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Wildflower plantings enhance the abundance of natural enemies in adjacent blueberry fields

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Abstract

Wildflower plantings can support local abundance of natural enemies, but their influence on biological control of pests in adjacent crop fields is less well documented. To test whether biological control is enhanced by these plantings, we established native, perennial wildflowers in areas adjacent to highbush blueberry fields. Once wildflowers were established we found greater abundance of natural enemies in the fields adjacent to wildflower plantings compared with those adjacent to unenhanced control field perimeters. Predaceous arthropods, including spiders, hoverflies, and lady beetles, were among the most common natural enemies observed and collected in the blueberry fields. Using corn earworm eggs, *Helicoverpa zea* (Lepidoptera: Noctuidae), as sentinel prey we found a similar pattern of biological control, with higher biological control services index values in fields adjacent to the wildflower plantings than in the unenhanced control fields. Our results provide evidence for the ability of wildflower plantings to support natural enemy populations in agricultural landscapes, and to potentially provide local enhancement of biological control in adjacent crops.

Key words: Conservation, insectary strips, predation, Vaccinium

1. Introduction

Beneficial insects provide valuable ecosystem services, including decomposition, pollination, and biological control that support agricultural production and human survival. Worldwide, pest suppression from insect natural enemies, such as predators and parasitoid wasps, is valued at over US\$400 billion annually (Costanza et al., 1997). The abundance and diversity of these insect natural enemies are generally higher in agricultural landscapes surrounded by uncultivated, natural habitat (Bianchi et al., 2006), whereas insecticide use, scarcity of flowering plants, and loss or fragmentation of habitat can all make agricultural systems resource-poor landscapes for these beneficial insects (Landis et al., 2000; Hendrickx et al., 2007; Wade et al., 2008). The lack of habitat and resources can cause declines in valuable biological control services and increase potential for pest outbreaks (Geiger et al., 2010; Chaplin-Kramer et al., 2011; Meehan et al., 2011). This trend may be reduced or reversed by enhancing habitat to provide natural enemies with alternate prey or hosts, a constant food supply, and appropriate microclimates. Increasing biological diversity within agricultural landscapes through the provision of additional floral resources to conserve natural enemies may also enhance or maintain other ecosystem services (Isbell et al., 2011; Kremen and Miles, 2012), such as creating bird habitat (Vickery et al., 2009), controlling erosion (Markwardt, 2005), and conserving native pollinators (Wratten et al., 2012). This latter benefit can also lead to enhanced crop yield (Blaauw and Isaacs, 2014a), which has the potential to stimulate further adoption of these practices by agricultural land owners.

Provision of habitat and resources to support beneficial insects can be used within conservation biological control programs to suppress pest populations (Pickett and Bugg, 1998; Landis et al., 2000; Jonsson et al., 2010). To enhance these insects, a diverse assemblage of flowering, non-crop plants can provide the pollen and nectar necessary to support populations

throughout the season (Landis et al., 2000; Wanner et al., 2006; Jonsson et al., 2008). These plants provide floral resources that are utilized by natural enemies as primary and alternative food sources (Wäckers et al., 2005; Jonsson et al., 2010), and can enhance their longevity, fecundity, and potential control of pests (Büchi, 2002; Berndt and Wratten, 2005; Lee and Heimpel, 2008).

In crop systems with limited habitat resources for natural enemies, increasing plant abundance and diversity through the addition of flowering plants and grasses is expected to increase natural enemy populations (Andow, 1991; Rebek et al., 2005; Sivinski et al., 2009; Thomson and Hoffmann, 2009) and enhance their provision of biological control services (Landis et al., 2000; Bianchi et al., 2006; Zurbrügg and Frank, 2006). There is less information on the relationship of these resources to the delivery of function rather than support of biodiversity (Letourneau et al., 2009; Letourneau et al., 2011), but we have recently found that natural enemy density and diversity increase with the area of native wildflower resources available, with a similar response of the natural control of an aphid pest (Blaauw and Isaacs, 2012).

