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The response of Apocrita wasp populations to insecticides in the Maine lowbush blueberry agroecosystem

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ABSTRACT

The relative abundances of Apocrita wasps associated with lowbush blueberry (*Vaccinium angustifolium* Ait.) were investigated in 33 blueberry fields throughout Washington County, Maine, USA. Wasps were captured during the springs and summers of 1997 and 1998 in Malaise traps erected along a transect in each field. A BACIP (before-after-control-impact-paired) experimental design was used to assess single applications of commonly used insecticides on predator and parasitoid wasp populations associated with lowbush blueberry fields. Insecticides were found to have a negative impact (time x treatment interaction, $P < 0.05$) on total wasp trap capture when comparing the change in wasp abundance before and after insecticide application periods between paired treated and non-treated fields. Relatively fewer wasps were captured in fields after treatment with insecticides compared to non-treated fields. These effects were found to be taxon-specific with the greatest negative impact observed for wasps belonging to the families Pompilidae and Braconidae.

KEYWORDS: Hymenoptera, parasitoid, predator, lowbush blueberry, *Vaccinium angustifolium*, insecticides, pest management

INTRODUCTION

Maine is the largest producer of lowbush blueberries (*Vaccinium angustifolium* Ait.) in the United States. In Maine, lowbush blueberries are currently harvested on more than 60,000 acres [1] with a 2014 yield of over 104 million pounds and a harvest value of $63 million [2]. Because of the wild nature and limited geographic distribution of this crop, research in the U.S. has been limited to cropping systems in Maine. According to the United States Department of Agriculture 2012 Census of Agriculture [3], the wild blueberry crop in Maine comprises 28% of the total blueberry acreage for the combined wild lowbush, cultivated highbush and rabbiteye blueberry crops in the United States. The wild lowbush blueberry crop has tripled over the past 20 years and is now harvested on over 60,000 ha in North America, averaging more than 82 million kg per year. More than one third of the wild lowbush blueberry crop is produced in Maine and almost two-thirds is produced in the Canadian provinces of New Brunswick, Newfoundland, Nova Scotia, Prince Edward Island, and Quebec [4].

Production starts with areas of forested land being cleared, burned, and treated with herbicides to enable the existing wild blueberry plants to be productive [5]. Lowbush blueberry has a clonal growth habit; it is a prostrate shrub that spreads through an underground network of rhizomes. Cultivation practices to date have focused on optimizing growth, namely by fertilization, irrigation, and prevention of losses resulting from diseases, weeds, and insect pests [6, 7].

Integrated pest management (IPM) techniques have been adopted by many lowbush blueberry growers for control of insect pests [6, 8]. Once insect pest

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populations attain economic threshold levels, a management tactic (most often an insecticide application) is usually employed [9]. Unfortunately, with the arrival of the invasive spotted wing drosophila in 2012, established blueberry IPM programs have been disrupted and more pesticide applications are being made to fields [10].

It is well documented that Apocrita wasps are important natural enemies of numerous insect pests in crops and forests worldwide [11, 12, 13, 14, 15, 16, 17]. In Maine lowbush blueberry, *Dusona sp.* and *Erromenus sp.* have been identified as parasitoids of the blueberry spanworm (*Itame argillacearia* (Packard) [16], and *Utetes richmondi* (Gahan), *Diachasma alloeum* (Muesebeck), and *Opius melleus* (Gahan) have been identified as parasitoids of the blueberry maggot (*Rhagoletis mendax* Curran) [18, 16, 19, 20]. Cutler *et al.* [21] have shown that wasps in the family Ichenumonidae can parasitize over 40% of blueberry spanworm populations in Canada. It is not known whether these parasitoids regulate populations of these blueberry insect pests in North America, but it is likely given that both parasitoids and pest species are native and thus have a long co-evolutionary history [20, 21]. Many species of wasp are highly sensitive to broad-spectrum insecticides and thus the toxic affect some insecticides have on wasp populations could make these control measures counter-productive [22, 23, 24, 25, 26, 27, 28, 29, 30, 31]. Insecticides may reduce insect pest populations, but if natural enemies of the targeted insect pests are being killed, then pest populations can quickly rebound (i.e. pest resurgence [32, 33, 34, 35]).

