

**HABITAT MANIPULATION TO ENHANCE
BIOLOGICAL CONTROL OF LIGHTBROWN
APPLE MOTH (*EPIPHYAS POSTVITTANA*)**

By

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requirements for the degree of

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Declaration of original contribution

I certify that this thesis does not incorporate, without acknowledgement, any material previously submitted for a degree or diploma in any university. It does not contain any material previously published or written by another person except where due reference is made in the text.

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Signature

31.03.04

Date

Publications produced from thesis

Chapters Two and Four of this thesis are reproductions of published refereed journal articles. Minor changes have been made to maintain consistency of format, spelling, added pictures and referencing style.

Chapter Two: Begum, M., Gurr, G.M., Wratten, S.D., Hedberg, P. & Nicol, N.I. (2004)

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Abstract

Trichogramma carverae Oatman and Pinto is mass-released for biological control of the leafroller pest, light brown apple moth, *Epiphyas postvittana* (Walker) in Australian vineyards. Parasitoid performance can, however, be constrained by a lack of suitable adult food and no information is available on the effect of nectar on the parasitism and longevity of *T. carverae*. To address this, the effect of alyssum, *Lobularia maritima* (L.) flowers on *E. postvittana* parasitism was studied in a vineyard experiment with and without releases of *T. carverae*. Egg parasitoid activity was assessed with *E. postvittana* egg ‘sentinel cards’ and no parasitism was recorded in plots without *T. carverae* releases. Where *T. carverae* were released, there was no significant enhancement of parasitism by the presence of *L. maritima* flowers.

Three hypotheses were subsequently tested to account for the lack of an effect: (i) *T. carverae* does not benefit from *L. maritima* nectar, (ii) *T. carverae* was feeding on nectar from other flowering plants (weeds) present in the vineyard, (iii) *T. carverae* was feeding on sugars from ripe grapes. A growth-cabinet experiment using potted *L. maritima* plants with and without flowers did not support hypothesis one. No parasitism was recorded after day two for *T. carverae* caged without flowers whilst parasitism occurred until day eight in the presence of flowers. A laboratory experiment with common vineyard weeds (*Trifolium repens*, *Hypochoeris radicata*, *Echium plantagineum*) as well as

L. maritima did not support hypothesis one but gave partial support to hypothesis two. Survival of *T. carverae* was enhanced to a small but statistically significant extent in vials with intact flowers of *L. maritima*, white clover (*T. repens*) and catsear (*H. radicata*) but not in vials with flowering shoots of these species from which flowers and flowering buds had been removed. Paterson's curse (*E. plantagineum*) flowers had no effect on *T. carverae* survival. In a laboratory study, punctured grapes significantly enhanced *T. carverae* survival compared with a treatment without grapes, supporting hypothesis three. *Trichogramma carverae* performance in the field experiment was probably also constrained by relatively cool and wet weather. Further work on the enhancement of *T. carverae* efficacy by *L. maritima* and other carbohydrate sources is warranted.

Greenhouse and field experiments were conducted to investigate whether *T. carverae* benefit from different groundcover plant species. Ten *T. carverae* adults (<24h after eclosion) were caged with different groundcover species and a control with no plant materials. *Epiphyas postvittana* egg sentinel cards were used to measure parasitism and longevity was recorded visually. Survival and realised parasitism of *T. carverae* was significantly higher in *L. maritima* than in *Brassica juncea*, *Coriandrum sativum*, shoots of these species from which flowers had been removed and nil control treatments. A similar experiment with *Fagopyrum esculentum* (with- and without-flowers) and a control treatment showed that survival was significantly higher in intact *F. esculentum* than in without-flower and control treatments. There was no significant treatment effect on parasitism in the early stages of that experiment, though parasitism was recorded in the presence of *F. esculentum* flowers for 12 days, compared with 6 days in other treatments. Higher parasitism was observed in intact *Borago officinalis* than in the flowerless shoot,

water only and no plant material control treatments in a third experiment. There was no significant treatment effect on parasitism. Fitted exponential curves for survival data differed significantly in curvature in the first, second and third experiments but the slope was a non-significant parameter in the second and third experiments.

In a second series of laboratory experiments, one male and one female *T. carverae* were caged with groundcover species to investigate male and female longevity and daily fecundity. Both male and female longevity in *F. esculentum* and *L. maritima* treatments were significantly higher than on shoots of these species from which flowers had been removed, and than in the control treatments. Daily fecundity was significantly greater in the intact *L. maritima* treatment than in all other treatments. Fitted exponential curves for daily fecundity differed significantly in position and slope but not in curvature. There was no significant treatment effect on longevity or parasitism when a male and female were caged with intact *B. juncea*, *B. officinalis* or without-flower of these species, nor in the treatment with no plant materials.

No parasitism was observed in a survey of naturally occurring egg parasitoids on two sites close to Orange and Canowindra in New South Wales, illustrating the importance of mass releases of *T. carverae* in biological control of *E. postvittana*. In an experiment on the Canowindra site, parasitism was significantly higher on day one and day two after *T. carverae* release when with-flower treatments were compared with without-flower treatments. Parasitism was significantly higher in the *F. esculentum* treatment than in *C. sativum*, *L. maritima*, vegetation without-flowers and control treatments on these dates. On day five, parasitism was higher in *C. sativum* than in all other treatments. There was no

significant increase in parasitism in a second experiment conducted on the Orange site. *Coriandrum sativum*, *F. esculentum* and *L. maritima* appear to be suitable adult food sources for *T. carverae* and offer some scope for habitat manipulation in vineyards

The adults of many parasitoid species require nectar for optimal fitness but very little is known about flower recognition. Flight cage experiments showed that the adults of *T. carverae* benefited from *L. maritima* bearing white flowers to a greater extent than was the case for light pink, dark pink or purple flowered cultivars, despite all cultivars producing nectar. Survival and realised parasitism on non-white flowers were no greater than when the parasitoids were caged on *L. maritima* shoots from which flowers had been removed. The possibility that differences between *L. maritima* cultivars were due to factors other than flower colour, such as nectar quality, was excluded by dyeing white *L. maritima* flowers by placing the roots of the plants in 5% food dye (blue or pink) solution. Survival of *T. carverae* was lower on dyed *L. maritima* flowers than on undyed white flowers. Mixing the same dyes with honey in a third experiment conducted in the dark showed that the low level of feeding on dyed flowers was unlikely to be the result of olfactory or gustatory cues. Flower colour appears, therefore, to be a critical factor in the choice of plants used to enhance biological control, and is likely to also be a factor in the role parasitoids play in structuring invertebrate communities.

Provision of nectar producing plants to increase the effectiveness of biological control is one aspect of habitat manipulation, but care needs to be taken to avoid the use of plant species that may benefit pest species. Greenhouse experiments were conducted to

investigate whether the adult *E. postvittana* and larvae benefit from nectar producing groundcover species. Newly emerged *E. postvittana* adults were caged with different groundcover species and a honey-based artificial adult diet. The longevity of male and female *E. postvittana* when caged with shoots of borage (*B. officinalis*) and buckwheat (*F. esculentum*) bearing flowers was as long as when fed a honey-based artificial diet. This effect was not evident when caged with shoots of these plants from which flowers had been removed. Longevity was significantly lower than in the artificial diet treatment when caged with coriander (*C. sativum*) or alyssum (*L. maritima*) irrespective of whether flowers were present or not.

There was no significant treatment effect on the lifetime fecundity of *E. postvittana*. A second experiment with mustard (*B. juncea*) (with- and without-flowers), water only and honey-based artificial adult diet showed no significant treatment effects on the longevity of male and female *E. postvittana* or on the lifetime fecundity of *E. postvittana*. The anomalous lack of a difference between the water and honey-based diet treatments precludes making conclusions on the value of *B. juncea* for *E. postvittana*.

Two greenhouse experiments were conducted to evaluate the effects of groundcover species on the larval development of *E. postvittana*. In the first experiment, larval mortality was significantly higher in *C. sativum*, and *L. maritima* than in *B. juncea*, *B. officinalis* and white clover (*T. repens*) a known host of *E. postvittana*. *Coriandrum sativum* and *L. maritima* extended the larval period. In *B. juncea* and *B. officinalis*, mortality did not differ from that in *T. repens*. In *F. esculentum*, larval mortality was significantly higher than in *T. repens*. A short larval period was observed on *B. juncea*, *B. officinalis* and

F. esculentum. Fitted exponential curves for larval mortality differed significantly in curvature between plant treatments. Similarly, successful pupation was significantly lower in *C. sativum*, *F. esculentum* and *L. maritima* than in *T. repens*. The percentage of successful pupation in *B. juncea* and *B. officinalis* did not differ from *F. esculentum* and *T. repens*. Fitted exponential curves for pupation differed significantly in curvature. A similar trend was observed in a second experiment with potted plants. The overall results suggest that *C. sativum* and *L. maritima* denied benefit to *E. postvittana* adults and larvae, so could be planted as vineyard groundcover with minimal risk of exacerbating this pest.

Overall results suggest that *T. carverae* require nutrients to reach their full reproductive potential and flowers provide such nutrients. *Lobularia maritima* and *C. sativum* may be considered “selective food plants” for *T. carverae* whereas *F. esculentum* appears to be a “non-selective food plant”; both *T. carverae* and *E. postvittana* benefited from it. Fruits such as grapes can be used as food resources in habitat manipulation and this merits further research. This result also suggests that within species flower colour is an important factor for flower selection in habitat manipulation.

Dedication

**To my mother and
the loving memory of my father**

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Table of contents

Declaration of original contribution	I
Publications produced from thesis	II
Abstract	III
Dedication	IX
Acknowledgments	X
Table of contents	XIV
List of tables	XVII
List of figures	XVIII
List of plates	XXII
Chapter One - General introduction	1
Australian vineyards	1
Arthropod pests of vines	1
Light brown apple moth and its pest status	2
Natural enemies of light brown apple moth	4
Current pest management	6
Habitat manipulation	8

Food supplements for parasitoids and other natural enemies	11
The selection of ground cover plant species	12
Biological control of light brown apple moth	13
Trichogrammatids in biological control	15
Research objectives.	17
Chapter Two - The effects of adult food on <i>Trichogramma carverae</i> and other natural enemies of <i>Epiphyas postvittana</i>: preliminary work	18
Introduction	18
Materials and methods	20
Results	30
Discussion	37
Chapter Three - The effects of adult food on <i>Trichogramma carverae</i> and other natural enemies of <i>Epiphyas postvittana</i>: follow-up studies	41
Introduction	41
Materials and methods	42
Results	57
Discussion	75
Chapter Four - Flower colour discrimination by <i>Trichogramma carverae</i> in habitat manipulation	78

Introduction	78
Materials and methods	81
Results	87
Discussion	94
Chapter Five -The effects of groundcover plant species on adult longevity and larval development of <i>Epiphyas postvittana</i>	97
Introduction	97
Materials and methods	98
Results	103
Discussion	113
Chapter Six – General discussion, recommendations for future research and conclusions	116
References	127
Appendices	148
Appendix One: Sample analysis of variance (ANOVA) table used for the analysis of parasitism data (Chapter Three, survival and parasitism experiment).....	148
Appendix Two: Sample multivariate analysis of variance (MANOVA) table used for the analysis of survival data (Chapter Two, weed and <i>L. maritima</i> experiment).....	149
Appendix Three: Sample exponential curves fitted for the survival data (Chapter Two,	

grape experiment one).....	150
Appendix Four: Sample Descriptive Statistics used for the analysis of an average and range numbers of eggs on the <i>E. postvittana</i> egg sentinel cards (Chapter Two, growth cabinet experiment materials and methods section).....	151

List of tables

Table 3. 1: Mean adult longevity of <i>T. carverae</i> when caged with <i>F. esculentum</i> and <i>L. maritima</i> (+ = shoots with flowers, - = shoots without flowers) and control (nil: no plant material).....	66
Table 3. 2: Mean adult longevity of <i>T. carverae</i> when caged with <i>B. juncea</i> and <i>B. officinalis</i> (+ = shoots with flowers, - = shoots without flowers) and control (nil: no plant material).....	69
Table 3. 3: Mean number of <i>E. postvittana</i> eggs predated in the Orange field experiment.....	71
Table 3. 4: Mean number of <i>E. postvittana</i> eggs parasitised by <i>T. carverae</i> in the Orange field experiment.....	72
Table 3. 5: Mean number of <i>E. postvittana</i> eggs predated in the Canowindra field experiment.....	73
Table 3. 6: Mean number of <i>E. postvittana</i> eggs parasitised by <i>T. carverae</i> in the Conowindra field experiments.....	74
Table 5. 1: Mean longevity and fecundity of <i>E. postvittana</i> when caged with shoots of different groundcover plants species (+ = shoots with flowers, - = shoots without flowers) and control (artificial adult food).....	104
Table 5. 2: Mean longevity and fecundity of <i>E. postvittana</i> when caged with shoots of <i>B. juncea</i> (+ = shoots with flowers, - = shoots without flowers), artificial food (positive	

control) and water control.....	105
Table 5. 3: Mean larval mortality and pupation of <i>E. postvittana</i> when caged with shoots of different ground cover plant species.....	106
Table 5. 4: Mean pupation of <i>E. postvittana</i> when caged with potted plants of different ground cover plant species.....	110

List of figures

Figure 2. 1: Survival of <i>Trichogramma carverae</i> when confined with flowering shoots (—▲—), shoots without flowers (—Δ—), or no plant material control (-----*-----): A = <i>Lobularia maritima</i> , B = <i>Trifolium repens</i> , C = <i>Hypochoeris radicata</i> , D = <i>Echium plantagineum</i> . Adjusted $R^2 = 85.5\%$. Points denote treatment means and lines denote fitted relationships.....	34
Figure 2. 2: Survival of <i>Trichogramma carverae</i> when confined with punctured grape (—▲—) or without grape (—Δ—): A = when water unavailable (adjusted $R^2 = 91.4\%$), B = when water available (adjusted $R^2 = 87.0\%$). Points denote treatment means and lines denote fitted relationships.....	36
Figure 3. 1: Mean survival of adult <i>T. carverae</i> when confined with: <i>B. juncea</i> with-flowers (—●—), <i>B. juncea</i> without-flowers (—○—); <i>C. sativum</i> with-flowers (—▼—), <i>C. sativum</i> without-flowers (—▽—); <i>L. maritima</i> with-flower (—▲—), <i>L. maritima</i> without-flowers (—Δ—) and control (-----*-----). Adjusted $R^2 = 98.1\%$. Points denote treatment means and lines denote fitted relationships.....	58
Figure 3. 2: Mean parasitism of <i>E. postvittana</i> by <i>T. carverae</i> when caged with: <i>B. juncea</i> with-flowers (☐), <i>B. juncea</i> without-flowers (▣); <i>C. sativum</i> with-flowers (■), <i>C. sativum</i>	

without-flowers (■); *L. maritima* with-flowers (■); *L. maritima* without-flowers (□) and control (□). Bars show the standard errors.....60

Figure 3. 3: Mean survival of adult *T. carverae* when confined with: *F. esculentum* with-flowers (—■—), *F. esculentum* without-flowers (—□—) and control (----*----). Adjusted $R^2 = 97.5\%$. Points denote treatment means and lines denote fitted relationships.....61

Figure 3. 4: Mean parasitism by *T. carverae* when caged with: *F. esculentum* with-flowers (■), *F. esculentum* without-flowers (□) and control (■). Bars show the standard errors...62

Figure 3. 5: Mean survival of adult *T. carverae* when confined with: *B. officinalis* with-flowers (—◆—), *B. officinalis* without-flowers (—◇—), control water (—+—) and control (----*----). Adjusted $R^2 = 97.1\%$. Points denote treatment means and lines denote fitted relationships.....64

Figure 3. 6: Mean parasitism by *T. carverae* when caged with: *B. officinalis* with-flowers (■), *B. officinalis* without-flowers (□); water control (■) and nil control (■). Bars show the standard errors.....65

Figure 3. 7: Mean daily fecundity of *T. carverae* when confined with different groundcover plant species: *F. esculentum* with-flowers (—■—), *F. esculentum* without-flowers (—□—); *L. maritima* with-flowers (—▲—), *L. maritima* without-flowers (—△—) and control (----*----). Adjusted $R^2 = 74.5\%$. Points denote treatment means and lines denote fitted relationships.....67

Figure 3. 8: Mean daily fecundity of *T. carverae* when confined with different groundcover plant species: *B. juncea* with-flowers (—●—), *B. juncea* without-flowers (—

○—); *B. officinalis* with-flowers (—◆—), *B. officinalis* without-flowers (—◇—) and control (—✱—). Bars show the standard errors.....69

Figure 4. 1: *Trichogramma carverae* adult emergence and subsequent survival when confined with different coloured *L. maritima* flowers or with shoots of the white-flowered cultivar with flowers removed: —▲— = white, flowers present; ----Δ---- = white, flowers removed; —□— = light pink, flowers present, —■— = dark pink, flowers present and —*— = purple, flowers present. Adjusted $R^2 = 90.2\%$. Points denote treatment means and lines denote fitted relationships.....88

Figure 4. 2: Parasitism rate of *E. postvittana* eggs by *T. carverae* for each egg release date when provided with different coloured *L. maritima* flowers: ■ = white, flowers present, ≡ = white, flowers removed (zero for all dates), □ = light pink, flowers present, ■ = dark pink, flowers present, ■ = purple, flowers present. Bars show the standard errors.....89

Figure 4. 3: *Trichogramma carverae* adult emergence and subsequent survival when confined with dyed and undyed white *L. maritima* flowers: —▲— = undyed white *L. maritima* flowers, —□— = dyed pink *L. maritima* flowers, —◆— = dyed blue *L. maritima* flowers. Adjusted $R^2 = 89.6\%$. Points denote treatment means and lines denote fitted relationships.....91

Figure 4. 4: *Trichogramma carverae* adult survival with dyed honey, undyed honey and water as food sources: —▲— = undyed 10% honey, —Δ— = 10% honey + 5% pink, ----□---- = 10% honey + 5% blue, —○— = water. Adjusted $R^2 = 73.6\%$. Points denote treatment means and lines denote fitted relationships.....93

Figure 5. 1: Mean cumulative larval mortality of *E. postvittana* when caged with shoots of

B. juncea (—●—), *B. officinalis* (----◇----), *C. sativum* (—▽—), *F. esculentum* (—■—), *L. maritima* (—▲—) and *T. repens* (—+—). Adjusted $R^2 = 94.9\%$. Points denote treatment means and lines denote fitted relationships.....107

Figure 5. 2: Mean cumulative pupation rates for *E. postvittana* when caged with shoots of

B. juncea (—●—), *B. officinalis* (----◇----), *C. sativum* (—▽—), *F. esculentum* (—■—), *L. maritima* (—▲—) and *T. repens* (—+—). Adjusted $R^2 = 94.9\%$. Points denote treatment means and lines denote fitted relationships.....109

Figure 5. 3: Mean cumulative pupation of *E. postvittana* when caged with potted plants of

B. juncea (—●—), *B. officinalis* (----◇----), *C. sativum* (—▽—), *F. esculentum* (—■—), *L. maritima* (—▲—) and *T. repens* (positive control) (—+—). Adjusted $R^2 = 99.4\%$. Points denote treatment means and lines denote fitted relationships.....112

List of plates

Plate 2. 1: Vineyard at the University of Sydney, Orange.....	21
Plate 2. 2: <i>Epiphyas postvittana</i> egg sentinel card: A = before release (arrow indicating egg mass) and B = after placement in the field.....	24
Plate 2. 3: An example of with-flower and without-flower cages: A = shoots with <i>Lobularia maritima</i> with-flowers, B = shoots of <i>L. maritima</i> from which flowers had been removed.....	27
Plate 2. 4: <i>Epiphyas postvittana</i> egg sentinel card after parasitism (arrow indicating black parasitised eggs).....	31
Plate 3. 1: Different groundcover plots at Orange: A = <i>Lobularia maritima</i> plot; B = Vegetation without-flowers plot; C = <i>Borago officinalis</i> plot and D = Control with bare earth plot.....	50
Plate 3. 2: A = Rosnay Estate vineyard at Canowindra; B = <i>Fagopyrum esculentum</i> plot; C = <i>Coriandrum sativum</i> plot.....	54

Chapter One - General introduction

Australian vineyards

Vineyards constitute Australia's largest horticultural industry and covered 158,594 ha in 2002 (Australian wine online, 2003). Wine exports have increased from \$13 million to \$2000 million in the 20 years to 2002. By 2008, wine exports are forecast to account for 67% of production, requiring approximately 18,000 hectares to be planted with wine grapes. Australia will earn an estimated Australian \$3.7 billion from those exports (Lewis, 2003). International pressures for environmental responsibility are increasing, and the 'clean and green' image of the Australian viticultural industry is a valuable component of its marketing success. It is critical to maintain this image for future expansion into the international market place. The National Vine Health Steering Committee identifies five major goals. One of these goals is to develop management strategies for established pests and diseases of national significance. Lightbrown apple moth is one such established pest, having long been of concern in Australian viticultural research (Roberts & McMichael, 2002).

Arthropod pests of vines

Seven major and 27 minor arthropod pest species are listed for Australian vineyards (Baker *et al.*, 1994). Mites (Acarina: Eriophyidae and Tenuipalpidae) mealybugs (Hemiptera:

Pseudococcidae) and lightbrown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), cause most of the damage (Bailey *et al.*, 1994). Three species of mites (*Brevipalpus* spp., *Colomerus vitis* Pagenstecher and *Calepitrimerus vitis* Nalepa) commonly cause damage in Australian vineyards (James & Charles, 1994). Two species of mealybugs (*Pseudococcus longispinus* (Targ.-Tozz.) and *P. calceolariae* (Maskell)) in southern Australia and another species (*P. affinis* (Maskell)) in Queensland and warm inland areas of Australia cause damage (Furness & Charles, 1994). Grape phylloxera (*Daktulosphaira vitifoliae* (Fitch)), which is considered to be the worst grape pest in the world, is found in Australia in some areas of Victoria and near Sydney and Corowa-Albury in New South Wales (Buchanan *et al.*, 1994). This project targets *E. postvittana* as it is considered to be a major pest (Childs, 1993). It also has local significance in the Central West of New South Wales.

Lightbrown apple moth and its pest status

Epiphyas postvittana is native to Australia (Baker *et al.*, 1994) and is also found in New Zealand, New Caledonia, the British Isles and Hawaii (Danthanarayana, 1975). In the U.S.A. and Canada it has been classified as a noxious insect (Sutherst *et al.*, 1997). It is a pest of a wide range of crop types including vine, citrus, pome and stone fruit crops, some fodder, vegetable crops, ornamentals and a variety of other plants. It is abundant in cooler regions of New South Wales, Victoria, South Australia and Tasmania. In Australia, and also in New Zealand, it is of major concern to the viticultural and pome fruit industries (Sutherst *et al.*, 1997).

Epiphyas postvittana has three-four generations in a year (Baker *et al.*, 1994).

Danthanarayana (1975) reported three distinct generations per year in Victoria: (i) summer generation (January - April) (ii) autumn-winter generation (May - September) and (iii) spring generation (October - December). A partial fourth generation may develop during the warm period from December. There are four generations per year in warmer areas: (i) winter generation (May - October), (ii) spring generation (November - December), (iii) early summer generation (January - February) and (iv) late summer generation (February - April) (Baker *et al.*, 1994). The winter generation larval stage is very long (Berndt, 2002) and larvae feed on ground cover plants such as broad-leaved weeds, medic and clover (Baker *et al.*, 1994).

Epiphyas postvittana can develop large populations. Around 1500 eggs per female is the maximum potential natality (Sutherst *et al.*, 1997) though only 14 –31% of eggs are laid because the actual fecundity is influenced strongly by temperature and the quality and variety of food plants (Sutherst *et al.*, 1997; Wearing *et al.*, 1991). Oviposition extends from 1 to 21 days and females lay >50% and >80% eggs by the sixth and tenth days respectively after emergence in the field (Wearing *et al.*, 1991). The female moth deposits 20-50 pale blue-green eggs on the upper surface of the leaves (Baker *et al.* 1994; Wearing *et al.*, 1991). The larvae pass through five or six instars (Baker *et al.*, 1994; Thomas, 1998; Berndt, 2002) or occasionally seven instars (Wearing *et al.*, 1991). The first instar larvae disperse before constructing a shelter on the underside of a leaf near the midrib or a vein (Danthanarayana, 1975). Larvae feed on the underside of leaves (Wearing *et al.*, 1991). They may construct typical leaf rolls by webbing leaves together, or may form a shelter among clusters of fruits or leaves, or may attach to fruit, leaves or buds. They continue to feed as they mature and eventually undergo pupation in the feeding sites (Danthanarayana,

1975).

On grapevines, damage is caused by larval feeding (Wearing *et al.*, 1991). The larvae feed on stems, leaves, buds and flowers and also cause internal damage by penetrating through the calyx (Wearing *et al.*, 1991). When feeding in grape bunches, they cause direct damage to the berries and make them more susceptible to infection by the fungal pathogen (*Botrytis cinerea*) (Bailey, 1997; Nair *et al.*, 1988). *Epiphyas postvittana* transmit botrytis fungus both externally and internally (Ferguson *et al.*, 1996). Externally they carry the spores with their body parts and transfer them when they move between berries in the bunch. Further, when they feed on healthy berries spores are introduced via their mouthparts. Most crop losses are caused by botrytis (Fowler *et al.*, 1999), following direct feeding of *E. postvittana* (Lo & Murrell, 2000). Therefore, it is important to control *E. postvittana* to reduce botrytis infestation. In Australia, crop loss is caused by both the spring and summer generations (Bailey, 1997; Buchanan, 1977). Therefore, *E. postvittana* populations depend largely on the climatic conditions as well as on grape varieties (Lo & Murrell, 2000). Crop loss can amount to \$2000/ha in a season, especially in the cooler grape growing areas in Australia (Buchanan, 1977).

