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Novel Pesticide Formulations For Locust And Grasshopper Control

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ABSTRACT

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Novel pesticide formulations for locust and grasshopper control

by Christopher Ian Bates

The primary question addressed was one of whether advanced formulation techniques, and more specifically microencapsulation, could be used to produce efficient formulations tailored for locust control. The key benefits of microencapsulation sought were an increase in persistence of a non-cumulative synthetic insecticide while reducing the toxicity to beneficial arthropods.

In the first phase of the investigation a number of insecticides of different chemical classes were assessed for suitability in microencapsulation. Emphasis was placed on developing oil based formulations appropriate for application with the controlled droplet application (CDA) sprayers commonly used for locust control.

A novel oil based formulation of the organophosphorus insecticide malathion was selected and variants developed for efficacy testing. Since it is clear that synthetic insecticides are unsuitable for all possible control situations, a novel oil based formulation of fungal conidia was also developed.

Malathion microcapsule formulations were tested for efficacy against nymphs of *Schistocerca gregaria* (Forskål) (Orthoptera: Acrididae) in the laboratory. The contact toxicity of dried malathion microcapsule residues on a glass substrate was found to be comparable to non-encapsulated malathion but having the benefit extended persistence.

A single variant of the malathion microcapsule formulation was tested for oral toxicity to *S. gregaria* nymphs and found to be between six and seven times less toxic than non-encapsulated malathion.

Finally, the same microcapsule formulation was assessed for toxicity to the indicator beneficial insect, *Aphidius colemani* (Viereck) (Hymenoptera: Braconidae), using a laboratory test in which the insects were exposed to dried microcapsule residues on glass. In this worst case scenario of continuous contact microencapsulation of malathion significantly increased the median lethal time to death.

Overall it was demonstrated in the laboratory that microencapsulated malathion has potential utility in locust control operations, future research should be aimed at optimising the wide range of microcapsule parameters to give the correct balance of persistence and toxicity to locusts while reducing the toxicity toward beneficial arthropods.

List Of Contents

List Of Tables

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

List Of Figures

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CHAPTER 1
GENERAL INTRODUCTION

1 Introduction To Locusts

The appellation locust is used to describe a number of species of Orthoptera Acrididae (short-horn grasshoppers) that exhibit phase polymorphism. This is the locusts' ability to undergo morphological, physiological, and behavioural change depending upon population density and was first identified by Uvarov (1921) for *Locusta migratoria*. Haskell (1992) defined the difference between locusts and grasshoppers as the locusts' ability to aggregate while grasshoppers almost never aggregate whatever their population density; although not definitive a list of the twelve species considered locusts is included as Table 1.1. It is noteworthy that not all locust species are a serious economic pest and some grasshopper species considerably more so, for example the Senegalese grasshopper *Oedaleus senaglensis* is an important pest in the Sahelian zone of West Africa (Cheke, 1990). The desert locust *Schistocerca gregaria* is arguably the most important locust pest species, exemplified by the 'eighth plague of Egypt' in 1300 BC and the 342 million US$ spent on control operations between 1986 and 1993 (Herok & Krall, 1995). This thesis will concentrate on the desert locust but the results should be broadly applicable to the control of other locust and grasshopper species.

The primary question addressed was whether advanced formulation techniques, and more specifically microencapsulation, could be used to produce efficient formulations tailored for locust control.

Table 1.1: Locust species and distribution (after Haskell, 1992).

Species	Distribution
Schistocerca gregaria Desert locust	Northern Africa, Arabia and Indian sub continent from 10 to 35° North
Schistocerca americana americana	Central America
Schistocerca americana paranensis	South America
Anacridium melanorhodon Sahelian tree locust	Found in a belt across Africa south of the Sahara, from about 10 to 20° North, but extending to the equator in the East
Anacridium wernerellum Sudanese tree locust	Found in a zone slightly further south than A. melanorhodon
Nomadacris septemfasciata Red locust	Africa, mainly south of the equator to about 30° South.
Patanga succinta Bombay locust	South West Asia
Melanoplus spretus Rocky mountain locust	North America (possibly extinct)
Chortoicetes terminifera Australian plague locust	Australia
Locusta migratoria Migratory locust	Different subspecies found in Southern Europe, Africa south of the Sahara, Malagasy Republic, Southern Russia, China, Japan, Philippines and Australia
Locusta pardalina Brown locust	South Africa
Dociostaurus maroccanus Morroccan locust	Middle East and Mediterranean countries

1.1 Locust Biology

1.1.1 Morphology, Anatomy and Physiology

The biology of locust and grasshopper species has been extensively reviewed by Uvarov (1966) and Chapman & Joern (1990); the information is briefly summarised in table 1.2.

Table 1.2: Locust and grasshopper physiology.

Morphology	Adult grasshoppers and locusts vary between 7 and 120 mm in length and have a body is composed of head, thorax and abdomen. The head contains compound eyes, mouth parts and antennae; the thorax has three pairs of legs (the hind pair optimised for jumping) and typically two pairs of wings; the abdomen contains eleven segments and genitalia at the rear that vary greatly from species to species.
Alimentary System	Composed of the mandibles that break vegetation into manageable portions, hypopharynx containing sensory hairs and salivary ducts, widening into the pharynx, oesophagus and crop. Digestion of food begins in the pharynx but continues with absorption processes in the midgut; the remaining matter passes through the pyloric valve into the hindgut where further absorption of water and inorganic ions occurs before excretion.
Circulatory System	The locust or grasshopper body is divided by two longitudinal diaphragms into three connected sinuses. Blood is pumped by a peristaltic action through the body only contacting the organs at the exterior.
Respiratory System	Ten pairs of openings (spiracles) on the thorax and abdomen allow air directly to the organs and muscles through trachea and air sacs. Circulation being achieved by muscular compression and release of the body.
Nervous System	The central nervous system incorporates the brain and ventral nerve chain that passes down the length of the body, branching to muscles and sense organs. The visceral nervous system includes various ganglia and the median nerve that controls spiracles, respiratory abdominal movements and wing movement. The remaining complicated bulk of nerve fibres is termed the peripheral nervous system.

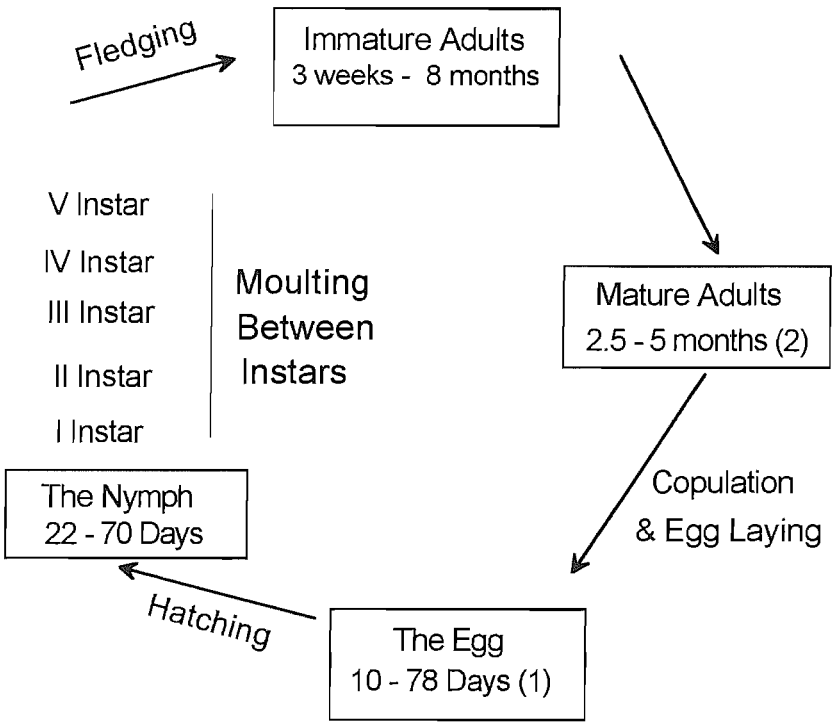
There are a number of weakness' in the locust body that may be exploited for control purposes. The three possible routes for control agents to enter the locust body are through the respiratory system for particles that are sufficiently small, orally and finally through the

cuticle (Matsumara, 1975). The hydrophobic insect cuticle is to a greater or lesser extent permeable to modern lipophilic insecticides and once within the insect body the highly developed nervous system is vulnerable to disruption. The insects hormonal mechanisms also offer a method of attack, an example being the insect growth regulator (IGR) diflubenzuron which interferes with chitin formation in the cuticle (van Emden, 1989) resulting in death or malformation. Finally the insects own natural enemies may, with the help of man, be made sufficiently active for control purposes. Examples of organisms suitable for biological control include protozoa, viruses and bacteria (Chapman & Joern, 1990). Possibly the most promising biological control agents are entomopathogenic fungi (Scherer et al, 1992; Mabbett, 1996; Streett, 1987) although some consider biological control agents to offer minimal potential overall (COPR, 1982).

1.1.2 Life Cycle

Locust and grasshoppers are exopterygote insects, the life cycle comprising an egg which hatches to give nymphs which then undergo between four and nine ecdyses (Uvarov, 1966) before fledging to adults with varying levels of wing development and flight capability. The life cycle of the desert locust is shown in figure 1.1 and a detailed description of the biology and behaviour will be limited to this species. It is noteworthy that solitarious phase desert locusts are capable of an extra instar between the normal III and IV instars shown in figure 1.1 (COPR, 1982). Features such as stage duration, instar duration, number of instars, etc. vary between locust species and as a result of changing environmental conditions. The desert locust exhibits an unstable polyvoltine cycle (Uvarov, 1977) capable of up to 4 generations per year, with no evidence of quiescence or diapause if environmental conditions become unfavourable (Wardhaugh et al, 1969).

Figure 1.1: The desert locust life cycle.



All duration's from COPR (1982)
except (1) from Wardhaugh (1964) and (2) from Steedman (1990).

Desert locust eggs are laid in soil within an egg pod that is 3 to 4 cm long, the bottom extending 10 cm down from the soils surface with which it is connected by a froth tube (Steedman, 1990). The number of eggs within each egg pod varies but between 70 and 80 is typical for the first laying by gregarious populations, 60 to 70 for the second laying and less than 50 for the third laying (Steedman, 1990). Gregarious females lay two or three egg pods and solitarious three or four, there is a seven to ten day interval of between layings but oviposition begins within two days of copulation (COPR, 1982). The duration of egg incubation is dependant on temperature; values from laboratory cultures include 11-13 days (Uvarov, 1966) and 16-17 days (Coppen, 1995) at 32C while field values of 9 to 70 days have been recorded depending on location (Steedman, 1990). The egg pods do not contain

sufficient moisture for the entire incubation period and must absorb a quantity equivalent to the egg pods initial weight from the surrounding soil (Uvarov, 1966). Field observations indicate 20 to 25 mm of rain is sufficient to ensure survival, although some soil can retain sufficient water to provide suitable breeding conditions for at least two months (COPR, 1982; Steedman, 1990).

Once incubation is complete the vermiform larva ruptures the egg cuticle by actively wriggling and then moves up the froth tube to the soil surface (Steedman, 1990; Uvarov, 1966). Synchronised hatching of an egg field is common with a duration of up to three days and peaks occurring three hours after sunrise (Steedman, 1990). This co-ordinated hatching is thought to be triggered by the soil temperature increasing to the point at which the hatching activity is possible (Uvarov, 1966) and indirectly by the effect of rain showers on soil temperature (Uvarov, 1977). During hatching the vermiform larva initiates a first moult that is completed on exit from the egg pod; the moult not being counted in the series of moults between instars.

The locust larva (commonly termed nymph or hopper) passes through five instars with moulting of the cuticle (ecdysis) between each. Indicators of instar include hopper size, development of tegmina and wings and the number of stripes on the compound eye (Uvarov, 1966). A stripe of pigmentation being added to the eye at each moult. After the final moult the emerging fledgling initially has soft, poorly pigmented cuticle and wings that harden and darken within hours (Uvarov, 1966). The fledgling adult remains sexually immature for a time and it is during this stage that the majority of population movements occur. Indeed maturation of the locusts may be delayed until the locusts migrate into areas suitable for successful reproduction (Norris, 1957). Laboratory populations of the desert locust may lay first during the third week of adult life but timing in the field depends on factors associated with rainfall (Norris, 1957; FAO, 1989) such as vegetation abundance (Carlisle & Ellis, 1965). The maturity within swarms is normally well synchronised and often between swarms within an area (Carlisle & Ellis, 1965), however the males commonly mature more rapidly than the females (Uvarov, 1977). Gregarious desert locusts undergo a change in colour from pink to yellow during maturation, caged populations being far more vividly coloured than in the field (Uvarov, 1966).

Copulation is achieved by the male desert locust mounting the female locust and locking together of the genitalia (Uvarov, 1966), the female locust remains free to feed or even

march (Popov, 1958). Duration of copulation may vary from minutes to greater than fourteen hours and the locusts may remain paired for over 48 hours (Popov, 1958). Parthenogenesis has been observed in the desert locust but is accompanied by reduced hatching success (Hamilton, 1955). Once a suitable egg laying site is located the female's abdomen is dug into the soil and when greatly extended oviposition occurs. Accessory glands at the base of the abdomen emit a frothy secretion that forms a pod around the eggs (Uvarov, 1966).

1.1.3 Fecundity and Mortality

The main causes of mortality in adult desert locusts are climate (Dempster, 1963; Greathead, 1966) and migration to areas incapable of supporting survival such as out to sea (Rainey, 1954). However, desert locusts of all developmental stages are subject to antagonists that are summarised in table 1.3.

Only limited information is available on the degree to which natural enemies control locust populations, from which generalised conclusions cannot be made (FAO, 1989). The primary sources of information on locust antagonists are Greathead (1963) and Prior & Greathead (1989). Other studies available include Stower & Greathead (1969), Roffey & Stower (1983), Ashall & Ellis (1962) and an extreme observation by Roffey and Popov (1968) that during an outbreak in 1967 mortality was approximately 92 percent between hatching and IV instar. Delays in the increase of parasite and predator population mean that although they may contribute to the decline of plagues they are unable to prevent the development of plagues (Prior & Street, in press).

Despite a potential multiplication rate of 200 times natural mortality limits the actual multiplication rate to approximately 10 to 20 times (Roffey & Popov, 1968; Hemming et al, 1979; COPR, 1982). Despite this the transition from recession to major plague the total numbers may increase by 10^5 in approximately two years (COPR, 1982).

Table 1.3: Natural enemies of the desert locust (COPR, 1982; Chapman & Joern, 1990; Greathead, 1966).

Life stage	Enemy	Notes
Adult /nymph	Fungi	Sporadic outbreaks usually associated with damp weather, may be rendered more efficient by control operations.
	Nematodes	Again associated with damp weather, large numbers within nymph and adult bodies may result in death.
	Flies	Hoppers are parasitised by the larvae of species such as *Blaesoxipha filipjevi* (Rohd) but large numbers of larvae are required to ensure mortality.
	Ants/spiders	Limited predatory effect.
	Mammals, amphibians and reptiles	Limited predatory effect.
	Birds	Important but erratic predator which is incapable of controlling the huge numbers of hoppers and adults in large gregarious populations.
Egg	Hymnoptera and diptera	The predators lay eggs in the surrounding soil or within the locust egg pod. Larvae attack the locust eggs eating/damaging some or all of the eggs. Varying degrees of predation recorded.
	Beetles	One species, *Trox procerus* (Harold) is capable of almost complete destruction of egg fields but occurrence is sporadic.

1.1.4 The Biological Basis of Phase Polymorphism.

Phase polymorphism exhibited by locusts was defined by Uvarov (1928) as that property of certain grasshopper species that renders them capable of existing as a series of forms differing from each other not only morphologically but also biologically. The variations in locust behaviour associated with phase polymorphism will be discussed later in this chapter but the biological effects are summarised in table 1.4.

It is noticeable that gregarious desert locust females lay fewer eggs than solitarious ones which appears contrary to the large increases in population observed. The hatchlings from gregarious females are however heavier and more resilient (Albrecht, 1962; Cheke, 1978)

making them more suited to survival in the arid recession area due to superior competitiveness for food (Dale & Tobe, 1990)) and reduction in predation (Cheke, 1978).

Table 1.4: Changes in morphology and physiology of the desert locust (*Schistocerca gregaria*) associated with phase transformation (from a review by Uvarov (1966) unless otherwise stated).

Characteristic	Effect of Phase.
Hopper Development	Crowded females lay eggs that produce larger hatchlings than isolated females. Solitarious hoppers may pass through six instars instead of the five usual for gregarious hoppers but the rate of development is reduced. Size, weight and activity of hoppers increased when gregarious.
Adult Morphology	Variation in size of head, prothorax, tegmen and legs with phase (Dirsh, 1953). Size increased in the gregarious phase.
Coloration	Hatchlings are darker in coloration when bred from crowded females. Hoppers are typically uniform green when solitarious but have a black pattern on a yellow or orange background when gregarious. Solitarious adults are pale grey or beige changing to pale yellow on maturation while gregarious adults are pink, changing to yellow (Steedman, 1990).
Reproduction	Reduced numbers of eggs in pods, numbers of pods and proportion viable eggs produced by gregarious females. The number of eggs per pod is theoretically limited by the number of ovarioles which is determined in the embryo. Sexual maturation occurs more rapidly for crowded females, 26 days before laying when crowded versus 34 days isolated (Norris, 1952).

It has been stated that phase polymorphism is an adaptation to the unstable seasonal aridity of the locust recession habitat (Kennedy, 1956; Kennedy, 1962). However, this theory has not received universal acceptance and an endocrine based control mechanism has been proposed (Dale & Tobe, 1990; Pener, 1983). Five likely groups of pheromones have been investigated by behavioural research, associated with the processes of gregarisation, maturation promotion, oviposition, solitarisation and maturation retardation (Loher, 1990).

1.2 Locust Behaviour

The behaviour of locusts, and most particularly desert locusts, is characterised by striking differences between the solitarious and gregarious phases (Hemming et al, 1979). Less is known about the solitarious phase behaviour of desert locusts due to the difficulties in making observations on low density populations (Uvarov, 1977).

1.2.1 Desert Locust Behaviour and Phase

Few locust species have come close to exhibiting the intensity of gregarious response found with the desert locust (Farrow, 1990). During the nymphal stage desert locusts form into well defined marching bands while solitarious hoppers remain diffuse and move only short distances (Ellis, 1959). Similarly in adulthood gregarious desert locusts form into stable cohesive swarms that fly by day (Uvarov, 1977) while adults in the solitarious phase fly mainly by night, the lower ambient temperatures restricting mobility (Hemming et al, 1979).

The transfer between the two extreme phases can occur relatively rapidly. Isolated gregarious desert locust hoppers begin changing to the solitarious phase by the process termed dissociation (Uvarov, 1928). Within hours of isolation solitarious behaviour is adopted (Roessingh & Simpson, 1994, a change to solitarious phase coloration occurs within days and after fledging the morphology will have changed sufficiently for the hopper to be considered solitarious. Conversely when solitarious hoppers are brought into close contact by environmental factors they can begin gregarisation within a few hours (Ellis, 1959) and then actively associate. The environmental factors that contribute to gregarisation are concentration within a restricted habitat (Roffey & Popov, 1968) and hatching of hoppers from densely laid egg pods. The dense laying by gregarious females may be forced by shortage of suitable areas and mutual attraction of the gregarious females (Uvarov, 1977; Roffey & Popov, 1968; FAO, 1989). If at a high enough density the hoppers begin to act gregariously soon after hatching and assemble into dense groups, marching begins two days after hatching with the bands covering between 2 and 12 miles in the period between hatching and fledging (Ellis & Ashall, 1957).

1.2.2 Gregarious Hopper Behaviour

Ellis & Ashall (1957) describe in detail the gregarious hoppers diurnal behaviour. Overnight the hoppers roost on plants, bushes or stones and at dawn may move to the eastern side of roosts to increase body temperature, before finally descending to form basking ground groups (COPR, 1982). Once the body temperature rises above 25C marching begins but should it exceed 40C, commonly at mid-day, the locusts again ascend into roosts (Steedman, 1990; COPR, 1982; Volkonsky, 1942). Control of body temperature is achieved solely by behavioural means such as sheltering in roosts and basking (Ellis & Ashall, 1957; Chapman, 1965). During the day locusts within bands exhibit continuous cycles of feeding, resting and marching (both walking and hopping) lasting 15 to 45 minutes each but within large bands the cycle may not be synchronised throughout. Within approximately 2 hours of sunset the hoppers form ground groups at the base of prominent plants, but may resume marching for up to 9.5 hours after sunset before finally ascending roosts.

The hoppers march in cohesive bands that vary from a few square metres to several square kilometres in size (Steedman, 1990). The size of hopper bands may vary by amalgamation or division but also increases with hopper development when numbers remain constant (Ellis & Ashall, 1957). The hopper density is highest when roosting or in quiescent ground groups, for example Ashall & Ellis (1962) noted densities of 30-50 /m^2 over areas of 385-2258 m^2 when roosting, compared to 15-20 hoppers /m^2 over 4348-35953 m^2 when actively marching. The distance travelled by bands increased for larger band sizes, reduced before moulting, was reduced by marching through denser vegetation, increased with increasing hopper development and varied with hopper density (Ellis & Ashall, 1957; Steedman, 1990).
A striking feature of hopper bands is the way in which band integrity is maintained. Hoppers reaching the edge of a band consistently turn back into the main body (Kennedy, 1945; Ellis & Ashall, 1957) due to 'gregarious inertia' (Kennedy, 1945). Uvarov (1977) summarised gregarious inertia as a combination of the intrinsic tendency of gregarious hoppers to movement, the interaction resulting in band cohesion, mutual stimulation resulting in synchronised marching at the same speed and finally the optomotor compensatory reactions that keep the hopper's courses parallel. The controlling influences on the direction of marching are not known with certainty (Uvarov, 1977) but it has been observed that displacement is usually down wind (Ellis & Ashall, 1957; Haskell et al, 1962), especially so

for small bands. Superimposed on the direction of travel imposed by external factors is that caused by gregarious inertia when faced with topographical or vegetation features. For example when a hopper band meets vegetation the leading hoppers may maintain course and climb the plant, this results in a loss of stimulation from surrounding hoppers while those travelling on bare ground around the plant maintain close contact and activity. This leads to the observations of bands deviating from the original course to follow the bare tracks between thick scrub vegetation (Kennedy, 1945). The degree of gregarious inertia within a band depends on the level of gregarisation. Bands with insufficient gregarious inertia may be dispersed when passing through dense vegetation (Roffey & Popov, 1968) and when two bands fuse the resultant direction of travel will be that of the larger and denser band (Kennedy, 1945).

1.2.3 Gregarious Adult Behaviour

The fledging of desert locust hoppers into adults is usually well synchronised within a band (Uvarov, 1977) but fledglings may remain marching with a band and initially make only short low flights. Ellis & Ashall (1957) observed groups of gregarious fledglings within bands make practice flights for three days until ready to make higher flights and be carried away by the wind. It has also been noted (Kennedy, 1951) that young fledglings react to even slight winds by settling, while more mature adults in denser swarms react to strong winds by continuing to fly with the wind.

The gradual departure of fledglings inevitably leads to small groups leaving the hopper band; the process of re-integration into swarms is currently unknown (Uvarov, 1977). Once formed, swarms of desert locusts may exhibit local wandering flights which in sufficiently gregarious populations leads to true migratory behaviour (Uvarov, 1977); the furthest flights/migrations being made before sexual maturation (Steedman, 1990; Rainey, 1963). There is a diurnal pattern associated with adult migratory flight similar to that of hopper bands (Gunn, Perry et al, 1948; Kennedy, 1951; Waloff & Rainey, 1951). The locusts spend the night roosting and in the morning bask either on the sunward side of the roost or in ground groups. An internal temperature of 20C must be attained before the locust is capable of flight (Gunn, Perry et al, 1948) but a number of stages commonly occur before mass departure. The order of stages may vary and the sequence may be aborted but typically the locusts initially undertake short random flights, collectively termed the milling stage, which

then gives way to the surging stage. Activity becomes more structured during the surging stage, flying groups exhibit a common rapidly changing internal orientation but there is no common orientation between groups. The orientation between groups becomes more co-ordinated leading to the streaming stage that is abruptly followed by a mass departure. of the swarm.

Only a proportion of the locusts within a swarm is airborne at any one time and the swarm progresses with a rolling action allowing the proportion of grounded locusts to rest and feed. Flying may continue after sunset but settling of swarms appears to be associated with a reduction in ambient temperature to below 23C (Gunn, Perry et al, 1948), the proportion of settled locusts increasing until the whole swarm is settled to roost.

Table 1.5: Behaviour and characteristics of *Schistocerca gregaria* swarms.

Data	Value	Reference
Locust airspeed	3 - 5 m/s	Gunn, 1979a
Locust flight speed	8 - 24 km/h	Uvarov, 1977
	16 - 19 km/h	Steedman, 1990
	15 - 20 km/h	Rainey, 1963
Maximum continuous endurance (estimated)	20 hours 12 hours	Rainey, 1963 Gunn, 1979a
Swarm movement	From a few km to over 100 km per day. Up to 3500 km per month	Steedman, 1990
Swarm area	0.03 - 150 km^2	Rainey, 1963
	Up to 600 km^2	Uvarov, 1977
Swarm density	1 - 14 locusts/m^3 flying	Gunn, Perry et al, 1948
	160 - 370 /m^2 roosting on the ground	
	Up to 1500 /m^2 roosting on trees	

From simple ground based observations it appears that all the locusts in a swarm fly with the same orientation, this erroneous impression being caused by localised orientation within groups (Rainey, 1963). Locusts reaching the edge of a swarm are noted to turn back into the swarm giving the remarkable swarm cohesion which has been observed for 1000's of miles; the sensory mechanisms of swarm cohesion are thought to be both visual and auditory (Uvarov, 1977).

Airborne swarms exhibit configurations varying between stratiform and cumuliform (Waloff & Rainey, 1951) depending on ambient conditions. Stratiform swarms are relatively low (up to 100m high) rolling 'curtains' of locusts with a dense leading edge and diffuse rear; they commonly occur in overcast conditions or in the late afternoon when the ground has cooled reducing convection. Cumuliform swarms appear as towers of locusts up to 1000 meters tall and are associated with convection currents from strong sunlight (Steedman, 1990). It is generally agreed that the direction of swarm movement is predominantly controlled by wind direction (Rainey, 1951; Rainey, 1963) and more specifically since wind speed typically increases with altitude there is an altitude above which the flying locust will be carried down wind irrespective of orientation (Farrow, 1990). Thus tall cumuliform swarms tend to downwind movement (Sayer, 1962) while the direction of low flying swarm may be influenced by locust response to ground features (Uvarov, 1977). Although the locusts are effectively carried by the wind movement of swarms is commonly at less than half the wind speed, this is due to the rolling action described previously (Rainey, 1963; Uvarov, 1977).

Swarms of the desert locust are able to migrate within an area of 29×10^6 km^2 which is approximately divided into two climatic zones by a central desert belt. Environmental conditions within the two zones are only suitable for locusts breeding in alternate seasons forcing locust migrations (Uvarov, 1977). The migration of acridoids was originally thought to be a passive reaction to hardship in the insects' environment but increasingly migration is seen as a positive mechanism for enhancing the insects' survival and reproduction (Farrow, 1990). Rainey (1951 & 1963) was the first to hypothesise that desert locusts are carried toward areas of wind convergence that are also associated with rainfall; convergence areas therefore concentrate locust populations in areas with the moisture required for successful reproduction and provision of vegetation for hoppers to feed on (Hemming et al, 1979; Bennett, 1976). The movements of convergence areas follow broadly reproducible patterns that can be estimated from synoptic weather maps (Rainey, 1963) but the patterns are insufficiently accurate to allow tracking or forecasting of migrations (Steedman, 1990). These cyclical weather systems also lead to a recognisable pattern of seasonal breeding (see Table 1.6) exemplified by the effects of the Inter-Tropical Convergence Zone (ITCZ). The ITCZ is the summer confluence of northerly and southerly winds that spans the desert locust

distribution area, the movements of which has a complex effect on desert locust movements (Farrow, 1990).

Key to monitoring and control of desert locusts is the rapid identification of populations that may generate a plague (Coppen, 1995), thus data on rainfall and the position of convergence zones would be of great value. It is unlikely that ground based weather observations would possess sufficient resolution over the large areas involved but satellite observations do offer another approach. It is not possible to detect the damage to vegetation by insects directly (Riley, 1989) but it is possible to monitor the condition and extent of vegetation (Hatfield & Pinter, 1993). Digitally processed data from satellite observations has, since 1975, been employed in the detection of potential outbreak areas by monitoring the key factors of rainfall and vegetation development (Hielkema, 1990). The data is however insufficient for the forecasting or tracking of locust swarms.

The final facet of the behaviour of adult desert locusts is mating and oviposition. The settling of desert locust swarms for mating and subsequent oviposition is not governed by the site's suitability for laying but more associated with the presence of mosaic vegetation, possibly due to the wider range of conditions available than in uniform vegetation (Norris, 1968).

The courtship of desert locusts has been studied (Loher, 1959 & 1961) but only in descriptive terms and with no reference to physiological effects. The male and female locusts pair off, which is sometimes accompanied by fighting among males (Popov, 1958), and copulation then occurs for between 3 and 14 hours.

The females search for the egg laying site by marching or flying but must lay within approximately 72 hours of copulation (Popov, 1958). Repeated probing of sand with the abdomen is used to select the site. At least 2% moisture must be present in the sand although a dry upper layer promotes digging, the warmest site will be preferentially chosen and laying near vegetation avoided (Norris, 1968). Gregarious females commonly lay in groups even when the ground is uniform in composition (Stower, Popov & Greathead, 1958); an unknown gregarising pheromone has been implicated (Whiteman, 1990).

1.2.4 Solitarious Behaviour

Far less is known about the behaviour of solitarious desert locust populations. Uvarov (1977) attributed this to the diffuse nature of solitarious desert locust populations making observations laborious and to the difficulty of assigning phase status because upon gregarisation behaviour may change far more rapidly than morphometrics (both adult and hopper). Locust hoppers in the solitarious phase actively avoid close interaction (Uvarov, 1966) but when forced into proximity phase transformation can begin within a few hours (Ellis, 1959). Solitarious adults are more resistant to gregarisation and on crowding there is no social grouping behaviour (Gillett, 1975).

Although the densities of locust in recession populations may be low the numbers may still surpass those in a swarm; examples include 30 adult locusts' /ha over 20,000 km^2 in Mauritania giving a total population in the tens of millions and a similarly sized scattered population of hoppers and fledglings in Chad at densities of 1200 to 4000 /ha over an area of 110 km^2 (Roffey, Popov & Hemming, 1968). Swarms and hopper bands have been reported during recessions but they were less than fully gregarious and could not be tracked (Waloff, 1966). It is likely that the bands were mere temporary assemblages that dispersed before or on reaching the adult stage.

One key property of non swarming populations is nocturnal flight and migration (Waloff, 1963; Roffey, 1963; Roffey & Popov, 1968), rather than the diurnal migration undertaken by gregarious populations. The recession area is approximately half the swarm invasion area and the northern limit is due to the minimum ambient temperature of 20C required for night flight (Waloff, 1962). Solitarious desert locusts will only fly during the day if disturbed and then only for short distances (Steedman, 1990) although some short, low daytime flights are made when sexually mature, possibly related to mating (Roffey & Popov, 1968).

Roffey (1963) made a series of observations on desert locust nocturnal flight in Saudi Arabia, the method employed was to shine an Aldis lamp into the night sky allowing manual counting locusts and estimation of height, track and course. Despite the obvious limitations it was discovered that take off occurred within 40 minutes of sundown, at heights above 10m and wind speeds above 4m/s the direction of flight was downwind, duration of flight was comparable to daytime flights and the locust flying density was equivalent to a low density diurnal swarm. A correlation between flight activity and moonlight was also suggested.

The nocturnal flights form a seasonal migration pattern between breeding areas (see Table 1.6), the relationship between migration pattern and convergence zones having been reviewed by Farrow (1990).

1.2.5 Desert Locust Plague Dynamics

Recession populations of the desert locust are restricted to the central arid portion of the distribution area covering $16x10^6$ km^2 and receiving an average annual rainfall of less than 200 mm (Steedman, 1990). Both solitarious and gregarious locust populations follow a rainfall governed pattern of seasonal breeding (Uvarov, 1977) shown in table 1.6. Outbreaks correlate with exceptional and/or drought breaking rains (Cheke & Holt, 1993; Farrow, 1990) but it must be noted that while outbreaks occur frequently progression to plague is rare. Since 1860 there have been eight major and two minor plagues (COPR, 1982; Steedman, 1990), the last one occurring in 1986-89 after a recession period continuing nearly twenty years. Plagues occur with an unpredictable frequency (Waloff, 1976: Cheke & Holt, 1993) but plague duration is less variable than recession period suggesting biological rather than environmental causes of plague decline (Waloff & Green, 1975). The total area liable to visitation by desert locust swarms during a plague is $29x10^6$ km^2 (Uvarov, 1977) although all areas are not infested at once. Instances of swarms reaching as far as the British Isles have been recorded (Rainey, 1954).

Table 1.6: Main seasonal breeding areas of the desert locust (*Schistocerca gregaria*) during recessions and plagues (COPR, 1982).

	Season	Breeding areas
Recession	November - December	Coastal plains bordering the Red Sea and Gulf of Aden, the Trucial and Batina coasts of SE Arabia, and the Mekran coast of Iran and Pakistan.
	January - June	West Sahara, Mauritania, South and central Algeria, the Frezzan of Libya, Tibesti of Chad, the Red Sea and Gulf of Aden coastal plains, South, Central and Eastern Arabia, the coastal plains and interior valleys of Iran and Pakistan, and South Afghanistan.
	July - October	Southern Sahara, interior and coastal areas of Ethiopia and South Arabia, the Deserts of Pakistan, and Rhajistan in North West India.
Plague	October - December	Coastal areas of the Red Sea and gulf of Aden, Somali peninsula, Kenya and Tanzania.
	February - June	NW Africa, coasts of Red Seas and Gulf of Aden, Somali peninsula, Kenya, SW, central and N Arabia, Middle East, Iran, Afghanistan, Pakistan and NW India.
	July - September	A belt between 12° and 20° North extending into Ethiopia, SW Arabia, Pakistan and India.

