓ/ha)	Atomiser blade angle	Track spacing	Av. wind speed (m/s)	Air temp. (°C)	Conidia conc.
1	6	2.5	25°	14	2 (E)	18	1.2×10^{12}
2	6	2.5	35°	14	2 (E)	23	1.2×10^{12}
3	2	1.0	35°	12	6 (N)	19	1.9×10^{12}
4	2	1.0	25°	12	7 (N)	20	1.9×10^{12}
5	2	1.0	25°	12	6 (N)	22	1.9×10^{12}
6	2	1.0	25°	12	2 (NE)	22	2.0×10^{12}
7	2	1.0	45°	12	2 (N)	27	2.0×10^{12}
8	6	2.5	45°	14	3 (S)	18	7.3×10^{11}
9	6	2.5	25°	14	3 (S)	21	7.3×10^{11}
10	6	2.5	25°	14	3 (S)	21	7.3×10^{11}

The concentration of conidia measured in samples from each myco-insecticide treatment is given in Table 7.3. All myco-insecticide samples also produced >90% germination, showing that there was nothing wrong with spore quality.

7.3.2.2 Efficacy

Meteorological conditions measured during the post-application mortality assessment period were hot and dry, which is typical of the Karoo in summer. Mean minima and maxima air temperatures thus measured 20 and 35°C, while soil surface temperatures measured 18 and 59°C respectively. Relative humidity fell from a mean maximum of 68% at night down to a mean minimum of 22% in the afternoons.

Efficacy of the myco-insecticide against enclosure samples, maintained in the field for ten days before being transferred to cages in Pretoria, is given in Table 7.4.

Table 7.4. Percentage mortality of L5 brown locust hoppers, maintained in field enclosures for 10d following aerial application of two different volume rates of the myco-insecticide.

Treatment No.	1	2	3	4	5	6	7	8	9	10	Control 1	Control 2
Applic. Rate (ℓ/ha)	2.5	2.5	1	1	1	1	1	2.5	2.5	2.5	-	-
Hopper samples =	62	85	202	135	71	89	92	118	184	151	76	96
% Mortality @10d	14	20	34	11	32	3	14	17	11	5	2	5
% Mortality @21d	71	82	97	86	82	29	56	97	98	95	16	14
% Mortality @28d	94	95	99	94	85	47	62	97	98	99	16	14
LT_{50} (days)	15	17	11	14	13	-	20	12	13	12	-	-
LT_{90} (days)	>21	>21	16	>21	-	-	-	16	17	17	-	-

Although the speed of mortality was initially slow at 10d, more than 90% control was achieved in 7 out of 10 treatments at 28d (Table 7.4). Median lethal time (LT_{50}) in these 7 bands averaged 13.4d, while an LT_{90} was achieved in 23.1d. Mortality was inadequate in bands 6 and 7. Mortality in the untreated controls averaged 14-16% after 28d.

Efficacy of the myco-insecticide against hopper samples, when removed after 24h from the above field enclosures and placed in cages for a 28d period, is shown in Table 7.5.

Table 7.5. Percentage mortality of L5 brown locust hoppers removed after 24h from the field enclosures and maintained in cages for 28d, following aerial treatments with the myco-insecticide agent.

	Cage sample obtained from corresponding enclosure treatment											
Treatment No.	Con.1	Con.2	1	2	3	4	5	6	7	8	9	10
Hopper sample (n=)	54	59	62	57	58	53	54	48	48	51	48	54
% mortality @ 10d	4	10	34	75	71	100	100	22	41	55	28	5
% mortality @ 21d	13	16	62	98	94	100	100	59	95	98	98	98
% mortality @ 28d	14	16	95	100	98	100	100	73	100	100	100	100

In several of the caged samples there was a marked increase in the speed of kill of insects compared with those kept under field conditions over the first ten days (Table 7.4). A pair-wise rank log statistic indicated significance differences (P= <0.001) in average survival time between samples in cages versus field enclosures in treatments 2, 6, 7 and 8. Mortality in control samples attained 14-16% after 28d.

7.3.2.3 Behaviour of treated bands

Hopper bands treated with the myco-insecticide agent at Wonderboom farm behaved exactly like control bands, feeding and marching 1-2km per day. Since there was no knock-down effect with the myco-insecticide agent, treated bands were soon lost as they marched off the farm. Hopper samples maintained in field enclosures showed few ill effects for 10d post-application and fed as avidly as untreated controls. Hoppers fledged into adults and enclosure samples had to be secured with netting in order to prevent escapes. However, food consumption in treated samples maintained in cages was observed to decline as locusts became sick and died.

There was evidently little persistence of the myco-insecticide agent under summer conditions at Wonderboom farm. Although low rates of mortality (10-30%) were recorded in all locust samples confined for 48h within implant cages in the spray plots and then kept under observation for a further 28d, no confirmed cases of mycosis by *M. anisopliae* var. *acridum* were recorded. These preliminary observations suggested that the myco-insecticide applied here did not readily persist on treated vegetation in the Karoo and did not cause mycosis under these conditions.