It is important to establish economic thresholds for *E. postvittana* in grapevines so that the grower can make decisions on the basis of these thresholds (Wood, 1997). The Australian Institute for Horticultural Development developed thresholds on the basis of egg masses and larvae (Clancy, 1997). Egg mass threshold is more than three unparasitised egg masses/1000 leaves and the corresponding larval threshold is more than eight larvae/50 shoots or four larvae/50 bunches.

Natural enemies of light brown apple moth

It is well documented that predators, parasites, bacterial disease and a nuclear polyhydrosis virus all reduce *E. postvittana* populations (MacLellan, 1973; Geier & Briese, 1980; Danthanarayana, 1983). In a study in Australian vineyards, the greatest mortality of *E. postvittana* occurred due to predator attacks and less than 2% emergence from 1000 eggs was recorded (Childs, 1993). Another study in pome fruit orchards found that the greatest mortality occurred between egg-hatch and the establishment of the larvae in webbing shelters (MacLellan, 1973). The fluctuations in population size of *E. postvittana* depend largely on the effects of predation on the early stages of development (Sutherst *et al.*, 1997). The primary cause of mortality of the various stages of development is predatory arthropods (Childs, 1993; Sutherst *et al.*, 1997).

In Australia, arthropod predators include lacewing larvae (*Micromus* spp.) (Neuroptera: Hemerobiidae), spiders (Araneae, especially Theridiidae), earwigs (*Forficula auricularia* L., Dermaptera: Forficulidae), predatory mites (Acarina) and some predatory bugs such as shield bugs (Hemiptera: Miridae) (Baker *et al.*, 1994; Danthanarayana, 1983). Among these, lacewing larvae and spiders are thought to be the most important for controlling *E. postvittana* in many Australian vineyards (Bailey, 1997). In addition, some wasps have been recorded as leafroller predators, such as German wasps (*Vespula germanica* (F.)) (Hymenoptera: Vespidae) (Thomas, 1965) and *Ancistrocerus gazella* (Panzer) (Hymenoptera: Eumanidae) (Harris, 1994b). Some of these predators benefit from floral resources and flowering groundcover plants can also moderate microclimate and provide shelter for them or for alternative prey. Therefore, groundcover plants offer scope to

increase the level of pest mortality by increasing the density of naturally present predators.

Seven larval and pupal hymenopteran parasitoids belonging to five families attack *E. postvittana* in Australia and there are also two dipteran larval parasitoids from the family Tachinidae (Danthanarayana, 1983). Sutherst *et al.* (1997) reported ten species of larval and pupal parasitoids that have been determined in Australia and occur in low numbers.

Wasps of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) are egg parasitoids of *E. postvittana*. In a study in southeastern Australian vineyards, three native species of *Trichogramma* were collected from *E. postvittana* (Glenn & Hoffmann, 1997). Among these three species, only *T. carverae* has been commercially released against *E. postvittana* as a bio-insecticide, though with variable results.

Current pest management

In Australian vineyards, arthropod pest management is highly variable. Some vineyards depend on pesticides, though others do not e.g., Rosnay Estate at Canowindra, New South Wales (Richard and Sam Statham, personal communication). Use of synthetic pesticides is a potential health hazard, causes environmental disruption and has adverse effects on beneficial insects. It has been reported that *E. postvittana* moths have also developed pesticide resistance in New Zealand (Suckling, 1984). Apart from this, it is difficult to control *E. postvittana* larvae with insecticides because webbed leaf rolls protect them and limit contact with insecticide sprays, especially in dense foliage and tight grape bunches late in the season (Danthanarayana, 1975).

In Australian vineyards, growers use a reduced amount of chemicals because of international export market pressure for more sustainable production methods (Braybrook, 2001) as well as public concern about health and environmental issues (Bennett, 2002). The use of the broad-spectrum insecticides, especially chlorpyrifos, on winegrapes has become severely restricted (Braybrook, 2001). However, synthetic chemicals have not yet been completely replaced by natural pest control (Reglinski & Kingston, 2001). In Australia, *E. postvittana* is the primary insect pest in most winegrape vineyards (Hibbert & Horne, 2001; Braybrook, 2001) and its management involves several options.

Some insecticides such as Mimic (tebufenozide) and Success Naturalyte (spinosad) have been developed as alternatives to chlorpyrifos and carbaryl but there are some winery restrictions (Braybrook, 2001). According to agrochemicals registered for use in Australian viticulture 2003/2004, Mimic should be used no later than four weeks before harvest and Success Naturalyte should be used no later than when bunches are hanging down (Bell & Danial, 2003). Mimic was registered in 1997 for control of *E. postvittana* in grapes. It has attempted to provide an extra tool in integrated pest management and insecticide resistance management. It controls *E. postvittana* larvae and is non-toxic to beneficial insects and mites. Success Naturalyte provides pest-specific control with a relatively low toxicity to non-target organisms, including many beneficial insects (Braybrook, 2001).

Bacillus thuringiensis (Bt) is a “biological insecticide” which is used to control *E. postvittana* (Childs, 1993). The success of Bt is dependent on timing as Bt is only effective on first to fourth instar larvae (Childs, 1993). Environmental factors such as high UV levels or rainfall also reduce the success of Bt (Hibbert & Horne, 2001). Frequent use

of Bt is also questionable because Bt resistance has been reported (Smith *et al.*, 1995).

Mating disruption pheromone traps have been used in Australia for a number of years in commercial vineyards with variable success (Bailey, 1997). Mating disruption using synthetic sex pheromones is effective only where the densities of *E. postvittana* are very low (Bailey, 1997).

The establishment and protection of beneficial insects such as predators and parasitoids is useful to control light brown apple moth. Egg parasitoids such as *Trichogramma carverae* Oatman and Pinto (Hymenoptera: Trichogrammatidae) have been identified as a potential biological control agent as well as an alternative to the chemical control of *E. postvittana*, but are not sufficiently common to control light brown apple moth with the naturally occurring population (Buchanan, 1977). *Trichogramma carverae* is now commercially available and has been released for control of *E. postvittana* in Australian vineyards. The recommended release rate is 60,000 wasps/ha and two to three releases per season are required, with each release costing Aus \$45/ha (Bailey, 1997). Timing is important because adult longevity is short, making it essential that the release coincides with the presence of *E. postvittana* eggs. In addition, the cost of release is very high. The use of *T. carverae* as an inundative biological control agent is questionable because of these factors. Therefore further research is required to explore the scope for habitat manipulation to reduce the precision required when releasing the parasitoid and to maximize the resulting parasitism of the pest.

Habitat manipulation

Habitat manipulation involves the diversification of an agricultural ecosystem and enhances the impact of endemic or native natural enemies and also of exotic agents (Gurr & Wratten, 1999). Recent reviewers (Gurr *et al.*, 1998; Wratten *et al.*, 1998; Gurr *et al.*, 2000; Landis *et al.*, 2000) have concluded that habitat manipulation is a very useful approach to pest management. Habitat manipulation increases the effectiveness of natural enemies through altering their habitat to improve resource availability. Habitat manipulation can be carried out within a crop or at the farm or landscape level (Landis *et al.*, 2000). Habitat manipulation enhances biological control by improving the availability of alternative foods (Jervis & Kidd, 1986; Idris & Graffius, 1995) such as nectar, pollen and honeydew and by providing shelter or a moderated microclimate (Thomas *et al.*, 1992; Smith *et al.*, 1996) and alternative prey or hosts (Kelly, 1987; Bugg *et al.*, 1987).

Many studies have observed the responses of natural enemies to increasing diversity in agroecosystems through habitat manipulation. For example, White *et al.*, (1995) found increased numbers of syrphids and decreased aphid populations in cabbage (*Brassica oleracea* L.) bordered with *Phacelia tanacetifolia* Bentham. Similarly, Smith & Papacek (1991) observed that the mite *Amblyseius victoriensis* (Womersley), a predator of the phytophagous eriophyid *Tegolophus australis* Keifer, obtained supplementary food from Rhodes grass (*Chloris gayana* Kunth) when orange (*Citrus aurantium* L.) growers retained it and allowed it to flower. Furthermore, within boundary windbreaks, *Eucalyptus torelliana* acted as a reservoir of these predators. Similar refuge exists within tea (*Thea sinensis* L.) production systems, where the plucking surface of the tea bush is stratified with the perennial bush. This perennial bush acts as a refuge for natural enemies (Kawai, 1997).

In local and larger scale landscapes, structure plays an important role in the effectiveness of natural enemies. For example, Landis & Hass (1992) observed that parasitism of the European corn borer *Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae) was higher along the field margins than in the field interiors. During the growing season, natural enemy populations can be suppressed by the high temperature and low humidity that are typical of field interiors. For example, Orr *et al.* (1997) found that the survival of augmentatively released *Trichogramma brassicae* Bezdenko was increased when ryegrass (*Lolium multiflorum* Lambert) was inter-planted in seed maize (*Zea mays* L.) fields to reduce the soil surface temperature. Similarly, Alderweireldt (1994) reported that spider densities increased dramatically when 10 to 12 cm deep holes were made in the soil surface. Obviously, the microclimate within these holes was different from the untreated soil.

In habitat manipulation, the presence of alternative prey can allow establishment of generalist predators before seasonal increase of pests takes place (van Emden, 1990). For example, Kozar *et al.* (1994) showed that the presence of alternative prey on weeds or in surrounding vegetation determined predator distribution among homopteran pests in apple. Similarly, many parasitoids require alternate hosts; one of the best examples is *Anagrus* (Hymenoptera: Mymaridae), an egg parasitoid of the grape leafhopper (*Erythroneura elegantula* Osborne) which overwinters on alternative hosts outside the grape (Doutt & Nakata, 1973).

In recent reviews on habitat manipulation in Australia, Gurr *et al.* (1998) identified that this approach has largely been neglected in Australia, where very little work has been

conducted. Most research in this area has been conducted in North America and Western Europe, although in New Zealand studies on this approach are increasing (White *et al.*, 1995). To date no researchers in Australia have investigated the enhanced field performance of *T. carverae* as well as of other natural enemies in vineyards.

Food supplements for parasitoids and other natural enemies

In a variety of ecosystems, research has shown that flower nectar and pollen can increase longevity, fecundity, flight propensity and realised parasitism of hymenopteran parasitoids (Idris & Grafius, 1995; Jervis *et al.*, 1996; Wäckers *et al.*, 1996; Drumtra & Stephen, 1999; Jacob & Evans, 2000; Berndt *et al.*, 2002). The provision of non-crop plant species within an agroecosystem could therefore play an important role in biological control (Zandstra & Motooka, 1978; Baggen *et al.*, 1999). For example, Jervis *et al.* (1992) noted that non-crop plants could play an important role in providing foods for adult parasitoids in the form of nectar and pollen. Similarly, Johanowicz & Mitchell (2000) reported that *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae) and *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) survived longer when they were fed alyssum.

Honeydew is a carbohydrate-rich food supplement for some parasitoids, although its nutritional quality varies with the homopteran source (Thompson & Hagen, 1999) and it is nutritionally less valuable than nectar. Therefore, provision of nectar by plants is important for parasitoids. Various plants such as cotton (*Gossypium hirsutum* L.) and faba bean (*Vicia faba* L.) produce extrafloral nectar, which is an important food supplement for adult parasitoids (Bugg *et al.*, 1989). Pollen is also an important food resource for various entomophagous insects; for example, adult syrphids need pollen for egg maturation and its

provision can result in an increased number of syrphids and reduced aphid populations (Hickman & Wratten, 1996). Food use by *Trichogramma* spp. has been studied to some extent (Hohmann *et al.*, 1989; Steidle *et al.*, 2001) but no work has attempted to look at the field performance of fed vs. unfed *T. carverae*; this was a key objective of this project.

One aspect of food supplement for natural enemies that appears not to have received prior attention is the potential role of carbohydrates from ripening fruits. Late in the growing season this may be a rich resource and could make provision of foods such as nectar superfluous. Exploring this possibility was another aim of this study.

The selection of groundcover plant species

The selection of appropriate groundcover plant species is critical to the success of the provision of floral resources for biological control agents of arthropod pests. Some plant species are less effective than others because of morphological characteristics of the flowers or nectar quality (Idris & Grafius, 1995). In habitat manipulation, factors considered important in the selection of plant species include duration of flowering (Lövei *et al.*, 1993). Wratten & van Emden (1995) stressed that pollen- and nectar-providing plants should be in flower at the time when pest populations begin to increase. In order to implement the planting of particular groundcover plant species in a cropping system, it is important to determine whether the plant species will be compatible with the cropping system and if it will flower when necessary and for a sufficiently long period. Other factors such as nectar quality and availability (Idris & Grafius, 1995), accessibility (Wäckers *et al.*, 1996), plant morphology and chemistry (Barbosa & Wratten, 1998) have been investigated but very few researchers have considered flower-colour. Some parasitoids are known to use

flower colour during food foraging (Wäckers & Lewis, 1994). Kevan (1973) reported that most flower-visiting parasitoids visit plants with white inflorescences. Similarly, Wäckers (1994) reported that during foraging, fed *Microplitis croceipes* (Cresson) preferred green leaves and food-deprived individuals preferred yellow targets. In contrast, some other researchers (Idris & Grafius, 1997; Wardle, 1990; Oliai & King, 2000) reported that the parasitoid was not influenced by the flower colour during food foraging. Despite the paucity of knowledge, flower colour is an important factor for plant selection in habitat manipulation and this is explained in the present study for *L. maritima*.

Other factors that have been considered during plant selection are agronomic tractability of plants (White *et al.*, 1995) competitive ability with weeds, cost and availability of seeds (Wratten & van Emden, 1995). Such negative aspects as: groundcover plants serving as an alternative host to an important plant pathogen, or having potential to become a noxious weed or the pest population benefiting with resulting increased crop damage have been considered (Gurr, 1994; Gurr *et al.*, 1998). For example, Baggen & Gurr (1998) reported that both the parasitoid, *Copidosoma koehleri* Blanchard (Hymenoptera: Encytridae) and its host, *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae) obtained benefit from certain groundcover plant species (e.g., *Coriandrum sativum* L., *Anethum graveolens* L. and the extrafloral nectaries of *Vicia faba* L.), necessitating the use of 'selective' food plants such as borage (*Borago officinalis* L.) that are fed upon only by the parasitoid. Wratten *et al.* (2000) commented on the risk of pest feeding as well as enhancement of the fourth-trophic level (i.e. enemies of biological control agents). Therefore, the selection of ground cover plant species needs to be done in a more careful and systematic manner.

Biological control of light brown apple moth

Biological control is a pest management method that is self-sustaining and presents a relatively low risk to humans and to the environment (Gurr & Wratten, 1999). The three approaches to biological control are classical, augmentative or inundative and conservation (van Driesche & Bellows, 1996). Classical biological control involves the introduction of new natural enemies into an area to control pests; these control agents breed to establish control over a wide area on a permanent basis e.g., vedalia beetle (*Rodolia cardinalis* (Mulsant)) (Coleoptera: Coccinellidae) in Californian citrus orchards (Doutt, 1964, cited by Gurr *et al.*, 2000). In augmentative or inundative biological control, the control agent does not persist from season to season and requires the repeated release of large numbers of natural enemies to control the pest - for example, the parasitoid wasp *T. carverae* that is released in Australian vineyards. Conservation biological control differs from the two other approaches and does not rely upon the introduction of new natural enemies in an area. Rather it creates a suitable ecological infrastructure to conserve and increase efficacy through habitat manipulation (Landis *et al.*, 2000).

Biological control of *E. postvittana* involves Bt, *T. carverae* and other natural enemies. *Bacillus thuringiensis* is an effective and safe biological insecticide that kills only the target pest (Stirrat, 1990). Use of *T. carverae* by vineyard managers has however been limited because of the high release cost (released wasps typically live for less than one week) and the need for two to three releases per season (Gurr *et al.*, 1998). Under these circumstances, conservation biological control can enhance the efficacy of *T. carverae* (Gurr *et al.*, 1998). When classical biological control is coupled with habitat manipulation techniques, the agent's requirements for nectar, pollen, a moderated microclimate or alternative hosts or

prey are met, allowing the agent to reach its full potential. The work proposed for this project will explore the potential utility of habitat manipulation strategies for control of *E. postvittana*, because no work has been conducted to date on biological control of *E. postvittana* through habitat manipulation in Australia.

Trichogrammatids in biological control

Trichogramma is a well known egg parasitoid and is widely used as an insect biological control agent worldwide (Stinner, 1977). The genus *Trichogramma* includes more than 100 species throughout the world (van Lenteren, 2000). They are present in agroecosystems where they attack eggs of Lepidopterans. Sometimes they are not present in sufficient numbers to control pests and need to be released to overcome these situations (Bennett, 2002). Every year, 32 million hectares of agricultural and forestland are treated with *Trichogramma* (van Lenteren, 2000) and more than 50 countries have investigated inundative releases for control of Lepidopteran pests (Smith, 1996). China, France, India, Russia Taiwan, Iran, The Philippines, Colombia and Peru have used *Trichogramma* extensively to control 28 different Lepidopteran pests (Hassan, 1993) attacking corn, rice, wheat, sorghum, sugarcane, sugar beet, cotton, soybean, cabbage, tomato, beans, apple, avocado, wine grape, pine and spruce forests and this list is increasing (van Lenteren, 2000). For economic reasons and due to the intensive use of pesticides, the application of *Trichogramma* is limited in Japan, South East Asia, South America, USA, Canada and Europe (van Lenteren, 2000).

Trichogramma brassicae has been used to control *Ostrinia nubilalis* Hübner (European corn borer) in Europe with the most consistent and best-documented success (Smith, 1996).

In India, parasitism of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) was high in sorghum, low in pigeonpea and failed in chickpea (Romeis *et al.*, 1999a; Romeis *et al.*, 1999b). Thus though many inundative releases of *Trichogramma* appear to be successful (Keller & Lewis, 1985), others have been only partially successful (Stinner, 1977) and parasitism rates correlate poorly with release rates (Andow *et al.*, 1995). This might be explained in several ways. One of the most important factors is the survival of *Trichogramma*, because after emergence *Trichogramma* does not persist very long in the field. The success of *Trichogramma* inundation depends largely on its survival. The lifespan of *Trichogramma* is very short (Newton & Odendahl, 1990) typically less than 7 days when no food source is available (Glenn & Hoffmann, 1997; Gurr & Nicol, 2000). The length of time *Trichogramma* is active is directly determined by its longevity (Mansfield & Mills, 2002) and this can constrain the numbers of host parasitised (Ashley & Gonzalez, 1974; Yu *et al.*, 1984). Many agroecosystems are devoid of food sources for *Trichogramma*. As a result, after mass release, *Trichogramma* either die due to starvation or spend time searching food sources before parasitising hosts (Lundgren *et al.*, 2002).

In the laboratory, *Trichogramma* longevity and fecundity is greatly increased with provision of food in the form of sugar (Gurr & Nicol, 2000; Hohmann *et al.*, 1988; McDougall & Mills, 1997; Leatemia *et al.*, 1995). Theoretically this improves biological control (Jervis *et al.*, 1996; Gurr & Wratten, 1999). *Trichogramma* spp. gets benefit from food (nectar/pollen) sources in the field as higher rates of parasitism have been recorded in cotton with extra-floral nectaries cotton compared with nectarless cotton (Treacy *et al.*, 1987). Similarly, the longevity of honey fed female *T. minutum* Riley increased 9-fold and fecundity 6-fold compared with unfed wasps (Yu *et al.*, 1984). Furthermore, intercropping

floral nectar increases parasitism by other minute parasitoids (Jervis *et al.*, 1996).

In Australia, seven *Trichogramma* species have been identified and *T. carverae* is commercially available for inundative release against *E. postvittana* in Australian vineyards. The inundation of *T. carverae* is questionable because of its field performance. This project aimed to test the benefits of providing floral nectar as food for *Trichogramma* through habitat manipulation.

Research objectives

The overall aim of this project was to investigate the extent to which groundcover plant species may enhance natural enemies, predators and parasitoids, particularly *T. carverae* a parasitoid of *E. postvittana*, through habitat manipulation, thus constituting the groundwork for the future development of a new pest management approach for *E. postvittana* in vineyards. The specific objectives of this research were:

- (1) To investigate the effect of sugar-rich exudates from ripening grapes on longevity and fecundity of *T. carverae* in the laboratory. This objective is addressed in chapter 2.
- (2) To screen a variety of potential groundcover plant species in the laboratory to determine which confer the greatest longevity and fecundity benefits to *T. carverae*. This objective is addressed in chapters 2 and 3.
- (3) To investigate the effect of the most promising groundcover plant species on *E. postvittana* and *T. carverae* in the field. This objective is addressed in chapters 2 and 3.
- (4) To investigate flower colour discrimination by *T. carverae* in habitat manipulation. This objective is addressed in chapter 4.

(5) To investigate the effects of groundcover plant species on adults and larvae of *E. postvittana* in the laboratory. This objective is addressed in chapter 5.

Chapter Two - The effects of adult food on *Trichogramma carverae* and other natural enemies of *Epiphyas postvittana*: preliminary work

Introduction

Conservation biological control aims to increase the impact of existing natural enemies rather than use inoculative or inundative releases of agents as in other forms of biological control (Barbosa, 1998). Much attention has been given to reducing the adverse effects of pesticides on pests' natural enemies but only in recent years have habitat manipulation approaches been explored in depth (Gurr *et al.*, 2000; Landis *et al.*, 2000). These include provision of shelter, alternative hosts or prey, or food plants from which nectar and pollen may be obtained (Landis *et al.*, 2000). Provision of such resources offers scope to enhance the fecundity, longevity and behaviour of natural enemies to increase their effectiveness. Manipulating non-crop plant species within an agroecosystem can have an important role in conservation biological control of pests (e.g., Zandstra & Motooka, 1978; Hickman & Wratten, 1996; Baggen *et al.*, 1999). Jervis *et al.* (1992) noted that non-crop plants could provide nutrients for adult parasitoids. Flower nectar can increase longevity, fecundity and parasitism rate by hymenopteran parasitoids (Idris & Grafius, 1995; Jervis *et al.*, 1996; Wäckers *et al.*, 1996; Drumtra & Stephen, 1999; Jacob & Evans, 2000; Berndt *et al.*, 2002). Similarly, some predators benefit from floral nectar. For example lacewings (Long *et al.*, 1998) and

Ancistrocerus gazella (Panzer) (Hymenoptera: Eumenidae) (Harris, 1994a) are known to feed on floral resources. Alyssum (*Lobularia maritima* (L) (Cruciferae) has been used in previous studies of parasitoid enhancement. For example, Johanowicz & Mitchell (2000) found that *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae) and *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) survived longer when they visited alyssum flowers.

Trichogramma carverae Oatman and Pinto (Hymenoptera: Trichogrammatidae) is a natural enemy of the light brown apple moth (LBAM), *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), a serious insect pest of Australian grapevines (Glenn & Hoffmann, 1997). Though recent damage estimates are not available, Buchanan (1977) estimated losses at up to \$2000/ha in a season, especially in the cooler grape growing areas. *Epiphyas postvittana* is a native Australian insect known to feed on 73 plant species. In cooler areas of Australia there are three generations in a year: (i) winter generation, (ii) spring generation and (iii) summer generation. There are four generations a year in warmer areas: (i) winter generation, (ii) spring generation, (iii) early summer generation and (iv) late summer generation (Baker *et al.*, 1994). Female moths lay scale-like eggs in masses of 20-50 on the upper surfaces of opened vine leaves (Baker *et al.*, 1994).

Trichogramma carverae, an endemic Australian parasitoid, is a particularly important target for enhancement by conservation biological control because inundative releases are already being used to augment natural populations in vineyards (Glenn & Hoffmann, 1997). These mass releases are, however, expensive and provision of appropriate adult food offers scope to increase the impact of a given release on pests though maximising fecundity, longevity and other aspects of the parasitoid's fitness. Some research has been done on the importance of

adult food for *Trichogramma* spp. The longevity of *Trichogramma* spp. is decreased when no sugar source is available (Newton & Odendael, 1990) and fewer hosts are parasitised by unfed females (Ashley & Gonzalaz, 1974). Somchoudhury & Dutt (1988) reported that parasitism by *T. perkinsi* Girault was increased significantly by access to flowers of Bengal gram (*Cicer arietinum* L.). *Trichogramma platneri* Ngarkatli survived longer when fed (Hohmann *et al.*, 1989). No previously published studies have evaluated the importance of nectar availability in the performance of *T. carverae*, although Gurr & Nicol (2000) examined the effects of a honey diet under laboratory conditions. They showed that the longevity of *T. carverae* (and *T. nr brassicae* Bezdenko) was improved by the availability of adult food. The present study investigated the effect of nectar from various flowers, including *L. maritima*, as well as other potential foods, on the longevity and parasitism of adult *T. carverae* in the field and laboratory.

Materials and Methods

Field experiment

Study Site

The experiment was carried out in the University of Sydney's Chardonnay vineyard at Orange, Australia (Plate 2.1). It was conducted in the 2.3 ha northern section of the vineyard in which no insecticides had been applied that season. Ripening grapes were present in the vineyard throughout the experimental period (see dates below). Of the 44 rows, numbers 5, 10, 15, 20, 25, 30, 35, 40 were designated as blocks. Each of the eight blocks comprised one replicate of each of four treatments. A factorial design was used, with/without flowers and with/without releases of *T. carverae*, giving four different

treatment combinations. Plots were 1.5 m long and 0.3 m wide, each separated by 30 m along the vine row.



Plate 2. 1: Vineyard at the University of Sydney, Orange.

Flower treatment

Differently coloured (white, light pink, dark pink and purple) cultivars of flowering *L. maritima* plants were purchased from a nursery (cvs. Small Flower White, Small Flower Pink, Plum Crazy, and Small Flower Purple, respectively; Oasis Horticulture Pty Ltd, Wimalee, Australia). A mixture of cultivars (eight Small Flower White, three Small Flower Pink, three Plum Crazy and two Small Flower Purple *L. maritima* plants; 16 plants in total) was planted into each plot allocated to the treatments with flowers. Plants were positioned

beneath vines in a single row, spaced 10 cm apart, on 2 April 2002. Plants were watered every day and 16 g of Yates 'Thrive' soluble fertilizer mixed with 9 L of water was applied once per week to each block.

