Evaluation of *Gratiana spadicea* (Klug, 1829) and *Metriona elatior* (Klug, 1829) (Chrysomelidae: Cassidinae) for the biological control of sticky nightshade *Solanum sisymbriifolium* Lamarck (Solanaceae) in South Africa.

THESIS

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by

MARTIN PATRICK HILL

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FRONTISPIECE

Top Row (Left to Right): *Gratiana spadicea* adults and egg case; *Gratiana spadicea* larvae; *Gratiana spadicea* pupae.
Centre: *Solanum sisymbriifolium* (sticky nightshade).
Bottom Row (Left to Right): *Metriona elatior* adults; *Metriona elatior* larvae; *Metriona elatior* pupae.

PUBLICATIONS ARISING FROM THIS STUDY

Parts of the research presented in this thesis, already accepted for publication are the following:

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ABSTRACT

Solanum sisymbriifolium (sticky nightshade) is a shrubby weed of South American origin that was introduced to South Africa at the turn of the century. Despite being indicative of disturbed habitats, the weed was found to be invasive in conservation, agricultural recreational and suburban areas; this, coupled with the failure of both chemical and mechanical control attempts suggested that the weed was a good candidate for biological control. A biological control programme which followed a standard protocol was initiated.

Observations suggested that *S. sisymbriifolium* dispersed primarily by seeds. Plants produced large quantities of fleshy fruit, favoured by frugivorous birds, which facilitated the rapid spread of the weed into new habitats. The seeds germinated quickly, especially in disturbed soil, often below the parent plant where they dropped from burst fruit, and along fences where birds roost.

The pre-introductory survey of the weed revealed that *S. sisymbriifolium* was attacked by a relatively small number of, mainly polyphagous, herbivorous insects. These were localised and sporadic in incidence and inflicted very little observable damage. The herbivore fauna of *S. sisymbriifolium* was depauperate even in relation to two other exotic weeds, *S. elaeagnifolium* and *S. mauritianum*, in South Africa.

The paucity of native herbivores on *S. sisymbriifolium* was ascribed to a combination of the weed's taxonomic distinctness from South African *Solanum* species, and the dense covering of glandular trichomes on its leaves. Although it was shown that the exudate produced by these glandular trichomes of *S. sisymbriifolium* seriously impeded the movement and feeding of native herbivores, there was not enough evidence to suggest that the glandular trichomes, alone could have been responsible the lack of herbivores on the weed.

Two leaf-feeding Cassidinae *Gratiana spadicea* and *Metriona elatior* were screened as agents for the biological control of *S. sisymbriifolium*. Favourable biological characteristics for both species included a high rate of increase, long-lived adults, many generations per year, and a high per capita feeding rate. Host range was investigated in larval survival tests and adult choice tests. The larvae of both species were reared through to the adult stage on

several of the native *Solanum* species tested, and also on eggplant (*S. melongena*). However, the survival of *G. spadicea* on the majority of these species was very low, suggesting that the beetles would be unlikely to attack them in the field. This was supported by the adult choice tests, where *G. spadicea* females displayed a strong oviposition preference for their natural host. In contrast, *M. elatior* larvae survived well on non-host plants, and the females selected several non-host species, including eggplant for oviposition. It was argued that the conflict of interests involving eggplant was overrated because eggplant is subjected to a stringent insecticide spray regime. Based on this evidence, permission for release was granted for *G. spadicea*.

The impact of native parasitoid host range extensions to weed biological control agents in South Africa was investigated. Native parasitoids were recorded from nearly half of the agent species that had established on their target weed. The level of concealment and taxon influenced susceptibility of the agents to parasitoid attack. Poorly concealed endophagous agents were most susceptible to attack, while exposed feeders were fairly free from attack. However, native parasitoids were reported not to strongly influence weed biological control agent populations and it was concluded that no agent should be rejected based only on its susceptibility to native parasitoid attack.

Finally, several predictions are made as to the potential success of *G. spadicea* on *S. sisymbriifolium* and some of the challenges facing the biological control of weeds are discussed.

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CHAPTER 1

GENERAL INTRODUCTION

The plant family Solanaceae contains some 3500 species in 84 genera worldwide (D'Arcy 1986). Of these, the genus *Solanum* is the biggest, contributing about 75% of the species. The genus *Solanum* is of cosmopolitan distribution, but has achieved its greatest diversity in the tropical and warm temperate regions of the southern hemisphere (Symon 1981). South America is regarded as the centre of speciation for *Solanum* (Symon 1981), supporting an estimated 1000-1100 species (Hunziker 1979). Other centres of speciation include Africa with 110 species (Jaeger and Hepper 1986) and Australia with 94 species (Symon 1981).

Solanum is fairly well represented in South Africa, where it comprises 51 native and 13 exotic species (Gibbs Russell *et al.* 1987). Plants within the genus often occupy disturbed sites and are rarely components of climax vegetation (Symon 1981), as a result of which, 15 native and 10 exotic species are regarded as problem plants (Wells *et al.* 1986). Three of the most important exotic weed species in South Africa are *Solanum elaeagnifolium* Cavanilles (satansbos), *S. mauritianum* Scopoli (bugweed) (Olckers and Zimmermann 1991), and more recently *S. sisymbriifolium* Lamarck (sticky nightshade).

Solanum sisymbriifolium is an erect annual or biennial shrub native to warm temperate South America (Symon 1981) and is an invasive weed of disturbed and pastoral land in South Africa (Nel 1988). The weed is in the subgenus *Leptostemonum* (Dunal) Bitter, which contains 68% of the native South African *Solanum* species (Jaeger and Hepper 1986), but

in the section *Cryptocarpum* Dunal (D'Arcy 1972), which is not naturally represented in Africa (Jaeger and Hepper 1986).

The taxonomy at the species level in *Solanum* is often not clear and synonymy is widespread within the genus (Symon 1981). *Solanum sisymbriifolium* has also been referred to as *S. balbisii*, *S. branaefolium*, *S. decurrans*, *S. formosum*, *S. inflatum*, *S. thouinii*, *S. viscidum* and *S. viscosum* (Manson 1967), as well as being confused with *S. aculeatissimum* Jacquin and *S. mauritianum* in older literature (Symon 1981). Common names for the weed include wild tomato (Nel 1988), sticky nightshade, viscid nightshade, decurrent-leaved nightshade and Balbis' nightshade (Manson 1967). In South Africa several Afrikaans names exist for the weed including "wildetamatie", "doringtamatie", "tamatiedissel" and "digdoringtamatie" (Nel 1988).

Solanum sisymbriifolium is associated with localized, short-term disturbances such as ploughed fields, road-sides, wastelands, landfills and cultivated crops in its region of origin, northwest Argentina, Brazil, Paraguay and Uruguay (Kvasina and de León 1985; Becker and Romanowski 1986; Becker and Frieiro-Costa 1988). The weed may have been introduced to South Africa as seeds in horse feed imported by the British military during the South African War around 1900 (Nel 1988). *Solanum sisymbriifolium* was also introduced to Australia (Symon 1981), China (An-ming 1986), India (Jain and Borthakur 1986; Sarat Babu *et al.* 1987), the southern United States of America (Barber 1933) and western Europe (Manson 1967; Morley 1975).

Solanum sisymbriifolium was introduced into European cultivation around 1800 as a novelty in French, German and Italian gardens, mainly because of its showy flowers and fruit (Morley 1975). It was grown in the Chelsea Physic Gardens in London and the Kew Botanical Gardens in the early 1800's, and the Royal Horticultural Society of London made an award to the weed in 1966 for its use as a greenhouse shrub (Manson 1967; Morley 1975). The roots of *S. sisymbriifolium* are resistant to some strains of the bacteria wilt, *Pseudomonas solanacearum* (Smith), and the nematode *Meliodogyne incognita*, and have been used as rootstocks for tomato in Japan (Matsuzoe *et al.* 1993). In addition, the ripe fruits are a rich source of the glycoalkaloid solasodine, a precursor material in the synthesis of corticosteroids and sex hormones, and an active ingredient in oral contraceptives (Pandeya *et al.* 1984).

The weed was introduced to the southern United States of America in the early 1900's, where it was grown as an ornamental (Barber 1933). It now plays an important role in the agricultural economy of eastern Georgia where it serves as an alternative host for the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), the tobacco budworm, *Heliothis virescens* (Fabricius) and the tomato worm, *Protoparce sexta* Johanssen (Barber 1933). The weed helps to maintain the populations of these three important pests of solanaceous crops when the crops are not available.

Initially, infestations of *S. sisymbriifolium* in South Africa must have been very localized, occurring around feed storage areas and transport routes, but spread steadily. The weed was first recorded in 1908 in Barberton in the Eastern Transvaal, and Johannesburg (Nel 1988) but is now widespread in the PWV region and Eastern Transvaal, and there are also infestations in the eastern Cape, Kwazulu-Natal, Orange Free State and in Swaziland (Figure 1.1).

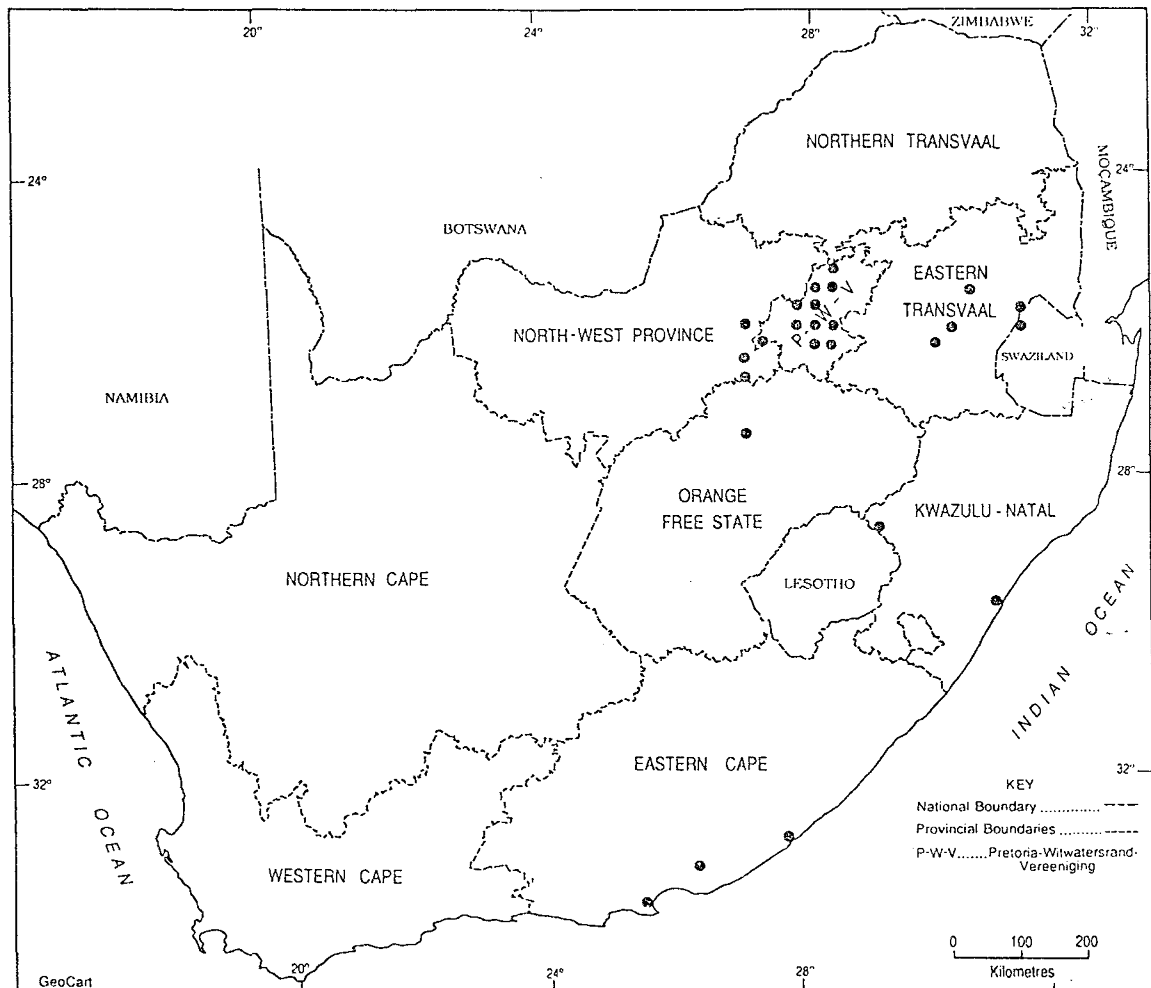


Figure 1.1 Distribution of *Solanum sisymbriifolium* in South Africa. The distribution records were obtained from the National Botanical Institute in Pretoria, and from T. Farrell (Weed inspector and extension officer, Resource Conservation, personal communication) and personal observations.

The weed is confined to the eastern regions of the country, Port Elizabeth in the Eastern Cape being the westerly extreme of its distribution. There could be two possible reasons for this pattern of distribution. Firstly, the weed might not have had the opportunity to invade the western regions of the country. This however is unlikely in view of the length of time (*ca.* 90 years) that the weed has been in the country (Nel 1988), and the fact that there is continual movement of people to and from the main centres in the western regions, and it is inevitable that some seed would have been transported in the soil of pot plants and such like.

The second, and possibly more feasible reason is that climatic conditions, and more specifically rainfall could have dictated the distribution of *S. sisymbriifolium* in South Africa. The eastern parts of the country are characterised by summer rainfall, and the Eastern Cape by an unpredictable, but essentially spring and autumn rainfall pattern (Mundy 1989). The Western Cape is characterised by winter rainfall and the western parts of the Orange Free State and North-West Province, and the Northern Cape are extremely arid regions. It could be that the winter rainfall, or lack of summer rainfall has prevented establishment of the weed in the Western Cape, and general lack of rainfall in the arid western regions. This would also tend to suggest that the Eastern Cape is probably a marginal region for the weed and as such not an entirely suitable area for biological studies of the weed (Chapter 2), and might explain why the weed fairly easily replaced by other vegetation in the Eastern Cape, but appears to maintain its populations in pasture lands in the Transvaal Highveld region, which is characterised by a climate similar to that of its native range in South America (S. Neser, Plant Protection Research Institute, personal communication).

In the first report of dense infestations of *S. sisymbriifolium* in the Transvaal, du Toit and van der Merwe (1941) warned of its invasive potential. Although the weed was relatively unknown in the southeastern Transvaal until 1980, by 1988, some 15 farms, in the vicinity of Carolina (26°04'S 30°06'E), Hendrina (26°10'S 29°40'E) and Breyten (26°18'S 29°59'E), had recorded dense infestations in pastoral lands (Nel 1988). Invasion of pastoral land was also observed in the Eastern Cape near Seaview (33°26'S 25°23'E).

Why is it that the weed has taken so long to become a problem in South Africa? Interestingly, the weed has been present in the Toowoomba region of Queensland (Australia) since 1921, but has only been recorded spreading in this region since the late 1970's (Symon 1981). Rather than making any assumptions as to the long term phenology of this weed, this behaviour is probably a product of a natural increase in the population density of the weed over time, coupled with an increasing number of suitable disturbed habitats for the weed caused through an increase in agricultural practices and poor land management. Indirect evidence for this is that *S. sisymbriifolium* has recently become a serious pest of agricultural lands in South America (Becker and Romanowski 1986; Becker and Frieiro-Costa 1988).

The majority of infestations in South Africa are in disturbed areas such as road-sides and construction sites. The weed, however, has also invaded undisturbed pasture lands, firebreaks, drainage culverts and areas cleared of other alien species. These infestations are increasing in both area and density (Nel 1988; T. Farrell, personal communication). Reproduction and dispersal of the weed occurs primarily by seeds. Plants produce large quantities of fleshy fruit, favoured by frugivorous birds, sheep and even children, which facilitates the rapid spread of the weed (Becker and Frieiro-Costa 1988; Nel 1988).

Solanum sisymbriifolium was proclaimed a noxious weed in South Africa under Act No. 42 of 1937 by proclamation No. 45 of 1941, whereby land owners are compelled to eradicate the weed by any methods (du Toit and van der Merwe 1941). Initially mechanical methods of control which included cutting plants off at the base were employed. However this stimulated coppicing from the cut stump and root stocks (du Toit and van der Merwe 1941). Farmers also tried discing, hoeing and ploughing the weed into the soil, but this only served to stimulate germination of the seeds (Nel 1988). Du Toit and van der Merwe (1941) emphasised that the weed should be cut before the flowering stage and then stacked, dried and burned. A further method of eradication, which has proved to be effective, was the use of a flame thrower for burning the plants down *in situ* (du Toit and van der Merwe 1941).

Chemical control of the weed has been attempted using the herbicide Roundup, but this is labour intensive, the costs were prohibitive and the results found to be doubtful at best (Nel 1988). A chemical control programme was initiated against *S. sisymbriifolium* around 1990, but was not a success (L. Stoffberg, Plant Protection Research Institute, personal communication) and to date no chemical has been registered against the weed.

The weed's apparent lack of utility, its increasing abundance on agricultural, recreational and suburban lands, its alien status and the failure of both mechanical and chemical control programmes, suggest that it might be a suitable candidate for a biological control programme.

Numerous species of *Solanum* are weeds in many countries of the world. However, none have been subjected to biological control programmes (Julien 1987). The biological control

of *S. elaeagnifolium* was considered in Australia, but rejected as unlikely to succeed (Wapshere 1988). One reason for the lack of biological control attempts on *Solanum* species could be the economic importance of the cultivated species in the Solanaceae (potato, tomato, eggplant and peppers) preventing the release of biocontrol agents because of perceived risks. However, the research undertaken in South Africa towards the biological control of *Solanum* weeds has been necessitated by the importance of these weeds in agricultural and conservation areas.

The biological control campaign against *Solanum* weeds in South Africa was initiated in the early 1970's because of the threat of *S. elaeagnifolium* (satansbos, silverleaf nightshade) to arable and pastoral lands in the Karoo, Orange Free State and northern Transvaal regions of the country (Henderson and Anderson 1966). In 1973, South Africa became the first country to import and test natural enemies against *Solanum* weeds, when five agents were imported to be tested for suitability against *S. elaeagnifolium* (Olckers and Zimmermann 1991). However, four agents were rejected, including the two tortoise beetles (Chrysomelidae: Cassidinae) *Gratiana lutescens* (Boheman) and *G. pallidula* (Boheman), because they were reared through on eggplant under quarantine conditions (Siebert 1975). The fruit-boring gelechiid, *Frumenta nephelomicta* Meyrick was released, but failed to establish (Neser and Siebert 1977).

After a further collecting trip for insects on satansbos in 1985, the two most promising candidates for the biological control of satansbos, the chrysomelids *Leptinotarsa texana* (Schaeffer) and *L. defecta* (Stål), were imported form North America (Hoffmann 1985). Because of the close relationship between the weed and crop species like potato (*S.*

tuberosum Linnaeus) and eggplant (*S. melongena* Linneaus), host specificity testing was intensive and spanned a number of years. Progress was constrained because the beetles survived on eggplant in the laboratory (Zimmermann 1987). However, further research showed that the beetles were not a threat to the crop because they did not attack it in their native country, and because the crop was already intensively sprayed for generalist pests in South Africa (Olckers and Zimmermann 1991). The two beetles were released on satansbos in 1992, making them the first biological control agents to be established on a solanaceous weed anywhere in the world.

The second species to warrant attention was *S. mauritianum* (bugweed) which had become a major weed of agriculture, forestry and conservation areas in Kwazulu-Natal and the Eastern Transvaal (Byford-Jones 1981). Although the plant is easily killed by herbicides, the extent of invasion made chemical control financially impractical (Hinze 1983). In 1984, three insect species were imported from Argentina but were later rejected because of a lack of host specificity (Neser *et al.* 1990). Surveys in Brazil in 1992 revealed a vast reservoir of potential agents that had not been found in Argentina (T. Olckers, Plant Protection Research Institute, personal communication). A number of these were imported into quarantine, the most promising of which are six species of *Platyphora* which are currently being tested (T. Olckers, personal communication).

The primary aim of my research was to investigate the potential for the biological control of *S. sisymbriifolium*, and more specifically to evaluate two potential agents (*Gratiana spadicea* Klug, 1829 and *Metriona elatior* Klug, 1829, Chrysomelidae: Cassidinae). The

research and also the thesis were structured according to a protocol for the biological control of weeds, adapted from that proposed by Harris (1971) and Andres *et al.* (1976):

1] Establish biology and invasiveness of the weed.

2] Conduct a pre-introductory survey of the weed.

3] Identify and import most promising biological control agents from the weed's country of origin.

4] Determine the biology and host specificity of the most promising agents.

5] Satisfying host specificity requirements, mass rear agent(s) and release.

6] Conduct post-release evaluation of the agent and weed populations.

A lack of phenological synchrony between a weed and a biological control agent can lessen the impact of the agent (Ehler and Andres 1983), so a good understanding of the biology of the weed is vital in any control attempt. Chapter 2, therefore, is an investigation into the basic phenology, and pollination and dispersal ecology of *S. sisymbriifolium*.

Goeden and Ricker (1986) suggest that the value of pre-introductory faunistic surveys during the planning of biological control campaigns are that they give an indication of whether the indigenous herbivores might influence the efficiency of introduced agents. Surveys of the fauna associated with weed species also obviates the testing and introduction of an agent that is accidentally already present on the weed in the country of introduction. Chapter 3 therefore describes the insect herbivore fauna associated with *S. sisymbriifolium* and compares it to the fauna associated with native and other exotic solanums in South Africa.

In a departure from the biological control protocol cited above, but closely related to the first step in the protocol, Chapter 4 attempts to explain the pattern of recruitment of insect herbivores onto *S. sisymbriifolium* in South Africa, in terms of the anti-herbivore defence system of the weed, and specifically the glandular trichomes on the leaves of the weed.

Chapters 5 and 6 are the focus of the thesis, and deal with the biology and host range of the two agents, *G. spadicea* (Chapter 5) and *M. elatior* (Chapter 6) in an evaluation of their potential for the biological control of *S. sisymbriifolium*. Goeden and Louda (1976) have suggested that biotic interference by predators and parasitoids have prevented the establishment of some potential biological control agents. Chapter 7 is a survey of the insects released as weed biological control agents in South Africa. It documents their susceptibility to native parasitoids and discusses the possibility of either of the agents becoming parasitised should they be released. Although steps five and six of the protocol fall outside the bounds of this study, they are nonetheless vital to the biological control programme.

The final discussion (Chapter 8) analyses the prospects for the biological control of *S. sisymbriifolium* in South Africa. Furthermore, critical problems facing the future of weed biological control as a whole are discussed.

CHAPTER 2

Biology of *Solanum sisymbriifolium* Lamarck in South Africa.

2.1 INTRODUCTION

A lack of phenological synchrony between a biological control agent and its target weed can lessen the impact of the agent (Ehler and Andres 1983). The key to many of the successful weed biological control programmes in South Africa has been a thorough understanding of the biology of the target weed (e.g. Campbell and van Staden 1983; Givelberg *et al.* 1984; Dennill 1987a; Kluge and Neser 1991). Crawley (1989), however, has pointed out that very few weed biological control programmes have been quantitatively assessed due to the lack of information on the biology and population dynamics of the weeds prior to the release of an agent. Dennill (1987a) has also stressed the need to understand weed biology but has cautioned against basing it on a single season's study.

This chapter describes the general biology of *S. sisymbriifolium*. In addition, the role of insect vectors in pollination, the influence of photoperiod on germination, and role of birds in the dispersal of the weed are discussed.

2.2 MATERIALS AND METHODS

2.2.1 Plant Phenology

The phenology of *S. sisymbriifolium* was studied at a large stand of the weed on a roadside embankment just outside Grahamstown, Eastern Cape Province (33°19'S 26°32'E) between January 1990 and April 1994. A fire denuded the entire embankment of vegetation in May

1991. By September 1991 about 300 *S. sisymbriifolium* seedlings had started to germinate whilst the rest of the embankment remained free of other vegetation. This event created an ideal opportunity to study the phenology of the weed as the age of the seedlings was known. On the 22nd September 1991, 50 of these seedlings were tagged at the site so that all plants were individually recognisable. The tagged plants were visited every month between September 1991 and December 1993 and the presence of flowers and fruit were recorded. In addition, between January 1992 and January 1993, all the fruit on the tagged plants were counted every two weeks and marked with a permanent ink pen (so that they would not be counted again) to quantify the fruit production of *S. sisymbriifolium* over that particular year.

To establish the number of seeds per fruit, the seeds in 100 *S. sisymbriifolium* fruit from the tagged plants were counted.

2.2.2 Pollination ecology

During the plant phenology studies (monthly between September 1991 and December 1993), observations were made on the visitors to *S. sisymbriifolium* flowers at the study site and on plants of 16 *Solanum* species growing in pots on the roof of the Biological Sciences Building, Rhodes University. All insects that visited the flowers were collected. The hind legs of these insects, with the pollen baskets or scopae, were removed as this pollen would not have been available for pollination. The remaining pollen was then dusted from the body of the insects and prepared for light microscopy using a technique proposed by Hill (1992) (Appendix I) to confirm that these insects had collected pollen from the plants they were visiting. A pollen reference collection of 30 of the locally-occurring *Solanum* species was made.

2.2.3 Germination and seed dispersal

Germination

Seeds were harvested from ripe (red) *S. sisymbriifolium* fruit and thoroughly washed in distilled water, as Mayer and Poljackoff-Mayber (1982) have shown that fruit pericarp of plant species within several families, including the Solanaceae, contained secondary compounds that inhibited the germination of seeds but could be removed by washing prior to any germination trials. This procedure, however, makes extrapolation from laboratory based germination trials to the field difficult. Distilled water was then added to the seeds and those that sank (the viable seeds (Campbell and van Staden 1983)) were air dried and stored at room temperature until required. All germination experiments were conducted within 2 weeks of harvesting the seeds.

The experiments were conducted under alternating temperature conditions of 15°C for 8 hours and 30°C for 16 hours in a constant environment room. Campbell and van Staden (1983) found this to be the most suitable temperature regime for germinating the seeds of *S. mauritianum* (the effects of different temperature regimes on the germination of *S. sisymbriifolium* seeds were not investigated). Seeds were placed in a 9mm petri-dish lined with filter paper. An initial watering with distilled water was followed by checks every 12 hours to keep the filter paper moist. Batches of seeds were exposed to one of the following light regimes for 30 days (initial studies showed that viable *S. sisymbriifolium* seeds all germinated within 20-25 days):

1] Constant dark

2] Constant light

3] Alternating light and dark (16 hours light and 8 hours dark)

Ten replicates of 100 seeds each were used for each light regime and three experiments were carried out. The differences in seed germination between the three light treatments were compared using the Kruskal-Wallis test as the data were not normally distributed (Sokal and Rohlf 1981). This was followed by a Dunn's Multiple Range test which indicated where any significant differences might lie in germination between light treatments (Steele and Torrie 1980).

Dispersal

Observations were made of the bird species feeding on *S. sisymbriifolium* fruit in the Eastern Cape and the Transvaal during the study (January 1990 to May 1994).

The gut passage time and the viability of *S. sisymbriifolium* seeds have an important bearing on the dispersal of the weed. Individuals of two of the bird species commonly observed feeding on the fruit (*viz.* Red-winged Starlings, *Onychognathus morio* Linnaeus and Cape White-eyes, *Zosterops pallidus* Swainson) were caught and subjected to feeding trials. Three individuals of each species were placed in a cage, starved for 3 hours to allow passage of any existing seeds in the gut, and presented with five halves of *S. sisymbriifolium* fruit. The seeds from the other five halves were washed and dried and used as controls in the germination trials. The time taken for the seeds to pass through the gut of the birds was recorded and the seeds in the droppings were collected and dried. Three replicates were conducted for each species of bird.

Seeds undergoing gut passage in frugivorous birds often have enhanced and more rapid germination (Fenner 1985). To test this in *S. sisymbriifolium*, the percentage and rate of

germination was compared between the seeds that had passed through the gut of the birds and the control seeds. Four replicates of 50 seeds each were used for each experiment with each bird species and for the accompanying controls. The seeds were germinated in petri-dishes on moist filter paper under a 16 hour light phase with a temperature of 30°C and an 8 hour dark phase at 15°C.

2.3 RESULTS

2.3.1 Plant Phenology

Solanum sisymbriifolium is a highly branched shrubby weed that grows to a height of about 1.5m within a year. The tagged seedlings that appeared at the study site by September 1991 after the fire in May 1991 were still present at the site in April 1994. From March 1994, the stand of *S. sisymbriifolium* appeared to be suppressed by other exotic vegetation, mainly black wattle (*Acacia mearnsii* De Wild.) and long leaved wattle (*A. longifolia* (Andr.) Willd.). By June 1994 the entire stand of *S. sisymbriifolium* had been replaced by other vegetation, and the few remaining isolated *S. sisymbriifolium* plants were restricted to the verges of the embankment. This replacement of *S. sisymbriifolium* by other exotic vegetation was also recorded at two sites in the Port Elizabeth area (33°58'S 25°38'E).

The ovate leaves were deeply lobed and turned from light green when first formed to much darker green. The weed did not die back in winter but maintained its leaves throughout the year both in the Eastern Cape and the PWV region and southeastern Transvaal where frost is common in winter. The stems, petioles, upper and lower surfaces of the leaves and the calyces were covered by spines (1 to 10mm in length) which were yellow to red in colour.

