Optimizing *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) releases to control European corn borer, *Ostrinia nubilalis* (Lepidoptera: Crambidae) in bell pepper

Anna Virginia Chapman

Thesis submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of:

Masters of Life Science
With a concentration in
Entomology

Thomas P. Kuhar
Peter B. Schultz
Carlyle C. Brewster

April 30, 2007
Blacksburg, Virginia

Keywords: *Trichogramma*, IPM, pepper, European corn borer

Optimizing *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) releases to control European corn borer, *Ostrinia nubilalis* (Lepidoptera: Crambidae) in bell pepper

Anna Chapman

Abstract

The effective dispersal ability of the egg parasitoid *Trichogramma ostriniae* Pang and Chen was assessed in potato fields on the Eastern Shore of Virginia in spring 2005 and 2006. Approximately 0.5 million *T. ostriniae* were released from a central release point in separate potato fields. Dispersal was monitored using yellow sticky card traps and European corn borer, *Ostrinia nubilalis* Hübner, egg mass sentinels. Adult *T. ostriniae* dispersed quickly throughout the 0.4 ha (1 acre) sampling area. Parasitism and sticky card captures were highest close to the release point and decreased with increasing distance. Sticky card data were a good fit to the diffusion model used ($r^2 > 0.90$) for all but two sampling dates. In 2005 parasitization peaked at 4 days post release with close to 40% of sentinels parasitized at 30m from the release point. The mean distance encompassing 98% (x_{98}) of *T. ostriniae* for both fields in 2005 was 27.5 (± 2.4) meters. For fields 1 and 2 in 2005, x_{98} for parasitism was 21 and 26 meters, respectively. In 2006 sticky card data fit the dispersal model moderately well ($r^2 > 0.77$) except for two sampling dates and dispersal was generally lower. The mean x_{98} value for sticky card data was 12.9 (± 0.9) meters. For parasitism, the x_{98} distances for field 1 and 2 were estimated at 8 and 10 meters, respectively. Correlation analysis showed no significant difference in the distributions between sticky card captures and sentinel egg mass parasitism.

In 2006, *T. ostriniae* were released in small pepper plots in Pennsylvania, Maryland and two locations in Virginia to evaluate the number of wasps needed per plant for effective control of European corn borer. Treatments included 0, 5, 20 and 50 wasps per plant. In each plot, parasitism was measured using 30

sentinel egg masses collected on 3 and 6 days post release. Parasitism was relatively low in Pennsylvania and Virginia and no significant effect from release density was observed. High rates of parasitization in the untreated control plot were observed in Maryland as well as one of the Virginia locations. Overall results show results show ambiguity in the data and high levels of natural parasitism occurring on *Ephestia* eggs sentinels.

In 2005 and 2006, several insecticides were evaluated for controlling *O. nubilalis* and impacting arthropod natural enemies in bell pepper. In addition, we compared the effectiveness of an integrated pest management program based around inundative releases of *T. ostriniae* to a conventional insecticide-based program for *O. nubilalis* control in multiple locations in the Mid- Atlantic US. To evaluate the insecticides, small plots of bell pepper were established at four locations in Virginia, Maryland, Delaware, and Pennsylvania. Insecticides were applied weekly from first fruit until final harvest (5 to 7 applications). Results indicated that the biorational insecticides, spinosad, indoxacarb, and methoxyfenozide provided comparable control of *O. nubilalis* as the broad-spectrum conventional insecticides, acephate, and lambda-cyhalothrin. At most locations, multiple sprays of lambda-cyhalothrin resulted in flares (outbreaks) of green peach aphids most likely from destruction of arthropod natural enemies. Indoxacarb also caused a similar aphid flare at one of the locations. For the IPM demonstration experiment, pepper plots were established at 5 locations in the Mid-Atlantic U.S. in 2005 and 2006. Treatments included: “conventional”, which involved weekly applications of acephate or lambda-cyhalothrin from first fruit until final harvest; 2) “IPM”, which included three or four inundative releases of *T. ostriniae* and a judicial application of methoxyfenozide only if lepidopteran pests exceeded action thresholds; and 3) an untreated control. No significant treatment effect was found in either year on cumulative number of marketable fruit or percentage of fruit damaged by lepidopteran pests. A significant treatment effect was found on peak numbers of green peach aphids, with the conventional insecticide approach causing aphid flares and the untreated control or IPM approach not having aphid pest problems. Inundative releases of *T.*

ostriniae may be a more environmentally-sound approach to managing *O. nubilalis* in peppers, although a comparison with conventional insecticides under greater lepidopteran pest pressure is still needed.

Acknowledgements

I would like to thank my committee members Tom Kuhar, Pete Schultz and Carlyle Brewster for their endless guidance and support. Each of them has contributed greatly to my education and professional experience. In addition, thank you to my many collaborators, the staff of the ESAREC as well as the HRAREC for your help in conducting field work and collecting data. This has been a rewarding and memorable experience and I am grateful to the Virginia Tech Department of Entomology for taking me in and challenging me as a student. Also, thank you to my family and my family of friends for being my inspiration and heart.

Table of Contents

List of Tables

List of Figures

Introduction

Bell peppers, *Capsicum annuum* L., are an extremely high value crop and are grown on more than 1,500 different vegetable farms in Delaware, Maryland, Pennsylvania and Virginia. European corn borer (ECB), *Ostrinia nubilalis*, is the most important insect pest of pepper in the region and is the target of numerous insecticide sprays each season. Most of the insecticides used in peppers are FQPA-targeted chemicals and broad-spectrum pesticides, which can induce severe secondary outbreaks of aphids due to the destruction of natural enemies. Reducing broad-spectrum insecticide use is a high priority for peppers from the standpoint of environmental stewardship and human safety.

Biological control and/or switching to more environmentally-friendly insecticides are two strategies for achieving this goal. *Trichogramma ostriniae*, a parasitoid of lepidopteran eggs, was recently introduced into the U.S., and has been shown to be highly effective for controlling ECB in sweet corn in the northeast. Recently, inundative releases of *T. ostriniae* were evaluated for control of ECB in bell pepper and caused high rates of ECB egg parasitism in the field and substantial reductions in cumulative fruit damage by the pest. The goal of this research was to advance and improve the use of *T. ostriniae* for integrated pest management in pepper. The objectives addressed in this research were:

1. to determine the effective dispersal ability of *T. ostriniae* after inundative releases
2. to determine the number of *T. ostriniae* to release per unit area for effective control of ECB
3. to evaluate the efficacy and relative impact of reduced-risk insecticides versus broad-spectrum insecticides on beneficial arthropods in pepper.

Literature Review

European corn borer

History and Significance

European corn borer (ECB), *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) is a major phytophagous pest throughout the United States. The moth was described by J. Hübner as *Pyralis nubilalis* in 1796 and was moved to the genus *Ostrinia* in 1957 (Beck 1987, Marion 1957). ECB is thought to be a native of Europe and brought to North America in the early 1900's on imported broom corn (Mason et al. 1996). Populations rapidly expanded due to the lack of natural enemies and immense acreage of maize in the U.S. (Beck 1987). Today, ECB is distributed throughout North America east of the Rocky Mountains (Mason et al. 1996). Although corn, *Zea mays,* is a preferred host plant, ECB is highly polyphagous and feeds on more than 200 plants (Beck 1987). It is a pest of many agricultural crops including beet, corn, cotton, cowpea, eggplant, lima bean, pepper, potato, snap bean, sorghum, swiss chard, and tomato (Capinera 2001). In bell pepper, ECB is the target of a preponderance of insecticide use, with growers in the Mid-Atlantic Region typically spraying insecticides every 5-10 days from 1st fruit until last harvest (Welty 1995; Kuhar and Speese 2001).

Life Cycle and Biology

Shortly after mating, *O. nubilalis* female moths deposit eggs on plants typically on the underside of leaves (Barlow and Kuhar 2004). During its two-week adult life span, female moths deposit about 350 eggs (Caffrey and Worthley 1927). However moths are most active from 4 to 6 days after mating (Elliot and Dirks 1979, Mason et al. 1996). Eggs are laid in clusters that hatch in 4 to 7 days (Mason et al. 1996) depending on temperature. The larval stage has five instars with the last measuring about 20-25 mm in length (Beck 1997). Larvae display negative phototaxis, positive thigmotaxis, and positive sacharotropism behavioral characteristics, which influence the orientation and pattern of feeding (Beck

1956a, b). Typically, larvae bore into petioles, stems, stalks, or fruit of host plants and are commonly not exposed to the external environment during feeding. Fifth instars will pupate or diapause overwinter in stalks, stems, or fruit (Beck 1987).

Based on genetic differences and environmental factors associated with geographical location, *O. nubilalis* completes one to four generations per year (Mutchmor and Beckel 1958, Showers 1976, Showers 1993). The three populations in North America include a northern univoltine, central bivoltine and southern multivoltine generation (Mason et al. 1996). In addition to voltinism, ECB shows phenotypic variation based on sex pheromones in North America (Klun 1975, Showers et al. 1975, Roelofs et al. 1985, Coates et al. 2004). There are two strains of ECB, the E-strain (New York type) and Z-strain (Iowa type), using mixtures of the female sex pheromone 98:2 and 1:99 of (E) and (Z)-11-tetradecenyl acetates, respectively (Glover et al. 1987). In the eastern United States including the Mid-Atlantic region, the two strains have overlapping distributions and have shown hybridization (Roelofs et al. 1985, Durant et al. 1995).

Crop Damage and Control Methods

With three generations per year, *O. nubilalis* is a season long pest of peppers. After a very brief period of leaf feeding, larvae quickly bore into fruit and stems. Larvae are only exposed to insecticide sprays from eclosion until tunneling, making pest control of ECB difficult. Direct damage is caused by the tunneling larvae which feed on the pericarp, placenta, and seeds of developing fruit (Jarvis and Guthrie 1972). In the Mid –Atlantic region, ECB is one of the most important pests of pepper and receives the preponderance of pest control measures (Boucher 2001). Moreover, tunnel holes frequently are entry points for fruit-rotting pathogens (Hazzard et al. 2001). If control measures are not taken in pepper, fruit damage can exceed 40 to 60% in Ohio (Welty 1995) and Virginia (Nault and Speese 2000, Kuhar and Speese 2002, Kuhar et al. 2003b).

In potato, *O. nubilalis* larvae tunnel into the base of stems causing them to break and create entry points for rot pathogens (Kennedy 1983). In most instances, potato plants can withstand low to moderate ECB injury without affecting tuber yield (Nault et al. 1996, 2001). Nonetheless, potato growers in Virginia and North Carolina typically apply one or two foliar insecticide sprays each year exclusively for *O. nubilalis* control (Nault and Kennedy 1996, Nault and Speese 2000).

Efforts to establish natural enemies of *O. nubilalis* have been ongoing since its introduction to North America (Clausen 1978). The exotic parasitoid species *Lydella thompsoni* Herting (Diptera: Tachinidae), *Macrocentrus grandii* Goidanich (Hymenoptera: Braconidae), and *Eriborus terebrans* Gravenhorst (Hymenoptera: Ichneumonidae), appear to be the primary parasitoids attacking ECB in North America (Mason et al. 1994). The greatest potential for biological control of ECB centers around the use of egg parasitoids. Killing ECB eggs on leaves before they hatch into tunneling larvae is a clear and logical approach to pest management.

Trichogramma

Overview

Trichogramma is a genus within family Trichogrammatidae comprised of minute polyphagous egg parasitoids. *Trichogramma* have been considered for biological control for over 100 years with the majority of progress coming from in the USSR and China prior to the1960's (Smith 1996). Initial work in the US was minimal due to the increased use of chemical insecticides in and around WWII. Since the 1960's renewed interest in developing *Trichogramma* as a biological control agent spurred research in mass rearing and inundative release programs (Smith 1996). In the past 30 years research into using *Trichogramma* for inundative releases has expanded into 50 or more countries with the majority of research focusing on lepidopteran pests. Control of lepidopteran pests has been investigated in a multitude of crops with the greatest success in corn against *Ostrinia* spp. and *Helicoverpa zea*. Naturally occurring *Trichogramma* parasitoids of ECB in North America include *T. minutum* Riley, *T. pretiosum* Riley, and *T. nubilale* Ertle and Davis. However, these species provide little natural biological control of *O. nubilalis* (Losey and Calvin 1995).

Trichogramma ostriniae

Trichogramma ostriniae Pang et Chen (Hymenoptera: Trichogrammatidae) is a parasitoid endemic to China where it is an effective natural enemy of the Asian corn borer, *Ostrinia furnicalis* Guenee (Zhang 1988, Hassan and Guo 1991). The species was first imported to the US in 1990 as a potential biological control agent of *O. nubilalis*. Pavlik (1993) and Hoffman et al. (1995) showed that *T. ostriniae* successfully parasitized *O. nubilalis* in laboratory conditions. Early testing in the field showed more than 97% parasitism of ECB eggs in sweet corn (Mason et al. 1996). Most research with *T. ostriniae* has focused on controlling ECB in sweet corn in the northeastern U.S. (Seaman et al. 1997, Wang and Ferro 1998, Wang et al. 1999, Wright et al. 2001, Hoffmann et al. 2002, Kuhar et al. 2002, Wright et al 2002, Kuhar et al. 2003a). Recently, Kuhar et al. (2004) assessed the effectiveness of *T. ostriniae* against *O. nubilalis* in solanaceous crops. In bell pepper, they demonstrated that four to five inundative releases of 30,000-50,000 *T. ostriniae* per .02 acre significantly reduced cumulative fruit damage. In release plots, ECB egg parasitism averaged 48.7% with a density of ~10 *T. ostriniae*/plant.

Justification

From the interest of human safety and environmental stewardship, there is strong impetus for long-term strategies and tactics to reduce reliance on multiple preventative applications of broad-spectrum insecticides in peppers. Most of the insecticides used in peppers are FQPA-targeted chemicals (USDA-NASS 2003). Given the success of biological control with *T. ostriniae* in corn and the potential for use in solanaceous crops, we propose to improve and optimize the use of *T. ostriniae* in integrated pest management (IPM) for pepper. Improving field efficacy of *T. ostriniae* will have an important effect on the development and adoption of alternative pest control by producers. Optimizing release strategies and integrating releases with existing pest management using reduced risk insecticides will help promote the use of *T. ostriniae* for control of ECB in pepper.

Literature cited

Barlow, V. M. and T. P. Kuhar. 2004. Distribution of egg masses of the European corn borer, *Ostrinia nubilalis*, in the bell pepper plant canopy. J. Entomol. Sci. 39: 670 – 672.

Beck, S. 1987. Developmental and seasonal biology of Ostrinia nubilalis. Agricultural Zool. Rev. 2: 59-96.

Beck, S. D. 1956a. The European corn borer, Pyrausta nubilalis (Hubn.), and its principal host plant. I. orientation and feeding behavior of the larva on the corn plant. Annals of the Entomological Society of America, 49: 552-558.

Beck, S. D. 1956b. nutrition of the European corn borer, Pyrausta nubilalis (Hubn.). IV. Feeding reaction of first instar larvae. Ann. Entomol. Soc. Am. 49: 399-405.

Boucher, T. J. 2001. General strategy of insect management. P. 63-66 In T. J. Boucher and R. A. Ashley (eds.), Northeast Pepper Integrated Pest Management (IPM) Manual. Univ. Conn. Coop. Ext., Stoors, CT.

Caffrey, D. J. and L. H. Worthley. 1927. A progress report on the investigations of the European corn borer. United States department of agriculture bulletin, 1476: 155.

Capinera, J. L. 2001. Handbook of vegetable pests. Academic, New York.

Clausen, C. P. 1978. Introduced parasites and predators of arthropod pests and weeds: A world review. U. S. Government Printing Office, Washington, D. C.

Coates, B. S., V. S. Douglas, and R. L. Hellmich. 2004. Geographic and voltinism differentiation among North American *Ostrinia nubilalis* (European corn borer) mitochondrial cytochrome c oxidase haplotypes. J. Insect Sci. 4: 1 - 9.

Durant, J.A., Fescemyer, H.W., Mason, C.E., and Udayagiri, S. 1995. Effectiveness of four blends of European corn borer (Lepidoptera : Pyralidae) sex pheromone isomers at three locations in South Carolina. J. Agric. Entomol. 12(4) : 241-253.

Elliot, W. M. and V. A. Dirks. 1979. Postmating age estimates for female European corn borer moths, Ostriniae nubilalis (Lepidoptera: Pyralidae) using time-related changes in sprematophores. Can. Entomol. 111: 1325-1335.

Glover, T. J., Tang, X. H., and W. L. Roelofs. 1987. Sex pheromone blend discrimination by male moths from E and Z strains of European corn borer. J. Chem. Ecol. 13(1): 143-151.

Hassan, S. A. and M. F. Guo. 1991. Selection of effective strains of egg parasites of the genus *Trichogramma* (Hym., Trichogrammatidae) to control the European corn borer *Ostrinia nubilalis* Hb. (Lep., Pyralidae). J. Appl. Entomol. 111: 335-341.

Hazzard, R. V., G. M. Ghidiu, and T. J. Boucher. 2001. European corn borer management. P. 67-76 In T. J. Boucher and R. A. Ashley (eds.), Northeast Pepper Integrated Pest Management (IPM) Manual. Univ. Conn. Coop. Ext., Stoors, CT.

Hoffmann, M. P., D. L. Walker, and A. M. Shelton. 1995. Biology of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) reared on *Ostriniae nubilalis* (Lepidoptera: Pyralidae) and survey for additional hosts. Entomophaga 40: 387 - 402.

Hoffmann, M. P., M. G. Wright, S. A. Chenus, and J. Gardner. 2002. Inoculative releases of *Trichogramma ostriniae* for suppression of *Ostrinia nubilalis* (European corn borer) in sweet corn: Field biology and population dynamics. Biol. Contr. 25: 249-258.

Jarvis, J. L. and W. D. Guthrie. 1972. Relation of horticultural characteristics of peppers to damage by larvae of the European corn borer. Iowa State Journal of Science 46: 463-470.

Kennedy, G. G. 1983. Effects of European corn borer (Lepidoptera: Pyralidae) damage on yields of spring-grown potatoes. J. Econ. Entomol. 76: 316-322.

Klun, J. A. 1975. Insect sex pheromones: intraspecific pheromonal variability of *Ostrinia nubilalis* in North America and Europe. Environ. Entomol. 4: 891 – 894.

Kuhar, T. P. and J. Speese. 2001. European corn borer control in peppers. Vegetable Growers News, Oct. 2001, Vol. 8.

Kuhar, T. P. and J. Speese. 2002. Evaluation of foliar insecticides for control of European corn borer in peppers, 2001. Arthrop. Manag. Tests. 27: E56.

Kuhar, T. P., M. G. Wright, M. P. Hoffmann, and S. A. Chenus. 2002. Life table studies of European corn borer (Lepidoptera: Crambidae) with and without inoculative releases of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae). Environ. Entomol. 31:482-489.

Kuhar, T. P., M. P. Hoffmann, and M. G. Wright. 2003a. Controlling European corn borer in vegetables with a parasitic wasp. Pesticide Outlook 14: 99-101.

Kuhar, T. P., J. Speese, R. J. Cordero, R. Y. Venkata, V. M. Barlow, and R. Cordero. 2003b. Evaluation of foliar insecticides for control of lepidopterous pests in peppers, 2002. Arthrop. Manag. Tests 28: E37.

Kuhar, T. P., V. M. Barlow, M. P. Hoffmann, S. J. Fleischer, E. A. Groden, J. Gardner, R. Hazzard, M. G. Wright, S. A. Pitcher, and P. Westgate. 2004. Potential of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) as a biological control agent of European corn borer (Lepidoptera: Crambidae) in solanaceous crops. J. Econ. Entomol. 97: 1209-1216.