7.4 DISCUSSION and CONCLUSION

The above trials demonstrated that the oil-based *M. anisopliae* var. *acridum* myco-insecticide agent could be successfully applied from ground and aerial application equipment and could sometimes produce good control of L5 brown locust hopper bands in the Karoo. The aerial trials were, in fact, the first demonstration that the myco-insecticide could be successfully applied from the air against a migratory locust target. Given suitable application conditions, the myco-insecticide regularly produced >90% control of brown locust hopper samples maintained in

cages - a level of kill comparable with that obtained with the best conventional insecticides against the brown locust. On these grounds, the myco-insecticide agent gained registration against the brown locust in South Africa under the proprietary name Green Muscle® in 1998.

However, there are clearly problems with its possible use in curative control operations. For example, the rate of kill achieved in the field by Green Muscle® was slow, with median lethal times of 10.3 and 13.4 days respectively for caged samples from the ground and aerial trials reported here. Indeed, in most cases >90% mortality was only achieved 3-4 weeks after application, which is regarded as unacceptable for brown locust control. By comparison, current protocols under Act 36 of 1947 for testing conventional insecticides against the brown locust in the Karoo stipulate 90% efficacy within 72h, before gaining registration.

7.4.1 Factors affecting speed of kill

The more rapid mortality observed with Green Muscle® in caged samples as compared to field samples after ten days was clearly demonstrated in the present study (*cf.* Tables 7.4 with 7.5). The effects of caging has since been shown to occur in a range of acridids treated with Green Muscle® and is apparently the result of temperature effects regulating the growth of the fungal agent inside its host. This has been referred to as 'behavioural fever', whereby infected insects in the field thermoregulate for extended periods and elevate their body temperature 2-3°C above normal (Blanford, Thomas and Langewald, 1998; Blanford and Thomas, 2000). This higher body temperature apparently exceeds the threshold temperature for growth required by *M. anisopliae* var. *acridum* and results in a substantial delay in the onset of mycosis within the host. According to Arthurs and Thomas (2000), this effectively delayed the onset of mycosis in the brown locust in the field for up to 70 days. In contrast, infected locusts kept in cages are partially shaded and are hence unable to thermoregulate to the same extent, which favours the onset of mycosis.

This temperature effect therefore artificially accelerated the rate of mortality of brown locust hoppers maintained in cages following treatment with Green Muscle®. Brown locust hoppers have been shown to be particularly active thermoregulators in the field, using postural adjustments and micro-habitat selection to maintain high body temperatures. The harsh

environmental conditions typical of the Karoo, coupled to the locust's thermoregulation behaviour, therefore poses a severe challenge for this pathogen's growth, resulting in variable performance against the brown locust (Blanford and Thomas, 2000).

7.4.2 Speeding up the rate of kill

Laboratory bioassays have demonstrated that the speed of kill achieved with Green Muscle® against L5 brown locust hoppers was directly proportional to the dose rate applied, with rates of 10^5-10^8 conidia/insect producing 100% mortality within 5-11d (Müller, 2000b). This work indicated that it may be possible to increase the speed of kill by applying a higher dose rate. However, given the current costs of conidial production and the technical problems associated with the application of such high dose rates, the prospects for using them appear impractical.

Development of fungal pathogens within the locust host is a biological process with very little difference between speed of kill produced by different isolates (Bateman *et al.*, 1996). It is thus unlikely that speed of kill can be significantly increased by genetic manipulation of isolates.

7.4.3 Secondary pick-up

Secondary pick-up of conventional insecticide residues from sprayed vegetation is known to be an important method of dose transfer in brown locust hopper control operations (Brown, 1988). Likewise, a prolonged exposure of hoppers to treated vegetation within spray plots is known to enhance the secondary pick-up of residual Green Muscle® from treated vegetation in humid habitats (Thomas, Langewald and Wood, 1997). However, samples of hoppers confined on treated vegetation in the Karoo for up to 72h did not show a greater, or more rapid mortality, than samples removed immediately after application (Table 7.2). Without a rapid knockdown action to confine sprayed hoppers within the treated area, the brown locust bands sprayed with the myco-insecticide typically descended from their roost and marched out of the small spray plots within 1-2 hours, thus allowing little time for additional pickup of conidia from treated vegetation. Secondary pick-up of Green Muscle® from treated vegetation is thus unlikely to be a significant source of additional dose transfer to brown locusts.

7.4.4 Persistence

Most synthetic pyrethroid and modern organophosphate insecticides used for locust control have relatively limited persistence in the environment, with measured half-lives of only a few days under field conditions (Everts, 1990). Moderate persistence (7-10 days) of Green Muscle® following application in some well vegetated and humid environments in West Africa has been shown to be a vital factor in dose transfer to grasshopper pests, due to the secondary pick-up of spore residues (Thomas, Wood, Langewald and Lomer, 1997). In contrast, preliminary studies with brown locust hoppers have shown poor persistence of the myco-insecticide under Karoo conditions, with a calculated half-life of only 3-4 days (LUBILOSA unpublished report). The sparse vegetation cover in the Karoo provides little shade protection from the high ultra-violet radiation, typical at the latitude and relatively high altitude of the Upper Karoo. This factor, combined with hot, dry conditions in the Karoo during summer could limit survival of conidia in the field. Unscreened *M.anisopliae* conidia are known to be highly vulnerable to ultra-violet radiation (Moore, Bridge, Higgins, Bateman and Prior, 1993).

