Climate Matching in the Colonisation of Biological Control Agents against *Chrysanthemoides monilifera* and *Marrubium vulgare*

Craig R. Clarke

Thesis submitted for the degree of Doctor of Philosophy

Department of Applied and Molecular Ecology
Faculty of Agriculture and Natural Resource Sciences
University of Adelaide

March, 2001

DECLARATION

This work contains no material which has previously been accepted for the award of any other degree or diploma in any university or other tertiary institution. To the best of my knowledge and belief, it does not contain any material previously published or written by another person, except where due reference has been made.

I give consent that this work may be photocopied or loaned from the University library.

Signed   Date   30.10.01

        Craig R. Clarke

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

ABSTRACT

Approximately 40% of releases of classical biological control agents targeting weeds fail to establish in the field and 70% fail to have a significant impact on the target weed (Julien *et al.*, 1984). Many theories have been proposed to explain these failures but, to date, little experimental research has been conducted to test them. In this project, several theories relating to the establishment of biological control agents were tested using two model systems, the bitou tip moth, *Comostolopsis germana*, for the control of the weed *Chrysanthemoides monilifera* and the plume moth, *Wheeleria spilodactylus*, for the control of horehound, *Marrubium vulgare*. The importance of climate matching, predation, release technique, laboratory adaptation and inbreeding to the establishment of these agents was investigated.

In both examples, it was believed that the moth populations failed to establish in South Australia because they were poorly adapted to South Australia's Mediterranean climate, particularly its dry, hot summers. The original imported populations of the bitou tip moth (from Durban, South Africa) were successfully established in coastal areas of New South Wales, which have a sub-tropical to warm temperate climate. The original imported population of the plume moth from southern France was established in the higher rainfall areas of north eastern Victoria, but apparently failed to establish in drier areas of south eastern Australia.

The hypothesis that the original populations of each insect imported into Australia failed to establish because they were poorly adapted to South Australia's climate was incorrect for the plume moth and only partially correct for the bitou tip moth.

The original populations of the bitou tip moth probably failed to establish in the field primarily due to ant predation. This insect population was also found to be poorly adapted to host plants that are suffering from moisture stress. This may have prevented their establishment even if ants had not been a problem. A population originating from Cape Town was better adapted to poor quality host plants, but this population also failed to prosper due to ant predation. The

relationship between the two bitou tip moth populations was not clear, they may be populations at either end of a cline or sibling species.

The plume moth insect populations under investigation were the original French collection (reared in the lab since 1992) and Spanish and French populations that were imported in 1996 and 1997, respectively. Because the Spanish population was obtained from an arid region, it was proposed that this collection would be better suited to South Australian conditions. Consistent with this hypothesis, the original French population established at fewer field sites and had a slower rate of population increase compared to the newly imported populations. However, results from laboratory studies have shown that the original French population was suffering from inbreeding depression and had almost lost their ability to enter an aestival diapause. In contrast to predictions on climate matching made on the basis of temperature and rainfall, the newly imported French and Spanish populations are both performing well in the field.

Chapter 1

Introduction and project aims

1.1 Introduction

Biological control has been practiced for centuries. The ancient Chinese are thought to have used the ant, *Oecophylla smaragdina*, to control certain pests of citrus and another ant, *Monomorium pharaonis*, to help in the control of pests of stored produce (Simmonds *et al.*, 1976). The modern era of "classical" biological control began in 1888 with the introduction of the predatory ladybird, *Rodolia cardinalis*, from Australia to the United States to control the cottony cushion scale, *Icerya purchasi*, a pest of citrus (Simmonds *et al.*, 1976). The first major classical biological control program against weeds commenced in 1902 with the introduction of phytophagous insects to Hawaii from Mexico to control *Lantana camara* (DeBach, 1964; Harley and Forno, 1992).

Classical biological control involves the introduction and establishment of natural enemies of exotic pest species to suppress the pest's population. The aim is not to try to copy the complex ecosystem from whence the pest came, merely to introduce a few key biotic agents that have been observed to attack the pest in its native range.

The range and density of a given population can be regulated by biotic or density dependent factors such as natural enemies, food supply and competition, and abiotic or density independent factors, such as climate. In a natural ecosystem, a population often remains relatively stable, fluctuating between certain upper and lower limits. This regulation occurs despite the fact that most organisms produce far more offspring than would be required to maintain their populations (Price, 1975). Biological control focuses on natural enemies in this regulation.

Within an organism's native range, it may inhabit all areas with suitable abiotic conditions, or its distribution and density may be regulated by natural enemies and/or interspecific competition from other species competing for the same resources.

The population of a phytophagous insect, which has evolved to utilise a specific plant species to complete its life cycle, is regulated by the abundance of that plant species, environmental conditions, competitors and its natural enemies (Debach, 1974). The population of an agent released for the biological control of a weed would be expected to increase until the population of the weed starts to decline. However, reviews of past biological control programs have shown this pattern of colonisation rarely occurs in practice. A significant proportion of released biological control agents either do not establish or have little impact on the target weed (Hall *et al.*, 1979; Julien *et al.*, 1984; Crawley, 1989, A; Cameron *et al.*, 1993).

Julien *et al.* (1984) evaluated 174 weed biological control projects conducted worldwide up to 1980 to control 101 weed species. A total of 499 releases of exotic invertebrates and fungi were made. Insects comprised 98% of the releases. Sixty four percent of released organisms established in the field and 29% were effective as control agents. These data omitted 43 releases whose fate was unknown at the time, most of which have since been reported as not established (Julien, 1989).

Cameron *et al.* (1993) evaluated biological control programs conducted in New Zealand up to 1991. A total of 341 species had been introduced into New Zealand for the control of insect pests and weeds. Thirty percent of the 341 species imported were not released due to rearing difficulties or ecological problems. For agents that were released, there was an establishment rate of 30.2% for entomophagous agents and 35.3% for phytophagous agents. Of the 70 programs where establishment was achieved, 37% resulted in some beneficial impact on the target pest.

Internationally the establishment rate for phytophagous agents appears to be higher than for entomophagous agents. Hall *et al.* (1979) calculated that the worldwide establishment rate of natural enemies against insects and arachnids was 34%, in contrast to 64% for phytophagous agents (Julien *et al.*, 1984). The difference may be due to the more transient nature of invertebrate pest populations (in time and space) and/or the greater intra-specific genetic variation among insect pest populations.

Overall rates of effectiveness relative to the number of biological control releases has declined over time (Julien 1989). One would think that success rates would have increased with an increase in knowledge. Julien (1989) stated that, "despite numerous studies on plant/herbivore interactions, few have contributed to the development of ecological theory". He concluded, as others have done (eg. Cullen, 1995), that the only way to improve success rates is through greater study of the ecology and population dynamics of weeds and their herbivores.

One factor which may have hindered improvement in success rates in recent times is the more stringent tests an agent must now pass before approval for release is granted (Greathead, 1997). The fear of releasing an agent that may have an effect on non-target species, based on potentially abnormal behaviour under artificial conditions in host specificity testing, may be eliminating many effective agents from consideration for release (Cullen, 1989). It has also been suggested that the easy biological control programs may have already been attempted. Julien *et al.* (1989) noted that the success rate for phytophagous agents was particularly high between 1910 and 1940, due to a few oligophagous insects controlling a range of cactus species. Lastly, Myers and Sabath (1980) suggested the decrease in success rates may be due to an increased emphasis on laboratory rearing.

A large number of hypotheses exist that aim to explain the failure of biological control agents to establish in the field. The more commonly discussed theories are: poor climate matching (Crawley, 1986); low genetic variability of the agent (reviewed by Roush, 1990); release method, number and timing (Hopper and Roush, 1993); laboratory adaptation (Mackauer,

12

1980; Myers and Sabath, 1980); aspects of the target weed's biology (Burdon *et al.*, 1980); disease and predation (Hoffman, 1980); competition with other natural enemies (Hoy, 1985); habitat stability (Beirne, 1975); and the lack of alternative hosts and rearing sites (Scott, 1992).

Hypotheses relating to the failure of biological control agents to establish, or to have an effect on the target pest, have generally been based on observations of field releases rather than sound experiments. A number of texts have listed the optimal characteristics of biological control agents and produced point scoring systems (eg. Harris, 1973). After reviewing a number of case studies where results have varied with location and agent, Cullen (1995) concluded that none have been satisfactory and the generalisations produced can be dangerous in neglecting the exceptions. Furthermore, experiments with classical biological control agents offer an ideal testing ground for hypotheses developed in the fields of ecology, evolutionary biology and population genetics.

Despite the apparent low success rate observed in classical biological control, the return on investment compares favourably with other pest control techniques. In California, it has been estimated that for every dollar spent on biological control there is a return of $US30 (Huffaker *et al.*, 1976). In Australia, Tisdell (1990) estimated the economic benefits of biological control targeting weeds to be over $1 billion. The fact that more emphasis has not been put on biological control may be because it is viewed as being unreliable (Beirne, 1985) and risky (Thomas and Willis, 1998). Successes are generally not well publicised, and are quickly forgotten by the public because agents go about their business quietly and their effects are gradual.

Reviews of past programs have suggested that climate matching is one of the more important factors responsible for the failure of agents to establish or have a significant impact on the target weed (Crawley, 1986; Cullen, 1995). Crawley (1986) found that poor climate matching was thought to have been an important cause of failure in 44% of weed biological control projects evaluated. Cameron *et al.* (1993) suggested that the large difference in establishment

rates between New Zealand (35% for phytophagous agents) and the rest of the world (64%) may be due to the difficulty in obtaining biological control agents from matching climates. However, the worldwide figure may also be inflated by the repeated release of a few highly successful agents against cacti and lantana.

Cullen (1995) reviewed 25 weed biological control programs where significant weed control was recorded in at least one country or habitat and failures were observed elsewhere. He found there were a total of 30 possible factors which may have resulted in the observed variation in the effectiveness of agents between countries or habitats. Effects of temperature or moisture on the agent or target weed were thought to be important factors resulting in the failure of agents to establish or suppress the weed in 14 of the 25 programs evaluated.

1.2 Climate matching

Organisms have evolved diverse ecological, behavioural and physiological adaptations to overcome the many problems they must face in their native environments (Tauber and Tauber, 1978). Because the environment in the native range of a biological control agent is never identical to the new environment where the agent is to be released, climate is thought by many to be an important impediment to the establishment of classical biological control agents. Practitioners of biological control generally agree that the chances of successfully establishing an agent are increased if the colonised environment matches the environment of the agent's native range and thereby the organism's climatic adaptation. However, there is disagreement about how important climate matching is and little guidance about how close a match is required. Is climate matching only important when transferring agents to areas where some climatic features are more extreme, or can climate also prevent establishment even when climates appear similar? If a close climate match is important, how can we assist with a population's adaptation to the new environment?

14

1.2.1 Reviews of past bio-control programs - importance of climate

Poor climate matching has commonly been used to explain the failure of particular biological control agents to establish or suppress the target pest (Crawley, 1986; Cullen, 1995)

To determine how important climate matching has been to the establishment of biological control agents, Leen (1991) analysed 527 records of releases, made with 30 species of phytophagous insects, conducted from a laboratory in California, USA, from 1944 to 1986. A comparison of source and release climates for the entire set of releases was made for agents that did establish, and for those that did not. Using mean temperature and rainfall values from Walter and Leith's Klimadiagramm-Wetatlas (1960-1967), there was found to be a significant difference between the source and release climates in both cases. Release climates where insects did establish were also compared to the release climates where the same agent failed to establish, and were not found to be significantly different, suggesting that climate was not an important factor in preventing establishment. However, Leen provided no details on her statistical analyses or data summaries.

Leen assumed that climatic factors were likely to have been responsible for poor establishment if the insects were released into a wide range of climates and successful establishment was achieved at some but not all sites, as was the case for *Coleophora* spp. released for the control of *Salsola australis* in the United States. However, without conducting experimental research, it would be difficult to rule out other factors in these examples. Conversely, Leen suggested that climatic factors were unlikely to be the cause of poor establishment in cases where the agent failed to establish at all sites, as was the situation for the importation of *Altica carduorum* from Switzerland to several areas of the United States. Leen found no significant difference between the source and release climates in this example. However, it is possible that some insect species are adapted to a wider range of environmental conditions than others.

Leen concluded from her analysis that climate has rarely hindered the establishment of phytophagous insects. However, it seems more likely that seasonal variation and extremes in climatic variables would have a detrimental effect on establishment rather than values around the mean, as in Walter and Leith's Klimadiagramm-Wetatlas. Climatic factors most likely to have an effect on establishment are extremes in temperature, low relative humidity and any variables which affect host quantity and quality. Insects show large differences in their ability to tolerate low temperatures. However, there is relatively little variability in the maximum temperatures their tissues can tolerate (Heinrich, 1981). A closer match may therefore be required where freezing temperatures occur in the colonised environment. The effect temperature has on insect development is not surprising, since most insects are ectothermic, with body temperatures close to ambient and processes occurring within the insect being governed by the laws of thermodynamics.

Reviews of past programs may well be useful in highlighting potential problems to target research, but they are limited by the quantity of releases and availability of accurate information, including the occurrence of atypical climatic conditions and microclimates. In a review of biological introductions in Canada, Beirne (1975) said it was difficult or impossible from the available data to separate the influences of geographic origin, host origin and release number. It would also be extremely difficult to separate other potential causes of establishment failures, such as laboratory adaptation and low genetic variation, without conducting well-designed experimental releases. Examples in the literature where poor climate matching has been confirmed experimentally as a factor inhibiting the establishment of a biological control agent are few and far between. In the vast majority of cases the reasons given for the failure of an agent to establish have been based solely on observations of the agent in the field rather than well-designed experiments.

1.2.2 Climatic adaptation - importance to bio-control

Organisms with a broad geographic distribution have at least occasionally evolved various biotypes that are adapted to particular climatic conditions (Flint, 1980). The selection of biotypes from climates which closely match the release environment is believed to enhance the likelihood of establishment. Diehl and Bush (1984) classified biotypes into several groups: non genetic polyphenisms, polymorphic variation, ecotypes, host races and sibling species. Each of these may be important to biological control (Caltagirone, 1985).

The ability of insects to live in environments ranging from hot deserts to arctic regions depends largely on the various levels of response to temperature (Heinrich, 1981). These responses range from immediate behavioural and physiological adjustments to long term seasonal synchronisations. Examples of how insects have adapted to their climate with behavioural, physiological and biochemical modifications are contained in texts by Hoffman (1985) and Heinrich (1981). In this section, examples from within the field of pest control will be discussed.

Callan (1969) suggested that biological control agents generally fall into two categories: those that do not vary over extensive geographic regions and are pre-adapted to a range of climates, and those that occur in discrete populations, each having specific climatic adaptations. If so, identification of the mechanisms which have led to these differences between species, and guidelines for their detection, would be useful to biological control. Factors that may have led to these differences include the insect's evolutionary history, the type of climates inhabited and the flow of genes across the range of an insect.

There are many examples where agents have been transferred successfully between dissimilar climates, suggesting that a close climate match is not required in all situations. An example is the collection of *Telenomous alsophilae* from Virginia, USA, and subsequent release in Colombia as a biological control agent for *Oxydia trychiata* (Bustillo and Drooz, 1977). In

another example, the gall midge, *Rhopalomia californica*, was collected from a winter rainfall, Mediterranean climate of the United States and released into the summer rainfall area of south eastern Queensland. The gall midge readily established on *Baccharis halimifolia* and was giving promising control of this weed until attacked by a native hymenopterous parasitoid (Julien, 1992).

The type of climate, especially the likelihood of extremes in climatic variables and presence of distinct seasons, in the collection and release areas will surely determine how close a climate match is required. For example, it is possible that a close climate match is not as critical when transferring agents from temperate areas to tropical areas, because the agent may simply act as if it's summer all year round, as was the case in the two examples given above. Problems may be more likely to arise when moving agents in the other direction, particularly if the agent's food supply is seasonal, or extremes of temperature or humidity occur in the temperate release location, which are generally not characteristics of tropical environments.

In a study of the climatic adaptations of tropical and temperate milkweed bugs (*Oncopeltus* spp.), tropical species showed no photoperiodically induced diapause and little migratory flight. In contrast, the temperate species exhibited short-day induced diapause and extensive migratory flight (Dingle, 1978). These results make sense, as it would be counter-productive for insects to take time out in diapause or use large amounts of energy migrating in areas where host plants are active throughout the year and seasonal extremes are generally absent, although differences in response to climatic variables are still highly possible between these tropical species. Vargas *et al.* (1996) evaluated the response to temperature of four fruit fly species (*Bactrocera cucurbitae, B. dorsalis, B. latifrons* and *Ceratitis capitata*), obtained from the tropical Hawaiian islands. All species were tolerant of temperatures from 16 to 32°C except *B. latifrons,* which had a capacity to survive only in a very narrow range of temperatures. All species originated from tropical regions of Africa, Asia or the Pacific and all species except *B. latifrons* have spread throughout almost all tropical and many temperate areas of the world (White *et al.*, 1992). Reasons were not suggested as to why these

differences were present, but may be due to the adaptation of each species to specific microclimates or other differences in their evolutionary history.

One of the more important variables facing an insect in temperate environments is seasonality, where there are regular fluctuations in temperature, light, moisture and natural enemies. As well as a direct climatic effect on the agent, climate matching may be important to completely synchronise the agent with the host, so as to gain maximum control of the pest (Cameron *et al.*, 1993). This assumes the host behaves in the same way in the release area as it does in the collection area. Insects can have four main phases in their life cycle: (1) reproduction, (2) growth and development, (3) dormancy, and (4) movement and migration. The timing of these phases is adapted to synchronise the insect with favourable physical and biotic conditions and to avoid unfavourable conditions, such as low moisture, extremes of temperature and low food supply (Tauber and Tauber, 1978). Because seasons are generally regular, insects are able to predict changes and undergo physiological and behavioural changes to prepare themselves for the approaching season. Environmental cues which insects use to respond to impending seasonal changes include photoperiod, temperature, food quality and quantity, moisture, population density and the host's physiological state (Tauber *et al.*, 1984).

Trioxys pallidus was imported into California from France for the control of the walnut aphid (*Chromaphis juglandicola*). The parasite readily became an important natural enemy in southern and coastal areas of California, but could not colonise northern California where the summers and winters are more severe. A population of the same parasite originating from Iran was later established in central and northern California. The Iranian population originated from an area with a climate similar to central California, although the winters were more severe (Messenger and Van Den Bosch, 1969).

There are several examples in the literature where the presence or absence of diapause, and the correct timing of diapause, have been shown to be important in the establishment of biological control agents. Collections of *Trioxys complanatus*, a parasite of the spotted alfalfa aphid,

were made from Iran, Italy and California. Each collection was compared to detect any differences in climatic adaptation. The Iranian and Californian ecotypes had a heat-induced, semi-protective diapause, while the Italian ecotype did not. All ecotypes entered cold-induced diapause, but the Italian ecotype had a lower temperature induction threshold (Flint, 1980). Field observations have noted the importance of the heat-induced aestival diapause for the success of *Trioxys complanatus* in central California and the failure of other non-diapausing parasites (van den Bosh *et al.*, 1964). The aestival diapause was thought to be important to overcome periods where hosts were in short supply and to escape high temperatures.

However, because diapause decreases the rate of population increase by removing a proportion of the population from reproduction and increasing generation times, it is only a benefit where environmental conditions threaten survival (Flint, 1980).

The presence of an unnecessary or poorly timed diapause may even hamper establishment and control of the weed. For example, the ragwort seedfly (*Pegohylemyia jacobaeae*) was imported from England in 1928 to the North Island of New Zealand. Poor synchronisation of the seedfly with the host plant was blamed for the lack of control achieved on the weed. Adult seedfly emerged up to six weeks prior to the start of flowering, resulting in competition for oviposition sites. Eighty to ninety percent of ragwort seeds escaped predation (Dymock, 1987).

In another example, the gorse seed weevil (*Apion ulicis*) only oviposits in spring, infesting ninety percent of gorse (*Ulex europaeus*) immature seed pods during this period. However, in warmer areas of New Zealand, many seed pods were produced before and after the weevil's reproductive period, reducing the effect the weevil had on the weed. This strain of weevil originated in southern England and was released in New Zealand in 1931. In England, gorse flowers over a much shorter time period (spring to mid summer), resulting in better synchrony between the weevil and its host (Hill *et al.*, 1991). It is possible the problem may have been overcome by making a collection of the weevil from a more suitable part of its native range.

Cotesia rubecula, a parasite of *Pieris rapae*, failed to survive in the eastern United States because it entered diapause too early in the autumn and therefore had insufficient fat reserves to support itself during diapause. The continuation of warm temperatures in the eastern United States after *C. rubecula* had entered diapause was thought to be the main factor leading to its failure to establish (Nealis, 1985). This collection of *C. rubecula* was imported from Vancouver, Canada. A further collection was imported from Beijing, China, and released in Massachusetts, where it did establish. The success of the Beijing ecotype was thought to be largely due to the good correlation of photoperiodic and climatic cycles between the two areas (Van Driesche, 1993). Collections of *C. rubecula*, originating from Europe, have also been successfully established in Canberra, Australia. The photoperiod required to induce diapause in this Australian population was 13 hours, versus 15-16 hours in the Vancouver population (Nealis, 1985). It is not known whether these differences were because the populations were imported from different climatic areas of Europe, or due to post colonisation adaptation, but the differences were important to the success of *C. rubecula* in these two environments. An earlier entry into diapause is required in Vancouver because autumn temperatures fall more quickly and severely. Further, hyperparasitism by *Tetrastichus galactopus* is substantial in Vancouver during September. Although larvae were observed to survive in temperatures as low as 11°C, cocoons spun in cold temperatures were poorly constructed and no insects survived.

A further example of the importance of a correctly timed diapause was found in a study of six geographic ecotypes of *Chrysoperla carnea*, which compared responses to temperature in post diapause development. The two populations which originated from areas with colder and less variable winter conditions had the lowest threshold for development, so that their development can resume while temperatures are still relatively low. The other populations originated from areas with milder and more variable winter temperatures. Higher developmental thresholds were characteristics of these populations, ensuring that development did not resume activity too early due to an early warm spell when food was not yet present. Differences in degree-days

required for development reflected differences in the onset and length of growing seasons in the different regions (Tauber and Tauber, 1978).

Intra-population variation in dormancy may be important in unpredictable or heterogeneous environments. In a strain of *Chrysoperla carnea*, 40 to 60% of the population entered an aestival diapause. Diapause induction was found to be related to food supply during the hot, dry summers. The remainder of the population continued to reproduce (Tauber *et al.*, 1984). Intra-population variation in the response of an insect to environmental variables may be a valuable attribute for a biological control agent, particularly if the release climate is not identical to that of the agent's native range, or if the agent is being released into a range of climatic types or unpredictable environments.

Precht (1973) divided the adaptive mechanisms of insects into two groups: modificational (non-genetic) and genotypic. In the case of a modificational adaptation, the response to an experimental temperature is dependent on the previous adaptive temperature, while in the case of genotypic adaptation, the response to an experimental temperature is independent of any previous temperature fluctuations. Modificational adaptive mechanisms, or the ability of an insect to respond quickly to temperature changes, may be important qualities for a biological control agent to possess.

Hopper *et al.* (1993) provided an extensive list of examples of variation between populations. They pointed out that very few studies have been conducted to confirm that the differences are genetic and not the result of maternal effects. Crosses that exclude maternal effects by evaluating the transmission of genetic information from the father are required.

Three major types of genetic mechanisms help determine the characteristics of diapause: Polygenic inheritance, supergenic inheritance and Mendelian inheritance (Tauber *et al.*, 1984).

Polygenic inheritance tends to result in co-dominance, with hybrid phenotypes intermediate between the parents. This can be illustrated by the hybridisation of different geographic strains of *Apanteles melanoscelus*, a parasite of the gypsy moth (Hoy, 1978). The critical photoperiod for diapause induction was 17 hr or less for a colony from Connecticut, United States, while it was 13 hr or less for colonies from France and Yugoslavia and for a hybrid of the French and Yugoslavian colonies. A triple hybrid between colonies from France, United States and Yugoslavia had a photoperiod induction length of 15 hr. This intermediate response is consistent with polygenic inheritance.

An example of Mendelian inheritance was demonstrated for two sibling species of *Chrysoperla* (Tauber *et al.,* 1984). Allelic differences at two autosomal loci resulted in one species being multivoltine (with facultative diapause cued by environmental conditions) and the other univoltine (with obligatory diapause after each generation).

Supergenic inheritance was demonstrated in *Drosophila littorallis* (Lumme, 1978). Several polygenes controlling diapause were closely linked and inherited as a "supergene". Responses to photoperiod were found to vary with the latitude of the population. Hybridisation of populations showed multivoltinism to be dominant. Allelic variation resulted in the observed gradation in photoperiod with latitude.

Most examples of poor climate matching have involved an insect's mal-adaptation to seasonal climatic variation and seasonal variation in host quality and quantity. Irregular stresses such as drought or aseasonal extremes in temperature are difficult to predict, therefore an insect's survival will depend on its immediate response. Many insects respond to such irregular stresses by quiescence, where development and reproduction are postponed until favourable conditions return, or by migrating to a more favourable environment (Tauber *et al.*, 1984). Many insects can also change aspects of their physiology and biochemistry to compensate for variation within the population-specific temperature range (Hoffmann, 1985). During periods of aseasonal stressful conditions, the survival of a proportion of the population in favourable

microclimates may carry the population through the difficult period. When introducing an agent to a new environment its geographic range will initially be limited, making it more susceptible to aseasonal stressful conditions.

In order to survive, insects must maintain their water content within certain critical limits. Regulation of spiracular and excretory water losses together with modifications in cuticular permeability play important roles in the maintenance of an insect's water and ion content (Kestler, 1985 and Hevert, 1985). No studies could be found implicating humidity in the failure of bio-control agents to establish.

If climate is found to be limiting the population of a biological control agent and no more suitable populations can be found, the question arises about whether an insect's tolerance to climatic extremes can be enhanced through genetic improvement. For example, the tolerance of *Aphytis lingnanensis* to extremes of temperature was improved after artificial selection for over 100 generations (White *et al.*, 1969). The response of biological control agents to climatic variables has been demonstrated to change following their introduction into a new environment. The temperature threshold of the cinnabar moth was found to have changed in less than 17 generations after release in California (Myers and Sabath, 1980). It is not known how commonly post release adaptation has occurred and whether adaptation following release is important to the success of an agent. This question will be discussed in more detail in chapter 7.

1.2.3 Climate modelling

Climatic models have been used to predict the suitability of biological control agents for release areas. Some computer modelling programs include BIOCLIM (Nix, 1986), CLIMEX (Sutherst *et al.*, 1985), GARP (Boston and Stockwell, 1994), CLIMATE (Pheloung, 1996) and HABITAT (Walker and Cocks, 1991).

A model based on development/temperature relationships was used to predict the suitability of seven ecotypes of *Microctonus hyperodae* for the control of Argentine stem weevil (*Listronotus bonariensis*) in New Zealand (Barlow *et al.*, 1994). The model was used to predict the number of parasitoid generations per year, the synchrony between the occurrence of parasitoid adults and susceptible pest stages, and the significance of differences in climatic adaptation between ecotypes. Small differences were observed in the development/temperature relationships between ecotypes, although these differences were not thought to be important for the success of the agent, except in the north of the North Island. In this area, it was proposed that the Brazilian ecotype would be more successful because it lacked a winter diapause. Warmer temperatures in this area during winter meant that a diapause would reduce population growth unnecessarily. The success of this model's predictions is not yet known.

CLIMEX is a program developed by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) in Australia to predict the potential distribution and abundance of a species. The program produces indices showing the potential response of an organism to environmental conditions. Separate indices estimate the potential of the organism to grow during favourable conditions and persist through adverse conditions (Sutherst *et al.*, 1985). Although this model is extremely useful, a major limitation is its use of long term averages, therefore the effects of atypical climatic events and microclimates cannot be modelled.

Perapion antiguum, collected from various climatic areas of South Africa, was extensively released across southern Australia for the biological control of *Emex* spp. Each collection was released in areas which matched the collection point climates as closely as possible. *P. antiguum* survived at four sites, three of which were in irrigated orchards and the fourth on Flinders Island, Tasmania. Failure of the weevil to survive at other sites was thought to be due to its inability to survive hot, dry summers (Julien, 1980). Scott (1992) used CLIMEX to confirm the hypotheses developed after the field releases. Information on the weevil's native distribution, phenology, abundance and development parameters were used in the model. The model confirmed that the failed release sites were not suitable for the weevil. Suitable sites

were predicted to be coastal areas of southern Australia, which included the successful site at Flinders Island. Scott (1992) stated that a weakness of CLIMEX was its use of long term climatic data which does not account for localised microclimates. All inland populations of *P. antiguum* in the western and eastern Cape Province, South Africa, were found along riverbanks, in irrigated fields or along roadsides, where there is runoff from the road. The weevil is most active after seeds are produced. The extended life of *Emex* spp. in these microclimates and in coastal areas was thought to be important for the weevil's survival. As well as the hot, dry summers, a lack of oversummering sites was thought to be a factor in the failure of the weevil to establish in some areas. In its native rangeland, it oversummers in various trees and shrubs, which were lacking at the Australian release sites.

This example highlights the importance of detailed monitoring of a potential agent in its native range, particularly any preference shown to specific microclimates which may restrict its potential distribution and usefulness as a control agent. It is possible that numerous releases of biological control agents have failed to establish because the microclimate of the release area was inappropriate.

Another practical problem with the implementation of models is that by the time all necessary data on the agent are obtained, approval for release has usually been issued and extra costs or effort involved with simply trying the release are minimal (Cruttwell McFadyen, 1991).

When predicting the potential range of a biological control agent in the release country, using the insect's native range and climate modelling programs, it is not possible to take into account the fact that key attributes of the insect may vary over its range. So, a population from one sampling location may not be adapted to the entire range.

Cruttwell McFadyen (1991) concluded, after reviewing climatic modelling, that climate modelling is a valuable predictive tool, but the predictions are no more reliable than the series of unproven, even if reasonable, assumptions on which they are based. Further testing of the

theories on which current climatic models are based is required, using experimental releases of biological control agents.

1.2.4 Other key attributes that vary between populations

Examples of variation in climatic adaptation between populations have been given in the preceding sections. Other attributes that may be important to biological control and which have been shown to vary between populations include dispersal, host finding ability, development time, insecticide resistance, fecundity, sex ratio, ability to avoid host defences, and host specificity (Roush, 1990; Hopper *et al.*, 1993; Edwards and Hoy, 1995).

1.2.5 Conclusions drawn from the literature

There are a few examples where poor climate matching has been confirmed to inhibit establishment or reduce the level of impact on the target pest. In contrast, there are a number of examples where agents have been successfully transferred between very different climates.

Thus, a close climate match is obviously not required in all situations. When is a close climate match required?

From the examples where poor climate matching has been confirmed to inhibit establishment, the important factors appear to be: (1) the lack of diapause or a poorly timed diapause to overcome climatic extremes such as freezing winters, hot dry summers and seasonality in host quantity and quality; and (2) a preference of the agent for certain microclimates, which was not considered when selecting release sites.

Some insect species appear to tolerate a wide range of climatic variables. This may be a requirement for the survival of insects originating from more variable climates. It may also arise from gene flow across the native range of an insect or other aspects of the insect's

27

evolutionary history. The identification of reasons why some insects have the ability to tolerate a wide range of climatic variables may prove to be useful. Such species could then be targeted by biological control workers. It is also possible that populations within the range of a species are adapted to a wide range of climatic variables, such as those originating from areas with more variable climates. It may be better to target these populations, assuming they do not possess a diapause that is unnecessary in the release location.

Although climate is clearly important, the importance of climate may have been overestimated in the past, particularly where no other obvious factors exist (McFadyen, 1985). Because it is extremely difficult to separate the many possible causes of establishment failures from observations alone, experiments identifying the reasons behind failures are required.

It should be possible to develop a set of general rules to consider, prior to transferring agents between different climates, based on a thorough review of past programs and experimental releases with different climatic ecotypes of agents.

1.3 Project Aims

The environment in the native range of a biological control agent is never identical to the release environment. Practitioners of biological control generally agree that the chances of successfully establishing an agent are probably increased if the colonised environment closely matches the environment in the area of collection and thereby matches the organism's climatic adaptation, although there is disagreement about how important climate matching is and how close a match is required (eg. McFadyen, 1985; Crawley, 1986; Leen, 1991). Is climate matching only important when transferring agents from areas with consistently mild climates to areas with climatic extremes, or can climate also prevent establishment even when climates appear similar? If a close climate match is important, can we assist with a population's

adaptation to the new environment by, for example, releasing populations with high genetic variation (van den Bosch, 1971)?

Because there are very few examples where the influence of climate matching on establishment has been tested experimentally, it is not clear how often poor climate matching has led to establishment problems and under what conditions problems may arise.

Experimental releases of the bitou tip moth (*Comostolopsis germana*) and the horehound plume moth (*Wheeleria spilodactylus*) offered an opportunity to investigate the importance of climate matching to the establishment of classical biological control agents. The bitou tip moth and plume moth were introduced into Australia for the control of *Chrysanthemoides monilifera*, bitou bush and boneseed, and *Marrubium vulgare*, horehound, respectively. Both agents were released in South Australia in the early 1990s, but had not performed as well as expected in the field. It was believed that their poor performance was because they were poorly adapted to South Australia's Mediterranean climate. This project aimed to test this hypothesis by comparing various populations of the plume and bitou tip moth collected from different climatic regions of their native range.

Chapter 2

The target weeds and the history of the biological control programs against them

2.1 Target no. 1: horehound

Marrubium vulgare (Lamiaceae), horehound, is one of 30-40 species of *Marrubium* native to Europe, Asia and north Africa (Young and Evans, 1986). Horehound is now spread throughout much of the temperate world. It is considered a weed in Australia, New Zealand, South and North America and in much of its native range. The weed was most likely introduced into Australia as a garden or medicinal herb, or possibly on livestock. It was first recorded in South Australia in 1841 and was considered naturalised by 1848 (Carter, 1990).

Horehound now occurs in all Australian states except the Northern Territory. The weed is estimated to have infested 6 million hectares of Victoria, 20 million hectares of South Australia and 13 thousand hectares of Western Australia (Carter, 1990). Horehound inhabits a range of climatic zones receiving greater than 200mm of rainfall per year. Horehound is drought tolerant. Consequently it is a considerable problem in semi-arid areas of southeastern Australia, where it has a competitive advantage over many pasture species.

Horehound occurs on a range of soil types, including mallee soils, red clay loams of Western Australia, red brown earths of South Australia, calcareous sands of South Australia, and well drained loams of western New South Wales (Carter, 1990). In Australia, it shows preference for alkaline soil types.

Figure 2.1 Horehound infestation during mid summer, Finders Ranges National Park, South Australia.

Compared to Australian horehound infestations, infestations of the weed in its native range are less dense and more scattered. Individual plants are considerably smaller and less vigorous. This difference in the growth of plants in Australia and its native range is thought to be due to the lack of natural enemies in Australia, as well as differences in environmental conditions and land management practices (Weiss *et al.*, 1994).

2.1.1 Growth and development

Horehound is an erect, spreading, bushy, perennial herb, up to 60cm in height (Jessop *et al.*, 1986). Plants may live for up to 20 years.

Seeds generally germinate in autumn, when conditions are cool and moist (Young *et al.*, 1986). A small proportion of seeds germinate through winter and spring. In the absence of cool, moist conditions, fluctuations of at least 15°C in diurnal temperatures will allow seeds to

germinate. A night temperature of 20°C and day temperature of 40°C was found to be optimal. Approximately 65% of seeds are dormant at maturity. After four months of storage, only 20 % of seeds remain dormant (Stritzke, 1975).

Seedlings are poor competitors, thus seedling survival is best in disturbed or grazed areas and on poor soils. In infertile soil types, horehound out-performs most pasture species (French, 1960). Increasing soil fertility reduces the competitive advantage of horehound, thereby reducing seedling survival (Carter, 1990). Horehound is often the first plant to colonise eroded areas (Carter, 1990). The establishment of horehound is aided by overgrazing. Horehound contains a bitter alkaloid which is unpalatable to stock, which therefore selectively graze around the horehound giving it a competitive advantage. Stock and native animals have been observed to feed on horehound during spring when the weed is actively growing, and when more palatable species are not present.

In the absence of competition, seedling growth is rapid during winter and spring. Plants may flower in the first year (Parsons and Cuthbertson, 1992). Established plants usually flower from July to December (Carter, 1990). Plants may produce up to 74,000 seeds per year (Forcella, 1985).

During times of severe water stress, mature plants die back to a woody crown (Figure 2.1). Regrowth occurs following the return of favourable conditions (Carter, 1990). This is a common occurrence, during summer and droughts, in the semi arid and Mediterranean areas of southeastern Australia.

2.1.2 Seed dispersal

Seeds are readily dispersed by humans and animals (Parsons and Cuthbertson, 1992). The calyx covering the seed bears hooks, enabling to seed to become attached to animals and

objects as they brush past the plant. Mature seeds may remain attached to the plant for several months until dislodged (Young and Evans, 1986).

2.1.3 Importance

Horehound is a weed of pastures, roadsides, wasteland and native vegetation. It can also occasionally be a weed of crops, however it does not persist under continued cultivation (Carter, 1990).

When present in pastures, horehound contaminates wool, taints meat and competes with desirable pasture plants. In 1988, horehound was estimated to cost the Australian wool industry $680,000 per year just through the contamination of wool by seeds (Sloane et al., 1989). The total cost of horehound to the Australian economy has not been estimated.

2.1.4 Control

Management practices for the control of horehound include herbicides, cultivation, pasture improvement, burning and biological control.

Burning can be used to stimulate germination and destroy mature plants. In one trial, burning reduced the number of viable seeds in the seed bank by 78% (J. Weiss, pers. comm.).

Applications of 2,4-D at approximately 2Kg/ha has been found to give greater than 90% control of horehound (Stritzke, 1975). Farmers in South Australia commonly use mixtures of 2,4-D with herbicides such as glyphosate and metsulfuron for control of horehound (R. Carter, pers. comm.). Work evaluating the integration of herbicides with the plume moth has found applications of 2,4-D at 0.5 to 1.5 Kg/ha reduced adult longevity and fecundity due to reduced flowering of the weed. Larval development and survival of the plume moth was not greatly affected by the herbicide application (Ainsworth, 1999).

Because horehound is a poor competitor in the seedling stage (Carter, 1990), pasture improvement and the sowing of desirable plants following cultivation, burning or herbicide application would aid in the control of the weed.

2.2 Biological control of horehound

A biological control program against horehound commenced in 1990. *Wheeleria spilodactylus* (Lepidoptera: Pterophoridae) (plume moth) was the first biological control agent to be released in Australia. Releases of the plume moth commenced in the summer of 1993/94.

At the start of this PhD project in 1996, the plume moth had been released at 19 sites across South Australia (Ceduna, Port Lincoln and Murray Bridge), Victoria (Swifts Creek, Wyperfeld National Park, Dandenong, Bacchus Marsh and Yarram) and New South Wales (Yanco, Tamworth and Michelago).

The moth was performing well only at sites with an annual rainfall exceeding 450mm per year (Weiss, 1996), while horehound occurs in rainfall zones as low as 200mm per year (Carter, 1990). The moth has been detected at several of the lower rainfall sites following release, but insect density was extremely low and unlikely to suppress the target weed (J. Weiss, pers. comm.).

The plume moth's native range is thought to be from northern Africa (Morocco) to central Europe. The plume moth population first released in Australia originated from Cape d'Agde, France. Cape d'Agde has a temperate climate with an annual average rainfall of 693mm and rarely experiences extremes in temperature due to a relatively close proximity to the sea. Horehound plants in this region have green foliage present throughout summer (J. Sagliocco, pers. comm.). Similarly, in areas where the plume moth was performing well in Australia, the

horehound plant remains green throughout the year (J. Weiss, pers. comm.) and these areas also rarely experience extremes in temperature.

The hot, dry summers which occur over much of southeastern Australia result in the horehound regularly dying back to a woody crown until rains return (Carter, 1990). This factor and high summer temperatures were believed to have hampered the establishment and population growth of the French ecotype of the plume moth. It was hypothesised that plume moth populations present in semi-arid regions of southern Europe would have developed mechanisms to cope with the die back of plants during summer and high summer temperatures (J. Weiss, pers. comm.). However, it is possible that the insects in the more arid regions of their native range do not possess additional mechanisms to overcome plant die back. Over the area of a horehound infestation, some plants will remain green even during very dry periods in arid areas, for example, along roadsides, in the shade of trees, or in dry creek beds. Even in the absence of an oversummering capability such as diapause, a proportion of the population may survive on these plants to carry it through the dry period.

The plume moth has 3 - 4 generations per year. The first generation emerges in spring, with subsequent generations occurring over summer and early autumn. Each generation takes between one and two months to complete, provided the host plant is suitable. First instars of the autumn generation over-winter in the leaf buds and complete their life cycle during the next spring (Weiss and Llippai, unpublished).

A second agent, the clear wing moth (*Chamaesphecia mysiniformis*), has also been released, but its fate is not known at present. If it is established in the field, this insect is likely to be the more effective agent in the drier regions of southeastern Australia. Most of its life cycle is completed below ground in the plant roots, making it less vulnerable to adverse environmental conditions and perhaps to predation, including inadvertant predation by grazing animals.

2.3 Target no. 2: *Chrysanthemoides monilifera*

Chrysanthemoidies is native to southern and coastal areas of Africa. Two species belong to the genus, *C. incana* and *C. monilifera*. Six sub-species of *C. monilifera* have been identified in southern Africa. They are described on the basis of the shape of their fruit and involucral bracts. Each sub-species has a well defined geographical range (Weiss, 1986).

Two of the six sub-species are naturalised in Australia, *C. monilifera ssp. monilifera* (boneseed) and *C. monilifera ssp. rotundata* (bitou bush).

Boneseed was originally imported into Australia as an ornamental (Weiss, 1986). It was first recorded in Sydney in 1852, in MacLeay's garden. It was subsequently grown as an ornamental in all states except the Northern Territory. Most of the present infestations are believed to have originated as garden escapees.

In South Africa, boneseed occurs in the South Western Cape province, from Knysna across to Capetown, in coastal and adjacent mountain environments (Weiss, 1995). This area has a Mediterranean climate. Climate types where boneseed exists in Australia range from Mediterranean to cool temperate. The main boneseed infestations occur in Victoria (Mornington Peninsula and You Yang Ranges), South Australia (Mount Lofty Ranges) and northeastern coastal areas of Tasmania. However, isolated populations occur over much of southeastern Australia, including southern New South Wales (Weiss, 1995).

In southern Africa, bitou bush occurs in coastal environments from Knysna along the east coast to Maputo in Mozambique (latitudes 34°00' to 26°00'). The bulk of this area is subtropical with predominantly summer rainfall. In Australia, bitou bush thrives in coastal environments from Tathra, on the New South Wales south coast, to the Queensland border, and has occurred as far north as Rockhampton (latitudes 36°45' to 23°00'), until recent eradication programs in Queensland. In 1982, bitou bush was present on 60% of the New

South Wales coast line and was the dominant species on 20% (Weiss, 1986). Bitou bush is believed to have been introduced into Australia in ballast dumped on the banks of the Hunter River (NSW) by South African ships. Between 1950 and 1970, bitou bush was used by mining companies and the New South Wales Soil Conservation Service to stabilise coastal sand dunes (Weiss, 1986).
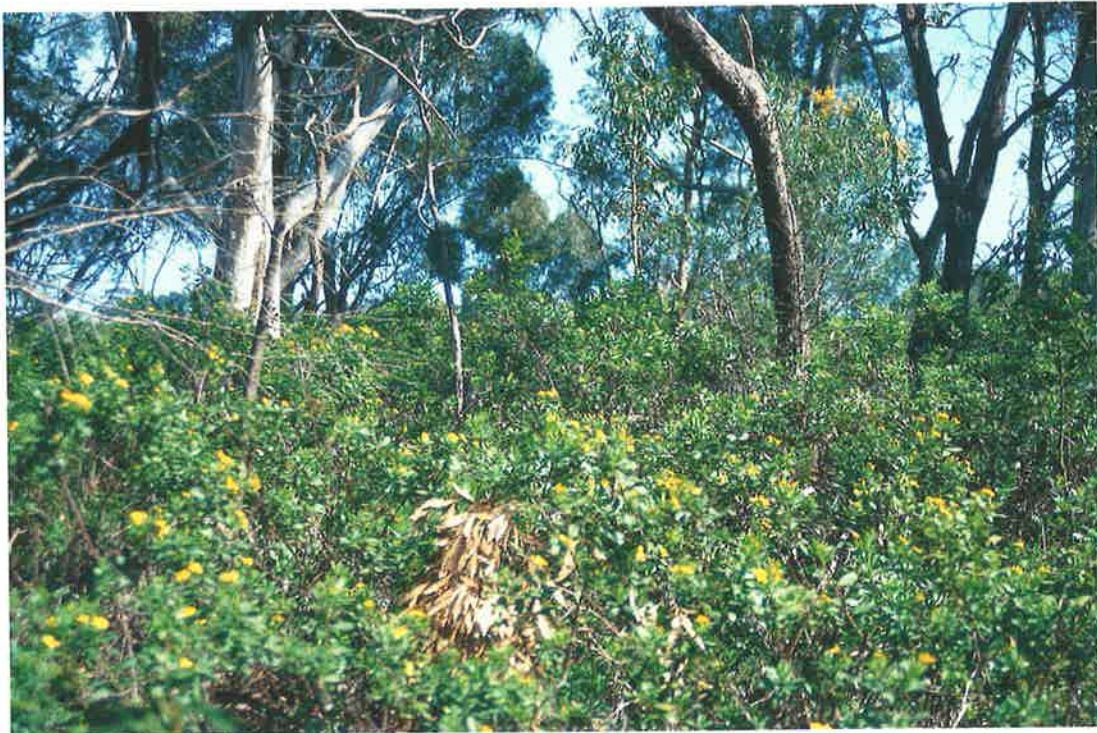
Figure 2.2 Boneseed infestation, Belair Recreation Park, South Australia.

The distribution of bitou bush and boneseed in Australia is explained partly by climatic preferences and partly by other ecological factors (Weiss, 1984). Boneseed is an erect shrub, allowing it to compete in forests (Figure 2.2), while bitou bush has a sprawling growth habit, suited more to wind swept environments such as coastal sand dunes. Boneseed is also better adapted to fire, making it better suited to the inland forests of Australia.

After studying the range of environments occupied by bitou bush in Australia, Howden (1985) concluded that there is considerable potential for the expansion of bitou bush in Australia.

Hybrids between sub-species occur where populations overlap. In Australia, transitional forms occur on the Mornington Peninsula (Victoria) and Avalon (New South Wales), where boneseed and bitou bush populations co-exist (Weiss, 1986, R. Adair, pers. comm.)

Chrysanthemoides monilifera also occurs in Sicily, Southern France, United States (California) and New Zealand, but is considered to be a noxious weed only in Australia (Parsons and Cuthbertson, 1992).

2.3.1 Growth and development

Both bitou bush and boneseed are evergreen perennials which do not exhibit a dormant period.

Flowering of boneseed occurs between July and December each year. Bitou bush can flower all year round, but mainly between April and July. The seeds of both sub-species are usually produced by allogamy. The ray florets are female and fertile, the disk florets are pseudo-hermaphrodite and the ovaries are abortive (Weiss, 1986).

Seed production in boneseed occurs during early summer. The seeds mature and fall to the ground during January and February. The seeds of bitou bush mature all year round but seed fall is greatest during June and September. Annual seed production has been observed to be up to 48,000 seeds/plant for bitou bush and 50,000 seeds/plant for boneseed (Weiss, 1986).

Boneseed germinates during autumn rains and seedling growth is then rapid over the winter/spring period. Seedlings usually take two to three years to reach reproductive maturity. However, on burnt areas seeds can be produced in the first year. Bitou bush seeds are able to germinate throughout the year, as long as there is sufficient moisture (Weiss, 1986).

Bitou bush seed has relatively low seed viability over time, with 0-2% of seed being viable after burial for three years. This figure increased slightly with increased depth of burial. In

comparison, 6-13% of boneseed seeds were viable after the same time period. Boneseed seeds have been shown to remain viable in the soil for up to ten years. Viability of laboratory stored seed was 24% for bitou bush and 42% for boneseed after three years (Weiss, 1984).

Boneseed seed germination takes place after the seed coat splits following weathering or fire. Temperatures of 100-150°C for a period of thirty seconds are required to germinate boneseed seeds with intact seed coats (Lane and Shaw, 1978).

2.3.2 Seed dispersal

The seeds of both sub-species have a fleshy pericarp, which is attractive to a number of animals. Cattle, rabbits, foxes and a range of birds are thought to be important dispersers of seed (Donkin and Gilmore, 1984). In the past, man has been one of the more important dispersal agents, cultivating the plants as ornamentals and re-vegetation species.