In addition to this, all parts of the plant, with the exception of the fruit, were densely pubescent with both stellate and glandular trichomes.

The inflorescences were up to 15cm long with 4 to 12 flowers per inflorescence (mean ± SD = 8.32 ± 4.49 flowers, n = 100 inflorescences). The flower buds started forming early in July and the first open flowers were noted by the middle of July (Figure 2.1). Flowers were recorded in every month of the year, but in all three study years there was a period (midwinter) when no flowers were present; this was later in 1990 than 1991 and 1992. The petals were pale blue early in the flowering season (July and August), but white for the rest of the flowering period. Individual flowers remained open for 2-3 days. The anthers were bright yellow, about 10mm in length (mean = 9.75 ± 2.37mm, n = 100 anthers) and tapered slightly towards the distal end of the flower. The five anthers formed a cone from which the style protruded in hermaphrodite flowers. The flowers opened immediately after dawn and remained open until dusk, except on days when the temperature exceeded 30°C where the flowers closed during the hottest part of the day (around noon) and reopened in the cooler afternoon.

Generally the flowers of *S. sisymbriifolium* were hermaphrodite, but some of the distal flowers on the inflorescences were female sterile (also referred to as male flowers). The female sterile flowers were easily distinguishable from the hermaphrodite flowers in that their styles were greatly reduced in length (2-6mm, n = 100 styles) in comparison to the styles of the hermaphrodite flowers (12-15mm, n = 100 styles) and in that their ovaries were non-functional (Whalen and Costich 1986). Female sterile flowers constituted a low proportion (6.26% of the flowers in 100 inflorescences) of the flowers at the study site.

Usually only the basal flowers on the inflorescences produced fruit. The immature fruit (green) were produced in early spring (August and September) and the bright red, ripe fruit were present in the weed population by November (Figure 2.1). Fruiting continued into winter, but no mature fruit were found after the end of June. The fruit were encased in large, spiny calyces and ranged in size between 1.2 and 2.6mm (mean = 1.78 ± 1.24mm, n = 100 fruit). The fruit contained a mean of 159.88 (± 52.41, range = 54 - 323, n = 100 fruit) straw coloured seeds. The tagged plants at the study site produced a mean of 297.96 ± 49.63 (n = 49 plants) fruit per plant during the year January 1992 to January 1993.

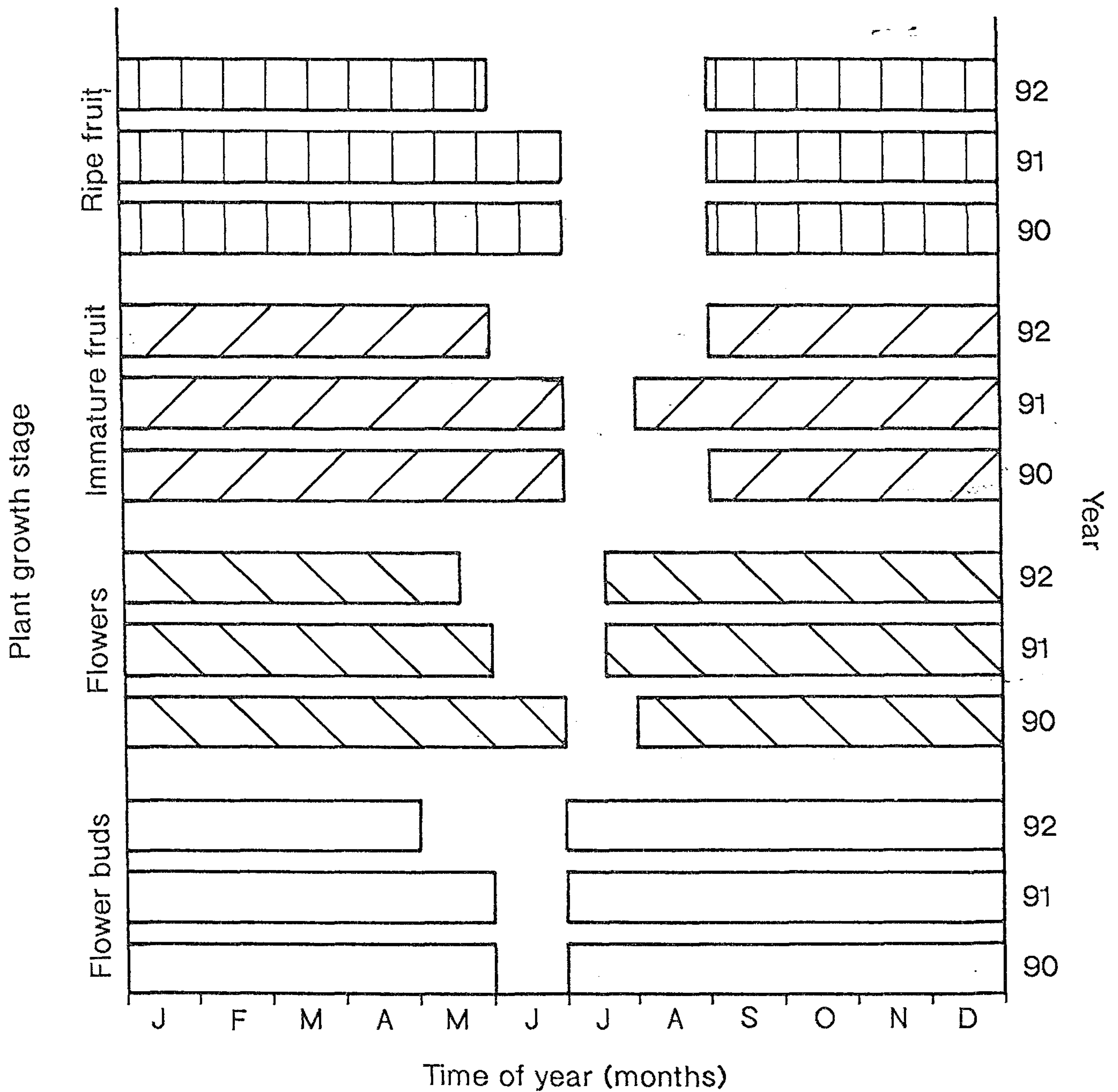


Figure 2.1 The annual flowering and fruiting phenology of *Solanum sisymbriifolium* at a site near Grahamstown, for the years 1990, 1991 and 1992.

2.3.2 Pollination ecology

A total of 12 potential pollinator species visited the flowers of *S. sisymbriifolium* (Table 2.1). Insects were only considered to be potential pollinators if the pollen dusted from the ventral surface (i.e. the pollen available for pollination (Buchman 1983)) of the thorax included *Solanum* pollen. Unfortunately, at both the light and scanning electron microscope level of resolution, the *Solanum* pollen could not be characterised at the species level.

Table 2.1 The potential pollinator species observed visiting the flowers of the weed *Solanum sisymbriifolium* and other *Solanum* species at two sites in the Grahamstown area.

Pollinator species	Plant species visited[a]
Anthophoridae	
Xylocopa flavorufa (De Geer)	Sis Acan
Xylocopa flavicollis (De Geer)	Sis Acan Ac Ri Li Mau In Pa
Xylocopa scioensis (Gribodo)	Sis Mau
Xylocopa caffra (Linnaeus)	Sis Acan Ri Li Mau
Amegilla acraensis (Fabricius)	Sis Acan Ri
Amegilla spilostoma (Cameron)	Sis Acan Mau Ri Li
Allodapula varigata (Smith)	Sis
Allodapula sp. (AcRh 714)[b]	Sis
Allodapula sp. (AcRh 715)	Sis
Halictidae	
Lasioglossum sp. (AcRh 721)	Sis Ri Li
Leuconomia sp. (AcRh 722)	Sis Mau
Poss. *Lipotriches* sp. (AcRh 723)	Sis

[a] Sis = *S. sisymbriifolium*, Acan = *S. acanthoideum* E. Meyer, Ac = *S. aculeatissimum* Jacquin, In = *S. incanum* Linnaeus, Li = *S. linnaeanum* Hepper and Jaeger, Mau = *S. mauritianum* Scopoli, Pa = *S. panduriforme* E. Meyer.

[b] Rhodes University accession number.

The potential pollinators were all solitary bees within the superfamily Apoidea. The three *Allodapula* species were recorded only on *S. sisymbriifolium*, while the other species were noted visiting other species of *Solanum*. The anthophorid species *Xylocopa flavicollis* was the most frequent visitor and also the insect that was recorded from the widest range of *Solanum* species.

The first pollinators were noted visiting the flowers in late August (late winter/early spring) and they continued to do so throughout the summer and into autumn (April); none visited the flowers in May, June and July.

When the bees alighted on the flowers, the four *Xylocopa* species and *Amegilla acraensis* curled the abdomen around all five of the anthers. The bees then vibrated their indirect wing muscles (Heinrich 1972) and "milked" each anther in turn by grasping the anther at its base with their mandibles and, in a series of short strokes directed towards the apex of the anther, caused the dehiscence of the pollen. The smaller *Amegilla* and *Allodapula* species curled their abdomens around one of the anthers and vibrated their indirect wing muscles and "milked" the anthers in much the same way as the larger bees. The weight of the bees on the flowers caused the flowers to point downwards. This facilitated the dehiscence of the pollen onto the bees, which were densely pubescent, and they were covered in the very fine pollen.

Once the pollen was on the bees, they mixed it with regurgitated nectar from some other source (Watmough 1974) and used their middle pair of legs to groom it into pollen baskets or scopae on the hind legs. However, some pollen remained in "safe" sites, the occiput of

the head, proboscideal fossa, the central line of the thoracic dorsum and between the coxal plates (Buchman 1983). This pollen was available for pollination.

While the large *Xylocopa* bees were on the flowers the stigma came into contact with the ventral surface of the thorax while they were collecting the pollen into their scopae and presumably pollen transfer from a previously visited flower occurred. The smaller bees (*Amegilla* and *Allodapula* species) did not appear to come into contact with the stigma as they curled their bodies around a single anther. It was unlikely that they play any significant role in pollination other than through accidental contact with the stigma. This whole process of buzz pollination was very rapid and the bees were on a single flower for no more that 15sec.

2.3.3 Germination and seed dispersal

Germination

Light or photoperiod had a marked influence on the germination of *S. sisymbriifolium* seeds. Very few seeds germinated in the dark, while a high percentage of the seeds germinated under the alternating light and dark regime (Table 2.2).

The Kruskal-Wallis test showed that the number of seeds germinated under each of the light treatments differed significantly ($p < 0.001$). The multiple range test showed that all three light treatments differed significantly from each other (Table 2.3).

Table 2.2 The median number of *Solanum sisymbriifolium* seeds germinated after 30 days under different light regimes. Each replicate contains 10 petri-dishes with 100 seeds in each.

Replicate	Median number of seeds out of 100 germinated		
	Constant dark	Constant light	16 hours light/ 8 hours dark
1	0.0	82.0	96.0
2	1.5	74.0	96.5
3	0.0	80.0	97.0
Total	0.0	79.5	96.5

Table 2.3 Results of the Dunn's Multiple Range test on the number of *Solanum sisymbriifolium* seeds germinated under the three different light regimes.

Treatment pair	q-Statistic	Sig. level
Constant dark and constant light	10.46	$p < 0.05$
Constant dark and alternating light and dark	11.88	$p < 0.05$
Constant light and alternating light and dark	7.31	$p < 0.05$

Dispersal

The seeds of *S. sisymbriifolium* were dispersed passively and actively. When the fruits became ripe, the spiny calyces folded back and the entire fruit was exposed. If the ripe fruit were not eaten by birds, they split after about a week and the seeds were shed around the parent plant. However, most of the fruit produced by *S. sisymbriifolium* were eaten by birds. Twelve species of birds were noted feeding on the fruit of the weed during the study (Table 2.4). In addition, Nel (1988) has recorded chats, francolins, partridges and quails feeding on *S. sisymbriifolium* fruit in the PWV region and the Eastern Transvaal.

Table 2.4 The bird species recorded feeding on the fruit of *Solanum sisymbriifolium*.

Birds recorded feeding on fruit		Region recorded
Common name	Specific name	
Blackeyed Bulbul	*Pyconontus barbatus* (Desfontaines)	E. Cape
Black Headed Oriole	*Oriolus larvatus* Lichtenstein	E. Cape
Blackcollard Barbet	*Lybius torquatus* (Dumont)	E. Cape
Cape Glossy Starling	*Lamprotornis nitens* (Linnaeus)	PWV
Cape Robin	*Cossypha caffra* (Linnaeus)	E. Cape
Cape White-eye	*Zosterops pallidus* Swainson	E. Cape
Crested Barbet	*Trachyphonus vaillantii* Ranzani	PWV
Indian Myna	*Acridotheres tristis* (Linnaeus)	PWV
Olive Thrush	*Turdus olivaceus* Linnaeus	E. Cape
Red-winged Starling	*Onychognathus morio* (Linnaeus)	E. Cape, PWV
Redfaced Mousebird	*Colius indicus* (Latham)	E. Cape, PWV
Speckled Mousebird	*Colius striatus* Gmelin	E. Cape

The gut passage times of the birds did not exceed 30 minutes (Table 2.5). The seeds took nearly twice as long to pass through the guts of the larger species tested (Red-winged Starling) than the smaller species (Cape White-eye).

Table 2.5 The gut passage times of *Solanum sisymbriifolium* fruit for two species of bird recorded feeding on the fruit in the field.

Bird species	Number of birds	Gut passage time (minutes)	
		Mean	Range
Red-winged Starling	9	24.03	20-27
Cape White-eye	9	13.07	9-16

The mean number of seeds germinating of those seeds that had passed through the guts of the Red-winged Starlings and the Cape White-eyes did not differ significantly from the control seeds (Starlings vs control: 46.17 ± 4.51 vs 45.58 ± 4.05, $t = 0.333$, $p = 0.742$, $n = 12$, t-test; White-eyes vs control: 45.75 ± 3.70 vs 44.50 ± 4.28, $t = 0.766$, $p = 0.452$, $n = 12$, t-test). However, the germination time (Table 2.6) of the seeds that had passed through the gut of both species of bird differed significantly from the control seeds (Mann-Whitney Rank Sum test: Red-winged Starlings vs control: $p < 0.001$; Cape White-eyes vs control: $p < 0.001$), although the differences were not large and probably of little biological significance.

Table 2.6 The germination time of *Solanum sisymbriifolium* seeds obtained directly from fruit and the two bird species tested.

Treatment	Germination time (days)	
	n	median
Red-winged Starling	566	17.0
Control	568	19.0
Cape White-eye	553	17.0
Control	547	18.0

2.4 DISCUSSION

The general biology of *S. sisymbriifolium* in South Africa is very similar to that described for the weed in other parts of the world. *S. sisymbriifolium* is regarded as a short-lived annual, or at most biennial, weed of disturbed areas and rarely of cultivated lands in its region of origin, *viz.* northwest Argentina, Brazil, Paraguay and Uruguay (Kvasina and de León 1985; Becker and Romanowski 1986; Becker and Frieiro-Costa 1988), and in most of its countries of introduction: Australia (Symon 1981), Europe (Morley 1975; Wakhloo 1975a), India (Sarat Babu *et al.* 1987) and the southern United States of America (Barber 1933). In South Africa, however, the weed is recorded as a perennial, once again of disturbed areas, but frequently also of cultivated lands (du Toit and van der Merwe 1941; Nel 1988).

Despite the weed's more perennial nature in South Africa, it should still be regarded as a pioneer species, exploiting temporal gaps in the natural vegetation. It appears to be able to germinate and survive well in areas unsuitable for other vegetation types. *S. sisymbriifolium* has a high reproductive potential in South Africa as it is visited by many pollinator species, fruits prolifically and has an effective dispersal system. In spite of this, it is easily suppressed by other vegetation, implying that while the weed might be apparent in space, it might not always be apparent in time.

The pollination ecology is similar to that of other species within the genus. *Solanum* is a conservative genus in terms of flower morphology and pollination (Buchman 1986). As the anthers of the flowers are poricidal (Symon 1981) the pollen is not readily available to generalist pollinators (Buchman 1986). There is evidence that many species of bees

(Apoidea: Apidae, Colletidae, Halictidae, Anthophoridae and Melittidae) and syrphid flies are able to manipulate the poricidal anthers of *Solanum* flowers (Linsley 1962; Michener 1962; Linsley and Cazier 1963; Watmough 1974; Bowers 1975; Free 1975; Symon 1979; Buchman 1983, 1986; Sarat Babu *et al.* 1987), thus being responsible for the pollination within this genus. The bees responsible for the pollination of *S. sisymbriifolium* in the Eastern Cape are members of the families Anthophoridae and Halictidae. These pollinators are polylectic, that is, they have been recorded as pollinators of several other plant families (Gess 1992). As the bees often visited several flowers on an individual plant before moving onto another one, the method of pollination appears to be prone to self fertilisation. However, self-sterility alleles are widespread in the genus *Solanum* and D'Arcy (1972) has reported self incompatibility in *S. sisymbriifolium.*

Andromonoecy, or the production of hermaphrodite and female sterile flowers, is a fairly widespread phenomenon in the genus *Solanum* (Whalen and Costich 1986). The mechanisms responsible for driving the evolution of andromonoecy in *Solanum* have been debated. The flowering and fruiting behaviour of *S. sisymbriifolium* supports the hypothesis that andromonoecy has arisen in the solanums as an internal mechanism to control fruit set, ensuring that energetic reserves for fruit development are not overextended (Symon 1979; Whalen and Costich 1986) while still producing plenty of pollen for pollinators. Wakhloo (1975a,b,c) found that high levels of potassium and kinetin increased the proportion of hermaphrodite flowers in *S. sisymbriifolium*, whereas an increase in gibberellic acid (GA_3) concentration promoted the formation of female sterile flowers. This suggests that the lack of hermaphrodite flowers at the distal end of an inflorescences could be a physiological

constraint, and would further explain why many of the hermaphrodite flowers on the distal ends of the inflorescences do not produce fruit.

The seed dispersal capabilities of *S. sisymbriifolium* are well suited to the transient habitat that it occupies, as there is both short range dispersal (fruit splitting) and long range dispersal (through birds). In many plant species, seeds that fall below the parent plants are subject to density-dependent mortality from the parent plants and other seedlings trying to germinate, resulting in reduced survival (Howe and Vande Kerckhove 1980; Coates-Estrade and Estrade 1988). This, however, would not apply to *S. sisymbriifolium* where it is an annual plant of disturbed areas, and the seedlings would not compete with their parent plants. In South Africa, however, where the weed appears to be longer-lived, suppression of seedlings by the parent plants might be expected.

Long range seed dispersal of *S. sisymbriifolium* by frugivorous birds is likely to promote colonization of a variety of habitats, some of which would be favourable and therefore enhance the spread of the weed. Indirect evidence to support this is provided by the seedlings that germinate along fences, under telephone cables, on the edges of plantations, and under roadside barriers (du Toit and van der Merwe 1941; Nel 1988). Oatley (1984) has shown that the Rameron Pigeon (*Columba arquatrix* Temminck and Knip) which is responsible for seed dispersal in the weed *S. mauritianum* in South Africa, has contributed greatly to the weed status of *S. mauritianum*. The large numbers of bird species seen feeding on the fruit of *S. sisymbriifolium* enhance its long range dispersal and ultimately its weed status in South Africa.

The germination success of *S. sisymbriifolium* was strongly enhanced by alternating light and dark photoperiods. Several authors have shown that both alternating light and dark, and alternating incubation temperatures promote germination within the Solanaceae (Wakhloo 1964; Porter and Gilmore 1976; Roberts and Lockett 1977, 1978; Campbell and van Staden 1983; Campbell *et al.* 1993). These laboratory-based results however are often not extrapolated to the field situation.

Solanum sisymbriifolium seeds in the soil appear to be under the same form of conditional dormancy, induced by constant dark conditions, that has been described for *S. mauritianum* seeds by Campbell and van Staden (1983). Under natural conditions, *S. sisymbriifolium* seeds germinate once the soil has been disturbed by fire, ploughing or bush clearing, or other agricultural practices (du Toit and van der Merwe 1941; Becker and Frieiro-Costa 1988; Nel 1988). A disturbance of the soil would bring a proportion of the seeds to the surface where they would be subjected to alternating light and dark conditions, and probably diurnal fluctuations in temperature, which would then stimulate them to germinate.

2.5 CONCLUSIONS

Early successional weed species, such as *S. sisymbriifolium*, are under strong selective pressure for rapid growth and the production of numerous, readily dispersed seeds (Cates and Orians 1975). The weed status of *S. sisymbriifolium* in South Africa is greatly enhanced by its copious fruit production, efficient dispersal capabilities and high percentage germination (in excess of 90% under laboratory conditions). In view of this, any biological control agent that attacks the reproductive structures of the weed would have the most impact on controlling its spread.

Annuals are generally unpredictable in space and time as they survive unfavourable seasons as seeds, grow rapidly, reproduce and die in relatively short time spans. Therefore, an individual plant is available to a herbivore for a only a short time (Janzen 1969, 1970). This has implications for the biological control of annual weed species, as the host plant (the weed) tends to be unpredictable in space and time. However, any agent that has had a long association with the weed in the country of origin would be cued into its spatial and temporal distribution. In addition, while in South America the above ground parts of the plant die back during the winter, regenerating from root stocks in the spring (Kvasina and de León 1985; Becker and Frieiro-Costa 1988), this behaviour was not noted in South Africa where the weed maintains its vigour throughout the winter, presenting any control agent with a more apparent host.

Ironically, the weed is often suppressed by dense infestations of exotic vegetation like wattle, as observed in the Eastern Cape. Removal of the wattle, by means of clearing or fires, results in a massive recruitment of *S. sisymbriifolium* seedlings; the plants in turn die back once the wattle regenerates. In these situations, the control of one invader merely enhances the status of another.

CHAPTER 3

The insect herbivore fauna associated with *Solanum sisymbriifolium* in South Africa.

3.1 INTRODUCTION

Introduced plant species provide ideal opportunities to study the recruitment of native phytophagous insects. One would expect indigenous plants to support a rich herbivore fauna, and introduced or exotic plants to support a poor herbivore fauna (Southwood 1961; Southwood *et al.* 1982). However, insects are often capable of finding and colonizing species of introduced plants quickly (100 years or less) (Strong 1974). The rate of colonization by phytophages is affected by the range of the introduced plant species, and the taxonomic, phenological, biochemical and morphological match between the introduced and native plants (Strong *et al.* 1984). South Africa is host to a number of native and exotic *Solanum* species, and Olckers and Hulley (1989a) envisage a local "pool" of phytophagous insects (termed *Solanum* oligophages) potentially capable of colonizing species within this genus. As related species of plant often present very similar cues to insects (Connor *et al.* 1980) one might expect *S. sisymbriifolium* to support a phytophage fauna similar to that of the native *Solanum* species.

The primary aim of this chapter was to conduct a survey of the insects associated with *S. sisymbriifolium*, in order to determine the contribution of native insects to the natural control of the weed, and hence the types of imported agents required to supplement their impact. Another aim was to determine whether any natural herbivores of *S. sisymbriifolium* had

already entered the country accidentally, as was the case with *Sesbania punicea* (Cavanilles) Bentham (Fabaceae) (Hoffmann and Moran 1988).

3.2 MATERIALS AND METHODS

3.2.1 Insect herbivores associated with *Solanum sisymbriifolium*.

Surveys of the insect herbivores feeding on *S. sisymbriifolium* were carried out in the Eastern Cape and Transvaal (PWV region and the Eastern Transvaal) (Table 3.1). Collections were made fortnightly from infestations around Grahamstown between February 1990 and November 1992 and opportunistically at other sites of infestation in the Eastern Cape and Transvaal between February 1990 and April 1994. During each collection, 20 or more plants were sampled. Individual plants were examined for ectophagous herbivores in the field and representative parts of the plants taken for further evaluation. Stems were dissected for borers, and the leaves for less obvious ectophagous and endophagous herbivores. Fruit and flowers were also collected, half of which were frozen for subsequent dissection and the remainder placed in emergence cages to allow any immature herbivores to complete their development. Only insects feeding or reproducing on the plants were recorded. Voucher specimens of unidentified species, referred to by their accession numbers, were placed in the Albany Museum (Grahamstown) and in the National Collection of Insects (Pretoria).

Table 3.1 Localities in Transvaal and Eastern Cape where *Solanum sisymbriifolium* was sampled.

Localities	Collections	Map reference
Eastern Cape		
Grahamstown	47	33°19'S 26°32'E
Port Elizabeth	5	33°58'S 25°38'E
Uitenhage	1	33°46'S 25°24'E
Transvaal		
Four-Ways	7	26°03'S 28°01'E
Olifantsfontein	9	25°57'S 28°08'E
Honeydew	4	26°09'S 27°55'E
Carolina	2	26°04'S 30°06'E

3.2.2 Comparison with the fauna of other *Solanum* species.

The insect herbivore fauna associated with *S. sisymbriifolium* was compared with that associated with two exotic (*S. elaeagnifolium* and *S. mauritianum*) and four native *Solanum* species from which more than 30 collections had been made (*viz. S. coccineum* Jacquin, *S. linnaeanum* Hepper and Jaeger, *S. rigescens* Jacquin and *S. panduriforme* E. Meyer) (all non *S. sisymbriifolium* data are take from Hulley and Olckers, unpublished data).

3.3 RESULTS

3.3.1 Insect herbivores associated with *Solanum sisymbriifolium*.

Twelve regularly-occurring phytophagous insects were collected on *S. sisymbriifolium* in the Eastern Cape and Transvaal (Table 3.2). The majority were polyphagous species, known also to attack families other than Solanaceae (Annecke and Moran 1982) although there were two *Solanum* oligophages (*Epilachna hirta* (Thunberg) and *Acanthocoris fasciolatus* Fabricius) (Table 3.2) (Olckers and Hulley 1989a, 1991; Hill *et al.* 1993).

Throughout South Africa the few herbivore species occurred in few samples and then in low individual numbers, so that the plants sustained very little insect damage. No leaf miners, stem borers or gall formers were recorded; all the herbivores were either exophytic, or endophytic within the fruit.

FOLIAGE

Coleoptera: Coccinellidae: *Epilachna hirta* (Thunberg)

Epilachna hirta was a fairly common phytophage found feeding externally on the leaves of *S. sisymbriifolium* throughout most of the year in both the Eastern Cape and Transvaal. It was the only species to inflict observable damage on the leaves. Feeding damage was characterised by track-like patterns on the surface of the leaves. Only adults were recorded on *S. sisymbriifolium* suggesting that this species utilises the weed as a secondary host. Evidence for this was the absence of the insect from *S. sisymbriifolium* samples taken in the Eastern Cape for most of 1991, when populations presumably preferred their native *Solanum* hosts which were readily available at this time.

This herbivorous ladybird has been recorded from most of the native and exotic *Solanum* species in South Africa (Olckers and Hulley 1989a). The reported status of *E. hirta* as a pest of potato crops (Jack 1913; Annecke and Moran 1982) and vegetable gardens (Ballard 1914) illustrates its wide host range.

Table 3.2 Insect herbivores associated with *Solanum sisymbriifolium* in the Eastern Cape and Transvaal. Single specimens found only in one sample were excluded. Incidence is reflected as the percentage of the total number of samples in each region in which the insect occurred.

Insect	Incidence			Other Hosts[a]
	Overall	E.Cape	Tvl	
FOLIAGE				
COLEOPTERA				
Coccinellidae				
Epilachna hirta (Thunberg)	24.0	28.3	13.6	Ri Co Li Ac Pa In Cf Ma El To
HEMIPTERA				
Coreïdae				
Acanthocoris fasciolatus F.	10.7	13.2	4.6	Ri Co Acan Li Ac Acul Pa In
Miridae				
Cyrtopeltis sp. (AcRh 674)	9.3	11.3	4.6	Acan Ac
Pentatomidae				
Nezara viridula (L.)	4.0	5.7	-	Polyphagous
Cixiidae				
Oliarus sp. (AcRh 686)	6.7	7.6	4.6	Unknown
FLOWERS				
THYSANOPTERA (various)	21.3	3.8	63.6	Polyphagous
FRUIT				
HEMIPTERA				
Lygaeidae				
Spilostethus furculus (H.S.)	4.0	3.8	4.6	Polyphagous
Coreidae				
Cletus pusillus Dallas	9.3	11.3	4.6	Unknown
DIPTERA				
Drosophilidae				
Zaprionus sp. (AcRh 471)	5.3	7.6	-	Polyphagous
Leucophenga sp. (AcRh 472)	4.0	5.7	-	Polyphagous
Tephritidae				
Ceratitis rosa (Karsch)	5.3	7.6	-	Polyphagous
LEPIDOPTERA				
Noctuidae				
Heliothis armigera (Hübner)	17.3	17.0	18.2	Polyphagous

[a] Ri = *S. rigescens*, Co = *S. coccineum*, El = *S. elaeagnifolium*
Li = *S. linnaeanum*, Ac = *S. aculeatissimum*, In = *S. incanum*,
Pa = *S. panduriforme*, To = *S. tomentosum* Linnaeus, Acan = *S. acanthoideum*, Cf = *S.* cf *acanthoideum*,
Acul = *S. aculeastrum* Dunal, Ma = *S. mauritianum*

Hemiptera: Coreidae: *Acanthocoris fasciolatus* Fabricius

The adults of this coreid were quite common in the samples taken in the Eastern Cape, but the number of insects per sample was very low, and it was only recorded from one sample taken in the Transvaal. No immature stages were recorded.