The existing vineyard floor was dominated by grasses but contained some broadleaved weeds. At the start of the experiment, a motorised brush cutter was used to remove the flowers and unopened flower buds from the weeds on either side of the plot and for a distance of approximately 10 m along the row from either end of each plot.

Effect of L. maritima on parasitism and predation rate

Capsules containing paper substrates bearing *Sitotroga cerealella* Oliver eggs parasitised by *T. carverae* were purchased from Bugs for Bugs, Bio Resources Pty. Ltd. Mundubbera, Queensland, Australia. Capsules were kept in an incubator at 28 °C. On 13 April 2002, after three days in the incubator, *T. carverae* was starting to emerge in an "indicator vial" prepared by the supplier such that adult eclosion occurred approximately 24 h before the adults in the rest of the consignment. All capsules were then taken out of the incubator and kept for 6 h at 22 °C in preparation for placement in the field. Rain, however, required postponement of release until 15 April. Capsules were stored in accordance with the supplier's directions in a refrigerator at 8 °C, except for 4 h at 22 °C immediately prior to release. Three capsules were placed in each plot allocated to the *T. carverae* treatment (i.e., in one plot with and one plot without flowers in each block). In each plot, two capsules were mounted with wire on a bamboo cane (0.6 m) pushed into the soil in the middle of the plot so that capsules were 0.3 m above the soil surface. The third capsule was stapled on to the vine leaves 1.5 m above the soil. A single, additional capsule enclosed in a ventilated

glass vial was positioned in row 40. Emergence of adults into this vial indicated the approximate emergence of adults from other capsules.

On 16 April 2002, within 24 h of *T. carverae* emergence, *E. postvittana* egg sentinel cards were stapled onto the upper surface of six vine leaves in each plot. *E. postvittana* eggs were obtained from the Department of Natural Resources and Environment, Victoria, Australia. Cards were prepared by cutting the plastic substrate into sections, each bearing an egg mass (Plate 2. 2A). Each section was stapled to a yellow paper sheet (5 × 1cm). Because acceptance of *E. postvittana* eggs by *T. carverae* is affected by age of host (Glenn & Hoffmann 1997), available *E. postvittana* egg masses were divided into two groups: green (younger) and orange (older). Microscopic examination (10×) was used to count the number of eggs in each mass and this was recorded on each paper sheet. Cards with green eggs bore an average of 19.35 (range, 4-55; with half bearing 11-25), whilst those with orange eggs bore an average of 20.21 (range, 4-48; with half bearing 13-26). In each plot, one card of each egg type was stapled to vine leaves 1 m from the soil at each end of the plot. An additional egg mass of each colour was stapled onto two central vine leaves 1.5 m above the soil (total, six cards per plot) (Plate 2. 2B). The spacing between green and orange egg sentinel cards was approximately 5 cm in each case. After 48 h, sentinel cards were recovered from the field and surviving eggs counted under a binocular dissecting microscope (10×) to determine predation rates. Each card was then placed in a small, sealed plastic bag and kept in the laboratory at room temperature (19 °C- 23 °C) until parasitised eggs became black and were then counted (Plate 2. 4). On 22 April 2002 (six days after the first release), fresh egg sentinel cards were placed and recovered after 48 h and handled as before.

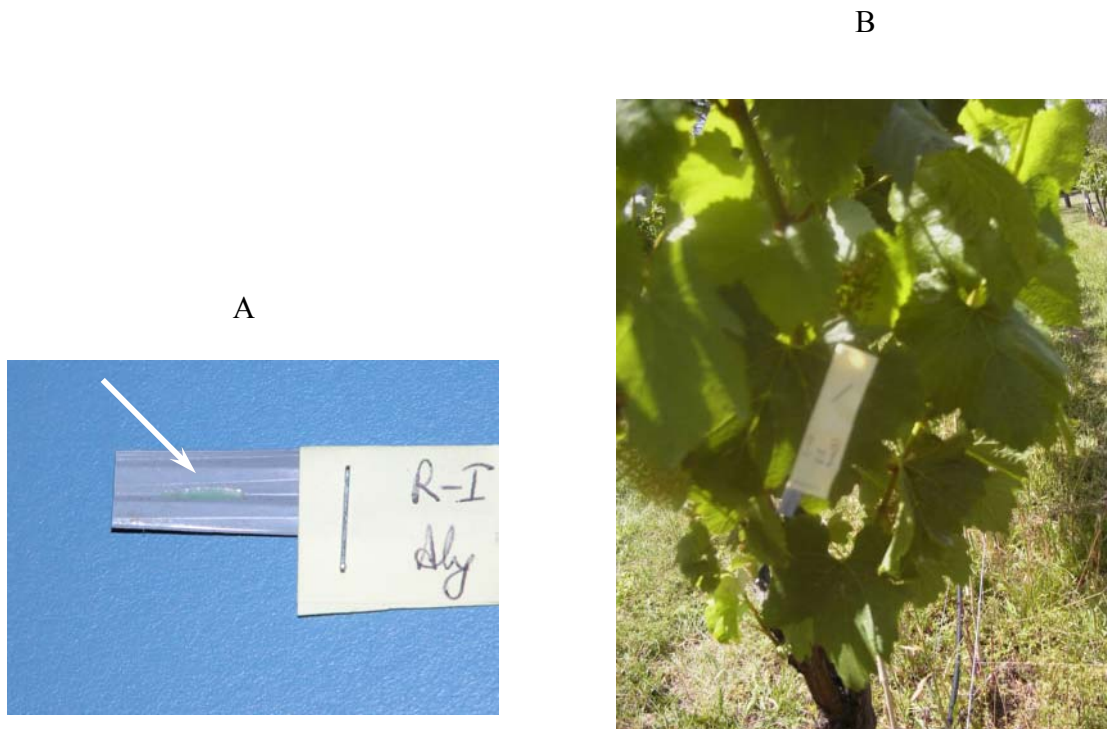


Plate 2. 2: *Epiphyas postvittana* egg sentinel card: A = before release (arrow indicating egg mass) and B = after placement in the field.

A rain gauge and maximum/minimum thermometer were placed in the field and data recorded daily at 9am and 5pm. A single *E. postvittana* sex pheromone trap was positioned in the south section of the vineyard, 20 m from the nearest point of the experiment, to monitor *E. postvittana* activity.

Data analysis

The numbers of eggs parasitised and predated were calculated separately for green and orange eggs. Numbers of green and orange eggs were tested as a covariate, found to be non significant and then excluded. A split-plot analysis of variance model was then used to compare the numbers of parasitised orange and green *E. postvittana* eggs on each date. With/without flowers and with/without release of *T. carverae* were used as main-plot factors and egg age as the sub-plot factor. GenStat release 6.1 (GenStat Committee, 2002) was used for analyses.

Growth cabinet experiment

Plant and insect material was sourced and prepared as described above. The experimental design was a randomised block with eight replicates and two treatments (with and without flowers). Each plot consisted of a white-flowering *L. maritima* plant (cv. Small Flower White) in a black plastic pot (850 ml). In the first treatment (without-flowers), all the flowers and unopened flower buds were removed prior to the start of the experiment. The second treatment used intact plants with flowers. Plants were covered with fine nylon mesh supported by a single bamboo cane in each pot and secured around the pot's rim with rubber bands. Half of a *T. carverae* capsule was attached to the bamboo cane, 20 cm above the potting mix surface. The experiment was conducted in a growth cabinet (Thermoline L+M, Smithfield, Australia) with a 16L: 8D photoperiod, 25L/15D°C temperature and 65L/55D% humidity. The start of emergence of adult *T. carverae* was determined by use of a segment of capsule held in a ventilated glass vial. On day two after emergence, three *E.*

postvittana egg sentinel cards (as used in the field experiment) were placed in each cage through the base of the mesh cover. These cards were recovered after 24 h. Cages were opened only during the dark and cool phase of the photoperiod when *T. carverae* would be inactive and escape would be minimised. Sentinel cards were secured to the bamboo cane by wire ties with two positioned 8 cm and one 15 cm above the potting mix. The mean number of eggs per card was 17.30 (range, 3-60; with half bearing 9-22 eggs). Fresh sentinel cards were similarly placed on days 5, 8, 11, 14, and 17. Recovered sentinel cards were kept in individual, sealed plastic bags in an unlit incubator at 23°C until parasitised eggs became black. They were then counted.

Data analysis

The numbers of eggs parasitised were calculated per plot and analysed using randomised block ANOVA on day two. A square root transformation $\sqrt{(x + 0.5)}$ was used to standardise the variance for pooled data from all dates and a comparison made between with- and without-flowers treatments using randomised block ANOVA. Square-root transformation was chosen rather than untransformed actual data after inspection of residuals.

Weed and *L. maritima* flowers experiment

Shoots of *Echium plantagineum* L., *Hypochoeris radicata* L. and *Trifolium repens* L. were collected from the vineyard in which the earlier experiment had taken place and *L. maritima* shoots (cv. Small Flower White) taken from greenhouse-grown plants. The cut ends of shoots were placed immediately in tap water and the experiment began within six hours of shoot collection. Each plant species was represented twice within each block: in an intact state and with the flowers removed from the shoots (Plate 2. 3A and B). Without-flower treatments

were used to control for the effect of plants on the microclimate within cages. An additional control treatment in each block contained no plant material. The experiment was replicated five times. In all flowering treatments, each shoot had sufficient buds to ensure continuous flowering throughout the experimental period. Plastic vials (5.5 × 4.5 cm) were used as flight cages. The bottom of each vial had a circular hole (1 cm diameter) through which the cut ends of shoots of the designated type were passed into water contained in a second vial (11 × 2.5cm) beneath the first. Shoots were sealed into the holes with non-setting adhesive. One quarter of a *T. carverae* capsule was placed into each vial. The top of the upper vial was then sealed with a sheet of tissue paper held in place with a rubber band. These flight cage assemblies were supported in racks. The numbers of live *T. carverae* present in each cage was assessed by microscopic examination (10×). Assessments were made daily for each cage until all its parasitoids had died. The experiment was laid out on a laboratory bench top and temperature ranged from 18.1-22.9°C with an average relative humidity of 56%.

A

B



Plate 2. 3: An example of with-flower and without-flower cages: A = shoots with *Lobularia maritima* with-flowers, B = shoots of *L. maritima* from which flowers had been removed.

Data analysis

A square root transformation $\sqrt{(x + 0.5)}$ was used for number of live insects on each day to standardise the variance. Square-root transformation was chosen rather than untransformed actual data after inspection of residuals. The effect of treatments was tested by a repeated measures approach multivariate analysis of variance (MANOVA). Because it is not possible to include in a MANOVA times where all replicates of one treatment yielded zeros, only the first nine days after the peak in insect counts were analysed, not the following four days when no *T. carverae* were living in one or more treatments. The significance of differences between treatments and days were determined using *F*-tests. The *F*-test in MANOVA is an approximation to the *F*-distribution. Exponential curves of the

form $y = A + BR^x$ (where y = number of live insects, x = number of days, A and B = linear parameters, R = survival rate) were also fitted to the data (omitting dates before the date with maximum wasp numbers) to compare the differences in shape and position for each treatment. First, an overall curve was fitted, then a set of curves with a different position (A) for each treatment, after that a set of curves with different position and slope (A and B) for each treatment and finally a set of curves with all (A , B and R) parameters separate. The steps defined above were accomplished automatically using the standard module for comparing non-linear regression in GenStat release 6.1. However, where curvature, representing the key biological parameter of insect survival, differed between treatments, comparison of the linear parameters becomes irrelevant. GenStat release 6.1 (GenStat Committee, 2002) was used for data analyses.

Grape experiment one

Table grapes (cv. Waltham Cross) were purchased from a supermarket and insect material obtained as described above. A randomised block design with ten replicates and two treatments (with and without grapes) was used. Glass vials (2×2.5 cm) with ventilated lids contained either a single grape punctured by a needle and suspended by a wire, or a wire with no grape (control). Twenty adult *T. carverae* (< 24 hours after eclosion) were released in each vial. Adult *T. carverae* were transferred to the vials using a gelatine capsule (size 00) (Tyco Healthcare Pty Ltd., Sydney, NSW, Australia) to avoid the need for anesthesia, allaying possible deleterious effects on behaviour or longevity. Within 24 h of emergence *T. carverae* do not fly (personal observation). Young adults were put on a white paper sheet (for ease of visibility) and each adult covered with half of a gelatine capsule. Adults crawled upwards inside the capsule, which was then lifted up and sealed with the other half

of the capsule. Adults were introduced into flight cages by separating the capsule halves and tapping the half containing the insect against the top of the vial. After adding 20 adults to each cage, the top was sealed and the assembly examined under a microscope (10×) to confirm that 20 live adults were present. The experiment was housed in a sealed plastic box (38 × 25 × 14 cm) in which a layer of free water maintained the relative humidity above 96%. The box was held at 24 – 25°C in an unlit incubator. Numbers of live adults were assessed by microscopic examination (10×) every 24 h until no live parasitoids were observed.

Data analysis

Data were analysed as described for the weed and *L. maritima* flowers experiment.

Grape experiment two

The with- and without-grape treatments, experimental design and execution was the same as for grape experiment one, except that a drop of water was added to each vial every day to avoid dehydration of *T. carverae*.

Data analysis

Transformed data were analysed as above.

Results

Field experiment

Predation of *E. postvittana* eggs did not differ significantly between with- and without-flower treatments on either date and was low, never exceeding 0.66%.

There was no significant effect of flowers on parasitism by *T. carverae* of *E. postvittana* eggs. Parasitism was recorded on *E. postvittana* sentinel cards only in plots in which *T. carverae* had been released except for one egg in one plot where wasps were not released. Overall rates of *T. carverae* parasitism were low, 1.06 ± 0.342 in with- and 0.87 ± 0.342 in without-flower treatments on the first release date. On the second release date, equivalent values were 1.31 ± 1.046 and 2.00 ± 1.046 respectively.

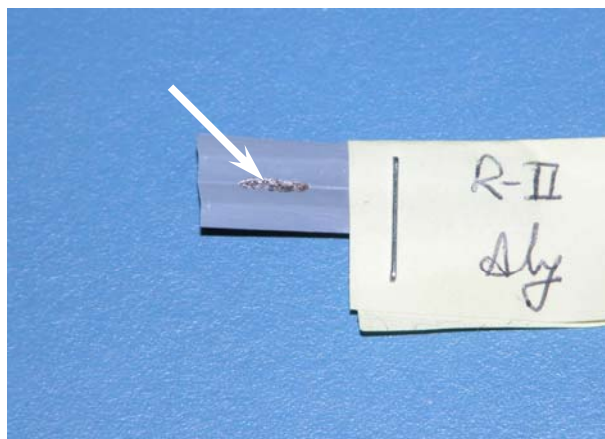


Plate 2. 4: *Epiphyas postvittana* egg sentinel card after parasitism (arrow indicating black parasitised eggs).

The age of *E. postvittana* eggs had no significant effect on parasitism. The mean parasitism was 0.87 ± 0.347 for younger (green) eggs and 1.06 ± 0.347 for older (orange) eggs on the first release date. Equivalent values for the second release date were 1.94 ± 0.798 and 1.37 ± 0.798 respectively.

During the nine-day period of monitoring *T. carverae* activity with sentinel cards, the mean daily rainfall was 3.17 mm with average daily temperatures of 29°C maximum and 10°C minimum. During the experimental period, an average of 4.67 *E. postvittana* adults were caught in the sex pheromone trap.

Growth cabinet experiment

Parasitism was significantly higher ($F= 9.75$; $df = 1, 7$; $P = 0.017$) in the with-*L. maritima* flower (4.75 ± 0.647) (mean \pm SE) than without-flower (1.89 ± 0.647) treatments when data from all dates were pooled. Parasitism was higher in the with-flower treatment on day two of the experiment (20.6 ± 4.76) compared with (5.6 ± 4.76) in without-flower treatment, though the significance of the treatment effect fell just outside the conventional threshold ($F= 4.96$; $df = 1, 7$; $P = 0.061$). On day five and day eight the mean numbers of eggs

parasitised in the with-flower treatment were 4.6 ± 1.64 and 3.0 ± 2.34 respectively. There was no parasitism in the control treatment (without flowers) on these dates, nor in either treatment on later dates.

Weed and *L. maritima* experiment

The MANOVA analysis of *T. carverae* survival showed significant interaction of treatment and time (days) ($F = 1.83$; $df = 72, 154$; $P < 0.001$). The mean number of live adults in the intact *T. repens* flower treatment on day one and day nine differed significantly ($P = 0.03$ and $P = 0.02$ respectively) from the control and the shoots without flower treatments. On day two, the numbers of live adults in the with-flower treatments were significantly ($P < 0.001$) greater than in the control treatment. The mean number of live adults in the intact *L. maritima* flower treatment on day two and nine differed significantly ($P < 0.001$ and $P = 0.02$ respectively) from the control values and also differed significantly from the shoots without flower treatment on day nine ($P = 0.02$). The mean number of live adults in the intact *H. radicata* flower treatment on day one and two and differed significantly from the control values and also differed significantly from the shoots without flower treatment on day nine. No treatment effects were significant for *E. plantagineum*.

Fitted exponential curves differed significantly in position ($F = 3.75$; $df = 8, 603$; $P < 0.001$) and slope ($F = 2.97$; $df = 8, 603$; $P = 0.003$) though curvature ($F = 1.63$; $df = 8, 603$; $P = 0.112$) was a non-significant parameter between the nine treatments (i.e. control and with-and without-flowers in each of the four flower species). Survival rates on intact flowers did not differ significantly between plant species (Figure 2.1). The daily survival

rates (\pm standard error) of *T. carverae* were: *T. repens*, 0.904 ± 0.0224 ; *L. maritima*, 0.888 ± 0.0235 ; *H. radicata*, 0.849 ± 0.0203 and *E. plantagineum*, 0.882 ± 0.0272 . In the treatments that used a stem from which flowers were removed, equivalent values were 0.860 ± 0.0225 ; 0.859 ± 0.0278 ; 0.825 ± 0.0240 and 0.833 ± 0.0308 respectively and in the control treatment, 0.836 ± 0.0307 .

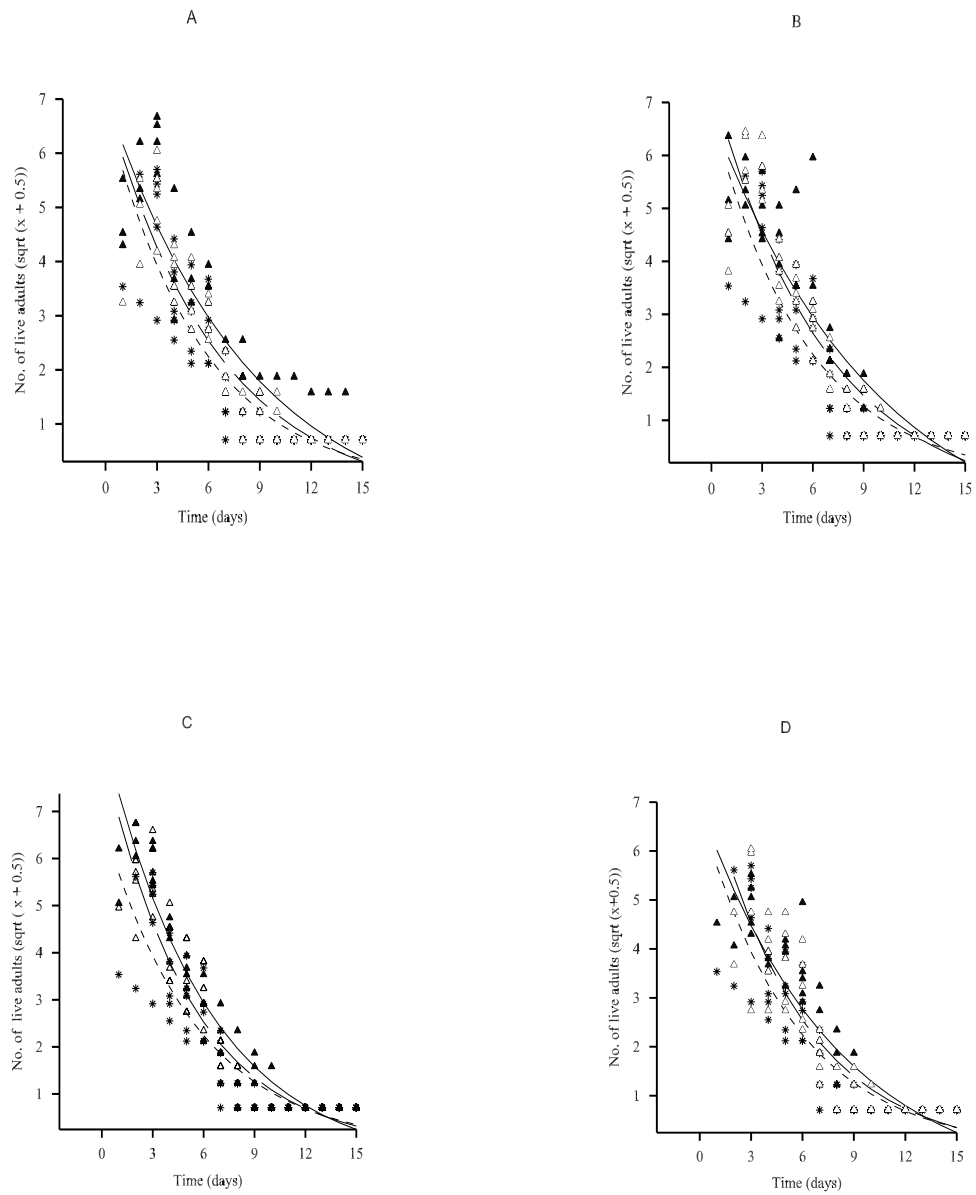


Figure 2. 1: Survival of *Trichogramma carverae* when confined with flowering shoots (—▲—), shoots without flowers (---△---), or no plant material control (----*----): A = *Lobularia maritima*, B = *Trifolium repens*, C = *Hypochoeris radicata*, D = *Echium plantagineum*.

Grape experiment one

Fitted exponential curves for treatments differed significantly in curvature ($F = 197.21$; $df = 1, 234$; $P < 0.001$). The survival rate (\pm standard error) of *T. carverae* in the with-grape treatment (0.776 ± 0.0150) was significantly higher than in the without-grape treatment (0.032 ± 0.0504) (Figure 2. 2A).

Grape experiment two

Fitted exponential curves for treatments differed significantly in curvature ($F = 57.22$; $df = 1, 174$; $P < 0.001$). The survival rate of *T. carverae* in the with-grape treatment (0.702 ± 0.0306) was significantly higher than in the without-grape treatment (0.320 ± 0.0440) (Figure 2. 2B).

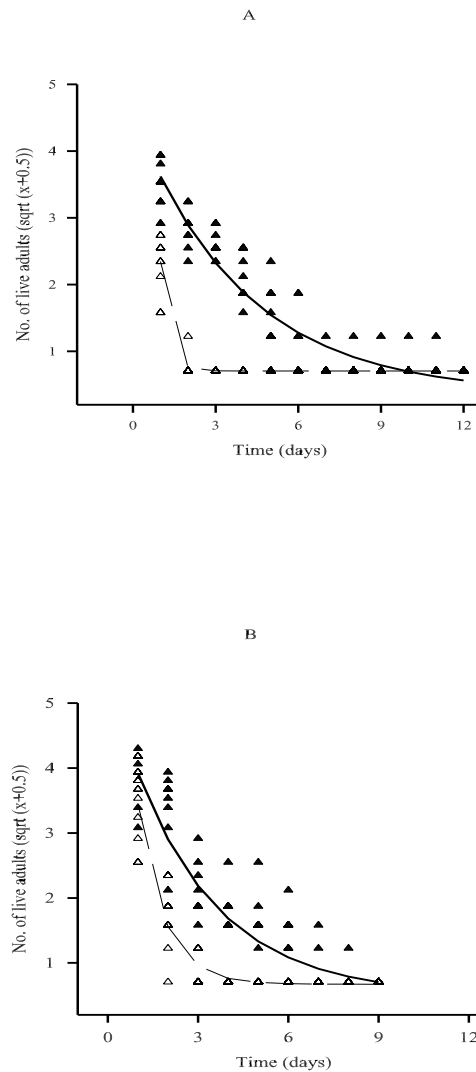


Figure 2. 2: Survival of *Trichogramma carverae* when confined with punctured grape (—▲—) or without grape (—△—): A = when water unavailable (adjusted $R^2 = 91.4\%$), B = when water available (adjusted $R^2 = 87.0\%$). Points denote treatment means and lines denote fitted relationships.

Discussion

The present field experiment constitutes the first non-laboratory assessment of the effect of host-egg age on parasitism by *T. carverae*. Though no effect of host-egg age was apparent, the very low overall levels of parasitism require this finding to be interpreted with caution. Glenn & Hoffmann (1997) found in the laboratory that *T. carverae* acceptance rate was highest (40 – 50%) for intermediate ages (green, 1-3 days old; yellow, 4-5 days old; orange, 6-7 days old; brown, 8 days old) of *E. postvittana* eggs, and lower for older eggs (black, >12 days old) (17%), followed by fresh eggs (<24 h old) (25%). Similarly, Miura & Kobayashi (1998) reported that *Trichogramma chilonis* Ishii preferred 24-h-old host (*Plutella xylostella* (L.)) eggs and acceptance was lower for older or younger hosts. The green eggs in the present study can be assumed to be younger than the orange ones and both were susceptible to field parasitism by *T. carverae*.

Lobularia maritima is recognized as a useful nectar-producing plant for some parasitoids (Chaney, 1998; Johanowicz & Mitchell, 2000). Many hymenopteran parasitoids live longer or parasitise more hosts eggs when they have access to nectar (Jervis *et al.*, 1996; Gurr & Nicol, 2000; Berndt *et al.*, 2002; English-Loeb *et al.*, 2003). Despite this, the present field experiment showed no significant enhancement of parasitism in the presence of this plant. It is likely that the relatively cool and moist conditions prevailing in the vineyard during the late-season experiment were important in constraining activity of *T. carverae* (and other natural enemies) as is evident from the low absolute levels of parasitism. Three other effects may also have operated to explain the lack of the effect of *L. maritima*:

- (i) *T. carverae* does not feed on *L. maritima*.