2.3.3 Importance

Boneseed and bitou bush are major weeds of native vegetation. They readily establish in disturbed areas and, although their spread and development is slow, they will also establish in undisturbed native vegetation. The presence of the weed results in a decline of aesthetic and biological qualities of native vegetation as well as preventing the efficient utilisation of areas such as forests, water catchment areas and coastal sand dunes (Weiss, 1986).

Because of their dense, bushy growth and prolific root production, boneseed and bitou bush have a competitive advantage over many native species, particularly in the seedling stage (Weiss and Noble, 1984). Several native plants have been observed to be displaced following the invasion of *C. monilifera*. Adverse effects on fauna are also likely due to the displacement of essential food plants (Weiss, 1986).

2.3.4 Control

The most successful control programs to date have involved an integration of herbicides, slashing or hand pulling and fire, followed by revegetation with desirable species and follow-up control of seedlings resulting from the seed bank or re-introductions (Weiss, 1993).

2.4 Biological control of *Chrysanthemoides monilifera*

To date six biological control agents have been released in Australia against boneseed and bitou bush.

1. Black boneseed beetle (*Chrysolina sp*)
2. Blotched boneseed beetle (*Chrysolina picturata*)
3. Painted boneseed beetle (*Chrysolina oberprieleri*)
4. bitou tip moth (*Comostolopsis germana*)
5. Bitou seed fly (*Mesoclanis polana*)
6. Lacy-winged seed fly (*Mesoclanis magnipalpis*)

None of the populations of the three *Chrysolina* species released across southeastern Australia have established. This is thought to be primarily due to predation by ants and spiders (R. Adair, pers. comm.). *M. polana* has established on bitou bush in New South Wales, after release at two sites in 1996. By 1998, the insect had dispersed over 1200km of the New South Wales coastline (Edwards *et al.*, 1999). *M. magnipalpis* was released on boneseed in 1998 in South Australia and Victoria, but its fate is not yet known.

In addition, a tortricid leaf roller is currently being evaluated for release against boneseed and bitou bush.

2.4.1 *Comostolopsis germana*

C. germana (Lepidoptera: Geometridae) (bitou tip moth) occurs from Natal to the South Western Cape province in South Africa on all sub-species of *Chrysanthemoides monilifera* and *C. incana.* Larvae of the bitou tip moth are found throughout the year in Natal province. In the Mediterranean climate of the South Western Cape province, larvae are mostly absent in winter (July). These differences are most likely due to climatic differences between the two areas (Adair and Scott, 1989).

Up to the commencement of this project in 1996, the bitou tip moth had been released at 79 sites across southeastern Australia. Post release inspections have been undertaken at 61 of these sites. The bitou tip moth had established at 36 of these sites and was colonising a further 6 sites. All 42 sites are located in New South Wales and Queensland, where *C. m. rotundata* is the dominant sub-species. All release sites where the bitou tip moth has established are located in coastal environments. The one exception is a small plot of boneseed and bitou bush located at the Keith Turnbull Research Institute, Frankston, Victoria, next to the tunnel houses where rearing of the bitou tip moth took place. The only obvious difference between this site and others in Victoria and South Australia is that the plants are actively growing throughout the year, due to regular watering and good soil. Apart from this site, the bitou tip moth has failed to establish elsewhere in South Australia, Victoria or Tasmania. The sites where failure occurred have been predominantly located in inland environments and infested with *C. m. monilifera.* Hot, dry conditions over summer combined with poor soil types at these locations result in poor growth of the boneseed during the summer period.

At several release sites in New South Wales, the released population has become more abundant on the more exposed areas of the foredune (Adair, pers. comm.). This is thought to be because predators are not as effective in this environment. It may also suggest this population of the bitou tip moth has a preference for the foredune microclimate.

41

Four collections of the bitou tip moth were originally made from South Africa, as shown in table 2.1.

Table 2.1 Importation details for the original bitou tip moth collections.

CSIRO collection no.	collection location	Weed sub-species present	No. insects imported
2330	Mtunzini, Natal	C. m. rotundata	51
2400	Cape St. Francis	C. m. rotundata	130
2362	Rondevlei	C. m. pisifera	35
2363	Rondevlei	C. m. pisifera	71

All populations were collected from coastal sub tropical environments, from *C. m. rotundata* and *C. m. pisifera*. The vast majority of releases were made with a mixture of collections 2400 and 2363 (Table 2.1). Most releases were made with second to fourth instar larvae. An average of 1800 larvae were released at each site at one time. The majority of sites have had at least one follow up release. No pattern could be found in the records of past releases indicating that a particular source population, release number, timing of release, or stage of the released insect was more successful than another (R. Adair, pers. comm.).

The failure of the bitou tip moth in South Australia and Victoria was hypothesised to be due to poor climate match of the agent and/or poor food quality during summer. The sub-species of the weed present at the South Australian sites may also be a factor, although early work by the Keith Turnbull Research Institute indicated that the insect population would readily develop on both weed sub-species (R. Adair, pers. comm.).

Chapter 3

Insect population origins, importation and maintenance

3.1 Introduction

Because organisms with broad geographic distribution can evolve "biotypes" or "ecotypes" adapted to particular climatic conditions (Flint, 1980), the selection of ecotypes from climates that match the release environment may enhance the likelihood of establishment. The bitou tip moth and plume moth insect populations used in this project had not previously been studied, so there were no known differences between them. They will therefore be referred to as populations or colonies.

To evaluate the importance of selecting agents from similar climates, populations of both the plume and bitou tip moths were collected from areas of their native range that more closely match South Australia's Mediterranean climate. These were compared to populations from the areas where the original populations were collected.

Classical biological control programs involve the collection of small numbers of individuals, often from only one or a few areas of the agent's native, or sometimes exotic, range. These populations are usually then reared under artificial conditions before being released in the field. Rearing of the agent prior to release is usually necessary to build up sufficient numbers and to remove any diseases or parasites and predators and to confirm identity.

Several concerns have been raised in regard to the importation and rearing process. One concern is that genetic variation in attributes that affect success is either lost or not collected during the importation and rearing process (Roush, 1990). During the rearing process,

inbreeding, selection and random genetic drift may also lead to genetic decay, loss of fitness, or the production of laboratory adapted ecotypes (Mackauer, 1976).

The rearing techniques employed in this project aimed to minimise any loss of fitness, although it is not clear what measures need to be taken to avoid problems during rearing, or how important genetic variation, inbreeding and drift have been to the establishment of biological control agents in the field. These questions will be discussed in detail in chapter 5.

3.2 Origins of the horehound plume moth populations

The first population of the plume moth imported and released in Australia was from Cape d'Agde, southern France. Material used for field releases was imported in 1992. A laboratory colony from this insect population was maintained continuously at the Keith Turnbull Research Institute (KTRI). This colony had not been contaminated with any other population, nor were field individuals reintroduced back into the colony. Six hundred insects from this colony were transported to Adelaide during October 1996, to start the "old French" laboratory colony.

Two further populations of the plume moth were imported into Australia for the purposes of this project. One was a new collection from Cape d'Agde, southern France ("new French" colony). The second was from Zaragoza, central Spain (Spanish colony). A summary of the importation details for the three insect colonies used in this project is given in Table 3.1. The insects were collected by Jean-Louis Sagliocco of CSIRO Entomology, based at Montpellier, France.

Table 3.1 Importation and rearing details for the three plume moth insect populations under evaluation.

Population name	Origin	Date imported	No. insects imported	Quarantine facility	Generations in lab prior to release
Old French	Cape d'Agde France	09.06.92	400	K.T.R.I.	21 - 26
New French	Cape d'Agde France	05.06.97	59	Adelaide	3
Spanish	Zaragoza Spain	17.07.96	50	Adelaide	5

There appear to be significant differences in climatic variables among overseas collection locations and the original release sites in Australia, as illustrated by two examples, Swifts Creek and Murray Bridge (Table 3.2). The moth could not be established at Murray Bridge using the old French population, despite repeated attempts with large release numbers made between late 1993 and 1996. In contrast, the moth was easily established at Swifts Creek by 1993 and by 1996 appeared to be having a significant impact on the horehound (J. Weiss, pers. comm.). Zaragoza experiences similar rainfall, hot summer temperatures and low humidity during summer as Murray Bridge. It was hypothesised that the Spanish population would be better adapted to the more arid areas of southeastern Australia, like Murray Bridge, where the horehound plants commonly die back to a woody crown over the summer period.

CLIMEX, a climate modelling program (Suthurst *et al.*, 1985), was used to compare the climates of the European collection locations with two of the original release sites, Murray Bridge and Swift Creek. CLIMEX produces a match index between 0 and 100 which indicates the similarities in climates. The larger the match index, the more similar the climates. Because climate data for Cape d'Agde were not present in the CLIMEX program, data for the nearest site, Marseille, France, were used in its place. Marseille is approximately 150 km east of Cape d'Agde.

Table 3.2 Climatic details for the two European collection locations, Zaragoza and Cape d'Agde, and two of the original Australian release sites, Murray Bridge and Swift Creek. Data were obtained from the Australian Bureau of Meteorology. (* - data not available).

	Zaragoza Spain	Cape d'Agde France	Murray Bridge South Australia	Swift Creek Victoria
Latitude	41.4 N	43.24 N	35.12 S	37.06 S
Longitude	001.01 W	003.41 E	139.27 E	147.35 E
Elevation (m)	258	95	15	685
Av. annual rainfall (mm)	314	693.4	346	684.2
Av. Jan. rainfall	23	54.1	16.5	52.9
Av. July rainfall	15	17	35.5	52.6
Av. no. days > 40°C	*	*	3.5	0.1
Av. no. days > 35°C	*	*	20	1.6
Av. no. days > 32.2°C	35	8.5	*	*
Av. no. days < 0°C	28	23.3	3	71
Av. max. Jan. temp.	10	10.6	28.6	26
Av. max. July temp.	31.1	27.8	16.1	10.1
Av. min. Jan. temp.	2.8	4.4	14.3	9.4
Av. min. July temp.	17.8	18.9	5.5	-0.02
Extreme max. (°C)	41.1	36.7	45	40
Extreme min. (°C)	-7.2	-10	-0.5	-11.7
Jan. 3pm relative humidity (%)	71	76	36	43
July 3pm relative humidity (%)	46	67	58	64

Table 3.3 CLIMEX match indices, comparing collection (Zaragoza and Cape d'Agde) and release locations (Murray Bridge and Swift Creek).

Match index (I) (0-100)	Release location	Zaragoza	Marseille (Cape d'Agde)
I total	Murray Bridge	63	63
	Swift Creek	57	64
I max. temperature	Murray Bridge	62	57
	Swift Creek	73	65
I min. temperature	Murray Bridge	75	70
	Swift Creek	52	51
I total rainfall	Murray Bridge	96	68
	Swift Creek	58	88
I rainfall pattern	Murray Bridge	92	91
	Swift Creek	89	80
I humidity	Murray Bridge	30	31
	Swift Creek	62	64

While the two release locations in Australia have a similar overall match (I total) to both collection locations, Murray Bridge has a total rainfall and rainfall pattern similar to Zaragoza, while Swift Creek has a total rainfall more similar to Cape d'Agde (Marseille) (Table 3.3). It was believed that the hot, dry summers experienced at Murray Bridge were a major factor in the failure of the old French population to establish at this site. The CLIMEX temperature match index is calculated using average monthly maximum and minimum temperatures and does not take into account temperature extremes. High temperatures may have had a direct effect on the insect, or an indirect effect on the insect by influencing the quality of the host plant. Zaragoza experiences summer temperatures that are more similar to Murray Bridge than Swift Creek (Table 3.2).

3.3 Maintenance of the plume moth laboratory cultures

3.3.1 Rearing

After importation, each insect population was firstly put through one generation in quarantine. In each case, late instar larvae were imported. These larvae were transferred to fresh potted plants at a density of only 2 insects per plant. Any insects that appeared sick were destroyed, along with their host plant. Surviving pupae were washed in 0.1% sodium hypochlorite, followed by a triple rinse in distilled water, and placed in clear plastic vials. Adults were allowed to mate in cages (60 x 60 x 40 cm), one pair per cage. A healthy, flowering horehound plant was placed in each cage. The number of pairs that successfully produced offspring was 22 for the new French population and 17 for the Spanish population. The number of pairs that were successfully mated for the old French population in their first generation was not known, as the imported insects were "mass mated". Leaves containing eggs were harvested and washed in 0.1% sodium hypochlorite plus 0.05% tween, followed by a triple rinse in distilled water. Emerging neonates were then transferred to fresh plants. When these insects were in

their pupal stage, they were again washed and removed from quarantine. The parentage of each insect was recorded and siblings were not mated to each other.

The three plume moth populations were reared in separate rooms. Two glasshouse rooms were available for each population; one room was maintained at 23°C and the other at 25°C.

Pupae were sexed and placed in open containers (12 x 18 x 7cm) on tissue paper. The containers were then placed in cages (0.8 x 0.6 x 1.0m) with 5 - 6 horehound plants. The number of pairs placed into each cage varied from 5 - 15, depending on the size and health of the host plants. Flowering plants were preferred to provide a food source for the adults. Artificial nectar sources were tried, but the moths were never observed to feed from any (various concentrations of honey solution were tested, with and without the addition of horehound water made from horehound leaves that were boiled in water for one minute). After 2 - 3 weeks, the cages could be removed and used for other matings. Nine mating cages were available for each population.

The males and females used in a mating cage always originated from different parents. A minimum of 200 individuals were used for the production of each new generation.

Maintaining healthy host plants was critical for producing large numbers of healthy insects. The horehound plants were grown in a sandy loam, with a pH of 8, in 20cm pots. The plants were watered so as to maintain the soil continually damp. When in the glasshouse, large variations in soil moisture appeared to make the plants more susceptible to root disease. The plants were fertilised with a soluble complete foliar fertiliser (Phosgen®) once a fortnight. Before plants became badly defoliated, fresh plants were placed alongside to allow larvae to crawl onto the fresh plant. Defoliated plants were cut back and allowed to re-grow outside.

The main pests of horehound in the glasshouse were glasshouse whitefly (*Trialeurodes vaporariorum*), two spotted mite (*Tetranychus urticae*), and the green peach aphid (*Myzus*

, *persicae*). Whiteflies were controlled with the parasitic wasp *Encarsia formosa* and yellow sticky traps. Two spotted mites were controlled with monthly releases of the predatory mites, *Phytoseiulus persimilis* and *Galendromus occidentalis* and, when necessary, the acaricide propargite at 0.15 gai/l. Green peach aphids were controlled with pirimicarb applied at 0.25 gai/l. Neither propargite or pirimicarb had any apparent detrimental effects on any stage of the plume moth at the rates used. Argentine ants (*Linepithema humile*) also caused problems and often established colonies in the pots. Before being moved into a glasshouse, pots were dipped in maldison (5 gai/10l) to destroy the ant colonies. After treatment with maldison, plants were held for two weeks before insects were placed onto them.

3.3.2 Disease

Soon after receiving the old French colony from the Keith Turnbull Research Institute, viral symptoms were observed in the colony. Infected larvae were pale in colour, often turning bright yellow. These colour changes persisted after death. Infected pupae were deformed and blackened. The disease was not identified conclusively, but inspections suggested that the larvae were infected by a cytoplasmic polyhedrosis virus (Professor Dudley Pinnock, then at the University of Adelaide, pers. Comm.). To remove any disease from the colony, eggs and pupae were washed initially with 0.05% Tween®, followed by 5% formalin, followed by a triple rinse in distilled water. All cages and instruments were washed in concentrated sodium hypochlorite. Rather than placing 5 - 15 pairs in large cages with six plants, 2 - 4 pairs were placed into smaller cages with one host plant, until disease symptoms were removed from the colony. The contents of cages with infected insects were destroyed. These treatments continued for two generations. Heat stress was observed to increase the visible disease symptoms. Every generation for the duration of the project, fifty randomly selected fourth instars were stressed by exposing them to 30ºC for 24 hours. No individuals were observed with viral symptoms following these treatments. During this process, the old French colony was put through a genetic bottleneck of approximately 140 individuals.

Before the viral symptoms were observed in the old French colony, a "practice" release was made with this population at the Cobbler Creek Recreation Park, Adelaide. The release was made on the 20th December 1996. Two hundred pupae were released in a tent, using the methods described in chapter 4. This site was later evaluated to give an estimate of the proportion of insects showing visual disease symptoms. The site was assessed for three generations after release. Each evaluation was timed so that the majority of insects were late instars. Three 100m transects were made through the release point. Two 0.1m^2 quadrats were randomly sampled every five metres. The total number of insects and the number showing visual viral symptoms were recorded. The proportion of infected insects declined until the third field generation, when no insects were observed with visual disease symptoms (Table 3.4). It cannot be concluded that the presumed virus was no longer present in the field population, but the results suggest the disease had become considerably less common under field conditions and may not have had a great influence on the establishment of this insect population in past field releases.

Table 3.4 Proportion of insects (old French population) showing visual viral symptoms in the field.

Generation	Transect number	% Insects showing viral symptoms	Total number of insects sampled
1	1	44	16
Sept. 1997	2	36	14
	3	44	9
2	1	13	15
Nov. 1997	2	15	13
	3	7	14
3	1	0	60
Jan. 1998	2	0	53
	3	0	34

3.4 Origins of the bitou tip moth populations

Two populations of the bitou tip moth were imported into Australia. Both were reared through their first generation at the University of Adelaide's quarantine facility. The first was collected from three coastal sites in the Natal province of South Africa, Durban, Shaka's Rock and St Lucia, from the sub-species bitou bush (*Chrysanthemoides monilifera rotundata*). The second population was found on boneseed (*C. m. monilifera)* at two sites near Cape Town, Rooiels and Bredasdorp (Table 3.5). Both populations were collected by Petra Mueller of CSIRO Entomology, Cape Town.

Table 3.5 Importation details for the two bitou tip moth populations under evaluation.

Colony name	Origin	Weed sub-species present at site	Date imported	No. insects imported
Cape Town	Rooiels	*C.m.monilifera*	03.12.96	65
	Bredasdorp	*C.m.monilifera*	03.12.96	34
Natal	Durban	*C.m.rotundata*	31.07.96	20
	Shaka's Rock	*C.m.rotundata*	31.07.96	16
	St Lucia	*C.m.rotundata*	31.07.96	49

There are significant differences between the climates of the South African collection sites, Durban and Cape Town, and the two representative examples of the original release sites in Australia, Adelaide and Port Kembla (Table 3.6). Establishment of the moths could not be achieved with releases made in January and April 1993 at the Adelaide site with moth populations collected from Durban. In contrast the colony readily established at Port Kembla, New South Wales. As mentioned in chapter 2, the original populations of the bitou tip moth imported into Australia in 1989 were collected in the Natal province, from bitou bush, and Cape Peninsula, from *C. m. pisifera.*

Table 3.6 Climate details for the two South African collection locations and two of the original Australian release sites, Adelaide, South Australia and Port Kembla, New South Wales (* - data not available).

	Durban, Natal Province	Cape Town, Cape Province	Adelaide (SA)	Port Kembla (NSW)
Latitude	29.50 S	33.54 S	34.92 S	34.48 S
Longitude	31.02 E	18.32 E	138.62 E	150.91 E
Elevation (m)	5	17	48	11
Av. annual rainfall (mm)	1008	509	555.1	1277.4
Av. Jan. rainfall	109	15	20.5	116.1
Av. July rainfall	28	89	84.6	62.6
No. days > 40°C	*	*	2.3	0.1
No. days > 35°C	*	*	17.5	1.6
No. days > 32.2°C	4	*	*	*
No. days < 0°C	0	0	0.1	0
Av. max. Jan. temp.	27	26	28.5	24.1
Av. max. July temp.	22	17	15.1	16.7
Av. min. Jan. temp.	21	16	16.6	18.4
Av. min. July temp.	11	7	7.4	9.8
Extreme maximum temperature (°C)	42	39	44.2	41.9
Extreme minimum temperature (°C)	4	-2	-0.4	1.1
Jan. 3pm relative humidity (%)	72	54	36	75
July 3pm relative humidity (%)	61	67	61	56

CLIMEX was used to compare the South African collection locations with two of the original release locations, Adelaide and Port Kembla. The CLIMEX match indices and the climate data show that Adelaide has a similar climate to Cape Town, while Port Kembla's climate is more similar to Durban's climate (Table 3.7). The main differences between Adelaide and Durban's climates are total rainfall, rainfall pattern and relative humidity. CLIMEX was not used to predict the potential distribution of the bitou tip moth in Australia using the known distribution in South Africa. These results would be likely to be meaningless since the climatic adaptation of the bitou tip moth is likely to vary over its large native range. A population from one locality is unlikely to be suited to such a wide range of climates.

Table 3.7 CLIMEX match indices, comparing collection and release locations for the bitou tip moth. The higher the value of the match index, the better the match.

Match index (I) (0-100)	Release location	Cape Town	Durban
I total	Adelaide	68	30
	Port Kembla	39	56
I max. temperature	Adelaide	84	60
	Port Kembla	82	57
I min. temperature	Adelaide	80	45
	Port Kembla	75	74
I total rainfall	Adelaide	77	51
	Port Kembla	51	78
I rainfall pattern	Adelaide	89	52
	Port Kembla	46	70
I humidity	Adelaide	51	13
	Port Kembla	32	59

Another climate modelling program, CLIMATE (Pheloung, 1996) was also used to compare the South African collection locations with Australia's climate regions. The results were consistent with the data generated by CLIMEX. Durban was shown to have a climate similar to coastal areas of New South Wales and southern Queensland (Figure 3.1). These regions coincide well with the areas where the original bitou tip moth population was established and where the bitou bush sub species of the weed is present. Cape Town was shown to have a climate similar to southern South Australia and southwestern Western Australia (Figure 3.2).

3.4.1 Rearing

The bitou tip moths were shipped from South Africa as late instars on plant cuttings. After importation, each population was first reared through one generation in quarantine. On arrival the insects were transferred to fresh cuttings, placed in aerated lunch box containers (12 x 18 x 7cm), one insect per container. These plant cuttings were changed every three days. Once the insects had pupated they were transferred to plastic aerated vials. Adults were allowed to mate in plastic cups (7cm diameter x 7cm height). A boneseed leaf was placed into each cup and a cotton wool ball, moistened with 15% honey solution, inserted into the lid. The cotton wool

ball was replaced every second day. Female moths laid their eggs onto the leaf and sides of the cup. Emerging neonate larvae were then transferred to a plant shoot with a fine paint brush, at a density of two per shoot. The number of pairs that was successfully mated in this first generation was 35 for the Natal population, with an average of 17.6 offspring per pair, and 31 for the Cape Town population, with an average of 19.9 offspring per pair. Pupae were then removed from quarantine. Both the eggs and pupae were washed in 0.1% sodium hypochlorite, followed by a triple rinse in distilled water.

Two glasshouse rooms, maintained at 25°C and 22°C, and one polyhouse, maintained at ambient temperature, were available for the rearing of each population. The procedure was similar to that used in quarantine. Pupae were collected from potted plants in the glasshouse, adults were mated in plastic cups, and the neonates placed back onto fresh potted plants. Each member of a pair originated from different parents. A minimum of 200 individuals was used for the production of each new generation.

Plants were grown in a sandy loam, pH 7, in 20cm pots. Plants were watered once a week in winter and at least three times a week in summer. A soluble complete foliar fertiliser (Phosgen®) was applied once a fortnight.

Boneseed and bitou bush plants grown in the glasshouse were infested by the same range of pest species as horehound plants. The main pest was glasshouse whitefly. The parasitic wasp, *Encarsia formosa*, and the yellow sticky traps merely slowed the whitefly population growth rate slightly. This meant that glasshouse plants had to be changed regularly with clean plants from outside, where whitefly didn't prosper. All whitefly-active insecticides registered in Australia were found to adversely affect the bitou tip moth.
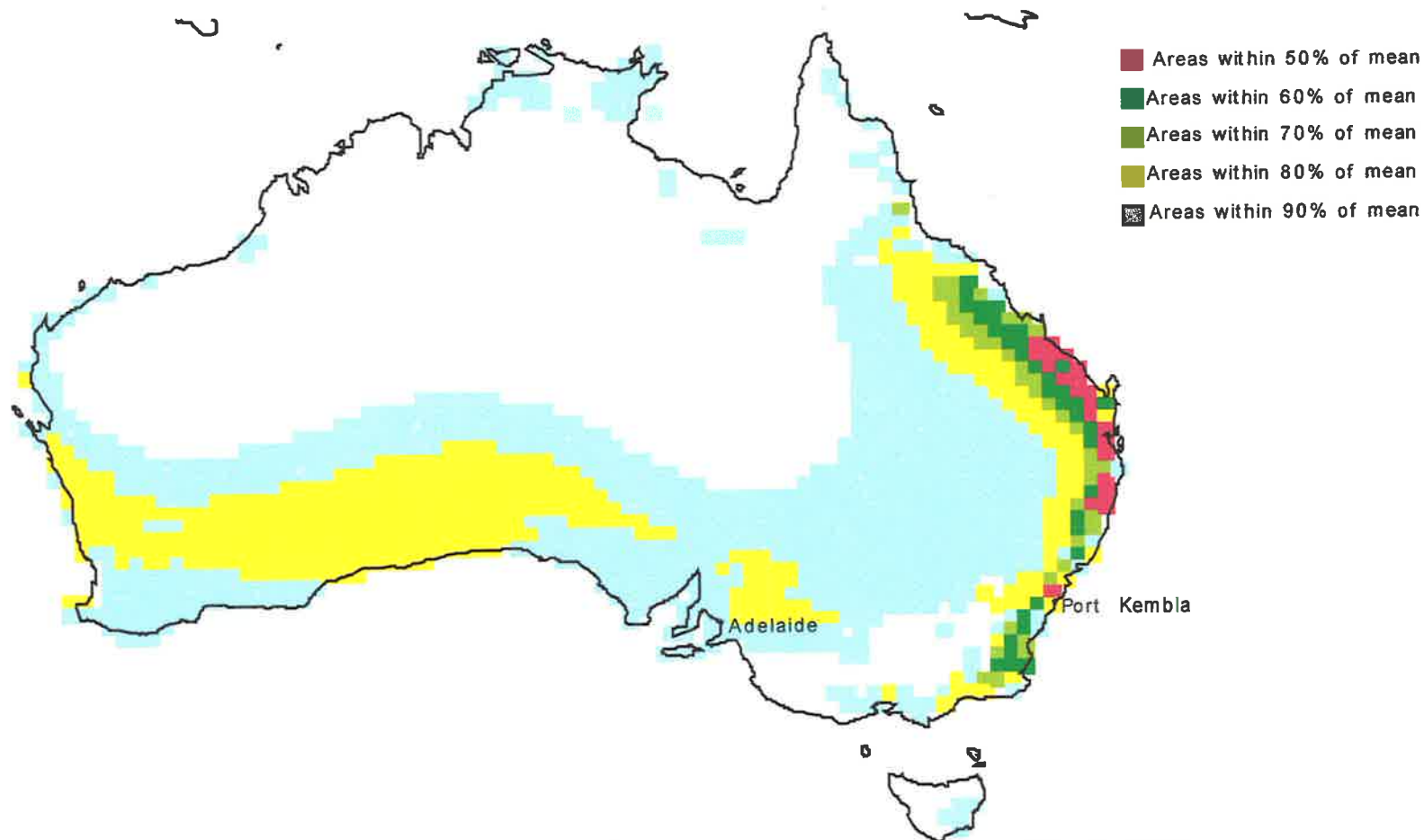
Figure 3.1: Comparison of Durban's climate with Australia's climatic regions, using CLIMATE. The darker shaded areas have a more similar climate to Durban.
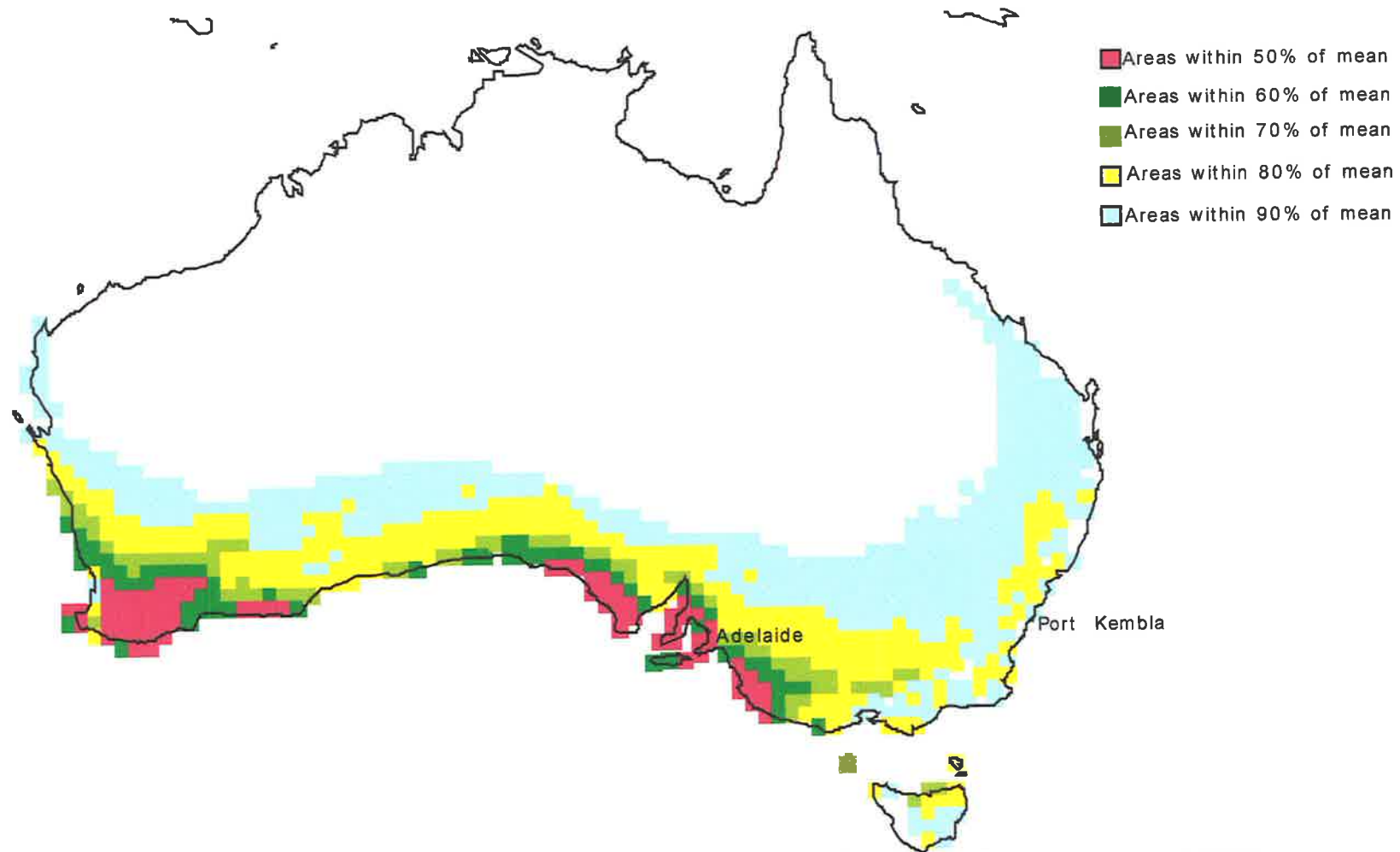
Figure 3.2: Comparison of Cape Town's climate with Australia's climatic regions, using CLIMATE. The darker shaded areas have a more similar climate to Cape Town.

Chapter 4

Climate matching in the plume moth: how close is close enough?

4.1 Introduction

Because the environments of collection and release locations are never identical, it has been proposed that climate may be one important impediment to the establishment of biological control agents (Crawley, 1986; Cameron *et al.*, 1993). The aim of the experiments outlined in this chapter was to test the hypothesis that the original population of the plume moth released in South Australia failed to establish in the field because it was poorly adapted to South Australia's Mediterranean climate. It was hypothesised that the French populations were poorly adapted to high temperatures and the die-back of plants during mid-late summer, and that a population originating from central Spain would be better adapted to these conditions. This hypothesis was tested by comparing three populations of the plume moth in laboratory studies (old French, new French and Spanish). The three populations were also evaluated in the field to determine if any detected differences were important.

4.2 Materials and methods

4.2.1 Experiment 1: the influence of temperature on egg development time

The aim of this experiment was to compare egg development times at various temperatures for the three insect populations.

For all three insect populations, ten adult pairs were mated at 25°C in small cages (30 x 30 x 30cm). Flowering horehound plants were placed into each cage. Leaves containing their eggs

were collected and placed into plastic aerated containers (8 x 15 x 10cm) in growth cabinets, set at 16, 20, 24, 28, 32 and 36°C. Eggs were between zero and six hours old when collected from the mating cages. This experiment was repeated four times to give four replicates. Eight to nineteen eggs were used for each replicate. The temperature cabinets were set at 14 hrs of light, 10 hrs dark (14 L: 10 D).

To evaluate the effect of humidity on percentage egg survival, the experiment was conducted at 70 - 80% and 40 - 50% humidity. To maintain the humidities in these ranges, trays containing saturated solutions of $NaCl$ and $NH_4NO_3+NaNO_3$ respectively were placed into the temperature cabinets (Winston and Bates, 1960). The humidity and temperature of each cabinet was checked daily.

The eggs were checked twice a day, at 8.00am and 6.00pm, and the time of hatch recorded.

The relationship between developmental time and temperature was firstly analysed with a restricted or residual maximum likelihood analysis (REML) using Genstat (Lane and Payne, 1996). The data were grossly unbalanced due to high egg mortality at the higher temperatures. Compared to an ANOVA, a REML analysis is better able to deal with unbalanced data. Like an ANOVA, a REML analysis allows consideration of the design of the experiment by enabling more than one source of extraneous variation to be included. Models are fitted with and without random effects (blocks and insect number); the significance of random effects can be determined by examining the change in deviance. The REML analysis indicated that the difference between replicates was small. The significance of the treatment effects was tested using a likelihood ratio test (Welham and Thompson, 1997).

The relationship between temperature, humidity and percent egg hatch was analysed with a multiple linear regression using Genstat. Genstat contains a number of non-linear regression models (exponential, logistic, generalised logistic, linear-by-linear, quadratic-by-linear, quadratic-by-quadratic, line plus exponential, double exponential, Gompertz, Fourier, double

Fourier, Gaussian, double Gaussian), that were routinely tried. A non-linear regression analysis and a regression using the cubic polynomial were conducted but these tests did not account for any more variation.

4.2.2 Experiment 2: influence of temperature on larval development

To compare the response of larval stages of the three plume moth populations to temperature, 30 neonates per population were placed onto healthy horehound cuttings in growth cabinets, set at 20, 25, 30 and 35°C. A temperature of 15°C was also attempted. However, after six weeks the larvae had not developed past their first instar. Due to a shortage of growth cabinets, this treatment was abandoned. Clearly 15°C was approaching their lower threshold of development. The temperature cabinets were set at 14 L: 10 D.

Because the health of the parents can have an influence on the quality of the progeny (Wellington, 1965), the parents of each of the insects used in this experiment were reared at the same temperature as their progeny, except that the parents of the insects exposed to 35°C were reared at 30°C, due to high mortality at 35°C. The 30 insects used for each treatment originated from at least six parents. The neonates were 0 - 12 hours old at the commencement of the experiment.

The base of the plant cuttings were placed in sealed vials filled with water. These cuttings were changed every second day. Larvae were carefully moved to a fresh cutting using a fine paint brush. One larva was placed onto each cutting.

The time for each insect to reach the pupal stage, their pupal weight and percent survival were recorded.

The humidity was promoted to near 70 - 80% inside the containers using a saturated solution of NaCl. Trays containing this solution were placed under each plant cutting.

The experiment was repeated three times at weekly intervals to give three replicates.

The relationships between developmental time and pupal weight, and temperature were firstly analysed with a REML analysis using Genstat. The REML analysis indicated that the difference between replicates was small for the developmental time data. The relationship between temperature and percent survival was analysed with a multiple linear regression using the cubic polynomial, using Genstat. A non linear regression analysis and a regression using the cubic polynomial were also conducted but these tests did not account for more variation.

4.2.3 Experiment 3: the influence of temperature on fecundity

To compare the fecundity of the three insect populations at different temperatures, ten adult pairs per temperature were mated in cages placed in growth rooms set at 20, 25, 30 and 35°C. Each cage was placed over a healthy non-flowering potted horehound plant. Only total fecundity was evaluated, the percentage of viable eggs was not determined in this experiment. The number of eggs was recorded ten days after mating or until the death of the female moth. More than 77% of eggs are laid in the first ten days after mating and the moths have one pre-oviposition day (J. Weiss and K. Lippai, unpublished).

Plume moth fecundity was observed to be dependent on cage size, plant health (size of leaves) and size, and the presence or absence of flowers. All experiments evaluating plume moth fecundity were conducted with the same size cages (30 x 30 x 60cm) and plants (20 - 30cm height). The diameter of the fourth leaf from the top was used as a gauge for plant health; plants with a leaf diameter of 3 - 4cm were used for experiments. None of the plants used for experiments were in flower.

All insects used in this experiment had been reared at 25°C.

The humidity of the growth rooms was maintained at 60 - 70%. This humidity was maintained by adding or removing large trays (90 x 60cm) of rock salt or water. The photoperiod in the rooms was 14 L: 10 D. The experiment was repeated four times at weekly intervals.

The relationship between temperature and fecundity was analysed with a simple linear regression using Genstat. A non-linear regression analysis and a regression using the cubic polynomial was also conducted but these tests did not account for more variation.

4.2.4 Experiment 4: effect of short bursts of a stressful temperature on larval survival

To compare the ability of the three insect populations to tolerate temperature extremes, 20 fourth instars were exposed to 55, 50, 45, 40, 35, 30, 15, 10, 5, 0 and - 5°C, in temperature cabinets, for a period of 12 hours. The 20 larvae were placed on plant cuttings in aerated plastic containers (12 x 18 x 7cm). The containers were lined with tissue paper to soak up excess moisture.

The insects used in this experiment had been reared in a glasshouse at 25°C. The insects exposed to the higher temperatures (30 - 55°C) were first exposed to 30°C for six hours. In Drosophila, it was found that insects can better tolerate heat shock if they are first exposed to a mild shock, due to the activation of heat shock proteins without the disruption of normal protein synthesis (Hoy, 1994). The twenty insects were selected at random from five rearing cages, four per cage. After the insects were exposed to the temperature extremes, they were placed back onto potted plants in a growth room at 25°C. The number of insects surviving to pupation was recorded.

The high temperature (30 – 55°C) treatments were conducted at 70 – 80% and 40 - 50% humidity. The low temperature treatments (-5 to 15°C) were conducted at 40 - 50% humidity only. The photoperiod was 14 L: 10D.

The experiment was repeated four times at weekly intervals. The relationship between temperature and larval survival was analysed with a logistic regression using Genstat.

4.2.5 Experiment 5: effect of water stress on host plant quality

To compare the three insect populations' ability to tolerate poor quality foliage, 20 neonate larvae from each population were placed onto two healthy and two water stressed plants, at a density of one insect per stem (ten per plant). The experiment was conducted in a growth room at 28°C, 40 - 50% humidity and 14 L: 10D.

The water-stressed plants were collected from the field (Port Gawler, South Australia) in late January, 1998. These plants were watered so as to maintain the foliage in a condition visually similar to that of field plants in late summer in the Adelaide area. The healthy plants were maintained so as to maintain the plants in a condition similar to that observed in the Adelaide region in early spring. Prior to the start of the experiment, three stems were removed from each plant to estimate moisture content. Moisture content was measured by weighing the stems prior to and after placing the stems in a drying cabinet (60°C) for 48 hrs. The average leaf diameter (fourth leaf from apex) and moisture content for the healthy plants were roughly twice as much as the poor quality plants (Table 4.1).

Table 4.1 Average leaf diameter and % moisture for healthy and water stressed plants used in the experiment.

Treatment	Block	Av. leaf diameter *	% Moisture
High water	a	2.24	76.4
	b	2.06	78.2
	c	2.42	74.1
Low water	a	0.88	42.9
	b	0.82	44.7
	c	0.78	37.9

* 4th leaf from the apex.

The 20 larvae were 0 - 12 hours old when transferred to the experimental plants. The insect populations were assigned to the plants randomly. The 20 larvae originated from five sets of parents, 4 from each parent.

Larval development rate, pupal weight and percentage survival were recorded. The experiment was repeated four times at weekly intervals.

The data were grossly unbalanced due to higher larval mortality on the low water plants. A REML analysis was therefore used to assess the relationship between host plant quality and developmental time and pupal weight. A one way ANOVA was used to assess the relationship between host plant quality and percent larval survival.

4.2.6 Experiment 6: diapause

Hibernal diapause

The plume moth was observed to have a hibernal diapause during winter and a facultative aestival diapause. No experiments were designed specifically to look at differences in the

three insect populations' hibernal diapause. However, differences were observed in the timing and trigger of diapause while rearing the colonies and conducting reciprocal crosses in cages located outdoors. These crosses were conducted during February and March 1998, and happened to coincide with the entry of each population into their winter diapause. Because of this, the crosses were no longer useful for their original purpose. For each population, ten sets of parents were mated individually in cages, at weekly intervals, for a period of eight weeks. The mating date where progeny were observed to diapause in the stem apex was recorded.

Aestival diapause

In the field during summer, first instar larvae were observed to halt development in the stem apex during periods of very hot weather and/or when host plants became severely water stressed. Whether this halt in development was a true diapause or quiescence was not determined. Further work determining the trigger for diapause induction and exit is required to answer this question. For the purpose of this thesis it will be called a diapause.

To compare the three insect populations' ability to diapause in the field during prolonged high temperatures, 80 neonate larvae of each insect population were placed onto four potted plants. These plants were placed into a growth room set at 12 hours of $35^{\circ}C$, followed by 12 hours at $25^{\circ}C$, 14 L: 10D. The four plants were treated as blocks. The insects were between 0 and 6 hours old at the start of the experiment and originated from at least 8 sets of parents. After six weeks the growth rooms were put back to $23^{\circ}C$ to allow all surviving insects to continue to develop to pupation. The percentage of insects that continued to develop at $35^{\circ}C / 25^{\circ}C$, halted development as neonate larvae at $35^{\circ}C / 25^{\circ}C$ and survived was recorded.

The data were analysed using Pearson chi-squared contingency tables. The influence of the replicates was first assessed and found to be non-significant (Pearson chi-squared value, 2.31, with 3 degrees of freedom). The replicates were then pooled and a comparison was made for the survival and proportion of insects entering diapause for each insect population.

4.2.7 Experiment 7: field releases

The three plume moth populations were compared in the field in ten release areas. Within each release area, the three populations were released at sites that were at least 500m apart, so that each population did not significantly spread into another during the time they were under evaluation. A distance of 500m was considered adequate on the basis of observations of dispersal by J. Weiss (pers. comm.). The ten release areas were spread across South Australia, from the Flinders Ranges, in the north of the state, to Robe in the southeast of South Australia (Table 4.2). The release areas differed considerably (often more than twofold) in plant fresh weight and moisture content, horehound plant density (Table 4.2), summer temperatures (Table 4.3), rainfall and relative humidity (Table 4.4). Spring plant moisture contents and winter temperatures varied to a lesser extent (Table 4.2).

So that the populations could be compared, each of the three sites within each release area were chosen for similar weed density, weed size, plant quality, infestation area, soil type, land use, vegetation type and climatic conditions. A further six release areas were abandoned due to differences in plant moisture content among the three sites. These differences first became apparent in summer and were due to variation in soil type among sites. All sites had a reasonably consistent stand of horehound over an area of at least 4 hectares, so that the dispersal of moths from the release point could be studied. The release point was placed in the middle of an infestation.

The plume moth releases were conducted between September 1997 and January 1998. Insects were placed into the field at all three sites within a release area on the same day. At each site, 200 pupae were released in field tents. The tents were made from white shade cloth, with dimensions of 3 x 2 x 1m. The tents were left standing for one month after release. The pupae were placed in an open plastic container on a brick, which had the sides treated with Tanglefoot® to prevent ants gaining access to the pupae. A white foam box with large holes cut in the sides was placed over the pupae to protect them from rain.

Table 4.2 Field release site details.

Site/population		February plant fresh wt (g/m²)	February plant % moisture	September plant fresh wt (g/m²)	September plant % moisture	Plants/ m²	% Weed ground cover	Land use	Soil type	Release date
Flinders 1	Sp	939	18.1	1810	54.2	2.2	56	national park	sandy loam/shale	06.11.97
	NF	949	18.9	1830	54.6	2.2	60	national park	sandy loam/shale	06.11.97
	OF	961	19.8	1860	54.3	2.3	62	national park	sandy loam/shale	06.11.97
Flinders 2	Sp	1247	25.3	2170	54.8	1.4	32	national park	sandy loam/shale	05.11.97
	NF	1222	22.1	2100	54.3	1.4	34	national park	sandy loam/shale	05.11.97
	OF	1169	22.9	2090	54.5	1.6	37	national park	sandy loam/shale	05.11.97
Flinders 3	Sp	1176	23.7	2070	56.5	1.1	38	national park	sandy loam/shale	05.11.97
	NF	1142	22.5	2100	55.7	1.0	38	national park	sandy loam/shale	05.11.97
	OF	1145	23.2	2100	55.2	1.4	40	national park	sandy loam/shale	05.11.97
Monarto	Sp	988	29.5	1910	55.5	2.3	36	recreational	sandy loam/limestone	04.09.97
	NF	1006	29.1	2030	54.7	2.3	31	recreational	sandy loam/limestone	04.09.97
	OF	1009	30.1	2060	52.9	2.7	33	recreational	sandy loam/limestone	04.09.97
Warooka	Sp	1998	35.3	2860	47.2	3.3	33	grazing	sandy loam/limestone	14.11.97
	NF	2069	38.5	2920	45.2	2.6	29	grazing	sandy loam/limestone	14.11.97
	OF	2007	37.9	2840	46.5	2.5	26	grazing	sandy loam/limestone	14.11.97
Adelaide	Sp	1489	35.5	2550	46.3	3.4	46	recreational	clay loam	19.09.97
	NF	1493	38.5	2460	48.8	3.7	47	recreational	clay loam	19.09.97
	OF	1423	37.9	2160	49.1	3.2	39	recreational	clay loam	19.09.97
Narrung 1	Sp	2263	39.6	3570	55.7	2.7	33	grazing	sandy loam	28.11.97
	NF	2229	39.9	3600	54.7	2.5	33	grazing	sandy loam	28.11.97
	OF	2181	39.9	3510	56.1	2.5	34	grazing	sandy loam	28.11.97
Narrung 2	Sp	2159	38.6	3530	55.8	3.1	40	grazing	sandy loam	22.01.98
	NF	2123	38.6	3580	54.2	3.4	37	grazing	sandy loam	22.01.98
	OF	2163	39.2	3650	54.0	3.4	41	grazing	sandy loam	22.01.98
Robe A	Sp	1959	44.9	3180	56.0	3.2	40	grazing	sandy loam	27.11.97
	NF	1939	44.3	3330	55.6	2.7	40	grazing	sandy loam	27.11.97
	OF	1951	43.7	3120	53.2	2.9	42	grazing	sandy loam	27.11.97
Robe B	Sp	2034	44.5	3240	53.4	2.9	45	grazing	sandy loam	27.11.97
	NF	1983	45.0	3060	54.6	3.1	44	grazing	sandy loam	27.11.97
	OF	2014	45.9	3150	54.9	3.1	42	grazing	sandy loam	27.11.97

Table 4.3 Temperature details for the field release areas.

Release Area	No. days max> 40C	No. days max.> 35C	No. days min.< 0C	Av. january max. temp.	Av. january min. temp.	Av. July max. temp.	Av. July min. temp.
Flinders 1	8	42	2	32.9	18.3	13.7	5.1
Flinders 2	8	42	2	32.9	18.3	13.7	5.1
Flinders 3	8	42	2	32.9	18.3	13.7	5.1
Monarto	6	26	3	28.6	14	15.1	4.1
Warooka	0	6	0	26.5	15.6	14.2	6.2
Narrung 1	1	9	2	25.9	14.2	14	5.5
Narrung 2	1	9	2	25.9	14.2	14	5.5
Adelaide	1	17	0	29.2	17.2	14.9	6.7
Robe A	0	0	0	22.1	13.8	13.2	7.7
Robe B	0	0	0	22.1	13.8	13.2	7.7

Table 4.4 Rainfall and humidity details for the field release areas.