It was first theorised by Johnston (1926) that outbreaks of the desert locusts were formed from solitarious locusts being concentrated in localised humid habitats and breeding over successive generations; detailed studies of this effect include Volkonsky (1942) and that of Roffey & Popov (1968). The latter describes an outbreak in the Tamesna area of the Niger and Mali republics during 1967, solitarious adult locusts moved from the Sahelian zone of Niger into Tamesna and were concentrated in green annual vegetation such as the Graminae *Tribulus* and the Cruciferae *Schowia* that covered less than 10% of the surface. The result was localised population densities of 50 to 1000 times that if evenly distributed and when combined with dense laying caused by restriction of suitable sites the successful cycles of breeding resulted in gregarisation of the locust population.

The major processes in upsurges were found to be concentration of locusts in suitable habitats, multiplication by successful breeding and subsequent gregarisation (Roffey & Popov 1968) although the stages may not be well defined or discrete. It has been argued that

outbreaks of desert locust may occur from small continuously gregarious populations surviving throughout recession periods (Rainey, 1963) but sufficient evidence is available to support the formation of outbreaks from initially solitarious populations (Uvarov, 1977). Progression of an outbreak into plague certainly requires a succession of successful breeding cycles but the failure of outbreaks to develop into plagues has a number of possible causes (Uvaov, 1977). These include mortality due to parasites and predators, adverse environmental conditions, the wind carrying adults to inhospitable areas such as seas and mountain tops, dissociation of gregarious hopper populations when passing through dense vegetation and finally control operations.

1.3 The Desert Locust As A Pest

Locusts still represent a significant international threat to crops and pasture world-wide (Skaf, Popov & Roffey, 1990). Approximately 12 locust species of varying economic importance exist over a geographical area ranging from Africa, Asia and the Americas to Australia (see table 1.1). In the case of the desert locust it presents no problem when solitarious and restricted to the recession area, the low density populations are integrated into the food chain and inhabit areas rarely used in agriculture (Herok & Krall, 1995). Transformation to transiens and then gregarious phase is triggered by exceptional and/or drought breaking rains (Cheke & Holt, 1993; Farrow, 1990). The invasion area covers more than 50 countries (Steedman, 1990) consisting of rangelands and agricultural areas that provide a domestic supply of food and cash crops for export (Herok & Krall, 1995). Some 400 species of plant have been credited as edible to the desert locust, although only a small proportion could be considered preferred food (Uvarov, 1977). An extensive list of cultivated or useful wild plants are attacked by the desert locust including staples such as barley, millet, maize, rice, sorghum, sugar cane and wheat (COPR, 1982; Steedman, 1990). The mass of food consumed varies with developmental stage but in typical laboratory populations a male consumes an average of 44g and a female 31g during their whole life (Davey, 1954). These values are likely to increase in the field and especially during migrations; it has been estimated that during migrations a moderate size (10 km^2) swarm of desert locusts would eat 1000 tonnes of fresh vegetation per day (COPR, 1982). A swarm

may also cause considerable damage by 'nibbling' through plant stems or by the sheer weight of numbers crushing plants when roosting.

Values are not available for the 1986-88 desert locust plague but between 1949 and 1957 the Food and Agriculture Organisation of the United Nations (FAO) estimates that for only 12 out of 40 affected countries' crops to the value of £17 million were destroyed, equivalent to £45 million in 1986 values (Steedman, 1990). Estimating the costs and benefits of control operations presents enormous difficulties, such as quantifying the environmental impact of control operations. A model constructed by Herok & Krall (1995) indicated that investments in desert locust control over the last fifty years have exceeded the potentially prevented losses. Despite undeniable economic losses the food harvests during plague years are not below average because the rains that promoted plagues also favoured crops, the primary problem was one of food distribution.

2 Locust Control

Control operations for desert locust upsurges and plagues still revolve around the large-scale use of insecticides to control outbreaks (Symmons, 1992). Large quantities of organophosphorus insecticides such as malathion and fenitrothion are currently used (MacCuaig, 1983) although historically, effective control was achieved by strip-spraying persistent organochlorines such as dieldrin (Bennett & Symmons, 1972; Courshee, 1959; Courshee, 1990). Use of organochlorine insecticides is prohibited because of their adverse environmental effects (MacCuaig, 1979) and the organophosphorus products in use have short persistence and must be sprayed directly onto locust infestations.

2.1 Pesticide Application Methods And Formulations

The main reason for formulation development is to transform the pesticide, which often has unsuitable physical properties, into a form that is easily handled, stable on storage and easily diluted. Contributory factors may include a requirement for reduced mammalian or plant toxicity, increased persistence, increased specificity, etc. The type of formulation employed is often dictated by the application method to be used.

Dusts

The simplest method of pesticide application is the spreading of a dust by hand. A technique employed for desert locust control with the insecticide γ-BHC, applied directly or to vegetation (Bennett & Symmons, 1972). The primary problems were of logistics, transporting the men and bulky dust to the treatment site. The dusting technique was still being used during 1986 with the insecticide fenitrothion (Kawasaki, 1990).
A dust formulation consists of particles less than 30 um in size and typically contains 0.5 to 10% active ingredient (Matthews, 1992).

Baiting

A bait formulation may be considered a development of dusting in that the insecticide powder is mixed with a food palatable to the target. Bait containing γ-BHC has been employed against both hoppers and adults of the desert locust (Bennett & Symmons, 1972) but exhibits the logistical problems in common with dusts. They also quickly become unpalatable on exposure to the elements and may have to 'compete' with freely available fresh vegetation.

Sprayable Formulations

Sprayable formulations are by far the most popular for locust control operations (Symmons, 1991). Traditionally, and particularly in the developed world, the application of pesticides has been achieved by gross dilution with water and atomisation using a simple pressure nozzle. The typical field conditions experienced in locust control operations makes the use of large quantities of water logistically unfeasible (Lloyd, 1959) but the technique found limited use during the 1986-89 desert locust plague (Skaf et al, 1990) and the 1984 Australian locust plague (Wright & Symmons, 1987).

In traditional spraying the need for gross dilution with water is imposed by the intrinsically poor efficiency of the method, for high efficiency the droplet size range must be narrow and suited to the use (Bals, 1976). Hydraulic spraying nozzles typically generate a droplet size range of 20 to 400 micron (Bals, 1969) which means each droplet is likely to be a massive under or overdose. It has been estimated that if theoretically perfect control were achieved then only 0.05% of the applied dose would be required; the inefficiency caused by carriage of small droplets away from the site by wind (exodrift) and run off or impaction of large droplets onto the soil (endodrift). (Himel, 1969).

In an attempt to improve efficiency the technique of controlled droplet application (CDA) spraying was developed, some times referred to as ultra low volume (ULV). Alternative methods of droplet formation are employed giving narrow droplet size ranges typified by centrifugal atomisation; the spray liquid is fed to a rotating surface and carried to the edge by centrifugal force where droplet formation occurs. Developments such as providing

grooves on a dished surface and teeth on the circumference aid droplet formation provided the disk is not flooded (Bals, 1976). Typical flow rates for a centrifugal atomiser would be 60 ml/min and 800ml/min for a conventional nozzle (Matthews, 1992) coupled with spray volumes of 1 to 3 l/ha and 300-500 l/ha respectively (Clayton, 1992). Further advantages of centrifugal atomisers are the ability to alter droplet size by varying the rotational speed, the simple compact nature of the equipment and that the equipment is suitable for manual operation, vehicle mounting or aerial spraying.

Before the availability of centrifugal atomisers the exhaust nozzle sprayer (ENS) was developed specifically to use ultra low volume application rates in locust control. The equipment is mounted on a vehicle and the exhaust gasses used to force the spray feed from a storage tank into a gaseous energy nozzle (Sayer, 1959). A typical flow rate was 1.2 l/min giving a use rate of 0.3 l/ha (Matthews, 1992) and the main disadvantages of ENS sprayers were in equipment maintenance.

For both centrifugal atomisers and ENS sprayers the wind is employed for even dispersal of the spray droplets. Overlapping swaths are sprayed perpendicular to the wind, the distance to impact depending upon droplet size and wind strength (Symmons, 1991). The formulations employed for ULV/CDA spraying are typically based on low volatility oils; water being ill suited due to droplet evaporation leading to loss of mass and increased drift outside the target area. Mixtures of oils can be employed, one oil conferring low volatility while the other dissolves the active ingredient. Barlow and Hadaway (1974) concluded that a ULV carrier should have a boiling point of at least 300C.

It is interesting to note that two of the most successful insecticides used against locusts, malathion and fenitrothion, are low volatility liquids suitable for ULV spraying without formulation. However, a ULV formulation of the carbamate pesticide bendiocarb has also been tested for locust control with favourable results (Symmons et al, 1989). The 'Ficam ULV' formulation (Cambridge Animal and Public Health Limited) comprised particles of insecticide suspended in an oil base.

Controlled Release Formulations

Controlled release formulations offer the opportunity to control the rate of active ingredient release after spraying a pesticide formulation; whether it be steady replenishment or sudden

triggered release. A range of methods is available for obtaining controlled release of active ingredients (Kydonieus, 1980) but when restricted to sprayable formulations, which are preferred in locust control (Symmons, 1991), then microencapsulation appears to offer the most utility. A microcapsule could be defined as a reservoir of liquid active ingredient surrounded by a coating of variable permeability and mechanical strength. Most commonly microcapsules for spray application are in aqueous suspension and have a particle size of 50 micron or less.

Microencapsulation of pesticides was first employed for pesticides by Vandagaer (1971) using an aqueous based formulation. Further advances, chiefly by the Monsanto company, resulted in increased active ingredient contents and utility (Beestman & Deming, 1981; Beestman & Deming, 1983; Becher & Magin, 1984). Additionally Monsanto developed the process for encapsulation of water soluble active ingredients with an oil continuous phase (Beestman, 1985a); because of the prior development of water based microcapsule suspensions' oil based formulations were termed 'reverse phase'.

No evidence was available that controlled release formulations have routinely been used for locust control operations; indeed the FAO recommend against use of microcapsule formulations (Symmons, 1991). Formulations previously available for testing were developed for traditional farm applications and not optimised for ULV application or the environmental conditions found in locust control. However, compared to a simple ULV formulation the extra components and processing necessary for a controlled release preparation will always cost more to produce on a LB for £ basis. Thus if microcapsule formulations are to be used in locust control operations a significant advantage must be proven.

Some possible advantages to the use of controlled release formulations have been identified in the literature (Phillips, 1968; Tsuji, 1990; Marrs & Scher, 1990). Firstly the persistence of the pesticide may be increased by a reduction in processes such as evaporation, degradation and leaching. Depending on release method the exposure of an insecticide may theoretically be restricted to certain types or sizes of insect and so protect non-target organisms. Toxicity of the pesticides to mammals, plants and fish may be reduced. Finally pesticide odour may be

masked to prevent a repellent effect and encapsulation may aid co-formulation of chemically incompatible components.

2.2 Methods of Desert Locust Control

It is apparent that the type of control operations must be suited to the developmental stage of the locust, which vary from immobile eggs through slow moving hopper bands to rapidly moving swarms.

It would appear that locust egg-fields offer a good opportunity for control due to the high population density and the extended period of availability but a number of operational problems make it impracticable. Firstly control must be achieved as the hoppers emerge, even though the eggs may hatch over a period of weeks. This requires use of environmentally damaging persistent insecticides or repeated spraying. Additionally only a small fraction of the laying sites are located by present methods (Symmons, 1991).

Two approaches are available for the control of hopper bands, either the direct spraying of bands or widespread dosing of vegetation in areas that are infested. When directly spraying hopper bands the target must first be located; as an example of scale it has been calculated that during a plague only 5000 km^2 of bands exist within 29 million km^2 of invasion area (Courshee, 1990). It has also been estimated that scouting with vehicles locates less than 10% of bands (Sayer, 1959). Once the bands have been located it must be decided whether to attack single bands or treat groups of bands in an infested area. Aerial spraying of individual hopper bands has proved more effective than ground spraying but aerial application to groups of bands (band zones) covering several square kilometre's is favoured (Bennett & Symmons, 1972; Symmons, 1991; Skaf et al, 1990; Courshee, 1990). The primary insecticide employed was the persistent organochlorine dieldrin, subsequently withdrawn due to environmental concerns. Fenitrothion, which is an organophosphorus compound, has been favoured in control of the Australian plague locust *Chortoicetes terminifera* but when use rates were accounted for it was found to cost eleven times more

than dieldrin and increase spraying time by a factor of three and a half (MacCuaig, 1979; Wright & Symmons, 1987; Symmons et al, 1989).

An alternative to direct spraying was the technique of "vegetation baiting" first described by Joyce in 1953 (Gunn, 1979b). The technique involved spraying a stomach acting insecticide onto vegetation likely to be eaten by locust hoppers. It was successfully developed using a combination of dieldrin and the exhaust nozzle sprayer (Sayer, 1959; Courshee, 1959), giving the advantages that one application of dieldrin could remain effective for up to a month and the pesticide could be rapidly applied from the air over large areas. A logical extension of the vegetation baiting method was the barrier spraying approach suggested independently by Mallamaire & Roy against desert locusts and Lloyd & Yule against red locusts (Gunn, 1979b). A series of strips were sprayed with insecticide, either in parallel or to form a box structure. The width of the barrier was chosen to ensure a locust ingested a lethal dose on crossing and the distance between barriers was chosen to ensure hopper bands met with a sprayed barrier during their development. A typical spray regime for aerial application was 2.8 litres of 20% dieldrin per kilometre in barriers 3 to 12 km apart (Bennett & Symmons, 1972).

Dieldrin was extremely successful in barrier spraying but widespread use was halted due to concerns over bioaccumulation; the situation has been covered in detail by MacCuaig (1979) and although his arguments may have a bias towards dieldrin the call for selected small scale use appears sensible. The point is put that a value judgement must be made as to whether the possible suffering due to poisoning is outweighed by the suffering due to starvation caused by a locust plague. With the halt in dieldrin use the barrier spraying method became effectively useless (Symmons, 1991). Attempts have been made to identify suitable replacement insecticides (FAO, 1978) with little success; insect growth regulators show promise (Symmons, 1991; Coppen, 1995) but require further development.

The attack of adult desert locusts differs from hoppers because of the increased mobility. Settled swarms may be sprayed employing ground or aerial equipment while flying swarms that can only be sprayed using aerial methods. The first task of locating swarms is far easier than in comparison with hopper bands; a single aircraft is capable of searching 50,000 km^2 in a day although swarms may subsequently be lost due to their great mobility (Symmons, 1992).

It is recommended that settled swarms of greater then one kilometre in size be sprayed from the air, in a similar manner to the attack of hopper bands (Symmons, 1991). However, for both the desert locust (Joyce, 1979) and the Red Locust *Nomadacris septemfasciata* (Lloyd, 1959; Gunn, 1979a) spraying of flying swarms is preferable to attack of settled swarms. Extensive data exists on pick up of spray droplets by flying locusts (Sawyer, 1950; Wootten & Sawyer, 1954; MacCuaig & Yeates, 1972) indicating high efficiencies with small droplets. Flying locusts pick up twice the amount of spray as settled ones and the flying locust is at least three times as vulnerable (Joyce, 1979). Thus even though the best techniques for swarm spraying are not yet known even a less than ideal technique should produce good results with only small amounts of pesticide (Symmons, 1991). It is clear that ULV application of insecticides from aircraft is the preferred option and that currently the lack of a suitably persistent insecticide for barrier spraying makes aerial spraying of flying swarms the only efficient method of desert locust control (Symmons, 1991 & 1992).

It has long been accepted that control of the locust (whether nymph or adult) must occur before it reaches cultivated lands (Gunn, 1979a), otherwise the crop will be destroyed before effective control can be mounted. Conversely it is impracticable to attack non gregarious locusts due to their low population densities (Bennett, 1976; Wright & Symmons, 1987). Therefore it has been suggested that the start of control operations should be timed to coincide with upsurge populations and prevent large swarms forming (Bennett, 1976; Roy, 1979, Hemming et al, 1979). This coupled with concerns over the environmental impact caused by spraying large quantities of short persistence insecticides such as fenitrothion (Everts, 1990) has made rehabilitation of barrier spraying desirable. Currently two candidates exist for barrier spraying, these are insect growth regulators (IGR) and microcapsule formulations of currently used insecticides. Both are inferior to dieldrin in that they are not cumulative and so require greater accuracy of application but current microcapsule formulations also posses insufficient persistence (Symmons, 1992).

2.3 Environmental Considerations Of Locust Control

Currently the only operationally effective method to combat locusts and grasshoppers is the use of chemical insecticide; either alternatives are not yet available or they are unsuitable for all circumstances (Everts, 1990). However, biological control methods are currently employed in combination with chemicals.
Between 1986 and 1990 an area of 1.4 million ha was sprayed with chemical insecticides during desert locust control operations (Everts, 1990). The main classes of chemical insecticide are organochlorine, organophosphorus, carbamate, synthetic pyrethroid, and insect growth regulator (IGR); all except IGRs act by interfering with the insects highly developed nervous system. The most common pesticides used in the control of desert locusts are proxpur (carbamate), fenitrothion, malathion (both organophosphorus) and deltamethrin (synthetic pyrethroid) (Herok & Krall, 1995). Organochlorines were heavily used for locust control but unacceptable environmental effects caused a total ban (Symmons, 1992) despite some calls for their reintroduction (Mackenzie, 1989).

Carbamate pesticides and their effects have been reviewed by the World Health Organisation (WHO, 1986a). There are more than 50 carbamate pesticides although these cover the classes' herbicide, nematocide, sprout inhibitor and insecticide. The carbamate ester derivatives normally used as insecticides are generally stable with low vapour pressure and low solubility in water but their properties vary to such a great extent that drawing general conclusions on their behaviour in the environment is not possible. However, the data indicates that bioaccumulation will only take place to a slight extent and toxicity to wildlife is low but some carbmamates may cause a significant reduction in non target organisms.

Organophosphorus insecticides and their effects have also been reviewed by the World Health Organisation (WHO, 1986b). In general the large numbers of organophosphorous insecticides (over 100) are only slightly soluble in water and exhibit low vapour pressures. The main route of degradation in the environment appears to be hydrolysis although the rate

of degradation is highly dependant on climatic conditions. Little information is available on the distribution environmental effects of organophosphorous insecticides.
The acute mode of action in both mammals and insects is by inhibiting acetylcholinesterase in the nervous system and subsequent build up of the neurotransmitter acetylcholine to toxic levels. Recovery may be slow due to the stability of the organophosphorylated enzyme.
The organophosphorous ester malathion is commonly used due to its broad range of effectiveness against sucking and chewing insects and low toxicity to warm blooded animals (Matolcsy, Nádasy & Andriska, 1988). This difference in toxicity towards insects and mammals is due to differing metabolism. Enzymatic oxidation to the highly toxic malaoxon occurs in insects while in mammals enzymatic hydrolysis converts malathion to a virtually non-toxic metabolite.

The history and properties of pyrethroid insecticides have been extensively reviewed by Elliot et al (1978). Synthetic pyrethroids are lipophilic compounds with low solubility in water, they possess high insecticidal activity although rapid knockdown may be followed by recovery if the dose is insufficient, and have oral toxicity's (rat) including 55 mg/kg for bifenthrin and >7000 mg/kg for bioresmethrin (RSC, 1991-4).
It has been concluded that in the case of the Australian plague locust (*Chortoicetes terminifera*) pyrethroids were insufficiently active to replace established organophosphorus insecticides (Edge & Casimir, 1975). When considering synthetic pyrethroids for control of desert locusts MaCuaig (1980) noted that non were suitable for application as a concentrated technical grade (possible with the organophosphorus compounds malathion and fenitrothion), thus the high insecticidal efficiency must also be offset against high cost.

IGR s are a novel group of insecticides which act by inhibiting the formation of insect cuticle (Cohen, 1987; Reynolds, 1987) resulting in mortality during ecdyses or malformation of the exoskeleton impairing further function and reproduction (Guyer & Neuman, 1988). Insect growth regulators are incorrectly named since no specific control is exerted and indeed the exact mode of action is unknown (Wilps & Nasseh, 1994). Efficacy with the desert locust was first demonstrated by van Daalen et al (1972) although due to the mode of action they are limited to attack of hoppers. They are characterised by oral or cuticular ingestion, low

toxicity to vertebrate species and extended persistence. Diflubenzuron for example has a rat oral LD50 of >4640 mg/kg (RSC, 1991-4) and a persistence of 5-6 weeks after spraying on Sahelian grassland (Sissoko, 1991). IGR s are non cumulative and degrade quickly in soil (Nimmo et al, 1984, 1986, 1990; Ost, 1987; Sundaram, 1991). It has been suggested that IGR s offer an alternative to organochlorine insecticides for barrier spraying (Symmons, 1991; Coppen, 1995) but the cost may be of concern (Prior & Streett, in press).

Little data is available on the environmental effects of the insecticides employed in locust control operations (Everts, 1990; Peveling et al, 1994; Mullie & Keith, 1993; van der Valk & Kamara, 1993) although outlines have been made of the studies required (Grant, 1989). A pilot study into the environmental effects of organophosphorus and IGR insecticides during locust and grasshopper control concluded that the organophosphorus compounds chlorpyrifos and fenitrothion may cause long term effects in the aquatic and terrestrial non-target fauna while the IGR diflubenzuron was relatively harmless under the same conditions (Everts, 1990). A similar trial investigated the effects of botanicals, insect growth regulators and entomopathogenic fungi on epigeal non-target arthropods (Peveling et al, 1994); there were no serious side-effects from any of the alternative control measures when compared to conventional insecticides.

Additionally it has been inferred that diflubenzuron is less hazardous to non-target beneficial invertebrates than other organophosphorus and synthetic pyrethroid insecticides (Murphy et al, 1994) by the database analysis of laboratory and field assays. It must be stressed however, that the technique is not predictive but merely indicative of the potential for harm.

Little is known of the specific environmental hazards associated with microcapsule formulations of insecticides but they would appear to present a greater risk to beneficial insects than conventional formulations due to the extended persistence. One of the earliest commercial products was PENNCAP-M®, an aqueous based methyl parathion microcapsule suspension (Ivy, 1971) with polyamide polyurethane copolymer microcapsule walls. In a review of field data concerning effects of PENNCAP-M® on entomophageous insects (parasitic and predatory) Dahl & Lowell (1984) found that for the most part insect populations were reduced by less than 50%. It was concluded that the effects on many parasitic and predatory insects were lessened due to the reduced use rates employed with PENNCAP-M®, especially if a degree of tolerance already exists in the population. What is

more important there were some indications that the commercial product PENNCAP-M® exhibits a degree of selectivity, beneficial insects remaining unharmed at use rates that achieve pest control (Koestler, 1980). However, it is important to note that bees may be at increased risk due to dried microcapsules being carried back to the hive with pollen (Koestler, 1980). This may be of special importance since unencapsulated malathion applied from the air using ultra low volume (ULV) equipment remained toxic to bees for up to 7 days (MacCuaig, 1983).

2.4 Biological Control

The technique of using biological agents for the control of locusts presents far less danger of adverse environmental impact, as demonstrated by database analysis of all available indicating pesticide effects on arthropod natural enemies (Theiling & Croft, 1989). Desert locusts possess a number of natural enemies (see table 1.3) but for the purposes of control operations pathogenic diseases offer the most promise because they allow spray application and do not require culturing of large numbers of insects or mammals.
Classes of pathogens include protozoa, viruses, rickettsia, nematodes and bacteria that all infect orally, and fungi that predominantly infect via the cuticle (Streett & McGuire, 1990; Burgess, 1981; Dillon & Charnley, 1986). Most of these natural enemies possess drawbacks when considered for use in control operations. Firstly no bacteria have been identified with significant activity against grasshoppers, including the most successful bacterium *Bacillus thuringiensis* (Zimmerman et al, 1994). Additionally the spores of Bacillus thuringiensis are susceptible to damage from strong solar radiation (Pinnock et al, 1977), high temperature and low humidity (Streett, 1987). Viruses appear safe for use as insecticides but their use is limited by the narrow range of host susceptibility and the inability to culture commercially *in vitro* (Streett, 1987). The highly effective rickettsia *Ricketsiella grylli* has been discounted for us as a control agent due to mammalian infectivity (Ignoffo, 1973). Protozoa have received significant attention as biological control agents, with some success in grasshoppers (Henry, 1977; Henry, 1982; Henry & Onsager, 1982). However, the protozoa *Nosema locustae* was tested against grasshoppers in the field with disappointing results (Johnson, 1989; Steedman, 1990). Commercial formulations of nematodes have been developed but

the high cost and a requirement for water during application makes them unsuitable (Prior & Streett, in press).

Several species of fungi offer potential as biological control agents for grasshoppers and locusts (Balfour-Browne, 1960; Streett, 1987; Bateman et al, 1994; Scherer et al, 1992) including *Metarhizum anisopliae*, *Metarhizum flavoviride, Beaveria bassiana, Verticilium lecanii* and finally *Entomophaga grylli* that has been responsible for dramatic reductions in grasshopper populations (Pickford & Riegert, 1964; Henry & Onsager, 1982). Some fungi are capable of cultivation (Ferron, 1981; Hall, 1981; Bateman, 1995) but all are limited by factors such as the very high ambient humidity required for spore germination/development, inactivation by UV light and a delay after application before control takes effect (Streett, 1987; Bateman & Thomas, 1996; van Emden, 1989). In the case of *M. flavoviride,* locust mortality is delayed at least 7 days post spraying and may be further postponed by adverse environmental conditions (Bateman & Thomas, 1996). Careful formulation of biological agents can help mitigate the detrimental effects of adverse environmental conditions. It has been demonstrated that suspending the fungal spores (conidia) in oil can improve efficacy at low ambient humidity (Prior et al, 1988, Bateman et al, 1993) and co-formulation of sunscreens may extend conidia viability when exposed to UV radiation (Moore et al, 1993). The key to maintaining conidial viability on storage for an oil based suspension was found to be minimisation of moisture and storage temperature (Moore et al, 1995, McClatchie et al, 1994).

Advantages to the use of fungal insecticides include infection of insects from contaminated surfaces (Bateman & Thomas, 1996), external sporulation of cadavers under suitable ambient conditions resulting in an epizootic (Scherer, 1992), host specificity (Scherer et al, 1992), reduction in feeding of locusts after infection (Moore et al, 1992) and possible co-application with chemical insecticides (van Emden, 1989). The first experiments using fungal pathogens for insect control were made in 1886 and the first commercial product was available in 1891 (van Emden,1989). However, commercial organisations may still experience difficulties in getting the technology widely accepted (Anon, 1996).

3 The Future Of Desert Locust Control

It was thought during the 1950s/60s that aerial spraying of dieldrin was the final answer to desert locust plagues (Roy, 1979). Since the withdrawal of dieldrin control operations have met with little success (Bennett, 1976; Rainey et al, 1979; Skaf et al, 1990; Showler & Potter, 1991), the reasons for this failure when compared to the successful control of other locust species include the huge recession area from within which upsurges can arise and the poor co-ordination of the resources available (Haskell, 1992; Schulten, 1989; Gunn, 1979a). Indeed the scale of emergency control operations during the latest plague (1986-89) may be related to the coincidental dissolution of tradition control organisations (Schulten, 1989).

Although the various control measures have been described they must be applied as part of a strategy. The three strategies identified for desert locust control are (Symmons, 1992):

1) Prevention of plagues by control during upsurge.
2) Elimination of plagues by destroying nearly all locusts.
3) Protection of crops only, allowing the plague to run its course. Typically used as an emergency measure in response to insect densities exceeding an economic threshold (Pledgely, 1993).

Currently it is attempted to carry out a policy of plague prevention but an inability to 'police' the whole recession area commonly causes fall back to upsurge containment and then finally crop protection (Symmons, 1992). There has been a great deal of debate over what control strategy should be adopted and what control methods should be employed to operate the strategy; currently there is no consensus and little hope of reaching one without greatly improving our understanding of population dynamics and economics. The following opinions have been published concerning strategy.

Despite being hampered by lack of information Herok & Krall (1995) were able to draw some conclusions on the economics of desert locust control. Firstly the investments in desert locust control over the last fifty years have exceeded the potentially prevented losses. Secondly no large scale famines have been caused by desert locusts in the last 50 years and

probably the last 150 years. While it is true that serious economic losses have been incurred due to crop losses the main problem has been one of national food distribution. Finally it was concluded that plague prevention and elimination were impractical. The suggested strategy comprised surveying of key areas from which plagues are known to emanate and concentration of control operations in important regions and for crop protection.

Prior & Streett (in press) considered the options for using entomopathogens in desert locust control. The main restrictions to the use of entomopathogens were a delay in mortality after spraying and unsuitability for barrier spraying, but unlike IGR's entomopathogens are effective against both nymphs and adults. Swarms are unsuitable targets for entomopathogens (unless in a very remote area); the highly mobile swarm may have moved to crop area during the delay between treatment and mortality. Attack of hopper bands was considered unfeasible due to the difficulty in locating them. Thus it was concluded that the use of entomopathogens is restricted to plague prevention as part of Integrated Pest Management Programs (IPM), where minimising use of conventional insecticides is the goal. For plague prevention to become feasible great improvements in the detection of gregarising populations would be required coupled with faster response.

Drawing on published data for locust numbers, spraying efficiencies, etc. Symmons (1992) concluded that plague elimination and crop protection are bound to fail. Aerial spraying of swarms was recommended for upsurge control because the loss of dieldrin made attack of gregarious hoppers ineffective.

Showler & Potter (1991) advocated 'strategic control' preventing plagues with a combination of scouting and selective attack. The method of attack should be chosen on site according to the local conditions and with reference to efficiency, environmental concerns, human safety, equipment availability, etc. .

Courshee (1990) and Mathews (1992) concluded that attack of hopper bands was ineffective but barrier spraying was the only technique easily justified in cost terms when compared to the damage done by locust plagues.

Skaf, Popov and Roffey (1990) concluded that during desert locust upsurges the technique of attacking hopper band zones from the air should be adopted because bands may remain available to attack for up to six weeks while swarms pass in one day. It was also concluded that given the problems inherent in locating hopper bands and aerial spraying of locust swarms, that plague prevention was unlikely unless a) dieldrin is used for barrier spraying, b) 50 times the necessary quantity of non persistent insecticides is used for large scale spraying of hopper bands or c) that there is a massive increase in swarm tracking and then aerial spraying when airborne or settled.

Finally, after analysis of the 1968 desert locust plague Bennett (1976) concluded that the most economical strategy for plague prevention might be one of 'upsurge elimination'. This is best described as allowing the natural upsurges of desert locust populations to occur unchecked until the threat of economic damage is certain and the gregarious population is highly concentrated. The advantage would be prevention of wasteful attack on localised population upsurges that die away from natural causes but it is doubtful that the current monitoring of locust populations would be sufficiently accurate and homogeneous to succeed.

Whatever the chosen control strategy the political realities must also be considered. Any strategy that allows gregarious populations to remain unthreatened is unlikely to gain favour (Bennett, 1976) and large organisations devoted to plague prevention are unlikely to survive the periods of inactivity between plagues (Symmons, 1992).

4 Aims And Objectives Of This Study

"After nearly twenty years with no major locust plague the desert locust came back with a bang in 1989, even invading the Caribbean after the first ever Atlantic crossing by a locust swarm. New methods of control are urgently needed." from (Haskell, 1992). This project was to approach the problem from an alternative perspective with the primary question of whether advanced formulation techniques, and more specifically microencapsulation, could be used to produce formulations tailored for locust control and which minimised environmental damage. Table 1.7 (after that of Jepson, unpublished) indicates what may be feasible by fitting the active ingredient and formulation to the control situation.

In the first phase of this project the aim was to develop a range of microcapsule formulations with properties projected to be suitable for locust control operations. Initially water based microcapsule formulations of the synthetic pyrethroid bifenthrin were developed and subjected to limited biological testing; however field testing of commercial aqueous based microcapsule formulations for desert locust control indicated their unsuitability (Jepson, personal communication). Emphasis was changed to microcapsule formulations based on low volatility oil; the physical properties of bifenthrin proved to be unsuitable so the organophosphorus insecticide malathion was adopted in its place.

The project's second phase consisted of biologically characterising of the oil based (reverse phase) malathion microcapsule formulations. Initially it was attempted to mimic field conditions in the greenhouse, applying the formulations to vegetation using commercial spinning disk sprayers and then exposing desert locust nymphs to the aged residues. Difficulties with the protocol forced a change to more strictly controlled laboratory tests concerned with persistence of deposits, cuticular toxicity and oral toxicity of the microcapsule formulations.
A priority of the project was to produce formulations with decreased environmental impact compared to currently used treatments; thus deposits of malathion microcapsule formulations were tested for toxicity to the indicator beneficial insect *Aphidius colemani*.

In the final phase of this project a formulation of *Metarhizium flavoviride* conidia was developed in collaboration with the International Institute of Biological Control. The formulation of living organisms offers a unique challenge in that the viability of the active ingredient must not be impaired while ensuring the physical stability, efficacy and serviceability.

Table 1.7 (over leaf): Environmentally Safe Strategies For The Chemical Control Of Locusts, Conceptual Flow Chart (after that of P. Jepson).

ENVIRONMENTALLY SAFE STRATEGIES FOR THE CHEMICAL CONTROL OF LOCUSTS: CONCEPTUAL FLOW CHART

	BIOLOGICAL TARGET					Emerging first instar hoppers		**ENVIRONMENTAL BENEFIT OVER CONVENTIONAL SPRAYING**
		Flying swarm	Settled swarm	Sedentary adults/ hoppers	Immigrant hopper bands	Sedentary species / forms	Migratory species / forms	
	CONTROL TACTIC	Aerial Spraying	Air or ground blanket spray	Air or ground blanket spray	Air or ground barrier spray	Air or ground blanket spray	Air or ground barrier spray	
PROPOSED CONTROL AGENT	Photo-labile synthetic pyrethroid	✸						Low bioavailability -low ground residue -rapid breakdown Low vertebrate toxicity Low operator risk
	Persistent Synthetic Pyrethroid		✸	✸				Low vertebrate toxicity Low operator risk
	Microencapsulated Organophosphorus			✸	?	✸		Reduce oral and dermal toxicity to vertebrates / operator Reduced leaching Greater selectivity
	Insect Growth Regulator			✸	✸	✸	✸	Low vertebrate toxicity Greater selectivity Low operator risk
	Entomopathogenic fungi	✸	✸	✸		✸		Low vertebrate toxicity Very selective Low operator risk

CHAPTER 2

FORMULATION OF THE ENTOMOPATHOGENIC FUNGI *Metarhizium flavoviride* FOR CONTROL OF THE DESERT LOCUST (*Schistocerca Gregaria* Forsk.).

