BIOLOGICAL CONTROL OF THE TARNISHED PLANT BUG, LYGUS LINEOLARIS (HEMIPTERA: MIRIDAE), BY PERISTENUS SPP. (HYMENOPTERA: BRACONIDAE), IN NEW YORK APPLE ORCHARDS

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Master of Science

by

Lora Anne Crampton

May 2007

ABSTRACT

Lygus lineolaris is a pest of many economically important crops. While the species comprising the *Peristenus pallipes* complex are native parasitoids of *L. lineolaris,* they do not provide adequate biological control of *L. lineolaris*. As a result, the European parasitoid, *Peristenus digoneutis,* was introduced in forage alfalfa. *Peristenus digoneutis* successfully dispersed from forage alfalfa to strawberries, suggesting that *P. digoneutis* may disperse into other high value crops. In apples, *L. lineolaris* feeding causes puncture wounds, scabs or cat facing on the fruit.

The success of a classical biological control introduction is dependent on both monetary and ecological costs and benefits. The dispersal and persistence of *P. digoneutis* into multiple crops is necessary for monetary benefit. Furthermore, if pesticides disrupt classical biological control no monetary gain can be made. In addition, an ecological cost of classical biological control is displacement of native species. The objectives of this study are to-1) establish the presence of the introduced parasitoid, *Peristenus digoneutis,* in New York apple orchards, 2) evaluate changes in rates of parasitism of *L. lineolaris* by native and introduced *Peristenus* spp. since the initial dispersal of *P. digoneutis* into western New York, 3) determine how four pesticide regimes- standard, reduced risk, organic and abandoned orchards- affect *Peristenus* spp. parasitism.

During this two year study *L. lineolaris* were collected from New York apple orchards treated with standard, reduced risk, organic insecticides. This is the first record of *P. digoneutis* parasitizing *L. lineolaris* in apple orchards. When compared to rates of parasitism collected from strawberries in 1999 there is an increase in the total *L. lineolaris* parasitism by *Peristenus* spp. in western New York. Additionally, there was a reduced rate of parasitism by native parasitoids in the *P. pallipes* complex,

suggesting that *P. digoneutis* may be competitively displacing natives. Insecticide treatment had a significant effect on rates of *Peristenus* spp. parasitism. When compared to standard insecticide regimens there was significantly higher parasitism in reduced risk when compared with standard orchards. Conversely, there was significantly less parasitism in organic orchards when compared to standard orchards.

BIOGRAPHICAL SKETCH

Lora lives in Oregon where she enjoys the long rainy winters, ice fishing and hang gliding.

Dedicated to 2/3 of a superhero: Susan Rottschaefer & Sarah Perdue

ACKNOWLEDGMENTS

I gratefully acknowledge funding from USDA-ARS (cooperative agreement 58-1926-0-039). I also owe many thanks to my advisor, Mike Hoffmann, and committee Kim Hoelmer and Greg Loeb. Thanks to Bryan Danforth and Brian Lazzaro, for generously allowing me to use their labs. A special thanks to Bill Day and Ian Merwin for their helpful suggestions and assistance. Many thanks to Jeffery Gardner for his encouragement and SAS help. Thank you to Sylvie Pitcher for all of the molecular help.

Many thanks to the 2nd floor entomology staff (Sue Ganey, Marian Hartill, Carol Hunter, David Smith, Janice Waller and Lisa) and Angela Pauchard for being helpful beyond their job descriptions. Thanks also to Linda Rayor and Ward Tingey for great teaching experiences. Very special thanks to Pepe for keeping the elevators from crushing me.

Thanks to all of the friends I make in Ithaca. I especially want to thank Susan Rottschaefer for more than I can express here. I also want to express gratitude to my family: Charles, Krista, Linda, and Sara Crampton. Thanks to my mother, who always has the highest expectations. Thanks to my father for all of his encouragement and introducing me to science. Thanks to my sisters for being strong, self-confident, successful role models. Finally, I wish to thank William Crampton, for believing and telling me I can do what ever I want.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

New York Apple Production

Apple production has a long history in New York; the first domestic apples, *Malus domestica* Borkhausen, were planted in the 16th century along the eastern seaboard. Today, New York produces an average of 25 million bushels of apples a year, ranking New York second only to Washington State in national apple production. The principle regions of apple production in New York are the Champlain Valley, Hudson Valley, Central Region, Lake Country and Niagara Frontier (New York Apple Association 2006). McIntosh, Empire, Red Delicious, Cortland, Golden Delicious, Red Rome, Idared, Crispin, Paula Red and Gala are the primary varieties grown in New York.

Lygus lineolaris ***(Palisot de Beauvois)***

The tarnish plant bug (TPB), *Lygus lineolaris* (Hemiptera: Miridae), is a polyphagous pest of over 322 plants, including over fifty economically important crops such as strawberries, raspberries, apples, herbs, tomatoes and cotton (Young 1986, Tingey and Pillemer 1977). Hammer (1939) proposed that TPB damage to apples was from oviposition; however, it is now apparent that most TPB damage is caused by adults feeding on the leaves, developing flowers, buds, and young apples (Boivin and Stewart 1982). It is difficult to assess the economic losses due to TPB leaf feeding. *Lygus lineolaris* feeding on developing flowers and fruit results in abscission of flower buds, fruit underdevelopment, and malformations (Prokopy and Hubbell 1981). Abscission of buds is of no economic consequence since only 2-5 % of blossoms are required to produce an adequate apple harvest; however, fruit underdevelopment and malformations are of economic importance since culling and downgrading result (Michaud et al. 1989 and Weires et al. 1985). Fruit malformations from TPB can be characterized as puncture wounds, scabs or cat facing. A four-year study found up to 12% malformations from TPB damage (Agnello et al. 2005). Apple varieties vary significantly in susceptibility (Weires et al. 1985).

TPB are damaging to apples because flower bloom coincides with adult dispersal from overwinter shelters. Since few other plants are in bloom, TPB are attracted to the early blossoming apple trees and TPB disperse frequently, seeking out flowering plants (Khattat and Stewart 1980). As such, TPB disperse from apples once their blossoms wane and understory foliage begins to bloom (Boivin and Stewart 1983).

Mated female TPB overwinter in sheltered environments such as hedgerows and margins of orchards. In NY *L. lineolaris* emerge from overwintering sites in April. After feeding, females lay small (1mm), cream colored eggs in understory plant tissue. Nymphs emerge about seven days later and undergo 5 instars. They begin life a bright green color, turning brown with maturation. TPB has from 2-5 generations per year depending on the climate, host and seasonal variation (Spangler et al. 1991).

During the nymph stage TPB is easily distinguished by five characteristic black dots, two pairs are behind the head and one is centered between the developing wing buds (Spangler et al. 1991). Adult TPB are 6-6.5 mm in length, greenish brown, with characteristic reddish brown marks on the wings and a small yellow triangle forming the scutellum on the dorsal surface of the abdomen (Liu et al. 2003).

Peristenus digoneutis *Loan*

In the case of TPB, which is endemic to North America (Kelton 1975), native parasitoids do not provide significant rates of parasitism to control TPB (Day 1987). In an effort to reduce populations of the widespread TPB, USDA scientists examined parasitoids of the European plant bug*, Lygus rugulipennis* Poppius (Hemiptera: Miridae). *Peristenus digoneutis,* a parasitoid of *L. ruglipennis* showed potential as a biological control agent of *L. lineolaris*. Classical biological control generally involves introducing natural enemies from endemic regions of the pest (Hayek 2004). In this case, *P. digoneutis* and *L. lineolaris* formed a new host association however, *L. lineolaris* and *L. ruglipennis,* are closely related and occupy the same ecological niche in North America and Europe, respectively.

Peristenus digoneutis was collected from France and successfully introduced to several alfalfa fields in the Northern United States (Day et al. 1990). Forage alfalfa fields were ideal sites to release *P. digoneutis* because the parasitoid had access to large populations of TPB, in a crop where insecticides were not used routinely, if at all. USDA scientists postulated that populations of *P. digoneutis* could build up in forage alfalfa then migrate to high value crops. Robust populations of *P. digoneutis* gradually developed in forage alfalfa, eventually reducing the total TPB population by 75% (Day 1996). The expectation for *P. digoneutis* to spread from the alfalfa fields to high value crops was supported by Tilmon and Hoffmann (2002) who demonstrated that *P. digoneutis* did spread to strawberries in New York State. There is an ongoing need to determine if other crops benefiting from the release of *P. digoneutis* (Day et al. 2003).

P. digoneutis females parasitize the nymphal stage of *Lygus lineolaris*. The female has a short ovipositor; therefore, she must perform an impressive display of acrobatics during oviposition. She braces with her forelegs extended dorsally as she thrusts her abdomen toward her nymphal victim. She lays a single egg that remains in the hemolymph for approximately 5 days before hatching. The first instar has fang-like falcate mandibles, thought to aide in the destruction of other parasitoids that may inhabit the host's hemolymph. Probably to avoid detection by the host's immune system the second instar remains within the skin of the first instar, which forms a characteristic tail. The third instar only remains within the host for a few hours, quickly exiting through the abdomen and dropping to the ground. The third instar burrows into the soil where it spins its cocoon within 24 hours. The cocoon and preimago will either undergo diapause for 8-10 months, or an adult will emerge within 10 days (Carignan et al. 1995). *Peristenus digoneutis* is bivoltine, adults are found all summer long (May-September) with peaks of abundance in early June and in late July (Loan and Bilewicz-Pawinska 1973, Tilmon 2001).

***Other introduced parasitoids of* Lygus lineolaris**

Another European species, *Peristenus rubricollis* (Thomson), was released into the U.S. at the same time as *P. digoneutis*. Preferring *Adelphocoris* spp., *P. rubricollis* is only occasionally found parasitizing *L. lineolaris* (Day 2005). In their review of the Nearctic species Goulet and Mason (2006) describe *P. conradi* Marsh, as a conspecific with *P. rubricollis*.

***Native Parasitoids of* Lygus lineolaris**

Peristenus pallipes is a species complex, found in the Holarctic region, consisting of nine species divided into two groups--*P. mellipes* and *P. dayi* (see Table 1.1). The *P. dayi* group consists of two species, *P. brausae* Goulet and *P. dayi* Goulet. The *P. dayi* group is rarely found on *L. lineolaris*, preferring *Adelphocoris lineolatus* (Goeze). There are seven species within the *P. mellipes* group: *P. carcamoi* Goulet , *P. mellipes* (Cresson) , *P. otaniae* Goulet, *P. pseudopallipes* Loan, *P. broadbenti* Goulet, *P. gillespiei* Goulet, and *P. howardi* Shaw. Within the *P. mellipes* complex three species (*P. carcamoi, P. gillespiei,* and *P. broadbenti*) do not utilize *L. lineolaris* as a host. The primary host of *P. howardi* is the western TPB, *L. hesperus* Knight, while the other three, *P. howardi, P. mellipies, P.otaniae* and *P. pseudopallipes*, are commonly found on *L. lineolaris* (Goulet and Mason 2006). Prior to Goulet and

Mason (2006), *P. pallipes, P. psuesdopallipes* and *P. howardi* were the only recognized species of the *P. pallipes* complex. Various species of the genus *Leiophron* are also occasionally found parasitizing *L. lineolaris*, however this study focuses on *Peristenus*.