Release Area	Av. annual rainfall (mm)	1998 annual rainfall	1998 January rainfall	1998 July rainfall	Av. January 3pm relative humidity	Av. July 3pm relative humidity
Flinders 1	310	412	46	127.6	25	51
Flinders 2	310	412	46	127.6	25	51
Flinders 3	310	412	46	127.6	25	51
Monarto	346	368	9.8	44.4	36	58
Warooka	446	455.6	9.6	55.4	40	69
Narrung 1	468	392.4	4.6	53.8	47	70
Narrung 2	468	392.4	4.6	53.8	47	70
Adelaide	555	546	6	65.4	36	61
Robe A	634	516.2	4.4	75.8	60	76
Robe B	634	516.2	4.4	75.8	60	76

The sites in lower rainfall areas were monitored for four generations and those in wetter areas for three generations. Because of the larger population increases and dispersal rates observed in the wetter areas, it would have been impractical to evaluate these sites for another generation using the same evaluation methods. The evaluations were timed so that the majority of insects were fourth or fifth instars when the insects were easier to detect (evaluation dates are given in appendix 1). However, a range of insect stages was usually present and a close inspection of the stem apex was required. To estimate the total number of insects and dispersal rate at each site, the sites were divided into concentric rings. A $0.1m^2$ quadrat was thrown randomly around each ring. The total number of insects within each quadrat was recorded. The presence or absence of horehound plants in the quadrats was also noted. The number of quadrats thrown in each ring was proportional to the area of that ring (Table 4.5). A minimum of 10 quadrat counts in the first 0 - 5m ring was needed to give a repeatable result.

Table 4.5 The number of $0.1m^2$ quadrats used at the various distances from the release point

Distance from release point	Area (m²) of ring	Quadrat number
tent area	6	4
0-5m	78.5	10
5-10m	235.7	30
10-20m	942.4	120
20-40m	3769.9	480
40-60m	6283.2	800
60-80m	8796.5	1120
80-100m	11309.7	1440
100-120m	13822.9	1761
120-140m	16336.3	2081
140-160m	18849.6	2401
160-180m	21362.8	2721
180-200m	23876.1	3042
200-220m	26389.4	3362

The sites were evaluated until two consecutive rings were encounted where no insects had been detected in the quadrats.

Ms Helena Oakey and Dr Ari Verbyla from the Department of Biometrics, University of Adelaide, applied the method used to analyse the field data. Each release site was analysed separately; the available memory of the Unix environment was insufficient to handle more than one site at a time. The response of interest, quadrat counts, are count data. A major problem with these count data was that the number of zeros obtained is greater than is predicted by accepted modeling tools. In this data set, the zeros were separated into two groups; (1) structural zeros, where no horehound plant and consequently no insects were present, and (2) sampling zero counts where a horehound plant was present but no insects. The structural zero counts give no information about the total number of insects present and can be excluded from the analysis. However they are needed to give an estimate of the horehound coverage of the site.

Even excluding structural zeros, there were far more zero counts than would be predicted by fitting a standard Poisson model. The approach used to model the data has been described as the two-part or Hurdle model (Welsh *et al.*, 1996). The first stage of this technique was to model the proportional presence (ignoring quantities) of insects within the quadrats using a logistic regression. The second stage was to model the mean number of insects per quadrat using a zero truncated Poisson distribution. These models were combined to estimate the overall mean number of insects per quadrat, at each distance from the release point. The methods for combining models and generating the standard error for each distance from the release point are discussed by Welsh *et al.* (1996).

In both stages the explanatory variables were distance (d_k), insect population (c_j) and generation (g_i). All variables were treated as categorial variables. In order to obtain unique estimates of the levels of a particular categorial parameter, contrasts between a baseline level

and other levels of the categorial parameter were used. The first category is taken as the baseline level and the other levels were compared to this baseline level.

When fitting the models, the importance of each explanatory variable was determined by fitting the maximal model which contained all the variables of interest and their interactions, then dropping each model term and determining the change in residual deviance (twice the log likelihood) after dropping the variable. The change in residual deviance was compared to a chi-squared distribution at the 5% level with degrees of freedom equal to the change in degrees of freedom. If the change in deviance was non significant at the 5% level, the term was removed from the model. Results of the logistic regression, zero-inflated Poisson and combination of models are given in appendix 2.

Because no software was available to fit a Zero Truncated Poisson model, a program was written by the Department of Biometrics to run in S-plus.

The final models fitted for each site were as follows:

Adelaide

model1 $\quad \log(P_{ijk}/1-P_{ijk}) = \mu + g_i + c_j + d_k + (cd)_{jk}$

model2 $\quad \log(\lambda_{ijk}) = \mu + g_i + c_j + d_k + (cd)_{jk}$

Monarto

model1 $\quad \log(P_{ijk}/1-P_{ijk}) = \mu + g_i + c_j + d_k + (cd)_{jk} + (gd)_{ik}$

model2 $\quad \log(\lambda_{ijk}) = \mu + g_i + c_j + d_k + (cd)_{jk} + (gc)_{ij}$

Narrung1

model1 $\quad \log(P_{ijk}/1-P_{ijk}) = \mu + g_i + c_j + d_k + (cd)_{jk} + (gd)_{ik} + (gc)_{ij} + (gcd)_{ijk}$

model2 $\quad \log(\lambda_{ijk}) = \mu + g_i + c_j + d_k + (cd)_{jk} + (gd)_{ik}$

Narrung2

    model1 $\log(P_{ijk}/1-P_{ijk}) = \mu + d_k$

    model2 $\log(\lambda_{ijk}) = \mu + c_j + d_k + (cd)_{jk}$

Warooka

    model1 $\log(P_{ijk}/1-P_{ijk}) = \mu + g_i + c_j + d_k + (cd)_{jk} + (gd)_{ik} + (gc)_{ij}$

    model2 $\log(\lambda_{ijk}) = \mu + g_i + c_j + d_k + (gd)_{ik}$

Flinders1

    model1 $\log(P_{ijk}/1-P_{ijk}) = \mu + g_i + c_j + d_k$

    model2 $\log(\lambda_{ijk}) = \mu + g_i$

Flinders2

    model1 $\log(P_{ijk}/1-P_{ijk}) = \mu + g_i + c_j + d_k + (gd)_{ik}$

    model2 $\log(\lambda_{ijk}) = \mu + g_i$

Flinders3

    model1 $\log(P_{ijk}/1-P_{ijk}) = \mu + g_i + c_j + d_k + (gd)_{ik}$

    model2 $\log(\lambda_{ijk}) = \mu + g_i + c_j + d_k + (gd)_{ik}$

RobeA

    model1 $\log(P_{ijk}/1-P_{ijk}) = \mu + g_i + c_j + d_k + (cd)_{jk} + (gd)_{ik} + (gc)_{ij} + (gcd)_{ijk}$

    model2 $\log(\lambda_{ijk}) = \mu + g_i + c_j + d_k + (cd)_{jk} + (gd)_{ik}$

RobeB

    model1 $\log(P_{ijk}/1-P_{ijk}) = \mu + g_i + c_j + d_k + (cd)_{jk} + (gd)_{ik} + (gc)_{ij} + (gcd)_{ijk}$

    model2 $\log(\lambda_{ijk}) = \mu + g_i + c_j + d_k + (cd)_{jk} + (gd)_{ik}$

where:

P_{ijk} is the probability that insects were present in a quadrat in the ith generation of the jth population at the kth distance.

$1\text{-}P_{ijk}$ is the probability that insects were not present in a quadrat in the ith generation of the jth population at the kth distance.

μ is the overall mean in the absence of explanatory variables.

λ_{ijk} is the expected number of insects in the ith generation at the kth distance for the jth strain.

From the predicted number of insects per quadrat ($0.1m^2$) for each distance from the release point (appendix 2), an estimate of the total number of insects at each site at a particular point in time was then calculated. This was calculated by multiplying the number of insects per quadrat by ten to give insects per square metre, then multiplying by the area of each concentric ring and the probability of plants in each concentric ring (calculated by dividing the number of quadrats with horehound plants by the total number of quadrats). The numbers in each concentric ring were then summed to give an estimate of the total number of insects present each generation. The variance of the total number of insects per concentric ring was calculated using the equation:

$$\text{Variance} = 10^2 \, \text{area}_k{}^2 (1/n(q_k)(1-q_k)(v_{ijk}{}^2+\sigma^2)+q_k{}^2\sigma^2)$$

Where q_k = the probability of plants in the kth distance

$\sigma^2 = Var(v_{ijk})$

Area$_k$ is the area for the kth distance.

A variance for the total number of insects per site was calculated from the variance calculated for each distance and the variance co-variance matrix, using the equation:

$$\text{Variance} = \sum_k \text{Var}(\text{T}_{ijk}) + 2\sum_{k<l}\text{Cov}(\text{T}_{ijk},\text{T}_{ijl})$$

Where $\text{Cov}(\text{T}_{ijk}, \text{T}_{ijl}) = 10^2 A_k A_l q_k q_l \text{Cov}(v_{ijk}, v_{ijl})$

Where A_k is the area of the kth distance, A_l is the area for lth distance, q_k is the probability of plants for the kth distance and T_{ijk} is the total number of insects per area.

4.3 Results

4.3.1 Experiment 1: the influence of temperature on egg developmental time

Development rates at the different temperatures and two humidities show little difference between insect populations (figures 4.1 and 4.2). Using the likelihood ratio test, the interaction term between humidity, temperature and insect population was found to be marginally significant (change in deviance=12.61, df=6, p=0.05) for egg development rates. The response variable developmental rate was transformed using the square root, owing to evidence of non-constant variance in the residual plot.
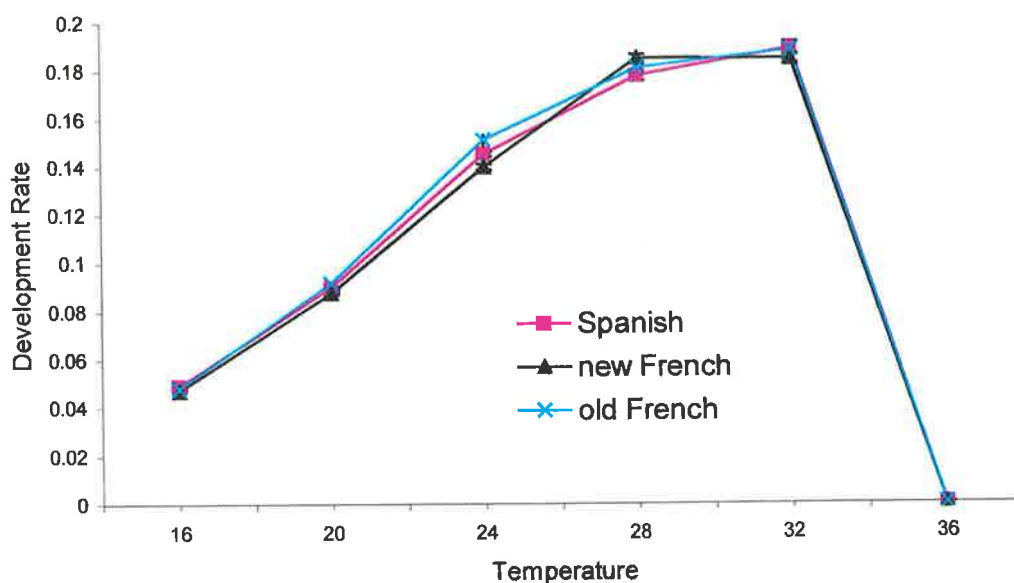
Figure 4.1 Egg development rates for the three plume moth populations, means ± sem, at 80% humidity.
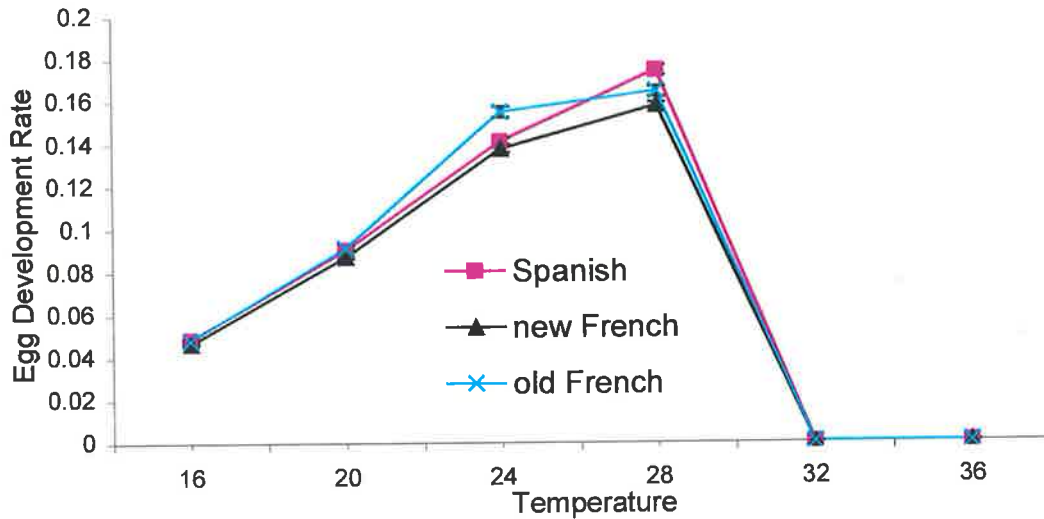
Figure 4.2 Egg development rates, mean ± sem, at 50% humidity.

There appeared to be no difference in the ability of the three insect populations to tolerate low humidity (Figures 4.3 and 4.4). At the lower humidity (40-50%) there was 100% egg mortality at 32 and 36°C. At both low and high humidity, there was a decrease in percent egg hatch with increasing temperature. Percentage egg hatch at the higher temperatures was greater under high humidity, but this was this case for all three insect populations. The linear regression equations for percent egg hatch at 40-50% humidity are very similar for all but the old French colony. The variation accounted for was 78.7%.

Spanish	%egg hatch = 191.2 - 5.226 (temperature)
new French	%egg hatch = 191.6 - 5.234 (temperature)
old French	%egg hatch = 108.7 - 2.968 (temperature)

There was no significant difference in the intercepts and slope between the new French and Spanish populations (t=0.02, t prob=0.982, df=5, 66 and t=0.01, tprob=0.992, df=5, 66). However, there was a significant difference in the intercepts and slope between the old French and the newly imported populations (t=4.22, t prob<0.001, df=5, 66 and t=3.1, t prob=0.003, df=5, 66).

The regression equations for percent egg hatch at the higher humidity (70-80%) were very similar except for the old French colony. The variation accounted for by these models was 62.4%.

Spanish   %egg hatch = 153.47 - 3.178(temperature)

new French   %egg hatch = 149.31 - 3.178(temperature)

old French   %egg hatch = 122.3 - 3.178(temperature)

There was no significant improvement in the proportion of variance accounted for (r^2) when fitting different slopes to each insect population (F=1.85, F prob=0.165, df=2, 66). The intercept for the old French population was significantly different from the two newly imported populations (t=4.71, t prob<0.001, df=3, 68). In contrast there was no significant difference in the intercept between the new French and Spanish populations (t=0.73, t prob=0.471, df=3, 68). The rate at which mortality increased with increasing temperature was slightly slower for the old French population.
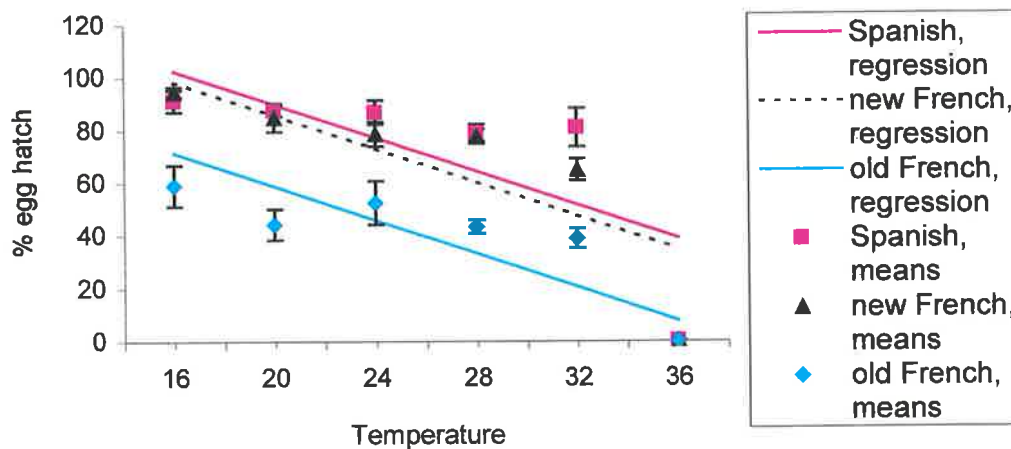
Figure 4.3 Percent egg hatch at 70-80% humidity, for the three plume moth insect populations. Means ± sem and linear regressions are shown.
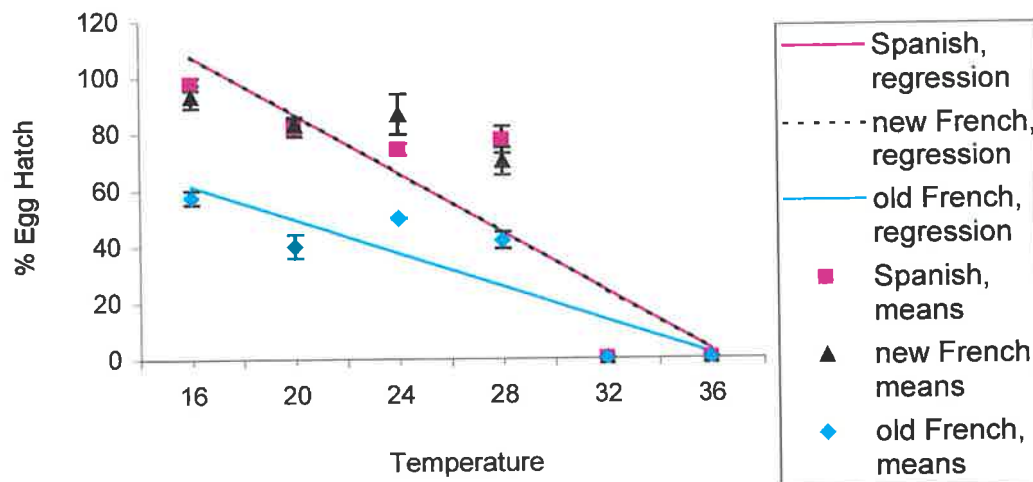
Figure 4.4 Percent egg hatch at 40-50% humidity, for the three plume moth insect populations. Means ± sem and linear regressions are shown.

4.3.2 Experiment 2: the influence of temperature on larval development

Significant differences in the response of each population to temperature were the result of differences at 35°C only (Table 4.6). At this temperature, the Spanish population had a higher development rate than the new French population and there was no development of the old French population due to complete mortality of larvae. Using the likelihood ratio test, the interaction term between temperature and insect population was found to be highly significant for development rates (change in deviance=53.99, df=5, p<0.001). The main effects of temperature (change in deviance=1484, df=3, p<0.001) and insect population (change in deviance=40.88, df=2, p<0.001) were also found to be significant.

Pupal weight was observed to decrease with increasing temperature. The interaction term between temperature and insect population for pupae weight was not significant at the 95% level (change in deviance=3.49, df=5). The main effects of temperature (change in deviance=169.1, df=3, p<0.001) and insect population (change in deviance=24.04, df=2, p<0.001) were found to be significant. Therefore, there was no significant difference between each insect population, in the rate that pupal weight decreased with increasing temperature.

The old French insect population had lower pupal weights at all temperatures, compared with the Spanish population (Table 4.7).

Table 4.6 Larval development rates (1/development time ± sem) for the three insect populations (* missing data due to very slow development rates, # missing data due to 100% larval mortality).

Insect population	Temperature (°C)				
	15	20	25	30	35
Spanish	*	0.017±.00013	0.035±.00029	0.038±.00042	0.023±.00052
new French	*	0.017±.00016	0.034±.00035	0.039±.00049	0.020±.00063
old French	*	0.016±.00009	0.033±.00038	0.038±.00042	#

Table 4.7 Pupal weights (mg ± sem) for the three insect populations (* missing data due to very slow development rates, # missing data due to 100% larval mortality).

Insect population	Temperature (°C)				
	15	20	25	30	35
Spanish	*	24.0±0.44	21.8±0.47	20.8±0.43	12.6±0.68
new French	*	23.6±0.46	21.0±0.38	20.1±0.34	9.9±0.45
old French	*	21.9±0.4	20.7±0.43	19.0±0.32	#

A linear regression using the cubic polynomial was used to describe the relationship between larval mortality and temperature, giving the following regression equations:

Spanish $\quad$ %survival $= 20.94(\text{temp}) - 0.4556(\text{temp})^2 - 141.8$

new French $\quad$ %survival $= 20.94(\text{temp}) - 0.4556(\text{temp})^2 - 146.8$

old French $\quad$ %survival $= 20.94(\text{temp}) - 0.4556(\text{temp})^2 - 159.72$

The percent variation accounted for was 88.1%, fitting different slopes for each population reduced this value to 71.9%. A linear regression accounted for only 7.9% of the variation. The old French population was found to have higher larval mortality compared with the other two populations. This difference was consistent over the range of temperatures tested, ie all three insect populations had a similar response to temperature (Figure 4.5), even though the old French population suffered higher mortality.
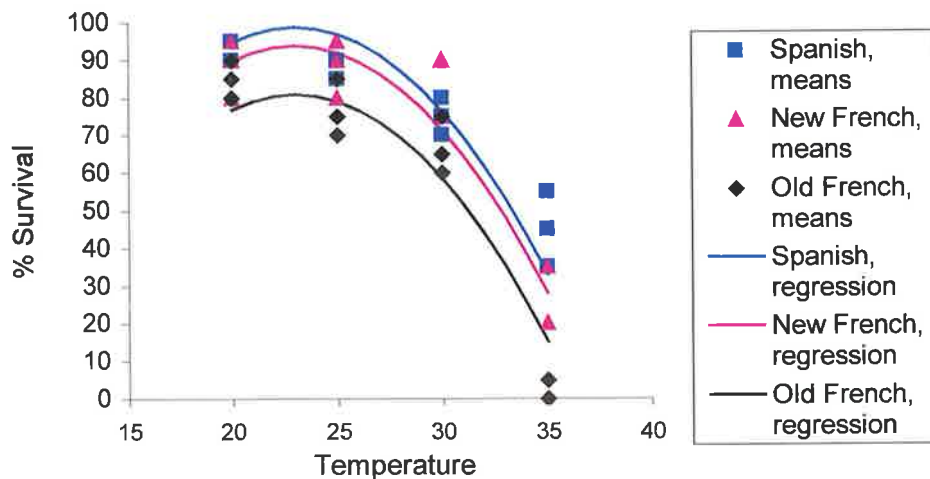
Figure 4.5 Percent larval survival of the three plume moth populations at 20 to 35°C. The replicate means and cubic polynomial regressions are shown.

4.3.3 Experiment 3: the influence of temperature on fecundity

There was a decline in the fecundity of insects from each population with increasing temperature (Figure 4.6). The linear regression equations for the three insect populations are given below.

Spanish	Fecundity = 131.99-3.26(temperature)
new French	Fecundity =141.99-3.633(temperature)
old French	Fecundity = 66.09-1.822(temperature)

The variation accounted for by this regression was only 43.9%. The regression equation using different slopes and intercepts for each insect population had a significantly higher r^2 value compared to the regression equations with the same slopes and different intercepts (F=8.314, df=2, 474, p<0.001). Non linear or polynomial regression did not account for more variation. The low r^2 value would be due to the observed large variation in fecundity between pairs, which may have been the result of factors such as larval nutrition and pupal weight.

The intercept and slope did not vary significantly for the new French and Spanish regression lines (t=0.76, t prob=0.45, df=5, 474 and t= 0.8, t prob=0.426, df=5, 474). There was, however, a significant difference in the intercept and slope values between the two newly imported populations and the old French population (t=5.76, df=5, 474, t prob=<0.001 and t=3.86, df=5, 474, t prob<0.001).
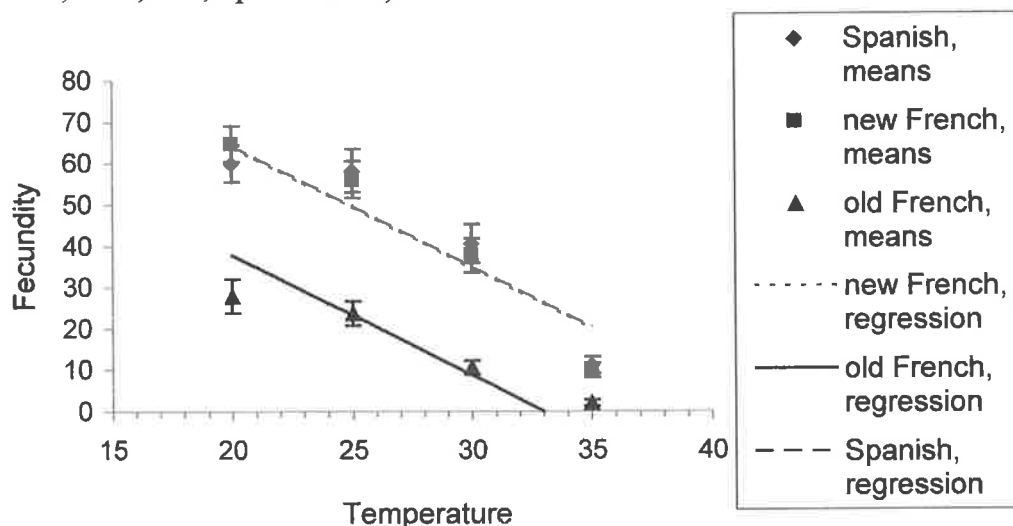
Figure 4.6 The fecundity of the three plume moth populations at 20 - 35°C. The linear regressions and means ± sem are shown.

At 20°C the old French population had a fecundity that was approximately 46% lower than the fecundity of the two newly imported populations. With increasing temperature, the old French population's fecundity decreased at a more rapid rate, suggesting that adults from the old French population were more susceptible to high temperatures (Figure 4.6).

4.3.4 Experiment 4: effect of short bursts of a stressful temperature on larval survival

There were no significant differences in ability of the three insect populations to tolerate short bursts of high temperatures (30-55°C) at either level of humidity (Figure 4.7 and 4.8).

A logistic regression was conducted on the data fitting curves with same slope and intercept, common slopes and separate intercepts, common intercepts and different slopes, and distinct slope and intercept.

For the experiment conducted at 70-80% humidity the percent of variation accounted for by fitting curves with common slope and intercept was 93.7. There was no significant improvement in the r^2 value when fitting curves with separate slopes or intercepts (F=-0.36, df=2, 66). The regression equation was:

$$\% \text{ survival}=-4.38+118.47/(1+EXP(0.2382(temperature-40.197)))$$

Similarly, at 40-50% humidity there was no difference in the ability of the three insect populations to tolerate short bursts of temperature between -10 and 55°C (F= 0.31, df=2, 126) (Figure 4.8). The regression equation was:

$$\% \text{ survival}=-1.23+102.81/(1+EXP(0.2798(temperature-41.016))$$

The r^2 value for this regression was 93.9%.
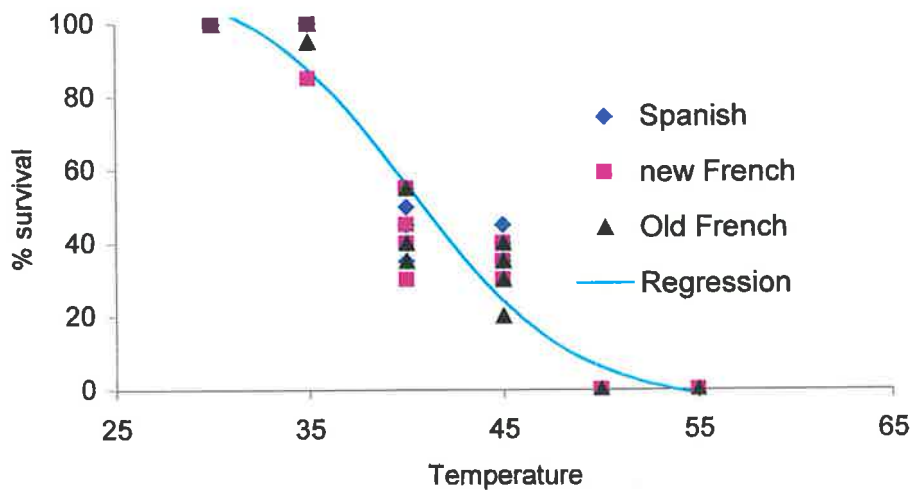
Figure 4.7 Insect survival (%) when exposed to stressful temperatures at 70-80% humidity, for a period of 12 hours. The logistic regression and replicate means are shown.
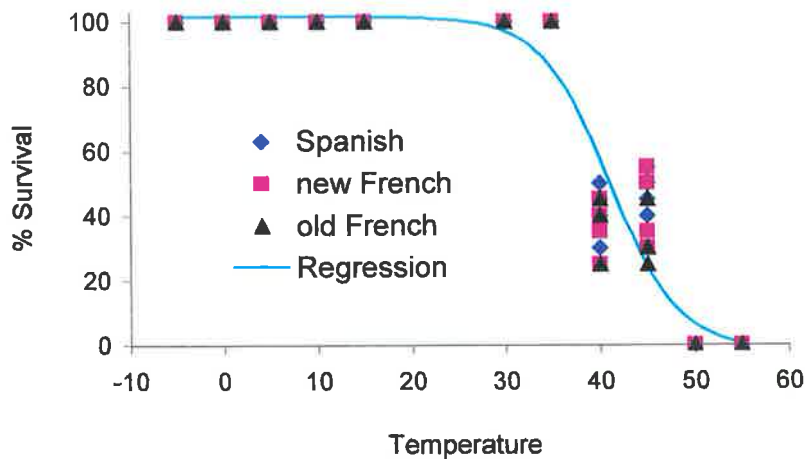
Figure 4.8 Insect survival (%) when exposed to stressful temperatures at 40-50% humidity, for a period of 12 hours. The logistic regression and replicate means are shown.

4.3.5 Experiment 5: effect of water stress on host plant quality

Using the likelihood ratio test the interaction term between water rate and insect source population was found to be non-significant for developmental times (change in deviance=5.08, df=2). The main effects of watering rate and insect population were found to be significant (change in deviance=12.21, df=1), indicating that for all three insect populations, developmental times were longer on water stressed plants, but there were no

significant differences among insect populations (Table 4.8). Using the likelihood ratio test, the interaction term between water rate and insect population was found to be significant for pupal weight (change in deviance=6.593, df=2). For all three populations, pupal weights were higher on the actively growing plants. On the moisture stressed plants, the Spanish population had significantly higher pupal weights, compared with the two French populations. However, the differences were very small. Similarly, the old French population had a higher pupal weight when reared on the actively growing plants, relative to the Spanish and new French populations (Table 4.8). The higher pupal weights of the old French population on actively growing plants may be explained by the lower numbers of insects present on each plant due to higher immature mortality.

Table 4.8 The development time, pupal weight and percent survival for insects reared on actively growing (high water) and moisture stressed (low water) horehound plants.

Insect population	Water rate	Development time (days) mean ± sem	Pupae weight (mg) mean ± sem	Percent survival mean ± sem
Spanish	high	25.96±0.35	21.82±0.48	81.67±6.01
	low	28.12±0.82	17.32±0.6	66.67±1.67
new French	high	27.7±0.4	21.7±0.4	78.33±6.67
	low	30.61±0.8	16.34±1.1	51.67±1.67
old French	high	27.05±0.42	23.35±0.56	61.67±6.01
	low	33.66±1.14	16.14±0.56	53.33±3.33

The interaction between insect population and water rate on insect survival was found to be non-significant (two way ANOVA, df=2, 10, F=2.16, F prob=0.166). For both the new French and Spanish populations, a significantly lower survival rate was observed on the moisture stressed plants, relative to their survival on actively growing plants (two way ANOVA, df=1, 10, F=20.93, F prob=0.001). The old French population also had a lower survival rate on moisture stressed plants. However, the difference was not significant, due to a low survival rate on the actively growing plants. Although there was no significant difference

(two way ANOVA, df=2, 10, F=7.0, F prob=0.013), the Spanish population's mean survival on the moisture stressed plants was higher than the two French insect populations' mean survival rate (Table 4.9).

4.3.6 Experiment 6: diapause

Hibernal diapause

The Spanish population's winter diapause was triggered by photoperiod and minimum temperature, while both of the French populations' winter diapause was triggered by photoperiod only and occurred earlier. Insects from both French populations, located in glasshouses heated to 23°C all year round and in unheated outside cages, commenced their winter diapause at the same time, indicating that temperature had no effect on the onset of diapause. In contrast, the Spanish population entered diapause six weeks earlier in the outside cages, compared to the heated glasshouse. In the glasshouse, Spanish moths mated after the 2nd of March produced offspring that went into diapause, while in the outside cages, insects entered diapause after the 6th of February. This suggests that for the Spanish populations, winter diapause is triggered by both photoperiod and minimum temperature. In the crosses conducted in the glasshouse and in outside cages during January - March 1998, crosses within the French populations made after the 18th of January produced offspring that went directly into diapause.

Aestival diapause

The proportion of insects entering summer diapause at 35°C varied significantly among insect populations (Pearson chi-square value = 29.53, 2 degrees of freedom, p<0.001). The Spanish population was found to have the highest proportion of insects entering diapause, followed by the new French population. Only 1.3% of the old French population entered diapause at 35°C. The total number of insects surviving through to pupation also varied

significantly among all three populations (Pearson chi-squared value = 45.73, 2 degrees of freedom, p<0.001). The Spanish population had the highest survival rate, followed by the new French population. Only 1.3% of the old French population survived (Table 4.9).

Table 4.9 The proportion of first instar larvae entering diapause and the proportion surviving through to pupation, when reared at a constant temperature of $35^{\circ}C$.

Insect population	% Insects in diapause mean ± sem	% Survival mean ± sem
Spanish	35.0±5.4	47.5±4.8
new French	23.8±3.8	26.3±3.8
old French	1.3±1.3	1.3±1.3

4.3.7 Experiment 7: comparison of populations in the field

There were no significant differences detected between the Spanish and the new French insect populations at any of the ten field release areas. There were, however, significant differences in the rate of establishment and population increase between the old French and the two newly imported insect populations (Figures 4.9-4.18), except for the Narrung 2 release area (Figure 4.16). The old French insect population performed particularly poorly in the lower rainfall areas (<400mm), where it failed to establish at 3 of the 4 sites (Figures 4.9-4.11) and the fate of the 4th site is still in doubt (Figure 4.12). In contrast, the newly imported insect populations failed to colonise only 1 of the 4 sites. At sites receiving an average rainfall greater than 400mm per year, the old French population has colonised all sites, but its annual population increase is approximately 75% lower than the population increase observed for the two newly imported insect populations (Table 4.10). For all three insect populations, the observed population increase was considerably greater in the high rainfall areas (Figures 4.13-4.18). It is not clear why the old French population was equivalent to the two newly imported populations at the Narrung 2 release sites. The Narrung 2 release was made during January 1998, two months after the other releases.

Table 4.10 The average population increase per generation for each insect population. The ten release sites have been divided into low, medium and high rainfall classes. The estimated annual increase was calculated using data from generations 2-4 only and assumed three generations per year.

Av. annual rainfall	Insect population	Generation number				Estimated increase/year
		1	2	3	4	
< 400mm	Spanish	1.3	7.4	0.8	6.6	39.1
(4 sites)*	new French	1.2	8.5	0.3	8.5	21.7
	old French	0.5	0.5	0	0.5	0
400-600mm	Spanish	2.1	18.1	11.7		>211.8
(4 sites)*	new French	2.1	21.2	10.9		>231.1
	old French	1.4	11.4	5.5		>62.7
> 600mm	Spanish	3.3	22.2	24.0		>532.8
(2 sites)*	new French	3.2	27.7	19.8		>548.5
	old French	2.5	20.1	5.7		>114.6

* Sites as listed in table 4.4.

Very low population increases were observed in the first field generations. This generation occurred in the release tent, which covered an area of only $6m^2$. High larval mortality was observed within the release tent due to a lack of food and plant buds, which give protection to the early instar larvae.

At the three Flinders Ranges release sites there was a decrease in population levels during the third generation. This generation coincided with an unusually hot/dry period during 1998. Horehound was one of the few understorey plants to retain any green foliage and, as a result, the euros and kangaroos had little choice but to feed on it and, of course, the diapausing insects were ingested along with the plant material. Flinders release area number one was particularly badly affected because there was a kangaroo camp in the middle of the weed infestation. Insects were not recovered from this area after this dry period.

The distribution of insects from the release point in the second field generation was generally leptokurtic (appendix 3, Figures 1-27). The exception was the Adelaide release site where the horehound infestation had a radius of only 40m (appendix 3, Figures 10-12). At the majority of sites the density distribution curves flattened during subsequent generations, with the density of insects decreasing close to the release point and increasing away from the release point. In contrast for the Monarto, Warooka and Flinders 3 sites the density distribution curves remained highly leptokurtic (appendix 3, Figures 1-3, 7-9 and 19-21). At the Monarto, Warooka and Flinders 3 release sites the health of the horehound decreased slightly moving away from the release point, due to changes in soil type. This may explain why the density distribution remained leptokurtic during generations 3 and 4, since areas further away from the release point would have a lower insect carrying capacity. The mean insect numbers per quadrat ($0.1m^2$) at each distance from the release point are shown in Appendix 2.

The extent of dispersion was greater in the higher rainfall areas (eg. Compare Figures 4-6 to 22-24, appendix 3). At the Robe sites insects had moved 220m after 3 generations, while at the Flinders release sites they had dispersed 120m after 4 generations. At all sites the extent of dispersion was increasing over time, except for generation 3 in the drier areas, apparently due to the dry conditions.
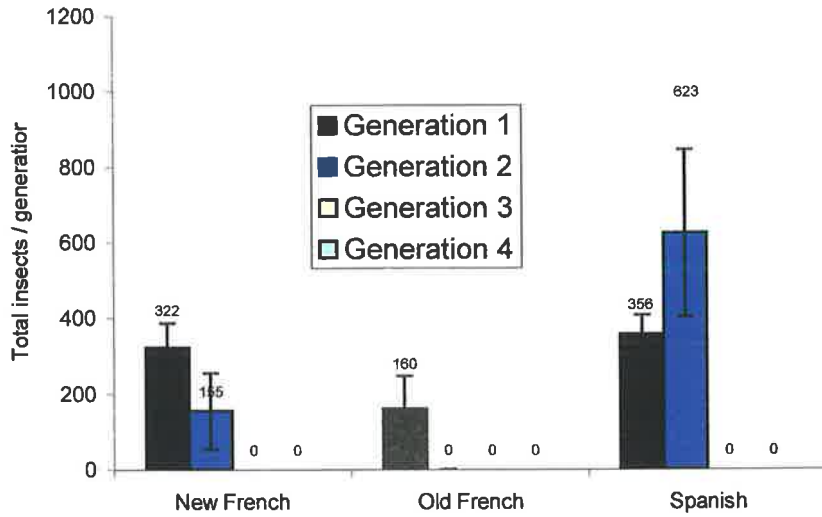
Figure 4.9 The estimated number of insects (± SE) present each generation for the first four field generations at the Flinders 1 release site (<400mm av. annual rainfall). The numbers over the histograms show the estimates for each location, many of which are zeros ("0").
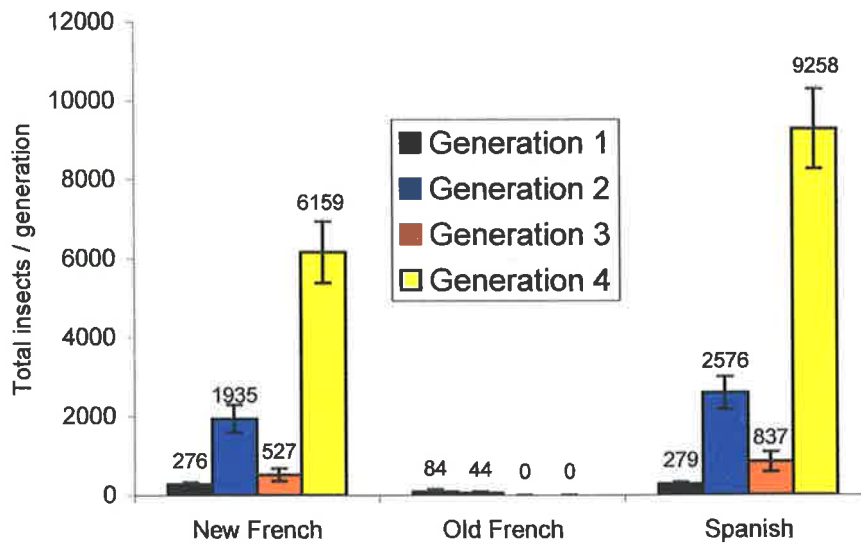
Figure 4.10 The estimated number of insects (± SE) present each generation for the first four field generations at the Flinders 2 release site (<400mm av. annual rainfall).
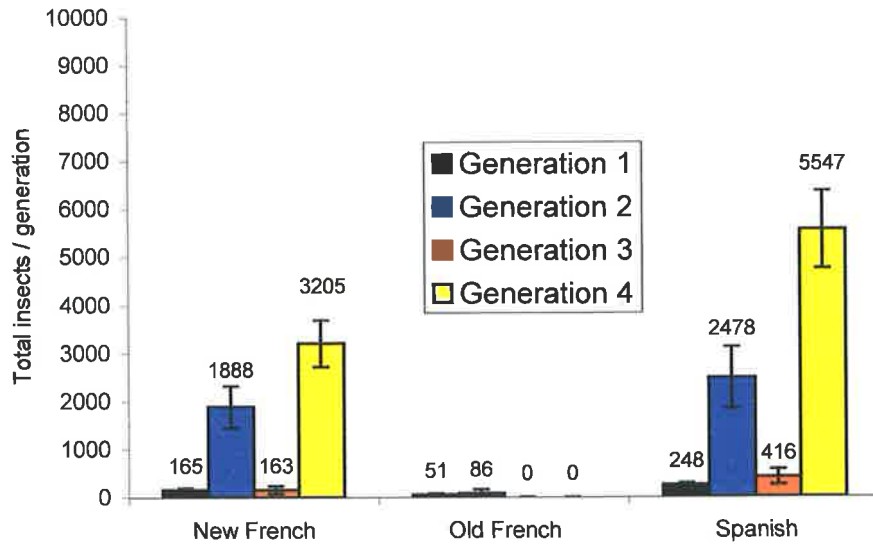
Figure 4.11 The estimated number of insects (± SE) present each generation for the first four field generations at the Flinders 3 release site (<400mm av. annual rainfall).
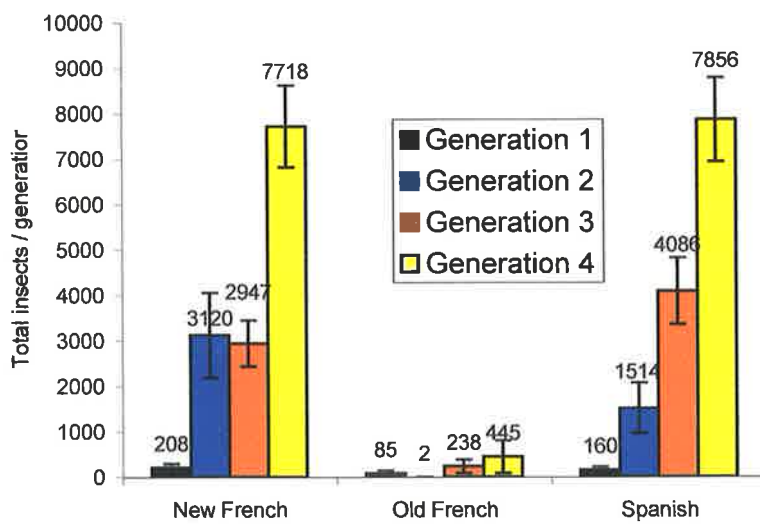
Figure 4.12 The estimated number of insects (± SE) present each generation for the first four field generations at the Monarto release site (<400mm av. annual rainfall).
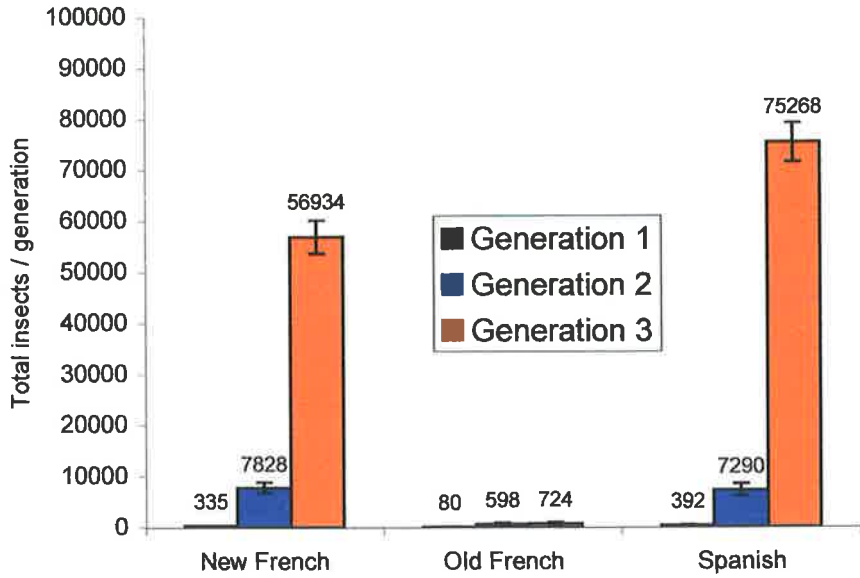
Figure 4.13 The estimated number of insects (± SE) present each generation for the first three field generations at the Warooka release site (400-600mm av. annual rainfall).
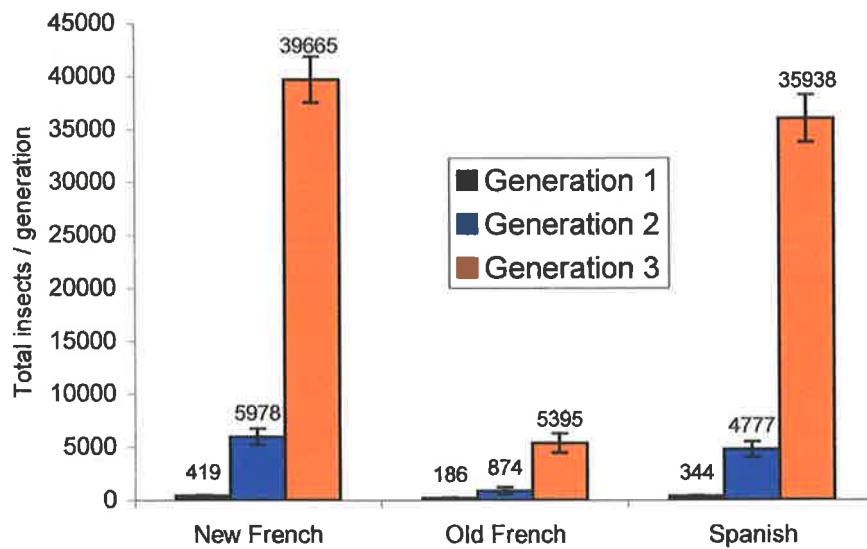
Figure 4.14 The estimated number of insects (± SE) present each generation for the first three field generations at the Adelaide release site (400-600mm av. annual rainfall).
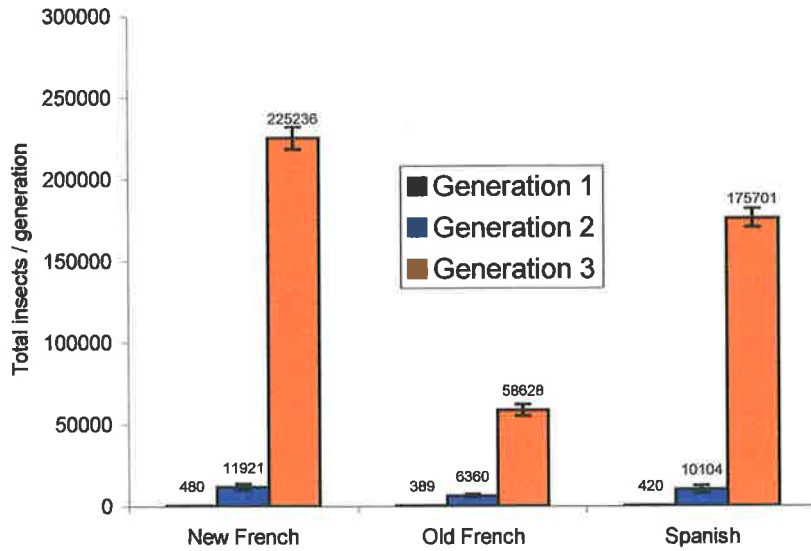
Figure 4.15 The estimated number of insects (± SE) present each generation for the first three field generations at the Narrung 1 release site (400-600mm av. annual rainfall).
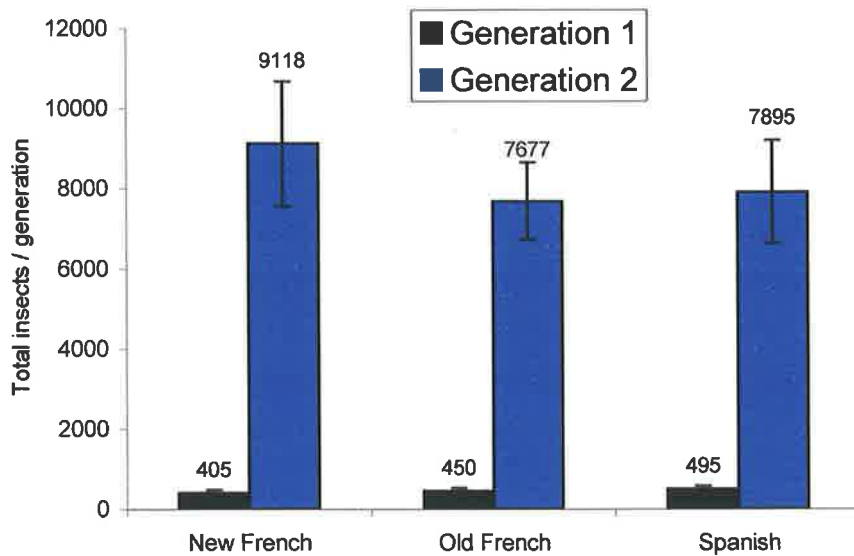
Figure 4.16 The estimated number of insects (± SE) present each generation for the first two field generations at the Narrung 2 release site (400-600mm av. annual rainfall).
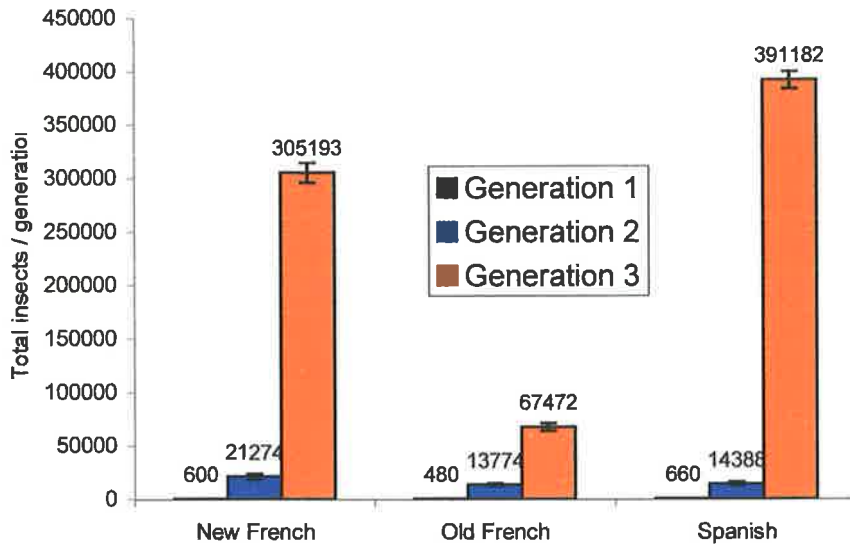
Figure 4.17 The estimated number of insects (± SE) present each generation for the first three field generations at the Robe A release site (>600mm av. annual rainfall).
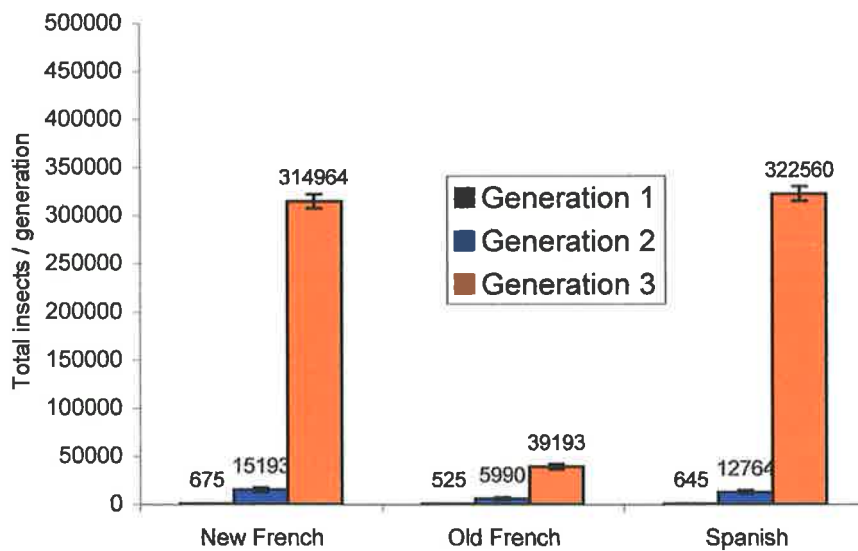
Figure 4.18 The estimated number of insects (± SE) present each generation for the first three field generations at the Robe B release site (>600mm av. annual rainfall).

