AUGMENTATION OF *TRICHOGRAMMA BRASSICAE* FOR CONTROL OF CRUCIFEROUS LEPIDOPTERA

J.G. Lundgren[1] and G.E. Heimpel[2]

[1]Department of Entomology, University of Illinois, Urbana, Illinois, U.S.A.
[2]Department of Entomology, University of Minnesota, St. Paul, Minnesota, U.S.A.

INTRODUCTION

Pesticides are the dominant method for controlling *Pieris rapae* (L.) and *Trichoplusia ni* (Hübner) in cruciferous crops because of a low market tolerance for pest damage and the lack of reliable alternative pest control options. In the northern United States, there are no native egg parasitoids that attack these pests, and over the past 30 years there have been several attempts to find a suitable species and strain of *Trichogramma* for this use (Oatman *et al*., 1968; Parker and Pinnell, 1972; Oatman and Platner, 1972; van Lenteren and Pak, 1984; Pak *et al*., 1989). In the most successful of these attempts, a strain of *Trichogramma evanescens* Westwood was able to suppress cruciferous Lepidoptera to acceptable levels in field trials (Parker *et al.*, 1971; Parker and Pinnell, 1972). Unfortunately, this strain was lost, and its identity is now in question (Pinto 1998); furthermore, research has not revealed a species of *Trichogramma* that inflicts comparable levels of mortality to crucifer pests. Preliminary data gathered in our laboratory suggested that a strain of commercially produced *Trichogramma brassicae* (Bezdenko) may be able to cause high levels of mortality in the eggs of *P. rapae* under field conditions (Fig. 1).

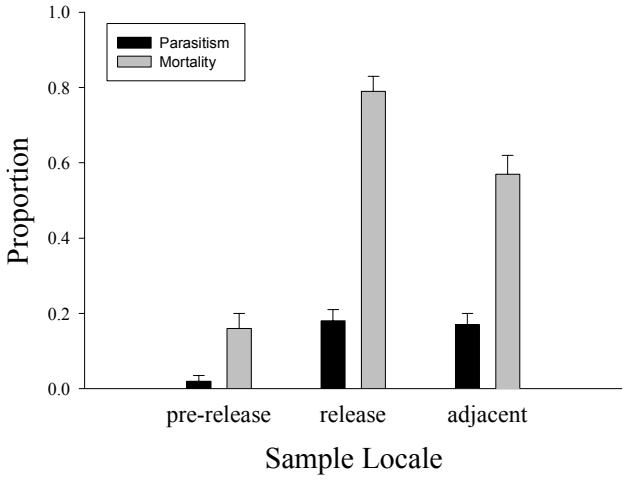

Figure 1. Mortality and parasitism (mean, SEM) of *Pieris rapae* (L.) eggs measured before (prerelease) and after (release and adjacent) releases of commercially produced *Trichogramma brassicae* (Bezdenko) in research plots in Minnesota.

The goal of this research was to evaluate the potential of commercially available *T. brassicae* to control *P. rapae* and *T. ni*. To determine the best release procedure, we compared egg parasitism and larval density resulting from point and broadcast releases of *T. brassicae*. We also evaluated whether concurrent applications of a sucrose solution could increase egg parasitism by *T. brassicae*. Point releases of *T. brassicae* were compared with applications of a *Bacillus thuringiensis* (*B.t.*) product (Dipel, an organically certified insecticide) and methomyl, (Lannate®, a carbamate insecticide approved for use on cabbage pests) in terms of reductions in pest larval populations and cabbage head weight.

MATERIALS AND METHODS

Study Site

Research was conducted at the University of Minnesota Experiment Station in Rosemount, Minnesota. Cabbage plants (*Brassicae oleracea* var. *capitata* L. "Gourmet") were transplanted as seedlings in late June of 1999 and 2000. In both years, four treatments with four replicates each were arranged in a complete randomized design and embedded in a 4.05 ha field of soybeans. Each plot had approximately 250 cabbage plants in eight rows of ca 30 plants each. Row spacing within plots was 1 m and plant spacing within rows was 0.4 m. Dimensions for each plot were 9.3 x 12.5 m. There was 48 m between plots north to south and 20 m between plots east to west.

Evaluation of Release Methods

We compared pest populations and egg parasitism in plots treated with point releases of *T. brassicae*, broadcast releases of *T. brassicae*, broadcast releases of *T. brassicae* coupled with weekly sugar sprays, and an untreated control. Weekly shipments of approximately 82,000 parasitized *Ephestia kuehniella* Zeller eggs were received from Beneficial Insectary (Redding, California) and kept at room temperature until *T. brassicae* began to emerge. Subsets of 1,000 to 5,000 parasitized eggs were deposited into four to five 36-ml vials and placed in the field to validate emergence. Voucher specimens were identified by Dr. John Pinto (Department of Entomology, University of California at Riverside) and deposited in the Insect Museum at the Illinois Natural History Survey (Champaign, Illinois, U.S.A.).