Table 1.1 Description of the *Peristenus pallipes* species complex, compiled from Goulet and Mason (2006).

Group	Species	Primary Host	Generations	Peak Occurrence
P. mellipes				
	P. carcamoi	*Lygus* spp.	univoltine	early June
	P. mellipes	*L. lineolaris*	univoltine	early-mid June
	P. otaniae	*L. lineolaris* and *Lygus* spp.	univoltine	late June-early July
	P. pseudopallipes	*L. lineolaris* and *L. vanduzeei*	univoltine	late July-early Aug
	P. broadbenti	*Lygus* spp.	univoltine	mid July
	P. gillespiei	*Lygus* spp.	univoltine	early June
	P. howardi	*L. hesperus*	multivoltine	mid June, late July, early Sept
P. dayi				
	P. brausae	*Adelphocoris lineolatus*	univoltine	late June-early July
	P. dayi	*Adelphocoris lineolatus*	univoltine	late May

Research objectives

The objectives of this study were to: 1) establish the presence of *P. digoneutis* in apple orchards, 2) determine if *Peristenus* spp. parasitism rates of *L. lineolaris* differ between active and abandoned orchards, 3) determine if *Peristenus* spp. parasitism rates were different between standard, reduced risk and organic pest management regimens and 4) assess if rates of parasitism by the native, *P. pallipes* and the introduced *P. digoneutis* have changed across regions of NY over time.

REFERENCES:

Agnello, A., J. Nyrop, H. Reissig, and D. Straub. 2005. Reduced-Risk Pest Management in apples using pheromone disruption and a selective pesticide program. USDA RAMP PROJECT final research report.

Boivin, G., and R.K. Stewart. 1982. Identification and evaluation of damage to McIntosh apples by phytophagous mirids (Hemiptera: Miridae) in southwestern Quebec. Can. Entomol. 114: 1037-1045.

Boivin,G., R. K. Stewart. 1983. Sampling technique and seasonal development of phytphagous mirids (Hemiptera:Miridae) on apple in southwestern Quebec. Ann. Entomol. Soc. Am. 76(3):359-364

Boivin, G., R. K. Stewart. 1983. Seasonal development and interplant movements of phytophagous mirids (Hempiptera: Miridae) on alternate host plants in and around an apple orchard. Ann. Entomol. Soc. Am. 76(4):776-780.

Carignan, S., Boivin, G., Stewart, R.K. 1995. Developmental biology and morphology of *Peristenus digoneutis* Loan (Hymenoptera: Braconidae: Euphorinae). Biological Control. 5: 553-560.

Day, W. H. 1987. Biological control efforts against *Lygus and Adelphocoris* spp. Infesting alfalfa in the United States, with notes on other associated mirid species. USDA-ARS 64. pp. 20-39.

Day, W. H., R.C. Hedllund, L.B. Saunders, D. Coutinot. 1990. Establishment of *Peristenus digoneutis* (Hymenoptera: Braconidae), a parasite of the tarnished plant but (Hemiptera: Miridae) in the USA. Environ. Entomol. 19, 1528-1533.

Day, W. H. 1996. An evaluation of biological control of the tarnished plant bug, *Lygus lineolaris* (Hemiptera:Miridae), in alfalfa by the introduced parasite *Peristenus digoneutis* (Hymenoptera: Braconidae). Environ. Entomol. 25: 512-518.

Day, W. H., A. T. Eaton, R. F. Romig, K. J. Tilmon, M. Mayer, T. Dorsey. 2003. *Peristenus digoneutis* (Hymenoptera: Braconidae), a parasite of *Lygus lineolaris* (Hemiptera: Miridae) in northeastern United States alfalfa, and the need for research on other crops. Entomological News. 114(2): 105-111.

Day, W.H. 2005. Changes in abundance of native and introduced parasites (Hymenoptera: Braconidae), and of the target and non-target plant bug species (Hemiptera: Miridae), during two classical biological control programs in alfalfa. Biological Control 33: 368-374.

Goulet H., Mason, P.G. 2006. Review of the Nearctic species of (Hymenoptera: Braconidae: Euphorinae) (Hemiptera: Miridae: Mirini). Zootaxa. 1323: 1-118pp.

Hammer. 1939. The tarnished plant bug as an apple pest. Journal of Economic Entomology. 32(2):259-264.

Hayek, A. 2004. Natural Enemies: an introduction to biological control. Cambridge University Press. Cambridge, U.K.

Kelton, L.A. 1975. The *Lygus* bugs (genus *Lygus* Hahn) of North America (Heteroptera: Miridae). Memoirs of the Entomological Society of Canada 95.

Khattat, A. R., R. K. Stewart. 1980. Population fluctuations and interplant movements of *Lygus lineolaris*. Ann. Entomol. Soc. Am. 73:282-287.

Liu, H., M. Skinner, B. L. Parker, W. H. Day. 2003. Recognizing tarnished plant bug damage: vegetables, fruits, herbs. University of Vermont, Entomology Research Laboratory brochure.

Loan, C. C., and T. Bilewicz-Pawinska. 1973. Systematic and biology of four Polish species of *Peristenus* Foerster (Hymenoptera: Braconidae, Euphorinae). *Environmental Entomology*, 2, 271–277.

Michaud, O.D., G. Boivin, R. K. Stewart. 1989. Economic threshold for tarnished plant bug (Hemiptera: Miridae) in apple orchards. Journal of Economic Entomology. 82(6):1722-1728.

New York Apple Association 2006. www.nyapplecountry.com/fastfacts.htm.

Prokopy, R. J., G. L. Hubbell. 1981. Susceptibility of apple to injury by tarnished plant bug adults. Environ. Entomol. 10(6): 977-979.

Spangler, S.M., R. W. Weires, A.M. Agnello. 1991. Tarnished plant bug. Cornell Cooperative Extension. Insect Identification Sheet No. 21.

Tilmon, K. J. 2001. Biological Control of *Lygus lineolaris* (Hemiptera: Miridae), a generalist herbivore, by native and introduced *Peristenus* (Hymenoptera: Braconidae). Ph.D. Thesis, Cornell University. Ithaca, New York, U.S.A. 125 pp.

Tilmon, K. J., Hoffmann, M.P. 2002. Biological control of *Lygus lineolaris* by *Peristenus* spp. in strawberry. Biological Control. 26:287-292.

Tingey, W.M. and E.A. Pillemer. 1977. *Lugus* bugs: crop resistance and physiological nature of feeding injury. Bull. Entomol. Soc. Am. 23:277-278.

Weires R. W., J. R. VanKirk, W. D. Gerling and F. M. McNicholas. 1985. Economic losses form the tarnished plant bug on apple in eastern New York. J. Agric. Entomol. 2(3): 256-263.

Young, O.P. 1986. Host plants of the tarnished plant bug, *Lygus lineolaris* (Heteroptera: Miridae). Ann. Entomol. Soc. Am. 79(4):747-762.

CHAPTER TWO

Biological control of *Lygus lineolaris* (Hemiptera: Miridae) by *Peristenus digoneutis* (Hymenoptera: Braconidae) in apples, a post introduction study in New York

Introduction

Classical biological control, the importation and release of natural enemies to control introduced (exotic) pests, has the potential to reduce pest numbers, and increase crop yield (McConnachie et al. 2003, Norgaard 1988, Ervin et al. 1983). In financial terms classical biological control is a cost-effective option for pest reduction. Cate (1990) estimated that for every dollar invested in classical biological control programs there is an estimated $30 return. Cullen and Whitten (1995) determined that the economic return from just four successful classical biological control projects exceeded the cost of research and implementation for all projects, including those that failed.

The successful establishment of a new natural enemy by way of classical biological control and consequent control of a pest has several advantages over insecticides. For example, once a natural enemy is introduced and becomes established the population is self perpetuating, making classical biological control a long term and cost-effective solution requiring minimal or no management. In contrast, insecticides often require repeated applications to keep pests below economically damaging levels. Such scenarios may result in selection of populations resistant to insecticides. Excessive use of insecticides may have ecological, monetary and human health costs (Beane Freeman et al. 2005, Eskenazi and Maizlish 1988, Rosenstock et al. 1991) that are rare with successful classical biological control (van Lenteren et al. 2003). To fully appreciate the impact of classical biological control a complete evaluation, including an examination of economic and ecological costs and benefits, is necessary.

Since it is difficult to predict the effectiveness of an introduced natural enemy (Hopper 1995, Louda et al. 2003), post introduction studies are needed to monitor and evaluate classical biological control agents. In addition to assessing its impact on the target pest species, evaluating the ecological impacts of an introduced natural enemy on non-target populations is also important (Louda et al. 2003). Taken together, post release studies can provide information on economic and ecological cost-benefits, dispersal, establishment and persistence all of which is valuable for evaluating the ultimate success of the introduction.

This post introduction study examines *Peristenus digoneutis* Loan, an introduced parasitoid of the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), and a complex of native *Peristenus* spp. parasitoids. Prior to the release of *P. digoneutis* several native species in the *P. pallipes* complex parasitized an average of 9% of *L. lineolaris* nymphs (Day 1990). This level of parasitism did not provide adequate control of *L. lineolaris* (Day 1990) so in the 1980's the U.S. Department of Agriculture began introductions of *P. digoneutis* as a classical biological agent for control of *L. lineolaris*.

The native *Peristenus pallipes* species complex consists of nine Holarctic species divided into two groups--*Peristenus mellipes* and *Peristenus dayi* (see Table 1.1). The *P. dayi* group is rarely found on *L. lineolaris*, preferring *Adelphocoris lineolatus* (Goulet and Mason 2006). Of the seven species within the *P. mellipes* group three are commonly found parasitizing *L. lineolaris*: *P. mellipes* (Cresson), *P. otaniae* Goulet and *P. pseudopallipes* Loan (Goulet and Mason 2006). This research examines native parasitoids at the taxonomic level of the *P. pallipes* species complex.

Peristenus digoneutis, the parasitoid selected for introduction, is a bivoltine, solitary, endoparasitoid endemic to Europe where it parasitizes a European plant bug, *Lygus rugulipennis* Poppius. This is an atypical example of a classical biological control effort because of the new parasitoid-host association formed between *P. digoneutis* and *L. lineolaris;* however, *L. lineolaris* is a close taxonomic relative and ecological homologue of *L. ruglipennis*.

Once released, *P. digoneutis* established in forage alfalfa at a New Jersey release site and has since dispersed as far north as Ontario, Canada and as far west as Ohio, U.S. (Day, personal communication). Robust populations of *P. digoneutis* have developed in forage alfalfa, and successfully dispersed and established in strawberries (Tilmon 2001), suggesting that *P. digoneutis* may be found in a range of cropping systems.

A second European species, *P. rubricollis* (Thomson) was also released at approximately the same time, to help control pests in the family Miridae (Day 1987). However, *Peristenus rubricollis* is only occasionally found parasitizing *L. lineolaris,* preferring another mirid, *Adelphocoris lineolatus* (Goeze), a pest of alfalfa (Day 2005).