4.4 Discussion

The laboratory experiments outlined in this chapter found few important differences between the new French and Spanish populations. However, there were large differences detected between the old French and the newly imported populations.

The results of the laboratory studies were consistent with the results of the field experiments, where there was little difference observed between the new French and Spanish population's but large differences observed between the old French and the two newly imported populations. The poor performance of the old French insect population in the field is likely to be the result of two factors. Firstly, as found in experiment 3, the old French insect population's mean fecundity was found to be 50% lower than the newly imported populations at 20°C, and 80% lower at 35°C. Secondly, in experiment 6, a very low proportion of the old French insect population (1.3%) were observed to enter diapause at high temperatures. The ability to cease development during stressful summer conditions would have been particularly important at the four low rainfall sites, which experience dry/hot summers. This factor, combined with the fact that the old French population's fecundity decreased at a more rapid rate with increasing temperature, explain the very poor performance of the old French population at sites which experienced hotter/drier summers (Figures 4.9-4.12). Even at the more southerly sites, which experience milder summers, the population increase per generation was considerably lower than the newly imported populations (Figures 4.17-4.18). This result was consistent with the results of laboratory experiment 3 for temperatures between 20 and 30°C. The old French population also suffered from higher larval mortality, irrespective of the rearing temperature (Figure 4.5). The reasons behind the observed differences between the old French and two newly imported populations are investigated in chapter 5.

There are several possible reasons for the large differences in population increase observed between the different climatic areas of South Australia. Firstly, and possibly most

importantly, there was a large difference in the quality and quantity of the host plant between the high and low rainfall areas, particularly during mid-late summer (Table 4.3). In the low rainfall areas, a maximum of two larvae were observed developing on each stem, despite the fact that large numbers of eggs were observed on each stem close to the release point, suggesting very high larval mortality due to a lack of quality food and buds to provide protection to early instars. On moisture stressed plants, larvae were only observed feeding on the shoot tips, not on the lower leaves and shoots. At the higher rainfall sites, greater than 10 larvae were observed to develop on individual stems. In experiment 5, plume moth larvae were generally found to have longer development times, smaller pupal weights and lower survival rates on moisture stressed plants. Secondly, the lower rainfall sites all experienced considerably higher temperatures (Table 4.4). In experiments 1, 2 and 3, egg and larval mortality rates were found to increase with increasing temperature and fecundity was found to decrease. A third factor would be that a higher proportion of the plume moth population would be spending the peak of summer in an aestival diapause in the lower rainfall areas due to higher temperatures and lower plant quality, thereby reducing the number of generations per year. Finally, greater variation in development rates was observed when the plume moth was exposed to stressful conditions. For example, on moisture stressed plants, the standard error of development times was at least twice that for actively growing host plants (Table 4.9). This could prevent or at least delay mating because fewer adults would be emerging at the one time. This would be compounded by the fact that adults live for a shorter period of time during hot, dry conditions when there is no food source available. While every effort was made to search the plants thoroughly, some insects in facultative aestival diapause may have been missed. This may have led to the underestimation of total insect numbers and differences between the old French and newly imported populations, particularly at the more arid sites.

The greater dispersion of insects in the higher rainfall areas may be due to: the larger populations present at these sites; environmental conditions such as lower temperature and availability of nectar, which would increase the longevity of the adults; and the general health and size of the adults, due to better larval nutrition.

Although the data may be ambiguous due to confounding effects of increased population size, it also appeared that there was an increase in the rate of dispersal (eg. Appendix 3, Figures 22-24 and Appendix 2, Table 9c). The increase in the rate of dispersal over successive generations may be due to increases in the density of the insect population, stimulating adults to travel further afield to avoid competition for their offspring. Allee effects may also be greatest on the periphery when the population is small, and decrease in importance as the population grows. Okubo (1988) observed that the rate of dispersal of an invader's range is initially slow but gradually increases until a constant rate of expansion is achieved. The Allee effect was used to explain this pattern of dispersal. An alternative hypothesis is that a very small proportion of the population is able or driven to travel long distances. As a population's size increases, the number of these individuals increases, giving an apparent increase in the average dispersal of the population.

The most significant differences detected between the new French and Spanish populations was their timing and trigger for their winter diapause and differences in the proportion of insects entering an aestival diapause at high temperatures. Neither of these differences appeared to have a great impact on the performance of the insect populations in the field, either because the field evaluation technique was not able to pick up subtle differences or because the differences were not important in these environments. Had the target weed been an annual, the timing of their winter diapause would be likely to be of much greater importance. Horehound is actively growing for most of the winter period. Twenty four percent of the new French population entered diapause when exposed to 35°C, while 35% of the Spanish population entered diapause. This difference was clearly not important to the establishment of the insects even in the harsh environment of the Flinders Ranges, because there were sufficient new French insects entering diapause to carry the population through the summer period. Horehound stays greener over the summer period in southern France and the area experiences milder summer temperatures relative to Zaragoza, Spain. This may explain why a slightly lower proportion of the new French population entered diapause at high temperatures. The presence of an aestival diapause would be important to avoid periods where

quality food is in short supply and possibly to avoid periods of high temperatures. Zaragoza typically has warmer autumn temperatures compared to southern France. The mean September maximum temperatures for Zaragoza and Cape d'Adge are 26.9° and 20°C respectively, which may explain the earlier entry of the French population into their winter diapause. The source latitudes (Table 3.2) alone would not be enough to explain these differences. Other differences observed between the two newly imported populations included slightly faster development times of the Spanish population at high temperature and slightly higher pupal weights of the Spanish population on poor quality plants. Again, these differences may affect each population in the field, but differences were too subtle to be picked up by the evaluation technique.

No attempt was made to quantify the impact of the plume moth on horehound, but early observations appear promising at the more southerly release sites that receive greater than 400mm of rain per annum and have milder summer temperatures. In the higher rainfall areas, the plume moth was observed to completely defoliate plants close to the release point, where the insect density was greatest. As a result, other vegetation was observed to become more prominent. The large decrease in insect density close to the release point during generation 3 at the Robe and Narrung release sites is likely to be due in part to the insect's effect on the weed (Appendix 3, Figures 13-15 and 22-27, and Appendix 2, Tables 3c, 9c and 10c), with the weed becoming less dense close to the release point. In the lower rainfall areas, no significant impact on the weed was observed over the period of evaluation. This may be due to the lower insect densities, feeding behaviour on moisture stressed plants, and lack of competition from other vegetation. A preliminary release was conducted at the Cobbler Creek Recreation Park during December 1996. By spring 1999 the area of ground covered by horehound over the infested area had been reduced by approximately 40%. This reduction is likely to have been due to the action of the plume moth, because management practices were not changed over this period and no atypical weather conditions were experienced. Unfortunately, the horehound has been replaced by other weeds, such as Paterson's curse and various annual grasses. The integration of the plume moth with other management techniques

and an evaluation of the impact of the moth is the subject of an investigation by Dr Nigel Ainsworth from the Keith Turnbull Research Institute, Department of Natural Resources, Victoria.

The results of the experiments conducted in this chapter do not support the hypothesis that the original releases of the plume moth failed because the insect population imported was poorly adapted to South Australia's Mediterranean climate. Both the new French and Spanish populations were equally well adapted to survive South Australia's stressful summer conditions, despite the fact that the Spanish population originates from a more stressful environment. The old French population was found to be poorly adapted to field conditions, but this is likely to be the result of problems arising during the importation and rearing process, rather than poor climate matching. This hypothesis is explored in chapter 5.

Chapter 5

Effects of lab rearing and inbreeding in the plume moth

5.1 Introduction

One potential problem that may occur during importation and rearing is that genetic variation in attributes that affect success is either lost or not collected (discussed by Roush, 1990).

The loss of genetic variation is only important to biological control if it affects attributes which are important to the success of the agent (Roush, 1990). Lists of desirable characteristics of agents have included categories such as searching ability, host specificity, degree of damage inflicted on the host, reproductive capacity, feeding behaviour, agent size, likelihood of hyperparisitism or disease, compatibility with other control agents and the agent's adaptability to the release environment (Harris, 1973; Goeden, 1983). The adaptability of the agent to the release environment and the presence of parasites and diseases is probably more important to establishment, while the other attributes are probably more important to the degree of control achieved. However, there is no clear evidence that successful agents have possessed a better range of these desirable characteristics than unsuccessful agents (Roush, 1990; Cullen, 1995).

5.1.1 Genetic variation within populations

In the preceding chapter, variation in attributes among insect populations was discussed. Important attributes which have been shown to vary within a population include developmental time, fecundity, longevity, male sterility, climatic adaptation and sex ratio (Hopper *et al.*, 1993). The central questions under discussion are (1) how many individuals should be collected to obtain the necessary amount of genetic variation in important attributes to allow

the population to thrive in the new environment, and (2) how should a laboratory colony be maintained to preserve this level of genetic variation?

In their review of biological control programs in New Zealand, Cameron *et al.* (1993) found that small gene pools were not necessarily detrimental. They found little correlation between the numbers of an agent imported and the degree of success. For example only two females and three males of *Aphelinus mali* were imported into New Zealand for the control of *Erisoma lanigerum*. Despite this, the project was a substantial success. Myers and Sabath (1980) also found that agents that have successfully established have had both high and low genetic variability. However, they assumed that populations with high founder numbers have higher levels of genetic variation.

If genetic variation in important attributes is critical to the successful establishment of an agent, then post release adaptation should be common where establishment has been achieved. High levels of genetic variation may only be critical where post release adaptation is required, ie. where the collected genotypes are not ideally suited to the release environment (Eccleston, 1996). However, in most environments, if not all, genetic variation may be critical to the long term survival of a population, even in the area of origin, to enable the population to survive unpredictable adverse environmental conditions.

There are only a few examples where post release adaptation has been shown to occur. It is not known how commonly post release adaptation occurs or whether it has been essential for establishment, because it has rarely been studied. Changes following release may sometimes be simply due to genetic drift. It is highly unlikely that an exotic agent is so well pre-adapted to the release environment that there is no need for any genetic fine tuning (Hoy, 1985), or maybe, populations are never so finely tuned, as their environments are continually changing. Dobzhansky and Ayala (1973) observed seasonal changes in allele frequencies in two populations of *Drosophila pseudoobscura* and *D. persimilis*, demonstrating that insect populations may be continually changing with their environment. All habitats undergo

fluctuations in environmental conditions, to varying degrees. Therefore, insect populations must already be adapted to survive in a range of environmental conditions, to lesser or greater extents. Insects from stressful or unpredictable climates often show greater variation in attributes such as developmental time, since they must "hedge their bets" to survive through any unpredictable adverse weather (Tauber *et al.*, 1984).

In an example of post colonisation adaptation, Eccleston (1996) found that the parasitoid, *Brachymeria intermedia,* had adapted to the cold winters of the northeastern United States. *B. intermedia* originates from Mediterranean areas of Europe. It appeared that the cold winters had selected for smaller insect size. In another example, the temperature threshold of the cinnabar moth was found to have changed in less than 17 generations after its release in California. The moth originated from France (Myers and Sabath, 1980).

In contradiction to Clausen's (1951) theory that if an agent has not succeeded within three years it never will, there are several examples where the agent's population has remained at a very low density for a number of years before finally reaching effective population levels. Peshken (1972) suggested these lag periods were the result of post colonisation adaptation. However, Hopper and Roush (1993) demonstrated, with models predicting dispersal and population growth, that these lag periods can be explained simply on demographic grounds, ie. at low densities individuals have difficulty finding mates.

Roush (1990) suggested establishment success may not improve with genetic population sizes greater than one hundred, because most important traits are polygenic. Nei *et al.* (1975) demonstrated theoretically that a sample size of 100 will collect 99.9% of the genetic variability of that population. Traits such as size, temperature tolerance, development rate and behaviour are controlled by multiple alleles at multiple loci (Falconer and Mackay, 1996). Because of this, Myers and Sabath (1980) suggested that although some alleles may be lost at some loci, genetic variability will not be greatly reduced when there are relatively few founders. Two important exceptions are pesticide resistance (Roush, 1990) and diapause, that

are often not polygenic (Tauber *et al.*, 1984). Research with *Drosophila* and housefly populations showed that genetic variance may actually increase following a single severe bottleneck (Carson, 1990), although some alleles, rare in the original population, may be lost.

There has been discussion regarding the possibility that some areas of an organism's native range may have higher genetic diversity than others and therefore may make more suitable collection locations. Remington (1968) argued that large central populations are more genetically diverse than marginal populations. Isolated populations or those on the periphery of the species distribution were termed marginal. It was suggested that large central populations are more likely to have suitable genotypes for use as biological control agents. Myers and Sabath (1980) discussed the possibility that central populations are more genetically diverse than marginal populations. They found that there were large differences between species in the comparative genetic variability of marginal versus central populations, demonstrating that large populations do not necessarily have more genetic diversity. The degree of genetic diversity within any one area of an insect's range may be more dependent on the type of environment in that area. Insect populations may need to have higher levels of genetic variation in areas with harsh or unpredictable environments.

Variation within populations may also have important implications for the timing of field collections, as well as the number collected. An example was given in the preceding chapter of variation in diapause induction within a population. Variation in diapause induction may be missed if a collection is made when a proportion of the population is in diapause.

The importance of genetic variation within a population of a biological control agent is unclear. Hopper *et al.* (1993) concluded that demographic barriers (such as failure to find mates) and disease may be more important causes of failure in biological control. Research is required to test current theories. Hopper and Roush (1993) suggested that experiments designed to measure the importance of genetic variation versus release numbers would be useful. In a survey of previous biological control attempts, Beirne (1975) found that the

chances of successfully establishing a biological control agent in Canada were improved with higher release numbers. He was unable to determine if this was due to increased genetic variation or increased ability of individuals to find mates and hosts.

5.1.2 Laboratory rearing

Rearing of an agent prior to release is necessary during the quarantine period to build up sufficient numbers and to remove any diseases, parasites or predators. The concern is that inbreeding, selection and random genetic drift during laboratory rearing may lead to genetic decay, loss of fitness, or the production of laboratory adapted ecotypes (Mackauer, 1976).

Genetic drift

Genetic drift is more of a concern when insect numbers are low and by chance some individuals contribute more to the next generation than others (Hopper *et al.*, 1993). In a study of allozyme variability in laboratory populations of *Aphidius ervi*, the effective population size was found to be approximately one half the number of individuals used to renew the cultures each generation, due to unequal reproductive contribution by individual females (Unruh *et al.*, 1983). Genetic drift can be detected by the changes in the frequency of neutral alleles (Roush and Hopper, 1995). The effect of genetic drift is likely to be minimal as population size increases (Roush, 1990).

Inbreeding

Inbreeding is also likely to be a concern when insect numbers are low or kept at constant levels (Roush, 1990). In normally outbreeding species, prolonged and close inbreeding is often harmful, due to reduced heterozygosity and the expression of lethal or semi-lethal recessive homozygotes (Mackauer, 1980). Roush (1990) estimated that inbreeding would have minimal

effect if the starting population was at least 20 individuals and the population was rapidly expanded and not kept for longer than 20 generations.

Attributes that strongly contribute to fitness tend to be most affected by inbreeding (Falconer and Mackay 1996). Inbreeding in *Drosophila melanogaster* was shown to affect fertility and viability, but not bristle number or body weight. Severe inbreeding depression was observed in cultures of *Heliothis virescens* after only one generation of brother-sister mating. Larval viability was particularly affected (Roush, 1986).

Hoy (1985) argued that it is premature to make assumptions about the effects of inbreeding on biological control. Of the many biological control agents, few have been studied genetically and much of the data on inbreeding is from diplo-diploid species. Most parasitic and many predatory biological control agents are haplo-diploid and, due to regular exposure of recessive alleles, are less likely to carry recessive alleles of poor fitness. Some species may well be adapted to inbreeding, due to large fluctuations in natural population levels, while others are not, as demonstrated by the development of abnormal sex ratios following inbreeding in some species (Hoy, 1985).

Ensuring that inbreeding and genetic drift are not major problems can be done by maximising the number of progeny produced, minimising variability in family sizes and avoiding the mating of closely related individuals. When inbreeding occurs slowly, selection will often remove deleterious recessive alleles as they become homozygous (Roush, 1990). In the laboratory where the insects may face a benign environment, the removal of non-lethal deleterious alleles may be slower than under field conditions.

Laboratory adaptation

The main concern during rearing has been that insects may become adapted to laboratory conditions through selection. In contrast to genetic drift and inbreeding, selection can occur in

large populations. As with genetic drift, selection could potentially result in the loss of attributes important to establishment (Roush and Hopper, 1995).

Bradyrrhoa gilveolella, a biological control agent for skeleton weed, was imported into Australia in 1973. The moth was reared on an artificial diet at 19°C. Rearing under conditions that more closely matched field conditions was found to be extremely difficult. During rearing, a bottleneck of between three and nine females occurred. Progeny were eventually released in 1975/76. Large numbers of the moth were released (22,000 first instar larvae, 639 mature larvae and 200 adults) at one site. The failure of this release was thought to be primarily due to the mal-adaptation of the population to fresh plants and field temperatures. Latter importations were more carefully reared to prevent laboratory adaptation and severe bottlenecks. Progeny from these colonies were far more successful in field releases (Cullen, 1980). Although preliminary experimental work suggested laboratory adaptation may have played a key role in the failure of the first colony, other factors were not excluded.

In laboratory reared *Heliothis virescens,* females from a 30 generation-old colony began fertile oviposition one day earlier than females from a 12 generation-old colony. On average, fertile oviposition began within three days for laboratory reared females, while wild females took greater than five days (Roush, 1986). Individuals from laboratory reared colonies that mate early and produce more offspring are likely to be favoured by selection. This demonstrates that laboratory adaptation can occur, but it is not clear that field establishment would be affected in this example. It would, however, have important implications for insects being reared for research purposes.

An example where laboratory adaptation would have almost certainly had important implications to field establishment was discussed by House (1967). *Pseudosarcophaga affinis* was reared at a constant temperature of 21°C for 200 generations. The incidence of pupal diapause dropped from 88% to almost none over this period, resulting in an increase in susceptibility to cold temperatures (House, 1967).

There are also several examples where no laboratory adaptation was found even when insects were reared under artificial conditions for long periods. One example was the rearing of *Bactra veruana* on an artificial diet for more than 35 generations. The ability of *B. verutana* to survive under field conditions was found to be unaffected (Garcia *et al.*, 1975).

In response to environmental conditions, insects may vary their behaviour and physiology, or their gene frequencies may change. The extent to which non-genetic acclimatisation has been involved during rearing is unclear (Mackauer, 1976).

Differences in the performance of laboratory reared insects may be the result of maternal effects or disease, as illustrated with the following example. *Muscidifurax raptor* that had been reared for seven generations were found to be larger, killed more house fly pupae, produced more progeny and had superior host finding abilities than populations reared for 94 generations (Geden *et al.*, 1992). These differences were found to be due to a maternally transmitted pathogen that was amplified during rearing and, to a lesser extent, to inbreeding depression.

The response of a population to selection depends on the selection pressure, genetic variance and the number of generations on which the selection pressure occurs. Thus, Roush (1990) suggested three methods for reducing the effect of selection in laboratory reared populations. Firstly, reduce the selection pressure by maintaining the population in conditions as close as possible to those present in the field. Secondly, minimise the number of generations involved. And lastly, minimise the genetic variance, through the production of several inbred lines. Roush and Hopper (1995) estimated that, for a diploid species, fifty family lines are required to preserve common alleles.

A major problem with laboratory rearing could be that laboratory colonies are generally maintained in constant environments, with no extremes in climatic variables. The proportion of an insect population that is adapted to survive adverse climatic conditions may therefore be lost or become less common after a prolonged period in the laboratory.

Selection during the rearing process has been used to advantage through the production of agents with genetically improved traits. An example was the selection of insecticide resistance in *Galandromus occidentalis*, a predator of spider mites (Roush and Hoy, 1981). In another example, the tolerance of *Aphytis lingnanensis* to extremes of temperature was improved after artificial selection for over one hundred generations (White *et al.*, 1969). Hoy (1990) reviewed the genetic improvement of natural enemies. She concluded that it is potentially a very useful tactic, but research is required to identify traits to target to improve the methodology of genetic manipulation and to better manage released populations.

Adaptation and inbreeding in plume moths

The old French population of the plume moth had a lower rate of population increase and established at fewer field sites compared with the new French population (chapter 4). It was hypothesised that this may be due to genetic problems resulting from inbreeding, drift and bottle-necks, and/or laboratory adaptation. The experiments and discussion in this chapter aimed to test these hypotheses.

5.2 Materials and methods

5.2.1 Experiment 1: reciprocal crosses

While rearing the three plume moth populations, it was observed that the old French population's fecundity was lower than that of the two newly imported populations. To determine if this was the result of genetic or non-genetic factors, reciprocal crosses were conducted between the populations.

For each cross, ten pairs were mated individually in cages placed into a growth room. The growth room was maintained at 23°C, 60 - 70% humidity and 14 hours of light. The cages

were 30 x 30 x 60cm^3. They were placed over healthy non-flowering horehound plants. All plants were of a similar size and health. The diameter of the fourth leaf from the apex was used as a gauge of plant health. All plants had an average leaf diameter of 3 - 4cm. The following crosses were conducted: Spanish (female) x Spanish (male), new French x new French, old French x old French, Spanish x new French, new French x Spanish, new French x old French and old French x new French.

The new French and Spanish insects used in the experiment had been reared in the laboratory for between 5 and 7 generations, while the old French population had been reared in the laboratory for between 23 and 28 generations.

The number of eggs was recorded after ten days. More than 77% of eggs are laid in the first ten days after mating and the moths have one pre-oviposition day (Weiss and Lippai, 1996). To gain an estimate of the proportion of viable eggs, leaves containing 20 eggs were harvested at random from each cage. The leaves were placed into plastic aerated containers (12 x 18 x 7cm), resting on paper towelling. The percentage of these eggs that hatched was recorded. In cases where fewer than 20 eggs were laid, all available eggs were collected.

The progeny from each of the 7 crosses were reared separately in large cages. The plants used in the mating cage were placed into the cages with two fresh plants, so that larvae could move from one plant to another. Pupae from these cages were harvested, weighed (females only) and placed individually in plastic vials. The emerging adults (F2) were then mated with each other, ensuring that each member of a pair originated from different parents. Ten pairs were mated for each cross. Their fecundity and percentage egg hatch was again recorded.

This experiment was repeated three times, at monthly intervals. The total fecundity and percent egg hatch data were analysed with an ANOVA followed by an LSD test using Genstat (Lane and Payne, 1996).

5.2.2 Experiment 2: fecundity of field-collected and lab-collected pupae

The aim of this experiment was to compare the fecundity of field- and laboratory-collected pupae for each insect population, to determine if the fecundity of the old French population had improved after being in the field for three generations.

One hundred pupae for each of the three populations were collected from field sites. The field-collected pupae were placed individually in aerated plastic vials. One hundred laboratory-reared pupae for each population were also placed in vials. Upon emergence, moths were mated in cages using the methods described in experiment 1. For each treatment ten pairs were mated.

The experiment was conducted using four replicates. For each replicate, the field collected material originated from different release areas. The release areas sampled for this experiment were Adelaide, Narrung 1, Monarto and Robe. Pupae were collected from the field, three generations after their release. The field-collected pupae were all collected during the first three weeks of September 1998. At this time of year horehound is actively growing, consequently the insects were very healthy.

The fecundity and percent egg hatch was recorded as for experiment 1. The data were analysed with a one-way ANOVA followed by an LSD test using Genstat (Lane and Payne, 1996). The percent hatch data were arc sine transformed prior to analysis, owing to evidence of non-constant residuals.

5.2.3 Experiment 3: inbred lines

Six inbred lines were created to determine the effect inbreeding had on fecundity over two generations. The insects used in this experiment originated from the laboratory Spanish colony. At the start of the experiment, the Spanish population had been reared in the laboratory for three generations. The original objective of this experiment was to compare the six inbred lines with progeny from the main lab colony in field releases. However, due to space restrictions in growth rooms these inbred lines had to be destroyed during February 1998 and field releases were not undertaken, but laboratory comparisons were still made.

Six pairs were mated individually in large cages (0.8 x 0.6 x 1.0m). Six potted horehound plants were placed into each cage. The F1 progeny were then allowed to mate randomly with their siblings within each cage to produce an F2 generation. Larvae were transferred onto fresh plants before plants became defoliated. Six pairs of F1 and F2 pupae were randomly selected from each of the six cages during the respective generations. Their fecundity was compared to pairs of insects collected at random from the main lab colony. Insects collected from the main colony originated from different grandparents. These matings were conducted using the methods outlined in experiment 1.

The entire experiment was conducted in two growth rooms at 25°C, 60 - 70% humidity and 14L: 10D. The total fecundity and percent egg hatch was recorded seven days after mating. The experiment was repeated four times between October 1997 and January 1998. The data were analysed with a two sample T-test using Genstat (Lane and Payne, 1996). The percent egg hatch data were transformed with an arc sine transformation.

5.2.4 Experiment 4: allozyme electrophoresis studies

The allozyme electrophoresis technique has been used for genetic identification, the investigation of genetic diversity and population structure, and phylogenetic reconstruction

(Hoy, 1994). The aim was to compare the genetic diversity of the three plume moth populations. This work was conducted with the assistance of Mr Mark Adams of the South Australian Museum.

For each of the three insect populations, 22 larvae were randomly sampled from the laboratory colonies. Each insect was assayed at a total of 42 loci (Table 5.1).

Table 5.1 Enzymes tested in the electrophoretic study of the plume moth (* indicates polymorphic allozymes).

Enzyme Symbol	E.C. number	E.C. name
Fdp*	3.1.3.11	Fructose bisphosphatase
G6dp*	1.1.1.49	Glucose-6-phosphate dehydrogenase
Gapd*	1.2.1.12	Glyceraldehyde-3-phosphate dehydrogenase
Gpi*	5.3.1.9	Glucose-6-phosphate isomerase
Gus*	3.2.1.31	Glucuronidase
Mdh*	1.1.1.37	malate dehydrogenase
Mpi*	5.3.1.8	Mannose-6-phosphate isomerase
PepA*	3.4.13.X	Dipeptidase
PepC*	3.4.13.X	Dipeptidase
PepD1*	3.4.13.X	Proline dipeptidase
PepD2*	3.4.13.X	Proline dipeptidase
Pgm1*	2.7.1.40	Phosphoglucomutase
Pgm2*	2.7.1.40	Phosphoglucomutase
Acon1	4.2.1.3	Aconitase hydratase
Acon2	4.2.1.3	Aconitase hydratase
Ada	3.5.4.4	Adenosine deaminase
Adh	1.1.1.1	Alcohol phosphatase
Ak	2.7.4.3	Adenylate kinase
Ap	3.1.3.1	Alkaline phosphatase
Fk	2.7.1.4	Fructokinase
Enol	4.2.1.11	Enolase
Est4	3.1.1.X	Esterase
Fum	4.2.1.2	Fumarate hydratase

Table 5.1 continued

Enzyme symbol	E.C. number	E.C. name
Got1	2.6.1.1	Aspartate aminotransferase
Got2	2.6.1.1	Aspartate aminotransferase
Glr	1.1.1.26	Glyoxylate reductase
Gpd	1.1.1.8	Glycerol-3-phosphate dehydrogenase
Gpt	2.6.1.2	Alanine aminotransferase
Hbdh	1.1.1.30	3-Hydroxybutarate dehydrogenase
Idh1	1.1.1.42	Isocitrate dehydrogenase
Idh2	1.1.1.42	Isocitrate dehydrogenase
Lap	3.4.11.1	Cytosol aminopeptidase
Ldh	1.1.1.27	L-Lactate dehydrogenase
Me	1.1.1.40	Malic enzyme
Ndpk	2.7.4.6	Nucleoside diphosphate kinase
PepB	3.4.11.X	Tripeptide aminopeptidase
Pgam	5.4.2.1	Phosphoglycerate mutase
6Pgd	1.1.1.44	Phosphogluconate dehydrogenase
Pgk	2.7.2.3	Phosphoglycerate kinase
Sordh	1.1.1.14	L-Iditol dehydrogenase
Tpi	5.3.1.1	Triose phosphate isomerase
Umpk	2.7.4.4	Nucleoside-phosphate kinase

Larvae were starved for 24 hours to ensure components of digested leaves did not interfere with the technique. The samples were stored in a -80°C freezer for two weeks before being processed.

Individual insects were homogenised in an equal volume of lysing solution (0.02 M Tris-HCL pH 7.4, containing 10mg NADP and 100µl 2-mercaptoethanol per 100ml). Allozyme variability was assessed by electrophoresis on cellulose acetate gels (Cellogel, Chemtron), using the methods outlined by Richardson et al. (1986).

Of the 42 loci tested, 13 were found to be polymorphic (Table 5.1). Allele frequency, expected and observed heterozygosity, and Nei genetic distances were calculated for these 13 loci using the Genpop program (Raymond and Rousset, 1995).

5.3 Results

5.3.1 Experiment 1: reciprocal crosses

The reciprocal crosses clearly demonstrated an inbreeding problem in the old French colony. In the initial generation (parents), crosses involving a female moth from the old French population had a significantly lower total fecundity and produced a lower proportion of viable eggs, compared to crosses within the new French and Spanish populations (Table 5.2). Significant differences were detected between crosses for total fecundity (one way ANOVA, df=6, 270, F=13.19, p<.001) and percent egg hatch (one way ANOVA, df=6, 230, F=15.57, p<.001).

Table 5.2 Total fecundity and percent egg hatch for the reciprocal crosses of the plume moth. Data for the parents, F1 and F2 generations are shown (Sp: Spanish, NF: new French and OF: old French insect population). Means±sem are given in the table. Means within a column followed by the same letter are not significantly different (P > 0.05) using the LSD test.

Cross	Parents		F1	F2
	total fecundity	% egg hatch	total fecundity	% egg hatch
Sp x Sp	49.5±4.5ab	90.4±1.7a	58.2±3.9a	92.1±1.9a
NF x NF	51.7±4.7a	89.7±1.4a	51.3±4.5a	89.4±3.0a
OF x OF	18.6±2.9c	48.2±5.0d	17.6±2.7c	54.1±5.5b
Sp x NF	50.1±5.1ab	64.9±6.7c	46.8±4.2ab	87.1±4.1a
NF x Sp	48.3±3.9ab	86.9±4.2a	38.8±4.0b	82.4±4.9a
NF x OF	40.4±3.7b	85.2±2.5a	53.4±4.3a	88.6±1.8a
OF x NF	19.9±2.4c	73.5±4.4bc	50.3±4.7a	88.8±1.9a

The depressed fecundity and fertility of the old French females could have been due to a maternal effect, but significant differences were detected in the resulting F1 generation for total fecundity (one way ANOVA, df=6, 270, F=10.91, p<0.001) and percent egg hatch (one way ANOVA, df=6, 231, F=14.22, p<0.001). When old French females were crossed with new

French males, their progeny (F1 generation) had a fecundity and percent egg hatch that was equivalent to crosses within the two newly imported populations (Table 5.2).

In the initial generation, the Spanish x new French cross produced significantly lower egg hatch compared to the crosses within the two newly imported populations. One of the four blocks had a very low egg hatch rate, while the remaining three blocks had a hatch rate similar to crosses within the Spanish and new French populations. This cross had a median hatch rate of 87.5%, while the mean was 64.9%. It is not clear why there was a problem with the hatch rate for this cross in the first block. The F1 offspring of the new French x Spanish cross also had a significantly lower fecundity compared with crosses within the two newly imported populations.

5.3.2 Experiment 2: fecundity of field-collected and lab-collected pupae

Significant differences were detected between laboratory and field collections for the three populations in fecundity (one way ANOVA, df=5, 231, F=16.18, p<0.001) and percent egg hatch (one way ANOVA, df=5, 202, F=42.25, p<0.001). For each comparison, individuals collected from field sites had significantly higher total fecundity compared to insects from the laboratory colonies (Table 5.3). Although there was a dramatic increase in the fecundity of the old French population after being in the field for three generations, the old French field colony still had a significantly lower fecundity compared to the Spanish and new French field colonies. There was no significant increase in the egg viability for the Spanish or new French populations after being in the field for three generations, while there was a dramatic increase in the egg viability of the old French field colony. The old French field colony had a hatch rate equivalent to that of the two newly imported populations. All females were found to reproduce.

Table 5.3 Comparison of fecundity and percent egg hatch for adults collected from the laboratory colonies and from the field. The field-collected individuals had been in the field for three generations. Means followed by the same letter are not significantly different (P>0.05) using the LSD test.

Insect population	Collection site	Total fecundity mean±sem	Maximum fecundity	% Egg hatch mean±sem
Spanish	field	72.8±5.3a	131	96.0±0.9ab
Spanish	lab	49.7±4.6b	99	90.5±1.7b
new French	field	78.0±5.0a	130	98.0±0.7a
new French	lab	48.0±4.2b	90	92.4±1.6ab
old French	field	58.5±5.3b	133	95.1±1.3ab
old French	lab	26.2±3.6c	92	52.7±5.6c

5.3.3 Experiment 3: inbred lines

In the F1 generation there was no significant difference in fecundity (t=0.38, df=46, p=0.707). Similarly in the F2 generation there was no significant difference in fecundity (t=1.26, df=46, p=0.216). In the F2 generation there was a significant reduction in egg viability in the inbred lines (t=2.42, df=43, p=0.02). The mean egg viability of the inbred line was seven percent lower than the out crossed line (Table 5.4). There were no significant differences in egg viability in the F3 generation (t=1.88, df=44, p=0.066).

Table 5.4 Comparison of fecundity and percent egg hatch for the inbred lines and out crossed individuals. Data for the F1-F3 generations are shown.

Cross	Fecundity (F1) mean±sem	% Egg hatch (F2) mean±sem	Fecundity (F2) mean±sem	% Egg hatch (F3) mean±sem
Inbred	101.0±6.5	87.0±2.3	95.4±5.7	83.5±2.5
Out crossed	105.0±9.3	94.1±1.5	106.1±6.4	90.0±2.4

5.3.4 Experiment 4: allozyme electrophoresis studies

The old French insect population had observed and expected heterozygosity that was approximately 40% lower than the expected heterozygosity of the two more recently imported insect populations (Table 5.5). Both the newly imported insect populations had very similar expected heterozygosity values. It would therefore be safe to assume that the old French insect population had similar levels of heterozygosity soon after the population was imported into Australia. The proportion of polymorphic loci and average number of alleles per loci were not found to vary greatly between the three insect populations (Table 5.5). Various studies (Nei, 1975; Leberg, 1992) have suggested that the proportion of polymorphic loci and average number of alleles per locus are more sensitive indicators of differences in genetic diversity between pre and post bottleneck populations, compared with heterozygosity, but that did not appear to be true in this case. The loss of heterozygosity was presumably due to bottlenecks rather than intentional inbreeding. The differences between expected and observed allozyme heterozygosity for the three insect populations across the 13 polymorphic loci were very small, suggesting the three laboratory colonies were not deviating from Hardy-Weinberg expectations (Table 5.5). The expected heterozygosity statistic gives the proportion of heterozygous individuals expected where there is random mating and no selection, mutation or immigration. The Nei genetic distances (Nei, 1978) were all very small (Table 5.6), which indicates that the three insect populations are very similar. No fixed differences were observed between the insect populations, although there appeared to be major differences at a few loci such as G6pd and PepC (Table 5.7).

Table 5.5 The observed and expected heterozygosity, proportion of polymorphic loci and the average number of alleles per locus for the three insect populations.

Insect population	Observed heterozygosity (±se)	Expected heterozygosity (±se)	Proportion polymorphic loci	Average number alleles per locus
Spanish	0.085±0.026	0.083±0.025	0.262	1.309
new French	0.076±0.026	0.081±0.027	0.190	1.238
old French	0.044±0.015	0.047±0.017	0.214	1.214

Table 5.6 Matrix of genetic distance coefficients between the three insect populations. Upper values are the Nei genetic distance, lower values are the percent fixed differences.

	old French	new French	Spanish
old French	-	0.015	0.006
new French	0	-	0.016
Spanish	0	0	-

Table 5.7 Allozyme frequencies, expressed as a percentage, at the 13 polymorphic loci, for the three insect populations.

Locus	Allele no.	Spanish	New French	Old French
Fdp	a	86	77	82
	b	14	23	18
G6pd	a	41	0	25
	b	59	100	75
Gapd	a	0	34	0
	b	100	66	100
Gpi	a	23	27	14
	b	77	73	86
Gus	a	0	8	0
	b	5	19	0
	c	95	73	100
Mdh	a	93	100	100
	b	7	0	0
Mpi	a	70	100	98
	b	30	0	2
PepA	a	14	21	7
	b	86	79	93
PepC	a	0	43	2
	b	100	57	98
PepD1	a	9	100	100
	b	91	0	0
PepD2	a	55	45	86
	b	20	30	14
	c	25	25	0
Pgm1	a	22	19	0
	b	64	81	72
	c	14	0	28
Pgm2	a	86	100	89
	b	14	0	11

5.4 Discussion

The observed low fecundity and egg viability of the old French population of the plume moth, relative to the two newly imported insect populations, was the result of genetic factors. Had non-genetic factors, such as disease, been the cause of the old French population's problems, the F1 offspring of crosses with the new French population (experiment 1) would also have had low fecundity and egg viability (Roush, 1990). The mean fecundity and egg viability of the old French colony were 65% and 47% lower, respectively, than the fecundity and egg viability of the new French population. The laboratory results were consistent with the population increase observed at field release sites in the wetter areas of South Australia (Robe, Narrung, Adelaide and Warooka) (Figures 4.13-4.18). These inbreeding results were supported by the results of the allozyme electrophoresis work, where it was found the old French population had a heterozygosity that was 43% lower than the two newly imported insect populations. The Nei genetic distances calculated from the allozyme data show that the three insect populations are genetically similar.

A major concern of biological control workers has been that, during the importation and rearing process, bottlenecks, genetic drift, inbreeding and laboratory adaptation may lead to a reduction in heterozygosity and fitness, an increase in the frequency of recessive deleterious alleles and mal-adaptation to field conditions, which may inhibit the colony from establishing in the field. A number of researchers have shown that bottlenecks, drift and inbreeding can result in decreased heterozygosity and fitness during laboratory rearing (eg. Roush, 1986; Unruh *et al.*, 1983 and Legner, 1979). However, it appears that these factors have not been shown to be important to the establishment of an agent in the field, prior to this study.

It is likely that the low fecundity, egg viability and heterozygosity of the old French population were the result of one or more bottlenecks which occurred during rearing for host specificity testing. Approximately 400 field-collected insects were imported from France to start the old French population, which was 8 times the number imported for the new French and Spanish

117

populations. It is therefore unlikely that the problems arose during collection and importation from France.

As well as having low fitness due to bottlenecks, it was found that the old French population was possibly suffering from laboratory adaptation (see chapter 4, experiment number 6). An extremely low proportion of the old French colony entered diapause (or quiescence) when exposed to high temperatures. Consequently survival was very low compared to the two newly imported populations. The loss of this high temperature-triggered diapause may have been the result of selecting against this trait while rearing. Diapausing insects cease to develop as first instars inside the plant tips. These insects are very difficult to find and consequently may not have been used to produce the next generation. It is also possible that the ability to diapause at high temperatures was lost during a bottleneck rather than by selection against this trait in the laboratory. Diapause is generally not polygenic (Tauber *et al.*, 1984), so this trait may be more easily lost from a colony. Another possibility is that the old French population was collected from the field at a time when a proportion of the population was in aestival diapause, and would therefore not have been collected. It is likely that the failure of the old French colony to enter an aestival diapause contributed to this colony's very poor performance at the field sites with lower rainfall and higher summer temperatures (Flinders and Monarto) (Figures 4.9 - 4.12). The remaining field sites experienced milder summers and better quality host plants during the summer period, so an aestival diapause would not have been as critical to their success at these sites.

In experiment 3, the plume moth (Spanish population) was put through a single severe bottle-neck (one pair) to try to replicate what had occurred with the old French population. No significant reduction in fecundity or egg viability was observed in the inbred lines during the F1 or F2 generation, although the inbred lines had a mean fecundity 4 - 10 % lower and mean egg viability 9% lower than pairs sampled from the main laboratory colony. The reduction in heterozygosity resulting from a bottle-neck not only depends on the size of the bottle-neck, but also on the rate of population growth after passing through the bottleneck (Nei, 1975). In this

experiment, the population was allowed to rapidly increase after the bottleneck. For each inbred line, between 50 and 100 F1 insects were mated to produce the F2 generation, and these insects were allowed to mate randomly in large cages. A bottleneck of a single pair gives an inbreeding coefficient (F) of 25% (Roush, 1990; Falconer and Mackay, 1996). This level of inbreeding would be expected to produce a depression in traits, such as fecundity, of about 10-20% (Roush, 1990). If this is the case for the plume moth, which is likely based on the results of experiment 3, the old French population is likely to have passed through more than one severe bottleneck, followed by periods of relatively slow population growth rates.

Using the following equation (Wright, 1931), it is possible to estimate the average effective population size for the laboratory colony of the old French population over the 25 generations it was reared in the laboratory.

$$H_t = H_i (1 - (1/2N_e))^t$$

Where H_t is the heterozygosity at time t, H_i is the initial heterozygosity, t is the number of generations in the laboratory and Ne is the effective population size. Using the values H_t = 0.047, H_i = 0.082 (average of the heterozygosity for the new French and Spanish populations) and t = 25, it is estimated that the average effective population size over the 25 generations was approximately 23. This seems reasonable for a laboratory colony being maintained at a moderate size over approximately 25 generations, as was recorded for the old French colony.

In experiment 2, the fecundity and egg viability of pairs sampled from field sites three generations after release were compared to that of insects sampled from the laboratory colonies. For all three insect populations, the field-collected insects had higher fecundity compared to the laboratory colonies. However, there had been a much greater increase in the fecundity of the old French population (56% compared to 32-39% for the other populations) after being in the field for three generations. There was also a dramatic improvement in egg viability (46% increase) for the field-collected old French population, while there was no

significant change for the other two populations. The field-collected old French population had an egg viability equivalent to the two newly imported populations. Therefore, at field sites where the old French population has colonised, selection seems to have lead to an improvement in the fertility of the old French population after only three generations. Although every effort was made to collect the field individuals ramdomly, sampling bias may led to a similar result through the population of more visible, healthier insects.

Biological control agents have often been observed to enter a lag phase following release. An alternative view to those discussed in the introduction is that the insect population is having to adapt back to field conditions, following a period in the laboratory. At a few of the sites where the plume moth was released in the early 1990s, the old French population was believed to have failed to establish (eg. Port Lincoln), but the moths appeared in large numbers 3-5 years following release. The population may be overcoming the problems resulting from laboratory rearing during this lag period.

In experiment 1, crosses between the French and Spanish populations, in both directions, on occasion, produced fewer eggs and had lower egg viability, compared with crosses within each population. This incompatibility was not consistent across all blocks. These results may be due to slight differences in mating behaviour or pheromones between the two populations. This may explain why the results were inconsistent.

The results of this chapter support the hypothesis that the old French colony was suffering from genetic problems arising during rearing, which were likely to be the result of low population size. These problems were important to the success of the population in the field. A PhD project, conducted by Jeanine Baker, University of Adelaide, is tackling this subject in more detail.

Chapter 6

Plume moth release technique: caged versus free release

6.1 Introduction

The use of tents in the releases (Figure 6.1), as discussed in chapter 4, was aimed at preventing adult plume moths from dispersing beyond the release site to a point where they would have difficulty detecting mates. However, the population increase observed in the first field generation was considerably lower than for following generations, suggesting that cages may have been detrimental to the colonisation of the insect, due to larval competition within the cages. The aim of the experiment discussed in this chapter was to compare releases made with and without cages.

Figure 6.1 Release tent set up for mock release of the horehound plume moth at Monarto Zoo, South Australia, staged for the media.

The Allee effect refers to a decrease in population growth rates when population sizes are small (Allee, 1931). Dennis (1989) argued that the Allee effect may result in individuals failing to find mates at low population densities. The Allee effect may therefore have important implications for the establishment of biological control agents (Hopper and Roush, 1993). In a review of biological control programs in Canada, Beirne (1975) found that the rate of establishment increased with increasing release numbers, suggesting that the Allee effect may be important. Cameron *et al.* (1993), in their review of biological control programs in New Zealand, also found that the rate of establishment increased with increasing release number, however, they noted that several very small releases had also established. This suggests that the release number required will be dependent on the attributes of the insect. Hopper and Roush (1993) produced a reaction-diffusion model to estimate the release number required. They found that the critical number of females required to establish a colony decreased hyperbolically as mate detection distance and net reproductive rate increased, and increased linearly as mean square displacement increased. It has also been suggested that the observed increase in success rate with increasing release number may be due to increased genetic variability within the released population. However, after analysing published data on collection and release numbers for parasitiods of Lepidoptera, Hopper and Roush (1993) found that the Allee effect was likely to be more important than a lack of genetic variation.

