Potential of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) for Biological Control of European Corn Borer (Lepidoptera: Crambidae) in Solanaceous Crops

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ABSTRACT We assessed the ability of Trichogramma ostriniae (Peng & Chen) to locate and parasitize Ostrinia nubilalis (Hübner) eggs in crops other than corn, and we evaluated the efficacy of inundative releases of the parasitoid in two solanaceous crops, pepper and potato. Despite a greater plant surface area to search, parasitism of *O. nubilalis* eggs was consistently higher in sweet corn than dicotyledonous crops such as pepper, snap bean, broccoli, potato, and melon, in choice and no-choice experiments. Nonetheless, in 2002 and 2003, we made four to five separate inundative releases of \approx 30,000 – 50,000 *T. ostriniae* per 0.02 ha in nine pepper fields in Virginia, Pennsylvania, and Massachusetts and compared *O. nubilalis* egg parasitization and fruit damage in those plots with spatially isolated nonrelease plots. Egg parasitization averaged 48.7% in T. ostriniae release plots, which was significantly higher than in nonrelease plots (1.9%). Also, cumulative pepper fruit damage averaged 8.7% in release plots, which was significantly less than in nonrelease plots (27.3%). In potatoes in 2002 and 2003, we made two releases of \approx 75,000 *T. ostriniae* per 0.2 ha in nine fields in Maine and Virginia and compared O. nubilalis damage in those plots with that in nonrelease plots. T. ostriniae releases significantly reduced the number of tunnel holes and number of *O. nubilalis* larvae in potato stems. We conclude that this parasitoid has great potential as a biocontrol agent for O. nubilalis in solanaceous crops.

KEY WORDS Ostrinia nubilalis, parasitoid, pepper, potato, biocontrol

Trichogramma ostriniae (Peng & Chen) (Hymenoptera: Trichogrammatidae) has received particular attention in recent years as a biological control agent of European corn borer, Ostrinia nubilalis (Hübner), in corn (Wang et al. 1999; Wright et al. 2001, 2002; Hoffmann et al. 2002; Kuhar et al. 2002, 2003a). The parasitoid is endemic to China where it is an effective natural enemy of the Asian corn borer, Ostrinia furnicalis Guenée (Hassan and Guo 1991, Smith 1996). Laboratory research has shown that T. ostriniae can successfully parasitize *O. nubilalis* eggs through most stages of embryonic development (Hoffmann et al. 1995) and sustain a relatively high fitness level after many generations of development on factitious hosts such as *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) or *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) eggs (Hoffmann et al. 2001). In addition, *T. ostriniae* is cold hardy and can survive prolonged storage (Smith 1996, Pitcher et al. 2002).

Field research with *T. ostriniae* in the United States has focused on releases in sweet corn in the northeast. Wang et al. (1997) and Wright et al. (2001) demonstrated proficient dispersal and host finding. In Massachusetts, Wang et al. (1999) achieved >40% parasitism of *O. nubilalis* eggs with four releases of \approx 34,000 *T. ostriniae* per hectare. More recently in New York, Hoffmann et al. (2002), Kuhar et al. (2002), and Wright et al. (2002) demonstrated that a single inoculative release of *T. ostriniae* (\approx 70,000 per hectare) could provide season-long parasitism of *O. nubilalis* eggs and reduce ear damage by 50%. Also, Hoffmann et al. (2002) demonstrated that *T. ostriniae* persisted in commercial fields after insecticide applications, that

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parasitism of *O. nubilalis* egg masses was not density dependent, and that *T. ostriniae* populations were distinctly female biased under field conditions.

In addition to sweet corn, O. nubilalis is a pest of other vegetable crops, including beet, cowpea, eggplant, lima bean, pepper, potato, snap bean, swiss chard, and tomato (Capinera 2001). In pepper, O. nubilalis larvae tunnel into and feed on fruit, causing direct damage, premature ripening, and entry points for fruit-rotting pathogens (Hazzard and Ghidiu 2001). If control measures are not taken in pepper, the percentage of damaged fruit can exceed 40-60% in Ohio (Welty 1995) and Virginia (Kuhar and Speese 2002, Kuhar et al. 2003b). The insect is difficult to control because larvae are only exposed to insecticide sprays from egg hatch until tunneling. In many regions, effective protection of fruit is only achieved with multiple insecticide applications beginning at early fruiting (Welty 1995, Hazzard and Ghidiu 2001).

