

Laboratory Evaluation of Commercial Trichogrammatid Products for Potential Use against *Plutella xylostella* (L.) (Lepidoptera: Plutellidae)

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In laboratory experiments we compared the mortality of *Plutella xylostella* L. eggs induced by six products of trichogrammatid egg parasitoids. Five of the species were commercially available, and the sixth is under development. Each of the six products represented a single species of trichogrammatid (*Trichogrammatoidea bactrae* Nagaraja, *Trichogramma pretiosum* Riley, *T. ostriniae* Pan & Chen, *T. platneri* Nagarkatti, *T. minutum* Riley, or *T. brassicae* Bezd.), concomitantly with any characteristics inherent with the commercialized culturing of the species (e.g., sex ratio and host upon which they were reared). Host mortality was assessed as the sum of percent parasitism and other direct induced mortality (e.g., host feeding). Three products (*T. bactrae*, *T. pretiosum*, and *T. minutum*) caused the highest mortalities (95 to 98%) of *P. xylostella* eggs, indicating that the focus of further greenhouse and field studies should be on these products. Inconsistent responses between shipments were observed within most of the products, indicating potential problems with quality control. Two products, *T. bactrae* and *T. pretiosum*, demonstrated the highest rates of mortality caused by parasitism of *P. xylostella* eggs (69 to 72%). Mortality caused by factors other than parasitism was high in two of the products. Two products, *T. minutum* and *T. platneri*, demonstrated the highest levels of nonparasitic mortalities (60 to 63%, or nearly two-thirds of the total mortality by each of these products). Important considerations for evaluating host mortality are discussed. © 1997 Academic Press

KEY WORDS: *Trichogrammatids*; *Plutella xylostella*; egg parasitoids; biological control.

INTRODUCTION

Plutella xylostella L. inflicts more than 1 billion dollars in losses per year on cruciferous crops worldwide (FAO, 1992; Talekar, 1992). Lack of diverse strategies of management has resulted in an almost total dependence on insecticides. Evidence of *P. xylostella* resistance to insecticides has been documented for most synthetic and even biologically based insecticides, including toxins generated by *Bacillus thuringiensis* Berliner (Sun *et al.*, 1978; Sun, 1992; Talekar and Shelton, 1993; Tabashnik, 1994). Future control of *P. xylostella* requires the development of new management strategies. Insect parasitoids are among the most promising alternatives (Lim, 1986; Talekar and Shelton, 1993).

Egg parasitoids of the family Trichogrammatidae are polyphagous natural enemies that attack many lepidopteran species including *P. xylostella*. Egg parasitoids are particularly valuable because they kill the pest before the damage occurs. Species like *Trichogrammatoidea bactrae* Nagaraja, *Trichogramma confusum* Viggiani, and *Trichogramma chilonis* Ishii are naturally occurring parasitoids of *P. xylostella* in Thailand (the first two) and Japan (Keinmeesuke *et al.*, 1992; Klemm *et al.*, 1992). Some of these and other species of trichogrammatids are commercially mass-reared and can be purchased in the United States and released in the field against *P. xylostella*. Several authors have pointed out the importance of carefully choosing trichogrammatid egg parasitoids before using them in the field (Pak and Van Lenteren, 1986; Hassan, 1990; Pak, 1991). Several trichogrammatid strains have been evaluated to control *Cydia pomonella* L., *Adoxophyes orana* F. R., and *Pandemis heparana* Schiff. in apple orchards (Hassan 1989); *Ostrinia nubilalis* Hb. in corn fields (Hassan and Guo 1991); and *Mamestra brassicae* (L.), *Pieris brassicae* (L.), *Pieris rapae* (L.), and *P. xylostella* in cabbage fields (Van Dijken *et al.*, 1986; Klemm *et al.*, 1992; Wührer and Hassan, 1993).

