Prediction of safety and effectiveness of a candidate biocontrol agent: quarantine evaluation of the root-feeding, Mexican flea beetle, *Longitarsus bethae*, for potential release against the noxious weed, *Lantana camara*, in Africa

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FRONTISPIECE

Longitarsus bethae **adult, eggs, larvae and pupae, with its host plant,** ***Lantana camara.***

DECLARATION

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ABSTRACT

A root-feeding, Mexican flea beetle, *Longitarsus bethae* Savini & Escalona 2005 (Coleoptera: Chrysomelidae: Alticinae), was evaluated in quarantine as a candidate biological control agent for the noxious weed, *Lantana camara* L. (Verbenaceae). The premise was that *L. bethae* would only be released if it could be ascertained that it was: (i) safe for non-target plants; (ii) likely to inflict significant damage on the target weed; and (iii) capable of surviving under the various (abiotic and biotic) environmental conditions in its new range.

In order to determine the safety of *L. bethae* as a biocontrol agent, host-specificity tests were carried out in a quarantine laboratory. Host-specificity studies indicated that *L. bethae* was adequately host-specific to be released against *L. camara* in South Africa (i.e. the biocontrol agent will attack the target weed without harmful side-effects on non-target plant species). To substantiate that *L. bethae* has the potential to suppress the target weed, the effects of egg density on leaf density, stem length, stem diameter, and below- and above-ground biomass accumulation of *L. camara* plants were measured. These studies demonstrated that initial egg densities beyond 200 per plant could inflict sufficient damage upon the roots to reduce plant growth vigour.

The abiotic factors that were likely to influence the beetle's reproductive performance and survival in the new range included soil characteristics (moisture content, texture, surface structure, levels of organic matter and presence of leaf litter) and climatic conditions (photoperiod, temperature and humidity). Laboratory studies showed that oviposition rates and survival of immature stages were found to be directly proportional to the clay content of the soil. Oviposition was higher on soils with

surface cracks than on soils with no surface cracks. Oviposition was lower in soils with elevated levels of organic matter than in soils with low organic matter content. Soil moisture had no influence on oviposition, but proportionately more adults emerged when larvae were confined in moderately moist soils than in very wet or very dry soils. The presence of leaf litter on the soil surface did not influence oviposition, but the percentage of eggs that became adults was higher in leaf litter-covered than in bare soil.

Of the climatic conditions, atmospheric humidity had no affect on the rate of oviposition but the proportion of eggs that hatched was highest at elevated humidity levels (>85% RH). Egg survival was higher at cooler (17°C) than at warmer (27°C) temperatures. Temperature and humidity regimes in the summer rainfall regions of South Africa where *L. camara* is a problem are anticipated to be well within the ranges that are tolerated by *L. bethae.*

Three biotic factors that could influence the survival and establishment of *L. bethae* in its new range were investigated, namely: sharing the host plant with another well-established agent (*Teleonemia scrupulosa,* Tingidae); predation on eggs and pupae by generalist, soil-dwelling, arthropod predators; and natural resistance of different varieties of *L. camara* to the beetles. The study showed that *L. bethae* females laid fewer eggs, and that larval survival was lower on *L. camara* plants that were already being utilised by large numbers of *T. scrupulosa*. Although the vast majority of potential predators of *L. bethae* were ants, significant correlation was found between relative density estimates of carabid beetles and predation upon *L. bethae* pupae,

particularly in leaf litter-covered treatments. Some varieties of *L. camara* inhibited oviposition by *L. bethae* and larval survival differed significantly among the varieties.

After identifying factors in isolation that affected *L. bethae*, a multi-factorial trial was carried out to examine how the various factors might act in unison, rather than in isolation, in order to predict the relative survival of *L. bethae* to adulthood under different conditions. Soil moisture and clay content had the most substantial effect on survival of *L. bethae* while the presence of *T. scrupulosa*, and the type of lantana variety serving as a host, had minimal influence on the beetles. The study also showed that the effects of some of the ecological factors, which were apparently important in isolation, were moderated when they operated in unison with other factors. Based on the results of the multi-factorial investigation, a survival-prediction equation was derived. This was used to identify three geographic regions which are likely to be either suitable, marginally-suitable or unsuitable for *L. bethae* in South Africa.

The overall evaluation is that *L. bethae* is safe to be released in South Africa, and it is predicted that, for the most part, the beetles are likely to cope with environmental and ecological conditions in the regions invaded by *L. camara*, and to inflict sufficient damage upon the target weed to markedly suppress its growth vigour. The knowledge gained from this investigation will also assist in the selection of release sites that are most likely to suit *L. bethae*, and thus ensure that mass-reared beetles are utilized most efficiently for biological control of the target weed.

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CHAPTER 1

General introduction

There is general agreement that the greatest challenge to increasing weed biological control success is improving techniques for selection of candidate agents (Wapshere, 1974; Myers, 1985; Cullen, 1989; Marohasy; 1998; McEvoy & Coombs, 2000). Although significant advances have been made in the methodology of host-specificity testing procedures (Sheppard, 2002; Briese, 2002), selection of effective biological control agents remains one of the least science-based activities in weed biological control programmes (McFadyen, 1998). The host-specificity procedure currently employed in biological control systems attempts to predict which host plants are likely to be attacked in the release environment. This procedure, however, neglects to screen control agents efficiently for effectiveness in the exotic range. Increasing concern about possible non-target effects of weed biological control (Greathead, 1995; Simberloff, 1996; McEvoy, 1996; Louda *et al.*, 1997; McEvoy & Coombs, 2000; Pemberton, 2000) requires that the total number of biocontrol agents utilised should be minimized, with the aim of reducing the risk to non-target plant species while maximizing the likelihood of effective weed control (McFadyen, 1998; McFadyen, 2002).

Biological control systems have been compared to a lottery in which the outcome of control organism introductions is so dominated by chance that the best course is to introduce as many control organisms as possible, leaving them to sort out which ones or combinations will prove most effective (Myers, 1985; McEvoy & Coombs, 2000).

For example, out of 18 insect agents intentionally released against lantana in South Africa during the past four decades, 12 have established (Baars & Neser, 1999; Simelane, 2002a), and none of these appears to have achieved consistent and widespread control of the weed. Given the public concern about risk to non-target species, the onus is on biocontrol scientists to channel their limited resources into agents that are not only adequately host-specific, but are also likely to establish and provide effective control of the target weed. Pre-release studies should therefore be expanded to show beyond reasonable doubt that the agent will be safe, establish, by virtue of having the ability to cope with ecological conditions in the release areas, and inflict significant damage on the weed.

Whilst it is generally accepted that post-release performance of an agent is difficult to predict with any degree of certainty, the influence of ecological factors should not be completely overlooked during the pre-release studies, as these factors ultimately determine the ecological range of an agent in the region to which it is introduced. For example, the *Aphthona* spp. root beetles that are being used to control spurge in North America are restricted to warm, open sites on dry, coarse soils with a steppe climate (Harris & McEvoy, 1995). Spurge growing in wet, vegetated depressions or in light shade is not attacked by the beetles (Harris & McEvoy, 1995). The cinnabar moth, *Tyria jacobaeae* L., is less common and more intermittent in shaded sites and its pupae are unable to survive in moist sites (Dempster, 1971). The herringbone leafminer, *Ophiomyia camarae* Spencer, released against lantana in South Africa, failed to establish in high altitude areas and is mainly confined to areas along the east coastal regions of the country (Simelane & Phenye, 2003; Simelane & Phenye, 2005). In the five years after its release into South Africa, the lantana mirid's (*Falconia*

intermedia Distant) rate of establishment and dispersal has been very low, despite the enormous amount of resources put into mass-rearing and distribution of the agent (Heshula, 2005). Therefore, an agent that fails to establish at all, or only does so in a very limited area, is not only posing unjustifiable risk to non-target plant species but is also a waste of resources.

Although over 40 insect agents have been released against lantana in 29 countries during the past century, lantana is frequently rated as the most unsuccessful target of biocontrol (Broughton, 2000). Taxonomically, lantana is a complex of many varieties, whose diversity has frustrated attempts at biocontrol by insects that show preferences for some varieties and lower ability to survive on others (Cilliers, 1983; Cilliers & Neser, 1991; Palmer & Pullen, 1995; Baars & Neser, 1999; Day & Neser, 2000; Broughton, 2000). The established agents in South Africa are mainly leaf-, fruit- or flower-feeding species whose populations are prone to drastic declines during autumn or winter when plant leaves abscise due to frost and drought (Harley *et al.*, 1979; Cilliers & Neser, 1991; Baars & Neser, 1999; Simelane & Phenye, 2003). In addition to these above-ground ecological constraints, below-ground factors, such as soil characteristics, could influence the efficacy of soil-dwelling biocontrol agents. Therefore, the development of techniques that can be used to identify ecological factors that might influence an agent's efficacy, and determine the extent of this influence on the agent, could improve our ability to predict the likely success or failure of biocontrol agents prior to their release.

The various approaches that are sometimes used to determine the potential efficacy of candidate biocontrol agents are usually generalizations which are not necessarily

applicable to specific biological control projects (Sheppard, 2002). The two basic approaches, namely: the identification of important and researchable attributes of the insect's biology (predicted from its taxonomy) (Harris, 1973; Sheppard, 2002), or eco-climatic matching between the insect's area of origin and the intended area of introduction (Wapshere, 1985; Sutherst & Maywald, 1999; Sheppard, 2002), are applied alone or in combination when assessing the agent's potential for suppressing the weed. Both taxonomic and biological attributes associated with success of biocontrol agents have many short comings, and are yet to be understood fully. Biological attributes (e.g. rate of population increase, multiple generations per year, etc.) are contained in the 12-point scoring system advocated by Harris (1973). Harris' scoring system does not only overlook the importance of the ecology of the agent, but is also biased against the initial selection of little-known species that might in fact be the most effective natural enemies (Goeden, 1983). The generalization that certain taxonomic groups of insects (e.g. cochineal insects, weevils, leaf beetles, gall flies and rusts) have a better track record as effective biocontrol agents is least science-based, and is characterised by a high number of exceptions. CLIMEX (Sutherst & Maywald, 1999) is a widely used tool for modelling the potential distribution of an organism in its country of introduction by inferring the new geographical range based on eco-climatic characteristics of locality records from the native range. However, certain weeds (e.g. lantana) grow under a diverse range of climates in their introduced areas, thereby limiting the effectiveness of many potential agents selected on this basis. Because of variation in the magnitude and complexity of ecological factors in the exotic range, the above approaches are not reliable predictors of the efficacy of candidate biocontrol agents in their exotic range. McEvoy & Coombs (2000) concluded that biocontrol workers usually have very limited knowledge of factors that

are likely to limit the effectiveness of control organisms, and that much of the knowledge is subjective.

The root-feeding flea beetle, *Longitarsus bethae* Savini & Escalona 2005 (Chrysomelidae: Alticinae), was collected from Mexico and selected as a potential biocontrol agent for *L. camara* in South Africa because of its presumed ability to damage the root system of its host, a niche currently not exploited by any of the previously introduced lantana biocontrol agents anywhere in the world. Rhizophagy was presumed on the basis of tentative identification to the genus *Longitarsus*. Potential efficacy was inferred from the knowledge that *Longitarsus jacobaeae* (Waterhouse) achieved excellent control of ragwort (*Senecio jacobaea* L.) in western USA (Hawkes & Johnson, 1978; Pemberton & Turner, 1990; McEvoy *et al.*, 1993).

Even if *L. bethae* proved to be adequately host-specific to *L. camara*, releasing it into the environment would be a risk not worth taking unless there were reasonable grounds that it would establish and become prolific in its new environment. The main purpose of this research programme was therefore three-fold: (i) to determine whether *L. bethae* is sufficiently host-specific to be released against lantana in Africa; (ii) to determine the potential impact of *L. bethae* on lantana; and (iii) to ascertain, and quantify the potential effect of ecological factors that could influence the abundance of *L. bethae*, so as to predict its relative efficacy in its new range. The premise was that *L. bethae* would only be released into South Africa if it could be ascertained that the beetle is capable of surviving under the ecological conditions in its new range, and likely to inflict significant damage on the target weed. Knowledge of the influence of ecological factors on the beetle would also help in the selection of suitable release

sites where *L. bethae* would be most likely to become established and have the greatest impact on the target weed.

CHAPTER 2

General materials and methods

2.1 *Origin and identity of* Longitarsus bethae:

Longitarsus bethae was collected as adults from the leaf canopy of several golden-orange, orangey-red and salmon-coloured horticultural selections of *Lantana camara* (known locally as Cinco Negritos) in the botanical garden (Jardin Borda) in Cuernavaca (18° 7' N, 99° 15' W), Morelos state, Mexico, in September and October 2000 (S. Neser & A.J Urban, Weeds Research Division, ARC-PPRI, personal communication). Taxonomic investigation of the beetle was arranged by Ms E. ('Beth') Grobbelaar of the Biosystematics Division, ARC-PPRI, and carried out by Prof. V. Savini and Mr H. Escalona of Museo del Instituto de Zoologia Agricola Francisco Fernandez Yepez, Facultad de Agronomia, Universidad Central de Venezuela, Maracay, Venezuela, culminating in its description as a new species (Savini and Escalona, 2005).

2.2 Quarantine glasshouse conditions

With the exception of the work reported in chapters 7 and 9, insect culturing and experimental studies reported in all the chapters were conducted in two quarantine glasshouse compartments under the conditions described here. The compartments had natural lighting supplemented from autumn (01 April) to spring (30 September) with quartz-halogen floodlighting for a photoperiod of 14:10 (L:D) h throughout the study period. Atmospheric humidity ranged between 60 and 90%RH. Temperature was

controlled by large air-conditioners and maintained at 28 ±2°C during the day and 22 ±2°C at night throughout the study.

2.3 Plant and insect cultures

Longitarsus bethae was reared on potted *Lantana camara* plants of variety 009 Light Pink. Unless indicated otherwise, variety 009 Light Pink was also used in all the trials because it is the most widespread and probably the most common in South Africa. Single plants propagated from shoot-tip cuttings were transplanted into large pots (10 *l*) and grown in a well-drained, rich medium of silty soil, sandy soil and compost mixed in a ratio of 2:1:1 by volume, respectively. Unless stated otherwise, this standard loamy soil was used in all experiments. Gauze cages (0.55 x 0.55 x 0.95 m) were used to confine 50 unsexed *L. bethae* adults with one plant during the oviposition period. Adults were allowed to feed and oviposit on each plant for up to 40 d before they were transferred to new ones. Plants were watered on daily basis to keep the soil moist, thereby preventing egg desiccation whilst facilitating larval mobility in the soil.

2.4 Measurement of soil moisture

Soil moisture in each pot was measured using a Hadeco soil moisture meter with readings ranging from 1 (very dry) to 8 units (very wet). The metal probe was planted vertically in each pot, with the tip 10 cm below the soil surface, and left in position throughout the course of the experiment. Through the use of this device, soil moisture regimes were maintained within 3-4, 5-6 and 7-8 units, representing low, moderate and high moisture levels, respectively. Meter readings were calibrated by taking soil

core samples from the surface to a depth of 10 cm, and oven-drying them at 100°C for 72 h to determine the percentage by mass of moisture in the soil (Table 2.1).

Table 2.1 Moisture content (% by mass) of the upper 10 cm of standard loamy soil (silty soil: sandy soil: compost :: 2:1:1 by volume) held at different moisture levels using the Hadeco soil moisture meter

	Soil moisture level		
	Low	Moderate	High
Meter reading	3-4	5-6	7-8
Moisture content (%)	11	16	20

2.5 Assessment of leaf damage caused by adult L. bethae

Evaluation of adult feeding damage was often done in conjunction with oviposition preference tests to determine leaf palatability of a plant, and thus the relative attractiveness of a plant exposed to a particular condition. Level of feeding damage was assessed using a 0-3 point scale (Simelane, 2005). The criteria of the ratings were: 0 = no visible feeding scars on the leaves; 1 = small punctures (exploratory feeding); 2 = small feeding holes (restrained feeding); 3 = large feeding holes (full feeding).

2.6 Collection of L. bethae *eggs*

Large quantities of eggs were required for various tests conducted in chapters 3 to 12. To concentrate oviposition, a group of approximately 400 newly emerged, unsexed *L. bethae* adults was enclosed in a gauze cage (0.55 x 0.55 x 0.95 m) with a single potted

plant grown in a large pot (10 *l*) for 8 d. The soil surface of each potted plant was first covered with a 3-cm-thick layer of sandy soil to facilitate egg recovery. Females started laying after a pre-oviposition period of about 5 d. From the 8th day onwards, eggs were extracted from the sand every 3 d using a sieve-floatation procedure (Southwood, 1978; Foster *et al.*, 1979). In this procedure, the sandy soil was poured off the surface of the potting soil, and was gently washed in the laboratory through a series of 4 stacked metal sieves (20 cm-diameter) of downwardly decreasing mesh size (1.00 mm, 0.4 mm, 0.2 mm and 0.1 mm). Eggs together with the soil matter of the same size were collected on the 0.2 mm sieve. The mixture of eggs and mineral matter collected in this sieve was thoroughly rinsed out into a flask and later passed through a filter funnel and the filtrate was checked for eggs under the microscope. Eggs were often stored on moist filter paper in closed Petri-dishes in cold storage (10-12°C) for not more 14 d to synchronize hatch in various experiments. Egg samples collected and maintained in the laboratory had a mean hatch of 58%.

2.7 Inoculation of plants with eggs

Eggs used for inoculating plants in various tests were collected from a group of cage-confined beetles as described in 2.5. In most cases, eggs were initially incubated on moist filter paper in closed Petri-dishes on the bench-top in a quarantine laboratory for 11 d at 25°C until the mandibulate stage, approximately 24 h before hatching. By means of a fine spray of water, the required number of eggs was washed into a horizontal slit in the soil, measuring 6 cm in length and 0.5 cm in depth. The slit was made by scraping a sharp knife across the soil surface near the base of the stem.

2.8 Extraction of immature stages from soil

When extracting immature stages of *L. bethae* from non-sandy soil, sieving alone was not sufficient because these life stages remained mixed with or obstructed by a mass of similar-sized mineral and organic matter. After the soil was gently washed through a series of 4 stacked sieves of decreasing mesh size (described in 2.5), soil or organic matter collected in all the sieves was transferred to a 500ml flask. The densities of mineral matter and biotic material are different so the immature stages were separated by floatation. A saturated solution of sodium chloride was added to the flask, mixed thoroughly and allowed to stand for 24 h. The resulting float was a mixture of all biotic material including eggs, larvae and pupae. Larvae and pupae, because of their white body colour, were easily identified and collected from the water surface using a fine brush. The mixture of eggs and organic material remaining in the float was decanted through a filter funnel and the filtrate was checked for eggs under microscope.

2.9 Extraction of larvae from roots

Some of the larvae were frequently not extracted by means of the soil washing and floatation technique, since they remained embedded inside the roots. To remove larvae inside the root cavities, the root crown and the roots were suspended in the air over a plastic basin half-filled with water under fluorescent lighting in the laboratory at room temperature (25°C) for 24 h. As the roots desiccated, they became unsuitable for larval occupation, resulting in the larvae emerging and falling into the basin of water. The floating larvae were later collected from the water surface using a fine brush.

2.10 Measurement of survival of immature stages

Experiments were generally conducted with plants growing in 17.5-cm square pots (capacity 2,5 *l*). Thirty days after inoculation with a known number of eggs, the surface of each pot was tightly covered by an isolation cage (30 x 30 x 40 cm) in order to capture adult beetles as they emerged from the soil. Adult progeny often emerged approximately 45 d after egg inoculation. The proportion of eggs that survived to adulthood was used as a measure of survival of immature stages under various biotic and abiotic conditions.

CHAPTER 3

Biology and host specificity of *Longitarsus bethae*

3.1 Introduction

Lantana camara remains a highly invasive species in many parts of the world despite an enormous amount of research effort expended over many years on attempts at biological control. Holm *et al.* (1977) rated lantana as one of the world's ten worst weeds. In South Africa it has invaded about 2.2 million hectares of forest plantations, watercourses and savanna (Cilliers, 1983). Mechanical measures and use of herbicides to control lantana have achieved limited success (Cilliers, 1983; Baars & Neser, 1999) and biological control is still considered to be the only cost-effective and sustainable solution to the problem.

The twelve introduced natural enemies of lantana that are established in South Africa (Baars & Neser, 1999; Simelane, 2002a; Simelane, 2002b) are generally insufficiently abundant to provide consistent control of lantana. Some of the established agents sometimes cause substantial damage on the weed, but this is always sporadic and localised (Cilliers & Neser, 1991; Baars & Neser, 1999). Most of the established agents are leaf-feeders that are subject to drastic population crashes during autumn or winter when the host plants defoliate due to frost or drought (Cilliers & Neser, 1991; Simelane & Phenye, 2003). The impact of the leaf feeding insects is also offset by the ability of lantana to compensate for leaf damage that is caused during summer

(Broughton, 2000), and this has motivated the South African biological control programme to seek natural enemies that attack parts of the plant other than leaves.

A root-feeding flea beetle, *Longitarsus bethae* Savini & Escalona 2005 (Chrysomelidae: Alticinae), was collected from *L. camara* in Mexico and introduced into quarantine in South Africa because it primarily develops on and damages the root system, a niche not exploited by any of the previously introduced lantana biocontrol agents anywhere in the world. The large reductions in agricultural yield, which are often attributed to root-feeding insects, provide evidence of their destructive capabilities (Brown & Gange, 1990; Blossey & Hunt-Joshi, 2003). Because root feeders attack part of the plant whose primary function is to absorb water, it is therefore highly likely that root feeding will have a detrimental effect on water relations of the plant (Gange & Brown, 1989; Brown & Gange, 1990). Although logistical difficulties have shifted the focus away from root feeders as weed biocontrol agents, their role in shaping plant fitness or plant communities cannot be overemphasised as below-ground tissue constitutes 30-90% of the total plant biomass (Nötzold *et al.,* 1998; Baars & Heystek, unpublished data; Williams & Madire, unpublished data; Mabuda & Thobagale, unpublished data). Accordingly, it is anticipated that *L. bethae* could make a substantial contribution to the biological control of *L. camara* in many countries around the world.

The genus *Longitarsus* is distributed worldwide, and is the largest in the sub-family Alticinae with almost 500 species (Konstantinov & Vandenberg, 1996). *Longitarsus* spp. that are closely associated with *Lantana* spp. are widespread in South and Central America (Winder & Harley, 1983; Palmer & Pullen, 1995). However, Jolivet &

Hawkeswood (1995) found that whilst some species within the genus *Longitarsus* had restricted host ranges, some were polyphagous. Two sub-species, namely: *L. columbicus columbicus* Harold 1876 and *L. columbicus centroamericanus* Bechyne 1960 were recorded on *Lantana* spp. in South and Central America, respectively (Bechyne, 1997; Baars, 2001). *Longitarsus howdeni* Blake is widespread on *L. camara* in Jamaica (Simelane, unpublished data). A taxonomic revision indicated that *L. bethae* was a new species (Savini & Escalona, 2005), and that its distribution outside the collection site in Mexico is yet to be established.

The results of host-specificity tests that were carried out on *L. bethae* in quarantine at Plant Protection Research Institute (PPRI), South Africa, are reported here. Results of investigations on certain biological attributes that should enhance the potential of *L. bethae* as an effective biocontrol agent for lantana are also reported.

3.2 Materials and methods

3.2.1 Biological studies

To observe aspects of the biology of *L. bethae*, ten pairs of adult beetles were kept in separate cages with potted lantana plants. Newly laid eggs were collected daily from a white nylon cloth that was tightly pinned onto the soil surface, covering the whole area around the base of the stem. The eggs were removed by rinsing and shaking the cloth gently in water in a basin. The eggs were sieved from the water using a 100 μm (= 0.1 mm) sieve and kept on moist filter paper in closed Petri dishes in a controlled environment room (25 ±2 °C; 70-80% RH; 16:8 h photoperiod) where they were monitored daily to check for eclosion. Larvae, initially released onto the roots of

plants grown in 2-*l* transparent pots, were monitored on a daily basis to examine their feeding behaviour and development. Except during observations, the transparent pots were covered with black paper to keep the larvae in the dark. Soil samples were taken every two weeks to determine the area and the depth within which the larvae were most abundant. In the same study, longevity of adults was determined.

3.2.2 Host-specificity studies

The host specificity of *L. bethae* was determined on a range of plant species that were selected on the basis of their taxonomic (Wapshere, 1974) and phylogenetic relatedness (Briese, 1996) to lantana or economic importance in southern Africa. Depending on the plant species, test plants were propagated in a nursery, either as cuttings or from seeds. Host-specificity tests were conducted on 52 test plant species in 11 families. These included 7 *Lantana* spp. and 16 other species in the same family (Verbenaceae), 13 species of Lamiaceae, 4 species of Solanaceae, 2 species of Poaceae, 2 species of Chenopodiaceae, 2 species of Asteraceae, 2 species of Fabaceae, and 1 species each from the Apiaceae, Brassicaceae, Curcubitaceae and Scrophulariaceae. Lamiaceae is the closest relative of Verbenaceae, hence its broad representation in the list of test plants. Identifications of *Lippia* species A and *Lippia* species B, with collectors' numbers 11 and 28, respectively (Retief, unpublished data), have not yet been provided by the South African National Biodiversity Institute (SANBI). The suitability of each plant species as a host for *L. bethae* was tested under one or more of no-choice, paired-choice, and multiple-choice conditions.

3.2.2.1 No-choice feeding and reproductive performance tests

No-choice tests of adult feeding and production of adult progeny were conducted on all of the 52 plant species. Six pairs of newly emerged adults (about 5 d old) were released into a gauze-covered cage (30 x 30 x 45 cm) containing a single potted test or control plant (*L. camara* variety 009 LP). After 25 d, all the adults were removed and counted to determine their survival on each test plant. At this stage the palatability of each test plant was evaluated by rating the leaf feeding damage using a 0-3 point scale described in chapter 2. After 50 d, plants were monitored daily to check for emergence of adults from each plant. Collection of emerging adults continued for 35 d, during which all adults were expected to have emerged. Differences in feeding damage and reproductive performance between plant species were tested using the Kruskal-Wallis non-parametric test.

3.2.2.2 Paired-choice feeding and reproductive performance tests

Plants that supported complete development of the flea beetle in the no-choice trials were selected for paired-choice feeding and reproductive performance tests. These included six *Lippia* species, *Aloysia citrodora* and two other *Lantana* spp., namely: *L. montevidensis* and *L. rugosa.* Two test plants of the same species and two control plants (*L. camara* variety 009 LP) were placed in a gauze-covered cage (55 x 55 x 95 cm). The plants were arranged in a square with the same species positioned diagonally opposite each other. Twenty pairs of adult beetles were released into each cage, and were removed after 25 d. After the beetles had been removed from each plant, adult feeding was assessed by scoring the leaf damage (Chapter 2). Individual plants were then tagged and covered by smaller plastic cages (30 x 30 x 45 cm) to capture beetles as they emerged from the soil. Comparisons between the target weed

and the non-target plant species were made using the non-parametric Mann-Whitney *U*-test.

3.2.2.3 Multiple-choice feeding and reproductive performance tests

The plant species that had been attacked and supported complete development of the beetles in paired-choice trials were subjected to multiple-choice adult feeding and reproductive performance tests. Three hundred newly emerged, unsexed adults were randomly released into a walk-in cage (4 x 4 x 2 m) in which seven potted plants of each of six *Lippia* species and *L. camara* variety 009 LP had been haphazardly arranged. Adult feeding was assessed after 25 d by scoring the leaf damage (Chapter 2). After rating the feeding damage, adults were removed from the walk-in cage and individual plants were covered with gauze-covered plastic cages (30 x 30 x 45 cm). Plants were monitored daily and numbers of emerging adults were recorded. Differences in feeding damage and reproductive performance between plant species were tested using a one-way analysis of variance on square root-transformed data. Comparisons of means were made using the Newman-Keuls test on square root-transformed data. Untransformed means and SEs are shown.

3.3 Results

3.3.1 Biology studies

Adults of *L. bethae* are golden-brown to dark brown in colour with enlarged hind femora and range in length from 1.5 to 1.9 mm (mean ±SE = 1.7 ±0.2 mm; n = 20). Like *L. albineus* (Foudras) (Huber, 1981), the sexes of *L. bethae* are easily distinguished because the larger abdomen of females extends well beyond the tip of

the elytra, exposing the last three tergites. The adults perforate the epidermis on either the upper or lower surface of the leaf and feed on the mesophyll tissue, producing a characteristic, irregular smattering of pits, which are small, rough edged, and of various sizes. Occasionally, adults feed on flower petals. On usable food plants, adults survived for up to 90 d. The beetles completed five generations per year in the laboratory.

Oviposition commenced after a pre-oviposition period of about 10 d, and continued for up to 50 d. Eggs are yellowish to light brown in colour, ellipsoidal in shape and about 0.3 mm long. The eggs were laid singly or in small clusters of up to four eggs in cracks in the surface of the soil within about 5 cm of the base of the stem of a plant growing in a 2.5-*l* pot.

Eclosion occurred after 11-14 d (mean ±SE: 12.3 ±1.6 d; n = 200) at room temperature (25°C). Early-instar larvae burrowed into rootlets and fed internally, producing elongate tunnels, which were most often found within the lower half of the 20-cm transparent pot. Late-stage larvae were observed feeding externally and removed the outer cortex of the rootlets or secondary roots. The fully-grown larvae moved upwards and pupated within 3 cm of the soil surface. Although the exact number of larval stages of *L. bethae* was not determined, Frick (1970), Ireson *et al.* (1991) and Jordan (1997) recorded three larval stages on *L. jacobaeae, L. flavicornis* and *L. quadriguttatus*.

Pupae were mostly found within a 20 cm radius of the root crown of a plant grown in a 20-*l* pot. Pupal length measured 1.5 ±0.1 mm (n = 10), and they remained white and

soft-bodied throughout their development. The adult-to-adult generation time ranged from 52 to 60 d (mean ±SE: 56 ±1.4 d; n = 55). The effects of feeding damage by *L. bethae* larvae on its host plant are described in Chapter 11.

3.3.2 Host specificity

3.3.2.1 No-choice feeding and reproductive performance tests

Of the 52 plant species that were tested in isolation cages, *L. bethae* laid eggs and produced adult progeny on 11 species (Table 3.1), all in the family Verbenaceae, namely: the target weed (*L. camara*), 3 other *Lantana* spp., 6 *Lippia* spp., and the ornamental lemon verbena (*Aloysia citrodora*). *Lantana camara* was the most suitable host, with 131.4 ±9.7 adults emerging per plant, compared to the other species which produced significantly fewer adults (χ^2 = 22.92; df = 10; P = 0.011), ranging from 0.8 to 22.3 per plant. All of the 14 cultivated crop species, belonging to eight families, were never utilised by the beetles during these trials. Fifteen plant species in the families Lamiaceae, Scrophulariaceae, Asteraceae, and 12 other species in the family Verbenaceae were not attacked. Although exploratory feeding by adults was evident on four other species in the Verbenaceae, adult progeny were not produced on these species.

Table 3.1 Plant species on which *Longitarsus bethae* was tested for adult feeding and reproductive performance in no-choice tests

Plant family/ *species*	n^a	Leaf feeding damage[b]	No. of adult progeny emerged	
			Range	Mean (±SE)
Verbenaceae				
Lantana camara L.	6	2.8 ±0.24a	97 - 152	131.4 ±9.7a
L. trifolia L.	5	1.6 ±0.24b	0 – 3	1.0 ±0.7b
L. montevidensis Briq.	5	2.0 ±0.32b	0 – 3	0.8 ±0.8b
L. rugosa Thunb.	5	2.4 ±0.24b	0 – 3	0.8 ±0.8b
Lippia rehmannii H. Pearson	5	2.4 ±0.24b	0 – 68	22.3 ±15.5b
L. wilmsii H. Pearson	5	2.6 ±0.24b	0 – 26	8.3 ±6.1b
L. scaberrima Sond.	5	1.4 ±0.24b	0 – 12	3.8 ±2.8b
L. javanica (Burm. F.) Spreng.	5	2.4 ±0.24b	0 – 4	0.8 ±0.8b
Lippia species A	5	2.2 ±0.37b	0 – 10	4.3 ±2.2b
Lippia species B	5	2.4 ±0.24b	0 – 9	3.8 ±2.2b
Aloysia citrodora Palau	5	1.4 ±0.24b	0 – 13	3.3 ±3.3b
Verbena brasiliensis Vell.	4	1.3 ±0.24b	0	0
L. mearnsii Moldenke	5	1.9 ±0.37b	0	0
L. dinteri Moldenke	4	1.6 ±0.24b	0	0
L. angolensis Moldenke	4	1.7 ±0.24b	0	0
V. bonariensis L.	4	0	0	0
V. tenuisecta Briq.	4	0	0	0
Duranta repens L.	4	0	0	0
Priva meyeri Jaub. & Spach	4	0	0	0
Phyla nodiflora (L.) Greene	4	0	0	0
Clerodendrum glabrum E. Mey.	4	0	0	0
Stachytarpheta mutabilis (Jacq.) Vahl.	4	0	0	0
S. cayennensis Vahl.	4	0	0	0
Lamiaceae				
Hemizygia obermeyerae Ashby	4	0	0	0
Teucrium trifidum Retz.	4	0	0	0
Lavandula spica L.	4	0	0	0
Nepeta cataria L.	4	0	0	0
Salvia greggi A. Grey	4	0	0	0
S. officinalis L.	4	0	0	0
S. elegans Vahl.	4	0	0	0
S. africana L.	4	0	0	0
Mentha piperita L.	4	0	0	0

[a]Number of cages (replicates) per plant species. [b] 4-point scale of 0-3, indicating non to full feeding by adults. Numbers followed by the same letter within each column do not differ significantly (χ^2 test: $P > 0.05$). Plants on which neither feeding damage nor adult emergence occurred were not analysed statistically.