The use of tents when releasing agents may therefore increase the chance of insects finding mates by decreasing mean square displacement. Whether tents are required or not would depend on the number of insects available for release at each site, their mate detection distance, net reproductive rate and mean square displacement. Tents may allow the available insects to be released at a larger number of sites, since fewer insects may be required per site. Releasing the biological control agent at a larger number of sites should decrease the time taken for the insect to be dispersed over the entire range of the target pest and lead to more rapid pest suppression. This would also minimise the effect of catastrophes from stochastic environmental variation at individual sites.

Another possible advantage with the use of cages is the exclusion of parasitoids and predators. Problems may arise with the use of cages if hosts are too scarce, a resource is lacking, or if the cages become to hot or wet (Van Driesche, 1993).

6.2 Materials and methods

This experiment was conducted with the new French collection of the plume moth and took place on a dairy property (Raucon) located 5km west of Narrung, South Australia. Each release technique (caged and uncaged) was repeated four times. All releases took place on the 22nd of January 1998. The eight release sites were at least 100m apart, so that they could be evaluated for two generations before an overlap of the populations occurred. The sites were all located in an area which had been fenced off for tree planting. The horehound in this area was relatively dense and uniform, as no attempt to control the weed had been made for several years. The eight sites were selected and a treatment randomly assigned to each site.

Two hundred pupae were released at each of the eight sites. For both treatments, the pupae were rested on tissue paper in an open plastic container (12 x 18 x 7cm). The container was placed on a brick, which had been treated with a ring of polybutene (tanglefoot®) to prevent ants from gaining access to the pupae. A white foam fruit box with large holes cut in the sides was placed over the pupae to protect them from rain and direct sunlight. The box was weighed down with rocks. The tented releases then had a tent made from black shade cloth placed over the box. The were 3 x 2m with a height of 1m. The tents were removed after two weeks. By this time the adults had mated and were nearing the end of egg laying.

The release sites were monitored for two generations using the methods described in chapter 4, experiment 7. The sites were evaluated on the 9th and 10th of September 1998 (generation 1) and the 23rd and 24th of December 1998 (generation 2).

The total number of insects per release site per generation was calculated by calculating the average number of insects/m^2 in each concentric ring, then multiplying by the area of the ring to give an estimated total number in each area. The totals for each area were then summed to give a site total, as in chapter 4.

Because the experiment was conducted using a completely randomised design, the population size and dispersal data could be analysed with the non-parametric Mann-Whitney rank test (Snedecor and Cochran, 1980).

6.3 Results

The size of the insect population present at the uncaged release sites was significantly greater than the population present at the caged release sites, during both generations 1 and 2 ($p = 0.014$ for each generation). The mean size of the plume moth populations released without cages was 5.6 times the size of the populations released with cages in generation 1 and 4.3 times larger in generation 2 (Table 6.1).

Table 6.1 Comparison of the total number of insects present during generations one and two (post release), for the caged and uncaged release sites of the plume moth.

Release technique	Total number of insects (mean ± sem)	
	Generation 1	Generation 2
Caged	431 ± 63.5	8049 ± 953.7
Uncaged	2434 ± 563.6	34911 ± 9388.9

The movement of the insect population from the uncaged release site was significantly greater than from the caged sites during generation 1 but not significantly different during generation 2 ($p = 0.014$ and $p = 0.1$, respectively). By the second generation, insects from the uncaged release sites had dispersed to an average distance of 75 ± 5m, while at the caged release sites insects had dispersed to an average distance of 35 ± 5m (Table 6.2).

Table 6.2 Comparison of the distance each new French population of the plume moth dispersed per generation, for the caged and uncaged release sites, based on the recoveries of larvae.

Release technique	Population movement/generation (m) (mean±sem)	
	Generation 1	Generation 2
Caged	0 ± 0	35 ± 5
uncaged	25 ± 5	50 ± 5.8

6.4 Discussion

The results of the study clearly show that the use of tents, with a release number of 200, was detrimental to the initial population growth rate and dispersal of the plume moth.

High levels of larval mortality were observed within the area of the release tent during generation 1, due to competition for food and shelter in plant tips which give the early instar larvae protection. After removal of the tents, late instar larvae were observed to crawl only as far as surrounding horehound stems, which were no more than 50cm from the edge of where the release tent had been situated.

This experiment was conducted in a relatively benign environment by South Australian standards. The use of tents is likely to have been more of a hindrance in the hotter/drier areas of the state, where the horehound is less dense and the presence of protected sites for early instar larvae is more critical.

If the caged adults laid the same number of eggs as those released without cages and there was a similar level of egg and larval mortality in the uncaged releases, then 406 neonates/m^2 would have emerged within the 6m^2 area of the release tent. The highest quadrat (0.1m^2) count

obtained in the releases discussed in chapter 4 was 33 (330 larvae/m^2) and this density led to the complete defoliation of a much denser infestation of horehound. Thus, even a very dense stand of horehound is clearly not capable of supporting the number of insects that could be produced in the tent (406 larvae/m^2). In the caged releases discussed in chapter 4, between 51 and 675 insects survived through to the last instar in generation 1 (Figures 4.9-4.18). It is likely that this number of insects could have been produced by releasing 50 or fewer pupae in each cage. Releasing fewer insects per caged site would have allowed more releases to be conducted.

The original releases of the plume moth made by the Keith Turnbull Research Institute were made with very large numbers of insects, typically one to three thousand insects per site. With the benefit of hindsight, this was clearly not the ideal release strategy. However, in the initial stages of a project before gaining information on the insect's dispersal, population increase, and mate finding distance, it may be wise to release larger numbers when establishing the initial nursery sites. The major limiting factor for the control program against horehound with the plume moth is their slow dispersal rate, rather than their rate of population increase (Clarke *et al.*, 2000). So, releasing fewer insects at more sites would have been a better tactic. The minimum number of individuals required per release site is currently being evaluated by Jeanine Baker, PhD student, Department of Applied and Molecular Ecology, University of Adelaide.

Because the plume moth does not travel long distances over its life and has high population growth rates and a mate detection distance of up to 8m (Leyson, 1999), it is unlikely that the Allee effect would be particularly important to the plume moth. The Allee effect would have a greater influence on insects that disperse widely and have small mate finding distances and population growth rates (Hopper and Roush, 1993).

An experiment evaluating different release numbers with and without tents would have been interesting to conduct to determine the minimum release number required using each release method. The minimum number of insects required per site may vary between the climatic zones

of the state, with more being required in areas with a harsh environment. It is possible that a slightly lower minimum release number may be required when using tents, allowing for a larger number of releases to be conducted. However, conducting releases without tents has the advantage of the moths dispersing more rapidly. Because it is relatively easy to rear large numbers of plume moth, cages cost money and the major limiting factor in the field is dispersal, uncaged releases would probably have been the better approach.

When dealing with biological control agents that are likely to be dramatically affected by the Allee effect, but where competition within the release tent is likely to create problems (as could be the case for the bitou tip moth), alternative strategies could include the removal of the tent after the majority of moths have mated but before the peak of egg laying occurs, or releasing fewer insects in a number of tents in close proximity to one another. Such efforts to evaluate release strategies for specific agents seem likely to pay large dividends in the efficiency of the colonisation process and rapid suppression of the target pest.

Chapter 7

Climate matching in the bitou tip moth: how close is not close enough?

7.1 Introduction

The bitou tip moth was originally released on boneseed and bitou bush across southeastern Australia, but established only on bitou bush in coastal areas of New South Wales. Environmental factors that may have hampered the establishment of the original bitou tip moth populations released in South Australia include: extremes in temperature, the sub-species of the weed present in South Australia and the vigour of the weed over the summer period. Unlike the east coast of Australia, where the original releases were successful, the areas where boneseed is present in South Australia generally have very low summer rainfall and poor, shallow soils, resulting in poor host plant quality during the summer/autumn period. South Australia also experiences greater temperature extremes and lower humidities compared to the east coast of Australia.

The objectives of the experiments outlined in this chapter were to compare the response of the two bitou tip moth populations to various climatic variables and to assess the importance of any differences in field studies. In so doing, we can test the hypotheses that (1) the original releases of the bitou tip moth failed because the insect population released (Natal population) was poorly adapted to South Australia's climate; and (2) that the Cape Town population would be better suited to South Australian conditions.

7.2 Materials and methods

7.2.1 Experiment 1: the influence of temperature on egg development time

The aim of this experiment was to compare the egg development rates and egg survival of the two insect populations at a range of temperatures.

For each insect population, adults were mated individually in cages at 16, 20, 24, 28, 32 and 36°C, with five pairs for each temperature and population. A six-leaf potted boneseed seedling was placed into each cage. The seedlings with eggs were then placed into temperature cabinets set at 16, 20, 24, 28, 32 and 36°C. The eggs were up to six hours old when placed into the temperature cabinets. The eggs were checked twice a day, at 8am and 6pm, and the time of hatch recorded. Eleven to 20 eggs were used for each treatment. The eggs for each treatment originated from at least three pairs of parents. The experiment was repeated four times, at weekly intervals during 1997.

The temperature cabinets were maintained at 40 - 50% humidity and 14 hours of light. The humidity was controlled using saturated solutions of $NH_4NO_3+NaNO_3$ (Winston and Bates, 1960). The solutions were placed into trays (30 x 45cm), with two trays per growth cabinet. Typically humidities at 3pm in the Adelaide area range from 30 - 50% over summer (Data obtained from the Australian Bureau of Meteorology).

The relationship between temperature and development rate (1/development time) was analysed with a restricted or residual maximum likelihood analysis (REML) using Genstat (Lane and Payne, 1996). The significance of the treatment effects was tested using a likelihood ratio test (Welham and Thompson, 1997). The relationship between egg survival and temperature was analysed using a logistic regression using Genstat (Lane and Payne, 1996).

7.2.2 Experiment 2: the influence of temperature on larval development

To compare the development of larval stages of the two bitou tip moth populations at various temperatures, thirty neonates per treatment were placed onto healthy boneseed cuttings, one larva per cutting. The plant cuttings were placed into temperature cabinets at 20, 25, 30 and 35°C. The larvae were up to six hours old at the start of the experiment. Due to a lack of space within growth cabinets, only four temperatures could be evaluated for this experiment.

The plant cuttings were 15cm in length and included the tips of the stems. The base of each cutting was placed in a sealed vial filled with damp tissue paper. The plant cuttings were changed every third day. Larvae were carefully transferred to fresh plant material using a fine brush. After 3 days, plant cuttings started to wilt at temperatures above 30°C. The larvae and plant material were placed into plastic aerated containers (12 x 18 x 7cm). The containers were lined with tissue paper to absorb excess moisture.

The temperature cabinets were maintained at 14 hours of light and 40 - 50% humidity, using saturated solutions of $NH_4NO_3+NaNO_3$. The solutions were placed into trays (30 x 45cm); two trays were placed into each growth cabinet.

The parents of the insects used in the experiment were reared at the same temperature to which their progeny were exposed. The 30 neonates used for each treatment originated from at least five pairs of parents.

The insects were checked once a day at 9.00am. The time for each insect to reach pupal stage, their pupal weight and % survival was recorded.

The experiment was repeated three times during 1997. The relationship between temperature and developmental rate (1/development time) was analysed with a residual maximum likelihood analysis using Genstat. The data were firstly transformed with a log transformation,

owing to non-constant variance in the residual plots. The relationship between pupal weight and temperature was analysed with a linear regression using Genstat. The relationship between larval survival and temperature was analysed using a squared polynomial regression using Genstat. The percent survival data were transformed with an arc sine transformation (Snedecor and Cochran, 1980).

7.2.3 Experiment 3: effect of short bursts of a stressful temperature on larval survival

To evaluate the ability of each population to tolerate temperature extremes, two separate experiments were conducted. The first evaluated the effect of high temperatures, this experiment was repeated at high and low humidities. The second evaluated the effect of low temperatures.

To compare the ability of larvae from the two bitou tip moth populations to tolerate short bursts of high temperatures, 20 fourth instar larvae were exposed to 55, 50, 45, 40, 35 and 30°C, in temperature cabinets for a period of twelve hours. The larvae were placed onto boneseed plant cuttings, which were placed into aerated plastic containers (12 x 18 x 7cm). Four insects and four plant cuttings were placed into one plastic container. The top 10cm of a boneseed stem was used. Before exposing the insects to the high temperatures, the larvae were allowed 12 hours to form a webbed tip on the plant cutting. To assess the influence of relative humidity, the high temperature (30 – 55°C) treatments were repeated at 70 - 80 and 40 - 50% humidity. The low humidities were maintained using saturated solutions of NH_4NO_3+$NaNO_3$ (Winston and Bates, 1960). The solutions were placed into trays (30 x 45cm), with two trays per growth cabinet. The high humidities were maintained by placing one tray of water into the temperature cabinets.

The experiment was repeated with low temperatures, -5, 0, 5 and 10°C. The low temperature treatments were conducted at 40 - 50 % humidity only.

The insects used in these experiments had been reared in a glasshouse, set at 25°C. The 20 insects were randomly selected from 5 rearing cages. The insects exposed to the high temperatures (30 - 55°C) were first exposed to a mild shock at 30°C for three hours. After the insects were exposed to the temperature extremes, they were placed back onto potted plants in a growth room set at 25°C. The number of insects pupating normally was recorded. The insects were exposed to 14 hours of light.

The experiments were repeated four times at weekly intervals during 1998. The relationship between temperature and larval survival, for the experiments conducted at 55 - 30°C, was analysed with a logistic regression using Genstat. The experiment evaluating the effect of low temperatures on insect survival was analysed with a quadratic polynomial regression, with data transformed by an arc sine transformation.

7.2.4 Experiment 4: survival during more realistic "heatwave" conditions

In experiment 3, larvae were exposed to 12 hours of high temperatures. Although this may detect differences in the tolerance of larvae to temperature extremes, it would be unlikely that insects would encounter such long periods of a high temperature in the field. The aim of experiment 4 was to evaluate the two insect populations' ability to tolerate more realistic heatwave conditions.

For each treatment, 20 fourth instars were exposed to 14 hours of 25°C, followed by 5 hours of 30°C, followed by 1 hour at a maximum temperature (35, 40, 45 or 50°C), followed by 4 hours at 30°C. This temperature regime was repeated for 4 days. The insects were placed into aerated plastic containers (12 x 18 x 7cm) on boneseed plant cuttings (top 10cm of plant shoot). Four insects and four plant cuttings were placed into each container. The plant cuttings were changed every two days. Larvae were carefully transferred to fresh cuttings using a fine paint brush.

The insects used in this experiment had been reared in a glasshouse at 25°C. The 20 insects were randomly selected from 5 rearing cages. After the insects were exposed to the temperature extremes, they were placed back onto potted plants in a growth room set at 25°C. The number of insects pupating normally was recorded. The growth cabinets were maintained at a humidity of 40 - 50% and 14 hours light.

The experiment was repeated four times at fortnightly intervals during 1998. The relationship between temperature and larval survival was analysed with a quadratic polynomial regression using Genstat. The data were transformed with an arc sine transformation.

7.2.5 Experiment 5: effect of plant sub-species and plant quality

The aim of this experiment was to compare the development of each insect population, and crosses between each population, on the two sub-species of *Chrysanthemoides monilifera* present in Australia (boneseed and bitou bush), and to evaluate each insect population's ability to tolerate water-stressed foliage.

For each treatment, 20 unfed neonates were placed onto two potted plants, one larva per growing tip. The experiment was conducted in a growth room at 25°C, 40 - 50% humidity and 14 hours light. The neonates were 0 - 12 hours old when transferred to the experimental plants and originated from four sets of parents. The experiment was repeated three times during 1997.

The two plant sub species were watered at a high and low rate. The high water plants were given half a litre of water three times a week. The low water plants were watered so as to maintain the plants in a condition visually similar to that of field plants in summer in the Adelaide area. Prior to starting the experiment, the watering rate for the low water treatments was gradually reduced over a period of four months. During the experiment these plants were given a quarter of a litre once a week. Prior to the start of the experiment two growing tips

(top 10cm) were removed from each plant and their moisture content estimated. Moisture content was measured by weighing the foliage prior to and after placing them in a drying cabinet for 48 hrs, at 60°C. It is interesting to note that the low water bitou bush treatments had a moisture content similar to the high water boneseed plants (Table 7.1). To compare the moisture content of experimental plants with field plants, field plants from the Belair field site were sampled once a month over a period of a year. At each sampling, twenty plant growing tips (10cm in length) were sampled from the site and the wet and dry weights determined (Table 7.2).

The high water boneseed plants used in the experiment had a moisture content similar to that present in the field during spring, when the plants are actively growing. The low water boneseed plants used in the laboratory experiment had a moisture content similar to that present in the field during the summer/autumn period.

Table 7.1 Moisture content in the growing tips (top 10cm of stem) of the experimental potted boneseed and bitou bush plants.

Treatment	Replicate	Average % moisture
1 boneseed, high water	a	78.5
	b	76.9
	c	77.8
2. boneseed, low water	a	68.8
	b	68.3
	c	69.3
3. bitou bush, high water	a	85.9
	b	83.8
	c	86.1
4. bitou bush, low water	a	77.4
	b	75.0
	c	75.4

Table 7.2 Dry weight and moisture content of the growing tips (top 10cm of shoot) of field boneseed plants present at the Belair Recreational Park.

Month	Average dry weight (g)	Average moisture content (%)
January	0.31	64.7
February	0.26	64.9
March	0.4	66.2
April	0.4	64.4
May	0.34	70.9
June	0.27	74.5
July	0.28	74.5
August	0.34	74.9
September	0.27	74.5
October	0.32	76.6
November	0.34	72.7
December	0.34	63.8

The larval development time and pupal weight for each insect was recorded. The relationships between plant sub-species and water rate, and development time and pupal weight were analysed with a residual maximum likelihood analysis using Genstat. The relationship between insect survival and temperature was analysed with an analysis of variance using Genstat. The residual plots for the percent survival data were inspected and a transformation of the data was not required.

7.2.6 Experiment 6: fecundity of moths reared on healthy and water stressed boneseed and bitou bush

In experiment 5, the pupal weights were found to vary depending on whether the insects had been reared on vigorous or water-stressed boneseed and bitou bush. The aim of this experiment was to compare the effect of plant quality and plant sub-species on the fecundity of the two tip moth populations.

For each insect population, one hundred insects were reared on five actively growing boneseed and bitou bush plants and five water-stressed boneseed plants. The plants were maintained as for experiment 5. Two plant tips were sampled from each of the five plants used for each treatment to give an estimate of the moisture content of the growing tips (Table 7.3). Pupae were harvested from the plants and placed individually into plastic aerated vials. For each treatment ten pairs were mated. The experiment was repeated three times during 1997, at monthly intervals. Each pair was mated in an aerated clear plastic cup (7cm diameter x 7cm height). A boneseed leaf was placed into each cup and a cotton wool ball, soaked in 15% honey solution, was inserted into the lid. The total number of eggs laid was recorded after seven days. In preliminary studies no eggs were observed to be laid beyond 7 days under these conditions.

Table 7.3 Average moisture content of the plant growing tip (top 10cm of plant stem), for the three treatments and replicates.

Treatment	Replicate	Average moisture content (%)
1. boneseed, high water	a	77.9
	b	77.7
	c	77.6
2. boneseed, low water	a	67.1
	b	67.1
	c	66.7
3. bitou bush, high water	a	85.2
	b	84.1
	c	85.6

The insects were reared and mated in a growth room set at 25°C, 40 - 50% humidity and 14 hours light. The relationship between fecundity, plant quality and plant sub-species was analysed with an analysis of variance using Genstat. The relationship between fecundity and female pupal weight was analysed with a linear regression using Genstat.

7.2.7 Experiment 7: field releases

The two bitou tip moth populations were compared in the field at eight release areas. Within each release area, the two populations were released at sites that were at least three kilometres apart, so that the insect populations did not meet for the period the sites were under evaluation. Table 7.4 shows the site details for the eight release areas and Tables 7.5 and 7.6 give the climatic data for the release areas. The density of plants and plant tips and the percent weed ground cover were estimated by randomly throwing ten $1m^2$ quadrats in the immediate area surrounding the release point (0-40m from release point).

So that the two insect populations could be compared, each of the sites within each release area were chosen for similar weed density, weed size, plant quality, infestation area, soil type, land use, vegetation type and climatic conditions. All sites had a reasonably even stand of boneseed over an area of at least 5 hectares, so that the dispersal of insects from the release point could be studied. The release point was placed in the middle of the weed infestation.

The field releases were conducted between May 1997 and April 1998. The two moth populations were released on the same day within each release area. At each site, 500 fourth instars were released over an area of approximately $100m^2$. One larva was placed into each plant growing tip using a fine paint brush. It was not practical to use tents for the release of the bitou tip moth. The sites had between 15 and 30 boneseed plant tips per m^2, so extremely large tents would have been required to cover sufficient plants.

Table 7.4 Site details for the sixteen bitou tip moth release sites.

Area number	Insect population	Site location	Plant tips/m2	Plants/m2	% Weed ground cover	Land use	Soil type	release date
1	Cape Town	Brown Hill creek	14.9	2.2	32.5	recreation park	clay loam	07.05.97
	Natal	Brown Hill creek	17.8	2.6	38.5	recreation park	clay loam	07.05.97
2	Cape Town	Belair	19.8	3.4	58	recreation park	clay loam	29.10.97
	Natal	Blackwood	21.2	3.7	60	council reserve	clay loam	29.10.97
3	Cape Town	Belair2	19.8	3.4	58	recreation park	clay loam	04.04.98
	Natal	Blackwood2	21.2	3.7	60	council reserve	clay loam	04.04.98
4	Cape Town	Morialta	26.7	4.4	65.5	recreation park	clay loam	19.09.97
	Natal	Morialta	29.5	4.6	70	recreation park	clay loam	19.09.97
5	Cape Town	Sth Para Reservoir	27.1	4.6	62	water catchment	clay loam	24.08.97
	Natal	Barossa Reservoir	28.7	4.5	67.5	water catchment	clay loam	24.08.97
6	Cape Town	Lyndoch	30.7	4.7	65.5	private land	sandy loam	30.09.97
	Natal	Lyndoch	30.3	4.8	70.5	private land	sandy loam	30.09.97
7	Cape Town	Waikerie	25.5	4.6	55	council reserve	sandy loam	13.11.97
	Natal	Berri	24.4	4.2	56	council reserve	sandy loam	13.11.97
8	Cape Town	Naracoorte	23.3	2.4	57	private land	sandy loam	28.11.97
	Natal	Lucindale	26.8	2.9	58	private land	sandy loam	28.11.97

Table 7.5 Rainfall and humidity data for the eight bitou tip moth release areas (Data obtained from the Australian Bureau of Meteorology).

Release area	Average annual rainfall (mm)	1998 annual rainfall	1998 January rainfall	1998 July rainfall	Average January 3pm relative humidity	Average July 3pm relative humidity
Brown Hill Creek	623	567.4	5.2	63	36	61
Belair/Blackwood	723	781.3	6.2	94	44	74
Belair/Blackwood2	723	781.3	6.2	94	44	74
Morialta	555	546	6	65.4	36	61
S[th] Para/Barossa Res.	757.2	456.2	11	92.6	33	73
Lyndoch	467.9	459.2	50.4	62.6	30	65
Waikerie/Berri	260	238.9	5	34.4	27	53
Naracoorte/Lucindale	581.7	487.6	16	78	36	68

Table 7.6 Temperature data for the eight bitou tip moth release areas (Data obtained from the Australian Bureau of Meteorology).

Release area	Number days max. > 40°C	Number days max. > 35°C	Number days min. < 0°C	Average January maximum temp.	Average January minimum temp.	Average July max. temp.	Average July min. temp.
Brown Hill Creek	1.7	13.9	0	27.8	16.2	14.1	7.7
Belair/Blackwood	0.6	8.7	0	26.6	14.9	12.5	7
Belair/Blackwood2	0.6	8.7	0	26.6	14.9	12.5	7
Morialta	2.4	17.9	0	28.5	16.6	15.1	7.4
Sth Para/Barossa Reservoir	1.1	10.4	29.5	27.2	11.3	12.3	3.1
Lyndoch	4.5	24.7	3.4	30	15.2	14.6	5.7
Waikerie/Berri	6.9	33.8	1.7	32.3	16.6	16.2	5.1
Naracoorte/Lucindale	2	13.4	8.4	27.9	12	14.2	4.8

The sites were monitored for four generations. To estimate the total number of insects and dispersal rates each generation, the sites were divided into concentric rings. A $1m^2$ quadrat was randomly thrown around each ring. The total number of insects in each quadrat was recorded. The number of quadrats thrown in each ring was proportional to the area of that ring (Table 7.7). A minimum of four quadrats in the centre 0-5m ring was needed to give a repeatable result. The sites were evaluated until three concentric rings were encountered in which no insects were detected. At each evaluation, surrounding areas were also inspected, to check that the insect population had not moved to a surrounding area. The evaluations were timed so that the majority of insects were 4th or 5th instars, making them easier to detect. Populations were maintained on potted plants located outdoors in suburban Adelaide to aid in the timing of the field evaluations.

Table 7.7 The number of $1m^2$ quadrats thrown at the various distances from the release point, at the bitou tip moth release sites.

Distance from release point (m)	Area of ring (m^2)	Quadrats thrown
0-5	78.5	4
5-10	235.7	12
10-20	942.4	48
20-40	3769.9	192
40-60	6283.2	320
60-80	8796.5	448
80-100	11309.7	576

From the raw data, an estimate of insect density (insects/m^2) could be calculated for each distance from the release point. An estimate for the total number of insects present at each site could then calculated by multiplying the area of each concentric ring by the estimated insect density and adding the values obtained for each concentric ring.

7.2.8 Experiment 8: predation by ants

While conducting and evaluating the bitou tip moth field releases, ants were observed to be preying on bitou tip moth larvae. The aim of these experiments was to quantify the level of larval mortality due to ants. The experiments were conducted in the Belair Recreation Park, Adelaide.

To compare the level of predation at different times of the year, the experiment was repeated three times, on the 30th October 1997, 11th February 1998 and 18th July 1998. For each insect population, 80 second instars were placed onto eight plants (10 per plant), with only one larva placed into each plant tip. Half the plants had polybutene (Tanglefoot®) applied to their base to prevent ants from moving onto the plants. The four plants used for each treatment were treated as four blocks. The number of larvae surviving after 14 days was recorded. The site was inspected every second day to ensure ants were not gaining access to the Tanglefoot treated plants. The sixteen plants were spread over an area of approximately $225m^2$. Treatments were assigned to the sixteen plants randomly.

The relationship between insect survival and the presence or absence of ants was analysed with an analysis of variance using Genstat. The data were transformed with an arc sine transformation.

7.3 Results

7.3.1 Experiment 1: the influence of temperature on egg development time

The Cape Town population had relatively higher development rates at the lower temperatures (16-20°C) and the Natal population had relatively higher development rates at the higher temperatures (24-32°C) (Figure 7.1). Using the likelihood ratio test the interaction term

between temperature and insect population was found to be highly significant (change in deviance=127.7, df=4, p<0.001). For both insect populations, egg development rate increased up to 28°C, but a decrease in development rate occurred after this temperature (Figure 7.1). Although there were statistically significant differences between the two populations, these differences were small and would be unlikely to be biologically important.
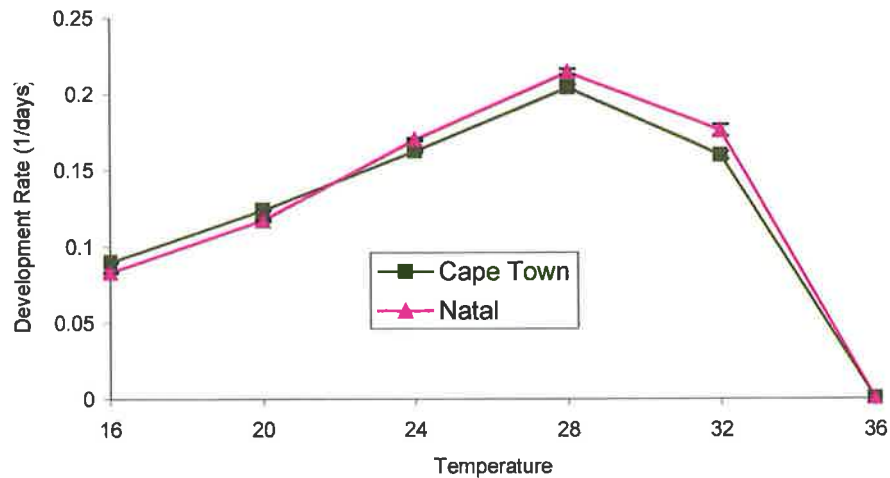
Figure 7.1 Egg development rates (means ± sem) at temperatures from 16 to 36°C, for bitou tip moth populations collected from Mediterranean (Cape Town) and sub-tropical (Natal) climates in South Africa.

The Cape Town population had slightly higher egg viability compared to the Natal population. This difference was consistent over all temperatures tested (Figure 7.2), suggesting that the differences in egg viability between the two insect populations were not due to a different tolerance to temperature extremes. The logistic regression equations used to assess the relationship between temperature and percent egg hatch are given below and accounted for 97.5% of the variation.

Cape Town   % hatch = 2.892+91.94/(1+EXP(4.0*(temperature-31.4)))

Natal   % hatch = -3.016+91.94/(1+EXP(4.0*(temperature-31.4)))

Fitting curves with different intercepts led to a significant increase in the percent of variation accounted for (F=340.19, df=1, 43, p<0.001). Fitting curves of different slopes increased the percent of variation accounted for by only 0.1%. There was a dramatic decrease in egg viability at temperatures of 32°C and above (Figure 7.2).
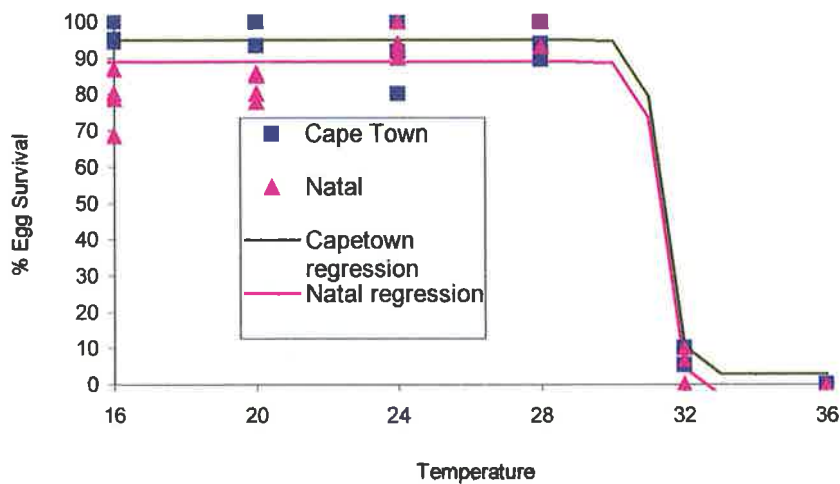
Figure 7.2 Percent egg survival at temperatures ranging from 16 to 36°C, for the two bitou tip moth populations. Means for the four replicates and the logistic regressions are shown.

7.3.2 Experiment 2: the influence of temperature on larval development

The only significant difference between the two populations was at the lower temperature (20°C). At 20°C larvae from the Natal population had a mean larval development time of 47.7±0.8 days compared with 39.1±0.5 days for the Cape Town population (Figure 7.3). Using the likelihood ratio test, the interaction term between temperature and insect population was found to be highly significant (change in deviance=79.26, df=3). For both populations development rate increased between 20 and 25°C and reached a plateau at 25 - 30°C and decreased at temperatures above 30°C (Figure 7.3). The fastest development rate for both insect populations occurs between 25 and 30°C.
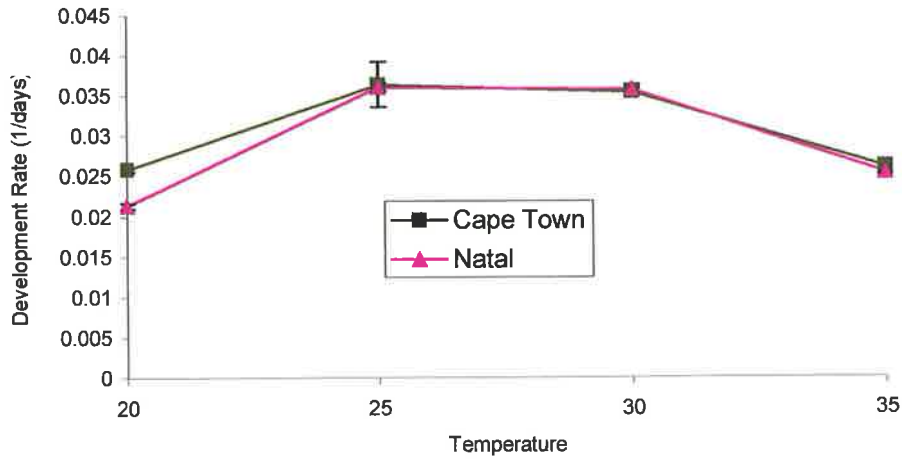
Figure 7.3 Larval development rates (means ± sem) for insects reared at 20 to 35°C, for the two bitou tip moth populations.

Pupal weight was found to decrease with increasing temperature (Figure 7.4). This relationship was described by the following equations:

Cape Town   Pupae weight = 20.135 - 0.3052(temperature)

Natal   Pupae weight = 19.619 - 0.3052(temperature)

The variation accounted for by these models was only 46%, suggesting that other factors, such as nutrition, affected pupae weight. Fitting curves with different intercepts accounted for significantly more variation (F=8.15, df=2, 389, p=0.005). Fitting curves with different slopes did not account for greater variation (F=0.01, df=1, 388, p=0.937).
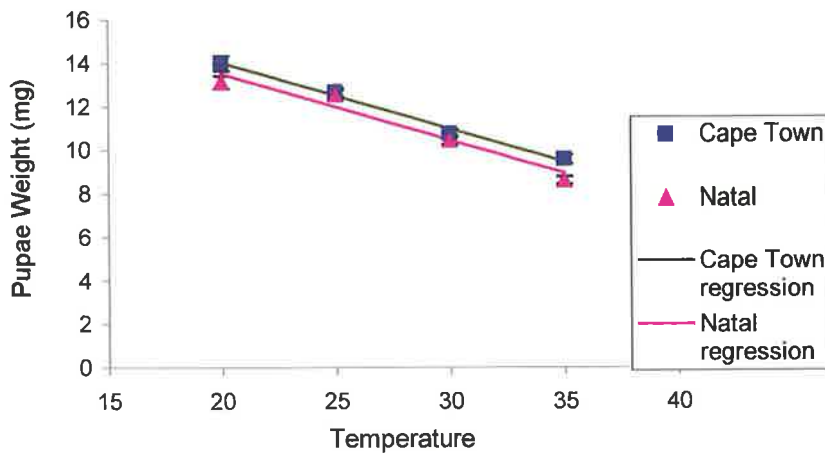
Figure 7.4 Pupal weights for insects reared at 20 to 35°C. Means ± sem and linear regressions are shown.

144

For both insect populations there was a decrease in larval survival with increasing temperature (Figure 7.5). The quadratic polynomial regressions used to describe the relationship between temperature and larval survival are given below:

Cape Town % survival = $15.9+3.659(\text{Temperature})-0.0845(\text{temperature})^2$

Natal % survival = $11.6+3.659(\text{Temperature})-0.0845(\text{temperature})^2$

Fitting curves with different intercepts accounted for a significant increase in variation (F=22.02, df=3, 20, p<0.001). The percent of the variation accounted for by fitting these models was 73.3%. Fitting curves with different slopes decreased the amount of variation explained to 63.5%.
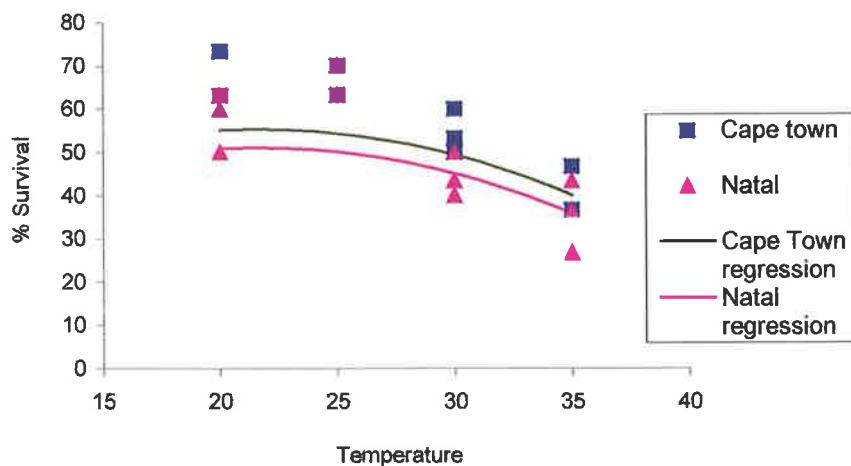
Figure 7.5 Larval survival (%) for the two bitou tip moth populations were at 20 to 35°C. The four back transformed replicate means and quadratic polynomial regressions (arc sin transformed) are shown.

The Cape Town population had higher pupal weights and rates of survival compared to the Natal population. These differences were consistent over all temperatures tested (Figures 7.4 and 7.5), suggesting that differences in pupal weight and survival between the two insect populations were not due to a different tolerance to temperature extremes.

7.3.3 Experiment 3: effect of short bursts of a stressful temperature on larval survival

In the experiment conducted at the high temperatures (30-55°C), there was a dramatic decline in larval survival at 45°C at both humidities (Figures 7.6 and 7.7). At 40-45°C there was slightly higher survival at the higher humidity, for both insect populations.
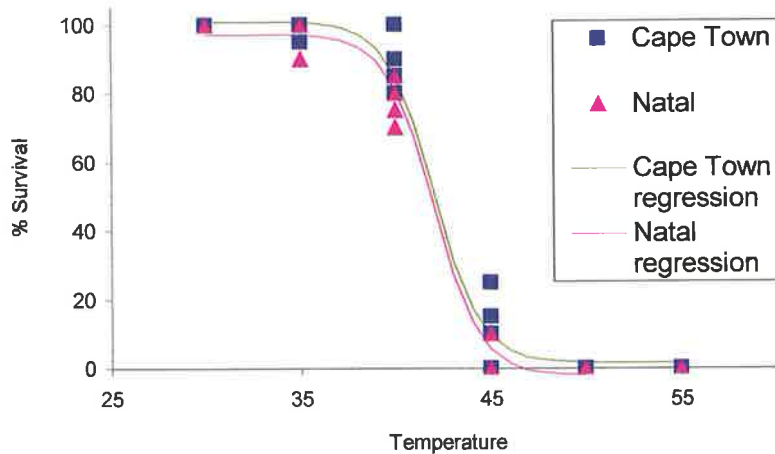
Figure 7.6 Percent survival of the two bitou tip moth populations when exposed to stressful temperatures (30 - 55°C) at 40-50% humidity, for a period of 12 hours. The four replicate means and logistic regressions are shown.
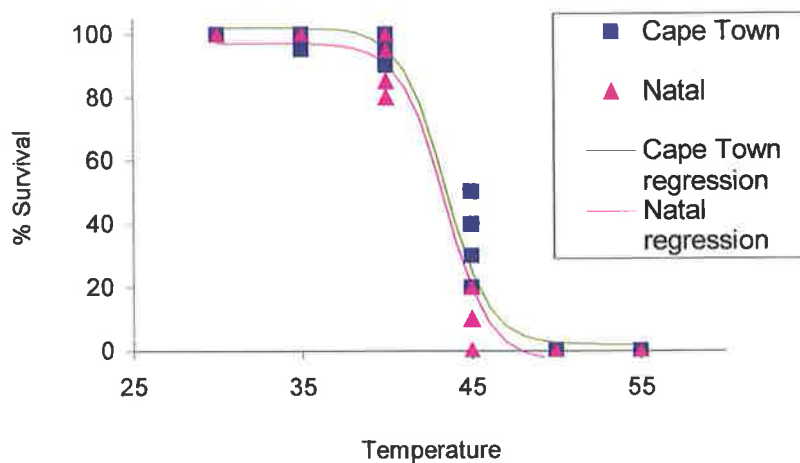
Figure 7.7 Percent survival of the two bitou tip moth populations when exposed to stressful temperatures (30 - 55°C) at 70-80% humidity, for a period of 12 hours. The four replicate means and logistic regressions are shown.

At both humidities the survival of the Natal population was slightly lower than for the Cape Town population. This difference was consistent over the range of temperatures tested (Figures 7.6 and 7.7). Therefore, the higher mortality of the Natal population is likely to have been due to factors other than temperature extremes.

The logistic regressions for the low humidity (40-50%) data are given below. The variation explained by fitting these models was 98.8%. Fitting curves with different slopes did not account for more variation (F=0.02, df=1, 42, p=0.903).

Cape Town   %survival = 1.693+99.31/(1+EXP(0.8257*(temperature-41.986)))
Natal   %survival = -2.057+99.31/(1+EXP(0.8257*(temperature-41.986)))

The logistic regressions at the high humidity (70-80%) (below) accounted for 97.8% of the variation. Fitting curves with different slopes did not significantly improve the amount of variation explained (F=1.03, df=1, 42, p=0.315).

Cape Town   %survival = 2.063+100.1/(1+EXP(0.7741*(temperature-43.422)))
Natal   %survival = -2.937+100.11/(1+EXP(0.7741*(temperature-43.422)))

At the lower temperatures there was a gradual decline in insect survival at temperatures below 5°C (Figure 7.8). The quadratic polynomial regression used to describe the relationship between temperature and insect survival is given below:

$$\% \text{ survival} = 79.61+2.689(\text{temperature})-0.1617(\text{temperature})^2$$

The variation accounted for when fitting this curve was 80.4%. Fitting curves with different slopes and/or intercepts for each insect population led to a decline in the percent of variation explained (F=0.289, df=3, 28). There was therefore no difference in the ability of the two insect populations to tolerate short bursts of low temperatures.
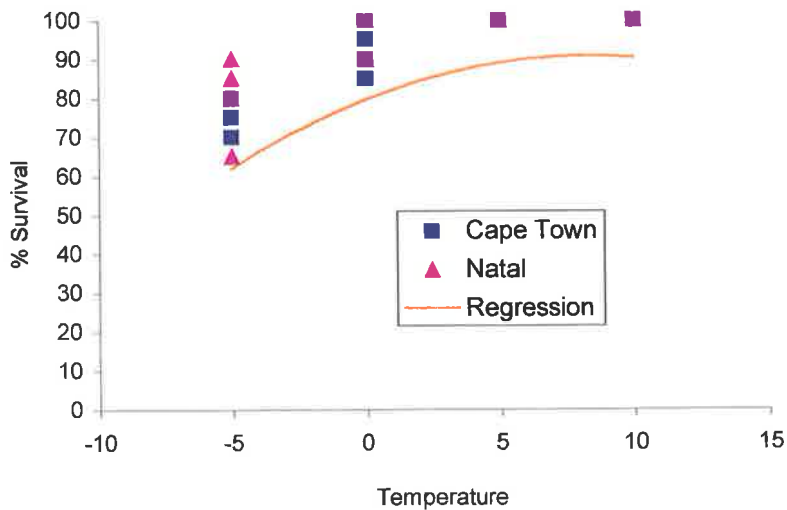
Figure 7.8 Percent survival of the two bitou tip moth populations when exposed to stressful temperatures (-5 - 10°C) at 40 - 50% humidity for a period of 12 hours. Back transformed replicate means and the quadratic polynomial regression is shown.

7.3.4 Experiment 4: survival during more realistic "heatwave" conditions

At maximum daily temperatures greater than 45°C, there was a dramatic decline in insect survival (Figure 7.9), but there was no difference in the ability of the two insect populations to tolerate simulated heatwave conditions. The quadratic polynomial regression used to describe the relationship between maximum daily temperature and insect survival is given below:

$$\% \text{ survival} = -684.4 + 41.7(\text{temperature}) - 0.5598(\text{temperature})^2$$

The variation accounted for when fitting this curve was 98.1%. Fitting curves with different slopes and/or intercepts for each insect population did not increase the percent of variation explained (F=0.248, df=3, 28).
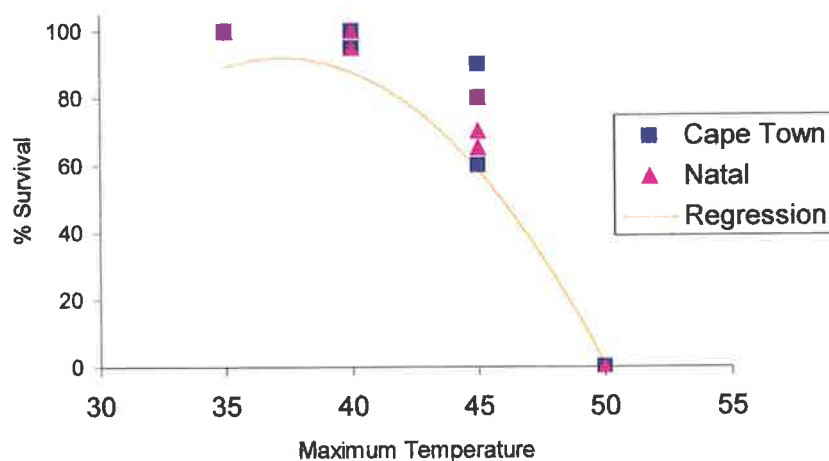
Figure 7.9 The percent survival of the two bitou tip moth populations when exposed to more realistic heatwave conditions for a period of four days. The maximum daily temperature (°C) is given on the x axis. The back transformed replicate means and quadratic polynomial regression are shown.

7.3.5 Experiment 5: effect of plant sub-species and plant quality

The Cape Town population had a shorter developmental time when reared on boneseed and conversely the Natal population took fewer days when reared on well watered bitou bush (Figure 7.10). The Cape Town population's development time was unaffected by plant quality (water rate) on either sub-species, while Natal larvae took approximately a week longer to develop on the moisture stressed plants than on actively growing plants on both sub-species of the weed. The development rates for the reciprocal crosses on boneseed were similar to that of the Cape Town population, and on bitou bush their development rate was similar to the Natal insect population (Figure 7.10). The reciprocal crosses therefore appeared to be equally well suited to both weed sub-species. Using the likelihood ratio test, the interaction term between insect population, plant sub-species and water rate for the response variate development time was found to be highly significant (change in deviance=70.04, df=3, $p<0.001$). All two way interactions were also found to be significant (change in deviance=620.1, df=7, $p<0.001$).

149

For the Natal insect population, the mean pupal weight was approximately 20% lower for insects reared on moisture stressed plants compared with insects reared on actively growing plants (Figure 7.11). For the Cape Town population or the reciprocal crosses there was no significant decrease in pupal weight with decreasing plant quality. The Cape Town insect population had lower pupal weights when reared on bitou bush and the Natal population had lower pupal weights on boneseed. These differences in pupal weights were similar for each insect population. The interaction term between insect population, plant sub-species and water rate, for the response variate pupal weight, was found to be not significant at the 95% level (change in deviance=1.594, df=3). The two-way interactions between insect population and plant sub-species, and insect population and water rate, were found to be significant (change in deviance=49.46, df=6, p<0.001 and change in deviance=26.47, df=6, p<0.001, respectively).