- (ii) *T. carverae* obtained sufficient nectar from weed flowers present in the vineyard.
- (iii) *T. carverae* was feeding on sugars exuding from ripening grapes.

Honeydew was not considered a significant food source because the insects responsible for its production are potentially serious vine pests, are generally very well controlled in Australian vineyards and were not present in detectable numbers in the study site.

Hypothesis one was tested in the growth cabinet experiment. This experiment demonstrated an increase realised parasitism by *T. carverae* and an increased longevity of *T. carverae* adults can be inferred, as previously observed for this species when fed a honey diet in the laboratory (Gurr & Nicol, 2000). Similarly, other researchers have reported that longevity (Johanowicz & Mitchell, 2000; Berndt, 2002) and lifetime fecundity (Berndt, 2002) were significantly higher when non-trichogrammatid parasitoids fed from *L. maritima*. The present study does not support hypothesis one.

The laboratory experiment using weed species and alyssum also failed to support hypothesis one and gave partial support to hypothesis two. The survival of *T. carverae* was increased significantly with access to flowers of *H. radicata* and *T. repens* (weed species) as well as with *L. maritima*. For *E. plantagenium* there was no enhancement of survival, indicating that some level of flower specificity applies to *T. carverae* feeding. Earlier studies have shown that wild flowers increase longevity of, and parasitism by, parasitoids (van Emden, 1963; Leius, 1967; Syme, 1975; Fitton & Walker, 1992; Zhao *et al.*, 1992; Idris & Grafius, 1995), though Hagley & Barber (1992) showed that some weed flowers are poor food sources for adult *Pholetesor ornigis* (Weed) (Hymenoptera: Braconidae), a parasitoid of

Phyllonorycter blancardella (Fabr.). The present results suggest that *T. carverae* may feed on nectar from some common vineyard weeds. This has significant practical implication but is unlikely to have occurred in the present field experiment because weed flowers had been mechanically removed from the vicinity of all plots. The small size of *T. carverae* suggests that it is unlikely that adults commuted from remaining weeds outside the plots (>10 m) and the sentinel cards placed within plots.

Hypothesis three was supported in experiments with punctured grapes. In the first of the two experiments (in which no water was provided to *T. carverae*) all parasitoids had died by day two unless given access to grape exudates, when they lived for up to six days. This result cannot be attributed solely to nutrients, as the water component may have been important in preventing desiccation even under the conditions of high humidity. The second experiment, in which water as well as exudate was provided to parasitoids, gave a very similar result. This indicates that nutrients within grape exudates are responsible for an increased survival of *T. carverae*. Previous researchers reported that carbohydrates increase longevity of *T. carverae* (Gurr & Nicol, 2000) and other parasitoids (e.g., Jacob & Evans, 2000). This result supports previous findings and hypothesis three. The broader significance of this finding is that it demonstrates that exudates from ripening grapes may be an important late-season food for *T. carverae* and possibly other natural enemies of vineyard pests, an effect that may also apply in other crop systems. In Australian vineyards, the summer generation of *E. postvittana* causes the majority of damage near harvest and leaves fruit highly vulnerable to botrytis, a fungal disease caused by *Botrytis cinerea* (Lo & Murrell, 2000). Late-season activity of parasitoids may reduce such damage and limit the winter generation of the pest population. The use of fruits as food sources for natural enemies has been little researched,

though Eijs *et al.* (1998) studied the effect of different food resources on drosophilid parasitoids. In *Asobara tabida* (Nees) and *Asobara rufescens* (Foerster) (Hymenoptera: Braconidae) fat reserves, which are highly correlated with lifespan (Ellers, 1996), were significantly higher when they fed from *Malus domestica* fruits. Potentially, fruits of various types could play a significant role in habitat manipulation, a factor largely ignored in research on conservation biological control. The value of fruits as late-season food fruit feeding does not, however, preclude a beneficial effect of habitat manipulation strategies that provide foods to natural enemies earlier in the season.

Chapter Three - The effects of adult food on *Trichogramma carverae* and other natural enemies of *Epiphyas postvittana*: follow-up studies

Introduction

Research in a variety of ecosystems has shown that many adult parasitoids use nectar and/or pollen as a food, and this is an important consideration for biological control (Jacob & Evans, 2000). Carbohydrate rich nectar is a source of energy, whereas pollen is a nutrient source for egg production in some parasitoids (Jervis *et al.*, 1996). Several laboratory and field experiments have shown that adult food increases the longevity and fecundity of parasitoids. For example, Berndt & Wratten (2001) have demonstrated that feeding on alyssum flowers (*Lobularia maritima* L.) by *Dolichogenidea tasmanica* (Cameron) increased their laboratory longevity six-fold over water fed animals. Similarly, *T. carverae* longevity increased when they were fed honey (Gurr & Nicol, 2000). The parasitism rate of *E. postvittana* increased significantly when buckwheat (*Fagopyrum esculentum* Moench) and faba bean (*Vicia faba* L.) was planted in an apple orchard (Irvin *et al.*, 2000). In addition, coriander (*Coriandrum sativum* L.) and faba bean increased the parasitism rate of the potato moth (*Phthorimaea operculella* (Zeller)) by *Copidosoma koehleri* Blanchard (Baggen & Gurr, 1998). The suppression of host populations of Lepidopteran pests is positively correlated with fecundity of parasitoid biological control agents (Lane *et al.*, 1999) and this may be constrained by a lack of suitable floral resources in many agroecosystems. These problems can be overcome through habitat manipulation by planting

flowering plants in agroecosystems. However, it is important to investigate the effect of floral resources on the longevity and fecundity of parasitoids in the laboratory before doing field trials (Jervis *et al.*, 1996; Gurr *et al.*, 2004). In the present study, experiments were conducted for laboratory assessment of the effect of groundcover plant species on the longevity and daily fecundity of *T. carverae* and the parasitism of *E. postvittana* by *T. carverae*. Field trials measuring the effect of groundcover plant species on egg parasitism and predation of *E. postvittana* were also conducted.

In the laboratory, two series of experiments were conducted. The first series measured survival and realised parasitism whilst the second series measured longevity of male and female *T. carverae* separately as well as daily fecundity of females. Two field trials were conducted in two different commercial Chardonnay vineyards during November and December 2003. One was a conventional vineyard at Orange (a cool district) and another an organic vineyard at Canowindra (a warmer district), New South Wales, Australia.

Materials and Methods

Laboratory assessment of the effect of groundcover plants on *T. carverae* longevity and parasitism

Plant and insect material

Plants of alyssum (*Lobularia maritima* L.: Cruciferae) (cv. Small White Flower), borage (*Borago officinalis* L.: Borginaceae) (cv. Borage), buckwheat (*Fagopyrum esculentum* Moench: Polygonaceae) (cv. Ikeda), coriander (*Coriandrum sativum* L.: Umbelliferae) (cv.

Macrocarpum) and mustard (*Brassica juncea* (L.) Czernj.: Brassicaceae)) (cv. Peacock Tail) were grown from seed in a greenhouse.

The source of *T. carverae* was as described in Chapter Two. The host insect, *E. postvittana*, was reared in the laboratory on an artificial diet modified from Shorey & Hale (1965) (cited by Glenn & Hoffmann, 1997; Thomson *et al.*, 2000; Bennett, 2002). A nucleus stock of *E. postvittana* eggs was obtained from a colony that was maintained by Rundle, B.J. at LaTrobe University, Victoria, Australia (personal communication). The following procedures (Glenn & Hoffmann, 1997; Thomson *et al.*, 2000; Bennett, 2002) were followed for culturing the host insect. For rearing purposes, 500ml plastic food containers were used; each container held larval diet and mature egg masses. This container was covered with a non-airtight lid beneath which a layer of paper tissue was stretched. After approximately four weeks, pupae or recently eclosed adults were collected and eight pairs of pupae (male and female) or moths (male and female) were placed in 250ml plastic coffee cups with vertical ridges for mating and oviposition. Adults were sexed on the basis of body size and wing characteristics and pupae on the abdominal segments (Baker *et al.*, 1994). Each cup was covered with clear plastic film with small ventilation holes, held in place by a rubber band (modified from Glenn & Hoffmann, 1997; Thomson *et al.*, 2000; Bennett, 2002). Adult diet (honey, 180ml; water, 1800ml; ascorbic acid, 10.8g (0.6%); sorbic acid, 1.8g (0.1%); paraben, 1.8g (0.1%) and 70% ethanol, 10ml; Rundle, B.J., La Trobe University, Victoria, Australia; personal communication)) was supplied to the moths via cotton wool balls. Females laid eggs on the vertical grooves of the inner surface of cups, which were then collected and stored at 4-6°C until needed. When eggs were required the cups were cut into strips containing one or two egg

masses. The colony was reared with a 16L: 8D photoperiod, 19°C temperature and an average relative humidity of 41%.

Survival and parasitism of *T. carverae*

This work investigated the effects of five plant species but because plants did not bloom synchronously a total of three experiments was conducted. In the first, a randomised block design with five replicates and seven treatments was used. The first three treatments comprised flowering shoots of *B. juncea*, *C. sativum* and *L. maritima* and the cut ends of these shoots were placed immediately in tap water until the experiment was initiated (within five hours of shoot collection). Each flowering shoot had several buds to ensure continuous flowering throughout the experimental period. A further three treatments consisted of the above plant species from which flowers and flower buds were removed. A final control treatment with no plant materials (nil) was used.

The second experiment used an identical design in which the treatments were *F. esculentum* (with- and without-flower) and control (nil). Similarly, in the third experiment, treatments were *B. officinalis* (with- and without-flower), water alone and control (nil). In these experiments, plastic vials (5.5 × 4.5 cm) were used as flight cages. In the bottom of each vial there was a small circular hole (1 cm diameter) through which the cut end of three shoots of the designated type were passed into water contained in a second vial (11 × 2.5 cm) beneath the first. Shoots were sealed into the holes with a non-setting adhesive (Blue-Tack). Ten adult *T. carverae* (< 24 h after eclosion) were released in each vial. *Trichogramma carverae* were transferred to the flight cages using gelatine capsules (size 00) (Tyco Healthcare Pty Ltd., Sydney, NSW, Australia), as described below, to avoid the need for the use of

anesthesia, allaying possible deleterious effects on behaviour or longevity. Within 24h of emergence, adult *T. carverae* do not fly (personal observation). Young adults were put on a white paper sheet (for ease of visibility) and each adult was covered with half a gelatine capsule. Adults crawled upward inside the capsule, which was then lifted up and sealed with the other half of the capsule. Adults were introduced into flight cages by separating the capsule halves and tapping the half containing the insect against a hole (2×1 cm) in the vial's wall. After adding ten adults, the hole was sealed with the adhesive described above and vial was the examined under a microscope ($10\times$) to confirm that ten live adults were present. The top of the upper vial was then sealed with a sheet of tissue paper held in place with a rubber band. All three experiments were laid on a greenhouse bench top with a 16L:8D photoperiod, 20°C L/16°C D temperature at an average relative humidity of 67% in the first experiment, 59% in the second and 47% in the third experiment. The number of live *T. carverae* in each flight cage was recorded every 24 h until no more live *T. carverae* were recorded.

Epiphyas postvittana egg sentinel cards were used to measure the parasitism rate. These were prepared by cutting the plastic oviposition substrate into strips, each bearing one egg mass. Strips were stapled individually to yellow paper sheets (5×1 cm). Microscopic examination ($10\times$) was used to count the number of eggs in each mass and this was recorded on each paper sheet. The mean number of eggs per card was 29.91 (range 12-68; half bearing 20-37 eggs) in the first experiment. In the second and third experiments the mean number of eggs per card was 23.57 (range 6-65; half bearing 15-26 eggs) and 19.87 (range 8-49; half bearing 12-27 eggs) respectively. One egg sentinel card was placed in each flight cage through a hole in the vial's wall to allow *T. carverae* oviposition to take place and was

recovered after 24h. The first batch of egg cards was placed when the experiment was first set-up (adults were < 24 h after eclosion). Egg cards were replaced every third day until all insects had died. On removal, egg masses were incubated at 23°C until parasitised eggs became black. They were then counted.

Data analysis

Data for the proportion of live insects on each day were angular transformed. Angular transformation is appropriate because of the large proportion of out-of-set insects. Multivariate analysis of variance (MANOVA) was used to test the significance of temporal effects. Where all replicates of one treatment were zeros analysis of variance (ANOVA) for each date was used. Exponential curves of the form $y = A + BR^x$ (where y = number of live insects, x = number of days, A and B = linear parameters, R = survival rate) were fitted to the data to compare the differences in position (A), slope (B) and curvature (R) for each treatment. First, an overall curve was fitted, then a set of curves with a different position (A) for each treatment, after that a set of curves with different position and slope (A and B) for each treatment and finally a set of curves with all (A , B and R) parameters separate. The steps defined above were accomplished automatically using the standard module for comparing non-linear regression in GenStat release 6.1. However, where curvature, representing the key biological parameter of insect survival, differs between treatments, comparison of the linear parameters becomes irrelevant. The number of eggs parasitised was calculated per cage. A square root transformation $\sqrt{(x + 0.5)}$ was used for this data to standardise the variance, which was analysed using randomised block ANOVA on each date. Square-root transformation was chosen rather than untransformed actual data after inspection of residuals.

Longevity and daily fecundity of *T. carverae*

This work aimed to investigate the effect of groundcover plant species on the daily fecundity of *T. carverae*. As in the earlier work, asynchronous flowering of plant species required the conduct of separate experiments. In the first, two plant species *F. esculentum* and *L. maritima* were tested. Four treatments (with-and without-flowers) as described in the above experiment and a control (nil: no plant material) treatment replicated ten times, were employed in a randomised block design. The second experiment used equivalent treatments for the plant species *B. juncea* and *B. officinalis*. Plastic vials were used as flight cages as described above. Each flight cage contained plant material and one male and one female *T. carverae*. The control (nil) treatment contained only *T. carverae*. *Trichogramma carverae* adults were sexed on the basis of antennal hairs using microscopic examination (10×) (Knutson, 1998). Long and abundant antennal hairs were present in males while hair was sparse and short in females. Adults (< 12 h after eclosion) were transferred to the flight cages as previously described. To measure daily fecundity, *E. postvittana* egg sentinel cards (preparation and placement as previously described.) were used. The mean number of eggs per card was 23.11 (range 6-76; half bearing 15-28 eggs) in experiment one and 26.22 (range 6-62; half bearing 15-35 eggs) in experiment two. Egg cards were replaced every 24 h until the female had died. In each flight cage, live adults were recorded every 24 h until both individuals were dead. Egg masses were subsequently incubated at 23°C until parasitised eggs became black. They were then counted. Both experiments were laid on a greenhouse bench top with 16L: 8D photoperiod, 20L/16D°C temperature. Relative humidity averaged 54% in the first experiment and 71% in the second experiment.

Data analysis

Longevity data were analysed using randomised block ANOVA. The number of eggs parasitised was calculated per cage. A square root transformation $\sqrt{(x + 0.5)}$ was used for these data to standardise the variance and analysed using randomised block ANOVA on each date. Square-root transformation was chosen rather than untransformed actual data after inspection of residuals. Exponential curves were fitted for experiment one but not in experiment two as all *T. carverae* died by the third day. Exponential curves of the form $y = A + BR^x$ (where y = number of eggs parasitised, x = number of days, A and B = linear parameters, R = rate of changes of parasitised eggs) were fitted to the data to compare the differences in position (A), slope (B) and curvature (R) for each treatment. First, an overall curve was fitted, then a set of curves with a different position (A) for each treatment, after that a set of curves with different position and slope (A and B) for each treatment and finally a set of curves with all (A , B and R) parameters separate. The steps defined above were accomplished automatically using the standard module for comparing non-linear regression in GenStat release 6.1.

Field evaluation of the effects of groundcover plants on performance of *T. carverae* and other natural enemies of *E. postvittana*.

Orange Field Experiment

The experiment was conducted in the University of Sydney's Chardonnay vineyard at Orange, New South Wales, Australia in the 2.3 ha northern section of the vineyard (Plate 2.1). The vineyard has a history of insecticides, herbicides and sulphur being applied each

year. The vegetation beneath vines consisted of broad-leaved weeds whilst between the rows there was grass and some broad-leaved weeds. A randomised block design with five replicates was used. Of the 44 rows in the vineyard, numbers 10, 15, 20, 25, 30 were designated as blocks. Plots were 1.5 m long and 0.6 m wide, located beneath two vines, and each plot was separated by 20 m along the vine row.

Plant treatments

Brassica juncea, *B. officinalis*, *C. sativum*, *F. esculentum* and *L. maritima* seeds were sown on 16 September 2003 and resown on 8 October 2003 to give prolonged flowering. *Lobularia maritima* and *B. officinalis* plots contained some plants that were transplanted from a greenhouse on 12 March 2003 (Plate 3.1A and C). These flowers bloomed during the experimental period. Greenhouse grown flowering *F. esculentum* plants were transplanted to the field on 25 November 2003 because the sown *F. esculentum* did not bloom. *Brassica juncea* and *C. sativum* plants also failed to flower, thus only *B. officinalis*, *F. esculentum*, and *L. maritima* treatments were retained along with treatments consisting of (i) weedy vegetation without-flowers and (ii) a control (cultivated bare earth) (Plate 3.1B and D). Before commencing the experiment, a motorised brush cutter was used to remove the flowers and unopened flower buds from the weeds on either side of plots and for a distance of approximately 10 m along the row from either end of each plot.

A



B



C



D



Plate 3. 1: Different groundcover plots at Orange, A = *Lobularia maritima* plot; B = Vegetation without-flowers plot; C = *Borago officinalis* plot and D = Control with bare earth plot.

Naturally occurring egg parasitoids

Before release of *T. carverae*, naturally occurring egg parasitoids were surveyed using *E. postvittana* egg sentinel cards (see Chapter Two for egg sentinel card descriptions). Egg cards were released on 26 November 2003 and recovered after 24h on 27 November 2003 because sulphur spraying was taking place on 28 November 2003. In each plot, three egg sentinel cards were released. One card was stapled to the vine leaves 1 m above the soil at each end of the plot. An additional card was stapled to a central vine leaf, 1.5 m above the soil. The mean number of eggs per card was 33.48 (range 12-76; half bearing 23-41 eggs). Recovered egg cards were kept in individual sealed plastic bags in an incubator at 23°C until parasitised eggs became black and were then counted.

Effect of groundcover plants on parasitism and predation rate

Trichogramma carverae was sourced as described in Chapter Two (field experiment). Capsules were kept in an incubator at 26°C. On 3 December 2003, after one day of incubation, *T. carverae* was starting to emerge in an indicator vial (see indicator vial description in Chapter Two, field experiment). All capsules were then taken out of the incubator and kept for four hours at room temperature in preparation for placement in the field. Four capsules were placed in each plot. Two were mounted with wire on a bamboo cane pushed into the soil in the middle of the plot so that capsules were 0.3 m above the soil surface. Two additional capsules were stapled on to the vine leaves 1.5 m above the soil. A single, additional capsule was positioned in row 30, enclosed in a ventilated glass vial so that timing of adult emergence from the other capsules could be assessed.

On 4 December 2003, within 24 hours of *T. carverae* emergence, *E. postvittana* egg sentinel cards (see Chapter Two field experiment for egg sentinel cards preparation) were stapled on the upper surface of the vine leaves in each plot. *Epiphyas postvittana* eggs were sourced as previously described. Only green (younger) egg masses were used and the mean number of eggs per card was 31.88 (range 11-90; with half bearing 23-39 eggs). In each plot, six egg sentinel cards were released as described for the field experiment in Chapter Two. After 48 hours (6 December, 2003) sentinel cards were recovered from the field and the surviving eggs counted under a binocular dissecting microscope (10×) to assess the predation rate. Each card was placed in a small sealed plastic bag and kept in an incubator at 23°C until parasitised eggs became black and were then counted. Additional batches of fresh egg sentinel cards were released on 7 and 10 December and recovered 9 and 12 December, respectively. Recovered cards were handled as before (Chapter Two).

A maximum/minimum thermometer and rain gauge were placed in the field and meteorological data was recorded daily. To monitor the activity of wild *E. postvittana*, a single sex pheromone trap was placed in the south section of the vineyard, 20 m from the nearest point of the experiment.

Data analysis

The numbers of eggs parasitised and predated were calculated per plot. The number of eggs per plot was used as a covariate and found to be non-significant and then excluded. Predation and parasitised data were analysed using randomised block ANOVA. With the parasitism data the contrast of with-flower (*B. officinalis*, *F. esculentum* and *L. maritima*) and without-flower (vegetation without-flowers and control) treatments was tested as part of

the treatment effect. If the treatment F -test was significant, treatments were compared using a Least Significant Difference (LSD) test. GenStat release 6.1 (GenStat Committee, 2002) was used for all data analyses.

Canowindra field experiment

The experiment was conducted in the “Rosnay Estate” Chardonnay vineyard at Canowindra, New South Wales, Australia (Plate 3. 2A). This 4.25 ha and two year old vineyard is managed under an organic system. The spray program includes: fortnightly applications of sulphur (2.5kg/ha), copper (1kg/ha) with Synertrol Horti oil and *Bacillus thuringiensis* applications. Weed management practices include: heavy grazing, flail mowing and straw mulch. Saffron thistle (*Carthamus lanatus* L.), prickly lettuce (*Lactuca serriola* L.) and Paddy’s lucerne (*Sida rhombifolia* L.) were the main weeds in the vineyard. The experiment was set up in a randomised block design with five treatments replicated five times. Of the 55 rows in the vineyard, numbers 5, 10, 15, 20 and 25 were designated as blocks. Plot size, position and spacing were as per the Orange field experiment.

A



B



C



Plate 3. 2: A = Rosnay Estate vineyard at Canowindra; B = *Fagopyrum esculentum* plot; C = *Coriandrum sativum* plot.

Plant treatments

Brassica juncea, *B. officinalis*, *C. sativum*, *F. esculentum* and *L. maritima* seeds were sown on 9 September 2003 and resown on 7 October 2003 to give prolonged flowering (Plate 3. 2B and C). *Coriandrum sativum* and *F. esculentum* bloomed during the experimental period and flowering *L. maritima* plants from the University of Sydney, Orange campus were transplanted there on 11 December 2003 because sown *L. maritima* did not bloom during the trial. *Brassica juncea* and *B. officinalis* also failed to flower so were disregarded. Additional treatments were the existing weedy vegetation without flowers and a control (cultivated bare earth). During the advanced stage of the trial, most of the *L. maritima* plants died in each plot. Plants were watered once a week. A motorised brush cutter was used to remove weed flowers and unopened buds, as per the Orange field, before commencing the experiment.

Naturally occurring egg parasitoids

Before release of *T. carverae*, naturally occurring egg parasitoids were surveyed as described in the Orange experiment. The mean number of eggs per card was 49.68 (range 18-86; half bearing 38-57). Egg sentinel cards were released on 15 December 2003 and, after 24 h, were recovered and handled as previously described.

Effect of groundcover plants on parasitism and predation rates

Trichogramma carverae and host eggs were sourced as described in the Orange field experiment. *Trichogramma carverae* release, *E. postvittana* egg sentinel card release and recovery methods were as per the Orange field experiment. On 16 December 2003, after one day of incubation, *T. carverae* were starting to emerge in an indicator vial (see indicator vial description in Chapter Two, field experiment). All capsules were then taken out of the

incubator and kept for three hours at room temperature and for another one and half hours in the car (in transit from Orange to Canowindra) in preparation for placement in the field. The first batch of egg sentinel cards was released on 16 December 2003 because *T. carverae* was starting to emerge in an indicator vial (one additional capsule was put into the indicator vial as before) after the finished capsules were released. The mean number of eggs per card was 34.39 (range 13-85; with half bearing 25-41 eggs). Recovered cards were handled as per the Orange field experiment. On 18 December, after 48 h, these cards were replaced with a fresh new batch of egg cards. The second batch was replaced on 20 December and recovered on 22 December 2003.

Rain gauge and Tinytag data logger (GLM, Version 2.8 Gemini Data Loggers (UK)) were placed in the field to record meteorological data. Tinytag data logger recorded temperature every 20 minutes during the whole experimental period. A single *E. postvittana* sex pheromone trap was placed as in the Orange field experiment, which captured male adults.

Data analysis

Data were analysed as per the Orange experiment. Predation and parasitism data were analysed using randomised block ANOVA. With the parasitism data the contrast of with-flower (*C. sativum*, *F. esculentum* and *L. maritima*) and without-flower (vegetation without-flowers and control) treatments were tested as part of the treatment effect.

Results

Laboratory assessment of the effect of groundcover plants on *T. carverae* longevity and parasitism

Survival and parasitism of *T. carverae*

In the first experiment that included *B. juncea*, *C. sativum* and *L. maritima*, the mean number of live adults in the with-flower treatments on days two to four differed significantly ($P < 0.001$), exceeding the control and the corresponding shoots without flower treatments. On day two, adults in the *L. maritima* with-flower treatment significantly exceeded the *B. juncea* with-flower treatment but not the *C. sativum* with-flower treatment. After that time, adult numbers in *L. maritima* were significantly higher than in both *B. juncea* and *C. sativum* with-flowers treatments and all other treatments. In the *C. sativum* with-flower treatment, adult numbers did not differ from those in the *B. juncea* with-flower treatment from day two to seven. No live *T. carverae* were recorded in any of these treatments except the *L. maritima* with-flower treatment after day seven. Adults were found in the *L. maritima* with-flower treatment until day 21. No adults were found in the control treatment after day three nor after day five in the without- flower shoot treatments.