Losey, J. E., and D. D. Calvin. 1995. Quality assessment of four commercially available species of *Trichogramma* (Hymenoptera: Trichogrammatidae). J. Econ. Entomol. 88: 1243 - 1250.

Marion, H. 1957. Complement a la classification et nomenclature des Pyraustidae d' Europ. L' Entomologiste. 13:130.

Mason, C. E., M. E. Rice, D. D. Calvin, J. W. van Duyn, W. B. Showers, W. D. Hutchison, J. F. Witkowski, R. A. Higgins, D. W. Onstad and G. P. Dively. 1996. European corn **borer**: Ecology and management. North Central Regional Extension Pub. No 327. Iowa State University.

Mason, C. E., R. F. Romig, L. E. Wendel, and L. A. Wood. 1994. Distribution and abundance of larval parasitoids of European corn borer (Lepidoptera:

Pyralidae) in the east central United States. Environ. Entomol. 23: 521 - 531.

Mutchmor, J. A., and W. E. Beckel. 1958. Importance of photoperiod and temperature in inducing diapause in the European corn borer, *Pyrausta nubilalis* (Hubn.). Nature 181: 204.

Nault, B.A., and G. G. Kennedy. 1996. Timing insecticide applications for managing European corn borer (Lepidoptera: Pyralidae) infestations in potato. Crop Prot. 15: 465-471.

Nault, B. A., N. M. French, and G. G. Kennedy. 1996. Influence of European corn borer (Lepidoptera: Pyralidae) damage to potato foliage availability on overwinerwinter survival of first generation Colorado potato beetle adults (Coleoptera: Chyrsomelidae) in North Carolina. J. Econ. Entomol. 89: 124-130.

Nault, B. A., J. Speese, G. G. Kennedy, J. Linduska, and G. Dively. 2001. Response of potato tuber yield to stem injury by European corn borer (Lepidoptera: Crambidae) in the Mid-Atlantic states. J. Econ. Entomol. 94: 1162-1169.

Nault, B. A. and J. Speese. 2000. Evaluation of insecticides to control European corn borer and corn earworm on fall-grown peppers, 1999. Arthrop. Manag. Tests. 25: E52.

Pavlík, J. 1993. Variability in the host acceptance of European corn borer, *Ostrinia nubilalis* Hbn. (Lep. Pyralidae) in strains of the egg parasitoid *Trichogramma* spp. (Hym. Trichogrammatidae). J. App. Entomol. 115: 77 - 84.

Roelofs, W. L., J. W. Du, X. H. Tang, P. S. Robbins, and C. J. Eckenrode. 1985. Three European corn borer populations in New York based on sex pheromones and voltinism. J. Chem. Ecol. 11: 829 – 836.

Seaman, A., M. Hoffmann, J. Gardner, and S. Chenus. 1997. Pilot testing of *Trichogramma ostriniae* releases in fresh market sweet corn for control of European corn borer, New York State Vegetable Project Reports Relating to IPM. NYS IPM Publ.

Showers, W. B. 1993. Diversity and variation of European corn borer populations, In: *Evolution of Insect Pest/patterns of Variation* (eds Kim KC, McPherson BA) pp. 287-309. Wiley and Sons Inc. New York, NY.

Showers, W. B., G. L. Reed, J. F. Robinson, and M. B. Derozari. 1976. Flight and sexual activity of the European corn borer. Environ. Entomol. 5: 1099 - 1104.

Showers, W. B., H. C. Chiang, A. J. Keaster, R. E. Hill, G. L. Reed, A. N. Sparks, and G. J. Musick. 1975. Ecotypes of the European corn borer in North America. Environ. Entomol. 4: 753 - 760.

Smith, S. M. 1996. Biological control with *Trichogramma*: advances, successes, and potential of their use. Ann. Rev. Entomol. 41:375-406.

Wang, B. and D. N. Ferro. 1998. Functional responses of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) to *Ostrinia nubilalis* (Lepidoptera: Pyralidae) under laboratory and field conditions. Environ. Entomol. 27: 752-758.

Wang B, D. N. Ferro and D. W. Hosmer, 1999. Effectiveness of *Trichogramma ostriniae* and *T. nubilale* for controlling the European corn borer *Ostrinia nubilalis* in sweet corn. *Entomologia Experimentalis et Applicata*. 91: 297-303.

Welty, C. 1995. Monitoring and control of European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae), on bell peppers in Ohio. J. Agric. Entomo1. 2:145-161.

Wright, M. G., M. P. Hoffmann, S. A. Chenus and J. Gardner. 2001. Dispersal behavior of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) in sweet corn fields: implications for augmentative releases against *Ostrinia nubilalis* (Lepidoptera: Crambidae). Biol. Contr. 22: 29-37.

Wright. M. G., T. P. Kuhar, M. P. Hoffmann and S. A. Chenus. 2002. Effect of inoculative releases of *Trichogramma ostriniae* on populations of *Ostrinia nubilalis* and damage to sweet corn and field corn. Biol. Contr. 23: 149-155.

Zhang, Z. 1988. *Trichogramma* spp. parasitizing the eggs of Asian corn borer *Ostrinia furnacalis* and its efficacy in Beijing suburbs, pp. 629 - 632, *Trichogramma* and other egg parasites. Les colloques de l" INRA, Paris.

Chapter 1

Dispersal of *Trichogramma ostriniae* in potato fields

Abstract

We investigated the dispersal ability of *Trichogramma ostriniae* Pang et Chen (Hymenoptera: Trichogrammatidae), a biological control agent of *Ostrinia nubilalis* (Lepidoptera: Crambidae) in commercial potatoes. The purpose of the study was to quantify dispersal of *T. ostriniae* after an inundative release to aid in determining the number of release points needed per unit area for effective biological control of *O. nubilalis* in solanaceous crops. A single release was made in two spatially separate potato fields on the Eastern Shore of Virginia in summer 2005 and 2006. From a central release point, dispersal was measured over a ~ 0.4 ha monitoring area. Each area contained 25 monitoring points at distances from 5 to 45 meters from the release point bearing a yellow sticky card and *O. nubilalis* egg sentinels to observe for adult wasps and parasitism, respectively. Results showed that *T. ostriniae* dispersed quickly up to 45m within 4 days in 2005 and 1 day in 2006. High rates of parasitization were recorded at this distance (20-50%) and decreased with increasing distance. The greatest numbers of *T. ostriniae* were recovered within 3-4 days of emergence Averaging the mean distance encompassing 98% of wasps for all fields, the majority of wasps dispersed within ~ 17 meters of the central release point. Based on these results multiple release points per 0.4 ha (or 1.0 acre) should be made for effective dispersal of *T. ostriniae* and control of ECB in solanaceous crops.

Introduction

Understanding the dispersal behavior of a parasitoid is important for developing effective augmentative release strategies and for assessing the spread and potential non-target effects of the species (Stern et al. 1965, Smith

1996; McDougall and Mills 1997, Orr et al. 2000). *Trichogramma ostriniae* Pang et Chen (Hymenoptera: Trichogrammatidae) is a tiny lepidopteran egg parasitoid endemic to China. Because of its effectiveness as a natural enemy of the Asian corn borer, *Ostrinia furnicalis* Guenée (Lepidoptera: Crambidae), the parasitoid was introduced into the United States in the early 1990s as a biological control agent of the European corn borer, *O. nubilalis* Hübner (Hassan and Guo 1991; Hoffmann et al. 1995). Although *T. ostriniae* has not been shown to overwinter and establish long-term in North America (Hoffmann et al. 2002, Wright et al. 2005), augmentative (mass) releases of the parasitoid have been shown to significantly reduce *O. nubilalis* damage to sweet corn (Wang et al. 1999; Wright et al. 2002; Hoffmann et al. 2002; Kuhar et al. 2002, 2003), peppers, and potatoes (Kuhar et al. 2004).

In sweet corn, Wright et al. (2001) demonstrated rapid dispersal of *T. ostriniae,* up to 180 m in 6 days and up to 230 m in 21 days after an inoculative release of 1 million wasps from a central release point. These authors also reported uniform parasitism of *O. nubilalis* egg masses up to 1 ha around a central release point. However, crop habitat can have a significant impact on the searching ability and dispersal behavior of *Trichogramma* (Thorpe 1985, Andow and Prokrym 1990). In particular, *T. ostriniae* appears to search for its host egg preferentially and/or more efficiently in a cornfield compared with a broadleaf vegetable crop such as bean, pepper, or potato (Kuhar et al. 2004) or a wooded habitat (Wright et al. 2005). The objective of this study was to assess the dispersal rate (behavior) of *T. ostriniae* after inundative releases in potato fields. Such information will help us to optimize augmentative release strategies for the parasitoid in solanaceous crops.

Materials and Methods

Experiments were conducted in May and June of 2005 and 2006 within two commercial potato fields each year located in Northampton County, VA. In each field, growers planted 'Atlantic' potatoes in early March on rows (hills) spaced 0.9 m apart with plants seeded ~0.28 m within rows. At the time of the experiments each year, potato plants were in bloom with a dense canopy within-rows and a plant height ranging from 0.5 to 0.9 m.

Field plot design

Within each field, a square plot that measured 4,096 m^2 or approximately 1 acre was marked off. Within each plot, 25 stations were marked off in a grid at varying distances from a central release point. In 2005, stations were placed at 1, 16, 23, 32, and 45 m from a central release point (Fig. 1-A), and in 2006, stations were placed 1, 5, 7, 9, 13, 18, 26, and 45 m from a central release point (Fig. 1-B).

At each station, a wooden tomato stake bearing a 15×15 cm yellow sticky card (Olson Products Inc., Medina, OH) was placed at a height equal to the plant canopy. In an adjacent row within 1 m of the stake, *O. nubilalis* egg mass sentinels on wax paper were pinned to the undersides of leaves on five individual potato plants. Sentinels were made by establishing a lab colony of *O. nubilalis* moths in cages and allowing females to oviposit on sheets of wax paper. A sentinel egg mass strip was made by cutting around one or two egg masses and gluing them to a small ~10 cm^2 strip of wax paper (Wright et al. 2001, 2002). For the experiment, the sentinel eggs and yellow sticky cards were placed in the fields a few days prior to parasitoid releases (to assess for "background noise" any natural *Trichogramma*), and at the time of parasitoid release.

Trichogramma releases: Shipments of *T. ostriniae* were obtained from M. P. Hoffmann at Cornell University, Ithaca, NY, USA. The lab colony, originally collected from northern China in the early 1990s, was maintained on sterilized eggs of *Ephestia kuehniella* under conditions of 16L:8D; 24±1°C; ~80% RH with access to undiluted honey for food. Before field releases, *T. ostriniae* were reared for four generations on *O. nubilalis* eggs and subsequently mass-reared using *E. kuehniella* eggs following the methods of Morrison (1985). Approximately 500,000 *T. ostriniae* females were released in each field using cardboard release containers with parasitized *E. kuehniella* eggs inside (Wright et al. 2001). Release cartons were perforated to allow *T. ostriniae* emergence and were fastened to a wooden stake bearing a small (30×30cm) plywood roof to shelter the release cartons from the weather. One central release point per plot was used.

***Data collection*:** Sticky cards and egg mass sentinels were collected from each station and replaced at one to three day intervals for up to 10 days or the duration of an adult wasp life cycle. Sticky cards were examined for numbers of *T. ostriniae* using a stereoscopic zoom microscope with 400× magnification. Collected sentinels were removed from wax paper and placed in gelatin capsules (size 00), held at room temperature in the laboratory until eclosion or emergence of adult parasitoids (Kuhar et al. 2002). A characteristic blackening of the vitelline membranes of host eggs is manifested during *Trichogramma* prepupal and pupal stages (Flanders 1937), thus "black" eggs were considered parasitized.

***Analysis of dispersal and parasitism*.** The method of fitting recapture data to the diffusion model described in Rudd and Gandour (1985) was used to estimate the extent to which *T. ostriniae* dispersed within the four potato fields that were studied. The diffusion model for insects moving in 1-dimensional space, such as along a row of crops, is

$$\partial y / \partial t = \frac{D\partial^2 y}{\partial x^2} - my \qquad (1)$$

The model describes the change in the number of individuals, *y*, at a distance, *x* from a release point at time, *t*. The constant *D* is the diffusion coefficient (distance^2 per time for the 1-dimensional case), and *m* is a removal constant for individuals because of emigration and/or mortality. The solution to equation 1 is a 1-dimensional normal distribution of the form,

$$y(x,t) = y_0 \frac{e^{mt} e^{-(x^2/4Dt)}}{2\sqrt{\pi D t}} \qquad (2)$$

where y_0 is the starting population and the other parameters are as described above for equation 1. The variance of this function is simply 2*Dt*.

The use of the diffusion model in equations 1 and 2 assumes that individual insects move at random and show no preferential directional movement in any one direction from the point of release point, *x*. That is, the model assumes that there are no external forces (e.g., wind) acting to influence the movement of individuals and resulting in drift or displacement in their movement (Rudd and Gandour 1985, Turchin and Thoeny 1993, Blackmer et al. 2004, Bancroft 2005, Puche et al. 2005). In the event that there is drift, equation 2 can be modified to include this effect, so that,

$$y(x,t) = y_0 \frac{e^{mt} e^{-(x-vt)^2/4Dt)}}{2\sqrt{\pi Dt}} \qquad (3)$$

with *v*, a parameter of constant velocity that moves the center of the distribution (Rudd and Gandour 1985). Equation 3 can easily be converted to a 1-dimensional probability density function of the normal distribution with the effects of mortality/emigration and drift by the total area underneath the curve equal to 1. This function can then be used as redistribution kernel (*K*) that describes the distribution of dispersal distances for the organism in 1-dimension (Neubert et al. 1995, Brewster and Allen 1997).

Although the dispersal arenas studied are 2-dimensional, for simplicity, in all of the analysis that follows, only the 1-dimensional case was considered since it is well known that any transect through a 2-dimensional normal distribution will be a normal distribution in 1-dimension (Allen and Gonzalez 1974).

As a first step in estimating diffusion coefficients, *D*, for *T. ostriniae* sticky trap data were fitted using a least squares method to a model given in Rudd and Gandour (1985) that represents the parametric form of half of the normal distribution. The model,

$$y_i = Ae^{-Bx_i^2} \qquad (4)$$

describes the relationship between the numbers of individuals dispersing, y_i and distances x_i. The constants *A* and *B* are estimates of the number of individuals at the release point (*x* = 0) and the proportional reduction in the number of those individuals with distance, respectively. One can then use *B* to estimate, *D*, as follows (Rudd and Gandour 1985):

$$D = \frac{1}{4Bt} \qquad (5)$$

The variance of dispersal is simply 2*Dt*. In addition, the distance that encompasses 98% of the activity or entity being influenced by diffusion (Bancroft 2005) can be calculated as:

$$x_{98} = 2\sqrt{4Dt} \qquad (6)$$

Equation 14 in Rudd and Gandour (1985) to estimate the mortality/emigration parameter, *m,*

$$m = \frac{-\ln\left(2A\sqrt{\pi Dt} / y_0\right)}{t} \qquad (7)$$

and then used the estimated value of *m* in

$$y(t) = y_0^{-mt} \qquad (8)$$

to determine the population of *T. ostriniae*, y_t, left within the field at the time of sampling, *t*.

Following the above analysis, a 1-dimensional redistribution kernel (*K*) was derived for *T. ostriniae* dispersal at each time point within each of the four fields. Several methods are available for estimating the redistribution kernels from observed (Neubert et al. 1995). Using methods described in Kot et al. (1996) to develop dispersal kernels for *T. ostriniae* from the observed release-recapture data. After obtaining each curve of the relationship of *T. ostriniae* trap catches with distance using equation 4, the curve was mirrored about the origin and divided by the total area underneath the curve to generate the probability density function with an area equal to 1. All of the curve fitting for the analyses described above were done using TableCurve 5.01 (SYSTAT Software Inc., Richmond, CA).

With respect to parasitism, the data collected on parasitization of *E. kuehniella* eggs by *T. ostriniae was used* to determine whether there was a significant spatial correlation between the distribution of *T. ostriniae* trap catches and parasitism. This test was done using the two-sample Cramér-von Mises test

(Syrjala 1996), which has the advantage of being insensitive to differences in total values of the samples in each of the two distributions to be compared and requires only that the samples for the two distributions are collected at approximately the same locations, as was done in the case of *T. ostriniae* sticky trap catches and parasitization of *O. nubilalis*. The null hypothesis for the two-sample Cramér-von Mises test is that there is no statistical difference between the distributions of *T. ostriniae* sticky trap catches and the distribution of parasitization of *O. nubilalis* in a field. The alternate hypothesis is that there is some unspecified difference between the pairwise distributions.

The two-sample Cramér-von Mises test is carried out by superimposing a Cartesian coordinate (X, Y) spatial grid over the two data sets. Data for each population is then normalized by dividing each observation by the sum of all of the observations. Starting at each corner on the spatial grid, four cumulative distribution functions are constructed from the normalized data for each of the data sets. A separate test statistic is calculated for each of the four cumulative distribution functions by taking the squared difference between the respective cumulative distribution functions for the two populations. An overall test statistic for the two original distributions (Ψ_1) is then calculated as the average of the four test statistics. Following this, 999 pseudo-random permutations of the data for the two populations are examined where for each permutation one of the observations from corresponding locations in the two spatial distributions is assigned randomly to the first population and the other to the second population. A new test statistic, Ψ_n (n = 1…999) is calculated after each permutation. The significance level (*P*-value) for the comparison is the proportion of the 1000 test statistics ($\Psi_n + \Psi_1$) that are $\geq \Psi_1$. The Cramér-von Mises analysis was carried out using a program written for MATLAB (Mathworks, Natwick, MA).

Results

2005 Study

Sentinels and sticky cards placed out before *T. ostriniae* releases in the two field detected no evidence of background activity for *Trichogramma* in the study area. The relationships between the number of sticky card captures and distance from the release point at different days after release are shown in Fig. 1.2A and Fig. 1.3A for Fields 1 and 2, respectively.   The fit of the sticky card data to the model in equation 4 was exceptionally good (r^2 > 0.90) for all except the data collected in Field 2 at 10 days after the release was made (r^2 = 0.78; Table 1).

The diffusion coefficients for the sticky card data on each of the sampling dates for the two fields are also shown in Table 1.1. As can be seen, *D* was relatively high at 1 day after release (67.31m/day), decreased thereafter to a mean (± S.E.) of 7.78 (± 0.84) m/day between days 4−8, and increased at 10 days after release.   One day after release in Field 1, *Trichogramma* were recaptured on sticky cards at 16, 23 and 32 meters from the release point. Within 4 days post release, wasps traveled up to 45m and persisted at this distance through the eighth day of sampling.

Despite the differences in *D* with sampling time the distance from the release point that encompassed 98% of the *T. ostriniae* females recaptured (x_{98}) was fairly similar for the different sampling dates, except again in Field 2 at 10 days after release (Tables 1.1). Mean x_{98} (± S.E.) across both fields on all sampling dates, except day 10 in Field 2, was 27.53 (± 2.39) m. The mean value for x_{98} is reflected in the shape of the redistribution curves shown in Fig. 1.2B and Fig. 1.3B for Fields 1 and 2, respectively that flattens out at the tail near the mean x_{98} distance.

The mean proportion of parasitized sentinels was 100% at the release point on all sampling dates after release in Field 1 and Field 2, except on day 10 in Field 2 (Fig. 1.4). On that date, no parasitism was observed at any distance. The mean proportion of parasitized sentinels was 10% at 16 and 23m from the central release point. In both fields, parasitization peaked at 4 days post release with close to 40% sentinels parasitized at 30 m from the release point. Using analysis similar to that carried out on the sticky card data estimates the distance from the release point that encompassed 98% of the parasitism (x_{98}) were approximately 21 m and 26 m for Field 1 and Field 2, respectively.