Described herein is the formulation of *Metarhizium flavoviride* conidia, for the purposes of locust control operations. An oil miscible flowable concentrate (OF) (Gifap, 1984) type formulation was chosen consisting of fungal conidia suspended in a paraffin oil with associated formulation additives. The theory and practice of formulating biological agents are discussed along with details of the laboratory test methods developed and requirements for storage stability.

1 Introduction

Since the withdrawal of dieldrin, large quantities of short persistence but broad spectrum insecticides have been used in locust control operations with a probable detrimental impact on non target fauna. Consequently there is an impetus towards developing safer alternatives, including control measures using biological organisms (Prior & Streett, in press). Three groups of control agents are currently being tested as alternatives to traditional insecticides in locust control (Peveling et al, 1994). These are insect growth regulators, botanicaly derived chemicals extracted from the seeds of various plants in the family Meliaceae, and finally entomopathogens as exemplified by entomopathogenic fungi which are mostly members of Deuteromycetes.

Experiments in insect control using fungal pathogens were begun in 1886 with a commercial product appearing in 1891 (van Emden, 1989). Several species of entomopathogenic fungi offer potential as biocontrol agents for grasshoppers and locusts (Balfour-Browne, 1960; Streett, 1987; Prior & Streett, in press) including *Metarhizium anisopliae*, *Metarhizium flavoviride*, *Beauveria bassiana* and *Verticilium lecanii*.

Advantages to the use of fungal insecticides include the ability of insects to acquire infection from contaminated surfaces (Bateman & Thomas, 1996), the external sporulation of cadavers

under suitable ambient conditions which can maintain epizootics (Scherer et al, 1992; Thomas, Ward & Lomer, 1995), reduction in flight and feeding of locusts after infection (Moore et al, 1992; Seyoum et al, 1994), possible co-application with chemical insecticides (van Emden, 1989) and unlike other biological control agents fungal insecticides are active by contact (Henry, 1977). Another important advantage is host specificity; Scherer et al (1992) found that *Metarhizium spp.* had varying degrees of specificity but the isolate being developed as a biological control agent rarely infected insect orders other than Orthoptera. Tests of *Beauveria bassiana* with epigeal, non-target arthropods indicated that at high humidity levels all test species became infected, despite this no reservations against introduction into grasshopper and locust control have been made (Peveling et al, 1994). The technique of using biological agents for the control of locusts presents far less danger of adverse environmental impact. Indeed database analysis of pesticide effects on arthropod natural enemies (Theiling & Croft, 1989) classified the entomopathogenic fungal insecticide *Beauvaria bassiana* as the least toxic of all pesticides.

A major disadvantage with the use of biological control agents is the inevitable delay in achieving mortality compared with chemical agents. *M. flavoviride* exhibits a delay of at least 7 days before locust mortality (Bateman & Thomas, 1996) but in that time locust feeding is reduced (Moore et al, 1992; Seyoum et al, 1994). Some fungi are capable of laboratory cultivation (Ferron, 1981; Hall, 1981; Bateman, 1995) but all are limited in use by factors such as the very high ambient humidity required for spore germination, and inactivation by UV light (Streett, 1987; Bateman & Thomas, 1996; van Emden, 1989; Moore et al, 1993).

Typically with a biopesticide the aim is inundative augmentation of an indigenous agent (Prior & Street, in press) in which case the role of formulation is identical to that for conventional insecticides:

1) Convert the active ingredient to a form which is suitable for application, i.e. evenly spreading small quantities of active ingredient over large areas. Most common types of formulation are water dilutable, but in the case of locust control ULV spraying of oil-based formulations is the accepted mode.
2) Ensure that the active ingredient remains in a usable form during extended storage. This includes both physical stability and chemical or biological stability.

3) Attempt to compensate for any deficiencies exhibited by the active ingredient. For example preventing inactivation by UV light, delayed efficacy from a particular physical form, short persistence, etc.
4) Maximise the insecticidal efficiency of the active ingredient.

It is impossible to develop an optimised pesticide formulation when concentrating only the more easily measured storage and dilution properties. A wider approach must be used that takes into account application and mode of action but which may require more complex and lengthy investigations.

The LUBILOSA (LUtte BIologique contre les LOcustes et SAuteriaux) program was begun following the discovery that deuteromycete fungi could be formulated in oil, giving an increase in efficacy (Prior et al, 1988; Bateman et al, 1993). The aim of the program was to develop a mycoinsecticide for the control of locusts and grasshoppers
Formulation of fungal conidia in oil offered a number of advantages additional to increased efficacy.

1) Oil-based formulations are compatible with existing CDA (controlled droplet application) equipment used extensively in locust control operations, which in turn bring increases in application efficiency compared to traditional application methods (Bateman, 1997).
2) Aerial conidia have a lipophilic surface making them far easier to suspend in oil (Bateman, 1994a).
3) The locust cuticle is also lipophilic allowing extensive spreading of impacting oil droplets over the insect to vulnerable areas and possibly increasing retention on the cuticle (Stathers et al, 1993).
4) Finally the infectivity of *M. flavoviride* is maintained at low ambient humidities (Prior et al, 1988) which may not be the case otherwise (Gillespie, 1988).

Before 1997 virtually all testing of mycoinsecticide formulations by LUBILOSA was performed using simple suspensions of conidia powder in vegetable or mineral oil. These formulations were prepared immediately before use and typically applied using CDA equipment. Initial field trials indicated that M. flavoviride conidia suspended in oil make an

efficient pesticide for use in locust control (Bateman et al, 1994; Scherer et al, 1992) but further development required identification and development of a suitable formulation. The purpose of this paper is to describe the development of a *Metarhizium flavoviride* conidia formulation suitable for (CDA) application as part of an integrated pest management (IPM) approach to locust control (Prior & Streett, in press). In the process of formulation development a number of test methods and specifications were developed which have also been described.

2 Mycoinsecticide formulation type

Myco-insecticide formulations for locust control should posses low mammalian and plant toxicity, biological and physical stability for up to one year and when diluted should have suitable properties for application with CDA equipment, especially low viscosity and low volatility (Bateman, 1994b).

Three strategies were identified for the formulation of *M. flavoviride* conidia. First and least favoured was the simple hand mixing of dry conidia powder with locally sourced oil immediately before application. The handling of dry spore powders can be problematical because they are more difficult to measure and mix than liquids and they also represent a small but significant risk of operator inhalation and explosion.

Secondly the conidia could be formulated in a similar manner to water dispersible granules (WG) but designed to disperse in oils; the key to this type of formulation would be ensuring rapid granule dispersion in the oil. When preparing conventional WG's the formulation typically contains milled active ingredient, dispersant powder and filler as a basis. The granules are prepared by dry mixing the components, adding water until a suitable consistency is obtained, extruding and then fluid bed drying. The dispersant acts as a binder giving a robust and dust free granule, while also preventing agglomeration of the particle suspension when dispersed in water. If the granule is too well bound due to too much dispersant, a dispersant of the wrong type or too much pressure during extrusion the granules will fail to disintegrate fully and then block application equipment.
To prepare oil dispersible granules new dispersants would have to be found with suitable binding properties and what is more important a volatile oil must be found which dampens the formulation sufficiently for extrusion while either being cheap and environmentally harmless or easily recoverable from the fluid bed drying stage. While oil dispersible granules were an interesting concept the engineering required was considered too radical in this instance.

Finally oil-based conidia suspensions could be developed either 'ready to use' or as a concentrate to be diluted with oil immediately before application. A ready to use formulation provides ease of use coupled with accurate control of the spore concentration and viscosity,

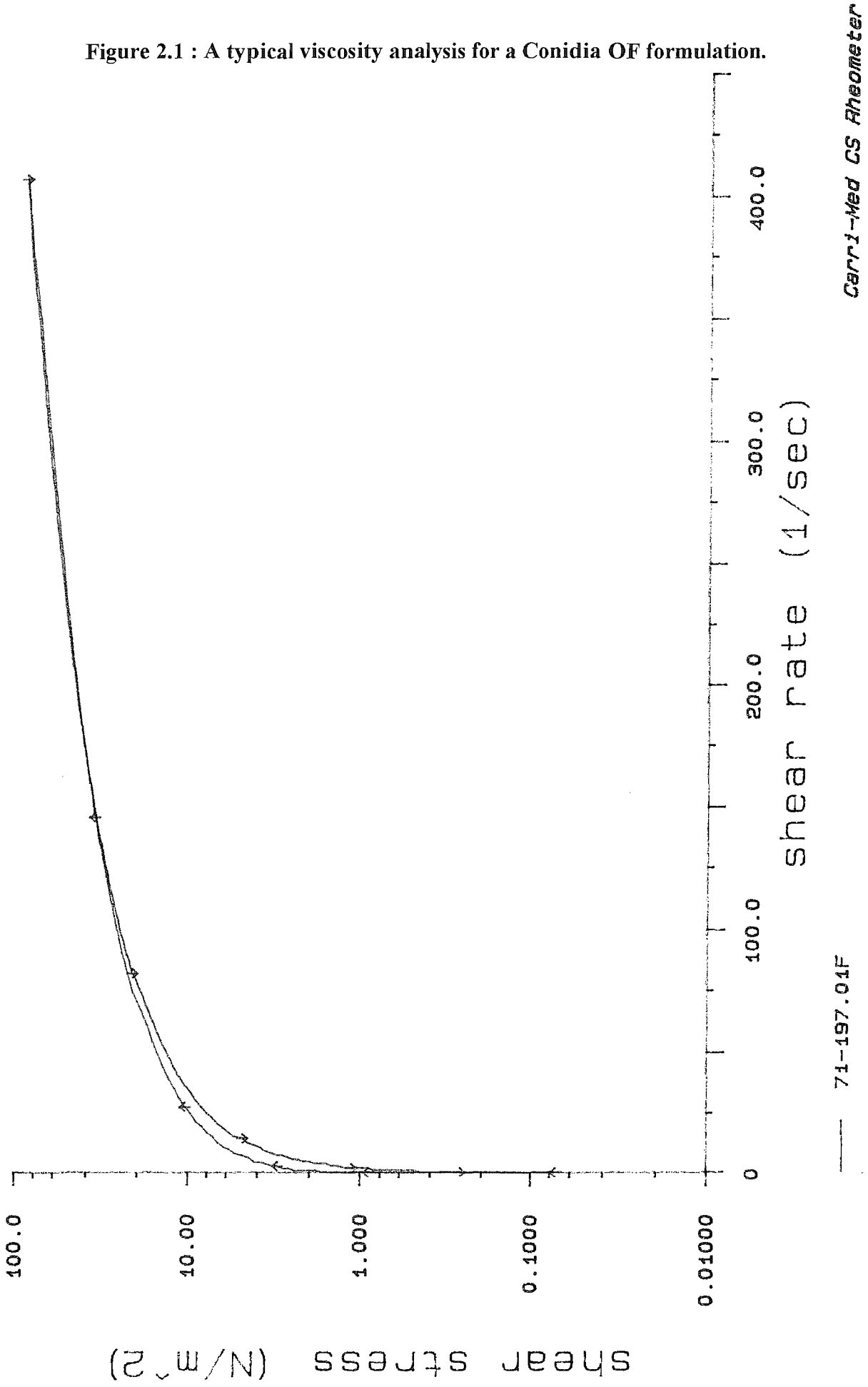
which are critical to application efficiency (Bateman, 1994b). Conversely a concentrate formulation minimises the amount of material to be shipped but requires local procurement of oils for dilution, with corresponding quality issues. The concentrate formulation type appeared most suitable for this application.

The primary consideration in the development of a mycoinsecticide formulation is that the active ingredient is a biological entity that may lose viability by formulation with incompatible components, this may be considered similar to chemical degradation of conventional insecticides. The factors controlling chemical stability within conventional insecticide formulations have been studied for many years and are well understood, but those governing stability of biopesticides are only now being investigated. For example, *M. flavoviride* conidia are incompatible with strong solvents; indeed some oils tested for use as carriers proved to be fungicidal (Bateman, 1995). Also it has been discovered that for conidia to retain biological viability on storage the moisture content of oil suspensions must be minimised (Moore et al, 1995).

The second priority of a pesticide formulation is to ensure physical stability. Suspensions of solids in liquids, such as the OF described herein, must be protected from agglomeration and sedimentation by the inclusion of dispersants and rheological modifiers respectively. The optimum dispersant and concentration can be found, for example, by measuring the viscosity of concentrated dispersions or the degree of particle agglomeration on dilution.

Suitable viscosity modifiers are identified using a combination of rheometry and stability testing at various temperatures; the aim is controlled thixotropy, i.e. to increase the formulation viscosity at the low shear equivalent to sedimentation without greatly increasing the viscosity at higher shear equivalent to pouring, shaking and pumping. A result from rheological analysis of a typical OF concentrate formulation is included in figure 2.1; when plotted in this manner viscosity is equivalent to the slope of the line. The use of creep and recovery tests at very low shear stress may yield further information gel structures and particle sedimentation (Schramm, 1994).

Figure 2.1 : A typical viscosity analysis for a Conidia OF formulation.



3 Specification for *M. flavoviride* conidia OF formulation.

Candidate formulations must be tested for their ability to meet a predetermined specification both initially and after storage at various temperatures. The CIPAC organisation (Collaborative International Pesticides Analytical Council) sets standardised test methods for pesticide formulations. Tests have been developed for aqueous-based particulate suspension concentrates but none were available for oil-based concentrates. Thus the following specification (Table 2.1) and tests were developed as a first approximation with the expectation that subsequent investigators will develop improved test methodologies.

Conidial Viability

Conidial viability was tested at the International Institute of Biological Control at Ascot (IIBC) using the method detailed in Moore et. al. (1995).

Appearance

The formulation should appear uniform, although slight syneresis is permissible. No sediment should be detected when probed with a thin metal implement. When shaken lightly any gellation caused by anti-sedimentation agents should disperse immediately.

Dispersion Test

To test dispersion a sample of formulation (typically 0.5g) was added to 100ml of paraffin oil (Isopar M, Exxon) contained within a stoppered 100ml measuring cylinder. The cylinder was inverted around its mid point up to sixty times and the number of inversions required for full formulation dispersion was noted. Each inversion was through an angle of 180° and back, once every two seconds, without any bouncing. It is advantageous to test the formulation at a dilution equivalent to the expected use rate, but the opacity may impair observation and lower dilution rates may be required.

Semi-Quantative Suspensibility

Firstly, 0.5g of formulation was dispersed in 100ml of paraffin oil using thirty inversions as described above. The dispersion was then transferred to a vessel with narrowed base and accurate volume markings (see figure 2.2). The dispersion was allowed to stand undisturbed

for one hour and the volume of sediment measured with the aid of a light beam passing through the liquid. The method may suffer from variability in sediment density and high concentrations could not be used because of excessive opacity.

Table 2.1: Specification for *M. flavoviride* conidia OF formulation.

Property	Value
Viability	> 80% germination
Appearance	Uniform liquid, although slight syneresis (2 to 3mm) allowable.
Sedimentation	None
Dispersion	< 30 inversions
Suspensibility	<0.05ml sediment or >90% suspended
Wet sieve (75µm)	<0.1% retention
Wet sieve (45µm)	<0.5% retention
Particle Size (Volume Distribution)	80% < 10µm 99.8 < 44µm 100% < 100µm

Gravimetric Suspensibility

The second suspensibility method overcame problems caused by opacity. The initial method was as described above but the dispersion was retained in a straight sided 100 ml measuring cylinder. After 3 hours a 10 ml aliquot was removed from the 50ml mark using a pipette and filtered using a pre-weighed Whatman GF/A filter paper. The filter paper was washed copiously with acetone and dried overnight in an oven at 37°C. The percentage suspensibility was calculated from the mass of solids on the filter paper and the theoretical quantity of solids in a 10ml aliquot. The latter was derived from the amount of formulation initially added. Advantages to this method include use of higher active ingredient concentrations and direct quantification of the suspended solids. Disadvantages include increased test duration and blocking of the filter paper at high formulation concentrations.

Wet Sieve Test

The wet sieve method entailed manually dispersing 5g of formulation in 100ml of Isopar M by stirring and then pouring through a 75 micron sieve, pre-wetted with paraffin oil. Any solid

residue was washed copiously with a stream of paraffin oil and then by a further acetone wash. The residues were washed into a pre-weighed Whatman GF/F or GF/A filter papers with acetone and then allowed to air dry at room temperature. The percentage sieve retention was calculated from the mass of solid residue on the filter paper and the original sample mass.

Particle Size

The particle size was measured using a laser particle size analyser after dilution of the sample in paraffin oil. In the case of the *Metarhizium* OF the maximum particle size was primarily determined by the quality of the conidia powder and was important in ensuring that the formulation did not sediment on storage. The specification agreed for the conidia powder was 80% <10 micron, 99.9% <60 micron and 100% <100 micron (volume distribution) as measured using a Malvern 2600 instrument. The result of a typical particle size analysis on a conidia OF formulation is included in table 2.2. Good agreement was found between the two instruments used for particle size analysis.

Table 2.2: Typical particle size analysis (micron) of a conidia OF formulation after dilution (prepared using a Galai Cis-1 laser particle size analyser).

Mean (nv)	3.4
Mean (vm)	6.0
Median	4.9
95% <	14.1

Storage Stability Testing

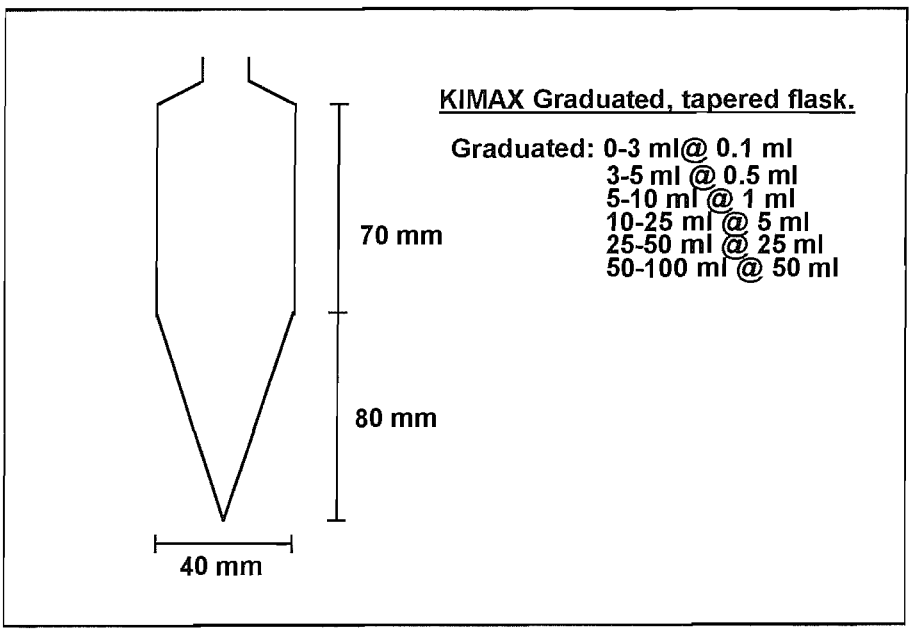
The formulation must continue to meet specification for the expected period of storage and over the range of expected storage conditions. The *Metarhizium* OF concentrate was developed to a point suitable for field trials but before a commercial formulation can be developed a more rigorous trial would be required. The test regimes are shown in Table 2.3. It is well known that elevated storage temperatures reduce the viability of fungal spores (McClatchie et al 1994; Hedgecock et al, 1995) but elevated temperatures have traditionally been used to predict the chemical and physical behaviour of conventional insecticide formulations over extended periods. For example two weeks' storage at 54C is considered

indicative of two years' storage at room temperature but when working with biological active ingredients' caution must be used in interpreting these results. A key factor in conidial viability is the presence of moisture and incorporation of moisture absorbing silica gel in the packaging of oil-based fungal suspensions is known to extend viability on storage (Moore et al, 1995 & 96).

Table 2.3: Storage Stability Testing.

Type	Temperature	Maximum Duration /weeks
Field Trial	4°C	2
	Room Temperature	4
	37°C	2
Commercial Formulation	4C	2
	Room Temperature	52
	25/40°C cycling	4
	30°C	30 days
	37°C	4
	54°C	2

Figure 2.2: Suspensibility test apparatus.



4 The results of formulation development

Previous screening of oils with *Metarhizium* conidia had indicated that selected vegetable oils, mineral oils and paraffin oils were suitable for use in conidia formulations (Stathers et al, 1994; Moore et al, 1995). For the purposes of formulation development a highly refined paraffin oil was chosen due to its high purity, the availability of various viscosity grades, minimal inter-batch variability and no requirement for preservatives.

Simple dispersions of conidia powder in paraffin oil exhibited particle agglomeration and rapid sedimentation. A range of dispersants was tested for effectiveness and fungicidal activity before a modified siloxane surfactant was chosen. Using this dispersant a spore concentration of 50% w/w was achievable without adversely effecting formulation viscosity although higher concentrations resulted in shear thickening (dilitancy).

It was found that using more viscous grades of paraffin oil failed to prevent conidia sedimentation so a wide range of viscosity modifying agents were tested. Finely divided silica may be used to thicken oils but it was found that all the candidate dispersants improved the silica dispersion and nullified any viscosity increases. Traditional gum based thickeners are unsuitable for non aqueous applications and various polymeric thickeners intended for oils were found to be ineffective. Mineral derived thickeners endowed excellent anti sedimentation properties to paraffin oil provided a polar 'activating' solvent was included, unfortunately suitable solvents such as propylene carbonate were very toxic to the spores. Finally an organic derivative of a smectite mineral was found that provided suitable rheological properties without requiring additional activators.

Even when all the components had been identified the relative proportions had to be optimised. For example a small increase in dispersant content to prevent agglomeration of the spores on dilution may result in an overall change of formulation viscosity. This may then require re-optimisation of the thickener content to prevent sedimentation or excessive gellation on long term storage. All the formulation components interact and a holistic approach must be taken to ensure success.

A final formulation was developed to the required standard containing 500g/l of conidia, suitable for dilution at the point of use (with kerosene) using the simple ratio of one part formulation to three parts diluent. The results of stability testing are included in table 2.4,

testing at IIBC confirmed no reduction in conidial viability on storage (compared to conidia powder in the presence of desiccant).

Table 2.4: The results of storage stability testing.

	Storage duration and temperature				
	Initial	2 weeks @ 4°C	2 weeks @ room temp.	2 weeks @ 37°C	4 weeks @ room temp
Appearance	Uniform with up to 3mm syneresis				
Sedimentation	None				
Dispersion	<30	<20	<20	<20	<25
Suspensibility (1 hour)	0.02	0.02	0.02	0.03	0.01
Wet sieve (75µm)	0.07	0.07	0.06	0.08	0.06

During field trials in Africa the formulation was successfully applied over an area of 800Ha although information on the degree of locust control achieved was not available at this time.

5 Discussion

Since the withdrawal of dieldrin and the adoption of indiscriminately toxic organophosphorous compounds for locust control it has become increasingly clear that alternative control methods are required. The use of biological active ingredients offers great selectivity and the potential to reduce the difficult to quantify environmental costs of locust control operations. However for their introduction to be successful their unique formulation requirements must be met.

Development of the *M. flavoviride* conidia formulation described in this chapter highlighted one of the fundamental problems of working with biological actives, their susceptibility to 'poisoning' by other formulation components. It must be noted that development of a water based conidia formulation would have allowed a wider range of formulation components to be considered but was inherently incompatible with the *M. flavoviride* conidia. An additional problem has been variation in the properties of the active ingredient, both between batches and on scale up of production.

In this instance a formulation was developed suitable for CDA application equipment, with sufficient storage stability for field trial use (including retention of biological viability). A wide range of prospective formulation components had to be screened for compatibility with the active ingredient and novel test methods had to be developed to quantify performance of the formulation.

Acceptance of this formulation for locust control will depend on the results of field trials examining factors such as storage stability in tropical climates (viability especially), ease of use and application, effectiveness in achieving control, perceived environmental benefit, and very importantly cost. Any cost penalty must be weighed against the environmental benefits which are extremely difficult to quantify; possibly legislation could be employed to modify the balance in favour of biological control methods. It must be noted however that the conidia OF formulation described is not a replacement for traditional chemical insecticides, if for no other reason than its delayed effect means it can only be used in preventative or upsurge control strategies.

Experimental work of interest in the future would include investigations into the effects of wetting, spreading and 'sticking' agents on the contact toxicity towards locusts or additives to protect conidia against the deleterious effects of ultra violet light.

CHAPTER 3
MICROCAPSULE FORMULATION DEVELOPMENT

1 Introduction

The purpose of this work was to develop novel formulations of conventional insecticides for locust control operations. The main aims were to improve efficacy while minimising deleterious environmental side effects. Controlled release formulation techniques such as microcencapsulation offered the required properties and to the author's knowledge, had never been developed specifically for locust control. Previous trials had tested the suitability of commercially available formulations for locust control (Holland & Jepson, 1996; Symmons, 1991).

A number of microcapsule formulations were investigated with various active ingredients before a single formulation type was developed for biological testing of efficacy, persistence and toxicity to indicator beneficial insects. The results of biological formulation testing will be described in subsequent chapters. A microencapsulated malathion formulation finally chosen for biological testing was particularly novel, this was because in contrast to all previously known microencapsulated formulations a non-aqueous core was encapsulated using an immiscible non-aqueous continuous phase. A water-based formulation of malathion was also developed with the intention of comparative testing.

A number of other microcapsule formulations were assessed before choosing the malathion formulation. A short investigation was made into microencapsulation of the insect growth regulator diflubenzuron and aqueous-based microcapsule formulations of the insecticide bifenthrin were developed to an advanced stage. Early biological testing of the bifenthrin microcapsule formulations with the desert locust (*Schistocerca gregaria)* gave poor results and field trials of commercial aqueous based formulations indicated fundamental difficulties in spray application under typical field conditions (Holland & Jepson, 1996).

During the process of microcapsule formulation development a very large number of different formulations were prepared, of those that were successful a large proportion was rapidly discarded as being flawed or leading to unprofitable avenues of investigation.

Presented in this chapter are selected formulations that exemplify the deductive approach that was employed, not all were successful. Details of the formulations are tabulated at the end of this chapter allowing the text to concentrate on the development path. For brevity commercial trade names are used for some formulation components and details of these materials are tabulated at the end of this chapter.

2 Introduction To Microencapsulation

A wide variety of formulation methods exist which give controlled release of an active ingredient (Wilkins, 1990; Kydonieus, 1980), but for the purposes of locust control any formulation must be capable of spray application under arduous field conditions and preferably using standard ultra low volume (ULV) equipment (Symmons, 1991). This restricts controlled release formulations to the microcapsule type, described most simply as micron sized reservoirs of active ingredient individually surrounded by a membrane of varying permeability and typically suspended in a liquid.
Microcapsule preparation methods may be segregated (Fanger, 1973; Marrs & Scher, 1990) into those which rely on physiochemical processes for membrane formation such as coacervation, those using mechanical devices to apply a coating and finally those that employ interfacial polymerisation. It was the authors opinion that interfacial polymerisation was superior due to a relatively simple preparation method, good batch reproducibility, easily variable microcapsule wall properties and acceptably high active ingredient contents.

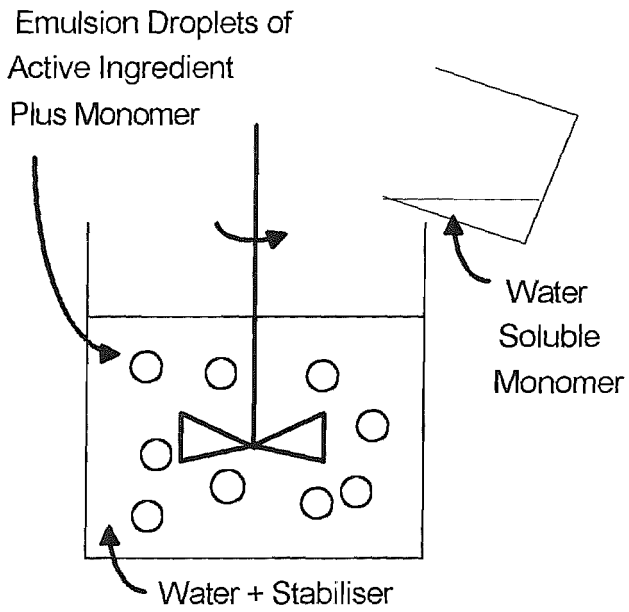
2.1 Encapsulation By Interfacial Polymerisation

Chemical encapsulation methods all follow the same processes (see Fig 3.1) with the main differences being in the type of reaction employed and the physical characteristics of the monomers. The active ingredient is first emulsified in a liquid continuous phase with which it is immiscible, hence the active ingredient must be a liquid or be liquefied by melting or dissolution. Monomers are mixed with the two immiscible emulsion phases and react at the interface forming a polymer insoluble in both phases The polymer membrane surrounds individual emulsion droplets resulting in discrete solid particles which have a liquid core.

For the encapsulation to be successful a surface active agent (surfactant) must be available in the aqueous phase to stabilise the emulsion and prevent agglomeration of the microcapsules when formed. High reaction temperatures may de-stabilise the emulsion and at high concentrations the emulsion may be prone to phase inversion. Microcapsule size depends on the emulsion droplet size before polymerisation with sizes of 1 micron up to several millimetres obtainable depending on the shear applied and the surfactant concentration.

Early microcapsule formulations were developed using an aqueous continuous phase while later advances gave formulations with an aqueous dispersed phase and oil continuous phase; these were thus termed 'reverse-phase' formulations. For the purposes of clarity, formulations with an aqueous continuous phase will be termed 'normal-phase'.

Figure 3.1: A graphical representation of normal-phase chemical microencapsulation



The chemical encapsulation is further subdivided by the type of polymerisation reaction used. The main subdivisions are addition polymerisation, polycondensation and finally in-situ polycondensation, wherein a single monomer effectively reacts with itself.

2.2 Interfacial Addition Polymerisation

This method differs from the remaining two in that a free radical mechanism is employed rather than the nucleophilic addition mechanism used in polycondensation. In practical terms there is little difference from the method outlined above, except that catalysts and high temperatures for long periods are commonly required to complete the encapsulation. A commercial example was the patent of Brynko (1961) for the preparation of 'carbonless copy paper'; dye containing microcapsules were coated on the reverse of paper and could be ruptured by the pressure of a stylus. It is noteworthy that the common impurities in pesticides, may interfere with the free radical generating catalysts used in these preparations (Marrs & Scher, 1990).

2.3 Interfacial Polycondensation

Wallace Carothers whilst working for the DuPont company was the first person to prepare polyamide (Nylon). Polyamide was the first example of the step growth polymers, so called because monomer units were sequentially added onto the ends of polymer chains. One of the more commonly known manifestations of this technology is the "nylon rope trick" (Morgan & Kwoleck, 1996). Two monomers are dissolved in two immiscible liquid phases and carefully combined so that one lies on top of the other. The monomers react at the interface forming a thin polymer membrane which may be drawn out of the liquid to form a continuous thread of polymer. It was a logical extension that if one phase is emulsified in the other then a polymer skin would form at the interface microencapsulating the emulsion droplets.

Examples of the polymer types that may be formed by this method are shown in table 3.1 and examples of the monomer types are shown in table 3.2. Reviews of condensation polymerisation methodology include Morgan (1959) and Wittbecker & Morgan (1996).

Table 3.1: A number of polymer types may be produced by combination of various monomers.

Organic Phase Monomer	Aqueous Phase Monomer	Polymer (linkage)	
Acid Chloride	Amine	Polyamide	(R-CO-NR_2)
Chloroformate	Amine	Polyurethane	(R-O-CO-NH-R)
Isocyanate	Alcohol	Polyurethane	
Sulfonyl Chloride	Amine	Polysulfonamide	(R-SO_2-NR_2)
Isocyanate	Amine	Polyurea	(R_2N-CO-NR_2)
Acid Chloride	Alcohol	Polyester	(R-CO-O-R)
Chloroformate	Alcohol	Polycarbonate	(R-O-CO-O-R)

It must be noted that for a polymerisation reaction to occur the monomers must be at least di-functional. If for example a monomer with a single amine group was used in polyamide preparation, it would react with acid chloride groups on the polymer chain leaving no site for further reaction. If a tri-functional monomer was employed then once it is incorporated in a polymer chain a site would still be available for reaction; this allows cross linking of the polymer leading to a denser and more durable polymer shell. The nature of the polymer wall may be further modified by using a mixture of monomers to produce a copolymer.

Table 3.2: Chemical formulas of typical microencapsulation monomers used in interfacial polycondensation

Monomer Type	Chemical Formula	Example
Acid chloride	R-CO-Cl	Adipoyl chloride
Chloroformate		1,3,5-Benzene trischloroformate
Isocyanate	R-NCO	2,4-Tolylene diisocyanate (TDI) Polymethylene polyphenylisocyanate (PAPI)
Sulfonyl chloride	R-SO_2-Cl	1,3-Benzenesulfonyl dichloride
Alcohol	R-OH	1,5-Pentanediol
Amine	R-NH_2 or R_2-NH	Diethylene triamine (DT) Ethylene diamine (ED)

Interfacial polycondensation was first employed for pesticides by Vandegaer (1971) using a variety of active ingredients and monomer combinations. Both normal and reverse-phase capsule suspensions were prepared but active ingredient levels were low because of the inefficient surfactants employed (such as polyvinyl alcohol and oil soluble soaps). Subsequent developments were mainly in the surfactant systems, resulting in higher concentration emulsions and long-term physical stability of capsule suspensions. Examples used in normal-phase encapsulation include lignin sulphonates (Beestman & Deming, 1981; Beestman & Deming, 1983) sulphonated naphthalene formaldehyde condensates and sulphonated polystyrenes (Becher & Magin, 1984), and finally water soluble alkylated polyvinyl pyrrolidone (Beestman, 1985a). Examples of reverse-phase microcapsule formulations include those of Lim & Moss (1981), Wallace & Tiernan (1966) and finally Beestman (1985b) that employed an oil soluble variant of alkylated polyvinyl pyrrolidone as surfactant. Active ingredient concentrations of greater than 480g per litre were obtainable for both normal and reverse-phase microcapsule suspensions.
When reviewing all microencapsulation techniques Marrs & Scher (1990) concluded that interfacial polycondensation was the most suitable for microencapsulating pesticides.