Although southern African tip wilters are known to cause wilting and die-back of the shoots of some plant species (Jacobs 1985), the level of infestation on *S. sisymbriifolium* was so low that the damage caused by the insect was negligible, and no severe wilting was observed. *Acanthocoris fasciolatus* has been recorded from most of the native *Solanum* species (Olckers and Hulley 1989a) and several other solanaceous species (Roberts 1930).

Hemiptera: Miridae: *Cyrtopeltis* sp. (AcRh 674)

This small mirid was observed feeding on the leaves, stems and the pericarp of damaged fruit. Despite the adults and nymphs being common in the samples taken from the weed in the Eastern Cape, the insect caused no observable damage. This species has also been recorded from *S. acanthoideum* in Natal, and *S. aculeatissimum* in the Eastern Cape. Its native hosts are unknown.

Hemiptera: Pentatomidae: *Nezara viridula* (Linnaeus)

Isolated adult individual green vegetable bugs were present in a few samples in the Eastern Cape, but caused no noticeable damage to the weed. This is a highly polyphagous, widespread pest in South Africa (Annecke and Moran 1982).

Hemiptera: Cixiidae: *Oliarus* sp. (AcRh 686)

The adults of this species were found feeding on the main veins of the leaves. They were not common, and appeared to do the plants no damage at all. Nymphs of this genus are subterranean and feed on roots (Jacobs 1985), although they were not found on *S. sisymbriifolium* roots. The native hosts of this species is unknown.

FLOWERS

Thysanoptera

Several species of thrips were recorded feeding on the tubular anthers of the weed. These were highly abundant and very common on the plants in the Transvaal, but less so in the Eastern Cape.

FRUIT

Hemiptera: Lygaeidae: *Spilostethus furculus* Herrich-Schaeffer.

This lygaeid was observed feeding on the seeds of fruit which had split open on the plants. It was observed in very few samples, and in low numbers within these samples, and would be unlikely to have any impact on the seed production of *S. sisymbriifolium*. *Spilostethus furculus* is a polyphagous phytophage, having been recorded on most of the native *Solanum* species (Hulley and Olckers, unpublished data), several other Solanaceous species, as well as species within the Lilaceae, Asclepiadaceae and Malvaceae (Slater and Sperry 1973).

Hemiptera: Coreidae: *Cletus pusillus* Dallas

This small, brown coreid was recorded feeding externally on the ripe fruit of the weed, presumably feeding on the seeds. Once again, only adults were recorded. Two other species within this genus have been recorded on native solanums; *Cletus* sp (AcRh 526) on *S.* cf.

gracile, *S. melongena* and *S. incanum*, and *Cletus* sp. (AcRh 556) on *S. incanum* (Hulley and Olckers, unpublished data). The native host range of *C. pusillus* is unknown.

Diptera: Drosophilidae: *Zaprionus* sp. (AcRh 471) and *Leucophenga* sp. (AcRh 472)
Despite being reared from the fruit of only a few samples in the Eastern Cape, these two drosophilids were very abundant in the fruit. They have been recorded from most of the native, and exotic *Solanum* species (Hulley and Olckers, unpublished data). Their sporadic occurrence in the fruit suggests they are using *S. sisymbriifolium* as a secondary host plant.

Diptera: Tephritidae: *Ceratitis rosa* (Karsch)
The Natal fruit fly, which was reported to maintain its winter numbers in the fruit of *S. mauritianum* (Ripley and Hepburn 1930, 1935), was reared from *S. sisymbriifolium* fruit harvested in the Eastern Cape. This strengthens the weed status of *S. sisymbriifolium*, as it may also facilitate the dispersal and rapid seasonal build up of fly populations.

Lepidoptera: Noctuidae: *Heliothis armigera* (Hübner)
The American bollworm had the most serious impact on the weed as it was responsible for severe fruit damage. The infestations of the bollworm on the fruit of *S. sisymbriifolium* were both sporadic and localised. When they occurred on the plants, fruit loss was usually in excess of 80%.

This insect has been recorded from most of the native and exotic *Solanum* species (Hulley and Olckers, unpublished data) and is a notorious polyphagous pest (Annecke and Moran 1982).

3.3.2 Comparison with the fauna of other *Solanum* species.

Solanum sisymbriifolium supported a poor herbivore fauna in comparison to both exotic and native *Solanum* species in South Africa (Table 3.3). When considering the regularly-occurring herbivores (those in at least 20% of the samples) it is evident that bugweed (*S. mauritianum*), satansbos (*S. elaeagnifolium*) and the native *S. coccineum* were all equally depauperate. The insects most common to this group of solanums were the so called *Solanum* oligophages (*E. hirta* and *A. fasciolatus*) and the generalist polyphages (*N. viridula*, *S. furculus*, the various thrips species and the fruit feeders).

Table 3.3 Comparison of the insect herbivore faunas of several native and exotic *Solanum* species.

Plant species		Number of herbivore species		Number of herbivore species in common with
	n[a]	All	20% of n[b]	*S.sisymbriifolium*
S. sisymbriifolium	75	12	2	-
Exotic				
S. elaeagnifolium	16	20	2	4
S. mauritianum	30	25	1	7
Native				
S. coccineum	33	28	3	3
S. incanum	40	60	6	6
S. linnaeanum	48	39	8	7
S. panduriforme	62	70	10	6

[a] = The number of collections on each of the plants.
[b] = The number of herbivore species in at least 20% of the samples.

3.4 DISCUSSION

Solanum sisymbriifolium in South Africa has an impoverished insect herbivore fauna. This is in contrast to South America where the plant supports an abundant insect herbivore fauna (H.E. Erb, personal communication) and in comparison to local *Solanum* species (Table 3.3 and Olckers and Hulley 1989a, 1991; Hill *et al.* 1993). No unintentionally introduced natural herbivores of *S. sisymbriifolium* were found in South Africa. The plants were normally vigorous and largely undamaged by insects, which indicated that native herbivores are not important in the natural control of this weed. No local insects are thus important enough to impose any constraints on the type of biological agent imported from South America for the biological control of *S. sisymbriifolium.*

Although exotic plants are attacked largely by polyphagous insect species (Andow and Imura 1994), recruitment by oligophagous species from some taxonomically or biochemically related plants can also be expected (Strong *et al.* 1984). The importance of local pools of preadapted herbivores in the establishment of herbivores on introduced plants was emphasized by Jermy (1988) and Zwölfer (1988). The local pool of *Solanum* herbivores appears not to be preadapted to colonize *S. sisymbriifolium.* This situation contrasts with that of thistle faunas in southern California (Goeden and Ricker 1986) where numerous thistle herbivores colonized the introduced thistle *Cirsium vulgare* (Save) Tenore.

Solanum sisymbriifolium, like *S. mauritianum,* recruits mainly polyphagous species (Olckers and Hulley 1989a). As with *S. mauritianum* (Olckers and Hulley 1989a, 1991) the ladybird *E. hirta* was the only native oligophage capable of inflicting noticeable damage on the foliage of *S. sisymbriifolium.* This apparent preadaptation to feed on *S. sisymbriifolium*

relates to a wide natural host range which incorporates numerous native and exotic species of *Solanum* as well as cultivated species like potato (*S. tuberosum*) and eggplant (*S. melongena*). However, *E. hirta* adults occurred in low numbers and no immature stages were recorded. *Solanum sisymbriifolium* therefore appears to be secondary host for *E. hirta* perhaps during shortages of its preferred hosts.

Many authors (e.g. Goeden and Ricker 1976; Connor *et al.* 1980; Strong *et al.* 1984) consider the taxonomic, biochemical and morphological match between exotic and native congenerics as a major determinant of herbivore recruitment by exotics. Olckers and Hulley (1991) ascribed the impoverished fauna of *S. mauritianum* to taxonomic isolation, as it belongs to the subgenus *Brevantherum* (Seithe) D'Arcy which is not represented in Africa. In contrast, *S. sisymbriifolium* belongs to the subgenus *Leptostemonum* (Dunal) Bitter which incorporates about 68% of native *Solanum* species (Jaeger and Hepper 1986). However, within this large subgenus, *S. sisymbriifolium* belongs to the section *Cryptocarpum* Dunal (D'Arcy 1972), which is not naturally represented in Africa (Jaeger and Hepper 1986). However, it seems that the answer to the relative freedom from attack of *S. sisymbriifolium* may lie rather with the nature of its physical defenses.

Solanum species are often reliant on glandular and non-glandular leaf trichomes (Levin 1973) for physical defence. While the majority of South African *Solanum* species have non-glandular leaf trichomes, *S. sisymbriifolium* leaves are richly supplied with glandular trichomes. The exudate produced by the glandular trichomes of some *Solanum* species seriously impede feeding by phytophagous species (Tingey and Gibson 1978; Gregory *et al.* 1986; Boiteau and Singh 1988; Neal *et al.* 1989). The novelty of glandular trichomes

among South African *Solanum* plants may be an important factor in affording *S. sisymbriifolium* immunity to attack by native insects.

CHAPTER 4

Glandular trichomes on the exotic *Solanum sisymbriifolium* Lamarck limit the recruitment of native South African insect herbivores.

4.1 INTRODUCTION

The pre-introductory survey on *S. sisymbriifolium* (Chapter 3) showed that the weed supported a very poor native herbivore fauna despite the presence of a local pool of *Solanum* oligophagous insects (Olckers and Hulley 1989a). The depauperate fauna on the weed was tentatively ascribed to its morphological uniqueness amongst the native *Solanum* species, and specifically to the much higher densities of glandular trichomes on its leaves in comparison to the native *Solanum* species.

The Solanaceae are well-equipped with defences against herbivory (Hsiao 1986). These defences might be physical (leaf hairs or trichomes) and/or chemical (alkaloids and other secondary compounds) (Tingey 1985). This has lead Drummond (1986) to suggest that relatively few insects attack the Solanaceae, and those that do have adaptations which allow them to circumvent these defences. The native South African pool of *Solanum* oligophages appear not to possess the necessary preadaptations to circumvent the anti-herbivore defence posed by the glandular trichomes on the leaves of *S. sisymbriifolium.*

Studies on plant resistance to insect attack have shown that the presence of leaf pubescence or trichomes is associated with low levels of insect attack (Poos and Smith 1931; Painter 1951; Beck 1965; Levin 1973; Carter 1982; Lyman and Cardona 1982). Trichomes are

widespread within the Solanaceae (Tingey 1985), and have been used as a taxonomic characteristic (Seithe 1962). While the non-glandular trichomes pose a mechanical barrier to insects, limiting access to feeding and oviposition sites (Levin 1973; Tingey 1985), and in some cases piercing the body wall of the would-be herbivores (Gilbert 1971; Pillemer and Tingey 1976), glandular trichomes interfere with locomotion and attachment of the herbivores to the plant, or physically entrap the insects (Tingey 1985).

Gregory *et al.* (1986) describe two forms each of both glandular and non-glandular trichomes within the genus *Solanum*. There are simple, spike-like trichomes and more complex stellate non-glandular trichomes, and type A and type B glandular trichomes. Type A glandular trichomes are short and have tetralobular, membrane-bound heads. Type B glandular trichomes are longer, simple trichomes and are tipped with an ovoid gland which continually discharges clear, viscous exudate containing fatty acid esters of sucrose (Gibson 1971a,b; Tingey and Laubengayer 1981; Neal *et al.* 1989). The exudate produced by the type A trichomes contains enzyme polyphenoloxidases and peroxidases which rapidly polymerize on contact with oxygen, encasing insect herbivores (Gregory *et al.* 1986; Neal *et al.* 1989).

There have been numerous published accounts of glandular trichomes within the genus *Solanum* offering resistance to a wide range of insect herbivores (e.g. Gentile and Stoner 1968a; Gentile *et al.* 1968; Gibson 1971b; Tingey and Gibson 1978; Tingey and Laubengayer 1981; Boiteau and Singh 1988). The majority of these studies have focused on the glandular trichomes of the wild potato (*Solanum berthaultii* Hawkes) with a view to hybridising it with potato (*S. tuberosum*) thereby enhancing the resistance of potato to the potato aphid (*Macrosiphum euphorbiae* (Thomas)), the green peach aphid (*Myzus persicae*

(Sulzer)), the greenhouse whitefly (*Trialeurodes vaporariorum* (Westwood)) and the potato leafhopper (*Empoasca fabae* (Harris)). A similar volume of literature exists on the attempts to hybridise tomato (*Lycopersicon esculentum* Mill.) with closely related glandular trichomate species to improve its resistance to generalist herbivores (reviewed in Duffy 1986).

Despite being a widespread defence throughout the plant kingdom, there have been very few reports of herbivorous insects circumventing trichomes. Rathcke and Poole (1975) showed how the ithomiid butterfly, *Mechanitis isthmia* Bates avoided the trichome defences of a species of *Solanum* by spinning a silk scaffolding over the tips of the trichomes on the undersurface of the leaves and using this as a platform from which to feed on the less protected edges of the leaves. Hulley (1988) reported that the caterpillars of the noctuid moth *Pardasena* sp. nr *diversipennis* mow the stellate trichomes on the leaves of their host plant *S. coccineum*, with their mandibles, prior to feeding. Siebert (1975) suggested that the tortoise beetle *Gratiana lutescens* nibbled off the trichomes from the leaves of their host plant *Solanum elaeagnifolium* before feeding.

There appears to be only one documented example of phytophagous insects circumventing glandular trichomes. Two genera (*Cyrtopeltus* and *Dicyphus*) within the subfamily Dicyphinae (Miridae: Hemiptera) show morphological and behavioural adaptations to overcoming the glandular trichomes on their Western Australian host plant *Drosera* (sundew) (Russell 1953; Southwood 1986). These mirids are long-legged and keep their bodies away from the plant surface, preventing them coming into contact with the trichome exudate. In addition, the angle between the tibia and the tarsus is large (>145°) so that only the apex of

the tarsus comes into contact with the plant surface. The "shepard's crook" shaped pretarsus was observed to grip the trichomes below the glandular head and in so doing avoiding the glandular exudate. On occasion when the mirids did get stuck to the trichomes, they had a technique of pulling and lifting the leg parallel to the body, and seemed efficient at freeing stuck limbs (Russell 1953). The mirids were frequently noted cleaning the exudate off of their limbs (Russell 1953; Southwood 1986).

The aim of the study described in this chapter was twofold, firstly to describe the leaf hairs on *S. sisymbriifolium* and to assess the extent of leaf pubescence on selected native and exotic *Solanum* species, and secondly, to compare the biologies of two tortoise beetle species (*Conchyloctenia tigrina* Oliv. and *Gratiana spadicea*) on both *S. sisymbriifolium* and *S. linnaeanum* (a native host of *C. tigrina*). The rationale behind choosing the species of insect was firstly, they are closely related (although *C. tigrina* is in the tribe Aspidomorphini and *G. spadicea* is in the tribe Cassidini of the subfamily Cassidinae (Spaeth 1914)), secondly they are of comparable size and have very similar biologies, and finally, they feed on closely related host species (*C. tigrina* on native *Solanum* species and *G. spadicea* on *S. sisymbriifolium*). However, *S. sisymbriifolium* has leaves which are very densely covered by glandular trichomes and the native *Solanum* species do not.

4.2 MATERIALS AND METHODS

4.2.1 Leaf pubescence of *Solanum sisymbriifolium* and other *Solanum* species.

Leaf specimens of *S. sisymbriifolium*, *S. acanthoideum*, *S. aculeatissimum*, *S. linnaeanum*, *S. rigescens*, *S. panduriforme* and *S. mauritianum* were viewed under a Wild M 3 dissection microscope at 60X magnification. In addition, the leaves of *S. sisymbriifolium*, *S.*

acanthoideum, *S. aculeatissimum*, *S. linnaeanum* and *S. rigescens* were viewed in a scanning electron microscope (the specimens were prepared using a standard freeze drying technique to prevent collapse of the tissue (Appendix II)). Both the upper (adaxial) and lower (abaxial) leaf surfaces of all species were viewed.

The trichome densities of the leaves of the above seven *Solanum* species were compared. The densities of trichomes per mm^2 were determined using a dissection microscope fitted with an Olympus OSM ocular micrometer, at 60X magnification. Trichome counts were made mid-way between the tip and the base of the leaves, about 2mm either side of the main vein. Only fully expanded leaves were used, and they were collected from different plants for each species; 25 counts were made from each surface of each species. Data were analyzed using a Kruskal-Wallis One Way Analysis of Variance on Ranks followed by Dunn's Multiple Range test where applicable (Sokal and Rohlf 1981).

4.2.2 Comparison of the biologies of *Gratiana spadicea* and *Conchyloctenia tigrina* on *Solanum sisymbriifolium* and *Solanum linnaeanum*.

Cultures of the two beetle species were established in a quarantine insectary with a photoperiod of 16:8 hours (light:dark). Temperatures fluctuated between 26 and 20°C in synchrony with the photoperiod and relative humidity ranged between 60 and 80%. Both cultures were maintained on potted specimens of their natural hosts. All behaviour and feeding experiments were conducted on potted plants to obviate possible atypical results with excised leaves (Jones and Coleman 1988).

Morphological differences

Scanning electron micrographs were made of the tarsi and mouthparts of adults and larvae of the two beetle species. Numerous studies have shown that glandular trichomes impede movement of insects by "gumming-up" their tarsi (in particular the tarsal claws) and cause starvation by occluding their mouthparts (e.g. Gentile and Stoner 1968a; Gentile *et al.* 1968; Gibson 1971a) so it follows that any major morphological adaptations should occur in these two regions.

Behavioural differences

Adults and larvae of *G. spadicea* and *C. tigrina* were observed moving and feeding on leaves of *S. sisymbriifolium* and *S. linnaeanum* under a dissection microscope using a cold light source. Beetles were sometimes starved for 12-24 hours prior to observation to stimulate immediate feeding. At least 20 hours of observations were made for each species, 10 hours for the larval and 10 hours for the adult stage.

Effect of glandular trichome exudate

A series of feeding trials were performed using both larvae and adults of both beetle species in which the exudate was removed from the *S. sisymbriifolium* leaves. Attached leaves of potted *S. sisymbriifolium* plants were swabbed with 75% ethanol to remove the glandular exudate and then rinsed in distilled water (Gentile *et al.* 1968; Gentile and Stoner 1968a,b; Dimock and Kennedy 1983; Hawthorne *et al.* 1992).

The exudate produced by the type B glandular trichomes was continuously discharged, but Gentile and Stoner (1968a) showed that repeated washing with 75% ethanol eventually

exhausted the stocks of the exudate in *S. berthaultii*. Here the leaves of *S. sisymbriifolium* were washed twice daily until no more type B exudate appeared (this usually took 4-5 days).

Five adults of each beetle species were confined on the washed leaves. Control beetles were confined to unwashed leaves. Twelve replicates were carried out for each beetle species. The beetles were confined to the leaves for 24 hours, after which leaf damage was estimated, and a score between 0 and 6 allocated (0 indicated no damage and 6 total destruction). It was ensured that leaves of the same approximate size were used for all feeding trials. Feeding on washed and unwashed leaves was compared using a Wilcoxon Signed Ranks test (Miller 1974). The experiments were repeated using twenty first instar larvae which were confined to the leaves for 48 hours.

This technique of allocating a score for feeding relies on the leaves of the two species being of similar thickness. To test this, 50 leaf discs (diameter 2cm) were cut from the leaves of both species, and weighed. The leaf discs of *S. linnaeanum* were 1.73 $\pm$ 0.82 times heavier than those of *S. sisymbriifolium*. Therefore the scores obtained by the beetles feeding on *S. linnaeanum* were multiplied by 1.73 so they could be directly compared with those obtained by the beetles on *S. sisymbriifolium*.

Washing the leaves with 75% ethanol could have had some influence on host recognition by the beetles or palatability of the leaves. To test this possibility, the experiments were repeated using washed and unwashed *S. linnaeanum* instead of *S. sisymbriifolium*. This not only provided a control for the ethanol treatment, but also allowed a comparison of feeding scores of the beetles on both hosts.

Larval survival and development periods

Twenty recently hatched, first instar larvae of both beetle species were placed on each species of plant. Larval survival was recorded weekly. Each experiment continued until all the surviving larvae had pupated. The time taken from hatching to pupation (development time) was recorded. Five replicates were conducted for each group of larvae on each test plant. Plants were changed every week to prevent excessive feeding damage. Larval survival and development time for both beetle species on both plant species were compared using a Kruskal-Wallis test, followed by a Dunn's Multiple Range test, where applicable (Sokal and Rohlf 1981).

Ideally a comparison of developmental rates of *C. tigrina* on *S. sisymbriifolium* with and without glandular trichome exudate (i.e. washed and unwashed) would have supplied the most useful information on the effect of the glandular trichomes on *C. tigrina*. However, as the exhausted exudate was replaced by the type B trichomes within a week, even after a thorough washing for several days, and the developmental periods are in the region of a month, this test could only have been achieved using a series of plants at different stages of washing, which was not practical under the laboratory conditions available.

4.3 RESULTS

In some of the tables that follow, means and standard deviations are quoted as a measure of central tendency as they provide an indication of the variability within the samples. However, the data were found to be not normally distributed and the non-parametric statistical tests used relied on the median values of the samples (Zar 1974).

4.3.1 Leaf pubescence of *Solanum sisymbriifolium* and other *Solanum* species.

All four of the trichome types described by Tingey (1985) and Gregory *et al.* (1986) within the genus *Solanum* were present on the leaves of some of the species investigated (only *S. acanthoideum* and *S. aculeatissimum* had all four types (Tables 4.1 and 4.2)). In all species considered, the veins of the leaves were far more densely trichomate than the rest of the leaf, presumably as protection against phloem-feeding insects.

Stellate trichomes occurred on both the adaxial and abaxial surfaces of all the species viewed. The stellate trichomes of *S. sisymbriifolium*, *S. aculeatissimum*, *S. acanthoideum*, *S. linnaeanum* and *S. rigescens* are referred to as porrect-stellate trichomes in Roe's (1967) classification of *Solanum* trichomes, and are radially symmetrical, consisting of a central ascending ray and 4-8 lateral rays, equally spaced around the axis in a horizontal plane (Figure 4.1). The stellate trichomes of *S. panduriforme* and *S. mauritianum* are referred to as multiangulate, without radial symmetry, consisting of 4-16 rays projecting at many angles from the axis. Both *S. panduriforme* and *S. mauritianum* had sessile (attached to the leaf surface) and stalked stellate trichomes. These formed two layers on the leaves, and explains the very high densities of stellate trichomes found on the surfaces of the leaves of these two species (Tables 4.1 and 4.2).

The stellate trichomes differed between species. Those of *S. linnaeanum*, *S. rigescens*, *S. panduriforme* and *S. mauritianum* were fairly small, and on closer inspection, contained fluid which might well have been cytoplasm (Levin 1973). The central ray of the stellate and simple trichomes of *S. sisymbriifolium*, *S. aculeatissimum*, and *S. acanthoideum* were segmented, and the joints appeared to be areas of disarticulation, which, when touched, were

easily broken, releasing a sticky exudate (Figure 4.1a,b), suggesting they are more than a purely physical defence.

Only *S. aculeatissimum* and *S. acanthoideum* possessed the tetralobular type A glandular trichomes, and only in low densities in comparison to the other types of trichomes (Tables 4.1 and 4.2) (Figures 4.1b and 4.2a,b). The type B glandular trichomes were present in all species, but not on the undersurface of the *S. mauritianum* leaves (Figures 4.1a,b and 4.2c,d,e,f). The type B trichomes of *S. sisymbriifolium*, *S. acanthoideum* and *S. aculeatissimum* also had segmented stalks, and were longer than the stalks of the type B trichomes of the other species (Figure 4.2). The leaves of *S. sisymbriifolium* and *S. acanthoideum* had significantly higher densities of these trichomes than the other species.

Small insects, (mainly Diptera and winged ants) were frequently found stuck to the exudate produced by both types of trichome. The glandular trichomes appear to give *S. sisymbriifolium* immunity to attack by red spider mite (*Tetranychus cinnabarinus* (Boisduval)), a very common pest on all native solanums, and the whitefly (*T. vaporariorum*), although many whiteflies were often able to escape the glandular trichomes because the powdery wax from their wings coated the trichomes. In spite of this, both mites and whiteflies were frequently observed stuck to both the type B and stellate trichomes. On occasion flea beetles (*Chaetocnema* sp. AcRh 465, a common herbivore of native *Solanum* species) were found stuck to the glandular trichomes. Scanning electron micrographs revealed that these insects were totally encased by the glandular exudate (Figure 4.3a,b).

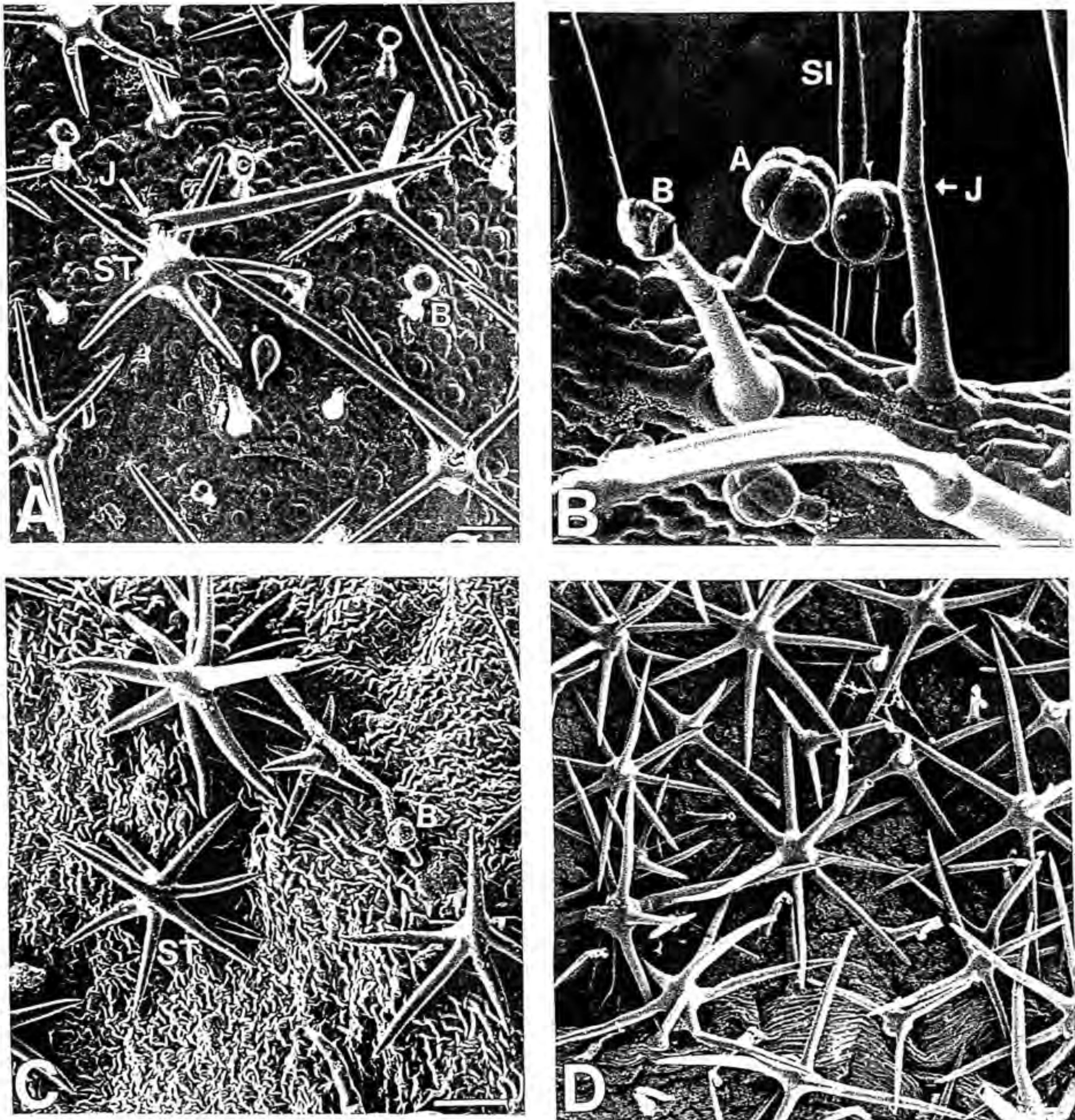


Figure 4.1 Scanning electron micrograph of the leaf surface of **A:** *Solanum sisymbriifolium* with the central ray of the glandular stellate trichome broken at the joint. **B:** *Solanum acanthoideum*, showing the joint in the glandular simple trichome. **C:** *Solanum linnaeanum* **D:** *Solanum rigescens*. **ST:** Stellate trichome, **B:** Type B glandular trichome, **A:** Type A glandular trichome, **SI:** Simple trichome, **J:** Joint in trichome. All scale bars = 100μm.