Table 3.1 (continued)

Plant family/ *species*	n^a	Leaf feeding damage[b]	No. of adult progeny emerged	
			Range	Mean (±SE)
M. spicata L.	4	0	0	0
M. longifolia L.	4	0	0	0
Plectranthus saccatus Benth.	4	0	0	0
Tetradenia riparia Benth.	4	0	0	0
Scrophulariaceae				
Mazus repens L.	4	0	0	0
Poaceae				
Zea mays L. (Maize)	4	0	0	0
Oryza sativa L.(Rice)	4	0	0	0
Fabaceae				
Phaseolus vulgaris L.(Bean)	4	0	0	0
Pisium sativum L. (Pea)	4	0	0	0
Solanaceae				
Solanum melongena L. (Egg plant)	4	0	0	0
Lycopersicon esculentum Mill. (Tomato)	4	0	0	0
Capsicum frutescens L. (Green pepper)	4	0	0	0
Solanum tuberosum L. (Potato)	4	0	0	0
Curcubitaceae				
Citrullus lanatus (Thunb) Mansf. (Watermelon)	4	0	0	0
Brassicaceae				
Brassica oleracea var *capitata* L. (Cabbage)	4	0	0	0
Chenopodiaceae				
Beta vulgaris var. *cicla* L. (Swiss Chard)	4	0	0	0
Beta vulgaris L. (Beetroot)	4	0	0	0
Apiaceae				
Daucus carota L. (Carrot)	4	0	0	0
Asteraceae				
Ageratina adenophora Sprengel (Crofton weed)	4	0	0	0
Lactuca sativa L. (Lettuce)	4	0	0	0

[a]Number of cages (replicates) per plant species. [b] 4-point scale of 0-3, indicating non to full feeding by adults. Numbers followed by the same letter within each column do not differ significantly (χ^2 test: $P > 0.05$). Plants on which neither feeding damage nor adult emergence occurred were not analysed statistically.

3.3.2.2 Paired-choice feeding and reproductive performance tests

In paired-choice tests involving *L. camara* and other susceptible plant species, the beetles showed a significant ($P < 0.05$) preference for *L. camara* (Table 3.2). The

less-preferred *Lippia* spp. included *Lippia rehmannii*, *Lippia* sp. A, *Lippia* sp. B and the ornamental *Aloysia citrodora.* The number of emerging beetles collected from these hosts did not exceed four while those collected from the *L. camara* plants ranged between 31 and 48 per plant. Although minor feeding damage by adults was evident on *Lippia wilmsii, Lippia scaberrima, Lantana rugosa* and the exotic *L. montevidensis,* neither larval feeding nor adult emergence was evident on these plant species.

Table 3.2 Relative rates of adult feeding and reproductive performance of *Longitarsus bethae* in paired-choice tests between *Lantana camara* and other susceptible plant species

Plant species	n^a	Leaf feeding damage[b] (Mean ±SE)	Adult progeny emerged	
			Range	Mean (±SE)
L. camara	5	2.8 ±0.24a	21 – 42	30.5 ±5.2a
L. camara		2.6 ±0.24a	26 – 50	37.6 ±4.9a
L. camara	4	2.8 ±0.20a	27-44	32.7 ±5.7a
Lippia wilmsii		1.4 ±0.24b	0	0b
L. camara	4	2.6 ±0.32a	26 – 32	27.5 ±2.0a
Lippia rehmannii		1.6 ±0.24b	0 - 4	1.8 ±1.2b
L. camara	4	3.0 ±0.00a	30 – 86	51.1 ±17.5a
Lippia 'species A'		1.2 ±0.12b	1 – 4	2.3 ±0.9b
L. camara	4	3.0 ±0.00a	18 – 65	40.7 ±13.7a
Lippia 'species B'		1.6 ±0.32b	0 – 4	1.3 ±1.3b
L. camara	4	2.8 ±0.20a	27 - 58	47.7 ±11.7a
Lippia javanica		1.0 ±0.00b	0 - 3	1.8 ±1.5b
L. camara	4	2.6 ±0.24a	28 - 44	34.0 ±5.0a
Lippia scaberrima		1.0 ±0.00b	0	0b
L. camara	4	3.0 ±0.00a	38 – 44	41.5 ±1.8a
Aloysia citrodora		1.2 ±0.12b	0 – 2	0.8 ±0.4b
L. camara	4	3.0 ±0.00a	36 - 58	46.0 ±6.4a
L. montevidensis		0b	0	0b
L. camara	4	3.0 ±0.00a	24 – 49	35.3 ±7.1a
L. rugosa		0.8 ±0.20b	0	0b

[a]Number of cages (replicates) per plant. [b]4-point scale of 0-3, indicating non to full feeding by adults. The means of test pairs followed by the same letter did not differ significantly at P = 0.05 by Mann-Whitney *U*-test.

2.3.2.3 Multiple-choice feeding and reproductive performance tests

Development was only completed on the target weed, *L. camara,* and three *Lippia* species (Table 3.3). *Lippia* spp. on which development was completed included *Lippia* sp. A, *Lippia* sp. B and *Lippia javanica.* *Lantana camara* was severely damaged by the adult beetles, and the number of emerging beetles recorded on lantana was significantly higher than on the three *Lippia* spp. ($F = 16.7$; df = 35; $P < 0.001$). Feeding damage by adults was negligible on *L. rehmannii*, *L. wilmsii,* and *A. citrodora,* and there was no evidence of larval development on these species. Examination of the rootlets on these species also failed to reveal any feeding damage by larvae.

Table 3.3 Relative rates of adult feeding and reproductive performance of *Longitarsus bethae* during multiple-choice tests with susceptible plant species

Plant species	No. of Plants per cage		Leaf feeding	No. of adult progeny emerged	
	Offered	Attacked	damage	Range	Mean (±SE)
Lantana camara	7	6	2.8 ±0.20a	11 - 53	31.7 ±7.6 a
Lippia 'species B'	7	3	1.0 ±0.32b	0 - 5	0.8 ±0.8 b
L. javanica	7	2	0.6 ±0.24b	0 - 3	0.5 ±0.5 b
Lippia 'species A'	7	2	0.6 ±0.24b	0 - 1	0.2 ±0.2 b
L. rehmannii	7	1	0.8 ±0.37b	0	0a
L. wilmsii	7	1	0.6 ±0.24b	0	0a
Aloysia citrodora	7	0	0	0	0a

Means within a column followed by the same letter did not differ at P = 0.05 by Newman-Keuls test on square root-transformed data. Untransformed means and SEs are shown.

3.4 Discussion

Cage conditions in laboratory situations interfere with natural host selection by insects, often resulting in the utilisation of hosts that would not be selected under outdoor conditions. The extended host ranges frequently found during the screening of potential weed biocontrol agents in the laboratory is now referred to as the physiological host range (i.e. the range of plant species that satisfy the feeding requirements of the test insect) (Cullen, 1989; Balciunas *et al.*,1996). This is contrasted with the ecological host range which is the range of plant species that the test insect can utilise while coping with biotic and abiotic stresses that occur under outdoor conditions. There are many examples of laboratory tests that have indicated a broader array of host plants than that utilised by the insects in the field (Zwolfer & Harris, 1971; Schroeder, 1983; Cullen, 1989; Wapshere, 1989; Clement & Cristofaro, 1995; Marohasy, 1998). The no-choice tests in this study suggested that *L. bethae* could physiologically utilise up to 10 plant species in the family Verbenaceae. However, the multi-choice tests indicated that the 'true' ecological host range of *L. bethae* is not that broad. A similar situation occurred with the herringbone leafminer, *Ophiomyia camarae* Spencer, which developed on four *Lippia* species during host range tests in the laboratory (Simelane, 2002a) but has never significantly attacked any of these species since becoming established in the field (Urban & Phenye, 2005). The disproportionately high number of beetles that emerged from eggs laid on the target weed (lantana) during the three sets of host range tests indicates strongly that lantana is by far the most suitable host for *L. bethae,* and that the other verbenaceous species would not serve as adequate hosts to any significant extent.

Although some verbenaceous species were slightly damaged by and supported development of *L. bethae* under laboratory conditions, the beetle is expected to be restricted to *L. camara* in the field. The laboratory results suggested that the feeding damage on and the progeny production from non-target species were so insignificant that they will, at best, be marginal hosts of *L. bethae* in the field. The low progeny production from non-target plant species indicates that they are unlikely to sustain field populations of *L. bethae* over time. It was therefore concluded that *L. bethae* does not pose a threat to either indigenous or cultivated verbenaceous plant species, and that it is adequately host-specific to be released against *L. camara* in South Africa.

CHAPTER 4

Influence of soil texture on preference and survival of *Longitarsus bethae*

4.1 Introduction

In South Africa, lantana is naturalised in regions that are characterised by a broad range of soil types, including clayey loam, silty loam and sandy loam. Adult oviposition preference and corresponding larval performance of the root-feeding flea beetle, *L. bethae*, among soil types could result in niche partitioning and restrict biological control to a range of particular soil types. Soil type has an effect on other edaphic factors, including water holding capacity of the soil, soil penetrability, soil temperature and soil flora and fauna (Macdonald & Ellis, 1990; Godfrey *et al*., 1995), and these could in turn influence oviposition preference and subsequent larval performance of *L. bethae.*

Many studies have examined the effect of soil physical properties on survival of soil-dwelling insects in agro-ecosystems, where disturbance by tillage implements alters the soil structure and other edaphic components (Turpin & Peters, 1971; Guston & Schumacher, 1989; Macdonald & Ellis, 1990; Brust & House, 1990; Godfrey *et al*., 1995). The multiplicity of factors involved, makes it difficult to develop reliable, simple models to describe relationships between individual soil factors and survival of insects. However, Turpin & Peters (1971) observed that the number of surviving larvae of the western corn rootworm (*Diabrotica virgifera virgifera* LeConte) increased as the clay percentage of the soil increased. Macdonald & Ellis (1990) later

discovered that larvae of western corn rootworm moved more than three times faster through silty clay or loam than through loamy sand, reducing the abundance of this pest in rough-textured soils. Turpin & Peters (1971) and Brust & House (1990) reported that sandy soils dried out quickly within the top10-cm of the soil profile, reducing the survival rate of the southern corn rootworm by increasing its vulnerability to desiccation. It has also been argued that the network of cracks in clay soils allows greater mobility of neonate larvae through the soil particles, allowing their access to rootlets, and consequently greater larval survival (Strnad & Bergman, 1987; Pacchioli & Hower, 2004). In another study, Guston & Schumacher (1989) concluded that soil texture, through its relationship with soil structure and pore size distribution, had a significant influence on larval survival.

The present study was undertaken to determine whether soil conditions influenced adult oviposition preferences and survival of immature stages of *L. bethae.* If soil conditions affect oviposition and subsequent larval development of *L. bethae*, and the range of soil types that are suitable for *L. bethae* can be determined, the extent of lantana infestations that occur in areas with suitable soils can be identified. If lantana is generally not associated with suitable soils, releasing the beetles could be a futile exercise with negligible prospects of success. Should a decision be made to release the beetles because lantana is associated sufficiently with suitable soils, release sites can be selected which will be most favourable for oviposition and larval development and thus enhance the chances of the agent becoming established and contributing to control of the weed.

Based on studies of other insect species (Kirk *et al.*, 1968; Turpin & Peters, 1971; Lummus *et al.*, 1983; Marrone & Stinner, 1984; Strnad & Bergman, 1987; Pacchioli & Hower, 2004), it was hypothesized that at least three factors might influence oviposition and larval development in *L. bethae*: soil texture; surface cracks and levels of organic content. Each of these was investigated in turn.

4.2 Materials and methods

4.2.1 Adult feeding and oviposition preference on different soil textures

A 3 x 4 factorial design was used to set-up an experiment to measure the effect of four soil textures at three 1 cm-soil depth increments. The soil textures, as determined by gravimetric analysis, were: clayey (5% sand, 39% silt and 55% clay; 1% organic matter), silty loam (9% sand, 68% silt and 21% clay; 2.2% organic matter), sandy loam (68% sand, 23% silt and 7% clay; 2.1% organic matter) and sandy soil (90% sand, 7.7% silt and 2% clay; 0.3% organic matter). Lantana plants were grown singly in pots containing each of the four soil textures. Twenty-four potted plants representing four groups, each with six plants growing in a particular soil type were placed randomly within a walk-in cage (4 x 4 x 2m). A group of 216 newly emerged, unsexed adults of *L. bethae* was released into the cage. The adults were confined for a period of 10 d, during which they fed on the plants and oviposited in the soil. During the 10-d period, adults found on each plant or on the soil surface of a plant were counted daily to determine the relative time spent on each type of pot. At the end of the 10-d period, the adults were removed and the potted plants were examined. Eggs were extracted from each of 1-cm layers of a 3-cm soil profile and counted. Leaf feeding damage by adults was also assessed (Chapter 2) on each plant.

4.2.2 Effect of soil cracks on oviposition

To test the hypothesis that surface cracks create better oviposition sites for *L. bethae*, ten pairs of newly emerged adults were confined in a gauze-covered cage (0.5 x 0.5 x 0.95 m) containing two plants, one with artificial cracks (test) and the other without cracks (control). On each test treatment plant, five vertical slits were made by scraping a sharp knife across the soil surface of each plant as described in chapter 2. The soil type used for both control and test plants was silty loam, containing 9% sand, 68% silt, 21% clay and 2.2% organic matter. After 10 d, the adults were removed, and eggs were recovered from within the depth of 3 cm of the soil surface. The total number of eggs laid during the 10-d period was compared between the test and the control plants. The experiment was replicated 10 times.

4.2.3 Relationship between oviposition and the amount of organic matter in the soil

To test whether organic matter influences oviposition, adult beetles were exposed to soils with five different levels of organic matter content. Silt and compost were mixed in ratios of 3:0, 3:1, 1:1, 1:3 and 0:3 to create soils with 0, 25, 50, 75 and 100% organic matter, respectively. Each of these media was placed at a depth of 3 cm over the exposed surface around each of five potted plants. The 25 plants were then arranged randomly in a walk-in cage (4 x 4 x 2 m) and exposed to 300 unsexed beetles for 10 d. After 10 d, the number of eggs laid per plant was determined. Effect of organic matter on oviposition was further determined in a no-choice situation. Five pairs of adult *L. bethae* were confined with a potted plant whose top 3 cm of soil comprised either pure organic matter or pure silt, in a gauze-covered cage (0.5 x 0.5 x 0.95 m).

4.2.4 Effect of soil texture on survival of L. bethae

Effect of soil texture on performance of *L. bethae* was measured by determining the survival of *L. bethae* from egg to adulthood on plants grown in four different soil textures used in the oviposition preference tests in section 4.2.1. Survival was measured by counting the newly emerged adults produced from an initial inoculation of 200 eggs per plant. The 200 eggs were placed along a vertical slit as described in chapter 2. Gauze cages (30 x 30 x 40 cm) were placed over individual plants to confine emerging beetles. Adults started emerging approximately 45 d after egg inoculation. Head capsule widths and body lengths of both sexes that emerged from each soil texture were measured, and, together with survival, were used to measure the suitability of the soil for *L. bethae.* Each soil texture was replicated 6 times.

4.2.5 Data analysis

Feeding scores and number of adults per plant were analysed using analysis of variance (ANOVA). Student's *t*-test was used to determine significance of difference between the number of eggs laid on cracked and those laid on uncracked soil surfaces. In order to stabilize the variance, the number of eggs laid and the number of adult progeny emerged from different soil textures were initially transformed to square-roots before being subjected to ANOVA. The transformed means were separated by Fisher's protected LSD. However, only untransformed means are presented in the tables. Correlation and regression analysis were conducted to determine relationship between the oviposition rate and the amount of organic matter in the soil.

4.3 Results

4.3.1 Feeding and oviposition preference on different soil textures

Soil texture had no effect on the extent of leaf feeding ($F_{(3,16)} = 0.293$; $P = 0.830$) and aggregation ($F_{(3,16)} = 0.023$; $P = 0.995$) by adult *L. bethae* (Table 4.1). However, soil texture significantly influenced ($F_{(3,14)} = 3.54$; $P = 0.042$) oviposition, with more eggs laid on clayey than on mixed silty and sandy loam soils (Table 4.2). The oviposition behaviour of the females was apparently not influenced by soil particle size as the numbers of eggs laid on clayey and sandy soils were not significantly different from each other.

Table 4.1 Number of adult *L. bethae* per plant and feeding preference on plants grown on different soil textures

Soil texture	Mean ±SE number of adults per plant	Level of feeding damage (Mean ±SE)[a]
Clayey soil	5.6 ±0.68	2.2 ±0.37
Silty loam soil	5.8 ±1.02	2.0 ±0.32
Sandy loam soil	5.8 ±0.67	2.0 ±0.32
Sandy soil	5.6 ±0.58	2.4 ±0.40

[a] 4-point scale of 0-3, indicating non to full feeding by adults.

Table 4.2 Number of eggs deposited by *L. bethae* at three soil depths on four soil types

Soil texture	Mean ±SE number of eggs laid per plant	Percentage of eggs laid at three soil depths		
		Upper 1 cm	Middle 1 cm	Bottom 1 cm
Clayey soil	246.3 ±63.8a	85	13.7	1.3
Silty loam soil	72.5 ±16.5b	100	0	0
Sandy loam soil	68.6 ±20.5b	70.1	29.9	0
Sandy soil	170.8 ±22.2ab	89.3	10.7	0

Means followed by the same letter within a column are not significantly different ($P > 0.05$; LSD test).

4.3.2 Effect of soil cracks on oviposition

The availability of cracks on the soil surface had a significant influence on the selection of oviposition sites by *L. bethae* ($t = 3.15$; $P < 0.05$; $n = 10$), with 162.7 ±25 and 54 ±18 (mean ±SE) eggs deposited on cracked and non-cracked soils, respectively.

4.3.3 Relationship between oviposition and the amount of organic matter in the soil

There was a strong negative relationship ($y = 137.9^{-0.07x}$; $r^2 = 0.59$; $P < 0.001$) between the percentage of organic matter in the soil and the number of eggs laid (Fig. 4.1). However, when beetles were confined in isolation cages and forced to lay on either pure compost or silt, they laid almost the same number of eggs under both conditions (pure organic matter: mean ±SE = 145.0 ±20.2, silt: 136.0 ±28.2, $t = 1.05$; $P = 0.35$; $n = 10$).

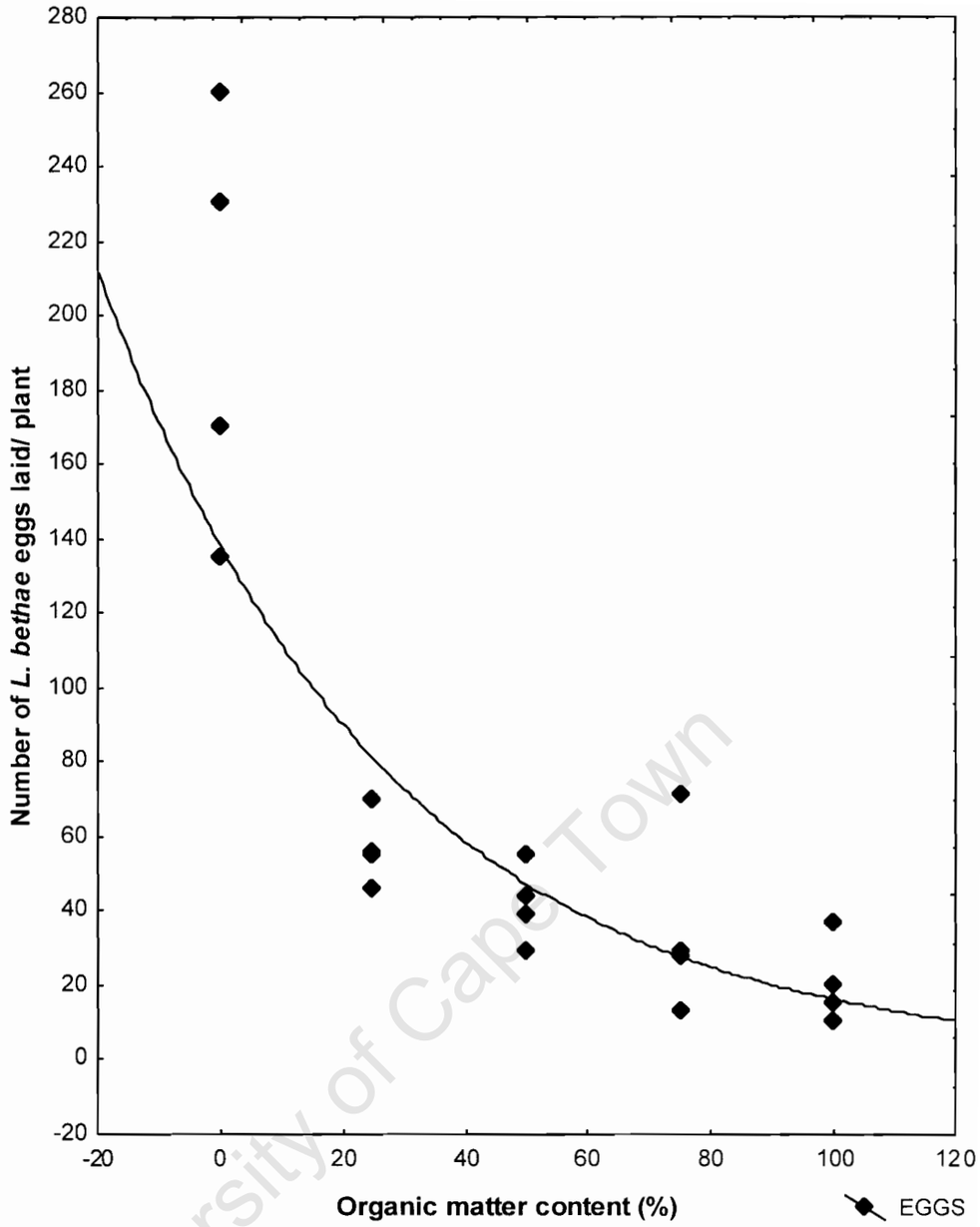

Fig. 4.1 Number of eggs laid in relation to the percentage of organic matter in the soil.

4.3.4 Survival of L. bethae *in different soil textures*

Survival from egg to adult stage was significantly ($F_{(3,14)} = 11.16$; $P < 0.001$) affected by soil texture (Table 4.3). Survival in clayey soils was more than 9-fold greater

compared with that in other soil textures. Development time from egg to adult stage did not differ significantly among the soil textures ($F_{(1,13)}$ = 1.56; P = 0.245) (Table 4.3).

Table 4.3 Emergence of adults and duration of development from egg to adult stages in four soil textures

Soil texture	Mean ±SE adults emerged per plant	Mean ±SE duration (d) from egg to adult stages
Clayey soil	87.4 ±20.3a	48.8 ±1.2a
Silty loam soil	9.6 ±1.18b	52.0 ±1.7a
Sandy loam soil	3.4 ±0.9bc	52.1 ±5.9a
Sandy soil	1.8 ±0.6c	58.0 ±4.5a

Means followed by the same letter within a column are not significantly different (P > 0.05; LSD test).

Adult *L. bethae* emerging from different soil textures did not vary significantly in body size (body length: $F_{(3,72)}$ = 0.656; P = 0.581, head capsule width: $F_{(3,72)}$ = 1.57; P = 0.21) (Table 4.4). Consistent with sexual dimorphism in body size, males were significantly smaller (body length: $F_{(1,72)}$ = 27.22; P < 0.05, head capsule width: $F_{(1,72)}$ = 13.73; P = 0.004) than females in all the soil textures.

Table 4.4 Mean ±SE body lengths and head capsule widths of *L. bethae* adults emerged from four soil textures

Soil texture	Mean ±SE body length (mm)		Mean ±SE head capsule width (mm)	
	Male	Female	Male	Female
Clayey soil	1.72 ±0.02	1.84 ±0.03	0.49 ±0.02	0.51 ±0.02
Silty loam soil	1.75 ±0.04	1.83 ±0.03	0.49 ±0.02	0.51 ±0.02
Sandy loam soil	1.73 ±0.03	1.83 ±0.04	0.47 ±0.02	0.52 ±0.02
Sandy soil	1.72 ±0.02	1.76 ±0.02	0.43 ±0.02	0.50 ±0.02

4.4 Discussion

The neonate larvae of *L. bethae* must initially find and feed on roots to ensure their establishment on the host plant. Therefore, the ability of female *L. bethae* to select oviposition sites that would be suitable for the survival of its immature stages could maximise the success rate of its establishment in the field. The results of the present study suggest that clayey and sandy soils were most preferred for oviposition. There are several possible interpretations for this result. Females might have selected oviposition sites in response to the availability of cracks that mainly characterised clayey soils (Kirk *et al.*, 1968). Cracks in clayey soils might have allowed physical access of females deeper into the soil profile, enabling them to lay as much as 15% of their eggs at the depth of 2-3 cm below the soil surface (Table 4.2). This appears to be consistent with previous studies where it was shown that the network of cracks in clayey soils attracts ovipositing adults, and subsequently allows first instar larvae greater mobility through the soil, increased access to roots, resulting in greater larval survival (Strnad & Bergman, 1987; Pacchioli & Hower, 2004).

During egg extraction from sand, females were often extracted together with eggs from within the upper 2 cm of the soil profile, suggesting that they had probably burrowed into the loose sandy soil to lay their eggs. Because sandy soils have poor moisture-holding capacity, survival of immature stages in such soils could have been reduced by water stress or desiccation (Hadley *et al.*, 1989). The rapid rate of soil drying, particularly within the top 5 cm of the soil profile of sandy soils could have caused eggs to desiccate and loose their viability. Studies in chapters 6 and 7 also showed that *L. bethae* eggs were highly vulnerable to desiccation, and that their mortality was increased under bare soil conditions or at lower relative humidities. Findings of Turpin & Peters (1971), Macdonald & Ellis (1990) and Brust & House (1990) also demonstrated that in sandy and other rapidly drying soils, desiccation had detrimental effects on both western and southern corn rootworms. Increased pupal mortality in coarse-textured soils was likely to have contributed to reduction of adult emergence as previous studies showed that pupae are also vulnerable to desiccation (Simelane, unpublished data). Sandy and other coarse-textured soils with abrasive soil particles are likely to have caused substantial physical damage to larvae, possibly reducing their survival (Hoback & Golick, 2000).

Whilst there was an apparent tendency of the beetles to avoid laying on organic matter-rich soils in a choice situation (4.3.3), oviposition on these substrates was not deterred in a no-choice situation. Therefore, the lack of oviposition shown in the multiple-choice experiment (4.3.1) might have been attributed to the amount of organic matter in silty or sandy loam soils, which are naturally richer in organic matter content than either clay or sand. However, as evidenced in the present study,

beetles would not have been deterred from laying on loamy soils under no-choice conditions. It is also assumed that organic matter content within a release site in the field is less likely to vary to the extent of presenting a clear choice for the ovipositing beetles.

The loamy soils used in the present study were inevitably pulverized when packed into plant pots during transplanting, thus producing a soil profile with poorer continuity of pores than one would expect in undisturbed loamy soils under field conditions (Pacchioli & Hower, 2004). In contrast, surface cracks were formed on clayey soils soon after transplanting, and this could have favoured oviposition and larval survival. Under natural field conditions, undisturbed loamy soils become more stable over time, forming structured pores (e.g. soil cracks, old root and earthworm channels) with continuity (Brady, 1974), and these are likely to enhance larval movement and survival.

The present study suggests that soil texture will be among the important environmental parameters influencing the population dynamics of *L. bethae* in the field. It will therefore influence prediction of establishment, future monitoring and management of the biocontrol programme for this agent, including the identification of optimum conditions for its establishment in the field.

CHAPTER 5

Influence of soil moisture on preference and survival of *Longitarsus bethae*

5.1 Introduction

Several studies have implicated soil moisture as one of the important edaphic factors affecting the performance of subterranean herbivorous insects. For example, southern corn rootworm (*Diabrotica undecimpunctata howardi* Barber, Coleoptera: Chrysomelidae: Galerucinae) laid 62% more eggs in irrigated than in non-irrigated systems (Brust & House, 1990). Weiss *et al.* (1983) reported that approximately 80% of eggs of western corn rootworm were found in the upper 10 cm of soil in irrigated fields while only 45% of eggs were found at this soil level under dry land conditions. Whilst western corn rootworm (*D. virgifera virgifera* LeConte) laid significantly more eggs in high-moisture subplots (Gustin, 1979), poor larval establishment and reduced populations of this beetle occurred when egg hatch took place under saturated soil conditions (Ridell & Sutter, 1995). Mortality of eggs and larval stages of southern corn rootworm was greater in non-irrigated than in irrigated systems (Brust & House, 1990). Similarly, larval and pupal mortality of *D. undecimpunctata* was significantly higher at lower (45-55%) plant available water content (PAW) than at higher PAW (70-100%) (Lummus *et al.*, 1983). Under drought conditions, when the upper surface of the soil became dry and hard, emergence of the pecan weevil, *Curculio caryae* Horn (Coleoptera: Curculionidae), was delayed (Schraer *et al.*, 1998). Marrone & Stinner (1983) observed that the movement of the bean beetle larva, *Cerotoma*

trifurcata Forster (Chrysomelidae: Galerucinae), was inhibited in very wet soils. Macdonald & Ellis (1990) found that when soils were very wet or very dry, larval establishment was reduced because of inhibition of larval movement, increased mortality, or both. Brust & House (1990) concluded that the rate of soil drying in the top 5-8 cm of soil profile was crucial for the survival of the southern corn rootworm and potential damage to crop roots.

In this study, adult aggregation, feeding, oviposition preference and survival of immature stages of *L. bethae* were determined under three (low, moderate and high) soil moisture regimes in the laboratory. It was hypothesised that females would choose soil moisture levels that would best suit egg hatch and subsequent larval performance in the soil. In terms of biological control, knowledge of the influence of soil moisture on survival of the beetles was considered an important aid to making releases at the most suitable sites and during the right season.

5.2 Materials and methods

5.2.1 Adult aggregation, feeding and oviposition preference under three soil moisture regimes

A 3 x 3 factorial design experiment was used to measure the effect of three soil moisture regimes at three soil depths on adult aggregation, feeding, and oviposition preference. A Hadeco soil moisture meter was implanted in each pot to monitor soil moisture level. Three regimes were maintained, representing low, moderate and high moisture levels, respectively (Chapter 2). A standard loamy soil (with 9% sand, 68% silt, 21% clay and 2.2% organic matter) was used at all moisture levels. A population

of 360 newly emerged unsexed adults of *L. bethae*, reared on potted lantana plants (variety 009 LP), were released into a walk-in cage (4 x 4 x 2 m) containing 30 potted plants with 10 of each having either low, moderate or high regimes of soil moisture. The pots were arranged randomly on the floor of the cage and the adult beetles were confined in the cage for a period of 10 days. After 10 days, adults were removed and counts were made of eggs that were extracted from each of three 1-cm soil layers. Adults found on each plant or soil surface below the same plant were counted daily, and leaf feeding damage by adults was assessed (Chapter 2) at the end of the experiment. The latter two parameters were used as additional measures of the relative moisture regime preferences of the ovipositing females.

5.2.2 Survival of L. bethae *from egg to adulthood under three soil moisture regimes*

Potted *L. camara* plants were maintained under low, moderate and high moisture regimes throughout the development of the immature stages of *L. bethae*. Each of the plants was initially inoculated with 200 *L. bethae* eggs. The effect of soil moisture on the survival of the immature stages was determined by counting the number of adults that emerged. Development time from egg to adulthood, and body size of adult progeny from each of the three moisture regimes, were also determined. Adults started emerging approximately 45 days after egg inoculation, and isolation cages (30 x 30 x 40 cm) were placed on individual plants to contain emerging beetles. Each soil moisture regime was replicated 5 times.

5.2.3 Data analysis

In order to reduce the variance, the number of eggs laid and the number of adult progeny emerged from different soil moisture regimes were initially transformed to

square-roots before being subjected to analysis of variance (ANOVA). The transformed means were separated by Fisher protected LSD. However, only untransformed means are presented in the tables.

5.3 Results

5.3.1 Adult aggregation, feeding and oviposition preference under three soil moisture regimes

The number of adults recorded on each plant, and the levels of feeding damage, were not significantly influenced by the level of soil moisture (number of adults: $F_{(2,12)} = 0.129$; $P = 0.88$, adult feeding: $F_{(2,12)} = 0.750$; $P = 0.493$) (Table 5.1). Females showed no decided oviposition preference for a particular soil moisture regime ($F_{(2,12)} = 0.654$; $P = 0.539$) (Table 5.2). Moisture levels had no influence on the depth of oviposition and over 95% of eggs, irrespective of soil moisture level, were deposited in the upper 1-cm layer of the soil (Table 5.2).

Table 5.1 Aggregation and feeding preference of *L. bethae* adults under three soil moisture regimes.

Soil moisture regime	Aggregation (adults/plant)	Adult feeding damage (index value, **mean ±SE**)
Low	5.0 ±0.8	3.8 ±0.2
Moderate	5.6 ±1.0	3.6 ±0.3
High	5.4 ±0.6	3.4 ±0.3

Table 5.2 Oviposition preference of *L. bethae* and egg depth distribution in soils maintained at each of three soil moisture regimes

Soil moisture regime	Oviposition preference (eggs/plant, mean ±SE)	Egg depth distribution (%) in		
		Upper 1 cm	Middle 1 cm	Bottom 1 cm
Low	351.4 ±78.0	99	1	0
Moderate	205.0 ±63.5	96	4	0
High	238.6 ±44.8	98	2	0

5.3.2 Survival of L. bethae *from egg to adulthood under three soil moisture regimes*

Survival to adulthood was significantly higher ($F_{(2,11)}$ = 9.98; P = 0.0035) in moderately moist than in very wet and very dry soils (Fig 5.1). Survival in moderate soil moisture regime was approximately 5 and10 times that in high and low moisture regimes, respectively. Development time from egg to adult stage was significantly ($F_{(2.8)}$ = 6.067; P = 0.05) delayed in wet soils, taking 4 and 5 days longer than in moderately moist and dry soils, respectively (Table 5.3). No significant difference in body length ($F_{(2,54)}$ = 0.24; P = 0.79) and head capsule width ($F_{(3,54)}$ = 2.57; P = 0.06) were observed among the beetles emerging from the three moisture regimes (Table 5.4). Females were, however, significantly (length: $F_{(1,54)}$ = 11.8; P = 0.001, width: $F_{(1,54)}$ = 3.54; P = 0.06) larger than males in all the treatments.