The target of these classical biological control efforts, *Lygus lineolaris,* is a polyphagous pest of many economically important crops in North America (Young 1986, Tingey and Pillemer 1977). In apples, adult *L. lineolaris* damage the fruit by feeding, which results in fruit malformations, which can be characterized as puncture wounds, scabs or "cat facing". A four-year study found up to 12% of fruit examined had damage caused from *L. lineolaris* feeding (Agnello et al. 2005). While *L. lineolaris* is considered a minor apple pest it is a good target for biological control because insecticides cannot be applied when the trees are being pollinated. This is also the time when *L. lineolaris* poses the greatest risk of damage to apples. Furthermore, *L. lineolaris* is also a good target for biological control because there is a linear relationship between adult *L. lineolaris* damage to apples and numbers of *L. lineolaris* (Michaud et al. 1989, Prokopy and Hubbell 1981). However, since *Peristenus* spp. are nymphal parasitoids it is also important that there is also a correlation between nymphal *L. lineolaris* parasitism rates by *Peristenus* spp. and nymphal density of *L. lineolaris* (Day 2005, Day 1996). As such, a reduction in *L. lineolaris* nymphal densities of by *P. digoneutis* should correspond to an overall reduction in adult densities, and consequently a reduction in apple damage by *L. lineolaris*.

The objectives of this research included determining 1) if *P. digoneutis* has been successfully in establishing in apple orchards in New York State, and if established, 2) the levels of parasitism in orchards under different pest management regimens and 3) how the frequency of native parasitoids in the *P. pallipes* species complex may have changed since the expansion of *P. digoneutis* into western New York.

Materials and Methods

Samples of *L. lineolaris* nymphs were collected from nine commercial apple orchards and three alfalfa fields located in five counties of New York (NY). In central NY orchards were in Tompkins County-West Haven Farm (WH) and Onondaga County-Apple Acres (AA) and Beak & Skiff (BS). In western NY Singer Farms (SF) and Russell Farm (RF) were located in Niagara County while Brown Farm (BF) and Lynn-Oakes (LO) were in Orleans County. In eastern NY Ulster County orchards were represented by Wright Brothers (WB) and Prospect Hill (PH). Nymphs collected from apple orchards in this study were also used for an analysis of the effect of pesticide regimen on parasitism of *L. lineolaris* by *Peristenus* spp. (for complete details see Chapter 3). The four regimens examined were abandoned- untreated orchards (ABD), standard- conventional orchards (ST), reduced risk- orchards use newer soft insecticides, designed to replace the soon to be lost standard products (RR), and organic- certified by the United States Department of Agriculture (OG), as summarized in Table 2.1. Several alfalfa (ALF) field were included in this study because rates of *Peristenus* spp. parasitism of *L. lineolaris* in this crop have been previously reported (Day 2005, Tilmon & Hoffmann 2002); as such, ALF served as a benchmark to compare with parasitism in apple orchards and to assess changes in parasitism rates and possible displacement of native parasitoids since the dispersal of *P. digoneutis* into western New York.

Table 2.1 Insecticide regimens sampled in this study. Note that all RR and one of the SF OG were only available in 2006. Orchards in central New York: Apple Acres (AA), Beak and Skiff (BS) and West Haven (WH). Western: Brown Farm (BF), Russell Farm (RF), Singer Farms (SF) and Lynn-Oakes (LO). Eastern New York: Wright Brothers (WB) and Prospect Hill (PH).

Insecticide regimen	Active ingredients of insecticides	Farms
Abandoned (ABD)	No treatments	BF BS WB
Standard (ST)	acetamiprid, carbaryl, chlorpyrifos, dimethyl-phosphorodithioate, endosulfan, etoxazole, esfenvalerate, etoxazole, fenpropathrin, gamma-cyhalothrim, kresoxim-methyl, lambda-cyhalothrin, methoxyfenozide, permethrin, phosmet, pyridaben, pyriproxyfen,	AA BS BF LO RF SF WB
Reduced Risk (RR) Only available in 2005	clofentizine, pyridaben, etoxazole, thiamethoxam, pyriproxyfen, imidacloprid, indoxacarb, acetamiprid, benzoic acid, spinosad and petroleum oil	AA BS BF LO RF WB
Organic (OG)	Certified organic by USDA- pyrethrum, rotenone, or ryania; or minerals, such as boric acid, cryolite, diatomaceous earth sulfur, copper, kaolin clay	SF- 2 OG PH WH

Nymphs were collected from the ground cover in an approximately 1 acre area located in the middle of each treatment regimen at each orchard. Ground cover consisted of *Dactylis* spp., *Trifolium* spp. *, Vicia* spp., *Hieracium* spp,. *Plantago* spp., *Taraxacum* spp., *Leontodon* spp., *Galium* spp., and grass. In OG(P) *Galium* spp. constituted about 80% of the ground vegetation. Orchards were sampled weekly (except when prevented by rain or insecticide applications) using a sweep net until either 24-30 *L. lineolaris* nymphs were collected or 2.5 hours had passed (whichever came first). Similarly, samples from alfalfa fields were collected by sweep net. In ALF, an area at least 30 ft. from the perimeter was swept with at least fifty passes of the net. All nymphs collected were preserved in 95% ethanol, and stored on ice until transported to the laboratory and transferred to a -20°C freezer.

Rates of parasitism were determined using PCR and restriction digests as described by Tilmon (2001). This technique allowed larval identification of *Peristenus* spp. in *L. lineolaris*. Briefly, the process used consisted of four steps; first - DNA was extracted from each *L. lineolaris* nymph and possible parasitoid within, second - polymerase chain reaction (PCR) with primers specific to *L. lineolaris* confirmed success of the extractions, third- another PCR with specific primers amplifed *Peristenus* spp. differentiating parasitized from unparasitized nymphs, fourth- parasitized samples were identified to species using a restriction enzyme that cut the PCR products of parasitized samples to a specific length, revealing the species or species complex of the parasitoid. For a complete description of the method see Tilmon et al. (2000). DNA was extracted using DNAzol, (Invitrogen Carlsbad, CA) following the manufacture's protocol except the 'Lysis step' was scaled down from 1ml to 100ul. Primers, C1-J-2252, C1-J-2183 and TL2-N-3014, where obtained from Integrated DNA Technologies (Coralville, IA). *Taq* polymerase and restriction enzyme were obtained from Promega (Madison WI).

During the time span of the research reported herein, Goulet and Mason (2006) published a major revision of the genus *Peristenus*; several new species were identified while other previously distinct species were combined. A significant change was the creation of the *P. pallipes* species complex, which includes *P. pallipes* and *P. pseudopallipes*. Since this is a recent revision, the nine new species within *P. pallipes* complex are not differentiated by the molecular identification technique used in this study, although *P. pallipes* and *P. pseudopallipes* can be differentiated by collection time because they occur at different times of the season (May = *P. pallipes*; July-August = *P. pseudopallipes*). There is, however, overwhelming evidence that all species are accounted for- at the taxonomic level of *P. pallipes* species complex: 1) the primers recognize *P. digoneutis* and *P. rubricollis* (formerly *P. conradi*) which are less closely related then species within the *P. pallipes* complex, so it is improbable that a species within *P. pallipes* complex will not be recognized by the primers, 2) Tilmon et al. (2000) could not differentiate the species previously identified as *P. pallipes* and *P. pseudopallipes,* which are now part of the *P. pallipes* complex indicating that the restriction digest is specific to the level of species complex, 3) Tilmon et al. (2000) showed no difference between the parasitism rates and species composition obtained by three different techniques-- rearing, dissection and the molecular identification-- this indicates that all parasitoids were represented using the molecular identification technique. Based on this evidence it is still reasonable to use the molecular identification technique changing only the names of the *P. pallipes* and *P. pseudopallipes* to *P. pallipes* complex, and *P. conradi* to *P. rubricollis.*

After the species composition of *Peristenus* was determined for 2005-2006, the data from western and eastern NY were used to compare with the 1997-1999 rates of parasitism compiled from Tilmon (2001). At that time *P. digoneutis* had recently expanded into western New York. The data were collected in strawberry and alfalfa fields in eastern (1997-1999) and western (1999) New York. Current eastern New York samples were collected to account for any differences that could be attributed to seasonal variation, sampling location or difference in parasitism between crops.

Statistical Analysis

χ^2 tests for homogeneity were used to assess regional and temporal changes in parasitism, and a Bonferroni correction for multiple comparisons was applied to the levels of significance. Statistical analyses were performed using SAS 9.1 (2003).

Results

Parasitism rates of *L. lineolaris* by *Peristenu*s spp. were relatively high over the two years of this study, exceeding 50% on 10% of the 203 samples collected. When analyzed according to the pest management regimens, parasitism of nymphs by *Peristenus* spp. over the two year study was 29.5% in the RR regimen, 17.8% in OG regimen, 27.4% in the ST regimen and 23.2% in ABD regimen.

Summed across all orchard treatments, alfalfa and all dates 24.9% of all nymphal stages of *L. lineolaris* collected were parasitized by *Peristenus* spp. Most of the nymphs were parasitized by the introduced *P. digoneutis* which were found in 21.7% of nymphs examined. The *P. pallipes* complex parasitized 2.1%, while *P. rubricollis* was represented by less than 0.1%. Multiple parasitism by *P. pallipes* complex and *P. digoneutis* was observed in 0.8% of the nymphs examined. The rates of parasitism by *Peristenus* spp. and species composition for all *L. lineolaris* nymphs examined are reported by week in Figure 2.1.

In both western and eastern New York *P. digoneutis* comprised most of the *Peristenus* found, parasitizing 18.9% and 17.4% of the *L. lineolaris* nymphs examined, respectively. *Peristenus pallipes* parasitized 3.8% of the *L. lineolaris* nymphs collected in eastern NY and 0.9% nymphs from western NY. Only 2 of the1034 nymphs collected from the east were parasitized by *P. rubricollis* and none were found in Western NY. Total parasitism in western NY was 20% and eastern NY was 23% (see Table 2.2)

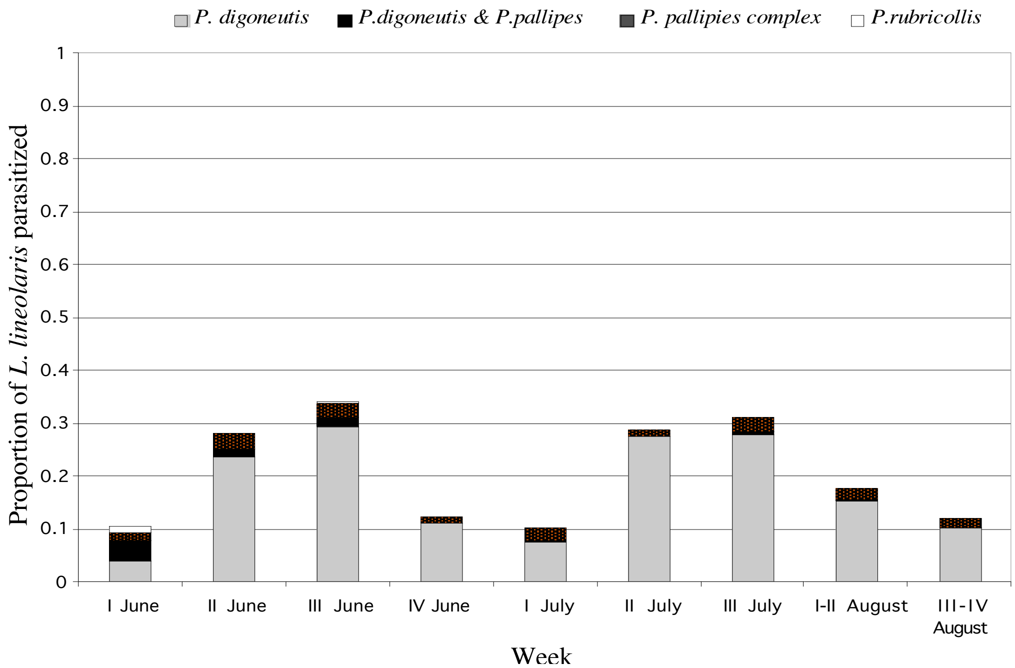

Figure 2.1. Species composition of *Peristenus* spp. parasitizing *L. lineolaris* nymphs collected from ALF, and apple orchards (ST, OG, ABD, RR) combined data collected in 2005 and 2006.

