Enhancing the effectiveness of the parasitoid *Diadegma semiclausum* (Helen): Movement after use of nectar in the field

Blas Lavandero a,⁎, Steve Wratten a, Parviz Shishehbor b, Sue Worner a

⁎ National Centre for Advanced Bio-Protection Technologies, Lincoln University, Canterbury, New Zealand

a Department of Plant Protection, Faculty of Agriculture, Shahid Chamran University, Ahwaz, Iran

Received 26 November 2004; accepted 29 April 2005

Available online 14 June 2005

Abstract

Evidence is required to show that parasitoids are able to move between floral subsidies and the crop in sufficient numbers, and sufficiently rapidly to improve pest suppression. Marking and tracking techniques can elucidate parasitoid movement and the spatial structure of their population, and therefore, help determine the crop area in which to manage. This, in turn, would help in the deployment of nectar and pollen as resource subsidies, as well as determining the optimal timing for their implementation. Over two successive seasons, experiments were carried out to determine the effect of buckwheat (*Fagopyrum esculentum*) on the nutritional status and movement of the parasitoid *Diadegma semiclausum* and parasitism rates on its host *Plutella xylostella*. The number of parasitoids decreased significantly with the distance from the flowers; however, there was no trend in plots without flowers. The percentage of fed females varied from 72.4 to 77.7% irrespective of treatment. Although the results suggest that the parasitoids can move 80 m in a short period, parasitism rates were lower in a non-flower treated plot separated from a flower treated plot by just 60 m. Parasitoid mobility alone will not give a complete indication of the spatial arrangement to follow to produce enhanced parasitism rates. There is a clear difference between how far a parasitoid can move and how far apart floral subsidies should be deployed.

© 2005 Elsevier Inc. All rights reserved.

Keywords: *Diadegma semiclausum*; *Plutella xylostella*; Parasitism rates; *Fagopyrum esculentum*; Rubidium; Movement

1. Introduction

The consumption of floral ‘resource subsidies’ (Tylianakis et al., 2004) has sometimes increased parasitism rates and decreased host densities in past studies (Berndt et al., 2002; Jervis et al., 1996; Landis et al., 2000; Tylianakis et al., 2004). As sugar sources can be highly variable in quantity, space, and time, the chances of finding an amount of sugar sufficient to increase longevity from a single feeding event can be critical for the forager’s fitness (Jervis and Kidd, 1999). Many parasitoid longevity studies suggest that adults need to locate food at least once per day to avoid starvation (Azzouz et al., 2004; Idris and Grafius, 1995; Irvin et al., in press; Siekmann et al., 2001), stressing the importance of frequent sugar sources to parasitoids. Therefore, the time spent looking for sugar sources in the field could be crucial for parasitoid survival. Also, evidence is required that the parasitoid is able to travel between the floral subsidies and the crop in sufficient numbers, and sufficiently rapidly to improve pest suppression. Marking and tracking techniques can aid in understanding parasitoid movement, and in determining the spatial structure of the population, and, therefore, help in deciding on the particular area to manage (Thomas, 2001). This, in turn, would help in the deployment of nectar and pollen as resource subsidies, as well as determining the optimal timing for their implementation (Gurr et al., 2003, in press; Wratten et al., in press).
The diamondback moth (*Plutella xylostella* (L.); Lepidoptera: Hyponomeutidae) is an important pest of brassica crops worldwide (Talekar and Shelton, 1993). High rates of parasitism (over 70%) have been recorded for *P. xylostella* in the North Island of New Zealand, principally by *Diadegma semiclausum* (Helen) (Hymenoptera: Ichneumonidae), with a lesser role for *Diadromus collaris* (Gravenhorst) (Hymenoptera: Ichneumonidae), producing significant reductions of *P. xylostella* populations (Todd, 1959). In the South Island of New Zealand, however, *P. xylostella* is not well controlled by parasitoids and regular applications of pesticides are made (Berry, 2000; Scott, 1984; Thomas and Ferguson, 1989). *D. semiclausum* is a common parasitoid in Europe, Africa, and Asia, and with *Cotesia pluteae* Kurdjumov (Hymenoptera: Braconidae) is considered to be one of the most important species attacking *P. xylostella* in the world (Talekar and Shelton, 1993). Although, both parasitoids can be effective, *D. semiclausum* is reported to be superior in locating and parasitizing the host (Chua and Ooi, 1986; Lim, 1986; Wang and Keller, 2002).