In the point release treatment, each release card (6.1 cm^2) had approximately 5,400 parasitized eggs. Cards were held individually in 2.4 liter glass jars with screened tops (1 mm^2 mesh size). A 70 mm diameter piece of filter paper was placed over the top of the screen until the release to prevent wasps from escaping (Heimpel *et al*., 2000). Fourteen jars were placed at release sites within each plot beneath "*Trichogramma* houses," inverted Styrofoam bowls suspended by three plastic forks. The 14 release sites were evenly distributed throughout the plots and were fixed throughout the summer.

For the broadcast release evaluation, the total mass of loose parasitized eggs, which represented an estimated 668,000 parasitized eggs, was divided into eight parts, and one part was released in each of the four replicates of the broadcast release and broadcast release + sucrose treatments. Each release consisted of 1.12 ± 0.02 g of eggs per plot, and groups of parasitized eggs were kept separate in 35 x 10 mm Petri dishes until wasp emergence. With the onset of wasp emergence, the unemerged parasitized eggs were put in a saltshaker that had all but one of its holes blocked. This single hole allowed 334 ± 41 (N = 8) eggs to escape with each shake. In broadcast release plots, each of the 250 plants in each plot received a single shake of parasitized eggs. Thus, approximately the same number of parasitized eggs was released per plot in the point release treatment and in the broadcast release treatments. In the broadcast release + sucrose treatment, sucrose was applied with a CO_2 sprayer once weekly directly after the release of *T. brassicae* at 64.5 kg/ha (2.04 kg/cm^2). This rate corresponds to 180 g of sugar per 2 liter bottle of water, or an 8% sugar solution by weight.

Point Releases Versus Insecticide Applications

Insecticide was applied based on thresholds involving Cabbage Looper Equivalents (CLEs) (Shelton *et al*., 1982) in which one CLE is equal to one *T. ni* larva or 1.5 *P. rapae* larvae. Treatment thresholds were three CLEs per 10 pre-heading plants and one CLE per 10 heading plants. Decisions to apply pesticides were made independently for each replicate of the two insecticide treatments. Consequently, the number and timing of applications made varied among the replicates of these two treatments. When thresholds were reached, Dipel or methomyl were applied at label rates within 24 hours. An average of 5.5 sprays of methomyl and 7.25 of Dipel were applied to plots.

Parasitoid releases in the point release treatment were made as outlined above. We began the season receiving our *T. brassicae* from a different insectary than used in previous work, although the new insectary reported their product to be the same strain. However, the new insectary misidentified its species of *Trichogramma*, and so *T. brassicae* was not actually released until 6 August, when we received shipments from the original insectary. For this reason, direct comparisons between the *Trichogramma* and insecticide treatments are not possible. The misidentified species was likely *Trichogramma minutum* Riley or *Trichogramma platneri* Nagarkatti, and we did not observe any parasitism of pest eggs by this species.

Data Collection and Analysis

Plots were sampled two or three times each week; 7 to 10 plants were sampled on each sample day in the release method study and five plants were sampled on each sample day in the insecticide comparison study. The numbers of *P. rapae* and *T. ni* eggs and larvae were determined for each sample date. A maximum of five eggs per plant were removed with a 12 mm diameter cork borer and placed together in a plastic Petri dish (6 cm diameter). All insects collected in the point release plots were kept separated based on their plant and plot of origin. In the point release treatment of the release method study, the distance from each sampled plant to the nearest release point was measured to the nearest centimeter. Eggs were brought back to the laboratory, excess cabbage foliage was removed from around the eggs, and eggs were placed in 1.5 ml microcentrifuge tubes until they hatched or parasitoids emerged. All specimens were reared on a laboratory bench at approximately 22 °C and 40% r. h. For the yield comparison in the insecticide comparison study, 10 cabbage heads per plot were chosen at random, harvested on 27 August and 3 September, and weighed to the nearest 0.023 kg.

Egg parasitism was defined as the number of eggs that yielded *T. brassicae* adults divided by the number of eggs that yielded either pest larvae or *T. brassicae* adults (i.e., dead eggs were excluded). Egg parasitism was compared among the four treatments in the release methods experiment using a repeated measures ANOVA, and the Tukey-Kramer means comparison method was used to reveal specific significant relationships among the treatments. Within the point release plots of the release method experiment, the distance to the nearest release site was compared with the rate of *P. rapae* egg parasitism by linear regression, pooled over sample dates.