In potato, O. nubilalis larvae damage the plant by tunneling near the base of stems causing breakage and lodging and entry points for stem rot pathogens such as Erwinia carotova (Kennedy 1983). Nault and Kennedy (1996) showed that O. nubilalis larvae exit and reenter a potato stem an average of 4.7 times during their development. In most cases, the potato plant can tolerate low-to-moderate levels of insect tunneling without impacting tuber yield (Nault et al. 1996, 2001). However, over the past 5 yr, pest pressure from O. nubilalis has increased in potato in Maine and other regions because growers are no longer applying broad-spectrum foliar insecticides to control Colorado potato beetle, Leptinotarsa decimlineata (Say) (Coleoptera: Chrysomelidae) (E.G. and T.P.K., unpublished data). Potato growers in North Carolina and Virginia routinely apply one to two foliar sprays of insecticides each year solely for O. nubilalis control (Nault and Kennedy 1996, Nault and Speese 2000).

Given the success of *T. ostriniae* in sweet corn, there is interest in its potential to control *O. nubilalis* in peppers and potatoes. The objectives of this study were to assess the ability of *T. ostriniae* to locate and parasitize *O. nubilalis* eggs in crops other than corn and to evaluate the efficacy of inundative releases of the parasitoid in pepper and potato.

Materials and Methods

Before use in these studies, we reared *T. ostriniae* for four generations on *O. nubilalis* and then onto *E. kuehniella* for mass production following the methods of Morrison (1985). The parasitoids were maintained at Cornell University, Ithaca, NY, under conditions of $25^{\circ}C/23^{\circ}C$; $\approx 80\%$ RH, and a photoperiod of 16:8 (L:D) h with access to undiluted honey (Hoffmann et al. 2001). For all experiments, we used release containers made from 150-ml cone-shaped paper cups that contained parasitized *E. kuehniella* eggs. Release cartons were stapled shut and perforated to allow *T. ostriniae* emergence.

Multiple-Crop No-Choice Experiment. In a preliminary experiment in 2001, we compared T. ostriniae parasitism in four different vegetable crops that were spatially isolated from each other by >50 m at the Cornell University Entomology Research Farm in Freeville, NY. The crops included: sweet corn (a mixture of 'Sprint', 'Dynamo', and 'Bonus' planted on 1 June with 0.9-m row spacing); potato (a mixture of 'Atlantic' and 'Seneca Gold' planted in mid-May on four-row strips); melon ('Athena' cantaloupe set on black plastic with drip irrigation); and sweet pepper (a mixture of 'Boynton' and 'Paladin' also set on black plastic with drip irrigation). Plant surface area was not assessed. On 1 August, all crops were producing fruit, and we released 1000 T. ostriniae in each of four separate 0.01-ha plots of each crop. Release cartons were fastened to plant stems in the field by using flagging tape. We obtained egg masses of O. nubilalis on wax paper from a colony at Cornell University and made sentinels by cutting around a section containing two or three egg masses and gluing them to a 2- by 5-cm strip of wax paper. We pinned 20 O. nubilalis sentinels to the undersides of leaves within 20 m from each release point. We collected sentinels on 3 and 6 August. Collected sentinels were carefully removed from the wax paper, placed in size 00 gelatin capsules, and held in the laboratory at room temperature where they were observed daily for parasitism. Because plantings of each crop were not replicated, data were not compared among crops. However, means and standard errors of percentage of parasitism were calculated to show the potential of T. ostriniae to find and parasitize eggs of O. nubilalis in different crops.

Multiple-Crop Choice Experiment. Also in 2001, at the same research farm, we compared parasitism of *O. nubilalis* among crops planted together. Sweet corn, pepper, snap bean, and broccoli were arranged in four randomized complete blocks, with each separated by >300 m to avoid parasitoid movement among blocks. Each block was 20 rows (0.9 m apart) by 20 m, and each crop occupied 10 rows by 10 m in a corner of the block. In early June 2001, we direct seeded a mixture of 'Primetime' and 'Sprint' sweet corn and 'Hystyle' snap bean. Approximately 2 wk later, we set transplants of 'Boynton' pepper and 'Southern Comet' broccoli in the field.