Fitted exponential curves for treatments differed significantly in curvature ($F = 115.10$; $df = 6, 133$; $P < 0.001$) (Figure 3.1). The survival rate (\pm standard error) of *T. carverae* on intact flowers was: *B. juncea*, 0.547 ± 0.0224 ; *C. sativum*, 0.592 ± 0.0203 and *L. maritima*, 0.922 ± 0.0105 . In treatments that used shoots from which flowers were removed, equivalent

values were 0.233 ± 0.0319 ; 0.247 ± 0.0317 and 0.391 ± 0.0275 , respectively and in the control treatment, 0.099 ± 0.0337 .

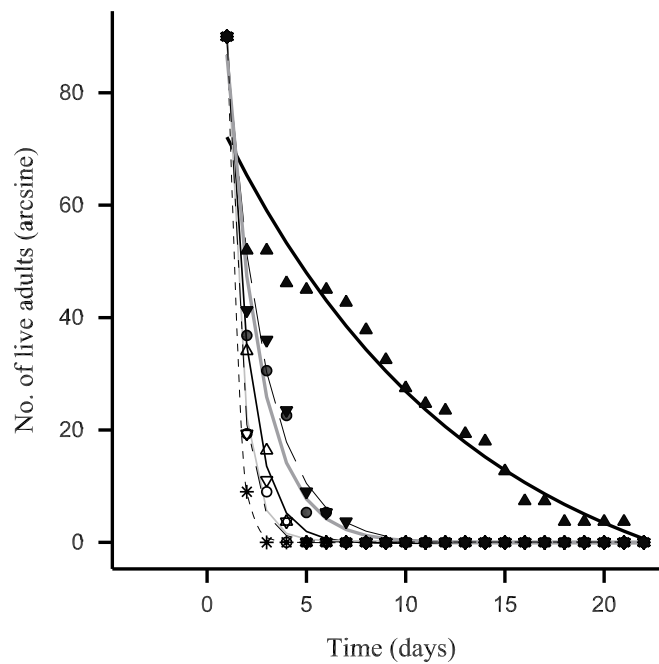


Figure 3. 1: Mean survival of adult *T. carverae* when confined with: *B. juncea* with-flowers (—●—), *B. juncea* without-flowers (---○---); *C. sativum* with-flowers (—▼—), *C. sativum* without-flowers (---▽---); *L. maritima* with-flower (—▲—), *L. maritima* without-flowers (---△---) and control (----*----). Adjusted $R^2 = 98.1\%$. Points denote treatment means and lines denote fitted relationships.

There was no significant treatment effect observed on day one and day three ($F = 0.56$; $df = 6, 24$; $P = 0.754$, $F = 2.36$; $df = 6, 24$; $P = 0.062$, respectively) though on day three considerably more parasitism was recorded in *L. maritima* than in *C. sativum* and no parasitism was observed in all other treatments. Numbers of eggs parasitised were significantly ($F = 5.44$; $df = 6, 24$; $P < 0.001$) higher in the *L. maritima* with-flower treatment than in other treatments on day six of egg sentinel cards, 2.36 ± 0.263 (mean \pm SE) compared with *C. sativum* with-flower 0.88 ± 0.263 treatment (Figure 3.2). There was no parasitism in the control, *B. juncea* (with- and without-flower), and without flower treatments of *C. sativum* and *L. maritima* on this date. On day 12, parasitism was recorded only in the *L. maritima* with-flower treatment and zero parasitism was recorded on day nine in all the treatments.

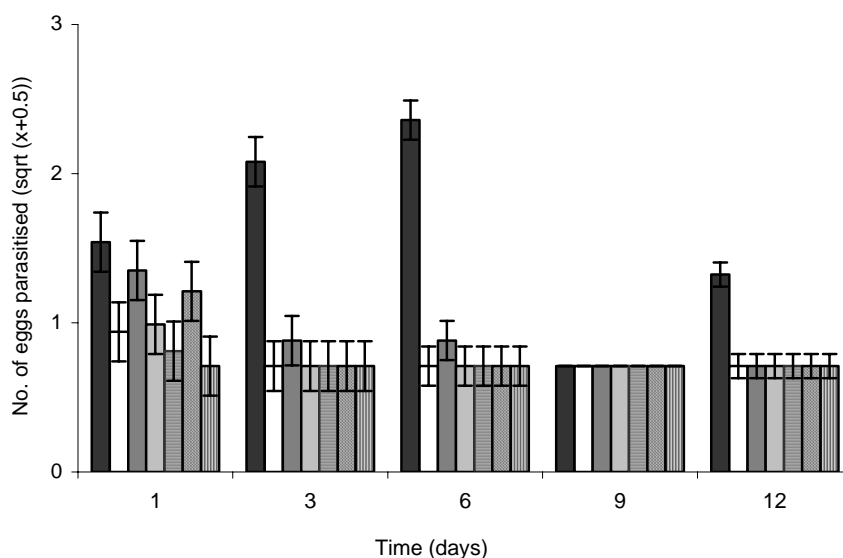


Figure 3. 2: Mean parasitism of *E. postvittana* by *T. carverae* when caged with: *B. juncea* with-flowers (■), *B. juncea* without-flowers (■); *C. sativum* with-flowers (■), *C. sativum* without-flowers (■); *L. maritima* with-flowers (■); *L. maritima* without-flowers (□) and control (■). Bars show the standard errors.

In the second experiment, which included *F. esculentum* (with- and without-flowers) and a nil control, the survival of *T. carverae* was affected significantly by the treatments. The mean number of live adults in the *F. esculentum* with-flower treatment on days three to six differed significantly ($P < 0.001$) from the control and the shoots without-flower treatment. Adults were found in the *F. esculentum* with-flower treatment until day 16. No adults were found in the control treatment after day four and after day eight in the without-flower treatment.

Fitted exponential curves for treatments differed significantly in curvature ($F = 76.78$; $df = 2,45$; $P < 0.001$) (Figure 3. 3). When caged with *F. esculentum* with-flowers, the survival rate (0.921 ± 0.0175) of *T. carverae* was significantly higher than without-flowers (0.582 ± 0.0277) and control (0.477 ± 0.0329) treatments.

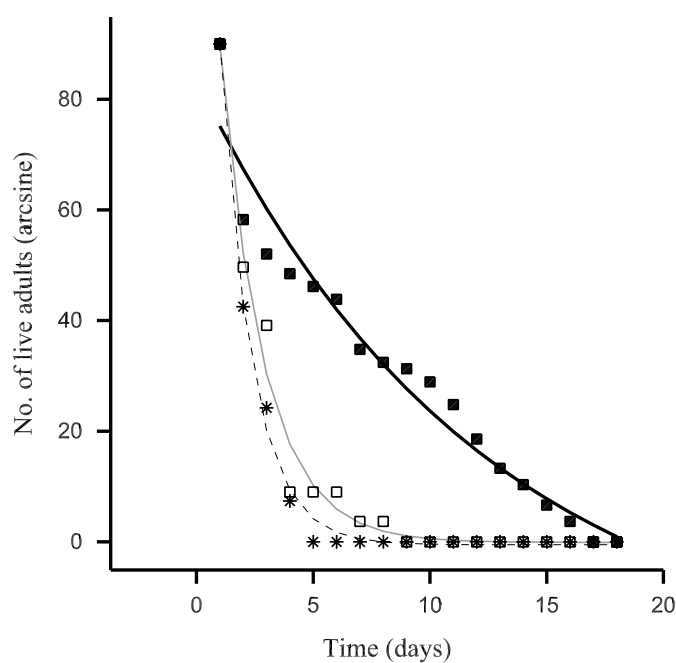


Figure 3. 3: Mean survival of adult *T. carverae* when confined with: *F. esculentum* with-flowers (—■—), *F. esculentum* without-flowers (—□—) and control (----*----). Adjusted $R^2 = 97.5\%$. Points denote treatment means and lines denote fitted relationships.

Parasitism was recorded in the *F. esculentum* with-flower treatment until day 12 but fell to zero in other treatments by day nine. There was no significant treatment effect on parasitism for within date comparisons (Figure 3. 4). There was no parasitism in the control after day three nor after day nine in the without-flower treatment.

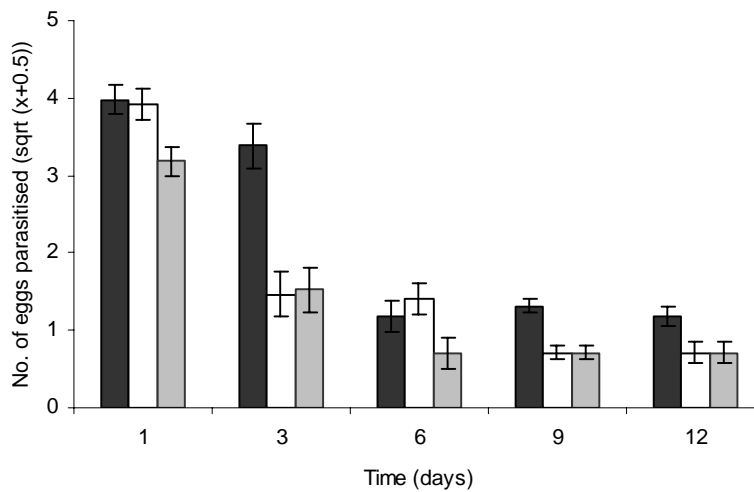


Figure 3. 4: Mean parasitism by *T. carverae* when caged with: *F. esculentum* with-flowers (■), *F. esculentum* without-flowers (□) and control (■). Bars show the standard errors.

In the final experiment that included *B. officinalis* (with- and without-flowers) and nil control the survival of *T. carverae* showed a significant treatment effect. The mean number of live adults in the *B. officinalis* with-flower treatment on day five and day six differed significantly ($P < 0.001$) from the shoots without flower treatments. No live *T. carverae* were recorded in control (nil) and water control on these dates. There were no significant treatment effects on day two. On day three and day four significantly higher ($P < 0.05$) number of adults were recorded in *B. officinalis* with-flower treatment than in both controls. No adults were found in the water and nil control treatments after day three and after day seven in the without flower treatment.

Fitted exponential curves for treatments differed significantly in curvature ($F = 7.24$; $df = 3, 28$; $P < 0.001$) (Figure 3. 5). The survival rate of *T. carverae* on *B. officinalis* with-flowers treatment was 0.744 ± 0.0450 ; without- flowers, 0.589 ± 0.0427 ; water control, 0.483 ± 0.0456 and nil control, 0.430 ± 0.0481 .

No significant treatment effect on parasitism was apparent. Parasitism was recorded only on days one and three (Figure 3. 6).

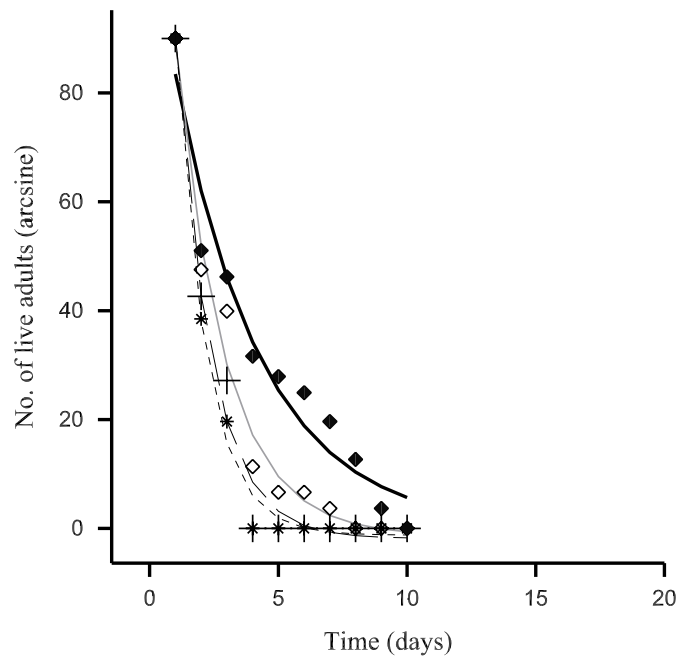


Figure 3. 5: Mean survival of adult *T. carverae* when confined with *B. officinalis* with-flowers (—◆—), *B. officinalis* without-flowers (—◇—), control water (- -+- -) and control (----*----). Adjusted $R^2 = 97.1\%$. Points denote treatment means and lines denote fitted relationships.

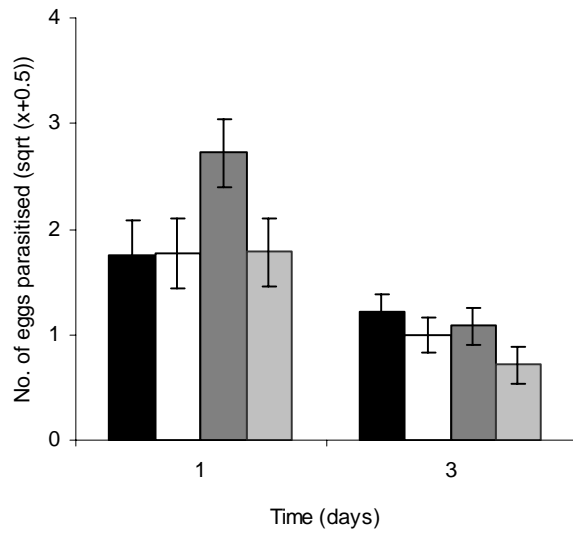


Figure 3. 6: Mean parasitism by *T. carverae* when caged with: *B. officinalis* with-flowers (■), *B. officinalis* without-flowers (□); water control (■) and nil control (■). Bars show the standard errors.

Longevity and daily fecundity of *T. carverae*

In the first experiment, which included *L. maritima* and *F. esculentum*, treatments significantly affected the longevity of male ($F = 27.19$; $df = 4,36$; $P < 0.001$) and female ($F = 22.98$; $df = 4, 36$; $P < 0.001$) *T. carverae* (Table 3.1). The mean longevity of male and female *T. carverae* with-flowers of *F. esculentum* and *L. maritima* was significantly higher than without-flower and control treatments.

Table 3. 1: Mean adult longevity of *T. carverae* when caged with *F. esculentum* and *L. maritima* (+ = shoots with flowers, - = shoots without flowers) and control (nil: no plant material).

Treatments	Male longevity (days)	Female longevity (days)
<i>F. esculentum</i> (+)	6.20c	6.60b
<i>F. esculentum</i> (-)	3.30ab	3.00a
<i>L. maritima</i> (+)	9.10d	9.10c
<i>L. maritima</i> (-)	3.70b	3.40a
Control (nil)	1.80a	1.70a
LSD	1.58	1.80

Means followed by the same letter do not differ significantly at $P < 0.05$

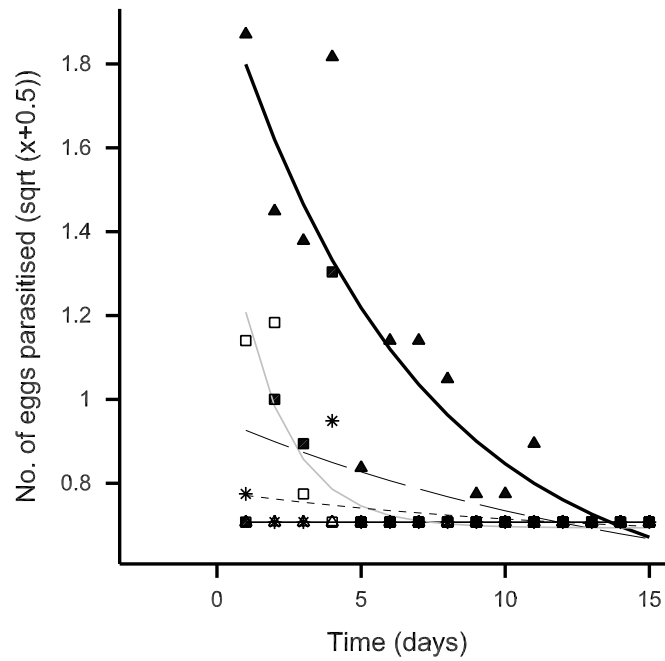


Figure 3. 7: Mean daily fecundity of *T. carverae* when confined with different groundcover plant species: *F. esculentum* with-flowers (—■—), *F. esculentum* without-flowers (—□—); *L. maritima* with-flowers (—▲—), *L. maritima* without-flowers (—△—) and control (----*----). Adjusted $R^2 = 74.5\%$. Points denote treatment means and lines denote fitted relationships.

Daily fecundity was significantly ($F = 2.76$; $df = 4,36$; $P = 0.043$) higher in the *L. maritima* with-flower treatment than in all other treatments on day one. After that no significant treatment effect was observed. Parasitism was recorded until day 11 in the *L. maritima* with-flower treatment but declined markedly in other treatments reaching zero by day four.

Fitted exponential curves for treatments differed significantly in position ($F = 21.28$; $df = 4, 60$; $P < 0.001$) and slope ($F = 18.97$; $df = 4, 60$; $P < 0.001$). Rates of change of number of parasitised eggs differed between with- and without-flowers treatment though overall curvature was non-significant ($F = 1.41$; $df = 4, 60$; $P = 0.241$) (Figure 3. 7).

In the second experiment, which included *B. juncea* and *B. officinalis*, the mean longevity of *T. carverae* did not differ significantly between treatments for either males ($F = 2.20$; $df = 4,36$; $P = 0.09$) or females ($F = 2.57$; $df = 4,36$; $P = 0.06$) (Table 3. 2).

Table 3. 2: Mean adult longevity of *T. carverae* when caged with *B. juncea* and *B. officinalis* (+ = shoots with flowers, - = shoots without flowers) and control (nil: no plant material).

Treatments	Male longevity (days)	Female longevity (days)
<i>B. juncea</i> (+)	3.00	3.10
<i>B. juncea</i> (-)	2.10	1.30
<i>B. officinalis</i> (+)	2.10	2.70
<i>B. officinalis</i> (-)	1.80	1.70
Control (nil)	1.20	1.50

No significant treatment effects.

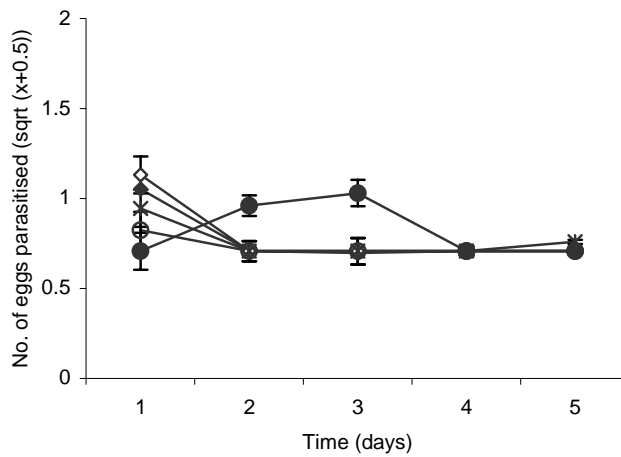


Figure 3. 8: Mean daily fecundity of *T. carverae* when confined with different groundcover plant species: *B. juncea* with-flowers (—●—), *B. juncea* without-flowers (—○—); *B. officinalis* with-flowers (—◆—), *B. officinalis* without-flowers (—◇—) and control (—⌘—). Bars show the standard errors.

There was no significant treatment effect on the daily fecundity of *T. carverae*. After day one no parasitism was recorded in *B. officinalis* with- and without-flowers treatments or in *B. juncea* without-flowers treatments (Figure 3. 8). In the *B. juncea* with-flower treatment, zero parasitism was recorded on day one. After that, parasitism was recorded until day four. In the control treatment, parasitism was recorded only on day one and only one egg was parasitised on day five.

Field evaluation of the effects of groundcover plants on performance of *T. carverae* and other natural enemies of *E. postvittana*.

Orange field experiment

There was no significant ($F=3.22$; $df=1,16$; $P=0.092$) treatment effect on predation of *E. postvittana* eggs on day one when with-flower treatments (*B. officinalis*, *F. esculentum* and *L. maritima*) were compared with without-flower treatments (vegetation without-flowers and control) treatments (Table 3. 3). Predation was significantly ($F=5.05$; $df=1,16$; $P=0.039$) higher in the pooled without-flower treatments on day three. No significant ($F=0.00$; $df=1,16$; $P=1.000$) treatment effect occurred on day six.

Table 3. 3: Mean number of *E. postvittana* eggs predated in the Orange field experiment.

Treatments	Mean predation		
	First release (day one)	Second release (day three)	Third release (day six)
<i>B. officinalis</i>	1.80	1.20	4.6
<i>F. esculentum</i>	3.40	1.00	6.2
<i>L. maritima</i>	1.80	3.60	6.6
Control (bare earth)	0.00	1.80	6.0
Vegetation (without-flowers)	0.60	7.00	5.6

No significant treatment effect on first and third release dates (second release, LSD = 3.606).

No parasitism by naturally occurring egg parasitoids was recorded from the release of *E. postvittana* egg sentinel cards. There was no significant effect of flower treatments on parasitism by *T. carverae* of *E. postvittana* eggs even when with-flower treatments (*B. officinalis*, *F. esculentum* and *L. maritima*) were pooled and compared with without-flower treatments (vegetation without-flowers and control) for days one, three and six ($F = 0.05$; $df = 1, 16$; $P = 0.820$, $F = 1.07$; $df = 1, 16$; $P = 0.315$, $F = 3.39$; $df = 1, 16$; $P = 0.084$, respectively). Parasitism declined markedly on day three and day six in each treatment and zero parasitism was recorded in *B. officinalis* and control treatments on day six (Table 3. 4).

Table 3. 4: Mean number of *E. postvittana* eggs parasitised by *T. carverae* in the Orange field experiment.

Treatments	Mean number of eggs parasitised		
	First release (day one)	Second release (day three)	Third release (day six)
<i>B. officinalis</i>	10.2	2.0	0.0
<i>F. esculentum</i>	10.4	6.2	4.4
<i>L. maritima</i>	22.6	10.8	6.0
Control (bare earth)	10.4	2.6	0.0
Vegetation (without-flowers)	15.2	3.4	0.2

No significant treatment effect.

During the period of surveying naturally occurring egg parasitoids, an average daily temperature of 27.5°C maximum and 5°C minimum was recorded. No rainfall was recorded and no *E. postvittana* adults were caught in the sex pheromone trap. During the ten-days period of monitoring *T. carverae* activity with sentinel cards, an average daily temperature of 34°C maximum and 10.8°C minimum was recorded. The mean daily rainfall was 5.8 mm and an average of 0.6 *E. postvittana* adults were caught in the sex pheromone trap.

Canowindra field experiment

On days one, two and five there were no significant treatment effects ($F = 0.13$; $df = 1, 16$; $P = 0.728$, $F = 0.00$; $df = 1, 16$; $P = 0.987$, $F = 0.36$; $df = 1, 16$; $P = 0.555$, respectively) on

predation of *E. postvittana* eggs when with-flowers (*C. sativum*, *F. esculentum* and *L. maritima*) treatments were pooled and compared with without-flower (vegetation without-flowers and control) treatments. Mean (\pm SE) predation rates tended to be numerically greater in *C. sativum* than in other treatments (Table 3. 5).

Table 3. 5: Mean number of *E. postvittana* eggs predated in the Canowindra field experiment.

Treatments	Mean numbers of eggs predated		
	First release (day one)	Second release (day two)	Third release (day five)
<i>C. sativum</i>	25.6	29.0	19.6
<i>F. esculentum</i>	13.6	6.6	7.6
<i>L. maritima</i>	3.6	9.8	4.8
Control (bare earth)	11.2	8.2	5.8
Vegetation (without-flowers)	12.6	22.4	6.4

No significant treatment effect.

No parasitism was recorded from surveying naturally occurring egg parasitoids. There was a significant effect of flower treatment on parasitism by *T. carverae* of *E. postvittana* eggs on day one ($F = 5.42$; $df = 1,16$; $P = 0.033$) and day two ($F = 5.25$; $df = 1, 16$; $P = 0.036$) when with-flowers (*C. sativum*, *F. esculentum* and *L. maritima*) treatments were compared with without-flowers (vegetation without-flowers and control) treatments (Table 3. 6). There was no significant treatment effect on day five. On day one and day two, mean parasitism

was higher in *F. esculentum* than in all other treatments. In *C. sativum*, parasitism declined over the course of the experiment but was still higher on day five than all other treatments.

Table 3. 6: Mean number of *E. postvittana* eggs parasitised by *T. carverae* in the Conowindra field experiments.

Treatments	Mean no of eggs parasitised		
	First release (day one)	Second release (day two)	Third release (day five)
<i>C. sativum</i>	10.4	10.2	6.80
<i>F. esculentum</i>	11.4	18.4	2.40
<i>L. maritima</i>	10.8	7.6	0.00
Pooled mean (with-flower treatments)	10.87	12.07	3.07
Control (bare earth)	0.8	0.8	0.00
Vegetation (without-flowers)	1.6	2.4	1.60
Pooled mean (without-flower treatments)	1.20	1.6	0.80
<i>P</i> (pooled mean comparison)	0.033	0.036	0.279

During the period of surveying naturally occurring egg parasitoids and eight-day period of monitoring *T. carverae* activity with sentinel cards, an average daily temperature of 25°C with maximum 44°C and minimum 13°C was recorded. The mean daily rainfall was 1.25 mm and an average of 11.25 *E. postvittana* adults were caught in the sex pheromone trap.

Discussion

The success of biological control in many systems, including the *E. postvittana*/*T. carverae* system, is determined by the reproductive success of parasitoids. Longevity is one of the determinants for reproductive success. Several researchers have determined that adult feeding increased longevity of adults (Gurr & Nicol, 2000; Berndt & Wratten, 2001). The present greenhouse study found that *L. maritima* fed *T. carverae* survived longer than those fed on *B. juncea*, *C. sativum* or no plant material (Figure 3. 1). This increased survival is in general agreement with other researchers' findings with non-trichogrammatid parasitoids (Johanowicz & Mitchell, 2000; Berndt & Wratten, 2001; Berndt, 2002). The second (Figure 3. 2) and third (Figure 3. 3) greenhouse experiments suggest that access to *F. esculentum* and *B. officinalis* flowers increased survival of *T. carverae* compared with access to shoots without-flowers or control treatments. Similarly, other researchers have found that *F. esculentum* and *B. officinalis* are good adult food resources for other Hymenopteran parasitoids (Baggen & Gurr, 1998; Stephens *et al.*, 1998). The effects of plant species on *T. carverae* could not be evaluated in a single experiment because of asynchronous flowering, so direct comparisons between separate experiments are not valid. It is clear however that several plant species significantly increase survival and realised parasitism of *T. carverae*, most dramatically *L. maritima*.