The results of the two-sample Cramér-von Mises analysis indicated that there was no statistically significant difference ($P > 0.05$) in the distribution of sticky card captures of *T. ostriniae* and the distribution of parasitism of *O. nubilalis* sentinel egg masses (Table 1.3).

2006 Study

Sentinels and sticky cards placed out before *T. ostriniae* releases in the two field detected no evidence of background activity for *Trichogramma* in the study area. The relationships between the number of sticky card captures and distance from the release point at different days after release are shown in Fig. 1.5A and Fig. 1.6A for Fields 1 and 2, respectively. The fit of the sticky card data to the model in equation 4 was reasonably good ($r^2 \geq 0.77$) in most cases except for the data collected in Field 1 at day 6 ($r^2 = 0.03$) and in Field 2 at 1 day 1 ($r^2 = 0.44$) after release of *T. ostriniae*, respectively (Table 1.2). Because of the poor fit to the model no useful assessment could be made of the diffusion coefficient *D* that may be calculated for these sampling dates.

For those sampling dates where the diffusion coefficient for the sticky card data was it was found to be much lower than those estimated for the study conducted in 2005. The highest level achieved was at 1 day after release in Field 1 (12.391m/day). For all the other sampling dates, the diffusion coefficient, *D,* was < 3 m/day. As such the mean (± S.E.) of D in the 2006 study, excluding dates when the fit to equation 4 was poor was 4.15 (± 2.07). Nevertheless, as was observed for the 2005 study, the distance from the release point that encompassed 98% of the *T. ostriniae* females recaptured (x_{98}) was fairly similar for the different sampling data for which equation 14 could be fitted (Table 1.2). Mean x_{98} (± S.E.) across both fields on all sampling dates was 12.88 (± 0.94) m. As expected, this value is much lower than was obtained for the 2005 study. However, again the mean value for x_{98} is reflected in the shape of the redistribution curves shown in Fig. 1.5B and Fig. 1.6B for Fields 1 and 2, respectively, that flattens out at the tail near the mean distance.

The mean proportion of parasitized sentinels was 100% at the release point on all sampling dates after release in Field 1 and Field 2, except on day 6 in Field 1 (Fig. 1.7). On that date, no parasitism was observed at any distance. In both fields, parasitization was still relatively high at 3 days post release with just over 40% of sentinels parasitized at 7 m from the release point. Estimates of the distance from the release point that encompassed 98% of the parasitism (x_{98}) were approximately 8 m and 10 m for Field 1 and Field 2, respectively. No two-sample Cramér-von Mises analysis was done to compare the distribution of sticky card captures of *T. ostriniae* and the distribution of parasitism of *Ostrinia nubilalis* sentinel egg masses.

Combining field data for each year, the relationship of distance to sticky card captures and sentinel egg mass parasitism was negative for both years. The regressions of distance to egg mass parasitism had very similar slopes and intercepts for both years (2005, $y = -0.2776 \ln(x) + 1.5290$; 2006, $y = -0.2608 \ln(x) + 1.3779$), showing similar dispersal behavior after releases in 2005 and 2006. There was no significant interaction between distance and time for all fields ($P > 0.05$).

Discussion

The results of this study show positive dispersal behavior characteristics for *T. ostriniae* in potatoes. In keeping with similar results from Wright et al. (2001) in sweet corn, we found *Trichogramma* dispersed rapidly over large distances in commercial potato fields. Despite the preference of *T. ostriniae* for corn over dicotyledonous plants (Kuhar et al. 2004), wasps successfully moved throughout a .4 ha area and reproduced within 45m of a central release point. Within 4 days in 2005 20% parasitism was observed at 45m and 33% within 1 day in 2006. Although high levels of parasitism were recorded at this distance, parasitism greatly decreased with increasing distance and thus searching area.

In the 2005 study the mean (± S.E.) distance in which 98% of the trapped parasitoids were found was 27.532 (± 2.39) m. This meant that the majority of parasitoids were trapped in an area equivalent to approximately 18% of the entire 4096 m^2 (64 m x 64 m) study area. This value was lower in the 2006 study. Considering the size of the sampling area in addition to plant structure, the surface area for a wasp less than 1mm in length was quite sizable. In field 1 2005, an estimated 2891 (~ 0.6% wasps of the original 500,000 released) remained in the field study area four days after emergence. All fields showed a dramatic decrease in the number of wasps (y_t) remaining in the sampling area after emergence. Despite low y_t values for each field, the *T. ostriniae* recovered were normally distributed with 20-80% parasitization recorded at ≥32 meters from the release point in 2005. The number of wasps remaining in the field is estimated from sticky card captures and does not reflect the number of wasps that may actually be present. This suggests that the decrease in parasitism with distance may not be the result of a lack of *T. ostriniae* presence, but perhaps the inability of *T. ostriniae* to locate sentinel egg masses in a much larger field area.

Boo and Yang (2000) proposed that *T. ostriniae* may orient to kairomone plumes from host moth scales and fly upwind in that direction. In addition *Trichogramma* are known to disperse either on their own or through phoresy on the host moth although this is largely undocumented (Smith 1996). ECB pressure was low in both years of the present study and the majority of wasps probably dispersed on their own. As *T. ostriniae* dispersed further away from the central release, host egg density decreased. Wajnberg (2003) found a significant increase in patch leaving tendency for *Trichogramma* that successfully oviposit in a host and/or reject a previously attacked host. The low abundance of native ECB egg masses and the low density of sentinel egg masses at greater distances may have increased localized searching or patch residence time of *T.ostriniae* and decreased their ability to find sentinel hosts. A possible benefit from decreased parasitism at larger distances is increased host selectivity. The highest rates of parasitism contained within the release site might tend to minimize non-target effects (Orr et al. 2000).

Weather conditions caused by wind and rain can affect the movement of parasitoids and their subsequent levels of parasitism. Weather data was not collected at the release sites, but was available from an airport weather station approximately 30 miles away. Numerous studies have shown parasitism significantly decreases in the upwind direction (Greatti 1995, Hsiao 1981, Smith 1988, Fournier 2000). Based on the location of our study sites in large open commercial fields and the trend in parasitism, it is likely that wind may have played an important role in *T. ostriniae* movement or influenced the substantial decrease in the number of wasps remaining in the field after sampling time t. However, this effect is not substantiated by the analysis. The fit of the data to equation 3 showed that the effect of drift (as measured by the parameter *v*) was negligible in describing the dispersal of the parasitoid.

In a study of attack by *T. pretiosum* on eggs of the cabbage looper, *Trichoplusia ni*, Allen and Gonzalez (1974) implied that the spatial pattern of attack could be used to infer the dispersal pattern of the parasitoid. The current study provides some evidence to support this position by Allen and Gonzalez (1974). The two-sample Cramér-von Mises analyses of the distribution of sticky card captures of *T. ostriniae* and the distribution of parasitism of *O. nubilalis* sentinel egg masses found no statistically significant difference between the pairwise distribution (Table 3). This meant that the two distributions were highly correlated in all of the cases examined so that high trap catches were usually obtained at locations where there were high levels of parasitism, and vice versa. As such, the spatial distribution of attack of *T. ostriniae* on *O. nubilalis* eggs could have been used to study the dispersal pattern of the parasitoid.

Some of the analysis done in this study also gives us a way to predict the distribution of the parasitoid some time after a release. This can be done using the method described in Brewster and Allen (1997) that requires information on the initial number of parasitoids at time, *t*, and the redistribution kernel (*K*) for the insect at time *t*. These two pieces of information can be used to develop an integrodifference equation model (Neubert et al. 1995, Kot et al. 1996, Brewster and Allen 1997),

$$Y_{t+1}(x) = \sum_{u=1}^{n} K(x-u) Y_t(u) \qquad (9)$$

to estimate the number of parasitoids at each location in a 1-dimensional spatial system at time $t+1$. In equation 9, Y_t and Y_{t+1} are the populations of parasitoid at time t and $t+1$, respectively, and $K(x-u)$ is the dispersal or redistribution kernel seen in Figs. 1.2B, 1.3B, 1.5, and 1.6B that describes the probability density of individuals moving from point *u* to *x* in the spatial system. The model for 2-dimensional dispersal is the logical extension to equation 9 (e.g., Brewster and Allen 1997). Population simulations with integrodifference equations can be done using the method described in Allen et al. (2001).

When applying our results for augmentive control of ECB, it is important to consider dispersal and parasitism in conjunction with ECB suppression. Inundative releases rely on rapid dispersal and uniform coverage of the target area. In 2005 and 2006 the greatest number of wasps recaptured occurred from 3-4 days after emergence. Timing or staggering releases could ensure that high numbers of *T. ostriniae* and thus high levels of parasitism persist during times of pest pressure. Inclement weather may also be a consideration for the timing and frequency of releases. In 2005 and 2006 parasitism of sentinel egg masses was not observed on sampling dates following inclement weather. Either through mortality or emigration, rainy conditions appeared to detrimentally affect parasitism. Averaging x_{98} values for all four fields, 98% of all wasps were captured within ~ 17m of the central release. Using this distance as a radius, the area encompassed by dispersing *T. ostrinae* equals ~ 0.1 ha More releases per ha, possibly 4 per 0.4 ha (or 1 acre), as well as multiple releases over the season would be needed for effective biological control of ECB in pepper.

Works Cited

Allen, J. C., and D. Gonzalez. 1974. Spatial attack pattern of *Trichogramma pretiosum* around release sites compared with a random diffusion model. Enviorn. Entomol. 3: 647-652.

Allen, J. C., C. C. Brewster, and D. H. Slone. 2001. Spatially explixit ecological models: a spatial convolution approach. Chaos, Solitons, Fractals 12: 333-347.

Boo, K. S., and J. P. Yang. 2000. Kairomones used by *Trichogramma chilonis* to find *Helicoverpa assulta* eggs. J. Chem Ecol. 26, 359-375.

Bancroft, J. S. 2005. Dispersal and abundance of *Lygus hesperus* in field crops. Environ. Entomol. 34: 1517-1523.

Blackmer, J. L., J. R. Hagler, G. S. Simmons, and L. A. Canas. 2004. Comparative dispersal of *Homalodisca coagulate* and *Homalodisca liturata* (Homoptera: Cicadellidae). Environ. Entomol. 33: 88-99.

Brewster, C. C. and J. C. Allen. 1997. Spatiotemporal model for studying insect dynamics in large-scale cropping systems. Enviorn. Entomol. 26: 473- 482.

Capinera, J. L. 2001. Handbook of vegetable pests. Academic, New York.

Flanders, S. E. 1937. Notes on the life history and anatomy of *Trichogramma*. Ann. Entomol. Soc. Am. 30: 304-308

Fournier, F. and G. Boivin. 2000. Comparative dispersal of Trichogramma evanescens and T. pretiosum (Hymenoptera: Trichogrammatidae) in relation to environmental conditions. Environmental Entomology 29: 55–63.

Greatti, M. and P. Zandigiacomo. 1995. Postrelease dispersal of Trichogramma brassicae Bezdenko in corn fields. Journal of Applied Entomology 119: 671–675.

Hassan, S. A. and M. F. Guo. 1991. Selection of effective strains of egg parasites of the genus *Trichogramma* (Hym., Trichogrammatidae) to control the European corn borer *Ostrinia nubilalis* Hb. (Lep., Pyralidae). J. Appl. Entomol. 111: 335-341.

Hoffmann, M. P., D. L. Walker, and A. M. Shelton. 1995. Biology of *Trichogramma ostriniae* (Hym: Trichogrammatidae) reared on *Ostrinia nubilalis* (Lep: Pyralidae) and survey for additional hosts. *Entomophaga* 40, 387-402.

Hoffmann, M. P., M. G. Wright, S. A. Pitcher and J. Gardner. 2002. Innoculative releases of *Trichogramma ostriniae* for suppression of *Ostrinia nubilalis* (European corn borer) in sweet corn: field biology and population dynamics. Biol. Control 25: 249-258.

Hsiao, K. J., 1981. The use of biological control agents for the control of the pine defoliator, *Dendrolimus punctatus* (Lep. Lasiocampidae) in China. Prot. Ecol. 2: 297-303.

Neubert, M. G., M. Kot, and M. A. Lewis. 1995. Dispersal and pattern formation in a discrete-time predator-prey model. Theor. Pop. Biol. 48: 7-43

Kot, M., M. A. Lewis, and P. van den Driessche. 1996. Dispersal data and the spread of invading organisms. Ecology 77: 2027-2042.

Kuhar, T. P., M. G. Wright, M. P. Hoffmann, and S. A. Chenus. 2002. Life table studies of European corn borer (Lepidoptera: Crambidae) with and without inoculative releases of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae). Environ. Entomol. 31:482-489.

Kuhar, T. P., M. P. Hoffmann, and M. G. Wright. 2003. Controlling European corn borer in vegetables with a parasitic wasp. Pesticide Outlook 14: 99-101.

Kuhar, T. P., Barlow, V. M., M. P. Hoffmann, S. J. Fleischer, E. A. Groden, J. Gardner, R. Hazzard, M. G. Wright, S. A. Pitcher, and P. Westgate. 2004. Potential of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) as a biological control agent of European corn borer (Lepidoptera: Crambidae) in solanaceous crops. J. Econ. Entomol. 97: 1209-1216.

Morrison, R. K. 1985. Mass production of *Trichogramma pretiosum* Riley. Southwest. Entomol. 8 (Suppl.): 28-35.

Nault, B. A., N. M. French II, and G. G. Kennedy. 1996. Influence of European corn borer (Lepidoptera: Pyralidae) damage to potato and foliage availability on overwintering survival of first generation Colorado potato beetle adults

(Coleopteran: Chrysomelidae) in North Carolina. J. Econ. Entomol. 89: 124-130.

Orr, D. B., C. Garcia-Salazar, and D. A. Landis. 2000. *Trichogramma* nontarget impacts: A method for biological control risk assessment. In: Follett, P.A., and Duan, J. J. (eds.) Nontarget Effects of Biological Control. Kluwer Academic Publishers, Boston, MA.

Puche, H. T.J. Weissling, R. Schnell, N. D. Epsky, and R. R. Heath. 2005. Estimating dispersal rate of the silky cane weevil (Coleoptera: Curculionidae). JEN 129: 293-299.

Rudd, W. G. and R. W. Gandour. 1985. Diffusion model for insect dispersal. J. Econ. Entomol. 78: 295-301.

Smith, S. M. 1988. Pattern of attack on spruce budworm egg masses by *Trichogramma minutum* (Hym: Trichogrammatidae) released in forest stands. Environ Entomol. 17: 1009-1015.

Taylor, R. A. J. 1978. The relationship between density and distance of dispersing insects. Ecol. Entomol. 3: 63-70.

Turchin, P., and W. T. Thoeny. 1993. Quantifying dispersal of southern pine beetles with mark-recapture experiments and a diffusion model. Ecol. Appl. 3:187-198.

Wajnberg, E., P. A. Gonsard, E. Tabone, C. Curty, N. Lezcano, and S. Colazza. 2003. A comparative analysis of patch-leaving decision rules in a parasitoid family. Journal of Animal Ecology 72: 618–626.

Wang, B., D. N. Ferro, and D. W. Hosmer. 1999. Effectivness of *Trichogramma osrtiniae* (Hymenoptera: Trichogrammatidae) in sweet corn fields: implications for augmentative releases against *Ostrinia nubilalis* (Lepidoptera: Crambidae). Biol. Control 22: 29-37.

Welty, C. 1995. Monitoring and control of European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae), on bell peppers in Ohio. J. Agric. Entomo1. 2:145-161.

Wright, M. G., M. P. Hoffman, S. A. Chenus, and J. Gardner. 2001. Dispersal behavior of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) in sweet corn fields: Implications for augmentive releases against *Ostrinia nubilalis* (Lepidoptera: Crambidae). *Biol. Control* 22, 29-37.

Wright, M. G., T. P. Kuhar and M. P. Hoffmann. 2002. Effect of innoculative releases of *Trichogramma ostriniae* on populations of *Ostrinia nubilalis* and damage to sweet corn and field corn. Biol. Control. 23: 149-155.

Wright, M. G., M. P. Hoffmann, T. P. Kuhar, J. Gardner, and S. A. Pitcher. 2005. Evaluating risks of biological control introductions: a probalistic risk-assessment approach. Biol. Contr. 35: 338-347.

Table 1.1 Variables and coefficients determined by fitting *Trichogramma ostriniae* release-recapture data collected in 2005 to a diffusion model.

Field	t^a	A^b	B^b	r^2	D (m²/day) [c]	x_{98} (m) [d]	m^e	Y_t [f]
1	1	29.0780	0.003714	0.98	67.31	32.82	6.3822	846
	4	152.9375	0.00879	0.99	7.11	21.33	1.2882	2891
	8	48.8095	0.00357	0.95	8.75	33.47	0.7306	1447
	Cumulative	231.2914	0.00619	0.98	5.05	25.42	0.5705	5210
2	4	201.6155	0.006588	0.93	9.49	24.64	1.1831	4402
	7	18.9083	0.006198	0.98	5.76	25.40	1.0098	426
	10	1.8759	0.001409	0.78	17.74	53.28	0.8639	89
	Cumulative	231.8831	0.009474	0.96	2.64	20.54	0.4774	4223

[a] Days after release of *T. ostriniae*; cumulative represents analysis done on the total number of parasitoids recaptured over all sampling days.

[b] Equation 4 in text; from Rudd and Gandour (1985)

[c] Equation 5 in text; from Rudd and Gandour (1985)

[d] Equation 6 in text; from Bancroft (2005)

[e] Equation 7 in text; from Rudd and Gandour (1985)

[f] Equation 8 in text; from Rudd and Gandour (1985)

Table 1.2 Variables and coefficients determined by fitting *Trichogramma ostriniae* release-recapture data collected in 2006 to a diffusion model.

Field	t^a	A^b	B^b	r^2	D (m^2/day)^c	x_{98} (m)^d	m^e	Y_t^{f}
1	1	108.8268	0.02018	0.92	12.39	14.08	5.9087	1358
	3	134.5224	0.03287	0.90	2.54	11.03	1.9802	1315
	6	1.3679	**-0.00020**	**0.03**	--	--	--	--
	Cumulative	243.2545	0.02528	0.90	1.65	12.58	0.8695	2712
2	1	5.8448	0.00154	**0.44**	--	--	--	--
	3	54.2935	0.03838	0.95	2.1714	10.21	1.5341	5015
	6	64.2217	0.02001	0.87	2.0752	14.14	1.0723	803
	9	17.9958	0.01791	0.77	1.5507	14.94	0.8499	238
	Cumulative	142.6592	0.02362	0.88	1.1759	13.01	0.6352	1645

[a] Days after release of *T. ostriniae*; cumulative represents analysis done on the total number of parasitoids recaptured over all sampling days.

[b] Equation 4 in text; from Rudd and Gandour (1985)

[c] Equation 5 in text; from Rudd and Gandour (1985)

[d] Equation 6 in text; from Bancroft (2005)

[e] Equation 7 in text; from Rudd and Gandour (1985)

[f] Equation 8 in text; from Rudd and Gandour (1985)

Table1.3 Results of Cramer von Mises two-sample analysis of *Trichogramma ostriniae* release-recapture data and parasitism of *Ostrinia nubilalis* egg masses.