The use of floral resources such as *Coriandrum sativum* L. (Umbelliferae) has been evaluated in cabbage crops in New Zealand for the attraction of hoverflies to reduce aphid infestations in the field (Morris and Li, 2000). When coriander was present in the field, the number of caterpillars of *P. xylostella* and *Pieris rapae* (L.) (Lepidoptera: Pieridae) decreased as compared with the control. In this case hoverfly larvae were responsible for the decrease in larval densities.

To deploy resource subsidies at the field level, researchers must have a good knowledge of selective resource subsidies, which could generate top-down effects. Previous studies in the laboratory found that *D. semiclausum* which had fed on buckwheat (*Fagopyrum esculentum* Moench (Polygonaceae)), increased their longevity 15-fold compared with water, and the potential fecundity doubled, without enhancing the fitness of the pest (B. Lavandero et al., unpublished). However, it is in the field where the effect of resource subsidies is most relevant. Therefore, this study deals with the effect of floral resources on parasitism rates and abundance of parasitoids. The use of the deployed resources is confirmed using rubidium chloride as a ‘self-marker.’ The following questions are addressed: Does the use of buckwheat positively affect the parasitoid such that parasitism rates can increase? How far does a parasitoid move after it feeds on these resources?

2. Materials and methods

2.1. Dispersal of parasitoids

Strips of broccoli plants 1 m wide and 50 m long were prepared in a certified organic farm (Biological Husbandry Unit, Lincoln University) field during November 2002. All vegetation around the strips were cleared by regular cultivation (Fig. 1). A strip 2 m long and 1 m wide of buckwheat (*F. esculentum*) was sown at one end of the broccoli strip. Spaces between buckwheat lines were left, and every 3 weeks a new line of buckwheat was sown to ensure the presence of flowers during the experiment.

The experiment was a randomized block design with two treatments: with and without buckwheat. The experimental unit consisted of a broccoli strip with marked plants and a set of sticky traps. All flowering vegetation was cleared from 50 m at each side of the margin. A total of four blocks were used, blocks being 180 m apart from each other. Each treatment within a block was separated 60 m from the other. A line of sticky traps (‘Trappit,’ Agrisense-BCS, Treforest Industrial Estate, Pontypridd, Mid Glamorgan, UK) was extended into the broccoli from the end of the buckwheat strip at 0, 5, 10, 15, 20, 25, 30, 35, and 40 m. Marked broccoli plants with *P. xylostella* third-instar larvae on a leaf, surrounded by...
an acetate funnel, were established at distances from the head of the strip matching the sticky trap positions. The funnel prevented larvae from falling off the leaves but still permitted the parasitoids to fly in and access the larvae. After 3 days, larvae were counted and taken to the laboratory. Larvae were reared in containers until pupation in a controlled environment room at 23 °C (±2 °C) and 16 L:8 D photoperiod. Parasitoids resulting from the larvae were identified and counted. Parasitism rates were calculated from the number of parasitoids emerged from the total larvae collected in the field. The traps were checked weekly between the 17 of January and 13 of February of 2003 and the number of parasitoids counted. Maximum parasitism rates were calculated at different distances as an estimate of the proportion of hosts available (see Hawkins, 1993).