When *T. carverae* longevity on these plant species (except *C. sativum*) was compared, it was found that both males and females benefited from *L. maritima* and *F. esculentum* compared with other treatments (Table 3. 1 and 3. 2). This experiment constitutes the first greenhouse assessment of the effect of groundcover species on the longevity of male and female

T. carverae. Other sections of this study (Chapters Two and Four) indicate that *L. maritima* increases longevity of *T. carverae*. The greenhouse experiment (survival and parasitism of *T. carverae*) in this chapter also indicates that *L. maritima* and *F. esculentum* increase longevity. The biological significance of this study is that long-lived females were found to have parasitised more host eggs than short-lived females. Therefore, female *T. carverae* with access to *L. maritima* or *F. esculentum* flowers survive longer and reach their full reproductive potential. If such laboratory results apply in the field, there would be clear implications for biological control.

Trichogramma pretiosum parasitised more host eggs when they accessed nectared cotton compared with nectarless cotton (Treacy *et al.*, 1987). Similarly, non-trichogrammatid parasitoids benefit from floral resources e.g., Irvin (1999) reported that female *Dolichogenidea tasmanica* (Cameron) had more eggs in their ovaries when fed *F. esculentum* flowers. The present study found that *T. carverae* parasitised a greater number of *E. postvittana* eggs when fed *L. maritima* flowers compared with *B. juncea*, *B. officinalis*, *C. sativum* and *F. esculentum* flowers. These results also suggest that *L. maritima* extended the oviposition period, supporting previous findings of this work (Chapter Two). Bennett (2002) found that the first 24 hours of egg laying represented only 20-25% of the egg laying capacity of a *T. carverae* female that survives for six days with host eggs. The longevity of *T. carverae* is very short, less than 7 days when no food source is available (Gurr & Nicol, 2000) but the present daily fecundity results suggest that *T. carverae* females need to survive longer than 7 days in order to deposit all their eggs. To survive longer they need food, and *L. maritima* provides such food. These results also

suggest that *T. carverae* remain fecund until close to death, which is an important consideration for habitat manipulation practitioners.

Surveys of naturally occurring egg parasitoids suggest that at least at the time of sampling, that activity of *E. postvittana* parasitoids was very low in the Orange and Canowindra fields. This illustrates the potential value of inundative releases of agents such as *T. carverae*.

In the Canowindra field experiment, the parasitism rate was greater in *C. sativum*, *F. esculentum* and *L. maritima* treatments compared with control and vegetation without-flowers treatments, indicating that *T. carverae* benefited from flower nectar. Irvin *et al.* (2000) reported that *E. postvittana* parasitism was greater when apple orchards were planted with buckwheat and faba bean plants. Overall results suggest that *T. carverae* shows a degree of flower selectivity behaviour and that *L. maritima*, *C. sativum* and *F. esculentum* are suitable plant species to provide food for *T. carverae*.

Chapter Four - Flower colour discrimination by *Trichogramma carverae* in habitat manipulation

Introduction

Colour is one of the most important cues for insect recognition of flowers (Menzel & Backhaus, 1991; Chittka & Menzel, 1992; Kevan *et al.*, 1996) and is well studied in relation to pollination biology (Menzel & Shmida, 1993; Heiling *et al.*, 2003). It is well documented that hymenopterans are important insect pollinators (Brown *et al.*, 1998) and that they discriminate between flowers by using signals such as colour, size, shape, patterns, odour and other characteristics (Gumbert, 2000). In addition, pollinators have demonstrated a capacity for association learning in relation to specific colours and rewards (Chittka & Menzel, 1992; Brown *et al.*, 1998; Oliai & King, 2000) and this ability allows them to identify and exploit profitable food sources (Chittka & Menzel, 1992).

Flowers are also important to non-pollinating insects such as many biological control agents. Parasitoids visit flowers for food and there is increasing evidence that many insect predators and parasitoids require access to nectar and/or pollen (Jervis *et al.*, 1993; Wäckers, 1994). Habitat manipulation approaches such as strips of flowers are sometimes used to maximise their biocontrol potential (Landis *et al.*, 2000). Adult parasitoids require food as an energy source for flight (Elton, 1966) or for egg production in synovigenic species (Jervis *et al.*, 1996). The ability of parasitoids to locate nectar sources is important in biological control agents, but despite this relatively little research has been done on the importance of visual

cues in the food/host location processes of parasitoids compared with the role of chemical stimuli (Weseloh, 1981; Takasu & Lewis, 1996). Some parasitoids have been shown to respond to colour and other visual cues (Vinson, 1976; Wäckers & Lewis, 1994).

It is well recognised in tri-trophic systems, that the first trophic level (crop) plays a significant role in mediating ecological interactions between hosts and parasitoids (Takabayashi, *et al.*, 1998; Verkerk, *et al.*, 1998). Plant attributes such as provision of nectar and pollen can affect abundance, survival, fecundity and development of natural enemies (Cortesero *et al.*, 2000). The selection of flower species is important to the success of the provision of nectar resources in habitat manipulation (Gurr *et al.*, 1998; Berndt, 2002). Factors considered important in floral attraction of insect natural enemies include nectar availability (Idris & Grafius, 1995) and accessibility (Wäckers *et al.*, 1996) and the duration of flowering (Lövei *et al.*, 1993), but very little attention has been given to flower colour. If, therefore, flower colour is important for the use of floral resources by parasitoids, such effects need to be better understood for optimising habitat manipulation approaches in conservation biological control.

Trichogramma carverae is one of the most important natural enemies of the light brown apple moth *E. postvittana*, a serious insect pest of Australian grapevines (Glenn & Hoffmann, 1997). In Australian vineyards, *T. carverae* is released to control *E. postvittana* (Glenn & Hoffmann, 1997) but at a cost of Aus\$ 45/ha for each of the two to three releases required per season (Gurr *et al.*, 1998). The longevity of *Trichogramma* spp. is very short when no sugar source is available (Newton & Odendal, 1990) and fewer hosts are parasitised by unfed females (Ashley & Gonzalas, 1974). Gurr & Nicol (2000) demonstrated

a dramatic reduction in survival of *T. carverae* when deprived of carbohydrate. It is important, therefore, that any factors affecting the availability of nectar for *T. carverae* are studied.

The present study investigated the effect of flower colour as a factor in the biological control efficacy of *T. carverae* and measured survival of and parasitism achieved by this trichogrammatid parasitoid when it had access to different coloured *L. maritima* flowers and a control treatment (shoots of white flowering *L. maritima* from which flowers were removed) under no-choice conditions. The study also investigated whether cultivar effects may have been associated with nectar quantity by determining the numbers of flowers without obvious nectar. To investigate whether flower colour was responsible for the differences observed in insect performance, an experiment was conducted involving dyeing plants of the white flowering cultivar of *L. maritima*. Finally, the study tested whether the non-preference for dyed *L. maritima* flowers was the result of negative olfactory or gustatory stimuli from the dyes.

Materials and Methods

Lobularia maritima flower colour experiment

Capsules containing paper substrates bearing *Sitotroga cerealella* Oliver eggs parasitised by *T. carverae* used in this experiment were purchased from Bugs for Bugs, BioResources Pty Ltd., Mundubbera, Queensland, Australia. Capsules were incubated at 28°C. After *T. carverae* started to emerge in an “indicator vial” all the capsules for this experiment were taken out of the incubator and kept at room temperature to await adult eclosion approximately 24 h later.

Alyssum, *Lobularia maritima* L. (Cruciferae) plants used in these studies were purchased from a nursery (Oasis Horticulture Pty Ltd., Wimalee, Australia). The experimental design was a randomised block with four replicates and five treatments. In this experiment, plastic vials (5.5 × 4.5 cm) were used as flight cages; each held three cut *L. maritima* shoots bearing either white, light pink, dark pink or purple flowers (cultivars: Small Flower White, Small Flower Pink, Plum Crazy, and Small Flower Purple, respectively). In all flowering treatments, each shoot had sufficient buds to ensure continuous flowering throughout the experimental period. A control treatment used shoots from white-flowered *L. maritima* from which flowers and unopened flower buds were removed. This treatment was used to control for the effect of plants on the microclimate within cages. The cut ends of shoots were placed immediately in tap water and the experiment began within 5 h of shoot collection. The cut end of each shoot was passed through a small circular hole (1 cm diameter) in the bottom of

each plastic vial into water contained in a second vial (11 × 2.5 cm) beneath the first. Shoots were sealed into the holes with non-setting adhesive (Blue-Tack) to prevent insect escape. The top of the upper vial was then sealed with a sheet of tissue paper held in place with a rubber band. The units were supported in laboratory racks. Each capsule (bearing *S. cerealella* eggs parasitised by *T. carverae*) was cut into eight segments, bearing approximately 125 eggs. One of these segments was placed into each flight cage via a hole (2 × 1 cm) in its wall, which was then sealed with the same adhesive. The experiment was laid out on a laboratory bench top. The numbers of live wasps in each flight cage was recorded every 24 h until no more live *T. carverae* were recorded.

To measure the parasitism rate, *E. postvittana* eggs were obtained from the Department of Natural Resources and Environment, Victoria, Australia. Sentinel cards were prepared by cutting the plastic oviposition substrate into sections, each bearing one intact egg mass, and stapling each section to a yellow paper sheet (5 × 1 cm). Because acceptance of *E. postvittana* eggs by *T. carverae* is affected by age of host (Glenn & Hoffmann, 1997), only green (younger) eggs were used. Microscopic examination (10×) was used to count the number of eggs in each intact mass and this was recorded on each paper sheet. The mean number of eggs per card was 20.96 (range 5-57; half bearing 13 – 27 eggs). On every third day after *T. carverae* emergence, one sentinel egg card was placed in each flight cage (except cages where no live adults were present) to allow *T. carverae* oviposition to take place and was recovered after 24 h. Egg masses were subsequently incubated at 23°C until parasitised eggs became black and could be counted.

Daily maximum and minimum temperatures were recorded and temperature ranged from 20.1 - 23.4°C with a relative humidity of 56% (measured by wet and dry bulb thermometer readings).

Data analysis

A square root transformation, $\sqrt{(x + 0.5)}$, was used for number of live insects on each day to standardise the variance. Square-root transformation was chosen rather than untransformed actual data after inspection of residuals. The effect of flower colour treatments was tested by a repeated measures approach, multivariate analysis of variance (MANOVA). Because it was not possible to include days when all replicates of one treatment were zero in the MANOVA, only the first eight days after the peak in insect counts were analysed, not the following six days when no *T. carverae* were living in one or more treatments. The significance of differences between treatments and days was determined using *F*-tests, although an *F*-test in MANOVA is only an approximation to the *F*-distribution. Exponential curves of the form $y = A + BR^x$ (where x = number of days, y = number of live insects, A , B = linear parameters, R = survival rate) were also fitted to the data (omitting dates before the date with maximum wasp numbers) to compare the differences in position (A), slope (B) and curvature (R) for each treatment. First, an overall curve was fitted, then a set of curves with a different position (A) for each treatment, after that a set of curves with different position and slope (A and B) for each treatment and finally a set of curves with all (A , B and R) parameters separate. The steps defined above were accomplished automatically using the standard module for comparing non-linear regression in GenStat release 6.1. However,

where curvature, representing the key biological parameter of insect survival, differs between treatments, comparison of the linear parameters becomes irrelevant.

The number of eggs parasitised was calculated per cage and then analysed using randomised block ANOVA on each date. For all of the above analyses, GenStat release 6.1 (GenStat Committee, 2002) was used.

Nectar availability test

Flowers of each cultivar were examined with a microscope at 20x magnification and nectar was recorded as present or absent. A total of 23 white, 8 light pink, 14 deep pink and 11 purple flowers were examined. The flowers used came from the same plants from which shoots were taken for other experiments.

Data analysis

Presence/absence data were analysed with a χ^2 - test using GenStat release 6.1 (GenStat Committee, 2002) to test for differences between cultivars.

Dyed *L. maritima* flower experiment

This experiment sought to determine whether flower colour is responsible for the differential effects on *T. carverae*. Plant and insect material was sourced as described above. Three treatments replicated four times were employed in a randomised block design. White-flowered *L. maritima* plants were removed from their pots and soil was washed from their roots before they were placed into water only or water with 5% food dye (pink or blue food

coloring; Queen Fine Foods Pty Ltd, Queensland, Australia). After 24 h, white flowers had assumed colours that to the human eye were similar to those of the naturally coloured flowers. Three flower-bearing shoots were cut from plants of each treatment (i.e., dyed pink, dyed blue or undyed) and placed in flight cages (vials) as previously described. A segment of capsule bearing eggs parasitised by *T. carverae* was also added and each vial sealed. In this experiment, the undyed white flowering treatment served as a comparison treatment. The experiment was laid out on a laboratory bench top. The number of live *T. carverae* present in each cage was recorded every 24 h until all insects had died.

Daily maximum and minimum temperatures were recorded and temperature ranged from 19.0 - 22.3°C with an average relative humidity of 52% (measured by wet and dry bulb thermometer readings).

Data analysis

A square-root transformed $\sqrt{(x + 0.5)}$ was used for number of live insects on each day to standardise the variance before carrying out MANOVA for the first five dates when there were live *T. carverae* in each treatment. Square-root transformation was chosen rather than untransformed actual data after inspection of residuals. The significance of differences between treatments and days was determined using *F*-tests. Exponential survivorship curves were fitted to the data, omitting the first date (when wasps were still eclosing) as day two had maximum wasp numbers. Curves were fitted to each treatment as described in the *Lobularia maritima* flower colour experiment. GenStat release 6.1 (GenStat Committee, 2002) was used for all analyses.

Dyed honey experiment

Insect material and food dye was sourced and purchased as described above. A randomised block design with five replicates and four treatments was housed in a sealed plastic box (38 × 25 × 14 cm) in which water maintained the relative humidity above 90% (measured by wet and dry bulb thermometer readings). The box was held at 28 ±1°C in an unlit incubator. Honey solution (10%) was dyed with the same dyes as above (dye concentration 5%) and treatments were 10% honey, 10% honey + 5% blue dye, 10% honey + 5% pink dye and water. The water control and undyed honey solution served as reference treatments against which the dyed treatments were compared. Ten adult *T. carverae* (< 24 h after eclosion) were released in each vial (5.5 × 4.5 cm). *T. carverae* adults were transferred to the flight cages (vials) using gelatine capsules (size 00) (Tyco Healthcare Pty Ltd., Sydney, NSW, Australia) as described below to avoid the need for the use of anaesthesia, allaying possible deleterious effects on behaviour or longevity. Within 24 h of emergence, *T. carverae* do not fly (personal observation). Young adults were put on a white paper sheet (for ease of visibility) and each adult covered with half of a gelatine capsule. Adults crawled upwards inside the capsule, which was then lifted up and sealed with the other half of the capsule. Adults were introduced into flight cages by separating the capsule halves and tapping the half containing the insect against a hole (2 × 1cm) in the vial's wall. After adding ten adults, the hole was sealed with the same adhesive as above and examined under a microscope (10×) to confirm that 10 adults were present. The diet, on soaked cotton wool, was inserted through this hole every second day. The tops of the vials were sealed as above. Each replicate was inspected every 24 hours until no live *T. carverae* were recorded. Inspections took place under red light to avoid the insects receiving any visual cues from the diet.

Data analysis

A square-root transformed $\sqrt{(x + 0.5)}$ was used for number of live insects on each day to standardise the variance and a MANOVA was conducted using the first six dates. Square-root transformation was chosen rather than untransformed actual data after inspection of residuals. Differences between treatments and times were tested using *F*-tests. Exponential survivorship curves were fitted to the data for each treatment as described above. GenStat release 6.1 (GenStat Committee, 2002) was used for these analyses.

Results

***Lobularia maritima* flower colour experiment**

The MANOVA analysis of *T. carverae* survival showed significant interaction of treatment and time (days) ($F = 3.61$; $df = 20, 32$; $P = 0.002$) (Figure 4.1). With access to white flowers, adults were evident for 13 days; on light pink, adults were evident for 10 days; in other treatments, no adults were evident after day eight. Fitted exponential curves for treatments differed significantly in curvature ($F = 19.20$; $df = 4, 234$; $P < 0.001$). The daily survival rate (survival rate \pm SE) of *T. carverae* in the white *L. maritima* (0.913 ± 0.0243) was significantly higher than in the other treatments (light pink, dark pink, purple and stem from which white flowers were removed), 0.689 ± 0.035 ; 0.704 ± 0.030 ; 0.640 ± 0.033 ; 0.672 ± 0.030 , respectively.

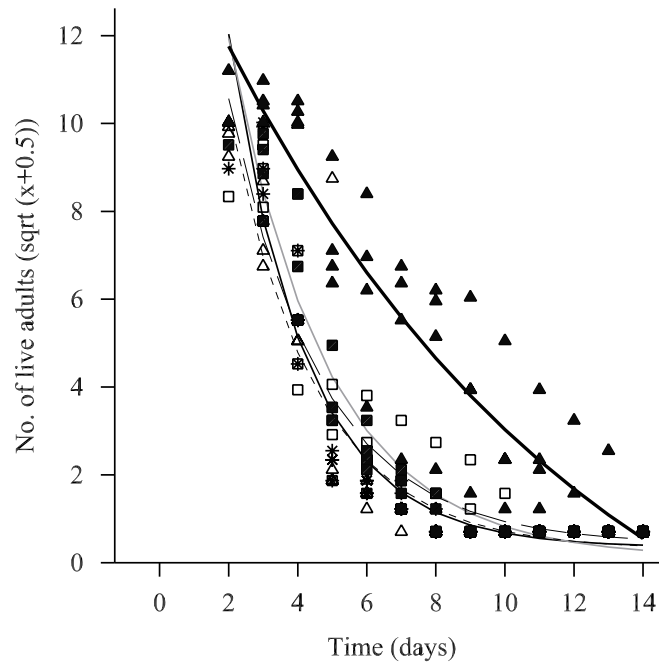


Figure 4. 1: *Trichogramma carverae* adult emergence and subsequent survival when confined with different colored *L. maritima* flowers or with shoots of the white-flowered cultivar with flowers removed: —▲— = white, flowers present; ----△---- = white, flowers removed; —□— = light pink, flowers present, —■— = dark pink, flowers present and —*— = purple, flowers present. Adjusted $R^2 = 90.2\%$. Points denote treatment means and lines denote fitted relationships.

Parasitism rates were significantly higher in the white-flower treatment than in other treatments on days three and six ($F = 14.37$; $df = 4, 12$; $P < 0.001$ and $F = 6.73$; $df = 4, 12$; $P = 0.004$, respectively) (Figure 4. 2). Parasitism had declined markedly in the white-flower treatment by the time of the ninth and twelfth day but no parasitism was recorded in other treatments at this time.

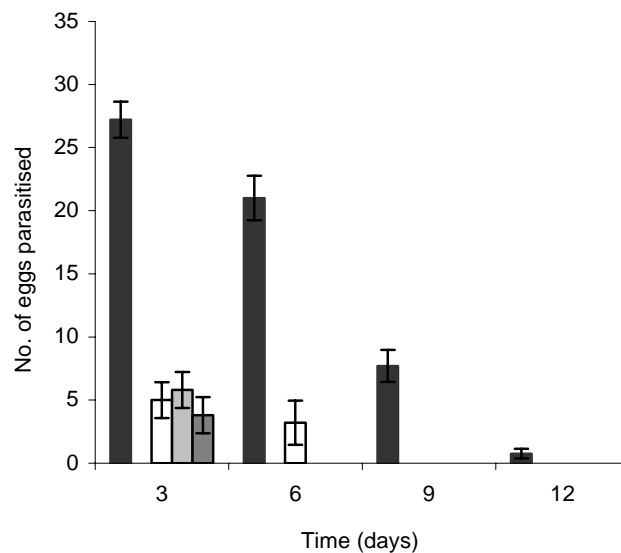


Figure 4. 2: Mean parasitism of *E. postvittana* eggs by *T. carverae* for each egg release date when provided with different coloured *L. maritima* flowers: ■ = white, flowers present, ▨ = white, flowers removed (zero for all dates), □ = light pink, flowers present, ■ = dark pink, flowers present, ■ = purple, flowers present. Bars show the standard errors.

Nectar availability test

The proportions of flowers without nectar (2/23, 1/8, 1/14, 1/11 for the white, light pink, dark pink, and purple, respectively) did not differ significantly ($\chi^2 = 0.15$; $df = 3$; $P = 0.985$).

Dyed *L. maritima* flower experiment

The survival of *T. carverae* showed a significant interaction between treatment and time (days) ($F = 39.86$; $df = 4, 10$; $P < 0.001$) (Figure 4. 3). When caged on non-dyed white *L. maritima* flowers, adults were evident for 10 days but in treatments in which white flowers were dyed either pink or blue, no adults were evident after day five. Fitted exponential curves for treatments differed significantly in curvature ($F = 24.50$; $df = 2, 111$; $P < 0.001$). *T. carverae* caged with non-dyed white *L. maritima* had a significantly higher survival rate (0.830 ± 0.038) than in the dyed pink (0.545 ± 0.042) and dyed blue (0.400 ± 0.058) treatments.

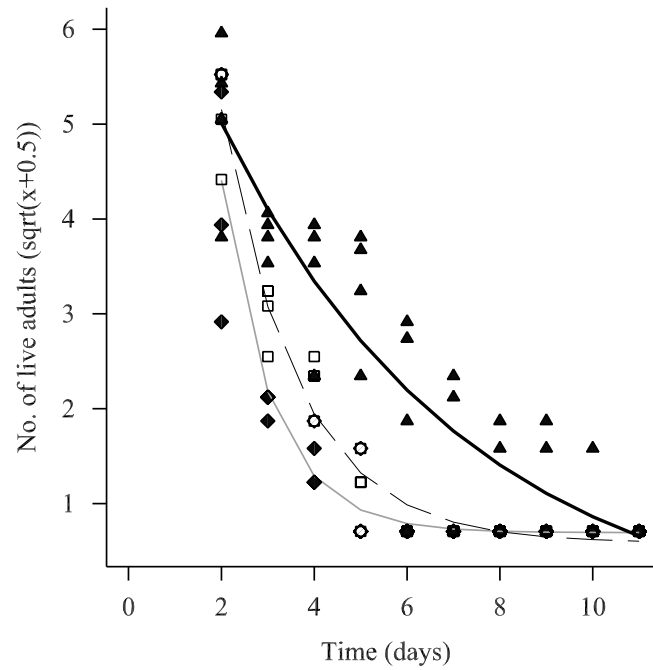


Figure 4. 3: *Trichogramma carverae* adult emergence and subsequent survival when confined with dyed and undyed white *L. maritima* flowers: —▲— = undyed white *L. maritima* flowers, - -□- - = dyed pink *L. maritima* flowers, —◆— = dyed blue *L. maritima* flowers. Adjusted $R^2 = 89.6\%$. Points denote treatment means and lines denote fitted relationships.

Dyed honey experiment

Using MANOVA for the first six dates did not show significant ($F = 0.83$; $df = 18, 20$; $P = 0.65$) interactions between treatments and time. The survival of *T. carverae* did not differ significantly between treatments (Figure 4. 4).

Fitted exponential curves for treatments differed significantly in curvature ($F = 8.24$; $df = 3, 168$; $P < 0.001$). The survival rate of *T. carverae* in the water treatment (0.302 ± 0.097) was significantly lower than the other three treatments (10% honey, 10% honey + 5% blue, 10% honey + 5% pink), 0.893 ± 0.070 ; 0.766 ± 0.068 ; 0.695 ± 0.070 ; 0.302 ± 0.097 , respectively.

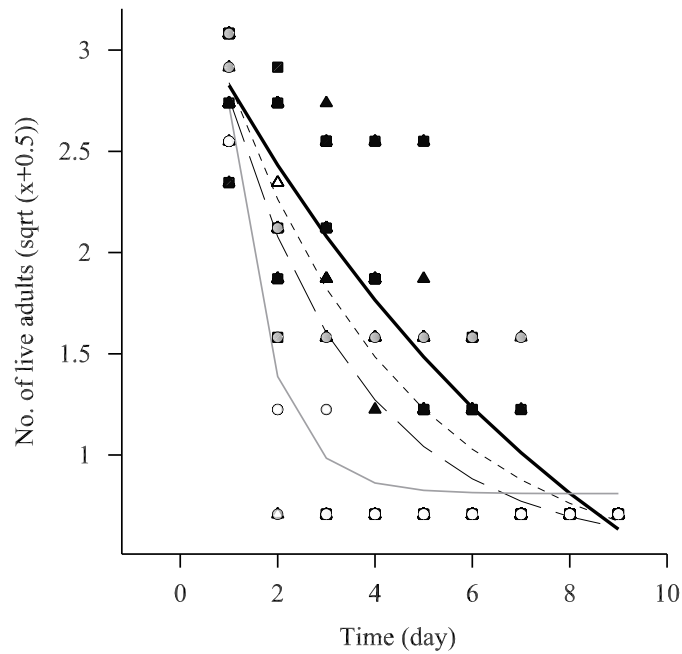


Figure 4. 4: *Trichogramma carverae* adult survival with dyed honey, undyed honey and water as food sources: **—▲—** = undyed 10% honey, **--△--** = 10% honey + 5% pink, **----□----** = 10% honey + 5% blue, **——○——** = water. Adjusted $R^2 = 73.6\%$.
Points denote treatment means and lines denote fitted relationships.