Table 2.2. Percent parasitism of *L. lineolaris* nymphs by *Peristenus* spp. in different regions of New York. Samples collected in 2005-2006 are from apple orchards and alfalfa fields. Data for 1997-1999 compiled from Tilmon (2001), and collected in strawberries and alfalfa.

Years	Region	N	Total Percent Parasitism
2005-2006			
	Eastern	1034	23.0
	Western	1585	20.0
1997-1999			
	Eastern	3048	19.7
	Western	818	12.3

There was no significant difference in total *Peristenus* spp. parasitism in Eastern NY between samples collected in 2005-2006 and those collected in 1997-1999 (χ^2 =4.12; df=2; p = 0.1274)(See Table 2.2). There was also no significant difference in total *Peristenus* spp. parasitism between eastern and western NY in the 2005-2006 samples (χ^2 =5.102; df=2; p = 0.078). However, in Western NY there was significantly higher total parasitism in samples collected in 2005-2006 when compared to those collected in 1997-1999(χ^2 =21.99; df=2; p < 0.001). When comparing western NY 2005-2006 data with 1999-1997 there is significantly more parasitism by *P. digoneutis* in the 2005-2006 samples(χ^2 =104.77; df=2; p < 0.001). Conversely there is no significant difference in *P. digoneutis* parasitism when comparing the eastern NY 2005-2006 and 1997-1999 samples (χ^2 =3.42; df=2; p = 0.181) (see Figure 2.2).

Discussion

This study presents the first data on *P. digoneutis* parasitizing *L. lineolaris* in apple orchards. Total parasitism by *P. digoneutis*, summed across time, region, orchard and treatment was 21.7%. Given that in alfalfa the total parasitism by *P. digoneutis* summed across region and time, was 21.9% and parasitism rates for *P. digoneutis* were similar in forage alfalfa at 20% (Day 2005), there is compelling evidence that *Peristenus* spp. has successfully established and possibly reached maximum levels of parasitism in New York apple orchards. The levels recorded in apples and alfalfa in western NY were comparable to those reported 6-7 year earlier in strawberries and alfalfa in eastern NY. *Peristenus digoneutis* has successfully expanded into a new crop environment; the persistence of classical biological control natural enemies in multiple habitats is a favorable attribute because the targeted pest will not be able to escape the natural enemy and multiple crops could receive direct economic benefit from the release. Of course no gains for classical biological control

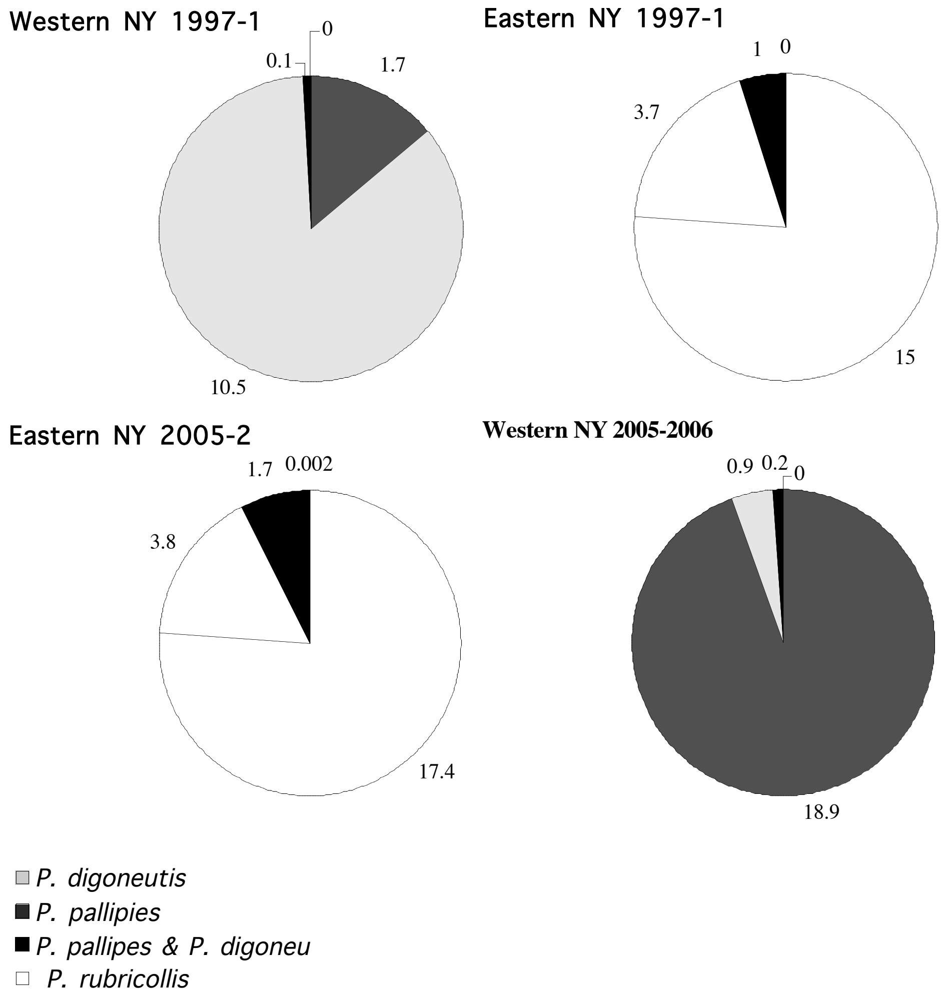

Figure 2.2. Species composition of *Peristenus* collected from *L. lineolaris* nymphs in different regions of New York over time. Samples collected in 2005-2006 are from apple orchards and alfalfa fields. Data for 1997-1999 collected in strawberries and alfalfa (compiled from Tilmon 2001). Note that the total percent of *L. lineolaris* parasitized is different for each region at each time (see Table 2.2).

can be made if the insecticide treatments disrupt the natural enemies, however, in this case *Peristenus* spp. was present in orchards that were actively sprayed with insecticides and in abandoned apple orchards that lacked treatments. Chapter three examines how treatments affected parasitism rates.

It is important to note the relationship between rates of parasitized *L. lineolaris* and apple damage by *L. lineolaris* is unknown. It is logical that a reduction in nymph population by parasitism would lead to a reduction in apple damage given that a reduction in nymph densities leads to a reduction in adult densities (Day 2005) and adult *L. lineolaris* density and damage to apples have a linear relationship (Michaud et al. 1989). Day et al. (2003) cautiously reported on corollary evidence of a relationship between rates of parasitism and *L. lineolaris* damage from a long term study of apple damage in New Hampshire. The study revealed similarities between a decrease in *L. lineolaris* damage to apples and a concurrent decrease in *L. lineolaris* numbers in alfalfa attributed to increased levels of *P. digoneutis* parasitism (Day et al. 2003). However, it is conceivable that other factors such as adult over-wintering success may play an important and even greater role in determining apple damage by *L. lineolaris*.

When tracking the effects that an introduced biological control agent has on native populations is important in helping to determine the ecological impact of classical biological control. Proportions of *Peristenus* spp. parasitism in western New York have changed dramatically since 1997-1999. The native *P. pallipes* complex has gone from parasitizing 10.5% of *L. lineolaris* nymphs in 1999 to parasitizing less than 1% of the nymphs in 2005-2006. Since species composition and rates of parasitism are not significantly different from 1997-1999 and 2005-2006 in eastern NY, it is logical to assume that none of the changes observed in western NY are due to sampling or seasonal differences. In addition to a decrease in parasitism by *P. pallipes* this study indicates there is in host competition through the season, as demonstrated by the instances of multiple parasitoids in one nymph (see Figure 2.1). It was possible to identify both the incidence and the species composition of multiple parasitism (several species parasitizing the same nymph) in this study because larvae were identified using a molecular technique. Rearing would not have reveal multiple parasitoids in the same host since only one parasitoid usually emerges from a nymph (Lachance 2001) and although dissection of nymphs would have detected multiple larvae it would not have been possible to identify species based on the morphology of the larvae. Seasonal overlap between native and introduced *Peristenus* was also observed indicating the native and introduced *Peristenus* spp. are not temporally isolated from competition.

The decrease in native parasitism reported here confirms initial findings by Tilmon and Hoffmann (2002) that suggested *P. pallipes* parasitism in western NY would decrease as *P. digoneutis* became established. The decrease in parasitism by native *Peristenus* spp. also mirrors the pattern that Day (2005) observed in forage alfalfa, where average *P. pallipes* complex parasitism before *P. digoneutis* release was 9% and dropped to 2% after *P. digoneutis* release. When taken together these studies reveal that the decrease in native *Peristenus* spp. parasitism is across habitat-strawberry fields, apple orchards, and alfalfa fields, and it extends across several regions- Delaware, New Jersey and New York. The significant decrease in native *Peristenus* spp., the seasonal overlap of native and introduced *Peristenus,* and the evidence of multiple parasitism together suggest that competition is a driving force for the decrease in *P. pallipes* complex parasitism of *L. lineolaris.*

While displacement has had a negative impact on abundance of the native *P. pallipes* complex attacking *L. lineolaris*, the most serious potential ecological cost of classical biological control, extinction of native species (Louda et al. 2002), is unlikely since they successfully utilize other hosts in the family Miridae (Day et al. 1992). For example the total parasitism, of the alfalfa plant bug, *Adelphocoris lineolatus,* increased after introduction of *P. digoneutis,* however proportions of *P. pallipes* complex attacking *Adelphocoris lineolatus* remained the same (Day 2005). Of course ecological impacts of classical biological control have to be balanced with the benefits; in this study, rates of parasitism by *Peristenus* spp. in western NY increased by 60% after the release of *P. digoneutis*.

REFERENCES:

Agnello, A., J. Nyrop, H. Reissig, and D. Straub. 2005. Reduced-Risk Pest Management in apples using pheromone disruption and a selective Pesticide program. USDA RAMP Project final research report.

Beane Freeman, L.E., M.R. Bonner, A. Blair, J. A. Hoppin, D. P. Sandler, J. H. Lubin. 2005. Cancer incidence among male pesticide applicators in the Agricultural Health Study cohort exposed to diazinon. Am J Epidemiol 162(11):1070–1079.

Cate, J. R. 1990. Biological control of pests and diseases: Integrating a diverse heritage. In: New directions in biological control: Alternatives for suppressing agricultural pests and diseases. Editors- R. E. Baker and P. E. Dunn. New York: Alan Liss. 23-43.