2.4 In-Situ Interfacial Polycondensation

The in-situ interfacial polycondensation method may be considered a development of the standard interfacial polycondensation. The process employs isocyanate monomers only, which hydrolyse in the aqueous phase to amines and then react with remaining isocyanate to give polyurea microcapsule walls (Scher, 1973, 1977 & 1978). There are a number of disadvantages to this method; the product is generally of low concentration to prevent microcapsule agglomeration, reaction times are long (even at elevated temperatures) and CO_2 gas is evolved by the reaction which has important repercussions for packaging of the product (Beestman & Deming, 1988; Beestman & Deming, 1981).

2.5 Release From Microcapsules

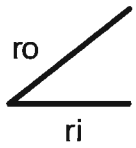
Fundamental to the use of microcapsule formulations is the ability to control release of the active ingredient by modifying the microcapsule wall structure (Bakan, 1976; Tsuji, 1990). For microcapsules containing insecticides the primary release processes would normally be diffusion or rupture but a number of other routes are available (see figure 3.2).

Microcapsule rupture as the main release process has been demonstrated for a polyurethane walled fenitrothion microcapsule formulation (Tsuda et al, 1987; Ohtsubo et al, 1987). The aqueous based microcapsule slurries were applied to surfaces by conventional spraying techniques and allowed to dry. Diffusion release of insecticide from the residues was negligible but trampling by German cockroaches (*Blatella germanica*) resulted in microcapsule rupture and contamination of the insect. Residual efficacy of the deposits was found to be closely related to wall thickness and microcapsule diameter.

Figure 3.2: Processes resulting in release of the active ingredient from microcapsules (after Bakan, 1976).

Process	Route	Cause		
Coating Fracture	External	Pressure	Shear	Abrasion
	Internal	Vapour Pressure	Osmotic Pressure	
Coating Degradation	Thermal	Melting	Decomposition	
	Solubility	Solvents	Micro-organisms	Enzymes
	U.V. Light			
Diffusion				

Figure 3.3: Release rate from a microcapsule, showing internal and external microcapsule radius (Baker & Lonsdale, 1975; Marrs & Scher, 1990).



$$\text{Release Rate} = \frac{\text{Mass}}{\text{Time}} = \frac{4\pi r_o r_i P(\Delta C)}{r_o - r_i}$$

$4\pi r_o - r_i$	:	Surface Area
$r_o - r_i$	:	Wall Thickness
D	:	Diffusion Coefficient
K	:	Distribution Coefficient
$P = D*K$	:	Permeability
ΔC	:	Concentration Gradient

Release by diffusion is described mathematically by a form of Fick's first law (figure 3.3). It is noticeable that the rate of release is zero order, giving constant release rate until close to depletion. Experimentally Ohtsubo et al (1989) found that the rate of diffusion release into water was proportional to the multiple of mass median diameter and wall thickness, which contradicts the theoretical Fick's law.

Table 3.3: Factors affecting diffusion release rate from microcapsules (Fanger, 1973 & Finch, 1985).

	Parameter	For Lower Permeability
Polymer Characteristics	Density	Increase
	Crystallinity	Increase
	Orientation	Increase
	Cross linking	Increase
	Porosity	Decrease
	Plasticiser level	Decrease
	Solubility parameter	Opposite to core
	Fillers	Increase (conditional)
Microcapsule Characteristics	Size	Increase
	Wall Thickness	Increase
	Configuration	Spherical as possible
	Conformity	Regular as possible
	Post treatments	Utilise
	Multiple coatings	Utilise
Environmental Characteristics	Storage temperature	Decrease
	Partial pressure differential	Decrease

A large number of factors may alter the rate of release from a microcapsule (see table 3.3) but most useful from the formulation point of view are wall thickness (varying quantity of monomers), cross linking and polymer type (varying monomer functionality), and microcapsule size (varying shear during preparation).

The measurement of microcapsule wall thickness is impractical, thus the wall thickness of formulations described in this chapter will be represented by a core to wall (c/w) ratio, which is the molar ratio of core phase monomer and active ingredient (including any solvent). It is possible to calculate a theoretical microcapsule wall thickness but the polymer

density must be known (Ohtsubo et al, 1987) or assumed (Koestler, 1980) and fundamentally the equation is still a ratio of the wall and core masses. Using the equation of Ohtsubo et al (1987) assuming a wall density of 1 kg/m^3 with a mass median diameter of 10 micron the microcapsule wall thickness for the 5:1 and 15:1 c/w reverse phase malathion formulations detailed later in this chapter would be 0.6 and 0.2 micron respectively. Ohtsubo et al quoted wall thickness' of between 0.07 and 0.28 micron for microcapsules with mass median diameters of 22 to 88 micron.
Tests examining the numbers of microcapsules ruptured by walking insects have indicated that microcapsule wall strength is proportional to the ratio of mass median diameter to the wall thickness (Ohtsubo et al, 1987 & 1989), the latter calculated as described above.

It is logical that the degree of cross linking can potentially have a great effect on the physical toughness of the microcapsule polymer. In this chapter the percentage polymer cross linking is calculated on the basis that if two di-functional monomers were reacted the cross linking would be zero, while if a di-functional monomer were reacted with tri-functional monomer the cross linking would be 100%.

3 Materials And Methods

A number of microcapsule formulations were developed, all employing interfacial polycondensation. The methods of formulation preparation are broadly described below although it must be noted that process conditions can greatly affect the results obtained. Since a primary factor in formulation choice is the physical properties of the active ingredient brief descriptions of the active ingredients are given to aid understanding of the formulation methods.

Malathion is an organophosphorus insecticide; the technical grade employed for encapsulation (approximately 95% pure) was a clear amber liquid with a melting point of 3°C, relatively low vapour pressure, a specific gravity of 1.2, low solubility in water and a moderate viscosity. Bifenthrin is a synthetic pyrethroid insecticide with a melting point of 70°C, a low water solubility and low vapour pressure. At the melting point of bifenthrin aqueous emulsions would be unstable and monomers prone to side reactions, thus the technical material was dissolved in a solvent before microencapsulation. Diflubenzuron is a urea type insect growth regulator with a melting point of approximately 230°C, a very low vapour pressure and very low solubility in most solvents.

All physical data was obtained from RSC (1994).

3.1 Preparation Method For Reverse-phase Malathion Microcapsules

The formulation of reverse-phase malathion microcapsules developed for this project significantly differed from all previously known reverse-phase (oil-based) formulations because the active ingredient to be encapsulated was not water soluble. Thus an 'oil' insecticide was encapsulated using an immiscible 'oil' as the continuous phase.

Initially the preparations were performed in single skin glass vessels placed in a water bath for coarse temperature control but subsequently a jacketed glass vessel was used which could be heated or cooled by circulating water. For some batches manual temperature measurements were taken but where possible an electronic temperature logger was used.

Temperature variation is an important indicator of exothermic polymerisation reactions. Emulsification and mixing were performed using Silverson type homogenisers.

Batch preparations were performed as follows. The continuous phase (Isopar paraffin oil) was mixed with a surfactant which is critical for emulsion formation and stabilisation of the transition between emulsion droplets and solid microcapsules. For both normal and reverse-phase microcapsule formulations these surfactants are normally polymeric in nature and work through steric interactions. It must be noted that for oil-based formulations the dielectric constant of the oil is typically so low that no charged species could be maintained, making traditional anionic and cationic surfactants completely ineffective (Rosen, 1978). The malathion technical was premixed with the first monomer (typically isocyanate) and then emulsified in the continuous phase. When a satisfactory droplet size was obtained the second monomer was added dropwise to the batch and shear maintained to aid reaction completion.

A Galai Cis-1 laser particle size analyser was available for measuring the particle size distribution of microcapsule formulations but it was discovered that the paraffin oil continuous phase made the analyses unreliable. Thus manual determinations of maximum microcapsule particle size were relied upon for reverse-phase formulations.

3.2 Preparation Method For Normal-phase Malathion Microcapsules

The preparation of malathion normal-phase microcapsules was performed similarly to that described above. Firstly the surfactant (typically Agrimer AL904) along with a silicone antifoam (such as Rhodorsil 426R) was dispersed in the water using a homogeniser. Malathion technical and the first monomer (typically an isocyanate) were premixed and then emulsified in the aqueous continuous phase. When the emulsion droplet size was suitable the second monomer (typically glycerol) was added dropwise. The batch was agitated with a low shear paddle stirrer for approximately four to five hours to ensure complete reaction. If agitation was not maintained for this period a secondary reaction occurred between the isocyanate monomer and water, releasing CO_2 gas and pressurising storage containers.

A large amount of work was performed investigating the physical stability of the normal-phase malathion formulations. This primarily involved incorporating dispersants to prevent microcapsule agglomeration and thickeners to prevent sedimentation. These additives were either incorporated dry or after premixing with water.

3.3 Preparation Method For Normal-phase Bifenthrin Microcapsules

The preparation method was similar to that described above but the bifenthrin technical was first dissolved in an aromatic solvent using ultrasonication. The Airvol 203 surfactant (polyvinyl alcohol) and Rhodorsil antifoam were dispersed in the water using high shear. The bifenthrin technical solution was emulsified in the aqueous continuous phase followed by the isocyanate monomer with which it was miscible. After a suitable period to allow mixing of the core phase and monomer the amine monomer was added dropwise and shear continued for a short period.

3.4 Preparation Method For Normal-phase Diflubenzuron Microcapsules

Technical grade diflubenzuron (Duphar) was found to possess low solubility in all solvents with physical properties suitable for microencapsulation. Thus the milled technical powder was suspended in water or paraffin oil before reverse or normal-phase microencapsulation. The method of preparation was as described above.

4 Experimental Results

Included at the end of this chapter are tables 3.8 to 3.13 which detail formulation compositions, characteristics and descriptions of trade names used throughout this chapter.

4.1 Malathion Reverse-phase Microencapsulation Results

As a preliminary to formulation development it was necessary to determine the miscibility of malathion technical with candidate continuous phases and monomers. Simple visual observations indicated that malathion was immiscible with water, paraffin oils and alkyl acetate solvents but was miscible with aromatic solvents and vegetable oils. Similarly tests with candidate monomers indicated miscibility with isocyanates, no miscibility with alcohol monomers and rapid reaction with amine monomers. This indicated that preparation of reverse-phase malathion microcapsules may be possible but the incompatibility with amine monomers prevented preparation of polyamide, polysulfonamide or polyurea walled microcapsules.

4.1.1 Feasibility

The first formulation prepared was 43-065 containing 25% malathion active ingredient and polyurethane microcapsule walls with a 5:1 core to wall ratio and 100 percent cross linking. Note that in previously published microencapsulation methods it was thought necessary for the monomers to be miscible with their respective continuous phase (Beestman & Demming, 1988). No monomer pair was available with suitable solubility characteristics for reverse malathion formulations, thus the second monomer was emulsified in the paraffin oil continuous phase. The route of monomer transport to the reaction site, that is the interface of the core phase with continuous phase, may be speculated upon. It is possible that the monomer emulsifies extremely well in the continuous phase and small emulsion droplets impact with the core phase droplets, secondly the monomer may have a very low but significant solubility in the continuous phase providing a dynamic transport route to the reaction site or finally the true process may be a combination of the two.

When finished formulation 43-065 was a low viscosity cream coloured liquid and microscopic examination revealed spheroids up to approximately 5 micron in diameter. A thick sediment formed on storage indicated formation of solid microcapsules and no signs of gas evolution were visible. No increase in batch temperature occurred during preparation indicating that the reaction was not highly exothermic. Use of the isocyanate monomer 2,4-Tolylene diisocyanate (TDI) in place of PAPI would have been preferable because previous experience indicated far greater reactivity; this was not feasible however because TDI was found to be miscible with both emulsion phases. Analysis using a Galai Cis-1 laser particle size analyser indicated a volume mean diameter of 4 micron and a maximum particle size of 7.5 microns.

As mentioned previously polymer type is a major factor in microcapsule release rate (Finch, 1985). Thus to increase the number of options available it was attempted to form microcapsules using polyester. Formulation 43-091 was prepared using adipoyl chloride monomer in the malathion phase and butanetriol as the second monomer; again the second monomer was immiscible with the paraffin oil continuous phase.
It was noted by microscopic examination that the emulsion prior to encapsulation was very unstable, the droplets coalescing rapidly when not under shear. The polymer forming reaction between the two monomers failed to occur under these reaction conditions (batch temperature of 24C) and the emulsion broke very rapidly upon removal of shear.

A series of batches were prepared investigating the possibility of increasing microcapsule particle size and use of a high viscosity paraffin oil (Isopar V) as a continuous phase. It was possible that the higher viscosity continuous phase would prevent sedimentation of microcapsules on storage.
Repeating formulation 43-065 using reduced shear during emulsification resulted in an increase in maximum microcapsule particle size to approximately 15 micron in diameter but the larger microcapsules were deformed and non spherical. Reducing the formulation surfactant content caused emulsion instability and only partial encapsulation of the active ingredient.

Use of Isopar V as the formulation continuous phase caused a reduction of microcapsule size to less than 1 micron, reduction of surfactant content again caused emulsion instability. Some batches exhibited an increase in final microcapsule size compared to the emulsion droplets immediately prior to addition of the second monomer; this suggested that microcapsule wall formation was far from instantaneous on addition of the second monomer.

For any microcapsule formulation to be commercially viable in locust control programs the active ingredient content must be maximised. Increased active ingredient content reduces costs during manufacturing and also reduces the volume of liquid which must be transported.

Formulations 43-135, 140, 141 and 142 were prepared containing 45, 55, 55 and 50% w/w malathion respectively. All of the formulations had a 5:1 core to wall ratio employing 100% cross-linked polyurethane. Note that as the active ingredient is increased the monomer content must be increased proportionally to maintain the core to wall ratio. Thus a small increase in active ingredient level can have a large impact on the relative volumes of the two emulsion phases and lead to phase inversion.

Formulations 43-135 and 142 were prepared successfully producing microcapsules of up to 10 microns in diameter Microscopic examination prior to reaction revealed rapidly coalescing emulsion droplets indicating unstable emulsions. Formulations 43-140 and 141 could not be prepared successfully due to phase inversion of the malathion emulsion which was confirmed by visual observation of dilution in the two phases. This was despite an increase in surfactant concentration in formulation 43-141.

It was concluded that the maximum active ingredient content of reverse-phase malathion microcapsules with polyurethane walls was approximately 50% w/w. Later formulations were reduced to 45% w/w because of extreme dilatancy developing on storage caused by the high concentration of suspended solids.

4.1.2 Preparation of formulations for biological testing.

The feasibility testing described above indicated that polyurethane walled malathion microcapsules could be prepared with acceptable physical properties and active ingredient contents. To optimise the release characteristics of reverse-phase malathion microcapsules two parameters were open to manipulation, the microcapsule wall thickness as indicated by the core to wall ratio and also the degree of polymer cross linking. The range of factors that have an effect on microcapsule release rate are shown in table 3.3 A number of varied formulations were prepared for subsequent biological testing as shown in table 3.4. Full formulations details are displayed in table 3.8.

Table 3.4: Formulations prepared for biological testing.

Formulation	Core to wall ratio	Cross linking
43-161& 211	5:1	100
60-109	5:1	100
43-177	5:1	75
43-237	7:1	100
43-250	10:1	100
43-253	15:1	100
60-052	5:1	100

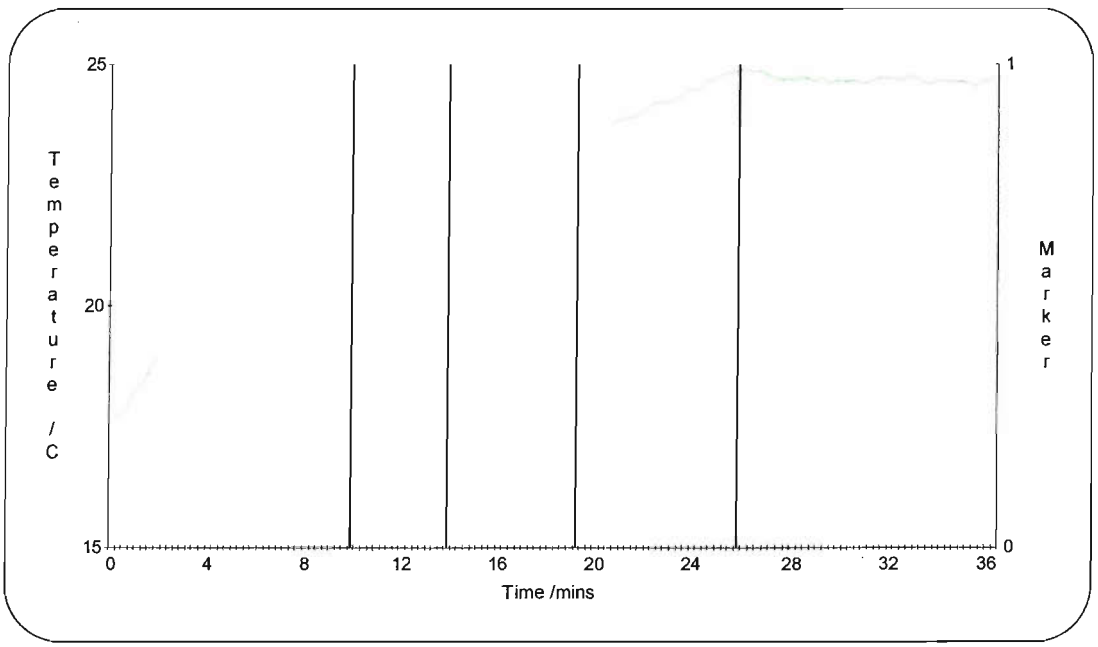
The reduction in cross linking of formulation 43-177 was achieved by replacing a proportion of the tri-functional PAPI 2027 isocyanate monomer with the di-functional Isonate M143 monomer and retaining the di-functional alcohol monomer. Formulation 60-052 was prepared containing the phase transfer catalyst dibutyltin dilaurate because it was suspected that increasing the reaction rate would alter the polymer density and consequently the microcapsule diffusion release rate.

All of the formulations were prepared successfully producing opaque cream coloured liquids of varying viscosities. Since non of the formulations contained viscosity modifiers the microcapsules sedimented rapidly on storage and the sediments had to be resuspended by stirring prior to testing. Microscopic examination revealed well formed microcapsules with

with maximum particle diameters of between 5 and 25 micron. When microscopically examined it was found that increase of core to wall ratios to 7:1, or greater, made the microcapsules prone to rupture when moderate pressure was applied to the microscope side, suggesting a weakening of the microcapsule wall.

In the case of formulation 60-109 the batch temperature was electronically recorded during preparation and is included in figure 3.4. Key stages in the preparation are marked by vertical lines, the first pair of markers indicating the period of technical/isocyanate emulsification while the second pair mark addition of the diol monomer. Note the steady temperature rise due to heat from the homogenisation process and slight changes due to incorporation of components at different temperatures and also note that there was no temperature increase on addition of the second monomer. This indicates that there was no rapid endothermic polymerisation reaction which can typically be witnessed with this type of microencapsulation.

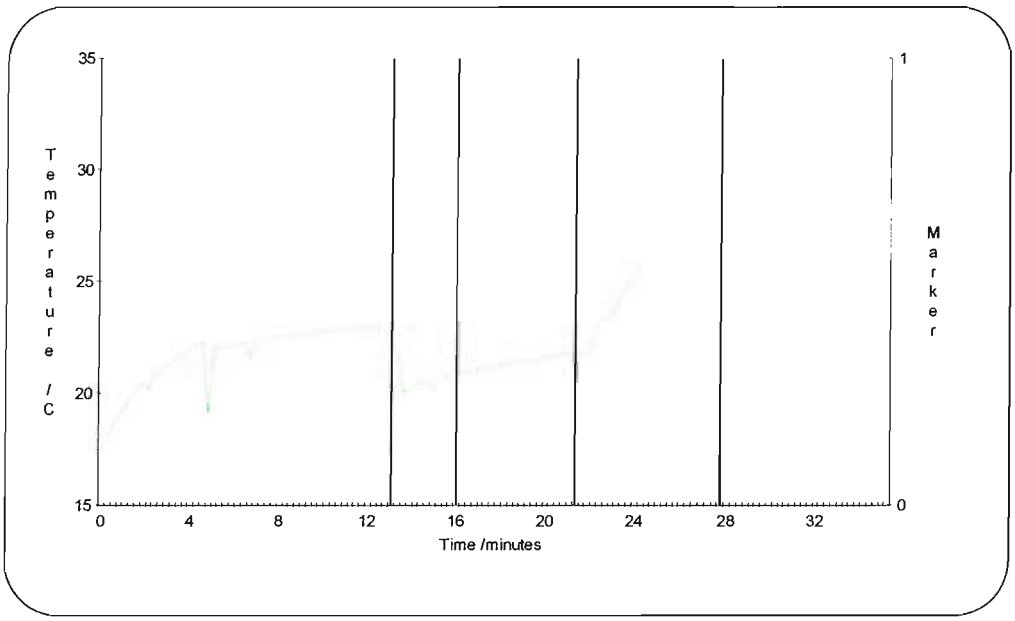
Fig 3.4: Temperature profile logged during the preparation of batch 60-109.



In the case of batch 60-052 the catalyst was premixed with the core phase while for 60-054 the catalyst was added to the formulation after emulsification of the core (technical and

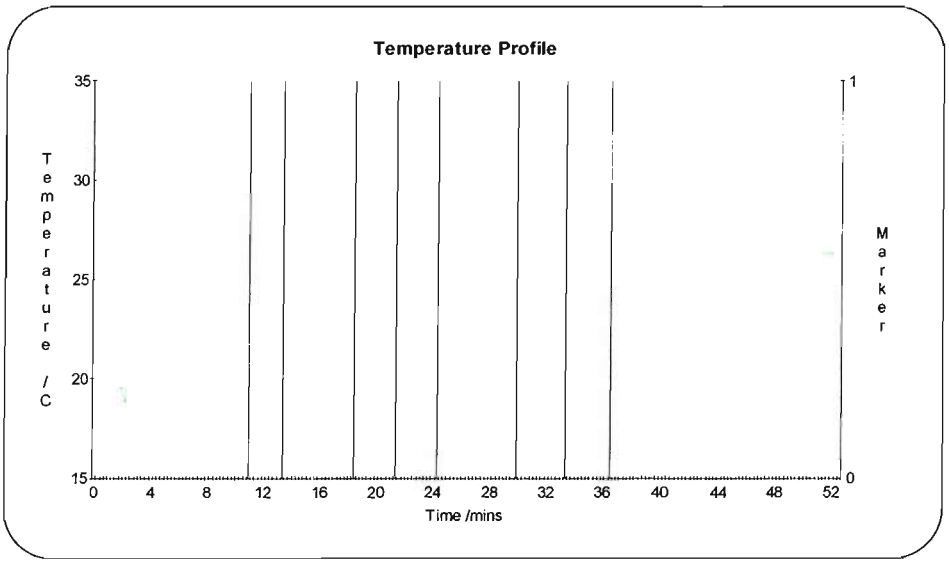
isocyanate premix) and addition of the second monomer. During the preparation of formulations 60-052 and 054 the batch temperature was also electronically recorded, the results are included in figures 3.5 and 3.6 respectively and indicate the effectiveness of the catalyst.

Fig 3.5: Temperature profile logged during the preparation of batch 60-052.



The first two lines in figure 3.5 mark the period of the technical/isocyanate monomer/catalyst premix addition while the remaining pair indicate addition of the second (diol) monomer. In contrast to formulations without catalyst an immediate temperature rise occurs on addition of the diol monomer.

Fig 3.6: Temperature profile logged during the preparation of batch 60-054.



The first four markers in table 3.6 were as per batch 60-052 but the fifth marker indicates addition of the catalyst as expressed in the formulation while the remainder signify addition of excess catalyst yielding successively less temperature rise.

It is clear from both batches that the catalyst does cause a rapid exothermic reaction, whether this was the same reaction as found in previous batches with greater rapidity or whether an alternative reaction was promoted is unknown.

4.1.3 Formulation Stability

The formulations described above were stored at room temperature and examined periodically. It was discovered that some formulations on extended storage developed a separate clear layer of liquid at their base. A large number of batches were examined for this effect.

25% ai, 10:1 c/w, approximately 30 weeks storage

The sample had formed a sediment, upper layer of opaque liquid and a layer of transparent yellow liquid below the sediment. Part of the lower liquid layer was removed and proved to be immiscible with Isopar M. Consideration of the formulation constituents forces the conclusion that the yellow liquid was unencapsulated malathion.

25% ai, 15:1 c/w, approximately 30 weeks storage

Sample as above but the layer of malathion was more substantial.

45% ai, 5:1 c/w plus catalyst, approximately 16 weeks storage

The sample had formed a deep sediment with thin upper layer of cloudy liquid and lower layer of malathion.

45% ai, 5:1 c/w plus catalyst and U.V. stabiliser, approximately 10 weeks storage.

A sediment had formed with an upper layer of cloudy liquid and no free malathion visible.

50% ai, 5:1 c/w, 75% cross-linked, approximately 46 weeks storage.

The sample was mainly a hard packed sediment with a small amount of upper cloudy liquid, no visible unencapsulated malathion.

25% ai, 5:1 c/w, 75% cross-linked, approximately 46 weeks storage.

Approximately equal quantities of sediment and upper cloudy liquid, no visible unencapsulated malathion.

45% ai, 5:1 c/w, 100% cross-linked, approximately 58 weeks storage.

Sample had sedimented with no visible unencapsulated malathion.

It was not considered feasible that a proportion of the malathion was not encapsulated during preparation and then remained as an emulsion for extended periods. Thus it was concluded that use of relatively thin microcapsule walls (large core to wall ratio's) or catalysts caused release of malathion technical into the continuous phase on storage.

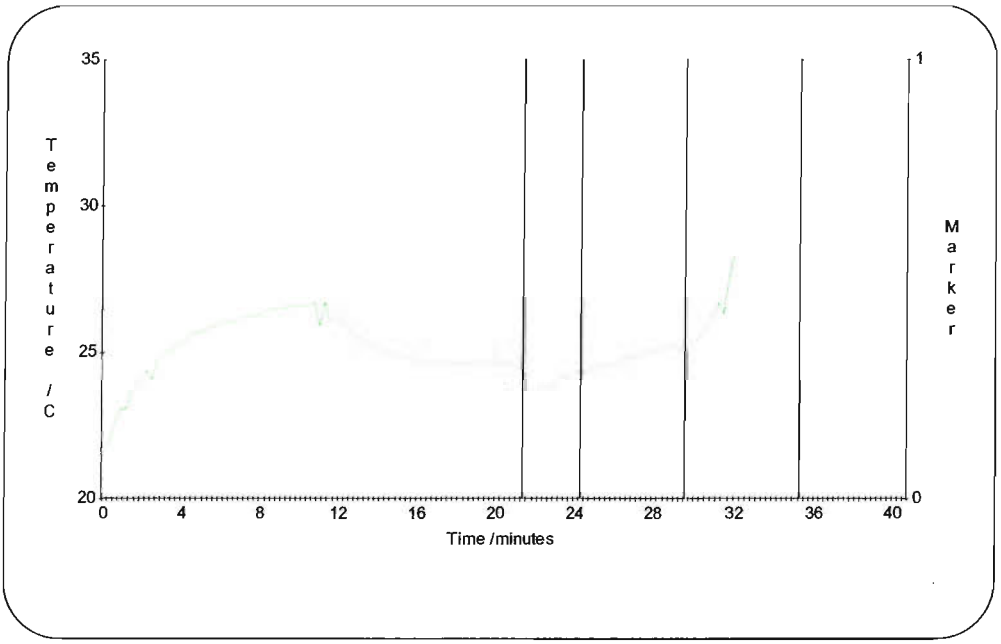
4.1.4 Incorporation Of UV Stabilisers

Ultra violet (U.V.) light is a common cause of pesticide degradation (RSC, 1994). Under typical field conditions for desert locust control operations it is feasible that degradation due to U.V. light would be an important factor. Thus the feasibility of incorporating U.V protectants within the formulations was investigated.
It was possible to disperse the U.V. protectants in the Isopar continuous after encapsulation but to maximise efficacy the additives were incorporated in the microcapsule core. A number of commercial additives were screened for physical properties, including Rhodialux A and Rhodialux D which were found to have a high solubility in malathion and low solubility in Isopar M.

Initially formulation 60-030 was prepared, very similar to previous formulations except for the addition of Rhodialux A dissolved in the core phase. The final product was found to contain microcapsules up to approximately 125 micron in diameter, from which it was surmised that the Rhodialux interfered with emulsion stability. A formulation was prepared with double the quantity of Antaron surfactant and although immediately after preparation the maximum particle size was 20 micron after one week the particle size had increased to 50 microns. Possibly the microencapsulation reaction rate had been reduced by the Rhodialux component, allowing the emulsion droplet size to increase before full reaction. Electronic logging of the formulation preparations had not been possible but manual observations indicated only a one degree temperature rise on addition of the second monomer.

Formulation 60-074 was prepared containing additional catalyst to speed the encapsulation reaction. In contrast to the previous formulations addition of the diol monomer was accompanied by a visible increase in batch viscosity. The batch temperature profile was recorded (figure 3.7) and shows a large temperature rise on addition of the diol monomer (between the second pair of markers). Microscopic examination revealed a maximum microcapsule diameter of approximately 7 micron, both initially and after one week.

Fig 3.7: Temperature profile logged during the preparation of batch 60-074.



It was concluded that the preparation of reverse-phase malathion microcapsules containing UV protectant was feasible but may have unpredictable effects on the microencapsulation process.

4.1.5 Scanning Electron Microscopy

Samples of malathion microcapsules were imaged with a scanning electron microscope (SEM) to give a greater understanding of microcapsule wall structure. A standard light microscope does not offer the depth of field or resolution necessary to observe surface details on microcapsules of the size prepared (Schramm, 1996).

Koestler (1980) microencapsulated a number of liquid active ingredients (including malathion) using interfacial polycondensation and then obtained images by SEM; the surface morphology was found to differ markedly between formulations and was regarded to be controlled by the emulsion interface characteristics during encapsulation. A highly irregular microcapsule surface is more likely to have imperfections than a smooth spherical one, with a corresponding effect on the critically important microcapsule release rate.

Samples of malathion microcapsules were imaged using a Jeol JSM-P15 table top SEM. In all cases the liquid samples were applied to Ciba Geigy / Spraying Systems oil sensitive paper and then allowed to dry before sputter coating with a conductive surface and imaging. The images are included in figure 3.8 along with details of magnification and scale bar length.

Image 1 shows a sample of formulation 43-211 applied to the substrate manually using a dropping pipette without prior dilution. Formulation 43-211 contained 50% w/w malathion active ingredient contained within a 5:1 c/w, 100% cross-linked polyurethane microcapsule wall. The image was taken at a magnification of 2000 times giving an equivalent scale bar length of 5 micron, note that the particle size varies from less than 1 micron in size to greater than 10 micron.

Images two to six show the same sample after dilution in Isopar M and application to the substrate using a spinning disk sprayer (Micron Ulva). Note that when compared to the previous image the application process has caused no widespread damage to the microcapsules. The images reveal the wide range of particle sizes and image six shows a comparatively rare deflated or ruptured microcapsule. It must be noted that the damage to the microcapsule may have been caused by the imaging process, exposure of the sample to a concentrated electron beam can lead to localised heating and loss of volatile components. Image seven shows the same sample but the surface had been purposefully distressed using a metal implement, in an attempt to rupture the microcapsules and reveal any internal structure. Little damage is visible indicating that the microcapsules are relatively robust.

Images eight and nine were taken from a sample of a 5:1 core to wall, 75% cross-linked formulation malathion microcapsule formulation broadly equivalent to 43-177, the sample having been diluted before application using a dropping pipette. Note the difference in the surface texture of the microcapsule wall, probably caused by the variation in monomers used to lower the amount of polymer cross linking.

From the SEM images it was concluded that reverse-phase malathion microcapsules had been successfully prepared and they were sufficiently robust to survive application with a typical ultra low volume / controlled droplet application device.

Fig 3.8: Images of malathion microcapsules obtained using a scanning electron microscope.

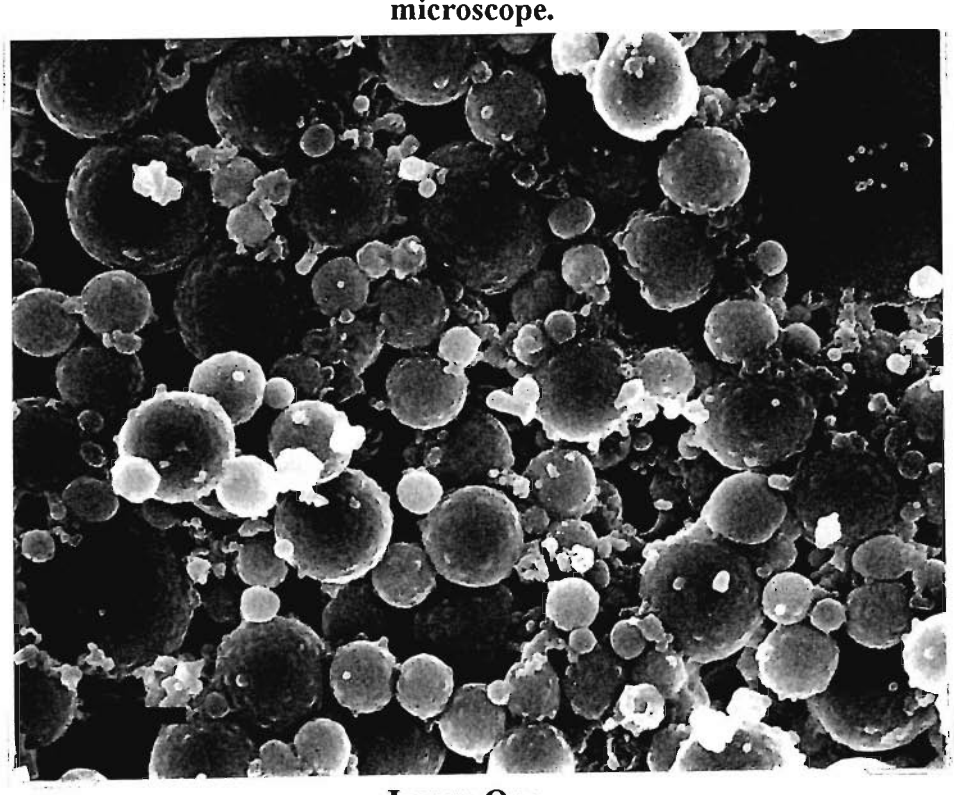


Image One

Scale bar equivalent to 5 μm. 2,000x magnification.