Our objectives were to evaluate commercially avail-

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able trichogrammatid products “off the shelf” for use against *P. xylostella* in laboratory trials and to assess the factors which cause mortality. These studies would serve as a base from which to conduct further tests in greenhouse and field situations of such commercially available products. It should be noted that, although each product was a different species of *Trichogramma*, the product's efficacy against *P. xylostella* could vary for reasons other than the species (e.g., what host the parasitoid was reared upon, rearing procedures which could influence sex ratio, etc). This dilemma is inherent in any product evaluation but is relevant to the commercial success of the product. The products were evaluated for their ability to cause host mortality through parasitism of the host as well as the mortality caused by factors other than parasitism (e.g., stinging the host egg, host feeding). Additionally, we evaluated the products for their consistency among different shipments. Results from this study will help to predict the potential for success of each product.

MATERIALS AND METHODS

Sampling procedure. We chose the products based on the trichogrammatid species and their availability from commercial suppliers in North America. Each product contained only one trichogrammatid species (*Trichogramma pretiosum*, *T. platneri*, *T. brassicae*, *T. minutum*, or *Trichogrammatoidea bactrae*). Additionally, we tested *Trichogramma ostrinae* because of its potential commercialization. Over a period of 1 year, three shipments of each product were tested as they were received by overnight express shipment. For ease of identification, the products are listed by species of trichogrammatid. *T. pretiosum*, *T. platneri*, and *T. bactrae* were supplied by Rincon Vitova insectaries (Ventura, CA) where they had been mass-reared on Angoumois grain moth eggs, *Sitotroga cerealella* Oliv. [Lep:Gelechiidae]. *Trichogramma brassicae* and *T. minutum* were supplied by Bio-Logicals (Guelph, Canada), where they had been mass-reared on Mediterranean flour moth eggs, *Ephestia kuehniella* Zell. [Lep:Pyralidae]. *T. ostrinae* was supplied by USDA APHIS (Mission, TX), where they were mass-reared on European corn borer, *O. nubilalis* Hübn. [Lep:Pyralidae] eggs. With the exception of the last shipment of *T. ostrinae*, suppliers were the same for each of the species and shipments throughout the duration of the experiment. The last “shipment” of *T. ostrinae* originated from the same source but was reared in the insectary at Cornell University on the same host species, *O. nubilalis*.

All trichogrammatid products were shipped while still in the host egg. Once received, parasitized host eggs were evenly distributed in ten 80 × 30 mm diameter glass tubes. Tubes were then covered with

tissue paper secured with a rubber band and the tissue paper was moistened with pure honey. Each glass tube contained about 3000 host eggs. Tubes were placed in rearing chambers at 25 ± 1°C, L:D 16:8, and 65% RH. After 12 h from the first recorded emergence, about 90% of the parasitoids were mated and ready to use. These adults were evaluated 12 to 24 h after they emerged.

Twenty samples of 25 individuals were taken from the glass tubes. The samples were presumed to be representative of the overall “quality” of the product and would encompass such factors as sex ratio, potential longevity of the individuals, etc. Samples were obtained by stimulating the insects to walk up the side of the tube toward a light source and through a 100 × 6 mm diameter glass pipette. The glass tube was covered with black plastic and laid on its side to facilitate counting the insects. Close observation of the pipette against a white background made it easy to count the number of insects inside. Once 25 insects had entered the pipette, the pipette was removed and capped with a cork and an empty pipette was inserted into the glass tube. Trichogrammatids inside the pipettes were then induced to walk into 60 × 23 mm diameter glass tubes, using the same procedure described above. This time, however, the glass tubes were located on top and placed on a perforated black box so the pipettes remained down and in the dark.

Eggs (1 to 6 h old) of *P. xylostella* were obtained from laboratory-reared moths (Geneva-88 strain) using the procedure of Shelton *et al.* (1991). On the day of the test, moths were induced to lay eggs on 1 × 7.5 cm aluminum foil strips previously dipped in autoclaved cabbage leaf juice. The aluminum foil strips were arranged on 6 × 3 cm white pieces of thin cardboard so moths laid their eggs on one side only.