Year	Field	t^a	Ψ_1	***P-value***
2005	1	1	0.0102	0.1990
		4	0.1630	0.5250
		8	0.0327	0.1260
		Overall	0.0076	0.2510
	2	4	0.3172	0.2140
		7	0.0032	0.2770
		10	--	--
		Overall	0.0073	0.2330
2006	1	1		
		3		
		6		
		Overall		
	2	1		
		3		
		6		
		9		
		Overall		

[a] Numbers are days after release of *T. ostriniae*; overall represents per-sampling day for the parasitoid density and average level of parasitism.

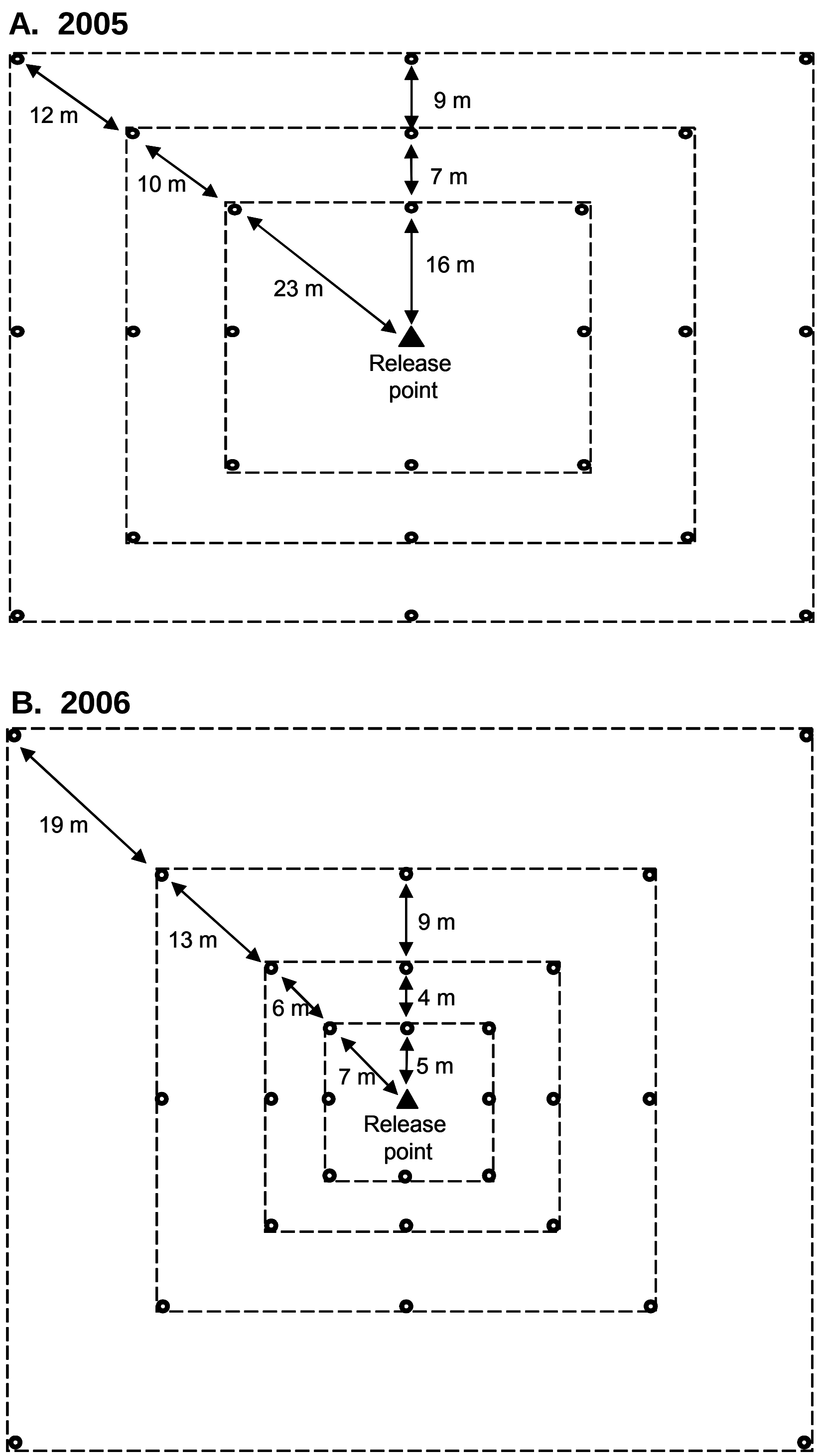

Fig. 1.1 Field plot design for *Trichogramma ostriniae* dispersal and parasitism study conducted in commercial potatoes in Virginia in 2005 and 2006. Each symbol represents the location where a sticky card was placed on a tomato stake and sentinel egg masses of *Ostrinia nubilalis* were fastened to potato leaves.

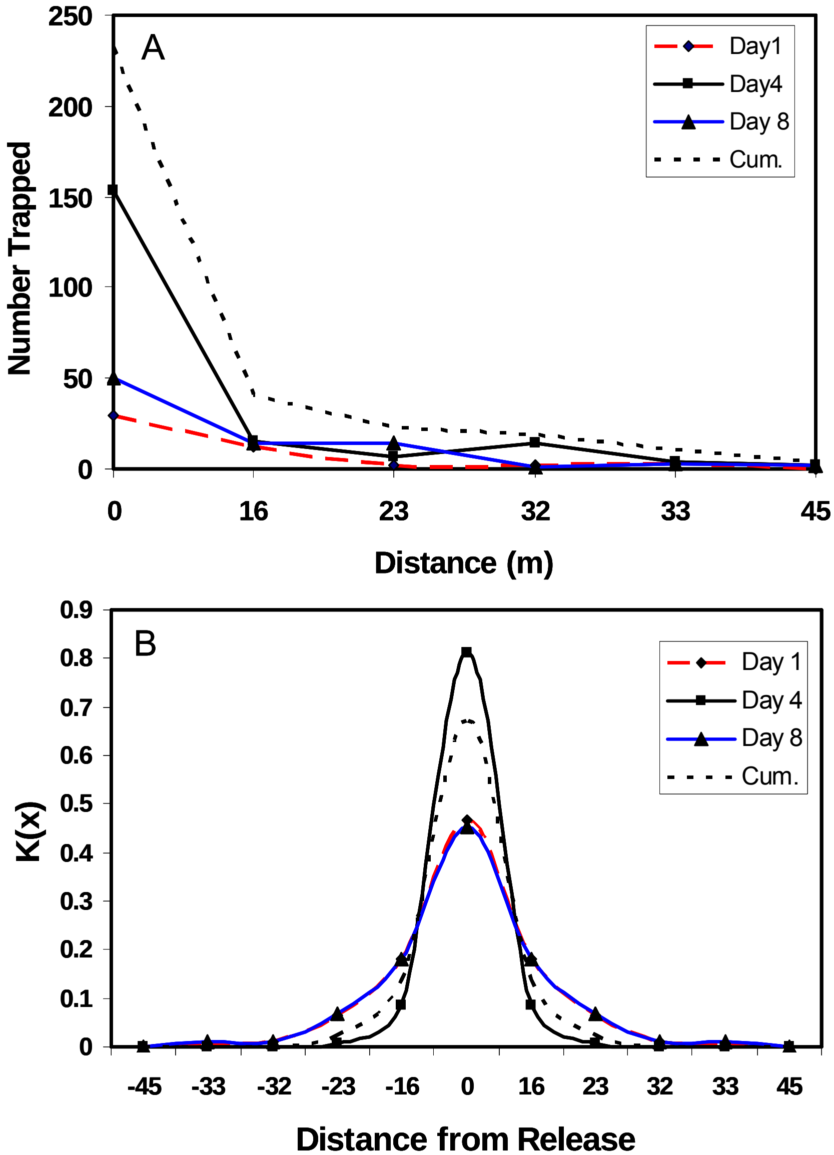

Fig. 1.2 *Trichogramma ostriniae* dispersal in Field 1 in 2005. (A) Total recapture-with-distance curves; (B) Corresponding redistribution kernels for each of the respective dispersal curves in A based on the diffusion model.

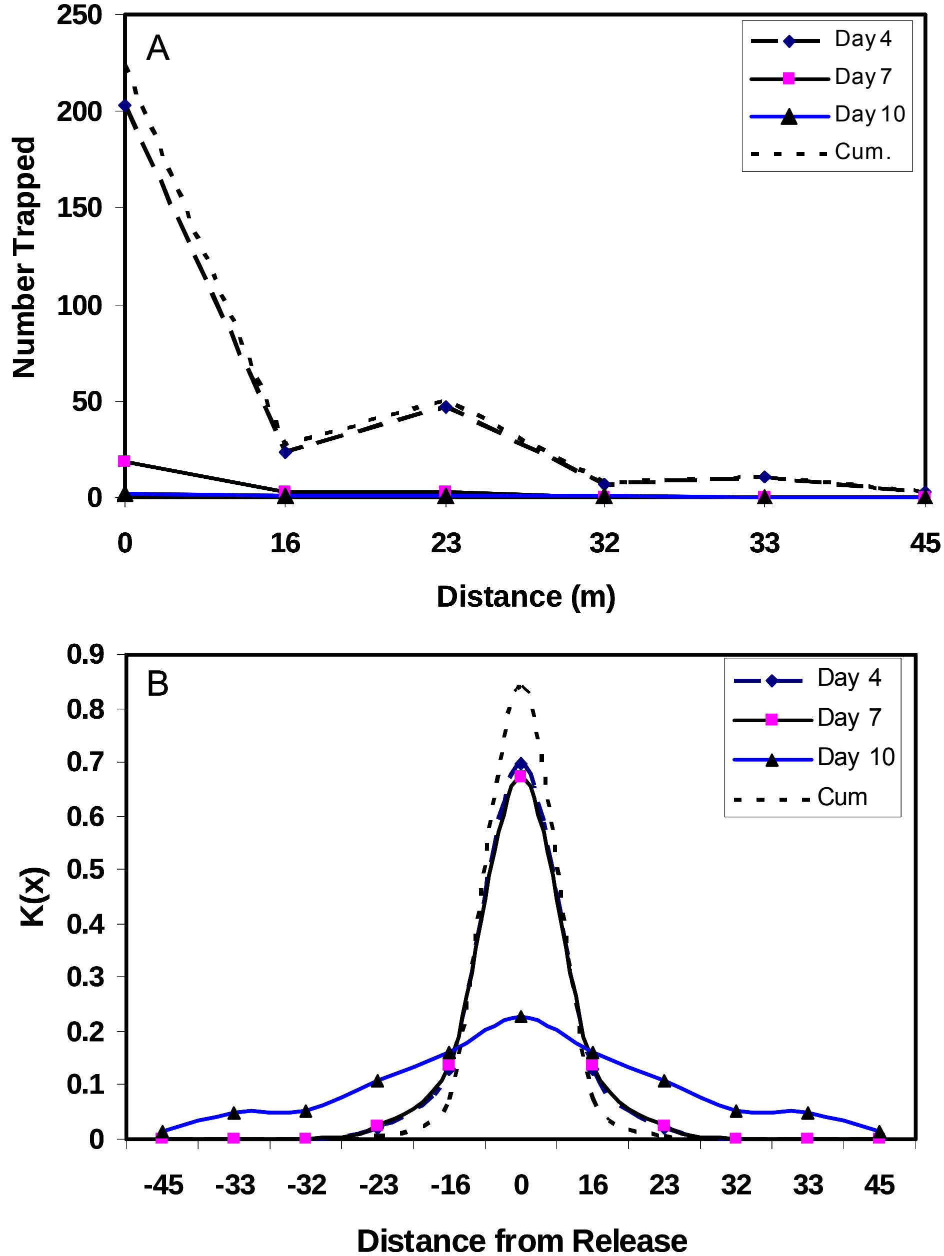

Fig. 1.3 *Trichogramma ostriniae* dispersal in Field 2 in 2005. (A) Total recapture-with-distance curves; (B) Corresponding redistribution kernels for each of the respective dispersal curves in A based on the diffusion model.

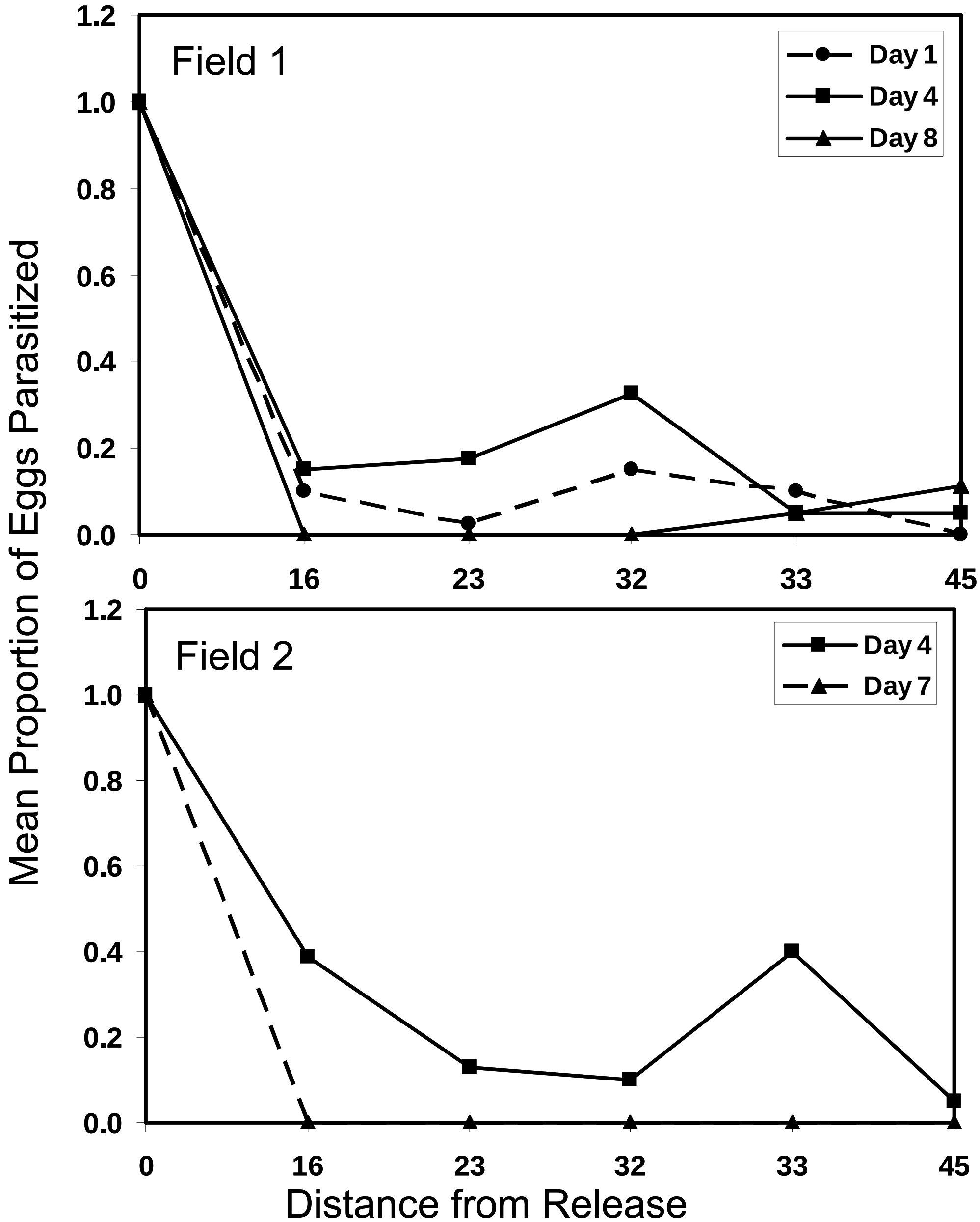

Fig. 1.4 Relationship of mean proportion of *Ostrinia nubilalis* egg masses parasitized and distance from release point on different sampling dates after release of *Trichogramma ostriniae*. Estimates of x_{98} were 20.80 and 25.67 m for Fields 1 and 2, respectively.

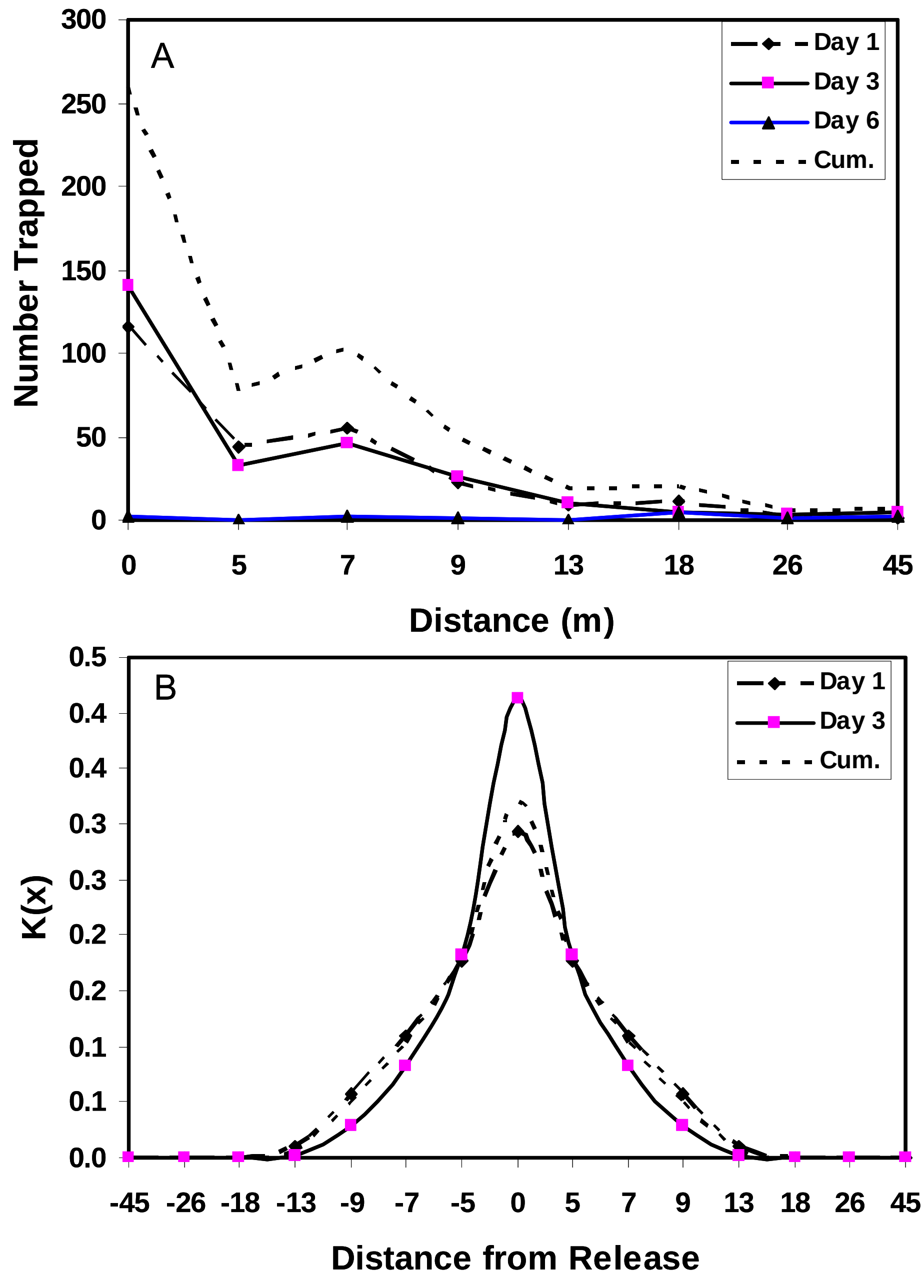

Fig. 1.5 *Trichogramma ostriniae* dispersal in Field 1 in 2006. (A) Total recapture-with-distance curves; (B) Corresponding redistribution kernels for each of the respective dispersal curves in A based on the diffusion model.