Cullen, J.M., M.J. Whitten. 1995. Economics of classical biological control: a research perspective. In: Biological control: benefits and risks. Editors- Hokkanen and Lynch. Cambridge, NY

Day, W. H. 1987. Biological control efforts against *Lygus* and *Adelphocoris* spp. Infesting alfalfa in the United States, with notes on other associated mirid species. USDA-ARS 64. pp. 20-39.

Day, W. H., R.C. Hedllund, L. B. Saunders, D. Coutinot. 1990. Establishment of *Peristenus digoneutis* (Hymenoptera: Braconidae), a parasite of the tarnished plant but (Hemiptera: Miridae) in the USA. Environ. Entomol. 19, 1528-1533.

Day, W. H., P. M. Marsh, R. W. Fuester, H. Hoyer, R. J. Dysart. 1992. Biology, initial effect, and description of a new species of *Peristenus* (Hymenoptera: Braconidae), a parasite of the alfalfa plant bug (Hemiptera: Miridae), recently established in the United States. Ann. Entomol. Soc. Am.85(4): 482-488.

Day, W. H. 1996. An evaluation of biological control of the tarnished plant bug, *Lygus lineolaris* (Hemiptera: Miridae), in alfalfa by the introduced parasite *Peristenus digoneutis* (Hymenoptera: Braconidae). Environ. Entomol. 25: 512-518.

Day, W. H., A. T. Eaton, R. F. Romig, K. J. Tilmon, M. Mayer, T. Dorsey. 2003. *Peristenus digoneutis* (Hymenoptera: Braconidae), a parasite of *Lygus lineolaris* (Hemiptera: Miridae) in northeastern United States alfalfa, and the need for research on other crops. Entomological News. 114(2): 105-111.

Day, W.H. 2005. Changes in abundance of native and introduced parasites (Hymenoptera: Braconidae), and of the target and non-target plant bug species (Hemiptera: Miridae), during two classical biological control programs in alfalfa. Biological Control 33: 368-374.

Ervin, R.T., L.J. Moffitt, D.E. Meyerdirk. 1983. Comstock Mealybug (Homoptera: Pseudococcidae): Cost Analysis of a Biological Control Program in California. J. Econ. Entomol. 76:605-609

Eskenazi, B., N. A. Maizlish. 1988. Effect of occupational exposure to chemicals on neurobehavioral functioning. In: Medical Neuropsychology: The Impact of Disease on Behavior. Editors- Butters M., Beers S.R., Tarter R.E., Edwards K.L., van Thiel D.H. New York: Plenum Press, 223–264.

Goulet, H., P.G. Mason. 2006. Review of the Nearctic species of (Hymenoptera: Braconidae: Euphorinae) (Hemiptera: Miridae: Mirini). Zootaxa. 1323: 1-118pp.

Hopper, K.R. 1995. Potential impacts on threatened and endangered insect species in the United States form introductions of parasitic Hymenoptera for the control in insect pests. In: Biological control: benefits and risks. Editors- Hokkanen and Lynch. Cambridge, NY.

Lachance, S., A. B. Broadbent, M. K. Sears. 2001. In-Host Compaetition of exotic and Native Parasitiods of the Tarnished Plant Bug (Heteroptera: Miridae). Environmental Entomology. 6:1158-1163.

Louda, S.M., R. W. Pemberton, M. T. Johnson, P. A. Follett. 2002. Nontarget effects- the Achilles' heel of biological control? Retrospective analyses to reduce risk associated with biocontrol introductions. Annu. Rev. Entomol. 48: 365-369.

McConnachie, A.J., M.T. de Wit, M.P. Hill, and M.J. Byrne. 2003. Economic evaluation of the successful biological control of *Axolla filiculoides* in South Africa. Biological Control. 28: 25-35.

Michaud, O.D., G. Boivin, R. K. Stewart. Economic threshold for tarnished plant bug (Hemiptera: Miridae) in apple orchards. Journal of Economic Entomology. 82(6):1722-1728.

Norgaard, R.B. 1988. The Biological Control of Cassava Mealybug in Africa. Amer. J. Agr. Econ. 366-371.

Prokopy, R. J., G. L. Hubbell. 1981. Susceptibility of apple to injury by tarnished plant bug adults. Environ. Entomol. 10(6): 977-979

Rosenstock, L., M.C. Keifer, W.E. Daniell, R. McConnell, K. Claypoole. 1991. Chronic central nervous system effects of acute organophosphate pesticide intoxication. The Pesticide Health Effects Study Group. Lancet 338(8761):223–227.

Tilmon, K. J., B.N. Danforth, W.H. Day, M.P. Hoffmann. 2000. Determining parasitoid species composition in a host population: A molecular approach. Ann. Entomol. Soc. Am. 93(3):640-647.

Tilmon, K. J. 2001. Biological Control of *Lygus lineolaris* (Hemiptera: Miridae), a generalist herbivore, by native and introduced *Peristenus* (Hymenoptera: Braconidae). Ph.D. Thesis, Cornell University. Ithaca, New York, U.S.A. 125 pp.

Tilmon, K. J., M.P. Hoffmann. 2002. Biological control of *Lygus lineolaris* by *Peristenus* spp. in strawberry. Biological Control. 26:287-292.

Tingey, W.M., E.A. Pillemer. 1977. *Lugus* bugs: crop resistance and physiological nature of feeding injury. Bull. Entomol. Soc. Am. 23:277-278.

van Lenteren, J.C., J. Bale, F. Bigler, H.M.T. Hokkanen and A.J.M. Loomans. 2006. Assessing Risk of Releasing Exotic Biological Control Agents of Arthropod Pests. Annu. Rev. Entomol. 51: 609-634.

Young, O.P. 1986. Host plants of the tarnished plant bug, *Lygus lineolaris* (Heteroptera: Miridae). Ann. Entomol. Soc. Am. 79(4):747-762.

CHAPTER THREE

Effect of insecticide regimens on biological control of *Lygus lineolaris* (Hemiptera: Miridae) by *Peristenus* spp. (Hymenoptera: Braconidae) in New York apple orchards

Introduction

Apple producers in New York and the northeastern (NE) US have formidable pest and disease pressures. There are over 700 known diseases and arthropod pests including oblique-banded leafroller, several species of mites, a suite of internal Lepidoptera pests, apple maggot, European sawfly, plum curculio, stinkbug, and tarnished plant bug (TPB) (New York Apple Association 2006). The intense disease and arthropod pressures, combined with the unpredictable and sometimes severe weather of the NE, create difficult growing conditions for New York apple producers, putting them at a disadvantage when compared to other apple growing regions of the U.S., especially those in western states.

To control insect pests apple growers utilize a range of chemical regimens. Standard growing practices rely heavily on broadspectrum organophosphate, carbamate, and pyrethroid insecticides. These insecticides have long caused concern, both for the risks to humans, the environment and beneficial arthropods (Beane Freeman et al. 2005, Eskenazi and Maizlish 1988, McConnell et al. 1994, Rosenstock et al. 1991). Scientific and public concern about organophosphate, carbamate and pyrethroid insecticides are great enough that Congress passed the Food Quality Protection Act (FQPA) in 1996 (Public Law 104-170) which calls for an eventual elimination of these insecticides from use in the United States (U.S.).

Given the importance of the organophosphate, pyrethroid and carbamate insecticides in controlling insect pests, apple growers need an alternatives to these widely used but soon-to-be proscribed insecticides. In response to this major change in the availability of these insecticides the Reduced-Risk Management Program, a collaborative effort consisting of scientists, growers, representatives from the pesticide industry, and the U.S. Department of Agriculture was formed to evaluate newer reduced risk insecticides in orchards that could replace the soon to be lost standard products. Reduced risk insecticides have been marketed as good alternative products because they are purported to be safer for the environment and more pest specific and they conform to the FQPA (Balazs et al. 1997, Pekar 1999, Hill and Foster 2003). Insecticide specificity allows practitioners to target pest species while avoiding damage to beneficial arthropods such as pollinators, natural enemies and non pests. Reduced risk insecticides, also known as soft pesticides, include Apollo® (clofentizine), Nexter® (pyridaben), Zeal® (etoxazole), Actara® (thiamethoxam), Esteem® (pyriproxyfen), Avaunt® (indoxacarb), Assail® (acetamiprid), Intrepid® (benzoic acid) and SpinTor® (spinosad) and dormant oil (petroleum oil). Despite their claim of being more selective, research into indoxacarb (Haseeb et al., 2004), imidacloprid, thiamethoxam (Williams et al. 2003, Nasreen et al. 2004) and spinosad (Nowak et al. 2001, Cisneros et al. 2002, Schneider et al. 2003) has shown them to be not as specific as originally believed (Stark and Banks 2001, Brunner et al. 2001). In addition, many reduced risk chemicals are significantly more expensive than standard products.

Another treatment option is producing apples under an organic regimen. While there are few large organic apple producers in NY there is increasing interest from growers and consumers in the burgeoning organic market across the U.S. (Peck et al. 2005). Organic apples receive a premium at market but organic growers are limited in what can be applied for arthropod and disease control. Certified organic producers can utilize non-synthetic, naturally derived compounds such as insecticides made from botanicals, elemental compounds (e.g. sulfur and copper), and physical barriers to battle pests. However, most of the organic compounds are more expensive than, and not as effective as, their synthetic counterparts.

As the Food Quality Act is implemented, and standard insecticides are banned, the increased cost of reduced risk insecticides will weigh heavily on apple growers. This creates an immediate need for research into alternatives that may reduce the need for insecticide sprays, such as biological control. In addition, research is needed to clarify how the new reduced risk insecticides affect non target species including natural enemies.

This study investigates, *Peristenus* spp., a group of nymphal parasitoids of the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois). *Lygus lineolaris* causes damage to apples by feeding on the developing flowers and fruit, resulting in abscission of flower buds, fruit underdevelopment, and malformations (Prokopy and Hubbell 1981). Abscission of buds is of no economic consequence; however, underdeveloped fruit and malformations can result in culling and downgrading (Michaud et al. 1989 and Weires et al. 1985). Fruit malformations from *L. lineolaris* can be characterized as puncture wounds, scabs or “cat facing” (Boivin and Stewart. 1982).

The *Peristenus* spp. parasitizing *L. lineolaris* are several species in the native *P. pallipies* species complex, and two introduced European species, *P. digoneutis* Loan and *P. rubricollis* (Thomson). The native species complex was not able to provide adequate biological control of *L. lineolaris* (Day 1990) so *P. digoneutis* was introduced from France in the 1980’s. After introduction into the U.S., robust populations of *P. digoneutis* developed in forage alfalfa, reducing the total *L. lineolaris* population in that crop by 75% (Day 2005). As expected, *P. digoneutis* expanded its geographic range and spread from the alfalfa fields to high value crops such as strawberries (Tilmon and Hoffmann 2002) and apples (see chapter two). *P. rubricollis* is only occasionally found parasitizing *L. lineolaris,* preferring other mirids in the *Adelphocoris* genus, especially the introduced European alfalfa plant bug, *A. lineolatus* (Goeze), which it was introduced to control (Day 2005).

The objective of this study was to examine the effect that pesticide regimens (organic, standard, reduced risk and abandoned) had on rates of parasitism by *Peristenus* spp. on *L. lineolaris* in apple orchards.