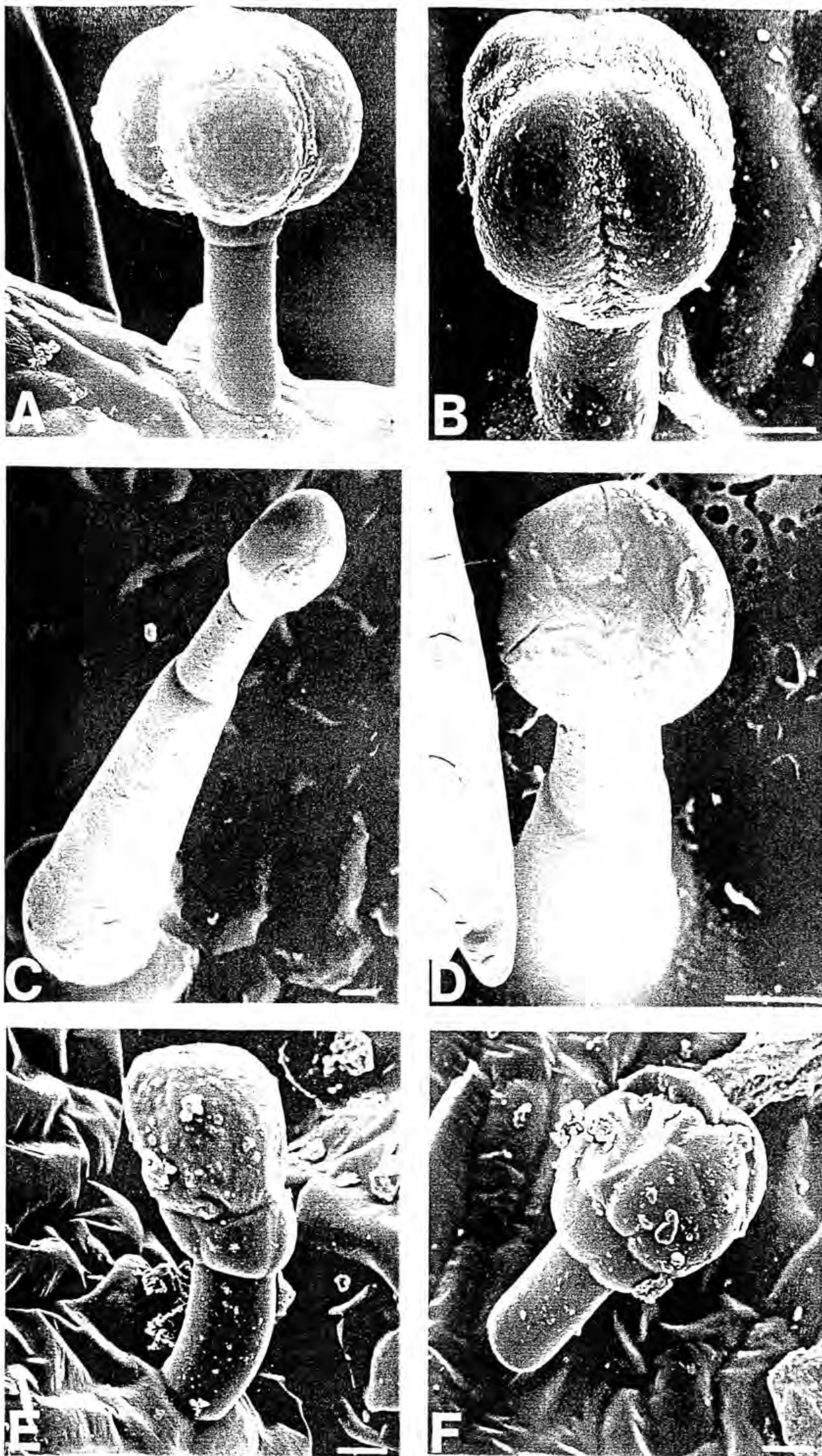


Figure 4.2 Scanning electron micrograph of type A glandular trichomes on the leaves of **A:** *Solanum acanthoideum*, **B:** *Solanum aculeatissimum*, and type B glandular trichomes on the leaves of **C:** *Solanum acanthoideum*, **D:** *Solanum sisymbriifolium*, **E:** *Solanum linnaeanum* and **F:** *Solanum rigescens*. All scale bars = 10μm

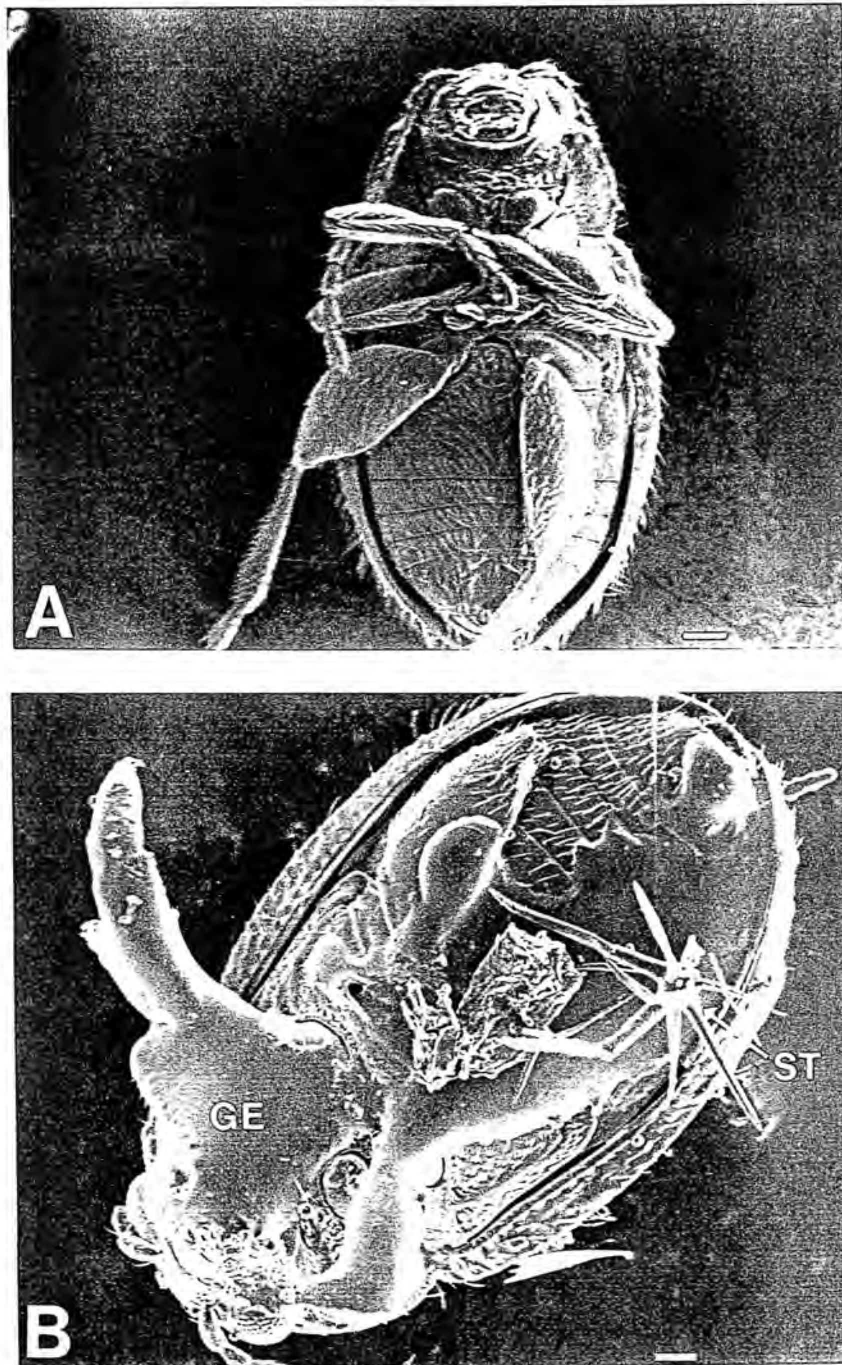


Figure 4.3 Scanning electron micrograph of the flea beetle, *Chaetocnema* sp. (AcRh 465), **A:** from its usual host *S. linnaeanum* and **B:** one that has become trapped by the glandular exudate produced by the trichomes of *S. sisymbriifolium*. **GE:** glandular exudate, **ST:** stellate trichomes. Both scale bars = 100μm.

Table 4.1 The number of different types of trichomes on the upper surface of the leaves of seven *Solanum* species.

Plant species	n	Number of trichomes (mean ± SD) (mm^2)[a]			
		Stellate	Simple	Type A	Type B
S. sisymbriifolium	25	14.08ab (3.92)	-	-	32.36a (5.44)
S. aculeatissimum	25	0.12c (0.33)	7.68a (3.38)	6.04 (0.41)	20.44ab (5.05)
S. acanthoideum	25	0.08c (0.28)	7.72a (0.43)	-	14.76bc (2.98)
S. linnaeanum	25	12.20a (2.22)	-	-	4.52de (1.45)
S. rigescens	25	9.60a (2.45)	7.44a (1.50)	-	8.36cd (2.00)
S. panduriforme	25	36.12bd (4.45)	-	-	7.32e (1.49)
S. mauritianum	25	85.64d (3.84)	-	-	1.28e (1.65)

[a] Means in columns not followed by the same letter differ significantly at the 5% level (Kruskal-Wallis test followed by Dunn's Multiple Range test).

Table 4.2 The number of different types of trichomes on the lower surface of the leaves of seven *Solanum* species.

Plant species	n	Number of trichomes (mean ± SD) (mm^2)[a]			
		Stellate	Simple	Type A	Type B
S. sisymbriifolium	25	10.44ab (2.95)	-	-	53.52a (7.85)
S. aculeatissimum	25	5.36b (2.38)	8.96a (7.61)	3.52a (2.86)	21.60ab (5.72)
S. acanthoideum	25	3.36b (2.16)	6.92a (2.29)	5.72a (1.81)	20.00b (3.96)
S. linnaeanum	25	24.24a (4.03)	-	-	2.92c (1.87)
S. rigescens	25	23.12a (2.32)	-	-	2.68c (2.87)
S. panduriforme	25	41.56c (4.82)	-	-	4.40c (1.94)
S. mauritianum	25	171.16d (5.84)	-	-	-

[a] Means in columns not followed by the same letter differ significantly at the 5% level (Kruskal-Wallis test followed by Dunn's Multiple Range test, except for the Simple and Type A categories which were compared using a Mann-Whitney Rank Sum test).

4.3.2 Comparison of the biologies of *Gratiana spadicea* and *Conchyloctenia tigrina* on *Solanum sisymbriifolium* and *Solanum linnaeanum.*

Morphological differences

The basic morphology of the mouthparts of both species was consistent with that described for many of the North American Cassidinae by Riley (1982, 1985, 1986) and the African Cassidinae by Borowiec (1985a,b,c). In both the adults and the larvae, at rest, the head is retracted into the prosternum, beneath the prosternal collar. The scanning electron micrographs of the mouthparts showed that there were no obvious morphological differences between *G. spadicea* and *C. tigrina* adults and larvae. There was certainly no marked

morphological differences suggesting any preadaptation of *G. spadicea* to the glandular trichomes of *S. sisymbriifolium*.

The terminal region of the tarsi of the larvae of both species was identical, and consisted of a single, simple tarsal claw. The tarsal claws of the adult beetles differed between the two species. *Gratiana spadicea* had simple, symmetrical tarsal claws (Figure 4.4a). The tarsal claws of *C. tigrina* were much broader and cup-shaped with comb-like structures composed of a series of teeth, arranged side by side, referred to as pectinate claws (Riley 1986). The teeth or pectines on the interior claw surface were much more pronounced than those on the external claw surface (Figure 4.4b).

The cup-shaped tarsal claws of *C. tigrina* were prone to getting clogged with the exudate produced by the stellate and type B glandular trichomes of *S. sisymbriifolium* (Figure 4c). Remarkably though, the fine hairs on the tarsi of both beetle species remained free on the exudate.

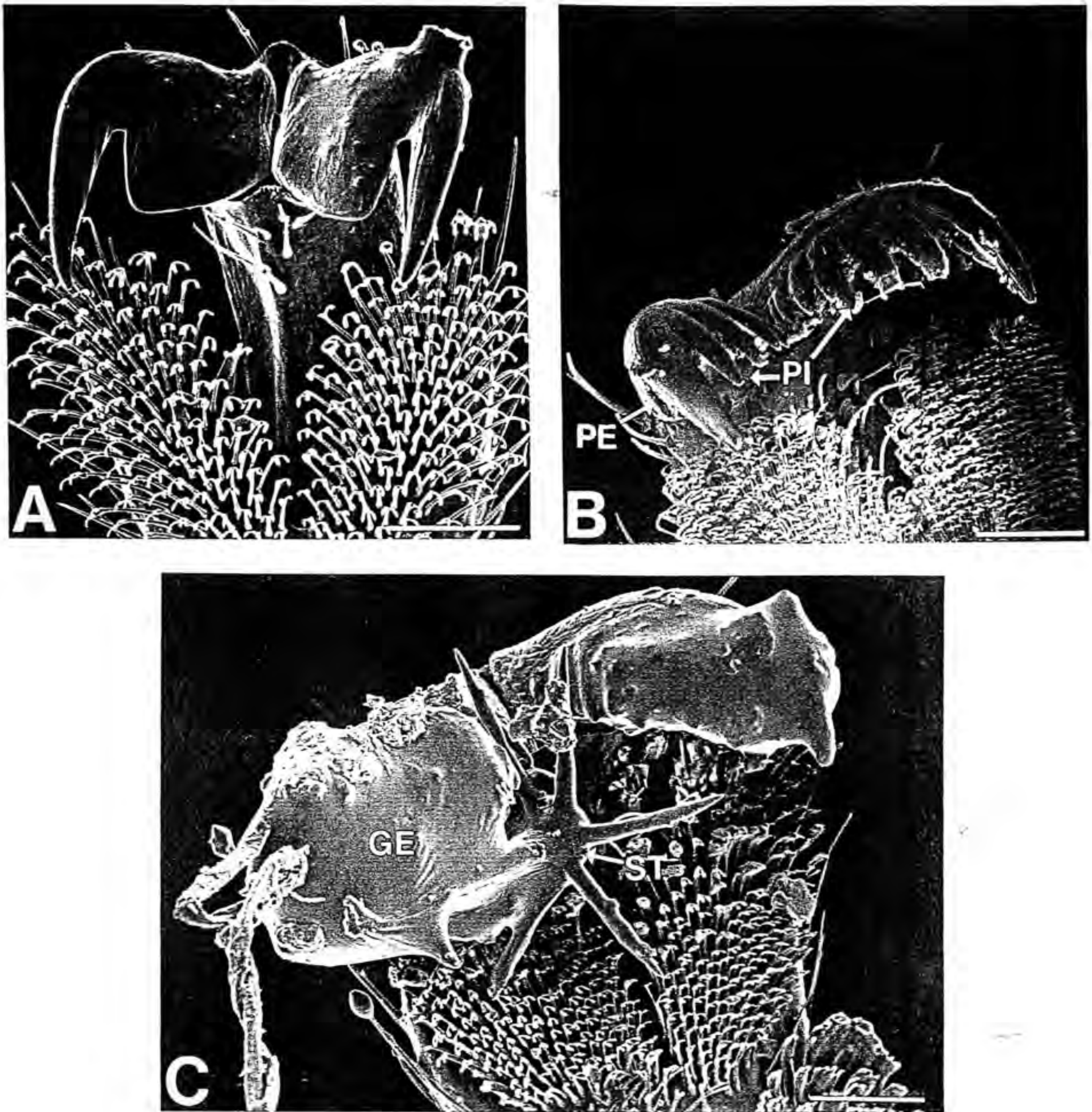


Figure 4.4 Scanning electron micrograph of the tarsal claws of **A:** *Gratiana spadicea* and **B:** *Conchyloctenia tigrina*. C: The tarsal claws of *C. tigrina* that had been walking on *S. sisymbriifolium*, and has become "gummed-up" with glandular exudate. **PE:** pectine of exterior claw surface, **PI:** pectine of interior claw surface, **GE:** glandular exudate, **ST:** stellate trichome. All scale bars = 100µm.

Behavioural differences

The initial reaction of *C. tigrina* on *S. sisymbriifolium* and *G. spadicea* on *S. linnaeanum* was to vacate the plants, presumably in search of a more suitable host. However, once forced to feed on a non-host plant, through hunger, the feeding behaviour of adults and larvae of both species on both plant species was essentially the same. The beetles removed an area of trichomes from the leaves before they commenced feeding, in much the same way as *Gratiana lutescens* on *S. elaeagnifolium* (Siebert 1975) and the noctuid on *S. coccineum* (Hulley 1988). The mandibles were used to bite off the trichomes as close to the surface of the leaf as possible. The excised trichomes were brushed off the mandibles by the maxillary palps and pushed to one side by the legs. Once an area had been cleared, the beetles began to feed, and ate through the leaf mesophyll to the layer of epidermis on the other side of the leaf, which was not eaten, thus avoiding the trichomes on that surface.

Stellate trichomes were found in the frass of both beetle species. This indicates that ingestion of the trichomes did occur despite attempts to avoid them, and that digestion of the trichomes did not occur. This is not surprising as Levin (1973) has stated that trichomes are composed almost entirely of lignin.

During feeding on *S. sisymbriifolium*, the beetles became covered with trichomes, in particular the head and thoracic regions. While *G. spadicea* adults and larvae seemed oblivious to this, continuing to feed, *C. tigrina* adults and larvae were continually grooming, using their forelegs to remove the trichomes stuck to the head region, and spent less time feeding in comparison to *G. spadicea*.

The movement of *C. tigrina* adults was clumsy on the leaves of *S. sisymbriifolium*, a result of their tarsal claw structure (Figures 4b, 4c). However, no hardening of glandular trichome exudate on the tarsi and mouthparts of the beetles, as described by Gentile and Stoner (1968a) and Gentile *et al.* (1968), was noted, suggesting that the exudate from the type B and stellate trichomes on *S. sisymbriifolium* did not contain the polyphenoloxidases and peroxidases that have been found in the type A glandular trichomes of other *Solanum* species (Gregory *et al.* 1986; Boiteau and Singh 1988). In addition, no symptoms of alkaloid poisoning, as described for several groups of insect in contact with the glandular trichomes of *Nicotiana* species (Thurston *et al.* 1966) and *Lycopersicon* species (Williams *et al.* 1980), were observed.

Effect of glandular trichome exudate

Washing the leaves with 75% ethanol and rinsing in distilled water appeared to have no effect on the insect's ability to recognise a host plant, and had no significant effect on the palatability of the plant, as the amounts of washed and unwashed *S. linnaeanum* leaf eaten by *C. tigrina* (both adults and larvae) and the amounts of washed and unwashed *S. sisymbriifolium* leaf eaten by *G. spadicea* (both adults and larvae) did not differ significantly (Tables 4.3 and 4.4).

Removal of the glandular trichome exudate from *S. sisymbriifolium* rendered it more susceptible to feeding damage by both *C. tigrina* adults and larvae. However, they both still ate significantly more of their usual host *S. linnaeanum*. *Gratiana spadicea* beetles, while unaffected by the washing of the leaves, also preferred their usual host (Tables 4.3 and 4.4).

Table 4.3 The feeding scores obtained by *Gratiana spadicea* and *Conchyloctenia tigrina* adults for washed and unwashed leaves of *Solanum sisymbriifolium* and *Solanum linnaeanum* eaten in 24 hours.

Insect on test plant	n[a]	Amount of leaf eaten (mean score (± SD))		Statistical comparison (p value)[c]
		Washed leaves[b]	Unwashed leaves[b]	
C. tigrina				
S. sisymbriifolium	12	2.63 (0.80)a	0.50 (0.52)a	0.010
S. linnaeanum[d]	12	5.17 (0.94)b	5.01 (1.03)b	0.997
G. spadicea				
S. sisymbriifolium	12	5.27 (0.72)b	5.48 (0.79)b	0.957
S. linnaeanum[d]	12	2.91 (0.67)a	2.58 (1.08)a	0.680

[a] Each replicate contained five adult beetles.
[b] Amounts in columns not followed by the same letter differ significantly at the 5% level (Kruskal-Wallis test, followed by Dunn's Multiple Range test).
[c] Mann-Whitney Rank Sum comparison of the results obtained for the two treatments.
[d] Feeding scores for *S. linnaeanum* were adjusted to allow for the difference in thickness of the leaves for the two species.

Table 4.4 Feeding scores obtained by *Gratiana spadicea* and *Conchyloctenia tigrina* larvae for washed and unwashed leaves of *Solanum sisymbriifolium* and *Solanum linnaeanum* eaten in 48 hours.

Insect on test plant	n[a]	Amount of leaf eaten (mean score (± SD))		Statistical comparison (p value)[c]
		Washed leaves[b]	Unwashed leaves[b]	
C. tigrina				
S. sisymbriifolium	12	1.53 (0.79)a	0.31 (0.29)a	0.023
S. linnaeanum[d]	12	3.92 (0.99)b	3.00 (1.21)b	0.728
G. spadicea				
S. sisymbriifolium	12	4.83 (1.07)b	4.59 (1.34)b	0.865
S. linnaeanum[d]	12	2.13 (0.88)a	1.79 (0.62)c	0.542

[a] Each replicate contained twenty first instar beetles.
[b] Amounts in columns not followed by the same letter differ significantly at the 5% level (Kruskal-Wallis test, followed by Dunn's Multiple Range test).
[c] Mann-Whitney Rank Sum comparison of the results obtained for the two treatments.
[d] Feeding scores for *S. linnaeanum* were adjusted to allow for the difference in thickness of the leaves for the two species.

Larval survival and development period

Larval survival of *C. tigrina* and *G. spadicea* was significantly higher on their natural hosts (*S. linnaeanum* and *S. sisymbriifolium* respectively) than on the non-hosts (Table 4.5). *C. tigrina* took significantly longer to develop to pupation on *S. sisymbriifolium* (the large standard error is a product of the small number of *C. tigrina* larvae pupating on *S. sisymbriifolium*). By comparison, although few *G. spadicea* larvae developed through to pupation on *S. linnaeanum*, the length of time to do so was not significantly greater than on *S. sisymbriifolium*, and *C. tigrina* on *S. linnaeanum*.

Table 4.5 Larval survival and development duration of *Gratiana spadicea* and *Conchyloctenia tigrina* larvae on *Solanum sisymbriifolium* and *Solanum linnaeanum*. Figures in parentheses indicate one standard deviation.

Insect on test plant	n[a]	Mean number of pupae/replicate[b]	Mean duration (days)[b]
C. tigrina			
S. sisymbriifolium	5	1.40 (0.55)a	52.73 (12.36)a
S. linnaeanum	5	15.33 (4.04)b	29.62 (7.43)b
G. spadicea			
S. sisymbriifolium	5	16.28 (3.45)b	31.15 (4.53)b
S. linnaeanum	5	2.21 (1.03)a	37.32 (6.59)b

[a] Each replicate contained twenty first instar larvae.
[b] Mean number of pupae and duration times in columns not followed by the same letter differ significantly at the 5% level (Kruskal-Wallis test, followed by Dunn's Multiple Range test).

4.4 DISCUSSION

The driving forces behind the evolution of leaf hairs has long been debated (Johnson 1975). The most popular school of thought is that leaf hairs increase the boundary layer around the leaves, reducing the amount of transpiration, and as such are an adaptation to an arid environment (Wooley 1964). However, the role of trichomes in herbivore defence is also well accepted (Levin 1973; Tingey 1985). The question of whether trichomes initially evolved as an adaptation to arid environments, or as a product of co-evolution with insect herbivores is not likely to be easily resolved, and has not been resolved by this study, although the trichomes on the leaves of *S. sisymbriifolium* have been shown to play a role in anti-herbivore defence. Possibly the most likely scenario is that they evolved as an adaptation to arid environments, and later acquired a parallel function of defence against herbivores. Glandular trichomes could support this idea, in that they appear to be of no benefit in reducing the amount of transpiration from the leaves.

The depauperate fauna associated with *S. sisymbriifolium* in South Africa was tentatively ascribed to the uniqueness of glandular trichomes among the native *Solanum* flora (Chapter 3). This study has shown that glandular trichomes are not uncommon among native solanums, but that they occur in very much greater densities on the leaves of *S. sisymbriifolium*, and the two other South American solanums investigated (*S. aculeatissimum* and *S. acanthoideum*). It is interesting that both *S. aculeatissimum* and *S. acanthoideum* also support very few insect herbivores in South Africa (Hulley and Hill, unpublished data).

The stellate type of trichomes within the Solanaceae are regarded as being non-glandular, offering a purely physical barrier to herbivores (Roe 1967; Duffey 1986; Gregory *et al.* 1986) and this appears to be the first report of their glandular nature. Several authors (Tingey and Gibson 1978; Tingey and Laubengayer 1981; Gregory *et al.* 1986) have shown that the type A and type B glandular trichomes complement each other in their defence against small insects. A similar situation occurs in *S. sisymbriifolium* where small insects (mites, aphids, small flies and wasps) were observed stuck to the viscous exudate produced by the type B glandular trichomes, and in their struggle to free themselves, broke off the stellate trichomes to be further covered with exudate.

The comparison of the two tortoise beetle species was designed to highlight any morphological or behavioural adaptations on the part of *G. spadicea* specifically for overcoming the defence posed by the glandular trichomes of *S. sisymbriifolium.* Tarsal morphology did differ between the two species. Riley (1986) believed that the cup-shaped tarsal claw, such as that in *C. tigrina*, is a derived character that has evolved twice independently in the Cassidinae, once in the Old World Aspidomorphini (to which *C. tigrina*

belongs), where it is regarded as a synapomorphic character, and again in certain of the genera of the *Charidotis* Group of the New World Cassidini, where it is regarded as an apomorphic character. The remainder of the Cassidini have simple, symmetrical, or asymmetrical claws, such as those of *G. spadicea*, and its fellow South American tortoise beetle, *Metriona elatior*. While it might be suggested that the tarsal claw structure of *G. spadicea* has evolved as a product of a long association with a plant bearing glandular trichomes, this study presented no evidence to support this. The tarsal claw structure of *G. spadicea* and *M. elatior* is shared by most species in the Tribe Cassidini. Apart from the unlikely event that the whole group are adapted to coping with glandular trichomes, it seems that their claw structure is simply a characteristic of this lineage which has made exploitation of glandular trichome-bearing plants possible.

The initial feeding behaviour of the two tortoise beetles on both *S. sisymbriifolium* and *S. linnaeanum* was similar, and similar to that observed for the noctuid *Pardasena* sp. nr *diversipennis* on *S. coccineum* (Hulley 1988) and *G. lutescens* on *S. elaeagnifolium* (Siebert 1975) in that the beetles cleared an area on trichomes prior to feeding. However, the feeding behaviour of the two tortoise beetles on *S. sisymbriifolium* did differ, in that despite both species becoming covered by the sticky exudate and trichomes, *G. spadicea* continued feeding, seemingly oblivious to the trichomes, whereas, *C. tigrina* indulged in extended bouts of grooming. The deleterious elements of the glandular trichomes on *C. tigrina* probably include behavioural stress associated with impaired mobility, metabolic costs incurred by energy expenditure during escape struggles, and possible starvation leading to reduced larval survival and greatly increased development periods caused by the grooming response and therefore lack of feeding.

Trichomes are common on the leaves of South African solanums, and in some cases reach extremely high densities. This is the case for *S. panduriforme*, which supports a rich native herbivore fauna, and on which *C. tigrina* is common, occurring in 40% of the faunal samples taken on this plant (Hulley, unpublished data). *Conchyloctenia tigrina* therefore certainly has the necessary adaptations to overcome the threat posed by trichomes of a non-glandular type, but the fact that it has not had a long association with a potential host with glandular trichomes has precluded the evolution of the necessary adaptations for overcoming the threat posed by this defence.

That the exudate produced by the glandular trichomes afforded *S. sisymbriifolium* a level of resistance to *C. tigrina* is evidenced by the fact that these beetles ate more foliage once the exudate had been removed. However, both adult and larval *C. tigrina* still consumed less washed *S. sisymbriifolium* than *S. linnaeanum*, suggesting that exudate produced by the glandular trichomes is only a part of the anti-herbivore defence system of the weed. Further evidence to suggest that trichomes form only part of the complex nature of *Solanum* anti-herbivore defence was that *G. spadicea* ate significantly more *S. sisymbriifolium* than *S. linnaeanum* (washed or unwashed), although the development times were similar on both plant species. In conclusion, glandular trichomes can be viewed as one of the factors limiting the recruitment of native herbivores onto *S. sisymbriifolium*.

On completion of this study, several avenues remain to be investigated. No attempt was made to characterise the chemistry of the trichome exudate; whether the exudate produced by the stellate glandular trichomes is analogous to that described for the type A glandular trichomes on other *Solanum* species (Tingey and Laubengayer 1981; Boiteau and Singh

1988) therefore remains unresolved. Many plants' glandular trichomes contain secondary compounds that are potentially toxic or deterrent to phytophagous insects (Duffey and Isman 1981), such as the (E)-ß-Farnesene released by the glandular trichomes of *S. berthaultii* (Gibson and Pickett 1983) that prevent *Solanum* oligophages from coming into contact with the leaf surface. This warrants further study.

There is no report of any attempt to confer the defences of *S. sisymbriifolium* onto either potato or tomato through hybridization to enhance the resistance of these crops to pests. Given the apparent novelty of the glandular stellate trichomes on this weed, the potential for hybridising or genetically engineering *S. sisymbriifolium* with either of these crops might be worth investigating.

CHAPTER 5

Biology and host range of *Gratiana spadicea* (Klug, 1829), a potential biological control agent for the weed *Solanum sisymbriifolium* Lamarck.