The Cape Town population had a lower rate of survival on bitou bush compared to boneseed. On actively growing plants the number of Cape Town insects surviving on bitou bush was 30% lower than on boneseed (Figure 7.12). Conversely, the Natal population had a lower survival rate on boneseed. On actively growing plants the number of Natal insects surviving on boneseed was 14% lower than on bitou bush. On moisture stressed boneseed, the number of Cape Town larvae surviving to pupation was 23% lower than on the actively growing plants, but there was no significant decrease in survival rate on bitou bush. For the Natal insect population the decrease was much greater. On both sub-species of the weed, there was a 50% reduction in the survival rate on water stressed plants. For the reciprocal crosses, there was also a decrease in the survival rate on water-stressed plants. The decrease was intermediate between the Cape Town and Natal populations when reared on bitou bush and smaller than the decrease in survival rate for the Cape Town and Natal populations, when reared on boneseed. The vast majority of insect deaths were observed to occur during their first instar. For the response variate percent survival, the three-way interaction between insect population, water rate and plant sub-species was found to be non significant (F=1.8, df=3, 30, p=0.169). The two-way interactions between insect population and water rate, and insect

population and plant sub-species, were both significant (F=9.81, df=3, 30, p<0.001 and

F=8.91, df=3, 30, p<0.001, respectively).
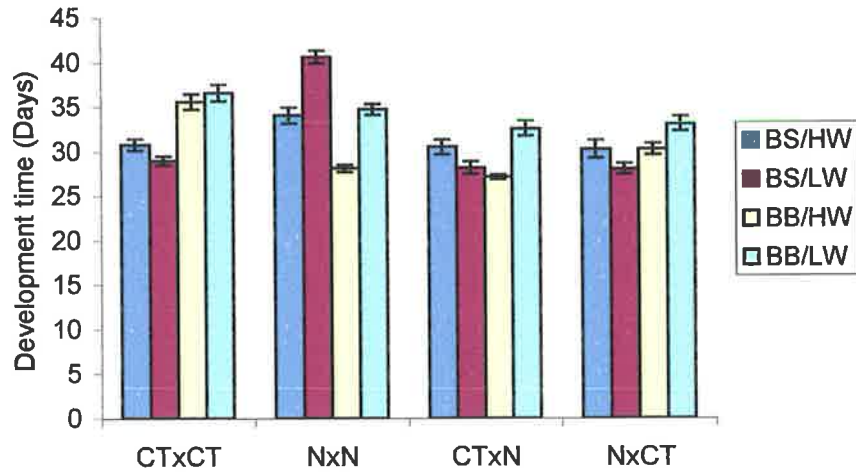
Figure 7.10 The development time (days) for the Cape Town (CT) and Natal (N) insect populations and crosses between them (female x male), reared on boneseed (BS) and bitou bush (BB), when watered at a high (HW) or low (LW) rate. Means ± sem are given.
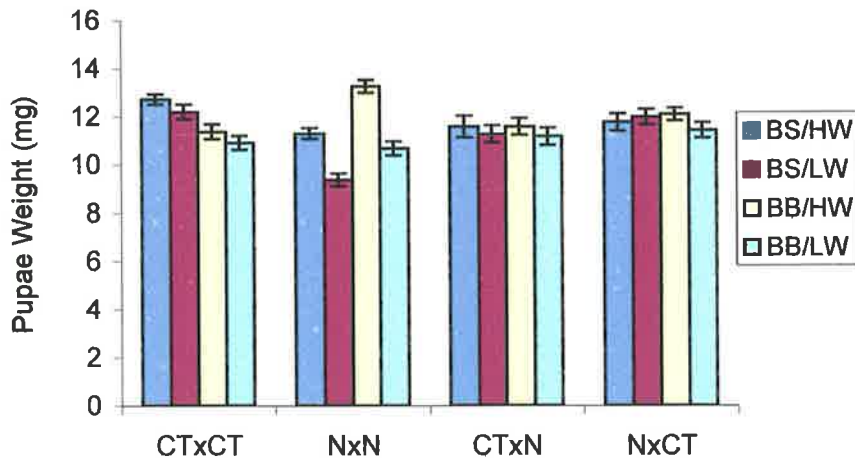
Figure 7.11 Pupae weights (mg) for the Cape Town (CT) and Natal (N) insect populations and crosses between populations (female x male), reared on boneseed (BS) and bitou bush (BB), when watered at a high (HW) or low (LW) rate. Means ± sem are given.
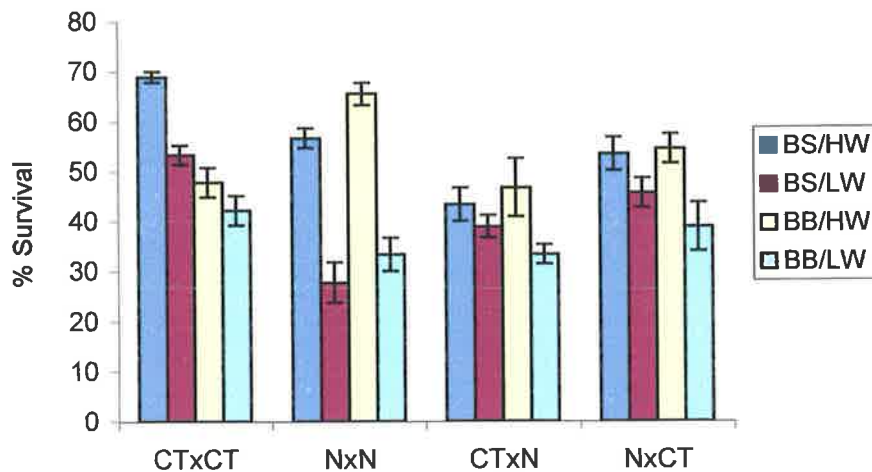
Figure 7.12 Percent survival for the Cape Town (CT) and Natal (N) insect populations and crosses between them (female x male), reared on boneseed (BS) and bitou bush (BB), when watered at a high (HW) or low (LW) rate. Means ± sem are given.

7.3.6 Experiment 6: fecundity of moths reared on healthy and water stressed boneseed and bitou bush

Although the mean fecundity of Natal moths reared on water stressed boneseed plants was at least 30% lower than other treatments (Table 7.8), the effect of water rate and plant sub-species on fecundity was found to be non significant (F=3.72, df=1, 172, p=0.055 and F=0.13, df=1, 172, p=0.72, respectively).

Table 7.8 The fecundity and female pupae weight for insects reared on boneseed and bitou bush watered at a high and low rate.

Insect population	Plant sub-species	Water rate	Female pupal weight mean ± sem	Total fecundity mean ± sem
Cape Town	boneseed	high	13.3±0.7	39.1±5.7
	boneseed	low	12.1±0.3	35.8±4.6
	bitou bush	high	11.8±0.3	32.8±5.8
Natal	boneseed	high	11.7±0.3	30.4±4.7
	boneseed	low	9.9±0.3	18.1±4.0
	bitou bush	high	13.4±0.5	40.4±6.4

The equation for the linear regression describing the relationship between female pupae weight and fecundity is given below. Data from both insect populations and all treatments were used in the analysis.

$$\text{Fecundity} = -33.1 + 5.481(\text{pupal weight})$$

The variation explained by this model was only 16.5%, indicating that female pupal weight had only a small influence on fecundity. Non-linear or polynomial regressions did not increase the percent of the variation explained.

7.3.7 Experiment 7: field releases

No insects belonging to the Natal population were recovered following release at any of the eight release sites across South Australia. Insects belonging to the Cape Town population were recovered at only three of the eight release sites. The total number of insects present at these three sites was estimated for the first four generations after release. There was a 6.8 - 9.7 fold increase in the population of the Cape Town insect population in the first field generation at the Belair2/Blackwood2 and Brown Hill Creek release sites. During the second field generation there was a dramatic decrease in the numbers of insects present, no insects were recovered at latter evaluations (Table 7.9). Ants were observed preying on all stages of the insect during spring 1997. This was believed to be the reason for the decline in numbers by the second evaluation. However, the failure of the Natal population to persist at any sites following release may also be attributed to the lack of fresh plant growth. The plants at these sites did not start to actively grow for three months after the release was made. At the third release site where Cape Town insects were recovered following release (Sth Para Reservoir/Barossa Reservoir site), only 196 insects were estimated to be present at the site during the first field generation. All these insects were recovered from a clump of boneseed plants located 15 - 20m from the release point on a small sandy rise. Subsequent evaluations of this site detected no insects.

Table 7.9 The estimated total number of insects present per generation at three of the bitou tip moth release sites for the Cape Town (CT) and Natal (N) insect populations. No insects were detected in evaluations conducted at the other five release sites.

Generation number (field)	Estimated total number of insects per site per generation					
	Belair2/ Blackwood2		Brown Hill creek		Sth Para Reservoir/Barossa Reservoir	
	CT	N	CT	N	CT	N
1	1936.7	0	1354.8	0	196.3	0
2	306.3	0	274.9	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0

At the two sites where there was initially an increase in the Cape Town insect population (Belair2/Blackwood2 and Brown Hill Creek), the distribution of the insects across the release site was leptokurtic in generation 1 (there was a rapid decrease in insect density moving away from the release point). The distribution of insects from the release point became less leptokurtic in the second generation (Figures 7.13 and 7.14). Because no insects were recovered from any Natal release sites and Cape Town insects were only recovered from 3 of the 8 sites, a statistical analysis of these data was not conducted.
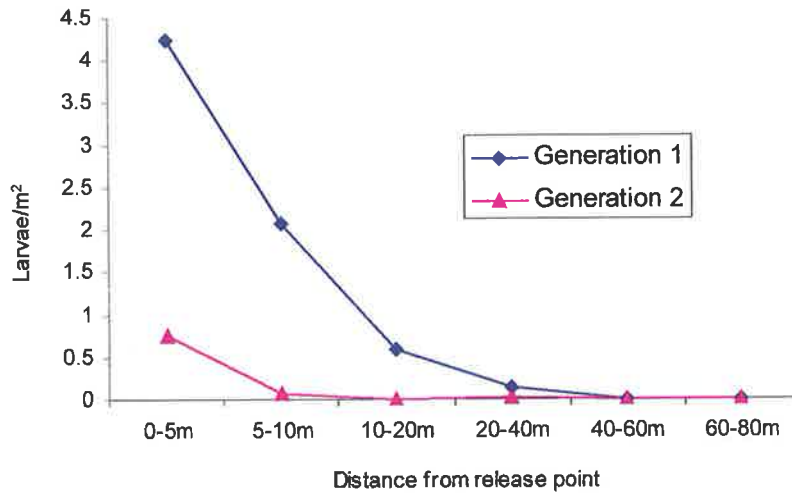
Figure 7.13 The density of bitou tip moth larvae (insects/m^2) at various distances from the release point, for the Cape Town population at the Belair 2 release site. Data for the first two field generations are shown.
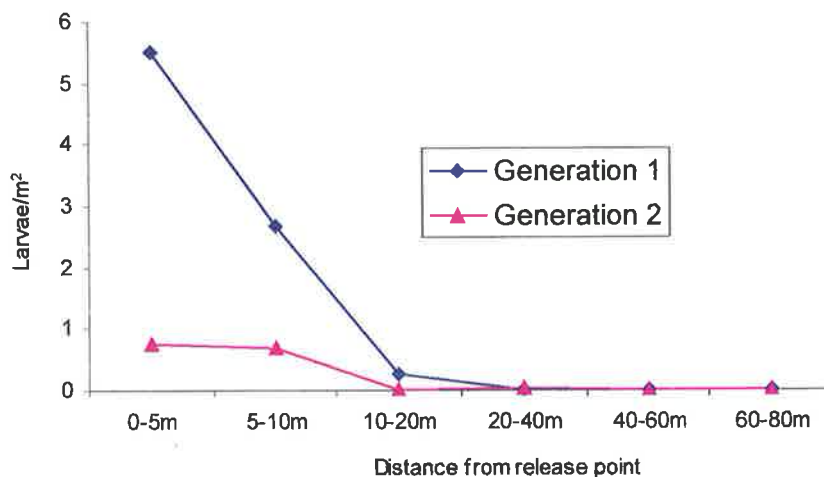
Figure 7.14 The density of bitou tip moth larvae (insects/m^2) at various distances from the release point, for the Cape Town population at the Brownhill Creek release site. Data for the first two field generations are shown.

7.3.8 Experiment 8: predation by ants

The proportion of insects surviving in the first experiment, conducted in October 1997, varied significantly between treatments that permitted or excluded ants ($F=40.88$, df=1, 9, $p<0.001$). There was also a significant difference between the Cape Town and Natal populations

(F=9.94, df=1, 9, p=0.012). In the presence of ants, the proportion of insects surviving in the field was 79% lower for the Cape Town population and 99% lower for the Natal population. In the absence of ants, the proportion of Cape Town larvae surviving was 52% higher than for the Natal insect population (Table 7.10). The two-way interaction between insect population and treatment was not significant (F=0.54, df=1, 9, p=0.481).

Table 7.10 Survival of 2nd instars in the field, in the presence and absence of ants (back transformed means±sem).

Insect population	Treatment	% Insect survival		
		October 1997	February 1998	July 1998
Cape Town	+ ants	15.0±9.6	17.5±8.5	70.0±7.1
	- ants	72.5±6.3	77.5±4.8	75.0±9.6
Natal	+ ants	0.5±0.5	15.0±11.9	50.0±4.1
	- ants	35.0±9.6	25.0±5.0	45.0±6.5

In the second experiment, conducted in February 1998, the proportion of insects surviving varied significantly between treatments (F=11.53, df=1, 9, p=0.008). There was also a significant difference between insect populations (F=5.4, df=1, 9, p=0.045). In the presence of ants, the proportion of insects surviving in the field was 77% lower for the Cape Town population and 40% lower for the Natal population. Even in the absence of ants, the proportion of Cape Town larvae surviving was 68% higher than for the Natal insect population (Table 7.10). The two-way interaction between insect population and treatment was again not significant (F=2.83, df=1, 9, p=0.127).

In the third experiment, conducted in July 1998, the effect of treatment was not significant (F=0.01, df=1, 9, p=0.913). Because ants are not active at this time of year, the Tanglefoot treatment had no effect on larval survival. However, the effect of insect population was still

significant (F=19.15, df=1, 9, p=0.002). The proportion of the Cape Town insect population surviving in the field was between 29 and 40% higher than the Natal population (Table 7.10).

7.4 Discussion

The results of the field releases were disappointing. Neither insect population established at any of the eight field release sites. The main reason for the failure of the bitou tip moth populations to establish in the field appears to be high levels of predation by ants (Table 7.10). However, even when ants were excluded or seasonally inactive, the Natal population of the bitou tip moth performed consistently more poorly than the Cape Town population.

Very high numbers of an unidentified black scale occur on boneseed across South Australia. This scale attracts large numbers of ants to the boneseed plants. The main species of ant observed to be feeding on the bitou tip moth belonged to the genus *Iridomyrmex*. Ants were observed preying on egg, larval and pupal stages of the bitou tip moth. At some sites (Morialta, Lyndoch and Waikerie/Berri), larvae were being preyed upon almost as fast as they were being released. Attempts were made to control ants in plots of boneseed at Belair, using baits (minced meat treated with various rates of maldison, pyrethrum and Amdro granular ant bait® (7.3g kg hydramethylnon)) and applications of insecticides (pyrethrum and permethrin) to the soil around the base of plants. None of the baits tested were effective in this situation and it was extremely difficult to get an even coverage of the insecticides on the ground in the type of terrain that boneseed infests, without spraying the boneseed foliage.

The two field releases where an initial increase in the numbers of the Cape Town insect population was observed were both conducted in early winter, when ant activity is very low. During spring, when there is increasing ant activity, a dramatic decrease in insect numbers was observed at both these sites (Table 7.9, generation 2, and Figures 7.13 and 7.14). Similarly in experiment 8, ants were found to be affecting the bitou tip moth during the spring

and summer when ant activity is high. A few Cape Town insects were recovered at the South Para Reservoir site on a small sandy rise during the first field generation. The small *Iridomyrmex* sp. was not observed on this clump of boneseed, which may have been due to the very sandy soil surrounding these plants.

Insects belonging to the Cape Town population were detected in post release evaluations at three of the eight release sites, while no insects were ever recovered from any of the Natal release sites, even for those sites where releases were conducted in winter when ant activity is low. In the experiment evaluating the impact of ants (experiment 8), there was a significantly higher survival rate for larvae from the Cape Town population compared to the Natal population, even in the absence of ants. In these field studies, the Cape Town insect population appeared, therefore, to be better suited to the environmental conditions present at the Adelaide Hills release sites.

Although there were statistically significant differences in the response of the two bitou tip moth populations to temperature, namely the slightly longer egg and larval development times at temperatures of 20°C and below (experiments 1 and 2), it is unlikely that these differences would be important to the establishment of the insect in the field. However, these small differences may have been important to the degree of weed control achieved had the insects established, since the Cape town population would be likely to have more generations per year and be more active over the winter period. There was also little difference in the ability of larvae from either of the two insect populations to survive short periods of freezing temperatures or heatwave conditions (experiments 3 and 4). Any differences in insect survival at high or low temperatures were consistent over the range of temperatures tested. Boneseed was used in these experiments. Many of the differences detected in experiments 3 and 4 are likely to be due to a different preference for host plant sub-species, rather than a different tolerance to temperature. Although the Cape Town population experiences greater extremes in temperature in its native range, both populations had an equivalent tolerance to temperature

extremes. Similarly, despite the fact that humidities are considerably higher in the Natal Province of South Africa, the Natal population was just as tolerant of hot/dry conditions.

Where there do appear to be important differences between the two insect populations is in their tolerance to moisture stressed host plants and preference for host plant sub-species. The Cape Town population showed a better performance on boneseed and the Natal population was stronger on bitou bush. Each insect population had shorter development times, higher pupal weights and higher survival on the sub-species from which they were collected in their native range. Pupal weight, developmental time and the survival of the Natal population was greatly affected by plant quality, on both sub-species of the weed. For the Cape Town population, pupal weight and developmental time were unaffected by plant quality. There was a lower survival rate on moisture stressed plants, but the observed decline in survival rate was not as dramatic as for the Natal insect population. Cape Town insects were observed to move around the host plant feeding considerably more than larvae from the Natal population. On moisture stressed plants the food within plant tips was often exhausted before the larvae completed their development. Once the food within the plant tips was exhausted, the Cape Town larvae were often observed feeding on exposed older leaves or the outer layers of plant stems, while the Natal larvae were rarely observed feeding outside the plant tip on either weed sub-species. In the glasshouse Cape Town larvae were often observed to complete their whole development on older exposed leaves.

No attempt was made to determine the reasons behind the different tolerances of the two bitou tip moth populations to moisture stressed plants. There are a host of possible explanations to account for this difference between the insect populations.

Apart from being digestible, absorbable and containing all nutrients required for development, the host plant will usually contain various allelochemicals. Some chemicals within a plant act as attractants and feeding stimulants for insects, and others are needed for the production of hormones, pheromones, kairomones and allomones, which are used in numerous processes

such as defence and reproduction. Some allelochemicals act as toxins by affecting the availability and utilisation of nutrients (Hagen et al, 1984). Some insects have adapted to feeding on nutrient-poor host plants. Adaptations include: selecting plant tissues with higher nutrient levels, low metabolic rates, manipulating the source-sink relationship in host plants and symbiotic relationships with micro organisms (Barbehenn *et al.*, 1984).

Changes that occur within plants during times of moisture stress that may affect insect development include: increased lignification and leaf toughness, changes in the concentration of the plant's chemical defences and salts, a decrease in water content, and changes in leaf nutrient levels and ratios. It is possible that the Cape Town population was better able to combat certain plant chemical defences and handle highly lignified plant tissue or it utilised water and nutrients more efficiently. Bitou bush has a higher moisture content compared with boneseed. Even when bitou bush plants were suffering from moisture stress as indicated by the dropping of mature leaves, their plant tips had a moisture content that was equivalent to actively growing boneseed plants (Table 7.1). Many allelochemicals classified as toxins or deterrents decline in concentration as a leaf ages, while compounds such as tannins, which are classified as quantitative resistance or digestibility reducing factors, increase in concentration as a leaf ages (Schoonhoven *et al.*, 1998). Cates (1980) suggested that host specific insects prefer young actively growing tissue while less specific insects prefer older plant tissue due to lower concentrations of allelochemicals in older leaves. Nitrogen is one of many important components of an insect's food. Nitrogen plays an essential role in all metabolic processes, cellular structure and genetic coding (Mattson, 1980).

Changes to the morphology of boneseed and bitou bush plants following water stress include increased pubescence and smaller more open plant tips which may leave the insect more exposed. Bitou bush has considerably hairier leaves than boneseed, particularly when the plants are suffering from water stress. In experiment 5, neonates from the Cape Town population were observed to have difficulty moving through hairs on the bitou bush leaves,

while Natal neonates appeared to have difficulty with the toughness of the boneseed leaves and open nature of the boneseed plant tips, particularly on water-stressed plants.

It would be interesting to know if the Cape Town insect population could be established on the east coast of Australia. Experimental releases on the east coast were not attempted because there were no known infestations of the weed that are not infested by the original population of the bitou tip moth released in Australia. The presence of the original moth population would make comparison of the two insect populations difficult because there may be differences in the ability of each insect population to mate and produce viable offspring with the existing insect population (see chapter 8). There were no results to suggest that the Cape Town population would be poorly adapted to the more benign environment of the east coast of Australia. Although the Cape Town population showed a preference for boneseed, the insect was able to successfully develop on both weed sub-species.

For biological control programs that are targeting pests that occur over a range of climates, and have limited resources restricting the number of insect populations able to be imported and maintained, it may be better to collect from areas with more variable, harsher climates, rather than from more benign, stable environments. Insects in these areas may be adapted to a wider range of conditions. In the case of the bitou tip moth, it may have been better to make the initial collections from the Cape Town area. However, as discussed in chapter 4, a closer climate match may be required in situations where the life cycle of the agent and target pest must be synchronised. Insects from harsh, unpredictable climates may also be more likely to have a diapause, which may not be necessary in more benign areas and may unnecessarily slow down the population growth of the insect.

In conclusion, ant predation was clearly the major reason behind the failure of the bitou tip moth to establish in South Australia. Whether the two bitou tip moth populations would have established in the field had ant predation not been an issue is unknown. However, the results suggest that the Cape Town population would have established on at least some of the release

161

sites. In the two releases of the Cape Town population conducted in early winter, the field populations were increasing at a rate of 7 - 10 fold per generation until spring, when ants became more active. The laboratory studies and the field experiments evaluating the impact of ants also suggested the population was well adapted to survive South Australia's environmental conditions during summer. The poor tolerance of the Natal population to moisture-stressed host plants may have prevented their establishment in South Australia and would have almost certainly led to a lower level of impact on the target weed had they established. In South Australia, the slower development of the Natal population in cooler temperatures would have also slowed their rate of increase and effect on the target weed. So it is likely that the match between the Natal and Adelaide climates was not close enough, although the Cape Town moths might have succeeded in the other direction, that is on the eastern Australian coast.

Chapter 8

Speciation in the bitou tip moth

8.1 Introduction

After the arrival of the two bitou tip moth populations in Australia, it became apparent that the two insect populations had a number of differences and could potentially be sibling species. For example, the majority of larvae from the Cape Town population (but not all) have a slightly more prominent striping pattern on their back. There were also several behavioural differences. Larvae from the Cape Town population were observed to move around the host plant considerably more than the Natal population and responded more aggressively to touch. The aims of the experiments discussed in this chapter were to assess the compatibility of the two insect populations and to determine their taxonomic relationship. This would be especially relevant to any proposals to release the two populations in close geographical proximity.

8.1.1 Importance of taxonomy to biological control

In preceding chapters, a number of examples have been given where important attributes have been shown to vary across the range of an insect. For this reason, the collection of insects from a number of regions of the host range may be of value. For example, where the agent is to be released over a wide range of latitudes, a number of collections may be required each with different diapause characteristics so that the agent and pest can be synchronised in each area. However, biotypes have occasionally been incorrectly identified, which has created problems in past biological control programs (Rosen and DeBach, 1973). Even in the one geographical location, biotypes can occur sympatrically (Diehl and Bush, 1984). There is, therefore, the potential for follow up collections to collect a different or mixture of biotypes.

The incorrect identification of biological control agents and the target pest has on occasion resulted in a considerable waste of resources. For example, successful control of California red scale in California could have been achieved 50 years earlier had the numerous species of *Aphytis* present in Asia been correctly identified. The species present in Asia were incorrectly identified as *A. chrysomphali*, which was already present in California and had been having little impact, so other more efficacious species were not introduced for some time (Rosen and DeBach, 1973). Schauff and LaSalle (1998) discuss a number of further examples where systematics could have assisted past biological control programs.

Because host specificity can vary between biotypes (Diehl and Bush, 1984), perhaps the greatest danger that may arise from the importation of different biotypes, without thorough testing, or the misidentification of a biological control agent, is the potential for adverse effects on non-target species. In an example of differences in host specificity between populations, two races of the weevil, *Rhinocyllus conicus*, were imported into California from Europe, for the control of various thistles. These were named the musk and the milk thistle races. The musk thistle race was found to attack several native, as well as exotic, species of thistle, while the milk thistle race was considerably more host specific. High levels of genetic and behavioural differentiation were found to exist between the two races, such as in egg placement (Unruh and Goeden, 1987).

Caltagirone (1985) recommended that during the collection of exotic natural enemies, material from different geographic areas or host plants should be kept separate and comparative studies conducted; newly imported material should be routinely tested for biotypes; and that more detailed studies be conducted on the biosystematics of key natural enemies.

8.1.2 Genetic variation and the mixing or hybridisation of populations

As mentioned in chapter 5, genetic variation and post release adaptation may be important when establishing an insect in a new environment (van den Bosch, 1971; Roush, 1990). If genetic variation is assumed to be important, the question of relevance here is: to increase the level of genetic variation in a population, should collections be released together, hybridised prior to release, or reared and released separately? The answer to this question would clearly depend on the compatibility of the insect populations, and whether or not genetic variation will actually be increased by the mixing or hybridising of insect populations.

Some have claimed that hybridisation prior to release is beneficial to improve vigour. Legner (1971) found expressions of hybrid vigour in F1 through F3 generations of crosses between strains of *Muscidfurax raptor,* a parasite of synanthropic flies. These strains were from climatically similar, but geographically isolated areas. Crosses of strains from temperate and tropical areas showed slight negative heterosis in the F2 generation. Legner (1971) suggested the production of synthetically superior strains through directed heterosis should be considered.

Bush and Hoy (1984) suggested that by rearing and releasing populations separately, higher genetic variability will be maintained, compared to a large single pooled colony. Maintaining separate colonies may reduce the potential for the loss of alleles due to genetic drift and selection.

Outbreeding depression may result during the crossing of insect populations due to hybrid dysgenesis (due to transposable P elements) or poor chromosomal pairing (Falconer and Mackay, 1996). Opponents of hybridisation have also argued that it may disrupt co-adapted gene complexes. Genes at many loci are selected for their combined effects on fitness. Therefore populations that are widely differentiated through adaptations to local conditions may show negative heterosis in the F2 generation (Falconer and Mackay, 1996). This may be

an issue if the populations are from very different environments, but is it likely to be an issue if the populations originate from similar but isolated environments? The answer to this question is possibly, since drift may cause divergence in characters such as pheromones and genital morphology, which are unrelated to climatic adaptation. In the field, barriers to hybridisation of sympatric biotypes include different courtship behaviour and pheromones, incompatibility of genitalia, gametic mortality, zygote mortality or hybrid inviability and hybrid sterility. The degree of depression can vary from high to negligible (Strickberger, 1995). Outbreeding depression appears to be more common in organisms that show more local adaptation or where the taxonomic status of the populations is under question (Frankham, 1995). Mackauer (1976) suggested keeping populations separate until their genetic compatibility was established.

8.2 Materials and methods

8.2.1 Experiment 1: reciprocal crosses - fecundity, % egg hatch and F1 survival

To test the reproductive compatibility of the two tip moth insect populations, ten pairs, for each cross, were mated individually in clear plastic cups (7cm diameter x 7cm height). Small holes were made in the lid of each cup. A boneseed leaf was placed into each cup and a cotton wool ball moistened with 15% honey solution was inserted into the lid. The experiment was conducted in a growth room at 22°C, 60-70% humidity and 14L: 10D.

The following crosses were conducted: Cape Town (female) x Cape Town (male), Natal x Natal, Cape Town x Natal and Natal x Cape Town. Lone Cape Town and Natal females were also placed into the cups to determine the number of eggs laid in the absence of males.

The number of eggs laid within the plastic cups was recorded after ten days. Under these conditions females were not observed to continue laying eggs longer than this period. The proportion of viable eggs was recorded.

To test the fertility of the F1 generation, twenty neonates (where available) were collected from each cup and transferred to potted boneseed plants using a fine paint brush. One larva was placed into each plant tip. Pupae were collected from these plants and placed into labelled aerated plastic vials. Emerging moths were mated (ten pairs per cross) using the same methods used for the parents. Each member of the pair originated from different parents. The experiment was repeated three times at monthly intervals.

To test the survival of the F1 generation at 22°C and 32°C, 100 neonates were randomly selected from each of the crosses and placed onto actively growing boneseed plants at a density of one per growing tip. Half the insects were placed into a growth room set at 22°C and half in a growth room set at 32°C, 60-70% humidity and 14L: 10D. The proportion of insects surviving to adulthood was recorded. The sex of the adults was also recorded to give the sex ratio.

The fecundity and percent hatch data were analysed with a one way ANOVA using Genstat. The percent hatch data were transformed with an arc sine transformation. The data from the unpaired females were not analysed with the remaining data. The percent larval survival data for the F1 generation were arc sine transformed and analysed with an analysis of variance using Genstat.

8.2.2 Experiment 2: allozyme electrophoresis studies

The primary aims of this allozyme electrophoresis study were to look for diagnostic loci to be used in hybridisation studies, and to estimate genetic distances and heterozygosity. This work was conducted with Mr Mark Adams of the South Australia Museum.

For each insect population, 20 late instars were randomly sampled from the laboratory colonies. Larvae were collected from five rearing cages, four from each cage. In a preliminary study the gut contents of the bitou tip moth larvae were found to interfere with the technique, larvae were therefore starved for 24 hours prior to being processed.

Individual insects were homogenised in an equal volume of lysing solution (0.02 M Tris-HCL pH 7.4, containing 10 mg NADP and 100μl 2-mercaptoethanol per 100ml). Allozyme variability was assessed by electrophoresis on cellulose acetate gels (Cellogel, Chemtron), using the methods outlined by Richardson *et al.* (1986). Each insect was assayed at a total of 41 loci. Of the 41 loci tested 27 were found to be polymorphic (Table 8.1).

Allele frequency, expected and observed heterozygosity, and Nei genetic distances were calculated using the Genpop program (Raymond and Rousset, 1995).

Table 8.1 Enzymes tested in the electrophoretic study of the bitou tip moth (* indicates polymorphic allozymes).

Enzyme Symbol	E.C. number	E.C. name
Acon1*	4.2.1.3	Aconitase hydratase
Acon2*	4.2.1.3	Aconitase hydratase
2ACP1	3.1.3.2	Acid phosphatase
Acyc*	3.5.1.14	Aminoacylase
Adh1	1.1.1.1	Alcohol phosphatase
Adh2*	1.1.1.1	Alcohol phosphatase
Ald	4.1.2.13	Fructose bisphosphate aldolase
Ao*	1.2.3.1	Aldehyde oxidase
Ap	3.1.3.1	Alkaline phosphatase
Dia	1.6.99.X	Diaphorase
Enol	4.2.1.11	Enolase
Est1*	3.1.1.X	Esterase

Table 8.1 continued

Enzyme Symbol	E.C. number	E.C. name
Est2*	3.1.1.X	Esterase
Est3*	3.1.1.X	Esterase
Fdp1*	3.1.3.11	Fructose bisphosphatase
Fdp2*	3.1.3.11	Fructose bisphosphatase
Fum	4.2.1.2	Fumarate hydratase
Glo	4.4.1.5	Lactoylglutathione lyase
Got1*	2.6.1.1	Aspartate aminotransferase
Got2*	2.6.1.1	Aspartate aminotransferase
Gpd1*	1.1.1.8	Glycerol-3-phosphate dehydrogenase
Gpd2*	1.1.1.8	Glycerol-3-phosphate dehydrogenase
Gpi*	5.3.1.9	Glucose-6-phosphate isomerase
Gpt*	2.6.1.2	Alanine aminotransferase
Hbdh	1.1.1.30	3-Hydroxybutarate dehydrogenase
Hex*	3.2.1.30	Hexosaminidase
Hk	2.7.1.1	Hexokinase
Idh1	1.1.1.42	Isocitrate dehydrogenase
Idh2	1.1.1.42	Isocitrate dehydrogenase
Mpi1*	5.3.1.8	Mannose-6-phosphate isomerase
Mpi2*	5.3.1.8	Mannose-6-phosphate isomerase
PepA*	3.4.13.X	Dipeptidase
PepB1	3.4.13.X	Dipeptidase
PepB2*	3.4.13.X	Dipeptidase
PepD1*	3.4.13.X	Proline dipeptidase
PepD2*	3.4.13.X	Proline dipeptidase
Pgam*	5.4.2.1	Phosphoglycerate mutase
6Pgd*	1.1.1.44	Phosphogluconate dehydrogenase
Pgm2*	2.7.1.40	Phosphoglucomutase
Pk	2.7.1.40	Pyruvate kinase
Tpi*	5.3.1.1	Triose phosphate isomerase

8.2.3 Experiment 3: isolation between insect populations under semi-realistic conditions

The aim of this experiment was to firstly determine if moths from either insect population showed a preference for ovipositing on either plant sub-species, and secondly to determine if given the choice under semi-realistic conditions, the two insect populations would mate with one another.

In experiment number 2, a fixed difference was detected at the *tpi* locus, enabling the two bitou tip moth populations and hybrids to be distinguished using the allozyme electrophoresis technique on a single allozyme.

The experiment was conducted in an enclosed glasshouse room (4 x 4m) maintained at 21-23ºC. Fifteen large potted boneseed plants were placed in one corner of the room and fifteen large potted bitou bush plants placed in the opposite corner. Each potted plant had at least 20 growing tips. Both plant sub-species were actively growing at the time of the experiment. Twenty virgin pairs from each population were released simultaneously into the centre of the room. When the majority of their progeny had reached the third instar (approximately 4 weeks), 50 larvae were randomly selected from each sub species of the weed and placed individually into Epindorph tubes.

The experiment commenced in October 1998 and a second replicate was completed in January 1999. The allozyme electrophoresis work was conducted in April 1999. Prior to analysis, the insects were stored in a -80°C freezer.

The relative proportions of each insect population present on each weed sub-species was analysed with a Pearson chi-squared test and the proportions of each insect population and hybrids recovered in total were analysed with a t-test using Genstat. The influence of replicates was found to be non-significant (Pearson chi square value=4.07, df=2, p=0.131).

8.2.4 Experiment 4: host specificity testing

Because doubts were raised regarding the taxonomy of the two bitou tip moth populations, comparative host specificity tests were undertaken with both insect populations. The original host specificity tests, undertaken by the Keith Turnbull Research Institute, were conducted with a mixture of insects originating from the Natal province and Port Elizabeth, South Africa.

The two *Chrysanthemoides* sub-species (boneseed and bitou bush) and three close relatives of *Chrysanthemoides* (*Calendula officinalis, Dimorphotheca sinuata and Osteospermum fruticosum*) were used in the host specificity testing. The three relatives are all garden plants present in the Adelaide area. Their inclusion in the tests was suggested by Dr David Cooke, a botanist with the South Australian Animal and Plant Control Commission.

Ten neonates were placed onto each plant. The number of insects that were observed to feed and the number surviving through to pupation was recorded. The experiment was repeated five times, at intervals of two days.

8.2.5 Taxonomy

Adults from both insect populations were sent to Dr Marianne Horak, CSIRO, Canberra for inspection. The genitalia of three males and three females from each insect population were inspected.

8.3 Results

8.3.1 Experiment 1: reciprocal crosses – fecundity, % egg hatch and F1 survival

There appeared to be considerable post mating reproductive incompatibilities between populations. In both the parental and F1 generation there were no significant differences in the mean fecundity of the four crosses (F=2.48, df=3, 114, p=0.064 and F=1.06, df=3, 114, p=0.368). However, there were significant differences in percent egg hatch in both the F1 (F=14.52, df=3, 108, p<0.001) and F2 generation (F=38.7, df=3, 107, p<0.001). In the F1 generation, egg viability for crosses between populations was significantly lower (approximately half) compared with crosses within populations. In the F2 generation the hybrids' egg viability was extremely low relative to crosses within the Cape Town or Natal populations (Table 8.2). In the F2 generation, the median percent egg hatch for the hybrids was zero (Table 8.2), demonstrating that the majority of pairs did not produce any viable offspring. Only 20±5.8% of F2 hybrid pairs produced offspring compared with 66.7±13.3% for the Cape Town population and 76.7±8.8% for the Natal population. It was observed that the inviable eggs produced by the hybrids were a mixture of infertile and black eggs, in which the latter indicated that death occurred to developing embryos. The inviable eggs produced by the within strain crosses were generally infertile only.

Lone females produced only very low numbers of eggs, all infertile (Table 8.2). Because there was no significant difference in the total number of eggs laid between inter- and intra-population crosses (all of which were ten-fold greater than for lone females), the adult females must have attempted mating in all crosses (Table 8.2).

Table 8.2 Total fecundity and percent egg hatch of the bitou tip moth for the reciprocal crosses and unpaired females. Data for the parents, F1 and F2 generation are shown (CT: Cape Town and N: Natal). Means ± sem are given in the table. Data from the unpaired females were not analysed with the remaining data.

Cross	Parents		F1		F2
Female x male	Fecundity	% egg hatch	Fecundity	% egg hatch	median % egg hatch
CT x CT	48.0±4.6	76.4±6.6	37.3±4.3	64.6±8.1	83.15
N x N	35.5±4.2	82.7±4.8	36.9±4.3	72.5±6.8	88.2
CT x N	51.1±5.0	31.5±6.7	45.4±5.1	5.5±2.6	0
N x CT	38.8±4.7	42.6±7.6	35.7±3.1	7.8±3.5	0
CT	2.9±0.8	0	3.6±0.8	0	0
N	3.5±0.9	0	2.9±0.7	0	0

For F1 mortality there was a significant interaction between temperature and cross ($F=4.16$, df=3, 14, $p=0.027$). At the low temperature (22°C) the hybrids had a higher mortality rate compared with the within population crosses. For all pairs, there was a significant increase in mortality at the higher temperature (32°C), but the increase in mortality was much greater for the hybrids (Table 8.3).

Table 8.3 F1 survival and the proportion of bitou tip moth females at 22 and 32°C.

Cross	F1 mortality (%) mean±sem		% females, mean±sem	
	22°C	32°C	22°C	32°C
CT x CT	24.7±3.5	39.3±2.9	49.7±4.4	57.2±3.1
N x N	25.3±3.5	36.7±3.5	54.5±2.5	52.0±5.3
CT x N	36.7±4.8	66.7±4.8	44.8±3.3	37.3±7.6
N x CT	33.3±3.5	69.3±6.4	46.7±3.7	39.5±2.1

The proportion of females in the F1 generation varied significantly among the four crosses ($F=5.22$, df=3, 14, $p=0.013$), temperature had no significant effect on the proportion of

females (F=0.79, df=1, 14, p=0.39). The hybrids had a lower proportion of females compared with the progeny of within population crosses (Table 8.3).

8.3.2 Experiment 2: allozyme electrophoresis studies

Allele frequencies were determined for 26 of the 27 polymorphic loci for the two insect populations (Table 8.4). The observed and expected heterozygosity, average number of alleles per locus and proportion of polymorphic loci did not vary significantly between the two insect populations (Table 8.5). For both populations, the difference between the observed and expected heterozygositiy was small, indicating the laboratory colonies were not deviating from Hardy-Weinberg expectations.

The Nei genetic distance and proportion of fixed differences was calculated to be 0.198 and 5 respectively, which lies in an ambiguous range for species and sub-species (M. Adams, pers. comm.). The two populations show some degree of divergence, especially at Tpi, Acon1 and Est3, but are still very similar. From these data alone it is difficult to determine the relationship of the two populations. The presence of fixed differences does suggest that gene flow has been limited between the two populations for some time.

Table 8.4 Allozyme frequencies, expressed as a percentage, at the 26 polymorphic loci, for the two bitou tip moth populations.

Locus	Allele no.	Cape Town	Natal
Acon1	a	90	0
	b	0	95
	c	10	5
Acon2	a	3	0
	b	52	100
	c	15	0
	d	30	0
Acyc	a	33	0

Table 8.4 continued

Locus	Allele no.	Cape Town	Natal
	b	34	5
	c	34	20
	d	0	20
	e	0	5
	f	0	45
	g	0	5
Adh2	a	9	6
	b	74	56
	c	2	0
	d	12	38
	e	3	0
Ao	a	0	10
	b	100	90
Est1	a	59	100
	b	41	0
Est2	a	100	69
	b	0	28
	c	0	3
Est3	a	0	44
	b	0	3
	c	0	53
	d	100	0
Fdp1	a	89	18
	b	6	75
	c	5	0
	d	0	7
Fdp2	a	3	0
	b	0	29
	c	18	3
	d	41	0
	e	0	32
	f	38	36
Got1	a	11	5
	b	89	95
Got2	a	17	0
	b	72	100

Table 8.4 continued

Locus	Allele no.	Cape Town	Natal
	c	11	0
Gpd1	a	100	94
	b	0	6
Gpi	a	15	60
	b	85	30
	c	0	10
Gpt	a	5	15
	b	85	85
	c	10	0
Hex	a	0	55
	b	100	45
Mpi1	a	84	0
	b	16	100
Mpi2	a	70	5
	b	30	95
PepA	a	20	0
	b	80	100
PepB2	a	3	0
	b	19	10
	c	3	0
	d	72	90
	e	3	0
PepD1	a	10	30
	b	0	30
	c	65	10
	d	20	0
	e	5	15
	f	0	15
PepD2	a	3	15
	b	97	85
Pgam	a	15	10
	b	85	90
6Pgd	a	0	3
	b	84	85
	c	16	12
Pgm2	a	11	0

Table 8.4 continued

Locus	Allele no.	Cape Town	Natal
	b	50	15
	c	0	5
	d	39	75
	e	0	5
Tpi	a	0	3
	b	0	97
	c	100	0

Table 8.5 The observed and expected heterozygosity, proportion of polymorphic loci and the average number of alleles per locus, for the two insect populations.

Insect population	Observed heterozygosity (±se)	Expected heterozygosity (±se)	Proportion polymorphic loci	Average number alleles/locus
Cape Town	0.177±0.037	0.184±0.036	0.488	1.902
Natal	0.176±0.037	0.180±0.038	0.512	1.927

8.3.3 Experiment 3: isolation between insect populations under semi-realistic conditions

The proportion of each insect population present on the two weed sub species varied significantly (Pearson chi square value=58.37, df=2, p<0.001). There was significantly higher numbers of the Cape Town population present on boneseed (84% of total Cape Town insects recovered) and significantly more of the Natal population present on bitou bush (84% of total Natal insects recovered) (Table 8.6).

Table 8.6 The number of hybrids, Cape Town and Natal insects recovered from the two weed sub-species, boneseed and bitou bush (mean±sem).

Insect population	Number of insects recovered	
	Boneseed	Bitou bush
Cape Town	32.0±1.0	7.0±0.0
Natal	6.0±2.0	37.5±1.5
Hybrid	12.0±3.0	5.5±1.5

There were significantly fewer hybrids recovered, compared to Cape Town insects (t=5.97, df=2, p=0.027) or Natal insects (t=6.67, df=2, p=0.022), but there were no significant differences in the total number of Cape Town and Natal insects recovered (t=2.88, df=2, p=0.102) (Table 8.6). Thus, even in a small green house, the moths also appeared to demonstrate a mating choice for their own population.

8.3.4 Experiment 4: host specificity testing

No insects from either insect population survived on any of the test plant species other than the two *Chrysanthemoides* sub-species, although a higher proportion of Cape Town insects showed feeding activity on a greater number of the test plant species. The Cape Town insect population feed on *C. officinalis* and *D. sinuata* and the Natal insect population feed on *C. officinalis* (Table 8.7). The insects that began feeding on *C. officinalis* and *D. sinuata* lived for less than one week and did not develop. The proportion of Cape Town insects feeding on *C. officinalis* was significantly higher compared to the Natal population (F=79.53, df=1, 4, p<0.001).

Table 8.7 The number of bitou tip moth larvae showing feeding activity and surviving on the two sub-species of *Chrysanthemiodes* and three close relatives of *Chrysanthemoides* present in Australia.

Plant species	Mean number of insects surviving to pupation (/10)		Number of insects observed feeding (mean±sem)	
	Cape Town	Natal	Cape Town	Natal
Chrysanthemoides monilifera monilifera	8	6.2	10±0	10±0
Chrysanthemoides monilifera rotundata	7.6	8.2	10±0	10±0
Calendula officinalis	0	0	6.8±0.7	1.6±0.5
Dimorphotheca sinuata	0	0	2.2±0.6	0±0
Osteospermum fruticosum	0	0	0±0	0±0

8.3.5 Taxonomy

In the male genitalia, the ventral part of the vinculum is distinctly shorter and broadly rounded in the Cape Town population, and the two bands of spines in the basal half of the valva are both curved with their distal ends approaching each other and, importantly, the space between the two ends has scattered small spines. In the Natal population the ventral part of the vinculum is longer, slender and subrectangular, and the two bands of spines in the basal half of the valva are less curved and distally more separated, and the space between their distal ends is without spines. The female genitalia have very few features but the signum in the Cape Town female is a small narrow sclerite with a row of about six sharp spine-like teeth, whereas it only has two large triangular teeth in the Natal female. There may be further differences in the male genitalia which need confirmation, with the Natal males possibly with more slender valvae and socii and the uncus less parallel-sided and widest just beyond the middle rather than in the basal half (M. Horak, pers. comm.).

8.4 Discussion

The results of experiment 1 clearly shows that the crossing of the two insect populations led to outbreeding depression. The F1 hybrids had high larval mortality and were only partially fertile.

The mortality of the F1 hybrids was approximately 30% higher than the within population crosses at 22°C, and 45% higher at 32°C. Thus, the degree of depression manifested in the F1 hybrids increased with increasing temperature. Similarly, Kidwell *et al.* (1977) found when crossing populations of *Drosophila* that low developmental temperatures tended to inhibit the expression of some dysgenic traits such as infertility.

Both reciprocal crosses produced significantly fewer females compared to the within population crosses. This difference could be explained by Haldane's rule, which states that the heterogametic sex (female) is most commonly lethal or sterile in the F1 generation of a cross between populations. A possible reason for this is that X-linked deleterious recessive alleles in heterogametic (XY) females are not masked by dominant alleles on the other X chromosome, as for the homogametic sex (Robinson, 1971). Alternatively, Hurst and Pomiankowski (1991) argued that it results from the loss of suppression of sex ratio distorters in the new nuclear cytotype of the hybrid.

The degree of depression observed for the reciprocal crosses in this case was equivalent in both directions. Often hybrid dysgenesis is found to be non-reciprocal, which is thought to be because hybrid dysgenesis is dependent on cytoplasm-chromosome interactions between strains (Kidwell *et al.*, 1977).

The outbreeding depression observed between the two bitou tip moth populations may be due to a number of factors, such as hybrid dysgenesis resulting from transposable P elements (Hoy, 1994) or poor chromosomal pairing. Some have argued that it may also result from the disruption of co-adapted gene complexes (Falconer and Mackay, 1996), although there is no

clear evidence that gene complexes are this fragile (Roush, 1990). Outbreeding depression is thought to be more common where there is greater genetic divergence and the taxonomic status of the populations is in question (Frankham, 1995).

In experiment 3, significantly higher proportions of the Cape Town population were recovered from boneseed and higher proportions of the Natal population from bitou bush. This suggests that the Cape Town population showed a preference for ovipositing on boneseed and the Natal population on bitou bush. However, it is likely that these differences were also due at least in part to the different rates of survival of each insect population on each weed sub-species (see experiment 5, chapter 7). In this experiment only 17.5% of the total insects recovered were hybrids, while 39% belonged to the Cape Town population and 43.5% belonged to the Natal population. If the moths from both populations mated at random and showed equal survival, one may have expected proportions of 25% each for the Cape Town and Natal populations and 50% for the hybrids. The low numbers of hybrids recovered may be explained by differences in mating behaviour or pheromones between the two populations resulting in fewer inter-population matings, and possibly higher mortality of the F1 hybrids.

Although both populations were found to be host specific, the Cape Town population was observed to show more feeding activity on some of the close relatives of *Chrysanthemoides*. Some insects, possibly including the Cape Town population of the bitou tip moth, have adapted to feeding on nutrient poor host plants. Adaptations include: selecting plant tissues with higher nutrient levels, low metabolic rates, manipulating the source-sink relationship in host plants and symbiotic relationships with micro organisms (Barbehenn *et al.*, 1984). The increased feeding activity on the close relatives of *Chrysanthemoides* may be because the Cape Town population possesses higher levels of some of these traits.

After inspecting the adult genitalia of the two insect populations, Dr Marianne Horak concluded that the two populations were very different, but from these data alone it was not possible to determine if they were sibling species or populations at either end of a cline.

Similarly with the allozyme data, the 5% fixed differences show that the populations have some degree of divergence but are still similar, with a Nei genetic distance of 0.198.

Mayr (1963) defined a species as a group of actually or potentially interbreeding natural populations which are reproductively isolated from other groups. Although this is a useful definition, it cannot be applied to asexual organisms and is difficult to use objectively in cases involving geographically isolated populations (Bush and Hoy, 1984). The fertility of hybrids between populations can range from nil to normal levels of fertility. At what point along this scale do populations become species? The relationship between the two bitou tip moth populations does not appear to be clear cut. The two populations may be sibling or sub-species or they may be simply be populations at either end of a cline, which in time may become distinct species. The bitou tip moth's native range stretches from Cape Town to the Natal Province, South Africa (Adair and Scott, 1989). Over this area, the populations are not continuous but fragmented, due to the patchy distribution of the host plant (R. Roush, pers. comm.). The only way to conclusively classify the two bitou tip moth populations would be to undertake further studies with collections made from areas between Cape Town and the Natal Province. But, from a practical point of view, because crosses between the two populations show outbreeding depression, it is important that they be treated separately. If ant predation had not been an issue, the chance of establishing the insect would almost certainly have been reduced had the two populations been hybridised prior to release. Even if the two populations were released in the same areas, there would seem to be a significant risk of poorly fertile hybrids occurring, which would have at least slowed population growth. Further, there would have been no obvious advantage to releasing both populations together, as suggested in the previous chapter. Ignoring the ant predation, it would have been best to import two popualtions, the Natal population for release along the east coast of Australia and a Cape Town population for release in southeastern Australia. Had only one importation been possible, the Cape Town population would have been the better bet.