Materials and Methods

Regimens

During this field study, conducted in the summers of 2005 and 2006, *L. lineolaris* nymphs were collected from apple orchards under four different arthropod management regimens; Organic (OG), Standard (ST), Reduced Risk (RR) and Abandoned (ABD). ST insecticide regimens are defined here as the current convention in apple production, which relies heavily on organophosphate, carbamate, and pyrethroid insecticides. The RR regimen (defined above) included the following insecticides (active ingredients) clofentizine, pyridaben, etoxazole, thiamethoxam, pyriproxyfen, indoxacarb, acetamiprid, benzoic acid, spinosad and petroleum oil. For complete RR spray records including rates and frequency of applications see Agnello et al. (2005). RR regimens were only available in 2005. The OG orchards selected were certified organic and followed guidelines mandated by the U.S. Department of Agriculture National Organic Program (Federal Register, 2000). Insecticides used in OG orchards included sulfur, copper and kaolin clay. The ABD orchards were typically no longer under any form of management and consequently had excessive weed populations and high infestations and damage by both insect pests and disease.

Orchards

The field sites consisted of nine commercial orchards located in three of the major apple producing regions of New York-- Hudson Valley, Central and Niagara (see Table 3.1). Six of the nine orchards were participants in the Reduced-Risk Management Program (2005 only). At these locations two approximately 10 acre blocks were under either the RR or ST treatment regimen in a split plot design. OG plots in the same regions were selected to compliment the split plot design. At Singer Farm, two OG plots were selected, they were labeled OG(P) and OG(G). The plot OG(G) was only available in 2005. In the Niagara region and Hudson Valley regions, OG plots were very close to the corresponding RR/ ST plots, sharing a common property line. However, the OG plot in Central New York (West Haven) was 45 miles from the closest RR\ST plot (Beak and Skiff) (see Figure 3.1). ABD plots were also selected from the same area, again two shared a property line with RR/ ST plots and the third was within 5 miles from an OG/ST plots (Singer Farm).

Table 3.1. Apple orchards and corresponding regimens used in the study. Note that Reduced Risk was only available in 2005.

<table>
<tr><th colspan="12">Central</th></tr>
<tr><td colspan="4">West Haven (WH)</td><td colspan="4">Apple Acres (AA)</td><td colspan="4">Beak and Skiff (BS)</td></tr>
<tr><td colspan="4">Organic</td><td colspan="4">Reduced Risk
Standard</td><td colspan="4">Abandoned
Reduced Risk
Standard</td></tr>
<tr><th colspan="12">Hudson Valley</th></tr>
<tr><td colspan="6">Wright Brothers (WB)</td><td colspan="6">Prospect Hill (PH)</td></tr>
<tr><td colspan="6">Abandoned
Reduced Risk
Standard</td><td colspan="6">Organic</td></tr>
<tr><th colspan="12">Niagara</th></tr>
<tr><td colspan="3">Singer Farm (SF)</td><td colspan="3">Russell Farms (RF)</td><td colspan="3">Lynn-Oaks (LO)</td><td colspan="3">Brown Farm (BF)</td></tr>
<tr><td colspan="3">Abandoned
Standard
Organic 2-OG(P) & OG(G)</td><td colspan="3">Reduced Risk
Standard</td><td colspan="3">Reduced Risk
Standard</td><td colspan="3">Abandoned
Reduced Risk
Standard</td></tr>
</table>

Figure 3.1. Map of New York State with apple orchards used in this study marked. The abbreviations are: AA- Apple Acres, BS-Beak and Skiff, BF-Brown Farm, RF-Russell Farm, SF-Singer Farm, LO- Lynn-Oakes, WB-Wright Brothers, WH-West Haven. Note that Prospect Hill (not shown) is at the same location as WB.

Field Sampling

In 2005 *Lygus lineolaris* nymph samples were collected weekly; except when prevented by inclement weather or pesticide regimens. In 2006 four weeks, corresponding with peak parasitism, were sampled. An area approximately 1 acre in size, located in the middle of each treatment block was sampled at each visit for TPB nymphs using a sweep net. Sweeping of the orchard ground cover continued until 24-30 TPB nymphs were collected or 2.5 hours passed. Ground cover was similar between regimens within orchards, with the exception of OG(P) treatment at SF. Typical ground cover consisted of *Dactylis* spp., *Trifolium* spp. *, Vicia* spp., *Hieracium* spp,. *Plantago* spp., *Taraxacum* spp., *Leontodon* spp., *Galium* spp., and grass. In OG(P) *Galium* spp. constituted about 80% of the ground vegetation. Nymphs collected in the sweep net were preserved in 95% ethanol, and stored on ice until transferred to a -20°C freezer.

Determining adult population size

There is evidence of a weak density-dependent relationship between numbers of *L. lineolaris* nymphs and *P. digoneutis* (Tilmon 2001, Day 2005). A density-dependent relationship is important in this study because if one of the regimens had significantly more nymphs than the other regimens the interpretation of the parasitism rates would change. Since adult and nymphal population densities are related (Day 2005) adult densities were used to establish relative *L. lineolaris* nymphal population sizes between treatments. Sticky cards were used to monitor relative *L. lineolaris* adult population levels in each treatment (Boivin et al. 1982. Boivin et al. 1983, Prokopy et al. 1982). A row within the 1 acre TPB collection area was arbitrarily chosen at the beginning of the study and each week thereafter five yellow 13.5 cm x 13.5 cm sticky cards, spaced 50 meters apart, were hung in trees approximately 0.75 meters above the ground. Cards were replaced each time the site was visited, wrapped in cellophane, and stored at 4°C until examined for TPB adults.

Determining Parasitism Rates

The molecular identification of *Peristenus* spp. was achieved using a technique developed by Tilmon et al. (2000). Briefly, DNA is extracted from each *L. lineolaris* nymph and possible parasitoid. Next Polymerase Chain Reaction (PCR) with primers specific to *L. lineolaris* are amplified, thereby confirming success of the extractions. Finally, another PCR with specific primers amplify *Peristenus* spp. genes differentiating parasitized from unparasitized TPB nymphs. Samples were stored at -20° C and used for an analysis in chapter one. DNA was extracted using DNAzol, (Invitrogen Carlsbad, CA) following the manufacture's protocol except the 'Lysis step' was scaled down from 1ml to 100ul. Primers, C1-J-2252, C1-J-2183 and TL2-N-3014, where obtained from Integrated DNA Technologies (Coralville, IA). Taq polymerase was obtained from Promega (Madison WI). PCR conditions, as outlined by Tilmon et al. (2000), were followed.

Statistical Analysis

A General Linear Model (GLM), with a log link and negative binomial distribution, was used to describe the adult *L. lineolaris* count number and test for treatment effects. Orchards were considered a repeated measure. The negative binomial distribution was selected instead of a Poisson distribution because the assumption of variance equaling mean was violated, indicating over dispersion. Variance in the negative binomial distribution is a dispersion parameter estimated by maximum likelihood.

Another GLM, with a logit link and a binomial distribution regression was used to analyze the relevance of treatment on parasitism of *L. lineolaris* by *Peristenus* spp. Again, orchards were considered a repeated measure. Since it is well established that there are two seasonal peaks in parasitism because *P. digoneutis* is bivoltine (Day 1992, Tilmon 2001, Goulet and Mason 2006) (see Figure 3.2), time was coded as a categorical value: this approach allows the magnitude of parasitism to change over the season. For the purpose of this study total parasitism (all *Peristenus* species combined) was used because a dichotomous response variable, (i.e. parasitized, not parasitized) is much easier to analyze than a response variable with multiple levels, such as species. Furthermore, for analyzing the affect of pesticide treatment on parasitism, *Peristenus* spp. was the unit of interest. All statistical analyses were preformed using SAS 9.1 (2003).

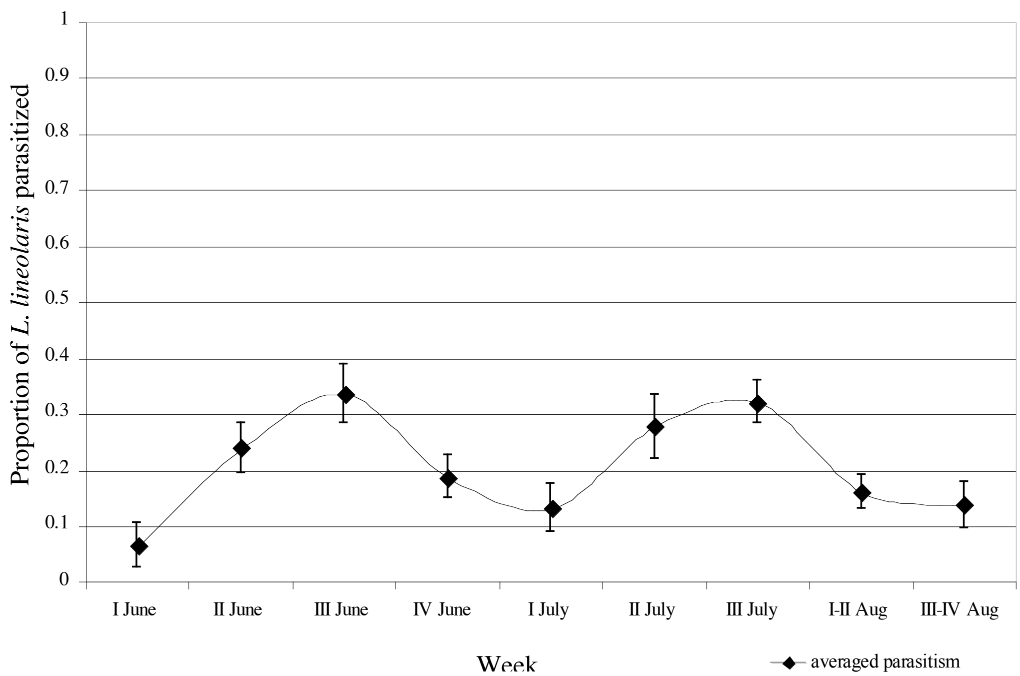

Figure 3.2. The average total parasitism at all sites, with standard error, of *L. lineolaris* by *P. digoneutis* for each week sampled during 2005-2006.

Results

Average parasitism, with standard error, for all treatments is reported in Figure 3.2. In addition, average parasitism, with standard error, is reported by treatment in Figure 3.3- 3.7. Total parasitism rates in pesticide regimens are reported by region and combined for both years (except RR and OG(G)), in Table 3.2.