5.1 INTRODUCTION

A leaf-feeding tortoise beetle, *Gratiana spadicea* (Chrysomelidae: Cassidinae), from Argentina, southern Paraguay, and Brazil was imported in 1989 for screening as a potential biological control agent for *S. sisymbriifolium.*

The Cassidinae are highly specialised in their feeding habits, and species in the subfamily are generally restricted to a limited group of host plants and in some instances to a single species (Maw 1976; Hsiao 1986). All eight species so far assigned to the genus *Gratiana* are restricted to the genus *Solanum* (Hsiao 1986; Becker and Frieiro-Costa 1988). This would suggest that *G. spadicea* has great potential as a biological control agent, especially as it is regarded as strictly monophagous in South America (Kvasina and de León 1985; Albuquerque and Becker 1986; Becker and Romanowski 1986; Becker and Frieiro-Costa 1988).

Siebert (1975) screened two species of *Gratiana*, *G. lutescens* and *G. pallidula*, as biological control agents for the exotic weed *S. elaeagnifolium* in South Africa. Both were rejected because several generations of each were reared through on eggplant (*S. melongena*) and a native *Solanum* (*S. linnaeanum*, referred to as *S. sodomeum* by Siebert (1975)). *Nuzonia pallidula* (Boheman) (the eggplant tortoise beetle) is a recorded pest of eggplant in the

southern United States of America (Rolston *et al.* 1965). Spaeth (1914) regards *Cassida pallidula*, *Nuzonia pallidula*, and *Gratiana pallidula* as conspecifics. This would explain the host range of *G. pallidula* as recorded by Siebert (1975).

The aim of this chapter was to describe the biology of *Gratiana spadicea*, and determine its host range under laboratory conditions.

5.2 MATERIALS AND METHODS

5.2.1 Biology of *Gratiana spadicea.*

All studies were conducted in a quarantine laboratory at 25 ± 2°C and 70 ± 5% relative humidity with a 16 hour photoperiod. Biological observations and host-range experiments were conducted on potted plants. All means are quoted with standard deviations.

5.2.2 Host range of *Gratiana spadicea.*

The host range of *G. spadicea* was determined using larval survival tests and adult choice tests.

Larval survival tests

Twenty newly hatched larvae that had not yet fed were placed on each of 29 test plant species, selected on the basis of economic importance and taxonomic relatedness to *S. sisymbriifolium* (Table 5.1). Larval survival, development time and pupal mass were compared between the plant species tested using a Kruskal-Wallis One Way Analysis of Variance on Ranks, followed by a Dunn's Multiple Range test (Zar 1974). There were ten replicates for each plant species tested.

Adult choice tests

The adult choice tests were designed to determine whether adult female *G. spadicea* were able to oviposit on non-host plants. These experiments were conducted in large glass-topped cages (60cm wide, 80cm long, and 85cm high). In each experiment, three *S. sisymbriifolium* plants and three of one of the non-host test plants were alternated in a circle within the cage so that the foliage overlapped. The plants were of similar size. Ten adult male and ten adult female *G. spadicea* that had recently eclosed from pupation and had not yet fed as adults (some insects have been shown to be influenced by prior experience (Traynier 1979)) were released in the centre of the circle of plants. After 7 days, the location of the adults and the position of any egg cases were recorded. There were three replicates for each test plant species. Comparisons between host and non-host plant species were made using a Mann-Whitney test (Zar 1974).

Table 5.1 Results of no-choice feeding trials with *Gratiana spadicea* larvae on selected test plants.

Test plant	Result[a]
A. Solanaceae	
Datura ferox[b] L.	0
Datura stramonium[b] L.	0
Nicandra physaloides[b] (L.)	0
Physalis peruviana[b] L.	0
B. Native **Solanum species**	
Solanum cf. *acanthoideum*	+
Solanum burchellii Dun.	0
Solanum coccineum[b] Jacq.	+
Solanum duplo-sinuatum Klotzsch	0
Solanum giftbergense Dun.	+
Solanum giganteum[b] Jacq.	0
Solanum incanum[b] L.	+
Solanum linnaeanum[b] Hepper & Jaeger	++
Solanum panduriforme[b] E. Mey.	+
Solanum rigescens[b] Jacq.	+
Solanum tomentosum L.	+
C. Exotic *Solanum* species	
Solanum acanthoideum E. Mey.	++
Solanum aculeatissimum Jacq.	+
Solanum elaeagnifolium Cav.	+
Solanum hispidum Pers.	+
Solanum mauritianum Scop.	0
B. Selected Vegetable Crops	
Brassicaceae	
Brassica oleracea var. *capitata* L. (cabbage)	0
Brassica oleracea var. *italica* L. (broccoli)	0
Brassica oleracea var. *botrytis* L. (cauliflower)	0
Chenopodiaceae	
Beta vulgaris var. *cicla* L. (spinach)	0
Asteraceae	
Lactuca sativa L. (lettuce)	0
Solanaceae	
Capsicum frutescens L. (chilli)	0
Capsicum annuum *L. (red and green pepper)*	0
Lycopersicon esculentum Mill. (tomato)	0
Solanum melongena L. (eggplant)	++
Solanum tuberosum L. (potato)	0

[a] 0 = No feeding or development.
+ = Less than 10% of larvae survived to pupation.
++ = Between 10.1% and 25% of larvae survived to pupation.
[b] Native weed of minor status (Wells *et al.* 1986)

5.3 RESULTS

In the tables that follow, means are quoted with standard deviations as a measure of central tendency as they provide an indication of the variability within the samples. However, as the data were not normally distributed, non-parametric statistical tests were employed which relied on the median values of the samples (Zar 1974).

5.3.1 Biology of *Gratiana spadicea.*

Egg

Female *G. spadicea* deposited egg cases on the foliage of *S. sisymbriifolium*. The egg cases consisted of vertically stacked translucent membranes, joined at the edges on one side. Between each two membranes there was a single egg, approximately in the middle. No eggs were found between the top two membranes, a position possibly most vulnerable to desiccation, and attack by parasitoids and predators (Muir and Sharp 1904). Of the 1032 egg cases on *S. sisymbriifolium*, 45.6% were deposited on the upper surface of the leaf, 38.4% on the under surface of the leaf, 10.0% in the growth tips of the plants, 4.2% on the stems and petioles, 1.1% on the flowers, and 0.7% on the fruit. Each egg case contained between 1 and 17 eggs with a mean of 5.93 ± 2.53 (n = 591) eggs per egg case.

In a sample of 591 egg cases from the laboratory culture, the mean length was 3.33 ± 0.49mm and the mean width 2.64 ± 0.51mm. The eggs were cylindrical, 1.27 ± 0.09mm in length and 0.66 ± 0.05mm (n = 80) in width. The mean incubation period was 6.83 ± 1.33 days (n = 544) (Figure 5.1). Of the 3510 eggs from the 591 egg cases, 2516 or 71.7% hatched. Of those that did not hatch, 17.2% developed fully but failed to hatch and 11.1% failed to develop at all.

Larval Instars

There were five larval instars in *G. spadicea.* The newly hatched larvae were white, but as soon as they began to feed they became green. The larvae were dorsoventrally flattened and each abdominal segment had lateral spines. As is usual in larval Cassidinae, two long caudal spines arose posteriorly on the eighth abdominal segment onto which the larval exuviae and excreta, and epidermal hairs from the leaves on which they fed were stacked using a protractile appendage into which the gut projected (Mata and Aravena 1926). The head of the larva was covered by the anterior edge of the prothorax. Apart from the size and the number of larval exuviae on the caudal spines, there appeared to be no external morphological differences between the five larval instars. The larvae ranged in length (to the end of the last abdominal segment, ignoring the caudal spines) from 1-1.5mm in the first instar to 5-6.5mm in the mature fifth instar (Table 5.2a) and in head capsule width from 0.3-0.4mm in the first instar to 0.8-1.2mm in the fifth instar (Figure 5.2b). Because there was so much overlap between instars with regard to body length and head capsule width, counting the number of larval exuviae on the caudal appendages proved to be the best method of distinguishing between the instars. In the late fifth instar larvae there was a non-feeding, sessile prepupal phase, which attached midventrally by means of a secretion to the surface of the plant. The duration of the larval instars varied between 2 and 4 days (Figure 5.1), and the time taken from hatching to pupation varied between 26 and 33 days.

The larvae fed on the surface or the edge of the leaves. Early instars seemed to feed more on the surface of the leaves, leaving small holes in the leaves close to the egg case. Later larval instars were voracious feeders and at high densities could skeletonize leaves. Abscission of badly damaged leaves was observed on potted plants.

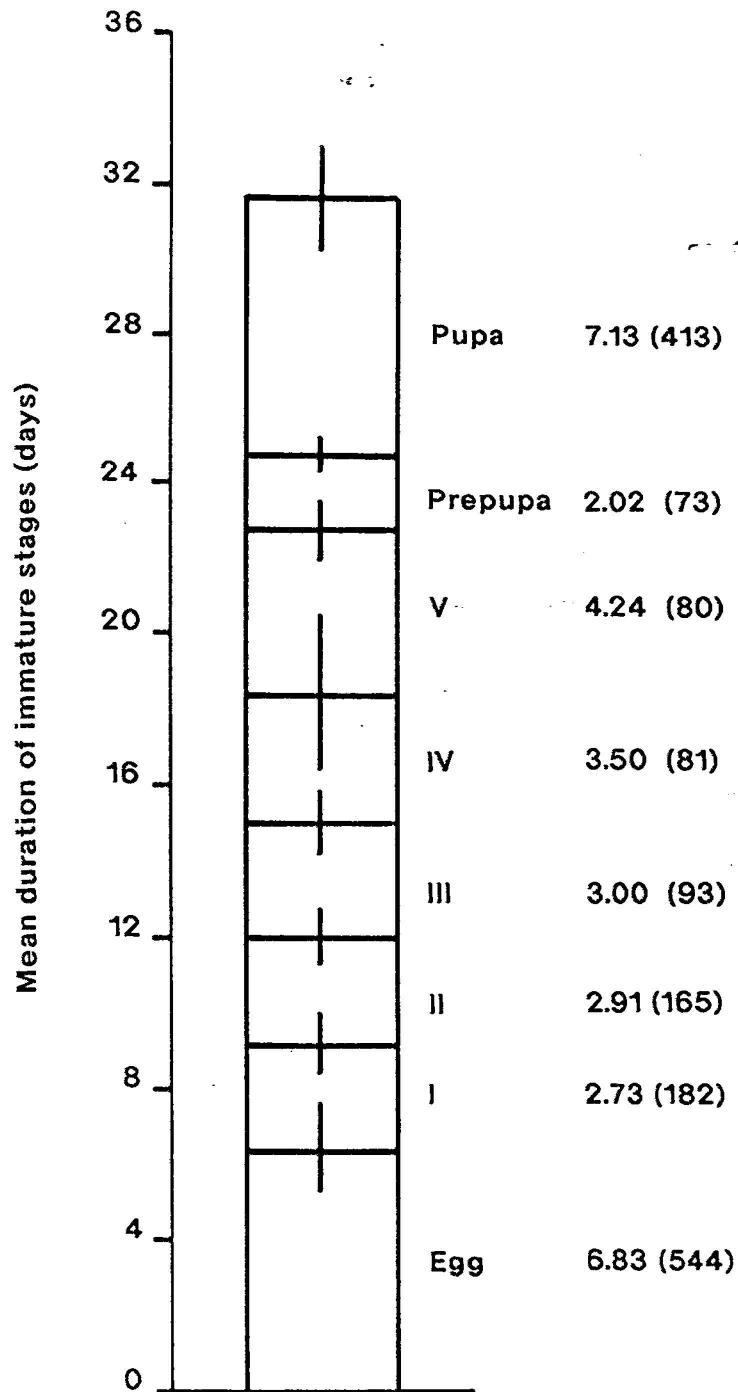
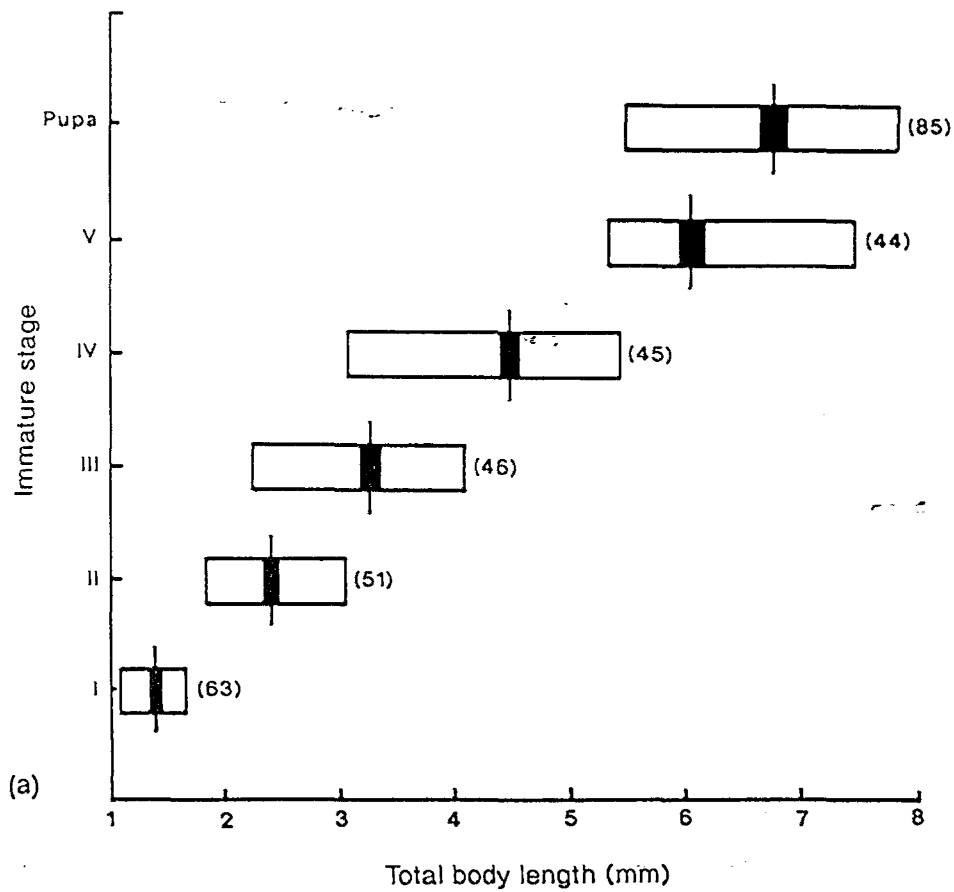


Figure 5.1. The duration of the five larval and the prepupal and pupal stages of *Gratiana spadicea* on *Solanum sisymbriifolium* in the laboratory. The vertical lines represent the standard deviation; the numbers in parentheses are the sample sizes. Biological studies were carried out at 25°C ± 2°C.

Pupa

The large pronotum, lateral expansion of some of the abdominal segments, and depressed shape were conspicuous pupal features. The pupae attached to the plant by means of a pad on the last abdominal segment. In a sample of 451 pupae, 30.6% were on the upper surface of the leaves, 9.3% on the under surface, 52.1% on the stems and petioles, 4.4% on the flowers, and 3.6% on the fruit. Pupal length varied from 4.5-7.2mm with a mean of 6.06 ± 0.39mm (n = 413) (Figure 5.2) and the head capsule width varied from 2.2-4.4mm with a mean of 3.40 ± 0.25mm (n = 413). The mean pupal weight for *G. spadicea* reared on *S. sisymbriifolium* was 25.22 ± 5.01mg (n = 356). The mean duration of pupation was 7.13 ± 0.59 days (n = 413) (Figure 5.1). The total development time of the immature stages (egg stage to adult eclosion from pupation) ranged between 34 and 42 days (mean development time was 36.53 ± 7.34 days, n = 100 larvae).



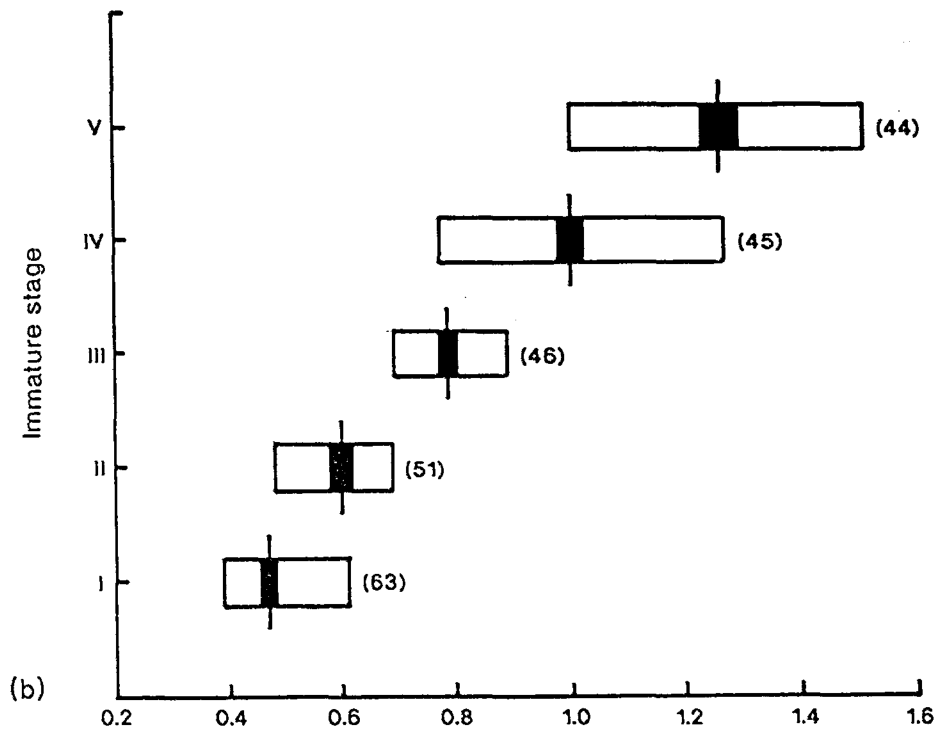


Figure 5.2 Total length (a) and head capsule width (b) of the immature stages of *Gratiana spadicea* reared on *Solanum sisymbriifolium* in the laboratory. The horizontal bars, vertical lines and shaded areas represent the ranges, means and standard deviations of the means respectively, the numbers in parentheses are the sample sizes.

Adult

On emergence, the teneral adults were a pale greenish yellow and the elytra were soft. The adults remained fairly still on the plant, close to the pupal exuviae until the elytra hardened and the beetles took on a green colour. The adult was diurnal and remained quiescent on the host at night.

It was easy to distinguish between the males and females. As pointed out by Becker and Romanowski (1986), males of *G. spadicea* have orange testes, visible through the ventral integument of the abdomen. In the female, a central, whitish area, comprising the pedicels and lateral oviducts, was present in this region. Adult female beetles were 6.41 ± 0.36 mm in length (n = 93), significantly longer than the male beetles which were 5.94 ± 0.45mm in length (n = 116, t = 9.04, p < 0.0001, t-test).

After emergence there was a pre-oviposition period of 7.73 ± 1.30 days (n = 20) in the females during which copulation occurred. The oviposition period lasted 66.97 ± 19.59 days (n = 20) during which the females laid a mean number of 1.01 ± 0.34 egg cases per day (n = 10). This was followed by a post-oviposition period of 3.77 ± 1.88 days (n = 20) before the females died. The number of egg cases laid per female varied between 44 and 118 with a mean of 80.80 ± 22.45 egg cases per female (n = 20). The fertility (lifetime production of larvae) of twenty females ranged between 120 and 519 larvae per female (mean fertility was 281.40 ± 126.31 larvae per female).

The mean adult longevity for female beetles was 79.62 ± 22.50 days (n = 20) which was significantly greater (t = 3.03, p = 0.007) than the mean longevity for males which was

56.01 ± 10.44 days (n = 20). Mean generation time for females was 116.15 ± 27.47 days (n = 20) and for males was 93.04 ± 15.32 days (n = 20).

Albuquerque and Becker (1986) have recorded five generations of *G. spadicea* per year in South America, and suggest that they diapause as sexually immature adults on surrounding vegetation. This is the common behaviour for the Cassidinae, and has been recorded in *G. pallidula* and *G. lutescens* (Siebert 1975) and for several other cassids by Paterson (1941).

5.3.2 Host Range of *Gratiana spadicea.*

Larval Survival Tests

At least a proportion of *G. spadicea* larvae completed their development on eight native *Solanum* species, four exotic *Solanum* species (*S. acanthoideum*, *S. aculeatissimum*, *S. elaeagnifolium*, and *S. hispidum*), and on eggplant (*S. melongena*) (Tables 5.1 and 5.2). However, on all but three of the non-target test plants, survival to pupation was restricted to less than 10% (Table 5.2). On the other plants tested (Table 5.1), very little feeding damage was noticed, and the larvae died within the first week without moulting.

Pupal mass and larval development duration did not differ significantly between the beetles reared on *S. sisymbriifolium* and those on most of the non-host plants (Table 5.2). However, no single measurement could give a reliable indication of the overall suitability of a host. Therefore, the host suitability index (Maw 1976) was provided by:

(pupal weight x percentage pupating)/development time

The index (Table 5.3) shows that *S. sisymbriifolium* is a far more suitable host for *G. spadicea* than any of the other plants tested.

Adult Choice Tests

Female *G. spadicea* showed a significant oviposition preference for their natural host (*S. sisymbriifolium*) (Table 5.4) and only a few egg cases were found on non-host plants. In addition, adults of both sexes were found significantly more often on *S. sisymbriifolium* (Table 5.4). Throughout the three experiments little or no feeding damage was noted on the non-host species.

Table 5.2 Development of *Gratiana spadicea*[a] on species of *Solanum*.

Host plant	Mean no. pupae/ replicate[b]	Percentage pupation	Mean pupal mass (mg)[b]	Mean duration (days)[bc]
S. sisymbriifolium	14.55a (0.81)	72.75	25.22a (1.12)	30.15a (5.70)
S. acanthoideum	7.06b (1.42)	35.30	22.27a (4.32)	34.46a (4.91)
S. melongena	3.92c (0.76)	19.60	22.26a (4.18)	35.12a (5.17)
S. linnaeanum	2.64c (0.98)	13.21	23.60a (4.20)	36.44a (6.86)
S. coccineum	1.63cd (0.70)	8.13	22.30a (4.22)	34.62a (5.67)
S. aculeatissimum	1.57cd (0.93)	7.85	19.00a (2.42)	42.73b (4.78)
S. rigescens	0.90d (0.43)	4.50	22.60a (4.17)	47.78ab (10.32)
S. elaeagnifolium	0.50d (0.45)	2.50	16.46ab (4.03)	55.00c (3.08)
S. hispidum	0.40d (0.22)	2.00	20.52a (2.24)	39.75a (4.57)
S. panduriforme	0.40d (0.38)	2.00	20.55a (5.05)	46.25ab (2.06)
S. tomentosum	0.30d (0.30)	1.50	21.60a (2.88)	35.12a (5.17)
S. incanum	0.30d (0.28)	1.50	15.70ab (2.30)	47.67b (0.58)
S. giftbergense	0.20d (0.13)	1.00	14.60b (0.52)	41.00a (1.41)
S. cf. *acanthoideum*	0.10d (0.10)	0.50	25.30a (-)	50.00c (-)

[a] Twenty larvae were used in each of ten replicates per test plant. The figures in parentheses represent the standard deviation.
[b] Means in columns not followed by the same letter differ significantly at the 5% level (Kruskal-Wallis test followed by Dunn's Multiple Range est).
[c] Development time in days from larval emergence to adult eclosion from pupation.

Table 5.3 Host suitability index of several species of *Solanum* for the biological control agent *Gratiana spadicea*.

Plant species	Host suitability	Percentage rating
S. sisymbriifolium	60.86	100.00
S. acanthoideum	22.81	37.48
S. melongena	12.42	20.41
S. linnaeanum	8.56	14.07
S. coccineum	5.24	8.61
S. aculeatissimum	3.49	5.74
S. rigescens	2.13	3.50
S. hispidum	1.03	1.69
S. tomentosum	0.92	1.51
S. panduriforme	0.89	1.46
S. elaeagnifolium	0.76	1.25
S. incanum	0.51	0.84
S. giftbergense	0.36	0.59
S. cf. *acanthoideum*	0.25	0.41

Table 5.4 Choice tests with adult *Gratiana spadicea* on *Solanum sisymbriifolium* and non-host plants[a].

Test pair	Mean number of egg cases (±SD)[b]	Mean number of adults (±SD)[b]
S. sisymbriifolium	37.50 (3.54)a	9.00 (2.00)a
S. sisymbriifolium	33.00 (10.82)a	10.00 (1.00)a
S. sisymbriifolium	35.67 (13.87)a	14.67 (3.22)a
S. acanthoideum	5.67 (1.53)b	5.33 (3.20)b
S. sisymbriifolium	52.00 (7.55)a	16.33 (1.16)a
S. melongena	2.33 (1.16)b	2.00 (1.00)b
S. sisymbriifolium	40.33 (7.51)a	14.33 (1.53)a
S. linnaeanum	0.33 (0.58)b	1.67 (1.53)b
S. sisymbriifolium	36.67 (10.60)a	14.67 (1.53)a
S. coccineum	0.67 (0.58)b	1.33 (1.16)b
S. sisymbriifolium	50.67 (4.04)a	15.33 (1.16)a
S. aculeatissimum	1.67 (0.58)b	1.33 (0.58)b
S. sisymbriifolium	38.67 (10.02)a	15.33 (1.53)a
S. rigescens	0.00 (-)b	1.00 (1.00)b
S. sisymbriifolium	42.50 (28.15)a	12.50 (1.92)a
S. hispidum	0.25 (0.50)b	1.50 (1.00)b
S. sisymbriifolium	51.67 (11.59)a	17.33 (2.08)a
S. tomentosum	0.00 (-)b	0.00 (-)b
S. sisymbriifolium	48.67 (18.93)a	14.33 (5.77)a
S. panduriforme	0.33 (0.58)b	2.00 (1.00)b
S. sisymbriifolium	51.57 (11.59)a	17.67 (1.53)a
S. elaeagnifolium	0.00 (-)b	0.67 (0.58)b
S. sisymbriifolium	36.67 (23.86)a	13.00 (6.93)a
S. incanum	1.00 (1.00)b	2.00 (2.65)b
S. sisymbriifolium	60.67 (16.29)a	17.67 (1.53)a
S. giftbergense	0.00 (-)b	0.00 (-)b
S. sisymbriifolium	36.67 (16.17)a	16.33 (2.52)a
S. cf. *acanthoideum*	0.67 (1.16)b	1.00 (1.73)b

[a] Mean number of *G. spadicea* egg cases per 10 females per week, and mean number of adult *G. spadicea* (of 20 used in each of three replicates).
[b] The means of test pairs not followed by the same letter differ at the 5% level (Mann-Whitney test).

5.4 DISCUSSION

It is widely accepted that the rearing of insects under restricted cage conditions can produce laboratory artifacts such as accelerated developmental rates and unusually high fertility, and that the results of starvation and even choice tests are often ambiguous as many insects feed readily on non-host plants (Harris and Zwölfer 1968; Zwölfer and Harris 1971; Wapshere 1974, 1989; Dunn 1978; Cullen 1990; Shepherd 1990). The biology of *G. spadicea* under laboratory conditions described above nevertheless compares favourably with that described for *G. spadicea* under natural conditions in its region of origin in warm temperate South America (Mata and Aravena 1926; Kvasina and de León 1985; Albuquerque and Becker 1986; Becker and Romanowski 1986; Becker and Frieiro-Costa 1988). This suggests that the laboratory conditions used for this work caused few artifacts, and were satisfactory for biological studies on this insect.

The biological characteristics of *G. spadicea* indicate that it has great potential as a biological control agent in that it has a high rate of increase, long-lived adults, several generations per year, and a high per capita feeding rate. *Gratiana spadicea* has been recorded in very high numbers on *S. sisymbriifolium* in Uruguay (Kvasina and de León 1985), Argentina (Mata and Aravena 1926), and Brazil (Becker and Frieiro-Costa 1988). According to Kvasina and de León (1985) *G. spadicea* has a marked effect on the weed in Uruguay, to the extent of reducing its reproductive capabilities. However *S. sisymbriifolium* is still regarded as a major weed in South America, which suggests that *G. spadicea* is not providing sufficient control. *Gratiana spadicea* is heavily parasitised in South America where up to 89.4% of the egg cases are attacked by a eulophid parasitoid, *Emersonella ooecia* De Santis (Becker and Romanowski 1986; Becker and Frieiro-Costa 1988). The

pupae are also susceptible to a chalcidid parasitoid in the genus *Brachymeria* (Kvasina and de León 1985). Release from these natural enemies might result in *G. spadicea* having a much greater effect on its host plant in South Africa. However, Siebert (1975) believed that the parasitoid complexes of local cassids on solanaceous plants might conceivably hamper the effectiveness of *G. lutescens*; the same might be true of *G. spadicea* (discussed in Chapter 7).