Host mortality tests. Bin and Vinson (1990) proposed criteria for assessing the different factors which affect the efficacy of parasitoids that attack host eggs that are laid singly. Total parasitoid impact is the sum of the percentage of parasitism (“parasitism efficiency” per Bin and Vinson) and other “direct parasitoid induced mortality” and is the total *P. xylostella* egg mortality corrected for host egg infertility. To subtract the mortality due to host egg infertility the Abbott (1925) procedure was used. Parasitism efficiency is equal to the portion of parasitized eggs (Bin and Vinson, 1990). Direct parasitoid-induced mortality is equal to the corrected host-egg mortality that results from parasitoid activity other than parasitization (Bin and Vinson, 1990), including mortality due to host predation and/or host suppression (drilling, venom injection, superparasitization, or pseudoparasitization).

To assess host mortality induced by the products, 200 (1 to 6 h old) *P. xylostella* eggs were placed together with the 25 (12 to 24 h old) trichogrammatid adults in 60 × 23 mm diameter glass tubes. The tubes were closed

with tissue paper (secured with a rubber band) and placed in a rearing chamber at $25 \pm 1^\circ\text{C}$, L:D 16:8, and 65% RH. No food was provided. Host mortality was estimated based on the number of parasitized eggs, the number of surviving eggs, and the number of male and female trichogrammatids placed in the glass tubes (the exact number of trichogrammatids and the sex ratio were assessed at the end of the trial). To reduce the error variability due to differences in number of parasitoids in each glass tube, the number of females and males was used as a covariate in the model. Eggs that turned black 7 days after they were exposed to the parasitoids were recorded as parasitized eggs. The number of wasps that would have emerged from the parasitized eggs was not counted since we were only interested in mortality induced by the released parasitoids. Surviving eggs were eggs from which larvae emerged after 5 days. Four control samples were used with each trial and consisted of 200 (1 to 6 h old) eggs of *P. xylostella* placed in empty 60×23 mm diameter glass tubes under the same environmental conditions as those of the treatments.

Statistical analyses. The six products plus the control constituted the main fixed effects of the experiment. Each one of the three shipments constituted the secondary random effect of the experiment, nested within products. There were seven treatments (=6 products + control) with three replications per treatment (=3 shipments) and 20 samples ($n = 20$) per shipment nested within products. Analyses were conducted using Minitab (1993) statistical software for nested design experiments. Pairwise mean separations were conducted using Tukey's method of multiple comparisons, with 95% family confidence coefficient (Neter *et al.*, 1990). Mean contrasts were conducted using Bonferroni's multiple comparison method with 95% family confidence coefficient (Neter *et al.*, 1990). The total number of parasitoids exposed in each glass tube counted as females or males was considered a covariate of the model (Neter *et al.*, 1990).

RESULTS AND DISCUSSION

Significant differences were observed among products when compared for total parasitoid impact ($F = 6.78$; $df = 5, 330$; $P = 0.003$), parasitism efficiency ($F = 5.93$; $df = 5, 330$; $P = 0.005$), or direct parasitoid-induced mortality ($F = 3.58$; $df = 5, 330$; $P = 0.033$). The products, *T. pretiosum*, *T. bactrae*, and *T. minutum*, killed ca. 95 to 98% of the *P. xylostella* eggs. All other products were significantly different from each other (Table 1). Those products containing *T. bactrae* and *T. pretiosum* demonstrated higher rates of parasitism of *P. xylostella* eggs than any other products (69 to 72%), followed by products containing *T. ostrinia*, *T. minutum*, *T. platneri*, and *T. brassicae* (Table 1). No signifi-

TABLE 1

Mean Percent (\pm SD) Parasitoid Impact, Percent Parasitism, and Direct Parasitoid-Induced Mortality