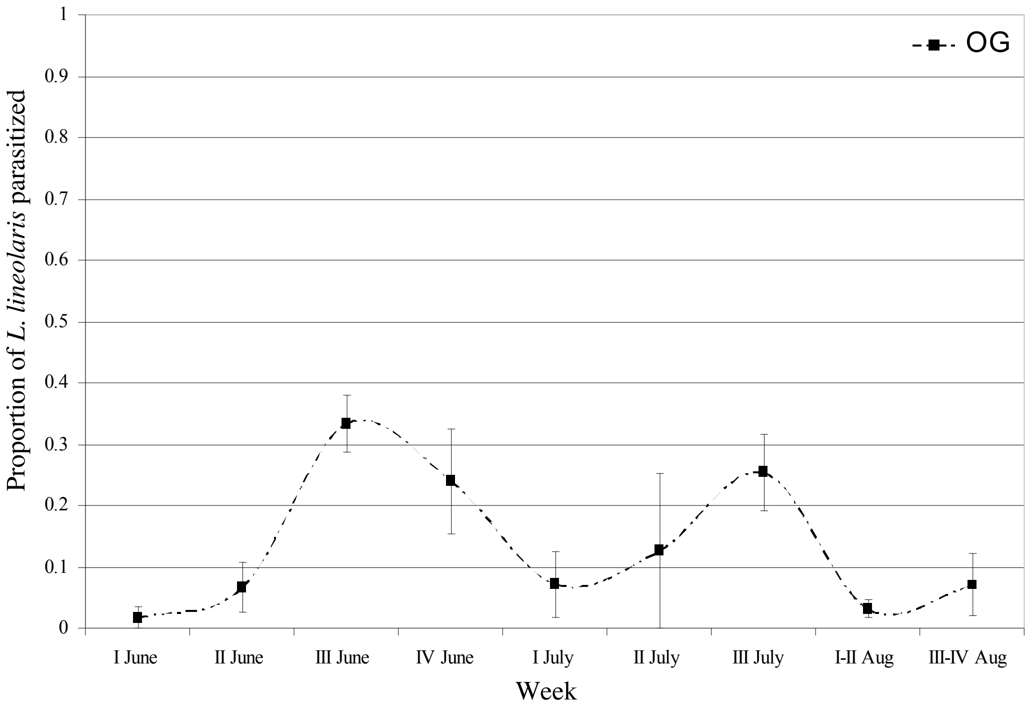

Figure 3.3. Average parasitism, +/- standard error, of *L. lineolaris* by *Peristenus* spp. for OG orchards over time. Data is combined from 2005 and 2006.

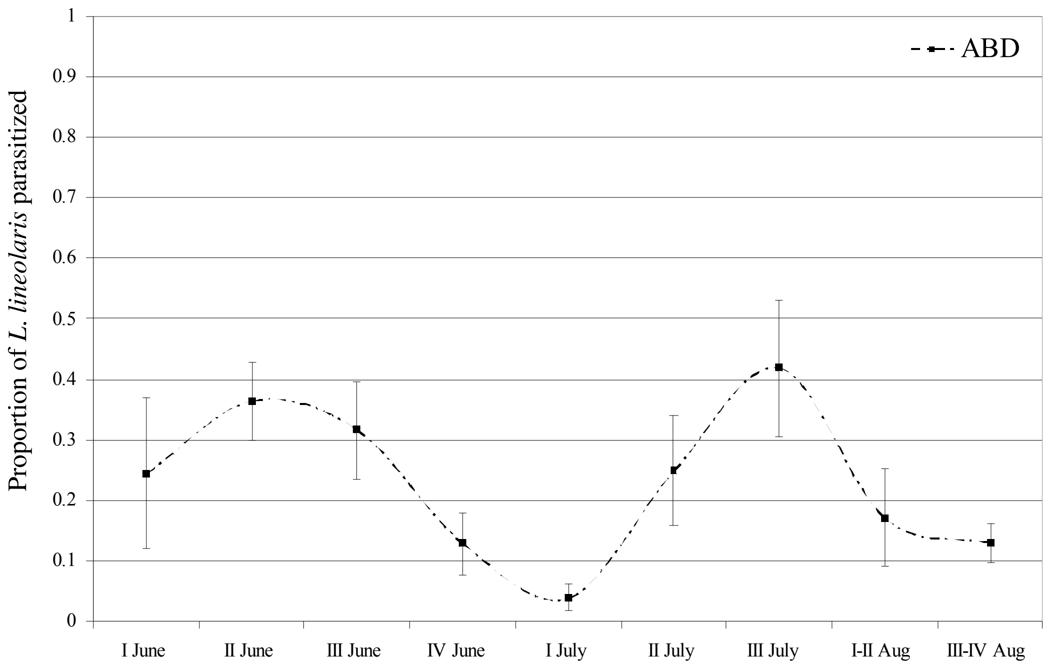

Figure 3.4. Average parasitism, +/- standard error, of *L. lineolaris* by *Peristenus* spp. for ABD orchards over time. Data is combined from 2005 and 2006.

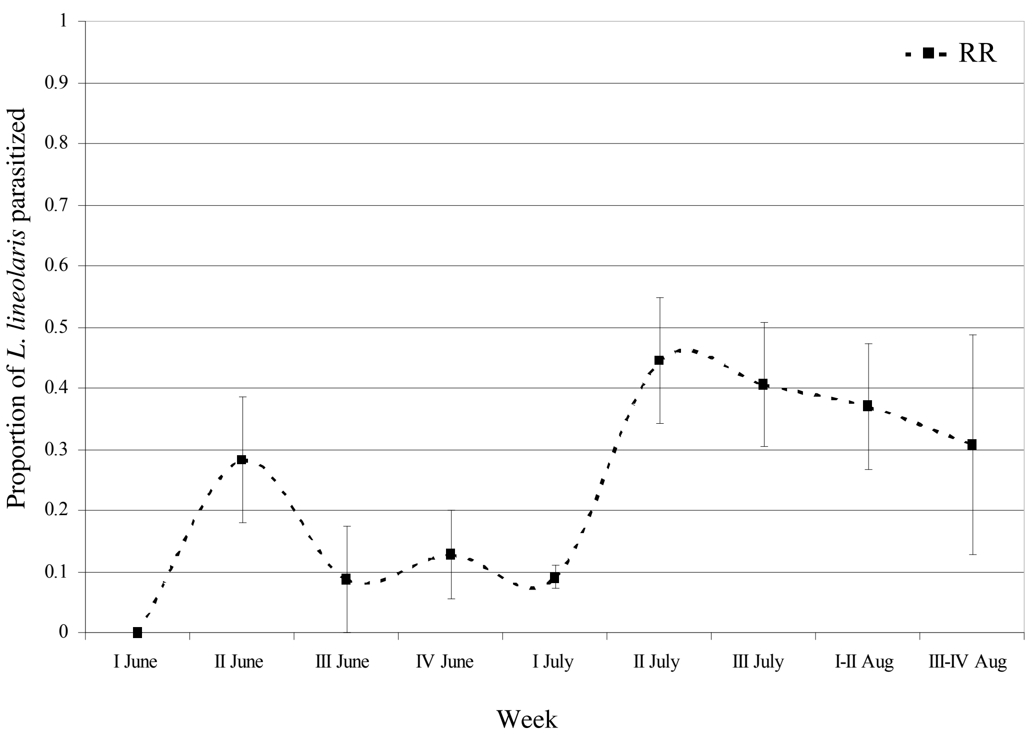

Figure 3.5. Average parasitism, +/- standard error, of *L. lineolaris* by *Peristenus* spp. for RR orchards over time. Data is combined from 2005 and 2006.

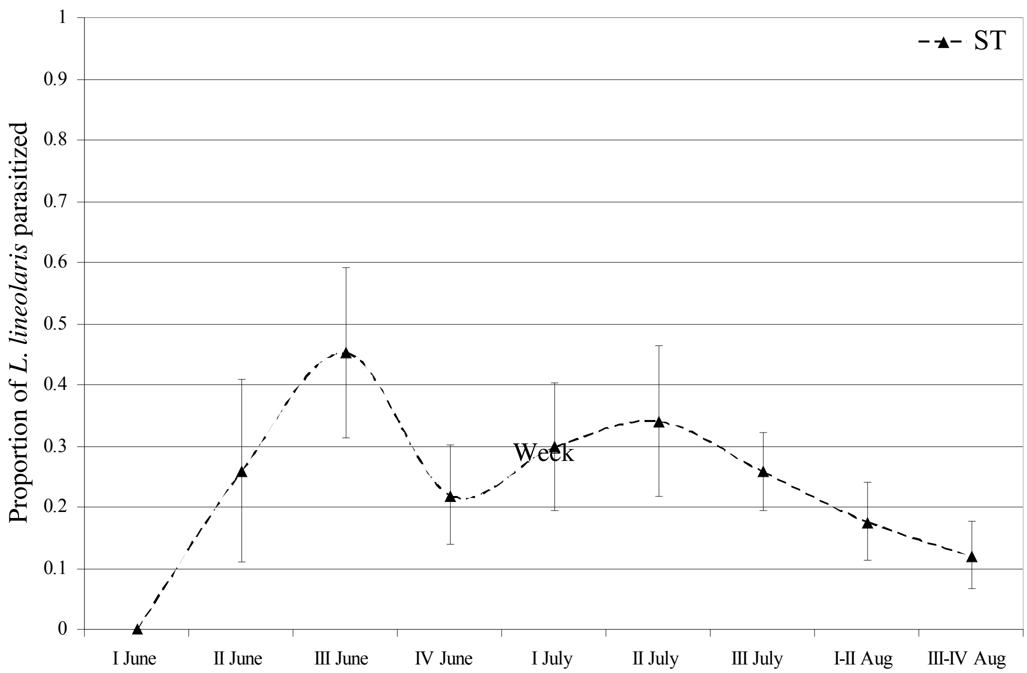

Figure 3.6. Average parasitism, +/- standard error, of *L. lineolaris* by *Peristenus* spp. for ST orchards over time. Data is combined from 2005 and 2006.

Table 3.2. Percent parasitism of *L. lineolaris* by *Peristenus* spp., in three apple producing regions of New York. Parasitism rates are given for each insecticide treatment of each region. Percents are of data combined across time and orchards. Data was collected in 2005 and 2006 for ST OG and ABD, data from RR was only collected in 2005.

Region of New York	**Treatment**	**N**	**Parasitism by *Peristenus* spp.**
Central			
	Abandoned	335	37.1 %
	Standard	441	37.6 %
	Reduced Risk	301	34.3 %
	Organic	192	17.7 %
Hudson Valley			
	Abandoned	265	23.4 %
	Standard	204	17.2 %
	Reduced Risk	69	36.2 %
	Organic	332	22.0 %
Niagara			
	Abandoned	256	12.5 %
	Standard	441	22.3 %
	Reduced Risk	349	24.1 %
	Organic	371	14.0 %

The logistic model for parasitism indicated that insecticide treatment was a significant predictor of parasitism (χ^2 =33.1; df=3; $p < 0.0001$). Total parasitism in ST regimens was not significantly different from that in ABD orchards (p=0.1063). However, OG farms had significantly lower likelihood of parasitism when compared with ST ($p < 0.0001$). The odds ratio indicates that the likelihood of encountering a parasitized *L. lineolaris* nymph in an OG orchard is 0.606 times that of encountering a parasitized nymph in ST orchard. Stated another way, a nymph encountered in an OG orchard is 39.4% less likely to be parasitized than a nymph encountered in a ST orchard (See Table 3.3). Reduced risk orchards had significantly higher likelihood of parasitism than ST orchards (p =.0413). The odds ratio indicates that a *L. lineolaris* nymph in RR orchard is 1.258 times more likely to be parasitized than a nymph in ST orchard. Restated, a nymph encountered in a RR orchard is 25.8% more likely to be parasitized than a nymph encountered in a ST orchard (see Table 3.3).

Table 3.3. Logistic regression of *Peristenus* spp. parasitism of *L. lineolaris* nymphs in apple orchards under different pesticide regimens. The parameter estimates are converted from log scale into odds ratios for ease of interpretation. The odds ratios for categorical variables are the likelihood of an event, in this case encountering a parasitized nymph, at a given level compared to the intercept, ST in this case.