In the insecticide study, the mean numbers of pest larvae per plant were compared among the pesticide treatments and the control using a repeated measures ANOVA, and the Tukey-Kramer means comparison method was implemented to reveal specific significant relationships among the treatments pooled over all dates. Also in the insecticide experiment, the numbers of pest larvae per plant after 6 August were compared between the *T. brassicae* treatment and the control, with a repeated measures ANOVA. In the release methods study, the mean numbers of *P. rapae* and *T. ni* larvae per plant were compared among the treatments with a repeated measures ANOVA. The mean head mass was calculated for each replicate plot, and the treatment means were then compared among the pesticide treatments and the negative control with ANOVA and a Tukey-Kramer means comparison. The mean head weight for the *T. brassicae* treatment was compared with the control using a t-test.

RESULTS

Evaluation of Release Methods

There was a significant effect of treatment on egg parasitism rates ($F_{3, 12} = 7.368$, $P = 0.005$). The seasonal mean parasitism rate also differed significantly among treatments ($F_{3, 12} = 6.97$, $P = 0.006$), and the point release had significantly higher rates of parasitism than the control (Table 1). The parasitism rates of the two broadcast treatments did not differ from the control (Table 1). In the point release plots, there was no correlation between egg parasitism and proximity to the release sites ($R^2 = 6x10^{-3}$, $F_{1, 199} = 1.27$, $P = 0.26$). Differences in larval populations were not significant among the treatments ($F_{3, 12} = 4.34$, $P = 0.732$).

Table 1. Mean egg parasitism rates of *Pieris rapae* (L.) and *Trichoplusia ni* (Hübner) by *Trichogramma brassicae* (Bezdenko) for treatments on cabbage at Rosemount, Minnesota, 1999 and 2000.

	Treatment	Mean proportion parasitized (SEM)a
Insecticide study	control	0
	point release	0.19 (0.04)
Release method study	control	0.15 (0.027) b
	point release	0.46 (0.040) a
	broadcast	0.31 (0.037) ab
	broadcast release + sucrose	0.24 (0.041) ab

[a] Information from different sampling dates is pooled. No inferential tests were applied to the means obtained in the insecticide study, and means among the four treatments in the release methods study were compared with the Tukey-Kramer means comparison (± = 0.05). Means followed by different letters differ significantly.

Point Releases Versus Insecticide Applications

Methomyl and *B.t.* were able to maintain *P. rapae* and *T. ni* larvae below economic thresholds, and both treatments significantly reduced the number of pests per plant relative to the control ($F_{2, 9} = 134.08$, $P < 0.001$; Fig. 2). The mean egg parasitism pooled over all sampling dates was 19%, and releases of *T. brassicae* did not reduce larval populations ($F_{1, 6} = 2.31$, $P = 0.18$). *Pieris rapae* and *T. ni* larvae had reached 10 larvae per plant when *T. brassicae* releases were begun (Fig. 2). Cabbage head weight did not differ significantly between the *T. brassicae* point release treatment and the control ($t = 1.33$, $df = 6$, $P = 0.23$) There was a significant relationship between treatment and head weight among the pesticide treatments and the control ($F_{2, 9} = 7.88$, $P = 0.01$), with the methomyl treatment having significantly higher head weights than the control.

DISCUSSION

We conclude that the commercially produced *T. brassicae* strain evaluated in this research is not competitive with either Dipel or methomyl as a control agent for cruciferous Lepidoptera. *Trichogramma brassicae* was unable to reduce *P. rapae* or *T. ni* larval populations in either experiment, despite 46% egg parasitism of *P. rapae* in the point release treatment. Egg parasitism was highest in the point-release treatment, possibly because *T. brassicae* dispersed throughout the small plots used in this experiment and because the *Trichogramma* houses may have offered some protection to pre-emerged *Trichogramma* from mortality factors. Sugar sprays did not affect the egg parasitism rate nor reduce