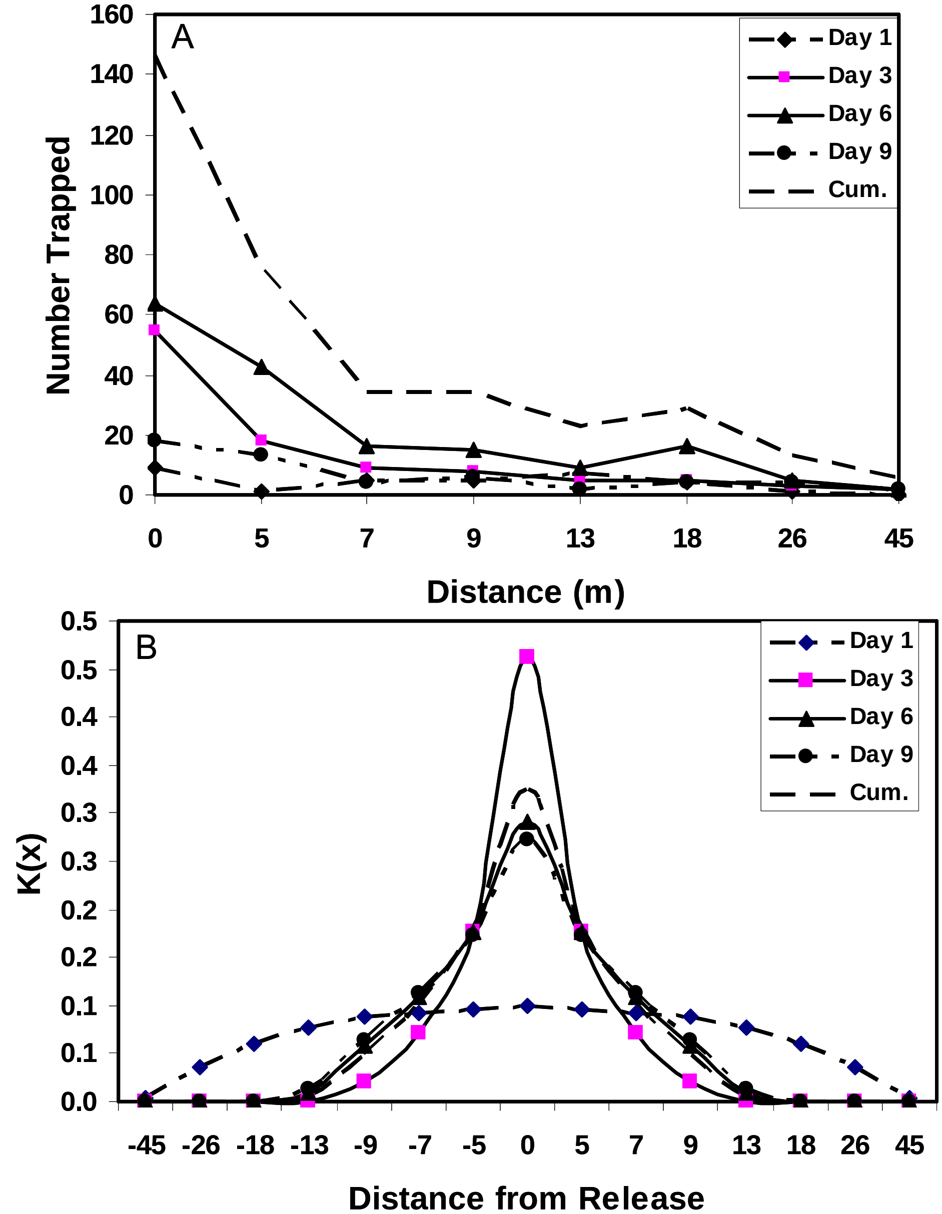

Fig. 1.6 *Trichogramma ostriniae* dispersal in Field 2 in 2006. (A) Total recapture-with-distance curves; (B) Corresponding redistribution kernels for each of the respective dispersal curves in A based on the diffusion model.

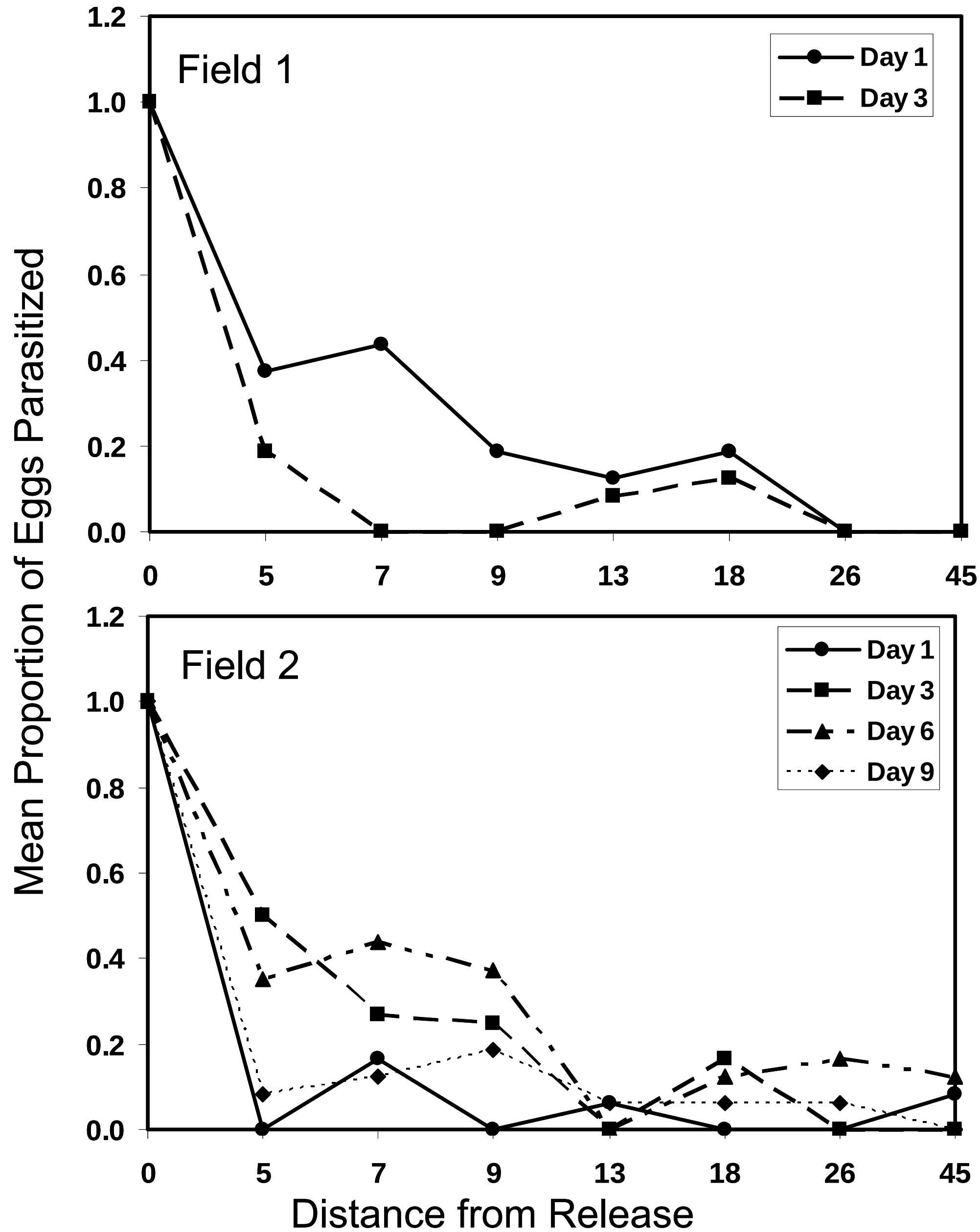

Fig. 1.7 Relationship of mean proportion of *Ostrinia nubilalis* egg masses parasitized and distance from release point on different sampling dates after release of *Trichogramma ostriniae*. Estimates of x_{98} were 8.35 and 10.24 m for Fields 1 and 2, respectively.

Chapter 2

Determining the optimal number of *Trichogramma ostriniae* to release per unit area in bell peppers for effective biological control

Introduction:

Developing effective release strategies for *Trichogramma* egg parasitoids is important to their success in biological control programs (Smith 1996). Knowledge of the proper release rate or density is one of the most important variables for devising effective release strategies. Smith (1996) suggested that ~80% parasitization is needed for effective biological control by *Trichogramma* species.

Trichogramma ostriniae, a native parasitoid of the Asian corn borer in China, was introduced to the U.S. in the early 1990s, and has shown success as a biological control agent of European corn borer, *Ostrinia nubilalis* Hübner (ECB). In sweet corn in Massachusetts, Wang et al. (1999) observed >40% parasitism of ECB eggs using four releases of ≈34,000 *T. ostriniae* per ha. In New York, Wright et al.(2002) demonstrated that a single inoculative release of *T. ostriniae* (≈70,000 per ha) could provide season-long parasitism of ECB eggs and reduce ear damage by 50%.

In bell peppers in the Mid-Atlantic states, four to five separate inundative releases of 30,000-50,000 *T. ostriniae* per 0.02 ha (~10 *T. ostriniae*/plant) resulted in ECB egg parasitization levels of 48.7% and fruit damage was reduced almost 70% (Kuhar et al. 2004). The objective of this study was to determine the optimal number of *T. ostriniae* to release per unit area for effective biological control of ECB in pepper. The aim is to determine what release rate achieves >80% parasitism of egg masses in the field.

Materials and Methods:

Experiments were conducted in summer 2006 in plots of bell peppers at four locations: Virginia Tech Eastern Shore Agricultural Research and Extension Center (AREC), Painter, VA; Virginia Tech Hampton Roads AREC, Virginia Beach, VA; University of Maryland Wye REC, Queenstown, MD; and the Russel E. Larson Research Farm, Rock Springs, PA. Each location represented one replicate in a randomized complete block experiment (n = 4). Four spatially-isolated plots of peppers (each ~0.03 ha and ~500 plants) were established at each location representing four separate *T.ostriniae* release densities: 0, 5, 20, and 50 wasps per plant. Individual plots were approximately 10 rows × 50 ft and spaced at least 500-ft apart in the field to minimize contamination by other plots.

To assess egg parasitism by *T. ostriniae* in the plots, sentinel egg masses were used due to the below average pressure from ECB in 2006 for the Mid-Atlantic region and the difficulty in finding natural *O. nubilalis* egg masses in peppers. Logistical problems in getting fresh *O. nubilalis* egg masses to each experiment location required use of an alternative host to *O. nubilalis* egg masses for sentinels. *Ephestia kuehniella* Zeller eggs are readily parasitized by *T. ostriniae* (Hoffmann et al., 1995) and are used to maintain colonies of *Trichogramma* commercially (Beneficial Insectary, Redding, CA). They can be sterilized, cold-stored and shipped commercially and were used as our alternative for this experiment. Sentinel egg masses were prepared using yellow sticky cards covered in sterilized *E. kuehniella* eggs. Sticky cards were cut into 1 square inch sections each containing hundreds of *E. kuehniella* eggs. Thirty individual sentinels were glued on wax paper and pinned to the lower leaf surfaces of 30 random plants within each plot. After three days, sentinels were collected from plants, placed in Petri dishes, and observed in the lab for parasitism. Sentinels were replaced at 3 days and collected again at 6 days post-release. Parasitization was recorded as the proportion of sentinels parasitized. If any number of the *Ephestia* eggs on the sentinel were parasitized, the entire sentinel was counted. The assumption was that if a *T. ostriniae* female found the sentinel strip and parasitized an egg, she would have also parasitized an *O. nubilalis* egg mass if it was present.

Data from the four locations were compiled for statistical analysis. The mean proportion of parasitized sentinels was reported for each location in the experiment.

Statistical significance between means was assessed using ANOVA (Statistix 8.0 Copyright (C) 1985-2003 Analytical Software).

Results:

At the two Virginia locations, we observed relatively low rates of parasitization and no significant effect from release density (Fig 2.1, 2.2). In Pennsylvania, the highest release density of 50wasps/plant resulted in a significantly greater mean proportion of parasitized sentinels than the 5wasps/plant and control plots at 3 days post release (Fig 2.3). The Maryland location overall had the highest rates of parasitization however statistically insignificant (Fig 2.4). Non-release control plots in Maryland and the Eastern Shore of Virginia had as much parasitism as plots receiving releases of *T. ostriniae*. Overall, results showed ambiguity in the data and high levels of natural parasitism occurring on *Ephestia* eggs at one of the locations.

Discussion:

It is important to address a number of quality control problems encountered during field studies in the experiment. Substituting *Ephestia* eggs for ECB for sentinel egg masses largely compromised results. *Ephestia* eggs were especially susceptible to loss from predation and inclement weather conditions. Often we observed ants and lacewing larvae actively feeding on *Ephestia* eggs on sentinels. Also, the sticky cards used to construct sentinels lost adhesive quality in rainy conditions and many sentinels were washed away with heavy precipitation. Sentinels that were recovered showed high levels of background parasitism on *Ephestia* as seen in the control plots (Fig 2.2, 2.4). In contrast, Kuhar et al. (2004) found that little to no natural egg parasitism (mean of 1.9 ±1.6% across eight fields) typically occurs on *O. nubilalis* egg masses in peppers in Virginia. Therefore, despite success in using *E. kuehniella* eggs for mass rearing *T. ostriniae*, their use for detecting and quantifying *T. ostriniae* parasitization levels in the field was not successful. However, it is worth noting that *E. kuehniella* egg could be considered for use in sentinels aiming to detect generalist natural enemies or perhaps, as an alternative food source to augment the presence of natural enemies. Given their susceptibility to parasitism and natural predation, *Ephestia* egg sentinels could be used as an indicator for natural enemies monitoring their distribution and possibly relative density within a field.

In addition to problems with sentinels, we also encountered quality control issue with shipments of *T. ostriniae* used in the experiment. The parasitoids arrived to each release site via overnight FedEx and on a number of occasions with wasps already emerging. Parasitoids were exposed to extremes in temperature during shipping which directly effected their development and disrupted the programming of emergence (Smith 1996). We explored methods such as ice packs included with shipments and obtaining younger parasitized eggs to try and prevent early emergence. However, these methods proved insufficient resulting in inaccuracies in the actual number of wasps released in each plot and thus the release densities.

A number of the problems encountered in this experiment can be addressed with suggestions for future improvements. Use of the target host ECB for sentinel egg masses was not possible in this experiment but would have been preferred. ECB is the preferred host of *T. ostriniae* and subject to little natural parasitism. Maintaining a colony of locally collected target pest species could not only provide a fresh supply of eggs for sentinels but might also alleviate logistical problems with interstate transport. Natural predation and parasitism could be alleviated with the use of mesh screens to exclude natural enemies other than *T. ostriniae*. Fournier (2000) used *E. kuehniella* eggs for sentinels to detect presence of *T. evanescens* and protected the eggs from predation using a 0.75mm mesh screen

Sentinel construction, specifically adhesives, is another key element to a more successful experimental design. In previous field studies we found that egg masses deposied by moths on wax paper and then glued to an additional piece of wax paper with Elmer's glue or another water based glue provided an effective sentinel. The two layers of wax paper seemed to provide a barrier for the water based glue and few sentinels were lost from inclement weather. Smith (1996) recognized the need for proper delivery of parasitoids in order to have good quality releases and pointed out that delivery problems most often occur during hot conditions and long distances traveled. Forecasting future weather conditions for transport and taking into consideration the effect this might have on emergence date could help guarantee more viable parasitoids at delivery. From the standpoint of grower adoption and implementation of a *Trichogramma* program, guaranteeing viable parasitoids is a major concern.

Literature Cited:

Fournier, F., and Bovin, G. 2000. Comparative dispersal of *Trichogramma evanescens* and *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) in relation to environmental conditions. Environ. Entomol. 29 (1): 55-63.

Hoffmann, M. P., D. L. Walker, and A. M. Shelton. 1995. Biology of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) reared on *Ostriniae nubilalis* (Lepidoptera: Pyralidae) and survey for additional hosts. Entomophaga. 40: 387-402.

Kuhar, T. P., V. M. Barlow, M. P. Hoffmann, S. J. Fleischer, E. A. Groden, J. Gardner, R. Hazzard, M. G. Wright, S. A. Pitcher, and P. Westgate. 2004. Potential of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) as a biological control agent of European corn borer (Lepidoptera: Crambidae) in solanaceous crops. J. Econ. Entomol. 97: 1209-1216.

Smith, S. M. 1996. Biological control with *Trichogramma*: advances, successes, and potential of their use. Annu. Rev. Entomol. 41: 375-406.

Wang B, D. N. Ferro, and D. W. Hosmer. 1999. Effectiveness of *Trichogramma ostriniae* and *T. nubilale* for controlling the European corn borer *Ostrinia nubilalis* in sweet corn. Entomol. Exp. Appl. 91: 297-303.

Wright, M. G., T. P. Kuhar, and M. P. Hoffmann. 2002. Effect of inoculative releases of *Trichograma ostriniae* on populations of *Ostrinia nubilalis* and damage to sweet corn and field corn. Biol. Control 23: 149-155.

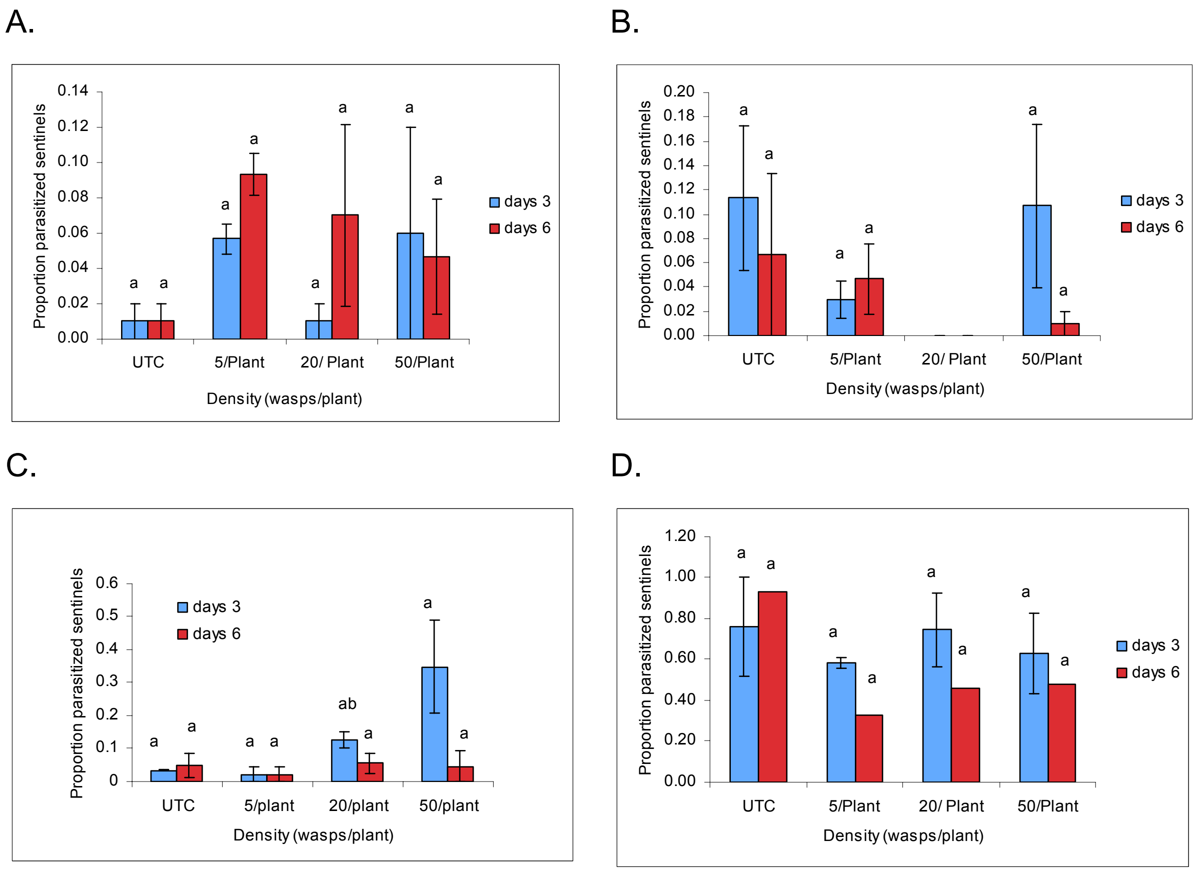

Figure 2.1 Mean (±SE) proportion parasitized *Ephestia kuehniella* egg sentinels by state (A = Virginia Beach, VA; B = Painter,VA; C = Rock Springs, PA; D = Queensland, MD). No significant treatment effect was found for all states excluding Penn using ANOVA.