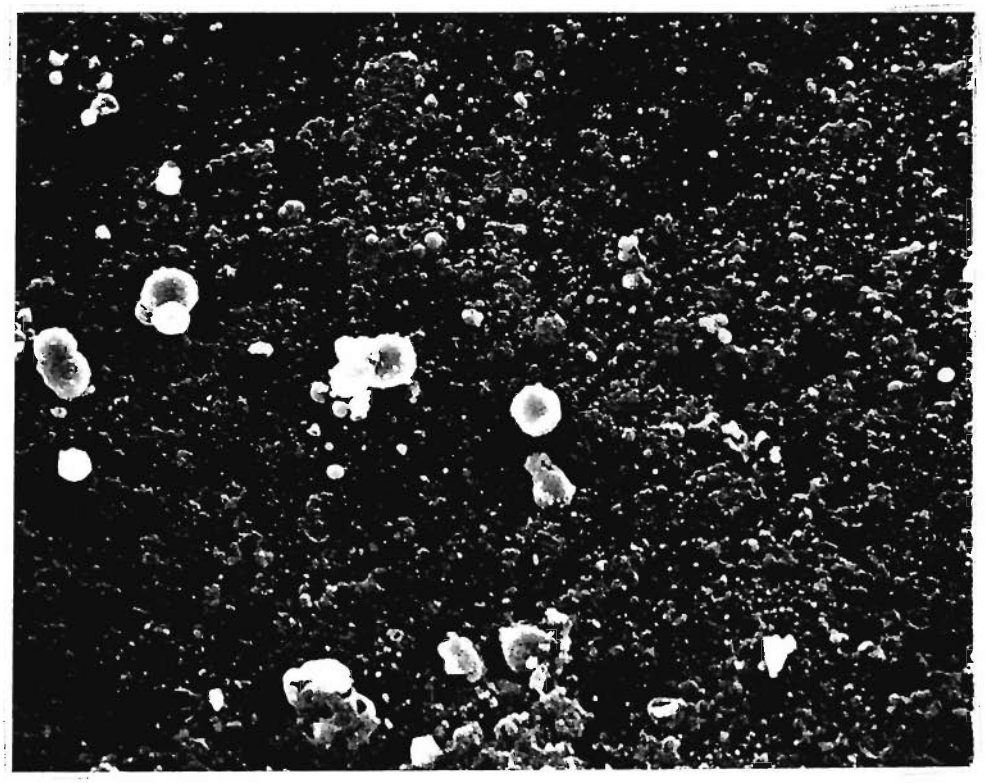


Image Two

Scale bar equivalent to 10 μm. 1,000x magnification.

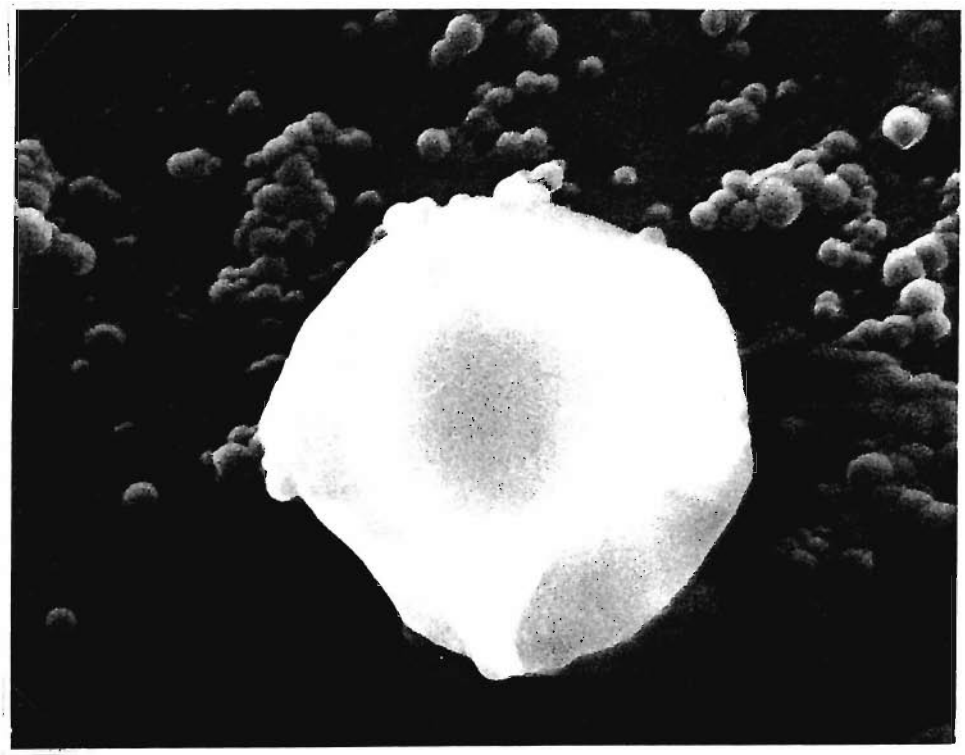


Image Three

Scale bar equivalent to 1 μm. 10,000x magnification.

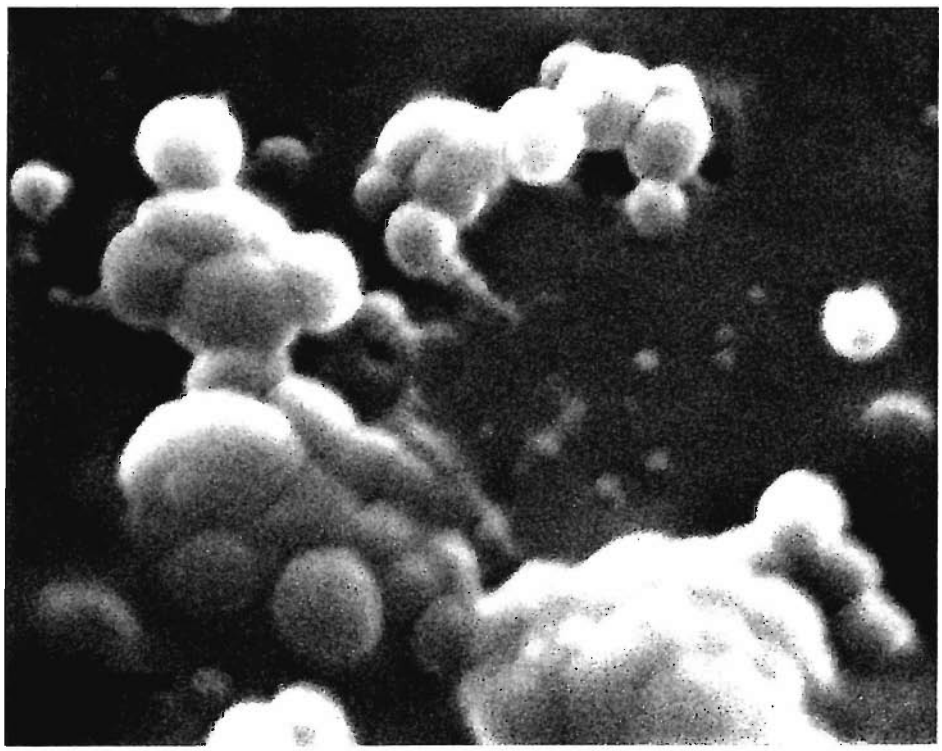


Image Four

Scale bar equivalent to 0.3 μm. 30,000x magnification.

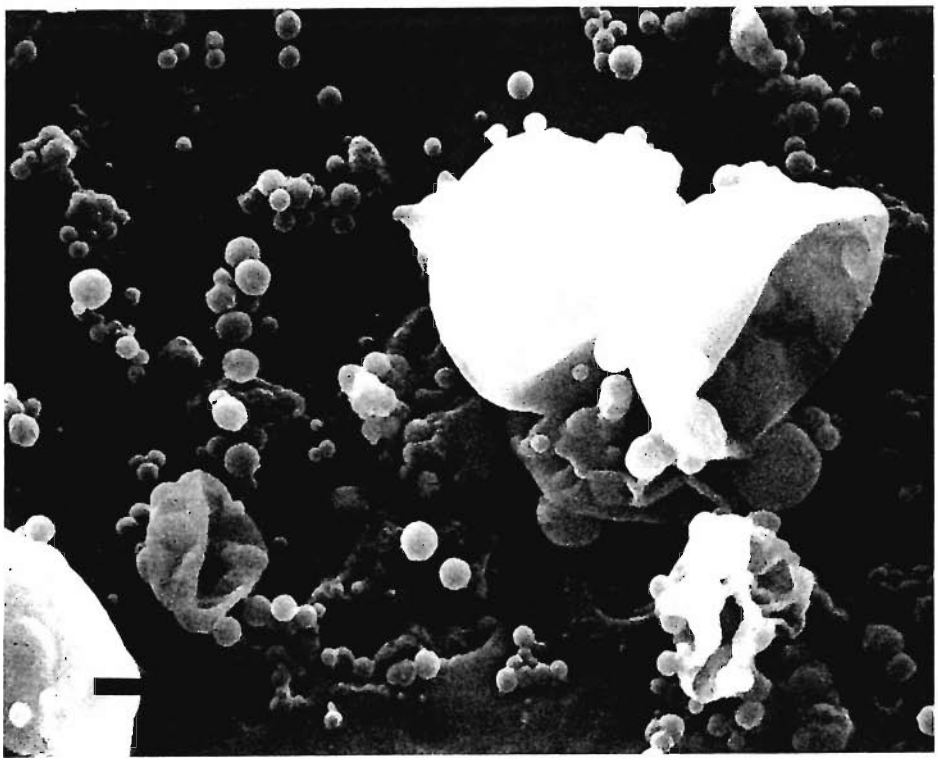


Image Five

Scale bar equivalent to 1.25 μm. 8,000x magnification.

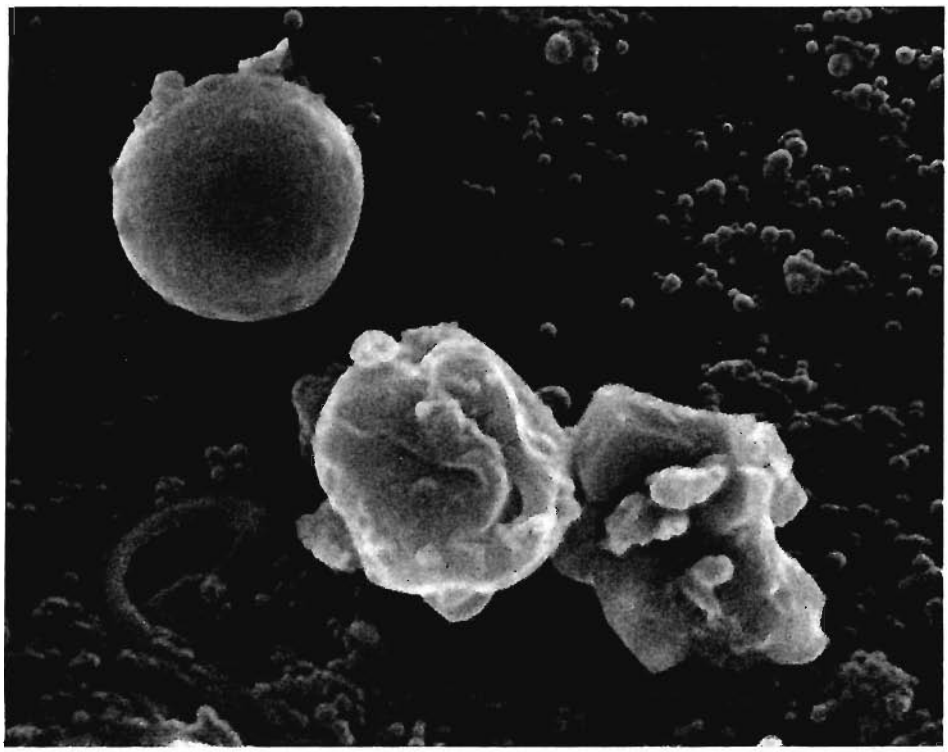


Image Six

Scale bar equivalent to 2 μm. 5,000x magnification.

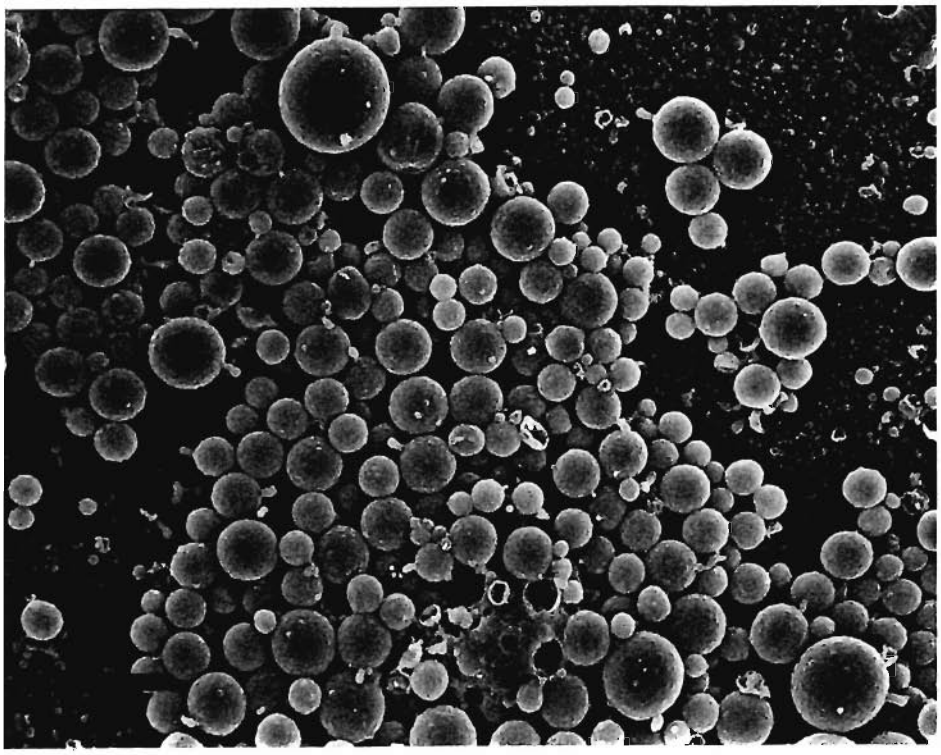


Image Seven

Scale bar equivalent to 10 µm. 1,000x magnification.

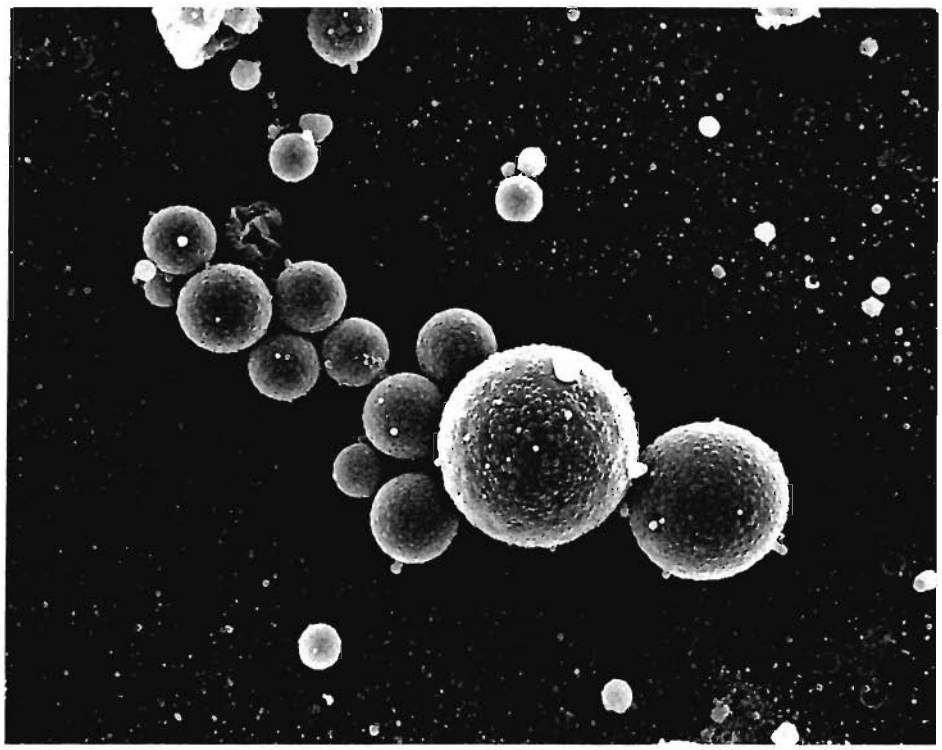


Image Eight

Scale bar equivalent to 10 µm. 1,000x magnification.

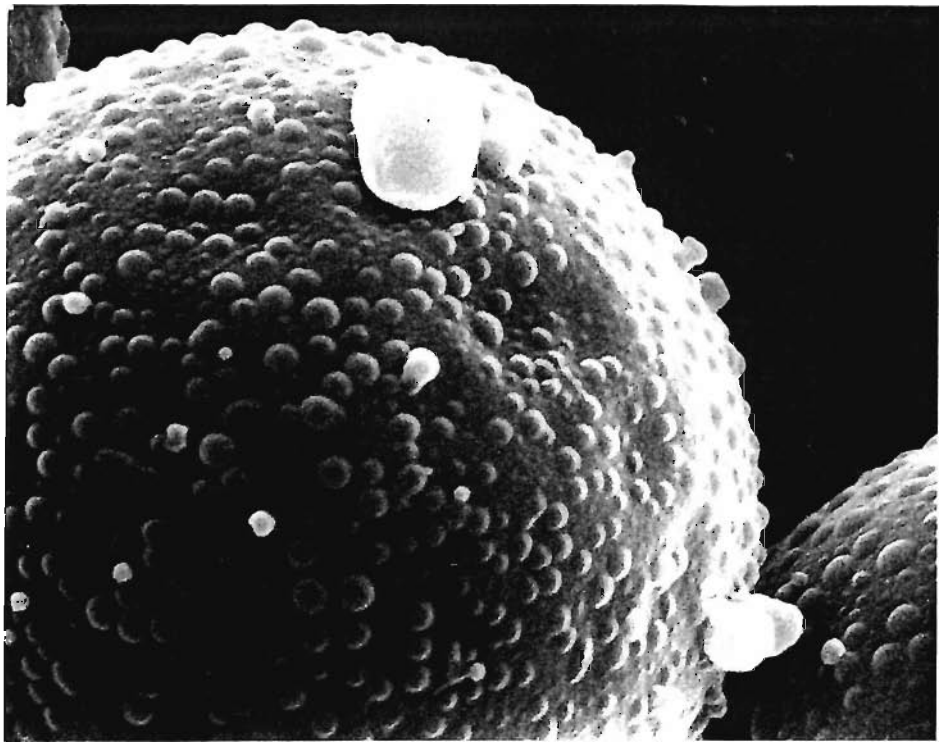


Image Nine

Scale bar equivalent to 2 μm. 5,000x magnification.

4.2 Malathion Normal-phase Microencapsulation Results

Aqueous based malathion microcapsules were developed with the intention of comparative biological testing against reverse-phase microcapsules. A large number of formulations were prepared and tested, of which a selection are included below.

4.2.1 Initial Feasibility Testing

A number of trial formulations were prepared to test the feasibility normal-phase malathion microcapsules. As previously described malathion was incompatible with amine monomers so candidate polycondensation polymers were restricted to polyurethane and polyester.

Firstly formulation 43-063 was prepared using isocyanate and diol monomers to give polyurethane walled microcapsules. A low viscosity opaque white liquid product was obtained and microscopic examination revealed well formed microcapsules up to 10 micron in size. Particle size analysis using a Galai Cis-1 laser analyser confirmed a volume mean diameter of 4.6 microns, median diameter of 4.4 microns and a maximum diameter of 10 microns. On storage the sample containers were found to pressurise due to a reaction between residue isocyanate monomer and the water continuous phase. This was despite continuing agitation for four hours after formulation preparation in an attempt to ensure complete reaction before storage.

Formulation 43-060 employed acid chloride and diol monomers to form polyester walled microcapsules. A suitable tri-functional monomer was unavailable so the microcapsules were prepared with no polymer cross linking with a probable corresponding reduction in physical robustness of the microcapsule. Again the product was a low viscosity white liquid but microscopic examination revealed microcapsules with grossly thickened and mishapened walls. The evidence suggested a reaction between the adipoyl chloride monomer and the alcohol groups on the polyvinyl alcohol (Airvol 203) surfactant, inclusion of the large polyvinyl alcohol molecules in the microcapsule polymer leading to thickened and presumably diffuse microcapsule walls. Formulation 43-061 was prepared in an identical manner except for the use of an alkylated polyvinylpyrrolidone surfactant (Agrimer AL904).

Microscopic examination revealed well formed microcapsules up to 10 micron in diameter with no visible wall thickening.

It was concluded that preparation of normal-phase malathion microcapsules was feasible and worthy of further investigation. Of particular concern was the lack of cross linking in the polyester walled microcapsules and gas evolution on storage of polyurethane walled microcapsule formulations.

4.2.2 Surfactant Selection

As described at the beginning of this chapter the main developments in chemical encapsulation methods have been related to surfactants. These fulfil the dual purpose of maintaining a stable emulsion prior to polymerisation and preventing interaction of the microcapsules as they form. A number of surfactants were screened for suitability and a selection is described below.

Formulation 43-069 was prepared using vinyl pyrrolidone/vinyl acetate copolymer (Agrimer VAS-64), vinyl pyrrolidone/styrene copolymer (Agrimer ST) or methyl vinyl ether/maleic anhydride (Agrimer AN-1200). Two of these surfactants were chemically related to alkylated polyvinyl pyrrolidone (Agrimer AL904) referred to in the literature (Beestman, 1985a).

The formulation prepared using Agrimer ST rapidly separated into two phases upon the removal of shear, the single change of surfactant compared to formulation 43-061 preventing formation of a microcapsule wall. Use of Agrimer AN-1200 resulted in formation of some polymeric solids but the protomicrocapsules were badly malformed and severely agglomerated. The same formulation using Agrimer VAS-64 resulted in a uniform low viscosity liquid product containing well formed microcapsules with a maximum particle diameter of 22 microns, volume mean diameter of 7.4 microns and a median diameter of 6.7 microns (Galai Cis-1).

Along with identifying a new surfactant for use in normal-phase microencapsulation these formulations indicate the great importance surfactant composition in the success or failure of microcapsule formation.

4.2.3 Alternative Monomers

Although successfully prepared, the previous formulations suffered from a lack of cross linking in the case of polyester walled microcapsules and post preparation outgassing for polyurethane microcapsules. The question had to be addressed as to whether alternative monomers could make an improvement.

Formulation 43-073 employed the di-functional 2,4-Tolylene diisocyanate (TDI) reacting with tri-functional glycerol giving fully cross-linked polyurethane walled microcapsules. Previous experience indicated that TDI was a far more reactive monomer than PAPI although it does present far more of a health risk, both during processing and from residues within the formulation. The product of formulation 43-073 was a low viscosity white liquid containing well formed microcapsules with a volume mean diameter of 3.8 microns, median diameter of 3.7 microns and maximum diameter of 8.5 microns. The formulation did evolve gas on storage and subsequent experiments indicated that a minimum of three hours low shear stirring was required after preparation to ensure full reaction of the isocyanate.

The monomers 1,5-pentanediol and glycerol were used in formulation 43-074 to give fully cross-linked polyester walled microcapsules. Again a low viscosity white liquid was produced containing well formed microcapsules with a volume mean diameter of 4.9 microns, median diameter of 4.5 microns and up to a maximum diameter of 12.5 microns.

The microcapsules in both formulations sedimented rapidly on storage but could easily be dispersed with light shaking. Microscopic examination indicated no agglomeration of the microcapsules had occurred in either case.

4.2.4 Improvement Of Physical Stability

All of the microcapsule formulations previously described suffered from storage instability common to all simple particulate suspensions, that is sedimentation and agglomeration. If the particle density differs from that of the suspending medium then the particles will sediment (negative sedimentation is often termed flotation or creaming). To prevent sedimentation the liquid density may be increased by adding soluble solids or the liquid viscosity may be increased to hinder particle movement. Agglomeration of suspended particles also tends to promote sedimentation; stabilisation is achieved by adsorption of dispersants to the particle surface such as protective colloids, steric stabilisers or ionizable groups.

The processes involved in the stability/instability of particulate suspensions have been extensively reviewed (Schramm, 1996; Everett, 1988; Tadros, 1993). In this instance an instrument (Malvern Zetasizer 2c) was employed to identify the effects of anionic dispersants on the microcapsule zetapotential value, which is the potential at a point close to the particles surface and may be considered indicative of the particles actual surface charge and polarity. Screening of dispersants using zetapotential measurements is far more efficient and rapid than manual screening (Misslebrook, 1991). Candidate anionic dispersants were identified for both polyester and polyurethane microcapsules but stability testing indicated that steric stabilisation from the Agrimer AL904 reaction stabiliser was sufficient without the use of anionic dispersants.

Additionally a Carrimed CSL rheometer was used to aid screening of viscosity modifiers to prevent microcapsule sedimentation. A wide range of viscosity modifying agents were tested for their ability to prevent sedimentation on storage without adversely affecting handling of the formulation (pumping, pouring, rinsing, etc.).

A commercial pesticide formulation must exhibit chemical and physical stability for up to two years over a wide range of temperature. For the purposes of biological testing the aim was to ensure physical stability for up to two weeks at room temperature and 54°C. A polyurethane walled microcapsule formulation was developed to this specification resulting

in formulation 43-119. Note that the viscosity modifiers were prepared as a premix in water to ensure full hydration of the gum molecules.

4.3 Bifenthrin Normal-phase Microencapsulation Results

Synthetic pyrethroids as a class of insecticide exhibit high toxicity to Desert Locusts (Ford & Reay, 1972; MacCuaig, 1980; Steedman, 1990) but are difficult to formulate for locust control applications (MacCuaig, 1980). The primary aim of this work was to discover if microencapsulation could extend the persistence of microencapsulated synthetic pyrethroids sufficiently for use in locust control operations.

As will be seen later in this chapter encapsulation of solids is feasible but the effect on release of active ingredient was uncertain, thus it was decided to encapsulate bifenthrin when in a liquid form. At the melting point of bifenthrin (70C) emulsions would be destabilised and polymerisation reactions in water unreliable so the active ingredient had to be dissolved in a solvent. The solvent Solvesso 200 was found to dissolve bifenthrin well and was compatible with microencapsulation. After a lengthy development period three formulations (43-018, 020 and 021) were prepared with varying microcapsule wall thickness' (5:1, 7:1 and 10:1 core to wall ratios respectively). The product of all three formulations was a moderate viscosity white liquid containing well formed microcapsules with particle size distributions shown in table 3.5 and active ingredient contents of 10% w/w as confirmed by HPLC assay

Table 3.5 Particle size analyses of normal-phase bifenthrin microcapsules.

	Particle Size /μm		
	43-020	43-021	43-022
Mean (nv)	3.4	3.1	3.1
Mean (vm)	5.2	4.8	4.9
Median	5.3	4.8	4.9
95/97% <	8.5	7.0	8.0
100% <	10.5	8.5	12.0

Formulations 43-020, 021 & 022 were tested by K. Gadgoud for efficacy with II instar Desert Locusts (*Schistocerca gregaria*) using both topical and ULV application, the results of which are included in Table 3.6. When topically applied to barley leaves and fed to locust hoppers at levels of between 0.5 and 1.25 ug ai per hopper a commercial suspension concentrate was found to produce higher mortality than all of the microcapsule formulations.

Table 3.6 Normal-phase bifenthrin microcapsules: mortality of II instar desert locust nymphs when exposed to diluted formulation topically applied to vegetation. (Performed by K. Gadgoud).

Formulations (core to wall Ratio)	Percentage Mortality At Dose (ug / hopper)			
	0.50	0.75	1.00	1.25
43-021 (10:1 c/w)	0	10	20	20
43-020 (7:1 c/w)	0	20	30	40
43-022 (5:1 c/w)	20	60	70	80
Suspension Concentrate	60	100	100	100
Blank Control	0	0	0	0

Table 3.7 Normal-phase bifenthrin microcapsules: mortality of II instar desert locust nymphs when exposed to diluted formulation applied to vegetation using ULV equipment at a rate of 0.6 mg ai per m^2. Data corrected for control mortality using Abbot's formula. (Performed by K. Gadgoud).

Formulations (core to wall Ratio)	Percentage Mortality (72 Hours After Exposure) At Each Age Of Spray Deposits.			
	Initial	5 Days Old	10 Days Old	15 Days Old
43-021 (10:1 c/w)	31.2	16.1	0.9	5.9
43-020 (7:1 c/w)	44.7	25.4	12.3	12.6
43-022 (5:1 c/w)	59.7	9.3	8.8	6.3
Suspension Concentrate	78.2	22.0	8.7	2.9

Similarly testing of the formulations after application to barley seedlings using a ULV sprayer revealed significant reduction in the toxicity of the microcapsules compared to a commercially available bifenthrin suspension concentrate formulation. A comparison of the quantities of microcapsules in suspension before and after spraying revealed a significant reduction in the numbers of microcapsules; the microcapsules were being 'filtered' from the

suspension and giving artificially low application rates. This effect was also probably responsible for the poor results obtained in the dosing trials.

Following these poor efficacy trial results and in consideration of the difficulties associated with application of aqueous based microcapsules in the field (Holland & Jepson, 1996) it was decided to change to use of oil-based reverse-phase microcapsule formulations. Bifenthrin was not suitable for reverse-phase encapsulation due to a significant solubility in all of the commonly available organic liquids (including alkanes, aromatic and oxygenated fluids) which would have been suitable for ULV application; the possibility of using silicone fluids was considered but the formulation cost would have been prohibitive for locust control.

4.4 Diflubenzuron Normal-phase Microencapsulation Results

Diflubenzuron belongs to the benzoylphenyl urea group of insect growth regulators. It exhibits a high toxicity to locusts, an extended persistence of up to six weeks under Sahelian conditions (Sissoko, 1991) and a low mammalian toxicity (company technical data). The following question was posed, was it possible to microencapsulate diflubenzuron and if so would the persistence be even further extended?
The microencapsulation of diflubenzuron using silicate and wax microcapsule had previously been reported (Sjogren & Thies, 1975) although the preparation method was not stated. The microcapsules that had been prepared possessed particle sizes far too large for use in spray application making them unsuitable for locust control operations.
Diflubenzuron presents a challenge to microencapsulate by chemical means due to its very high melting point and very low solubility in most solvents (including water). The only approach available was to encapsulate a suspension of solid diflubenzuron in oil or water. While a reference to the microencapsulation of suspensions was located (Bakan, 1976) details of the preparation method had been omitted; thus encapsulation diflubenzuron by chemical means was considered sufficiently novel to attempt.

The technique employed is demonstrated with formulations 43-030 and 43-053 which were normal (water based) and reverse phase (oil-based) capsule suspensions respectively.

The diflubenzuron technical in formulation 43-030 was suspended in the aromatic solvent Solvesso 200 prior to encapsulation. The preparation was completed successfully to give a uniform low viscosity white liquid in which microscopic examination revealed well formed microcapsules up to approximately 7 micron in diameter.

Before microencapsulating, the diflubenzuron technical in batch 43-053 was dispersed in water using a dispersant (Aerosol DP0545) to stabilise the suspension. The preparation was completed successfully but microscopic examination indicated that some of the crystalline active ingredient had transferred to the oil phase of the formulation. This may have been due to the suspension of the hydrophobic technical in water not being sufficiently stable or possibly destabilisation of the dispersant by the high pH of the amine monomer.

It was concluded that the technique offered promise but was not worthy of investigation in this project. The primary disadvantages were the low active ingredient contents of the formulations and concerns over the method and rate of active ingredient release from the microcapsules.

5 Discussion

No evidence has been found that controlled release insecticide formulations have been specifically developed for locust control. Previous trials with microcapsule formulations had been aimed at testing 'off the shelf' formulations in the hope that the complex release characteristics of formulations developed for Western agriculture would impart some benefit to locust control (Holland & Jepson, 1996). This is despite the ULV application method used in locust control differing fundamentally from the high volume application techniques almost exclusively used in Western Agriculture (Matthews, 1992). Unsurprisingly on the basis of these trials the Food and Agriculture Organisation of the United Nations has recommended against the use of controlled release formulations in locust control operations (Symmons, 1991)

During the microcapsule development phase of this project four formulation types were developed to different degrees. The formulations were diflubenzuron containing microcapsules, normal phase bifenthrin microcapsules, normal phase malathion microcapsules and most importantly reverse phase malathion microcapsules.

The feasibility of normal and reverse phase encapsulation of diflubenzuron was only very briefly investigated. In contrast to almost all other microcapsule formulations the physical properties of diflubenzuron forced the encapsulation of slurries of solid active ingredient. Although partially successful the active ingredient levels of the formulations would always be comparatively low, the release rate characteristics would be unpredictable, any reduction in ecological toxicity would be of questionable importance and finally any extension in persistence would be of limited virtue.

Chronologically normal phase microcapsules containing bifenthrin were the first to be developed, and developed to give a good degree of physical storage stability. A number of formulations with differing microcapsule wall thickness' were tested for toxicity to desert locust nymphs but all gave poor results in comparison to a conventional suspension concentrate formulations.