On 13 August, we estimated total plant surface area of each crop within a block by measuring leaf and stem area from five randomly selected plants and multiplying by the number of plants within the block. Also on 13 August, we released \approx 5,000 *T. ostriniae* females into the center of each block at the juncture of the four crops. On 13, 17, and 21 August, we pinned O. nubilalis egg mass sentinels to the undersides of leaves on 25 randomly selected plants of each crop per block. After 3 d in the field, we collected the sentinels and assessed for parasitism as described in the previous study. We used analysis of variance (ANOVA) (PROC GLM, SAS Institute 1999) to analyze differences in plant surface area and proportion of parasitism. Proportion parasitism data were arcsine square-root transformed before analysis to stabilize variance (Ott 1984). Dif-

Table 1. Crop information and T. ostriniae release o	ation and T. ostrini	ae release dates	lates for each location of the pepper experiments in 2002 and 2003	and 2003	
Location	Variety	Transplant date	Crop production method	T. ostriniae release dates	Harvest dates
2002 Eastern Shore 1, VA	'Paladin'	18 June	Ground with overhead irrigation	17, 23, 30 July, and 6, 22 Aug.	16 Aug. and 10 Sept.
Eastern Shore 2, VA Tidewater, VA	'Paladin' 'Paladin'	18 June 19 June	Ground with overhead irrigation Single rows on black plastic with drip-line	17, 23, 30 July, and 6, 22 Aug. 17, 23, 30 July, and 6, 22 Aug.	16 Aug. and 10 Sept. 16 Aug.
Virginia Beach, VA	'Paladin'	18 June	irrigation Ground with drip-line irrigation	17, 23, 30 July, and 6, 22 Aug.	16 Aug. and 4 Sept.
Landisville, PA	'King Arthur'	3 June	Double-staggered rows on black plastic with drip	11, 24 July, and 7, 14, 30 Aug.	24 July, 7, 14, 21, 27 Aug. and 3, 10, 17 Sept.
Rock Springs, PA	'King Arthur'	10 June	irrigation Double-staggered rows on black plastic with drip irrigation	15, 26 July, and 6, 13, 29 Aug.	6, 15, 20, 30 Aug., and 5, 12, 18 Sept.
2003 Eastern Shore. VA	'Paladin'	18 Iune	Ground with overhead irrigation	16. 23. 30 Iuly, and 6 Aug.	14. 21 Aug. and 2 Sept.
Virginia Beach, VA South Deerfield, MA	'Paladin' 'Aristotle'	24 June 4 June	Ground with drip-line irrigation Double-staggered rows on black plastic with drip	16, 23, 30 July, and 6 Aug. 30 July, and 6, 12 and 19 Aug.	12, 20 Aug., and 1, 9 Sept. 15, 25 Aug., and 9 and 24 Sept.
			irrigation		

ferences in percentage parasitism among crops were tested using Fisher's least significant difference (LSD) at the $\alpha < 0.05$ level of significance.

Evaluations in Pepper. We conducted an experiment at six locations in 2002 and three locations in 2003 (Table 1). Each location represented a replicate in the experiment. We established T. ostriniae release and nonrelease plots (each ≈ 0.02 ha and ≈ 300 plants) of bell pepper at each location. Release and nonrelease plots were separated by at least 200 m. No insecticides were applied to pepper plots. We made four or five separate releases of $\approx 30,000-50,000$ T. ostriniae per release plot (Table 1). Release densities varied somewhat on each date because of differences in percentage of emergence of the parasitoids. Beginning on the day of first release until last harvest, we inspected 50–100 plants approximately weekly in each plot for O. nubilalis egg masses. Egg masses were collected from the field, placed in gel caps (size 00), and held at room temperature in the laboratory until O. nubilalis eclosion or emergence of adult parasitoids.

Peppers were hand harvested and evaluated for damage in August and September (Table 1). At each harvest, we inspected a sample of 100 fruit per plot for insect injury. We dissected any infested fruit and recorded the number of *O. nubilalis* or other insect larvae present. We pooled data across years to increase the sample size for statistical analysis. We used paired *t*-tests (SAS Institute 1999) at the P < 0.05 level of significance to analyze potential treatment differences in proportion of egg parasitism and cumulative proportion of fruit infested at harvest. Proportion of parasitism data were arcsine square-root transformed before analysis to stabilize variance (Ott 1984).