The need for more sustainable methods to control insect pests has stimulated interest in the conservation of native natural enemies [24, 36, 37, 38, 39, 40]. Shaw and Hochberg [41] argue that the conservation of parasitic wasps has been seriously neglected, and that growers should develop a management philosophy that enhances the beneficial Hymenoptera. As many insect pests of lowbush blueberry are indigenous to Maine, it can be argued that the best biological control approach would be to conserve native wasp populations [16, 24, 34, 36]. For blueberry growers to enhance wasp populations in an effort to manage insect pests in a sustainable and environmentally-friendly manner, it stands to reason that they would need to know how insecticides impact beneficial wasp populations. The objective of our study was to investigate the response of the Apocrita wasp community to insecticide applications in lowbush blueberry during the 1997 and 1998 fruiting cycle.

MATERIALS AND METHODS

Site description

This study was conducted in selected lowbush blueberry fields during their fruiting cycle (lowbush blueberry fields are usually managed to produce a crop every other year), within Washington County, Maine, USA. The majority of blueberry fields in this study were owned and managed by Jasper Wyman & Son or Cherryfield Foods, Inc. Some fields, however, were owned and managed by independent farmers who agreed to participate in the study. In all fields, data collection occurred for only one season, because it is standard commercial practice to rotate a field out of production every other year (prune cycle) by either burning or mowing it after harvest [42]. Each blueberry field and its bordering forest were considered as an individual experimental unit. Thirty-three sites were arbitrarily selected for this study: eighteen in 1997 and fifteen in 1998 (Table 1). Field sizes ranged from less than 1 to 71 ha. Some fields were isolated and were completely surrounded by forest. Many fields were only partially surrounded by forest because they were situated immediately next to other lowbush blueberry fields or roads. More details on the study sites can be found in the work of Karem [43].

Trap design

In a preliminary study in Penobscot Co., ME during the fall of 1996, a number of different insect traps were used to evaluate their efficiency in trapping wasps. Results of this study suggested that the use of malaise traps is an effective method for trapping wasps in blueberry fields [43]. Malaise traps were used to sample insects in blueberry fields and forest stands bordering the perimeter of those fields. Traps were constructed of a vinyl mesh material (8 threads/cm) with a pore size of 0.08 cm^2. The lower intercept panels were made by sewing two pieces of black mesh material (102 cm high x 91 cm wide) together to form a “+” shape when viewed from above. The upper collecting hood was made by sewing four triangular pieces of white mesh material

Table 1. Description of the 18 field sites studied in 1997, Washington County, Maine.

Year	Field ID	Regional block	Field size (ha)	% Field edge forested	Application date	Insecticide	Rate (l/ha)
1997	1	I	3.2	50	none	none	none
1997	2	I	14.6	70	5/19/97	Biobit XL[1]	1.17 l
1997	3	I	49.8	60	7/12/97	Sniper[2]	0.58 l
1997	4	I	32.4	40	5/19/97	Biobit XL	1.17 l
1997	5	I	16.2	40	7/12/97	Sniper	0.58 l
1997	6	I	2.4	100	5/19/97	Biobit XL	1.17 l
1997	7	I	23.9	70	7/12/97	Sniper	0.58 l
1997	8	II	13.8	40	5/19/97	Biobit XL	1.17 l
1997	9	II	15.8	0	7/12/97	Sniper	0.58 l
1997	10	II	4.0	40	5/19/97	Biobit XL	1.17 l
1997	11	II	3.2	60	7/12/97	Sniper	0.58 l
1997	12	III	4.0	50	5/19/97	Biobit XL	1.17 l
1997	13	III	10.1	70	7/12/97	Sniper	0.58 l
1997	14	III	6.9	60	7/16/97	Imidan[3]	0.70 l
1997	15	III	3.0	80	7/16/97	Imidan	0.70 l
1997	16	III	11.3	70	7/18/97	Imidan	0.70 l
1997	17	III	5.7	50	7/18/97	Imidan	0.70 l
1997	18	III	1.2	90	7/17/97	Sniper	0.58 l

[1] *Bacillus thuringiensis,* Kurstaki strain
[2] azinphos-methyl
[3] phosmet

(50 cm high x 66 cm base) together to form a pyramid-shaped section that was placed over the lower panels. A 1.52 m length of hardened EMT® steel conduit was used to support the trap. A collecting cup was seated on top of the conduit. The trap was secured to the ground using tent stakes and guy-lines. A small block of Vapona® was placed in the collecting cups of each malaise trap as a killing agent.