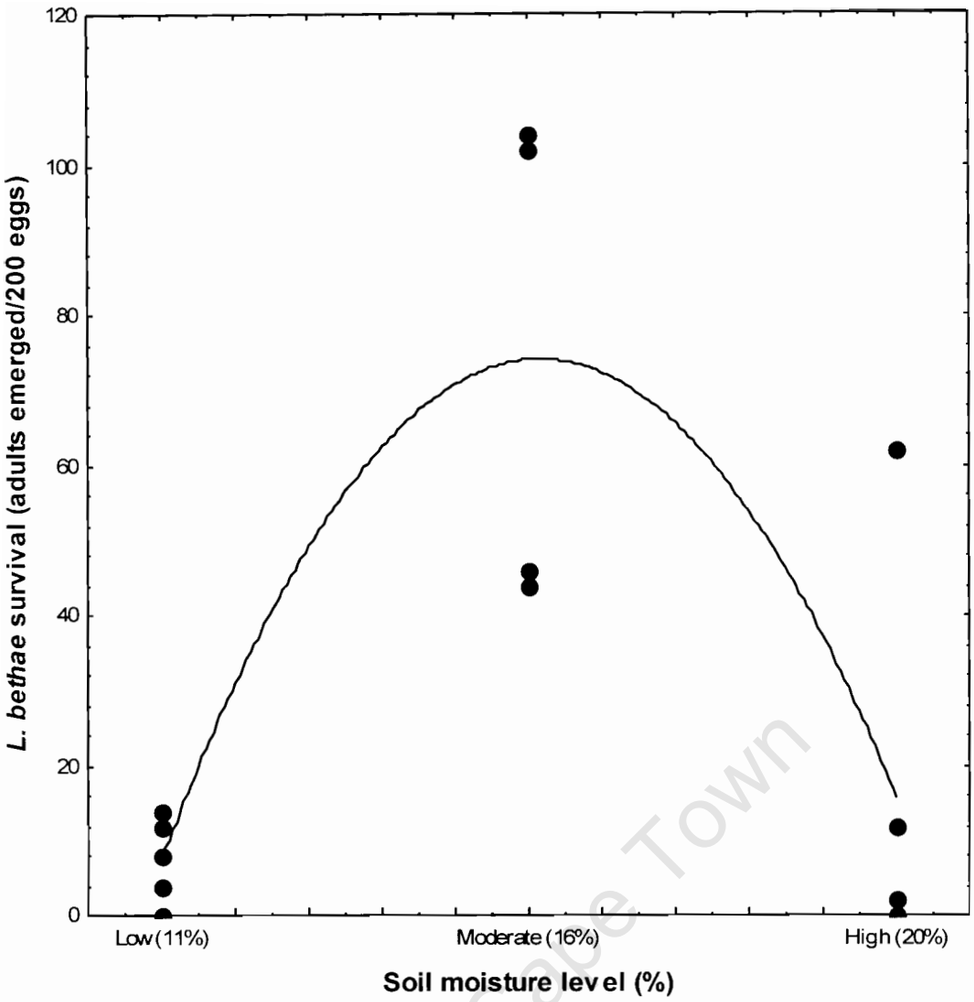

Fig. 5.1 Survival of *L. bethae* from egg to adulthood under three soil moisture regimes, from an initial inoculation of 200 eggs/plant.

Table 5.3 Body size (length and head capsule width) and duration of development of adult *L. bethae* emerging from soil of three moisture regimes

Soil moisture regime	Body length (mm, mean ±SE)		Head capsule width (mm, mean ±SE)		Duration of development (d, mean ±SE)
	Male	Female	Male	Female	
Low	1.8 ±0.04	1.8 ±0.03	0.5 ±0.02	0.5 ±0.02	47.10 ±0.34b
Moderate	1.7 ±0.01	1.8 ±0.04	0.5 ±0.02	0.5 ±0.02	48.85 ±1.01b
High	1.7 ±0.02	1.8 ±0.04	0.4 ±0.02	0.5 ±0.02	52.78 ±1.38a

5.4 Discussion

Although the present study showed no statistically significant influence of soil moisture on oviposition by *L. bethae*, survival and development of the immatures was found to be highly dependent on soil moisture. Gustin (1979) also found no significant effect of soil moisture on oviposition by western corn rootworm. Rather than soil moisture, the nature of the soil surface (e.g. soil cracks) and soil texture seemed to be most likely to influence the selection of oviposition site (Chapter 4).

Studies in chapters 4 and 6 showed that oviposition occurred within the upper 2 cm, and overwhelmingly in the uppermost 1 cm of the soil profile, a layer that is prone to rapid drying. Therefore, the presentation of the dynamics of soil moisture in this study is relatively simplistic, as it does not account for changes in survival that could probably result from changes in moisture at or very near the soil surface. The results of the experiment in chapter 7 showed that eggs were highly susceptible to desiccation, and that over-exposure to dry conditions could be detrimental to their

survival. Furthermore, the survival from egg to adult stage was much higher in leaf litter-covered treatments where the rate of soil drying was low compared with that in bare-surface treatments (Chapter 6). Early instars, because of their small size, possess a large surface-to-volume ratio, and therefore experience greater evaporative loss in dry soil than later instars (Edney, 1977). Riedel & Sutter (1995) also found that if soils were saturated during peak egg hatch, larval establishment was greatly hampered, eventually reducing adult emergence of western corn rootworm. Therefore, both desiccation in very dry soils and poor aeration in water-filled pores of wet soils could have inhibited larval movement and prevented them from finding roots, thereby reducing their survival.

In moist, subtropical areas of the Limpopo, Mpumalanga, KwaZulu-Natal and Eastern Cape provinces of South Africa where lantana is presently naturalized and abundant, heavy infestations of lantana often form dense thickets with closed canopies, resulting in leaf litter accumulation on the ground (Simelane, personal observation). Under such conditions, the rate of soil drying and soil surface temperature could be reduced, thereby conserving soil moisture required for increased survival of *L. bethae*. Given a favourable soil texture in such habitats, the prospect of *L. bethae* establishment could be increased.

Studies in this thesis have demonstrated that other abiotic factors that are associated with low soil moisture (e.g. soil of a sandy texture, bare ground and low atmospheric humidity) have a detrimental effect on the survival of *L. bethae.* These findings are consistent with previous studies in which soil moisture was found to have a significant influence on the survival of immature stages of various root-feeding herbivores

(Gustin, 1979; Lummus *et al.*, 1983; Marrone & Stinner, 1983; Marrone & Stinner, 1984; Macdonald & Ellis, 1990; Tauber *et al.*, 1994; Ridell & Sutter, 1995). Because soil moisture is highly dependent on soil texture, the development of a simple explanatory and predictive model of survival of *L. bethae* as a function of the combined effect of various ecological factors, including soil moisture and texture, was undertaken in chapter 12.

CHAPTER 6

Influence of leaf litter on preference and survival of *Longitarsus bethae*

6.1 Introduction

Host and habitat selection by adult female insects forms a crucial part of the life cycle of insect species whose ability to disperse is limited during the immature stages (Leather, 1985; Mayhew, 1997). *Longitarsus bethae* larvae can burrow for short distances but need to be close to the roots of a suitable host plant in order to reach food and survive. The larvae are therefore reliant on their eggs being placed in suitable positions by the ovipositing females.

The eggs of some insects are also vulnerable when exposed to environmental extremes. For example, egg survival of two tiger beetles (*Cicindela circumpicta* and *C. togata* (Coleoptera: Cicindelidae)) was higher under shaded conditions than on sunny soil surfaces for the duration of the incubation period (Hoback & Golick, 2000). Similarly, the immature stages of *L. bethae* are highly susceptible to desiccation in dry soils (Chapter 5) and there was a substantial decline in the viability of *L. bethae* eggs that were kept below 75% RH for more than 24 h (Chapter 7).

Besides egg mortality, very dry soils can present major problems for neonate larvae, including death due to desiccation stress and an inability to burrow through hard, dry soil to reach the roots of the host plant (Hadley *et al.*, 1989). Both oviposition and pupation of *L. bethae* occur within the top 3 cm of the soil profile, where the soil dries

relatively rapidly in the absence of precipitation (Simelane, 2005), rendering the immature stages highly vulnerable to desiccation. The pupal stage of *L. bethae* is also vulnerable to desiccation, and no pupae completed their development when held at an atmospheric humidity of or below about 60% RH (Simelane, unpublished data).

The detrimental effects of desiccation could be reduced by leaf litter, which curbs water loss through evaporation and allows soil to retain moisture for much longer than equivalent, exposed soils. It was hypothesized that *L. bethae* will maximize the chances of their offspring surviving by ovipositing preferentially in sites where there is shade and/or a covering of leaf litter (mulch) over the soil surface. In order to test this hypothesis, measurements were made to determine: (i) the influence of leaf litter on oviposition site preference and the depth at which eggs are deposited into the soil; (ii) egg hatch of *L. bethae* under two soil surface conditions (leaf litter-covered or bare ground); and (iii) the influence of leaf litter on the survival of *L. bethae* from egg to adult stage.

6.2 Materials and methods

6.2.1 Adult feeding and oviposition of L. bethae *under two soil surface conditions*

A group of newly emerged adults of *L. bethae* were given a choice of two soil surface conditions, that is, with or without a leaf-litter cover, on which to lay their eggs. Heat-sterilized dry lantana leaves were placed on the soil surface of 10 potted lantana test plants (variety 009 Light Pink) to serve as mulch. The soil type used for both control and treatment plants was silty loam soil containing 9% sand, 68% silt, 21% clay and 2.2% organic matter. The soil surfaces of 10 similar control plants were left

bare (unmulched). Plants with either of the two soil surface conditions were randomly placed in a walk-in cage (4 x 4 x 2 m), and were confined with a group of 200 adult beetles for a period of 12 days. To determine whether the surface cover influenced the depth at which eggs were deposited into the soil, eggs laid in each 1-cm stratum within the uppermost 3-cm of the soil profile were extracted and counted. Adult feeding damage levels on the plants were assessed (Chapter 2) as an indication of the relative amount of time spent by adults on each plant type.

6.2.2 Effect of leaf litter on survival of L. bethae *eggs*

To determine the effect of leaf litter on egg survival, a group of newly laid (24-hour-old) *L. bethae* eggs was allowed to develop and hatch on either bare or leaf litter-covered soil. Eggs were buried at a depth of 0.5 cm in silty loam soil contained in plastic rectangular containers (9 x 7 x 4 cm). To ensure adequate drainage in each container, the base of the container was perforated with multiple fine holes. Each container was 80% filled with soil. Ten treatment dishes with 40 eggs each were covered with leaf litter for 15 days. Another 10 (control) containers with the same number of eggs were exposed to the same conditions except that the soil surface was left bare during the incubation period. The containers of both treatment and control were placed on an exposed platform to ensure adequate exposure to sunlight. An equal amount of water (50ml) was applied on daily basis on both control and treatment containers. After 15 days, which was beyond the incubation period of *L. bethae* eggs under these laboratory conditions, the unhatched eggs and egg shells were recovered from the soil using the standard sieve and floatation technique. Egg hatch was confirmed by counting the number of egg shells recovered from each container. Comparison of percentage egg hatch was made between bare and leaf litter-covered

soil treatments.

6.2.3 Effect of leaf litter on survival of immature stages of L. bethae

To determine the influence of leaf litter on survival of *L. bethae* larvae and pupae, comparisons of adult emergence, duration of development and adult body size were made between leaf litter-covered and bare soil surfaces. Two hundred eggs were seeded into the soil (Chapter 2) around each of twenty plants. The soil surface around 10 of the plants was covered with heat-sterilized dry lantana leaves (mulch treatment) while that of 10 control plants was left bare during the course of larval and pupal development. An equal amount of 500 ml of water was applied onto each plant daily throughout the study. Gauze-covered isolation cages (30 x 30 x 40 cm) were used to cover individual plants in order to capture beetles as they emerged from the soil. Adults started emerging approximately 45 days after egg inoculation. Survival to adulthood was determined by counting the newly emerged beetles from each plant. The body size (body length and head capsule width) and duration of development from egg to adulthood were measured, and these were used as additional measures of the suitability of conditions in the soil during larval and pupal development. The experiment was replicated 10 times, with each plant representing a replicate.

6.2.4 Data analysis

Unless otherwise indicated, the data presented in the tables are the actual number of *L. bethae* individuals. These data were transformed to square-roots and subjected to Student's *t*-test to make comparisons of feeding, oviposition, egg hatch and survival of *L. bethae* between the two leaf litter conditions.

6.3 Results

6.3.1 Adult feeding, oviposition and egg hatch under two soil surface conditions

Leaf feeding damage did not differ significantly between plants growing under the two ground cover conditions (Table 6.1). The presence of leaf litter on the soil surface did not influence oviposition by *L. bethae,* and the number of eggs laid in the ground under both conditions was almost the same (Table 6.1). However, percentage egg hatch was significantly higher (46.5%) in leaf litter-covered than in bare soil (22.8%) (Table 6.1). The depth of oviposition was almost the same on both ground cover conditions, with 95 and 89% of eggs found in the top 1 cm of the leaf litter-covered and bare ground surfaces, respectively (Table 6.2).

Table 6.1 Effect of leaf litter on adult feeding, oviposition and egg hatch in *L. bethae*

Parameter of *L. bethae* performance	Soil surface condition		Statistic	
	Leaf litter-covered	Bare	*t*	*P*
Adult feeding damage (index) (mean ±SE)	2.7 ±0.2	2.8 ±0.2	0.5	0.62
Oviposition (eggs/plant) (mean ±SE)	154 ±29.9	171.6 ±24.4	0.46	0.65
Egg hatch (out of 40) (mean ±SE)	18.6 ±1.9	9.1 ±1.2	4.26	**0.0006**

Table 6.2 Effect of leaf litter on depth distribution of *L. bethae* eggs in uppermost 3 cm of soil profile

Soil stratum	Depth distribution of eggs, with soil surface			
	Leaf litter-covered		Bare	
	Mean (no./plant)	Fraction (%)	Mean (no./plant)	Fraction (%)
Top1 cm	69	95.3	59.8	89
Middle 1 cm	3	4.0	4.8	7
Bottom 1 cm	0.5	0.75	0.5	4

6.3.2 Effect of leaf litter on survival of immature stages of L. bethae

Leaf litter cover had a significant influence on survival of *L. bethae* larvae and pupae (Table 6.3). Survival was 36% in leaf litter-covered soil compared with 20% in bare soil. No significant difference in the duration of development was observed between the leaf litter-covered and bare soil treatments. Although males were smaller (body length: $F = 9.15$, $P = 0.005$, $n = 36$; head capsule width: $F = 10.12$, $P = 0.003$, $n = 36$) than females, the body size of adults of each gender emerging from the two ground cover conditions was not significantly different (body length: $F = 0.01$, $P = 0.92$, $n = 36$; head capsule width: $F = 0.92$, $P = 0.35$, $n = 36$).

Table 6.3 Effect of leaf litter on survival of immature stages of *L. bethae*, from 200 eggs/plant

Parameter of *L. bethae* performance	Soil surface condition		Statistic	
	Leaf litter-covered	Bare	*t*	*P*
Number of adults emerged (mean ±SE)	72.3 ±5.6	40.2 ±4.4	4.49	**0.0003**
Duration of development (d) (mean ±SE)	46.5 ±1.0	47.4 ±0.7	0.77	0.45

6.4 Discussion

Although the presence of leaf litter did not influence oviposition by *L. bethae*, it approximately doubled survival of eggs and other immature stages. Environmental conditions, namely temperature and soil moisture, were more stressful in bare than in leaf litter-covered soils (Godfrey *et al.*, 1995), particularly within the uppermost 1-cm stratum of the soil profile, where the majority of eggs were laid. As also demonstrated in chapters 5 and 7, eggs and larvae are particularly susceptible to moisture stress, which could have contributed significantly to the mortality of both eggs and first-instar larvae in rapidly drying bare soil. In bare soils, newly hatched larvae could be killed either by desiccation stress (Hadley *et al.*, 1989) or starvation due to failure to burrow sufficiently within the hard dry soil to locate the roots of the host plant.

It is conceivable that mortality due to the absence or sparseness of leaf litter cover will occur less often in the moist subtropical regions of South Africa, where heavy infestations of lantana often create closed canopies and a layer of leaf litter, thereby reducing the rapid loss of moisture from the soil (Simelane, personal observation). Grass cover or leaf litter which often surrounds isolated lantana plants in the field could also provide sufficient shade for eggs laid around the stems, and thus reduce the levels of desiccation that the eggs are exposed to. Conservation of soil moisture by leaf litter cover could facilitate both egg development and movement of first-instar larvae during their crucial endeavour to locate host plant roots in the soil. However, prolonged drought, even in the presence of leaf-litter cover, could cause the upper surface layers of the soil to become extremely dry, causing eggs to desiccate and larvae to die.

Whilst shade may be necessary in the conservation of soil moisture required for egg development and larval survival, the accumulation of leaf litter could, however, provide a niche for occupation by undesirable predatory species (Brust & House, 1990) that may reduce survival of immature stages of *L. bethae.* Studies in chapter 9 showed that predation of *L. bethae* pupae was higher in leaf litter-covered than in bare soil.

CHAPTER 7

Influence of temperature, photoperiod and humidity on oviposition and egg hatch of *Longitarsus bethae*

7.1 Introduction

Climatic conditions in the area of release of biological control agents can influence whether establishment succeeds or fails and can determine whether the agent species will proliferate sufficiently to have an impact on the target weed (Debach & Rosen, 1991; Dent, 1991). In order to forecast the range of conditions that will suit an agent species, information is needed on the development, survival and reproduction of the insects under different climatic conditions. Of these, temperature probably has the greatest influence on the geographic distribution and abundance of insects (Howe, 1967; Campbell *et al.*, 1974; Kramer *et al.*, 1991).

Longitarsus bethae predominantly overwinters in the egg stage (Simelane, unpublished data). About 75% of eggs taken from plants that had been exposed to ovipositing females during autumn, failed to hatch until late September when temperatures were increasing during spring. Some early- and middle-stage larvae were also found in the roots during winter, suggesting that larval stages could over-winter as well. For *L. bethae* to succeed, egg hatch should coincide with the onset of summer rains, because soil moisture has been shown to influence both egg development and larval survival (Chapters 5).

The development of a degree-day (DD) model for predicting the time of egg hatch in the field could be useful in determining the likelihood of larvae appearing when soil conditions are suitable. The threshold temperature for egg hatch was therefore determined, and a DD model was developed in the laboratory, to predict the time of egg hatch.

Photoperiod is another abiotic factor that affects the biology and behaviour of insects, sometimes resulting in the development of seasonal morphs (McPherson, 1974; Gorman *et al.*, 1997; Goehring & Oberhauser, 2002; Danks, 2003). Photoperiod is probably the main factor regulating reproductive diapause (McPherson, 1975; Chocorosqui & Pinizzi, 2003). A decline in reproductive output of *L. bethae* was observed during four successive autumn seasons in a naturally-lit quarantine glasshouse in Pretoria, suggesting induction of reproductive diapause by shortening day length (Simelane, unpublished data). Therefore, a study to examine the effect of day length on *L. bethae* was undertaken to determine whether photoperiod affected oviposition, percentage egg hatch and duration of egg development.

Relative humidity is a third climatic factor that could also influence survival of *L. bethae* eggs, because the eggs are deposited on or very near the soil surface (Chapters 4, 5 and 6) where they are subject to greater fluctuations in humidity than at greater depths. Desiccation could be particularly problematic in a year with a relatively warm winter, if the DD requirements are met and embryonic development commences well before the onset of the seasonal rains in summer. For these reasons, the influence of atmospheric humidity and soil moisture on oviposition and egg viability were investigated.

7.2 Materials and methods

7.2.1 Degree-day requirement and effect of temperature on egg development

Developmental rates and percentage survival of *L. bethae* eggs were determined in five separate temperature-controlled growth-chambers set at constant temperatures of 11, 17, 22, 27 and 32°C, respectively. Temperatures in each growth chamber were checked regularly with a digital thermometer. Newly laid eggs (< 24 h old) were placed in batches of 50 on moist filter paper in petri dishes. Five dishes were housed in each of the growth chambers (giving five replicates at each temperature) and were checked on a daily basis to record incubation periods and the proportion of eggs that hatched. The threshold temperature for egg development was estimated by the x-intercept method derived from the linear regression of incubation period against temperature (Arnold, 1959). In this method, it was assumed that the rate of egg development was linear over the temperature range tested, and that the x-intercept of the regression line indicated the minimum temperature at which egg development occurred. The DD above the temperature threshold required to complete egg development was calculated as the reciprocal of the slope of the fitted regression line (i.e., DD = $1/x$). A negative exponential curve was used to describe the relationship between the temperature and the duration of egg development. To obtain a graph for the exponential curve, a non-linear regression of the form $t = a^{-bx}$ was used, where t = duration in days, and x = temperature (°C) (Statistica, 2004). The parameters a and b were estimated by regression. The survival curve of eggs kept under constant temperatures was graphically interpolated as a parabola ($y = ax^2 + bx + c$) (Fornasari, 1995).

7.2.2 Effect of photoperiod on oviposition and egg hatch of L. bethae

A comparative study of the influence of day length on oviposition and subsequent egg hatch was conducted under two photoperiodic regimes (16:8 h L:D and 8:16 h L:D) in the laboratory. Ten pairs of newly-emerged *L. bethae* adults were enclosed with a potted lantana plant (009 LP variety) in a clear plastic cage (30 x 30 x 40 cm) covered with plastic gauze, and kept in a growth-chamber for 10 d under a constant temperature (28 ±1°C), relative humidity (RH) of 40-60%, and at either long (16:8 h) or short (8:16 h) photoperiod. Sand was placed at 3-cm thickness over the soil surface of each pot to facilitate the recovery of eggs. The experiment was replicated 10 times, with each cage (plant) representing a replicate. Eggs were collected from each plant, and sub-samples in batches of 100 were placed on moist filter paper in a closed petri dish and kept at room temperature (25 ±1°C), and in complete darkness inside a ventilated black box till they hatched. Comparison was made of egg counts, pre-eclosion period and percentage egg hatch between the two photoperiodic regimes. Student's *t*-test was used determine significances of differences between responses to the two photoperiodic regimes.

7.2.3 Effect of atmospheric humidity on oviposition

Ten pairs of newly emerged adults were housed in isolation cages (30 x 30 x 40 cm) with one plant grown in 17.5-cm square pots (capacity 2,5 *l*), and were placed in glasshouses with either low (30 to 45% RH) or high (70 to 95% RH) humidities for a period of 14 d. In both humidity regimes, the temperature ranged between 22 ±2°C at night and 28 ±2°C during the day, and the photoperiod was 14:10 h (L:D). Water (~200ml) was applied on daily basis on both control and treatment plants. In each isolation cage, eggs were laid in the sand covering the soil surface to a depth of 3 cm.

After a 14-d oviposition period, eggs were recovered from the sand and counted to compare the effect of the two humidity regimes.

7.2.4 Effect of relative humidity on egg hatch

Relative humidities were maintained at constant levels within glass, screw-capped jars (10 x 10 x 20 cm) with saturated solutions of salts or mixtures as described by Winston and Bates (1960). The jars were kept in a temperature-controlled growth-chamber set at a constant (25°C) temperature. Six relative humidities were achieved: 21±1% with sodium hydroxide (NaOH); 45±1% with pure honey; 64±1% with 25 ml honey + 10 g sugar; 74±1% with sodium chloride (NaCl); 86±1% with potassium chloride (KCl); and 95% with potassium dichromate ($K_2Cr_3O_7$). Vial caps measuring 2 cm in diameter, were used to expose 50 newly laid *L. bethae* eggs (24 h old). The vial caps with eggs were placed on a mesh platform raised 5 cm above the salt solution and left for 3, 6, 9, and 12 d before being transferred onto moist filter paper in a petri dish. The eggs were then incubated at 25°C on moist filter papers to determine viability after exposure to the various humidity levels for different lengths of time. The experiment was repeated three times. Data were subjected to analysis of variance (ANOVA), and Fisher's protected least significant difference (LSD) was applied for separation of means (Statistica 6.1, 2004). The square-root transformation of counts did not change the significance of the analysis; thus the results and means from untransformed data are reported.

7.3 Results

7.3.1 Degree-day requirement and effect of temperature on egg development

The effect of temperature on the rate of development of *L. bethae* eggs is presented in Figure 7.1. At 12°C, eggs failed to hatch. Percentage egg hatch increased rapidly with increase in temperature from 17 to 27°C, for which the regression equation was found to be $y = 0.0056x - 0.063$ ($r^2 = 0.9759$). Calculated as the reciprocal of the slope of this regression line, DD required for complete embryo development was found to be 178.6 d ° above the threshold temperature. The regression line showed that the temperature had to be over the threshold (T^o) of 11.3°C for egg development. The relationship between duration of egg development and temperature was best described by a negative exponential equation ($t = 125.7^{-0.086x}$), where t = duration in days, and x = temperature, which shows that developmental times decreased with increasing temperatures.

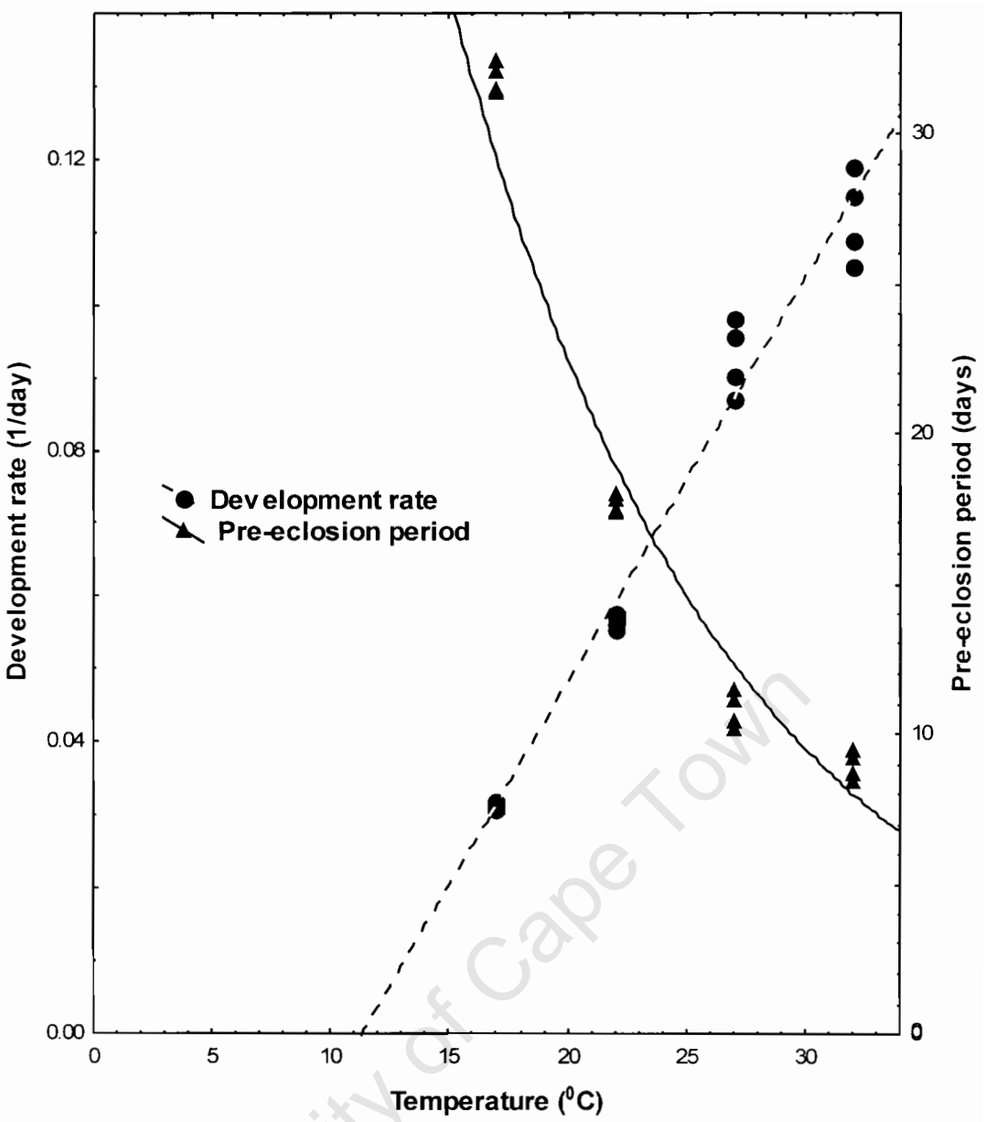

Fig. 7.1 Duration of development (pre-eclosion period) and rate of development of *L. bethae* eggs under constant temperature.

The survival of eggs kept under constant temperature is graphically interpolated as a parabola ($y = -0.45x^2 + 60.66x - 177.57$) (Fig. 7.2). Survival of *L. bethae* eggs varied from 12 to 65% at 32 and 17°C, respectively, and was optimum at approximately 23°C. Although pre-eclosion period was longer at 17°C, egg survival at 17 and 22°C,

did not differ significantly, averaging 56 and 55%, respectively. A significant decline in egg survival was observed when temperature increased from 27 to 32 °C ($F = 53.61$; $P < 0.001$; $n = 50$), decreasing from 45% at 27°C to 27% at 32°C. One hundred percent mortality was observed when eggs were exposed to a constant temperature of 37°C, indicating that the upper lethal temperature limit was between 32 and 37°C.

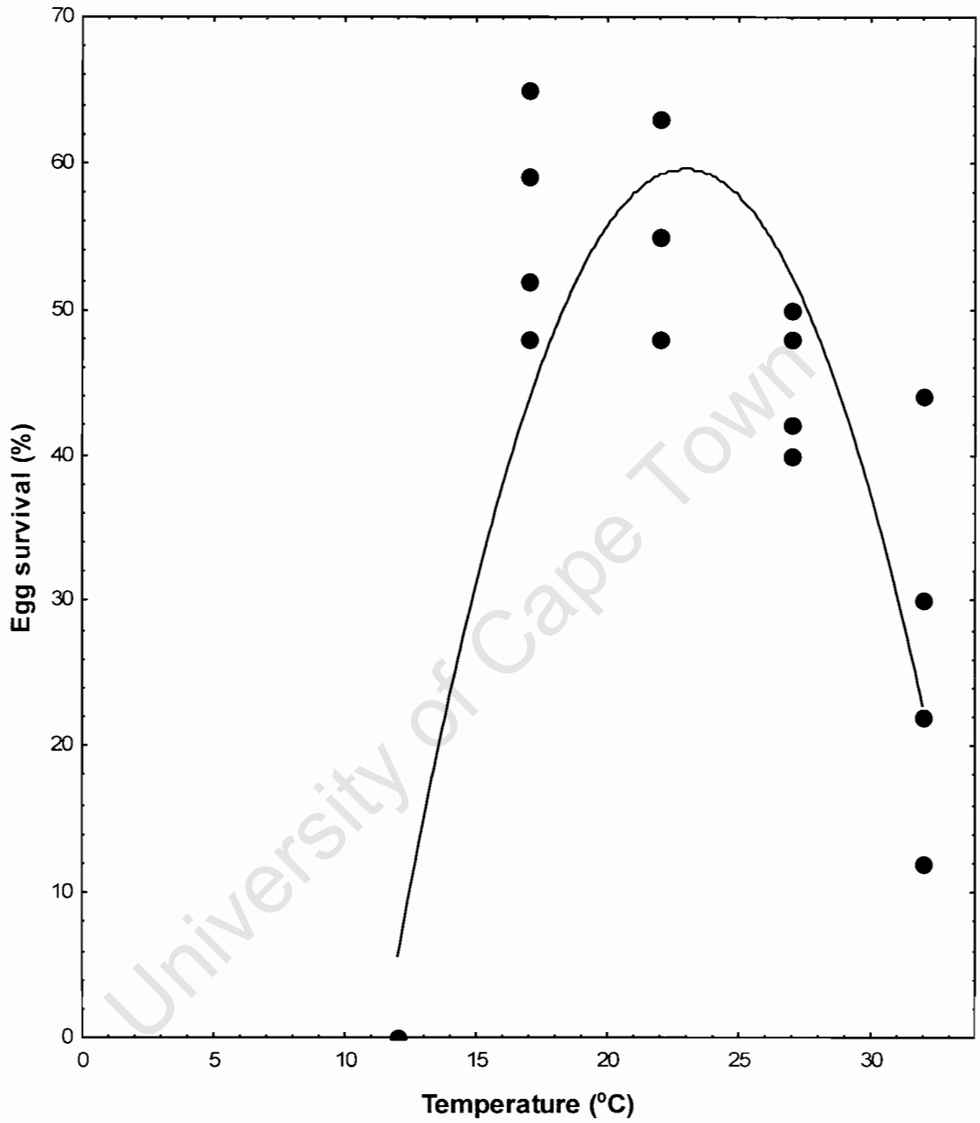

Fig. 7.2 Percentage survival of *L. bethae* eggs under constant temperatures.

7.3.2 Effect of photoperiod on oviposition and egg hatch of L. bethae

There was no significant difference in number of eggs laid by females under the short and long photoperiod regimes during the 10-d period (Table 7.1). Exposure of newly emerged adults to either short or long day-length during oviposition had no effect on subsequent egg-hatch. Neither percentage hatch nor incubation period were affected by photoperiod regime (Table 7.1). Production of viable eggs also showed that mating activity was not hampered by the reduction of photoperiod.

Table 7.1 Oviposition of *L. bethae* during a 10-d period and subsequent rate of egg development and hatch under two photoperiodic regimes. Data are mean ±SE.

Oviposition or egg development parameter	Long day-length (16:8) (L:D)	Short day-length (8:16) (L:D)	*t*-value	*P*
Oviposition (eggs/plant)	276 ±31	265 ±37	0.21	0.83
Pre-eclosion period (d)	12.4 ±0.58	12.5 ±0.54	0.29	0.78
Egg hatch (%)	59.7 ±4.2	61.3 ±3.7	0.34	0.74

7.3.3 Effect of atmospheric humidity on oviposition

Oviposition rates were almost the same at both low and high humidity regimes (t = -0.255; P = 0.80). During the 14-d period, a group of 10 females laid 745.1 ±38.4 (±SE) and 759.9 ±43.1 eggs under low and high humid conditions, respectively.