2.2. Cold anthrone test

In the same experiment as above, females were tested for fructose in their guts, using the cold anthrone test, to estimate the percentage of individuals in each treatment that was fed. Suction sampling, using a motorized suction sampler with a catching area of 0.05 m², was carried out twice during the season, in the broccoli strips. A total of four points per strip (0, 10, 20, and 40 m from the edge) were sampled three times for 30 s each. Insects were placed in a 1.5 ml plastic tube on ice during collection in the field and then taken to the laboratory, and frozen at −80 °C until examination. The tubes were then placed on a grid, 200 μl of anthrone reagent was added to each, and insects were crushed with a plastic pestle. The plastic pestle was rinsed twice in deionized water between samples, and every 10 samples the rinsed pestle was dipped into an empty tube with anthrone reagent to check for possible cross-sample contamination (for more details see Olson et al., 2000). The control samples stayed yellow and the positive samples turned dark green. The presence or absence of feeding in all samples was determined after an hour. Because the cold anthrone test does not detect the low levels of fructose present in the unfed insect (Steppuhn and Wackers, 2004), fructose detection ability and behaviour of most insects (Corbett et al., 1996; Jackson et al., 1988; Stimmann et al., 1973). Rubidium chloride moves readily in the plant tissue and substitutes for potassium (Berry et al., 1972). Consequently, insects that feed on nectar or pollen of a rubidium-sprayed plant obtain a higher than background concentration of the element (Long et al., 1998), which can be detected by the use of atomic absorption spectrometry.

Using a motorized knapsack sprayer, RbCl (Meta purity = 99%; Sigma–Aldrich, USA) was sprayed every 2 weeks on the flowers, using a concentration of 1000 ppm. To ensure that the insects marked had fed on buckwheat nectar, spraying was done before sunrise, to minimize any topical marking. Once the plants had dried (1–2 h later), the sticky buckets were placed in the field for 4 days, and were then taken to the laboratory to analyse. Petroleum-ether was used to separate the insects from the sticky surface, *D. semiclaustrum* were counted, sexed, and placed into 1.5 ml plastic tubes.

Parasitoid samples were prepared by a method similar to that of Corbett et al. (1996). Insects were dried in an oven maintained at 30 ± 2 °C for 2 days. After drying, 150 μl of concentrated aristar HNO₃ (69%) was added to each sample and returned to the oven for another 2 days. Hydrogen peroxide (H₂O₂, 30%) was then added (150 μl) to the samples for an additional 2 days. Samples were then diluted to complete a 1.5 ml sample.

After sample preparation, the rubidium content was measured in a graphite furnace by atomic absorption (AA) spectrometry using a GBC 909 AA with a Pal 3000 autosampler. Volumes of 10 μl were automatically pipetted by a programmable sample dispenser into a graphite tube where the sample was heated to a temperature of 1500 °C. The rubidium content of each sample was then measured by summing the amount of energy absorbed at 780.0 nm. Total absorbance was integrated over a period of 2s, with the absolute rubidium concentration established by calibration of a standard solution of 10 ppb. The spectrophotometer automatically prepared five standards starting at 10–2 ppb. Samples were measured twice and the average concentration of these was...
considered to be the sample rubidium content. Samples were re-done if the percentage of relative standard deviation (%RSD) of the absorbances was greater than 5%. A tomato leaf standard (NIST) with a rubidium concentration of 14.89 ± 0.27 mg/K was used as a reference for the analysis, to ensure the accuracy of the readings. An insect was considered marked if its rubidium level exceeded the mean background plus three standard deviations (Stimmann, 1974); this threshold gives a conservative Type I error rate of 0.13% and was calculated to be 0.786 ng/insect (0.290 + 3 SD).

2.4. Statistical analysis

The accumulated parasitoid numbers trapped were analysed using a generalized linear model routine, and the significances of the main effects and interactions determined. The mean value of all the dates combined per replication was used for each distance and treatment, to determine the effect regardless of the sampling date. Regression analysis was then preformed separately for each treatment to determine the effect of number over distance after checking for normality and homogeneity of the variances. Parasitism rates, proportion of fed parasitoids and the proportion of rubidium marked insects per distance was analysed using a logistic regression. All statistical analysis was performed using SAS (version 6.11).