Chapter 3

Integration of chemical and biological control of European corn borer in bell pepper in the Mid-Atlantic United States

ABSTRACT

In multiple locations in the Mid-Atlantic U.S., we evaluated the efficacy of several insecticides at controlling *Ostrinia nubilalis* Hübner and impacting arthropod natural enemies in bell pepper. Also in bell pepper, we compared the effectiveness of an integrated pest management program based around inundative releases of *Trichogramma ostriniae* Pang and Chen to a conventional insecticide-based program for *O. nubilalis* control. For the insecticide evaluations, small plots of bell pepper were established at four locations in Virginia, Maryland, Delaware, and Pennsylvania. Insecticides were applied weekly from first fruit until final harvest (5 to 7 applications). Results indicated that the biorational insecticides, spinosad, indoxacarb, and methoxyfenozide provided comparable control of *O. nubilalis* as the broad-spectrum conventional insecticides, acephate, and lambda-cyhalothrin. At most locations, multiple sprays of lambda-cyhalothrin resulted in flares (outbreaks) of green peach aphids most likely from destruction of arthropod natural enemies. Indoxacarb also caused a similar aphid flare at one of the locations. For the IPM demonstration experiment, pepper plots were established at 5 locations in the Mid-Atlantic U.S. in 2005 and 2006. Treatments included: “conventional”, which involved weekly applications of acephate or lambda-cyhalothrin from first fruit until final harvest; 2) “IPM”, which included three or four inundative releases of *T. ostriniae* and a judicial application of methoxyfenozide only if lepidopteran pests exceeded action thresholds; and 3) an untreated control.. No significant treatment effect was found in either year on cumulative number of marketable fruit or percentage of fruit damaged by lepidopteran pests. A significant treatment effect was found on peak numbers of green peach aphids, with the conventional insecticide approach causing aphid flares and the untreated control or IPM approach not having aphid pest problems. Inundative releases of *T. ostriniae* may be a more environmentally-sound approach to managing *O. nubilalis* in peppers, although a comparison with conventional insecticides under greater lepidopteran pest pressure is still needed.

KEY WORDS *Ostrinia nubilalis*, *Trichogramma ostriniae*, pepper, biocontrol, integrated pest management, biorational insecticides

Introduction

Sweet bell pepper [*Capsicum annuum* L.] can be a valuable vegetable crop grossing nearly 500 million dollars annually in the U.S. (NASS 2006). Commercial growers often have a large financial investment at stake once plants begin to fruit in the field, and under such circumstances, often apply insecticides preventatively and liberally to protect the pepper fruit from insect pests. Pepper growers in the Mid-Atlantic U.S. generally apply the organophosphate acephate for two sprays (maximum allowed per crop per season), then switch to a pyrethroid insecticide for several additional applications usually every 5-10 days until final harvest (T. P. Kuhar, *unpublished 2005 grower survey data*). This conventional (preventative spraying) approach may result in five to ten insecticide applications per crop. Although it is generally effective from a pest management standpoint (Welty 1995, Kuhar and Speese 2002, Kuhar et al. 2003), it has numerous drawbacks associated with unwarranted insecticide applications including potential buildup of pesticide residues on fruit, destruction of important natural enemies in the agro-ecosystem, environmental and human health risks, and reduced profits.

For pepper growers in the northeastern and central U.S., European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), is the primary target of insecticide sprays (Welty 1995, Hazzard et al. 2001). The insect has two to three generations per year and is typically a season-long pest risk to bell pepper. Egg masses are deposited on the undersides of leaves (Barlow and Kuhar 2004) and usually hatch in three to ten days (Mason et al. 1996). After a brief period of leaf feeding, larvae bore into fruit if available or stems if no fruit or only tiny immature fruit are present (Hitchner and Ghidiu 2006). Direct damage is caused by the tunneling larvae that feed on the pericarp, placenta, and seeds of developing fruit (Jarvis and Guthrie 1972). Moreover, tunnel holes frequently are entry points for fruit-rotting pathogens such as *Erwinia caratovora* (Hazzard et al. 2001). If control measures are not taken in bell pepper, fruit damage can range from 40 to 60% (Welty 1995, Kuhar and Speese 2002, Kuhar et al. 2003). Even greater fruit damage can occur to mature (colored) bell peppers because of the increased time that fruit are in the field (Welty and Vitanza 2005, Barlow 2006). *Ostrinia* *nubilalis* is particularly difficult to control because larvae are only exposed to insecticide sprays from egg hatch until tunneling.

Other insects that may infest pepper fruit include the noctuid pests, *Helicoverpa zea* (Boddie), *Spodoptera exigua* Hübner, *S. frugiperda* (J. E. Smith), and pepper maggot, *Zonosemata electa* (Say) (Diptera: Tephritidae) (Boucher and Ashley 2001). However, Kuhar et al. (2004) found that this complex of pests only accounted for about 5% of insects found attacking bell pepper fruit in Virginia, with most (95%) being *O. nubilalis*. In addition, green peach aphid, *Myzus persicae*, can be an important indirect pest of peppers by feeding on plant juices, reducing plant vigor, vectoring plant viruses, and depositing honeydew, where black sooty mold fungus may grow (Boucher 2001). Green peach aphid has a tremendous reproductive capability, and can quickly build to damaging levels if populations are not contained by natural enemies (van Emden et al. 1969). Multiple applications of broad-spectrum insecticides such as pyrethroids kill off natural enemies, but not aphids, and cause most (if not all) green peach aphid outbreaks in bell pepper (Boucher 2001). Therefore, reducing or even eliminating the use of broad-spectrum insecticides for *O. nubilalis* control would significantly reduce the pest potential of aphids in peppers, in addition to improving environmental stewardship, human safety, and food safety associated with reduced pesticide usage.

Biological control of *O. nubilalis* eggs using augmentative releases of *Trichogramma* parasitoids may provide an alternative to reliance on insecticides (Smith 1996). *Trichogramma ostriniae* Pang and Chen (Hymenoptera: Trichogrammatidae) was introduced into the U.S. from China in the early 1990s (Hoffmann et al. 1995), and has shown tremendous promise for controlling *O. nubilalis* in corn (Wang and Ferro 1998; Wang et al. 1999; Wright et al. 2001; Hoffmann et al. 2002; Kuhar et al. 2002; Wright et al. 2002; Kuhar et al. 2003) and solanaceous crops (Kuhar et al. 2004). After four to five inundative releases of *T. ostriniae* in bell pepper, *O. nubilalis* egg parasitism averaged around 50% and cumulative fruit damage was reduced almost 70% compared with non-release control plots (Kuhar et al. 2004). However, under heavy pest pressure, pepper fruit damage still exceeded 10% in the *T. ostriniae* release plots.

The use of reduced-risk (biorational) insecticides as an alternative to organophosphates and pyrethroids is another less detrimental approach for controlling

O. nubilalis in pepper. In recent years, a few narrow-spectrum (more lepidopteran specific) insecticides have been registered for use on bell pepper in the U.S. including spinosad (Spintor®, Dow AgroSciences), methoxyfenozide (Intrepid®, Dow AgroSciences), and indoxacarb (Avaunt®, E.I. DuPont de Nemours). Spinosad is a biological insecticide produced by a soil actinomycete. It is a mixture of two macrocyclic lactone compounds called spinosyns (spinosyns A and D), that are active on specific insect pests, in particular Lepidoptera (Sparks et al. 2001), but are generally less toxic to natural enemies (Cisneros et al. 2002). Methoxyfenozide is an insect growth regulator (IGR) that belongs to the Diacylhydrazine chemical group. It is an ecdysone agonist that interferes with molting and other processes in insects, specifically Lepidoptera (Bylemans 2003). Indoxacarb is an oxadiazine insecticide that was recently registered for use on peppers. Indoxacarb and its metabolite decarbomethoxyllated JW062 have multiple actions on sodium channels, neuronal nicotinic acetylcholine receptors, and GABA receptors (Narahashi 2001). Spinosad, methoxyfenozide, and indoxacarb provide effective control of most lepidopteran pests, while generally having greatly reduced toxicity to natural enemies (Brunner et al. 2001, Elzen 2001, Bauer et al. 2003, Hewa-Kapuge et al. 2003, Hill and Foster 2003, Schneider et al. 2003, Studebaker and Kring 2003a, 2003b, Carton et al. 2003, Haseeb et al. 2004, 2005, Villanueva and Walgenbach, 2005).

Herein we evaluated strategies to reduce broad-spectrum insecticide use on bell pepper. In one experiment, the efficacy and relative impact on beneficial arthropods of spinosad, methoxyfenozide, and indoxacarb were compared with the conventional broad-spectrum insecticides acephate and lambda-cyhalothrin; and in a second experiment, the efficacy of a *T. ostriniae*-based biological control program was compared with a conventional insecticide-based program in bell pepper in the Mid-Atlantic U.S.

Materials and Methods

Insecticide efficacy field experiments: In summer 2005, we conducted small-plot field experiments on bell pepper at four locations: Virginia Tech Eastern Shore Agricultural Research and Extension Center (AREC), Painter, VA; University of

Delaware REC, Georgetown, DE; University of Maryland Wye REC, Queenstown, MD; and the Russell E. Larson Research Farm, Rock Springs, PA. Details on the pepper crop production at each location are found in Table 3.1. Each field plot consisted of treatments laid out in a randomized complete block design with four replications of each of six insecticide treatments. Individual plots were 4 rows wide by 6-m long with >3-m alleys between plots. In general, crops were established and maintained using standard agricultural procedures including fertilizer, herbicide, and fungicide applications according to a commercial vegetable production manual for the Mid-Atlantic U.S. (Kuhar et al. 2006b). Insecticide treatments included: 1) indoxacarb at 0.072 kg [AI]/ha (Avaunt 30WG; E. I. du Pont de Nemours and Co., Wilmington, DE); 2) methoxyfenozide at 0.112 kg [AI]/ha (Intrepid 2F; Dow AgroSciences LLC, Indianapolis, IN); 3) spinosad at 0.026 kg [AI]/ha (SpinTor 2SC, Dow AgroSciences LLC, Indianapolis, IN); 4) acephate at 1.09 kg [AI]/ha (Orthene® 97SG; Valent USA Corporation, Walnut Creek, CA); 5) lambda-cyhalothrin at 0.03 kg [AI]/ha (Syngenta Crop Protection Inc., Greensboro, NC); and 6) an untreated control. Insecticides were applied with a CO_2 backpack sprayer with a one-row boom having 3 hollow-cone nozzles per row (one over the top and one drop nozzle on each side) delivering 39 gpa at 40 psi. Treatments were applied weekly from 1st fruit until final harvest. Dates of applications for each location are presented in Table 3.1. On three post-spray sample dates, aphids were counted on 50 randomly-picked leaves per plot and relative samples of natural enemies (generalist arthropod predators and parasitoid species) were made by slowly walking the entire length of one middle row per plot and vacuuming plants using a Craftsman™ 200 mph leaf blower-vac fitted with a fine mesh bag on the intake tube of diameter 12 cm. After each sample was collected, bags were sealed and placed in a freezer for several days. Any dirt or plant debris was removed from each sample and all insects were stored in 70% ethanol for later identification and sorting into natural enemy groups such as: ladybird beetles; predatory bugs; parasitic hymenoptera; lacewings; predatory diptera; spiders, and other.

On three separate dates during a one month span in late summer (Table 3.1), all market-sized fruit were harvested from the middle two rows of each plot and assessed for insect damage. *Ostrinia nubilalis* damage to bell pepper can manifest itself as either reduced number of harvested fruit from rotting, or percentage of harvested fruit exhibiting damage. The cumulative number of marketable fruit and the percentage damaged by insects were recorded. Damaged fruit were dissected and inspected for any insects present.

Data were analyzed using analysis of variance (Statistix Analytical Software 2003) to test for significant treatment effects on marketable fruit yield, proportion damaged fruit, peak density of aphids on leaves, and relative abundance of predominant natural enemies. In order to stabilize variances, prior to analysis, all proportion damage data were arc-sine square-root transformed, aphid density data were $\log_{10}$ x-transformed, and natural enemy data were square root (x + 0.5)-transformed because of the presence of zeros. If the treatment source of variation was significant, differences among treatment means were tested using Fisher's Protected LSD at the $P \leq 0.05$ level of significance.

Integrated pest management demonstration experiments: In summer 2005 and 2006, we established plots of bell peppers at five locations: Virginia Tech Eastern Shore Agricultural Research and Extension Center (AREC), Painter, VA; Virginia Tech Hampton Roads AREC, Virginia Beach, VA; University of Delaware REC, Georgetown, DE; University of Maryland Wye REC, Queenstown, MD; and the Russell E. Larson Research Farm, Rock Springs, PA. The same locations were used in both years. Each location represented a replicate (n = 5) in a randomized complete block experiment that was analyzed separately by year. Three spatially-isolated plots of peppers (each ~0.03 ha and ~500 plants) were established at each location. Details on planting date, variety, and production practices at each location are presented in Table 3.2. In general, crops were established and maintained using standard agricultural procedures including fertilizer, herbicide, and fungicide applications following a commercial vegetable production manual designed for the Mid-Atlantic U.S. (Kuhar et al., 2006b).

At each location, the three pepper plots were randomly designated as: 1) "conventional", which involved weekly applications of acephate or lambda-cyhalothrin from first fruit until final harvest; 2) "IPM", which included three or four inundative releases of *T. ostriniae* and a judicial application of methoxyfenozide only if lepidopteran pests exceeded action thresholds; and 3) an untreated control. In the IPM plot, we released 100,000 *T. ostriniae* per acre on three or four dates (Table 3.2) using perforated cardboard release cartons and methods described in Kuhar et al. (2004). The parasitoids were obtained from M. P. Hoffmann (Cornell University, Ithaca, NY), details on the *T. ostriniae* colony maintenance can be found in Hoffmann et al. (1995, 2001). Insect monitoring followed guidelines outlined in Boucher and Ashley (2001), and involved blacklight or pheromone trap monitoring of *O. nubilalis* and *Helicoverpa zea* (Lepidoptera: Nocutidae) moths and weekly inspections of 40 plants per plot for aphids and eggs or larvae of lepidopteran pest species. In the conventional plot, weekly insecticide applications commenced at first fruiting of the crop, and included two applications of acephate at 1.09 kg [AI]/ha (Orthene® 97SG; Valent USA Corporation, Walnut Creek, CA) followed by four to five applications of lambda-cyhalothrin at 0.03 kg [AI]/ha (Syngenta Crop Protection Inc., Greensboro, NC). Insecticide spray dates at each location are shown in Table 3.2.

When they reached marketable size, pepper fruit were hand-harvested and evaluated for damage periodically from Jul to Oct (Table 3.2). At each harvest, we inspected fruit for insect injury and recorded the total number damaged and marketable. Data were pooled across the five locations and analyzed by year using analysis of variance (Statistix 8.0 Analytical Software, 2003) to test for significant treatment effects on marketable fruit yield, proportion damaged fruit, and peak density of aphids on leaves. Proportion data were square-root transformed prior to analysis in order to stabilize variance. Differences among treatment means were tested using Fisher's Protected LSD at the $P \leq 0.05$ level of significance.

Results

Insecticide efficacy field experiments: Green peach aphid, *M. persicae*, infested pepper plots at all four locations, but did not reach damaging levels in the untreated control plots on any sample date at any location. However, after four or more insecticide applications, there was a highly significant treatment effect on numbers of aphids at three of the four locations: Virginia ($f = 23.43$; $df = 5, 23$; $P < 0.0001$); Pennsylvania ($f = 6.35$; $df = 5, 23$; $P < 0.0024$); and Delaware ($f = 7.99$; $df = 5, 23$; $P =$

0.0008). In Virginia and Pennsylvania, aphid densities were significantly higher in the lambda-cyhalothrin plots compared with any other insecticide treatment or the control, and in Delaware, aphid densities were significantly higher in the lambda-cyhalothrin and indoxacarb plots compared with any other insecticide or the control (Table 3.3). Additional applications of these insecticides resulted in even greater aphid densities on leaves, while control plot aphid densities remained low.

Lepidopteran pest pressure was relatively low across all locations and there was no significant treatment effect on cumulative number of marketable (undamaged) fruit in Maryland (f = 0.67; df = 5, 23; P = 0.6534), Pennsylvania (f = 1.80; df = 5, 23; P = 0.1730), and Virginia (f = 0.27; df = 5, 23; P = 0.9243). However, there was a significant treatment effect on the percentage of fruit damaged by *O. nubilalis* in Pennsylvania (f = 6.35; df = 5, 23; P < 0.0024) and Virginia (f = 7.56; df = 5, 23; P < 0.0010); where the untreated control plots at both locations had more damage than any of the insecticide treatments, and no differences were found among the insecticide treatments (Table 3.3). No significant treatment effect on fruit damage was found at the Maryland location (f = 1.56; df = 5, 23; P = 0.2308). Fruit damage and concomitant number of marketable fruit was not assessed at the Delaware location.

Arthropods from the vacuum samples of the pepper plots were not identified to species, but rather identified to the lowest taxonomic unit that we felt comfortable with in order to classify it into a natural enemy grouping. The predominant natural enemies collected were parasitic hymenoptera (mostly Chalcidoidea, Braconidae, and Ichneumonidae), predatory bugs (mostly Anthocoridae - *Orius* spp.), ladybird beetles (Coccinelidae), and spiders (Aranea). There was no significant treatment effect at any location on cumulative numbers of parasitic hymenoptera or predatory bugs (Figs. 3.1B and 3.1C). In Virginia, there was a significant treatment on cumulative number of ladybird beetles (f = 4.60; df = 5, 15; P < 0.020), with the untreated control plots having more beetles than the lambda-cyhalothrin and acephate plots (Fig. 3.1A). In Delaware there was a significant treatment effect on cumulative number of spiders (f = 2.82; df = 5, 15; P ≤ 0.0545), with the untreated control having more spiders than the lambda-cyhalothrin, acephate, or methoxyfenozide plots (Fig. 3.1D). Similarly in Pennsylvania there was a significant treatment effect on number of spiders (f = 3.00; df = 5, 15; P ≤

0.0452), with the untreated control having more spiders than lambda-cyhalothrin, acephate, methoxyfenozide, or indoxacarb (Fig. 3.1D).