Insect sampling

Wasp populations in each field site were sampled during the summers of 1997 or 1998. Three traps were deployed along a linear transect established within each field site. Transects extended from a point 10 m beyond the field edge out to the interior of each field. Three field locations (A, B, and E) were established along each transect for the positioning of traps. Trap A was located at the end of the transect 10 m beyond the field border into the adjacent forest, trap B was located at the field border, and trap E was located at the field interior (near the center). In this study, trap capture was pooled among locations within a field. In 1997, traps were set during the week of 26-5-97 and checked once a week while blueberry plants were in bloom until the week of 23-6-97. Thereafter, traps were checked every other week until the week of 21-7-97, one week before harvest. In 1998, traps were set during the week of 11-5-98 and checked every other week until the week of 27-7-98, one week before harvest. All insects were collected during each field visit and returned to the University of Maine for sorting, pinning, and identification.

Insect identification

All insects of the suborder Apocrita (ants, bees, parasitic and non-parasitic wasps) except those of the family Formicidae (ants) were sorted from the collection cups of all traps. These insects were then further divided by removing all members of the superfamily Apoidea (bees). A reference collection of parasitic and non-parasitic wasps was developed using these specimens. Wasp specimens were initially identified to superfamily, family or subfamily. Identification of all wasps to the species level was impractical due to limited taxonomic expertise. Instead, wasps from this study were classified into morphologically distinct taxa (morphs) based on external morphological characteristics. This method has been used in other studies as an alternative to formal insect species identification in order to get relative estimates of the abundance and richness of insect communities [44]. Identification to superfamily, family, or subfamily and sorting to morphospecies was performed by J. E. Karem and D. Ngollo. Morphospecies were further identified to genus or species by Dr. John Luhman of the Minnesota Department of Agriculture. As a result, some morphospecies represent a single wasp species, and some represent a species complex. Voucher specimens are curated at the Maine State Museum in Augusta, Maine.

Data analysis

Data sets were constructed to represent the following variables estimated at the site level and summed over the season: 1) the number of all wasps captured at each site; 2) the number of each morphospecies captured at each site; and 3) the amount and frequency of all insecticides applied to each blueberry field during the sampling duration.

The insecticides Biobit XL (*Bacillus thuringiensis* Kurstaki, 1.2 kg/ha), Imidan 2.5 EC (Phosmet, 1.5 L/ha), and Sniper 2-E (Azinphosmethyl, 1.0 L/ha) were applied to fields while insects were being collected for this study (Table 2). In 1997, 12 fields had one of these insecticides applied between the 5th and 6th collections (12-7-97 through 18-7-97), and 6 fields did not receive insecticide. In 1998, 9 fields had one of the insecticides applied between the 5th and 6th collections (17-7-97 and 18-7-97), and 6 fields did not receive insecticide (Table 2). Preliminary analyses were performed using linear regression (PROC GLM, SAS for Windows 8.1 [45]) to assess the impact that the number of insecticide applications had on the overall abundance of wasps in blueberry fields within a year. A replicated BACI (before-after-control-impact) design analysis [46, 47] was conducted to determine the experimental effect of a single insecticide application on wasps. This analysis requires a randomized complete block design for a multivariate repeated measures analysis (PROC GLM, SAS for Windows 8.1) to examine the effect of insecticides on wasp abundance before (collection 1-5) and after the insecticide period (collection 6) in treated versus untreated paired fields. Variables were transformed as appropriate to normalize the data and stabilize the variance. A significant time-by-treatment interaction indicated that the number of insects captured is not consistent before and after treatment with insecticide for treated versus non-treated fields.