7.4.5 Current brown locust control tactics

During brown locust outbreaks in the Karoo, each mobile control spray team may typically spray between 10-30 roosting locust targets during a single morning. Locust control operators use the rapid knock-down effect produced by Decis® to act as a visible marker to determine the outcome of spraying (Brown and Kriel, 1994). Spray operators will therefore experience severe problems distinguishing between treated and untreated bands, following application of a slow-acting product with no knock-down action, such as Green Muscle®. Most Karoo farmers also expect a fast kill of locusts in order to protect their grazing from further damage and a rapid knockdown action will also be required for direct crop protection. However, Green Muscle® may be suitable for controlling locusts in large-size conservation areas or for the suppression of the early stages of an outbreak where speed of kill may not be so important.

7.4.6 Environmental impact

The Decis 7UL formulation, currently applied against the brown locust is known to have a high initial impact against non-target invertebrates in the Karoo and kills >95% of acridids within a

few hours of application (Steward, 1998; Roux, 1998). In contrast, Green Muscle® is known to produce minimal environmental impact on non-target organisms, as compared to conventional insecticides (Peveling, Attignon, Langewald and Ouambana, 1999) and is thus considered to be the most environmentally benign acridicide currently available (FAO, 2000; Peveling, 2001). However, isolates of *M. anisopliae* var. *acridum* originated from grasshopper hosts, which thus makes these agents pathogenic to most non-target grasshoppers (Peveling *et al.*, 1999). Since there is a rich diversity of grasshopper fauna in Karoo, characterised by a high number of endemic genera and species, of which many are flightless, any disease organism affecting these insects is of concern. The less vagile, wingless grasshoppers in the Karoo are known to be slow to recolonise areas treated with Decis® (Steward, 1998) and the same could occur following application of Green Muscle®. A risk assessment of Green Muscle® on Karoo grasshoppers is required, especially if broad-acre spraying tactics are introduced.

7.4.7 Future use of Green Muscle®

Green Muscle® has been used operationally on a small-scale for the control of grasshopper pests in West Africa, while the adoption of Green Muscle® technology for red locust control in its flooded grassland outbreak areas in central Africa is currently ongoing (e.g. Price, Müller, Brown, D'Uamba and Jone, 1999). Likewise, in Australia, an indigenous strain of *M. anisopliae* var. *acridum,* which is locally produced under the name, Green Guard®, has been used in large-scale operations for the control of the Australian plague locust, *Chortioicetes terminifera* (Walker), at dose rates of 25-50g/ha (Hunter, Milner and Spurgin, 2001).

However, what is very clear in the case of the brown locust, is that the current control tactics of spot treatment of thousands of individual band targets by ground spraying, cannot possibly accommodate the slow rate of kill produced by Green Muscle®. As mentioned previously, locust control operators will find it impossible to distinguish between treated and untreated band targets. The mobility of brown locust hopper bands also makes them difficult control targets for the pathogen. Whether an alternative application strategy for Green Muscle® can be developed, as suggested by Thomas and Blanford (1998) for managing the brown locust, remains to be seen.

7.5 BENEFITS and CONSTRAINTS

7.5.1 Potential benefits

- Low environmental impact of Green Muscle® against non-target organisms compared with conventional insecticides.
- Greatly reduced hazard to personnel during handling and spray application.
- Green Muscle® has been registered under Act 36 of 1947 and is commercially produced by Biological Control Products, (Pty), Ltd., Pinetown, KwaZulu Natal, South Africa.
- Green Muscle® myco-insecticide can be applied through conventional spray apparatus.
- The product has strong environmental credibility factors associated with its operational use.

7.5.2 Potential constraints

- Slow and variable speed of kill against the brown locust under Karoo conditions.
- No knockdown action following application to mark treated bands will lead to confusion and re-spraying by locust officers.
- Current cost of conidia at recommended dose rates is high (±30US Dollars per ha) and there is currently limited capacity for mass produce.
- Facilities would have to be provided to store conidia under refrigerated conditions in the Karoo and dry spore powder, or spore concentrate, has to be formulated in paraffin oil shortly before use, which would create serious problems for locust control officers.
- Myco-insecticides are not suitable for direct crop protection where rapid control is usually required and there would be negative feedback from farmers requiring rapid control.
- Environmental impact of Green Muscle® against the rich diversity of grasshoppers in the Karoo is currently unknown.
- New application strategies will have to be developed and perfected.

CHAPTER 8

Microbial control agents

8.1 INTRODUCTION

Apart from entomopathogenic fungi, a range of other pathogenic micro-organisms have been isolated from grasshoppers and locusts, including various bacteria, entomopoxviruses, microsporidia, protozoa, rickettsiae and nematodes (Uvarov, 1977; Henry, Wilson, Oma and Fowler, 1985; Streett and McGuire, 1990; Prior and Streett, 1997). Most of the known pathogens of locusts already occur in the main outbreak areas, but natural epizootics are rare due to the ecological limitations inherent in the spread of pathogens in locust populations (Prior and Greathead, 1989). Attempts at biocontrol of acridids by the inundative augmentation of micro-organisms, such as the microsporida, *Nosema locustae* Canning, entomopathogenic bacteria (*Coccobacillus acridiorum* d'Herelle and *Serratia marcesens* Bizio), and entomopoxviruses have met with mixed success (Streett, Woods and Erlandson, 1997). Biological control of locusts with pathogens has long been considered impractical as even the most promising pathogens require expensive mass production and augmentative release techniques to be developed.