7.3.4 Effect of relative humidity on egg hatch

Longitarsus bethae eggs were sensitive to aridity, and no eggs hatched after exposure to humidities below 63% for more than 3 d at 25°C (Table 7.2). Only 1.5% eggs hatched when they were exposed to 63 ±1% RH for up to 3 d. About 47.5% of eggs hatched when exposed to 74 ±1% RH for 3 d, but viability declined when the eggs were kept at this humidity level for more than 3 d, with only 3% hatching after nine days of exposure to 75% RH. Optimal egg survival was observed when eggs were kept between 85 and 95% RH for up to 12 d. Although a slight decline in egg hatch was observed on the 12th day at 85 ±1% RH, the viability of eggs kept at 95% RH did not decline during the 12-day pre-eclosion period. Humidity had no significant influence on the duration of the pre-eclosion period (Table 7.3).

Table 7.2 Percentage survival of *L. bethae* eggs kept at six constant humidity levels and at a constant temperature (25°C) for 3 to 12 d

Atmospheric humidity (% RH)	Viability of eggs (%, mean ±SE) after exposure for			
	3 d	6 d	9 d	12 d
21-22	0	0	0	0
45-46	0	0	0	0
65-66	1.5 ±1.0*	0	0	0
74-75	47.5 ±5.2a	24.8 ±3.8b	3.0 ±1.3c	2.0 ±0.8c
86-87	52.5 ±3.2a	43.8 ±4.8a	49.0 ±3.4a	40.5 ±2.62b
95	51.0 ±5.8a	48.5 ±4.1a	50.0 ±5.0a	52.5 ±5.0a

Means within the same humidity level followed by the same letter are not significantly different, P = 0.05, using LSD test. * = Value was not included in statistical analysis.

Table 7.3 Pre-eclosion period of *L. bethae* eggs kept at six constant humidity levels and at a constant temperature (25°C) for 3 to 12 d

Atmospheric humidity (% RH)	Pre-eclosion period (d, mean ±SE) of eggs kept at constant humidity for 3 to 12 d			
	3 d	6 d	9 d	12 d
21-22	–	–	–	–
45-46	–	–	–	–
65-66	–	–	–	–
74-75	12.2 ±0.2	12.9 ±0.1	12.6 ±0.2	12.7 ±0.2
86-87	12.3 ±0.2	12.1 ±0.1	12.4 ±0.2	12.3 ±0.3
95	12.2 ±0.1	12.6 ±0.2	12.5 ±0.2	12.6 ±0.3

- = Complete mortality before egg hatch.

7.4 Discussion

The establishment of biological control agents depends upon a number of factors, including climatic conditions in the area of release being conducive to the survival and growth of insects (Dent, 1991; Debach & Rosen, 1991). In the current study, both humidity and temperature had an effect on egg development. Although climatic factors interact under natural conditions, the present study suggests that both temperature and humidity could play some role in the population dynamics of *L. bethae*. In the event that eggs are the over-wintering stage, as shown in previous investigations (Simelane, unpublished data), the DD model determined in the present study will enable prediction of egg hatch in the field. The data analysis showed that the temperature threshold for eggs to hatch was 11.3°C, and yet no egg hatch was observed at 12°C. A reason for this discrepancy may be that continuous exposure to low temperatures allows some embryonic development but not the physiological

adjustments necessary for eclosion of neonates from the egg (Force & Messenger, 1964). The complete arrest of egg hatch during constant exposure to 12°C and resumption of normal development on restoration to 25°C may be of some use in preserving and distributing the agent during a mass-release programme. However, there are limitations on how long this type of manipulation can be exploited because mortality of *L. bethae* eggs increased after constant exposure to 12°C for more than two months. (Simelane, unpublished data).

Photoperiod alone did not induce reproductive diapause. The diapause phase observed during autumn in a naturally-lit glasshouse could have been a result of several factors operating in concert such as photoperiod and temperature signals (Zaslavski *et al.*,1999), and the condition of the host plant (Goehring & Oberhauser, 2002; Perdikis *et al.*, 2004). Low soil temperature may either delay the development or trigger diapause of immature stages of *L. bethae* during winter months.

Whilst the study showed that *L. bethae* will lay within a wide range of atmospheric humidity, eggs need high humidity or damp surface layers of soil for successful development. For this reason, during periods of low rainfall under normal summer temperatures, egg survival will probably be adversely affected, especially in exposed habitats where the surface of the soil becomes extremely dry. It seems probable that favourable temperature and humidity levels necessary for egg survival will prevail only during the wet summer season. As demonstrated in chapters 5 and 6, most of the unfed first-instar larvae and pupae could be eliminated by desiccation stress which prevails in dry soil conditions. Because differing levels of humidity did not influence

the period of egg development, the timing of egg hatch would be dictated by temperature, regardless of the levels of humidity.

In order to fully understand the population dynamics of this agent, future investigations should endeavour to give a full description of the phenology of *L. bethae,* which will take into account the effects of temperature on development of all life stages, including pre-oviposition and oviposition periods. To formulate a field emergence model for *L. bethae*, a field-based DD model similar to that developed by Skinner *et al.* (2004), whereby plants with soil cores containing an overwintering stage were held at constant temperatures, should be developed after the insect has been released into the environment.

CHAPTER 8

Effect of herbivory by *Teleonemia scrupulosa* on the utilization of a shared host, *Lantana camara*, by *Longitarsus bethae*

8.1 Introduction

The weed, *Lantana camara,* is currently a host to at least 12 introduced insect herbivores in South Africa (Baars & Neser, 1999; Simelane, 2005). These are mainly leaf-, flower- and shoot-attacking insects. One of these, the sap-sucking lacebug, *Teleonemia scrupulosa* Stäl (Heteroptera: Tingidae), has been rated as the most damaging and widely distributed herbivorous insect throughout the geographic range of lantana in South Africa (Cilliers & Neser, 1991; Baars & Neser, 1999). It is therefore probable that, if released in South Africa, adults of the root-feeding flea beetle, *L. bethae*, will frequently encounter plants that are either damaged by, or harbouring, *T. scrupulosa*.

Several studies have demonstrated that feeding damage by one or more insect herbivores can lead to changes in the chemical composition of the plant foliage, thereby altering the suitability of the plant for survival, growth, fecundity and development of any subsequent herbivores (Fowler & Lawton, 1985; Harrison & Karban, 1986; Gange & Brown, 1989; Karban & Myers, 1989; Moran & Whitham, 1990; Masters & Brown, 1992; Inbar *et al.*, 1995; Salt *et al.*, 1996; Bezemer *et al.*, 2003; Dam *et al.*, 2005). For example, the moth, *Orgyia vestusta* (Lepidoptera: Lymantriidae), attains lower pupal weights and produces fewer eggs when fed on

branches of bush lupine, *Lupinus arboreus* Sims, that had been damaged by another moth, *Platyprepia virginalis* (Lepidoptera: Arctiidae), earlier in the season (Harrison & Karban, 1986). In species in which immature instars have limited dispersal ability, females may maximize their fitness by avoiding plants that are unsuitable for larval development and survival (Pilson & Rausheer, 1988; Wise, 2002). Females of the potato leaf beetle, *Leptinotarsa juncta* Germar (Chrysomelidae), show a strong oviposition preference for undamaged plants, and this behaviour has presumably been selected for because their larvae develop more slowly when feeding on plants already hosting the root-feeding flea-beetle, *Epitrix fuscula* Crotch (Chrysomelidae), (Wise & Weinberg, 2002). Cohabitation on the same plant is not always detrimental, as shown by Williams & Myers (1984), who found that fall webworms grew faster and attained higher pupal weights when fed on leaves of *Alnus rubra* L. previously attacked by western tent caterpillars than when fed on non-infested leaves.

Although the larval stages of *L. bethae* will occupy a niche (i.e. roots) not utilised by any of the other introduced biological control agents in South Africa, indirect competition may occur because of overall declines in foliar quality induced by other herbivores using various other plant parts, either simultaneously or sequentially (Masters & Brown, 1992; Inbar *et al.*, 1995; Bezemer *et al.,* 2003). Such interactions have occurred in other biological control programmes where more than one species of agent has been released, but none of these examples has ever had a detrimental outcome for the biological control programme, because the agents are, for the most part, able to cope with detrimental interactions, and their combined damage is often greater than that which either species would cause alone (McEvoy, 1984; Crawley,

1989; Pemberton & Turner, 1990; Harris, 1991; McEvoy *et al.*, 1993; Saner *et al.*, 1994; Hoffmann & Moran, 1998).

It was hypothesized that *L. bethae* will compete indirectly with *T. scrupulosa,* but that the detrimental effects will be minimised because *L. bethae* females will be able to detect and avoid plants that are hosting, or have already been damaged by colonies of the tingid. To test these assumptions, the influence of the presence of *T. scrupulosa,* or its damage, on the aggregation, feeding and oviposition preference and larval survival of *L. bethae* were examined.

8.2 Materials and methods

8.2.1 Aggregation, feeding and oviposition preferences of adult L. bethae *on plants damaged by* T. scrupulosa *adults*

To obtain *L. camara* plants with different levels of *T. scrupulosa* damage, individual potted plants were confined in cages (55 x 55 x 95 cm) with either three, six or nine *T. scrupulosa* adults for a period of 5 d. Five plants were exposed to each density of the tingid and five plants were kept free of *T. scrupulosa* adults to serve as undamaged controls. After the *T. scrupulosa* adults had been removed, all of the plants were placed in a walk-in cage (4 x 4 x 2 m) in which 250 unsexed *L. bethae* adults (5 d old) were released and left for 12 d. The number of *L. bethae* adults on each plant was recorded daily to determine the relative attractiveness of the plants in each damage category. The plants were then removed and inspected for feeding damage by *L. bethae* adults. Oviposition intensity was determined by counting eggs extracted from the soil surface of each plant pot.

8.2.2 Aggregation and oviposition preference of L. bethae *on plants harbouring* T. scrupulosa *nymphs*

Five plants were confined in a cage (55 x 55 x 95 cm) with a density of either three, six or nine pairs of *T. scrupulosa* adults for a period of five days to facilitate oviposition on the plants. After five days, the adults *T. scrupulosa* were removed and the plants were kept for another 10 d to allow egg hatch and development of *T. scrupulosa* nymphs to reach the second nymphal stage. When approximately 80% of the nymphs had moulted into the second stage, all of the plants were randomly placed in a walk-in cage (4 x 4 x 2 m), and were exposed to 250 newly emerged (< 5 d old) *L. bethae* adults for 20 days. Five healthy plants were also among this mix to serve as uninfested controls. During the 20-day period, *L. bethae* adults fed and laid eggs. Feeding and oviposition preference among plants and the relative number of *L. bethae* adults per plant were measured as described in experiment 8.2.1.

8.2.3 Survival of L. bethae *from egg to adulthood on plants infested with* T. scrupulosa *nymphs*

The effect of *T. scrupulosa* infestation on the survival of the immature stages of *L. bethae* was determined by counting the number of *L. bethae* adults emerging from plants initially infested with *T. scrupulosa* at population densities of either three, six, or nine pairs of adults per plant. Exposure of plants to *T. scrupulosa* lasted for five days to facilitate oviposition. After the removal of the *T. scrupulosa* adults, each plant was inoculated with a population of 260 *L. bethae* eggs. Isolation cages (30 x 30 x 40 cm) were placed on individual plants to confine and capture adult progeny of both agents as they emerged from the individual plants. Adult progeny of both agents were counted and removed on a daily basis to minimize over-exploitation of the plants. The

experiment was terminated 70 d after egg inoculation, that is, when all the surviving *L. bethae* had completed their development. Head capsule widths and body lengths of both sexes of newly emerged *L. bethae* adults, and their duration of development from egg to adult, were determined and used as additional measures of the suitability of the *T. scrupulosa*-infested plants. Each treatment was replicated five times, with single plants representing a replicate.

8.2.4 Data analysis

Regression analyses were conducted to determine relationships between the population density of lace-bug nymphs and oviposition intensity by the flea beetle. This procedure was also used to determine the relationship between the density of *T. scrupulosa* nymphs and the survival of *L. bethae* from egg to adult. Analysis of variance was used to determine the statistical significance of differences in feeding, oviposition, duration of development and body size of the flea beetles reared on plants damaged by or infested with *T. scrupulosa* at different population levels. Fisher's protected least significant difference (LSD) was applied to separate means. Data were initially square root-transformed to reduce problems of unequal variances. However, untransformed results are presented.

8.3 Results

8.3.1 Aggregation, feeding and oviposition preferences of adult L. bethae *on plants damaged by* T. scrupulosa *adults*

During the 12-d period, an inverse correlation was apparent between the intensity of aggregation, feeding and oviposition by *L. bethae* and the intensity of prior damage to

the plants by *T. scrupulosa* (Table 8.1), but these trends were not quite statistically significant ($P > 0.05$).

Table 8.1 Aggregation, feeding and oviposition preferences of *L. bethae* among plants previously damaged by *T. scrupulosa*. Data are mean ±SE

Damage intensity (initial no. *T. scrupulosa* adults/plant)	Aggregation (no. *L. bethae* adults/plant)	Feeding (damage level by *L. bethae*)	Oviposition (*L. bethae* eggs/plant)
0 (Control)	3.2 ±0.33	2.5 ±0.28	163.8 ±20.4
Low (6)	3.3 ±0.46	2.8 ±0.25	164.5 ±32.4
Moderate (12)	2.6 ±0.50	2.5 ±0.28	152.8 ±36.8
High (18)	2.0 ±0.44	2.3 ±0.25	78.8 ±44.7

8.3.2 Aggregation and oviposition preference of L. bethae *on plants harbouring* T. scrupulosa *nymphs*

A substantial number of *L. bethae* adults gradually migrated from highly to less *T. scrupulosa*-infested plants during the 20-d period (Fig. 8.1). The number of flea beetles that had colonized heavily infested plants was 6.0 ±0.15 (mean ±SE) on the 2nd day, and gradually declined to 0.6 ±0.58 on the 20th day. In contrast, the number of adult *L. bethae* that had colonized each uninfested (control) plant was 4.7 ±0.35 on the 2nd day, and increased to 7.1 ±0.33 on the 20th day. The emigration of flea beetles from *T. scrupulosa*-infested plants explained the significant ($P < 0.05$) decline in the rate of oviposition by the flea beetles on infested plants during the same period (Fig.

8.2). The number of eggs deposited per plant was negatively correlated ($y = 178.2 - 0.77x$; $r^2 = 0.64$; $P < 0.001$) with the number of *T. scrupulosa* nymphs per plant, with female *L. bethae* laying, on average, 62.9 ±8.2 ($n = 9$) and 199.0 ±19.3 ($n = 6$) eggs on infested and uninfested plants, respectively, during the 20-d period.

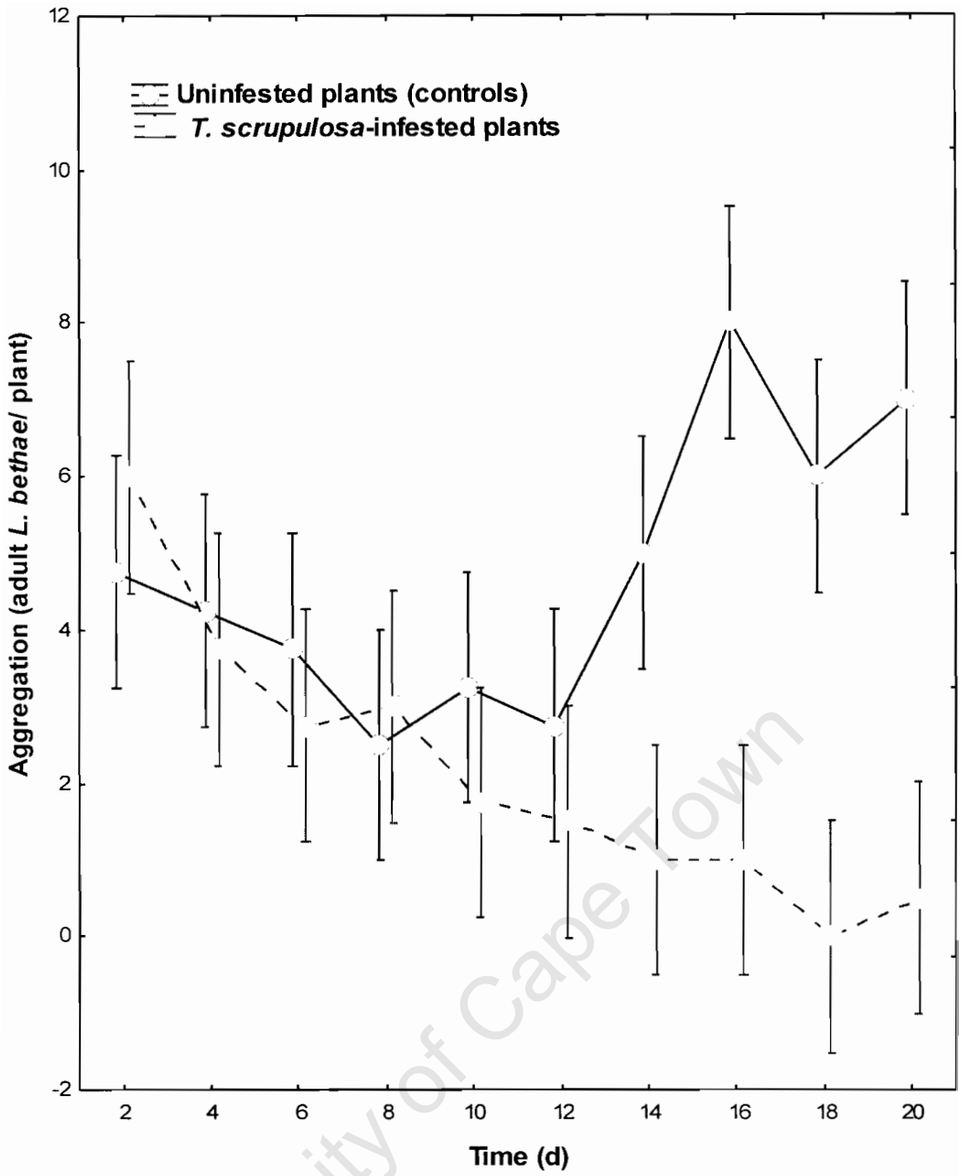

Fig. 8.1 Migration of adult *L. bethae* from *T. scrupulos*a nymph-infested (test) to uninfested (control) plants during a 20-d period.

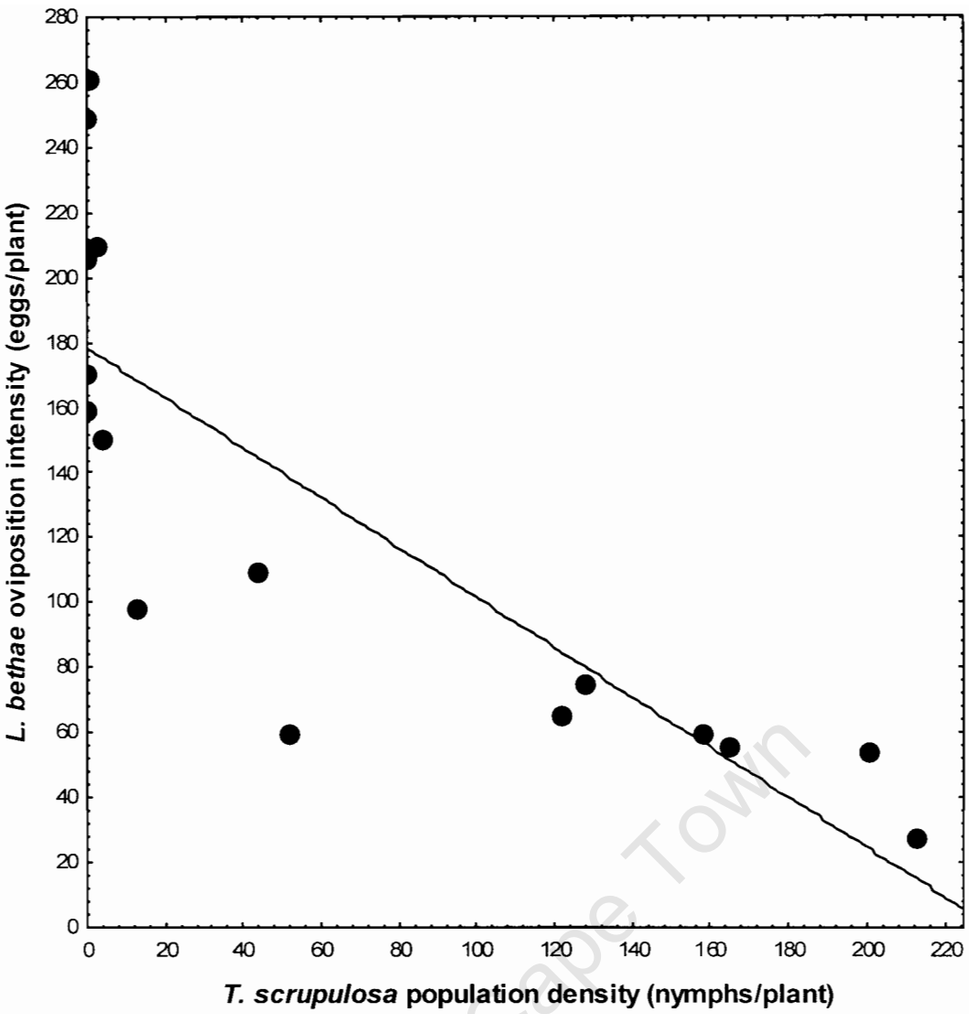

Fig. 8.2 Relationship between oviposition intensity of *Longitarsus bethae* and population density of *Teleonemia scrupulosa* nymphs on the same plant.

8.3.3 Survival of L. bethae *from egg to adulthood on plants infested with* T. scrupulosa *nymphs*

An increase in the number of *T. scrupulosa* nymphs present per plant was associated with a significant decline in the percentage survival of *L. bethae* from egg to adulthood ($y = 54.6 - 0.32x$; $r^2 = 0.64$; $P = 0.0003$) (Fig. 8.3). Survival of *L. bethae* on uninfested plants ranged from 18.5 to 27.7% (mean ±SE: 21.3 ±2.0%) compared with 1.5 to 7.3% (4.3 ±1.0%) on highly *T. scrupulosa*-infested plants. The duration of development was not influenced by the population density of *T. scrupulosa* on the plants ($F_{(3,12)} = 1.59$; $P = 0.24$) (Table 8.2).

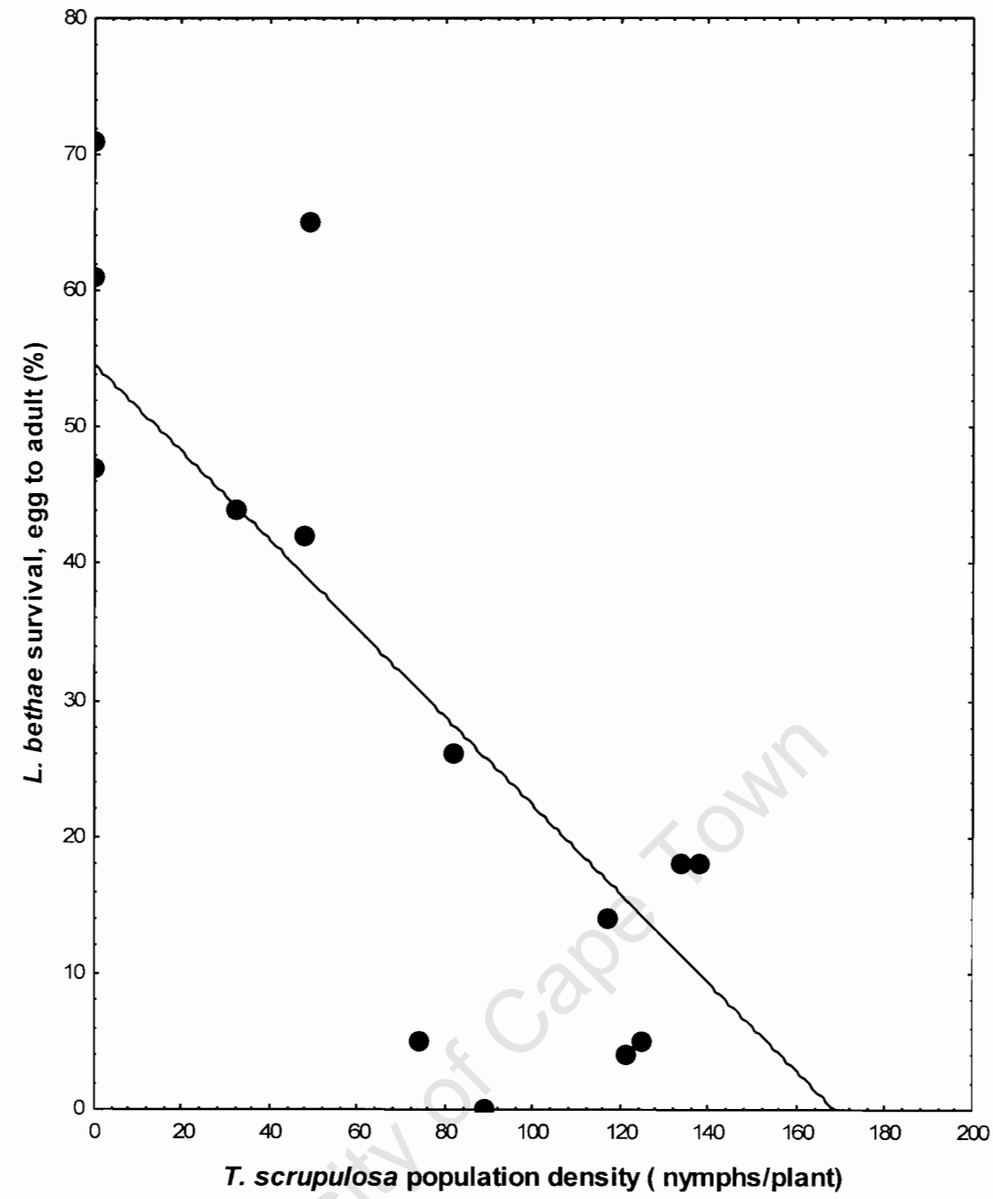

Fig. 8.3 Relationship between percentage survival of *Longitarsus bethae* from egg to adulthood, and population density of *Teleonemia scrupulosa* nymphs on the same plant.

Table 8.2 Duration of development of *Longitarsus bethae* from egg to adult on plants infested with *Teleonemia scrupulosa* nymphs at various population densities

T. scrupulosa population density (initial no. adults/plant)	Duration of development of *L. bethae*, egg to adult (d)	
	range	mean ±SE
Control (0)	40 – 42	40.93 ±0.76
Low (6)	39 – 43	40.73 ±0.83
Moderate (12)	41 – 44	41.94 ±0.78
High (18)	41 – 45	43.11 ±1.06

The study showed no variation in body length and head capsule width among newly emerged flea beetles reared on plants infested at various levels with *T. scrupulosa* nymphs (body length: $F_{(3,72)} = 1.44$; $P = 0.24$, head capsule width: $F_{(3,72)} = 1.94$; $P = 0.13$) (Table 8.3). However, females were significantly larger (body length: $F_{(1,72)} = 14.22$; $P = 0.0003$, head capsule width: $F_{(1,72)} = 6.66$; $P = 0.012$) than males in all the treatments.

Table 8.3 Effect of *Teleonemia scrupulosa* population density on size of *Longitarsus bethae* adults. Data are mean ±SE

T. scrupulosa population density (initial no. adults/plant)	Size (mm) of adult *L. bethae*			
	Body length		Head capsule width	
	Male	Female	Male	Female
Control (0)	1.84 ±0.04	1.90 ±0.03	0.49 ±0.02	0.52 ±0.02
Low (6)	1.76 ±0.02	1.90 ±0.03	0.44 ±0.03	0.53 ±0.02
Moderate (12)	1.76 ±0.04	1.89 ±0.03	0.45 ±0.01	0.47 ±0.02
High (18)	1.85 ±0.05	1.91 ±0.04	0.49 ±0.02	0.51 ±0.02

8.4 Discussion

During the course of the experiment, the *L. camara* plants gradually deteriorated due to damage caused by the *T. scrupulosa* nymphs, causing the flea beetles to emigrate from them and aggregate on less-infested or uninfested plants. The overall trend was that uninfested or less-infested plants that enabled better larval survival were frequently chosen as oviposition sites in preference to those that were highly infested with *T. scrupulosa* and provided poor conditions for larval survival. The emigration of *L. bethae* as a result of dense *T. scrupulosa* infestations explains the reduction in oviposition by *L. bethae* onto such plants. These findings are consistent with previous studies that demonstrated that herbivore damage could deter subsequent feeding and oviposition, and retard larval growth of other specialist insects (Fowler & Lawton, 1985; Harrison & Karban, 1986; Gange & Brown, 1989; Karban & Myers, 1989; Moran, 1990; Masters & Brown, 1992; Inbar *et al.*, 1995; Salt *et al.*, 1996; Bezemer *et al.*, 2003; Dam *et al.*, 2005).

Although the present study indicated that the abundance of *T. scrupulosa* might have a negative impact on *L. bethae* larvae, it is likely that this will occur less often in nature since the flea beetles will emigrate from plants with high densities of lace bugs to aggregate and oviposit on plants with lower infestations. In the event that *L. bethae* flourishes and becomes abundant in the field, the side-effect of root-feeding damage on plant primary and secondary chemistry, and the growth and development of above-ground herbivores (such as *T. scrupulosa*) remains uncertain. However, Dam *et al.* (2005) found that root-feeding can significantly alter the nutritional quality of the shoots and change secondary chemical levels, thereby hampering the performance of specialist shoot feeders. Nonetheless, McEvoy *at al.* (1993) and Saner *et al.* (1994) downplayed the issue of competition as a factor limiting successful biocontrol of weeds. Instead, Crawley (1989), Harris (1991) and Hoffmann & Moran (1998) found that better control could be obtained by cumulative stress imposed by several control agents that attack the plants in a temporal sequence or attack different plant parts. Indeed, the introduction of the root-feeding flea beetle, *Longitarsus jacobaeae* Waterhouse, and the leaf-feeding cinnabar moth, *Tyria jacobaeae* L., in the states of Oregon and California resulted in a substantial decline in field populations of ragwort, *Senecio jacobaeae* L. (McEvoy, 1984). Other examples of enhanced weed control from the combined action of natural enemies in biological control systems include prickly-pear cacti in California (Goeden *et al.*, 1967), ragwort in the Pacific Northwest (Hawks & Johnson, 1978; McEvoy, 1984; Pemberton & Turner, 1990) and skeleton weed in Australia (Groves & Williams, 1975). McEvoy (1984) and McEvoy *et al.* (1993) found that root-feeders increased in abundance and their attack subsequently resulted in population crashes of their host plant despite simultaneous attack by

folivores. Therefore, the combined effect of the two agents (*T. scrupulosa* and *L. bethae*) under natural field conditions is expected to be complementary, particularly if lantana is attacked at different times or in different spatial locations.

CHAPTER 9

Influence of arthropod generalist predators on immature stages of *Longitarsus bethae*

9.1 Introduction

Despite disruption of the soil by tillage implements, crop rotations and application of soil pesticides, which often cause the decline of soil-dwelling predator populations and their prey items in agro-ecosystems (Brust *et al.*, 1986), ground predators are considered to be important control agents of agricultural pests (Lang, 1999; Jones & Bradford, 2001; Vichitbandha & Wise, 2002; Cardinale *et al.*, 2003; Rypstra & Marshall, 2005; Sunderland *et al.*, 1995). Studies by Stinner, *et al.*, 1984, Brust *et al.*, (1986) and Brust & House (1990) demonstrated that predator activity is much greater in no-tillage than in conventional tillage systems. Lantana ecosystems are likely to be stable and non-disruptive for most ground-dwelling arthropods, including generalist predators that may reduce overall densities of root-feeding herbivores such as *L. bethae.*

Previous studies indicated a strong relationship between forest canopy and arthropod predator abundance, species richness and family diversity, and that forest clearing or deforestation often results in loss of arthropod species richness (Watt *et al.*, 2002; Morris *et al.*, 1999). Similarly, lantana-infested areas are often characterised by high plant densities, forming canopies that could enhance predator species richness and composition. This could have profound implications on the population dynamics of

lantana biocontrol agents such as *L. bethae*. Although the studies described in chapter 6 demonstrated that egg and larval survival of *L. bethae* was higher in leaf-litter-covered than in bare soils, there is evidence that leaf litter creates a habitat that fosters an increase in predator abundance and activity (Brust *et al.*, 1986; Brust & House, 1990; O'Neal *et al.*, 2005; Watt *et al.,* 2002).

Insect herbivores living below ground are subject to attack by a wide range of species of predators, so it is logical to consider predation in the context of a community of natural enemies. Previous studies have shown that some carnivorous beetle larvae and spiders are active throughout the year, and that these could reduce populations of both active and over-wintering stages of below-ground herbivores (Juen *et al.*, 2003; Ruano, *et al.*, 2004). Ants were reported to constitute 67.6% of all the ground predators of sugarcane soil pests (Cherry, 2003). Similar findings by Morris *et al.* (1999) showed that more individuals of ants and more spider species were captured in olive plantation than all other groups.

It was hypothesized that a suite of generalist predators will play a substantial role in the population dynamics of *L. bethae* in South Africa, and that the degree to which this happens will be influenced by the presence of accumulated leaf litter within stands of *L. camara*. To test this prediction, samples were taken from within *L. camara* stands to identify potential predators of *L. bethae*. The destructiveness of these predators on the immature stages *L. bethae* was measured in the field and in the laboratory to determine the possible impact that predation might have in terms of the successfulness of the biological control agent.