When topically applied to vegetation using a mechanical pipette, the commercial suspension concentrate formulation gave greater locust mortality than all the microcapsule formulations. It was theorised that microcapsules agglomerated on dilution and were selectively removed by the pipette. It is interesting to note however that the microcapsule formulation efficacy increased steadily with increasing wall thickness. Variations between formulations other than wall thickness were minor, suggesting that the microcapsule wall thickness did alter formulation toxicity.

Further tests with desert locust nymphs used a spinning disk sprayer to apply microcapsule deposits to the vegetation. Immediately after application the commercial suspension concentrate formulation again gave superior mortality but after five days a microcapsule formulation with intermediate wall thickness was superior and after 15 days all the microcapsule formulations gave greater mortality. Statistical analysis of nymphal mortality by K. Gadgoud indicated no significant difference between the microcapsule and suspensions concentrate formulation types ($F_{3,12}$=0.30, P=n.s.). Concern had been expressed that the microcapsules were hindered when passing through the sprayer, giving depleted residues on the vegetation. A significant reduction of microcapsule numbers was found after spraying when quantifying microcapsule suspensions using a haemacytometer ($F_{1,4}$=48.0,P<0.01), the numbers of microcapsules reducing as wall thickness increased. The poor efficacy trial results combined with the operational difficulties of applying aqueous based formulations using ULV equipment in tropical conditions (Holland & Jepson, 1996) caused a change in emphasis to oil based formulations for which bifenthrin was unsuitable.

Formulations of normal phase malathion microcapsules were developed, primarily with the intention of comparative testing against reverse phase malathion microcapsules. However, lack of time prevented biological testing of the normal phase formulations. The importance of surfactants in stabilising microcapsule preparation was demonstrated by the screening of chemically similar polymeric materials.

The main formulation development concerned reverse phase malathion microcapsules and a range of formulations was prepared for biological testing with varying microcapsule wall properties. Extended storage stability was not considered necessary for the purposes of

laboratory biological trials and although the microcapsules sedimented rapidly on standing the sediment could easily be redispersed when required.
It was noted that formulations with relatively thin microcapsule walls, or those that employed catalysts during preparation, released malathion after storage for tens of weeks. This would have important implications for field use and may limit the scope for tailoring microcapsule properties for efficacy, persistence, selectivity, etc.
Samples of malathion microcapsules were imaged using a scanning electron microscope which is capable of resolving far smaller features than a simple light microscope (Schramm, 1996). Importantly it was confirmed that spray application by a ULV spinning disk sprayer did not damage the microcapsules. Differences in microcapsule surface texture were discernible between formulations employing different monomers but it was not possible to determine what effect these differences would have on release of the active ingredient.

The biological suitability of reverse phase malathion microcapsules for locust control required investigation and will be addressed in subsequent chapters. Important considerations were that microencapsulation had not reduced the toxicity of malathion to locusts, that persistence of malathion deposits had been increased by microencapsulation, and that toxicity to other organisms had been minimised.
When surveying the literature it can be seen that relatively few commercially available microcapsule formulations exist (Wilkins, 1990). This is undoubtedly due in part to cost; that is the cost of development, production, raw materials and a cost imparted due to reduced active ingredient levels. It is likely that a microcapsule formulation would incur increased costs from all of these sources due to its increased complexity, this would be especially important in the case of locust control since technical grade malathion may be applied directly using ULV equipment (MacCuaig, 1983). Little information was available on the economics of microcapsule formulation but sources included Harbeston (1975) and Finch (1985).
Given that cost is not an advantage of microcapsule formulations other advantages must exist to make their use viable. As stated in chapter one, possible advantages of microcapsule formulations include the following (Phillips, 1968; Tsuji, 1990; Marrs & Scher, 1990). Firstly the persistence of the pesticide may be increased by a reduction in

processes such as evaporation, degradation and leaching. Depending on release method the exposure of an insecticide may theoretically be restricted to certain types or sizes of insect and so protect non-target organisms. Toxicity of the pesticides to mammals, plants and fish may be reduced. Finally pesticide odour may be masked to prevent a repellent effect and encapsulation may aid co-formulation of chemically incompatible components.

Now that the preparation of reverse phase malathion microcapsules has been demonstrated as feasible any advantages must be proved. It is theorised that advantages will lie in the areas of increased persistence giving increased application intervals and possibly allowing reintroduction of barrier spraying and increased insect selectivity leading to reduced environmental impact.

Table 3.8: Formulations and their microcapsule properties.

Formulation	Active Ingredient Content (%w/w)	Core to wall ratio	Cross Linking (%)	Microcapsule Polymer
Malathion reverse-phase microcapsules				
43-065	25	5:1	100	Polyurethane
43-091	25	5:1	100	Polyester
43-135	45	5:1	100	Polyurethane
43-140	55	5:1	100	Polyurethane
43-142	50	5:1	100	Polyurethane
43-161 & 211	50	5:1	100	Polyurethane
60-109	45	5:1	100	Polyurethane
43-177	25	5:1	75	Polyurethane
43-250	25	10:1	100	Polyurethane
43-253	25	15:1	100	Polyurethane
60-052 & 054	45	5:1	100	Polyurethane
60-030	50	5:1	100	Polyurethane
60-074	45	5:1	100	Polyurethane
Malathion normal-phase microcapsules				
43-063	25	5:1	100	Polyurethane
43-060	25	5:1	0	Polyester
43-061	25	5:1	0	Polyester
43-069	25	5:1	0	Polyester
43-073	25	5:1	100	Polyurethane
43-074	25	5:1	100	Polyester
43-119	25	5:1	100	Polyurethane
Bifenthrin normal-phase microcapsules				
43-020	10	7:1	100	Polyurea
43-021	10	10:1	100	Polyurea
43-022	10	5:1	100	Polyurea
Diflubenzuron microcapsules				
43-030	10	5:1	100	Polyurea
43-053	10	5:1	100	Polyurea

Table 3.9: Malathion reverse-phase microcapsule formulation details

Component	Formulations (%w/w)						
	43-091	43-135	43-140	43-141	43-142	60-030	60-074
Malathion Technical (95% w/w ai)	26.32	47.37	57.89	57.89	52.63	52.63	47.37
Adipoyl Chloride	2.92						
PAPI 2027		9.75	11.92	11.92	10.83	10.83	9.75
1,2,4-Butanetriol	1.13						
1,5-Pentanediol		4.48	5.48	5.48	4.98	4.98	4.48
Antaron V216	4.00	2.00	2.00	4.00	2.00	2.00	2.42
Rhodialux A						0.50	0.50
Dibutyltin Dilaurate							0.05
Isopar M	65.63	36.40	22.71	20.71	29.56	29.06	35.43

Component	Formulations (% w/w)						
	43-161 & 211	60-109	43-177	43-237	43-250	43-253	60-052& 054
Malathion Technical (95% w/w ai)	52.63	47.37	26.32	26.32	26.32	26.32	47.37
PAPI 2027	10.83	9.75	4.30	4.10	2.87	1.91	9.75
Isonate M143			1.24				
1,5-Pentanediol	4.98	4.48	2.32	1.78	1.24	0.83	4.48
Antaron V216	2.00	2.43	4.10	2.00	4.38	4.50	2.42
Dibutyltin dilaurate							0.05
Isopar M	29.56	35.97	61.72	65.80	65.19	66.44	35.93

Table 3.10: Malathion normal-phase microcapsule formulation details

Component	Formulations (%w/w)						
	43-063	43-060	43-061	43-069	43-073	43-074	43-119
Malathion Technical (95% w/w ai)	26.32	26.32	26.32	26.32	26.32	26.32	26.32
PAPI 2027	6.07						
Adipoyl Chloride		2.92	2.92	2.92		2.92	
2,4-Tolylene Diisocyanate (TDI)					2.78		2.78
1,5-Pentanediol	2.49	1.66	1.66	1.66			
Glycerol					0.98	0.98	0.98
Airvol 203	4.00	4.00					
Agrimer AL904			4.00		4.00	4.00	4.00
Candidate Emulsifier				4.00			
Rhodorsil 426R	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Veegum							0.35
Xanthan Gum							0.09
Proxel GXL							0.10
Distilled Water	60.62	64.60	64.60	64.60	65.42	65.28	64.88

Table 3.11: Bifenthrin normal-phase microcapsule formulation details

Component	Formulations (%w/w)		
	43-020	43-021	43-022
Bifenthrin Technical (92% w/w ai)	10.59	10.59	10.59
Solvesso 200	10.59	10.59	10.59
2,4-Tolylene Diisocyanate (TDI)	2.24	1.57	3.13
Diethylene Triamine	0.88	0.62	1.24
Airvol 203	4.00	4.00	4.00
Rhodorsil 426R	0.40	0.40	0.40
Xanthan Gum	0.20	0.20	0.20
Proxel GXL	0.10	0.10	0.10
Distilled Water	71.00	71.90	69.80

Table 3.12: Diflubenzuron microcapsule formulation details

Component	Formulations (%w/w)	
	43-030	43-053
Diflubenzuron Technical (air milled)	10.00	10.00
Solvesso 200	10.00	
2,4-Tolylene Diisocyanate (TDI)	3.26	1.12
Diethylene Triamine	1.29	0.44
Airvol 203	4.00	
Antaron V216		10.00
Rhodorsil 426R	0.40	
Aerosol DP0545		5.00
Distilled Water	71.05	10.00
Isopar M		63.44

Table 3.13: Description of formulation components

Component	Use	Notes
Adipoyl Chloride	Monomer	*
PAPI 2027	Monomer	Polymethylene polyphenylisocyanate*
Isonate M143	Monomer	Diphenylmethane diisocyanate*
2,4-Tolylene diisocyanate	Monomer	*
1,2,4-Butanetriol	Monomer	*
1,5-Pentanediol	Monomer	*
Glycerol	Monomer	*
Diethylene triamine	Monomer	*
Airvol 203	Surfactant	Partially hydrolised polyvinyl alcohol (Air Products, Walton on Thames, Surrey)
Antaron V216	Surfactant	Polyvinyl pyrrolidone (ISP, Guildford, Surrey)
Agrimer AL904	Surfactant	Alkylated vinyl pyrrolidone (ISP, Guildford, Surrey)
Agrimer VAS-64	Surfactant	Vinyl pyrrolidone/vinyl acetate copolymer (ISP, Guildford, Surrey)
Agrimer ST	Surfactant	Vinyl pyrrolidone/styrene copolymer (ISP, Guildford, Surrey)
Agrimer AN-1200	Surfactant	Methyl vinyl ether/maleic anhydride copolymer (ISP, Guildford, Surrey)
Rhodialux A	UV Protectant	(Rhone-Poulenc, Stockport, Cheshire)
Rhodorsil 426R	Antifoam	(Rhone-Poulenc, Stockport, Cheshire)
Dibutyltin Dilaurate	Catalyst	*
Isopar M	Continuous Phase	Paraffin oil (Exxon Chemical, Fareham, Hants)
Solvesso 200	Solvent	Aromatic solvent (Exxon Chemical, Fareham, Hants)
Proxel GXL	Antibacterial agent	(Zeneca Biocides, Blackley, Manchester)
Veegum	Viscosifier	(Vanderbilt Company Inc., Norwalk US)
Xanthan gum	Viscosifier	*

*= Ex Aldrich Chemical Co.

CHAPTER 4

INITIAL TESTING OF MICROENCAPSULATED MALATHION FOR EFFICACY, PERSISTENCE AND TOXICITY TO AN INDICATOR BENEFICAL INSECT AND LOCUSTS.

1 Introduction

The reverse phase malathion microcapsule formulations developed in the first phase of this project required testing for suitability in locust control operations. Three trials were performed, two to test the efficacy and persistence against II instar Desert Locust (*Schistocerca gregaria*) nymphs and the third to test the relative toxicity of the microcapsule formulation to a beneficial insect.

The toxicity of malathion formulations to locusts and Carabidae could have been tested by with great accuracy by topical application or direct dosing (Busvine, 1971). However, it was decided to forgo some accuracy in favour of realism. For testing efficacy and persistence with locust nymphs it was decided to apply residues to vegetation and allow simultaneous cuticular and dermal exposure. For testing toxicity with Carabidae, treatments were applied to a sand surface. While it is true that the dose acquired for each insect is unknown the effects of behaviour on residue transfer are automatically included. This may be of great importance for microcapsule formulations where microcapsule rupture by trampling or chewing could be the primary release mechanism (Tsuda et al, 1987; Ohtsubo et al, 1987).

A number of application methods were considered. It is known in the literature for insecticide residues to be applied to vegetation by simple dipping (Busvine, 1971) and a surface layer of insecticide contaminated sand could be have been prepared by hand mixing (Critchley, 1972). However, in the pursuit of test realism it was decided to apply treatments by droplet impaction.
The test arenas intended for beneficial insect testing were suitable for use with a Potter spray tower which has been specifically developed to obtain homogeneous spray deposits (Potter, 1952). The size and area of vegetation used when testing with locusts was unsuitable for treatment by a Potter tower. For all experiments it was decided to use the

type of controlled droplet application (CDA) equipment commonly used in locust control operations.

Various apparatus are cited in the literature for applying pesticides to vegetation but were unreliable or complex (Busvine, 1971). Parr & Busvine (1948) combined a spinning disk sprayer with a motorised track but this would appear to offer little advantage over practised manual application. After completion of these experiments Bateman (1994a) published details of a motorised track sprayer fitted with a modified Micro Ulva sprayer; the spinning disk was largely screened and only a homogeneous flat fan of droplets was emitted rather than the more typical hollow cone. In all the experiments described below a standard Micro Ulva sprayer was used, allowing the droplets to sediment under gravity rather than the wind assisted overlapping swath technique commonly used in the field (Steedman, 1990).

Available data on microcapsule formulation testing with locusts is limited. Steedman (1990) indicated that in field testing microencapsulation approximately doubled persistence but reduced initial kill. No details were included of test protocol or location. Holland & Jepson (1996) field tested normal phase microcapsules of fenitrothion, chlorpyrifos and diazinon against locusts and although initial efficacy was equivalent to unencapsulated fenitrothion no measure of persistence could be obtained.

The effects of microencapsulated insecticides on beneficial insects are poorly understood. One of the earliest commercial microcapsule formulations of an insecticide was PENNCAP-M® containing methyl-parathion. It was rapidly discovered that bees were at increased risk due to dried microcapsules being carried back to the hive with pollen (Koestler, 1980), however if label recommendations are adhered to the risk may actually be decreased (Dahl & Lowell, 1984).

There are also some indications that PENNCAP-M® exhibits a degree of selectivity, beneficial insects remaining unharmed at use rates that achieve pest control (Koestler, 1980). Dahl & Lowell (1984) briefly reviewed the effects of microencapsulated methyl parathion on entomophageous insects; it was concluded that the reduced effects on parasitic and predatory insects may make microcapsule formulations of use in integrated pest management (IPM) programs.

2 Materials And Methods

All technical grade malathion used in these trials, including microcapsule preparations, was obtained from Cheminova Agro A.S. and contained 95% w/w malathion active ingredient. Stocks of *S. gregaria* nymphs were cultured as described in Coppen (1995).

2.1 Trial One: Efficacy and persistence of a malathion microcapsule formulation against *Schistocerca gregaria* nymphs.

The aim of this investigation was to determine the efficacy and persistence of malathion microcapsule residues on vegetation when used for the control of *Schistocerca gregaria* nymphs. Treatments were applied to barley seedlings grown in trays and II instar *S. gregaria* were exposed to treated foliage at various times following spraying. In line with typical locust control operations the treatments were applied using CDA spray equipment.

2.1.1 Preparation Of Seedlings

Initially sixty seed trays (46 cm x 27.5 cm x 8 cm) were filled approximately 40 mm deep with John Innes No. 2 potting compost and then covered with a thin layer of barley seeds (60 ml volume) before adding a further layer of compost approximately 25 mm deep. The trays were placed in a glass house (minimum 13°C in the day and 8°C at night) for ten days, after which the seedlings had attained a height of approximately 10 to 14 cm. The growth was very variable within trays.

2.1.2 Spray Application

The aim was to apply an even coverage of each treatment, at a use rate of 900 g malathion a.i./ha (0.09 g of malathion per m^2) and equivalent coverage of Isopar M in the control treatment. Initial examinations of a Micro-Ulva spinning disk sprayer indicated that a swath width of approximately one metre was generated when using five 1.5V batteries to power the sprayer. The applied voltage controls the speed of the spinning disk and the droplet size generated (Shell), thus it is important that the voltage does not vary during trials.

The flow rate of each treatment through the sprayer (fitted with a yellow flow regulator) was measured with a stationary disk and used to calculate the required transition speed over the long axis of a rectangle 1m wide by 3m long marked on a flat surface. The sprayer was held at a height of approximately one metre and carried over the spray area; the walking pace was adjusted with practice to ensure that the required application rate was obtained. The seedling trays were placed within the marked area, the sprayer spinning disk started with the machine inverted (no flow) at one edge of the area and then quickly turned upright to allow flow as the sprayer was carried manually over the marked area at the practised speed. The droplets were allowed to sediment onto the vegetation in static air.
The sixty trays were divided into three groups and sprayed with either Isopar M as a blank control treatment, malathion technical diluted in acetone (12.5% w/w a.i.) or formulation 43-161 diluted in Isopar M paraffin oil (50% w/w a.i., 5:1 c/w ratio and 100% cross linked polyurethane microcapsules diluted to 12.5% w/w a.i.).
It was assumed in the case of the malathion technical treatment that the acetone evaporated leaving no residues or plant damage and thus required no blank control treatment of its own. An error was made during spray application of the malathion microcapsule treatments leading to an additional application of Isopar M containing no malathion. This extra application was unlikely to modify the test results.

The primary method of controlling flow rate when using a Micro-Ulva sprayer is by using flow restrictors with varying orifice diameters. The possibility that this restriction would cause a filtration effect when spraying suspensions of particles such as microcapsules was of concern. Thus it was felt prudent to measure the number of microcapsules in the spray liquid before and after passing through the sprayer. First it was attempted to use a haemacytometer to count the number of microcapsules per volume of spray liquid, before and after passing through a stationary Micro-Ulva. The counting of microcapsules was hampered by the relatively wide size range of the particles and the operator's perception that although the numbers of microcapsules were similar although the maximum particle size may have reduced. Considering that the volume of a spheroid increases as the cube of the radius, the removal of larger microcapsules during spraying would disproportionately affect the amount of active ingredient reaching the target. Thus samples of spray liquid were

removed for HPLC assay both before and after passing through a stationary Micro-Ulva spray head.

2.1.3 Post Treatment and Assessment

After spraying the trays were covered with vented propagator lids and returned to the same conditions as during growth.
The toxicity of spray residues to II instar *S. gregaria* nymphs was assessed at various intervals. Five trays of each treatment type (fifteen in total) were removed to a locust culture room after 0, 3 and 8 days for the introduction of seven II instar nymphs into each tray. The condition of the hoppers was visually assessed after approximately 24 and 48 hours' exposure.

2.2 Trial Two: Investigating the toxicity of malathion microcapsule residues (on sand) to *Agonum dorsale* (Coleoptera: Caribidae)

The aim was to measure the toxicity of a malathion microcapsule formulation to the beneficial insect *Agonum dorsale*. A test protocol was employed derived from that employed commercially for assessment of pesticide effects on ground beetles (Heimbach, 1991).

2.2.1 Initial Preparations

The *A. dorsale* used in the test were obtained at the Leckford estates near Winchester, Hampshire and no differentiation of sexes made. Twenty clear plastic arenas of 10 cm diameter were prepared with perforated lids and filled approximately one centimetre deep with sterile sand, loaded with water at 70% of maximum capacity. Six beetles were placed in each arena without food and stored for two days at a temperature of approximately 21C while exposed to 14 hours' darkness and 10 hours' light. It was attempted to make the lighting diffuse as required in the original protocol by placing an opaque cover between the overhead fluorescent lights and the containers. After the two days' storage any water loss due to evaporation was replenished; with the beetles remaining in-situ a domestic hand

operated sprayer was used to apply a fine water mist until the average initial mass was re-attained.

2.2.2 Spray Application

The twenty containers were divided into four groups of five. The first group of five remained untreated and the second 'blank' group was treated with both acetone and Isopar M paraffin oil. The remaining two groups were treated with malathion technical diluted in acetone or a reverse phase malathion microcapsule formulation diluted in Isopar M paraffin oil, containing 12.5% w/w malathion active ingredient. The microcapsule formulation was 43-161, containing 50% w/w malathion active ingredient in 100% cross linked polyurethane microcapsule walls with a 5:1 core to wall ratio.
The treatments were applied using a Micro-Ulva spinning disk sprayer, in a similar manner to that described for trial one, using an application rate equivelant to 900g/ha. For this experiment the five containers for each treatment were placed in the central square meter of the marked area to allow the sprayer to stabilise before the swath reached the target. A portion of moist cat food (trade name 'Delicat') food was placed in each arena before spraying and the beetles left in situ during spraying. No attempt was made to prevent spray deposition on the container walls and it was noted that flexing of the container walls gave voids in the sand surface that may have sheltered some or all the beetles during spraying. HPLC analysis of the malathion technical and microcapsule treatments before and after passing through a static sprayer confirmed that the required active ingredient level had been achieved.

2.2.3 Post treatment and Assessment.

The arenas were replaced in the controlled environment specified above and assessed after 2 hours, 4 hours, 1, 2, 4, 7 and 10 days. The moisture content of the sand was replenished and the food replaced at the second day and subsequent assessments.

When assessed the beetles were classified as live or dead on the basis of movement. Some difficulties were met due to beetles inhabiting voids in the sand and in these situations it was attempted to solicit movement by gentle nudging with a rounded probe. The food was in

the form of cubes and so on replacement could be classified as consumed, partially consumed or uneaten.

2.3 Trial Three: Efficacy and persistence of a malathion microcapsule formulation against *Schistocerca gregaria* nymphs.

This second trial with *S. gregaria* nymphs was performed using a protocol modified in response to difficulties found in the trial one. Most fundamentally the swath width under these spraying conditions was found to be half of that assumed in the first trial.

2.3.1 Preparation Of Seedlings

Forty-five clear plastic containers (10 cm diameter and 5 cm deep) were pierced in the base to allow drainage and filled with approximately 4 cm of John Innes No.2 potting compost. A 10 ml volume of barley seeds was spread evenly over the surface and then coated with a further thin layer of compost. The pots were stored in a greenhouse environment (day minimum of 13C and night minimum of 8C) for 11 days with regular watering. At the end of this period a foliage height of 10 to 14 cm was attained.

2.3.2 Spray Application

The pots were divided into three groups of 15 and sprayed with a blank treatment, technical grade malathion diluted in acetone to 10% w/w a.i. or a reverse phase malathion microcapsule treatment diluted in Isopar M paraffin oil to 5% w/w a.i. An application rate equivalent to 900g malathion per hectare was employed. For the malathion technical treatment the acetone diluent was assumed to evaporate shortly after spraying, without residue, and hence did not require a blank treatment.
Malathion microcapsule formulation 43-211 was used containing 50% malathion active ingredient encapsulated in 100% cross linked polyurethane walls with a 5:1 core to wall

ratio. Both the malathion technical and microcapsules were diluted to 12.5% w/w active ingredient.

The application method was similar to that described above for trial one. Investigations into the swath width obtained from a Micro-Ulva using oil soluble dyes and oil sensitive paper indicated a width of approximately 0.5m; half of that assumed for the first two trials. The height of the spray head above ground level was also reduced from one meter to 50 cm, to allow more reproducible droplet coverage. Thus a rectangular area of 0.5m by 3m was marked and the 15 pots of vegetation for each treatment placed in the central 0.5m. The purpose of the empty areas at the beginning and end of the spray axis (longest axis) was to provide a stable droplet pattern while depositing on the vegetation.

Spray coverage was monitored using sections of oil sensitive paper amongst the pots of vegetation. Samples of spray liquid were taken from the sprayer outlet (with the disk stationary) and assayed for malathion content by HPLC.

2.3.3 Post Treatment and Assessment

After treatment the vegetation was replaced in the greenhouse under the conditions described above and covered to prevent accidental overhead watering.

At zero, five and nine days post spraying five pots of vegetation from each treatment type were removed to a locust culture room. Each pot was placed in a vented propagator and ten II instar *S. gregaria* nymphs introduced. Mortality of the locusts was visually assessed after approximately 24 and 48 hours' exposure.

3 Results

3.1 Trial One

HPLC assay of the spray liquid before and after passing through a Micro-Ulva was performed which confirmed that less than 3% of the active ingredient was removed from the microcapsule suspension during spraying.

Difficulties were experienced in making assessments of nymph mortality. This was primarily due to the quantity of vegetation hiding nymphs, difficulties in locating rapidly darkened corpses against a soil background and possible escape of locusts. Thus the data is presented in table 4.1 as percentage mortality of locusts located and the total number located (35 insects were originally added per treatment). It is recognised that escaped locusts would skew the results to higher mortality values but the effect would be constant over all treatments and assessment dates.

Table 4.1: Trial One - Mortality of II instar *S. gregaria* exposed to residues of malathion technical and microcapsules.

Treatment	Percentage nymphal mortality at time after first exposure. (numbers of hoppers located)					
	Initial		Four day old residues		Nine day old residues	
	24h	44h	29h	49h	25h	47h
Blank	0 (28)	3 (27)	3 (32)	0 (33)	6 (35)	3 (33)
Technical	100 (30)	100 (29)	55 (20)	47 (19)	4 (26)	23 (34)
Microcapsules	17 (30)	23 (30)	0 (23)*	0 (25)*	0 (28)	10 (31)

* = Only 28 nymphs available for testing in four replicates.

3.2 Trial Two

Samples of spray liquid had been taken before and after passing through a Micro-Ulva sprayer with the disk stationary. Assay of malathion content by HPLC confirmed both that the required concentration of malathion was present and microcapsules were passing through the sprayer without restriction.

Throughout the ten days of observation no mortality was observed in any of the untreated arenas (numbered 1 to 5) or those sprayed with a blank treatment (arenas 6 to 10).

Mortality observed from the remaining malathion technical and microcapsule treatments is included in table 4.2. The sand surface of one malathion microcapsule replicate was accidentally disturbed shortly after spraying and was thus discarded.
The data in table 4.2 was not suitable for presentation as total percentage mortality per treatment due to the great variation in mortality between replicates.

Table 4.2: Trial Two: Mortality of *Agonum dorsales* exposed to malathion technical and microcapsules.

Time After First Exposure	Beetle Mortality (Six insects per replicate)									
	Malathion Technical					Malathion Microcapsules				
	11	12	13	14	15	16	17	18	19	20
2 hr	4	0	0	0	0	0	0	0	N/A	0
4 hr	5	0	0	0	0	0	0	0	N/A	2
1 day	6	1	0	0	0	0	0	4	N/A	6
2 days	6	1	0	0	0	0	0	4	N/A	6
4 days	6	1	0	0	0	0	0	6	N/A	6
7 days	6	1	0	0	0	0	0	6	N/A	6
10 days	6	1	0	0	0	0	0	6	N/A	6

When replaced the food was assessed as uneaten or gnawed/disassembled, in no case had all the food been totally consumed. Table 4.3 displays the percentage of replicates in which the food had been eaten. Notice that despite the beetle mortality remaining almost constant after two days the number of replicates in which food had been consumed had decreased. This was especially noticeable in the untreated replicates.

Table 4.3: Trial Two: Feeding of *Agonum dorsales* during toxicity testing.

Time After First Exposure	Percentage of replicates in which food had been consumed.			
	Untreated	Blank	Malathion Technical	Malathion Microcapsules
2 days	100	80	40	25
4 days	80	80	60	25
7 days	40	80	40	0
10 days	20	60	40	0

3.3 Trial Three

Aassay of spray liquids by HPLC indicated that the correct quantities of malathion were present in each treatment and that passage through the Micron-Ulva sprayer did not 'filter' the microcapsules. Samples of the paraffin oil blank were tested for malathion content and trace quantities detected. It is not known whether the source of the contamination (approximately 0.1% w/w) was from the spray equipment or from the glassware used in the analysis. Such low levels of malathion are unlikely too have influenced the experiments outcome.

Sections of oil sensitive paper placed amongst the sample containers indicated a uniform droplet coverage across the spray area but possible reduction in the number of droplets deposited at the end of the spray axis. It was also noted that the Isopar M blank and malathion technical treatments appeared to produce smaller droplets than the malathion microcapsule treatment.

The percentage mortality of II instar *S. gregaria* nymphs exposed to the deposits is shown in table 4.4. At each assessment ten nymphs were contained with each treated pot of vegetation and five replicates were tested per treatment. For one treatment, at one test date 51 locusts were tested accidentally instead of the intended 50.

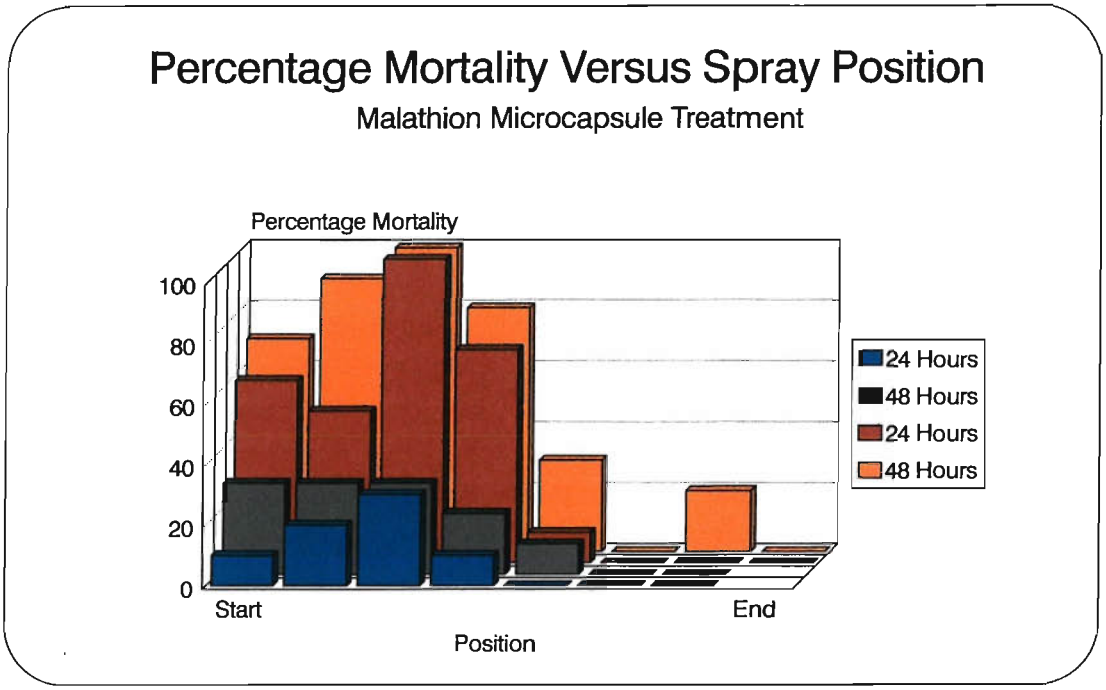
Table 4.4: Trial 3: Mortality of locusts exposed to treated vegetation one day post spraying.

Treatment	Percentage nymphal mortality at time after first exposure.					
	Initial		Five day old residues		Nine day old residues	
	25h	48h	24h	48h	25h	48h
Blank	0	0	0	0	0	0
Technical	96*	98*	32	40	0	6
Microcapsules	58	68	6	16	8	12

*= 51 nymphs tested in total

A great variation in mortality between replicates was observed. Figure 4.1 shows mortality data plotted against the position of the replicate when sprayed, irrespective of residue age. It can be seen that superimposed on the reduction in mortality due to ageing of the deposits there is a pattern of increased mortality for replicates at the beginning of the spray line. Treatment of mortality data obtained from the malathion technical replicates gave similar results of increased mortality for the replicates at the start of the spray axis.

Figure 4.1: Trial 3: Plot of nymphal mortality versus position during spraying.



4 Discussion

All the three trials suffered from a fundamental problem associated with the method of spray application but problems specific to each trial will be highlighted first.

In trial one the density of barley foliage within each tray was greater than expected but very uneven, this lead to difficulty in locating locust nymphs for mortality assessment. The foliage also grew significantly during the experiment's duration, possibly leading to exaggerated growth dilution effects. Finally the propagator lids used to contain the locust nymphs were a poor fit onto the seed trays in which the vegetation was grown; this gave the possibility of nymphs escaping and caused further uncertainty in the assessment of mortality.

For trial three growth of the vegetation in small pots made observation of locusts more reliable due to less shielding by vegetation and good contrast between corpses and the propagator trays.

Trial two suffered from two deficiencies, firstly despite close control of the sand's moisture content some replicates exhibited a small amount of standing surface water with unknown effects on the transfer and degradation of malathion residues. Secondly the sand substrate was placed in containers with flexible side walls, which caused spaces below the sand surface in which some beetles could hide both during and after spraying. These holes appeared to be a preferred location for the beetles and would have caused variation in exposure of the beetles to malathion residues.

For all three of the trials it was attempted to make the spray deposits as similar as possible to those that would be generated in the field, hence use of a Micro-Ulva sprayer. However all three trials were flawed due to the use of a Micro-Ulva a spinning disk sprayer without wind assistance. In the first two trials the swath width was incorrectly assumed to be 1m wide while in reality a swath 0.5m wide was generated, which caused some replicates to be under dosed with a corresponding effect mortality.

Trial three benefited from further testing of the swath width generated under the imposed application conditions and use of oil sensitive paper indicated that droplet density was

approximately uniform across the spray area. However, the oil sensitive paper also indicated a reduction in the number of droplets along the spray axis which was substantiated by a pattern superimposed on the nymphal mortality obtained from replicates of both the malathion technical and microcapsule treatments.

Typically when spinning disk sprayers are used in the field the spray is generated in swaths at 90° to the prevailing wind, the droplets moving downwind as they sediment under the influence of gravity until impacting on the ground or vegetation. Incremental spraying of overlapping swaths is employed to compensate for irregularities in droplet deposition (Steedman, 1990). It is clear that for all three of the trials that the inhomogeneity of spray from a spinning disk sprayer was grossly underestimated. Similar problems have been encountered by other groups and in one case a modified spinning disk sprayer was employed with a spray track to give homogeneous spray coverage (Bateman, 1994a).