However, global concern over the extensive application of broad-spectrum, but non-persistent, insecticides during the 1980s for control of acridoid pests, particularly desert locusts and Sahelian pest grasshoppers, provided a strong impetus to search for alternative, more target-specific, biological control agents to lessen dependence on synthetic insecticides (Prior and Greathead, 1989). The development and commercialisation of the *M. anisopliae* var. *acridum* myco-insecticides for locust control in Africa and Australia (Green Muscle® and Green Guard®) has also proved that pathogens can become viable products and has in turn stimulated applied research into the potential of other pathogens as biological control agents.

Various strains of the spore forming bacteria, *Bacillus thuringiensis* Berliner (Bt), have been used successfully as microbial insecticides against certain Lepidoptera, Coleoptera and Dipteran

pests (Zelazny, Goettel and Keller, 1997). Bt has been the most successful microbial control agent to date and has been commercialised world-wide, although none of the Bt strains evaluated have proved sufficiently pathogenic against Orthoptera to merit further attention (Streett and McGuire, 1990; Zelazny *et al.*, 1997). The low pH (<pH8) of the Orthoptera gut evidently prevents the endotoxin crystals of most known Bt strains from dissolving and becoming activated under the acid conditions (Prior and Greathead, 1989).

However, from a commercial point of view, the possibility of finding strains of Bt with activity against Orthoptera is highly attractive because of the considerable industrial expertise currently available in mass production, formulation and application of Bt products (Prior and Greathead, 1989; Zelazny *et al.*, 1997). During the screening of various Bt strains by the Biotechnology Programme, Council for Scientific and Industrial Research (CSIR), Pretoria, moderate control of house-hold cockroaches was achieved with selected acid-tolerant Bt strains in the laboratory. Although none of these Bt strains eventually proved commercially viable against cockroaches, the opportunity arose to screen some of the more promising strains against the brown locust.

The entomopoxviruses (EPVs) are a group of occluded DNA viruses specific to insects and have excited considerable interest as possible insect biocontrol agents (Streett *et al.*, 1997), although a relatively small number of EPVs have so far been isolated from grasshoppers (Street and McGuire, 1990; Streett *et al.*, 1997). Under field conditions, horizontal transmission of grasshopper EPVs is apparently by consumption of infected cadavers, after which the virus spheroids break down in the grasshopper gut due to the action of digestive proteases and the virons are released. The virons fuse with the midgut epithelial cells, enter the haemolymph and infect the fat body tissues (Street and McGuire, 1990).

Although the EPVs tend to debilitate rather than kill their grasshopper hosts, some are considered to offer potential as microbial control agents since they can be readily formulated in bait and can tolerate the dry conditions that are common in areas with traditional grasshopper problems. Small-scale field trials of EPV baits have been undertaken against certain grasshopper pests with varying success (Streett, 1987; Streett *et al.*, 1997).

One promising entomopoxvirus isolated from the pest grasshopper, *Oedaleus senegalensis* Krauss, in West Africa, was shown to have potential for the control of some rangeland grasshopper pests in the western USA (Streett, 1987). The *O. senegalensis* EPV suspension was thus imported under quarantine into South Africa and screened against the brown locust.

8.2 MATERIALS and METHODS

8.2.1 Test insects

Samples of freeze-dried spores of five strains of Bt were supplied by the Biotechnology Programme, CSIR, Pretoria, and screened against laboratory-reared L3 brown locust hoppers, derived from field collected parents. Hoppers were reared in wire-gauze locust cages, described previously. These hoppers were fed a diet of green wheat seedlings and dry wheat bran and were kept at a temperature range of 26-30°C and under a 12h photoperiod. Hoppers were starved for 24h prior to the experiments in order to standardise their feeding response.

8.2.1.1 Bt bait bioassay

Batches (100g) of each of the five Bt baits were prepared by mixing the Bt spores into dry wheat bran at a standard dose rate of 1mg spores/g bran. Each Bt bait was assayed against three separate batches of ten hoppers, containing both sexes, placed in sterilised wire-gauze cages, measuring 40x25x25cm. Each batch of ten hoppers were provided with 5g Bt bait, placed in a petri dish (5cm diameter) in the middle of the locust cage. The bait was also slightly moistened with a few drops of distilled water to make it more acceptable to locusts. Hoppers were exposed to the 5g quantity of the Bt baits for 24h. They were then removed to clean cages and fed their usual diet of green wheat seedlings and dry wheat bran. Three batches of control hoppers (n=10) were fed a diet of untreated wheat bran (5g per cage) under the same conditions.

In a second experiment, three batches of L3 brown locust hoppers (n=10) were exposed continuously to the five Bt baits, fed *ad lib.* over a 21d period. They were also provided with green wheat seedlings to supplement their diet during this period.