Integrated pest management demonstration experiments

In both years, lepidopteran pest pressure to bell peppers was moderately low to low at all five Mid-Atlantic locations. *Ostrinia nubilalis* was the primary insect pest encountered and weekly moth catch at each location and year is shown in Fig. 3.2. In 2005, conventional plots averaged 7 insecticide applications (2 acephate sprays + 5 pyrethroid sprays) compared with <1 insecticide spray of methoxyfenozide in the IPM plots. In 2006, conventional plots averaged 5.8 insecticide applications (2 acephate sprays + additional pyrethroid sprays) compared with no insecticide sprays in the IPM plots.

Number of marketable fruit. There was no statistically significant treatment effect on cumulative number of marketable fruit in 2005 (f =1.37; df = 2, 6; P = 0.3035) or 2006 (f =0.7; df = 2, 6; P = 0.5239). However, in 2005, across the five locations, conventional plots tended to have the most marketable fruit followed by the IPM plots and then the untreated control plots (Fig. 3.3A).

Percentage of fruit damaged. There was no statistically significant treatment effect on percentage of fruit damaged by lepidopteran larvae in either 2005 (f = 0.15; df = 2, 6; P = 0.8649) or 2006 (f =2.60; df = 2, 6; P = 0.1351). Percentage of damaged fruit averaged between 4 and 12% among the treatments with the untreated control plots typically having the most damage numerically (Fig. 3.3B).

Aphids. There was a significant treatment effect on peak density of green peach aphids in 2005 (f =5.71; df = 2, 6; P < 0.041) and nearly a significant effect in 2006 (f =3.70; df = 2, 6; P < 0.0896). In both years, the conventional insecticide plots had the most aphids, and no differences were found between the IPM and untreated control plots (Fig. 3.3C).

Discussion

In the two years of our study, the biorational insecticides, spinosad, indoxacarb, and methoxyfenozide provided similar control of *O. nubilalis* in bell pepper as the conventional acephate or lambda-cyhalothrin treatments. However, lepidopteran pest pressure was relatively low in our study. Nonetheless, similar efficacy results with some or all of the aforementioned insecticides on bell peppers have been shown by Nault and Speese (2000), Kuhar and Speese (2002), Kuhar et al. (2003, 2006a), and Sorenson and Cooke (2004). In contrast, Welty and Vitanza (2005) found acephate to be the superior insecticide under extreme *O. nubilalis* pest pressure on mature red bell pepper in Ohio. Timing of the insecticide applications is critical for *O. nubilalis* control. Most insecticides do not kill the egg stage, and after hatch, larvae quickly tunnel into plants where they are protected from chemical sprays. An insecticide such as acephate could exhibit greater efficacy against *O. nubilalis* because of a longer residual in the field compared with the newer biorationals.

Green peach aphid did not reach damaging levels in the untreated control plots, or in plots treated with acephate, spinosad, or methoxyfenozide. In contrast, at most locations, multiple sprays of the pyrethroid lambda-cyhalothrin caused severe aphid flares. Also at one location, multiple sprays of indoxacarb caused similar aphid flares. Destruction of arthropod natural enemies by insecticides was likely the cause for the aphid flares (van Emden et al. 1969, Croft and Brown 1975). Lambda-cyhalothrin and acephate are highly toxic to most arthropod natural enemies (Bauer et al. 2003). However, acephate provides excellent control of green peach aphid, but pyrethroid insecticides, such as lambda-cyhalothrin, do not control the pest (Speese 1994, 1996). Therefore, multiple sprays of lambda-cyhalothrin or any pyrethroid will typically exacerbate green peach aphid problems (Boucher 2001). Our data showed that at least in one field, multiple sprays of indoxacarb could result in similar aphid outbreaks. Indoxacarb does not control aphids, but has been shown to be moderately toxic to some natural enemies including predatory hemipterans (Bauer et al. 2003) and ladybeetles (Smith 1996, T. P. Kuhar, *unpublished data*). A number of factors can influence insecticide efficacy in the field. For instance, Hill and Foster (2000) found that spinosad was extremely toxic to the hymenopteran parasitoid *Diadegma insulare* in leaf dip assays; however, in field experiments they found that *D. insulare* parasitism of diamondback moth larvae was not affected by spinosad applications (Hill and Foster 2003). Relatively rapid degradation of surface residues in the field would definitely improve the compatibility potential with natural enemies. This would likely be the case with spinosad degradation by photolysis (Viktorov et al. 2002). In addition, the translocation and translaminar properties of some insecticides make them available in the host plant tissues for control of leaf feeders, but surface residues disappear quickly, thus making them safe for parasitoids and most natural enemies (Hoy and Cave 1985, Brunner et al. 2001).

Our vacuum collections of natural enemies from the insecticide plots did not reveal significant treatment differences for counts of most natural enemies. We believe our field plot design was probably inadequate to properly assess arthropod natural enemy densities. Because of field space constraints, the border areas (and alleys) between our insecticide plots were restricted to ~3 m, which in hind site was probably not large enough. Most predators and parasitoids are highly mobile organisms, and it is likely that continuous re-colonization of sprayed plots by various species affected our results. Baur et al. (2003) reported similar concerns of predator reestablishment in field plots.

Our demonstration plots in the Mid-Atlantic Region of IPM versus conventional-insecticide based programs in bell pepper did not reveal significant treatment effects on cumulative number of marketable fruit or percentage fruit damage. This was clearly a result of low lepidopteran pest pressure across the two years of our study. Inundative releases of the parasitoid, *Trichogramma ostriniae*, have been shown to significantly reduce *O. nubilalis* damage in bell pepper (Kuhar et al. 2004). Unfortunately, in our study, we were unable to determine if this pest management approach can provide comparable control of *O. nubilalis* as a conventional insecticide approach. It should be noted however, that the conventional insecticide treatment resulted in significantly higher densities of green peach aphids on peppers compared with either the untreated control plots or the *Trichogramma*-based IPM plots. Thus, depending on the degree of pest pressure, *T. ostriniae* may provide a more environmentally-sound pest management option for *O. nubilalis* in peppers.

References

Barlow, V. M. 2006. Use of the egg parasitoid wasp, *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) as a biological control agent of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Crambidae) in bell pepper: An approach to integrated pest management. Ph.D. Dissertation. Virginia Polytechnic Institute and State University.

Barlow, V. M. and T. P. Kuhar. 2004. Distribution of egg masses of the European corn borer, *Ostrinia nubilalis*, in the bell pepper plant canopy. J. Entomol. Sci. 39: 670-672.

Baur, M. E., J. Ellis, K. Hutchinson, and D. J. Boethel. 2003. Contact toxicity of selected insecticides for non-target predaceous hemipterans in soybeans. J. Entomol. Sci. 38: 269- 277.

Boucher, T. J. 2001. General strategy of insect management. P. 63-66 In T. J. Boucher and R. A. Ashley (eds.), Northeast Pepper Integrated Pest Management (IPM) Manual. Univ. Conn. Coop. Ext., Stoors, CT.

Boucher, T. J. and R. A. Ashley (eds.). 2001. Northeast Pepper Integrated Pest Management (IPM) Manual. Univ. Conn. Coop. Ext., Stoors, CT.

Brunner, J. F., J. E. Dunley, M. D. Doerr, E. H. Beers. 2001. Toxicity and pharmokinetics of insect growth regulators and other novel insecticides on pupae of *Hyposoter didymator* (Hymeoptera: Ichneumonidae), a parasitoid of early larval instars of lepidopteran pests. J. Econ. Entomol. 94, 1075-1084.

Bylemans, D., L. de Maeyer, A. Auwerkerken, H. de Craen, J. W. Wijsmuller, and D. Peeters. 2003. Methoxyfenozide, a reliable IPM compatible compound against Lepidoptera in pome fruit and vegetables with sterilising, ovicidal and larvicidal efficacy on codling moth. Comm. Agric. Appl. Biol. Sci. 68: 189-202.

Carton, B., G. Smagghe, and L. Tirry. 2003. Toxicity of two ecdysone agonists, halofenozide and methoxyfenozide, against the multicoloured Asian lady beetle *Harmonia axyridis* (Col., Coccinellidae). J. Appl. Entomol. 127, 240-242.

Cisneros, J., D. Goulson, L. C. Derwent, D. I. Penagos, O. Hernandez, and T. Williams. 2002. Toxic effects of spinosad on predatory insects. Biol. Contr. 23: 156-163.

Elzen, G. W. 2001. Lethal and sublethal effects of insecticide residues on *Orius insidiosus* (Hemiptera: Anthocoridae) and *Geocoris punctipes* (Hemiptera: Lygaeidae). J. Econ. Entomol. 94: 55-59.

Haseeb, M., T. X. Liu, and W. A. Jones. 2004. Effects of selected insecticides on *Cotesia plutellae*, endoparasitoid of *Plutella xylostella*. BioControl 49, 33-46.

Haseeb, M., H. Amano, and T. X. Liu. 2005. Effects of selected insecticides on *Diadegma semiclausum* (Hymenoptera: Ichneumonidae) and *Oomyzus sokolowskii* (Hymenoptera: Eulophidae), parasitoids of *Plutella xylostella* (Lepidoptera: Plutellidae). Insect Sci. 12: 163-170.

Hazzard, R. V., G. M. Ghidiu, and T. J. Boucher. 2001. European corn borer management. P. 67-76 In T. J. Boucher and R. A. Ashley (eds.), Northeast Pepper Integrated Pest Management (IPM) Manual. Univ. Conn. Coop. Ext., Stoors, CT.

Hewa-Kapuge, S., S. McDougall, and A. A. Hoffmann. 2003. Effects of methoxyfenozide, indoxacarb, and other insecticides on the beneficial egg parasitoid *Trichogramma* nr. *brassicae* (Hymenoptera: Trichogrammatidae) under laboratory and field conditions. J. Econ. Entomol. 96: 1083-1090.

Hill, T. A., and R. E. Foster. 2000. Effect on insecticides on the diamondback moth (Lepidoptera: Plutellidae) and its parasitoid Diadegma insulare (Hymenoptera: Ichneumonidae). J. Econ. Entomol. 93: 763-768.

Hill, T. A., and R. E. Foster. 2003. Influence of selected insecticides on the population dynamics of diamondback moth (Lepidoptera: Plutellidae) and its parasitoid, *Diadegma insulare* (Hymenoptera: Ichneumonidae), in cabbage. J. Entomol. Sci. 38: 59-71.

Hitchner, E. M., and G. M. Ghidiu. 2006. Fruit size and infestation by European corn borer, *Ostrinia nubilalis* Hubner, in bell pepper. J. Veg. Sci. 12: 101-107.

Hoffmann, M. P., D. L. Walker and A. M. Shelton. 1995. Biology of *Trichogramma ostriniae* (Hym.: Trichogrammatidae) reared on *Ostrinia nubilalis* (Lep.: Pyralidae) and a survey for additional hosts. Entomophaga 40:387-402.

Hoffmann, M. P., P. R. Ode, D. L. Walker, J. Gardner, S. van Nouhuys and A. M. Shelton. 2001. Performance of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) reared on factitious hosts including the target host, *Ostrinia nubilalis* (Lepidoptera: Crambidae). Biol. Contr. 21: 1-10.

Hoffmann, M. P., M. G. Wright, S. A. Chenus, and J. Gardner. 2002. Inoculative releases of *Trichogramma ostriniae* for suppression of *Ostrinia nubilalis* (European corn borer) in sweet corn: Field biology and population dynamics. Biol. Contr. 25:249-258.

Hoy, M. A., and F. E. Cave 1985. Laboratory evaluation of avermectin as a selective acaricide for use with *Metaseiulus occidentalis* (Nesbitt) (Acarina: Phytoseiidae). Exp. Appl. Acarol. 1: 139-152.

Jarvis, J. L., and W. D. Guthrie. 1972. Relation of horticultural characteristics of peppers to damage by larvae of the European corn borer. Iowa State Journal of Science 46:463-470.

Kuhar, T. P. and J. Speese. 2002. Evaluation of foliar insecticides for control of European corn borer in peppers, 2001. Arthropod Manag. Tests. 27: E56.

Kuhar, T. P., M. G. Wright, M. P. Hoffmann, S. A. Chenus. 2002. Life table studies of European corn borer (Lepidoptera: Crambidae) with and without inoculative releases of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae). Environ. Entomol. 31:482-489.

Kuhar, T. P., J. Speese, R. J. Cordero, R. Y. Venkata, and V. M. Barlow, and R. Cordero. 2003. Evaluation of foliar insecticides for control of lepidopterous pests in peppers, 2002. Arthropod Manag. Tests 28: E37.

Kuhar, T. P., Barlow, V. M., M. P. Hoffmann, S. J. Fleischer, E. A. Groden, J. Gardner, R. Hazzard, M. G. Wright, S. A. Pitcher, and P. Westgate. 2004. Potential of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) as a biological control agent of European corn borer (Lepidoptera: Crambidae) in solanaceous crops. J. Econ. Entomol. 97: 1209-1216.

Kuhar, T. P., A. V. Chapman, and E. M. Hitchner. 2006a. Evaluation of insecticides for lepidopteran pest control on bell peppers, 2005. Arthropod Manag. Tests 31: E43.

Kuhar, T. P., S. B. Phillips, R. A. Straw, S.B., C. M. Waldenmaier, and H. P. Wilson. 2006b. Commercial Vegetable Production Recommendations. Virginia Coop. Ext. Pub. No. 456-420.

Mason, C. E., M. E. Rice, D. D. Calvin, J. W. Van Duyn, W. B. Showers, W. D. Hutchinson, J. F. Witkowski, R. A. Higgins, D. W. Onstad, and G. P. Dively. 1996. European corn borer: Ecology and management. North Central Regional Extension Pub. No 327. Iowa State University.

Narahashi, T. 2001. Recent progress in the mechanism of action of insecticides: pyrethroids, fipronil and indoxacarb. J. Pesticide Sci. 26: 277-285.

National Agricultural Statistics Service, 2006. Census of Agriculture. Historical Data. Electronic publication. Accessed Oct. 2, 2006. http://www.usda.gov/nass/pubs/histdata.htm

Nault, B. A. and J. Speese. 2000. Evaluation of insecticides to control European corn borer and corn earworm on fall-grown peppers, 1999. Arthropod Manag. Tests. 25: E52.

Schneider, M.A., G. Smagghe, A. Gobbi, and E. Viñuela. 2003. Toxicity and pharmokinetics of insect growth regulators and other novel insecticides on pupae of *Hyposoter didymator* (Hymeoptera: Ichneumonidae), a parasitoid of early larval instars of lepidopteran pests. J. Econ. Entomol. 96: 1054-1065.

Smith, S. M. 1996. Biological control with *Trichogramma*: advances, successes, and potential of their use. Ann. Rev. Entomol. 41:375-406.

Sorensen, K. A. and D. G. Cooke. 2004. European corn borer, corn earworm, and beet armyworm control with insecticides, 2002. Arthr. Manag. Tests. 29: E58.

Sparks, T. C., G. D. Crouse, and G. Durst. 2001. Natural products as insecticides: the biology, biochemistry and quantitative structure-activity relationships of spinosyns and spinosoids. Pest Manag. Sci. 57: 896-905.

Speese, J. 1994. Soil and foliar treatments to control insects in peppers, 1993. Arthr. Manag. Tests. 19: 106-107.

Speese, J. 1995. Foliar sprays to control insects on spring peppers, 1995. Arthr. Manag.Tests. 21: 137.

Statistix 2003. Statistix 8.0 Analytical Software for Windows.

Studebaker, G. E., and T. J. Kring. 2003a. Effects of insecticides on *Orius insidiosus* (Hemiptera: Anthocoridae) measured by field, greenhouse and Petri dish bioassays. Florida Entomol. 86: 178-185.

Studebaker, G. E., and T. J. Kring. 2003b. Effects of various insecticide residues in cotton on gender and developmental stage of the insidious flower bug (Hemiptera: Anthocoridae). J. Entomol. Sci. 38: 409-419.

Van Emden, H. F., V. F. Eastop, R. D. Hughes, and M. J. Way. 1969. The ecology of *Myzus persicae*. Annu. Rev. Entomol. 14: 197-270.

Viktorov, A.V., E. N. Preshokov, and V. A. Driniaev. 2002. Comparative analysis of degradation of native avermectins and spinosyns by UV-HPLC. Antibiot. Khimioter. 47: 6-10.

Villanueva, R. T., and J. F. Walgenbach. 2005. Development, oviposition, and mortality of *Neoseiulus fallacis* (Acari: Phytoseiidae) in response to reduced-risk insecticides. J. Econ. Entomol. 98, 2114-2120.

Wang, B. and D. N. Ferro. 1998. Functional responses of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) to *Ostrinia nubilalis* (Lepidoptera: Pyralidae) under laboratory and field conditions. Environ. Entomol. 27: 752-758.

Wang B, D. N. Ferro, and D. W. Hosmer. 1999. Effectiveness of *Trichogramma ostriniae* and *T. nubilale* for controlling the European corn borer *Ostrinia nubilalis* in sweet corn. *Entomol. Exp. Appl*. 91: 297-303.

Welty, C. 1995. Monitoring and control of European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae), on bell peppers in Ohio. J. Agric. Entomol. 2: 145-161.

Welty, C. and S. Vitanza. 2005. Insecticide efficacy evaluation of European corn borer control in bell pepper, 2004. Arthr. Manag. Tests. 30: E52.

Wright, M. G., M. P. Hoffmann, S. A. Chenus, and J. Gardner. 2001. Dispersal behavior of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) in sweet corn fields: implications for augmentative releases against *Ostrinia nubilalis* (Lepidoptera: Crambidae). Biol. Contr. 22: 29-37.

Wright, M. G., T. P. Kuhar, M. P. Hoffmann, and S. A. Chenus. 2002. Effect of inoculative releases of *Trichogramma ostriniae* on populations of *Ostrinia nubilalis* and damage to sweet corn and field corn. Biol. Contr. 23: 149-155.

Table 3.1 Crop information, insecticide application, and harvest dates for each location of the bell pepper insecticide efficacy experiment conducted in the Mid-Atlantic Region of the United States in 2005.

Location	Variety	Transplant date	Crop production method	Insecticide application dates	Harvest and fruit damage evaluation dates
Eastern Shore, VA	'Paladin'	14 June	Ground with overhead irrigation	21, 28 Jul, and 3, 9, 16, 23, 31 Aug	11, 22 Aug, and 7 Sept
Georgetown, DE	'Aristotle'	27 May	Single rows on black plastic with drip-line irrigation	16, 22, 28 Jun, 6, 13, 20, 27 Jul, and 5 Aug	12, 26 Jul, and 15 Aug
Queenstown, MD	'Paladin'	16 June	Single rows with overhead irrigation	24 Jul, 1,8,15,21,29 Aug, and 8 Sept.	19 Jul, 3 and 15 Aug
Rock Springs, PA	'King Arthur'		Double-staggered rows on black plastic with drip irrigation	25 Jul, and 1, 9, 15, 22, 29 Aug	20 Aug, and 1, 16 Sept

Table 3.2 Crop information and pest management application dates for each location of our bell pepper integrated pest management demonstration experiments conducted in the Mid-Atlantic Region of the United States in 2005 and 2006.