In an effort to see whether the impact of insecticides was greater on certain wasp taxa than others, the same analysis was applied to each of the 4 families that were represented by selected wasp morphospecies (Table 3). The abundance of some morphospecies was not sufficient to perform this analysis on individual morphospecies, and hence morphospecies were grouped together by family. No analysis could be performed on morphospecies belonging to Chrysididae or Vespidae because an insufficient number of these wasps were recovered after the insecticide period. All wasp family data were transformed as necessary with the natural logarithm.

RESULTS

Biobit XL (2 pints/acre), Sniper 2-E (1 pint/acre) and Imidan 2.5 EC (1.5 pints/acre) were used to treat fields in this study (Table 2). All three of the insecticides were considered in the aggregate in our analyses. Even though Biobit XL (*B. thuringiensis* toxin) is not directly toxic to Hymneoptera, it is toxic to many of their prey and hosts and hence indirect effects could occur. Therefore, our analyses measured both direct and indirect effects simultaneously. It was not possible to factor out independent effects with our experimental design since Biobit was applied in the same fields as Sniper.

Initial analyses suggested no significant relationship between the number of insecticide applications on overall logarithm-transformed wasp trap capture in 1997 ($F_{(1,17)} = 3.45$, $P = 0.075$) or 1998 ($F_{(1,14)} = 1.53$, $P = 0.233$). Knowing that the impact on wasp abundance was being affected by other factors such as field size and floral abundance and diversity [17], a repeated-measures analysis was performed to

Table 2. Description of the 15 field sites studied in 1998, Washington County, Maine.

Year	Field ID	Regional block	Field size (ha)	% Field edge forested	Application date	Insecticide	Rate (l/ha)
1998	19	III	1.6	30	none	none	none
1998	20	III	5.9	60	7/17/98	Sniper[2]	0.58 l
1998	21	III	9.5	50	none	none	none
1998	22	II	5.7	40	7/17/97	Imidan[3]	0.70 l
1998	23	II	2.0	80	7/17/97	Imidan	0.70 l
1998	24	II	16.6	40	7/17/97	Imidan	0.70 l
1998	25	II	44.5	60	7/17/97	Imidan	0.70 l
1998	26	I	3.2	50	none	none	none
1998	27	I	0.8	70	none	none	none
1998	28[1]	I	68.8	50	8/03/98	Sniper	0.58 l
1998	29[1]	I	28.3	100	8/03/98	Sniper	0.58 l
1998	30	I	12.1	50	7/18/97	Imidan	0.70 l
1998	31	I	2.0	70	7/18/97	Imidan	0.70 l
1998	32	I	70.9	70	7/18/97	Imidan	0.70 l
1998	33	I	0.8	80	7/18/97	Imidan	0.70 l

[1] Fields considered untreated for data analyses since insecticide was applied after final insect collection date.
[2] azinphos-methyl
[3] phosmet

Table 3. Thirteen morphospecies of parasitic and non-parasitic wasps identified from all wasps captured in malaise traps.

Morphospecies ID	Family	Subfamily	Genus	Species
BM2	Diapriidae	N/A	N/A	N/A
BM3	Chrysididae	N/A	N/A	N/A
BM5	Vespidae	Vespinae	N/A	N/A
BM6	Pompilidae	N/A	N/A	N/A
BM7	Ichneumonidae	Ophioninae	*Ophion*	N/A
BM8	Ichneumonidae	Tryphonina	*Netelia*	*chloris, blantoni, ttarsata*
BM9	Ichneumonidae	Campoplegina	*Dusona*	*laminata, montrealensis, variabilis*
BM11	Braconidae	Microgastrinae	*Microplitis*	N/A
BM12	Braconidae	Cheloninae	*Phanerotoma*	N/A
BM13	Ichneumonidae	Banchinae	*Banchus*	*flavescens*
BM14	Ichneumonidae	Cryptinae	*Aptesis*	*incompta*
BM16	Ichneumonidae	Ichneumoninae	*Cratichneumon* *Barichneumon*	*pteridis, rubricoides, flavipectus* *soror, excesior*
BM17	Ichneumonidae	Banchina	*Exetastes*	*abdominalis*