Species	Parasitoid impact ^a	Percent parasitism	Direct induced mortality
<i>T. pretiosum</i>	98.0 \pm 4.3 a	69.4 \pm 14.0 a	28.6 \pm 13.7 b
<i>T. bactrae</i>	95.5 \pm 11.0 a	71.9 \pm 13.1 a	23.6 \pm 11.1 b,c
<i>T. minutum</i>	94.9 \pm 10.3 a	32.0 \pm 22.8 c	62.9 \pm 34.4 a
<i>T. platneri</i>	86.0 \pm 22.7 b	25.8 \pm 13.6 c,d	60.2 \pm 26.8 a
<i>T. ostrinia</i>	66.0 \pm 29.3 c	43.6 \pm 26.5 b	22.4 \pm 12.0 b,c
<i>T. brassicae</i>	41.6 \pm 27.5 d	22.4 \pm 18.9 d	19.2 \pm 14.5 c

^a Means within the same column and followed by the same letter are not significantly different (Neter *et al.*, 1990) ($P > 0.05$ family confidence coefficient, Tukey method of multiple comparisons).

cant differences in percentage of parasitism were found between products containing *T. bactrae* and *T. pretiosum*; *T. minutum* and *T. platneri*; and *T. platneri* and *T. brassicae*. The order in which some of these products are ranked coincides with the findings of Klemm *et al.* (1992) and Wührer and Hassan (1993) in their studies of host acceptance. Although products containing *T. minutum* and *T. platneri* caused high mortality in the host eggs, both showed relatively low parasitism (Table 1). These results suggest the importance of another kind of host mortality inflicted by the parasitoids in addition to parasitization. This "direct parasitoid-induced mortality" (host predation or host suppression) was most pronounced with the products containing *T. minutum* and *T. platneri* (ca. 60 to 63%, Table 1).

Significant differences were observed among shipments within at least one product when compared for parasitoid impact ($F = 20.1$; $df = 12, 330$; $P < 0.0001$), percentage of parasitism ($F = 23.6$; $df = 12, 330$; $P < 0.0001$), or direct parasitoid-induced mortality ($F = 44.9$; $df = 12, 330$; $P < 0.0001$). Parasitoid impact was most consistent between shipments of products containing *T. pretiosum* and *T. bactrae* (Fig. 1). The largest inconsistencies of percentage of parasitism were with *T. minutum* and *T. ostrinia*. Percentage of parasitism was somewhat inconsistent between shipments of products that showed consistently high parasitoid impact (*T. pretiosum* and *T. bactrae*), suggesting that these species could compensate for loss of parasitism with other mortality factors. Parasitoid-induced mortality was significantly different between shipments of all products, except for *T. bactrae* and *T. ostrinia*. With the products containing either *T. bactrae*, *T. pretiosum*, *T. minutum*, or *T. platneri* lower direct parasitoid-induced mortality coincided with a higher percentage of parasitism and vice versa; however, with *T. ostrinia* and *T. brassicae* this was not the case.

Significant differences were observed among products when compared for percentage of females