The cages were placed in partial shade conditions in a glasshouse maintained at a constant 25°C. Air temperature and relative humidity at cage level was monitored continually with a data logger. Mortality in the two feeding treatments was monitored daily for 21 days.

8.2.2 *Oedaleus senegalensis* entomopoxvirus

O. senegalensis entomopoxvirus (OsEPV) suspension was supplied by the USDA Agricultural Research Service, Rangeland Insect Laboratory, Bozeman, Montana, USA. Frozen virus suspension was imported into South Africa under quarantine conditions in compliance with the South African Department of Agriculture's regulatory Directorate of Plant and Quality Control (import permit No. 14/2/2/1(9/21/172)). The suspension was kept frozen for six weeks at -10°C until locust hoppers became available for experimentation.

8.2.2.1 Entomopoxvirus bioassay

OsEPV suspension was evaluated against a sample of L3-L4 brown locust hoppers (n=30), the progeny of gregarious populations collected in the Karoo and reared as described during the Bt experiments. Virus was also evaluated against L3 and L4 hoppers (n=30) of the African migratory locust, *L. migratoria migratorioides* R.& F., and L4 and L5 hoppers (n=30) of the local grasshopper, *Oedaleus nigrofasciatus* (De Geer), collected in the field near Bothaville in the Free State Province. The later two species were then maintained in the laboratory on a diet of green wheat seedlings and were kept at a temperature range of 26-30°C and under a 12h photoperiod. Evaluation of the virus against the *Oedaleus* grasshoppers was undertaken to compare virulence against a species from the same genus as the original OsEPV grasshopper host. Hoppers were kept separately in glass vials, measuring 110x25mm, and were starved for 24h in order to standardise their feeding response.

The frozen virus suspension was thawed overnight and thoroughly mixed by means of a sonic mixer. One droplet (10µℓ) of OsEPV suspension, containing approximately $1x10^6$ spheroids, was applied by means of a Hamilton micro-syringe to the centre of individual discs (8mm diameter) cut from young maize leaves. Droplets were allowed to dry and single leaf discs were fed to individual hoppers in the glass vials. Only insects that consumed the entire leaf disc within a 8h

period were included in the experiment. Hoppers were then removed from the vials and placed in two groups of 15 insects in wire-gauze locust cages, described above. Batches of control hoppers of each species (n=30), fed maize discs without virus, were also set up. Cages were then placed in the quarantine glasshouse and hoppers were fed green wheat seedlings and dry wheat bran for the following 24 days. Cages were monitored for any hopper mortality or disease. Air temperature and R.H. in the glasshouse was monitored with a data logger.

In a second experiment, L4-L5 brown locust hoppers (n=20) were given a double dose of OsEPV by feeding them 20μℓ droplets of virus suspension (2 x 10^6 spheroids) on maize discs. Mortality over a 24d observation period in the quarantine glasshouse was compared with untreated controls (n=20) maintained under identical conditions.

8.3 RESULTS

8.3.1 Bt bait

The virulence of the five Bt baits, fed to L3 brown locust hoppers over a 24h exposure, is given in Table 8.1.

Table 8.1. Cumulative percentage mortality of L3 brown locust hoppers fed different Bt baits for 24h (n=30 hoppers per treatment).

Days p.a.	Cumulative % mortality					
	Bt HD1	Bt 42	Bt 3	Bt 55	Bt 65	Control
3	0	0	0	0	0	0
6	3	10	0	0	7	3
9	3	13	3	0	7	10
12	3	13	3	0	7	13
15	3	13	3	0	7	13
18	3	17	7	0	7	13
21	3	17	7	0	10	13

Hoppers were observed feeding on all five Bt baits, proving that the bait formulations were acceptable to hoppers. However, none of the Bt baits produced significant mortality compared with untreated controls (ANOVA test; F=1.23, 5 d.f., P>0.05). The 3-17% mortality recorded in the Bt treatments and controls over the following 21d could be attributed to the stress of handling or other unknown causes. There was also no evidence of abnormal behaviour in any of the treated insects or controls. Air temperature in the glasshouse during the trials averaged 24°C (range 21-27°C) and R.H. averaged 40% (25-60%).

Mortality of brown locust hoppers following continuous exposure to the five Bt baits over a 21d period is given in Table 8.2.

Table 8.2. Cumulative percentage mortality of L3 brown locust hoppers (n = 30) fed Bt baits continuously over a 21d observation period.

Days	Cumulative % mortality					
	Bt HD1	Bt 42	Bt 3	Bt 55	Bt 65	Control
3	0	0	0	0	0	0
6	8	0	3	5	3	0
9	13	5	5	10	3	0
12	13	8	5	10	3	0
15	13	8	5	10	3	0
18	13	8	5	10	3	3
21	13	8	5	10	3	3

No significant mortality of hoppers fed Bt bait continuously for 21d was evident compared with untreated controls (ANOVA test, F=1.69; 5 d.f., P>0.05) (Table 8.2). Surviving test hoppers fledged into adults at the end of the experiment.

8.3.2 *O. senegalensis* entomopoxvirus

Efficacy of the OsEPV suspension against 3-4th instar hoppers of the brown locust, African migratory locust and *O. nigrofasciatus* grasshoppers is given in Table 8.3.