9.2 Materials and methods

9.2.1 Relative abundance and potential impact of arthropod predators on L. bethae *eggs and pupae*

Heat-sterilized soil was added to 20 containers (9 cm in diameter and 2 cm high) with their bases removed and replaced with a fine mesh to facilitate drainage while retaining eggs and other immatures of *L. bethae*. One hundred and fifty *L. bethae* eggs (less than 2 d old) were spread evenly on the soil surface of each of 10 containers, and were covered by a thin (0.5 cm) layer of soil. Ten pupae of *L. bethae* were also placed at 0.5 cm below the soil surface in each of 10 other identical containers. Both sets of containers were placed under a patch of lantana plants, approximately 1m tall, in such a way that the top edge of each container was level with the surface of the surrounding soil. Half of the containers of both eggs and pupae were covered with leaf litter while the other half were left bare for three consecutive days. Water was applied as a fine spray daily onto the soil surface of all treatments to prevent desiccation of the immature stages. A total of ten pitfall traps, each measuring 8 cm in diameter and placed 50 cm away from leaf litter-covered and bare ground treatments of both eggs and pupae, were set up in order to sample potential predators of the immature stages of *L. bethae*. Each trap contained a solution of water, ethylene glycol and detergent (Wendell, 1975), and was sunk to a depth at which the top edge was at ground level. The eggs and pupae recovered from each container at the end of the 3-d period were counted and recorded. At the same time, arthropods caught in the pitfall traps were taken to the laboratory and samples were drained through paper towels, sorted, counted and identified by relevant specialists from the Biosystematics Division of the ARC-Plant Protection Research Institute of

South Africa and the Iziko South African Museum, Cape Town. Survival of eggs and pupae in the leaf litter-covered containers was compared with that in the bare-soil treatment. Within 10 d of completion of the experiment, an insecticide was applied twice onto the soil at the experimental site, and the lantana plants under which eggs and pupae were placed were destroyed to prevent any unintentional escape of *L. bethae* into the environment. All field experiments were carried out in an abandoned pasture owned by the Agricultural Research Council at Rietondale Research Centre (S25°43'36.7"; E23°14'03.3"), Pretoria, South Africa, in October 2004.

9.2.2 Predation upon L. bethae *eggs and pupae by* Technomyrmex *sp. ants in the laboratory*

A 10-cm diameter petri dish containing a small amount of honey to attract ants was placed below lantana plants at Rietondale Experiment Farm in Pretoria. After 24 h, a considerable number of *Technomyrmex* sp. were foraging in the dishes. Batches of one hundred randomly selected ants were removed and confined in dishes with 100 *L. bethae* eggs that had been sprinkled haphazardly and covered with a 0.5-cm thick layer of soil. At the same time, 10 pupae of *L. bethae*, also buried at 0.5 cm, were confined separately with 20 *Technomyrmex* sp. The dishes with ants and their potential prey item (eggs or pupae) were held in the laboratory in 5-*l* ant-proof containers for 24 h at a temperature regime which ranged between 20 ±2 and 28 ±2°C, at night and during the day, respectively, and with a photoperiod of 16:8 h (L:D). Eggs were recovered from each petri-dish after 24 h and percentage predation of both eggs and pupae during a 24-h period was determined from the number of surviving individuals. The experiment was replicated 10 times.

9.2.3 Effect of Technomyrmex sp. *ants on survival of* L. bethae *from egg to adulthood*

A number of *Technomyrmex* sp. ants, initially attracted by a meat bait placed on 10 potted lantana plants in a lantana-infested area in Pretoria, were confined in ant-proof cages (0.55 x 0.55 x 0.95 m) for 5 d. Plants were initially smeared with concentrated honey to provide ants with a source of carbohydrates whilst they formed nests in the soil. When holes surrounded by excavated soil particles, which were the sign of ant colony establishment, became visible on the soil surface, each plant was inoculated with 250 *L. bethae* eggs. Eggs used in the study were 12 d old, with head capsule visible on the embryo within, and were therefore expected to hatch within 24 h. The plants were confined individually in the ant-proof cages for 70 d, and watered daily until all adult *L. bethae* progeny emerged. Newly emerged adults were collected between the 48th and the 70th day. The same procedure was followed using five control plants, which had been rendered ant-free by submerging their pots in water for 24 h in order to eradicate soil-inhabiting ants. On completion of the experiment, ants were recovered from the soil using a method similar to the egg collection technique described in chapter 2. The relationship between the total number of ants recovered and the number of the newly emerged adults was determined.

9.2.4 Data analysis

The data were subjected to multiple regression analysis and correlation techniques to discern which arthropod predator contributed the most to either egg or pupal predation. Regression analysis was also used to determine the relationship between the relative number of ants and the number of newly emerged adults. Student's *t*-test was used to determine the statistical significance of differences in egg or pupal predation between the two (leaf litter-covered and bare soil) treatments.

9.3 Results and discussion

9.3.1 Relative abundance and potential impact of arthropod predators on L. bethae *eggs and pupae*

A total of 1428 ground-dwelling arthropods which are potential predators of the immature stages of *L. bethae* were caught in the 10 pitfall traps that were placed under lantana plants at Rietondale Experiment Farm during the 3-d period (Table 9.1). Of these, the vast majority were ants, which made up 75.5% of the individuals in the total catch. Among the ants, the genus *Technomyrmex* was clearly dominant, making up 96.5% of the individuals of the two ant genera found in the traps. The predominance of ants is not unusual and they are frequently the most abundant predatory arthropods in soil (e.g. Cherry, 2003; Morris *et al.*, 1999). The second and third most abundant predatory groups were ground-beetles (Carabidae) (both adults and larvae) and predatory mites (Erythraeidae), which constituted 13.2 and 6.0% of the total catch, respectively. Predator catches also included a small number of spiders (Lycosidae and Gnaphosidae) and rove-beetles (Staphylinidae), which formed 4.1 and 1.2% of the total catch, respectively. The pitfall traps might have excluded or underestimated the abundance of small and less-mobile predators which were less likely to be trapped than large and more-mobile organisms (Juen *et al.,* 2003).

Table 9.1 Relative abundance of different types of arthropod predators caught in pitfall traps in Rietondale Experiment Farm, Pretoria

Predator taxon	Total number of individuals
Ants (Hymenoptera: Formicidae):	
Technomyrmex sp.	1040
Anoplolepis sp.	38
Beetles (Coleoptera):	
Ground-beetles adults (Carabidae)	61
Ground-beetle larvae (Carabidae)	128
Rove-beetles (Staphylinidae)	17
Mites (Arachnida: Acari: Prostigmata):	
Erythraeidae	85
Spiders (Arachnida: Araneae):	
Wolf-spiders (Lycosidae)	39
Flat-bellied ground-spiders (Gnaphosidae)	20
Total	1428

Predation upon eggs in both leaf litter-covered and bare soil treatments was almost identical, reaching approximately 38% in three days under both conditions (Table 9.2). Predation upon pupae was substantially higher than that upon eggs under both conditions. More pupae disappeared from the leaf litter-covered than from the bare soil (Table 9.2), declining by 74 and 56%, respectively. This trend is consistent with findings from other studies, which showed that an increase in the litter layer relates to an increase in the number and diversity of predators, making prey items under such

conditions more vulnerable (Brust *et al.* 1986; Brust & House, 1990; O'Neal *et al.*, 2005).

Table 9.2 Comparison of numbers of eggs and pupae of *L. bethae* recovered from leaf litter-covered and bare soil treatments in the field after 3 d

Life stage	Number exposed (individuals/site) at (no. of sites)	Number recovered (mean ±SE) (and % mortality) after 3 d under		Data analysis		
		Leaf litter	Bare soil	*t*-value	df	*P*-value
Eggs	150 (10)	91.7 ±7.4 (38.9)	92.1 ±7.5 (38.6)	0.04	18	0.97
Pupae	10 (10)	2.6 ±0.4 (74)	4.4 ±0.4 (56)	3.18	18	**0.005**

Mortality numbers in parentheses give the percentage of eggs or pupae that disappeared and were presumed to have been consumed by generalist predators.

A multiple regression analysis showed that egg predation was not significantly correlated with the presence of any particular type of predator (Table 9.3), suggesting that no single predator detected was associated with the significant removal of *L. bethae* eggs, and therefore none of those listed could be regarded as potential key-predators of eggs. The disappearance of the eggs (38% in 3 d) could have been due to combined predation by several predator groups captured in the pitfall traps (Table 9.1). The possibility of predation by microscopic soil-dwelling predators, such as nematodes, entering the previously sterilized soil through the fine mesh base of the buried container cannot be ruled out. The situation differed for pupae, in that the multiple regression analysis showed a strong correlation between the number of

carabid adults trapped and the number of *L. bethae* pupae consumed (Table 9.4). This demonstrates that carabid adults are likely to be key-predators of *L. bethae* pupae in the field. The data also showed a negative correlation between the number of spiders and ants trapped, and the number of pupae consumed. That an increase in the spider and ant population was related to a reduction in pupal consumption, could have been due to intra-guild predation, which is known to reduce natural enemy impacts on herbivore populations (Finke & Denno, 2003; Finke & Denno, 2005). For example, the effectiveness of predacious mirid bugs in controlling planthoppers was reduced as result of intra-guild predation by wolf-spiders, and planthopper population growth was positive in the presence of both predators despite the fact that each predator alone caused a decrease in planthopper population growth (Finke & Denno, 2005).

Table 9.3 Multiple regression analysis of the number of *L. bethae* eggs consumed, as a function of the abundance of ants, predatory mites, spiders, carabid adults, carabid larvae and staphylinids in the field

Predictor	Coefficient	Standard error	t|	P-level
Intercept (βo)		100.8109	-0.0567	0.9583
Ants	0.705274	0.8511	0.9487	0.4127
Predatory mites	0.426725	1.6459	0.7848	0.4898
Spiders	0.281411	7.4082	0.4302	0.6960
Carabid adults	-0.359758	5.4263	-0.5916	0.5957
Carabid larvae	0.298068	3.1531	0.45325	0.6811
Staphylinids	-0.089374	10.0063	-0.1766	0.8710

Table 9.4 Multiple regression analysis of the number of *L. bethae* pupae consumed, as a function of the abundance of ants, predatory mites, spiders, carabid adults, carabid larvae and staphylinids in the field

Predictor	Coefficient	Standard error	*t*	*P*-level
Intercept (*ßo*)		11.8465	7.1121	0.0057
Ants	-0.8542	0.1000	-3.0011	0.0576
Predatory mites	0.2868	0.1934	1.3779	0.2620
Spiders	-1.1480	0.8705	-4.5838	**0.0194**
Carabid adults	0.9055	0.6376	3.8889	**0.0301**
Carabid larvae	0.7230	0.3705	2.8715	0.0639
Staphylinids	0.1629	1.1758	0.8413	0.4619

9.3.2 Predation upon L. bethae *eggs and pupae by* Technomyrmex *sp. ants in the laboratory*

On average, only 14 out of the 100 eggs presented to 100 ants in each container were consumed within 24 h (Table 9.5). Predation upon pupae by 20 ants of the same species was up to 100%, and averaged 76% over the same period of 24 h. Although predation rates in the laboratory cannot be directly extrapolated to field conditions, where the composition of predators and their abundance is uncontrolled, predation upon pupae under both laboratory and field conditions was substantially higher than that upon eggs. Eggs suffered less predation, probably because their smaller size rendered them less readily detected by predators, while pupae placed under similar conditions were evidently more readily detected.

Table 9.5 Predation upon *L. bethae* eggs and pupae by *Technomyrmex* sp. ants during a 24-h period in the laboratory

Life stage	Number exposed (individuals/dish) and no. of reps (dishes)	Number eaten within 24 h		Predation (%)
		Range	Mean ±SE	
Eggs	100 (10)	0-30	13.7 ±3.9	13.7
Pupae	10 (10)	4-10	7.6 ±0.3	76.0

9.3.3 Influence of Technomyrmex *sp. ants on survival of* L. bethae *from egg to adulthood*

There was a strong negative correlation between numbers of *Technomyrmex* sp. per plant and the number of *L. bethae* that survived to adulthood ($y = 56.8^{-0.03x}$; $r^2 = 0.707$; $P < 0.05$) (Fig. 9.1). Survival of adult *L. bethae* decreased from 27 to 4.5%, at 0 and 60 ants per plant, respectively. Survival was almost zero at a density of 100 ants per plant. Whilst this high correlation indicates the potential of the predatory ants to reduce populations of the immature stages of *L. bethae*, the laboratory experiments may have represented a worst case scenario in that the ant colonies confined on potted plants had no alternative prey items. The survey carried out at Rietondale Experiment Farm to determine the relative abundance of ant nests under lantana plants indicated that only two out of twenty plants (10%) had signs of ant nests within a 50-cm radius of the trunk of the plant (Simelane, unpublished data). Therefore, the laboratory findings do not justify major concern about potential failure of establishment of this agent as a result of these predatory ants. Rather, a suite of generalist predators could suppress the populations of *L. bethae* in the field.

Although the present study indicates a possible influence of some ground predators on *L. bethae*, further studies are needed to demonstrate the impact of these predators on *L. bethae* over a longer period of time and at different sites within the geographic and ecological range of lantana. For example, a sharp increase in relative abundance of a predator at a certain time of the year may have a significant influence on the population dynamics of *L. bethae* in the field. Furthermore, entomopathogenic organisms such as fungi, bacteria and viruses are known to attack and kill a number of root-feeding insect larvae, and these are expected to influence the population dynamics of *L. bethae* in the field (Brown & Gange, 1990; Blossey & Hunt-Joshi, 2003).

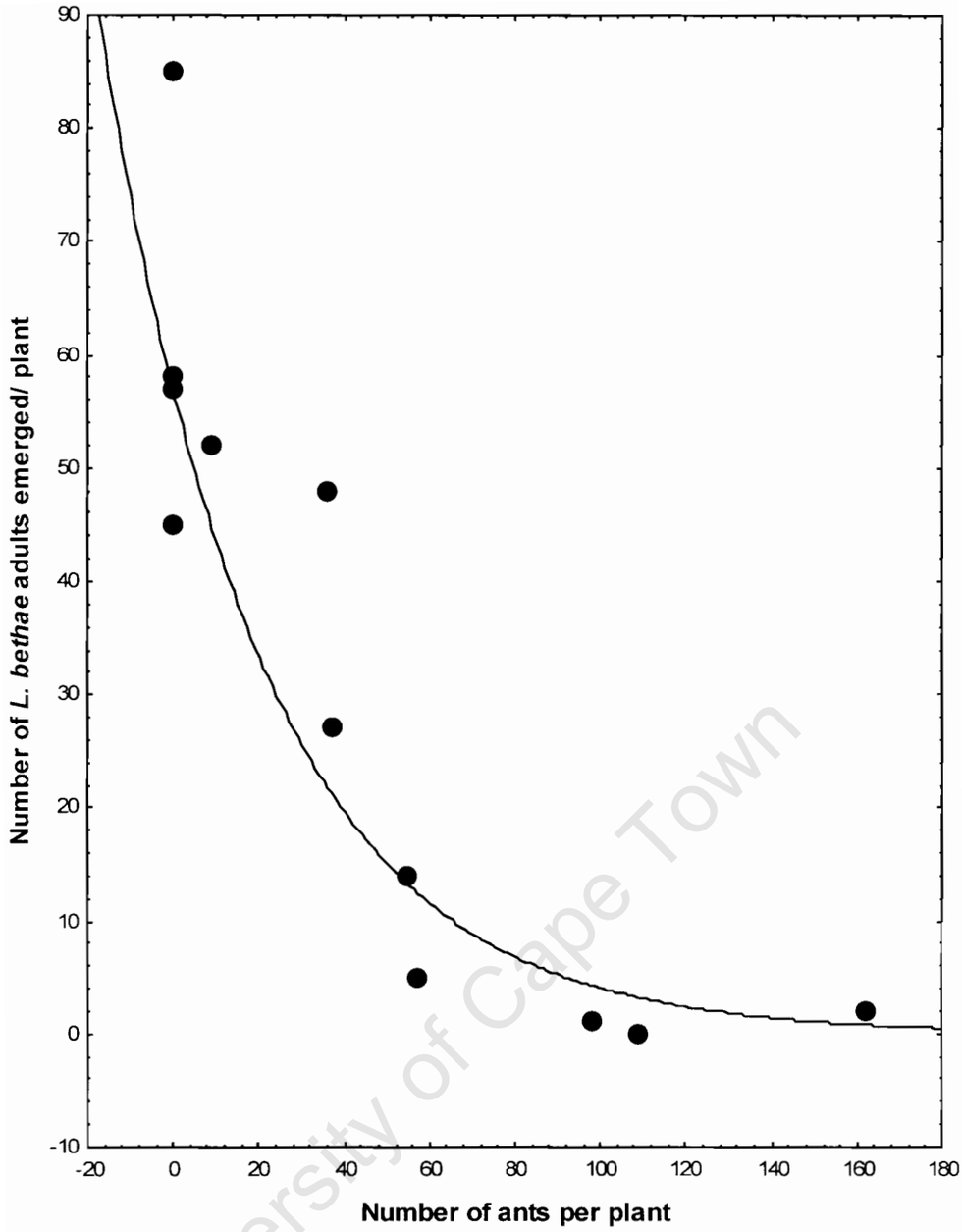

Fig. 9.1 Relationship between survival of *Longitarsus bethae* from fully developed egg to adult, and *Technomyrmex* sp. ant intensity.

CHAPTER 10

Suitability of some *Lantana camara* varieties as host plants for the root-feeding agent, *Longitarsus bethae*

10.1 Introduction

The cultivation and deliberate hybridisation of *Lantana camara* L. with other entities for more than a century outside its native range has resulted in hundreds of phenotypes and polyploid hybrids being produced and cultivated world wide, with up to 40 occurring in South Africa alone (Graaff, 1996). This hybridisation process produced genetic changes that have substantially reduced the suitability of the newly formed phenotypes for the co-evolved natural enemies associated with the parental genotypes in their native range. The failures of lantana biological control programmes in many countries have frequently been attributed to incompatibility between some lantana phenotypes and the introduced biological control agents (Harley *et al.*, 1979; Cilliers, 1983; Neser & Cilliers, 1989; Cilliers & Neser, 1991; Day & Neser, 2000).

The limited effectiveness of the lantana lace-bug, *Teleonemia scrupulosa* Stäl, in Australia, Fiji, Guam, Hawaii, Micronesia and South Africa is argued to be associated with the wide range of lantana phenotypes, some of which cannot be utilized as hosts by the bug (Harley *et al.*, 1979; Denton *et al.*, 1991; Neser & Cilliers, 1989). The gall-forming tephritid fly, *Eutreta xanthochaeta* Aldrich, failed to establish in South Africa apparently because the insect agent and the South African lantana biotype on which it was released were incompatible (Cilliers & Neser, 1991). The reproductive

performance of the mirid, *Falconia intermedia* Distant, varied almost 15-fold between lantana varieties (Urban & Simelane, 1999). Suppression of reproductive capacity of lantana by the eriophyid mite, *Aceria lantanae* (Cook), varied significantly between lantana varieties, ranging from 10 to 95% in the South African varieties, and from 0 to 30% in the Australian varieties (Urban *et al.*, 2003). Whilst agent preferences for certain phenotypes of the target weed have been strongly implicated in the overall outcome of biological control programmes against lantana by some researchers (Harley *et al.*, 1979; Cilliers, 1983; Neser & Cilliers, 1989; Cilliers & Neser, 1991; Day & Neser, 2000), the incompatibility issue has been downplayed by others (Broughton, 2000; Baars & Heytek, 2003). For example, field studies in Australia (Broughton, 2000) and in South Africa (Baars & Heytek, 2003) showed that a wide range of lantana varieties were equally acceptable and susceptible to at least five of the biocontrol agents that are established in both countries.

Compatibility between the agent and the target weed could impinge on the chances of successful establishment and eventual effectiveness of *L. bethae* as a biocontrol agent of lantana in South Africa. As a result, a study was undertaken to determine the influence of nine varieties of lantana on the feeding and oviposition preferences of the adult and survival of the immature stages of *L. bethae.* Identification of the most suitable varieties for *L. bethae* was also seen as a potential aid in the selection of release sites at which the host phenotypes would be best suited to the biocontrol agent.

10.2 Materials and methods

10.2.1 Test plants and insects, and laboratory conditions

Nine varieties of lantana and one species of *Lippia* were selected for the trials, including: 009 Light Pink; 018 Dark Pink; 113 Dark Pink; 015 White Yellow; 017 Orange Red; 150 Orange; 029 White Pink; 021 Total Pink; Richmond Pink (Australian lantana variety); and *Lippia wilmsii*. The eight South African *L. camara* varieties were selected because they represented a wide range of morphological features (notable growth form, morphology of leaves and flower colour) that are present over the geographic range of lantana in this country. Variety Richmond Pink was supplied by the Alan Fletcher Research Station, Brisbane, Australia for research purposes. *Lippia wilmsii,* a close relative of lantana, is one of the indigenous verbenaceous species widely distributed in South Africa. Single plants were transplanted into medium-sized pots (11 x 11 x 10 cm). *Longitarsus bethae adults* and eggs used in the trials were reared on lantana variety 009 Light Pink.

10.2.2 Adult aggregation, feeding and oviposition preference on different lantana varieties

Adult aggregation, feeding and oviposition preference tests were conducted concurrently in a quarantine glasshouse on plants that had reached a height of about 25 cm and had developed approximately 150 leaves with at least one flower. Sand was placed to a depth of 3 cm on top on the soil around each plant to facilitate extraction of eggs. Fifty plants, comprising five plants of each of the selected lantana varieties and *Lippia wilmsii*, were placed randomly in a walk-in cage (4 x 4 x 2 m), and exposed to 500 newly emerged, unsexed, adult *L. bethae* for a period of 10 d. The

number of beetles per plant was recorded every second day to determine the relative attractiveness of each variety to the insects. Adult feeding damage was assessed on the 10th day. To measure oviposition preference, the eggs laid under each plant were extracted, counted and compared among the different varieties.

10.2.3 Survival of the immature stages of L. bethae *on different lantana varieties*

Eggs of *L. bethae* were placed in batches of 220 around potted plants of the nine lantana varieties and *Lippia wilmsii.* The eggs were about 12 d old, with the embryo having a visible head capsule, and were ready to hatch within 24 h. After 40 d, individual plants were enclosed in isolation cages (30 x 30 x 45 cm) to capture beetles as they emerged from the soil. The number of beetles that emerged from each plant was recorded to determine the suitability of each variety for survival of the immature stages. Duration of development to adulthood was determined by counting the number of days between egg inoculation and emergence of adults. Body length and head capsule width of both sexes that developed on each plant variety were measured and compared. The experiment was replicated five times, with each plant of each variety representing a replicate.

10.2.4 Data analysis

Analysis of variance (ANOVA) was used to determine the statistical significance of differences in aggregation, feeding damage, oviposition and survival between varieties. In order to stabilize the variance, data were transformed to square-roots before being subjected to ANOVA. The transformed means were separated by Fisher's protected LSD. The square-root transformation of duration of development and progeny body size data did not change the significance of the analysis; thus the

results from untransformed data are reported. To examine the relationship between oviposition preference and survival, oviposition counts were plotted against survival of *L. bethae* on each variety. The relationship between oviposition and survival was described by linear regression, with survival as the dependent variable. Only untransformed means are presented in all the tables.

10.3 Results

10.3.1 Adult aggregation, feeding and oviposition preference on different lantana varieties

In the multiple-choice feeding trials, the intensity of feeding by adult *L. bethae* during the 10-d period was almost the same on seven lantana varieties, and was significantly higher than that recorded on variety 018 Dark Pink, 017 Orange Red and *Lippia wilmsii* ($F_{(9,30)} = 3.43$; $P = 0.005$) (Table 10.1). The number of adult *L. bethae* that were found every second day on the plants during the same period also differed significantly ($F_{(9,30)} = 2.77$; $P = 0.017$) among the varieties, and aggregation was heaviest on varieties 150 Orange, 009 Light Pink and 113 Dark Pink. Variety 150 Orange was the most preferred for oviposition ($F_{(9,30)} = 2.39$; $P = 0.035$), with females laying almost twice as many eggs on this variety as on 113 Dark Pink, the second most preferred variety. Oviposition was generally greater on *L. camara* varieties than on the species (*Lippia wilmsii*) belonging to a neighbouring genus.

Table 10.1 Aggregation, feeding and oviposition preference of *L. bethae* adults among nine different *L. camara* varieties and *Lippia wilmsii* during a 10-d exposure period. Data are mean ± SE

Plant variety	Aggregation (adults/plant)	Feeding damage level	Oviposition (eggs/plant)
150 Orange	7.95 ±1.15a	3.00 ±0.00a	176.5 ±44.63a
113 Dark Pink	5.08 ±1.05abc	2.75 ±0.25ab	90.00 ±25.69b
017 Orange Red	2.80 ±0.70bc	2.00 ±0.41bc	81.25 ±36.96b
Richmond Pink	4.40 ±1.02bc	2.50 ±0.29abc	74.75 ±23.44b
015 White Yellow	3.48 ±1.03bc	2.25 ±0.25abc	71.25 ±32.91b
009 Light Pink	5.75 ±0.89ab	3.00 ±0.00a	62.25 ±14.52b
029 White Pink	2.75 ±0.59c	2.25 ±0.25abc	51.00 ±18.16b
021 Total Pink	2.63 ±0.51c	2.25 ±0.25abc	47.75 ±22.50b
Lippia wilmsii	3.83 ±1.08bc	2.00 ±0.41bc	30.25 ±12.24b
018 Dark Pink	2.95 ±0.56bc	1.75 ±0.25c	27.25 ±10.19b

Means within a column followed by the same letter are not significantly different.

10.3.2 Survival of the immature stages of L. bethae *on different lantana varieties*

Survival to adulthood differed significantly among the lantana varieties ($F_{(9,30)}$ = 16.68; $P < 0.01$) (Table 10.2). The number of adult progeny that developed from an initial inoculation of 220 eggs was highest (mean ±SE: 90.25 ±5.39) on variety 150 Orange, and lowest on the Australian variety Richmond Pink (1.25 ±0.95) and *Lippia wilmsii* (4.75 ±2.84). Duration of development varied significantly among the varieties ($F_{(9,27)}$ = 6.5; $P < 0.01$). The longest (53.5 ±1.7 d) duration of development was recorded on variety 029 White Pink while the shortest (42.4 ±0.7 d) was recorded

on variety 115 White Yellow. Although variation in body size among varieties was less significant, males emerging from variety 150 Orange were significantly smaller than those from 021 Dark Pink, and slightly smaller than those from other varieties (body length: $F_{(8,162)} = 3.99$; $P = 0.05$, head capsule width: $F_{(8,162)} = 1.16$; $P = 0.33$) (Table 10.3). The average body size of females produced on 150 Orange was, however, the same as that of those produced on other varieties. Generally, males were significantly smaller than females (body length: $F_{(8,162)} = 8.73$; $P = 0.0036$, head capsule width: $F_{(8,162)} = 10.82$; $P = 0.001$) in all the varieties. The data showed no significant interaction between variety and size of adult progeny (body length: $F_{(8,162)} = 0.83$; $P = 0.583$, head capsule width: $F_{(8,162)} = 1.78$; $P = 0.09$).

Table 10.2 Survival of the immature stages of *L. bethae* (adult progeny produced from an inoculum of 220 eggs) and the duration of development from mature egg to adult stage on different *L. camara* varieties. Data are mean ± SE

Plant Variety	Survival of immatures (adult progeny/plant)	Duration of development (d)
150 Orange Red	90.3 ±5.39a	49.1 ±1.51ab
113 Dark Pink	52.0 ±6.89b	49.1 ±1.19ab
015 White Yellow	36.5 ±8.80bc	42.4 ±0.69c
021 Total Pink	26.5 ±3.52bcd	51.0 ±1.24ab
029 White Pink	23.3 ±5.63cd	53.5 ±1.73a
017 Orange Red	22.5 ±5.20cd	45.8 ±1.07bc
009 Light Pink	19.3 ±5.69cd	47.3 ±0.71bc
018 Dark Pink	16.5 ±7.64d	48.1 ±0.31ab
Lippia wilmsii	4.8 ±2.84e	48.51 ±1.76ab
Richmond Pink	1.3 ±0.95e	**

Means within a column followed by the same letter are not significantly different (P > 0.05; LSD test).
** Duration of development on this variety was not determined due to the insufficient number of samples.

Table 10.3 Size of *L. bethae* adults that developed on different *L. camara* varieties and *L. wilmsii*. Data are mean ± SE

Plant variety	Size of male adult progeny (mm)		Size of female adult progeny (mm)	
	Body length	Head capsule width	Body length	Head capsule width
150 Orange Red	1.75 ±0.03b	0.41 ±0.02b	1.83 ±0.03a	0.51 ±0.02a
113 Dark Pink	1.81 ±0.03ab	0.50 ±0.02a	1.88 ±0.03a	0.47 ±0.02a
015 White Yellow	1.82 ±0.03ab	0.50±0.02a	1.91 ±0.05a	0.52 ±0.03a
021 Total Pink	2.00 ±0.03a	0.50 ±0.02a	1.91 ±0.03a	0.49 ±0.03a
029 White Pink	1.91 ±0.03ab	0.50 ±0.03a	2.00 ±0.03a	0.52 ±0.02a
017 Orange Red	1.86 ±0.04ab	0.50 ±0.03a	2.00 ±0.03a	0.53 ±0.07a
009 Light Pink	1.80 ±0.06ab	0.50 ±0.03a	1.90 ±0.03a	0.51 ±0.02a
018 Dark Pink	1.89 ±0.03ab	0.50 ±0.02a	1.90 ±0.03a	0.47 ±0.02a
Lippia wilmsii	1.70 ±0.04ab	0.50 ±0.02a	1.80 ±0.05a	0.51 ±0.03a
Richmond Pink	*	*	*	*

Means within a column followed by the same letter are not significantly different (P > 0.05; LSD test).
*Body length and head capsule width were not measured on this variety due to the insufficient number of samples.

9.3.3 Preference-performance correlation

When oviposition counts (Table 10.1) were plotted against survival (Table 10.2) of *L. bethae* per variety, a significant positive correlation was obtained ($y = -7.73 + 0.52x$; $r^2 = 0.738$; $p = 0.0015$) (Fig.10.1).

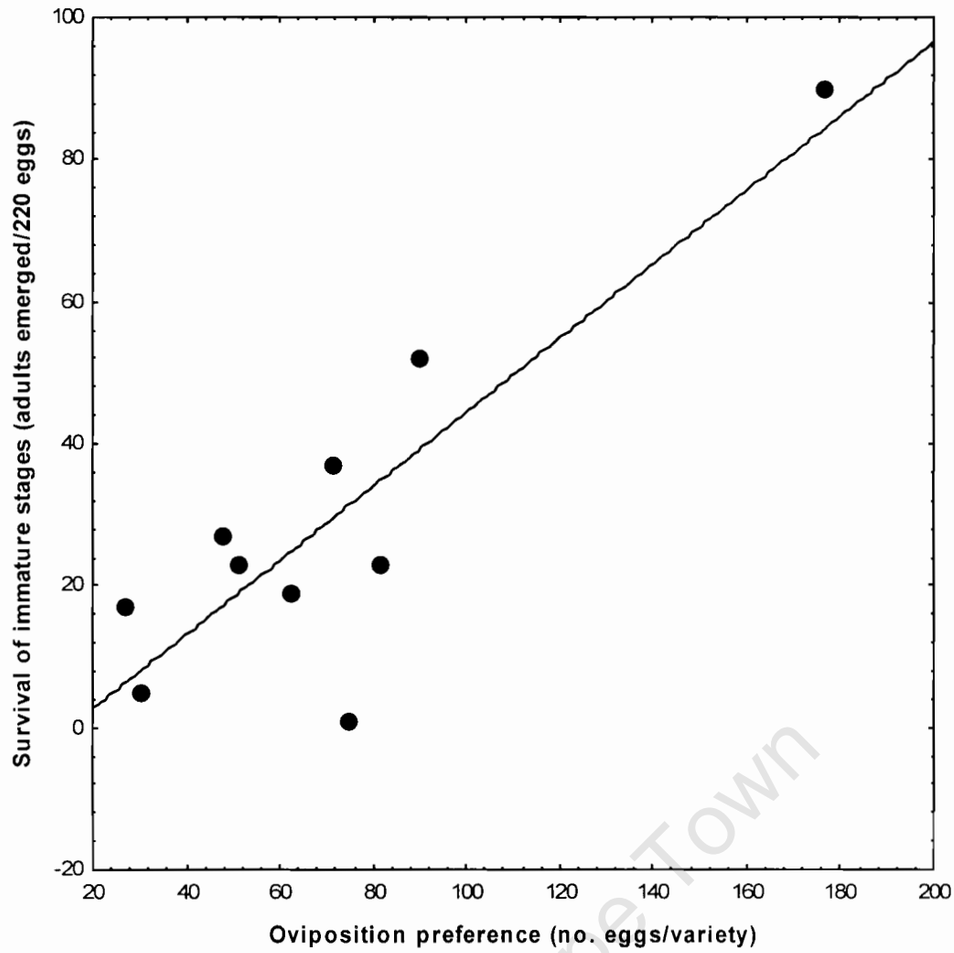

Fig. 10.1 The relationship between oviposition preference and survival of the immature stages of *L. bethae* per variety.

10.4 Discussion

The present study shows that aggregation, feeding, oviposition and survival of immature stages of *L. bethae* are significantly influenced by the variety of *L. camara.* The results also show that there was a significant positive correlation between oviposition preference of *L. bethae* and offspring survival performance on the different varieties. This is consistent with optimal oviposition theory (Jaenike, 1978; Matteson, 1980), often referred to as the preference-performance hypothesis. The

theory states that oviposition preference should correlate with host plant suitability for offspring development, as females are assumed to maximise their fitness by ovipositing on high quality hosts. For example, the results showed that both oviposition and survival were substantially high on varieties 150 Orange and 113 Dark Pink. Likewise, some varieties, e.g. 018 Dark Pink, were less preferred for oviposition, and also less suitable for survival of the immature stages. Positive correlations between oviposition preference and offspring performance have also been shown for different insect species (Craig *et al.*, 1989; Howlett *at al.*, 2001; Forister, 2004). The results suggest *L. bethae* will deposit eggs preferentially under varieties on which its immatures are more likely to flourish, and that the flea beetle populations will increase more rapidly in areas where the most suitable lantana varieties predominate.