compare within and between field temporal changes in wasp abundance, before and after insecticide applications in paired treated and non-treated fields. There was a significant time (before *vs.* after) x insecticide treatment interaction in 1998 ($F_{(1,88)} = 6.50$, $P = 0.017$). Results from 1997 were not significant ($F_{(1,106)} = 3.94$, $P = 0.056$), but are notable and consistent with the significant trend observed in 1998. In 1997, non-treated fields showed large increases in trap captures, ranging from 18-53%, after the treatment period, compared to fields treated with insecticides which showed minimal increases in trap captures (Fig. 1). A somewhat different temporal trend was observed in 1998. The number of wasps decreased in both treated and untreated fields, but the decrease was substantially greater in insecticide-treated fields. Fields treated with at least one insecticide exhibited decreases in trap capture ranging from 31% to 42%, but non-treated fields showed minimal change (Fig. 1).

We found evidence to suggest that the response of wasp families (Diapriidae, Braconidae, Ichneumonidae and Pompilidae) to insecticide applications varied significantly. Pompilidae appear to suffer a substantial negative impact from insecticide application. A highly significant time x treatment effect was observed with pompilids in 1997 ($F_{(1,34)} = 10.56$, $P = 0.005$) and 1998 ($F_{(1,28)} = 11.43$, $P = 0.005$). The number of pompilid wasps trapped in treated fields consistently decreased after the application of insecticides while the number trapped in non-treated fields increased more than 100% for both years after the times of applications (Fig. 2).

Braconidae also appear to be lower in treated sites than non-treated sites after applications of Imidan and Sniper insecticides. In 1997, a significant time-by-treatment effect was not detected at a 0.05 rejection level ($F_{(1,34)} = 3.39$, $P = 0.084$), but the difference between the number of Braconid wasps captured in non-treated versus treated fields was substantial (Fig. 2). After the application period, the number of Braconid wasps captured in non-treated fields increased approximately three times that of treated fields (Fig. 2). In 1998, a time-by-treatment effect was detected ($F_{(1,28)} = 9.82$, $P = 0.008$), and fields treated with insecticides showed about a 71% decrease in the number of Braconid wasps recovered from traps following application whereas untreated fields exhibited a 14% increase (Fig. 2).

There was no statistical evidence for decreases in the Diapriidae due to insecticide applications in 1997 ($F_{(1,34)} = 2.64$, $P = 0.124$) or 1998 ($F_{(1,28)} = 1.59$, $P = 0.229$), nor the Ichneumonidae in 1997 ($F_{(1,34)} = 1.04$, $P = 0.323$) or 1998 ($F_{(1,28)} = 2.57$, $P = 0.133$) due to insecticide application (Fig. 3).

DISCUSSION

Exposure to insecticides is thought to be a key factor in determining the abundance and species