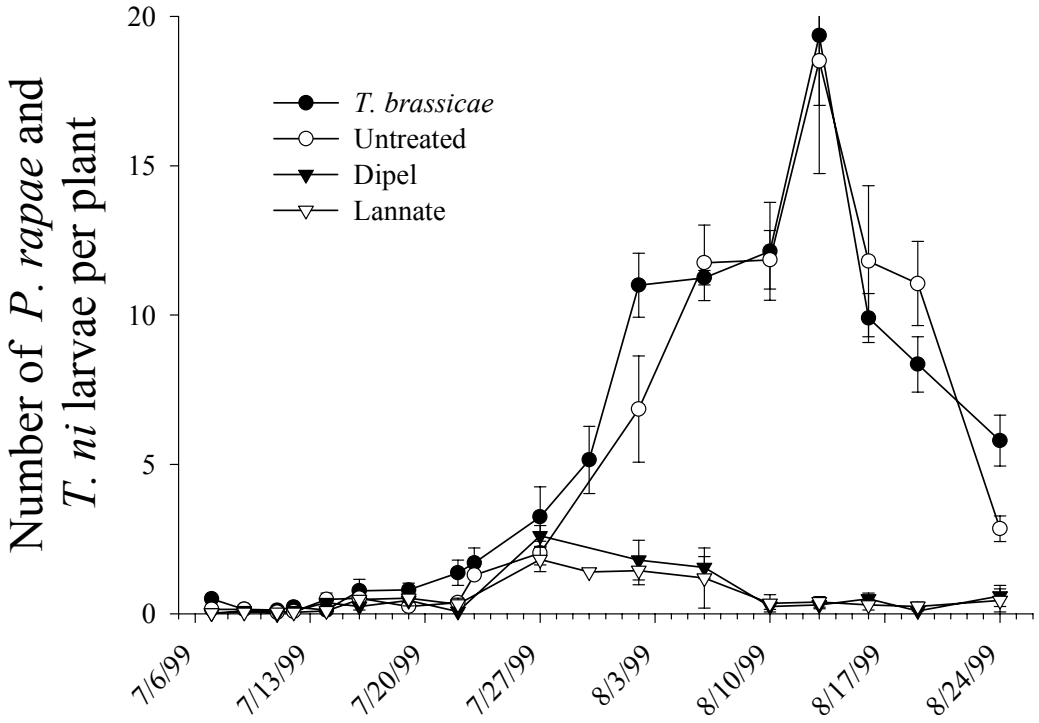

Figure 2. The seasonal abundance of *Pieris rapae* (L.) and *Trichoplusia ni* (Hübner) larvae in the insecticide study. *Trichogramma brassicae* (Bezdenko) was released weekly after 6 August.

P. rapae or *T. ni* larval populations relative to the unsprayed release treatment or the control. Several factors may have contributed to the low egg parasitism rates observed in our study, including the host selection and foraging behaviors of the commercially available *T. brassicae* strain and the disappearance (mortality and/or dispersal) of wasps before they could parasitize all of the available hosts. Finally, this study illustrates the importance of accurate identification of the biological control agent for the ultimate adoption of the recommendations proposed by biological control scientists.

In our study, egg parasitism was less than 50%, despite the availability of hosts, and *T. brassicae* did not increase the rate of egg parasitism in response to increases in host egg density in any of the treatments. Host recognition varies among *Trichogramma* strains (Pak and De Jong, 1987; Nasr and Shonouda, 1998), and there may be alternative species of *Trichogramma* or strains of *T. brassicae* that are more appropriate control agents of *P. rapae* and *T. ni* in cabbage fields. Nevertheless, parasitism of *P. rapae* eggs by the commercially produced strain of *T. brassicae* was observed in all of the treatments, though it did not result in significant reductions of pest larvae. Life table analyses of *P. rapae* and *T. ni* might be useful in determining how many eggs must be destroyed before larval populations are reduced to acceptable levels.

Trichogramma likely feeds on nectar or other sugar sources in the field (Treacy *et al*., 1987), and this fact makes it difficult to explain why sugar sprays did not enhance egg parasitism by *T. brassicae* in the field. Perhaps *T. brassicae* cannot consume sucrose once it has dried, that weekly sprays were not frequent enough to improve *T. brassicae* fitness in the field, or that the increased the number of predators observed in the sugar spray plots (J. G. L. unpublished data) increased mortality of *T. brassicae*.

Misidentification of the *T. brassicae* culture resulted in a complete absence of egg parasitism in the first part of the insecticide study, and this likely reduced any cumulative effects on pest populations from the recruitment of wasps from parasitized pests that may have otherwise occurred over the season. Misidentification of natural enemies is not restricted to the insectary or species used in this study; at least one shipment of *Trichogramma* was misidentified by an insectary in research conducted by Lundgren and Heimpel (2003), and insectary produced *Chrysoperla rufilabris* Burmiester was misidentified as *C. carnea* (Stephens) in another quality control study (O'Neil *et al*., 1998). Efforts are underway to develop an identification process using sequences of the internally transcribed spacer (ITS2) of *Trichogramma* ribosomal DNA (Stouthamer *et al*., 1999), which will allow quick and accurate identification of *Trichogramma* by people who are not taxonomic specialists in this group.