The atypical instances, in which a particular variety supported high levels of survival to adulthood but was less preferred for oviposition, may support the hypothesis that some plant secondary chemicals are more effective in deterring oviposition than in curbing larval development (e.g. Leather, 1985; Mayhew, 1997; Valladares & Lawton, 1991). This asymmetry between oviposition preference and larval performance allows for the possibility that ovipositing females may avoid a variety that is suitable for larval development (Leather, 1985; Leather *et al.*, 1987). Mayhew (1997) argued that such a pattern is a regular feature of insect behaviour, and given the potential suitability of some varieties for larval development, this may provide potential for host range expansion. On the other hand, host-use patterns may also evolve on varieties that favour strong oviposition coupled with poorer larval performance (e.g. Richmond Pink and 017 Orange Red), by larval performance

improving on the host after thorough natural selection (Futuyma & Peterson, 1985). As Leather *et al.* (1987) suggested, as long as some larvae are able to complete their development on the chosen hosts, then the overall result is a net increase in the population.

Preference for variety 009 Light Pink in multiple-choice experiments may, however, suggest that prior experience with this variety during rearing might have influenced aggregation, feeding and possibly oviposition (Papaj & Prokopy, 1989). Because the adult beetles used in the preference tests had been reared on variety 009 Light Pink for at least 18 generations, there could have been some selection for a host plant biotype, with the beetles developing a greater preference over time for the variety on which they were reared.

Whilst the size of the emerging adult males appeared to be reduced by poorer larval performance on a certain variety (150 Orange), the size of the emerging adult females did not differ significantly among varieties. This suggests that the inherent nutritional and resistance qualities of the varieties are unlikely to compromise the reproductive capacity (fecundity) of the surviving females. The exceptionally heavy oviposition intensity on variety 150 Orange could possibly have lead to intra-specific competition in the exceptionally dense population of larvae on this variety. That the female adults are almost invariably slightly larger than the male adults, suggests that the same gender-linked size difference must apply to their larvae. It appears that, under conditions of unusually high population density, and intra-specific larval competition, the slightly bigger and stronger female larvae consume food at the expense of the

slightly smaller and weaker male larvae, with a consequent slight stunting of the growth of the males.

Although phytophagous insects often display strong preferences for particular plant species or varieties in response to a suite of chemical and physical cues associated with the plants or their environment (Dethier, 1954; Thorsteinson, 1960; Ehrlich & Raven, 1964; Ehrlich & Murphy, 1988; Evans, 1988; Lapis & Borden, 1993), the role of these cues in the finding of preferred varieties by *L. bethae* remains uncertain. Research to identify and quantify these cues should be considered in future investigations. Nevertheless, the chances of establishment and successful control of *L. camara* are expected to be much greater on varieties that were preferred for oviposition and provided better survival of the immature stages of *L. bethae*.

CHAPTER 11

Effect of population density and larval instar of *Longitarsus bethae* on vegetative growth and biomass of *Lantana camara*

11.1 Introduction

It is logical to assume that root herbivores have the greatest capacity to shape plant life cycles, plant fitness or plant communities because they attack the underground plant organ which constitutes 30 to 90% of the total plant biomass and performs the crucial function of absorption of water and inorganic nutrients (Nötzold *et al.*, 1998; Baars & Heystek, unpublished data; Williams & Madire, unpublished data; Mabuda & Thobagale, unpublished data). The large reductions in agricultural yield, which are often attributed to root-feeding insects, provide evidence of their destructive capabilities (Brown & Gange, 1990). Despite the huge potential of root feeders for reducing plant population growth, they have received far less consideration in biological control of weeds than warranted, because of the inherent difficulties of observing and manipulating them (Blossey & Hunt-Joshi, 2003). In acknowledgement of the importance of root herbivores, a root-feeding flea beetle, *Longitarsus bethae*, was introduced into quarantine in South Africa in a bid to complement the impact of the rather ineffective above-ground insect agents already established on lantana (Cilliers & Neser, 1991; Baars & Neser, 1999; Simelane, 2002a; Simelane, 2005). Several species in the genus *Longitarsus* have been used with great success as biological control agents of some weed species in several countries (Frick, 1970; Pemberton & Turner, 1990; Jordan, 1997).

Most of the insect herbivores that have been introduced into South Africa for biological control of lantana are leaf feeders (Baars & Neser, 1999), with fewer affecting the flowers and fruits. In certain parts of the country, these biocontrol agents are frequently subjected unfavourable environmental conditions which lead to drastic population declines during autumn or winter when the plants become defoliated due to frost or drought (Cilliers & Neser, 1991; Simelane & Phenye, 2003). Furthermore, lantana has the capacity to compensate for defoliation caused by insects during summer (Broughton, 2000). Larvae of *L. bethae* remain alive in the soil during the winter months (Simelane, unpublished data), making them ideally suited to build up numbers rapidly and sustain control of the weed in its introduced range.

Longitarsus bethae larvae break off root hairs and excavate cavities in the roots of their host plant while feeding. Heavily damaged roots usually die distally (Simelane, 2005). The potential problems arising from root attack by insects include: drought stress to the plant caused by pruning of the root system (Andersen, 1987; Hou *et al.*, 1997); loss of reproductive output (Dutcher & All, 1979) and an increase in the plant's susceptibility to infection by soil-borne pathogens (Jin *et al.*, 1992; Caesar, 2003). Root feeding by insects may directly reduce the following: food reserves such as carbohydrates (Dintenfass & Brown, 1988), synthesis of numerous growth hormones (Skene, 1975) and plant stability, resulting in lodging (Gray & Tollefson, 1987). Therefore, the combined effect of the above- and below-ground insect agents could aggravate and accelerate plant damage, reducing the weed's growth rate and possibly population density. Successful biological control of *Senecio jacobaeae* L. in the coastal regions of Oregon (McEvoy, 1985) and northern California (Pemberton & Turner, 1990) was due to the synergistic action of the leaf-feeding cinnabar moth,

Tyria jacobaeae L., and the root-feeding flea beetle, *Longitarsus jacobaeae* Waterhouse.

Even though feeding damage by larvae of *L. bethae* has a visible effect on lantana under laboratory conditions, no data are available on the effects of larval population density and instar on the vegetative growth and below- and above-ground biomass of the plant. As part of the process of attempting to predict the potential effectiveness of *L. bethae* for biological control of *L. camara* in South Africa, the destructiveness of the insect needed to be quantified, so as to confirm that, when they have coped with the biotic and abiotic environmental constraints, they will also have an impact on the vigour of the target weed.

11.2 Materials and methods

11.2.1 Experimental set-up and procedure

Lantana (variety 009 Light Pink) plants used in the trials were grown on sterilized silty loam soil, with approximately 27% sand, 48% silt and 26% compost. Soil was sterilised at 60°C for 24 h to eliminate potential soil-dwelling predators of *L. bethae.* Plants were grown in medium-sized square pots (11 x 11 x 10 cm). The experiment was a two-factorial design: egg density (at four levels: 0, 100, 200 and 300 eggs per plant) and larval instar (first, second and third instar). When plants had reached a height of approximately 20 cm and a leaf density of about 200 leaves per plant, they were inoculated with eggs at one of the four densities. *L. camara* cultivar 009 Light Pink was used in all the experiments. The cultivar is widespread along the ecological zone of lantana in South Africa.

To ensure that data from different treatments were comparable, leaf numbers, stem diameter, stem height and plant biomass were counted or measured at the beginning of the experiment. Eggs placed in the plant pots were about 12 d old, with the embryo's head capsule visible, and were therefore ready to hatch within 24 h. Based on observations from other studies, (Simelane, unpublished data), by the 15th, 30th and 45th day, almost all *L. bethae* larvae would have moulted into second instar, third instar and pupa, respectively. Therefore, to measure the effect of larval population density and larval instar, leaf density, stem height, stem diameter, and dry mass of both above- and below-ground plant parts were determined on the15th, 30th and 45th day after inoculation with eggs. Below- and above-ground plant material were separated and put into paper bags, oven dried at 80°C for 72 h, and weighed. Each treatment level, that is, each level of each factor, was replicated three times.

11.2.2 Data analysis

Plant growth and biomass were compared using analysis of variance (ANOVA). Data were square root-transformed before analysis; however, the untransformed values are reported here. The transformed means were separated by Fisher's protected LSD, and a significance level of P = 0.05 was used for all analyses.

11.3 Results

11.3.1 Effect of larval population density and instar on leaf density

Both initial egg density and larval stage had a significant impact on leaf density of *L. camara* plants (Fig. 11.1). Although leaf number increment was not significantly

affected by larval population density during the first-instar period ($F_{(3,8)}$ = 3.24; P = 0.41), the larval populations that developed from initial cohorts of 100, 200 and 300 eggs per plant significantly ($F_{(3,8)}$ = 6.89; P = 0.013) reduced leaf number increment by 34, 32 and 35%, respectively, compared with the controls, during the second-instar period. By the end of the third larval period, root-feeding damage had caused yellowing and defoliation, significantly reducing the leaf number increment on plants inoculated with 200 and 300 eggs by 32 and 54%, respectively, compared with the controls ($F_{(9,32)}$ = 1.66; P = 0.014). The inoculation of 100 eggs per plant had no statistically significant effect on leaf density by the end of the third larval instar. The overall impact of low-, medium- and high-density populations of *L. bethae* larvae completing their development on the roots of *L. camara* plants indirectly caused leaf number increment losses of 37, 77 and 127%, respectively (Fig. 11.1).

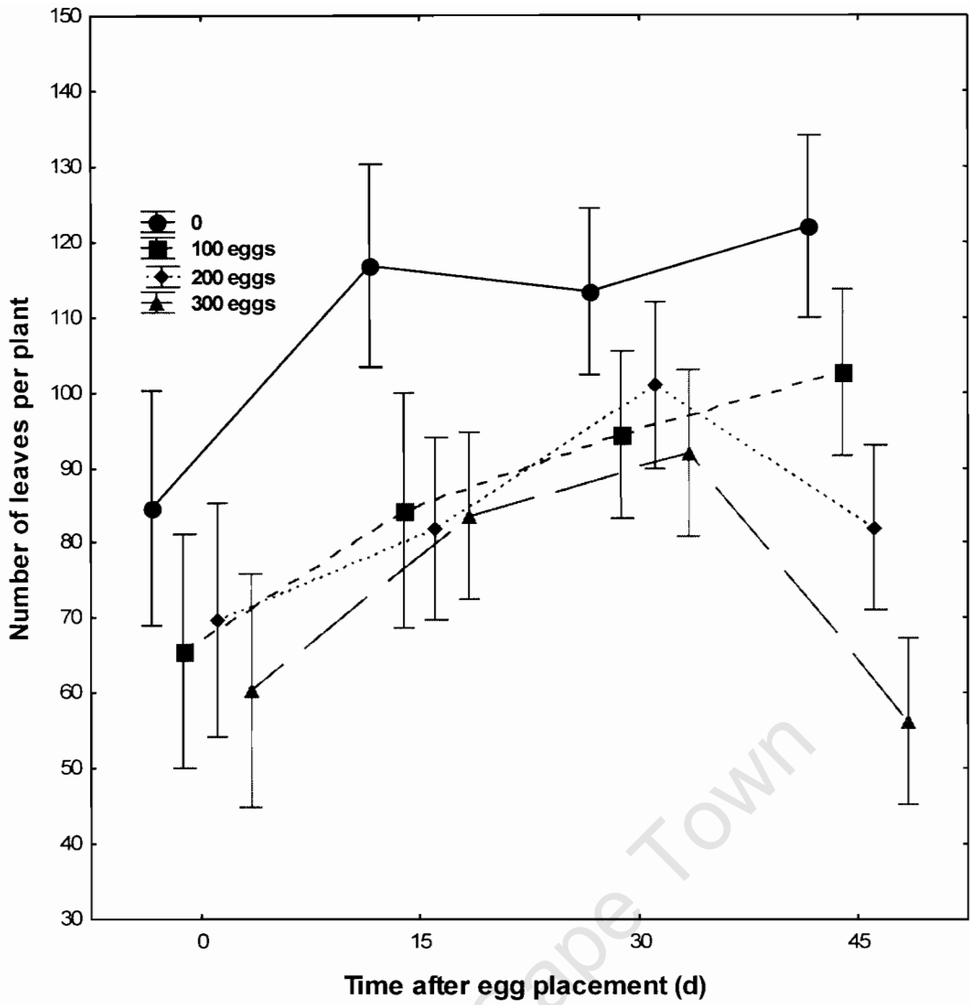

Fig. 11.1 Number of leaves (mean ±SE) on potted *Lantana camara* plants under which *Longitarsus bethae* eggs were placed at four different densities. Counts made on the 15th, 30th and 45th day coincided with the moulting of the first, second and third larval instars, respectively.

11.3.2 Effect of larval population density and instar on plant height

Prior to egg placement, the heights of all the plants used in the study were not statistically different ($F_{(3,8)} = 0.167$; $P = 0.92$) from each other (Fig. 11.2). There was no significant difference in plant height by the end of the first larval period ($F_{(3,8)} = 1.61$; $P = 0.26$). Although stem growth was slightly reduced compared with the

controls at all egg placement density levels during the second larval period, none of these reductions were significant ($F_{(3,8)} = 2.68 = P = 0.12$). By the end of the third larval period, the height of plants which had received 200 and 300 eggs were significantly ($F_{(3,8)} = 18.10$; $P = 0.0006$) shorter than those of the controls. An initial density of 100 eggs per plant, had no significant effect on height, compared with the controls, by the end of the last larval instar. The overall impact of low-, medium- and high-density populations of *L. bethae* larvae completing their development on the roots indirectly caused stem height increment losses of 5, 29 and 54%, respectively (Fig. 11.2).

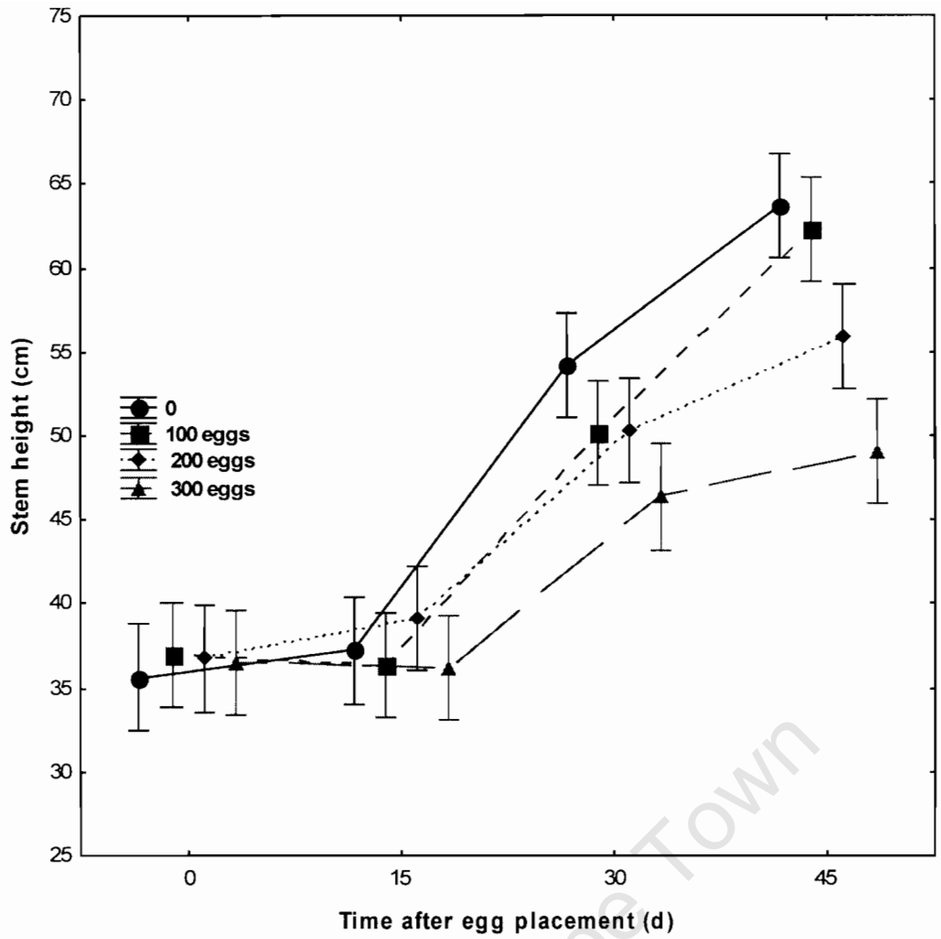

Fig. 11.2 Stem height (mean ±SE) of potted *Lantana camara* plants under which *Longitarsus bethae* eggs were placed at four different densities. Counts made on the 15th, 30th and 45th day coincided with the moulting of the first, second and third larval instars, respectively.

11.3.3 Effect of larval population density and instar on stem diameter

The first-instar larvae had no effect on radial growth of stems at all population density levels ($F_{(3,8)}$ = 1.03; P = 0.42) (Fig. 11.3). During the second larval period, radial growth was significantly reduced at all larval population levels ($F_{(3,8)}$ = 5.91; P = 0.0065). By the end of the third larval period, radial growth of all test treatment plants had been significantly reduced, compared to that of the control plants, ($F_{(3,8)}$ = 29.14;

P = 0.00012) by 25, 29 and 32% at the low, medium and high larval population density levels, respectively. The overall impact of low-, medium- and high-density populations of *L. bethae* larvae completing their development on the roots indirectly caused stem diameter increment losses of 48, 65 and 71%, respectively (Fig. 11.3)

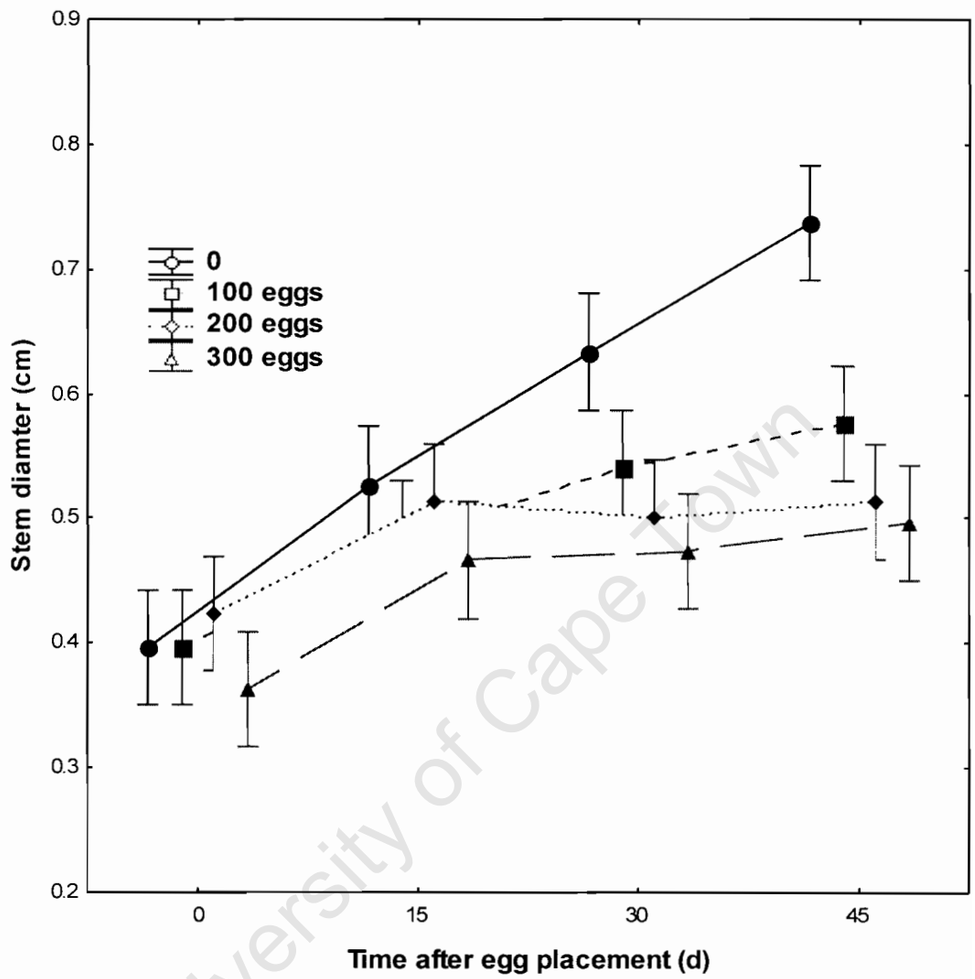

Fig. 11.3 Stem diameter (mean ±SE) of potted *Lantana camara* plants under which *Longitarsus bethae* eggs were placed at four different densities. Counts made on the 15th, 30th and 45th day coincided with the moulting of the first, second and third larval instars, respectively.

11.3.4 Effect of larval population density and instar on above-ground dry-mass

The accumulated above-ground dry-mass was reduced, but not significantly, during the first larval period at all the larval population density levels ($F_{(3.8)} = 1.20$; $P = 0.70$) (Fig. 11.4). A significant reduction in the accumulated above-ground dry-mass, compared with the controls, was observed on the plants with the highest larval density at the end of the second larval period ($F_{(3,8)} = 10.96$; $P = 0.0033$). Although the study showed slight reductions in the accumulated above-ground dry-mass in the low- and medium-density treatments at the end of the second larval period, these did not differ significantly from that of the control. By the end of the third larval period, the accumulated above-ground dry-mass had been significantly ($F_{(3.8)} = 2.41$; $P = 0.014$) reduced by 44% in the high larval-density treatment, and 12.0 and 37.5% in low- and medium-density treatments compared with the controls during the same period, respectively. The overall impact of low-, medium- and high-density populations of *L. bethae* larvae completing their development on the roots indirectly caused above-ground dry-mass increment losses of 27, 84 and 99%, respectively (Fig. 11.4).

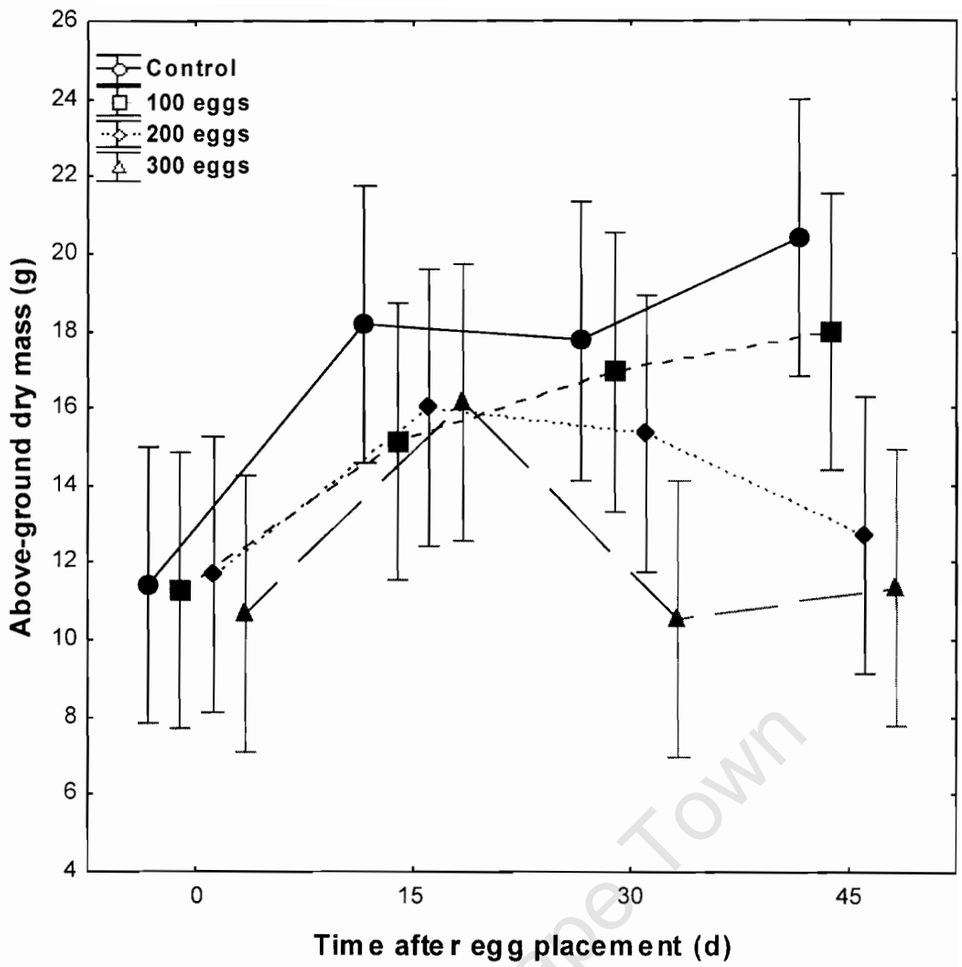

Fig. 11.4 Above-ground dry-mass (mean ±SE) of potted *Lantana camara* plants under which *Longitarsus bethae* eggs were placed at four different densities. Counts made on the 15th, 30th and 45th day coincided with the moulting of the first, second and third larval instars, respectively.

11.3.5 Effect of larval population density and instar on below-ground dry-mass

The below-ground dry-mass was not affected by early instars at all egg inoculation levels ($F_{(3,8)}$ = 0.27; P = 0.85) (Fig. 11.5). Inoculation of 300 eggs per plant significantly reduced accumulated below-ground dry-mass during the second and third larval periods, compared with the controls (second-instar period: $F_{(3,8)}$ = 7.12; P = 0.012, third-instar: $F_{(3,8)}$ = 0.5.88; P = 0.02). At the end of the second-instar period,

the accumulated below-ground dry-mass in 100-, 200- and 300-egg treatments was 5, 13 and 41%, respectively, less than the controls. By the end of the third larval period, accumulated below-ground dry-mass in 100-, 200- and 300-egg treatments was 12, 17 and 44%, respectively, less than the controls. However, the below-ground dry-mass at both 100 and 200-egg inoculation levels was not significantly different from that of the control at the end of the third larval period. The overall impact of low-, medium- and high-density populations of *L. bethae* larvae completing their development on the roots directly and indirectly caused below-ground dry-mass increment losses of 32, 45 and 119%, respectively (Fig. 11.5).

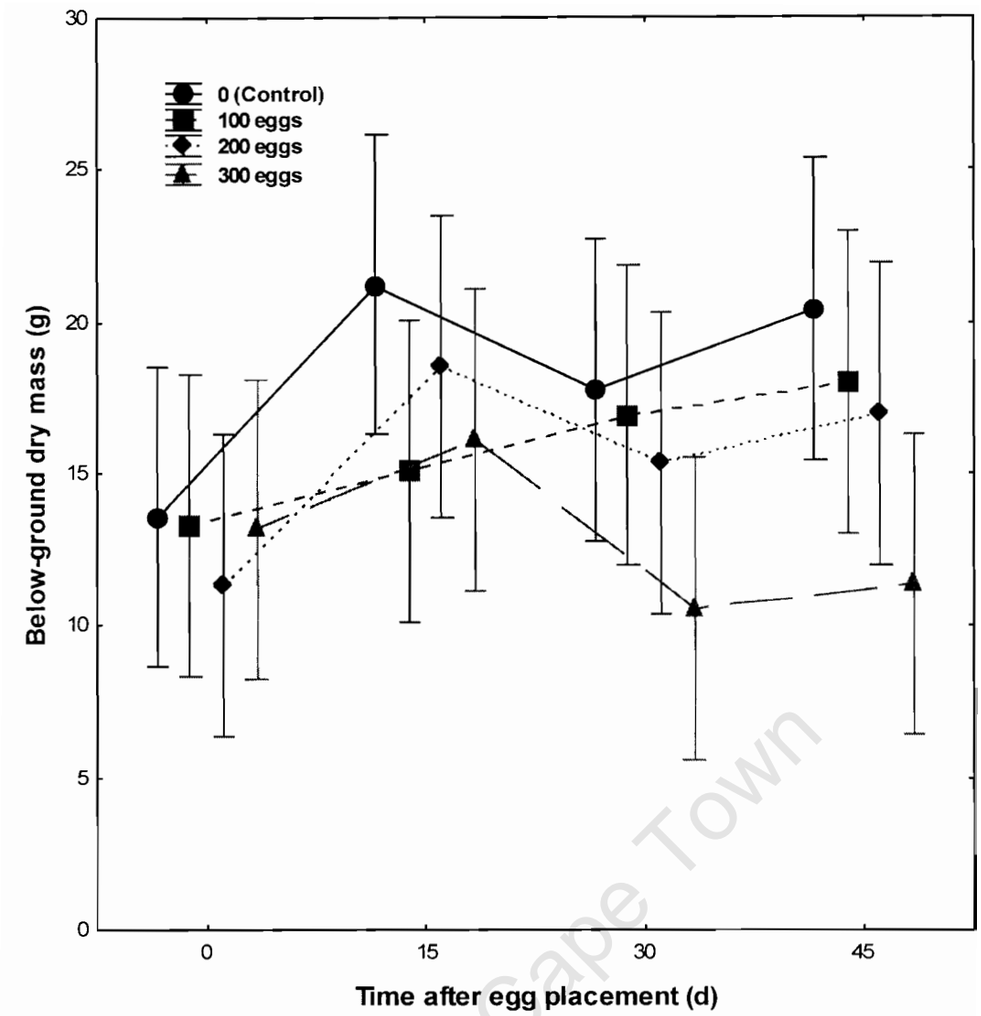

Fig. 11.5 Below-ground dry-mass (mean ±SE) of potted *Lantana camara* plants under which *Longitarsus bethae* eggs were placed at four different densities. Counts made on the 15th, 30th and 45th day coincided with the moulting of the first, second and third larval instars, respectively.

11.4 Discussion

The data show that, under certain levels of attack, feeding damage by *L. bethae* larvae can significantly reduce leaf number increment and eventually even the number of leaves, plant height increment, stem diameter increment, and biomass accumulation

both of subterranean and aerial parts. For all of the parameters that were measured, there were usually no differences between the plant parameter values of controls and those of the three different larval population densities by the end of the first larval instar. There were significant differences in vegetative growth and plant biomass accumulation by the end of second and third instars, showing that larval populations developing from initial densities of 200 to 300 eggs per plant could inflict sufficient physical damage to the roots to reduce plant growth. Very high populations of *L. bethae* larvae completing their development on the roots of small *L. camara* plants can markedly stunt the growth of the weed, by causing growth increment losses in the number of leaves, stem height, stem diameter, above-ground dry-mass, and below-ground dry-mass, of up to 127, 54, 71, 99 and 119%, respectively.

Occasionally, inconsistent changes in certain plant parameters in response to larval population density were observed during the larval periods. Although this was not clearly understood, it is likely that defoliation, which occurred on some plants during the last stage of the experiment, could have contributed to the inconsistent reduction of dry-mass accumulation in some test treatment plants. A subsequent root compensation response in test treatment plants might have been the cause of their recovery during the third larval instar (Simberloff *et al.*, 1978), thereby producing the inconsistent results.

The indirect effect of larval injury on flower production proved to be impossible to measure because the small plants used in this study did not produce enough flowers under laboratory conditions. Also, bigger plants maintained in the nursery, because of their considerable growth vigour, would probably have been less responsive to lower

larval population densities. To make a dent in their vegetative and reproductive growth, bigger plants would have required very large doses of egg inoculations. The current egg-rearing capacity was, unfortunately, insufficient to meet these levels of inoculation. However, it is conceivable that under field conditions, high larval populations will be attained that would proportionally impair the ability of bigger plants to grow and produce flowers or set seeds. Furthermore, through their significant reduction in the growth increment of other plant parts, especially leaves, larval feeding damage could also have an indirect impact on the reproductive capacity of the plant.

Although unfavourable below-ground factors (e.g. insufficient soil moisture, unsuitable soil texture and the presence of soil-dwelling predators) are likely to keep the populations of the immature stages in check (Chapter 9) and probably limit the effectiveness of this agent in controlling lantana, the immature stages of *L. bethae* will escape unfavourable above-ground weather conditions (e.g. frost and drought) and aerial natural enemies that often cause population crashes of the leaf-feeding agents in many inland areas of South Africa. Furthermore, the interaction of this root-feeder and the above-ground agents such as *T. scrupulosa* (Chapter 8) is expected to have a complementary impact on plant biomass, thereby increasing the chances of successful biological control of lantana in South Africa. This will be particularly true if the two agents attack lantana at different times or at different locations (McEvoy *et al.*, 1993).

Overall, the ability of *L. bethae* larvae to both directly suppress root growth and indirectly suppress leaf and stem growth of *L. camara*, indicates that this flea beetle may have a considerable impact on the weed's invasiveness, and that it could make a

substantial contribution to the biological control of lantana in South Africa and elsewhere.

Chapter 12

Towards predicting relative survival of *Longitarsus bethae* at ecologically different sites

12.1 Introduction

There is a general acceptance that the post-release performance of biological control agents cannot be predicted with certainty before the agents have been deployed. At the same time, there is considerable concern that insufficient efforts are being made to determine, before release, the suitability of new environments for prospective agents. Almost without exception, releases are made on a trial and error basis, with the consequences that the agent may fail to establish or be restricted to certain sites. The trial and error approach may also increase the number of agents necessary for successful control, with the concomitant risks that undesirable non-target effects may accrue (Simberloff & Stiling, 1996; McEvoy, 1996; Louda *et al.* 1997; McEvoy & Coombs, 2000; Pemberton, 2000). One way to address these issues is to study how prospective agents respond to different environmental and ecological factors under controlled experimental conditions. Such studies should identify factors that are most likely to have an influence on the development and survival of the agent species in question, as has been done in this study on *L. bethae*.

This study showed that two biotic (sharing the host plant with another well-established agent (*Teleonemia scrupulosa,* Tingidae) and plant varietal resistance) and two abiotic (soil moisture and texture) factors, amongst others, had a significant influence on the development of *L. bethae*. The real challenge is to determine how

the factors might act in unison, as happens under natural conditions, rather than in isolation. The primary objective of the present study was therefore to develop a model to explain and predict relative survival of *L. bethae*, based on the combined effect of sharing the host plant with another well-established agent, plant varietal resistance, soil moisture and soil texture.

Such a model could be used to determine whether, and to what extent, conditions in the distribution of the target weed are suited to the new agent. Such knowledge could then be used as part of the decision-making process, initially to decide whether to release the agent or not. It could also be used to select release sites where the agent is most likely to establish and have a substantial impact on the weed. With these considerations in mind, a set of experiments was performed to determine how *L. bethae* responds to different combinations of environmental conditions, and the results were used to develop a model for the performance of the beetle in different regions of South Africa.