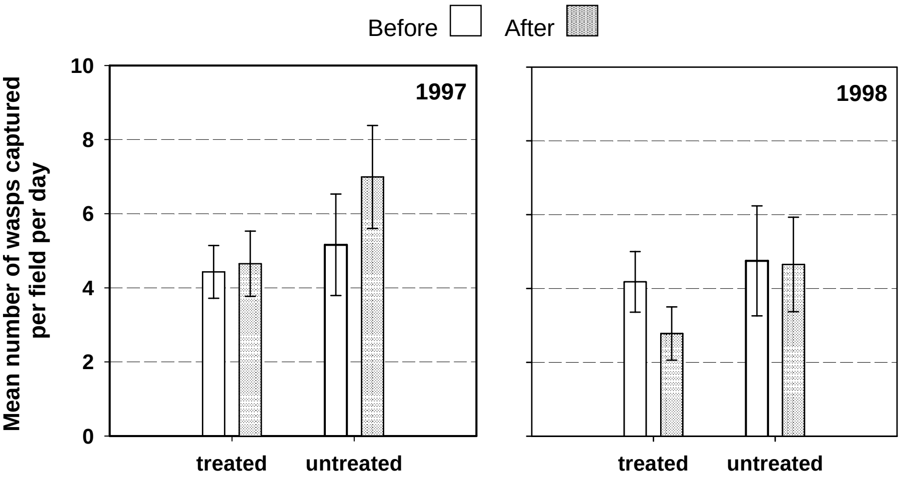

Fig. 1. The mean number of total wasps captured daily before and after the insecticide application periods in treated (n = 12 in 1997; n = 9 in 1998) and untreated fields (n = 6 in 1997; n = 6 in 1998). Bars represent one standard error of the mean (S.E.).

composition of parasitoid assemblages within an agricultural landscape [37, 48, 49], and may even determine the diversity and abundance of parasitoid communities [35, 50]. In particular, organophosphate insecticides have been found to be particularly harsh on many species of wasps [51, 52, 53]. In Maine blueberry, predaceous and parasitic wasp abundance varied between sites as much as six fold [17]. Therefore, we used a proxy of insecticide exposure (insecticide applications) in lowbush blueberry in an attempt to assess the impact on overall wasp abundance between fields, and to determine whether the impact varied among different wasp taxa at the family level.

The insecticides used during this study, Biobit XL, Imidan and Sniper, when considered as an aggregate appear to have substantial negative impacts on overall wasp populations in lowbush blueberry (Fig. 1). Further, pompilid and braconid wasps appear to be far more sensitive to the toxic effects of these insecticides than diapriids and ichneumonids. Noticeable decreases in pompilid populations in treated fields (while non-treated fields showed substantial population increases) during both years may be the result of both direct and indirect toxic affects on these wasps (Fig. 2). Prey spider populations may also be reduced by these insecticide applications that could influence the