Table 8.3. Cumulative percentage mortality of hoppers of the brown locust (BL), African migratory locust (AML) and *O. nigrofasciatus* (O.nig.), fed OsEPV suspension.

			Days after treatment							
Species	**n =**	**OsEPV dose**	3	6	9	12	15	18	21	24
BL	30	1×10^6	0	3	3	3	3	3	3	3
BL control	30	-	0	0	0	0	3	3	3	3
BL	20	2×10^6	0	0	0	0	5	5	5	5
BL control	20	-	0	5	5	5	10	10	15	15
AML	30	1×10^6	0	0	0	0	0	0	3	3
AML control	30	-	0	0	0	3	3	3	3	3
O.nig.	30	1×10^6	0	0	0	0	0	0	0	0
O.nig control	30	-	0	0	0	3	3	3	3	3

No detectable infection was produced by OsEPV in any of the test species, with the dose rates of spheroids evaluated. Highest mortality was, in fact, recorded in the untreated controls due to infection by the common bacterial disease of caged locusts, *S. marcescens*. The surviving hoppers subsequently fledged to adults. Mean air temperature and R.H. in the cages over the 24d observation period was 24°C (range 18-27°C) and 38% R.H. (range 22-46%) respectively.

8.4 DISCUSSION and CONCLUSION

Despite reports of toxicity against cockroaches, the five Bt strains proved ineffective against L3 brown locust hoppers at the dosage rates investigated. No significant mortality of hoppers was achieved compared with untreated controls when fed Bt bait for 1d or for 21d. These results further confirm the lack of virulence of the Bt strains evaluated so far against acridids (Zelazny *et al.*, 1997). However, the virulence of any Bt strains isolated from acridids in the field, or strains genetically modified to become more acid tolerant, obviously merit further investigation.

The standard dose rate of OsEPV spheroids produced no detectable infection of L3 and L4 hoppers of the brown locust, African migratory locust, or the related grasshopper, *O.nigrofasciatus*, over a 24d observation period. The lack of pathogenicity of the OsEPV in this study was disappointing, as the viability of the OsEPV suspension was confirmed before being

exported to South Africa and the presence of spheroids in the OsEPV suspension was confirmed under phase contrast microscope by staff of the Virology Section, ARC-PPRI. There was also no reason to doubt the intake of OsEPV into the gut of the test insects. However, entompoxvirus are known to be relatively host specific (Zelazny *et al.*, 1997) and the virulence of the OsEPV against acridids other than its direct host grasshopper may be low.

The future development of EPVs as microbial agents against locusts is uncertain, as the isolates currently available produce slow and variable kill and have to be produced by expensive culture in living host insects. A thorough search for additional virulent isolates is required and mass production technology has to be developed (Zelazny *et al.*, 1997). The epizootiology of grasshopper diseases also requires more study to examine the dynamics of natural infections and the factors governing disease development in grasshopper populations.

8.5 BENEFITS and CONSTRAINTS

8.5.1 Potential benefits

- Microbial control agents are considered host-specific with minimal impact on non-target organisms and with reduced health hazards for man.
- Augmentative release of microbial agents may raise background levels of host-specific pathogens in the environment to a level whereby secondary cycling of these agents could provide increased background levels of control.

8.5.2 Potential constraints

- Efficacy of current microbial agents is variable and speed of kill is usually very slow.
- Survival and virulence of microbial agents under field conditions requires more study.
- Microbial agents are currently difficult and expensive to produce.

CHAPTER 9

Discussion and Recommendations

9.1 Evaluation of alternative control methods

The brown locust has proved a formidable pest problem over the past 100 years due to the high frequency of outbreaks and rapid gregarisation of locust populations in the Karoo. Since the days when only mechanical methods were available to protect crops and pastures from the ravages of locusts there has been significant progress in locust control. Sophisticated insecticides and advanced application methods are now available which are more effective and safer to the operator and to the environment than the arsenic, organochlorine and organophosphate compounds used in the past. Today we have the technical capacity to manage locusts, although the high costs, formidable logistics and manpower resources associated with conducting effective control campaigns, present new challenges. Nevertheless, the brown locust still has the potential to produce plagues and to threaten food security across the entire southern African region. On the other hand there is also an urgent demand for more environmentally benign and cost effective methods of managing locust outbreaks.

The above thesis presents various methods of controlling the brown locust as possible alternatives to conventional spot application of broad-spectrum insecticides. The alternative methods were examined within the context of the current control strategy of targeting hopper bands, rather than adult swarm control. Once optimal dose rates and application techniques had been developed, most of these alternatives eventually proved capable of killing brown locust hoppers. However, pure killing action is only a part of what constitutes an effective locust control agent. The pros and cons to the operational use of each alternative method under outbreak conditions were highlighted at the end of each Chapter. Expanding on these initial assessments, the seven alternative methods investigated here were then critically evaluated according to a range of operational performance criteria (Table 9.1). They were also compared against the current performance levels achieved with conventional spot treatment with synthetic pyrethroid insecticides applied from either knapsack sprayers or vehicle mounted sprayers (Table 9.1).

Table 9.1. Evaluation of alternative brown locust control methods under various performance criteria.