Chapter 9

Conclusions and general discussion

Why do so many releases of classical biological control agents fail to establish? Has poor climate matching been as common a problem as many people have believed, or has it just been a convenient scapegoat where no other obvious factors exist? Clearly, there are a large number of possible reasons why agents fail to establish in the field. Within this project alone, four causes of establishment failures have been identified (six if ingestion of plants by kangaroos and accidental cultivation of release sites by farmers are included). Although all projects are different and factors that are important in one project may be irrelevant in another, it should be possible to develop a general set of guidelines based on reviews of past programs and research such as this.

9.1 Reasons for the poor establishment of the original plume moth and bitou tip moth populations

The hypothesis that the original population of the plume moth released into South Australia failed to establish because it was poorly adapted to South Australia's hot/dry summers was rejected. A population recently imported from the original collection location in southern France was equally as well adapted to the range of climate types present in South Australia (semi-arid to temperate) as a population originating from the harsh climate of central Spain (Zaragosa). Both populations established at 9 of the 10 release sites. The population at the 10th site failed because the host weed was heavily grazed by kangaroos during a drought. The original French population released in Australia (old French population) was found to be poorly adapted to the areas of the state that experience warmer and drier summers, probably due to laboratory adaptation, given that two newer collections from Europe were well adapted

to the field. During the time the old French insect population was reared in the laboratory there may have been unintentional selection against aestival diapause (or quiescence). In both the newly imported insect populations, 24-35% of the population entered diapause at 35°C, while only 1% of the old French population entered diapause. The plume moth enters diapause as early instar larvae in the growing tips of plants. These insects are hard to find, and as a result diapausing insects may have been overlooked during rearing and not selected for the following generations. An aestival diapause would have been essential in the northern areas of South Australia to avoid periods of hot/dry conditions, which resulted in scarcity of quality food. Even in the cooler/wetter areas of South Australia, the old French population of the plume moth had a lower rate of population increase and dispersal compared to the two newly imported populations. In laboratory studies, the old French population's fecundity was 50% lower than the two newly imported populations at 20°C and 80% lower at 35°C. This was found to be the result of inbreeding depression and/or population bottlenecks. This difference in fecundity observed in the laboratory was similar to the difference in population increase observed at many of the field sites.

Differences were detected between the Spanish and new French populations, but they were not shown to be important in the field. The largest difference detected was in the timing and trigger of their winter diapause. Had the target weed been an annual, this difference may have been critical.

In the case of the bitou tip moth, the hypothesis that the original population (Natal population) failed to establish in South Australia because it was poorly adapted to South Australia's Mediterranean climate was only partially correct. The primary reason was ant predation. Had ant predation not been an issue, it is likely that the Natal population would have failed to establish or suppress the weed because it was poorly adapted to moisture-stressed host plants and the sub-species of the weed present in South Australia. An insect population originating from Cape Town was better adapted to moisture-stressed host plants and the sub-species of the weed present. Despite the fact that Natal does not often experience temperature extremes

and Cape Town does, the Natal population seemed as well adapted to survive extremes in temperature as the Cape Town population. However, the Natal population developed more slowly at lower temperatures.

9.2 Climate matching

The results of this study suggest that where the release climate has large seasonal variations in temperature and rainfall, and corresponding seasonal variation in host quality or quantity, populations should be sought from areas with similar seasonal variations.

This outcome is consistent with past research. In previous studies, a close climate match has been found to be important to establishment and degree of weed control where the release climate has large seasonal variations in temperature (eg. Van den Bosh, 1964; Tauber and Tauber, 1978; Flint, 1980; Nealis, 1985; Van Driesche, 1993) and host quantity and quality (eg. Dymock, 1987; Hill *et al.*, 1991), which are not present in the collection location. In these situations, populations should be collected that are synchronised with the seasons and target pest of the release location (Cameron *et al.*, 1993). Care should also be taken to avoid populations that are adapted to specific microclimates, as was demonstrated in the example of *Emex* spp. given by Scott (1992).

How close a match is required may depend on how extreme the climatic variables are, the degree of variation between the seasons and how critical it is to synchronise the agent with the target pest and seasons of the release climate. Because both target weeds were perennial, synchronisation with the target weed was not critical and, in the case of the plume moth, a facultative diapause overcame hot/dry periods where there was poor host quality.

Targeting insects with a facultative rather than obligatory quiescence or diapause may be an advantage in unpredictable areas such as northern South Australia, where stressful conditions may strike at any time. The importance of a facultative aestival diapause in the plume moth

was demonstrated with the failure of the old French population in the northern areas of South Australia.

In projects such as the biological control program against *Chrysanthemoides monilifera*, where the weed exists over a wide range of climate types (sub-tropical to Mediterranean), it may be better to select populations from Mediterranean environments which are adapted to more variable climates and possibly a wider range of conditions. These populations should be well adapted to the Mediterranean release locations and, in sub-tropical environments, they may simply act as if it is spring all year round. In projects where agents have been successfully transferred between very dissimilar climates, the agent has usually been imported from a temperate to a tropical or sub-tropical environment, as was the case with the exportation of *Telenomous alsophilae* from Virginia, USA., to Columbia (Bustillo and Drooz, 1977) and transfer of *Rhopalomia californica* from a Mediterranean area of the USA to a sub-tropical region of Queensland, Australia (Julien, 1992). However, if the population enters a diapause in environments where it is superfluous, this will unnecessarily slow population growth rates and their resulting effects on the target pest (Flint, 1980). The collection and maintenance of only one Mediterranean population would reduce the time involved and facilities required.

As well as affecting the establishment of an agent, a poor climate match may also reduce the impact on the target weed by reducing the agent's population growth rates. Had the two tip moth populations established, it is likely the Natal population would have reached much lower population densities, due to higher mortality and development rates on moisture-stressed boneseed and slower development rates at low temperatures. A poor climate match may also result in the poor synchronisation of the host's life cycle with that of the biological control agent, thereby reducing the effect of the agent on the target pest, as was demonstrated in the biological control program against gorse in New Zealand (Hill *et al.,* 1991).

Because of the lack of accurate information, some reviews of past programs may have overstated the importance of climate (eg. Crawley, 1986; Cullen, 1995). As the reviewers have

indicated, these surveys of anecdotal examples can only be used as a guide to identify areas where more detailed research is required. Without experimental releases, the old French population of the plume moth may have been another case added to the poor climate matching list. While others may have underestimated climate's importance (eg, Leen, 1991), because the synchrony of the agent with the pest and seasons and climatic extremes were not fully considered in her analysis.

The study conducted with the bitou tip moth demonstrates the importance of checking the taxonomic status of multiple collections. The incorrect identification of insect populations may waste time and resources (Rosen and DeBach, 1973), or lead to the importation of a biotype with a wider host range (Diehl and Bush, 1984).

9.3 Insect quality and genetic variation of the agent

The results of this study reinforce Mackauer's (1976) ideas regarding insect quality. There have been a number of studies demonstrating that laboratory adaptation, inbreeding, drift and bottlenecks can occur during the importation and rearing process (eg. House, 1967; Cullen, 1980; Roush, 1986; Geden et al., 1992), but, to my knowledge, no previous studies have demonstrated that these processes have been critical to the establishment of a biological control agent.

It is unclear whether the low fecundity and genetic variability of the old French population of the plume moth was due to bottlenecks, inbreeding, or a combination of both and what level of inbreeding or bottleneck size occurred. It is also unclear if the plume moth is particularly sensitive to inbreeding or bottlenecks, or if some particularly dramatic events occurred while rearing this insect population. A PhD project, conducted by Jeanine Baker, University of Adelaide, is tackling some of these questions.

Answers to these questions are required so we know how to avoid problems such as this in the future. For insects that are sensitive to inbreeding and bottlenecks, it may be good practice to replace the laboratory colony with fresh field material periodically (Mackauer, 1980). Because the cost of additional importations is small in relation to the cost of failure, it may be worthwhile to make further importations following the completion of the host specificity testing. Once the insect is established in the field, the laboratory colony could be replaced with field material at regular intervals.

The reduction of the old French population's ability to enter an aestival diapause may have been due to unintentional selection against this trait in the laboratory, or because there was no selection in favour of aestival diapause in the laboratory. This example reiterates Mackauer's (1980) concerns regarding laboratory rearing.

To increase the level of genetic variation within a population of a biological control agent, it has been proposed that it may be of benefit to hybridise populations from different regions of the agent's host range (Legner, 1971). But, as suggested by Mackauer (1976), the results of the bitou tip moth study clearly demonstrate that the compatibility of the populations should first be assessed.

9.4 Release technique

The initial releases of the plume moth were made with considerably more insects than would be required to achieve establishment. So that the bio-control agent can be colonised over the entire range of the target pest as quickly as possible, it is important that the minimum number of insects required to gain establishment be released at each site, so the insect can be released at as many sites as possible. Releasing at a larger number of sites will also minimise the effects of stochastic environmental variation (Shea and Possingham, 2000).

Using an analysis of past programs and their reaction-diffusion model, Hopper and Roush (1993) estimated that around 1000 insects are required per release site to ensure establishment of parasitic wasps. Because insects vary in their dispersal rate, mate finding distance and net population increase, the minimum release number required will vary between insects. Unfortunately, because it takes time to determine the behaviour of the agent and the number of insects required per site, the rule of thumb approach is required at least initially.

In their reviews of past bio-control programs, Beirne (1975) and Cameron et al. (1993) found that the establishment rate increased with increasing release numbers, suggesting that the Allee effect may be important. One method of minimising the Allee effect is the use of release tents. Because the plume moth does not travel over long distances, has a high population growth rate and a mate detection distance of up to 8m (Leyson, 1999), the use of tents seems unnecessary for this species. In fact, the use of tents was found to hamper initial population growth and dispersal, when releasing 200 pupae. Perhaps the release of 50 pupae within tents, or 100-200 pupae without tents, would have been a better release strategy. Whether or not tents are of benefit would depend on the distances dispersed by insects, mate finding distance and the availability of resources in the tent area. It may also depend on the availability of insects, as tents may allow the release of fewer individuals at each site.

The major limiting factor in the control program against horehound with the plume moth was the plume moth's slow dispersal rate, rather than its population increase. It was estimated that at current rates of increase and dispersal, it will take 27 - 500 years for the moth to distribute itself across South Australia's 20 million hectares of horehound from the existing release sites, while the population growth rates determined in this project suggest that there will be enough insects to cover the entire area with horehound suppressing densities in less than five years (Clarke et al., 2000). The control of horehound will therefore benefit from further redistribution of the plume moth. The cost of horehound to the state's economy has not been estimated, but is likely to run into the millions of dollars per year. There is therefore considerable financial incentive to distribute the moths as quickly as possible. Unfortunately,

there is little funding in Australia for redistribution or post release evaluation of biological control agents.

9.5 Predation

In native ecosystems there is little that can be done to combat predation of biological control agents. In the bitou tip moth project, a number of methods for controlling ants around the release site were tried, but none were successful. Even if ants could be controlled around the release sites and the insect was established, predation would almost certainly have prevented the population from building to levels that would have suppressed the target weed. The ants were attracted to boneseed plants by an unidentified black scale insect. This is a further example of how two phytophagous insects can have a negative impact on one another. The black scale would also have made the boneseed less attractive to the tip moth by reducing fresh tip growth.

Additional agents for the control of boneseed in South Australia should only be considered if they have mechanisms to overcome ant predation. For example, the bitou seed fly's (*Mesoclanis sp.*) larval stages occur within the flower buds and seed and therefore should not be greatly affected by ant predation. In contrast, the tortricid leaf roller currently being evaluated is likely to be vulnerable to ant predation.

Crawley's (1989a) review of past bio-control projects found that insect families with the poorest establishment record are often external feeders, such as Lepidoptera, while many of the best establishers were internal feeders, such as Diptera. It was suggested that internal feeders were better protected from predators. But, without thorough investigation, it would not be possible to rule out other factors, eg the internal feeders may have been better protected from adverse environmental conditions. Whatever the reason, if there is a choice it may be better to target agents that are better protected from predators. Crawley (1989a) noted that

there may be a trade off between establishment success and degree of pest control with internal and external feeders.

The plume moth was observed to suffer little from predation. Ants and a pentatomid bug were occasionally observed feeding on pupae.

9.6 Further Research

This study highlights the need for past failures to be evaluated experimentally. It is extremely difficult to distinguish the many possible causes of establishment failures based on observation alone. For example, in the case of the plume moth, distinguishing between poor climate matching and laboratory adaptation would only be possible by experimentally comparing the laboratory population with a newly imported colony, collected using the same procedures from the same location. Without this research, the Spanish population of the plume moth would have been released alone and its good performance in the field would probably have been believed to have been because it was better adapted to South Australia's climate. This would have given bio-control workers a false perception of the importance of climate matching and more importantly, underestimated the problems associated with the importation and rearing process.

The correct identification of failures is important, not only to improve the way future programs are conducted, but also to overcome the problem at hand.

A major problem, as always, is lack of funding. Interested parties such as farmer groups are reluctant to fund projects aimed at determining reasons for the failure of an agent to establish. Funding for bio-control programs is generally only sufficient to cover exploration, importation, host specificity testing and the initial releases.

191

The literature is full of hypotheses and reviews of past programs aimed at explaining the failure of bio-control agents to establish (eg. Hall *et al.*, 1979; Mackauer, 1980; Julien *et al.*, 1984; Crawley, 1989a; Roush, 1990; Leen, 1991; Cameron *et al.*, 1993; Hopper and Roush, 1993; and Cullen, 1995). As the authors indicate, retrospective studies are important to identify areas to target research, but what is needed now are further experimental releases of agents to identify the reasons behind the failure of particular biological control agents to establish, and the importance of factors such as founder size. This research will need to be conducted with a range of different bio-control agents and in a range of environments. Only then will we be able to develop a more robust set of guidelines and models to improve establishment success rates.

Appendix 1

Plume moth field evaluation dates

Release area	Release date	Evaluation 1	Evaluation 2	Evaluation 3	Evaluation 4
Flinders 1	06.11.97	15.12.97	07.02.98	19.08.98	09.01.99
Flinders 2	05.11.97	16.12.97	08.02.98	20.08.98	08.01.99
Flinders 3	05.11.97	16.12.97	09.02.98	18.08.98	07.01.99
Monarto	04.09.97	14.10.97	05.01.98	12.08.98	21.12.98
Warooka	14.11.97	23.12.97	30.01.98	03.09.98	-
Adelaide	19.09.97	26.10.97	12.01.98	15.09.98	-
Narrung 1	28.11.97	02.02.98	09.09.98	23.12.98	-
Narrung 2	22.01.98	09.09.98	23.12.98	-	-
Robe A	27.11.97	05.02.98	12.09.98	28.12.98	-
Robe B	27.11.97	06.02.98	11.09.98	27.12.98	-

Appendix 2

Plume moth field data analysis

Adelaide

Stage 1- Logistic Regression

Table 1a: The probability of plume moths being present in each quadrat at the Adelaide site for each species, distance and generation.

New French

Distance	Generation		
	1	2	3
tent	0.999		
0-5m		0.785	0.993
5-10m		0.568	0.981
10-20m		0.310	0.946
20-40m		0.019	0.428

Old French

Distance	Generation		
	1	2	3
tent	0.999		
0-5m		0.406	0.964
5-10m		0.166	0.886
10-20m		0.004	0.134
20-40m		0.001	0.045

Spanish

Distance	Generation		
	1	2	3
tent	0.999		
0-5m		0.758	0.992
5-10m		0.522	0.977
10-20m		0.123	0.845
20-40m		0.016	0.385

Adelaide

Stage 2- Zero-Inflated Poisson

Table 1b: Predicted mean for quadrats showing insects present for the Adelaide site.

New French

Distance tent	Generation		
	1	2	3
tent	6.994		
0-5m		3.028	6.067
5-10m		1.383	2.771
10-20m		1.253	2.511
20-40m		0.904	1.812

Old French

Distance tent	Generation		
	1	2	3
tent	3.104		
0-5m		2.819	5.648
5-10m		0.566	1.135
10-20m			0.607
20-40m			0.607

Spanish

Distance tent	Generation		
	1	2	3
tent	5.731		
0-5m		3.627	7.269
5-10m		1.768	3.544
10-20m		1.278	2.561
20-40m		0.823	1.649

Adelaide

Stage 3 - Combining Models

Table 1c: Overall Predicted Means (Standard Errors) per quadrat for the Adelaide site.

New French

Distance tent	Generation		
	1	2	3
tent	6.988 (1.327)		
0-5m		2.376 (0.502)	6.025 (0.716)
5-10m		0.785 (0.162)	2.718 (0.277)
10-20m		0.389 (0.066)	2.376 (0.153)
20-40m		0.017 (0.004)	0.775 (0.075)

Old French

Distance tent	Generation		
	1	2	3
tent	3.102 (0.835)		
0-5m		1.146 (0.492)	5.445 (0.836)
5-10m		0.094 (0.043)	1.006 (0.223)
10-20m		-	0.081 (0.038)
20-40m		-	0.027 (0.013)

Spanish

Distance tent	Generation		
	1	2	3
tent	5.727 (1.194)		
0-5m		2.750 (0.639)	7.209 (0.866)
5-10m		0.923 (0.197)	3.463 (0.345)
10-20m		0.157 (0.038)	2.165 (0.168)
20-40m		0.013 (0.003)	0.635 (0.069)

Monarto

Stage 1- Logistic Regression

Table 2a: The probability of plume moths being present in each quadrat at the Monarto site for each species, distance and generation.

New French

Distance	Generation			
	1	2	3	4
tent	0.750			
0-5m		0.414	0.625	0.389
5-10m		0.399	0.589	0.388
10-20m		0.086	0.194	0.301
20-40m		0.013	0.108	0.131
40-60m			0.020	0.091
60-80m				0.046
80-100m				0.013
100-120m				0.012

Old French

Distance	Generation			
	1	2	3	4
tent	0.500			
0-5m		0.260	0.278	0.404
5-10m		0.115	0.115	0.209
10-20m		<0.001	<0.001	<0.001
20-40m		0.001	0.001	0.020
40-60m			<0.001	<0.001
60-80m				<0.001
80-100m				<0.001
100-120m				<0.001

Spanish

Distance	Generation			
	1	2	3	4
tent	0.750			
0-5m		0.456	0.811	0.756
5-10m		0.290	0.656	0.614
10-20m		0.030	0.146	0.366
20-40m		0.003	0.051	0.113
40-60m			0.005	0.047
60-80m				0.030
80-100m				<0.001
100-120m				0.009

Monarto

Stage 2- Zero-Inflated Poisson

Table 2b: Predicted mean for quadrats showing insects present for the Monarto site.

New French

Distance tent	Generation			
	1	2	3	4
0-5m	4.621	6.361	1.997	3.086
5-10m		1.603	0.503	0.778
10-20m		1.231	0.386	0.597
20-40m		1.852	0.581	0.899
40-60m			0.544	0.841
60-80m				0.425
80-100m				0.317
100-120m				0.001

Old French

Distance tent	Generation			
	1	2	3	4
0-5m	2.821	0.002	1.029	1.502
5-10m		0.001	0.491	0.717
10-20m				
20-40m				

Spanish

Distance tent	Generation			
	1	2	3	4
0-5m	3.563	2.943	2.139	1.932
5-10m		2.247	1.634	1.476
10-20m		1.745	1.269	1.146
20-40m			0.799	0.722
40-60m				0.634
60-80m				0.715
80-100m				NA
100-120m				0.874

Monarto

Stage 3- Combining Models

Table 2c: Overall Predicted Means (Standard Errors) per quadrat for the Monarto site.

New French

Distance tent	Generation			
	1	**2**	**3**	**4**
	3.466 (1.355)			
0-5m		**2.632** (1.142)	**1.249** (0.386)	**1.202** (0.516)
5-10m		**0.640** (0.229)	**0.297** (0.078)	**0.302** (0.097)
10-20m		**0.105** (0.046)	**0.075** (0.021)	**0.180** (0.042)
20-40m		**0.024** (0.013)	**0.063** (0.014)	**0.118** (0.023)
40-60m			**0.011** (0.004)	**0.076** (0.016)
60-80m				**0.019** (0.006)
80-100m				**0.004** (0.003)
100-120m				**<0.001** <0.001

Old French

Distance tent	Generation			
	1	**2**	**3**	**4**
	1.411 (0.898)			
0-5m		**<0.001** 0.007	**0.287** (0.200)	**0.606** (0.320)
5-10m		**<0.001** 0.001	**0.057** (0.041)	**0.150** (0.091)
10-20m				**NA** NA
20-40m				**0.020** (0.010)
40-60m				
60-80m				
80-100m				
100-120m				

Spanish

Distance tent	Generation			
	1	2	3	4
	2.672 (1.100)			
0-5m		1.342 (0.606)	1.734 (0.410)	1.460 (0.358)
5-10m		0.652 (0.270)	1.072 (0.238)	0.907 (0.207)
10-20m		0.052 (0.031)	0.185 (0.057)	0.420 (0.085)
20-40m			0.041 (0.012)	0.081 (0.018)
40-60m				0.030 (0.009)
60-80m				0.022 (0.008)
80-100m				NA NA
100-120m				0.007 0.007

Narrung Site 1

Stage 1- Logistic Regression

Table 3a: The probability of plume moths being present in each quadrat at Narrung Site 1 for each species, distance and generation.

New French

Distance tent	Generation		
	1	2	3
	1.000		
0-5m		1.000	0.833
5-10m		0.333	1.000
10-20m		0.246	0.623
20-40m		0.183	0.766
40-60m		<0.001	0.674
60-80m			0.672
80-100m			0.292
100-120m			0.125
120-140m			0.088
140-160m			0.035
160-180m			0.008

Old French

Distance tent	Generation		
	1	2	3
	1.000		
0-5m		1.000	0.714
5-10m		0.267	0.824
10-20m		0.250	0.352
20-40m		0.020	0.398
40-60m		<0.001	0.286
60-80m			0.083
80-100m			0.009
100-120m			0.002
120-140m			<0.001
140-160m			<0.001
160-180m			<0.001

Spanish

Distance tent	Generation		
	1	2	3
	1.000		
0-5m		0.714	1.000
5-10m		0.353	0.786
10-20m		0.222	0.667
20-40m		0.082	0.621
40-60m		<0.001	0.661
60-80m			0.593
80-100m			0.260
100-120m			0.098
120-140m			0.036
140-160m			0.019
160-180m			<0.001

Narrung Site 1

Stage 2- Zero-Inflated Poisson

Table 3b: Predicted mean for quadrats showing insects present for Narrung Site 1.

New French

Distance	Generation		
	1	2	3
tent	7.997		
0-5m		11.345	6.375
5-10m		1.874	4.202
10-20m		1.060	3.422
20-40m		1.214	2.754
40-60m			2.462
60-80m			2.217
80-100m			1.959
100-120m			1.449
120-140m			1.843
140-160m			1.552
160-180m			0.607

Old French

Distance	Generation		
	1	2	3
tent	6.490		
0-5m		6.326	3.555
5-10m		1.579	3.540
10-20m		1.160	3.748
20-40m		1.139	2.583
40-60m			2.183
60-80m			1.199
80-100m			0.984
100-120m			2.232
120-140m			
140-160m			
160-180m			

Spanish

Distance tent	Generation		
	1	**2**	**3**
0-5m	6.994		
5-10m		16.646	9.353
10-20m		1.540	3.454
20-40m		1.236	3.994
40-60m		0.988	2.241
60-80m			2.574
80-100m			1.859
100-120m			1.731
120-140m			1.594
140-160m			1.856
160-180m			0.513

Narrung Site 1

Stage 3- Combining Models

Table 3c: Overall Predicted Means (Standard Errors) per quadrat for Narrung Site 1.

New French

Distance tent	Generation		
	1	**2**	**3**
	7.997 (1.413)		
0-5m		**11.344** (1.223)	**5.313** (1.192)
5-10m		**0.625** (0.239)	**4.202** (0.513)
10-20m		**0.261** (0.063)	**2.132** (0.274)
20-40m		**0.222** (0.037)	**2.109** (0.115)
40-60m			**1.660** (0.083)
60-80m			**1.490** (0.066)
80-100m			**0.571** (0.045)
100-120m			**0.181** (0.022)
120-140m			**0.162** (0.021)
140-160m			**0.054** (0.011)
160-180m			**0.005** (0.002)

Old French

Distance tent	Generation		
	1	**2**	**3**
	6.490 (1.269)		
0-5m		**6.326** (0.793)	**2.539** (0.707)
5-10m		**0.421** (0.198)	**2.916** (0.504)
10-20m		**0.290** (0.072)	**1.320** (0.247)
20-40m		**0.023** (0.011)	**1.029** (0.097)
40-60m			**0.624**

Distance tent			
60-80m			(0.061) **0.099** (0.017)
80-100m			**0.008** (0.004)
100-120m			**0.005** (0.004)
120-140m			
140-160m			
160-180m			

Spanish

	Generation		
	1	**2**	**3**
Distance tent	**6.993** (1.319)		
0-5m		**11.890** (3.074)	**9.353** (1.111)
5-10m		**0.544** (0.205)	**2.714** (0.549)
10-20m		**0.275** (0.073)	**2.662** (0.322)
20-40m		**0.081** (0.020)	**1.392** (0.102)
40-60m			**1.70** (0.087)
60-80m			**1.102** (0.057)
80-100m			**0.451** (0.037)
100-120m			**0.156** (0.020)
120-140m			**0.067** (0.014)
140-160m			**0.010** (0.003)
160-180m			

Narrung Site 2

Stage 1- Logistic Regression

Table 4a: The probability of plume moths being present in each quadrat at Narrung Site 2 for each species, distance and generation.

New French

	Generation	
	1	2
Distance		
tent	1.000	
0-5m		1.000
5-10m		0.821
10-20m		0.132
20-40m		0.017
40-60m		<0.001

Old French

	Generation	
	1	2
Distance		
tent	1.000	
0-5m		1.000
5-10m		0.821
10-20m		0.132
20-40m		0.017
40-60m		<0.001

Spanish

	Generation	
	1	2
Distance		
tent	1.000	
0-5m		1.000
5-10m		0.821
10-20m		0.132
20-40m		0.017
40-60m		<0.001

Narrung Site 2

Stage 2- Zero-Inflated Poisson

Table 4b: Predicted mean for quadrats showing insects present for Narrung Site 2.

New French

	Generation	
	1	2
Distance tent	6.742	
0-5m		11.428
5-10m		1.126
10-20m		1.065
20-40m		1.365
40-60m		

Old French

	Generation	
	1	2
Distance tent	7.496	
0-5m		5.647
5-10m		2.185
10-20m		2.318
20-40m		0.023
40-60m		

Spanish

	Generation	
	1	2
Distance tent	8.248	
0-5m		8.713
5-10m		1.519
10-20m		1.365
20-40m		0.874
40-60m		

Narrung Site 2

Stage 3- Combining Models

Table 4c: Overall Predicted Means (Standard Errors) per quadrat for Narrung Site 2.

New French

Distance tent	Generation 1	Generation 2
tent	6.742 (1.294)	
0-5m		11.428 (1.278)
5-10m		0.925 (0.194)
10-20m		0.140 (0.047)
20-40m		0.023 (0.009)
40-60m		

Old French

Distance tent	Generation 1	Generation 2
tent	7.496 (1.366)	
0-5m		5.647 (0.786)
5-10m		1.794 (0.324)
10-20m		0.305 (0.088)
20-40m		<0.001 0.003
40-60m		

Spanish

Distance tent	Generation 1	Generation 2
tent	8.248 (1.435)	
0-5m		8.713 (1.115)

Distance		
5-10m		**1.247** (0.219)
10-20m		**0.180** (0.049)
20-40m		**0.015** (0.007)
40-60m		

Warooka Site

Stage 1- Logistic Regression

Table 5a: The probability of plume moths being present in each quadrat at the Warooka site for each species, distance and generation.

New French

	Generation		
	1	**2**	**3**
Distance			
tent	1.000		
0-5m		0.860	0.974
5-10m		0.587	0.805
10-20m		0.241	0.654
20-40m		0.100	0.426
40-60m		0.024	0.167
60-80m		<0.001	0.120
80-100m			0.029
100-120m			0.005
120-140m			<0.001
140-160m			<0.001
160-180m			<0.001

Old French

	Generation		
	1	**2**	**3**
Distance			
tent	0.750		
0-5m		0.355	0.351
5-10m		0.167	0.087
10-20m		0.013	0.013
20-40m		<0.001	<0.001
40-60m		<0.001	<0.001
60-80m		<0.001	<0.001
80-100m			<0.001
100-120m			<0.001
120-140m			
140-160m			
160-180m			

Spanish

Distance	Generation		
	1	**2**	**3**
tent	1.000		
0-5m		1.000	1.000
5-10m		0.584	0.868
10-20m		0.217	0.727
20-40m		0.047	0.349
40-60m		0.018	0.192
60-80m		<0.001	0.212
80-100m			0.038
100-120m			0.007
120-140m			0.037
140-160m			<0.001
160-180m			0.008

Warooka

Stage 2- Zero-Inflated Poisson

Table 5b: Predicted mean for quadrats showing insects present for the Warooka site.

New French

Distance	Generation		
	1	**2**	**3**
tent	5.579		
0-5m		6.435	6.435
5-10m		1.729	3.182
10-20m		0.812	2.662
20-40m		0.879	2.515
40-60m		0.724	1.705
60-80m			1.602
80-100m			1.236
100-120m			0.876
120-140m			
140-160m			
160-180m			

Old French

Distance	Generation		
	1	2	3
tent	1.778		
0-5m		2.051	2.051
5-10m		0.551	1.014
10-20m			0.848
20-40m			
40-60m			
60-80m			
80-100m			
100-120m			
120-140m			
140-160m			
160-180m			-

Spanish

Distance	Generation		
	1	2	3
tent	6.536		
0-5m		7.539	7.540
5-10m		2.026	3.728
10-20m		0.952	3.119
20-40m		1.030	2.947
40-60m		0.849	1.997
60-80m			1.877
80-100m			1.448
100-120m			1.026
120-140m			1.540
140-160m			1.365
160-180m			

Warooka

Stage 3- Combining Models

Table 5c: Overall Predicted Means (Standard Errors) per quadrat for the Warooka Site.

New French

| Distance tent | Generation | | |
	1	2	3
	5.578 (0.783)		
0-5m		5.532 (1.072)	6.265 (0.731)
5-10m		1.015 (0.218)	2.560 (0.350)
10-20m		0.196 (0.046)	1.742 (0.176)
20-40m		0.088 (0.019)	1.071 (0.090)
40-60m		0.018 (0.006)	0.285 (0.034)
60-80m			0.193 (0.025)
80-100m			0.036 (0.009)
100-120m			0.005 (0.003)
120-140m			
140-160m			
160-180m			

Old French

| Distance tent | Generation | | |
	1	2	3
	1.333 (0.475)		
0-5m		0.728 (0.370)	0.720 (0.352)
5-10m		0.092 (0.054)	0.088 (0.058)
10-20m			0.011 (0.009)
20-40m			

Spanish

Distance	Generation		
	1	**2**	**3**
Distance tent	**6.536** (0.921)		
0-5m		**7.538** (0.898)	**7.540** (0.826)
5-10m		**1.182** (0.271)	**3.236** (0.381)
10-20m		**0.206** (0.052)	**2.269** (0.210)
20-40m		**0.049** (0.013)	**1.030** (0.096)
40-60m		**0.015** (0.005)	**0.384** (0.045)
60-80m			**0.397** (0.040)
80-100m			**0.055** (0.013)
100-120m			**0.007** (0.004)
120-140m			**0.056** (0.014)
140-160m			**<0.001** (<0.001)
160-180m			

Flinders Site 1

Stage 1- Logistic Regression

Table 6a: The probability of plume moths being present in each quadrat at Flinders Site 1 for each species, distance and generation.

New French

Distance	Generation			
	1	**2**	**3**	**4**
Distance tent	0.865			
0-5m		0.170	<0.001	<0.001
5-10m		0.042	<0.001	<0.001
10-20m		<0.001	<0.001	<0.001

Old French

Distance	Generation			
	1	**2**	**3**	**4**
tent	0.428			
0-5m		0.023	<0.001	<0.001
5-10m		0.005	<0.001	<0.001
10-20m		<0.001	<0.001	<0.001

Spanish

Distance	Generation			
	1	**2**	**3**	**4**
tent	0.956			
0-5m		0.413	<0.001	<0.001
5-10m		0.130	<0.001	<0.001
10-20m		<0.001	<0.001	<0.001

Flinders Site 1

Stage 2- Zero-Inflated Poisson

Table 6b: Predicted mean for quadrats showing insects present for Flinders Site 1

New French

Distance	Generation			
	1	**2**	**3**	**4**
tent	6.210			
0-5m		1.287		
5-10m				
10-20m				

Old French

Distance	Generation			
	1	**2**	**3**	**4**
tent	6.210			
0-5m				
5-10m				
10-20m				

Spanish

Distance	Generation			
	1	**2**	**3**	**4**
Distance tent	6.210			
0-5m		1.287		
5-10m		1.287		
10-20m				

Flinders Site 1

Stage 3- Combining Models

Table 6c: Overall Predicted Means (Standard Errors) per quadrat for Flinders Site 1.

New French

Distance	Generation			
	1	**2**	**3**	**4**
Distance tent	**5.372** (1.084)			
0-5m		**0.219** (0.142)		
5-10m				
10-20m				

Old French

Distance	Generation			
	1	**2**	**3**	**4**
Distance tent	**2.660** (1.452)			
0-5m				
5-10m				
10-20m				

Spanish

Distance tent	Generation			
	1	2	3	4
0-5m	5.939 (0.849)			
5-10m		0.531 (0.237)		
10-20m		0.168 (0.094)		

Flinders Site 2

Stage 1- Logistic Regression

Table 7a: The probability of plume moths being present in each quadrat at Flinders Site 2 for each species, distance and generation.

New French

Distance	Generation			
	1	2	3	4
tent	0.972			
0-5m		0.875	0.453	0.765
5-10m		0.327	0.099	0.480
10-20m		0.073	0.048	0.187
20-40m		0.222	0.008	0.054
40-60m		<0.001	<0.001	0.059
60-80m				0.012
80-100m				0.001
100-120m				0.001
120-140m				<0.001

Old French

Distance	Generation			
	1	2	3	4
tent	0.296			
0-5m		0.078	0.010	0.038
5-10m		0.006	0.001	0.011
10-20m		0.001	0.001	0.003
20-40m		<0.001	<0.001	<0.001
40-60m		<0.001	<0.001	<0.001
60-80m				<0.001
80-100m				<0.001
100-120m				<0.001
120-140m				<0.001

Spanish

Distance	Generation			
	1	2	3	4
tent	0.981			
0-5m		0.914	0.556	0.831
5-10m		0.424	0.143	0.583
10-20m		0.106	0.070	0.258
20-40m		0.033	0.013	0.079
40-60m		<0.001	<0.001	0.086
60-80m				0.018
80-100m				0.002
100-120m				0.002
120-140m				<0.001

Flinders Site 2

Stage 2- Zero-Inflated Poisson

Table 7b: Predicted mean for quadrats showing insects present for Flinders Site 2

New French

Distance	Generation			
	1	2	3	4
tent	4.736			
0-5m		1.204	0.911	1.313
5-10m		1.204	0.911	1.313
10-20m		1.204	0.911	1.313
20-40m		1.204		1.313
40-60m				1.313
60-80m				1.313
80-100m				1.313
100-120m				
120-140m				

Old French

Distance	Generation			
	1	2	3	4
tent	4.736			
0-5m		1.204		
5-10m				
10-20m				
20-40m				
40-60m				
60-80m				
80-100m				
100-120m				
120-140m				

Spanish

Distance	Generation			
	1	2	3	4
tent	4.736			
0-5m		1.204	0.911	1.313
5-10m		1.204	0.911	1.313
10-20m		1.204	0.911	1.313
20-40m		1.204	0.911	1.313
40-60m				1.313
60-80m				1.313
80-100m				1.313
100-120m				1.313
120-140m				

Flinders Site 2

Stage 3- Combining Models

Table 7c: Overall Predicted Means (Standard Errors) per quadrat for Flinders Site 2.

New French

Distance tent	Generation			
	1	2	3	4
	4.604 (0.709)			
0-5m		1.054 (0.156)	0.413 (0.162)	1.004 (0.182)
5-10m		0.394 (0.108)	0.091 (0.047)	0.630 (0.136)
10-20m		0.088 (0.027)	0.043 (0.018)	0.245 (0.049)
20-40m		0.027 (0.008)		0.071 (0.014)
40-60m				0.077 (0.013)
60-80m				0.015 0.004
80-100m				0.002 (0.001)
100-120m				
120-140m				

Old French

Distance tent	Generation			
	1	2	3	4
	1.403 (1.1011)			
0-5m		0.094 (0.085)		
5-10m				
10-20m				

Spanish

Distance tent	Generation			
	1	2	3	4
	4.648 (0.707)			
0-5m		1.100 (0.141)	0.507 (0.171)	1.091 (0.149)
5-10m		0.510 (0.123)	0.130 (0.065)	0.765 (0.135)
10-20m		0.128 (0.038)	0.064 (0.026)	0.339 (0.060)
20-40m		0.040 (0.012)	0.012 (0.006)	0.104 (0.020)
40-60m				0.113 (0.018)
60-80m				0.023 (0.006)
80-100m				0.003 (0.002)
100-120m				0.002 (0.002)
120-140m				

Flinders Site 3

Stage 1- Logistic Regression

Table 8a: The probability of plume moths being present in each quadrat at Flinders Site 3 for each species, distance and generation.

New French

Distance	Generation			
	1	2	3	4
tent	0.977			
0-5m		0.793	0.239	0.399
5-10m		0.279	0.098	0.432
10-20m		0.021	0.021	0.132
20-40m		0.010	<0.001	0.036
40-60m		<0.001	<0.001	0.019
60-80m				0.010
80-100m				0.006

Old French

Distance	Generation			
	1	2	3	4
tent	0.541			
0-5m		0.095	0.009	0.180
5-10m		0.011	0.003	0.020
10-20m		0.001	0.001	0.004
20-40m		<0.001	<0.001	0.001
40-60m		<0.001	<0.001	<0.001
60-80m				<0.001
80-100m				<0.001

Spanish

Distance	Generation			
	1	2	3	4
tent	0.982			
0-5m		0.832	0.289	0.462
5-10m		0.334	0.123	0.495
10-20m		0.027	0.027	0.164
20-40m		0.013	<0.001	0.046
40-60m		<0.001	<0.001	0.025
60-80m				0.013
80-100m				0.008

Flinders Site 3

Stage 2- Zero-Inflated Poisson

Table 8b: Predicted mean for quadrats showing insects present for Flinders Site 3

New French

	Generation			
	1	2	3	4
Distance tent	2.822			
0-5m		2.989	0.413	0.983
5-10m		0.677	0.699	0.686
10-20m		0.637		0.619
20-40m				0.975
40-60m				0.926
60-80m				0.485
80-100m				1.027

Old French

	Generation			
	1	2	3	4
Distance tent	1.557			
0-5m		1.649		
5-10m				
10-20m				
20-40m				
40-60m				
60-80m				
80-100m				

Spanish

	Generation			
	1	2	3	4
Distance tent	4.207			
0-5m		4.456	0.615	1.466
5-10m		1.009	1.042	1.022
10-20m		0.950	0.874	0.923
20-40m		0.273		1.453
40-60m				1.381
60-80m				0.723
80-100m				

Flinders Site 3

Stage 3- Combining Models

Table 8c: Overall Predicted Means (Standard Errors) per quadrat for Flinders Site 3

New French

Distance tent	Generation			
	1	**2**	**3**	**4**
	2.757 (0.566)			
0-5m		**2.370** (0.509)	**0.099** (0.070)	**0.393** (0.163)
5-10m		**0.189** (0.067)	**0.068** (0.044)	**0.296** (0.079)
10-20m		**0.013** (0.009)		**0.082** (0.023)
20-40m				**0.035** (0.009)
40-60m				**0.018** (0.005)
60-80m				**0.005** (0.002)
80-100m				**0.006** (0.004)

Old French

Distance tent	Generation			
	1	**2**	**3**	**4**
	0.842 (0.448)			
0-5m		**0.157** (0.121)		
5-10m				
10-20m				
20-40m				
40-60m				
60-80m				
80-100m				

Spanish

Distance tent	Generation			
	1	**2**	**3**	**4**
0-5m	4.132 (0.824)	3.706 (0.780)	0.177 (0.128)	0.677 (0.279)
5-10m		0.337 (0.118)	0.128 (0.083)	0.506 (0.130)
10-20m		0.026 (0.017)	0.023 (0.015)	0.151 (0.041)
20-40m		0.004 (0.002)		0.067 (0.017)
40-60m				0.035 (0.010)
60-80m				0.009 (0.004)
80-100m				

Robe Site A

Stage 1- Logistic Regression

Table 9a: The probability of plume moths being present in each quadrat at Robe Site A for each species, distance and generation.

New French

Distance	Generation		
	1	2	3
tent	1.000		
0-5m		1.000	1.000
5-10m		1.000	0.947
10-20m		0.506	0.932
20-40m		0.139	0.578
40-60m		0.016	0.454
60-80m		<0.001	0.463
80-100m			0.261
100-120m			0.133
120-140m			0.186
140-160m			0.215
160-180m			0.092
180-200m			0.022

Old French

Distance	Generation		
	1	2	3
tent	1.000		
0-5m		1.000	0.667
5-10m		0.333	0.850
10-20m		0.219	0.553
20-40m		0.045	0.308
40-60m		0.002	0.191
60-80m		<0.001	0.201
80-100m			0.088
100-120m			0.027
120-140m			0.003
140-160m			<0.001
160-180m			<0.001
180-200m			

Spanish

Distance	Generation		
	1	**2**	**3**
tent	1.000		
0-5m		1.000	0.800
5-10m		0.714	0.938
10-20m		0.243	0.938
20-40m		0.156	0.637
40-60m		0.033	0.519
60-80m		<0.001	0.639
80-100m			0.747
100-120m			0.289
120-140m			0.149
140-160m			0.172
160-180m			0.009
180-200m			0.001

Robe Site A

Stage 2- Zero-Inflated Poisson

Table 9b: Predicted mean for quadrats showing insects present for Robe Site A

New French

Distance	Generation		
	1	**2**	**3**
tent	10.000		
0-5m		18.072	10.500
5-10m		2.056	5.355
10-20m		1.221	4.024
20-40m		1.289	2.547
40-60m		0.726	2.478
60-80m			2.554
80-100m			2.264
100-120m			1.980
120-140m			2.176
140-160m			1.826
160-180m			1.425
180-200m			0.923

Old French

Distance tent	Generation		
	1	**2**	**3**
0-5m	7.997	8.026	4.663
5-10m		1.180	3.073
10-20m		0.717	2.364
20-40m		0.994	1.962
40-60m		0.553	1.889
60-80m			2.039
80-100m			1.390
100-120m			1.318
120-140m			0.008
140-160m			
160-180m			
180-200m			

Spanish

Distance tent	Generation		
	1	**2**	**3**
0-5m	11.000	14.055	8.170
5-10m		1.677	4.369
10-20m		1.150	3.790
20-40m		1.187	2.343
40-60m		0.699	2.386
60-80m			2.169
80-100m			2.628
100-120m			1.973
120-140m			1.668
140-160m			1.495
160-180m			0.503
180-200m			0.008

Robe Site A

Stage 3- Combining Models

Table 9c: Overall Predicted Means (Standard Errors) per quadrat Robe Site A.

New French

Distance tent	Generation		
	1	2	3
	9.999 (1.583)		
0-5m		18.070 (1.475)	10.498 (1.042)
5-10m		2.056 (0.243)	5.073 (0.558)
10-20m		0.618 (0.091)	3.752 (0.244)
20-40m		0.179 (0.031)	1.472 (0.099)
40-60m		0.012 (0.005)	1.125 (0.074)
60-80m			1.183 (0.0642)
80-100m			0.592 (0.048)
100-120m			0.263 (0.030)
120-140m			0.405 (0.030)
140-160m			0.393 (0.026)
160-180m			0.131 (0.014)
180-200m			0.020 (0.005)

Old French

Distance tent	Generation		
	1	2	3
	7.997 (1.414)		
0-5m		8.025 (1.015)	3.109 (0.999)
5-10m		0.393 (0.142)	2.612 (0.409)
10-20m		0.157 (0.038)	1.306 (0.177)
20-40m		0.045	0.605

Distance			
	(0.013)	(0.071)	
40-60m	**0.001**	**0.360**	
	(0.001)	(0.044)	
60-80m		**0.410**	
		(0.040)	
80-100m		**0.122**	
		(0.018)	
100-120m		**0.036**	
		(0.009)	
120-140m		**<0.001**	
		(<0.001)	
140-160m			
160-180m			
180-200m			

Spanish

	Generation		
	1	**2**	**3**
Distance tent	**10.999**		
	(1.660)		
0-5m		**14.053**	**6.533**
		(1.372)	(1.662)
5-10m		**1.198**	**4.096**
		(0.257)	(0.533)
10-20m		**0.280**	**3.556**
		(0.064)	(0.223)
20-40m		**0.186**	**1.495**
		(0.030)	(0.092)
40-60m		**0.023**	**1.238**
		(0.007)	(0.072)
60-80m			**1.387**
			(0.057)
80-100m			**1.964**
			(0.062)
100-120m			**0.570**
			(0.035)
120-140m			**0.248**
			(0.022)
140-160m			**0.258**
			(0.021)
160-180m			**0.005**
			(0.002)
180-200m			**<0.001**
			(<0.001)

Robe Site B

Stage 1- Logistic Regression

Table 10a: The probability of plume moths being present in each quadrat at Robe Site B for each species, distance and generation.

New French

Distance	Generation		
	1	**2**	**3**
tent	0.750		
0-5m		1.000	0.857
5-10m		0.722	0.947
10-20m		0.286	0.959
20-40m		0.133	0.946
40-60m		0.002	0.701
60-80m			0.528
80-100m			0.527
100-120m			0.296
120-140m			0.125
140-160m			0.093
160-180m			0.004
180-200m			0.001
200-220m			0.008

Old French

Distance	Generation		
	1	**2**	**3**
tent	0.750		
0-5m		1.000	0.500
5-10m		0.353	0.700
10-20m		0.197	0.478
20-40m		<0.001	0.243
40-60m		<0.001	0.147
60-80m			0.147
80-100m			0.014
100-120m			<0.001
120-140m			<0.001
140-160m			<0.001
160-180m			0.037
180-200m			0.019
200-220m			<0.001

Spanish

	Generation		
	1	**2**	**3**
Distance tent	1.000		
0-5m		1.000	1.000
5-10m		0.611	0.941
10-20m		0.258	0.944
20-40m		0.181	0.783
40-60m		<0.001	0.584
60-80m			0.531
80-100m			0.626
100-120m			0.319
120-140m			0.062
140-160m			0.048
160-180m			0.037
180-200m			0.019
200-220m			<0.001

Robe Site B

Stage 2- Zero-Inflated Poisson

Table 10b: Predicted mean for quadrats showing insects present for Robe Site B

New French

	Generation		
	1	**2**	**3**
Distance tent	15.000		
0-5m		17.078	8.908
5-10m		1.202	5.231
10-20m		0.965	3.918
20-40m		1.001	2.364
40-60m		3.921	2.057
60-80m			1.969
80-100m			1.861
100-120m			1.793
120-140m			1.769
140-160m			1.321
160-180m			0.715
180-200m			0.003
200-220m			0.273

Old French

Distance	Generation		
	1	2	3
tent	11.667	8.974	4.681
0-5m		0.783	3.408
5-10m		0.625	2.537
10-20m			1.915
20-40m			1.615
40-60m			1.610
60-80m			0.550
80-100m			
100-120m			
120-140m			
140-160m			
160-180m			
180-200m			
200-220m			

Spanish

Distance	Generation		
	1	2	3
tent	10.750		
0-5m		10.947	5.710
5-10m		1.082	4.707
10-20m		0.827	3.357
20-40m		1.153	2.721
40-60m			2.436
60-80m			2.159
80-100m			2.039
100-120m			2.090
120-140m			1.513
140-160m			1.594
160-180m			0.926
180-200m			0.690
200-220m			

Robe Site B

Stage 3- Combining Models

Table 10c: Overall Predicted Means (Standard Errors) per quadrat for Robe Site B.