12.2 Materials and methods

12.2.1 Multi-factorial survival trial

A four-factorial randomized block design experiment was arranged to determine how four environmental factors acting in combination might influence survival of the immature stages of the beetle. The four factors were:

(i) *Clay content* (*CC*). Three different soil textures were used: clayey (5% sand, 40% silt and 55% clay), loamy (68% sand, 25% silt and 7% clay) and sandy (90% sand, 8% silt and 2% clay)

(ii) *Soil moisture* (*SM*). Soil moisture was maintained at low (Hadeco soil moisture meter reading: 3-4 units), moderate (5-6 units) and high levels (7-8 units) during the course of the experiment, in accordance with the methods described in chapters 2 and 5.

(iii) Plant *cleanliness* (*PC*): Half the plants were caged (0.5 x 0.5 x 0.95 m) with three pairs of field-collected *Teleonemia scrupulosa* adults for 5 d. During this period, the *T. scrupulosa* females deposited eggs which were the founders of colonies of *T. scrupulosa* on the plants. After removal of the original *T. scrupulosa* adults, each plant was inoculated with 150 *L. bethae* eggs as described in chapter 2. When the newly emerged *T. scrupulosa* nymphs had developed to the adult stage, they were removed from the plants to avoid over-exploitation.

(iv) *Lantana variety* (*LV*): Four lantana varieties were selected from those whose influence on survival of *L. bethae* had already been determined in chapter ten. Ranked according to their relative suitability to *L. bethae*, these varieties were 150 Orange, 021 Total Pink, 009 Light Pink and 018 Dark Pink.

Potted (7 cm diameter x 5 cm deep) plants of each of the four lantana varieties (150OR, 021TP, 009LP and 018DP) were grown separately in soils of three different textures (clayey, loamy and sandy), maintained at three different soil moisture levels (low, moderate and high) and either with or without exposure to *T. scrupulosa* (nil and moderate). The trial thus comprised 72 treatments, simulating ecologically different 'sites', and each treatment was replicated three times. Each plant was housed in a cage (0.95 x 0.5 x 0.5 m) in order to capture the *L. bethae* adults that

emerged. The relative survival (RS) of the immature stages of *L. bethae* was calculated as percentage survival from the number of adults obtained from an inoculum of 150 eggs per plant.

12.2.2 Derivation of L. bethae *survival model*

Relative survival (RS) of *L. bethae* was modelled as a multiple linear regression, dependent upon functions of four independent environmental variables, namely: soil moisture (sm), clay content (cc), plant cleanliness (pc) (i.e. presence or absence of *T. scrupulosa*), and lantana variety (lv). Thus:

$$\mathbf{RS = a + b(f_{sm}) + c(f_{cc}) + d(f_{pc}) + e(f_{lv})} \qquad \text{(equation 1)}$$

where a is the y-intercept , and b, c, d and e are the coefficients of a function (f) of the relevant ecological variables. It was not permissible to use the *absolute* values of these environmental parameters, because, whilst the relationships between survival and clay content, plant cleanliness and lantana variety were all known to be directly proportional and therefore linear (Tables 4.3, 10.3 and Fig. 8.3), that between survival and soil moisture was known to be parabolic (Table 5.4). To overcome this difference, the absolute values of the environmental parameters of each site were first transformed into component relative survival values (rs) (Table 12.1), ranging from 0 to 1, calculated as decimal fractions of the maximum survival. Equation (1) then became:

$$\mathbf{RS = a + b(rs_{sm}) + c(rs_{cc}) + d(rs_{pc}) + e(rs_{lv})} \qquad \text{(equation 2)}$$

Table12.1. Relative survival values (rs) of *L. bethae* at a 'site', calculated from the site values of each of four ecological variables when operating separately (Tables 4.3, 5.4, 10.3 and Fig 8.3)

Ecological Variable	Value at 'site'	Relative survival value (rs) of *L. bethae*
Soil moisture (**SM**)	Low (3-4 Hadeco units)	rs_{sm} 0.11
	Moderate (5-6 units)	rs_{sm} 1.00
	High (7-8 units)	rs_{sm} 0.21
Clay content (**CC**)	High (clayey soil, 55%)	rs_{cc} 1.00
	Low (loamy soil, 7%)	rs_{cc} 0.04
	Very low (sandy soil, 2%)	rs_{cc} 0.02
Plant cleanliness (**PC**)	Clean (uninfested)	rs_{pc} 1.00
	Moderate (infested)	rs_{pc} 0.40
Lantana variety	150 Orange Red (LV_1)	rs_{lv} 1.00
	021 Total Pink (LV_2)	rs_{lv} 0.29
	009 Light Pink (LV_3)	rs_{lv} 0.22
	018 Dark Pink (LV_4)	rs_{lv} 0.18

The data from the 216 measurements (3 replicates from each of the 72 'sites') were subjected to multiple regression and correlation analyses, to quantify the importance of the ecological variables in explaining the survival of *L. bethae.* The relationship between component relative survival (rs) and actual survival (RS) was described by correlation coefficients, with RS as the dependent variable. The multiple regression analysis supplied the values of the coefficients (a to e) for the survival equation (Equation 3).

12.2.3 Prediction of performance of L. bethae *in South Africa*

Soil texture (specifically clay content), annual rainfall and lantana distribution data were obtained from the Institute for Soil, Climate and Water (ARC-ISCW), the South African Weather Bureau (SAWB) and the Southern African Plant Invaders Atlas (SAPIA) of Plant Protection Research Institute (ARC-PPRI), respectively. Clay content, rainfall and lantana distribution maps were generated by Geographic Information System (GIS) software (2005). The clay map illustrated two categories of (>15 and <15%) clay content while the rainfall map illustrated three categories (>700, 400-700 and < 400 mm per annum) within the ecological zone of lantana. The three maps were superimposed to divide the lantana-infested area of South Africa into six ecological zones (Table 12.2). Estimated values of relative survival for soil type and moisture level in each of the categories are shown in Table 12.2. Based on their apparent lack of significance when the four factors were operating in concert, the relative survival values for plant cleanliness and lantana variety were not expected to vary significantly from site to site, and were therefore fixed as 0.8 and 0.9, respectively. The survival prediction equation (Equation 3) was then used to predict relative survival of *L. bethae* in each of the six ecological zones. Based on their predicted relative survival values, the six ecological zones were ranked and mapped as three main zones (suitable, marginally suitable and unsuitable) of differing performance of *L. bethae* in South Africa. Zones in which relative survival was predicted to be over 20%, between 12 and 20%, and less than 12% were regarded as suitable, marginally suitable and unsuitable, respectively.

CLIMEX, a climate matching programme that is frequently used to infer areas suitable for growth and development of an organism elsewhere in the world (Sutherst

& Maywald, 1999), was also used to map areas in Africa that are climatically suitable for *L. bethae.* Although the culture of *L. bethae* originated from the town of Cuernavaca in Mexico (Chapter 2), matching was based on the nearby town of Puebla, at similar altitude and latitude, for which climate data were available on CLIMEX, and all available climatic parameters were employed. A qualitative comparison was made, of potential distributions of *L. bethae* predicted by the model developed here and that by CLIMEX.

Table 12.2 Estimated values of relative survival components for clay content (rs_{cc}), soil moisture (rs_{sm}), plant cleanliness (rs_{pc}) and lantana variety (rs_{lv}) in six different environmental zones of South Africa

Ecological zone	Value of ecological variable				Estimated value of relative survival component			
	Rainfall (mm/ year)	Clay content (%)	Plant cleanliness	Lantana variety	rs_{sm}	rs_{cc}	rs_{pc}	rs_{lv}
A_1	>700	>15	High	009LP	1.0	0.9	0.8	0.9
A_2	400-700	>15	High	009LP	0.6	0.9	0.8	0.9
A_3	<400	>15	High	009LP	0.2	0.9	0.8	0.9
B_1	>700	<15	High	009LP	1.0	0.4	0.8	0.9
B_2	400-700	<15	High	009LP	0.6	0.4	0.8	0.9
B_3	<400	<15	High	009LP	0.2	0.4	0.8	0.9

12.3 Results and discussion

12.3.1 Effect of each environmental variable, operating in the presence of others

The effect on survival of *L. bethae*, of each environmental variable operating in the presence of others, was qualitatively approximately the same as when it operated alone. *L. bethae* survival was strongly associated with soil moisture and clay content, weakly with plant cleanliness (i.e. freedom from infestation with *T. scrupulosa*) and

hardly at all with lantana variety. In the presence of the other environmental variables, survival was much higher on plants grown on clayey (mean ±SE: 24.4 ±1.7%) than on sandy (13.0 ±1.2%) or loamy soils (8.4 ±0.9%). Survival was much higher on moderately moist (22.9 ±1.7%) than on very wet (12.2 ±1.4%) or very dry soils (10.7 ±1.0%). Survival was slightly higher (16.9 ±1.3%) on clean than on moderately *T. scrupulosa*-infested plants (13.7 ±1.1%). In contrast to the above, survival was not higher on the lantana variety 150R (13.1 ±0.87 %) than on 021TP (16.2 ±0.87%), 009LP (18.4 ±0.87%) or 018DP (13.5 ±0.87%).

However, it was found that, when operating in concert, the differential between relative survival and maximum survival, in response to the different values of each ecological variable, was moderated considerably (Table 12.3).

Table 12.3 Relative survival of *L. bethae* in response to values of ecological variables operating alone [a] (data from Tables 4.3, 5.4, 10.3 and Fig 8.3) and in concert [b] (second paragraph above)

Ecological variable	Values of ecological variable	Relative survival in response to values of ecological variable operating	
		alone [a]	in concert [b]
Soil moisture	Low : Moderate : High	0.10 : 1.00 : 0.21	0.45 : 1.00 : 0.58
Clay content (%)	2 : 7 : 55	0.02 : 0.04 : 1.00	0.61 : 0.38 : 1.00
Plant cleanliness	Moderate : Max.	0.23 : 1.00	0.82 : 1.00
Lantana variety	018DP:009LP:021TP:15OR	0.18: 0.21: 0.29: 1.00	0.73: 1.00: 0.88: 0.71

12.3.2 Derivation of L. bethae *survival model*

Results of ANOVA of the multiple regression model (Table 12.4) indicated that the model contained variables that were significant ($P < 0.001$) in predicting the relative survival of *L. bethae.* Correlation analyses showed that percentage survival of *L. bethae*, in the presence of other environmental variables, was highly significantly ($P < 0.01$) correlated with relative survival (rs) due to clay content ($r = 0.50$) and rs due to soil moisture ($r = 0.43$) (Table 12.4). Compared with soil texture and moisture, the effect of plant cleanliness was only just significant ($P = 0.03$) while that of lantana varieties was insignificant ($P > 0.05$). Substitution of the coefficients from the multiple regression analysis (Table 12.5) of the data from the multifactorial survival trial into the generalized survival relationship (Equation 2) gave the multiple linear regression model that best explained the variation in survival of *L. bethae*, namely:

$$\mathbf{RS = 2.48 + 13.87(rs_{sm}) + 14.18(rs_{cc}) + 4.69(rs_{pc}) - 3.68\ (rs_{lv})} \quad \text{(equation 3).}$$

Therefore, the four-variable survival model that was developed here (Equation 3) can be used to predict the relative performance of *L. bethae* at different sites, after measuring the two key variables alone, namely soil moisture and clay content.

Table 12.4 ANOVA for the multiple linear regression of percentage survival of *L. bethae* against the relative survival value of 72 'sites' based on the ecological model (Equation 3)

Source	Df	SS	MS	*F*	*P*
Model	4	16121.98	4030.94	43.84	<0.0001
Error	211	19399.50	91.94		
Corrected total		35521.48			

Table 12.5 Multiple regression analysis of percentage survival (RS) of *L. bethae* from egg to adulthood as a function of relative survival values (rs) derived from soil moisture (rs_{sm}), clay content (rs_{cc}), plant cleanliness (rs_{pc}), and lantana variety (rs_{lv}) when operating separately

Predictor	Coefficient	Standard error	$\|t\|$	P
y-intercept	2.48	2.07	1.20	0.231
$rs_{\text{lantana variety}}$	-3.68	1.95	-1.88	0.061
$rs_{\text{plant cleanliness}}$	4.69	2.18	2.15	0.032
$rs_{\text{soil moisture}}$	13.87	1.64	8.46	<0.0001
$rs_{\text{clay content}}$	14.18	1.43	9.93	<0.0001

$$\mathbf{RS = 2.48 + 13.87(rs_{sm}) + 14.18(rs_{cc}) + 4.69(rs_{pc}) - 3.68\ (rs_{lv})}$$

$$\mathbf{r^2 = 0.4435}$$

12.3.3 Prediction of performance of L. bethae *in South Africa*

On the assumption that average soil moisture level is largely determined by annual rainfall level, an attempt was made to map zones of differing predicted performance of *L. bethae* across South Africa (Figure 12.1). When the maps of clay content (>15 and <15%), rainfall (>700, 400-700 and < 400 mm/annum) and lantana distribution were superimposed, the six resulting ecological zones were grouped and mapped into three main zones as shown in Table 12.6.

Table 12.6 Predicted relative survival (RS) of *Longitarsus bethae* in six different environmental zones of South Africa, obtained by substituting estimated values of relative survival components for clay content (rs_{cc}), soil moisture (rs_{sm}), plant cleanliness (rs_{pc}) and lantana variety (rs_{lv}) (Table 12.2) in the survival prediction equation {$RS = 2.48 + 13.87(rs_{sm}) + 14.18(rs_{cc}) + 4.69(rs_{pc}) - 3.68\ (rs_{lv})$}

Ecological Zone	Predicted relative survival	
	RS value	Zone
A1 (>700mm rainfall & >15% clay content)	29.5	Suitable (Black shade)
A2 (400-700mm rainfall & >15% clay content)	24.0	Suitable (Black shade)
A3 (<400mm rainfall & >15% clay content)	18.4	Marginally suitable (Dark grey)
B1 (>700mm rainfall & <15% clay content)	22.5	Suitable (Black shade)
B2 (400-700mm rainfall & <15% clay content)	16.9	Marginally suitable (Dark grey)
B3 (<400mm rainfall & <15% clay content)	11.4	Unsuitable (Light grey shade)

The zones predicted to be suitable, marginally suitable or unsuitable for *L. bethae* survival are indicated by black, dark grey and light grey shading (Fig. 12.1). Comparison of the map of lantana distribution in South Africa (Henderson, 2001) with the map of areas suitable for *L. bethae* (Fig. 12.1) indicated that the suitable zones constitute over 50% of the geographic range of lantana, and this is wide enough to justify the release of this agent in South Africa. Within the limitations of this initial model (Equation 3), performance is predicted to be good in places such as the high rainfall areas of Mpumalanga and KwaZulu-Natal provinces (Fig 12.1). For the most part, the inland provinces of Gauteng and North West were predicted to be marginally suitable or unsuitable for the survival of *L. bethae.* Some of the lowveld regions in Mpumalanga, KwaZulu-Natal and Limpopo provinces were also predicted to be marginally suitable or unsuitable for *L. bethae.* Insufficient rainfall is likely to limit the performance of *L. bethae* in some of the inland provinces and the lowveld regions, rendering these regions less suitable for the survival of this biocontrol agent.

Fig 12.1 Geographic distribution of different zones of predicted relative survival of *Longitarsus bethae* in South Africa based on percentage clay content and annual rainfall. Black, dark grey and light grey shades indicate areas that are suitable, marginally suitable and unsuitable for survival of *L. bethae*, respectively.

Based on CLIMEX, the potential geographic distribution of *L. bethae* covers the entire geographic zone of lantana in South Africa (Fig12.2).

Fig.12.2 The predicted distribution of *L. bethae* in Africa using CLIMEX.

A comparison of the potential distributions of *L. bethae* generated by CLIMEX (Fig. 12.2) and that by the current predictive model (Fig 12.1) highlights major differences. The predictive model developed here shows that the occurrence of *L. bethae* in South

Africa will occupy only a proportion of the area predicted by CLIMEX. A similar observation was made by Julien *et al.* (1995) who found that the geographic distribution of the flea beetle (*Agasicles hygrophila* Selmon and Vogt) in Australia was just a small fraction of the CLIMEX-predicted distribution. Use of CLIMEX alone produced the prediction that *Aerenicopsis championi* was likely to establish on *Lantana camara* along the entire coast of Queensland, Australia (Palmer *et al.* 2000), but the agent failed to establish there (Day *et al.,* 2003), apparently due to factors other than climate. The real value of CLIMEX as a predictive model is that it will show areas where species will probably not be able to exist. However, within regions that are shown to be climatically suitable, there could be other key-factors (e.g. soil type) that may account for the agent's establishment and geographic distribution. To predict the establishment of an insect agent in the field more precisely, it is crucial to first carefully define the major ecological factors that are likely to play a significant role in the insect's survival, and use those to develop a predictive model as has been done in this study on *L. bethae*. This can only be achieved through the use of properly designed experiments to demonstrate the direct effect of various environmental factors on the performance of a biological control agent.

The model developed in the present study explains 44% of the variation in survival of *L. bethae* observed in the laboratory. In the field, the multiple additional gradients that come into play when additional environmental variables interact will make it more difficult to predict the performance of *L. bethae* precisely. For example, soil surface cracks (Chapter 4) will increase performance, whilst predation (Chapter 9) will decrease the performance of *L. bethae.* Thermal accumulation determines the seasonal development dynamics of insects (Tauber *et al.*, 1994), and in areas with

higher temperature, faster development of *L. bethae* (Chapter 7) will expose its predation-susceptible eggs and pupae for a shorter time, but predators will be greater in variety, abundance, activity and therefore possibly also have a greater impact on *L. bethae*. Root (1973) found that an increase in background vegetation could make herbivores less effective by decreasing their search efficiency or increasing levels of mortality due to natural enemies. Therefore, an improved model would need to incorporate all these additional variables to enable more accurate predictions. The end result would be a more valuable tool for predicting establishment and impact of *L. bethae* in the field. Zalucki & Furlong (2005) suggested that a model of this nature should be tested against independent data sets, including the species' geographic distribution or long time series data for a site. However, such data are unavailable before the insect agent is released from quarantine.

Nevertheless, the initial model developed in the current study provides some insight into and understanding of how the interaction of key-variables determines the performance of *L. bethae*. The real advantage of such a predictive model for biological control is that it can be used, in combination with host-specificity tests (Chapter 3) and potential impact studies of a biocontrol agent on the target weed (Chapter 11), to demonstrate the chances of success of a biological control agent prior to its release from quarantine. This will also minimise the number of agents necessary for successful control, thereby decreasing the likelihood of non-target effects of control organisms. At the very least, a model of this sort will allow efficient distribution of an agent by initially releasing it onto sites predicted to be environmentally suitable for its survival.

CHAPTER 13

General discussion and conclusions

The calls for improving selection of effective weed biocontrol agents prior to release have been increasing in frequency and intensity (Wapshere, 1974; Myers, 1985; Cullen, 1989; Marohasy; 1998; McFadyen, 1998; McEvoy & Coombs, 2000; Sheppard, 2002; Briese, 2002). In an attempt to address this issue, carefully designed experiments were carried out to demonstrate that the biocotrol agent (*L. bethae*) will be safe, establish by virtue of having the ability to cope with important ecological conditions in the release areas, and inflict significant damage on the target weed (*L. camara*).

Host-specificity tests conducted in chapter 3 demonstrate beyond reasonable doubt that *L. bethae* will be restricted to *L. camara* in the field. The limited development on some indigenous *Lippia* and *Lantana* spp. may be explained by the tendency of host-specific insects to expand their host ranges under laboratory conditions (Cullen, 1989; Balciunas *et al.*, 1996). The considerable impact of root-feeding damage on plant growth and biomass (Chapter 11) provides evidence that under favourable environmental conditions for the beetle, attacks could contribute to reductions in lantana population growth. Blossey & Hunt-Joshi (2003) reported that 54% of the released root-feeding biocontrol agents contribute to the suppression of the invasive plant population versus 34% of the above-ground biocontrol agents. While results from pot trials can provide valuable information on specific aspects of plant/ herbivore interactions, they provide relatively little information about the effects of

herbivores on plant community dynamics (Brown & Gange, 1990; Dhileepan, 2002). Therefore, the assumption that *L. bethae* would reduce the population density of its hosts may not strictly hold, particularly because of complexities and magnitude of abiotic factors in the release areas.

When studied independently, a number of ecological factors indicated possible effects on survival of *L. bethae* (Chapters 4 to 10). However, when these factors were operating in concert in a multivariate model (Chapter 12), only soil moisture and texture were strongly associated with *L. bethae* survival while the others were moderated considerably. For soil moisture, this is consistent with studies conducted in chapters 4, 6 and 7 which showed a stronger trend for greater mortality of *L. bethae* under abiotic conditions associated with rapid moisture loss (e.g. sand, bare soil and low humidity). Ireson *et al.* (1991) also found that low summer rainfall was a key-factor that restricted establishment of *Longitarsus flavicornis* (Stephens) in Tasmania from 1979 to 1984, while wetter summers occurring at some sites in 1985/86 supported population increases. The increase in clay and the corresponding decrease in sand content, which is directly related to increase in soil moisture holding capacity, also affects the survival of and accounts for increased abundance of a number of root-feeding insects (Morrone and Stinner, 1984; Strnad and Bergman, 1987; Riedell and Sutter, 1995; Pacchioli and Hower, 2004). It is therefore conceivable that a combination of fine-textured soil and other conditions that inhibit rapid water loss will increase the survival of and damage to the root system by *L. bethae*. Preliminary evidence suggests that in moist subtropical eastern regions of South Africa where lantana is presently naturalized and abundant, lantana often forms dense thickets with closed canopies, resulting in leaf litter accumulation on the ground (Simelane,

personal observation). Under such conditions, the rate of soil drying and soil surface temperature could be reduced, thereby conserving soil moisture required for increased survival of *L. bethae*.

Because of logistical constraints, temperature and other climatic factors were not incorporated into the multivariate experiment (Chapter 12), and this is by no means an implication of their insignificance in the survival of *L. bethae*. Crawley (1989) found that climate was important in 44% of the failures of biocontrol agents to establish or control weeds. Studies in Chapter 7 clearly demonstrate that both humidity and temperature have significant influence on embryonic development, and these could play some role in the population dynamics of *L. bethae*. The study found that percentage egg hatch increased rapidly with increases in temperature from 17 to 27°C and was optimum at approximately 23°C. This appears to be within the range of summer soil temperatures (16-29°C) recorded by the author at a lantana-infested farm (S25°43'36.7"; E23°14'03.3"), Pretoria, South Africa, between October 2004 and March 2005. Therefore, summer temperature and humidity regimes in the high rainfall regions of South Africa where *L. camara* is a problem are anticipated to be well within the ranges that are tolerated by *L. bethae* eggs.

The multivariate model (Chapter 12) showed that biotic factors such as herbivory by a well-established agent (*T. scrupulosa*) and varietal resistance are unlikely to restrict establishment and abundance of *L. bethae* in the field. While a few studies have indicated that the use of multiple agents in weed biocontrol systems may actually reduce the likelihood of success through the process of competitive exclusion (Julien & Griffiths, 1998; Denoth *et al.*, 2002), the present study suggests that *L. bethae* is

likely to cope with detrimental interactions, and that the combined damage with the foliage feeder (*T. scrupulosa*) could result in cumulative stress on the plant. There is also evidence that above-ground herbivores, even at high population levels, are unable to prevent the build-up of under-ground herbivore populations and the population collapse of their host plants (Gange & Brown, 1990). Multiple agent releases have caused cumulative stress on various weeds, resulting in biocontrol successes in a number of weed biocontrol systems (McEvoy, 1985; Crawley, 1989; Pemberton and Turner, 1990; Harris, 1991; McEvoy *et al.*, 1993; Saner *et al.*, 1994; Hoffmann and Moran, 1998).

Although reasons for failure of some biocontrol agents to establish or effectively control lantana in South African and Australia have been attributed to differences in phenotypes of the weed (Neser & Cilliers, 1989; Cilliers & Neser, 1991; Day & Neser, 2000; Broughton, 2000), data in chapter 12 suggest that this biotic factor is unlikely to significantly reduce the survival of *L. bethae* in the field. While chances of survival and establishment are expected to be greater on varieties that were preferred for oviposition and provided better survival of the immature stages, this is not expected to jeopardize the overall biocontrol success *L. bethae* in the field.

Logistical difficulties prevented the inclusion of predation, parasitism and diseases in the multivariate experiment in chapter 12, and yet these could significantly influence the population dynamics of *L. bethae* in the field. Although root feeders, by virtue of their feeding niche, occupy a safe refuge from aerial predators and parasitoids (Blossey & Hunt-Joshi, 2003), a number of studies suggests that soil dwelling predators may greatly reduce populations of root feeders (Sunderland *et al.*, 1995;

Lang, 1999; Jones & Bradford, 2001; Vichitbandha & Wise, 2002; Cardinale *et al.*, 2003; Juen, *et al.*, 2003; Cherry, 2003; Rypstra & Marshall, 2005), hence their use as biocontrol agents of arthropod pests. Although the data were collected over a short period of time, studies in chapter 9 indicate a potential influence of some ground-dwelling predators (e.g. carabid beetles) on *L. bethae*. A number of soil-born entomopathogenic nematodes, fungi, bacteria and viruses attack and kill root-feeding larvae, and some are used as biocontrol agents of arthropod pests (Poprawski *et al.*, 1985; Shetlar *et al.*, 1988; Kard *et al.*, 1988; Blossey & Ehlers, 1991). Although it is intuitively obvious that entomopathogenic organisms could hamper the efficacy of root-feeding insect agents, recorded instances of this occuring are rare. Long-term studies are therefore needed to assess how natural enemies and entomopathogenic organisms may shape interactions of root feeding insect agents and their host plants.

While the main purpose of the study was to predict the effectiveness of *L. bethae* prior to release, the value of the model developed in chapter 12 extends beyond this. The model defines the geographic areas where the chances of success of *L. bethae* will be low. The model may thus be helpful in making decisions about future control strategies of the target weed in these areas. For example, a decision to import and screen additional insect species that may be more adapted to areas deemed unsuitable for *L. bethae* can be made promptly. While the model of Julien *et al.* (1995) is also capable of defining areas where an agent would have some impact on the weed, their model was only based on climatic suitability of the agent using CLIMEX. Unlike the model developed here, CLIMEX predicts with a broader accuracy (Chapter 12), and that it does not take into account the other key-factors (e.g. soil conditions) that may account for the agent's establishment and geographic distribution. There are instances

where a biocontrol agent has either failed to establish (Day, 2003) or established on a small fraction of the CLIMEX-predicted distribution (Julien *et al*., 1995).

The assessment of the potential impact of *L. bethae* on vegetative growth and biomass production of *L. camara* demonstrated that this insect is likely to exhibit sufficient herbivore pressure on this rapidly growing weed. The combined effect of this root feeder and the above-ground biocontrol agents such as *T. scrupulosa* is expected to have a complementary effect on plant biomass, thereby increasing the chances of successful biological control of lantana in South Africa.

All indications are that the importance of all edaphic characteristics lies in their influence upon the ability of the soil to retain moisture, which ultimately determines the amount of moisture available for survival of the immature stages of *L. bethae*. Therefore, good establishment and population increase of *L. bethae* may be expected on soils with high moisture-retention capacity, such as fine-textured or clayey soils. Conversely, ecological factors that are associated with rapid water loss (e.g. sand, bare ground and low atmospheric humidity) will have a detrimental effect on the survival of *L. bethae* in the field.

Longitarsus bethae is expected to cope with a suite of biotic ecological constraints (e.g. herbivory by an established agent and varietal resistance). As demonstrated in the study, the effects of both herbivory by a well-established agent and lantana variety are likely to be moderated considerably or become insignificant when various ecological factors operate in concert in the field. While predation by ground-dwelling arthropods should be expected upon the eggs and pupae of *L. bethae,* lying on or just

under the surface of the soil, predation by above-ground natural enemies is expected to be negligible upon the root-chewing larvae, occupying a safer refuge further below the surface.

Although the predictive model developed in the study is based on only four variables (soil texture, soil moisture, cleanliness of the host plant and lantana variety), it provides some insight into and understanding of how the interaction of ecological variables determines the performance of *L. bethae*. Based on this model, it is predicted that the distribution of *L. bethae* could cover over 50% of the geographic range of lantana in South Africa.

Given the evidence that *L. bethae* is host-specific, likely to cope with ecological constraints to establish on more than half of the geographic range of lantana in South Africa, and inflicts markedly substantial damage on the weed, it is strongly justified to release this biocontrol agent into this country. Demonstration of safety and relative efficacy of a biocontrol agent prior to release, as has been done in this study on *L. bethae*, could reduce the number of control organisms necessary for successful control of the weed, thereby reducing the overall risk of non-target affects.

REFERENCES

ANDERSEN, D. C. 1987. Below-ground herbivory in natural communities: A review emphasizing fossorial animals. *Quarterly Reviews of Biology* **62**: 261-286.

ARNOLD, C.Y. 1959. The determination and significance of the base temperature in a linear heat unit system. *American Society of Horticultural Science* **74**: 430-435.

BAARS, J-R. 2001. Biology and laboratory culturing of the root-feeding flea beetle, *Longitarsus columbicus columbicus* Harold, 1877 (Chrysomelidae: Alticinae), a potential natural enemy of *Lantana camara* (Verbenaceae) in South Africa. *Entomotropica* **16**: 149-155.

BAARS, J-R. & HEYSTEK, F. 2003. Geographical range and impact of five biocontrol agents established on *Lantana camara* in South Africa. *BioControl* **48**: 743-759.

BAARS, J-R & NESER, S. 1999. Past and present biological control initiatives of *Lantana camara* (Vervenaceae) in South Africa. *African Entomology memoir* **1**: 21-33.

BALCIUNAS, J. K., BARROWS, D.W. & PURCELL, M.F. 1996. Comparison of the Physiological and Realized host-ranges of a biological control agent from Australia for the control of the aquatic weed, *Hydrilla verticillata*. *Biological Control* **7**: 148-158.

BENCHYNE, J. 1997. Evaluacion de los datos sobre los Phytophaga daninos en Venezuela (Coleoptera) Parte I & II. Savini, W. (Ed) Boletin de Entomologia Venezolana, Serie Monografias No.1, Noviembre 1997.

BEZEMER, T. M., WAGENAAR, R, N. M. VAN DAM, N.M. & F. L. WACKERS. 2003. Interactions between above- and below-ground insect herbivores as mediated by the plant defense system. *Oikos* **101**: 555-562.

BLOSSEY, B. & EHLERS, R-U. 1991. Entomopathogenic nematodes (*Heterorhabditis* spp. and *Steinernema anomali*) as potential antagonists of the biological weed control agent *Hylobius transversovittatus* (Coleoptera: Curculionidae). *Journal of Insect Pathology* **58**: 453-454.

BLOSSEY, B. & HUNT-JOSHI, T.R. 2003. Belowground herbivory by insects: influence on plants and aboveground herbivores. *Annual Reviews of Entomology* **48**: 521-547.

BRADY, N.C. 1974. The nature and properties of soils (8th edition). Macmillan Publishing Co. New York.

BRIESE, D. T. 1996. 1996. Phylogeny: can it help us to understand host choice by biological weed control agents? *In* V. C. Moran and J. H. Hoffmann (eds). Proceedings of the IX International Symposium on Biological Control of Weeds, pp. 63-70. Stellenbosch, South Africa.

BRIESE, D.T. 2002. The centrifugal phylogenetic method used to select plants for host-specificity testing of weed biological control agents: can and should it be modernized?. *In* H. Spafford Jacob & Briese (eds). Improving the selection, testing and evaluation of weed biological control agents, pp. 23-33. University of Western Australia, Perth, Western Australia.

BROUGHTON, S. 2000. Review and evaluation of lantana biocontrol programs. *Biological Control* **17**: 272-286.

BROWN, V.K. & GANGE, A.C. 1990. Insect herbivory below ground. *Advances in Ecological Research* **20**: 1-58.

BRUST, G.E., STINNER, B.R & McCARTNEY, D.A 1986. Predator activity and predation in corn agroecosystems. *Environmental Entomology* **15**: 1017-1021.

BRUST, G.E. &. HOUSE, G.J. 1990. Effects of soil moisture, non-tillage and predators on Southern corn rootworm (*Diabrostica undecimpunctata* Howard): survival in corn agroecosystem. *Agriculture, Ecosystem and Environment* **31**: 199-216.

CAMPBELL, A., FRANZER, B.D. GILBERT, N. GUTIERREZ, A.P & MACKAUER, .M. 1974. Temperature requirements of some aphids and their parasites. *Journal of Applied Ecology* **11**: 431-438.

CAESAR, A.J. 2003. Synergistic interaction of soilborne plant pathogens and root-attacking insects in classical biological control of an exotic rangeland weed. *Biological Control* **28**: 144-153.

CARDINALE, B.J, CHAD, C.T., GROSS, K. & IVES, A.R. 2003. Biodiversity and biocontrol: emergent impact of a multi-enemy assemblage on pest suppression and crop yield in an agroecosytem. *Ecology Letters* **6**: 857-865.

CHERRY, R. 2003. Effect of harvesting and replanting on arthropod ground predators in Florida sugarcane. *Florida Entomologist* **86**: 49-52.

CHOCOROSQUI, V.R. & PANIZZI, A.R. 2003. Photoperiod influence on the biology and phonological characteristics of *Dichelops melacanthus* (Dallas, 1851) (Heteroptera: Pentatomidae). *Brazilian Journal of Biology* **63**: 141-150.

CILIESRS, C.J. 1983. The Weed, *Lantana camara* L., and the insect natural enemies imported for its biological control into South Africa. *Journal of Entomological Society of Southern Africa* **46**: 131-138.

CILLIERS, C.J & NESER, N. 1991. Biological control of *Lantana camara* (Verbenaceae) in South Africa. *Agriculture, Ecosystem and Environment* **37**: 57-75.

CLEMENT, S.L. & CRISTOFARO, M. 1995. Open-field tests in host-specificity determination of insects for biological control of weeds. *Biocontrol Science and Technology* **5**: 395-406.

CRAIG, T. P., ITAMI, J.K. & PRICE, P.W. 1989. A strong relationship between oviposition preference and larval performance in a shoot-galling sawfly. *Ecology* **70**: 703-727.