Floral resources have been used previously in crop systems to increase parasitoid populations and enhance parasitism of pests (Jonsson et al., 2010; Gontijo et al., 2013). For example, Irvin et al. (2006) found increased parasitism rates and decreased densities of light-brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) in apple orchards close to plantings of buckwheat (*Fagopyrum esculentum* Moench) and sweet alyssum (*Lobularia maritima* L. Desv.). Several studies support the importance of floral resources for enhancing parasitoid life histories and their provision of biological control services, but most of these projects have been short-term and have used exotic, annual flowers (Fiedler et al., 2008). In contrast, native perennial flowering plants are adapted to the local environment, provide long-

term support of beneficial insects, are less likely to become invasive, and may increase native biodiversity in agricultural landscapes (Stephens et al., 2006; Fiedler and Landis, 2007b; Isaacs et al., 2009), but few, long-term studies have investigated these plants for the enhancement of insect natural enemies and their services (Fiedler et al., 2008). Conservation plantings that use a mix of native perennial flowering plant species to provide floral resources for an extended period of time (Fiedler and Landis, 2007a; 2007b) are expected to enable natural enemies to remain near crop fields even when prey/host densities are low (Olson et al., 2005) or to provide refuge when fields are sprayed for pest control (Walton and Isaacs, 2011). It is important to note that additional food resources provided by the establishment of wildflower plantings to help conserve natural enemy populations may provide resources and refuge to insect herbivores, including some pests (Wäckers et al., 2007).

Highbush blueberry, *Vaccinium corymbosum* L., is a perennial fruit crop native to eastern North America with a community of native and exotic insect pests (Retamales and Hancock, 2012). Populations of the blueberry aphid, *Illinoia pepperi* (MacGillivray) (Hemiptera: Aphidae), are suppressed by insect natural enemies such as syrphids, coccinellids, chrysopids, and *Orius* spp. (Whalon and Elsner, 1982). Parasitism of blueberry maggot, *Rhagoletis mendax* Curran (Diptera: Tephritidae), larvae by braconid wasps can reach 50% in unmanaged fields (Stelinski et al., 2004). Finally, ground cover can enhance ground beetle abundance and lead to increased prey removal in blueberry fields (O'Neal et al., 2005a; O'Neal et al., 2005b). As highbush blueberry is often managed using intensive pest control approaches, providing resource-rich habitats for natural enemies outside of the crop field may be needed to avoid non-target effects of insecticides on natural enemy populations. Thus, highbush blueberry is a suitable system to measure the effects of local habitat and resource manipulation on natural enemies and pest suppression.

Assessing natural pest control in the field can be challenging due to insecticide use to control pests (Kidd and Jervis, 2007; Sarvary et al., 2007). However, surrogate pest organisms can be used to assess predator activity within field settings. For example, using sentinel corn earworm eggs, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), Werling et al. (2011) determined that predation was strongly related to generalist predator activity, which was enhanced as plant diversity increased within the landscape surrounding the sentinel eggs.

To determine the impact of perennial floral resources on insect natural enemies and the level of biological control services in blueberry fields, we compared insect natural enemy abundance and diversity within field margins that were enhanced with native wildflower plantings or not enhanced. Removal of sentinel prey items as well as natural enemy and pest abundance were measured in the crop fields.

2. Materials and methods

2.1. Site preparation

In 2009, a single wildflower planting was established at each of five highbush blueberry (*Vaccinium corymbosum* L.) farms in southwest Michigan, USA. The plantings were rectangular in shape, ranging from 0.06 to 1.01 ha in size (mean area = 0.36 ± 0.17 ha), and were established in open areas within 3 m of a border of blueberry fields. At each farm, the blueberry field adjacent to the wildflower planting was paired with a control field of the same cultivar and management program but with a typical perimeter of a drive lane and mown grass at least 9 m wide (mean area = 0.14 ± 0.03 ha), without the addition of sown native wildflowers. These control perimeters were separated from the enhanced field border by an average distance of 287.4 m (range 175 to 490 m). The landscapes at a 1 km radius surrounding the sites were

relatively consistent, with $55.3 \pm 4.1\%$ semi-natural habitat (forest and grassland) around the wildflower plantings and $59.3 \pm 8.1\%$ around the control perimeters.

A perennial wildflower seed mix (Michigan Wildflower Farm, Portland, MI) was developed, consisting of 15 species of Michigan native wildflowers with sequential bloom periods that together span May through October and have been shown to be attractive to natural enemies (Fiedler and Landis, 2007a) (Table 1). To reduce competition with weed grasses and invasive plants, and to provide fuel for potential controlled burns, three native grass species were also included in the seed mix (as advised by Wildtype Native Plant Nursery, Mason, Michigan). Details of the establishment, maintenance, and assessment of these wildflower plantings is provided in Blaauw and Isaacs (2014a).