New French

Distance	Generation		
	1	2	3
tent	11.250 (0.655)		
0-5m		17.077 (1.442)	7.635 (1.454)
5-10m		0.868 (0.185)	4.955 (0.559)
10-20m		0.275 (0.063)	3.759 (0.234)
20-40m		0.133 (0.024)	2.238 (0.090)
40-60m		0.009 (0.010)	1.443 (0.066)
60-80m			1.040 (0.054)
80-100m			0.981 (0.045)
100-120m			0.531 (0.034)
120-140m			0.221 (0.021)
140-160m			0.123 (0.014)
160-180m			0.003 (0.002)
180-200m			<0.001 (<0.001)
200-220m			0.002 (0.001)

Old French

Distance	Generation		
	1	2	3
tent	8.750 (0.927)		
0-5m		8.974 (1.044)	2.340 (1.014)
5-10m		0.276 (0.101)	2.386 (0.474)
10-20m		0.123	1.212

		(0.034)	(0.196)
20-40m			**0.465**
			(0.064)
40-60m			**0.237**
			(0.035)
60-80m			**0.237**
			(0.030)
80-100m			**0.007**
			(0.003)
100-120m			
120-140m			

Spanish

	Generation		
	1	**2**	**3**
Distance tent	**10.749**		
	(1.640)		
0-5m		**10.946**	**5.709**
		(1.115)	(0.707)
5-10m		**0.661**	**4.430**
		(0.162)	(0.555)
10-20m		**0.213**	**3.167**
		(0.052)	(0.219)
20-40m		**0.209**	**2.133**
		(0.032)	(0.106)
40-60m			**1.422**
			(0.078)
60-80m			**1.146**
			(0.058)
80-100m			**1.276**
			(0.051)
100-120m			**0.666**
			(0.040)
120-140m			**0.094**
			(0.014)
140-160m			**0.076**
			(0.012)
160-180m			**0.034**
			(0.007)
180-200m			**0.013**
			(0.004)
200-220m			

Appendix 3

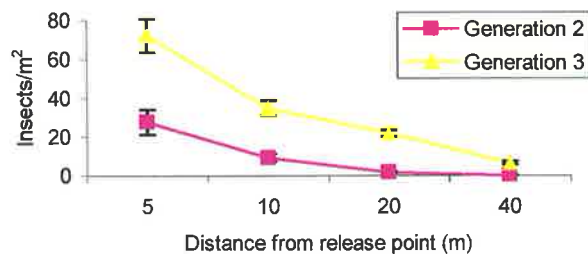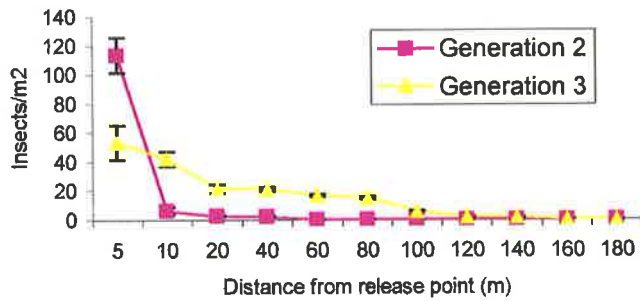## Dispersion of the plume moth from the release point
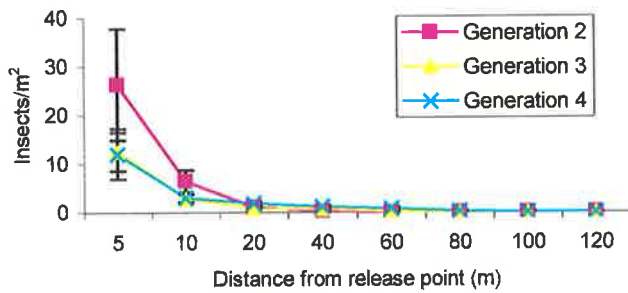
Figure 1. Dispersal of the new French population from the Monarto release site. The mean number of insects per m² at each distance from the release point and standard errors are given.
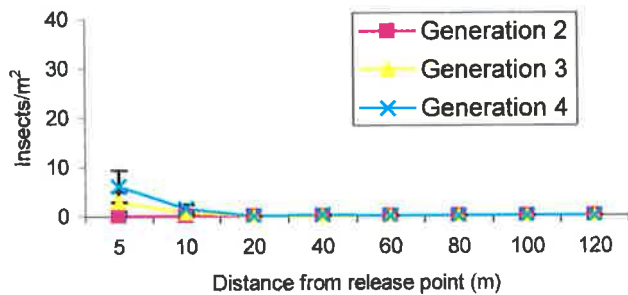
Figure 2. Dispersal of the old French population from the Monarto release site. The mean number of insects per m² at each distance from the release point and standard errors are given.
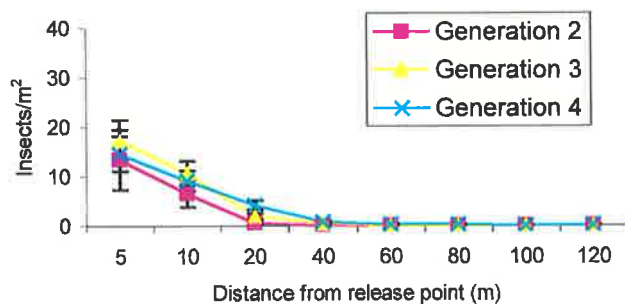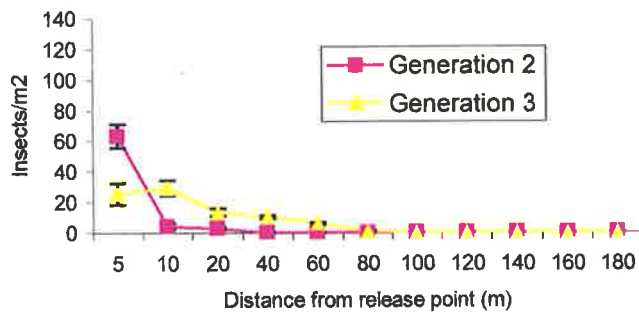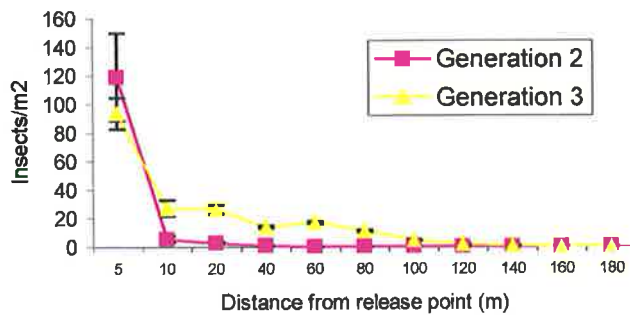

Figure 3. Dispersal of the Spanish population from the Monarto release site. The mean number of insects per m² at each distance from the release point and standard errors are given.
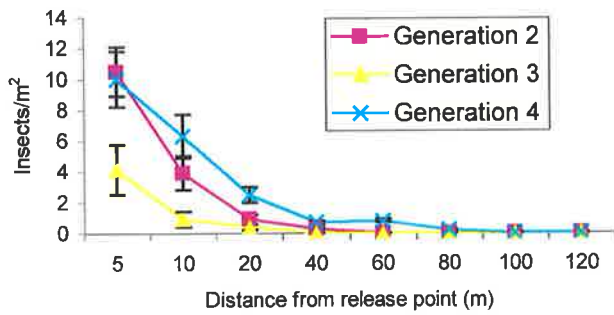
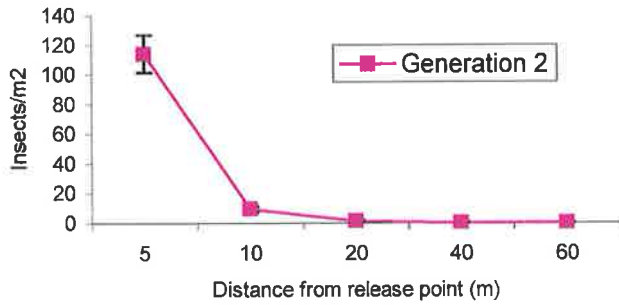**Figure 4**. Dispersal of the new French population from the Flinders 2 release site. The mean number of insects per m² at each distance from the release point and standard errors are given.
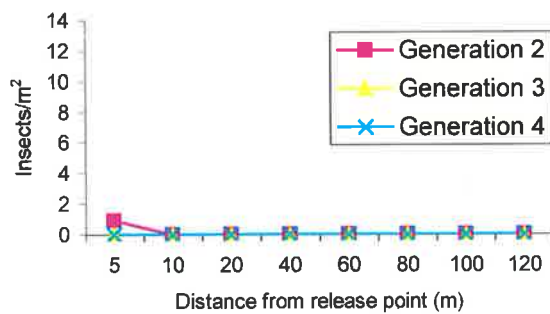
Figure 5. Dispersal of the old French population from the Flinders 2 release site. The mean number of insects per m² at each distance from the release point and standard errors are given.
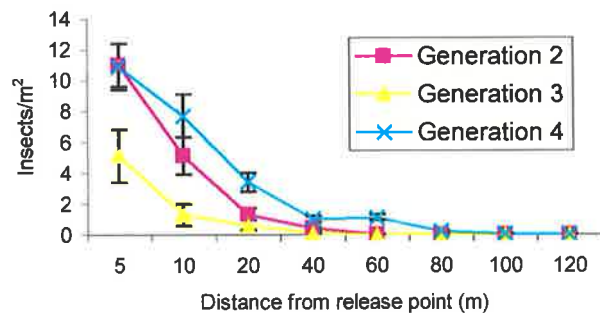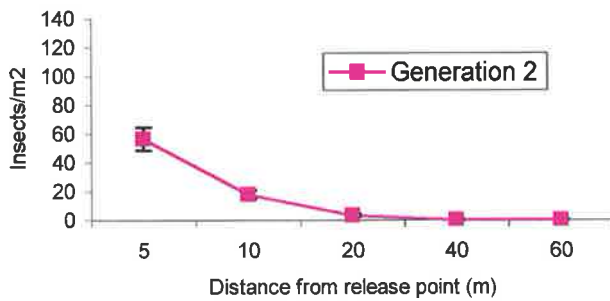

Figure 6. Dispersal of the Spanish population from the Flinders 2 release site. The mean number of insects per m² at each distance from the release point and standard errors are given.
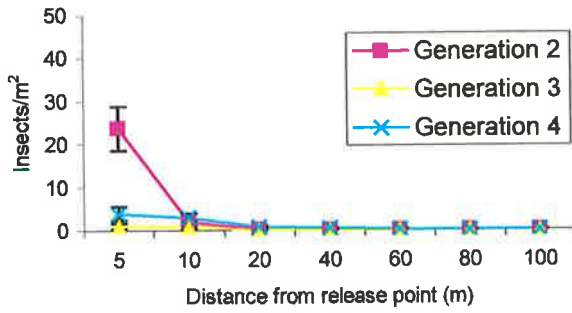
Figure 7. Dispersal of the new French population from the Flinders 3 release site. The mean number of insects per m² at each distance from the release point and standard errors are given.
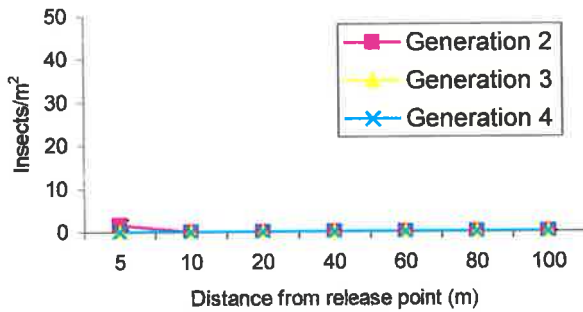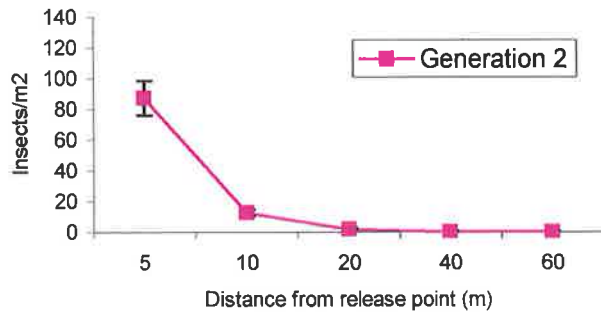

Figure 8. Dispersal of the old French population from the Flinders 3 release site. The mean number of insects per m² at each distance from the release point and standard errors are given.
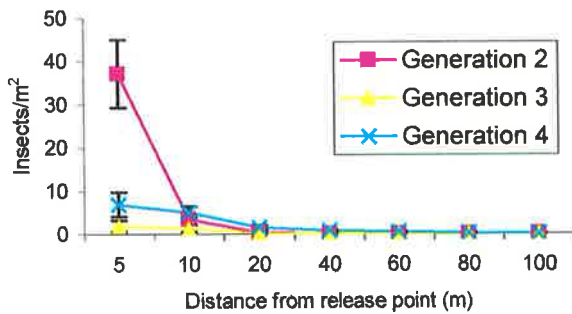
Figure 9. Dispersal of the Spanish population from the Flinders 3 release site. The mean number of insects per m² at each distance from the release point and standard errors are given.
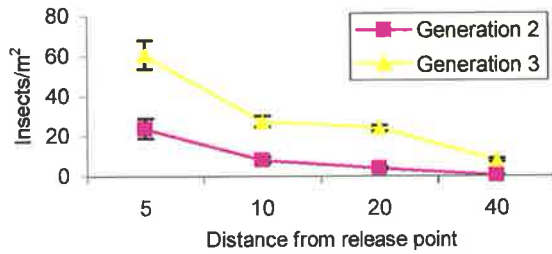
Figure 10. Dispersal of the new French population from the Adelaide release site. The mean number of insects per m² at each di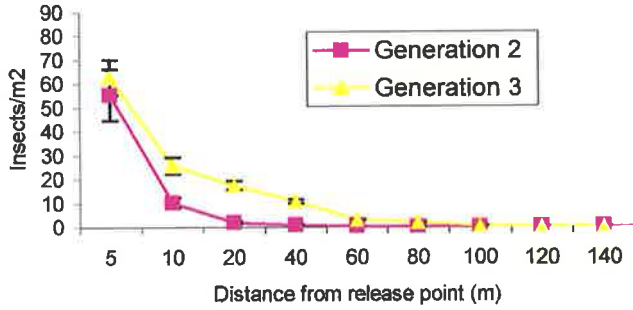stance from the release point and standard errors are given. The limit of the horehound infestation at the Adelaide site was 40m.
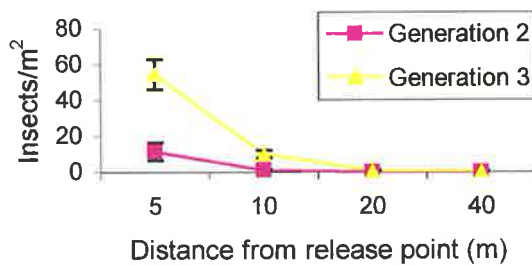
Figure 11. Dispersal of the old French population from the Adelaide release site. The mean number of insects per m² at each distance from the release point and standard errors are given. The limit of the horehound infestation at the Adelaide site was 40m.
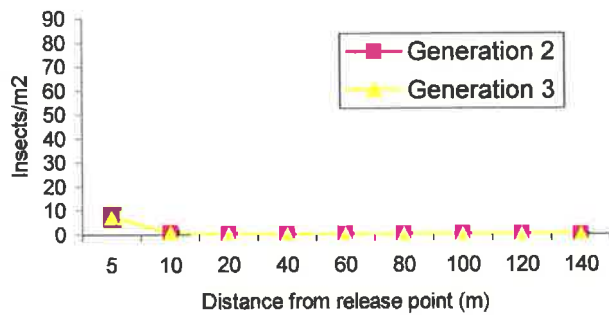

Figure 12. Dispersal of the Spanish population from the Adelaide release site. The mean number of insects per m² at each di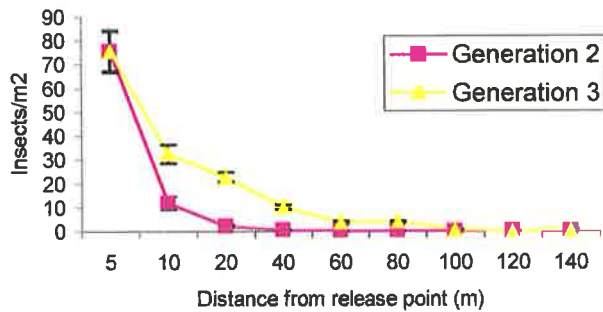stance from the release point and standard errors are given. The limit of the horehound infestation at the Adelaide site was 40m.

Figure 13. Dispersal of the new French population from the Narrung 1 release site. The mean number of insects per m^2 at each distance from the release point and standard errors are given.

Figure 14. Dispersal of the old French population from the Narrung 1 release site. The mean number of insects per m^2 at each distance from the release point and standard errors are given.
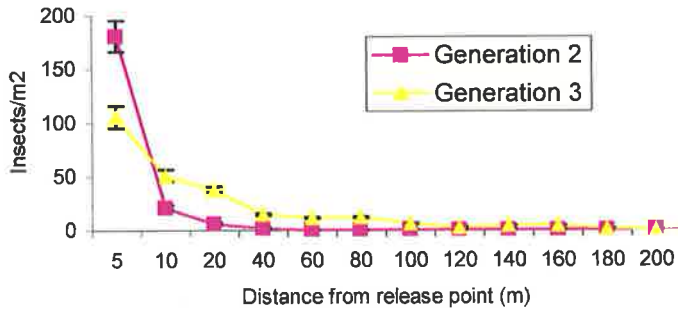

Figure 15. Dispersal of the Spanish population from the Narrung 1 release site. The mean number of insects per m^2 at each distance from the release point and standard errors are given.

Figure 16. Dispersal of the new French population from the Narrung 2 release site. The mean number of insects per m^2 at each distance from the release point and standard errors are given.
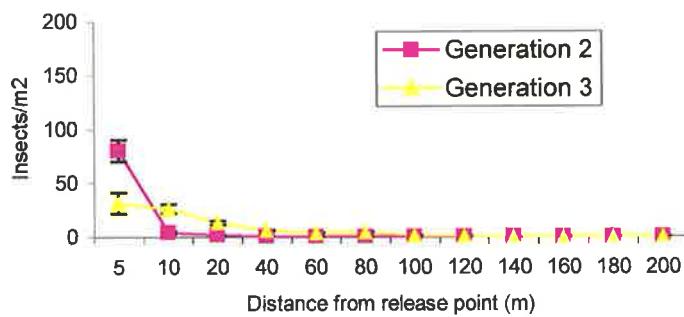

Figure 17. Dispersal of the old French population from the Narrung 2 release site. The mean number of insects per m^2 at each distance from the release point and standard errors are given.
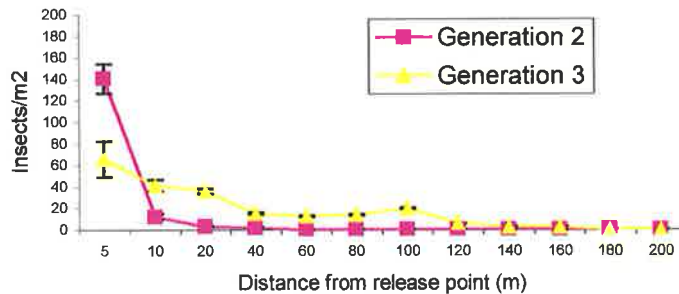

Figure 18. Dispersal of the Spanish population from the Narrung 2 release site. The mean number of insects per m^2 at each distance from the release point and standard errors are given.

Figure 19. Dispersal of the new French population from the Warooka release site. The mean number of insects per m^2 at each distance from the release point and standard errors are given.

Figure 20. Dispersal of the old French population from the Warooka release site. The mean number of insects per m^2 at each distance from the release point and standard errors are given.

Figure 21. Dispersal of the Spanish population from the Warooka release site. The mean number of insects per m^2 at each distance from the release point and standard errors are given.
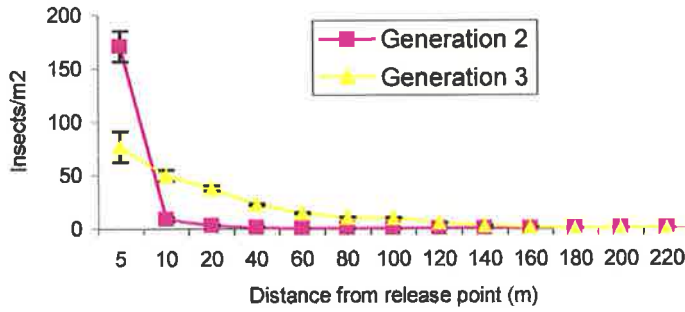
Figure 22. Dispersal of the new French population from the Robe A release site. The mean number of insects per m² at each distance from the release point and standard errors are given.
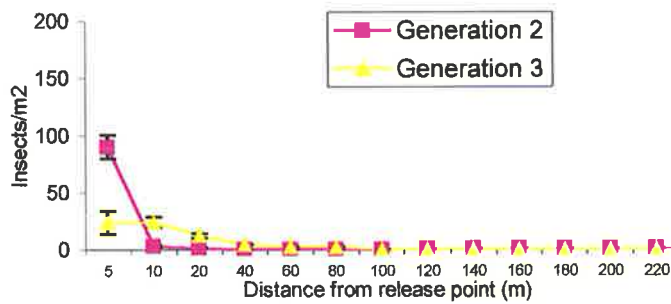

Figure 23. Dispersal of the old French population from the Robe A release site. The mean number of insects per m² at each distance from the release point and standard errors are given.
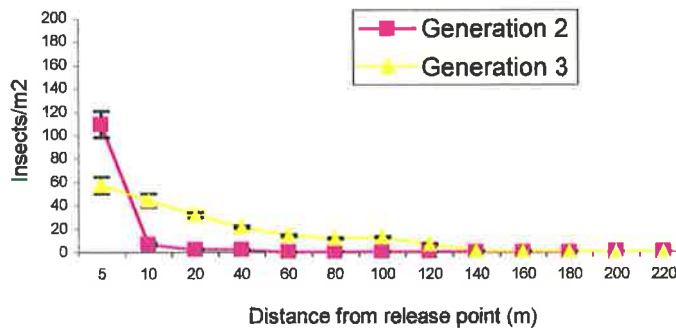

Figure 24. Dispersal of the Spanish population from the Robe A release site. The mean number of insects per m² at each distance from the release point and standard errors are given.

Figure 25. Dispersal of the new French population from the Robe B release site. The mean number of insects per m^2 at each distance from the release point and standard errors are given.

Figure 26. Dispersal of the old French population from the Robe B release site. The mean number of insects per m^2 at each distance from the release point and standard errors are given.

Figure 27. Dispersal of the Spanish population from the Robe B release site. The mean number of insects per m^2 at each distance from the release point and standard errors are given.

References

Adair, R. J. and Scott, J. K. (1989) The life history and host specificity of *Comostolopsis germana* Prout, a biological control agent of *Crysanthemoides monilifera.* Bull. Ent. Res. 79, 649-657.

Ainsworth, N. (1999) Potential for integrated control of horehound *Marrubium vulgare* L. by the plume moth *Wheeleria spilodactylus* (Curtis) and 2,4-D. Biocontrol Science and Technology 9, 355-364.

Allee, W. C. (1931) Animal Aggregations: A Study in General Sociology. University of Chicago Press, Chicago.

Annadurai, R. S. (1989) Reproductive potential in terms of quantitative food utilisation of *Zygogramma bicolorata* (Coleoptera: Chrysomelidae) on *Parthenium hysterophorous* (Asteraceae). Proceedings of the 7th International Symposium on Biological Control of Weeds, Rome, Italy, 1988.

Barbehenn, R. V., Reese, J. C. and Hagen, K. S. (1984) The food of insects. In Ecological Entomology. Edited by C.B. Huffaker and A.P. Gutierrez. John Wiley and Sons. Second edition, 83 - 121.

Barlow, N. D., Goldson, S. L. and McNeill, M. R. (1994) A prospective model for the phenology of *Microctonus hyperodae,* a potential biological control agent of argentine stem weevil in New Zealand. Biocontrol Science and Technology 4, 375-386.

Beirne, B. P. (1975) Biological control attempts by introductions against pest insects in the field in Canada. Canadian Entomologist 107, 225-236.

Beirne, B. P. (1985) Avoidable obstacles to colonisation in classical biological control of insects. Canadian Journal of Zoology 63, 743-747.

Bernays, E. A. (1985) Arthropods for weed control in IPM systems. In Biological Control in Agricultural IPM Systems. Edited by M. A. Hoy and D. C. Herzog. Academic press, 373-391.

Boston, T. and Stockwell, D. R. B. (1994) Interactive species reporting, mapping and modeling using the world wide web. Second International WWW Conference 94: Mosaic and the Web.

Burdon, J. J., Marshal, D. R. and Groves, R. H. (1980) Aspects of weed biology important to biological control. Proc. 5th Int. Symp. Biol. Control Weeds, Brisbane, Australia, 1980, 21-29.

Bush, G. L. and Hoy, M. A. (1984) Evolutionary processes in insects. In, Ecological Entomology. Edited by Huffaker and Rabb. J. Wiley and Sons inc, 247-278.

Bustillo, A. E. and Drooz, A. T. (1977) Cooperative establishment of a Virginia (USA) strain of *Telenomus alsophilae* on *Oxydia trychiata* in Columbia. Journal Economic Entomology 70, 767-770.

Callan, E. M. (1969) Ecology and insect colonisation for biological control. Proceedings of the Ecological Society of Australia 4, 17-31.

Caltagirone, L. E. (1985) Identifying and discriminating among biotypes of parasites and predators. In Biological Control in Agricultural IPM Systems. Edited by M.A. Hoy and D.C. Herzog. Academic press, 198-200.

Cameron, P. J., Hill, R. L., Bain, J. and Thomas, W. P. (1993) Analysis of importations for biological control of insect pests and weeds in New Zealand. Biocontrol Science and Technology 3, 387-404.

Carson, H. L. (1990) Increased genetic variation after a population bottleneck. Trends in Ecological Evolution 5, 228-230.

Carter, R. J. (1990) Biology and control of horehound, *Marrubium vulgare.* Proceedings of the 9th Australian Weeds Conference, Adelaide, South Australia 1990, 382-386.

Cates, H. C. (1980) Feeding patterns of monophagous, oligophagous and polyphagous insect herbivors: The effect of resourse abundence and plant chemistry. Oecologia 46, 22-31.

Center, T.D. and Durden, W.C. (1981) Release and establishment of *Sameodes aldiguttalis* for the biological control of waterhyacinth. Environmental Entomology 10, 75-80.

Chaboudez, P. and Sheppard, A. W. (1995) Are particular weeds more amenable to biological control? - a reanalysis of mode of reproduction and life history. Proceedings of the 8th International Symposium on Biological Control of Weeds, Canterbury, New Zealand, 1992.

Clarke, C. R., Baker, J., Keller, M. A. and Roush, R. T. (2000) Biological control of horehound: lessons from South Australia. Plant Protection Quarterly 15, 29-32.

Clausen, C. P. (1951) The time factor in biological control. Journal of Economic Entomology 44, 1-9.

Crawley, M. J. (1986) The population biology of invaders. Philosophical Transactions of the Royal Society London. Series B 314, 711-731.

Crawley, M. J. (1989) Plant life history and the success of weed biological control projects. Proceedings of the 7th International Symposium on the Biological Control of Weeds, Rome, Italy.

Crawley, M. J. (1989a) The successes and failures of weed biocontrol using insects. Biocontrol News and Information 10, 213-223.

Cruttwell McFadyen, R. E. (1991) Climate modelling and the biological control of weeds:- one view. Plant Protection Quarterly 6, 14-15.

Cullen, J. M. (1980) Considerations in rearing *Bradyrrhoa gilveolella* for control of *Chondrilla juncea* in Australia. Proceedings of the 5th Symposium on Biological Control of Weeds. Brisbane, Australia, 233-239.

Cullen, J. M. (1989) Current problems in host-specificity screening. Proceedings of the 7th International Symposium on the Biocontrol of Weeds. Rome, Italy, 27-35.

Cullen, J. M. (1995) Predicting effectiveness: fact and fantasy. Proceedings of the 8th International Symposium on the Biological Control of Weeds. Canterbury, New Zealand. Edited by E. S. Delfosse and R. R. Scott. DSIR/CSIRO, Melbourne 103-109.

DeBach, P. (1964) The scope of biological control. In: DeBach, P. (ed) Biological control of insect pests and weeds. London; Chapman and Hall, 844.

DeBach, P. (1974) Biological Control by Natural Enemies. Cambridge University Press.

Dennis, B. (1989) Allee effects: population growth, critical density, and the chance of extinction. Natural Resource Modeling 3, 1-58.

Diehl, S.R. and Bush, G.L. (1984) An evolutionary and applied perspective of insect biotypes. Annual Review of Entomology 29, 471-504.

Dingle, H. (1978) Migration and diapause in tropical, temperate and island milkweed bugs. In Evolution of Insect Migration and Diapause. Edited by Dingle, H.. Springer-Verlag. 254-276.

Dobzhansky, T. and Ayala, F. J. (1973) Temporal frequency changes of enzyme and chromosomal polymorphisms in natural populations of *Drosophila*. Proc. Nat. Acad. Sci. USA 70, 680-683.

Dodkin, M. J. and Gilmore, A. M. (1984) Species and ecosystems at risk - a preliminary review. Proceedings of the Conference on *Chrysanthemoides monilifera,* Port Macquarie, Australia, 33-52.

Downing and Moilliet (1967) Relative densities of predacious and phytophagous mites on three varieties of apple trees. Canadian Entomology 99, 738-41.

Dymock, J. J. (1987) Population changes of the seedfly, *Pegohylemyia jacobaeae*, introduced for biological control of ragwort. New Zealand Journal of Zoology 14, 337-342.

Eccleston, J. A. (1996) An investigation into the role of adaptation in the establishment of *Brachymeria intermedia* (Hymenoptera: Chalcididae) in the United States. Thesis for Doctor of Philosophy, Cornell University.

Edwards, O. R. and Hoy, M. A. (1995) Random amplified polymorphic DNA markers to monitor laboratory-selected, pesticide-resistant *Trioxys pallidus* after release into three California walnut orchards. Environmental Entomology 24, 487-496.

Edwards, P. B., Holtkamp, R. H. and Adair, R. J. (1999). Establishment and rapid spread of the bitou seed fly, Mesoclanis polana Muro (Diptera: Tephritidae), in eastern Australia. Australian Journal of Entomology. 38, 148 - 150.

Falconer, D. S. and MacKay, T. F. C. (1996) Introduction to quantative genetics. 4th edition. Longman.

Flint, M. L. (1980) Climatic ecotypes in *Trioxys complanatus,* a parasite of the spotted alfalfa aphid. Enviromental Entomology 9, 501-507.

Forcella, F. (1985) Weed Research 25, 181-191.

Frankham, R. (1995) Conservation Genetics. Annual Review of Genetics 29, 305-327.

French, R. J. (1960) South Australian Journal Agriculture 63, 533-537.

Garcia, C. and Frick, K. (1975) *Bactra verutana*, a possible biological control agent of purple and yellow nutsedge: large scale rearing on artifitial diet. Annals of the Entomological Society of America 76, 837-48.

Geden, C. J., Smith, L., Long, S. J. and Rutz, D. A. (1992) Rapid deterioration of searching behaviour, host destruction and fecundity of the parasitoid *Muscidifurax raptor* in culture. Annals of the Entomological Society of America 85, 179-187.

Goeden, R. D. (1983) Critique and revision of Harris' system for selection of insect agents in biological control of weeds. Protection Ecology 5, 287-301.

Greathead, D. J. (1997) An introduction to the FAO code of conduct for the import and release of exotic biological control agents. Biocontrol News and Information 18, 117-124.

Huffaker, C. B. and Messenger, P. S. (1976) Theory and practice of biological control. Academic Press.

Hagen, K. S., Dadd, R. H. and Reese, J. (1984) The food of insects. In Ecological Entomology. Edited by C.B. Huffaker and R.L. Rabb, John Wiley and Sons, 79-112.

Hall, R. W. and Ehler, L. E. (1979) Rate of establishment of natural enemies in classical biological control. Bulletin of the Entomological Society of America 25, 280-282.

Harley, K. L. S. and Forno, I. W. (1992) Biological control of weeds, a handbook for practitioners and students. Inkata Press.

Harris, P. (1973) The selection of effective agents for the biological control of weeds. Canadian Entomology 105, 1495-1503.

Heinrich, B. (1981) Insect Thermoregulation. John Wiley and Sons.

Hevert, F. (1985) Water and salt relations. In Environmental Physiology and Biochemistry of Insects. Edited by K. H. Hoffman. Springer-Verlag.

Hill, R. L., Gourlay, A. H. and Martin, L. (1991) Seasonal and geographic variation in the predation of gorse seed, *Ulex europaeus* L., by the seed weevil *Apion ulicis* Frost. New Zealand Journal of Zoology 18, 37-43.

Hoffman, J. H. (1980) Release of *Tucumania tapiacola* in Sth Africa against *Opuntia aurantiaca*: the value of detailed monitoring. Proceedings of the 5th symposium of Biological Control of Weeds, Brisbane, Australia, 1980, 367-373.

Hoffman, K. H. (1985) Enviromental Physiology and Biochemistry of Insects. Springer-Verlag.

Hopper, R. K. and Roush, R. T. (1993) Mate finding, dispersal, number released and the success of biological control introductions. Ecological entomology 18, 321-331.

Hopper, K. R., Roush, R. T. and Powell, W. (1993) Management of genetics of biological control introductions. Annu. Rev. Entomology 38, 27-51.

House (1967) The decreasing occurrence of diapause in the fly *Pseudosarcophaga affinis* through laboratory reared generations. Canadian Journal of Zoology 45, 149-153.

Howden, S.M. (1985) The potential distribution of bitou bush in Australia. Proceedings of a conference on *Chrysanthemoides monilifera*, 1985, 53-64.

Hoy, M. A. (1978) Variability in diapause attributes of insects and mites: some evolutionary and practical implications. In Evolution of Insect Migration and Diapause. Edited by H. Dingle. Springer-Verlag, 101-126.

Hoy, M. A. (1985) Improving establishment of arthropod natural enemies. In Biological Control in Agricultural IPM Systems. Edited by M. A. Hoy and D. C. Herzog, Academic press.

Hoy, M. A. (1990) Genetic improvement of arthropod natural enemies: becoming a conventional tactic? In New Directions in Biological Control: Alternatives for Suppressing Agricultural Pests and Diseases. Alan R. Liss, inc., 405-417.

Hoy, M. A. (1994) Insect Molecular Genetics. An introduction to principles and applications. Academic Press, San Diego, California.

Hurst, L. D. and Pomiankowski, A. (1991). Causes of sex ratio bias may account for unisexual sterility in hybrids: a new explanation of Haldane's rule and related phenomena. Genetics 128, 841-858.

Huffaker, C. B., Simmonds, F. J. and Laing, J. E. (1976). The theoretical and emperical basis of biological control. In Theory and Practice of Biological Control, edited by C. B. Huffaker and P. S. Messenger, Academic Press, 41-78.

Jessop, J. P. and Toelken, H. R. (1986) Flora of South Australia, part 3. Woolman, Adelaide, South Australia, page 1200.

Julien, M. H. (1980) A discussion of the limited establishment of *Perapion antiquum* and a review of the current status of biological control of *Emex* spp. in Australia. Proceedings of the 5th Symposium on Biological Control of Weeds. Brisbane, Australia, 507-514.

Julien, M. H. (1989) Biological control of weeds worldwide: trends, rates of success and the future. Biocontrol News and Information 10(4), 299-306.

Julien, M. H. (1992) Biological control of weeds. A world catalogue of agents and their target weeds. CAB international.

Julien, M. H., Kerr, J. D. and Chan, R. R. (1984) Biological control of weeds: an evaluation. Protection Ecology 7, 3-25.

Kestler, P. (1985) Respiration and water loss. In Environmental Physiology and Biochemistry of Insects. Edited by K. H. Hoffman. Springer-Verlag.

Kidwell, M. G., Kidwell, J. F. and Sved, J. A. (1977) Hybrid dysgenesis in *Drosophila melanogaster*: a syndrome of aberrant traits including mutation, sterility and male recombination. Genetics 86, 813-833.

Kuniata, L. S. (1994) Importation and establishment of *Heteropsylla spinulosa* for the biological control of *Mimosa invisa* in Papua New Guinea. International Journal Pest Management. 40, 64-65.

Lane, D. and Shaw, K. (1978) The role of fire in boneseed (*Chrysanthemoides monilifera* (L.) Norlindh) control in bushland. Proceedings of the 1st Conference of the Council of Australian Weed Science Societies.

Lane, P. W. and Payne, R. W. (1996) Genstat for windows: an introductory course. Oxford University Press, New York.

Leburg, P. L., 1992 Effects of population bottlenecks on genetic diversity as measured by allozyme electrophoresis. Evolution 46, 477-494.

Leen, R. (1991) Climatic associations and establishment of biological control of weed insects. Proceedings of the Syposium on Exotic Pest Plants. Miami, Florida (1988), 189-195.

Legner, E. F. (1971) Observations on hybridization and heterosis in parasitoids of synpanthropic flies. Annals of the Entomological Society of America 65, 254-263.

Legner, E. F. (1979) Prolonged culture and inbreeding effects on reproductive rates of two pteromalid parasites of muscoid flies. Annals of the Entomological Society of America 72, 114-118.

Leyson, M. (1999) Courtship and mating of the horehound plume moth, *Wheeleria spilodactylus*. Honours Thesis, Department of Applied and Molecular Ecology, University of Adelaide.

Lumme, J. (1978) Phenology and photoperiodic diapause in northern populations of *Drosophila*. In, Evolution of Insect Migration and Diapause. Edited by H. Dingle. Springer-Verlag, 145-170.

Mackauer, M. (1976) Genetic problems in the production of biological control agents. Annual Review of Entomology 21, 369-385.

Mackauer, M. (1980) Some aspects of quality and quality control of biological control agents during insectary propagation. Proc. 5th Int. Symp. Biol. Contr. Weeds, Brisbane, Australia. 1980, 207-220.

Maddox, D. M. and McCready, R. M. (1975) Preliminary studies of the effects of an induced phosphate deficiency on carbohydrate-nitrogen ratios in *Alternanthera philoxeroides* and on the feeding of *Agasicles hygrophila*. Entomophaga 20, 409-419.

Mattson, W. J. (1980) Herbivory in relation to plant nitrogen content. Annual Review of Ecology and Systematics 11, 119-161.

Mayr, E. (1963). Animal Species and Evolution. Harvard University Press, Cambridge.

McFadyen, R. E. (1985) Larval characteristics of *Cactoblastis* spp. and the selection of species for biological control of prickly pears. Bulletin of Entomological Research 75, 159-168.

Meggs, J.M. (1995) Biotic resistance to a Chrysomelid beetle. Honours thesis, Monash University.

Messenger, P. S. and Van Den Bosch, R. (1969) The adabtability of introduced biological control agents. In Biolgical Control. Edited by C. B. Huffaker. Plenum Publishing Corporation, New York 68-92.

Myers, J. H. and Sabath, M. D. (1980) Genetic and phenotypic variability, genetic variance and the success of establishment of insect introductions for the biological control of weeds. Proceedings of the 5th Symposium on Biological Control of Weeds, Brisbane, Australia, 91-102.

Nealis, V. (1985) Diapause and the seasonal ecology of the introduced parasite, *Cotesia* (*Apanteles*) *rubecula* (Hymenoptera: Braconidae). Canadian Entomology 117, 333-342.

Nei, M., Maruyama, T. and Chakraborty, R. (1975) The bottleneck effect and genetic variability in populations. Evolution 29, 1-10.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89, 583-590.

Nix, H. A. (1986) A biogeographical analysis of Australian Elapid snakes. Atlas of Australian Elapid snakes. Australian Flora and Fauna Series. Edited by R. Longmore, 8, 4-15.

Okubo, A. (1988) Diffusion type models for avian range expansion. In Acta XIX Congress Internationals Ornithologici Volume 1. National museum of Natural Sciences. University of Ottawa Press, 1038-1049.

Parsons, W.T. and Cuthbertson, E.G. (1992) Noxious weeds of Australia. Inkarta Press, 496-499.

Peschken, D. K. (1972) *Chrysolina quadrigemina* introduced from California to British Columbia against the weed *Hypericum perforatum:* comparison of behaviour, physiology and colour in association with post-colonisation adaptation. Canadian Entomology 104, 1689-1698.

Pheloung, P.C. (1996) CLIMATE: a system to predict the distribution of an organism based on climate preferences, Agriculture Western Australia.

Precht, H. (1973) Temperature and life. Springer-Verlag.

Price, P. W. (1975) Insect Ecology. J. Wiley and Sons.

Raymond, M. and Rousset, F. (1995) Genpop (version 1.2). A population genetics software for exact test and ecumenicism. Journal of Heredity 86, 248-249.

Remington (1968) The population genetics of insect introductions. Annual Review of Entomology 13, 415-426.

Richardson, B.J., Baverstock, P.R. and Adams, M. (1986). Allozyme Electrophoresis; a handbook for animal systematics and population studies. Academic Press, Sydney.

Robinson, R. (1971) Lepidoptera Genetics. Pergamon Press.

Room, P. M. and Thomas, P. A. (1985) Nitrogen and establishment of a beetle for biological control of the floating weed *Salvinia* in Papua New Guinea. Journal of Applied Ecology 22, 139-156.

Rosen, D. and DeBach, P. (1973) Systematics, morphology and biological control. Entomophaga 18 (3), 215-222.

Roush, R. T. and Hoy, M. A. (1981) Genetic improvement of *Metaseiulus occidentalis*: selection with methomyl, dimethoate and carbaryl and genetic analysis of carbaryl resistance. Journal of Economic Entomology 74, 138-141.

Roush, R. T. (1986) Inbreeding depression and laboratory adaptation in *Heliothis virescens* (Lepidoptera: Noctuidae). Annals of the Entomological Society of America 79, 583-587.

Roush, R. T. (1990) Genetic variation in natural enemies: critical issues for colonisation in biological control. In Critical Issues in Biological Control. Edited by M. Mackauer, L.E. Ehler, and J. Roland, Intercept, 263-288.

Roush, R. T. and Hopper, K. R. (1995) Use of family lines to preserve genetic variation in laboratory colonies. Annals of the Entomological Society of America 88, 713-717.

Schauff, M. E. and LaSalle, L. (1998) The relevance of systematics to biological control: protecting the investment in research. Procceedings 6th Australasian Applied Entomological Research Conference. The University of Queensland, Brisbane, Australia, 425-436.

Schoonhoven, L. M., Jermy, T. and van Loon, J. J. A. (1998) Insect - plant biology from physiology to evolution. Chapman and Hall. First edition.

Scott, J. K. (1992) Biology and climatic requirements of *Perapion antiquum* in southern Africa: implications for the biological control of *Emex* spp. in Australia. Bulletin of Entomological Research 82, 399-406.

Scott, J. K. and Adair, R. J. (1992) Selection of biological control agents for *Chrysanthemoides monilifera*, a weed of protected areas in Australia. Proceedings of the 8th Symposium on the Biological Control of Weeds, Canterbury, New Zealand. Edited by Delfosse and Scott, 1995, 61-67.

Shea, K. and Possingham, H. P. (2000) Optimal release strategies for biological control agents: an application of stochastic dynamic programming to population management. Journal of Applied Ecology 37, 77-86.

Simmonds, F. J., Franz, J. M. and Sailer, R. I. (1976) History of biological control. In Theory and Practice of Biological Control. Edited by Huffaker and Messenger. Academic Press.

Simmons, D.M. and Flint, P.W. (1986) Variation in *Chrysanthemoides monilifera* in Eastern Australia. Weed Research 26, 427-432.

Sloan, Cook and King Pty Ltd (1988) The economic impact of pasture weeds, pests and diseases on the Australian wool industry. Australian Wool Corporation, Melbourne.

Snedecor, G. W. and Cochran, W. G. (1980) Statistical Methods. 7th edition. Ames, Iowa. Iowa State University.

Spencer, N. R. (1995) Biological weed control: the plant-insect interaction. Proceedings of the 8th International Symposium on Biological Control of Weeds, Canterbury, New Zealand, 1992.

Strickberger, M. W. (1995) Evolution. Second Edition. Jones and Bartlett Publishers.

Stritzke, J. F. (1975) Germination characteristics and chemical control of horehound. Journal of Range Management 28, 225-226.

Sutherst, R. W. and Maywald, G. F. (1985) A computerised system for matching climates in ecology. Agriculture, Ecosystems and Environment 13, 281-299.

Tauber, M. J. and Tauber, C. A. (1978) Evolution of phenological strategies in insects: a comparative approach with eco-physiological and genetic considerations. In Evolution of Insect Migration and Diapause. Edited by H. Dingle. Springer-Verlag, 53-71.

Tauber, M. J., Tauber, C. A. and Masaki, S. (1984) Adaptions to hazardous seasonal conditions: dormancy, migration and polyphenism. In Ecological Entomology. Edited by Huffaker and Rabb. John Wiley and Sons, 149-183.

Thomas, M. B. and Willis, A. J. (1998) Biocontrol - risky but necessary? TREE 13, 325-329.

Tisdell, C. A. (1990) Economic impact of biological control of weeds and insects. In Critical Issues in Biological Control. Edited by M. Mackauer; L. E. Ehler and J. Roland. Intercept, 301-306.

Unruh, T. R. and Goeden, R. D. (1987) Electrophoresis helps to identify which race of weevil, *Rhinocyllus conicus,* has transferred to two native southern California thistles. Environmental Entomology 16, 979-983.

Unruh, T.R., White, W., Gonzalez, D., Gordh, G. and Luck, R.F. (1983) Heterozygosity and effective population size in laboratory populations of *Aphidius ervi*. Entomophaga 28, 245-258.

Van den Bosch, R. (1971) Biological control of insects. Ann. Review of Ecology and Systematics 2, 45-66.

Van den Bosch, R., Hom, R., Matteson, P., Frazer, B.D., Messenger, P.S. and Davis, C.S. (1978) Biological control of the walnut aphid in California: Impact of the paracite, *Trioxys pallidus*. Hilgardia. 47(1), 1-13.

Van Driesche, R. G. (1993) Methods for the field colonisation of New Biological control agents. In Steps in Classical Arthropod Biological Control. Edited by R. G. Van Driesche and T. S. Bellows. Lanham, Entomological Society of America.

Vargas, R. I., Walsh, W. A., Jang, E. B., Armstrong, J. W. and Kanehisa, D. T. (1996) Survival and development of immature stages of four Hawaiian fruit flies reared at five constant temperatures. Annals of the Entomological Society of America 89, 64-69.

Walker, P. A. and Cocks, K. D. (1991) HABITAT: A procedure for modeling the disjoint environment envelope for a plant or animal species. Global Ecology and Biogeography Letters 1, 108-18.

Weiss, J. (1996) The biological control of horehound in southern Australia. Progress report to the South Australian Animal and Plant Control Commission. Unpublished.

Weiss, J. and Lippai, K. (1996) The biology and host specificity of the horehound plume moth, *Pterophorous spilodactylus;* a biological control agent for horehound (*Marrubium vulgare*). Unpublished.

Weiss, J. and Sagliocco, J. (1994) Horehound, *Marrubium vulgare* - a global perspective. Presentation to the South Australian Animal and Plant Control Conference, 1994.

Weiss, P. W. (1984) Seed characteristics and regeneration of some species in invaded coastal communities. Australian Journal of Ecology 9, 99-106.

Weiss, P. W. (1986) The biology of Australian weeds 14. *Chrysanthemoides monilifera.* Journal of the Australian Institute of Agricultural Science 52, 127-134.

Weiss, P. W. (1993) Ecology of *Chrysanthemiodes monilifera* in relation to control. Proceedings of a National Workshop on *Chrysanthemoides monilifera*. Port Macquarie, April 1993.

Weiss, P. W. (1995) Distribution of *Chrysanthemoides* in Australia and South Africa. Bitou Bush and Boneseed Bulletin, Number 7, August 1995.

Welham, S. J. and Thompson R. (1997) Likelihood ratio tests for fixed model terms using residual maximum likelihood. Journal of the Royal Statistical Society 59, 701-714.

Wellington, W. G. (1965) Some maternal influences on progeny quality in the western tent caterpillar, *Malacosoma pluviale* (Dyar). Canadian Entomologist 97, 1-14.

Welsh, A. H., Cunningham, C. F., Donnelly, C. F. and Lindenmayer, D. B. (1996) Modelling the abundance of rare species: statistical models for counts with extra zeros. Ecological modelling 88, 297-308.

White, E. B., DeBach, P. and Garber, M. J. (1969) Artificial selection for genetic adaptation to temperature extremes in *Aphytis lingnanensis* compere. Hilgardia 40, 161-192.

White, I. M. and Elson-Harris, M. M. (1992) Fruit flys of economic significance: their identification and bionomics. CAB, Oxon, UK.

Winston, P. W. and Bates, D. H. (1960) Saturated solutions for the control of humidity in biological research. Ecology 41, 232-237.

Wright, S. (1931) Evolution in Mendelian populations. Genetics 28, 114-138.

Young, J. A. and Evans, R. A. (1986) Germination of white horehound (*Marrubium vulgare*) seeds. Weed Science 34, 266-270.