Location	Variety	Transplant date	Crop production method	Insecticide application dates in "conventional" plots	*Trichogramma ostriniae* release dates in "IPM" plots	Harvest and fruit damage evaluation dates
2005						
Eastern Shore, VA	'Paladin'	14 June	Ground with overhead irrigation	21, 28 Jul, and 3, 9, 16, 23, 31 Aug	21 Jul, and 2, 12 Aug	11, 22 Aug, and 7 Sept
Virginia Beach, VA	'Paladin'	6 June	Ground with drip-line irrigation	22, 29 Jul, and 4, 12, 19, 30 Aug	22 Jul, and 3, 12 Aug	26 Jul, 4, 16 Aug, and 1 Sept
Georgetown, DE	'Aristotle'	27 May	Single rows on black plastic with drip-line irrigation	16, 22, 28 Jun, 6, 13, 20, 27 Jul, and 5 Aug	29 Jun, and 12, 19 Jul	12, 26 Jul, and 15 Aug
Queenstown, MD	'Paladin'	16 June	Single rows with overhead irrigation	24 July, 1, 8, 15, 21, 29 Aug, and 8 Sept		19, 26 Aug, and 16 Sept
Rock Springs, PA	'King Arthur'	13 June	Double-staggered rows on black plastic with drip irrigation	25 Jul, and 1, 9, 15, 22, 29 Aug	25 Jul, and 3, 12, 20 Aug	20 Aug, and 1, 16 Sept
2006						
Eastern Shore, VA	'Paladin'	7 June	Ground with overhead irrigation	28 Jul, and 4, 11, 18, 25 Aug, and 5 Sept	3, 10, 17 Aug	14, 24, and 5 Sept
Virginia Beach, VA	'Paladin'	7 June	Single rows on black plastic with drip-line irrigation	29 Jul, and 3, 11, 17, 24, 31 Aug	3, 10, 17 Aug	5, 17, 29 Aug
Queenstown, MD	'Paladin'	6 June	Single rows with overhead irrigation	12, 20, 31 July, 17, 24 Aug and 4 Sept	4, 12 Aug	27 July, 11 and 23 Aug
Georgetown, DE	'Aristotle'	?	Single rows on black plastic with drip-line irrigation	8, 15, 22, 29 Jul, and 5 Aug	19, 27 Jul, and 3, 14 Aug	26 Jul and 8, 21 Aug
Rock Springs, PA	'Paladin'	8 June	Single rows on black plastic with drip irrigation	24, 31 Jul, and 7, 14, 21, 28 Aug	21, 28 Jul and 4 Aug	7, 17, 24, 31 Aug and 7 Sept

Table 3.3 Results of small-plot insecticide efficacy experiments conducted on bell peppers at four locations in the Mid-Atlantic U.S. in 2005. All insecticides were applied ~weekly beginning at first appearance of fruit until final harvest.

Georgetown, Delaware

Treatment	Rate kg [AI]/ha	Mean± SE no. green peach aphids per 10 leaves			Cumulative no. marketable fruit per plot	% of fruit insect damaged
		After 4 sprays	After 6 sprays	After 7 sprays		
Lambda-cyhalothrin	0.03	44.2 ± 33.2 a	81.2 ± 23.8a	139.4 ± 38.0 a	NA	NA
Acephate	1.09	0.0 ± 0.0 b	0.2 ± 0.1 b	0.2 ± 0.2 b	NA	NA
Spinosad	0.026	0.8 ± 0.42 b	0.8 ± 0.4 b	3.4 ± 0.7 b	NA	NA
Indoxacarb	0.072	7.1 ± 5.2 ab	55.3 ± 39.7 a	139.8 ± 84.5 a	NA	NA
Methoxyfenozide	0.112	0.7 ± 0.41b	0.5 ± 0.4 b	2.9 ± 1.1 b	NA	NA
Untreated control	-	0.9 ± 0.51b	1.0 ± 0.3 b	2.0 ± 0.4 b	NA	NA

Salisbury, Maryland

Treatment	Rate kg [AI]/ha	Mean± SE no. green peach aphids per 10 leaves after 3 sprays	Cumulative no. marketable fruit per plot	% of fruit with insect damage
Lambda-cyhalothrin	0.03	1.3 ± 0.44 a	29.5 ± 8.5 a	29.2 ± 7.8 a
Acephate	1.09	0.0 ± 0.0 a	24.0 ± 2.6 a	26.6 ± 7.9 a
Spinosad	0.026	1.6 ± 0.4 a	23.4 ± 6.0 a	29.7 ± 2.3 a
Indoxacarb	0.072	15.5 ± 13.4 a	21.9 ± 7.2 a	17.3 ± 5.9 a
Methoxyfenozide	0.112	1.7 ± 0.5 a	37.3 ± 17.2 a	13.8 ± 6.1 a
Untreated control	-	1.1 ± 0.9 a	15.6 ± 5.8 a	33.1 ± 7.5 a

Rock Springs, Pennsylvania

Treatment	Rate kg [AI]/ha	Mean± SE no. green peach aphids per 10 leaves			Cumulative no. marketable fruit per plot	% of fruit with insect damage
		After 2 sprays	After 5 sprays	After 6 sprays		
Lambda-cyhalothrin	0.03	0.1 ± 0.1 a	21.9 ± 10.4 a	35.9 ± 7.9 a	141.0 ± 14.2 a	4.8 ± 1.1 b
Acephate	1.09	0.0 ± 0.0 a	0.0 ± 0.0 b	0.0 ± 0.0 b	155.3 ± 5.6 a	8.5 ± 2.4 b
Spinosad	0.026	0.5 ± 0.2 a	4.6 ± 1.2 b	4.6 ± 4.2 b	157.3 ± 11.1 a	5.9 ± 2.5 b
Indoxacarb	0.072	0.5 ± 0.3 a	1.9 ± 1.0 b	0.3 ± 0.1 b	177.3 ± 8.9 a	4.8 ± 0.1 b
Methoxyfenozide	0.112	0.4 ± 0.3 a	2.4 ± 0.5 b	13.0 ± 12.2 b	169.3 ± 6.6 a	7.2 ± 1.7 b
Untreated control	-	0.4 ± 0.3 a	0.4 ± 0.2 b	0.0 ± 0.0 b	144.8 ± 13.7 a	17.2 ± 2.6 a

Painter, Virginia

Treatment	Rate kg [AI]/ha	Mean± SE no. green peach aphids per 10 leaves			Cumulative no. marketable fruit per plot	% of fruit with insect damage
		After 3 sprays	After 6 sprays	After 7 sprays		
Lambda-cyhalothrin	0.03	0.6 ± 0.4 a	76.3 ± 25.6 a	158.0 ± 46.7 a	177.6 ± 14.4 a	2.5 ± 1.6 b
Acephate	1.09	0.0 ± 0.0 a	0.0 ± 0.0 b	0.0 ± 0.0 b	184.7 ± 29.5 a	0.5 ± 0.5 b
Spinosad	0.026	0.2 ± 0.1 a	0.0 ± 0.0 b	0.5 ± 0.5 b	169.2 ± 18.5 a	2.3 ± 0.6 b
Indoxacarb	0.072	0.2 ± 0.1 a	0.8 ± 0.3 b	3.2 ± 1.7 b	180.2 ± 14.9 a	1.0 ± 0.6 b
Methoxyfenozide	0.112	0.2 ± 0.2 a	0.3 ± 0.3 b	0.7 ± 0.7 b	170.7 ± 2.5 a	3.3 ± 1.3 b
Untreated control	-	0.7 ± 0.6 a	0.0 ± 0.0 b	0.4 ± 0.4 b	167.2 ± 17.0 a	10.5 ± 0.6 a

Means within a column with a letter in common are not significantly different according to ANOVA and Fisher's Protected LSD at the P = 0.05 level of significance.

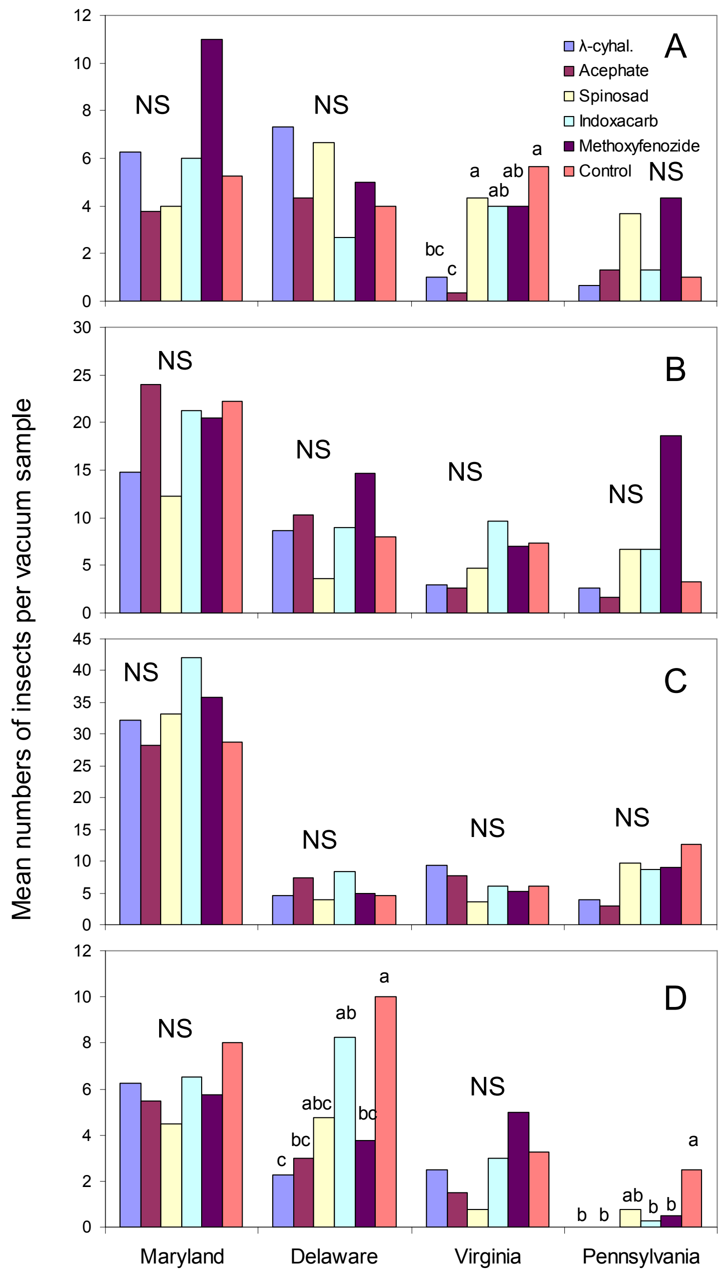

Fig. 1

Figure 3.1 Numbers of selected natural enemies collected from vacuum samples of pepper plots treated with various insecticides at four locations in the Mid-Atlantic States. All insecticides were applied weekly beginning at first fruit until final harvest.

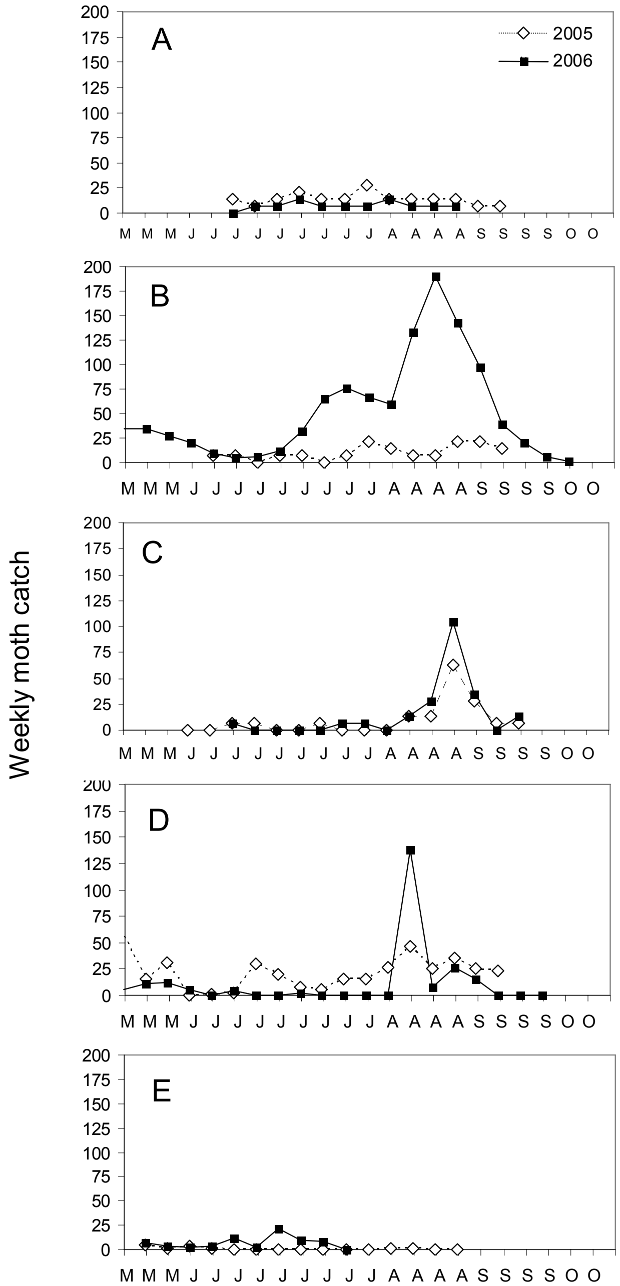

Fig. 2.

Figure 3.2 Weekly moth catch of *Ostrinia nubilalis* in blacklight or pheromone traps located at A) Rock Springs, PA; B) Queenstown, MD; C) Georgetown, DE; D) Painter, VA; and E) Virginia Beach, VA in 2005 and 2006.

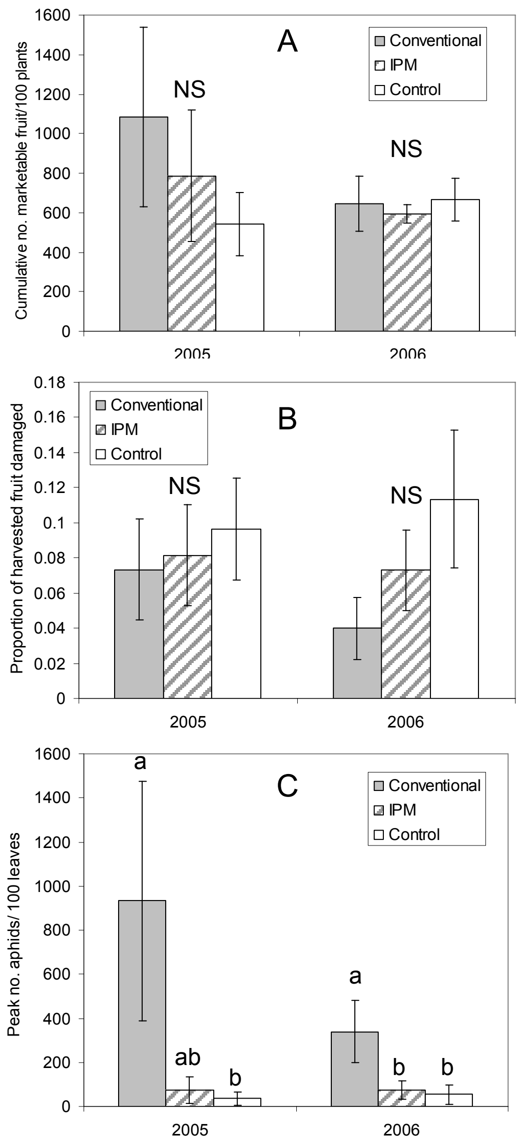

Fig. 3

Figure 3.3 Cumulative yield of marketable fruit (a); percentage of fruit damaged by lepidopteran pests (b); and green peach aphid densities (c) from bell pepper demonstration plots in the Mid-Atlantic States (n = 5 locations per year) under three different pest management strategies. Conventional strategy included weekly insecticide (acephate or lambda-cyhalothrin) applications from first fruiting until final harvest. The IPM strategy focused on three or four inundative releases of *Trichogramma ostriniae* to control *Ostrinia nubilalis* plus 0, 1 or 2 applications of the IGR insecticide, methoxyfenozide.

Research Summary:

The overall objective of this research was to improve the use of *Trichogramma ostriniae* for control of European corn borer (ECB), *Ostrinia nubilalis*, in integrated pest management in pepper. Optimizing releases of *T. ostriniae* and integrating more environmentally-friendly insecticides were two strategies explored to address the research objective.

Inundative releases of approximately 0.5 million *T. ostriniae* were made in separate potato fields on the Eastern Shore of Virginia in 2005 and 2006. Results showed that *T. ostriniae* dispersed quickly throughout the 0.4 ha sampling area. In 2005, wasps dispersed up to 32 meters within 1 day. In 2005 and 2006, wasps dispersed up to 45 meters within 4 days from central releases. High rates of parasitization (20-50%) were recorded at this distance and decreased with increasing distance. The relationship of distance to sticky card captures and sentinel egg mass parasitism was negative for both years. Cumulative displacement of sticky card captures and parasitism showed directionality in dispersal. Comparing cumulative displacement and cardinal direction, it appeared that wind conditions were likely a primary factor in the directionality of dispersal. Despite drift and decreasing parasitism with distance, *T. ostriniae* effectively dispersed throughout 0.4 ha with the highest rates of parasitization occurring within 16m of the central release. Multiple release points or approximately 1 release per 0.10 ha (= 4 release points per acre) should be made for effective control of ECB.

Release densities of 0, 5, 20, and 50 *T. ostriniae* per plant were tested in four spatially-isolated plots of peppers at four locations. At the two Virginia locations, we observed relatively low rates of parasitization and no significant effect from release density. In Pennsylvania, the highest release density of 50 wasps/plant resulted in a significantly greater mean proportion of parasitized sentinels than the 5 wasps/plant and untreated control plots at 3 days post release. Untreated control plots in Maryland and the Eastern Shore of Virginia had as much parasitism as plots receiving releases of *T. ostriniae* indicating high levels of natural egg parasitism in the fields. Overall, results showed ambiguity in the data and high levels of natural parasitism occurring on *Ephestia* eggs. A number of experimental design and quality control issues negatively affected the accuracy of the experiment. The difficulty in assessing optimal *T.ostriniae* release rates can be improved and more research is needed to establish effective release strategies for *Trichogramma* egg parasitoids with respect to release density.

The insecticides evaluated in this research consisted of indoxacarb, methoxyfenozide, spinosad, acephate, lambda-cyhalothrin and an untreated control. The biorational insecticides, spinosad, indoxacarb, and methoxyfenozide provided similar control of *O. nubilalis* in bell pepper as the conventional acephate or lambda-cyhalothrin treatments. No significant treatment effect was found on cumulative number of marketable (undamaged) for insecticide efficacy field experiments or IPM demo plots. However, there was a significant treatment effect on the percentage of fruit damaged by *O. nubilalis* in Pennsylvania and Virginia. Untreated control plots at both locations had the highest damage and no differences were found among the insecticide treatments.

The predominant natural enemies collected in pepper were parasitic hymenoptera (mostly Chalcidoidea, Braconidae, and Ichneumonidae), predatory bugs (mostly Anthocoridae - *Orius* spp.), ladybird beetles (Coccinelidae), and spiders (Aranea). In Virginia, lambda-cyhalothrin and acephate significantly reduced cumulative number of ladybird beetles compared with untreated control plots. In Delaware, lambda-cyhalothrin, acephate, and methoxyfenozide significantly reduced the cumulative number of spiders compared with untreated control plots. Similarly in Pennsylvania, lambda-cyhalothrin, acephate, methoxyfenozide, and indoxacarb significantly reduced the number of spiders, compared with untreated control plots. Collections of natural enemies from the insecticide plots did not reveal significant treatment differences of most natural enemies. Considering most predators and parasitoids are highly mobile organisms and the potential for re-colonization of sprayed plots, we believe border areas and/or spacing between insecticide plots is critical when surveying for natural enemies.

In both years, multiple sprays of lambda-cyhalothrin caused severe aphid flares. In one field, multiple sprays of indoxacarb resulted in similar aphid outbreaks. There were significantly greater numbers of aphids in conventional insecticide plots compared with IPM and untreated control plots in 2005 and nearly a significant effect in 2006. At three of the four locations, aphid densities were significantly higher in the lambda-cyhalothrin plots compared with any other insecticide treatment or the control.