Parameter	DF	Estimate	Standard Error	Odds Ratio	p
Intercept	1	-1.0189	0.2105	.	$p < 0.0001$
Abandoned	1	-0.1790	0.0737	0.836	$p = 0.1063$
Organic	1	-0.5002	0.0788	0.606	$p < 0.0001$
Reduced Risk	1	0.2318	0.0779	1.258	$p = 0.0413$
Standard	0	.	.	.	.

The Pearson chi-squared test revealed the negative binomial regression model was an adequate fit (χ^2 =1055.6; df=881; χ^2 / df =1.2); in addition, pesticide treatment was a significant predictor of adult *L. lineolaris* count (χ^2 =35.99.; df=3; p < 0.0001). There was no statistically significant difference between the numbers of *L. lineolaris* in ST and RR orchards (p=0.1518). There are significant differences between OG and ST (p<0.0001) and between ABD and ST (p= 0.0015). The positive ABD estimate indicates that ABD orchards had more *L. lineolaris* adults than ST orchards. OG orchards also have more *L. lineolaris* than ST, which is described by the positive OG parameter estimate (see Table 3.4).

Table 3.4. General linear model with log link and negative binomial distribution of adult *L. lineolaris* numbers in apple orchards under different pesticide regimens. λ is the parameter estimate. When λ is negative the parameter it represents (in this case number of *L. lineolaris* in that insecticide treatment) is less then the intercept (ST in this case). When λ is positive, the parameter it represents is more than the intercept.

Parameter	DF	λ	Standard Error	χ^2	p
(Intercept)	0	0.3095	0.1366	.	p=0.0235
Abandoned	1	0.6876	0.2161	10.13	p=0.0015
Organic	1	0.8569	0.2090	16.81	p<0.0001
Reduced Risk	1	-0.2965	0.2069	2.05	p=0.1518
Standard	0	.	.	.	.
Dispersion	1	4.5655	0.3399	.	.

Discussion

An intriguing result of this study is that there was no significant difference in parasitism between the ABD orchards and the ST orchards. This indicates that the *Peristenus* spp. parasitoids were able to attack *L. lineolaris* nymph hosts in the ST insecticide environment as effectively as in the ABD environment where no insecticides were applied. However this result should be interpreted with caution since the relative adult density data reveals that ABD orchards had significantly higher numbers of *L. lineolaris.* Given the density dependent relationship between parasitism and nymph populations, higher population density in ABD should be accompanied by higher parasitism rates, which could have masked a difference in the parasitism between ST and ABD.

The regression comparison between ST and RR insecticide regimens was significant; a nymph encountered in a RR orchard is 25.8% more likely to be parasitized than a nymph in a ST orchard. This result is bolstered by the relative adult density data that indicated density of *L. lineolaris* in RR and ST orchards was not significantly different. This study indicates that, in the case of *Peristenus* spp, the reduced risk regimen has a less disruptive effect on biological control than the standard insecticide regimen. This result is not surprising when reports that reduced risk chemicals are less toxic to non target insects are considered (Balazs et al. 1997, Pekar 1999, Hill and Foster 2003).

A compelling result in this study is that a nymph encountered in an OG orchard was 39.4% less likely to be parasitized than a nymph encountered in a standard orchard. OG orchards had significantly higher *L. lineolaris* densities relative to in ST orchards. The higher OG adult density relative to ST strengthens the aforementioned result. Given that parasitism is density dependent it is expected that parasitism rates would be higher in OG than in ST, since the opposite is true it suggests that insecticide regimen could had an even stronger effect than indicated by the odds ratio.

While this study indicates that rates of parasitism are significantly higher in ST orchards when compared to OG apple orchards Tilmon and Hoffmann (2002) found the opposite effect in OG and ST strawberries. In their study *Peristenus* parasitism was 5-6.5 times more likely in an OG strawberry field than in a conventional (ST) strawberry fields. A key difference between these studies is that the OG strawberry fields sampled did not have pesticides applied, whereas all of the OG apple orchards applied USDA certified organic pesticides. This is evidence that the application of OG pesticides can have a negative impact on *Peristenus* spp. Furthermore, a model by Kovach et al. (1992) of apple production in New York suggests that the impact of OG apple production on the environment is greater than the impact of ST apple production. This evidence suggests the lower likelihood of parasitism by *Peristenus* spp. in OG orchards is due to OG pesticides; however, further research is required to establish cause and effect relationships of the organic pesticides on beneficial arthropods.

REFERENCES:

Agnello A., J. Nyrop, H. Reissig, and D. Straub. 2005. Reduced-Risk Pest Management in apples using pheromone disruption and a selective Pesticide program. USDA RAMP PROJECT final research report.

Balazs, K., M. Molnar, G. Bujaki, I. Gonda, D. Karacsony, J. Bartha. 1997. Possibility and problems of organic apple growing in Hungary. Biological Agriculture and Horticulture 15, 223-232.

Beane Freeman, LE, M.R. Bonner, A. Blair, J.A. Hoppin, D. P. Sandler, J. H. Lubin. 2005. Cancer incidence among male pesticide applicators in the Agricultural Health Study cohort exposed to diazinon. Am J Epidemiol 162(11):1070–1079.

Boivin, G., R.K. Stewart. 1982. Identification and evaluation of damage to McIntosh apples by phytophagous mirids (Hemiptera: Miridae) in southwestern Quebec. Can. Entomol. 114: 1037-1045.

Boivin, G., R. K. Stewart. 1983. Sampling technique and seasonal development of phytphagous mirids (Hemiptera:Miridae) on apple in southwestern Quebec. Ann. Entomol. Soc. Am. 76(3):359-364

Boivin,G., R. K Stewart. 1983. Seasonal development and interplant movements of phytophagous mirids (Hempiptera: Miridae) on alternate host plants in and around an apple orchard. Ann. Entomol. Soc. Am. 76(4):776-780.

Brunner J.F., J. E. Dunley, M. D. Doerr, E. H. Beers. 2001. Effect of pesticides on *Colpoclypeus florus* (Hymenoptera: Eulophidae) and *Trichogramma platneri* (Hymenoptera: Trichogrammatidae), parasitiods of leafrollers in Washington. Journal of Economic Entomology. 94(5):1075-1084.

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

Day, W. H., R.C. Hedllund, L.B. Saunders, D. Coutinot. 1990. Establishment of *Peristenus digoneutis* (Hymenoptera: Braconidae), a parasite of the tarnished plant but (Hemiptera: Miridae) in the USA. Environ. Entomol. 19, 1528-1533.

Day, W. H., P. M. Marsh, R. W. Fuester, H. Hoyer, R. J. Dysart. 1992. Biology, initial effect, and description of a new species of *Peristerus* (Hymenoptera: Braconidae), a parasite of the alfalfa plant bug (Hemiptera: Miridae), recently established in the United States. Ann. Entomol. Soc. Am.85(4): 482-488.

Day, W.H. 2005. Changes in abundance of native and introduced parasites (Hymenoptera: Braconidae), and of the target and non-target plant bug species (Hemiptera: Miridae), during two classical biological control programs in alfalfa. Biological Control 33: 368-374.

Eskenazi, B, N.A. Maizlish. 1988. Effect of occupational exposure to chemicals on neurobehavioral functioning. In: Medical Neuropsychology: The Impact of Disease on Behavior (Butters M., Beers S.R., Tarter R.E., Edwards K.L., van Thiel D.H., eds). New York:Plenum Press, 223–264.

Goulet, H., P.G. Mason. 2006. Review of the Nearctic species of (Hymenoptera: Braconidae: Euphorinae) (Hemiptera: Miridae: Mirini). Zootaxa. 1323: 1-118pp.

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

Hill, T.A., 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. Journal of Entomological Science 38, 59-71.

Kovach J., C. Petzoldt, J. Degni, J. Tette. 1992. A method to measure the environmental effect of pesticides. N.Y Food Life Sci. Bull. No. 139.

Michaud, O.D., G. Boivin, R. K. Stewart. Economic threshold for tarnished plant bug (Hemiptera: Miridae) in apple orchards. Journal of Economic Entomology. 82(6):1722-1728.

Nasreen, A., G.M. Cheema, M. Ashfaq, M.A. Saleem. 2004. Survival of *Trichogramma chilonis* ishii (Hymenoptera: Trichogrammitidae) after exposure to different insecticides: Laboratory studies. Pakistan Journal of Zoology 36, 79-82.

Nowak, J.T., K.W. McCravy, C.J. Fettig, C. W. Berisford. 2001. Susceptibility of adult hymenopteran parasitoids of the Nantucket pine tip moth (Lepidoptera: Tortricidae) to broad-spectrum and biorational insecticides in a laboratory study. Journal of Economic Entomology 94, 1122-1127.

New York Apple Association 2005. www.nyapplecountry.com/fastfacts.htm.

Peck, G.M., P.K. Andrews, C. Richter, J.P. Reganold. 2005. Internationalization of the organic fruit market: The case of Washingtion State's organic apple exports to the European Union. Renewable Agr. Food Sys. 20:101-112.

Pekar, S., 1999. Effect of IPM practices and conventional spraying on spider population dynamics in an apple orchard. Agriculture Ecosystems and Environment 73, 155-166.

Prokopy, R. J., G. L. Hubbell. 1981. Susceptibility of apple to injury by tarnished plant bug adults. Environ. Entomol. 10(6): 977-979.

Rosenstock L, M.C. Keifer, W.E. Daniell, R. McConnell, K. Claypoole. 1991. Chronic central nervous system effects of acute organophosphate pesticide intoxication. The Pesticide Health Effects Study Group. Lancet 338(8761):223–227.

Schneider, M.I., G. Smagghe, A. Gobbi, E. Vinuela. 2003. Toxicity and pharmacokinetics of insect growth regulators and other novel insecticides on pupae of Hyposoter didymator (Hymenoptera: Ichneumonidae), a parasitoid of early larval instars of lepidopteran pests. Journal of Economic Entomology 96, 1054-1065.

Stark J.D., J. E. Banks. 2001. "Selective" pesticides: Are they less hazardous to the environment? BioScience. 51(11): 980-982.

Tilmon, K. J., B.N. Danforth, W.H. Day, M.P. Hoffmann. 2000. Determining parasitoid species composition in a host population: A molecular approach. Ann. Entomol. Soc. Am. 93(3):640-647.

Tilmon, K. J. 2001. Biological Control of *Lygus lineolaris* (Hemiptera: Miridae), a generalist herbivore, by native and introduced *Peristenus* (Hymenoptera: Braconidae). Ph.D. Thesis, Cornell University. Ithaca, New York, U.S.A. 125 pp.

Tilmon, K. J., M.P. Hoffmann. 2002. Biological control of *Lygus lineolaris* by *Peristenus* spp. in strawberry. Biological Control. 26:287-292.

Williams, L., III, L.D. Price, V. Manrique. 2003. Toxicity of field-weathered insecticide residues to *Anaphes iole* (Hymenoptera: Mymaridae), an egg parasitoid of *Lygus lineolaris* (Heteroptera: Miridae), and implications for inundative biological control in cotton. Biological Control 26, 217-223.

Weires R. W., J. R. VanKirk, W. D. Gerling, F. M. McNicholas. 1985. Economic losses form the tarnished plant bug on apple in eastern New York. J. Agric. Entomol. 2(3): 256-263.