In response to these difficulties all future tests employed topical application or a Potter spray tower in a more controlled laboratory environment. This greater control of test conditions was achieved at the expense of some 'realism' in the test conditions.

The deficiencies in protocol and spray application make analysis of data from the three trials suspect. However, in general, for trials' one and three concerning the mortality of *S. gregaria* nymphs exposed to malathion technical and microcapsule residues it appeared that the malathion technical treatment consistently achieved higher mortality than the microcapsule treatment.

Trial two was to assess the toxicity of malathion technical and microcapsule residues to the beneficial insect *A. dorsale*. If it is assumed that replicates displaying high mortality received the intended droplet coverage then it can be concluded that both treatments were very toxic to *A. dorsale*.

CHAPTER 5

TESTING OF MALATHION MICROCAPSULE RESIDUES ON GLASS WITH THE DESERT LOCUST *S. gregaria.*

The initial testing of reverse phase malathion microcapsule residues (detailed in chapter 4) failed primarily due to uneven deposits produced by a Micron Ulva sprayer. In response to this simplified test protocols were developed to investigate the cuticular and oral toxicity's of microcapsule formulations independently. This chapter details the testing of the cuticular toxicity of microcapsule formulations to nymphs of the desert locust *Schistocerca gregaria*.

1 Introduction

The increased complexity of interactions of controlled release formulations, coupled with an inability to predict adequately the important external processes, such as meteorology, make the evaluation and development of controlled release formulations particularly problematical (Pfister & Bahadir, 1990). Due to this, initial laboratory experiments play an important role in ensuring the procedure and performance of later large scale trials.

An example of making good use of laboratory testing is the development of microencapsulated fenitrothion for cockroach control (*Blatella germanica*) (Tsuda et al, 1987; Ohtsubo et al, 1987 & Kawada et al, 1994). From a combination of chemical analysis and bioassay of dried residues it was found that release by diffusion was negligible and the primary release mechanism was microcapsule rupture due to a combination of insect trampling and eating. The microcapsule wall strength, and hence resistance to rupture, was found to be proportional to the ratio of median microcapsule diameter and wall thickness; theoretically the ratio could be optimised for one size or type of insect. The bioassay protocol consisted of spraying dilute microcapsule suspensions onto plywood panels (application method not revealed), allowing to dry for 24 hours at room temperature and then exposing cockroaches to the deposits for two hours.

Similar microcapsule formulations containing fenvalerate (Ohtsubo et al, 1989) were tested against the diamond back moth *Plutella xylostella* and the tobacco cutworm *Spodoptera litura* by application to cabbage and potato leaves respectively. For initial efficacy tests the leaves were simply dipped in dilute suspensions of microcapsules but for persistence testing

the suspensions were sprayed onto the plants by an unspecified method and leaves removed at set periods for testing. It was concluded that the main release mechanism for dried residues was microcapsule rupture by the crawling caterpillar insects. The efficacy was again related to the ratio of mass median microcapsule diameter and wall thickness but also there appeared to be an optimal wall thickness for extended persistence. Aquatic toxicity testing with *Oryzias latipes* indicated that the microcapsule toxicity (compared to unencapsulated active ingredient) was proportional to the mass median microcapsule diameter multiplied by the wall thickness.
It must be noted that all the formulations in the examples described above were aqueous based (normal phase) giving benefits in minimal background toxic effects from the diluent.

For the purposes of the experiments described below particular attention was paid to choosing the formulation application method. It would have been possible to dip or smear treat a surface but it was felt that the form of the residues would differ from those produced in the field (Busvine, 1971). Topical application of droplets to the locust cuticle was rejected due to the following factors. Firstly only initial efficacy can be tested with no measure of residue persistence, secondly toxicity is influenced by the site of droplet application (Ahmed & Gardiner, 1968) and finally the insecticide diluent can have an overriding effect on mortality (Busvine, 1971). For the tests described in this chapter a Potter spray tower (Potter, 1952) was adopted for the application of malathion and reverse phase malathion microcapsules to glass plates. The spray apparatus consisted of a concentric jet nozzle suspended above a vertical metal tower and a sample carrying platform at the base which was capable of being raised into position. The design was aimed at providing an even air pressure over the target area and the minimum of turbulence consistent with giving an even droplet deposit. An earthed metal tower was used to prevent unpredictable effects due to the variable electrostatic charges naturally developed by droplets. Reported experiments with distilled water (Potter, 1952) indicated a consistent droplet deposition over a range of ambient temperatures and humidities.

When choosing a substrate it was recognised that it may affect the toxicity of residues due to factors such as porosity and absorbency. To improve the test realism, vegetation could

be used as the substrate but immediately this imposes problems of reproducibility and obtaining a sufficiently large homogeneous surface. For bioassay tests it is common to use either filter paper or glass as substrate (Busvine, 1971), the latter was chosen in this case.

In summary the test method used for these experiments involved application of the spray treatments to glass plates using a Potter spray tower and subsequent confinement of a single II instar *S. gregaria* nymph over each treated surface. The mortality of the locusts was monitored visually after increasing periods of exposure to the pesticide deposits, both malathion microcapsules and technical grade active ingredient. It was intended that the primary route of malathion ingestion would be through the cuticle although no attempt was made to block the locust mouth parts.

The first set of experiments investigated the effect of microcapsule wall composition on locust mortality rate, both initially and after ageing of the malathion deposits. Then it was attempted to determine the maximum persistence of a single malathion microcapsule formulation, compared to malathion technical, when exposed to typical laboratory ambient conditions. It is accepted that the absolute persistence of deposits in the harsher field environment would differ markedly from those obtained with this experiment but the relative increase in persistence will be indicative of performance in the field.

2 Materials And Methods

The testing was divided into two phases. The first phase investigated the effects of microcapsule formulation on efficacy, specifically variations in microcapsule wall thickness and type. The second phase was designed to find the persistence of malathion microcapsule deposits in comparison with technical grade malathion, using biological testing to monitor persistence rather than chemical analysis.

The nymphs used in this experiment were cultured as described in Coppen (1995) and the bioassay performed under the same environmental conditions as culturing. Stocks of technical grade malathion (95% w/w active ingredient) were obtained from Cheminova Agro and used both for microcapsule preparation and biological testing. A Potter spray tower was used for residue application (Burkhard Manufacturing Company Ltd., Rickmansworth, Hertfordshire).

2.1 Trial One: Investigation into the effects of microcapsule formulation factors on the toxicity of residues on glass to *S. gregaria* nymphs - both initially and after ageing.

2.1.1 Spray Application

A Potter spray tower fitted with a two fluid nozzle was used to apply treatments to 10 cm square glass plates. An air pressure of 3.5 PSI was used and the spray volume (measured by Gilson pipette) adjusted to give a deposit mass of 0.018g liquid per plate, this gave a use rate equivalent to 900 g malathion/ha when applying 5% ai w/w dilution's of technical grade malathion or microencapsulated malathion.

The malathion microcapsule formulations were diluted in Isopar M (Exxon Chemical Company) before application and control treatments of unencapsulated malathion were applied diluted in the solvent Solvesso 200 (Exxon Chemical Company). Blank treatments of Solvesso 200 and Isopar M were tested (0.018g liquid per plate) along with untreated plates. Ten replicate glass plates were tested per treatment.

Five malathion microcapsule formulations were tested and are described in table 5.1. The microcapsule formulations all contained polyurethane walled microcapsule which were 100% cross linked. The microcapsule wall thickness was varied between a thickest of 5:1 core to wall ratio and a thinnest of 15:1. Batch 60-052 employed a catalyst during preparation to speed microcapsule wall formation, giving a probable increase in wall density and corresponding effect on release rate (see Chapter 1).

Table 5.1: Microcapsule formulations used in cuticular testing.

Formulation	Core to wall ratio	Active Ingredient %w/w
43-211	5:1	50
43-237	7:1	25
43-250	10:1	25
43-253	15:1	25
60-052*	5:1	45

* = Employed catalyst to speed microcapsule wall formation.

All treated glass plates were allowed to dry overnight at room temperature before immediate bioassay or storage followed by bioassay. Ten replicate plates were made of each treatment and the plates tested only once, repeat sets being prepared for each storage period. The plates were stored horizontally in stacks with the treated surface uppermost, spacers were used resting on areas of the plates that were not exposed to the locusts during testing. The storage environment was characterised by high air flow (a fumecupboard), typical ambient laboratory temperatures and diffuse sunlight.

2.1.2 Bioassay

The toxicity of spray residues was tested by containment of a single II instar *S. gregaria* nymph over each treated surface and visually observing mortality. The nymphs were contained using inverted transparent plastic cups (284 ml capacity and 9 cm diameter opening) with ventilation holes pierced in the base. On initial placement the nymphs were forced to contact the treated glass surface by tapping of the plastic container but then the locusts were free to climb the inside of the cup.

Visual assessments were made of locust mortality. Locusts climbing the container walls were obviously alive; if the insect was standing motionless on the treated surface the container was lightly tapped to check response. No food was administered to the insects during the 48h assessment period.

2.2 Trial Two: Investigation into the comparative persistence of malathion microcapsule residues on a glass surface.

2.2.1 Spray Application

The method of spray application was as described above. The treatments consisted of a malathion microcapsule formulation diluted in Isopar M, technical grade malathion diluted in Solvesso 200, blank Isopar M and blank Solvesso 200. Malathion microcapsule formulation 60-109 was employed containing 45% w/w malathion active ingredient encapsulated in 100% cross linked polyurethane microcapsules with a 5:1 core to wall ratio.

Dilution and application rates were as described above. Thirty replicates were prepared for each treatment and stored under typical ambient laboratory temperatures in diffuse sunlight. In contrast with trial one the plates were stored in still air rather than the forced air flow of a fumecupboard.

2.2.2 Bioassay

In contrast with trial one the treated plates were repeatedly tested for efficacy against locust nymphs. The bioassay method was identical to that described for trial one.

2.3 Assay of malathion and malathion microcapsule treatments by high performance liquid chromatography (HPLC).

Malathion formulations were assayed using a Hewlett Packard 1050LC instrument fitted with a 5µm ODS Spherisorb 2 column, UV/visible wavelength detector, isocratic pump, column heater and autosampler.

The required quantity of sample was added to a volumetric and diluted with eluent (55% water, 45% acetonitrile). The mixture was ultrasonicated for at least one hour to ensure rupture of the microcapsules and even mixing of the sample. The samples were allowed to

equilibrate with room temperature before filling of the volumetric, mixing and filtration using glass fibre filter papers (Whatman GF/F). Samples of 10 to 20 µl volume were injected and resolved using a column temperature of 30C and eluent flow rate of 1.5 ml/minute. The malathion content was determined using a 210 nm wavelength (550 nm reference) and by comparison to a malathion analytical standard analysis.

3 Results

3.1 Trial One

Table 5.2: Toxicity and persistence testing of 5:1 c/w ratio malathion microcapsules and technical with II instar Desert Locust hoppers.

Residue Age/ Days	Treatment	Percentage Mortality At Time After First Exposure			
1		2 hours	3 hours		24 hours
	Unsprayed	0	0		0
	Solvesso 200	0	0		0
	Isopar M	0	0		0
	Malathion	0	90		100
	Malathion Microcapsules	0	70		100
14		2 hours	3 hours		24 hours
	Unsprayed	0	0		0
	Solvesso 200	0	0		0
	Isopar M	0	0		0
	Malathion	0	0		100
	Malathion Microcapsules	10	70		100
35		2 hours	3 hours	7 hours	24 hours
	Unsprayed	0	0	0	0
	Solvesso 200	0	0	0	0
	Isopar M	0	0	0	0
	Malathion	0	0	0	0
	Malathion Microcapsules	0	10	90	100

Table 5.3: Toxicity testing of 7:1 c/w ratio malathion microcapsules and technical with II instar Desert Locust hoppers.

Residue Age/ Days	Treatment	Percentage Mortality At Time After First Exposure		
1		2 hours	3 hours	24 hours
	Unsprayed	0	0	0
	Solvesso 200	0	0	20
	Isopar M	0	0	0
	Malathion	70	90	100
	Malathion Microcapsules	50	100	100

The 7:1 core to wall formulation 43-237 proved to be physically unstable on storage, thus no further testing of the formulation was possible.

Table 5.4: Toxicity and persistence testing of 10:1 c/w ratio malathion microcapsules and technical with II instar Desert Locust hoppers.

Residue Age/ Days	Treatment	Percentage Mortality At Time After First Exposure			
1		2 hours	3 hours		24 hours
	Unsprayed	0	0		0
	Solvesso 200	0	0		0
	Isopar M	0	0		40
	Malathion	90	100		100
	Malathion Microcapsules	60	100		100
14		2 hours	3 hours	7 hours	24 hours
	Unsprayed	0	0	0	0
	Solvesso 200	0	0	0	0
	Isopar M	0	0	0	0
	Malathion	0	0	20	40
	Malathion Microcapsules	0	0	100	100
35		2 hours	3 hours	6 hours	32 hours
	Unsprayed	0	0	0	0
	Solvesso 200	0	0	0	0
	Isopar M	0	0	0	0
	Malathion	0	0	0	0
	Malathion Microcapsules	0	0	50	100

Table 5.5: Toxicity and persistence testing of 15:1 c/w ratio malathion microcapsules and technical with II instar Desert Locust hoppers.

Residue Age/ Days	Treatment	Percentage Mortality At Time After First Exposure				
1		2 hours	3 hours			24 hours
	Unsprayed	0	0			0
	Solvesso 200	0	0			0
	Isopar M	0	0			0
	Malathion	30	90			100
	Malathion Microcapsules	30	60			100
14		2 hours	3 hours	5 hours	10 hours	24 hours
	Unsprayed	0	0	0	0	0
	Solvesso 200	0	0	0	0	0
	Isopar M	0	0	0	0	0
	Malathion	0	10	10	40	70
	Malathion Microcapsules	20	40	80	100	100
42		2 hours	3 hours	6 hours		27 hours
	Unsprayed	0	0	0		0
	Solvesso 200	0	0	0		0
	Isopar M	0	0	0		0
	Malathion	0	0	0		0
	Malathion Microcapsules	10	30	90		100

Table 5.6: Toxicity and persistence testing of 5:1 c/w ratio malathion microcapsules (with catalyst) and technical with II instar Desert Locust hoppers.

Residue Age/ Days	Treatment	Percentage Mortality At Time After First Exposure			
14		2 hours	3 hours	7.5 hours	32 hours
	Unsprayed	0	0	0	10
	Solvesso 200	0	0	0	0
	Isopar M	0	0	0	0
	Malathion	0	0	0	0
	Malathion Microcapsules	0	10	90	100
35		2 hours	3 hours	6 hours	24 hours
	Unsprayed	0	0	0	0
	Solvesso 200	0	0	0	0
	Isopar M	0	0	0	0
	Malathion	0	0	0	0
	Malathion Microcapsules	0	30	80	100

Where possible χ^2 analysis (Bailey, 1959) was performed on the data in tables 5.2 to 5.6, the analyses were however hampered by the small number of insects tested in each instance and also by the need to vary observation times in some cases. The data for malathion microcapsule treatments was tested for heterogeneity over the period of storage for each treatment and the results included in table 5.7.

Table 5.7: Statistical (χ2) testing of locust mortality data - heterogeneity of mortality data for microcapsule treatments over total storage period.

Treatment	Table	Heterogeneity χ^2	d.f.	Signif.[a]
5:1 c/w microcapsules	5.2	2.43	4	ns
10:1 c/w microcapsules	5.4	30.00	4	s
15:1 c/w microcapsules	5.5	2.11	4	ns
5:1 c/w microcapsules with catalyst	5.6	2.03	2	ns

[a] Significance level: ns = not significant ($P > 0.05$), s = significant ($P < 0.05$)

Mortality data from the technical grade malathion treatments in tables 5.2, 5.4 and 5.5 was treated in the same manner with heterogeneity being significant ($P < 0.05$) in all cases. The reduction in mortality on storage is made obvious by the lack of mortality after 5/6 weeks storage.

It would have been desirable to test the heterogeneity of the data for multiple treatments obtained at one test date, the data would not however support such an analysis.

3.2 Trial Two

The data obtained from trial two is presented in tables 6.8 and 6.9.

Table 5.8: Persistence testing of malathion with II instar Desert Locust hoppers.

Storage / Days	Number of insects tested	Percentage Mortality At Time After First Exposure /hours					
		Malathion					Blank
		0.5	1	2	3	24	24
1	30	0	3	43	73	100	0
7	30	0	3	47	80	100	0
14	29	0	3	55	90	100	0
29	16	0	0	13	69	81	0
35	29	0	0	7	14	83	0
42	30	0	0	3	10	67	0
70	30	0	0	0	0	17	3
95	26	0	0	0	0	3	3

Note that in some cases insufficient locusts were available to test all of the treated plates. When percent mortality was plotted Vs the age of the malathion deposit figure 5.1 was obtained.

Figure 5.1: Residual exposure of malathion to II instar *S. gregaria* : Percent mortality Vs age of deposit at various locust exposure times.

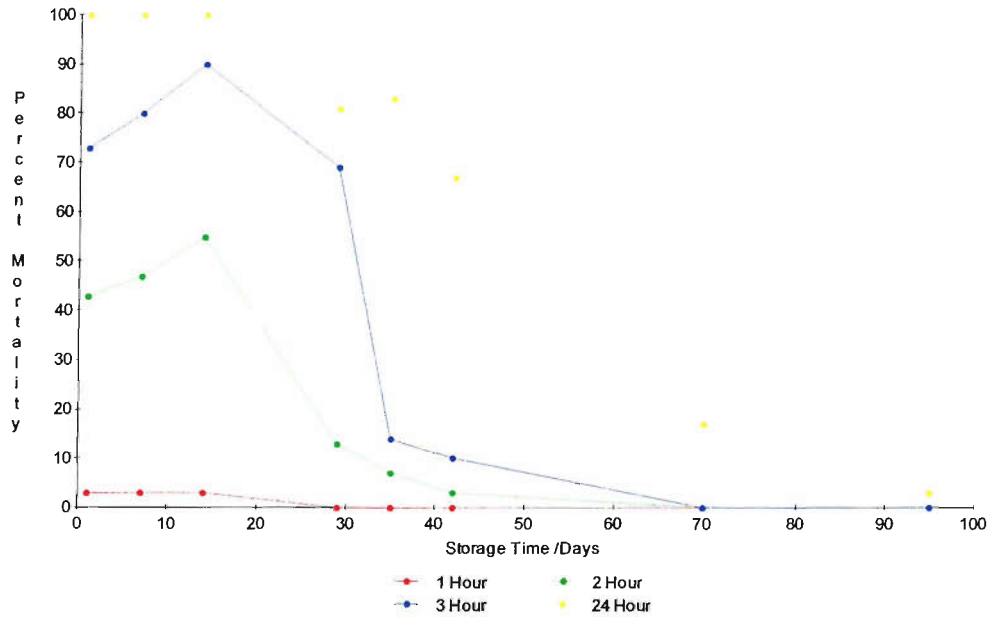
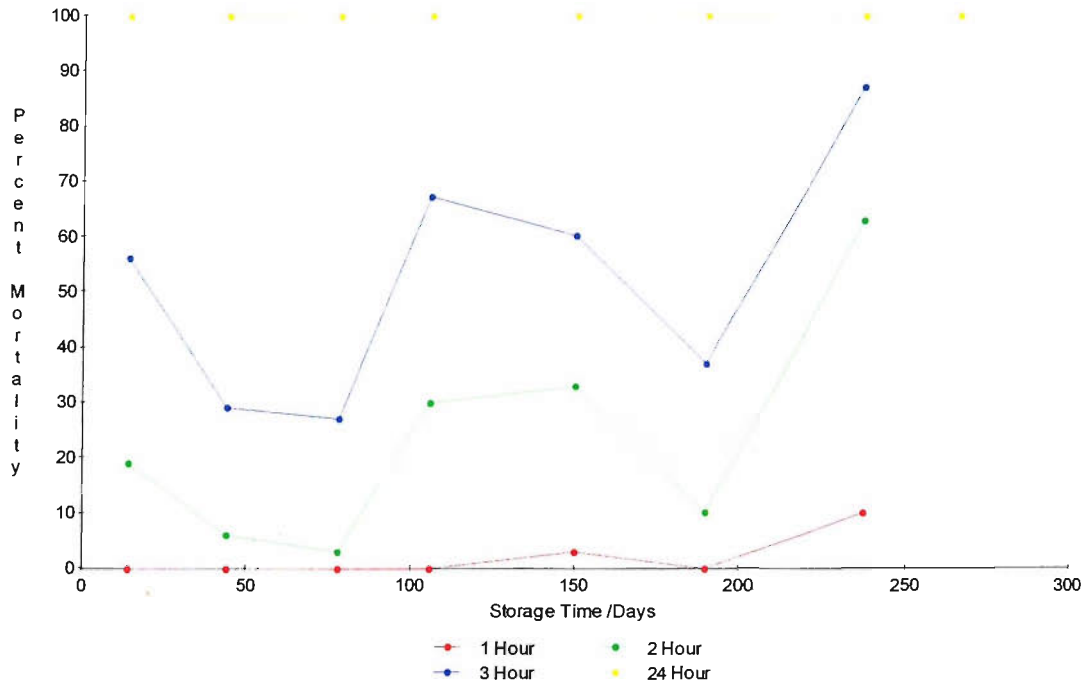


Table 5.9: Persistence testing of 5:1 c/w malathion microcapsule formulation with II instar Desert Locust hoppers.

Storage / Days	Number of insects tested	Percentage Mortality At Time After First Exposure /hours					
		Malathion Microcapsules					Blank
		0.5	1	2	3	24	24
14	16	0	0	19	56	100	0
44	17	0	0	6	29	100	0
78	30	0	0	3	27	100	0
106	30	0	0	30	67	100	0
150	30	0	3	33	60	100	3
190	30	0	0	10	37	100	0
237	30	0	10	63	87	100	13
266	30	0	N/A	N/A	N/A	100	13

The data in table 5.9 was plotted in the same manner as that for technical grade malathion and included as table 5.2.

Figure 5.2 Residual exposure of malathion microcapsules to II instar *S. gregaria* : Percent mortality Vs age of deposit at various locust exposure times.



3.3 Results of HPLC assays

Following completion of the tests the formulations were assayed by HPLC to ensure that the active ingredient had not chemically degraded on storage. The results are shown in table 5.10. All of the microcapsule formulations contained over the expected active ingredient level but this can be at least partially attributed to the elevated levels of active ingredient in the technical (nominal 95% w/w ai). The actual use rate of malathion shown above for each treatment was calculated based on this data and should be considered when the mortality data is interpreted.

Table 5.10: Assays of malathion microcapsule formulations and technical employed for testing.

Sample	% ai	Predicted % ai	Use Rate g ai/ha	% increase
Technical (Batch No. 31111-V)	96.2	95		
Technical (Batch No. 40414-V)	97.5	95	900	0
43-211	52.7	50	943	5
43-250	27.5	25	990	10
43-253	27.9	25	1,004	12
60-052	51.3	45	1,034	15

4 Discussion

Information in the literature relating to the increase in persistence due to microencapsulation is limited, but includes the following. Ivy (1972) reported that polyamide microcapsules of methyl-parathion for agricultural use persisted for five to seven days with several insect types compared with one or two days' persistence from a conventional emulsifiable concentrate formulation. Marrs & Scher (1990) reported microcapsules of lambda-cyhalothrin developed for control of public health pests. They gave effective control of *B. germanica* for eight weeks at half the rate of a conventional wettable powder formulation effective for only four weeks (residues on concrete). Tsuji (1990) tested polyurethane walled fenitrothion microcapsules against *Spodoptera litura* and found that persistence was highly dependant on the ratio of microcapsule wall thickness and mass median diameter. Some microcapsule formulations maintained full efficacy after over thirty days while a conventional emulsifiable concentrate formulation gave negligible control after three days.

Tests of microcapsule formulations with locusts are even fewer. Field trials were briefly reported by Steedman (1990) in which microcapsules approximately doubled the persistence of an active with a reduction in immediate kill. Holland & Jepson (1996) field tested normal phase microcapsules of fenitrothion, chlorpyrifos and diazinon against locusts and although initial efficacy was equivalent to unencapsulated fenitrothion no measure of persistence could be obtained.

The complete lack of data available on the performance of reverse phase microcapsule formulations and the complexity of microcapsule interaction with extrinsic factors makes the results of these experiments all the more important. Before discussing the results however a number of observations will be made concerning the test protocols.

During development of the protocols described above it was first attempted to apply malathion diluted in acetone to the glass surface and test locust mortality before complete drying. It was found that the acetone residues caused nymph mortality. Death caused by exposure to malathion residues (both microcapsules and active ingredient) was preceded by massive uncoordinated flexing of muscles while acetone residues caused complete

immobility, often the locusts dying on their feet. For this reason all residues were allowed to dry at least overnight before testing and the solvent Solvesso 200 was adopted for dilution of malathion technical.

It must be recognised that a number of factors may cause these experimental results to differ from those found in the field. Firstly glass is a totally artificial surface and vegetation, unlike glass, is likely to absorb malathion which is not encapsulated. Secondly malathion residues may be diluted by vegetation growth, a factor which may become significant over extended durations. Thirdly the droplet deposits may differ in nature from those produced by controlled droplet application (CDA) methods. Fourthly the contribution of mortality from malathion vapour was probably higher in the enclosed test arenas than in the open. Fifthly, in trial two, after successive testing of the same residues dried secretions from dying nymphs began to accumulate on the glass surface with an unknown effect on the residues. Finally residues in the field would be subjected to far harsher environmental conditions of temperature and sunlight and so the relative importance of degradation compared to evaporation of malathion may differ.

In the results from trial one although absolute value of residue persistence some trends with variation in microcapsule wall characteristics could be discerned within the data (tables 6.2 to 6.6). The data indicates that loss of activity from the malathion deposits was far more rapid than for the malathion microcapsule treatments. All microcapsule treatments remained 100% effective over the 5 week period while the malathion had very reduced activity after 2 weeks and none after 5 weeks. Control mortality was low throughout the experiments, rising to a maximum of 40% mortality in one isolated instance (table 5.4, one day after spraying) possibly due to contamination during spraying. Typically control mortality of 20% or less is considered adequate (Busvine, 1971).

Mortality from the unencapsulated malathion deposits varied slightly between individual experiments, which can be attributed to varying susceptibility between batches of locusts. However the data in table 5.6 shows a premature loss of activity from the glass plates treated with malathion.

The statistical analysis of the data from trial one indicates that there was no significant reduction in locust mortality when exposed to malathion microcapsule residues on glass plates over the 5/6 week storage period tested, except for the 10:1 core to wall ratio formulation. It was expected that the reduction in wall thickness, indicated by an increase in core to wall ratio, would give less protection for the active ingredient against losses due to evaporation and degradation. The data however indicates that this was not the case. It is possible that the single significant case of heterogeneity from the 10:1 core to wall ratio formulation was due to variations in locust susceptibility, but this is thought to be unlikely.

The bioassay results from trial two demonstrated an increase in residue persistence due to microencapsulation of malathion, (tables 6.8 & 6.9). Effective control of locust nymphs was extended two and a half times that of unencapsulated malathion with 100% mortality (24h exposure) still being achieved at termination of the experiment.
When compared to trial one the persistence of both malathion and microencapsulated malathion was increased despite identical application rate and locust age. This was probably due to the conditions under which the glass plates were stored. The treated plates for trial one were stored in a continuously running laboratory fumecupboard while those for trial two were stored in still air. From this it can be theorised that evaporation plays a large part in the loss of residue efficacy.

The data from trial two is displayed in graphical form in figures 6.1 and 6.2. When considering the data from 24 hour locust exposure the mortality from unencapsulated malathion exhibits a steady reduction in efficacy after approximately 20 days. It was attempted to generate from the data a persistence LT_{50} value by probit analysis but heterogeneity was significant at the 95% confidence limit. Residues of microencapsulated malathion exhibited no corresponding reduction in toxicity over the entire test period.

The toxicity of malathion microcapsule residues to locust nymphs has been demonstrated. An increased persistence of malathion microcapsule deposits compared to unencapsulated malathion has also been demonstrated.

CHAPTER 6

THE ORAL TOXICITY OF MICROENCAPSULATED MALATHION TO NYMPHS OF THE DESERT LOCUST *Schistocerca gregaria* (Forskål) (Orthoptera: Acrididae).

1 Introduction

Since the prohibition of organochlorine insecticides due to their damaging environmental effects large quantities of short persistence pesticides are now used as contact insecticides to treat desert locust (*Schistocerca gregaria*) infestations (Symmons, 1991). The highly persistent organochlorine insecticides such as dieldrin had the advantage that they could be used in the twin techniques of vegetation baiting and barrier spraying (Courshee, 1990). Dieldrin was aerially applied onto vegetation in diffuse, widely spaced parallel bands and the band spacing chosen to ensure marching nymphal bands met at least one band during development. The cumulative insecticidal effect and extended persistence of dieldrin ensured that marching bands of nymphs would be controlled for weeks from one application.

Concern over the environmental effects of blanket spraying short persistence insecticides (Everts, 1990, Grant, 1989, Mackenzie, 1988) makes a return to barrier spraying desirable if an alternative to dieldrin could be found. The insect growth regulators (IGR's) such as diflubenzuron may be suitable (Coppen, 1995) but care is required when applying due to the non cumulative nature; the barrier dose and width must be sufficient to ensure nymph mortality during a traverse (Steedman, 1990).

It is theoretically possible that the increased persistence obtained with microencapsulation of insecticides (DeSavigny & Ivy, 1973) could make them suitable for barrier spraying, subject to the same provisos as IGR's. Regardless, an increase in persistence coupled with greater selectivity (Koestler, 1980) would make microcapsule formulations desirable for blanket spraying.

The aim of this phase of the project was to ensure that microencapsulation of malathion did not reduce the toxicity to locusts when orally ingested. It is well known that microencapsulation may reduce the oral and dermal toxicity of insecticides (Ivy, 1972) and a reduction in the efficacy may be caused by overly thick microcapsule walls (Tsuji, 1990). However, insects such as locusts have a crop in the digestive tract (Uvarov, 1966) which may assist in the rupture of orally ingested microcapsules (Tsuji, 1990).

Experiments were described in the previous chapter investigating the toxicity of dried malathion microcapsule residues to desert locust nymphs. It is probable that the primary route of ingestion was absorption through the locust cuticle although oral ingestion and respiration of vapours was not prevented. For this phase of the project it was necessary for locust nymphs to ingest known doses of formulation in as realistic manner as possible under controlled laboratory conditions. In a review of the techniques for testing insecticides Busvine (1971) identified three methods for administering liquid stomach poisons; induced feeding where droplets are placed on the insect's mouth parts, forced feeding and free feeding for which the insect was allowed to feed *ad libitum* on poisoned bait. It was noted with the latter that unless single insects were fed small quantities of food the precise quantity ingested would be unknown or at best variable. Examples are known of testing 'synthetic' poison baits with grasshoppers (Kenaga, E.E. et al, 1965) but it was preferred to test droplets on vegetation for their similarity to spray deposits.

The protocol adopted was similar to that employed by Coppen (1995) for testing insect growth regulators against *S. gregaria* nymphs. A droplet of dilute formulation was placed on a single leaf and fed to a single locust with no attempt made to prevent the insect from contacting the insecticide before swallowing it. Due to logistical considerations the testing was divided into groups of doses. Malathion technical was tested first and applied using a syringe based Arnold Hand Applicator but the syringe based application method proved to be unsuitable for dosing of the malathion microcapsule treatments due to particle sedimentation. Thus a positive displacement pipette was adopted for microcapsule dosing which ensured complete dispensing of the microcapsules due to its mode of action.

2 Materials And Methods

The II instar *Schistocerca gregaria* nymphs used in this experiment were cultured as described in Coppen (1995) and the bioassay performed under the same environmental conditions as culturing. Stocks of technical grade malathion (95% w/w active ingredient) were obtained from Cheminova Agro and used both for microcapsule preparation and biological testing.

2.1 Malathion Technical Treatment

Before testing the technical grade malathion was diluted in alkali refined rape seed oil (Croda Chemicals Limited). The test protocol was as follows. Petri dishes with a diameter of 9 cm were filled with moderately damp sand and the surface smoothed. Single young barley leaves were freshly cut to a length of approximately 4 cm and then placed upright in the centre of the sand. A single droplet of malathion technical, or oil blank, was applied just below the leaf tip using an Arnold Hand Applicator (Burkhard Manufacturing Company Ltd., Rickmansworth, Hertfordshire) fitted with a 1 ml glass syringe. Rape seed oil was used in preference to solvents such as Solvesso 200 due to its probable lower inherent toxicity.

The dose of malathion applied to the leaf was varied using droplet volume and degree of dilution (on a mass/mass basis). Droplet volumes of between 0.25 to 1.0 µl were applied to the leaves and the average droplet mass used to calculate the exact dose. Single II instar desert locust nymphs were trapped over each petri dish with ventilated clear plastic drinks cups (284 ml capacity with 9 cm diameter opening) and the locusts allowed to eat at will. The nymphs had been starved for between one and four hours before use to increase appetite.

Locust mortality and consumption of the vegetation were assessed visually at increasing periods after exposure of the locusts. Any replicate in which the vegetation had not been completely consumed and the locust survived was disregarded.

2.2 Malathion Microcapsule Treatment

Testing of reverse phase malathion microcapsules was performed using the protocol described above for technical grade malathion. For a range finding experiment a 6% w/w solution of the dispersant Antaron V213 in Isopar M was employed as the diluent and blank treatment but for all subsequent tests neat Isopar was used. The dispersant solution was used to prevent agglomeration of the diluted microcapsule suspensions but proved unnecessary.
Formulation 60-109 was employed for the experiment containing microcapsules with 5:1 core to wall ratio and 100% cross linked polyurethane wall. When assayed the formulation contained 47% w/w malathion.

Initial studies with the Arnold Hand Applicator indicated that the syringe basis was unsuitable, the microcapsules sedimented within the syringe during use and the needle tended to block depleting the applied droplet of microcapsules. A positive displacement pipette proved more suitable and was employed throughout.
For the range finding experiment 2 µl volume droplets were employed for dosing but the low surface tension of the Isopar M droplet on the leaf allowed the liquid to spread downwards toward the sand base. For all the remaining experiments a 1 µl droplet volume was used.