Discussion

The present study is the first to show an effect of flower colour on a trichogrammatid and is likely to have implications for the biological control efficacy of *T. carverae*. The survival and realised parasitism of *T. carverae* were significantly greater with access to white flowers than with *L. maritima* cultivars with flowers of other colours and a control treatment (shoots of white flowering *L. maritima* from which flowers and flowering buds were removed). The presence of white flowers is obviously important for *T. carverae*. Kevan (1973) reported Ichneumonidae visited white flowers though other researchers (Wardle, 1990; Idris & Grafius, 1997; Oliai & King, 2000) have reported that during flower selections, parasitoid were not guided by flower colour. Lukianchuk & Smith (1997) examined the foraging behaviour of *T. minutum* on artificial and natural surfaces and concluded that foliage colour did not influence female *T. minutum*. Similarly, Keller (1985) demonstrated that it is unlikely that female *Trichogramma* spp. were influenced by visual characteristics because they are polyphagous and host plant visual characteristics varied greatly.

Parasitic Hymenoptera may use colour contrast cues. Wäckers (1994) showed that food-deprived *Microplitis croceipes* parasitoids preferred yellow targets when foraging, while sugar-fed individuals preferred green leaves. In the current experiment, performance of *T. carverae* was greatest in the presence of white flowers. One possible reason for this effect is that white coloured flowers may have given the greatest contrast against green foliage. The reflectance peak of most green leaves is generally lower than that of white flowers (Kevan *et al.*, 1996).

Results demonstrate that choice of cultivar (within a plant species) is potentially important, leading to outcomes that range from a marked benefit to parasitoid fitness to no benefit. The cultivar effects observed could result if the nectar quantity of the white flowering cultivar is superior to that of other cultivars, and not directly related to flower colour. To investigate this, the number of flowers without obvious nectar was determined for each cultivar, but the proportions did not differ significantly. Further studies involved dyeing plants of the white flowering cultivar of *L. maritima*. The survival of *T. carverae* was greater on non-dyed white *L. maritima* than on dyed flowers of white *L. maritima*. This result shows that factors such as the nectar quality were not in play and suggests that *T. carverae* uses flower colour as an important visual cue. Beach *et al.* (2003) demonstrated that nectar use may be influenced by gustatory responses and suggested that some food sources act as mild feeding deterrents. Similarly, Wäckers (1994) found that parasitic Hymenoptera showed innate responses to food odour. It is therefore possible that the dyes used to manipulate flower colours in the present study were responsible for impaired *T. carverae* survival in blue- and pink-dyed treatments. In the follow-up experiment, however, in which honey solution was dyed, survival of *T. carverae* did not differ when they were fed blue- or pink-dyed honey solution and non-dyed honey solution. Survival was significantly lower in the water control treatment. Collectively, these results suggest that *T. carverae* performances on dyed *L. maritima* flowers were the result of visual rather than olfactory or gustatory cues.

Accordingly, cultivar choice profoundly influences the use of 'floral subsidies' (a type of food resource subsidy) (Polis & Strong, 1996) by *T. carverae*. Resource subsidies can enhance natural enemy performance in biological control (Kean *et al.*, 2003) via effects on longevity (Heimpel *et al.*, 1997; Johanowicz & Mitchell, 2000) fecundity (Yu *et al.*, 1984)

female-based sex ratio of parasitoid offspring (Berndt *et al.*, 2002) and spatial distribution (Thomas *et al.*, 1992). Knowledge of colour discrimination in *T. carverae*, as well as other natural enemies, is critical for the development of optimal habitat manipulation strategies. Overall, results indicate that flower colour is important for *T. carverae* and that, because of the observed effect on survival and parasitism, this may affect biological control efficacy. Such effects are unlikely to be confined only to this trichogrammatid species or this family and further work with Hymenoptera and other flower-feeding biological control agents is warranted. Providing resource subsidies (Polis & Strong, 1996) to parasitoids and predators is an effective and fast-expending component of conservation biocontrol (Landis *et al.*, 2000). Success of 'classical' biological control has been only 10% from the 1880s to the close of the 20th century (Gurr *et al.*, 2000). Identifying and addressing constraints on biological control agent performance offers scope for increasing the success rate. The ecological aspects of biological control need to be better understood, and the role of flower colour in tri-trophic-level interactions is clearly important and merits further research.

Chapter Five - The effects of groundcover plant species on adult longevity and larval development of *Epiphyas postvittana*

Introduction

The supply of adult food is an important factor in determining the efficacy of parasitoids as biological control agents (van Lenteren *et al.*, 1997; Takasu & Lewis, 1996) but many adult Lepidoptera also feed on floral nectar (Kevan & Baker, 1984). When floral resources are made available to natural enemies in a cropping system this resource may also act as a nectar source for a pest (e.g., Baggen & Gurr, 1998). *Epiphyas postvittana* is known to benefit from access to alyssum flowers or honey compared with only water in the laboratory (Irvin, 1999; Gu & Danthanarayana, 1990). Further, *E. postvittana* larvae are polyphagous and feed on broad-leaved weeds, medic and clover during winter (Baker *et al.*, 1994). Therefore, *E. postvittana* larvae may use vineyard groundcover plants as a food source. It is important, therefore, to assess whether this form of habitat manipulation would provide a benefit for adult or larval *E. postvittana* before recommending the establishment of groundcover plants in vineyards.

Therefore, the objective of this study was to investigate whether the adults or larvae of *E. postvittana* benefit from groundcover plant species that, in other sections of this work, have been evaluated as resources for *T. carverae*.

Materials and Methods

Effect of groundcover plants on the longevity and lifetime fecundity of *E. postvittana*

Plant species did not bloom in synchrony so two experiments were conducted in the green house to test plants as they flowered.

Effect of groundcover plants on *E. postvittana* longevity and lifetime fecundity

Plant materials and *E. postvittana* were sourced as in Chapter Three. A randomised block design with ten replicates and nine treatments was used. The first four treatments used flowering shoots of alyssum (*Lobularia maritima* L.; cv. Small Flower White), borage (*Borago officinalis* L.; cv. Borage), buckwheat (*Fagopyrum esculentum* Moench.; cv. Ikeda) and coriander (*Coriandrum sativum* L.; cv. Macrocarpum). To ensure continuous flowering throughout the experimental period, each shoot had several flowers. A further four treatments consisted of the above plant species from which flowers and flower buds were removed. A positive control treatment with *E. postvittana* reared on adult food (honey, 180ml; water, 1800ml; ascorbic acid, 10.8g (0.6%); sorbic acid, 1.8g (0.1%); paraben, 1.8g (0.1%) and 70% ethanol, 10ml. Rundle, B., La Trobe University, Victoria, Australia, personal communication)) was used as a comparison treatment. Cotton wool balls were moistened with this solution and placed in each replicate. These were re-moistened every second day using a hypodermic syringe. Plant shoots were collected from plants grown in a green house and immediately placed in tap water until the experiment began (within five hours of shoot collection). Plastic vials were used as flight cages; a description of the method used is given in Chapter Three (see survival and parasitism of *T. carverae*

experiment). The plant material and cotton in the control treatment was replaced once per week. In each cage one piece of plastic coffee cup (4.5×2.5 cm) was provided as a female oviposition substrate. One male and one female both newly emerged (< 24 h after eclosion) adults *E. postvittana* were placed in each cage. Males and females were identified on the basis of body size and wing characters (Baker *et al.*, 1994). The top of each cage was sealed with tissue paper using the same method described in Chapter Three. In each flight cage, the number of live individuals was recorded at 24 h intervals until all had died. After the death of the female, the flight cage, oviposition substrate, test plant foliage and stems were examined for *E. postvittana* eggs using microscopic examination ($10\times$) and the number was recorded. If the male was alive it was transferred to a new vial with fresh plant material. The experiment took place in a green house maintained a 16L: 8D photoperiod, 20°C L/ 16°C D temperature and an average relative humidity of 54%.

Data analysis

Longevity data were analysed using randomised block ANOVA. A square root transformation $\sqrt{(x + 0.5)}$ was used to standardise the variance for lifetime fecundity data and analysed using randomised block ANOVA. Square-root transformation was chosen rather than untransformed actual data after inspection of residuals. If the treatment F-test was significant, treatments were compared using a Least Significant Difference (LSD) test. GenStat release 6.1 (GenStat Committee, 2002) was used for all data analyses.

Effect of *B. juncea* on *E. postvittana* longevity and lifetime fecundity

This experiment was designed and conducted in the same way as the previously described experiment. The treatments in this experiment were mustard (*Brassica juncea* L. (Czernj));

cv. Peacock Tail) with- and without-flowers and two types of control. One was the previously described artificial adult food and the second was water alone. This experiment was conducted in the same greenhouse with a 16L: 8D photoperiod 20°C L /16°C D temperature and an average relative humidity of 60%.

Data analysis

Data were analysed in the same way as described above.

Effect of groundcover plants on larval development of *E. postvittana*

Effect of groundcover plants on *E. postvittana* larvae: excised shoot experiment

Plant material and *E. postvittana* larvae were sourced as described above, with the addition of white clover (*Trifolium repens* L.: Leguminosae) plants which were collected from the University's Orange Campus and grown in the greenhouse. A randomised block design with five replicates and seven treatments was used. Blocks had one replicate each of *B. juncea*, *B. officinalis*, *C. sativum*, *F. esculentum*, *L. maritima* and *T. repens* and an artificial larval diet (positive control). Shoots with flowers and leaves of these species were placed in flight cages in the same way as described above. Ten neonate (< 24 h old) *E. postvittana* larvae were introduced into each cage and the top of each vial was sealed as described above. The number of dead larvae was assessed every 24 h using microscopic examination (10×). Counting continued until all larvae either died or underwent pupation (the total observation period was 78 days). Plant materials were replaced every week. Pupae were collected once per week. This experiment was conducted on the greenhouse bench top where 16L: 8D photoperiod, 20°C L/16°C D temperature and an average humidity of 57% were maintained.

Data analysis

Levels of larval mortality in each replicate at the end of the experiment's 78 day duration were subject to angular transformation, then analysed using randomised block ANOVA. Angular transformation is appropriate because of the large proportion of out-of-set insects. Larval mortality data were tested using MANOVA but it was not possible to analyse dates where all replicates of one treatment had the same values such as when all larvae had died in all replicate. Exponential curves of the form $y = A + BR^x$ (where x = number of days, y = mean cumulative number of dead larvae, A , B = linear parameters, R = mortality rate) were fitted to the temporal data to compare the differences in position (A), slope (B) and curvature (R) for each treatment (see Chapter Three). First, an overall curve was fitted, then a set of curves with a different position (A) for each treatment, after that a set of curves with different position and slope (A and B) for each treatment and finally a set of curves with all (A , B , and R) parameters separate. The steps defined above were accomplished automatically using the standard module for comparing non-linear regression in GenStat release 6.1. Data from the artificial diet control treatment was discarded from the data analysis because all larvae died as a result of the diet drying out.

The pupation data in each replicate at the end of the experiment were subject to angular transformation then analysed using a randomised block ANOVA. In a separate analysis, the number of pupae on each date was angular transformed then analysed using MANOVA. Only five pupal collection dates were analysed because MANOVA cannot be used where all replicates of one treatment yielded zeros. Significant differences between treatments and days were determined using F -tests. The F -test in MANOVA is an approximation to the F - distribution. Exponential curves were fitted to the temporal data to compare the

difference in position (*A*), slope (*B*) and curvature (*R*) for each treatment as pupae curves as described above. GenStat release 6.1 (GenStat Committee, 2002) was used for all data analyses.

Effect of groundcover plants on *E. postvittana* larvae: intact plant experiment

Plant and insect material were sourced as described above. A randomised block experimental design was used with five replicates. Each replicate consisted of a potted plant of *B. juncea*, *B. officinalis*, *C. sativum*, *F. esculentum*, *L. maritima* or *T. repens*. Pots were 850ml and the plants were covered with fine nylon mesh supported by a single bamboo cane and secured around the pot's rim with rubber bands. Twenty neonate (< 24 h old) larvae of *E. postvittana* were introduced into each cage. They were checked every week after the first six weeks and all pupae present on each occasion were collected. The experiment was conducted on the greenhouse bench top and environmental conditions were as stated above.

Data analysis

The numbers of pupae in each replicate at the end of the experiment were subject to angular transformation then analysed using ANOVA. Angular transformation is appropriate because of the large proportion of out-of-set insects. The numbers of pupae present on each collection date were angular transformed. Differences between treatments and times were tested using MANOVA (Multivariate analysis of variance was not possible where all replicates of one treatment were zeros). The data were then analysed using randomised block ANOVA at each date. Exponential curves were fitted to the data and compared as described above. GenStat release 6.1 (GenStat Committee, 2002) was used for all data analyses.

Results

Effect of groundcover plants on the longevity and lifetime fecundity of *E. postvittana*

Effect of groundcover plants on *E. postvittana* longevity and lifetime fecundity

The treatments significantly affected the longevity of male ($F = 3.81$; $df = 8,72$; $P < 0.001$) and female ($F = 4.79$; $df = 8,72$; $P < 0.001$) *E. postvittana* (Table 5.1). The mean longevity of female *E. postvittana* with- and without-flowers of *C. sativum* and *L. maritima* treatments was significantly lower than in the *F. esculentum* with-flower and control (artificial diet) treatments. Female longevity in the *C. sativum* and *L. maritima* with-flowers treatments was not significantly greater than in the corresponding treatments without flowers, though there was a similar lack of significant difference between the two *B. officinalis* treatments for females but not for males, for which longevity was increased by presence of flowers. Longevity in the with-flower treatment of *B. officinalis* was significantly higher than that in *C. sativum* treatments and the without-flower treatments of *L. maritima* and *F. esculentum*. The longevity of male *E. postvittana* in the *C. sativum* and *L. maritima* treatments with- and without-flowers treatments differed significantly from the *B. officinalis* with-flowers and control treatment, but not from the *B. officinalis* and *F. esculentum* without-flower treatments. The longevity of female *E. postvittana* in the *F. esculentum* treatment with-flowers was significantly greater than the equivalent without-flower treatment. In contrast, male longevity in the *F. esculentum* treatment with-flowers did not differ from the without-flowers or control treatments. Female longevity did not differ in *B. officinalis* with-flower and without-flower treatments. Male longevity in *B. officinalis* treatment with-flowers was

significantly higher than the without-flower treatment and did not differ significantly from the *F. esculentum* with-flower and control treatments.

Lifetime fecundity of *E. postvittana* did not differ significantly between treatments ($F = 1.19$; $df = 8.72$; $P = 0.115$; Table 5. 1).

Table 5. 1: Mean longevity and fecundity of *E. postvittana* when caged with shoots of different groundcover plants species (+ = shoots with flowers, - = shoots without flowers) and control (artificial adult food).

Treatments	Male longevity (days)	Female longevity (days)	Lifetime fecundity back-transformed (sqrt-transformed)
<i>B. officinalis</i> (+)	13.00e	16.40bc	147.12 (12.15)
<i>B. officinalis</i> (-)	6.80ab	11.80ab	131.29 (11.48)
<i>C. sativum</i> (+)	6.00a	10.00a	80.50 (9.00)
<i>C. sativum</i> (-)	6.90abc	9.30a	56.81 (7.57)
<i>F. esculentum</i> (+)	9.80bcde	18.20c	75.86 (13.28)
<i>F. esculentum</i> (-)	7.30abc	10.10a	123.82 (11.35)
<i>L. maritima</i> (+)	7.80abc	12.90ab	156.50 (12.53)
<i>L. maritima</i> (-)	8.40abcd	9.70a	78.18 (8.87)
Control	11.60de	18.50c	184.46 (13.60)
LSD ($P = 0.05$)	3.427	4.828	ns

Means followed by the same letter do not differ significantly ($P < 0.05$).

Effect of *B. juncea* on *E. postvittana* longevity and lifetime fecundity

Longevity did not differ significantly for female ($F = 0.34$; $df = 3, 27$; $P = 0.795$) or male ($F = 2.31$; $df = 3, 27$; $P = 0.099$) *E. postvittana* (Table 5.2). Lifetime fecundity of *E. postvittana* did not differ significantly between treatments ($F = 1.19$; $df = 3, 27$; $P = 0.331$; Table 5.2).

Table 5. 2: Mean longevity and fecundity of *E. postvittana* when caged with shoots of *B. juncea* (+ = shoots with flowers, - = shoots without flowers), artificial food (positive control) and water control.

Treatments	Male longevity (days)	Female longevity (days)	Lifetime fecundity back-transformed (sqrt-transformed)
<i>B. juncea</i> (+)	10.40	10.30	84.69 (9.23)
<i>B. juncea</i> (-)	8.90	9.30	36.10 (6.05)
Control (artificial food)	9.50	11.10	97.51 (9.90)
Control (water)	13.20	10.40	122.27 (11.08)

No significant treatment effects.

Effect of groundcover plants on larval development of *E. postvittana*

Effect of groundcover plants on *E. postvittana* larvae: excised shoot experiment

Larval mortality rate differed significantly ($F = 4.86$; $df = 5,20$; $P = 0.005$) between treatments (Table 5. 3). Mortality was significantly higher in *C. sativum* *F. esculentum* and *L. maritima* than in *T. repens*. Mortality in *B. juncea* and *B. officinalis* treatments differed significantly from the *C. sativum* and *L. maritima* but not from the *F. esculentum* and *T. repens*. Within a few days of the experiment's commencement the artificial diet dried and all the larvae in this treatment died.

Table 5. 3: Mean larval mortality and pupation of *E. postvittana* when caged with shoots of different ground cover plant species.

Treatments	Mean larval mortality back-transformed (angular transformed)	Mean pupation back-transformed (angular transformed)
<i>B. juncea</i>	49.65% (44.8) ab	50.35% (45.2)bc
<i>B. officinalis</i>	50.00% (45.0)ab	50.00% (45.0)bc
<i>C. sativum</i>	90.45% (72.0)c	9.55% (18.0)a
<i>F. esculentum</i>	80.37% (63.7)bc	19.63% (26.3)ab
<i>L. maritima</i>	92.50% (74.1)c	7.51% (15.9)a
<i>T. repens</i>	42.87% (40.9)a	57.13% (49.1)c

Means (angular transformed) followed by the same letter do not differ significantly at $P < 0.05$, (dead larvae and pupae, L.S.D = 19.97 (angular transformed)).

Fitted exponential curves for larval mortality treatments differed significantly in curvature ($F = 30.86$; $df = 5, 450$; $P < 0.001$) (Figure 5. 1). The daily larval mortality rates (\pm standard error) were: *B. juncea*, 0.997 ± 0.0051 ; *B. officinalis*, 0.996 ± 0.0042 ; *C. sativum*, 0.920 ± 0.0063 ; *F. esculentum*, 0.989 ± 0.0025 ; *L. maritima*, 0.986 ± 0.0028 and *T. repens*, 0.963 ± 0.0045 .

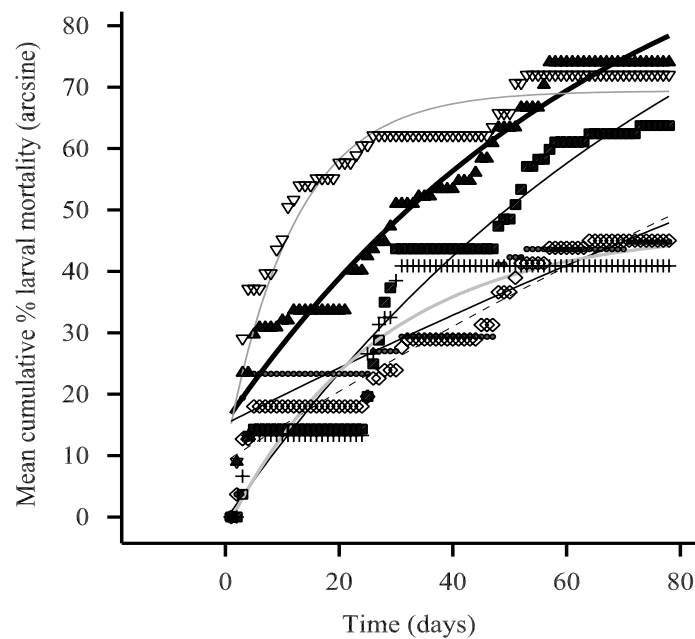


Figure 5. 1: Mean cumulative larval mortality of *E. postvittana* when caged with shoots of *B. juncea* (—●—), *B. officinalis* (----◇----), *C. sativum* (—▽—), *F. esculentum* (—■—), *L. maritima* (—▲—) and *T. repens* (—+—). Adjusted $R^2 = 94.9\%$. Points denote treatment means and lines denote fitted relationships.

Pupae were first found in *L. maritima* after eight weeks, seven weeks in the case of *C. sativum*, six weeks in *B. juncea*, five weeks in *F. esculentum* and *B. officinalis* and four weeks in *T. repens*. Pupation rate differed significantly ($F = 4.86$; $df = 5, 20$; $P = 0.005$) between treatments and was lower in *C. sativum*, and *L. maritima* than in *B. juncea*, *B. officinalis* and *T. repens* (Table 5. 3). In *F. esculentum*, pupation rate was significantly lower than on *T. repens* but did not differ from other treatments. The MANOVA analysis of pupation data showed a significant interaction of treatment and time (days) ($F = 1.88$; $df = 25, 61$; $P = 0.024$). The mean number of pupae in the *C. sativum*, *F. esculentum* and *L. maritima* treatments on the fourth, fifth, sixth, seventh and eighth collection dates were significantly ($P < 0.0001$; $P < 0.001$; $P < 0.001$; $P = 0.002$; $P = 0.004$, respectively) lower than was observed for *B. juncea*, *B. officinalis* and *T. repens*.

Fitted exponential curves for pupation data differed significantly in curvature ($F = 2.61$; $df = 5, 25$; $P < 0.05$) (Figure 5. 2). The pupation rates (\pm standard error) were: *B. juncea*, 0.908 ± 0.0201 ; *B. officinalis*, 0.966 ± 0.0117 ; *C. sativum*, 0.868 ± 0.0617 ; *F. esculentum*, 0.982 ± 0.0253 ; *L. maritima*, 0.951 ± 0.0322 and *T. repens*, 0.927 ± 0.0222 .

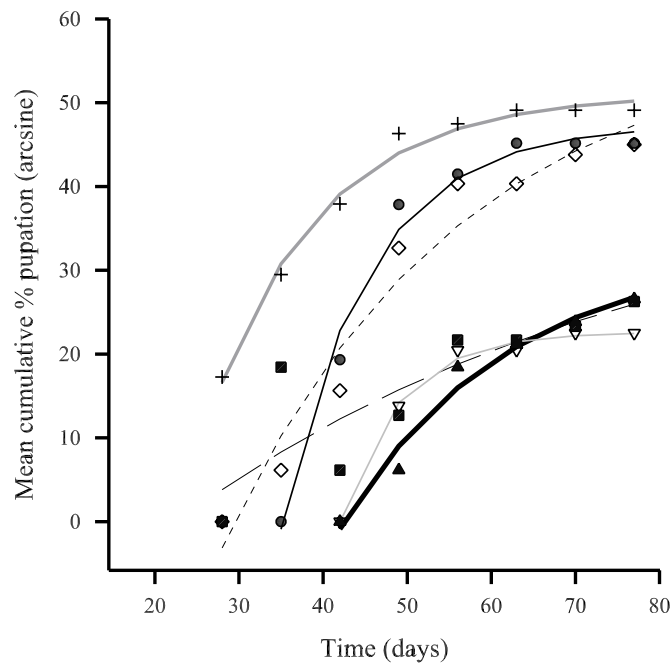


Figure 5. 2: Mean cumulative pupation rates for *E. postvittana* when caged with shoots of *B. juncea* (—●—), *B. officinalis* (----◇----), *C. sativum* (—▽—), *F. esculentum* (—■—), *L. maritima* (—▲—) and *T. repens* (—+—). Adjusted $R^2 = 94.9\%$. Points denote treatment means and lines denote fitted relationships.

Effect of groundcover plants on *E. postvittana* larvae: intact plant experiment

Pupae were first found in *L. maritima* after seven weeks and six weeks in the remaining treatments, but the number was variable. Only two pupae were found in *C. sativum*, in contrast to 11 in *B. officinalis*, 28 in *B. juncea*, 33 in *F. esculentum* and 37 in *T. repens*.

Pupation rate was significantly ($F = 7.88$; $df = 5, 20$; $P < 0.001$) lower in *C. sativum* and *L. maritima* than in *B. juncea*, *B. officinalis*, *F. esculentum* and *T. repens* treatments (Table 5. 4). In *B. officinalis*, pupation rate was significantly lower than in the *T. repens* treatment.

Table 5. 4: Mean pupation of *E. postvittana* when caged with potted plants of different ground cover plant species.

Treatments	Pupation rate back-transformed (angular transformed)
<i>B. juncea</i>	62.44% (52.2)bc
<i>B. officinalis</i>	37.40% (37.7)b
<i>C. sativum</i>	6.02% (14.2)a
<i>F. esculentum</i>	63.11% (52.6)bc
<i>L. maritima</i>	9.14% (17.6)a
<i>T. repens</i>	74.39% (59.6)c

Means (angular transformed) followed by the same letter do not differ significantly at

$P < 0.05$. (L.S.D = 20.26 (angular transformed)).

Pupation trends showed significant interaction of treatment and time (days) ($P < 0.001$). The mean number of pupae in the *C. sativum* and *L. maritima* treatments on the second, third and fourth collection dates was significantly ($P < 0.001$) lower than for *B. juncea*, *B. officinalis*, *F. esculentum* and *T. repens*. On the first date no pupae were recorded in *L. maritima* and the number recorded on *C. sativum* was lower than from *B. juncea*, *F. esculentum* and *T. repens*.

Fitted exponential curves for pupation differed significantly in curvature ($F = 5.20$; $df = 5, 12$; $P = 0.009$) (Figure 5. 3.). The pupation rates (\pm standard error) were: *B. juncea*, 0.818 ± 0.0359 ; *B. officinalis*, 0.861 ± 0.0326 ; *C. sativum*, 0.856 ± 0.0647 ; *F. esculentum*, 0.884 ± 0.0330 ; *L. maritima*, 0.100 ± 0.0240 and *T. repens*, 0.880 ± 0.0271 .