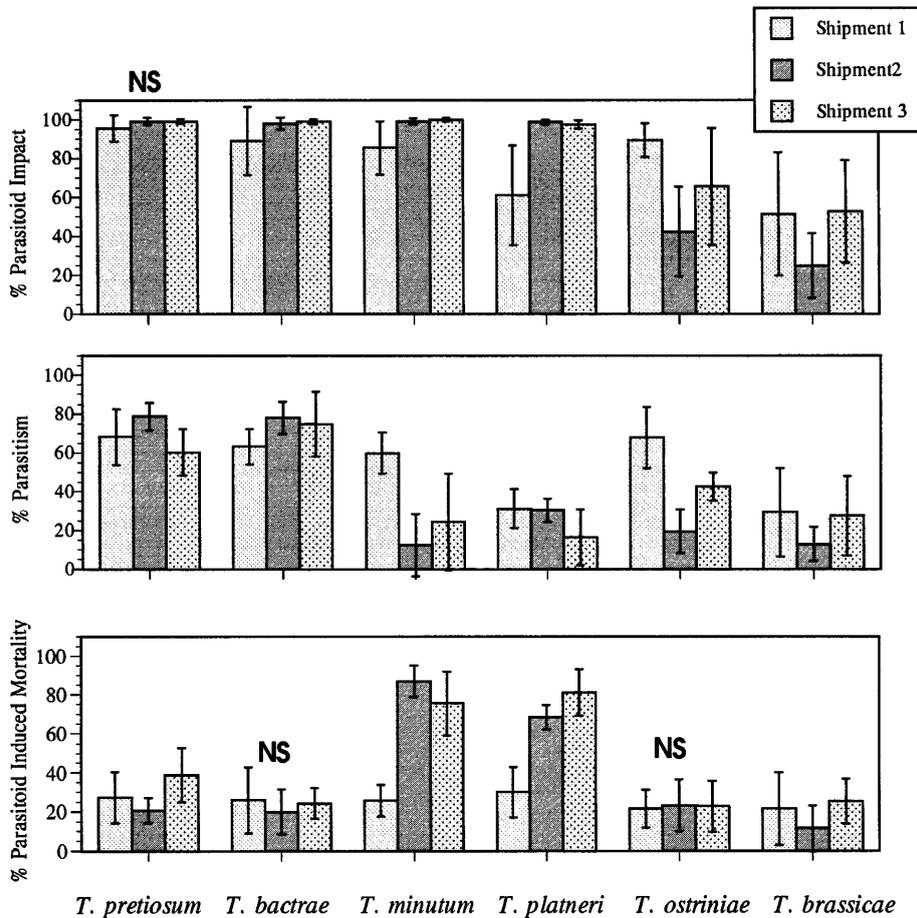


FIG. 1. Mean parasitoid impact, percentage of parasitism, and direct parasitoid-induced mortality per shipment. NS, No significant differences between shipments, F test ($df = 2, 330$; $P \geq 0.05$).

($F = 107.54$; $df = 5, 348$; $P = 0.000$). Products containing *T. platneri* and *T. ostriniae* had the highest percentage of females, 90.8 to 96.1%, respectively, followed by products containing *T. bactrae* (88.8%), *T. minutum* (81.1%), *T. brassicae* (65.3%), and *T. pretiosum* (59.1%). No significant differences in percentage of females were found between products containing *T. platneri* and *T. ostriniae* and *T. ostriniae* and *T. bactrae*. Products containing *T. platneri* and *T. ostriniae* had the highest percentages of females but inflicted the lowest rates of parasitism and parasitoid-induced mortality. The product containing *T. bactrae*, however, also had a high percentage of females and inflicted among the highest rates of parasitism and parasitoid-induced mortality. Thus, there is not a clear relationship between high percentages of females and high parasitoid-induced mortality. Significant differences were observed among shipments within at least one product when compared for percentage of females ($F = 17.23$; $df = 12, 348$; $P = 0.000$). Percentage of females was not consistent between shipments of all products except those containing *T. platneri* and *T. ostriniae*.

Products containing trichogrammatids are being sold

commercially and this study examined six such products for their use against *P. xylostella* eggs. Each of the products contained a different trichogrammatid species. We suspect that differences in host mortality caused by each product were primarily a reflection of the species it contained, but other factors such as the host the species was reared on may have influenced the efficacy of a particular parasitoid. Additionally, rearing conditions as well as packing and shipment practices may also influence the efficacy of the product by influencing the sex ratio or longevity of the adults. Still, commercial users of these products should have some indication of how well these products may perform. As a first step in a longer term effort to utilize trichogrammatids for commercial control, we were interested in assessing which products may warrant further testing against *P. xylostella* eggs. Further testing should be conducted in greenhouse and field situations which will take into account other parameters, such as habitat selection, which will affect the parasitoid's ability to find its host. Our assessments were conducted in the laboratory under what we believed were conditions which would maximize the efficacy of species (e.g., fresh

eggs and confined arenas in which the adults would contact the host eggs). If products containing a particular species showed no parasitism or direct induced mortality under these conditions, we assume that they will not be able to under field conditions. This assumption may be incorrect if habitat strongly influences parasitism, but may be a requirement when many species are to be examined. Likewise, if some products demonstrated high host mortality, this would have been good evidence for further studies examining how they perform on a larger scale and whether their impact could be improved by such procedures as rearing them on other hosts or under different environmental conditions. Based on our results, we are now in a better position to select products for further evaluation based on the following conclusions.