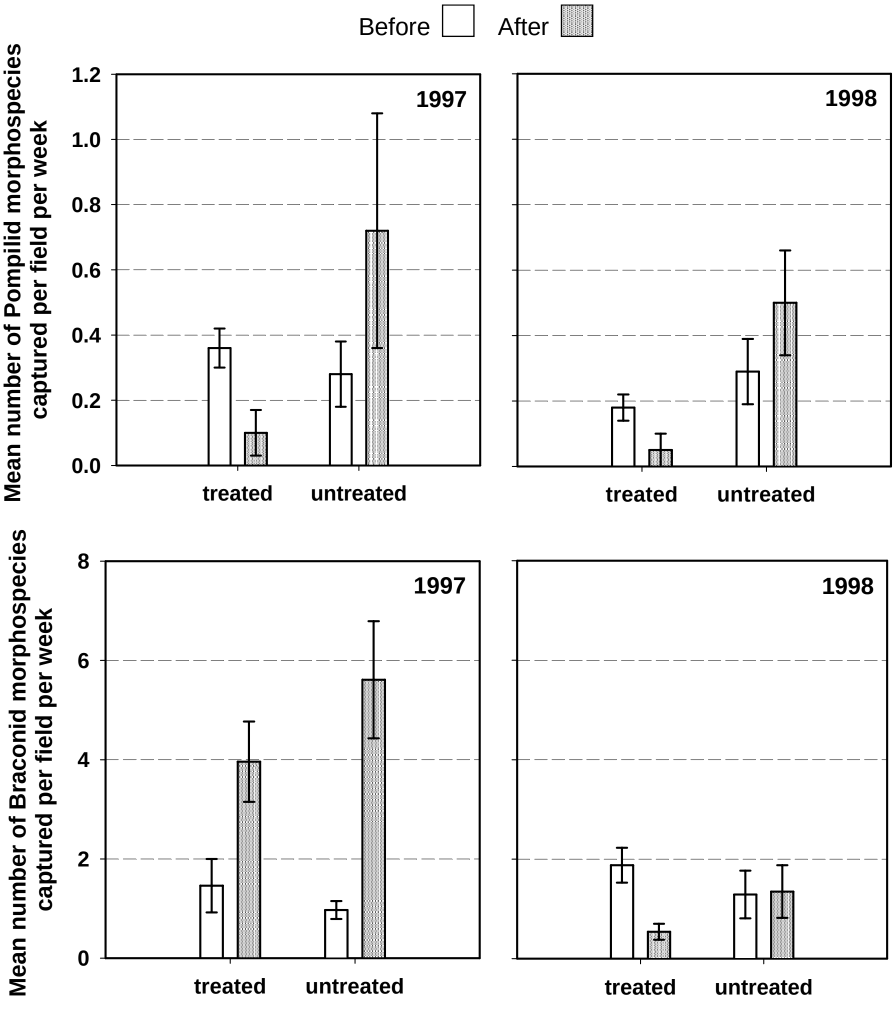

Fig. 2. The mean number of Pompilid and Braconid morphospecies captured weekly before and after the insecticide application period in treated (n = 12 in 1997; n = 9 in 1998) and untreated fields (n = 6 in 1997; n = 6 in 1998). Bars represent one standard error of the mean (S.E.).