2.2. Natural enemies and herbivores in field perimeters

Natural enemies and insect herbivores in the wildflower plantings and in the unplanted control perimeters were vacuum sampled once each month from May to September in 2010 and 2011. Each site was sampled five times for 30 seconds each (2.5 min total) on each sampling date using a modified reversed-flow leaf blower (BG 56 C-E; Stihl, Waiblingen, Germany) with a fine white mesh bag (150 μ m, The Cary Company, Addison, IL) placed over the intake to capture insects (Fiedler et al., 2008). To limit the bias of sampling due to vegetation height (Hossain et al., 1999), the five 30 s sampling periods were distributed throughout the wildflower plantings and mown control perimeters, being sure to sample from areas that were in bloom. The number of natural enemies and insect herbivores collected via vacuum samples were recorded and identified to family, with the exception of parasitoid wasps (grouped as Parasitica) and *Orius* spp., as well as the blueberry pests Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae); cranberry fruitworm, *Acrobasis vaccinii* Riley (Lepidoptera: Pyralidae); cherry

fruitworm, *Grapholita packardii* Zeller (Lepidoptera: Tortricidae); and blueberry maggot, *R. mendax*. Only the most commonly collected and/or agriculturally important natural enemies in the blueberry system (10) and insect herbivores (7) (Table 2) from the three-year period were used for abundance analyses (Figure 1). Voucher specimens were deposited in the Albert J. Cook Arthropod Research Collection at Michigan State University.

Because the wildflower plantings were at different stages of establishment in each year, the catches of natural enemies and insect herbivores in the wildflower plantings and control perimeters were compared separately for each year between the two treatments (independent variable) using a generalized linear model (GLM) with Poisson distribution, overdispersion parameter estimated by Pearson Chi-square/DF, and estimated by maximum likelihood (JMP, Version 8, SAS Institute Inc., Cary, NC). Count data for each year were pooled per sampling date for each week throughout the sampling period. The diversity of natural enemies and insect herbivores (Shannon's index, H (Wani et al., 2005)) of the specific insect groups of interest from vacuum samples were also compared separately for each year between the two treatments using a Mann-Whitney U test to allow for non-normal distribution of data (SPSS, Version 20, IBM Corp., Armonk, NY).

2.3. Natural enemies and herbivores in crop fields

To determine the effect of floral resource plantings on natural enemies and herbivores in the adjacent crop fields, visual samples were taken every two weeks from May through September during 2009-2011. At each field, 10 blueberry bushes (8 bushes in 2009) along the crop edge were observed for 1 min each, repeating this sampling at another 10 bushes located 15 m into the crop interior parallel to the border (8 bushes in 2009). Insects were sampled in fields adjacent to the wildflower plantings as well as those adjacent to the control field perimeters. The

number of natural enemies and insect herbivores observed on the blueberry plants was recorded and identified as described above for the vacuum samples.

Additional natural enemy and insect herbivore sampling was conducted with yellow sticky traps deployed for seven days each month from May-September of each year. Each of the crop fields adjacent to wildflower plantings and those adjacent to control perimeters received eight yellow sticky traps: four traps along the crop edge and four 15 m into the interior, placed approximately 1-2 m high within bush canopies. After one week deployed in the field, traps were collected and taken back to the laboratory for evaluation. Insects were identified to major taxonomic groups as described above.

Natural enemy and insect herbivore densities observed on blueberry plants per 10 min sample (8 min in 2009) as well as the densities of insects collected per sticky trap were compared between treatments using a GLM as described above (JMP, Version 8). Data were analyzed separately for each year (natural log + 1 transformation for non-normal data), pooling the abundance data from the crop edge and from the interior for each of the two treatments. Shannon diversity values (based on grouping described above) for the natural enemy and insect herbivore communities from observation and sticky trap samples were compared separately for each year between the two treatments using Mann-Whitney *U* tests (SPSS, Version 20).

2.4. Biological control services

To determine how wildflower plantings affect biological control in crop fields, in 2011 and 2012 corn earworm eggs, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) were used as sentinel prey. Corn earworm eggs are predated upon by several generalists predators, including coccinellids, *Orius* spp., carabids, and various spider species (Lesiewicz et al., 1982; Pfannenstiel and Yeorgan, 2002; Pfannenstiel, 2008). Eggs were obtained from a commercial