Evaluations in Potato. In 2002, we established T. ostriniae release and nonrelease plots (0.2 ha) of potato at four locations: the Eastern Shore Agricultural Research and Extension Center near Painter, VA; two University of Maine Research Farms, Roger's Farm in Old Town, ME, and Aroostook Farm in Presque Isle, ME; and a commercial farm in Presque Isle, ME. We established and maintained potato crops according to standard grower practices. In Maine, we monitored O. nubilalis moth activity by using wire cone traps containing the "E" and "Z" strain pheromone lures. Beginning at first moth catch (early July), we sampled 50 random plants per plot each week for O. nubilalis egg masses. Additionally, each week from 15 July to 19 August, we installed \approx 30–100 sentinels in each plot by pinning them to the undersides of randomly selected plants. In Virginia, we did not sample for natural *O. nubilalis* egg masses, but we did install \approx 30 sentinels in each plot weekly from 23 May to 12 June. On 22 May and 12 June in Virginia, and 15 and 22 July in Maine, we released 75,000 T. ostriniae per plot. We collected O. nubilalis sentinels and natural egg masses weekly and assessed for parasitism as described above.

In 2003, we established *T. ostriniae* release and nonrelease plots (0.2 ha) of potato at five locations: four commercial fields near Cape Charles, VA, and Aroostook Farm in Presque Isle, ME. We sampled for natural

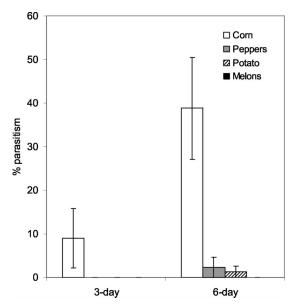


Fig. 1. Parasitism of sentinel *O. nubilalis* egg masses $(\pm SE)$ after simultaneous releases of 1000 *T. ostriniae* into replicated plots of four vegetable crops spatially isolated from each other near Freeville, NY.

O. nubilalis egg masses by examining 100 plants per plot on 3 and 10 June in Virginia and 7 and 30 July in Maine to assess for parasitism. On 14 May and 5 June in Virginia and 17 and 24 July in Maine, we released 75,000 *T. ostriniae* per plot.

In mid-June in Virginia and in late August in Maine of both years, before potato plants dried down, we examined 200 randomly selected potato stems per plot for signs of *O. nubilalis* infestation. We dissected any stems with tunnels and counted *O. nubilalis* larvae present. We pooled data across years to increase the sample size for statistical analysis. We used paired *t*-tests (SAS Institute 1999) at the P < 0.05 level of significance to analyze potential treatment differences in number of stems with tunnels and number of *O. nubilalis* larvae per 200 stems.

Results

Multiple-Crop No-Choice Experiment. At 3 d after *T. ostriniae* were released, parasitism of *O. nubilalis* egg masses was low in all crops, and the only sentinel egg masses that were parasitized were collected from the sweet corn plots (Fig. 1). At 6 d after release, parasitism of *O. nubilalis* egg masses was detected in the sweet corn, pepper, and potato plots, with sweet corn having a much higher parasitism than all other crops.

Multiple-Crop Choice Experiment. On the date of release of *T. ostriniae*, total plant surface area was different among crops (F = 17.4; df = 3, 15; P < 0.001), with sweet corn having the greatest surface area (167.6 ± 25.3 m²), followed by snap bean (118.7 ± 6.3 m²), and then broccoli (41.0 ± 2.2 m²) and pepper (32.7 ± 1.8 m²), which did not differ from each other. Differences in parasitism of *O. nubilalis* egg masses

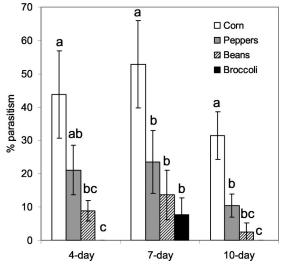


Fig. 2. Percentage of *O. nubilalis* sentinel egg masses parasitized (\pm SE) after a single release of 5,000 *T. ostriniae* into a 0.02-ha block of four vegetable crops at Freeville, NY. Bars with the same letter are not significantly different, Fisher's protected LSD, *P* < 0.05.

occurred among the crops at 4 d (F = 4.09; df = 3, 15; P < 0.05), 7 d (F = 4.13; df = 3, 15; P < 0.05), and 10 d (F = 8.34; df = 3, 15; P < 0.05) after *T. ostriniae* release. Parasitism was significantly higher in sweet corn than other crops, followed by pepper, snap bean, and then broccoli (Fig. 2).