CRAWLY, M.J. 1989. The successes and failures of weed biocontrol using insects. *Biocontrol News and Information* **10**: 213-223.

CRAWLEY, M.J. 1989. The success and failures of weed biocontrol using insects. *Biocontrol News and Information* **10**: 213-223.

CULLEN, J. M. 1989. Current problems in host-specificity screening. *In* "Proceedings VII International Symposium on Biological Control of Weeds, Rome, Italy, 6-11 March 1988" (E. S. Dalfosse, Ed.), pp. 27-36.

DAM, N.M., RAAIJAMAKERS, C.E. & VAN DER PUTTEN, W.H. 2005. Root herbivory reduces growth and survival of the shoot-feeding specialist, Pieris rapae on Brassica nigra. *Entomologia Experimentalis et Applicata* **115**: 161-170.

DANKS, H.V. 2003. Studying insect photoperiodism and rhythmicity: components, approaches and lessons. *European Journal of Entomology* **100**: 209-221.

DAY, M.D. & NESER, S. 2000. Factors affecting the biological control of lantana in Australia and South Africa. In: Proceedings X International Symposium of Biological Control of Weeds, 4-14 July 1999, Montana State University,

Bozeman, Montana, USA. N.R. Spencer (ed), pp. 897-908.

DAY, M.D., WILEY, C.J., PLAYFORD, J. & ZALUCKI, M.P. 2003. Lantana: Current Management Status and Future Prospects. Australian Centre for International Agricultural Research, Canberra, Australia.

DEBACH, P. & ROSEN, D. 1991. Biological control by natural enemies (2nd ed). Cambridge University Press, Cambridge, UK.

DEMPSTER, J.P. 1971. The population ecology of the cinnabar moth, *Tyria jacobaeae* L. (Lepidoptera: Arctiidae). *Oecologia* **7**: 26-67.

DENT, D. 1991. Insect Pest Management. CAB International, Londan.

DENTON, G.R.. MUNIAPPAN, W.R. & MARUTANI, M. 1991. Status and natural enemies of *Lantana camara* in Micronesia. *Tropical Pest Management* **37**: 338-344.

DETHIER, V.G. 1954. Evolution of feeding preference in phytophagous insects. *Evoulution* **8**: 33-54.

DHILEEPAN, K. 2002. The centrifugal phylogenetic method used to select plants for host-specificity testing of weed biological control agents: can and should it be modernized?. *In* H. Spafford Jacob & Briese (eds). Improving the selection, testing and evaluation of weed biological control agents, pp. 51-60. University of Western Australia, Perth, Western Australia.

DINTENFASS, L.P. & BROWN, G.C. 1988. Influence of larval clover root curculio (Coleoptera: Curculionidae) injury on carbohydrate reserves and yield of alfalfa. *Journal of Economic Entomology* **81**: 1803-1809.

DUTCHER, J.D. & ALL, J.N. 1979. Damage impact of larval feeding by the grape root borer in a commercial concord grape vineyard. *Journal of Economic Entomology* **72**: 159-161.

EDNEY, E.B. 1977. Water balance in land arthropods. Springer, New York.

EHRLICH, P.R & RAVEN, P.H. 1964. Butterflies and plants: A study in co-evolution. *Evolution* **18**: 586-608.

EHRLICH, P.R & MURPHY, D.D. 1988. Plant chemistry and host range in insect herbivores. *Ecology* **69**: 908-909.

EVANS, W.G. 1988. Chemically-mediated habitat recognition in shore insects (Coleoptera: Carabidae: Hemiptera: Saldidae). *Journal of Chemical Ecology* **14**: 1441-1454.

FINKE, D.L. & DENNO, R.F. 2003. Intra-guild predation relaxes natural enemy impacts on herbivore populations. *Ecological Entomology* **28**: 67-73.

FINKE, D.L. & DENNO, R.F. 2005. Predator diversity and functioning of ecosystem: the role of intra-guild predation in dampening trophic cascades. *Ecology Letters* **0**: 12-17.

FORCE, D.C. & MESSENGER, P.S. 1964. Duration of development, generation time, and longevity of three hymenopterous parasites of *Therioaphis maculata*, reared at various constant temperatures. *Annals of Entomological Society of America* **57**: 405-413.

FORISTER, M.L. 2004. Oviposition preference and larval performance within a divergent lineage of Lycaenid butterflies. *Ecological Entomology* **29**: 264-272.

FORNASARI, L. 1995. Temperature effects on embryonic development of *Aphthona abdominalis* (Coleoptera: Chrysomelidae), a natural enemy of *Euphorbia esula* (Euphorbiales: Euphorbiceae). *Environmental Entomology* **24**: 720-723.

FOSTER, R.E., RUESINK, W.G & LUCKMANN,W.H. 1979. Northern corn rootworm egg sampling. *Journal of Economic Entomology* **72**: 659-663.

FOWLER, S.V. & LAWTON, J.H. 1985. Rapidly induced defenses and talking trees: the devil's advocate position. *American Naturalist* **126**: 181-195.

FRICK, K.E. 1970. *Longitarsus jacobaeae* (Coleoptera: Chrysomelidae), a flea beetle for biological control of tansy ragwort: host plant specificity studies. *Annals of Entomological Society of America* **35**: 71-77.

FUTUYMA, D.J & PETERSON, S.C. 1985. Genetic variation in the use of resources by insects. *Annual Reviews of Entomology* **30**: 217-238.

GANGE, A. C. & BROWN, V.K. 1989. Effects of root herbivory by an insect on a foliar-feeding species mediated through changes in the host plant. *Oecologia* **81**: 38-42.

GODFREY, L.D., MEINKE, L.J. WRIGHT, R.J & HEIN, G.L. 1995. Environmental and edaphic effects on western corn rootworm (Coleoptera: Chrysomelidae) overwintering egg survival. *Environmental Entomology* **88**: 1445-1454.

GOEDEN, R.D. 1983. Critique and revision of Harris' scoring system for selection of insect agents in biocontrol of weeds. *Plant Ecology* **5**: 287-301.

GOEDEN, R.D., FLESCHNER, C.A & RICKER, D.W. 1967. Biological control of prickly pear cacti on Santa Cruz Island, California, *Higardia* **38**: 579-606.

GOEHRING, L. & OBERHAUSER, K.S. 2002. Effects of photoperiod, temperature, and host plant age on the induction of reproductive diapause and development time in *Danaus plexippus*. *Ecological Entomology* **27**: 674-685.

GORMAN, M.R., FREEMAN, D.A. & ZUCKER, I. 1997. Photoperiodism in hamsters: Abrupt versus gradual changes in day length differentially entrain morning and evening circadian oscillators. *Journal of Biological Rhythms* **12**: 122-135.

GRAAF, J.L. 1986. *Lantana camara,* the plant and some methods for its control.

South African Forestry Journal, March: 26-30.

GRAY, M.E. & TOLLEFSON, J.J. 1987. Influence of tillage and western and northern corn rootworm (Coleoptera: Chrysomelidae) egg populations on larval populations and root damage. *Journal of Economic Entomology* **80**: 911-915.

GREATHEAD, D. 1995. Benefits and risks of classical biological control. *In* H.M.T. Hokkanen and J.M. Lynch: Biological Control: *Benefits and Risks*. Cambridge University Press, Cambridge, U.K. 53-63.

GROVES, R.H. & WILLIAMS, J.D. 1975. Growth of skeleton weed (*Chondrilla juncea* L.) as affected by growth of subterranean clover (*Trifolium subterraneum* L.) and infection by *Puccinia chondrillina* Bubak & Syd. *Australian Journal of Agricultural Research* **26**: 975-983.

GUSTON, R.D. 1979. Effects of two moisture and population levels on oviposition of the western corn rootworm. *Environmental Entomology* **8**: 406-407.

GUSTON, R.D. & SCHUMACHER, T.E. 1989. Relationship of some pore parameters to movement of first-instar western corn rootworm (Coleoptera: Chrysomelidae). *Environmental Entomology* **18**: 343-346.

HADLEY, N.F., KNISLEY, C.B., SCHULTZ , T.D. & PEARSON, D.L.. 1989. Water relations in tiger beetle larvae (Cicindela marutha): correlations with habitat microclimate and burrowing activity. *Journal of Arid Environments* **19**: 189-197.

HARLEY, K.L.S., KERR, J.D. & KASSULKE, R.C. 1979. Effects in S.E. Queensland during 1967-1972 of insects introduced to control *Lantana camara*. *Entomophaga* **24**: 65-72.

HARRIS, P. 1973. The selection of effective agents for the biological control of

weeds. *Canadian Entomologist* **105**: 1495-1503.

HARRIS, P. 1991. Classical biocontrol of weeds: its definition, selective of effective agents and administrative political problems. *Canadian Entomologist* **123**: 827-849.

HARRIS, S. & KARBAN, R. 1986. Effects of an early-season folivorous moth on the success of a later-season species, mediated by a change in the quality of the shared host, *Lupinus arboreus* Sims. *Oecologia* **69**: 354-359.

HARRIS, P. & McEVOY, P. 1995. The predictability of host utilization from feeding tests and suggested improvements for screening weed biocontrol agents. *In* E.S. Delfosse (ed.). Proceedings of the Eighth International Symposium on Biological Control of Weeds, pp. 125-131. CSIRO, Melbourne, Australia.

HAWKES, R.B & JOHNSON, G.R. 1978. *Longitarsus jacobaeae* aids moth in the biological control of tansy ragwort. Pp. 193-196. *In:* Proceedings of the IV International Symposium on Biological Control of Weeds. Freeman, T.E. (ed), Gainesville, Florida, Institute of Food and Agricultural Science, University of Florida, 30 August-2 September 1976, Florida.

HESHULA, U-N. L. P. 2005. Establishment and impact of the sap-sucking mirid, *Falconia intermedia* (Distant) (Hemiptera: Miridae), on *Lantana camara* (Verbenaceae) varieties in the Eastern Cape Province, South Africa. MSc Thesis. Rhodes Unversity, Grahamstown.

HENDERSON, L. 2001. Alieen weeds and Invasive plants: A complete guide to declared weeds and invaders in South Africa. Plant Protection Research Institute, Agricultural Research Council, South Africa.

HOBACK. W.W. & GOLICK, D.A. 2000. Salinity and shade preferences result in oviposition defferences between sympatric tiger beetle species. *Ecological*

Entomology **15**: 180-187.

HOFFMANN, J.H. & MORAN, V.C. 1998. The population dynamics of an introduced tree, *Sesbania puniceae*, in South Africa, in response to long-term damage caused by different combinations of three species of biological control agents. *Oecologia* **144**: 343-348.

HOLM, L. G., PLUCKETT, D.L., PANCO, J.V. & HERBERGER, J.P. 1977. "The World's Worst Weeds." University Press of Hawaii, Honolulu, HI.

HOU, X., MEINKE, L.J. & ARKEBAUER, T.J. 1997. Soil moisture and larval western corn rootworm injury: Influence on gas exchange parameters in corn. *Agronomy Journal* **98**: 709-717.

HOWE, R.W. 1967. Temperature effects on embryonic development in insects. *AnnualReviews of Entomology* **12**: 15-42.

HOWLETT, B.G., CLARK, A.R & MADDEN, J.L. 2001. The influence of leaf age on oviposition preference of *Chrysophtharta bimaculata* (Olivier) and the establishment of neonates. *Agricultural and Forest Entomology* **3**: 121-127.

INBAR, M., ESHEL, A. & WOOL, D. 1995. Interspecific competition among phloem-feeding insects mediated by induced host-plant sinks. *Ecology* **76**: 1506-1515.

IRESON, J.E., FRIEND, D.A, HOLLOWAY, R.J. & PETERSON, S.C, 1991. Biology of *Longitarsus flavicornis* (Stephens) (Coleoptera: Chrysomelidae) and its effectiveness in controlling ragwort (*Senecio jacobaeae* L.) in Tasmania. *Journal of Australian Entomological Society* **30**: 129-141.

JAENIKE, J. 1978. On optimal oviposition behaviour in phytophagous insects. *Theoretical Population Biology* **264**: 701-707.

JIN, X., MORTON, J. & BUTLER, L. 1992. Interactions between *Fusarium avena*ceum and *Hylastinus obscurus* (Coleoptera: Scolytidae) and their influence on root decline in red clover. *Journal of Economic Entomology* **85**: 1340-1346.

JOLIVET, P & HAWKENSWOOD, T.J. 1995. Host plants of Chrysomelidae of the world. Backbys Publishers, Leiden.

JONES, T.H., BRADFORD, M.A. 2001. Assessing the functional implications of soil biodiversity in ecosystems. *Ecological Research* **16**: 845-858.

JORDAN, T. 1997. Host Range of *Longitarsus quadriguttatus* (Pont. 1765) (Coleoptera: Chrysomelidae), an agent for the biological control of hand's tongue (*Cynoglossum officinale* L., Boraginaceae) in North America. *Journal of Applied Entomology* **121**: 457-464.

JUEN, A., STEINBERGER, K-H & TRAUGOTT, M. 2003. Seasonal change in species composition and size distribution of epigeic predators in a small field. *Entomologia Generalis* **26**: 259-275.

JULIEN, M.H., SKARRATT, B. & MAYWALD, G.F. 1995. Potential geographical distribution of alligator weed and its biological control agent *Agasicles hydrophila*. *Journal of Aquatic Plant Management* **33**: 55-60.

KARBAN, R. & MYERS, J.H. 1989. Induced plant responses to herbivory. *Annual Review of Ecology and Systematics* **20**: 331-348.

KARD, B.M., HAIN, F.P. & BROOKS, W.M. 1988. Field suppression of three white grub species (Coleoptera: Scarabaiedae) by the entomogenous nematodes Steinernema feltae and Heterorhabditis heliothidis. *Journal of Economic Entomology* **81**: 1033-1039.

KIRK, V.M., CALKINS, C.O & POST, F.J. 1968. Oviposition preference of western corn rootworm for various soil surface conditions. *Journal of Economic Entomology* **61**: 1322-1324.

KONSTANTINOV, A & VANDENBERG, N. 1996. Handbook of Paleartic flea beetles (Coleoptera: Chrysomelidae: Alticinae). *Contributions on Entomology* **1**: 237: 439.

KRAMER, D.A. STINNER, R.E & HAIN, F.P. 1991. Time versus rate in parameter estimation of nonlinear temperature-dependant development models. *Environmental Entomology* **20**: 484-488.

LANG, A., FILSER, J. & HENSCHEL, J.R. 1999. Predation by ground beetles and wolf spiders on herbivorous insects in a maize crop. *Agriculture, Ecosystem and Environment* **72**: 189-199.

LAPIS, E.B. & BORDEN, J.H. 1993. Olfactory discrimination by *Heteropsylla cubana* (Homoptera: Psllidae) between susceptible and resistant species of *Leucaena* (Leguminosae). *Journal of Chemical Ecology* **17**: 83-90.

LEATHR, S.R. 1985. Oviposition preference in relation to larval growth rates and survival in the pine beauty moth moth, *Panolis flammea. Ecological Entomology* **10**: 213-217.

LEATHER, S.R., WATT, A.D & FORREST, G. I. 1987. Insect-induced chemical changes in young lodge pole pine (*Pinus contorta*): the effect of previous defoliation on oviposition, growth and survival of the pine beauty moth, *Panolis flammea. Ecological Entomology* **12**: 275-281.

LOUDA, S.M., KENDALL, D. CONNOR, J. & SIMBERLOFF, D. 1997. Ecological effects of an insect introduced for the biological control off weeds. *Science* **277**: 1088-1090.

LUMMUS, P.F., SMITH, J.C. & POWELL, N.L. 1983. Soil moisture and texture effects on survival of immature southern corn rootworms, *Diabrostica undecimpunctata howardi* Barber (Coleoptera: Chrysomelidae). *Environmental Entomology* **12**: 1529-1531.

MACDONALD, P.J & ELLIS, C.R. 1990. Survival time of unfed, first-instar western corn rootworm (Coleoptera: Chrysomelidae) and the effects of soil type, moisture and compaction on their mobility in the soil. *Environmental Entomology* **19**: 666-671.

MAROHASY, J., 1998. The design and interpretation of host-specificity for weed biological control with particular reference to insect behaviour. *Biocontrol News and Information* **19**: 13N-15N

MARRON, P.G. & STINNER, R.E. 1983. Bean leaf beetle, *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae): physical factors affecting larval movement in the soil. *Environmental Entomology* **12**: 1283-1285.

MARRON, P.G. & STINNER, R.E. 1984. Influence of soil physical factors on survival and development of the larvae and pupae of the bean leaf beetle *Cerotoma trifurcata* (Coleoptera: Chrysomelidae). *Canadian Entomologist* **116**: 1015-1023.

MASTERS, G.J. & BROWN, V.K. 1992. Plant-mediated interactions between two spatially separated insects. *Functional Ecology* **6**: 175-179.

MATTESON, W.J. 1980. herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics* **11**: 119-161.

MAYHEW, P.J. 1997. Adaptive patterns of host-plant selection by phytophagous insects. *Oikos* **79**: 417-428.

McEVOY, P.B. 1985. Dipression in ragwort (*Sinecio jacobaeae*) abundance following introduction of *Tyria jacobaeae* and *Longitarsus jacocaeae* on the central coast of Oregon. *In*: Proceedings of the Sixth International Symposium of Biological Control of Weeds, pp. 57-64, 1984, Vancouver, Canada. Delfosse, E.S. (ed). Agriculture, Canada.

McEVOY, P.B. 1996. Host specificity and biological pest control. *BioScience* **46**: 401-405.

McEVOY, P.B & COOMBS, E.M. 2000. Why things bite back: unintended consequences of biological weed control. *In* P.A. Follet and J.J Duan (eds.). Nontarget effects of biological control, pp. 167-194. Kluwer Academic puplishers, Boston, Massachusetts, USA.

McEVOY, P.B., RUDD, N.T. CAROLINE, S.C. & MANUELA, H. 1993. Disturbance, competition, and herbivory effects on ragwort *Senecio jacobaeae* populations. *Ecological Monographs* **63**: 55-75.

McFADYEN CRUTTWELL, R.E. 1998. Biological control of weeds. *Annual Review Of Entomology* **43**: 369-393.

McFADYEN CRUTTWELL, R.E. 2002. Does Ecology help in the selection of biocontrol agents? *In* H. Jacob Spafford and D.T. Briese (eds.), Improving the selection, testing and evaluation of weed biological control agents, pp. 5-9.University of Western Australia, Perth, Western Australia.

McPHERSON, J.E. 1974. Photoperiod effects in a Southern Illinois population of the *Euscchistus tristigmus* complex (Hemipterra: Pentatomidae). *Annals of Entomological Society of America* **67**: 943-952.

McPHERSON, J.E. 1975. Effects of developmental photoperiod on adult morphology in *Euscchistus tristigmus* Say (Hemipterra: Pentatomidae). *Annals of*

Entomological Society of America **68**: 1107-1110.

MORAN, N.A. & WHITHAM, T.G. 1990. Interspecific competition between root-feeding and leaf-galling aphids mediated by host-plant resistance. *Ecology* **7**: 1050-1058.

MORRIS, T.I.M., CAMPOS, N.A.C., KIDD, M.A. & SYMONDSON, W.O.C. 1999. Dynamics of the predatory arthropod community in Spanish olive groves. *Agricultural and Forest Entomology* **1**: 219-228.

MYERS, J.H. 1985. How many insect species are necessary for successful biocontrol of weeds? *In* E.S. Delfosse (ed.). Proceedings of the Sixth International Symposium on Biological Control of Weeds, pp. 77-82. Agriculture, Canada, Ottawa.

NESER, S. & CILLIERS, C.J. 1989. Work towards biologial control of *Lantana camara*: Perspectives. In: Proceedings VII International Symposium on Biological Control of Weeds, Delfosse, E.S. (ed) Rome, 1988. Instituto sperimentale per la Patalogia Vegetale Minestero dell Agricolture e delle Foreste, Rome, Italy, pp. 363-369.

NÖTZOLD, R., BLOSSEY, B. & NEWTON, E. 1998. The influence of belowground herbivory and plant competition on growth and biomass allocation of purple loosestrife. *Oecologia* **113**: 82-93.

O'NEAL, M.E., ZONTEK, E.L, SZENDREI, Z., LANDIS, D.A. & ISAACS, R. 2005. Ground predator abundance affects prey removal in highbush blueberry (*Vaccinium corymbosum*) fields and can be altered by aisle ground covers. *BioControl* **50**: 205-222.

PALMER, W.A. & PULLEN, K.R. 1995. The phytophagous arthropods associated with *Lantana camara, L. hirsuta, L. urticifolia, and L. urticoides*

(Verbenaceae) in North America. *Biological Control* **5**: 54-72.

PALMER, W.A., WILSON, B.W. & PULLEN, K.R. 2000. Introduction, rearing and host range of *Aerenicopsis championi* Bates (Coleoptera: Cerambycidae) for the biological control of *Lantana camara* L. in Australia. *Biological Control* **17**: 227-233.

PACCHIOLI, M.A. & ARTHUR, A.A. 2004. Soil and moisture effects on the dynamics of early instar clover root curculio (Coleoptera: Curculionidae) and biomass of alfafa root nodules. *Environmental Entomology* **33**: 119-127.

PAPAJ, D.R. & PROKOPY, R.J.1989. Ecological and evolutionary aspects of learning in phytophagous insects. *Annual Reviews of Entomology* **34**: 315-50.

PEMBERTON, R. W. 2000. Predictable risk to native plants in weed biological control. *Oecologia* **125**: 489-494.

PEMBERTON, R. W. & TURNER, C.E. 1990. Biological Control of *Senecio jacobaeae* in Northern California, an enduring success. *Entomophaga* **35**: 71-77.

PERDIKIS, D.CH, LYKOURESSIS, D.P. & ECONOMOU, L.P. 2004. Influence of light-dark phase, host plant, temperature, and their interactions on the predation rate in an insect predator. *Environmental Entomology* **33**: 1137-1144.

PILSON, D. & RAUSHER, M.D.1988. Clutch size adjustment by swallowtail butterfly. *Nature* **333**: 361-363.

POCCHIOLI, M.A. & HOWER, A.A. 2004. Soil and moisture effects on the dynamics of the early instar clover root curculio (Coleoptera: Chrysomelidae) and biomass of alfafa root nodules. *Environmental Entomology* **33**: 119-127.

RIEDEL, W.C. & SUTTER, G.R. 1995. Soil moisture and survival of western corn

rootworm in field plots. *Journal of Kansas Entomological Society* **68**: 80-84.

ROOT, R.B. 1973. Organization of plant-arthropod association in simple and diverse habitat: the fauna of collards (*Brassica oleracea*). *Ecological Monographs* **43**: 95-124.

RUANO, F., LOZANO, C. GARCIA, P., PENA, A. TINAUT, A. PASCUAL, F. & CAMPOS, M. 2004. Use of arthropods for the evaluation of the olive-orchard management regimes. *Agricultural and Forest Entomology* **6**: 111-120.

RYPSTRA, A. L. & MARSHALL, S.D. 2005. Augmentation of soil detritus affects spider community and herbivory in a soybean agroecosystem. *Entomologia Experimentalis at applicata* **116**: 149-157.

POPRAWSKI, T.H., MARCHAL, M., ROBERT, P.H. 1985. Comparative susceptibility of *Otiorhynchus sulcatus* and *Sitona lineatus* (Coleoptera: Crculionidae) early stages of five entomopathogenic hyphomycetes. *Environmental Entomology* **14**:557-561.

SALT, D.T., FENWICK, P. & WHITTAKER, J.B. 1996. Interspecific herbivore interactions in a high CO_2 environment: root and shoot aphids feeding on *Cardamine*. *Oikos* **77**: 326-330.

SANER, M. A., PHILIPPE, J. & HEINZ M-S. 1994. Interaction among two biological control agents and the developmental stage of their target weed, *Dalmatian toadflax, Linaria dalmatica* (L.) Mill. (Scrophulariaceae). *Biocontrol Science and Technology* **4**: 215-222.

SAVINI, V. & ESCALONA, H.E. 2005. A new species of *Longitarsus* Latreille, 1825 from Mexico (Coleoptera, Chrysomelidae, Alticinae, Longitarsini). *Zootaxa* **956**: 1-7.

SCHRAER, S.M., HARRIS, M., JACKMAN, J.A. & BIGGERSTAFF, M. 1998.

Pecan weevil (Coleoptera: Curculionidae) emergence in a range of soil types. *Environmental Entomology* **27**: 549-554.

SCHROEDER, D., 1983. Biological control of weeds. *In* " Recent Advances in Weeds Research" (W. W. Fletcher, Ed.), pp. 41-78. CAB International, Tucson, AZ.

SHEPPARD, A.W. 2002. Prioritizing agents based on predicted efficacy: Beyond the lottery approach. *In* H. Jacob Spafford and D.T. Briese (eds.), Improving the selection, testing and evaluation of weed biological control agents, pp. 11-21.University of Western Australia, Perth, Western Australia.

SHETLAR, D.J, SULEMAN, P.E. & GEORGIS, R.1988. Irrigation and use of entomogenous nematodes, *Neoalectana* spp. and *Heterorhabditis heliothidis* (Rhabdita: Steinernematidae and Heterorhabditidae), for control of Japanese beetle (Coleoptera: Scarabaeidae) grubs in turfgrass. *Journal of Economic Entomology* **81**: 1318-1322

SIMELANE, D.O. 2002a. Biology and host range of *Ophiomyia camarae*, a biological control agent for *Lantana camara* in South Africa. *BioControl* **47**: 575-585.

SIMELANE, D.O. 2002b. Adding a fly to the mix. *Plant Protection News* **60**; 6-7.

SIMELANE, D.O. 2005. Biological Control of *Lantana camara* (Verbenaceae) in South Africa: targeting a different niche with a root-feeding agent, *Longitarsus* sp. *BioControl* **50**: 375-387.

SIMELANE, D.O. & PHENYE, M.S. 2003. Preliminary assessment of release and establishment of lantana herringbone leafminer, *Ophiomyia camarae* Spencer (Diptera: Agromyzidae), in South Africa. Pp 447-450. *In*: Proceedings of the XIIIth International Symposium of the biological Control of Weeds. (Eds)

Cullen, J.M., D.T. Briese, D.J. Kriticos, W.M. Londsdale, L. Morin and J.K. Scott, CSIRO Entomology, 27 April - 5 May 2003, Canberra, Australia.

SIMELANE, D.O.& PHENYE, M.S. 2005. Suppression of growth and reproductive capacity of the weed Lantana camara (Verbenaceae) by Ophiomyia camarae and Teleonemia scrupulosa (Heteroptera: Tingidae). Biocontrol Science and Technology **15**: 153-163

SIMBERLOFF, D., BROWN, B.J., LOWRIE, S.1978. Isopod and insect root borers may benefit Florida mangroves. *Science* **201**: 630-632.

SIMBERLOFF, D. & STILING, P.1996. How risky is biological control? *Ecology* **77**: 1965-1974.

SKENE, K.G.M. 1975. Cytokinin production by roots as a factor in the control of plant growth. Pp 365-396. *In:* The development and function of roots. J.G. Torrey and D.R. Clarkson (Eds.). Academic Press, New York.

SKINNER, L.C., RAGSDALE, D.W. HANSEN, R.W., CHANDLER, M.A. & MOON, R.D. 2004. Temperature-dependant development of overwintering *Aphthona lacertosa* and *A. nigriscutis* (Coleoptera: Chrysomelidae): two flea beetles introduced for biological control of leafy spurge, *Euphorbia esula. Environmental Entomology* **33**: 147-154.

SOUTHWOOD, T. R. E. 1978. Ecological Methods. Chapman and Hall, New York.

STATISTICA ENTERPRISE SYSTEMS, TECHNOLOGY. 2004. Statistica 6.1 for Windows, Tulsa Oklahoma.

STINNER, B.R., MCCARTNEY, D.A. & RUBINK, W.L. 1984. Some observation of ecology of the stalk borer *Papaipema nebris* (Lepidopetera: Noctuidae) in no-tillage ecosystem. *Journal of Georgia Entomological Society* **19**: 229-234.

STRNAD, S.P. & BERGMAN, M.K. 1987. Movement of first-instar western corn

rootworm (Coleoptera: Chrysomelidae) in soil. *Environmental Entomology* **15**: 839-842.

SUNDERLAND, K.D., DE SNOO, G.R. DINTER, A. HANCE, T. HELENIUS, J. JEPSON, P. KROMP, B. & LYS, J-A. 1995. Density estimation of invertebrate predators in agroecosystems. In Toft, S. B. Ulber (eds): Arthropod natural enemies in arable land. Density, spatial heterogeneity and dispersal. *Acta Jutlandica* 70: 133-162, Aarhus, Denmark.

SUTHERST, R. W. & MAYWALD, G.F. 1999. CLIMEX. 1999. Predicting the effects of climate on plants and animals: CD-ROM. CSIRO Publishing, Collingwood, Australia.

TAUBER, M.J., TAUBER, C.A. & NYROP, J.P. 1994. Soil moisture and postdormancy emergence of Colorado potato beetle (Coleoptera: Chrysomelidae): descriptive model and field emergence patterns. *Environmental Entomology* **23**: 1485-1496.

THORSTEINSON, A.J. 1960. Host selection in phytophagous insects. *Annual Review of Entomology* **5**: 193-218.

TURPIN, F.T. & PETERS, D.C. 1971. Survival of southern corn rootworm larvae in relation to soil texture. *Journal of Economic Entomology* **64**: 1448-1451.

URBAN, A.J. & PHENYE, M.S.1999. Impact of *Ophiomyia camarae* (Diptera: Agromyzidae) on *Lantana camara* (Verbenaceae): prediction and realization. *In*: Proceedings XVth Entomological Society of Southern Africa, Villet, M.H. (ed), 10-13 July 2005, Grahamstown, South Africa. p. 84.

URBAN, A.J. & SIMELANE, D.O. 1999. Performance of a candidate biological control agent, *Falconia intermedia* (Hemiptera: Miridae), on selected biotypes of *Lantana camara* (Verbenaceae). In: Proceedings XIIth Entomological

Society of Southern Africa, van Rensberg, J.b.J. and J. van den Berg (eds), 12-15 July 1999, Potchefstroom, South Africa. p. 120.

URBAN, A.J., SIMELANE, D.O, MPEDI, P.F, NESER, S & CRAEMER, C. 2003. Varietal resistance within lantana (*Lantana camara*) reduces potential impact of mirid (*Falconia intermedia*) and flower gall mite (*Aceria lantanae*). In: Proceedings of the XIIIth International Symposium of the biological Control of Weeds. (Eds) Cullen, J.M., D.T. Briese, D.J. Kriticos, W.M. Londsdale, L. Morin and J.K. Scott, 27 April – 5 May 2003, CSIRO Entomology, Canberra, Australia.

URIAS-LOPEZ, M. A., MEINKE, L.J. HIGLEY, L.G. & HAILE, F.J. 2000. Influence of the western corn rootworm (Coleoptera: Chrysomelidae) larval injury on Photosynthetic rate and vegetative growth of different types of maize. *Environmental Entomology* **29**: 862-867.

VALLADARES, G. & LAWTON, J.H. Host-plant selection in the holly leaf-miner: Does mother know best? *Journal of Animal Ecology* **60**: 227-240.

VICHITBANDHA, P. & WISE, D.H. 2002. Spiders and carabid beetles are abundant generalist predators that prey upon insect pests of agricultural crops. *Agricultural and Forest Entomology* **4**: 31-38.

WAPSHERE, A.J. 1974. A strategy for evaluating the safety of organisms for biological control of weeds. *Annals of Applied Biology* **77**: 201-211.

WAPSHERE, A. J. 1985. Effectiveness of biological control agents for weeds: present quandaries. *Agriculture Ecosystem and Environment* **13**: 261-280.

WAPSHERE, A.J.1989. A testing sequence for reducing for reducing rejection of potential biological control agents for weeds. *Annals of Applied Biology* **112**: 515-526.

WATT, A.D., STORK, N.E. & BOLTON, B. 2002. The diversity and abundance of ants in relation to forest disturbance and plantation establishment in Southern Cameroon. *Journal of Applied Ecology* **39**: 18-30.

WEISS, M.J., MAYO, Z.B. & NEWTON, J.P. 1983. Influence of irrigation practices on the spatial distribution of corn rootworm (Coleoptera: Chrysomelidae) eggs in the soil. *Environmental Entomology* **12**: 1293-1295.

WENDELL, L.M. 1975. Plastic pitfall trap. *Environmental Entomology* **4**: 596.

WILLIAMS, S.K. & MYERS, J.H. 1984. Previous herbivore attack of red alder may improve food quality for fall webworm. *Oecologia* **57**: 298-302.

WINDER, J.A. & HARLEY, K.L.S. 1983. The phytophagous insects on lantana in Brazil and their potential for biological control in Australia. *Tropical Pest Management* **29**: 346-362.

WINSTON, P.W. & BATES, D.H. 1960. Saturated solutions for the control of humidity in biological research. *Ecology* **41**: 232-237.

WISE, M.J. & WEINBERG, A.M. 2002. Prior flea beetle herbivory affects oviposition preference and larval performance of a potato beetle on their shared host plant. *Ecological Entomology* **27**: 115-122.

WOLFSON, J.L. & MURDOCK, L.L. 1987. Suppression of larval Colorado potato beetle growth and development by digestive proteinase inhibitors. *Entomologia experimentalis et applicata* **44**: 235-240.

ZALUCKI, M.P. & FURLONG, M.J. 2005. Forecasting *Helicoverpa* populations in Australia: A comparison of regression based models and a bio-climatic based modelling approach. *Insect Science* **12**: 45-56.

ZASLAVSKI, V.A., ZINOVJEVA, K.B, UMAROVA, T.Y.A. & REZNIK, S.Y.A. 1999. Induction of circadian rhythm synchronized by photoperiod and

thermoperiod with direct influence of light and temperature in adult eclosion rhythm determination in two species of the genus Trichogramma (Hymenoptera: Trichogrammatidae). *Entomological Review* **78**: 3-14

ZWOLFER, H. & HARRIS, P. 1971. Host specificity determination of insects for biological control of weeds. *Annual Review of Entomology* **16**:159-178.