A successful biological control programme relies on the phenologies of the biocontrol agent and its host (the weed) coinciding (Ehler and Andres 1983). However, as the biology of the agent is invariably elucidated under the constraints of a quarantine laboratory, or determined in the country of origin, one can only predict how it might behave once released. *S. sisymbriifolium*, by virtue of its dense, perennial infestations, offers *G. spadicea* an apparent host in South Africa (Chapter 2), in contrast to the situation in South America, where the weed is an annual of unpredictable environments (Becker and Frieiro-Costa 1988). However, the agent is successful at tracking new host plant patches in South America (Becker and Frieiro-Costa 1988), and it is quite conceivable that *G. spadicea* would have no trouble cuing into the large infestations of *S. sisymbriifolium* in South Africa. In addition, while in South America the weed is not available in winter, forcing *G. spadicea* into diapause (Kvasina and de León 1985; Becker and Frieiro-Costa 1988), it maintains its vigour throughout winter in South Africa (Chapter 2), presenting the agent the opportunity of maintaining its population through winter.

Gratiana spadicea in South America is strictly monophagous (Kvasina and de León 1985; Albuquerque and Becker 1986; Becker and Romanowski 1986; Becker and Frieiro-Costa

1988). This was found not to be the case, at least under laboratory conditions, in South Africa, where some larvae developed on 13 other *Solanum* species including the economically important *S. melongena* (eggplant). The selection of plants fed upon in the laboratory may be considered the potential host range of the insect, whereas the realized host range in the field is subject to a number of additional factors such as choice constraints and availability of food, adult and larval biology, parasitoids, and predators. It does not necessarily follow from these laboratory results that non-target plants (native *Solanum* species and eggplant) are in danger of being attacked in the field. In support of this, the host suitability index (Maw 1976) clearly showed that *S. sisymbriifolium* was by far the most suitable host for *G. spadicea*.

Adult-choice tests under laboratory conditions tend to be very conservative in that the arrangement of test plants in cages can induce females to oviposit on non-hosts (Marquis and Braker 1987). However, even where the foliage of host and non-host plant intertwined, *G. spadicea* females found the non-host plants very poor substitutes as oviposition sites. It may be argued that even though a small proportion of larvae might be able to develop on certain non-host plants, these plants are not in serious danger of attack because the adult females are unlikely to oviposit on them.

The potential effectiveness of *G. spadicea* as a biological control agent was assessed using a scoring system proposed by Harris (1973) (Table 5.5). This scoring system was devised to eliminate the least promising agents before host specificity testing was started. Although the scoring system was used here after all the host specificity testing had been completed and in spite of Goeden's (1983) critique and revision of the scoring system, it gives an

indication of the potential of *G. spadicea* as a biological control agent for *S. sisymbriifolium*. *Gratiana spadicea* obtained a total score of 22 compared to about 30 for an effective species such as *Chrysolina quadrigemina* (Suffr.) on *Hypericum perforatum* L. and 9 for an ineffective one such as *Altica carduorum* Guer. against *Cirsium arvense* (L.) (Harris 1973). The breakdown of the analysis for *G. spadicea* indicates that its apparent inability to control *S. sisymbriifolium* in South America (Albuquerque and Becker 1986) and minimal direct damage to the plant, contribute the lowish score. However, empirical information on some of these points was lacking (such as the impact of heavy feeding damage by the beetles on the weed's reproductive potential), and a higher score is possible with further information.

Table 5.5 Effectiveness of *Gratiana spadicea* as a weed biological control agent for *Solanum sisymbriifolium*[a].

Criteria	Actual score	Possible max score
1. Host Specificity	1	3
2. Direct Damage Inflicted	2	5
3. Indirect Damage Inflicted	?	3
4. Phenology of Attack	4	4
5. Number of Generations	2	4
6. Number of Progeny/Generation	1	2
7. Extrinsic Mortality Factors	?	4
8. Feeding Behaviour	2	2
9. Compatibility	2	2
10.Distribution	6	6
11.Effectiveness	0	6
12.Size	2	4
Total	22	45

[a] Based on Harris' scoring system (Harris, 1973).

Solanum melongena is widely cultivated in the Americas, yet *G. spadicea* has not been recorded as a pest on this crop in Argentina (Hayward 1958), Brazil (Costa Lima 1968), or the southern United States of America (Rolston *et al.* 1965). In addition, *G. spadicea* has not been recorded in neglected, and therefore unsprayed *S. melongena* cultivations in the Americas, despite extensive surveys (S. Neser, personal communication). Nevertheless, the fact that at least a proportion (albeit small) of *G. spadicea* larvae were able to develop on an economically important *Solanum* (eggplant) must be considered. In South Africa, none of the many phytophagous insects of native *Solanum* species (Olckers and Hulley 1989a) are recorded pests of *Solanum* crops, except for the coccinellid *Epilachna hirta* (Annecke and Moran 1982). However, the majority of native *Solanum* insects will attack *S. melongena* (Olckers and Hulley 1989a). In South Africa insects in eggplant cultivations are effectively controlled by insecticides (Olckers and Zimmermann 1991). In the unlikely event of *G. spadicea* extending its host range to include eggplant, it will almost certainly also be controlled by the existing insecticide regime and should therefore be no more of a problem than indigenous insects. This suggests that eggplant is not in serious danger of attack by *G. spadicea* in South Africa.

Of the other non-hosts that were able to support development of *G. spadicea*, four, *S. acanthoideum*, *S. aculeatissimum* (T. Olckers, personal communication), *S. elaeagnifolium*, and *S. hispidum* are exotic in South Africa (Gibbs Russell *et al.* 1987) and are not a cause for concern. The remaining eight native solanums are recorded as problem plants in South Africa (Wells *et al.* 1986). Native solanums generally have sparse and patchy distributions and thus seem less vulnerable to oligophagous insects, which tend to attack the most abundant hosts available (Harris 1990). Furthermore, as *S. sisymbriifolium* belongs to the

section *Cryptocarpum* of *Solanum* (D'Arcy 1972), which is not naturally represented in South Africa, the taxonomic distinctness of the native *Solanum* species from *S. sisymbriifolium* might further preclude *G. spadicea* from attacking them in the field as they might not present the necessary host finding stimuli for the agent.

5.5 CONCLUSIONS

The general biology of *G. spadicea* suggests that in the absence of other herbivores (Chapter 3) it will exert pressure on *S. sisymbriifolium* by reducing the above-ground biomass and fruit production of the weed, and as such is a promising biological control agent. The conflict of interest involving eggplant has been previously addressed during the successful application for release of two species of *Leptinotarsa* (Coleoptera: Chrysomelidae) on *S. elaeagnifolium* (Olckers and Zimmermann 1991; Olckers and Hulley 1994; Olckers and Zimmermann 1994). The outcome of this application, in conjunction with the above study, clearly indicates that the conflict of interest with eggplant is not likely to be a real problem.

Our perception of native solanums is that they are regarded as weeds and thus of low conservation status. In the unlikely event that some damage is inflicted on these in the field, it may well be perceived as a fair trade-off for the predicted impact on an aggressive exotic weed. The situation can be compared to that of the nodding thistle *Carduus nutans* L. (Asteraceae) in Canada and the USA, where the weevil *Rhinocyllus conicus* Froel. (Curculionidae) was released even though it was known to attack native thistles (Harris 1990). This resulted in successful control of the weed and negligible damage to native thistles. We predict that, even assuming a worst-case scenario, the same will be true for *G. spadicea* in South Africa.

The data presented here have supported a request for the release of *G. spadicea* on *S. sisymbriifolium*, which was granted in March 1994. Forty sexually mature adults, 20 males and 20 females were released at the site near Grahamstown on the 7th May 1994. This was an opportunistic release, and in view of the time of year of release, they were not expected to establish. In spite of a very severe winter, two adults were found at the release site in late September 1994, suggesting that they have been able to overwinter successfully. Further, large scale releases will be made in late spring (November) 1994.

CHAPTER 6

Biology and host range of *Metriona elatior* (Klug, 1829) a second potential biological control agent for *Solanum sisymbriifolium*.

6.1 INTRODUCTION

A second potential biological control agent for *S. sisymbriifolium*, *Metriona elatior*, also a leaf-feeding tortoise beetle (Cassidinae), was collected on the weed in the Misiones area of Argentina (S. Neser, personal communication). It was imported into quarantine in 1992. This beetle is also within the tribe Cassidini which is the largest and taxonomically most complex tribe of the Cassidinae (Riley 1986). Synonymy appears to be widespread in the Cassidini, and *M. elatior* has also been known as *Cassida elatior* (Riley 1982). *C. elatior* is the type-species of the genus *Metriona* by designation of Spaeth (1914).

There are some potential hazards of introducing more than one agent onto one weed species. It is not clear what role competitive exclusion has played in the outcome of weed biological control projects, but it should be considered, especially when the introduced species attack the same plant structure, as is the case with *G. spadicea* and *M. elatior*. Interspecific competition among control agents, leading to competitive exclusion, is believed to have contributed to the low rate of establishment of certain weed biological control agents (De Bach 1964). However, whether or not such competition could actually prevent a natural enemy complex from controlling the weed has not been demonstrated in the field (Ehler and Andres 1983; Walter 1988). Furthermore, an increase in the number of natural enemy species might reduce the amount of control effected by an individual species (due to

competition), but still increase the amount of control of the weed by the complex of natural enemy species.

Zwölfer (1973) studied interactions between the insects infesting the flower heads of the thistle *Carduus nutans* (L.) in Europe. He concluded that if the insects were to be used for biological control of the weed, it would be best for the sequence of introductions to proceed first with the intrinsically inferior weevil *Rhynocyllus conicus* Froelich. If this insect proved ineffective, the more superior insects would follow without danger of competitive exclusion.

The hypothesis of interspecific competition leading to competitive exclusion, as described above, relies on the assumption that the phytophagous insects are food limited. While this is notoriously difficult to prove in the field, it is very unlikely to be the case with *G. spadicea* and potentially *M. elatior* on *S. sisymbriifolium*, as the weed is under minimal pressure from native insects (Chapter 3).

Very little literature exists on *M. elatior*; however, Costa Lima (1968) has recorded the beetle on sweet potato (*Ipomoea batatas* (L.) Lam. (Convolvulaceae)) and *S. aculeatissimum*, in addition to *S. sisymbriifolium* in Brazil. Three other members of the genus *Metriona* have been recorded as pests of sweet potato: *M. bicolor* (Barrows 1979), *M. bivittata* (Stearns 1933) and *M. circumdata* (Raman and Ganesan 1992), all in North America. However, as Riley's (1986) revision of the Cassidini excludes the genus *Metriona* from North America, restricting it to South America, the taxonomic relatedness of these three species to *M. elatior* is uncertain, although they presumably belong to closely related genus. Sweet potato is grown as a crop in South Africa, especially in the Eastern Transvaal (Annecke and Moran

1982), the area which also supports the highest infestations of *S. sisymbriifolium* (see Figure 1.1). That *M. elatior* is a recorded pest of the crop in its country of origin is cause for concern in terms of its suitability for biological control.

The aim of this chapter, as in Chapter 5, was to determine the biology and host range of *M. elatior*, thereby assessing its potential as a biological control agent for *S. sisymbriifolium* in South Africa.

6.2 MATERIALS AND METHODS

The methods employed to determine the biology and host range of *M. elatior* under laboratory conditions were the same as those used for *G. spadicea* (Chapter 5), and are not repeated. In some instances the sample sizes differ, but these are indicated within the results.

6.3 RESULTS

Once again, in the tables that follow, means are quoted with standard deviations as a measure of central tendency as they provide an indication of the variability within the samples. However, as the data were not normally distributed, non-parametric statistical tests were employed which relied on the median values of the samples (Zar 1974).

6.3.1 Biology of *Metriona elatior*.

The biology of *M. elatior* was very similar to that of *G. spadicea* and consistent with that described for other Cassidinae (Muir and Sharp 1904; Siebert 1975; Olckers and Hulley 1989b).

Egg

The female *M. elatior* laid papery egg cases on the surface of the *S. sisymbriifolium* leaves, usually against the main vein (96.6% on the undersurface and 3.4% on the upper surface of the leaves (n = 652 egg cases)). The *M. elatior* egg cases were very similar to those of *G. spadicea*, but larger, 4.10 ± 0.33mm in length and 3.60 ± 0.56mm in width. The egg cases contained between 1 and 12 eggs with a mean of 5.24 ± 2.04 eggs per egg case (n = 159 egg cases). The pale yellow eggs were cylindrical, 1.43 ± 0.27mm in length and 0.72 ± 0.19mm wide (n = 100 eggs). The mean incubation period was 8.16 ± 2.02 days (n = 144 egg cases) (Figure 6.1). Of the 833 eggs in 159 egg cases, 678 (81.39%) hatched. Of those that did not hatch, 85 (10.20%) had developed fully, and 70 (6.41%) had failed to develop at all.

Larval Instars

The larvae were pale yellow, and there were five larval instars (the fifth instar included a non-feeding prepupal stage). Once again, apart from the size and the number of exuviae on the caudal spines, there appeared to be no external morphological differences between the larvae in the different. The larvae ranged in length (to the end of the last abdominal segment, ignoring the caudal spines) from 1.05-1.65mm in the first instar to 5.36-7.49mm in the full-grown fifth instar (larger than the larvae of *G. spadicea*) (Figure 6.2a). Head capsule width varied between 0.39-0.61mm in the first instar to 1.00-1.51mm in the fifth instar (Figure 6.2b). Because there was overlap between instars with regard to both head capsule width and body length, counting the number of larval exuviae on the caudal spines proved the best method of distinguishing between instars. The duration of the larval instars

varied between 3.42 and 6.31 days (Figure 6.1). The time taken from hatching to pupation varied between 24 and 33 days (n = 100 larvae).

The early instar larvae fed on the surface of the leaves, close to the egg case. The later instars were voracious feeders, and fed on both the surface and the edges of the leaves, and at high densities caused abscission of badly damaged leaves.

Pupae

The pupae were similar to those of *G. spadicea*, but generally larger, and pale yellow, with a dorso-lateral black stripe. The pronotum, which concealed the head, was expanded as a smooth shield, bearing fine black spines. A prominent feature of the pupae were two pairs of black spines on the anterior edge of the pronotum. In a sample of 300 pupae, 84.33% were on the lower leaf surface, 10.33% on the upper leaf surface, and 5.33% on the stems and petioles. The pupae varied in length between 5.46 and 7.85mm (mean = 6.81 ± 0.53mm, n = 85) and the pronotum width varied between 3.00 and 4.59 (mean = 3.92 ± 0.31mm, n = 85). The mean pupal mass for *M. elatior* reared on *S. sisymbriifolium* was 30.00 ± 7.51mg (n = 85). The mean duration of pupation was 6.95 ± 1.48 days (n = 85) (Figure 6.1). The total development time of the immature stages (egg stage to adult eclosion from pupation) ranged between 40 and 46 days (mean development time was 42.56 ± 6.27 days, n = 63 larvae).

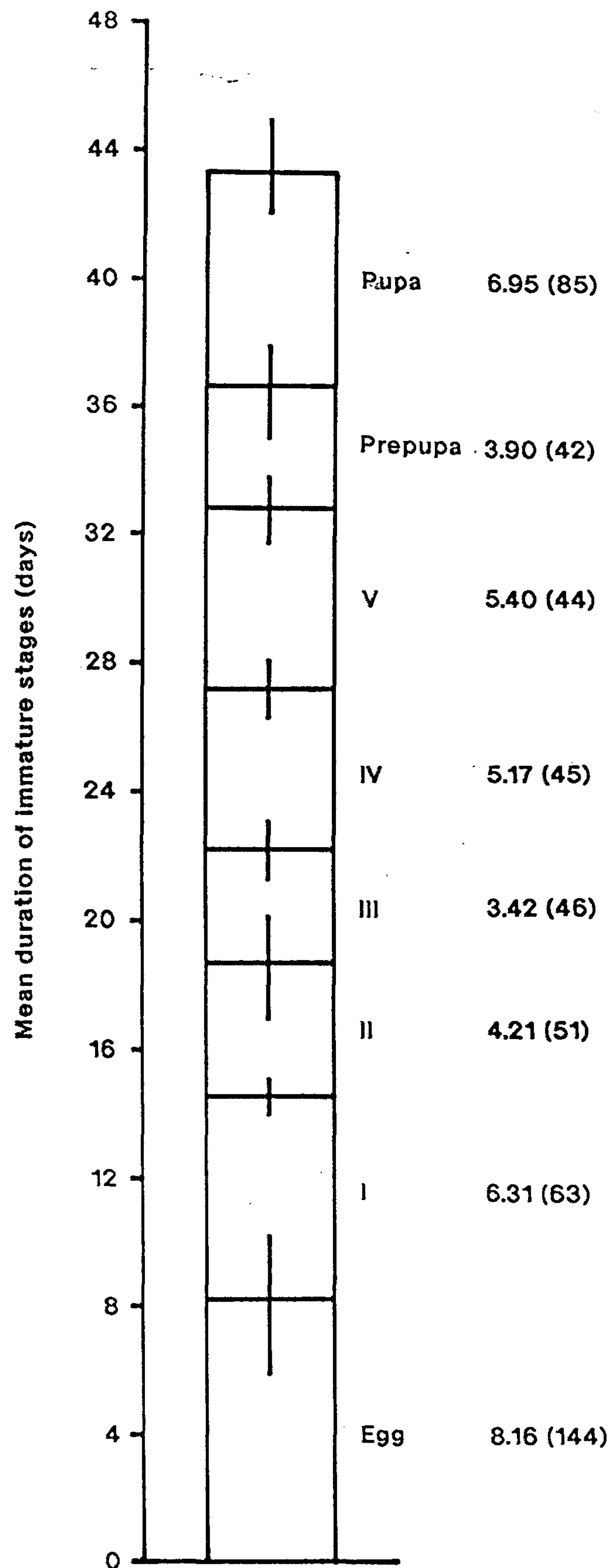
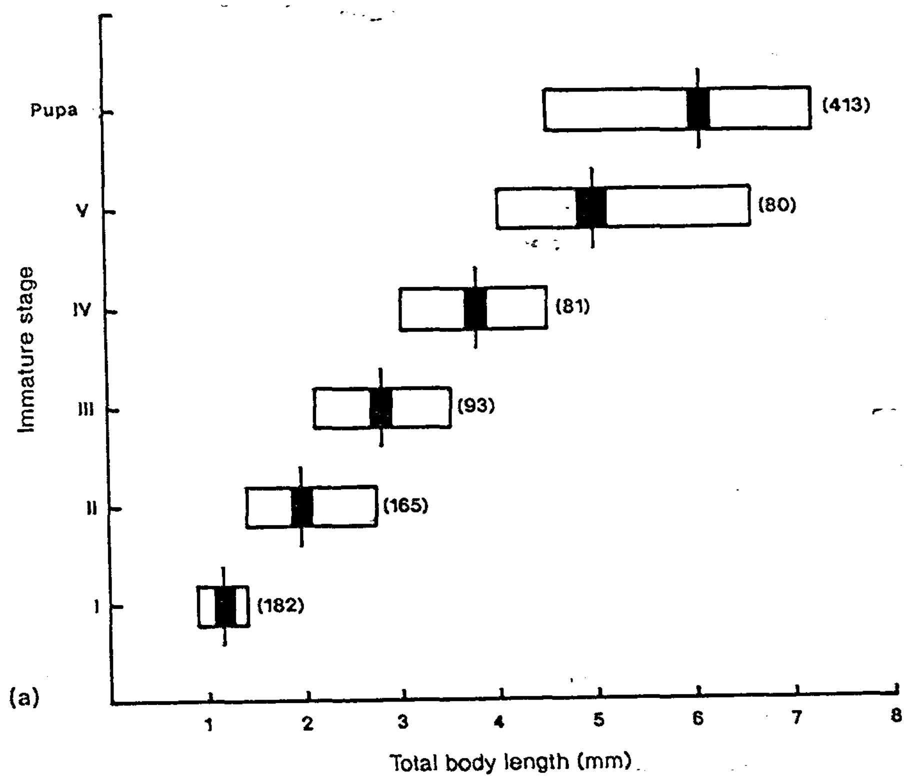


Figure 6.1 The duration of the immature stages of *Metriona elatior* on *Solanum sisymbriifolium* in the laboratory. The vertical lines represent the standard deviation of the means. The numbers in parentheses are the sample sizes. Biological studies were carried out at 25°C ± 2°C.



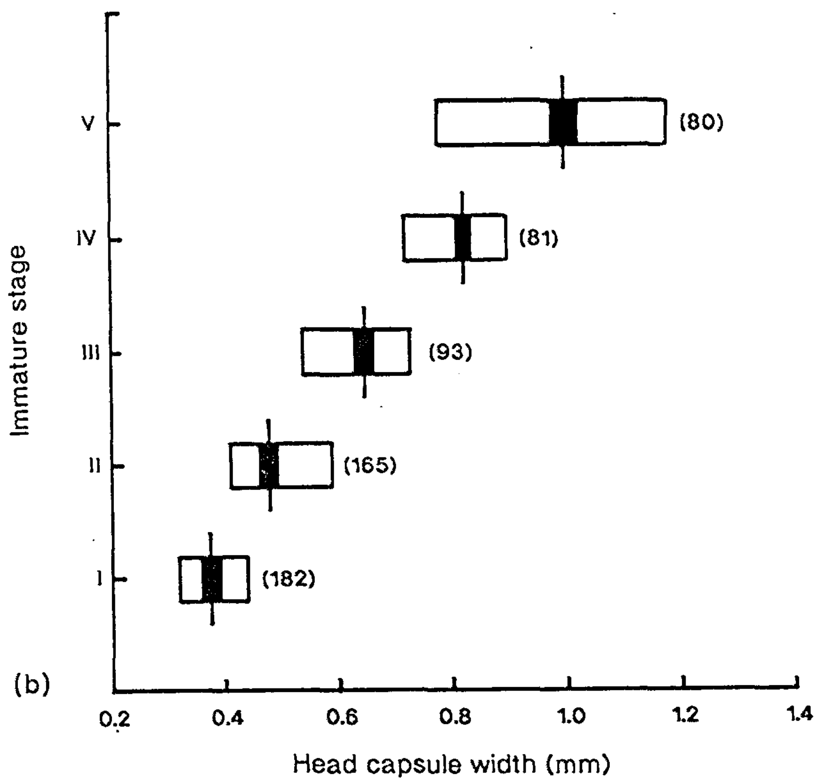


Figure 6.2 Total length (a) and head capsule width (b) of the immature stages of *Metriona elatior* reared on *Solanum sisymbriifolium* in the laboratory. The horizontal bars, vertical lines and shaded areas represent the ranges, means and standard deviations of the means respectively, the numbers in parentheses are the sample sizes.

Adult

On emergence, the *M. elatior* adults behaved in much the same way as *G. spadicea*, remaining fairly still on the leaves until the elytra hardened, and changing from pale yellow to a dull olive green, which usually took two days. Unlike *G. spadicea*, it was not possible to sex the adults externally, and it was necessary to catch the adults during copulation to successfully sex them. The adult female beetles were 7.71 ± 0.28mm (n = 30) in length, significantly larger than the males which were 6.93 ± 0.35mm (n = 30) in length (z = 2.85, p = 0.004, Mann-Whitney test).

The pre-oviposition period of *M. elatior* was considerably longer than that of *G. spadicea*, 20.32 ± 4.18 days (n = 20 pairs of male and female), during which copulation frequently occurred. The oviposition period lasted 89.72 ± 34.62 days (n = 20 females) during which the females laid 0.97 ± 1.22 egg cases per day. The post-oviposition period lasted 7.91 ± 6.34 days before the females died. The number of egg cases laid per female varied between 31 and 109 with a mean of 60.45 ± 37.89 egg cases per female (n = 20 females). The fertility (number of larvae) of twenty females ranged between 92 and 379 larvae per female (mean fertility was 179.72 ± 107.35 larvae per female, n = 20 females).

The mean longevity for female beetles was 116.43 ± 22.35 days (n = 30), while that for males was 72.94 ± 16.75 days (n = 30). As with *G. spadicea*, the females were significantly longer lived than the males (z = 3.45, p = 0.002, Mann-Whitney test). The mean generation time for females was 162.46 ± 39.56 days (n = 20 beetles) and 115.78 ± 22.35 days (n = 19 beetles) for males.

In the absence of any cited information, *M. elatior* is likely to diapause as the sexually immature adult on surrounding vegetation. Diapause will probably coincide with the onset of colder weather, shorter day length and a possible reduction in the nutritional value of the host plants.

6.3.2 Host Range of *Metriona elatior.*

Larval Survival Tests

Metriona elatior has a fairly wide *Solanum* host range in comparison with *G. spadicea* (Table 6.2). The larvae were reared through from first instar to adult on 10 of the 11 native solanums tested, all five of the exotic solanums tested, and on the economically important *S. melongena* (eggplant) and *Lycopersicon esculentum* (tomato). On eight of the non-host plants, including eggplant, more than 30% of the larvae were reared through to adults (Tables 6.1 and 6.2).

Table 6.1 Results of the no-choice feeding trials with *Metriona elatior* larvae on selected test plants.

Test plant	Result[a]
A. Native *Solanum* species	
Solanum cf. *acanthoideum*	+++
Solanum burchellii	++
Solanum coccineum[b]	++
Solanum duplo-sinuatum	++
Solanum giftbergense	+
Solanum giganteum[b]	0
Solanum incanum[b]	++
Solanum linnaeanum[b]	+++
Solanum panduriforme[b]	++
Solanum rigescens[b]	+++
Solanum tomentosum	+
B. Exotic *Solanum* species	
Solanum acanthoideum	++++
Solanum aculeatissimum	++++
Solanum elaeagnifolium	+
Solanum hispidum	+++
Solanum mauritianum	+
C. Selected Vegetable Crops	
Brassicaceae	
Brassica oleracea var. *capitata* (cabbage)	0
Brassica oleracea var. *italica* (broccoli)	0
Brassica oleracea var. *botrytis* (cauliflower)	0
Chenopodiaceae	
Beta vulgaris var *cicla* (Spinach)	0
Asteraceae	
Lactuca sativa (lettuce)	0
Convolvulaceae	
Ipomoea batatas (sweet potato)	0
Solanaceae	
Capsicum frutescens (chilli)	0
Capsicum annuum (red and green pepper)	0
Lycopersicon esculentum (tomato)	+
Solanum melongena (eggplant)	+++
Solanum tuberosum (potato)	0

[a] 0 = No feeding or development
+ = Less than 10% of larvae survived to pupation.
++ = Between 10.1% and 25% of larvae survived to pupation.
+++ = Between 25.1% and 50% of larvae survived to pupation.
++++ = More than 50% of larvae survived to pupation.
[b] Native weed of minor status (Wells *et al.* 1986).

Table 6.2 Development of *Metriona elatior*[a] on species used in the no-choice larval host specificity tests.

Host plant	n	Mean no.pupae/ replicate[b] (±SD)	Percentage pupation	Mean pupal mass (mg)[b] (±SD)	Duration (days)[b,c] (±SD)
S. sisymbriifolium	10	7.5 (2.2)ab	75	30.00 (7.51)abc	34.84 (4.26)ab
S. acanthoideum	10	6.6 (2.3)ab	66	30.40 (6.65)abc	35.68 (4.18)abc
S. aculeatissimum	10	6.5 (2.1)ab	65	34.94 (6.27)abc	34.68 (4.76)a
S. melongena	10	4.5 (2.7)ab	45	31.38 (6.51)abc	34.34 (4.33)a
S. rigescens	10	4.1 (1.7)ab	41	28.50 (5.28)ab	39.30 (2.16)abcd
S. cf. *acanthoideum*	10	3.9 (1.3)a	39	29.06 (6.27)abc	44.70 (4.26)d
S. linnaeanum	10	3.8 (2.3)a	38	27.95 (4.80)abc	37.82 (3.71)abcd
S. hispidum	10	3.2 (2.3)a	32	28.22 (7.12)a	38.78 (7.28)abcd
S. burchellii	5	3.2 (1.9)a	32	27.54 (4.47)acd	40.00 (1.51)abcd
S. panduriforme	10	1.9 (1.7)c	19	26.30 (6.59)abc	42.71 (6.17)cd
S. coccineum	7	1.9 (1.4)c	13	26.25 (5.38)acd	48.71 (8.24)d
S. duplo-sinuatum	10	1.8 (1.1)c	18	27.84 (5.82)abc	42.88 (4.97)cd
S. incanum	10	1.5 (1.5)cd	15	26.06 (4.88)abc	43.00 (6.02)abcd
S. tomentosum	8	1.0 (1.1)cd	8	28.95 (3.19)abc	42.29 (4.68)d
L. esculentum	10	0.8 (1.0)cd	8	21.45 (3.50)a	44.00 (3.82)abcd
S. giftbergense	6	0.5 (0.8)cd	3	20.23 (1.31)abc	52.67 (3.06)d
S. mauritianum	10	0.3 (0.7)cd	3	19.00 (1.74)a	49.67 (2.52)bcd
S. elaeagnifolium	10	0.2 (0.4)cd	2	19.65 (0.64)a	51.00 (1.41)abcd

[a] Ten larvae were used in each of the replicates per test plant species. The figures in parentheses represent the standard deviation.

[b] Means in columns not followed by the same letter differ significantly at the 5% level (Kruskal-Wallis test followed by Dunn's Multiple Range test).

[c] Development in days from larval emergence to adult eclosion from pupation.

The larval developmental period might give an indication of the relative chemical and nutritional suitability of a host for an insect. The total period of development of *M. elatior* on the various non-host plants gave no clear result in this respect (Table 6.2), and therefore a comparison of the developmental periods of each of the *M. elatior* instars on selected non-host plant species was made (Table 6.3). Once again, there was no trend in the duration period of the immature stages, although the larvae reared on *S. burchellii* generally took longer to develop. As expected, the duration of the non-feeding stages (prepupa and pupa) did not differ significantly.