3. Results

3.1. Abundance

The effect of distance and treatment were not significant (deviance = 3.10 df = 1, p = 0.08; deviance = 46.64, df = 1, p = 0.085, respectively), but the interaction between distance and treatment significantly affected the mean number of D. semiclausum parasitoids found in yellow sticky traps (deviance = 35.1, df = 1, p < 0.001). However, on average the number of parasitoids trapped per sticky trap seemed greater in the non-flower compared with the flower treatment (289.75 ± 41.2 and 233.22 ± 27.19, respectively). The number of parasitoids decreased significantly with distance from the flowers as compared with no trend in the plots without flowers (Fig. 2).

3.2. Parasitism rates

There were highly significant effects of treatment ($\chi^2 = 5.229$, df = 1, p = 0.022) and non-significant effects of distance, and the interaction (p = 0.484 and p = 0.989, respectively) on the proportion of P. xylostella larvae parasitized by D. semiclausum. The percentage of parasitized larvae in the flower treatment was more than twice that in the non-flower treatment (22.5 ± 0.93 and 9.9 ± 0.43, respectively). Maximum parasitism rates for the flower treatment were 75% whereas the no-flower treatment was 40%. Maximum parasitism seems to decrease with distance from flowers whereas in the non-flower treatment parasitism seems to be greatest closer to the edges (Fig. 3).

3.3. Anthrone test

There were no significant responses to treatment ($\chi^2 = 0.2483$, df = 1, p = 0.6183, n = 65) on the proportion...
of fed females. The percentage of fed females varied from 72.4 to 77.7% irrespective of treatment.

3.4. Mark-capture

Buckwheat plants remained marked 9 days after spraying with rubidium with levels declining to normal by day 15. Although the parasitoid densities in the field were low, the average percentage of marked insects was 18.6%.

There was no significant difference between the distances from flower patches in the proportion of marked insects with rubidium ($\chi^2 = 0.0683, df = 1, p = 0.7939$) (Fig. 4). The mean proportion of marked insects ranged from 0.045 to 0.257. The proportion of marked insects at 80 m from the rubidium source was still 0.139. There was no apparent trend of the proportion of marked insects throughout the 80 m transect.

4. Discussion

The deployment of buckwheat flowers had a different relative effect on the interaction of parasitoid abundance with distance compared with the control treatments. These results suggest that buckwheat is attracting *D. semiclausum*. A similar attraction to buckwheat borders that were perpendicular to cucumber and squash rows was observed by Platt et al. (1999). In that study, density of tachinid flies and hymenopteran wasps decreased as distance from the buckwheat increased.

Although no statistical differences were found in the number of trapped parasitoids per treatment, the higher number of parasitoids trapped in the non-flower treatment could lie in the fact that parasitoids were collected using yellow sticky traps. This is a confounding factor, as parasitoids are known to respond to yellow primarily when they are food-deprived. The lower number of parasitoids collected in the buckwheat field may therefore not mean that fewer parasitoids were present, but rather reflect a lower degree of food deprivation among parasitoids. Similarly, this may also have affected the spatial distribution pattern in the buckwheat field, as the accumulation of parasitoids in the proximity of the buckwheat edge may be partly due to a higher incidence of food-seeking individuals.

However, in the broccoli, contrary to what was expected, most of the females analysed for fructose were positive irrespective of the treatment. Although aphids were not present at the beginning of the experiment in the field, later during that season they were found on the plants and could have acted as a sugar source for the parasitoids in the control plots. In a similar study with parasitoids in cabbage, by detecting sugar type using HPLC, Wackers and Steppuhn (2003) concluded that only parasitoids collected from the flower margin had a raised concentration of nectar sugars with no traces of honeydew sugars, while no evidence of nectar feeding was found in parasitoids in the control plots. Nevertheless, in this present study, the presence of buckwheat in the broccoli-strip doubled the parasitism rates compared with the control. Similarly adding buckwheat to an apple orchard increased parasitism levels of leafrollers by *Dolichogenidea tasmanica* (Cameron), although in this study it was also found that leafroller numbers were higher, compared to a control without buckwheat (Stephens et al., 1998). Later laboratory experiments with *Do. tasmanica* have shown not only that buckwheat can enhance leafroller fitness but that it is a potential host plant for oviposition (see Irvin et al., in press). Another example in vineyards showed that by providing buckwheat flowers, parasitism rate of grape leafhoppers (*Erythroneura* spp.) was increased (English-Loeb et al., 2003). In a similar way, proximity to buckwheat floral patches significantly increased rates of parasitism of *Aphidius rhopalosiphi* and *Diaeretiella rapae* (*Hymenoptera: Aphidiidae*) in a wheat field (Tylianakis et al., 2004).