insectary (French Agricultural Research, Lamberton, MN, USA), where the eggs had been oviposited on sheets of paper towel. Using methods similar to Werling et al. (2011), standardized egg clusters were created by cutting 1 x 2 cm strips of paper towel containing approximately 30 eggs each (range: 15–50). The paper towel strips were then glued to 3 × 5 cm pieces of cardstock using water-soluble glue (Elmer's Glue-All®, Borden, Inc., Columbus, OH, USA). The number of eggs per card was counted and recorded. Eggs were freeze-killed to prevent hatching and then deployed at the five blueberry farms by stapling the cards to the underside of blueberry foliage at roughly 1 m height. At each site, three egg cards were placed at each of three positions: in the wildflower planting, along the edge of the crop field, and 15 m into the interior of the crop field. One of the egg cards at each position was covered with a fine white mesh bag (150 µm, The Cary Company, Addison, IL) to exclude predators, whereas the other two egg cards remained open and exposed to potential predators. The covered egg cards were utilized to control for egg loss due to handling and environmental forces. Using the same methods, egg cards were also placed at the three corresponding locations in crop fields adjacent to the control perimeter at each farm.

Biological control services were measured by comparing the number of eggs present before being placed into the field with the number of eggs (after egg predation by natural enemies in the field) after four days. The biological control services index (BSI) (Gardiner et al., 2009) calculated from these results can vary from 0 to 1, with values increasing as the level of predation increases (Blaauw and Isaacs, 2012). This approach was used to measure biological control services during 4-day periods in June, July, and August in 2011 and 2012 for covered and exposed egg cards.

Biological control services were evaluated by taking the mean of the BSI values across the three sample dates to determine season-averaged egg predation for each sample location

(only by treatment for covered egg cards). The season-averaged BSI values were compared separately for each year between treatments and field locations using a Mann-Whitney *U* test to allow for non-normal distribution of data (SPSS, Version 20, IBM Corp., Armonk, NY).

3. Results

3.1. Wildflower planting establishment

As demonstrated in Blaauw and Isaacs (2014a), throughout the duration of this study, the percent cover and the density per square meter of seeded plants was greater within the wildflower plantings than in the control mown grass field margins, and it increased over time.

3.2. Natural enemies and herbivores in field perimeters

Vacuum sampling along the crop perimeters revealed that in the first year of sampling, the density of natural enemies was slightly, but significantly lower in wildflower plantings than in the control perimeters ($\chi^2=6.14$, $P=0.013$; Figure 1), with lower densities of Coccinellidae, Parasitica, and Staphylinidae (Table 2). During 2011, the overall abundance of natural enemies collected in wildflower plantings increased, but was not significantly different from the control perimeters ($\chi^2=1.05$, $P=0.31$; Figure 1). Specific predator groups, such as Araneae, Cantharidae, and Syrphidae, were collected in significantly higher numbers in the wildflower plantings compared to the control perimeters (Table 2). Staphylinidae were the only natural enemy group to remain significantly greater in the control treatment. When comparing the natural enemy communities collected in the two types of field perimeters, the diversity index (*H*) was slightly greater for the flower plantings during both years, but not significantly different from the grassy perimeters (Table 3).

A diverse community of insect herbivores and pests was also collected via vacuum sampling from the wildflower plantings and control perimeters. The densities of herbivore groups collected from the perimeters were not significantly different between the two treatments in either of the two years of this study (2010: $\chi^2=1.77$, $P=0.18$; 2011: $\chi^2=1.50$, $P=0.22$; Figure 1). When assessing the recorded insect herbivores, in 2010 the densities of Cercopidae and Chrysomelidae were significantly greater in the flower treatments (Table 2). In 2011, Cercopidae densities were again greater in wildflower plantings, whereas Chrysomelidae and Cicadellidae densities were greater in control perimeter samples (Table 2). Of the agriculturally important blueberry pests monitored during this study (*P. japonica*, *A. vaccinii*, *G. packardii*, and *R. mendax*), only *P. japonica* were collected in the vacuum samples, and only in 2011, and their densities were similar in the two treatments (Table 2). Comparing the insect herbivore communities collected from the two types of perimeters, insect diversity was slightly higher each year in the wildflower treatment, but there was never a statistically significant difference (Table 3).

3.3. Natural enemies and herbivores in the crop fields

During 2009 while the plantings were becoming established, there was no difference between treatments in the densities of natural enemies observed in crop fields adjacent to the control or flower treatments (Control: 6.1 ± 0.8 insects/min; Flower: 6.1 ± 0.6 insects/min; $\chi^2 = 0.0006$; $P = 0.98$). During the following two years significantly greater densities of insect natural enemies were observed in fields adjacent to the wildflower plantings compared to fields adjacent to the control perimeters (2010: $\chi^2=5.65$, $P=0.017$; 2011: $\chi^2=8.57$, $P=0.003$; Figure 1). In 2010, the greater natural enemy densities observed in crop fields adjacent to wildflower plantings were dominated by Coccinellidae, Parasitica, and Syrphidae, whereas in 2011, Cantharidae, Parasitica,

and Syrphidae had significantly greater densities (Table 4). In neither year were there any natural enemy groups significantly greater in crop fields adjacent to the control perimeters than the flowering perimeters.