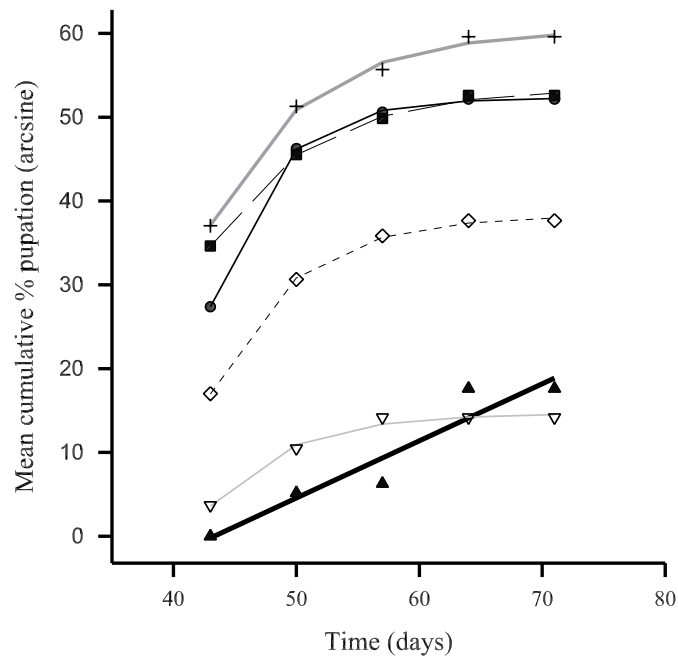


Figure 5. 3: Mean cumulative pupation of *E. postvittana* when caged with potted plants of *B. juncea* (—●—), *B. officinalis* (----◇----), *C. sativum* (—▽—), *F. esculentum* (—■—), *L. maritima* (—▲—) and *T. repens* (positive control) (—+—). Adjusted $R^2 = 99.4\%$. Points denote treatment means and lines denote fitted relationships.

Discussion

Gu & Danthanarayana (1990) showed that honey increases longevity of *E. postvittana*. In the present study, the relatively low longevity of female *E. postvittana* in the treatments without-flowers suggested that *E. postvittana* were food deprived. Depressed longevity in *C. sativum* and *L. maritima* with-flowers treatments indicates that these flowers do not provide a suitable food for adult *E. postvittana*. This result contradicts the findings of Irvin (1999) who found that when this Lepidopteran had access to *L. maritima* flowers or honey both longevity and fecundity were significantly increased compared with water. Male *E. postvittana* benefited from *B. officinalis* and *F. esculentum* flowers whilst female longevity was increased in the presence of flowers of the latter. This indicates that *B. officinalis* and *F. esculentum* provide nutrition for adult *E. postvittana* that is equivalent to the honey-based artificial diet. Other flower species in the first experiment, *L. maritima* and *C. sativum*, appeared not to benefit lepidopteran longevity. In the second experiment neither male nor female longevity was improved by access to honey-based adult diet compared with the water only diet. This lack of effect is unexplained and its anomalous nature means that conclusions on the effect of *B. juncea* flowers (which appeared to provide no benefit) must be treated with caution.

Epiphyas postvittana adults obtain both water and nutrients when feeding upon nectar and the experimental design used in the first experiment did not include a water only control to indicate the relative importance of each. It is clear, however, that provision of *B. officinalis* and *F. esculentum* as floral resources for natural enemies in a vineyard may increase the

longevity of *E. postvittana*. In the absence of evidence to the contrary, *Brassica juncea* also must be considered as a possible food source for this pest.

A risk associated with the use of nectar producing plants in vineyards is to increase the fitness of natural enemies. *Epiphyas postvittana* larvae may benefit from such groundcover plant species. Suckling *et al.* (1998) reported that larvae feed on weeds commonly found in or near the vineyards. Danthanarayana (1975) studied the rates of larval development of *E. postvittana* on curled dock (*Rumex crispus* L.), plantain (*Plantago lanceolata* L.) and apple (*Pyrus malus* L.) leaves at constant temperatures and concluded that rate of growth was faster than the rate observed for larvae fed on capeweed (*Arctotheca calendula* (L.) Levyns) on which all larvae died within a few days. In the present study, the larval developmental period of *E. postvittana* was extended on *C. sativum* and *L. maritima* compared with other plant species. This result suggests that *C. sativum* and *L. maritima* are poor hosts of *E. postvittana*. In contrast, *B. juncea* and *T. repens* were suitable hosts.

The overall results suggest that *C. sativum* and *L. maritima* denied any benefit to *E. postvittana* adults and that both are poor hosts of larvae. Baggen & Gurr (1998) suggested the value of “selective food plants”. In that study, “selective food plants” were those that increased the fitness of the hymenopteran parasitoid *Copidosoma koehleri* Blanchard whilst denying benefit to its pestiferous host, the potato moth (*Phthorimaea operculella* (Zeller)). The present study is the first equivalent work on a *T. carverae* / *E. postvittana* conservation biological control system and has value in suggesting that *C. sativum* and *L. maritima* may be useful vineyard groundcover species that do not benefit the adults or larvae of the key pest *E. postvittana*. The polyphagous nature of this pest’s larvae contrasts with the

Solanaceae-specific nature of *P. operculella* larvae. This fact made it important to test larval feeding rather than only adult feeding as done by Baggen & Gurr (1998). This represents a significant methodological advance in the development of conservation biological control towards targeted approaches and away from shotgun approaches (Gurr *et al.*, 2004).

Chapter Six- General discussion, recommendations for future research and conclusions

General discussion

The Australian wine industry is anticipated to earn Aus \$ 4.5 billion in annual sales of wine by 2025, but consumer demand for the development of sustainable vineyard management systems is increasing. One of the major problems of this industry is the use of synthetic pesticides for the control of pests. Biological control is one way to reduce pesticide use and to develop more sustainable vineyard management systems. Conservation biological control offers scope to increase the effectiveness of natural enemies (predators and parasitoids) and to reduce pest damage through habitat manipulation.

This study has investigated the effect of floral resources on the natural enemies of *E. postvittana*, especially the egg parasitoid *T. carverae*, in the vineyard environment, as well as laboratory assessment of the fitness of *T. carverae* in the presence of floral resources of various types. As an endemic Australian egg parasitoid, *T. carverae* is a particularly important target for enhancement by conservation biological control, because inundative releases are already being used to augment natural populations in vineyards. These mass releases are, however, expensive. Provision of appropriate adult food offers scope to increase the impact of a given release on pest densities through maximising fecundity, longevity and other aspects of the parasitoid's fitness but screening work of the type undertaken in this work is important to identify the most appropriate food plant species. The

study also investigated whether *E. postvittana* adults and larvae get any benefit from these floral resources, for habitat manipulation without such prior work can have adverse outcomes (e.g., Baggen & Gurr, 1998; Stephens *et al.*, 1998).

Prior to this study, available literature was reviewed (Chapter One). Some studies have been done in a variety of ecosystems on the importance of food for adult parasitoids (van Emden, 1963; Jervis *et al.*, 1996; Berndt *et al.*, 2002). For example, *Trichogramma perkinsi* Girault parasitised more host eggs when they accessed flowers of Bengal gram (*Cicer arietinum* L.) (Somchoudhury & Dutt, 1988) and feeding increased the longevity of *T. platneri* Ngarkatli (Hohmann *et al.*, 1989). There is no indication in the literature that adult *T. carverae* require food to reach their potential effectiveness in the field. Only Gurr & Nicol (2000) have previously studied the effect of a honey diet under laboratory conditions. The following sections analyse the contribution of each piece of work to the overall aims of this project.

Effects of adult food on *T. carverae* and other natural enemies: preliminary work

The preliminary work of this project reported in Chapter Two aimed to investigate the effect of adult food on the natural enemies (predators and parasitoids) of *E. postvittana*, particularly the egg parasitoid *T. carverae* both in the laboratory and field. The field study showed that both green (younger) and orange (older) eggs of *E. postvittana* are susceptible to parasitism by *T. carverae*. Other researchers have demonstrated that *T. carverae* and other *Trichogramma* spp. have host age preferences (Glenn & Hoffmann, 1997; Miura & Kobayashi, 1998). This result suggested that host-egg age has no effect on parasitism, and this is the first non-laboratory assessment of host age effect on parasitism by *T. carverae*. This observation is clearly of relevance to future researchers working with this parasitoid but

has practical relevance also in suggesting that the ‘window of opportunity’ during which releases of *T. carverae* may effectively be made is wider than would be the case if there was a strong age effect.

In this study, *L. maritima* was used as a nectar source because it is well researched as a nectar producing plant for some other parasitoids (e.g., Chaney, 1998; Irvin, 1999; Johanowicz & Mitchell, 2000; Berndt, 2002). In the initial field experiment, *L. maritima* did not lead to any enhancement of parasitism. Possible reasons may be cool and moist conditions prevailing in the vineyard during late-season and the activity of *T. carverae* (and other natural enemies) being constrained by the environmental conditions. For example, van Steenburgh (1934) observed that *Trichogramma* parasitoid do not take flight during rain, so precluding foraging for host eggs. Similarly, Fournier & Boivin (2000) assessed the dispersal patterns of two trichogrammatid species with 16 environmental variables and concluded that these egg parasitoids are very sensitive to climatic conditions. Other than the likely suppressing effect of the environmental conditions that prevailed in the preliminary field experiment, three hypotheses were tested to explain the lack of the effect of *L. maritima* on parasitism by *T. carverae*.

A growth cabinet experiment tested hypothesis one. Results showed that *L. maritima* increased realised parasitism by *T. carverae*. This result is broadly consistent with previous findings for other Hymenoptera and does not support the hypothesis that *T. carverae* does not feed on *L. maritima* (hypothesis one). This finding showed that habitat manipulation for this natural enemy offers scope to improve the management of *E. postvittana*.

Hypothesis two was tested with weed species and *L. maritima* in the laboratory. *Trichogramma carverae* survived longer when they were fed *Hypochoeris radicata*, *Trifolium repens* and *L. maritima*. *Echium plantagineum* did not give any enhancement of survival, indicating that some flower selectivity applies to *T. carverae* feeding. This result supports hypothesis one and partially supports the hypothesis that *T. carverae* may feed on nectar from some common vineyard weeds (hypothesis two). This result has some implication in suggesting that if vineyard managers were prepared to tolerate flowering dicotyledon weeds in the vineyard, there may be benefits for natural enemies of *E. postvittana* and possibly other pests. It is important to consider, however, that information in which plant species should be tested or added is critical in optimising vineyard management.

In the laboratory, punctured grape experiments showed that *T. carverae* feeds on exudates from ripening grapes (hypothesis three). *Trichogramma carverae* longevity was increased when they accessed grape exudates, which might be an important late-season food for *T. carverae* as well as other natural enemies of vineyard pests. The significance of this finding is that the late-season activity of parasitoids may reduce crop damage and limit the winter generation of *E. postvittana* populations. This result indicates more generally that fruits can play an important role in habitat manipulation, but very little research has been done on the uses of fruits as food for natural enemies in conservation biological control research and this merits closer consideration by future habitat manipulation researchers.

Effects of adult food on *T. carverae* and other natural enemies: follow-up studies

The work presented in Chapter Three represents follow-up studies from Chapter Two. The greenhouse experiment showed that survival and realised parasitism of *T. carverae* were

significantly higher in the presence of *L. maritima* than in *C. sativum* and *B. juncea* or in without-flowers of these species or in the nil control treatments. These results are consistent with the preliminary work of this study (Chapter Two). Both the second and the third experiment in that series suggested that survival of *T. carverae* was enhanced by nectar from *F. esculentum* and *B. officinalis* flowers though there was no significant increase in parasitism. Other researchers have also reported that *B. officinalis* and *F. esculentum* are both good nectar sources for other parasitoids (Stephens *et al.*, 1998; Berndt *et al.*, 2002): *L. maritima* had a particularly marked effect on survival and realised parasitism suggesting it may be a good choice for use as a habitat manipulation tool.

The greenhouse assessment of the effect of groundcover plant species on male and female longevity and the daily fecundity of *T. carverae* showed that males and females both survive longer when they were fed on *L. maritima* compared with *F. esculentum*. Male and female longevity did not differ when they were fed on *L. maritima* whereas in *F. esculentum* the females survived longer than the males. Other Hymenopteran female parasitoids e.g., *Dolichogenidea tasmanica* (Cameron), survived longer when they fed on *F. esculentum* compared with water fed females (Irvin *et al.*, 1999) and similarly, *Cotesia marginiventris* (Cresson) survived longer on *L. maritima* compared with water fed females; (Johanowicz & Mitchell, 2000).

The daily fecundity results suggest that *L. maritima*-fed female *T. carverae* can deposit all their eggs before death, which indicates that *L. maritima* increases the oviposition period; that result also showed that *L. maritima* increased daily fecundity. Similarly, Bennett (2002)

reported that honey fed *T. carverae* oviposit more eggs per day than unfed females. This result illustrates the value of providing supplementary food for *T. carverae*.

Egg sentinel card surveys of egg parasitoid activity showed no evidence of wild populations in the Orange and Canowindra sites, suggesting that inundative releasing of *T. carverae* may be justified. The Orange field result showed no significant flower effect on parasitism, though mean values of parasitism were higher in *L. maritima* until day six than in *B. officinalis*, *F. esculentum*, vegetation without-flowers and control treatments. This result was also an indirect field level measurement of the longevity of *T. carverae*. A similar result was found in the Canowindra field, where parasitism was significantly higher in the *C. sativum*, *F. esculentum* and *L. maritima* treatments compared with the vegetation without-flowers and control treatments. In the *B. officinalis* treatment parasitism was no greater than in the vegetation without-flowers and control treatments, which indicates that *T. carverae* exhibits some flower selectivity, a conclusion that was tentatively drawn in relation to weed flowers in Chapter Two.

Flower colour discrimination by *T. carverae*

The work reported in Chapter Four explored in greater detail the phenomenon of flower selectivity in *T. carverae*. Survival and realised parasitism of *T. carverae* were significantly higher when they fed on white *L. maritima* flowers than when they fed on *L. maritima* cultivars of other colours or on white flowers that were artificially dyed. Many researchers reported that parasitoids (Wardle, 1990; Oliai & King, 2000) or, more specifically, trichogrammatids (Keller, 1985; Lukianchuk & Smith, 1997) were not guided by flower colour or visual characteristics. The observed differences between cultivars may be the

results of nectar quantity, but the present result showed that the proportions of nectar quantity did not differ significantly between white and other colour cultivars of *L. maritima*. The survival of *T. carverae* was greater on non-dyed white *L. maritima* than on dyed flowers of white *L. maritima*. This result suggested that during flower selection *T. carverae* are guided by flower colour not by the nectar quality. Some researchers have demonstrated that nectar use may be influenced by gustatory responses (Beach *et al.*, 2003) and that parasitic hymenopterans showed innate responses to food odour (Wäckers, 1994). The results obtained from the dyed honey experiment clearly suggested that *T. carverae* uses visual cues though some role of olfactory and gustatory cues may still be involved. The overall results of this study show for the first time in a conservation biological control system that within-plant species flower colour is an important factor. This finding has important significance for flower selection in habitat manipulation because the biological control efficacy of *T. carverae* is largely affected by its survival and rate of parasitism. These types of effect are unlikely to be confined to trichogrammatid species alone, so further research on hymenopteran and other flower-feeding biological control agents is important.

The effects of groundcover plant species on *E. postvittana*

The experimentation reported in Chapter Five showed that *E. postvittana* did not get any benefit from *C. sativum* or *L. maritima* at any stage (adult and larval). This result contradicts earlier New Zealand findings, Irvin (1999) and suggests that in the Australian vineyard system these plant spp. may be planted without risk of inadequately benefiting *E. postvittana*. Adults benefited from *B. officinalis* and *F. esculentum* flowers constituting a warning against field use. *B. juncea* also must be treated with caution, as the results for this experiment failed to conclusively show no benefit for *E. postvittana*.

Epiphyas postvittana larvae are polyphagous and feed on weeds commonly found in Australian vineyards (Suckling *et al.*, 1998) so there is a clear risk of the groundcover plants introduced for habitat manipulation providing a benefit to pest larvae. In the present study a long larval period was observed in the *C. sativum* and *L. maritima* treatments, indicating that these plant species are not good hosts for *E. postvittana* larvae. On the other hand, *B. juncea* and *T. repens* appeared to be suitable hosts. Baggen & Gurr (1998) suggested the value of the selective food plant and such selectivity is potentially important to make conservation biological control more strategic. The present results suggested that *C. sativum* and *L. maritima* are relatively selective food plant species, for whilst *T. carverae* benefited from these plants little benefit was apparent for larvae and adults of *E. postvittana*. The selectivity of these plant species suggested that they may be useful vineyard groundcover species in future habitat manipulation attempts to enhance biological control of *E. postvittana* in Australian vineyards.

Recommendations for future research

This work was undertaken to gather knowledge that may lead to an improvement in the field performance of *T. carverae* and other natural enemies of *E. postvittana* in the vineyards, through habitat manipulation. In California, 22 flowering plant species were ranked for their potential use in field insectaries including: *B. juncea*, *B. officinalis*, *C. sativum*, *F. esculentum* and *L. maritima* (Chaney, 1998). These plant species were not only attractive to the beneficial insects; they were also agronomically compatible with the grape crops. For

example, *L. maritima* does not interfere with crops or machinery because of its low growth and does not need to be replanted yearly. Accordingly these plant species were chosen for the study. The laboratory and field experiments results suggest that *C. sativum*, *F. esculentum* and *L. maritima* increased *T. carverae* effectiveness in the field. *Epiphyas postvittana* did however, benefit from *F. esculentum*. Therefore, *C. sativum* and *L. maritima* are the best groundcover plant species identified in the present study for vineyards.

Several aspects will have to be considered before commercial scale establishment of flowering plants in the vineyards through habitat manipulation. The first considerations include: are they agronomically tractable? Are they compatible with the grape crop or are crop yields affected by these plants? How well will they compete with weed competitions? Are other pests benefited by these groundcover species? Further research is required to address these questions. Stephens *et al.* (1998) reported that buckwheat sown as an understorey in apple orchards enhanced *Anacharis* sp. (Hymenoptera: Figitidae) a parasitoid of the brown lacewing (*Micromus tasmaniae* (Walker)). Therefore, it is also important to investigate the effect of groundcover species on the fourth trophic level (natural enemies of the pests' natural enemies). It is also important to consider if they increase any disease resulting in elevated crop damage.

Other considerations for future researchers are to select the best flower species as well as to consider accessibility of nectar and pollen by natural enemies (parasitoids/predators). During flower selection the following aspects should be considered: flower colour, nectar quality, availability, diurnal production and total output as well as flower architecture. It is also necessary to determine flower spacing, and the management of flowering period.

Finally, before recommending the establishment of an additional plant species into the vineyards or any other cropping environment it is important to determine that it is not a host of other pest species such as grapevine moth (*Phalaenoides glycine* Lewin), mites, mealybugs etc. Accordingly *E. postvittana* management through habitat manipulation requires the coordinated study of a range of vineyard management system components.

Conclusions

This study constitutes the first phase of work required for habitat manipulation to improve the field performance of *T. carverae* and, potentially, other natural enemies of *E. postvittana* in Australian Vineyards. The most conclusive outcome of this study is that *C. sativum* and *L. maritima* appear to be relatively selective groundcover species for *T. carverae* that are unlikely to benefit *E. postvittana*. This shows good scope for habitat manipulation to improve the field performance of this important biological control agent. *Fagopyrum esculentum* is a good nectar source for both *T. carverae* and *E. postvittana* so is likely to be a less suitable groundcover choice for habitat manipulation. The present study constitutes the first report of the effect of different groundcover species on the daily fecundity and male and female longevity of *T. carverae*. This study also presents the first non-laboratory assessment of the effect of host-egg age on parasitism by *T. carverae*. Another finding of this study is that exudates from ripening grapes may be an important late-season food for *T. carverae* or may be for other natural enemies of vineyard pests. Therefore fruit can play an important role in habitat manipulation. The most important general finding of this study is the first report of flower colour discrimination by *T. carverae*, which is an important factor

for the development of habitat manipulation strategies for this and most likely for other biological control agents.

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Appendices

Appendix One: Example of analysis of variance (ANOVA) table of the type used for the analysis of parasitism data (Chapter Three, longevity and parasitism experiment).

***** ANALYSIS OF VARIANCE *****

VARIATE: SQRPARA_16_07_03

SOURCE OF VARIATION	D.F.	S.S.	M.S.	V.R.	F	PR.
REPLICATE_1 STRATUM	4	1.7858	0.4465	1.27		
REPLICATE_1.*UNITS* STRATUM						
TREATMENT_1	6	11.4599	1.9100	5.44	0.001	
RESIDUAL	24	8.4216	0.3509			
TOTAL	34	21.6673				

***** TABLES OF MEANS *****

VARIATE: SQRPARA_16_07_03

GRAND MEAN 0.97

TREATMENT_1	ALY	ALY (-)	CON	CORI	CORI (-)	MUS	MUS (-)
	2.36	0.71	0.71	0.88	0.71	0.71	0.71

*** STANDARD ERRORS OF DIFFERENCES OF MEANS ***

TABLE	TREATMENT_1
REP.	5
D.F.	24
S.E.D.	0.375

*** LEAST SIGNIFICANT DIFFERENCES OF MEANS (5% LEVEL) ***

TABLE	TREATMENT_1
REP.	5
D.F.	24
L.S.D.	0.773

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

VARIATE: SQRPARA_16_07_03

STRATUM	D.F.	S.E.	CV%
REPLICATE_1	4	0.253	26.1
REPLICATE_1.*UNITS*	24	0.592	61.2

Appendix Two: Example of multivariate analysis of variance (MANOVA) table of the type used for the analysis of longevity data (Chapter Two, weed and *L. maritima* experiment).

***** MULTIVARIATE ANALYSIS OF VARIANCE *****

*** REPLICATE STRATUM ***

***** WARNING FROM MANOVA: ERROR SSP MATRIX SINGULAR. *****

** REPLICATE . _UNITS_ STRATUM ***

*** TREATMENT ***

DATES 14/6 TO 22/6 INCLUDED IN MANOVA

DATES 23/6 ONWARDS CONTAIN ZERO MEANS

*** TESTS ***

WILK'S LAMBDA: 0.02315

APPROXIMATE CHI SQ: 116.74 ON 72 D.F.

APPROXIMATE F TEST: 1.83 ON 72 AND 154 D.F. AND FMAN 0.0003375

PILLAI-BARTLETT TRACE: 2.463

ROY'S MAXIMUM ROOT TEST: 0.8098

LAWLEY-HOTELLING TRACE: 7.053

Appendix Three: Example of exponential curves fitted for the survival data (Chapter Two, grape experiment one).

***** NONLINEAR REGRESSION ANALYSIS *****

RESPONSE VARIATE: SQRWASPS
 EXPLANATORY: DATE
 GROUPING FACTOR: TREATMENT, ALL PARAMETERS SEPARATE
 FITTED CURVE: $A + B * (R^{**}X)$
 CONSTRAINTS: $R < 1$

*** SUMMARY OF ANALYSIS ***

	D.F.	S.S.	M.S.	V.R.	F PR.
REGRESSION	5	157.75	31.55070	509.50	<.001
RESIDUAL	234	14.49	0.06192		
TOTAL	239	172.24	0.72069		
CHANGE	-1	-12.21	12.21206	197.21	<.001

PERCENTAGE VARIANCE ACCOUNTED FOR 91.4

STANDARD ERROR OF OBSERVATIONS IS ESTIMATED TO BE 0.249

* MESSAGE: THE FOLLOWING UNITS HAVE LARGE STANDARDIZED RESIDUALS:

UNIT	RESPONSE	RESIDUAL
25	1.581	-3.24
61	1.581	-3.24
125	2.345	3.25
173	0.707	-3.40
205	2.915	-2.96

* MESSAGE: THE RESIDUALS DO NOT APPEAR TO BE RANDOM;
 FOR EXAMPLE, FITTED VALUES IN THE RANGE 0.564 TO 0.707
 ARE CONSISTENTLY SMALLER THAN OBSERVED VALUES
 AND FITTED VALUES IN THE RANGE 0.709 TO 0.759
 ARE CONSISTENTLY LARGER THAN OBSERVED VALUES

* MESSAGE: THE ERROR VARIANCE DOES NOT APPEAR TO BE CONSTANT:
 LARGE RESPONSES ARE MORE VARIABLE THAN SMALL RESPONSES

*** ESTIMATES OF PARAMETERS ***

	ESTIMATE	S.E.
R TREATMENT G -	0.0316	0.0504
B TREATMENT G -	51.9	82.6
A TREATMENT G -	0.7069	0.0250
R TREATMENT G+	0.7756	0.0150
B TREATMENT G+	4.199	0.107
A TREATMENT G+	0.3651	0.0846

*** ACCUMULATED ANALYSIS OF VARIANCE ***

CHANGE	D.F.	S.S.	M.S.	V.R.	F PR.
+ DATE	2	97.81695	48.90848	789.80	<.001
+ TREATMENT	1	26.89281	26.89281	434.28	<.001
+ DATE.TREATMENT	1	20.83168	20.83168	336.40	<.001
+ SEPARATE NONLINEAR	1	12.21206	12.21206	197.21	<.001
RESIDUAL	234	14.49042	0.06192		
TOTAL	239	172.24391	0.72069		

Appendix Four: Example of Descriptive Statistics of the type used for the analysis of an average and range numbers of eggs on the *E. postvittana* egg sentinel cards (Chapter Two, growth cabinet experiment materials and methods section).

Column1

Mean	17.29513889
Standard Error	0.696695171
Median	13
Mode	8
Standard Deviation	11.82330911
Sample Variance	139.7906383
Kurtosis	1.297507404
Skewness	1.330560925
Range	57
Minimum	3
Maximum	60
Sum	4981
Count	288
Largest(72)	22
Smallest(72)	9
Confidence Level(95.0%)	1.371280559