Although all six trichogrammatid products parasitized *P. xylostella* eggs, *T. pretiosum*, *T. bactrae*, and *T. minutum* consistently caused the highest mortalities of *P. xylostella* (95 to 98%). The results obtained with *T. pretiosum* and *T. bactrae* coincide with those found by Klemm *et al.* (1992) and Wührer and Hassan (1993). Most of the *P. xylostella* egg mortality caused by *T. pretiosum* and *T. bactrae* was due to parasitism (69 to 72%). These two cultures showed little variation, compared with the other species, among shipments, indicating a more consistent quality of the parasitoid cultures.

According to Klemm *et al.* (1992), *T. bactrae* is a naturally occurring parasitoid of *P. xylostella* eggs in Thailand and was ranked first in host suitability tests on *P. xylostella* eggs. *T. pretiosum*, on the other hand, occurs naturally in the United States (Pinto *et al.*, 1992) but has a lower ability to reproduce in *P. xylostella* eggs than *T. bactrae* (Klemm *et al.*, 1992). Since our study also showed high efficacy of *T. bactrae*, this suggests that the positive results of both studies were not uniquely due to the particular insect cultures studied. Consequently, *T. bactrae* may offer the best possibilities for further testing in the field.

Host mortalities recorded with the product containing *T. minutum* were as high as those for the products containing *T. pretiosum* and *T. bactrae* (95 to 98%). The mortality recorded with *T. minutum*, however, was inflicted in a different manner. Approximately two-thirds of the mortality inflicted by *T. minutum* was in the form of direct induced mortality (63%). *T. minutum* killed 95% of *P. xylostella* eggs, but only parasitized about one-third of them. This kind of host predation or host suppression behavior has been observed with other egg parasitoids, such as *Edovum puttleri* Grissell, (Hym: Eulophidae) (Ruberson *et al.*, 1987, 1991). Host feeding has also been observed by Ruberson (1993) in studies of host preference using *T. pretiosum*. The parasitism efficiency and the direct induced mortality (total and per female) of *T. minutum* were highly variable between shipments but significantly different

between species. These results suggest that *T. minutum* (like *T. pretiosum*) may be useful for inundative releases where control is produced by the insects released, not the offspring. We observed that this behavior could result from the change of host and that successive generations on the new host will result in higher levels of parasitization. However, further tests are necessary to adequately test this hypothesis.

Although the product containing *T. platneri* caused as much direct induced "nonparasitic" mortality as *T. minutum*, the total mortality caused by *T. platneri* was significantly lower. *P. xylostella* egg mortality by *T. platneri* was reasonably high but not as good as that by the other three products discussed above. Two of three shipments of *T. platneri* caused high levels of host predation or host suppression and their quality was comparable to those in *T. bactrae*, *T. pretiosum*, and *T. minutum*. Such data suggest that *T. platneri* may warrant further testing.

In this study the products containing *T. ostrinia* and *T. brassicae* provided the lowest levels of *P. xylostella* egg mortality. Neither product caused considerable mortalities nor demonstrated consistent responses between shipments. The *T. brassicae* studied were too variable in all shipments to consider suitable for further evaluations. Whether this variation was due to the species or rearing methods is not clear. *T. ostrinia* ranked 17th (from 27 species) in host acceptance tests in a study by Klemm *et al.* (1992), but produced 74% parasitism in cabbage transplants placed in a screen-house Shelton *et al.* (1992). In this study only one shipment of *T. ostrinia* had a strong response. Thus, based on what is presented in the literature about *T. ostrinia* and the lack of consistency in our tests, other species may be preferable for further testing.

In most studies examining the suitability of trichogrammatids, more importance is given to parameters like host parasitization than to the total inflicted mortality. However, measuring parasitism alone may underestimate the potential of some species like *T. minutum* and *T. platneri* that caused considerable host mortality without parasitization. Thus, to assess the effectiveness of a given parasitoid it may be more suitable to evaluate host mortality rather than percentage of parasitized eggs.

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