Evaluations in Pepper. In 2002, heavy pest pressure from O. nubilalis occurred at all four Virginia locations and moderate pressure occurred at the Pennsylvania locations (Fig. 3). In 2003, low-to-moderate pest pressure from O. nubilalis occurred at all three locations. Very little $(1.9 \pm 1.6\%)$ parasitism occurred in O. nubilalis egg masses collected from nonrelease (control) plots, whereas $48.7 \pm 6.8\%$ of the egg masses collected from release plots were parasitized by *T. ostriniae* (Table 2). This difference in parasitization was highly significant (t = 8.54, df = 8, P < 0.0001). At harvest, the percentage of fruit damaged averaged $27.3 \pm 6.3\%$ in nonrelease plots and was significantly lower (t = 4.17, df = 8, P = 0.0031) in release plots, averaging $8.7 \pm 1.6\%$ (Table 2). Of the insects found in pepper fruit, 95% were identified as O. nubilalis. The remaining 5% were the noctuid pests, Helicoverpa zea (Boddie), Spodoptera exigua Hübner, Spodoptera frugiperda (J.E. Smith), and Spodoptera ornithogalli (Gueneé) or pepper maggot, Zonosemata electa (Say) (Diptera: Tephritidae).

Evaluations in Potato. In both years, very few natural *O. nubilalis* egg masses were encountered in the potato plots and sentinels had very little (<1%) parasitism. Thus, parasitism data were not analyzed. The number of potato stems damaged by *O. nubilalis* was significantly less in *T. ostriniae* release plots (t = 3.00, df = 8, P = 0.0171; Table 3). Similarly, the number of *O. nubilalis* larvae per 200 stems was significantly less

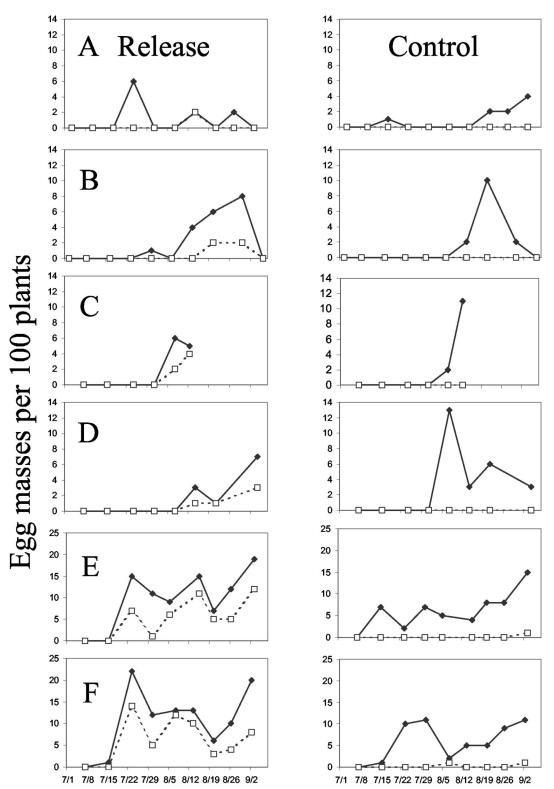


Fig. 3. Total numbers of *O. nubilalis* egg masses (represented by the black diamond) and egg masses parasitized by *T. ostriniae* (represented by open square) in release and control plots of pepper in 2002 from the following locations: (A) Landisville, PA; (B) Rock Springs, PA; (C) Tidewater, VA; (D) Virginia Beach, VA; (E) Eastern Shore 1, VA; and (F) Eastern Shore 2, VA.

Table 2. European corn borer egg parasitization and damage to sweet pepper with and without inundative releases of T. ostriniae

Location	% parasitization of total collected ECB egg masses		% cumulative harvested fruit damaged by ECB	
	Release	Control	Release	Control
2002				
Eastern Shore 1, VA	54.9	0.3	14.0	53.0
Eastern Shore 2, VA	56.7	2.6	18.0	65.5
Tidewater, VA	52.1	0.0	9.0	19.0
Virginia Beach, VA	46.3	0.0	5.5	26.0
Landisville, PA	12.5	0.0	8.5	20.6
Rock Springs, PA	20.0	0.0	3.6	16.7
2003				
Eastern Shore, VA	58.1	0.0	8.4	14.2
Virginia Beach, VA	60.9	0.0	8.5	13.3
South Deerfield, MA	76.9	14.3	3.2	17.2
Mean \pm SE	$48.7\pm6.8\%$	$1.9 \pm 1.6\%^a$	$8.7\pm1.6\%$	$27.3 \pm 6.3\%$

ECB, European corn borer.

^{*a*} Significant difference between *T. ostriniae* release and control according to paired *t*-test (P < 0.05).

in T. ostriniae release plots (t = 2.39, df = 8, P = 0.0439).