Performance criteria	Synthetic pyrethroids	Barrier treatments	Insecticide baits	Myco-insecticides	IGRs	Other pathogens	Mechanical control	Natural enemies
Reliable efficacy in field	++++	+++	++	+	+	+	++	+ +
Speed of kill in field	++++	++++	+++	+	+	+	++	+
Practicality / ease of use	++++	++++	++	++	+++	+	+	+
Safety to non-targets	++	+	+++	++++	+++	++	+++	++++
Operator safety	++	+++	++++	++++	+++	++	++	-
Registered / availability	++++	++++	+++	+++	+	+	-	-
Competitively priced	++	++	++++	+	++	-	-	-
Targets: Hoppers only (++) or hoppers & swarms (++++)	++++	++	++	++	++	++		-
For small (+), medium (++) & big outbreaks (++++)	++++	++++	+	++	++	++	+	-
Index of overall performance	83%	75%	67%	56%	50%	33%	30%	22%

Key to ranking values: ++++ = excellent; +++ = good; ++ = average; + = poor.

Only Adonis® barrier treatments and baiting showed sufficient promise, if applied under special conditions or in certain niche situations, to warrant consideration as possible alternatives to conventional spraying. However, no alternative control method was considered sufficiently flexible and effective enough under all field conditions to entirely replace the current spot treatment with pyrethroid insecticides. Fast acting ULV insecticides will hence continue to provide the backbone of the brown locust control programme for the foreseeable future.

Although various IGR products and *Metarhizium* myco-insecticides are currently being used operationally against certain locust and grasshopper pests in different parts of the world, they were found to be unsuitable for operational use against gregarious brown locust populations. Preliminary observations showed that the feeding behaviour and high mobility of brown locust bands did not favour the application of IGR barrier treatments, while the harsh environmental conditions of the Upper Karoo and the active thermoregulation behaviour of the brown locust hopper stage was detrimental to the Green Muscle® myco-insecticide. Likewise, mechanical control, natural enemies or microbial insecticides do not currently offer viable control options for large-scale brown locust outbreak suppression.

In conclusion, Adonis® barrier treatments and insecticide baits were considered to have sufficient potential to be incorporated into an IPM system for use against brown locust hopper bands in certain situations. Adonis® barriers are recommended for possible use in the remote areas of the Northern Cape Province where the locust reporting network is currently weak or where access to large areas of land is becoming increasingly difficult, e.g. military testing grounds, empty farms and locked gates. Baits have potential for use on a small-scale in conservation areas where a reduced environmental impact is required or for use by rural communities on municipal land. The areas of the Karoo where barrier treatments and baits could potentially be used as an alternative to conventional spraying should be identified and mapped within a geographical information system (GIS), followed by large-scale operational trials

9.2 The need to modernise brown locust control

Brown locust control strategy has historically targeted the ground based hopper stage, especially the late instar "rooibadjie" hopper bands, using the 'commando' system of mobilising local farmers and their workers. However, the tracking down and spraying of thousands of individual hopper bands using vehicles has long been considered an inefficient use of manpower and resources. Records dating back to the 1960s and confirmed again in the 1990s, showed that spraying hopper bands utilised up to 90% of the locust control budget, but only accounted for 10% of the actual number of locusts controlled, whereas spraying roosting adult swarms accounted for 90% of the population (Cilliers, Van Someren-Greve and Lea, 1964; Steenkamp, Botha and Van der Westhuizen, 1997). The targeting of hopper bands as the main control strategy is thus considered to be relatively ineffective in some areas of the Karoo and there is an urgent need to develop a more modern integrated management approach based on sound scientific principles. The management options available and whether locusts should be controlled at all, are discussed below.

9.3 LOCUST CONTROL OPTIONS

9.3.1 Abandon brown locust control entirely

Historical records show that uncontrolled brown locust plagues in the past all eventually collapsed due to a combination of factors: the build-up of natural enemies and disease, unfavourable climatic conditions or as a result of the exodus of swarms into reception areas which were unsuitable for further breeding, eg. Mountains of Lesotho and the wetter areas of the Transkei. Earlier plague cycles were then always followed by comparatively long recession periods, since the swarming populations had all vacated the optimum breeding areas in the Karoo (Lea, 1968). By not controlling locusts it can be argued that outbreaks will always come to an end naturally and that the money saved on control could then be used to compensate the crop losses. However, uncontrolled locust plagues in the early part of the 20th Century posed a serious threat to agricultural production in South Africa, causing hardship to farming communities. With a strategic emphasis on maintaining food security in South Africa, the Government is highly unlikely to jeopardise agricultural production or neglect its international obligations with neighbouring countries by abandoning management of the brown locust.

Although there is still debate on whether locusts and grasshoppers should be chemically controlled to protect rangeland grazing (Peveling, 2001), there can be no doubt regarding the need to control these pests in order to protect vulnerable staple food crops such as maize, wheat and millet (Krall, Peveling and Ba Diallo, 1997; Peveling, 2001). Likewise in the Karoo, the brown locust problem will have to be continually managed, so that damage caused to food crops and pastures is prevented or reduced below economic levels. The damage that brown locusts cause to Karoo pasture and the economic benefits of controlling locusts have been described by Coetzee (1994).