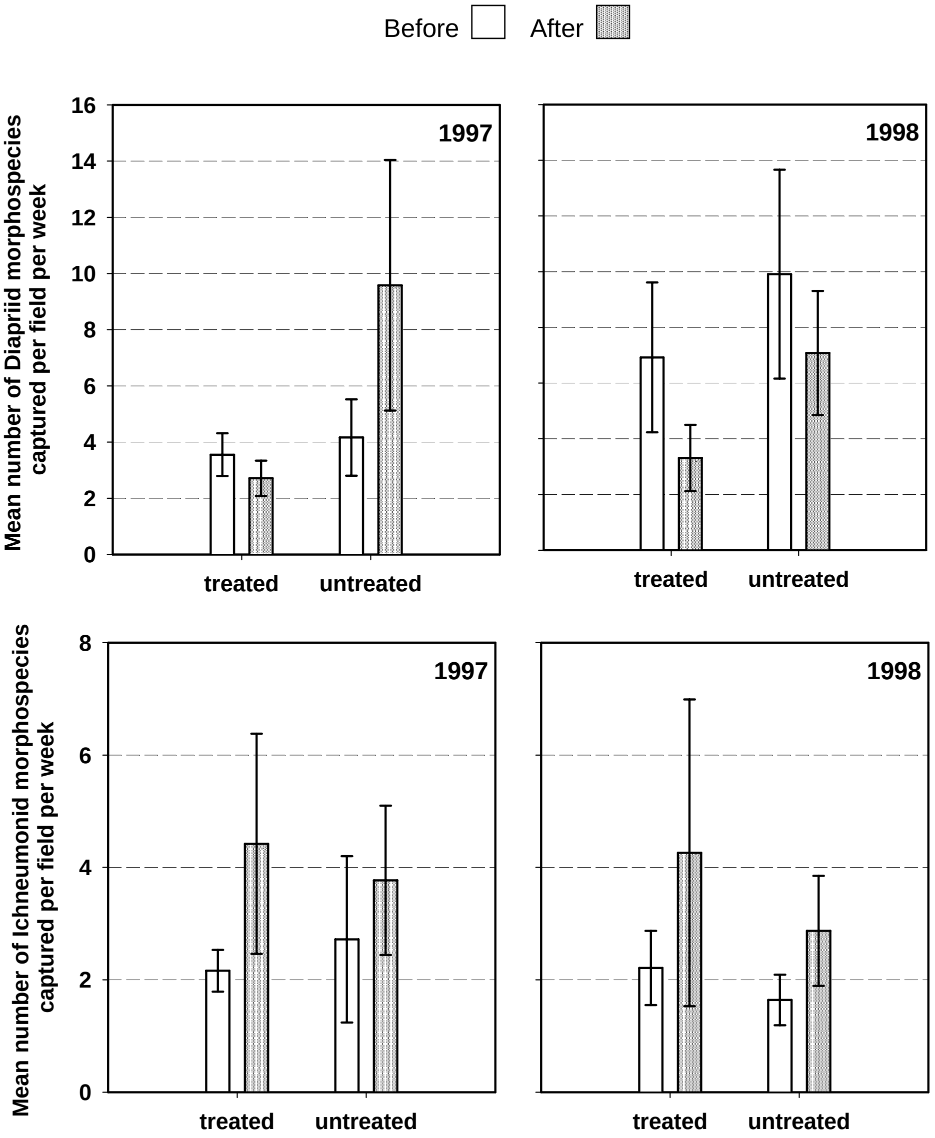

Fig. 3. The mean number of Diapriid and Ichneumonid morphospecies captured weekly before and after the insecticide application period in treated (n = 12 in 1997; n = 9 in 1998) and untreated fields (n = 6 in 1997; n = 6 in 1998). Bars represent one standard error of the mean (S.E.).

abundance of pompilids in treated fields [54]. Studies of the effects on braconid wasps by insecticides in agricultural cropping systems seem to be more common. A number of researchers have found braconids to be very sensitive to a variety of insecticides used in agricultural landscapes [25, 29, 55, 56]. For example, Tillman [24] examined a number of insecticides and found all except the moderately toxic carbamate insecticide thiodicarb were extremely toxic to a species of *Microplitis* (*Microplitis croceipes*).

Organophosphate insecticides such as Imidan and Sniper may not have the lethal effects on diapriids that they appear to have on braconids and pompilids, perhaps because diapriids were mostly captured within the field border [57] and may avoid exposure to insecticides applied in the field that were applied primarily to the interior of the fields (Fig. 3). It is also interesting that ichneumonid populations did not decrease significantly as a result of insecticides (Fig. 3). Ichneumonids may not be as sensitive to these organophosphate insecticides as braconids and pompilids, as suggested by Williams *et al.* [58] with the insecticide Spinosad®. However, it may be important to investigate wasps at a finer taxonomic resolution. Some ichneumonids (i.e. *Aptesis incompta* Townes) may have a reduced exposure to insecticides because in Maine blueberry landscapes they primarily inhabit the forested border like diapriids [57], while others may suffer from insecticide exposure because they are primarily found in the interior of blueberry fields foraging for host insects (i.e. *Ophion* spp.) [57].

Considering that past research has identified the important role of natural enemy biodiversity in agriculture [25, 35, 59, 60, 61, 62, 63], conserving and promoting populations of native parasitic and predatory natural enemies that prey upon lowbush blueberry insect pests should be an integral part of lowbush blueberry pest management. The results of our study suggest that efforts in managing agroecosystems for biodiversity for the purpose of enhancing wasp diversity and abundance might be facilitated by blueberry growers minimizing exposure of wasps to persistent insecticides. Considering the negative impact that insecticides had on braconid morphospecies in this study (Fig. 2), blueberry growers should consider alternative insecticides that have been found less toxic to wasps such as spinosyns [9]. Insecticide toxicity may be of particular concern with two genera from this study, *Microplitis* sp. and *Phanerotoma* sp. (Hymenoptera: Braconidae), as they are widely recognized as beneficial crop parasitoids in numerous other systems [25, 64, 65, 66, 67]. Biological controls such as *Bacillus thuringiensis* and *Beauveria bassiana* could be effective in reducing particular insect pests without harming beneficial wasp populations [18].

CONCLUSION

Conservation biological control is likely to be the most environmentally friendly and inexpensive pest control method available to blueberry growers. Ironically, the practice of conserving natural enemies has been possible since the start of blueberry cultivation, but is a relatively new management tool compared to the more traditional IPM tactics developed over the past two decades for lowbush blueberry production [6]. However, with the arrival of the spotted wing drosophila, convincing growers to reduce pesticide use to conserve natural enemies may prove more difficult. The degree of its success depends on the amount of reliable and useful information generated from field surveys such as this one that focuses on the ecology of native natural enemies. As it appears that some wasp taxa respond differently to insecticides, this information and further toxicology studies are important to develop management strategies that will promote populations of beneficial wasps associated with the blueberry field interior.

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CONFLICT OF INTEREST STATEMENT

The authors certify that they have no conflict of interest concerning any of the data collected and statistical analysis and conclusions reported in the results and discussion.

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