3 Results

3.1 Malathion Technical Treatment

Firstly a 'range finding' experiment was performed. Doses of 0, 0.1, 1, 10 or 100 µg of malathion active ingredient were fed to locust nymphs with ten replicates for each dose. An ambient temperature of 26C was recorded during dosing and a droplet volume of 1 µl was

employed for all dose levels. Mortality of the nymphs was assessed 24 and 48 hours after exposure to the dosed vegetation. From this crude experiment it was determined that the LD_{50} value for unencapsulated malathion was between 1 and 10 µg per nymph, with no control mortality.

A series of four trials was then performed to produce mortality data within the required dose range. Logistical considerations forced using four individual hatchings of locusts. The results are shown in table 6.1.
Note that replicates in which the vegetation had not been consumed were disregarded, hence the variation in numbers of replicates.

Table 6.1: Mortality of II instar *S. gregaria* nymphs versus dose of malathion fed to them.

Trial	Droplet Volume /µl	Malathion Dose /µg per nymph	Percentage Mortality (No. of replicates)	
			24 h	48 or 56 h
A	1.00	3.99	93 (29)	100 (30)
A	0.75	2.99	93 (27)	93 (30)
A	0.50	2.00	86 (22)	76 (29)
A	0.25	1.00	63 (19)	59 (29)
D	1.00	0.98	0 (26)	0 (30)
D	1.00	0.97	0 (30)	0 (30)
D	1.00	0.95	7 (28)	10 (30)
C	1.00	0.94	22 (23)	21 (29)
C	1.00	0.89	16 (25)	14 (28)
C	1.00	0.84	4 (25)	3 (29)
B	1.00	0.80	13 (24)	12 (26)
B	0.75	0.60	8 (25)	7 (28)
B	0.50	0.40	0 (26)	4 (27)
B	0.25	0.20	0 (22)	0 (29)
Combined	1.00	0.00	0 (45)	0 (55)

The final assessments of trials A, C and D were at 48 hours while trial B had a final assessment at 56 hours. The ambient temperature during dosing was 27C for trials A and B

and 29C for C and D. It was noted during trial D that although the nymphs were the same age as for previous tests they appeared both larger and more vigorous.

It was apparent from the data that trials C and D were inconsistent with trials A and B. Whether this be due to ambient temperature during the test or differences in insect susceptibility is not known. Statistical analysis (see table 6.2) was performed on the data, both as a whole and with trials C and D excluded.

Table 6.2: The results of probit analysis on the mortality of nymphs after oral ingestion of malathion.

Data	Probit Slope	Probit Intercept	LD_{50} (±SE) (Detransformed) (µg malathion per nymph)	Heterogeneity Chi Square (d.f.) Signif.[a]
24 h A+B	3.942	-0.364	1.24 (0.19)	12.09 (6) ns
24 h All	4.678	-0.867	1.53 (0.87)	47.07 (12) s
48/56 A+B	4.107	-0.316	1.19 (0.33)	9.61 (6) ns
48/56 All	4.830	-0.810	1.47 (0.43)	55.26 (12) s

[a] Significance level: ns = not significant ($P > 0.05$), s = significant ($P < 0.05$)

3.2 Malathion Microcapsule Treatment

As described above for the malathion technical treatment, a 'range finding' experiment was performed initially. Locust nymphs were fed a 0, 0.1, 1, 14 or 143 µg dose of encapsulated malathion with ten replicates per dose. An ambient temperature of 32C was recorded at the trial's commencement and a droplet volume of 2 µl employed for all dose levels. Assessment after 21 and 52 hours indicated the LD_{50} value lay between approximately 1 and 14 µg malathion per nymph. Mortality from the blank treatment had risen to 50% after 52 hours but it must be noted that the blank treatment for the 'range finding' experiment only contained a small quantity of dispersant as described in the method above.

A full range of doses was then tested with increased replication the results of which can be seen in table 6.3.

Table 6.3: Mortality of II instar *S. gregaria* nymphs versus dose of microencapsulated malathion fed to them.

Trial	Malathion Dose /µg per nymph	Percentage Mortality (No. of replicates)	
		24 h	48 h
D	14.1	81 (21)	89 (28)
D	11.3	100 (22)	96 (27)
C	10.9	63 (27)	77 (30)
D	10.4	81 (16)	81 (27)
E	10.3	65 (26)	80 (30)
E	10.2	57 (28)	67 (30)
C	10.0	44 (27)	46 (28)
E	9.0	39 (23)	50 (30)
C	8.7	23 (30)	30 (30)
B	7.6	38 (24)	52 (29)
B	6.7	41 (22)	56 (25)
B	5.9	29 (21)	37 (27)
B	4.4	13 (23)	17 (29)
A	4.2	17 (24)	31 (29)
A	3.3	12 (26)	27 (30)
A	2.2	0 (27)	3 (29)
A	1.0	0 (25)	0 (30)
Combined	0.0	0 (46)	2 (48)

The ambient temperatures during dosing were 27C for trials A and C and 28C for the remainder. The dose value indicates the mass of malathion active ingredient applied; the formulation having been assayed by High Performance Liquid Chromatography at 47.3% w/w malathion (see previous chapter).

The data in table 6.3 was subjected to probit analysis giving the results shown in table can be seen in table 6.4.

Table 6.4: The results of probit analysis on the mortality of nymphs after oral ingestion of reverse phase malathion microcapsules.

Data	Time/h	Probit Slope	Probit Intercept	LD_{50} (±SE) (Detransformed) (µg malathion per nymph)	Heterogeneity Chi Square (d.f.) Signif.[a]
A+B+C+D	24	6.323	-6.246	9.72 (N/A)	24.22 (9) s
	48	3.681	-3.263	7.70 (N/A)	34.53 (10) s
A+B+D	24	5.834	-5.309	8.13 (1.32)	10.93 (6) ns
	48	4.146	-3.390	6.57 (1.70)	11.16 (7) ns
A+B+D+E	24	5.603	-5.336	8.96 (1.36)	14.81 (9) ns
	48	3.804	-3.202	6.95 (2.13)	17.82 (10) ns

[a] Significance level: ns = not significant ($P > 0.05$), s = significant ($P < 0.05$)

N/A = not available

3.3 Validation

In consideration of the change in dosing equipment between the two treatment types and the large number of nymph generations over which the trial spanned it was deemed necessary to check that the LD_{50} values for the two treatments were directly comparable, irrespective of the application method.

An experiment was performed (see table 6.5) to compare the effects of the two types of dosing equipment as well as investigating variation in locust susceptibility. Doses of 2.5 µg and 1.2 µg per nymph were equivalent to the previously measured LD_{90} and LD_{50} values respectively.

Table 6.5: A comparison of nymphal mortality obtained using two dosing methods at two dose levels.

Malathion Dose /µg per nymph	Dosing equipment	Percentage Mortality (No. of replicates)	
		24 h	48 h
2.4	Pipette	14 (7)	10 (10)
2.4	Hand Applicator	44 (9)	44 (9)
1.3	Pipette	0 (10)	0 (10)
1.2	Hand Applicator	0 (10)	0 (10)
0.0	Pipette	0 (8)	0 (9)

4 Discussion

The toxicity of the reverse phase malathion microcapsule formulation was found to be between six and seven times less than that of malathion technical alone. Within the limits of error no differences were found for mortality values at 24 and 48 hours after first exposure, for both treatments.

The microcapsules employed for these tests (formulation 60-109) possessed a relatively thick polyurethane wall (5:1 core to wall ratio) allied with 100% cross linking. Testing of an identical formulation (except for a slight difference in active ingredient content) indicated that a 5:1 core to wall ratio was sufficient to increase greatly the persistence of dried residues (see chapter 5). If required, it is feasible that the microcapsule wall thickness could be reduced sufficiently to improve the oral toxicity to locusts while still maintaining enhanced persistence of residues. The effect of nymph development stage on the relative efficacy of malathion microcapsules is unknown, but possibly the larger insects digestive tract would be more efficient at rupturing microcapsules.

Available literature on the toxicity of malathion to *S. gregaria* nymphs includes (MacCuaig, 1983) an LD50 value of 60ug/g for malathion technical and 72ug/g for a 10% dilution when administered to V instar Desert Locusts. Average insect masses for II and V instar are 50-80 mg and 1000-1200 mg respectively (Steedman,1990). A sample of 30 II instar nymphs from the stocks used in this experiment (killed by storage at sub zero temperatures) had an average mass of 48 mg (s.d. = 11.6 mg). Unfortunately it is not possible to calculate literature values for nymphs of a comparable age to those used in these studies.

Logistical considerations forced the testing of the two treatments to be tested consecutively and to be split into groups of doses with individual hatchings. Obviously this is not ideal because of the possibility of locust susceptibility varying over the experiment's duration. Of the intrinsic factors affecting toxicity, the age and growth stage were constant while no restrictions were placed on the natural distribution of insect size and sex. A test to validate

using the two types of application equipment (with reduced replication) indicated that the susceptibility of the culture to malathion had altered between testing of malathion technical and microcapsule treatments. Also the mortality response differed for the two application methods with identical doses of malathion, despite rigorous calibration of droplet masses. In all cases it must be noted that the steepness of the malathion technical dose response curve would exaggerate slight variations in insect susceptibility and dosing method.

Control mortality from dosing the diluent alone was low throughout the experiments and well below the commonly accepted limit of 20% (Busvine, 1971). The one exception was the range finding experiment with malathion microcapsules; in this case the elevated control mortality may have been due to the 6% w/w solution of the dispersant Antaron V213 used as diluent and blank treatment. For all other microcapsule testing pure Isopar M paraffin oil was used as the blank treatment.

Overall it may be concluded that the thick walled microcapsule formulation tested was less efficacious as a stomach poison for locust control than malathion technical. However, a reduction in microcapsule wall thickness may rectify this deficiency without having a large detrimental impact on residue persistence and beneficial toxicity.

In the next chapter the toxicity of malathion microcapsule residues to the indicator beneficial insect *Aphidius colemani* was investigated.

CHAPTER 7

THE TOXICITY OF RESIDUAL FILMS OF MICROENCAPSULATED MALATHION TO THE BENEFICIAL INSECT *Aphidius colemani.*

1 Introduction

Currently there are no alternatives to chemical insecticides suitable for locust control (Symmons, 1992). The persistent organochlorine insecticides such as dieldrin were highly efficient for locust control but have been withdrawn due to environmental concerns (Steedman, 1990). Due to these two facts large quantities of broad-spectrum short persistence insecticides are now employed with doubtful impact and possible harmful effects on the environment (Symmons, 1991). Some investigations have been made into the environmental effects of locust control (Everts, 1990; Mullie & Keith, 1993; Niassy, Beye & van der Valk, 1993; van der Valk & Kamara, 1993) and the required environmental impact studies have been planned (Grant, 1989). When considering impact of locust control operations on non-target, beneficial invertebrate populations Murphy et al (1994) attempted to merge the data from laboratory and field tests with indicator organisms to rank common insecticides in order of hazard.

In the sphere of locust control the term beneficial arthropod may be applied to predators and parasites but also to the natural enemies of grain pests and to pollinators (van der Valk, 1990). Large numbers locust antagonists are known (Greathead, 1963; Prior & Greathead, 1989) but the importance of individual beneficial arthropod taxa in the Sahelian ecosystem is little understood (van der Valk, 1990).

It is recognised that selective pesticides are urgently required for use in integrated control programs (Hassan et al, 1994) but the question was posed could reformulation of an existing insecticide have an impact on the toxicity to non-target organisms? In this instance a non aqueous microcapsule formulation of the organophosphorus insecticide malathion was tested for acute toxicity to the indicator species *Aphidius colemani*. With so little known about Sahelian beneficial arthropods *A. colemani* was chosen for availability and ease of handling.

The effects of microencapsulated insecticide formulations on beneficial insects are poorly documented and the data that does exist relates to aqueous based formulations. One of the earliest commercial products was PENNCAP-M®, an aqueous based methyl parathion microcapsule suspension (Ivy, 1971) with polyamide polyurethane copolymer microcapsule walls. In a review of field data concerning effects of PENNCAP-M® on entomophageous insects (parasitic and predatory) Dahl & Lowell (1984) found that for the most part insect populations were reduced by less than 50%. It was concluded that the effects on many parasitic and predatory insects were lessened due to the reduced use rates employed with PENNCAP-M®, especially if a degree of tolerance already exists in the population. What is more important there were some indications that the commercial product PENNCAP-M® exhibits a degree of selectivity, beneficial insects remaining unharmed at use rates that achieve pest control (Koestler, 1980). However, it is important to note that bees may be at increased risk due to dried microcapsules being carried back to the hive with pollen (Koestler, 1980). This may be of special importance since unencapsulated malathion applied from the air using ultra low volume (ULV) equipment remained toxic to bees for up to 7 days (MacCuaig, 1983).

One difficulty is that extrapolation of the results from temperate test conditions to typical Sahelian conditions may be misleading (Grant, 1989) and indeed we cannot with certainty transfer the knowledge gained from simple laboratory tests to a complex natural ecosystem (Müller, 1990). However laboratory tests were required to test any improvement in selectivity caused by microencapsulation.

Insects of the order hymenoptera and particularly parisitoids are understood to be very vulnerable to the effects of pesticides (Elzen, 1989), especially the adult stage of the life cycle (Stary, 1970). Since 1975 the International Organisation of Biological Control/West Palearctic Regional Section (IOBC/WPRS) (Hassan, 1989) has held the main discussions on developing test methods for determining the risk to beneficial insects from chemical control methods (Jepson, 1993b). It was concluded for the purposes of this project that a standardised test method, of the type developed by IOBC/WPRS, was not required since the primary aim was to compare the toxicity of malathion technical and a microcapsule

formulation to a beneficial insect rather than produce an overall indication of environmental risk.

A basic test method was adopted exposing adults of *Aphidius colemani* to dried deposits on a glass substrate. In previous tests of this type the aqueous based insecticide deposits were allowed to dry at room temperature for approximately one hour before testing (Longley, 1995; Mead-Briggs, 1992), due to the use in this instance of oil/solvent based applications a longer period of drying was required.

Laboratory tests of this type represent a worst case situation (Brown et al, 1990) in which the insect is confined to the treated surface. The exposure of insects to pesticide deposits is partially controlled by insect factors such as activity levels, behaviour and the degree of contact with the treated surface (Jepson et al., 1990; Jepson 1993a; Wiles & Jepson, 1993). Additionally exposure is modified by the chemical and physical nature of the pesticide deposits; it must be noted in the context of this project that both treatments are non aqueous and thus may differ markedly in physical properties from the more common aqueous based treatments. Finally exposure of the insect to the pesticide is modified by interaction of the deposit and substrate; adsorption, absorption and evaporation reduce exposure to the insect and vary depending on the substrate (Adams et al, 1987; Arnold & Briggs, 1990). When testing an aqueous formulation of deltamethrin with *Aphidius rhopalosiphi* using a similar method, Longley (1995) found residues on glass to be between 1.3 and 1.8 times more toxic than residues on vegetation and it was also found that LT50 values decreased proportionally with increasing concentration. It must be noted that a simple residual exposure test is too variable to allow comparison between different insect species (Jepson, 1993b).

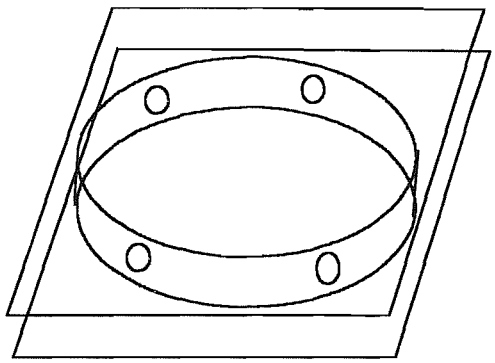
2 Materials And Methods

The test method adopted was modified from that of Mead-Briggs (1992).

2.1 Test Arena

The test arena shown in figure 7.1 consisted of a section of plastic pipe sandwiched between two treated glass plates and held with elastic bands.

Fig 7.1: Experimental apparatus employed for testing treatments with *Aphidius colemani*.



The section of plastic pipe had a diameter of 10.5 cm diameter, height of 2.5 cm and was drilled with five holes of 10 mm diameter. Three of the holes were covered on the inside with gauze to prevent escape of insects while allowing ventilation. One hole was employed for addition of the insects and subsequently blocked with cotton wool, the tip of which had been soaked in a 2:1 honey:water solution. A rubber bung pierced with a hypodermic syringe needle was fitted in the remaining hole and used to provide humidified air from a small capacity pump and dreschel bottle filled with water.

Before each experiment the glass plates were washed in a commercial machine at 45C and then soaked in detergent solution (Decon 90, Decon manufacturing Ltd.) for 30 minutes followed by thorough rinsing. The sections of plastic pipe were also cleaned in detergent solution.

2.2 Spray Application

The glass plates described above were treated with insecticide or blank diluent using a Potter spray tower (Potter, 1952) manufactured by Burkhard Manufacturing Company Ltd., Rickmansworth, Hertfordshire. The Potter spray tower was fitted with a twin fluid nozzle pressurised at 3.5 PSI and the deposit mass controlled by adjusting the volume of spray liquid (measured by Gilson pipette). The apparatus was adjusted to give a deposit mass of 0.018g liquid on a 10x10 cm glass plate; equivalent to a field rate 900 g malathion/ha when applying 5% w/w active ingredient dilution's of technical grade malathion or microencapsulated malathion. Once calibrated the process was used to treat 12x12 cm glass plates for use in the test arena described above.

The treatments tested were malathion microcapsules, malathion technical, blank and unsprayed plates. Blank treatments comprised glass plates sprayed consecutively with the diluents of the two malathion treatments. The malathion microcapsule formulations were diluted in Isopar M (Exxon Chemical Company) before application and control treatments of unencapsulated malathion were applied diluted in the solvent Solvesso 200 (Exxon Chemical Company). A number of malathion use rates were tested varying between 5 times field rate (FR) and 1/75th of FR, achieved by keeping deposit mass constant and altering the malathion content of the spray liquid. Reverse phase (oil based) microcapsule formulation 60-109 was used throughout (see chapter 3), containing 47.3% malathion active ingredient. The microcapsules had a relatively thick wall (5:1 core to wall ratio, see chapter 3) composed of 100% cross linked polyurethane.
The treated glass plates were allowed to air dry at room temperature overnight before assembling into test arenas with treated surfaces innermost. Note that an extended drying time was required when compared with the more typically tested aqueous treatments.

2.3 Insect Testing

Due to logistical considerations the use rates were grouped together and tested with individual batches of insects. For each treatment, at each use rate, thirty insects were tested divided among three test arenas. For each group of tests, blank treatments were tested with the same replication and where possible unsprayed plates were tested with the same replication.

The *A. colemani* were obtained from Koppert Biological Systems and stored at 4C until ready for use. Ten insects were carefully placed in each test arena using a soft artists brush with no attempt being made to identify and separate the insect sexes. An ambient temperature of 3 to 4C was used while handling the insects to render them immobile. The test arenas were transported to a controlled environment room (17-20C, 16h light and 8h dark) and connected to the air supply that was necessary to prevent accumulation of insecticide fumes within the cell.

The condition of the insects was visually monitored periodically and classified as live (upright and/or moving) or dead (immobile and prostrate). No evidence was witnessed that the deposits were adhesive which would have lead to physical damage of the insect.

Probit analysis was performed on the mortality data to obtain dose-response statistics (Finney, 1971).

3 Results

In most cases no control mortality was evident but in all cases it was below 10% of the population and thus within the commonly accepted maximum of 20% (Busvine, 1971). No differences were noted between blank treatments and unsprayed plates.

It was attempted to extend the data to lower malathion use rates but over the extended experimental duration 'natural' mortality became an important factor.

Table 7.1: Toxicity of malathion and malathion microcapsule residues to *Aphidius colemani* with varying use rate.

Dose of malathion applied[a]	Probit mortality regression equation[b]	LT50 (±SE) Hours	Heterogeneity χ^2 (df) Significance[c]
Malathion Technical			
5 x FR		< 0.58	
FR		≤ 0.83	
1/5 FR	-9.18 + 9.04x	0.62 (±0.03)	0.34 (3) ns
1/10 FR	-9.34 + 9.11x	0.62 (±0.03)	0.18 (3) ns
1/10 FR	-11.53 + 8.68x	1.34 (±0.09)	11.07 (5) s
1/10 FR	-12.10 + 9.90x	0.89 (±0.04)	2.05 (5) ns
1/30 FR	-14.73 + 10.00x	1.57 (±0.07)	1.60 (5) ns
1/50 FR	-3.16 + 3.62x	2.98 (±0.28)	12.79 (14) ns
1/50 FR	-19.99 + 11.61x	2.36 (±0.10)	0.43 (4) ns
1/75 FR	-15.59 + 9.86x	2.04 (±0.10)	0.47 (6) ns
Malathion Microcapsules			
5 x FR	-12.88 + 9.55x	1.24 (±0.06)	1.54 (6) ns
FR	-28.08 + 16.73x	1.58 (±0.04)	0.88 (6) ns
1/5 FR	-18.74 + 11.96x	1.61 (±0.05)	2.48 (7) ns
1/5 FR	-6.37 + 5.73x	1.60 (±0.09)	4.68 (14) ns
1/10 FR	-16.93 + 9.78x	2.91 (±0.13)	2.39 (7) ns
1/10 FR	-8.30 + 6.26x	2.22 (±0.13)	2.02 (9) ns
1/10 FR	-1.08 + 2.33x	6.78 (±3.3)	20.51 (6) s
1/50 FR		8 - 24	

[a] Assumed field rate (FR) of 900 g malathion per hectare.

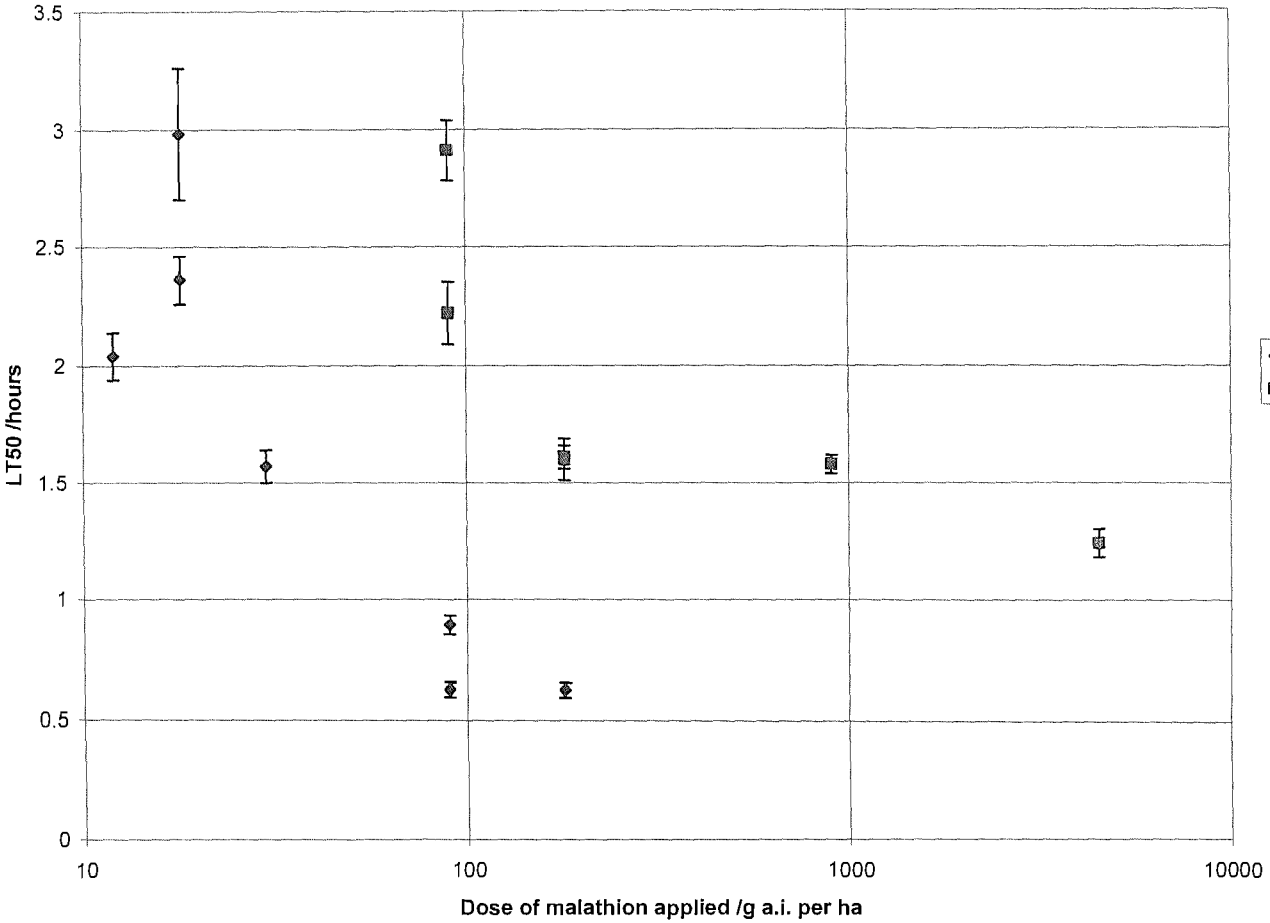
[b] Probit mortality regression equation: Y is the probit mortality, a is the intercept value, b is the slope of the probit line and X is time in minutes.

[c] Significance level : ns = not significant, s = significant ($P < 0.05$).

Note that for both treatments the use rate was calculated based on assay of the concentrated liquid by high performance liquid chromatography (HPLC).

The LT50 values were plotted versus use rate with the results shown in figure 7.2, error bars indicate the standard error associated with each value.

Fig 7.2: Plot of LT50 values (hours) obtained from testing malathion microcapsules and technical with Aphidius colemani.



Error bars indicate standard error associated with each value.

4 Discussion

As previously stated laboratory tests of this nature represent a worst case scenario (Brown et al, 1990) with the insect being confined to the treated surface. Despite this the microencapsulated malathion displayed a significant increase of median lethal time to death (LT_{50}) of one to two hours compared to malathion technical. In the field where insects are free to leave the treated area, or shelter on untreated surfaces, this selectivity is likely to be of importance in the survival of populations. It is unlikely that selectivity could be further increased by thickening the microcapsule wall without a detrimental reduction in oral toxicity to locusts (see chapter 6). It is possible that co-formulation with chemicals repellent to beneficial insects could improve selectivity without reducing efficacy (Matthews, 1992).

Although these results are promising the effect of substrate type may modify the results when tested in the field (Busvine, 1971). For these laboratory tests non-absorbent glass was employed as substrate, while in the field residues are most likely to be encountered on vegetation which is likely to be partially absorbent to the lypophilic malathion. The question arises whether aged microcapsule residues would be a more efficient source of malathion to beneficial insects than aged and partially absorbed malathion. Vegetation was not used as the substrate in these tests due to the great variability in surface characteristics and degradation of the plant tissue over the extended test duration's associated with lower use rates.

Finally when considering the use of these formulations in the field it must be recognised that tests of this nature only indicate acute toxic effects. Microcapsule formulations by their nature increase the residence time of the active ingredient in the environment with unknown chronic effects on beneficial arthropod populations.

In summary these experiments have indicated that reverse phase malathion microcapsules are capable of selectivity in contact toxic effect towards insect species. The range of this selectivity toward non-target organisms requires further investigation but is beyond the scope of this project.

CHAPTER 8
GENERAL DISCUSSION

This dissertation consists of laboratory trials aimed at developing insecticide formulations optimised for the control of locusts. The microencapsulation of a number of insecticides was investigated and a range of malathion containing formulations tested for efficacy and toxicity to an indicator beneficial insect.

Formulations of *Metarhizium flavoviride* fungal conidia were developed for application to locust populations using ultra low volume application equipment (Chapter 2).
Development of these formulations presented an additional challenge in that survival of the fungal spores depended on preparation with non 'toxic' formulation aids. Due to the novel nature of these oil based formulations a number of new test methods had to be developed and also a specification to ensure continued stability; this was successfully achieved although selection of formulation components for low toxicity to the conidia was only possible using an empirical approach.
A slightly modified version of the formulation is shortly to be commercialised under the trade name Green Muscle™, indicating the success of this phase of the project.

Although formulations of locust pathogens offer obvious environmental benefits the delay in locust mortality after application ensures that formulations of synthetic organic insecticides will still be required. One fundamental problem associated with locust control is it's extended cyclical nature, unfortunately the prospect of having large sales volumes once every five or more years is insufficient incentive to commercially develop bespoke formulations tailored to locust control.
With these problems in mind the next phase of the project (Chapter 3) investigated the use of controlled release formulations for locust control . The technique of microencapsulation appeared particularly suitable, offering as it does the potential to increase formulation persistence and also reduce the toxicity to beneficial insects. A number of synthetic insecticides were investigated but finally a novel formulation was developed of the

organophosphorous insecticide malathion, encapsulated by interfacial polycondensation and suspended in a mineral oil continuous phase. A range of similar malathion formulations were prepared with differences in microcapsule wall structure achieved by variation of monomer type and quantity, along with the use of catalysts to modify the rate of polymer deposition and hence density. Since the rate of active ingredient release from a microcapsule is dependant on the polymer characteristics it was expected that the formulations would exhibit differing persistence and toxicity to beneficial insects, this was the subject of subsequent chapters.

The initial biological experiments grouped in chapter 4 were concerned with the testing of both formulation efficacy with the desert locust (*Schistocerca gregaria*) and toxicity to an indicator beneficial insect species. One of the main aims of the efficacy testing was to ensure, as far as possible, that the results were a true reflection of what would be found in the field. Therefore a spinning disk, ultra low volume (ULV) sprayer was used to apply the oil based malathion microcapsule formulations to trays or pots of vegetation, using unencapsulated malathion as a control treatment.
It became clear that the methodology suffered from fundamental flaws. Firstly using a spinning disk sprayer without wind assistance lead to heterogeneous droplet coverage on the vegetation and secondly using relatively large quantities of vegetation (for this type of test) caused difficulties in assessment of locust mortality. The beneficial insect test was performed using the same ULV sprayer, again resulting in uneven dosing of the formulation. For both types of test no meaningful conclusions could be drawn from the results. In all of the subsequent tests the need for realism was abandoned in favour of closer control of the experimental conditions.

In the next experimental phase the cuticular toxicity of microencapsulated malathion to *S. gregaria* nymphs was tested. A Potter spray tower was used to apply even deposits of formulation or active ingredient to glass slides and then a single locust nymph was confined over the treated surface. Glass was chosen for the substrate in preference to vegetation because it allowed the testing of deposit persistence after ageing, but it is clear that the fate of spray deposits on glass may differ from those on a leaf surface. Also it must be noted that no attempt was made to block the nymph's mouth parts so a proportion of oral

ingestion may have occurred, possibly during 'grooming'. Unfortunately due to the logistical constraints of pursuing a part-time project the testing had to performed with consecutive treatments split over multiple locust hatchings.

The test results clearly indicated that the microencapsulation of malathion did not reduce the toxicity of spray deposits to locust nymphs. Additionally it was clear that microencapsulation did increase deposit persistence, although an absolute value of persistence increase could not be obtained because the microcapsule deposits exhibited no reduction in toxicity at termination of the study. Even though ambient conditions in the field differ markedly from those found inside a laboratory (sun light, temperature, humidity, etc.) the comparative increase in persistence is still valid. Studies with differing microcapsule wall construction indicated no major differences in deposit persistence, possibly because the chosen malathion use rate was too high.

Following confirmation of the cuticular toxicity of malathion microcapsules the oral toxicity to locust nymphs was similarly investigated (chapter 6). A malathion microcapsule formulation or technical grade malathion was applied to single barley leaves which were then fed to individual locust nymphs, thus providing a known dose. Again the treatments had to be tested consecutively and using multiple hatching's of locust nymphs due to the logistic considerations of a part time project.

The malathion microcapsule formulation was found to be between six and seven times less toxic than that of technical grade malathion. Obviously for use in locust control operations this is less than ideal but the experiments in chapter 5 had indicated that microencapsulation did not reduce the cuticular toxicity. It is feasible that the microcapsule wall thickness could be reduced in future to increase the oral toxicity to locusts but care is required that persistence is not reduced or the toxicity to beneficial insects increased.

In the final phase the acute toxicity of malathion microcapsule residues to an indicator beneficial insect was tested. Under European Commission of Council Directive 91/414/EEC concerning the placing of plant protection products on the market, the toxicity of formulations to beneficial insects must be tested prior to sale. The regulations indicate that parasitic wasps of the genus *Aphidius*, commonly found in the European cereal

ecosystem are suitable. Although not native to locust habitats the species *Aphidius colemani* was used as an indicator species in these tests.

The test protocol involved exposure of *A. colemani* to glass surfaces bearing dried insecticide deposits. The glass plates were constructed into a container with the treated surfaces innermost and the insects contained within provided with food and a fresh air supply. This type of test represents a worst case scenario where the insects are incapable of moving away from the treated surface, even so microencapsulation of malathion gave an increase of median lethal time to death of one to two hours. Although the results are promising they do not indicate the chronic effects of exposure and in no way indicate the overall environmental impact of this new microcapsule formulation

To summarise two novel formulations were developed specifically for use in locust control operations, one an oil based 'flowable' formulation of fungal conidia and the other an oil based microcapsule suspension (CS) containing malathion. The malathion microcapsule formulation was found to be efficacious against desert locust nymphs by both cuticular and oral ingestion routes and also exhibited reduced toxicity to an indicator beneficial insect. Importantly the formulation also exhibited a large increase in persistence of spray applied deposits compared to the unencapsulated active ingredient.

The question arises, what implications do these results have for locust control? Firstly the fungal conidia formulation is to be used commercially in the near future and so it's impact in control operations will be directly measurable. Secondly with respect to the malathion CS formulations although the laboratory test results were promising unfortunately it was not possible within this project to perform field testing. Only through field testing could the benefits of microencapsulation be proved and the microcapsule wall properties optimised. The key aim for the future would be to optimise a formulation for use in the highly efficient technique of aerial barrier spraying developed using the now prohibited organochlorine insecticides; this would require from the formulation high oral toxicity to locust nymphs, long spray deposit persistence on vegetation and reduced toxicity to beneficial insects. It is hoped that this project will prompt further work on the use of advanced formulation techniques for locust control.

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