9.3.2 Update the current commando system

In areas such as the Eastern Karoo the commando control system still functions satisfactorily, whereas in the more remote western areas of the Arid Karoo it has become less effective due to demographic factors associated with depopulation of the farming areas. If the commando system is still to be applied throughout the Karoo, it will have to be modernised in order to improve the capacity to locate locust targets, such as by implementing an effective locust monitoring and early warning system. An updated and refined commando system would require additional manpower for conducting operations in the remote areas, requiring a substantial increase in financial resources.

To sum up, none of the alternative control methods evaluated to date has the potential to entirely replace conventional ULV spot spraying, and fast-acting synthetic insecticides will thus remain the mainstay of brown locust control. However, it is recommended that the most effective alternatives, especially barrier treatments and baits, incorporated into an integrated pest management (IPM) programme, could offer a wider range of locust management solutions for selected problem areas. These include remote areas with a high number of unoccupied farms, in recognised conservation areas, on organic farms producing venison products, or on farms where conservation-minded landholders object to the current use of conventional insecticides. On the other hand, synthetic contact insecticides would still have to be applied for the control of adult swarms.

9.3.3 Abandon hopper control and target adult swarms only

Using modern spray aircraft to target young swarms as they mill around and aggregate into large-sized targets is the standard control strategy in various countries, such as Australia and Madagascar. Apart from the high work rate of spray aircraft, adult locusts are also known to be easier to kill with insecticides than late instar hoppers, which has important consequences for reduced insecticide application and environmental impact. Aircraft would only control swarms once a threshold number or size of swarm targets had been reached and once cereal crops were threatened. The co-ordinated air to air spraying of flying swarms using low dose rates of synthetic pyrethroid insecticides, accurately applied from aircraft equipped with 'SATLOC' navigational guidance and computer regulated flow rate systems, could effectively control even large-scale locust plagues.

A limited number of hard working, small to medium capacity spray aircraft, such as the Piper Pawnee, Gippsland GA200C, Cessna C-188 and spray helicopters, could intercept large-size swarm targets as they aggregated and migrated east and south-east along their traditional flyways out of the Upper Central Karoo. Aircraft operating from strategic centres in the Eastern Karoo would attack swarms once they entered a 100-150km wide buffer 'no fly' zone stretching along the Orange river. However, this "Maginot Line" concept would require accurate real time information on swarm numbers, trajectories and displacements, so that the control capacity would not be overwhelmed. Outbreaks in the Great Karoo could be controlled with aircraft stationed at aircraft stationed at the nearest airstrip. Small-scale outbreaks may even be managed with microlight aircraft equipped with appropriate ULV spray gear, as has been demonstrated in trials by PPRI.

9.3.4 Integrated locust management strategy

The most effective strategy for combating brown locust outbreaks in future probably lies in modernising the commando system of hopper control, supported by the judicious use of aircraft for aerial swarm control. An integrated management strategy combining ground and aerial tactics would hiave the flexibility and capacity to deal efficiently with all locust emergencies.

The current commando system is probably only viable in the Central Karoo (eg. Hanover, De Aar, Hopetown and Philipstown Districts) where there is a high density of resident farmers who are able to report outbreaks and where good communications exist in order to enable control teams to respond rapidly to emergencies. Hopper bands in the remote western areas of the northern Cape Province should be controlled using passive barrier treatments of fipronil, applied by vehicle or aircraft. Locust intervention in all areas should only be initiated once an economic or strategically important infestation threshold has been reached. This will require real time surveys of suspected outbreak areas by vehicle or by microlight spotter aircraft.

Apart from implementing a barrier treatment strategy, scattered outbreaks throughout most of the Western and Central Karoo should be left to fledge into adult swarms. These will later aggregate into large-sized swarm targets, which can be more easily controlled with the aid of spray aircraft. The NDA locust depots at De Aar and Upington would act as command centres receiving information on locust targets from survey teams and farmers and relaying this to the spray pilots. The decision to initiate and terminate aerial control operations would be the responsibility of a campaign director based at forward command centres.

The farming community in the Karoo will therefore still remain a vital component of the anti-locust control operations and will be heavily involved in gathering and forwarding information on locust populations and movements during outbreaks. To strengthen this information gathering system, an educational and information campaign should be undertaken in order to inform the farming community as to what type of locust information is required. The NDA should consider setting up a toll-free telephone system to encourage people to report locusts, as undertaken by the Australian Plague Locust Commission in Canberra.

9.4 RECOMMENDATIONS

It is recommended that an IPM strategy for brown locust control be developed within the framework of a geographical information system (GIS). Barrier treatments and baiting are viable alternative options for brown locust control and areas where these tactics could be effectively deployed in the Karoo should be identified and delimited. Conventional fast-acting synthetic insecticides, especially pyrethroids, will continue to be the mainstay of brown locust control. Outbreaks in the Eastern Karoo could continue to be managed by an upgraded Commando system, incorporating improved locust survey and reporting methods so as to develop an effective early warning information system. However, the management of outbreaks over most of the remote Northern Cape Province should be undertaken with properly equipped spray aircraft and experienced pilots, targeting the young swarms either in flight or on their roosts using track guidance and satellite navigation systems.

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