Table 6.3 Comparison of the developmental rates of the immature stages of *Metriona elatior* on selected *Solanum* species[a].

Test plant species	n	Duration of immature stages of *M. elatior* (days ± SD)						
		I	II	III	IV	V	Prepupa	Pupa
S. sisymbriifolium	42	6.31ab (0.60)	4.21a (1.76)	3.42a (0.89)	5.17a (0.81)	5.40a (0.02)	3.25a (0.12)	6.95a (1.48)
S. acanthoideum	31	5.26b (0.42)	4.76a (0.42)	4.97b (0.57)	5.21a (0.75)	5.05a (0.56)	3.24a (0.59)	8.01a (1.30)
S. aculeatissimum	20	5.48b (0.73)	4.75a (0.57)	4.38b (1.11)	4.18a (0.78)	5.51a (0.73)	2.61a (0.45)	7.44a (0.78)
S. melongena	21	5.31b (0.67)	4.79a (0.52)	4.93b (0.34)	4.70a (0.58)	4.80a (0.41)	3.76a (0.64)	7.62a (1.01)
S. linnaeanum	26	6.23ab (0.57)	5.33b (1.28)	6.25c (0.58)	6.37b (0.85)	5.73a (0.84)	3.08a (0.74)	7.45a (1.33)
S. rigescens	22	5.34b (0.32)	5.80b (0.49)	5.56b (0.26)	6.36b (0.63)	6.13a (0.56)	3.00a (0.53)	7.70a (1.53)
S. panduriforme	17	5.25b (0.31)	5.42b (0.56)	5.55b (0.68)	6.51b (0.86)	6.93b (0.65)	2.87a (0.91)	8.23a (1.23)
S. incanum	10	5.40b (0.21)	6.62b (0.78)	4.99b (0.61)	6.59b (0.40)	7.25b (0.76)	3.62a (1.14)	7.99a (1.45)
S. burchellii	10	7.21c (1.35)	7.61c (0.94)	6.87c (1.64)	8.05c (1.35)	8.11c (1.39)	3.60a (0.81)	8.15a (0.70)

[a] Means in columns not followed by the same letter differ significantly at the 5% level (Kruskal-Wallis test followed by Dunn's Multiple Range test).

There was much overlap in pupal mass and development period between the plant species tested and a host suitability index (Maw 1976: pupal mass x percentage pupation / developmental period) was used to determine the most suitable host for *M. elatior*. The index (Table 6.4) shows that three exotic species (*S. sisymbriifolium*, *S. acanthoideum* and *S. aculeatissimum*) were equally suitable hosts for the agent. *S. melongena* was also a suitable host for the agent, and four native species (*S. rigescens*, *S. linnaeanum*, *S.* cf. *acanthoideum* and *S. burchellii*), and the exotic *S. hispidum* would probably support populations of *M. elatior*, in the presence of a preferred host. This contrasts the situation for *G. spadicea* (see Table 5.3) where the two most suitable hosts after *S. sisymbriifolium* (*S. acanthoideum* and *S. melongena*) obtained index scores 38% and 20% respectively of the *S. sisymbriifolium* index score.

Table 6.4 Results of the host suitability index for *Metriona elatior*, reared on several species of *Solanum*.

Plant species	Host suitability	Percentage rating
S. aculeatissimum	65.49	100.00
S. sisymbriifolium	64.58	98.61
S. acanthoideum	56.23	85.86
S. melongena	41.12	62.73
S. rigescens	29.73	45.40
S. linnaeanum	28.08	42.88
S. cf. *acanthoideum*	25.35	38.71
S. hispidum	23.29	35.56
S. burchellii	22.03	33.64
S. panduriforme	11.70	17.86
S. duplo-sinuatum	11.69	17.85
S. incanum	9.09	13.88
S. coccineum	7.01	10.70
S. tomentosum	5.48	8.37
L. esculentum	3.90	5.96
S. giftbergense	1.15	1.76
S. mauritianum	1.15	1.76
S. elaeagnifolium	0.77	1.18

Adult Choice Tests

Female *M. elatior* laid significantly more egg cases on *S. sisymbriifolium* than on the non-host plants, with the exception of *S. aculeatissimum* and *S. acanthoideum* (Table 6.5). However, *S. melongena*, *S. linnaeanum*, and *S. hispidum* served as fairly consistent oviposition sites. Adults also recognised *S. acanthoideum*, *S. aculeatissimum*, *S. melongena*, *S rigescens* and *S. hispidum* as feeding sites, and adult feeding damage was recorded on these plants and *S.* cf. *acanthoideum*, *S. linnaeanum*, *S. coccineum*, *S. burchellii*, and *S. incanum*.

Table 6.5 Results of the adult choice tests of *Metriona elatior* on *Solanum sisymbriifolium* and several non-host plant species[a].

Test pair	Mean number of egg cases (±SD)[b]	Mean number of adults (±SD)[b]
S. sisymbriifolium	20.33 (7.21)a	6.67 (2.08)a
S. sisymbriifolium	22.67 (5.53)a	12.33 (4.51)a
S. sisymbriifolium	27.00 (5.20)a	9.00 (3.00)a
S. aculeatissimum	19.67 (3.21)a	6.33 (1.53)a
S. sisymbriifolium	24.33 (3.46)a	11.00 (3.64)a
S. acanthoideum	20.00 (3.53)a	7.00 (4.36)a
S. sisymbriifolium	46.67 (5.21)a	9.33 (4.04)a
S. melongena	12.00 (4.73)b	7.67 (3.79)a
S. sisymbriifolium	39.33 (2.54)a	9.67 (2.08)a
S. rigescens	4.33 (3.21)b	6.33 (5.13)a
S. sisymbriifolium	31.67 (5.29)a	12.33 (4.51)a
S. cf. *acanthoideum*	4.67 (1.16)b	5.67 (1.53)b
S. sisymbriifolium	26.33 (6.00)a	10.00 (4.58)a
S. linnaeanum	7.67 (3.79)b	5.33 (1.53)b
S. sisymbriifolium	17.00 (4.58)a	10.67 (5.03)a
S. hispidum	8.33 (3.51)b	7.33 (2.31)a
S. sisymbriifolium	32.00 (12.53)a	13.00 (5.29)a
S. burchellii	2.00 (1.73)b	3.67 (1.16)b
S. sisymbriifolium	22.67 (7.57)a	10.67 (1.16)a
S. panduriforme	0.67 (1.16)b	3.00 (3.61)b
S. sisymbriifolium	27.67 (8.08)a	11.67 (3.56)a
S. coccineum	2.33 (1.53)b	4.67 (1.53)b
S. sisymbriifolium	24.67 (19.55)a	9.33 (2.08)a
S. duplo-sinuatum	1.67 (1.53)b	2.67 (3.06)b
S. sisymbriifolium	22.33 (11.24)a	7.67 (1.53)a
S. incanum	0.33 (0.58)b	3.67 (0.58)b
S. sisymbriifolium	30.00 (12.29)a	14.00 (3.46)a
S. tomentosum	3.00 (1.73)b	2.33 (1.16)b
S. sisymbriifolium	32.00 (11.79)a	12.33 (0.58)a
L. esculentum	3.33 (2.08)b	1.67 (2.08)b
S. sisymbriifolium	27.67 (8.02)a	15.00 (2.00)a
S. giftbergense	0.33 (0.58)b	1.33 (0.58)b
S. sisymbriifolium	28.33 (6.51)a	14.33 (1.53)a
S. mauritianum	0.00 (-)b	0.67 (0.58)b
S. sisymbriifolium	25.00 (6.25)a	13.00 (3.61)a
S. elaeagnifolium	0.00 (-)b	0.33 (0.58)b

[a] Mean number of *M. elatior* egg cases per 10 females per week, and mean number of adult *M. elatior* (of 20 used in each replicate).
[b] The means of test pairs not followed by the same letter differ at the 5% level (Mann-Whitney test).

6.4 DISCUSSION

The biology of *M. elatior* is very similar to that of *G. spadicea*, as is evidenced by their relative scores using the scoring system of biological control agents proposed by Harris (1973) (Table 6.6). Goeden (1983) states that Harris' system is not capable of predicting accurate scores for those agents that have not been released elsewhere, or about which little is known from their country of origin, as a number of criteria used in the system are unknown, and suggests that for a first time candidate the maximum possible score should be 40 as opposed to 46. Taking this into consideration, both *G. spadicea* and *M. elatior*, according to Harris (1973), should be suitable for testing as biological control agents for *S. sisymbriifolium*. In the only other study of this nature, Maw (1976) attributed a total score of 19 for *Cassida hemisphaerica* Hbst. (Cassidinae), an agent under considered for the control of *Silene cucubalis* Wibel, in Canada.

A comparison of the scores of the two agents (Table 6.6) reveals that it is the longer generation times, and therefore fewer generations per year, and very localised distribution of *M. elatior* on *S. sisymbriifolium* in South America (S. Neser, personal communication) that attributes to its lower score. On the other hand, *M. elatior* obtains a maximum possible score for criterion 1 as it is not strictly monophagous in its country of origin (Costa Lima 1968), which implies minimal insect-plant homeostasis (Pimentel 1963) and therefore greater potential as a biological control agent (Harris 1973; Hokkanen 1989; Hokkanen and Pimentel 1989; Pimentel and Hokkanen 1989). This hypothesis, however, has not been widely accepted (see Goeden 1983; Goeden and Kok 1986), and is discussed in Chapter 8. Despite the criticisms of Goeden (1983), Harris' (1973) system does provide a good framework to assess the biological characteristics of weed biological control agents.

Table 6.6 Effectiveness of *Metriona elatior* as a weed biological control agent for *Solanum sisymbriifolium*[a].

Criteria	Score of *M. elatior*	Score of *G. spadicea*	Maximum possible score
1. Host Specificity	3	1	3
2. Direct Damage Inflicted	2	2	5
3. Indirect Damage Inflicted	?	?	3
4. Phenology of Attack	4	4	4
5. Number of Generations	2	4	4
6. Number of Progeny/Generation	1	1	2
7. Extrinsic Mortality Factors	?	?	4
8. Feeding Behaviour	2	2	2
9. Compatibility	2	2	2
10. Distribution	2	6	6
11. Effectiveness	0	0	6
12. Size	2	2	4
Total	20	24	45

[a] Based on Harris' scoring system (Harris 1973).

This study showed *M. elatior* to be an oligophagous herbivore on a number of native and exotic *Solanum* species, and the economically important eggplant (*S. melongena*) under quarantine laboratory conditions. However, contrary to Costa Lima's (1968) findings, sweet potato (*I. batatas*) did not support larval development and therefore was not a host for *M. elatior*.

In contrast to the results of the specificity testing of *G. spadicea* (Chapter 5), where the non-host plants on which development of the larvae occurred were very inferior hosts in comparison to *S. sisymbriifolium*, and unlikely to support field populations of the agent, several of the test plants here appeared to be equally suitable hosts for *M. elatior*.

Furthermore, one of the strongest arguments in favour of releasing *G. spadicea* was the inability of the females to recognise non-hosts as oviposition sites. Female *M. elatior*, although they oviposited on fewer non-hosts than larvae were able to develop on, nevertheless accepted a number of non-hosts for oviposition.

6.5 CONCLUSIONS

Despite the fact that the two agents are so similar, the arguments that were used to motivate for the release of *G. spadicea* can not be applied to *M. elatior*. It could be argued that the wide host range of *M. elatior*, and their ability to recognise supposedly non-hosts as oviposition sites, are merely products of the restricted cage conditions under which they were tested. However, the results presented here are so convincing compared to those of *G. spadicea* on non-hosts that they are likely to represent a realistic situation.

That *M. elatior* survives well on several of the exotic solanums could be seen as a fortuitous by-product of releasing *M. elatior*. However, it does indicate that the agent is not monophagous. Despite the evidence presented in Chapter 5, the high survival rate and number of egg cases laid on eggplant are unacceptable for a biological control candidate. Although it has been shown that the conflict of interest involving eggplant have been overrated (Chapter 5), the release of an agent that appears to be a realistic and strong threat to an economically important crop is unacceptable. Therefore, based on the data presented here, the release of *M. elatior* for the biological control of *S. sisymbriifolium* is not suggested.

CHAPTER 7

Host-range extension by native natural enemies onto weed biological control agents in South Africa: Implications for *Gratiana spadicea.*

7.1 INTRODUCTION

In the biological control of weeds, introduced insect herbivores released from their parasitoids have great potential for increasing in number and thus for reducing the populations of the target weed (Dennill 1985; Moran *et al.* 1986). Insect herbivores are attacked by large complexes of parasitoids (Cornell and Hawkins 1993); however, biological control agents may also be susceptible to attack by native parasitoid species. Cornell and Hawkins (1993) suggested that introduced herbivores should initially be attacked mostly by generalist parasitoids which are able to extend their host ranges more easily from their native hosts to the new herbivore than are the more host specific species. Askew and Shaw (1986) suggested that specialist parasitoids might then more slowly evolve appropriate traits to exploit this novel resource. The feeding niche of a host (manner of feeding and the plant structure fed on) can influence the number and type of parasitoid species in the parasitoid complex (Askew and Shaw 1986; Hawkins and Lawton 1987; Gross and Price 1988; Price and Pschorn-Walcher 1988; Hawkins 1988; Hawkins *et al.* 1990; Gross 1991). In general, poorly concealed endophagous insects support more parasitoid species, while species that are either more mobile (ectophagous herbivores) or better concealed support fewer parasitoid species (Hawkins and Gross 1992). Endophagous hosts support parasitoid complexes containing large proportions of generalists because the parasitoids tend to locate the plant

structure, and not the actual insect, whereas external feeders support relatively more specialists (Hawkins 1990; Hawkins *et al.* 1992; Cornell and Hawkins 1993).

Goeden and Louda (1976) investigated the impact of predators, parasitoids, and disease on insects imported as weed biocontrol agents. They found that the non-establishment of any weed biocontrol agents could not be ascribed solely to the activities of indigenous parasitoids, and that parasitoids and disease had far less severe consequences on populations of biocontrol agents than predation.

Apart from the study of Pettey (1948) on the native parasitoid complex attacking *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae), introduced to South Africa for the biological control of *Opuntia ficus-indica* (L. Miller) (prickly pear cactus), the effect of native parasitoids on weed biocontrol agents introduced to South Africa has not been studied. Siebert (1975) recorded host-range extension by native parasitoids onto *Gratiana pallidula* and *G. lutescens* (introduced to South Africa for testing as control agents for *Solanum elaeagnifolium* (satansbos), but not released) under quarantine conditions and suggested that the parasitoid complexes of native cassidines might hamper the effectiveness of these agents should they be released.

The Cassidinae are prone to attack by egg parasitoids of the family Eulophidae and pupal parasitoids of the family Chalcididae (e.g. Einser *et al.* 1967; Carroll 1978; Ward and Peinkowski 1978; Windsor 1987; Olmstead and Denno 1992). In Brazil, the eulophid egg parasitoid, *Emersonella ooecia* De Santis, 1983, was recorded in 89.4% of some 2300 *Gratiana spadicea* egg cases (Becker and Romanowski 1986). Becker and Frieiro-Costa

(1988) have also recorded *E. ooecia* from field populations of *G. spadicea*. In addition, they found that at least one egg in 95% of the egg cases investigated had been sucked dry by a mirid bug, *Tupicoris cincticornis* (Stål, 1860). It is quite possible that the egg and pupal parasitoids associated with native tortoise beetles (Olckers and Hulley 1989b), and the mirid, *Cyrtopeltis* sp. (AcRh 674), recorded feeding on *S. sisymbriifolium* (Chapter 3) might also attack *G. spadicea* in the field.

The aims of this chapter were, firstly, to investigate reported host-range extensions by native parasitoids to weed biocontrol agents in South Africa, and specifically to determine whether differences in parasitoid richness on biocontrol agents could be explained by obvious differences in the level of concealment of the agent and agent taxonomy. Secondly, to try and determine experimentally the possible threat to *G. spadicea* populations posed by native parasitoids and predators.

7.2 MATERIALS AND METHODS

7.2.1 Host-range extension by native parasitoids.

The number of phytophagous insect species released as biological control agents of weeds in South Africa was established from Julien (1987). Questionnaires were sent to the researchers responsible for releasing any biological control agents on weeds in South Africa.

Each host/agent was initially classified into one of seven life-style categories based on the habits of the immature stages of the agents. The classification is a variant of standard ones regularly used for herbivorous arthropods (Hawkins and Lawton 1987; Hawkins 1990; Gross 1991; Hawkins *et al.* 1992):

(1) External chewers

(2) Leaf rollers

(3) Mixed endophagous/ectophagous life-style

(4) Case bearers

(5) Leaf miners

(6) Gall formers

(7) Stem, flower, fruit, and seed borers

Because several categories had small sample sizes we followed the example of Cornell and Hawkins (1993) and combined several of them, yielding:

(a) Ectophages (1, 2, and 3)

(b) Poorly concealed endophages (4 and 5)

(c) Well concealed endophages (6 and 7).

Including the mixed endophage/ectophage lifestyle (3) into the ectophage classification (a) was justified because the larval stages of the two agents concerned were predominantly ectophagous.

7.2.2 Predator and Parasitoid trials involving *Gratiana spadicea.*

All of the predator and parasitoid trials were conducted in the quarantine insectary with a photoperiod of 16:8 hours (light:dark), temperatures fluctuated between 26 and 20°C in synchrony with the photoperiod, and relative humidity ranged between 60 and 80%.

The mirid *Cyrtopeltis* sp. (AcRh 674) was collected from infestations of *S. sisymbriifolium* in the environs of Grahamstown. Five adult mirids were placed into a 9cm diameter petri

dish containing 10 *G. spadicea* egg cases, on moist filter paper. The petri dishes were left for 24 hours, after which the mirids were removed and the eggs were allowed 10 days to hatch. After 10 days, the egg cases were dissected. A control was conducted simultaneously, using the same experimental design, but without the addition of the mirids. The experiment was repeated on 20 occasions.

Parasitoids for egg and pupae trials were obtained as follows: egg cases and pupae of the tortoise beetles *Conchyloctenia tigrina* feeding on several native *Solanum* species and *Aspidomorpha tecta* Boh. feeding on morning glory (*Ipomoea purpurea* (L.) Roth (Convolvulaceae)) were collected from field populations around Grahamstown. Both the egg cases and the pupae were placed in petri dishes on moist filter paper, and the emerging parasitoids were collected. Both *C. tigrina* and *A. tecta* were attacked by a eulophid egg parasitoid (*Tetrastichus* sp. AcRh 520 and *Tetrastichus* sp. AcRh 689 respectively) and a chacidid pupal parasitoid (*Brachymeria* sp. AcRh 518 and *Brachymeria* sp. AcRh 690 respectively). All parasitoids were maintained in petri dishes on moist filter paper, and sustained on saturated sugar water for at least 10 days before use.

Parasitism of egg cases

Twenty *Tetrastichus* sp. (AcRh 520) parasitoids were introduced to a petri dish containing 10 *G. spadicea* egg cases and left until all the parasitoids had died (4-6 days), after which they were removed and the egg cases left to hatch out. Ten days after the parasitoids had all died, the egg cases were dissected to establish how many larvae had emerged. A control experiment was conducted simultaneously using the egg cases of the natural host of *Tetrastichus* sp. (AcRh 520), *C. tigrina*, and further controls, using the same experimental

design, but without the parasitoids, were performed. This experiment was repeated on five occasions.

The trial was repeated using a second *Tetrastichus* sp. (AcRh 689), its natural host, *A. tecta*, was used as the control.

Parasitism of pupae

Ten *Brachymeria* sp. (AcRh 518) parasitoids were introduced to a petri dish containing five *G. spadicea* pupae (pupae less than 2 hours old were used) and left until all the parasitoids had died, after which the parasitoids were removed and the adult beetles allowed to eclose. Any pupa from which an adult beetle did not emerge, was dissected. A control experiment was conducted simultaneously using pupae of the natural host of the parasitoid, *C. tigrina*. Once again, controls were conducted for both species by not introducing the parasitoids. This experiment was repeated on five occasions.

The trial was repeated using a second *Brachymeria* sp. (AcRh 690), its natural host, *A. tecta* was used as the control.

7.3 RESULTS

7.3.1 Host-range extension by native parasitoids.

Information was obtained for all of the insects released as biological control agents of weeds in South Africa. In most cases there had been little opportunity for quantitative post-release evaluation; so all of the results presented here should be interpreted with caution as they are likely to change as more post-release samples are accumulated for the agents.

A total of 62 insect species have been released as biological control agents against some 22 weed species in South Africa. Of those released, 40 (64.5%) have became established, 21 (33.9%) have not establish, and for one release (1,6%) it is too soon to tell. Sixteen (40.0%) of those agents established are known to be parasitized (Table 7.1). One (4.8%) of the agents that did not establish is known to have been parasitized.

Table 7.1 The biological control agents that have established on weeds in South Africa and that have subsequently been parasitized by native parasitoids, and an estimate of their levels of parasitism.

Weed	Agent	Parasitoid	Level of parasitism[a]	Reference
Acacia longifolia (Andr.) Willd.	*Trichilogaster acaciaelongifoliae* (Froggatt) Hymenoptera Pteromalidae	*Antistrophoplex* sp. *Torymus* sp. Torymidae	0.5-1.6%	Dennill (1987b)
	Melanterius ventralis Lea Coleoptera Curculionidae	*Pteromalus* sp. Pteromalidae	Low	D.Donnelly pers. comm.
Ageratina adenophora (Spreng.) King & Robinson	*Procecidochares utilis* Stone Diptera Tephritidae	Unidentified Hymenoptera	Common	S.Neser pers. comm.
Hakea gibbosa (Smith) Cav. and *Hakea sericea* Schrader	*Erytenna consputa* Pascoe Coleoptera Curculionidae	Braconidae Hymenoptera	Low	S.Neser pers. comm.
Hakea sericea	*Carposina autologa* Meyrick Lepidoptera Carposinidae	Trichogrammatidae Hymenoptera	Low	S.Neser pers. comm.
Hypericum perforatum L.	*Zeuxidiplosis giardi* Kieffer Diptera Cecidomyiidae	*Antistrophoplex* 3 spp. *Torymus* sp. *Mesopolobus* sp. Unidentified Pteromalidae	0.4-11.8%	Gordon & Kluge (1991)

TABLE 7.1 continued

Weed	Agent	Parasitoid	Level of parasitism[a]	Reference
Lantana camara L.	*Calcomyza lantanae* Frick Diptera Agromyzidae	Unidentified Hymenoptera	High	S.Neser pers. comm.
	Hypena strigata (F.) Lepidoptera Noctuiidae	Unidentified Hymenoptera	Low	Oosthuizen (1964)
	Octotoma scabripennis Guèrin-Mèneville Coleoptera Chrysomelidae	Unidentified Hymenoptera	Low	S.Neser pers. comm.
	Ophiomyia lantanae (Froggatt) Diptera Agromyzidae	Braconidae (1 sp.) Cynipidae (1 sp.) Eupelmidae (1 sp.) Eulophidae (*Euderus* sp.)	Low	Oosthuizen (1964) C.Cilliers pers. comm.
	Uroplata giradi Pic Coleoptera Chrysomelidae	Unidentified Hymenoptera	Low	S.Neser pers. comm.
Opuntia aurantiaca Lindley	*Mimorista pulchellalis* (Dyar) Lepidoptera Pyralidae	*Hockeria* sp. *Invreia* sp. Chalcididae Hymenoptera	Low	J.H.Hoffmann pers. comm.

TABLE 7.1 continued

Weed	Agent	Parasitoid	Level of parasitism[a]	Reference
Opuntia ficus-indica (L.) Miller	*Cactoblastis cactorum* Bergroth Lepidoptera Phycitidae	*Trichogrammatoidea lutea* *Trichogrammatoidea* sp. *Bracon hebetor* *Pseudoperichaeta* sp. *Brachymeria* sp. *Invreia* sp. *Euchalcidia* sp. *Pimpla* sp. *Phorocea blepharipa* Ichneumonidae (6 spp.) Chalcididae (9 spp.)	0.04-5.2%	Pettey (1948) Robertson & Hoffmann (1989)
Prosopis spp.	*Algarobius prosopis* (LeConte) Coleoptera Bruchidae	10 spp. of Hymenopteran parasitoid	Low (0.3%)	J.H.Hoffmann pers. comm.
	Algarobius bottimeri Kingsolver Coleoptera Bruchidae	As above	As above	J.H.Hoffmann pers. comm.
Sesbania punicea Bentham	*Neodiplogrammus quadrivittatus* (Olivier) Coleoptera Curculionidae	Braconidae Hymenoptera	Low	J.H.Hoffmann pers. comm.

[a] Level of parasitism at the population level.

In all but two agents it was the larval stage that was attacked by native parasitoids. One of the exceptions was *Carposina autologa* Meyrick released against *Hakea sericea* Schrader in which only the exposed egg stage of the agent was attacked; this agent was placed in the ectophage category. The other exception was *C. cactorum*, released against *O. ficus-indica* and *O. aurantiaca* Lindley, from which egg, larval and pupal parasitoids were reared. Of the 23 native parasitoids reared from *C. cactorum*, two were recovered from the egg stage, two from the larval stage, and the remaining 19 species of parasitoids from the exposed pupal stage (Pettey 1948). As a result this agent was placed in the exposed or ectophagous category.

There is a strong indication that the susceptibility to attack of different orders of agents varied (Table 7.2). Despite the very small sample sizes, and ignoring the single hymenopteran agent released, the dipteran and lepidopteran biocontrol agents seemed more susceptible to attack by native parasitoids than members of the Coleoptera. The hemipteran agents, on the other hand, were not attacked.

Analysis of parasitism in relation to guild membership suggests that poorly concealed and even well concealed endophages were more susceptible to attack by native parasitoids than ectophages (Table 7.3).

Table 7.2 Analysis by Order of the biological control agents that were parasitized after release on weeds in South Africa.

Agent	Number released	Number established	Number parasitized	% P[a]
Coleoptera	29	20	7	35.00
Lepidoptera	16	7	4	57.14
Hemiptera	10	7	0	0.00
Diptera	6	5	4	80.00
Hymenoptera	1	1	1	100.00
Total	62	40	16	40.00

[a] % P = percentage of the number of **established** agents that were parasitised.

Table 7.3 The guilds of the established biocontrol agents released onto weeds in South Africa and their levels of parasitism.

Guild of insect	Agents	Parasitized	% P[a]	Mean number of parasitoids/agent (±SD)[b]
Ectophages	15	3	20.00	8.67 (±13.28)
Poorly concealed endophages	8	5	62.50	2.73 (±3.45)
Well concealed endophages	17	8	47.06	4.33 (±3.57)

[a] % P = percentage of the different guilds of agents that were parasitised.
[b] mean number of parasitoid species recorded on each of the parasitised biological control agents within each of the three guilds.

Table 7.4 Analysis by Family of the biological control agents that were parasitised after release on weeds in South Africa.

Agents	N R[a]	N E[b]	N P[c]	% P[d]
Coleoptera				
Curculionidae	14	13	3	23.08
Chrysomelidae	8	3	2	66.67
Cerambycidae	3	1	0	0.00
Bruchidae	2	2	2	100.00
Apionidae	1	1	0	0.00
Bruprestidae	1	0	0	-
Lepidoptera				
Pyralidae	5	3	1	33.33
Noctuidae	3	1	1	100.00
Arctiidae	2	1	0	0.00
Gelichiidae	2	0	0	-
Carposinidae	1	1	1	100.00
Phycitidae	1	1	1	100.00
Geometridae	1	0	0	-
Pterophoridae	1	0	0	-
Hemiptera				
Dactylopidae	5	5	0	0.00
Tingidae	3	1	0	0.00
Pseudococcidae	1	1	0	0.00
Aphidae	1	0	1	100.00
Diptera				
Tephritidae	3	2	1	50.00
Agromyzidae	2	2	2	100.00
Cecidomyiidae	1	1	1	100.00
Hymenoptera				
Pteromalidae	1	1	1	100.00

[a]N R = Number of agents released, [b]N E = Number of agents established, [c]N P = Number of established agents parasitised, [d]% P = Percentage of established agents parasitised.

The post-release surveys revealed that the native parasitoids that had thus far extended their host-range to include biocontrol agents did so within three years after release. The majority of the parasitoids reared from the weed biological control agents had been identified only to the family level and therefore the native host ranges of the parasitoids were not determined. It is assumed that all the parasitoids reared from the agents have native, South African, hosts.

Table 7.5 Number of species of parasitoid per released biological control agent on weeds in South Africa.

Number of parasitoids	Agents
1	8
2	3
3	0
4	1
5	0
6	1
> 6	3

7.3.2 Predator and Parasitoid trials involving *Gratiana spadicea*.

The mirid, *Cyrtopeltis* sp. (AcRh 674) is a facultative predator, preying on other insects where available. They were observed to insert their mouthparts into the *G. spadicea* egg cases and presumably suck out the contents of the enclosed eggs. Of the 200 egg cases used in this experiment, none produced any larvae, and on dissection it was revealed that the eggs were collapsed and the contents had been sucked dry. In contrast, 82.7% of the eggs in the control egg cases hatched, 10.1% developed but failed to hatch, and 7.2% failed to develop.