Comparing maximum parasitism in the flower and no-flower treatments as an estimate of the proportion of hosts available (Hawkins, 1993) can give an idea of the possible success of this parasitoid and others in controlling their hosts (Hawkins et al., 1993). Hawkins and Cornell (1994) found that no control was achieved by a parasitoid in its original location when maximum parasitism rates were below 36%. Since maximum parasitism rates increased from 40 to 75% (Fig. 3), the chance of *D. semiclausum* successfully decreasing *P. xylostella* population increases. This decrease in ‘enemy-free-space’ (Jeffries and Lawton, 1984) can be explained by the increase in the number of parasitoids closer to the flower patch (Fig. 2). A greater number of parasitoids exploiting an area would increase the popu-
locations’ searching ability. However, interpretations must be cautious as in Hawkins et al. (1993) model some parasitoid species that had maximum parasitism rates of over 75% failed to control the host population (five out of 75 cases). Only by collecting long-term host-population data there will be assurance of success or failure. Nevertheless, deployment of buckwheat in the field could add to an IPM program to control P. xylostella.

There was no decline in the number of insects marked with rubidium in the field with distance from the rubidium source in a 4-day period. This gives evidence of the high mobility after feeding of D. semiclau сумму in the field. A similar study using fluorescent dyes detected movement of D. semiclau сумму up to 81 m, following harvest of a dyed field of broccoli (Schellhorn et al., 2004). Another study showed how the parasitoid Venturia canescens (Gravenhorst) could reach distances up to 45 m after release (Desouhant et al., 2003).

Contrary to Siekmann et al. (2004), who suggested that parasitoids are not expected to search for flowers over long distances, D. semiclau сумму in the current work, in a 4-day period, reached distances of over 80 m after feeding from a flower source and, possibly, returned to this source. The fact that parasitoids can move so far could possibly explain why in the previous year it was found that more than 70% of the insects fed even in the control treatment, since aphids were not present during most of the experiment.

Rubidium proved to be a valuable marker for studying movement of D. semiclau сумму. Previous studies (B. Lavandero, unpublished) using fluorescent dyes failed to recapture any parasitoids in a 40 m transect. Other studies with rubidium and predatory insects in sorghum and cotton found that 5.58 and 7.14%, respectively, were positive for elevated rubidium concentration in a 10–24 m transect (Prasifka et al., 2001). Spraying just 1000 ppm maintained a steady mark during the sampling period. The marker was also useful to confirm nectar use of the parasitoids at the field level. Since this parasitoid does not host feed and buckwheat has not been observed in the field to harbour any sap-sucking insects, the only way to receive the higher rubidium concentrations would be by nectar feeding on rubidium-sprayed plants.

Although these results suggest that the parasitoids would move through a 80 m transect in a short period of time, parasitism rates were lower in a no-flower treated plot separated from a flower treated plot by just 60 m. Mobility studies alone will not give an indication of the spatial arrangement to follow to produce enhanced parasitism rates. As shown here, there is a clear difference in how far a parasitoid can move and how far the floral subsidies should be deployed. Further studies should consider a marker that could not only mark the adults but also the parasitized larvae, to determine the spatial scale over which enhanced parasitism occurs.

Acknowledgments

We thank the helpful comments from the referees. For field logistics, we thank Don Heffer from the Field Service Centre, Lincoln University. We also acknowledge the very helpful field assistants Guillaume Caillot, Chris Lowe, and Tom Birkett. Research was funded by Lincoln University and NZAID Study Award.

References


Jackson, C.G., Cohen, A.C., Verdugo, C.L., 1988. Labeling Anaphes ovijentatus (Hymenoptera: Mymaridae), and egg parasite of Lygus