ACKNOWLEDGMENTS

We thank Seth Bomgren, Amanda Jacobson, Sam Lockner, Katie Vogt, Tim Wheeler, and the staff of the Rosemount Experiment Station for their assistance in producing this research. Gary Pahl provided cabbage transplants in 2000. Beneficial Insectary provided the correctly identified strain of *Trichogramma brassicae*. Dr. John Pinto identified our sample of *T. brassicae*. Abbot Laboratories and Dow AgroScience provided the pesticides. This research was funded by the North American Pesticide Impact and Assessment Program and the Minnesota Department of Agriculture's Biological Control Granting Program. This research has been supported in whole or in part by the University of Minnesota Agricultural Experiment Station.

REFERENCES

Heimpel, G. E., D. A. Andow, J. G. Lundgren, and S. Mahr. 1999. Releasing *Trichogramma* wasps from commercial insectaries. NCR-125 Biological Control Fact Sheet, Web-Based Document.

Lundgren, J. G. and G. E. Heimpel. 2003. Quality assessment for three species of *Trichogramma* and the first report of in a commercially-produced strain of *Trichogramma*. *Biological Control* 26: 68-73.

Nasr, F. N. and M. L. Shonouda. 1998. Evaluation of the effect of host and non-host kairomones on the behavior of the egg parasitoid *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae). *Mitteilungen aus der Biologischen Bendesanstalt fur Land- und Forstwirtschaft* 356: 7-12.

Oatman, E. R. and G. R. Platner. 1972. Colonization of *Trichogramma evanescens* and *Apantales rubecula* on the imported cabbageworm on cabbage in southern California. *Environmental Entomology* 1: 347-351.

Oatman, E. R., G. R. Platner, and P. D. Greany. 1968. Parasitization of imported cabbageworm and cabbage looper eggs on cabbage in southern California, with notes on the colonization of *Trichogramma evanescens*. *Journal of Economic Entomology* 61: 724-730.

O'Neil, R. J., K. L. Giles, J. J. Obrycki, D. L. Mahr, J. C. Legaspi, and K. Katovich. 1998. Evaluation of the quality of four commercially available natural enemies. *Biological Control* 11: 1-8.

Pak, G. A. and E. J. De Jong. 1987. Behavioral variations among strains of *Trichogramma* spp.: host recognition. *Netherlands Journal of Zoology* 37: 137-166.

Pak, G. A., T. G. van Heiningen, F. A. N. van Alebeek, S. A. Hassan, and J. C. van Lenteren. 1989. Experimental inundative releases of different strains of the egg parasite *Trichogramma* in Brussels sprouts. *Netherlands Journal of Plant Pathology* 95: 129-142.

Parker, F. D. and R. E. Pinnell. 1972. Effectiveness of *Trichogramma* spp. in parasitizing eggs of *Pieris rapae* and *Trichoplusia ni*. 1. Field studies. *Environmental Entomology* 1: 785-789.

Parker, F. D., F. R. Lawson, and R. E. Pinnell. 1971. Suppression of *Pieris rapae* using a new control system: mass releases of both the pest and its parasite. *Journal of Economic Entomology* 64: 721-735.

Pinto, J. D. 1998. Systematics of the North American Species of *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae). *Memoirs of the Entomological Society of Washington* No. 22.

Shelton, A. M., J. T. Andaloro, and J. Barnard. 1982. Effects of cabbage looper, imported cabbageworm and diamondback moth on fresh market and processing cabbage. *Journal of Economic Entomology* 75: 742-745.

Stouthamer, R., J. Hu, F. J. P. M. van Kan, G. R. Platner, and J. D. Pinto. 1999. The utility of internally transcribed spacer 2 DNA sequences of the nuclear ribosomal gene for distinguishing sibling species of *Trichogramma* (Hymenoptera: Trichogrammatidae). *BioControl* 43: 421-440.

Treacy, M. F., J. H. Benedict, M. H. Walmsley, J. D. Lopez, and R. K. Morrison. 1987. Parasitism of bollworm (Lepidoptera: Noctuidae) eggs on nectaried and nectariless cotton. *Environmental Entomology* 16: 420-423.

van Lenteren, J. C. and G. A. Pak. 1984. Can we use *Trichogramma* spp. to control Lepidoptera in cabbage? *Mitteilungen aus der Biologischen Bendesanstalt fur Land- und Forstwirtschaft* 218: 119-135.