The parasitoid trials produced the opposite result: no parasitoids were reared from any of the *G. spadicea* egg cases or pupae, or the control egg cases and pupae, suggesting that there was a problem with the technique used. On dissection of the egg cases, any larvae that did not emerge were malformed, and not parasitised, and all of the pupae produced adults (experimental and control).

Eulophid egg parasitoids attacking tortoise beetles have been shown to be phoretic, recognising the ovipositing female rather than the egg case itself (Carroll 1978; Becker and Romanowski 1986). Furthermore, host plants provide important cues for host location by parasitoids (Vinson 1976; Clark 1984; van Alphen and Vet 1986). The restricted experimental conditions of the parasitoid trials would have bypassed several host finding steps needed by the parasitoids to cue into the hosts. This could explain why no parasitism of the control animals (the natural hosts) occurred. Siebert (1975) did record parasitism of *G. pallidula* and *G. lutescens* under laboratory conditions; however, the details of his experimental design and quantification of results have not been recorded.

7.4 DISCUSSION

Forty percent of the insects introduced to South Africa and established as biological control agents for weeds have been parasitised by native parasitoids. This figure is a conservative estimate due to the lack of post-release data for some of the agents.

The levels of parasitism that have been recorded from the weed biocontrol agents were very low (Table 7.1). Even *C. cactorum*, which supports many parasitoid species and in spite of heavy predation of the eggs by ants (Robertson 1988), is a successful biocontrol agent

(Pettey 1948) and does not appear to be strongly affected by its natural enemy load. This suggests that for the native parasitoids the introduced agents are not favoured hosts and the parasitoid populations would probably not be maintained on the novel hosts. Two exceptions to this however are *Calcomyza lantanae* Frick which sustained a high percentage of parasitism on *Lantana camara* L. hampering its effectiveness as a biological control agent, and *Procecidochares utilis* Stone which was released in South Africa in 1984 against Croften weed (*Ageratina adenophora*). Despite establishing well, *P. utilis* has had limited success against the weed (J.H. Hoffmann, personal communication) and parasitoids were commonly recorded from it (S. Neser, personal communication) (Table 7.1). Bennett (Plant Protection Research Institute, unpublished data) suggests that the effectiveness of *P. utilis* as a control agent for *A. adenophora* might have been severely hampered by host extension by native parasitoids, as it was in Queensland, Australia, where population of the agent was severely reduced by eight native Australian parasitoid species (Dodd 1961).

In the cases of weed biocontrol reviewed by Goeden and Louda (1976), with the exception of *P. utilis* on Croften weed, parasitism appeared to be inconsequential to the degree of weed control attained. These results support the data we have presented above.

The biocontrol agents quickly (within 3 years of release) accumulate native parasitoids. These results parallel expectations for the accumulation of herbivores on introduced host plants (Strong *et al.* 1984) and suggest that common theoretical principles may govern the assembly of communities at both trophic levels (Cornell and Hawkins 1993). In the same way, the accumulation of specialist parasitoids should be much slower and will probably span evolutionary rather than ecological time scales (Cornell and Hawkins 1993). However,

introduced herbivores may have a probability of accumulating new parasitoids little or no different from indigenous herbivores. That, complications to such theoretically-expected patterns exist is illustrated by this study, which has shown that a combination of taxon (order) and guild (manner of feeding) had a significant influence on the susceptibility of the biocontrol agents to host-range extension by native parasitoids. In spite of the small sample sizes, it appeared that some orders were more susceptible to parasitoid attack than others. The low levels of parasitism in the Hemiptera can be ascribed to the fact that five of the seven established agents are in the family Dactylopidae (cochineal insects released on various cacti) (Table 7.4). No parasitoids have been recorded from any cochineal species (Goeden *et al.* 1967; Zimmermann *et al.* 1979), introduced and in their natural ranges. It is possible that carminic acid, which is an anthraquinone (Baranyovits 1978) and gives the characteristic red colour to the body contents of the cochineal insects, is an effective deterrent to parasitoids (Moran 1980).

The results also supported the hypothesis that the susceptibility of herbivores/biocontrol agents to attack is influenced by their feeding niche. Interactions between herbivores and their host plants provide important stimuli for host location and selection by parasitoids (Vinson 1976; van Alphen and Vet 1986). In this study, the poorly concealed endophage category was susceptible to attack by native parasitoids. These endophages are often sedentary and thus unable to move away from the chemical cues provided by their feeding. In addition, they are highly visible and afforded minimal protection from attack (Hawkins 1988).

Well concealed endophagous species generally support low numbers of parasitoid species (Hawkins 1988) in that they provide few outward clues to their location and are physically protected by plant tissue (Gross 1991). Hawkins (1990) suggested that concealed herbivores are attacked by generalist parasitoids that cue into the plant structure containing the insect, rather than the insect itself. In this study, however, a high percentage of well concealed biocontrol agents were attacked, and they supported a fairly rich parasitoid fauna. This could be a result of generalist parasitoids being able to extend their host range from native herbivores relatively quickly (Cornell and Hawkins 1993).

Externally feeding herbivores are able to move away from the telltale signs of their activities (Askew and Shaw 1986) and they are attacked by more specialist parasitoid complexes (Hawkins 1990). It is very likely that the relative immunity from attack afforded to the externally feeding weed biocontrol agents in South Africa is short-lived and given evolutionary time, the native parasitoids will become physiologically, behaviourally, and ecologically adapted to externally feeding biocontrol agents.

Although determining the host-range of a particular parasitoid is difficult (Askew and Shaw 1986), information regarding the host ranges of native parasitoids could be of great value when considering the introduction of a weed biological control agent which has closely related species in the country of introduction. Unfortunately this information is mostly not available at present.

7.5 CONCLUSIONS

Based on the data presented here, I do not advocate that any weed biocontrol agent be rejected due to its potential susceptibility to attack by native parasitoids. Even if, under quarantine conditions, an agent was shown to be highly susceptible to native parasitoids, I would still suggest the release of that agent. Results obtained under quarantine conditions tend to be conservative, and the behaviour of a parasitoid in the laboratory in relation to one species of host should be interpreted with care as its performance in the field may be affected by the availability of alternative hosts and its host finding procedure (Askew and Shaw 1986); in addition, it appears that even heavily parasitised species can be successful. However, I do suggest that post-release surveys to determine host-range extension by native parasitoids should not be regarded as a luxury, but should be conducted as a matter of course, since these extensions serve as convenient natural experiments to test ideas about the assembly of ecological communities, which will be of importance in predicting the fate of biological control agents when more information has accumulated.

What are the possible implications of this study for *G. spadicea* released into South Africa as a biological control agent for *S. sisymbriifolium*? The glandular trichomes on the weed (Chapter 4) will afford *G. spadicea* a certain amount of immunity to generalist predators such as ants. In addition, the faecal shield borne on the anal fork of the larvae has been shown to be an effective deterrent against generalist predators (Rolston *et al.* 1965; Eisner *et al.* 1967; Olmstead and Denno 1992, 1993). However, the egg cases of *G. spadicea* are very likely to be attacked by *Cyrtopeltis* sp. in the field, much as it is by *T. cincticornis* in South America (Becker and Frieiro-Costa 1988). This predation may hamper efforts to establish the beetle.

The similarity in biology between *G. spadicea* and *C. tigrina* implies that the parasitoids associated with the latter are likely to have the necessary adaptations for finding and parasitising both the egg cases and pupae of *G. spadicea*. Despite the contradictory laboratory evidence, I predict that within 3 years of release *G. spadicea* will support both *Tetrastichus* sp. (AcRh 520) and *Brachymeria* sp. (AcRh 518) at the same levels as those found in *C. tigrina*, and probably the same levels as they do of their native parasitoids in South America.

CHAPTER 8

GENERAL DISCUSSION

8.1 Prospects for successful biological control of *Solanum sisymbriifolium* by *Gratiana spadicea.*

The objective of this research was to investigate the potential for the biological control of the South American weed *Solanum sisymbriifolium* in South Africa. The methods employed to do this were the standard ones used in biological control programmes in South Africa, and followed a tried and tested protocol (Chapter 1). This study revealed that *S. sisymbriifolium*, whilst found predominantly in disturbed habitats, was invasive in certain parts of the country, and as such, cause for concern. The weed's copious seed production, and methods of dispersal and germination enhanced its invasive potential (Chapter 2), suggesting that it would not be controlled by conventional chemical and mechanical means. Furthermore, the pre-introductory survey (Chapter 3) showed that the weed was under only limited pressure from native herbivores, possibly a result of its taxonomic distinctness from native *Solanum* species, and its dense covering of glandular leaf hairs (Chapter 4). These considerations indicated that the weed might be a suitable candidate for biological control and the main focus of the research was the evaluation of two leaf-feeding tortoise beetles for the control of *S. sisymbriifolium.* While *G. spadicea* proved to be a suitable agent for release against the weed (Chapter 5), *M. elatior*, as a result of its wide *Solanum* host range, including eggplant (*S. melongena*) under laboratory conditions, was not considered for release (Chapter 6). This research also showed that biological control agents released in South Africa were

not likely to be immune from attack by native parasitoids, but that the effect of native parasitoids on these agents was negligible (Chapter 7).

The imminent release of *G. spadicea* affords one the opportunity to make certain predictions as to its ultimate impact on *S. sisymbriifolium* which could be tested in post-release evaluations. There are three areas in which one can make predictions, and these are: the establishment of the agent, its damage to the target weed, and the risk of releasing it.

What factors might influence the establishment of *G. spadicea* on *S. sisymbriifolium*? While this is the first release of a tortoise beetle (Cassidinae) against a weed in South Africa, four species have been released as biological control agents elsewhere, and three of these introductions have failed to establish (Julien 1987). The fourth, *Cassida rubiginosa* Müller was accidentally introduced to North America from Europe and feeds on three species of exotic thistle in Canada and a further four species in northern United States of America. However, this insect was able to complete its life cycle on artichoke (*Cynara scolymus* L.) which has precluded exploitation of it as a biological control agent in North America (Ward and Pienkowski 1978; Tipping 1993). In addition to these examples of classical biological control using members of the Cassidinae, several unsuccessful attempts have been made to establish five native Canadian species of tortoise beetle against two species of bindweed (*Convolvulus arvensis* L. and *C. sepium* L.) in Canada (Julien 1987) as "new associations" (e.g. Pimentel and Hokkanen 1989, and discussion below).

Despite the contradictory evidence in Chapter 7, it is very likely that *G. spadicea* will be attacked by the same eulophid egg parasitoids and chalcidid pupal parasitoid species that

attack native tortoise beetle species, specifically those of *Conchyloctenia tigrina*. Although it is not likely that these parasitoids will prevent the establishment of *G. spadicea*, they might hamper its effectiveness as an agent, as in the case of *C. rubiginosa*. This species was established on seven exotic thistle species in Canada and North America, but its effectiveness was severely limited by native parasitoids and its control of the thistles was negligible (Julien 1987).

The performance of other members of the Cassidinae does not therefore auger well for the possibility of successful control of *S. sisymbriifolium* by *G. spadicea*. However, as each system has its own suite of variables predictions of this sort are often unreliable.

The second question concerned the effectiveness of the beetle against the weed. How effective will *G. spadicea*, as a folivore, be against *S. sisymbriifolium*? To have an impact on a short-lived weed, such as *S. sisymbriifolium*, an agent must reduce seed set, either directly by attacking the flowers or fruit, or indirectly by attacking the non-reproductive parts of the weed to such an extent that the reproductive potential is reduced (Huffaker 1964; Harris and Zwölfer 1968). There is no purpose in introducing agents that, although host specific, do the target weed no such damage, especially in view of the potential risks involved. *G. spadicea* did cause sever damage to the leaves of the weed in the laboratory (Chapter 5). However, just how effective an agent will be remains difficult to predict from the laboratory situation even with the aid of Harris' (1973) scoring system and Goeden's (1983) revision thereof, but the following considerations are helpful.

According to Odum (1971) host plant populations cannot tolerate much feeding damage from their exploiters because host resources are limited. In general, roughly 10% of the resources of the host are passed on to exploiters, and the host utilises most of its energy and other resources for its own growth, maintenance and reproduction. As a result, even a small amount of herbivore feeding pressure can influence the abundance and distribution of host populations (Harris and Zwölfer 1968; Odum 1971). Furthermore, the competitive position of a weed species will be a function of the conditions for germination, seedling establishment, growth, production of seeds and their viability (Huffaker 1964). Returning to *S. sisymbriifolium*, the damage caused by *G. spadicea* might not appear significant in the laboratory, but it may be adequate in tipping the scale against the weed in the field. If a biological control agent places a weed under stress by its feeding activity this may also encourage additional damage to the weed by fungal, bacterial and other disease organisms (Huffaker 1964; Harris 1973).

Evidence for the effectiveness of the agent may also come from its region of origin, where *G. spadicea* occurs in high densities on the weed, and causes extensive damage to the foliage resulting in abscission of the leaves (as it does under laboratory conditions in South Africa (Chapter 5)). Kvasina and de León (1985) state that in some areas of South America, *G. spadicea* inflicts sufficient herbivory on the weed to reduce its reproductive potential. So it seems that there is a good chance of success on these bases.

So, if we assume that *G. spadicea* has established and has had an impact on the infestations of *S. sisymbriifolium*, what are the potential risks involved? How does one answer the perennial question: "What will **your** insects eat once they have finished eating the weed?".

Firstly, it must be assumed that herbivorous insects, in the absence of pressure from natural enemies (Chapter 7) are the prime factor in regulating plant populations (Brues 1946). It must be emphasised that biological control is not a "quick fix", and that the insect normally effects a gradual reduction in the weed numbers. Eradication over large areas is rarely, if ever achieved (Andres *et al.* 1976), so there will be very little selection pressure on *G. spadicea* to find an alternative host. Secondly, the chance of a basic dietary change which will cause the well-established *G. spadicea* to adopt a new host in South Africa is no less or greater than the chance of such change occurring amongst the thousands of innocuous native species.

In addition to this, Dethier (1954) has shown that the evolution from polyphagy to a restricted diet has been far more common than the reverse. Although the evolution from monophagy to secondary polyphagy has occurred it appears as though most change is towards specialisation. Since specialisation is viewed as a deepening rut in evolution, it may be concluded that while there can be no guarantee that an insect will not accept a new host, those which have long progressed in this rut of high specificity are unlikely to escape from it (Huffaker 1964). Since the insects chosen for biological control are of this type only, the safety of this method is assured. In addition, if an insect belongs to a taxon (species group, section, subgenus, or higher taxon) that is restricted to plants in one taxon (as *Gratiana* is to the genus *Solanum* (Maw 1976)), it indicates that the insect taxon has speciated on the plant taxon and that over a long time and usually wide geographical area the insect taxon has not exploited groups of other plants (Zwölfer and Harris 1971; Andres *et al.* 1976).

Despite being, in the main, highly specialised in their feeding habits (Maw 1976; Hsiao 1986) and therefore ideal biological control candidates, there are examples of Cassidinae being rejected as control agents due to a lack of host specificity. *Cassida hemisphaerica* Hbst. was screened as a biological control agent for the European weed bladder campion (*Silene cucbalis* Wibel) in North America, but was rejected because it survived on several species of ornamentals under laboratory conditions (Maw 1976). There is also the example of *Gratiana lutescens* and *G. pallidula*, considered for the biological control of *S. elaeagnifolium* in South Africa, but rejected as agents because they were successfully reared on eggplant (*S. melongena*). *Metriona elatior* can now also be added to this list (Chapter 6).

The testing of *G. spadicea* exposed a classical conflict of interests situation of the type that has long troubled the biological control of weeds. The evidence presented here (Chapters 5 and 6) supports the notion that *S. melongena*, possibly by virtue of artificial selection, is a "neutral" host for solanaceous insects (Olckers and Zimmermann 1991). If this is the case, it is highly unlikely that any suitably host specific insects will be found for the biological control of *Solanum* weeds in South Africa. Olckers (1988) suggested that the biological control of exotic solanums in South Africa had reached a crossroads and suggested two possible options. Firstly, to abandon research into the biological control of exotic solanums, and rely on conventional chemical and mechanical methods to control the weeds. Alternatively, he proposed that the problem could be approached through a thorough investigation into the status of cultivated *Solanum* species in South Africa, and suggested that the insecticidal regimes of cultivated *Solanum* species would be found to preclude them

from attack by biological control agents released against exotic *Solanum* species. Subsequent studies have supported this expectation.

The perceived conflict of interest involving *S. melongena* has now been shown to be overrated (Olckers and Hulley 1994; Olckers and Zimmermann 1994), and this new scientific approach to the biological control of weeds has resulted in permission for release of two species of *Leptinotarsa* against *S. elaeagnifolium* (Olckers and Hulley 1994) and *G. spadicea* against *S. sisymbriifolium* (Chapter 5), despite the fact that they were able to complete larval development on eggplant under the restricted conditions of the laboratory. It was suggested (Chapter 5) that the extended host range shown by *G. spadicea* was probably an artifact of the laboratory conditions and of no biological significance.

The three questions posed above may thus be answered by the following predictions: *G. spadicea* will establish on *S. sisymbriifolium*, despite the presence of native parasitoids which will have the necessary preadaptations to extend their host ranges to include the agent; it will exert sufficient herbivore pressure on the weed to reduce its reproductive potential, possibly curbing the spread of the weed; and it presents negligible risk to native or economically important *Solanum* species.

8.2 Some of the challenges facing the biological control of weeds.

Huffaker (1959, 1964) and Wilson (1964) both suggested that the results of laboratory based larval starvation tests as indicators of host specificity were unreliable and should be interpreted with care. Wilson in fact called for a relaxation of these stringent tests and stated that the importance of the weed, the difficulty in controlling it by other means and the

importance of other plants attacked during the starvation tests should also be taken into account. Since then several other authors have followed Huffaker's lead, and the potential for aberrant results produced by these tests is now widely accepted (e.g. Harris and Zwölfer 1968; Zwölfer and Harris 1971; Dunn 1978; Wapshere 1974, 1989; Cullen 1990; Shepherd 1990).

There have been several attempts to make these larval starvation tests more biologically relevant, in particular in the selection of test plants. Generally, the methods used today to choose the test plants follow Wapshere's (1974) centrifugal phylogenetic method. This method, used in the present study, involved testing the potential agent on plants closely related to the weed (native *Solanum* species) and then progressing to more distantly related plants (other Solanaceae). This method assumes that closely related plants have similar morphological and biochemical characters and that the insects cue in to the same characters that taxonomists deem important for the systematics of the group. However, host recognition and larval feeding might depend largely on a single chemical or physical character which has arisen independently in several plant taxa (Zwölfer and Harris 1971; Wapshere 1974). One can obviously not predict, or test this, but it is fortunately unlikely that unrelated plants will provide all of the necessary host finding and feeding stimuli (Andres *et al.* 1976). Harris and Zwölfer (1968) suggested that selected, unrelated crops, especially those grown sympatrically with the weed, should also be tested.

What, in view of their shortcomings, are the advantages of using larval starvation tests? Firstly, because of the conservative nature of the tests, any plant which does not support larval development in the laboratory is most unlikely to support the agent in the field.

Secondly, these tests may be used to reassure the general public and quarantine officials of the host specificity of the agent (Harris and Zwölfer 1968; Zwölfer and Harris 1971; Andres *et al.* 1976). Finally, and most importantly, biological studies of the agent, including larval starvation tests, supply the researcher with a thorough understanding of how the particular system works (Cullen 1990; Shepherd 1990).

There are several alternatives to relying on laboratory based starvation tests as a measure of host specificity. Firstly, more emphasis should be given to host records of the agent in its country of origin (Huffaker 1959; Wapshere 1989). Possibly the most compelling piece of evidence that supported the request for release of *G. spadicea* was that has not been recorded in neglected and therefore unsprayed eggplant cultivations in South America, where it is regarded as monophagous (Kvasina and de León 1985; Albuquerque and Becker 1986). There are, however, drawbacks to this approach, the insect might be relatively unknown in its country of origin, and there might be no host records for it, as was the case with *Metriona elatior* (Chapter 6) in which case one is forced to rely on the results of laboratory tests.

Secondly, the host specificity testing should ideally be conducted in the country of origin of the weed, without the restriction of quarantine laboratories (Shepherd 1990). The potential problem with this approach, apart from its high cost, is that not all of the plants required for testing would necessarily be available; for example, none of the native South African *Solanum* species against which *G. spadicea* and *M. elatior* were tested occur naturally in South America, and import restrictions would certainly prevent testing them in South America.

Thirdly, and possibly the most realistic alternative to larval starvation tests is to provide the agent with the most natural conditions possible within the limitations of quarantine. To start with, this would include testing potential agents on potted test plants in large cages and not on bouquets, cut leaves or leaf discs in petri dishes (Jones and Coleman 1988). Whereas under natural conditions insects will only feed on hosts that provide them with the necessary recognition stimuli insects tend to feed on any host that lacks feeding inhibiters under cage conditions (Harris and Zwölfer 1968).

Generally only the physiological host range of an agent is considered, the behavioural and ecological aspects to host range are not investigated (Cullen 1990). What is often neglected is that host specificity does not only entail suitability for larval development, but also host plant finding and acceptance for feeding and oviposition by the adult female (Harris and Zwölfer 1968; Zwölfer and Harris 1971). It appears that the more the host finding stimuli are bypassed during laboratory host specificity tests, the more conservative the tests become (first instar larval survival trials are the most conservative). This has lead Wapshere (1989) to propose a testing sequence where the agent is tested at the stage where most of the host finding cues have been bypassed (usually the first instar larval stage) and the plants not fed on by the agent can be regarded as safe from attack and need not be tested further. Those plant species not disregarded at this stage are used in less conservative tests which might involve larval choice tests and so on, until the insect is presented with as many host finding cues as possible under restricted conditions. This approach was followed in this study, where both agents were presented with sufficient host selection cues during the adult choice tests to obtain a realistic indication of their host ranges. This approach assumes that the researcher has a thorough understanding of the host finding mechanism of the agent.

Many biological control agents for weeds are rejected, and of those not rejected, many fail to establish (Harris 1973) and since research on each insect represents a substantial cost in time and money, selection of the best agents from the country of origin of the weed is essential (Andres *et al.* 1976). Therefore, any system that eliminated potentially poor agents prior to their importation for screening could reduce the cost of biological control considerably. There have been several attempts to produce a system that will improve the efficiency of selection of good and rejection of poor weed biological control agents.

Harris (1973), as mentioned above, proposed a system of selecting biological control agents in which the effectiveness of a potential agent is scored according to its general biology and previous success as an agent. However, many of the categories used in the scoring system are not known prior to the detailed biological studies that the system is trying to obviate, and as a result, the system is biased against poorly studied, or unknown insects (Goeden 1983). Harris' system was used to score the two insects in this study, but only after all of the laboratory trials had been completed, to try and get an idea of how their scores compared with those obtained for other agents (Chapters 5 and 6). It is felt that the scores for *G. spadicea* and *M. elatior* were underestimates of their potential as control agents for *S. sisymbriifolium* because information for several categories was lacking. Goeden (1983) produced a revision of Harris' system, which also requires a great deal of familiarity with the biology of the insect. Neither system has really streamlined selection of agents as intended, and in spite of these attempts, there is still a tendency to release host specific agents, regardless of their effectiveness scores (Goeden 1983).

Biological control agents are traditionally selected from the centre of origin of the weed species, where the greatest variety of specialised natural enemies of the weed is allegedly found (DeBach 1964). Hokkanen and Pimentel (1984) proposed a novel approach for the selection of biological control agents. They suggested that insects that have had a long association with a plant are not likely to be good biological control agents because the plant-insect system has evolved towards a state of interspecific homeostasis, they term such a relationship an old association. They state that the balance evolved in the old association between the insect and the weed might prevent them from being effective biological control agents and suggest that agents be selected from relatives of the weed species (Hokkanen 1989; Hokkanen and Pimentel 1989; Pimentel and Hokkanen 1989). This new association would lack this interspecific homeostasis and allow the insect to better exploit the weed which presumably does not have the necessary defences against the agent. In this study, this would entail isolating an insect from another *Solanum* species and importing it for screening as an agent for *S. sisymbriifolium* on the assumption that it would be a more effective agent than *G. spadicea* because *S. sisymbriifolium* has not previously been exposed to it. However, what Hokkanen and Pimentel (1989) don't seem to emphasise sufficiently is the need for adaptation on the part of the insect since the plant might have its own suite of defences (for example glandular trichomes) to which the insect has not been exposed, and to which it would need to be preadapted in order to exploit the weed. For example, new association agents are taken from plant species closely related to the weed (in this case possibly *Solanum acanthoideum* or *S. aculeatissimum*) and might therefore be able to overcome the glandular trichomes (both these South American solanums have a heavy covering of glandular trichomes, see Chapter 4). However, does this represent any less of a homeostatic equilibrium with the weed than *G. spadicea*?

Hokkanen and Pimentel (1989) have shown that the success of biological control programmes using new associations to be 75% greater than old associations. However, their analyses were based on very few examples from the biological control of weeds, and 8 of the 12 weed examples included the same agent, *Cactoblastis cactorum* on opuntias (Dennill and Moran 1986). The biological control of the *Opuntia* species are not typical of weed biological control programmes (Moran *et al.* 1986) and if these examples were excluded from the analyses, the data strongly supported the use of old associations (Goeden and Kok 1986). Goeden and Kok (1986) further questioned the host specificity of new association agents, but Pimentel and Hokkanen (1989) and Dennill and Hokkanen (1990) have provided enough evidence to show that the risks associated with host specificity are no greater in new associations than in old associations.

It appears that new associations are equally easily established, equally effective and safe for the biological control of weeds. The achievement of success in biological control is, of course, dependent on the release of agents from their natural enemies, as well as the degree of homeostasis between host and agent (Dennill and Hokkanen 1990). However, the release of an old association agent from its natural enemies would constitute enough of a shift in the insect weed homeostasis to represent a new association. Hokkanen and Pimentel (1989) do not recommend an abandonment of old associations in the control of weeds, but suggest new associations as an additional approach for selecting agents.

Despite these attempts to improve the testing methods and efficiency of weed biological control, the techniques are essentially the same as they have always been (Huffaker 1964)

and are likely to remain so. The real risk of these laboratory based tests is our ignorance of the nature of host specificity rather that the methods employed (Huffaker 1959).

8.3 CONCLUSION

Solanum sisymbriifolium is an aggressive weed which is currently not controlled by conventional means, although mechanical clearing and herbicides might still be important in its control. Any biotic pressure exerted by *G. spadicea* could augment these control measures, by reducing the above-ground biomass and fruit production of the weed. *G. spadicea* should therefore not necessarily be seen as a possible solution on its own, but as part of an integrated management scheme.

The exclusion of biological control from the management of *S. sisymbriifolium* would require continuous and costly herbicide treatments within cropland situations, while infestations outside the croplands would be likely to remain untreated. Further spread of the weed would thus be certain, aggravating an already serious situation in the southeastern Transvaal.

Finally, the idea of introducing exotic insects is still a foreign concept to the man in the street. Biological control workers should not underestimate the privilege they enjoy in understanding trophic relations, host specificity and other concepts that make it possible to see that it is very unlikely that weed biological control agents will extend their host ranges and themselves become pests (Waage and Greathead 1988).

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APPENDIX I

Preparation procedure for the removal of pollen from insects.

1] Place insect into a 50ml glass centrifuge tube.

2] Add 20ml 10%NaOH and place in a heated water bath (80-90°C) for 10 minutes, stirring occasionally.

3] Strain and wash through a 200µm mesh sieve, using distilled water. The insect is removed before filtration and is not crushed into the sieve.

4] Centrifuge and decant the supernatant. All centrifuging is done at 300rpm for 3min.

5] Wash 3 times with distilled water. On the last wash stain with 4 drops of aqueous Safranine stain.

6] Add 5ml tertiary butyl alcohol (TBA) to each tube. Stir, centrifuge and decant.

7] Transfer suspension to labelled vials using TBA, centrifuge and decant.

8] Add glycerol, equal in amount to sediment. Store uncapped for 24 hours to allow TBA to evaporate.

Mounting slides

1] Clean and label slides.

2] Pick up pollen with a small block of glycerine jelly.

3] Place glycerine jelly on centre of slide and pass slide over a warm surface (40-50°C) to melt jelly.

4] Dip warm glass rod into paraffine wax. Apply a strip of the wax to the slide around the jelly.

5] Lower a cover slip onto the jelly. If the slide is inverted the pollen grains well settle near the surface of the cover slip, making microscope viewing easier.

APPENDIX II

Preparartion of leaf specimens for scanning electron microscopy involving a freeze drying technique.

1] Pour 200ml of liquid nitrogen into a polystyrene cup. Place in a vacuum chamber and evacuate until the nitrogen has solidified. Maintain under vacuum until required.

2] Admit air to vacuum chamber, remove cup of sub-cooled nitrogen and rapidly quench freeze specimens by plunging them as fast as possible into the nitrogen. Allow to freeze for at least 60 seconds.

3] Remove specimens from the nitrogen and place in specimen tube on aluminium foil boats. Lower into a dry ice bath, connect to the vacuum pump and evacuate. Allow the ice to sublime under vacuum at a temperature of -40°C to -70°C until the specimens are completely dry.

4] Disconnect from the vacuum pump, admit air and remove from specimen tube. Mount leaf specimens on stubs, metal coat and view in scanning electron microscope.