Discussion

Smith (1996) suggested that Trichogramma find host eggs by random searching, and authors have hypothesized that parasitism is inversely proportional to the size (Ables et al. 1980, Burbutis and Koepke 1981, Wang et al. 1997) and complexity (Andow and Prokrym 1990, Gingras and Boivin 2002) of the host plant. In general, the greater the plant surface area to search, the lower the parasitism rate. In our multiplecrop experiments, however, sweet corn had the greatest plant surface area, yet the highest parasitization of O. nubilalis egg masses by T. ostriniae. Plant complexity may have been a factor, with corn having a fairly simple monocotyledonous architecture with relatively few connections of leaves, buds, and stems (Gingras and Boivin 2002). Nonetheless, we believe that our results suggest a habitat selection or preference by T. ostriniae for corn over dicotyledonous plants. Smith (1996) suggested that Trichogramma species, in general, are much more habitat specific than host specific. This has been demonstrated in Trichogramma pretiosum Riley and Trichogramma minutum Riley (Altieri et al. 1982, Keller et al. 1985, Thorpe 1985). Some host plants provide volatile cues (kairomones) that arrest and stimulate searching and parasitism in *Trichogramma* (Altieri et al. 1982, Noldus 1989). More research into the environmental cues (visual and chemical) used by *T. ostriniae* for host finding is needed to help explain these differences.

Although dicotyledonous plants may not be the preferred habitat or most efficient habitat for finding hosts, our study showed that four to five inundative releases of T. ostriniae in pepper resulted in significant O. nubilalis egg parasitism and concomitant reductions in fruit damage. Pepper fruit damage averaged 8.7% in our T. ostriniae release plots, which is comparable with peppers sprayed up to six times with standard insecticides such as acephate, spinosad, methoxyfenozide, indoxacarb, or pyrethroids (Kuhar and Speese 2002, Kuhar et al. 2003b). Moreover, in potatoes, two releases of \approx 75,000 *T. ostriniae* per 0.2 ha significantly reduced the number of tunnel holes and number of *O. nubilalis* larvae in potato stems. Based on our knowledge of the scientific literature, these results demonstrate one of the most efficacious uses of a parasitoid to control O. nubilalis in peppers or potatoes. Working with a similar species, Trichogramma *nubilale* Ertle & Davis, in sweet pepper in Delaware,

Transfer	No. tunnel holes/200 stems		No. O. nubilalis larvae/200 stems	
Location	T. ostriniae release	Control	T. ostriniae release	Control
2002				
Aroostook Farm, Presque Isle, ME	74	125	62	104
Roger's Farm, Old Town, ME	4	28	10	18
Commercial Farm, Presque Isle, ME	31	67	31	67
Eastern Shore, VA	16	12	8	4
2003				
Aroostook Farm, Presque Isle, ME	80	100	54	67
Cape Charles 1, VA	22	19	13	11
Cape Charles 2, VA	10	10	4	4
Cape Charles 3, VA	5	46	5	26
Cape Charles 4, VA	4	19	3	8
Mean \pm SE	27.3 ± 9.9	47.3 ± 13.9^{a}	21.1 ± 7.5	34.3 ± 12.0

^{*a*} Significant difference between *T. ostriniae* release and control according to paired *t*-test (P < 0.05).

Tolerance for crop injury plays an important role in the level of O. nubilalis control required. Potato can withstand reasonable levels of O. nubilalis tunnel injury without significant yield loss (Nault and Kennedy 1996, Nault et al. 2001), but tolerance for *O. nubilalis* in pepper is much lower because the marketable portion of the crop is directly injured (Hazzard and Ghidiu 2001). Therefore, combined O. nubilalis mortality rates from parasitism and other factors would need to be higher in pepper than potato for economic control. European corn borer eggs and early instars are attacked by naturally occurring predators, parasitoids, and pathogens (Mason et al. 1996). These natural enemies along with abiotic factors contribute to a relatively high natural mortality (60-90%) of O. nubilalis eggs and early instars in corn (Frye 1972, Showers et al. 1978, Ross and Ostlie 1990). In sweet corn, Kuhar et al. (2002) showed that 60-80% of O. nubilalis eggs and early larvae perished from natural causes and that increasing egg parasitism to 37% with augmentative releases of T. ostriniae increased the total mortality to >90%. This resulted in a concomitant 50% reduction in ear damage.

Thus, depending on the degree of pest pressure, the tolerance for injury in the host plant and the market, and the level of natural control, *T. ostriniae* may provide an effective management option for *O. nubilalis* in solanaceous crops. More work is needed to optimize release rates and strategies, as well as compatibility with other management tactics such as insecticides.

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