Assessing the diversity of natural enemies observed in crop fields in 2009, there was no difference between the two treatments (Control: $H = 0.8 \pm 0.05$; Flower: $H = 0.8 \pm 0.05$; $U = 2933$; $P = 0.36$), but natural enemies were significantly more diverse in crop fields adjacent to wildflower plantings in 2010 and 2011 (Table 3).

In addition to observations, natural enemies were sampled in crop fields using yellow sticky traps. Similar to the observation data, in 2009 there was no significant difference in the density of natural enemies collected per sticky trap in fields adjacent to either treatment (Control: 7.9 ± 0.4 insects/trap; Flower: 8.5 ± 0.4 insects/trap; $\chi^2 = 1.26$; $P = 0.26$). In 2010 and 2011, significantly greater densities were collected in fields adjacent to the flower treatments (2010: $\chi^2=6.35$, $P=0.012$; 2011: $\chi^2=15.86$, $P=0.0001$; Figure 1).

Evaluating the specific natural enemy groups collected via sticky traps, in 2010 the densities of Carabidae, Coccinellidae, Formicidae, Parasitica, and Syrphidae were significantly greater in crop fields adjacent to the flower treatments (Table 5). Similarly, in 2011, Carabidae, Parasitica, Staphylinidae, and Syrphidae had significantly greater densities in the crop fields adjacent to the wildflower plantings (Table 5). In contrast, the diversity of natural enemies collected with sticky traps was similar in the two treatments in 2009 (Control: $H = 0.9 \pm 0.03$; Flower: $H = 0.9 \pm 0.04$; $U = 19686$; $P = 0.79$) and thereafter (Table 3).

Observations of insect herbivores in crop fields indicated that their density did not differ between the two treatments in 2009 (Control: 2.0 ± 0.7 insects/m²; Flower: 3.6 ± 0.7 insects/m²; $\chi^2 = 0.045$; $P = 0.83$), 2010, or 2011 (2010: $\chi^2=1.86$, $P=0.17$; 2011: $\chi^2=0.009$, $P=0.92$; Figure 1). Consistent with the overall insect herbivore density in 2010, there were no individual herbivore

groups that varied significantly between treatments. Conversely, in 2011 significantly more Cercopidae were observed in fields adjacent to the wildflower plantings, and of the agriculturally important blueberry pests monitored during this study, only *R. mendax* was observed to have significantly lower densities in crop fields adjacent to the flower perimeters (Table 4). Assessing the diversity of the observed insect herbivore community, there was no significant difference between the two treatments in 2009 (Control: $H = 0.2 \pm 0.04$; Flower: $H = 0.3 \pm 0.04$; $U = 3089$; $P = 0.68$) or in 2010, whereas in 2011 a less diverse community of insect herbivores was observed in fields adjacent to the flower perimeters (Table 4).

Insect herbivore density and diversity in crop fields were also measured on the yellow sticky traps, and these were similar between treatments in 2009 (Control: 21.3 ± 2.6 insects/traps; Flower: 25.7 ± 3.3 insects/traps; $\chi^2 = 1.13$; $P = 0.29$), with this pattern continuing in subsequent years (2010: $\chi^2 = 2.06$, $P = 0.15$; 2011: $\chi^2 = 0.27$, $P = 0.61$; Figure 1). Although the total herbivore density was not different between the two treatments in 2010, the density of Chrysomelidae was significantly greater in crop fields adjacent to wildflower plantings and the density of *P. japonica* was lower in fields adjacent to the perimeter with wildflowers (Table 5). Similarly, in 2011 significantly greater densities of Chrysomelidae, Cercopidae, and Miridae were observed in fields adjacent to the wildflower plantings, whereas *P. japonica* and *R. mendax* were observed to have significantly lower densities in crop fields adjacent to the wildflower-enhanced perimeters (Table 5). Assessing the diversity of the insect herbivore community collected via sticky trap sampling, there was no significant difference between the two treatments in 2009 (Control: $H = 0.3 \pm 0.02$; Flower: $H = 0.3 \pm 0.02$; $U = 19846.5$; $P = 0.89$) or in 2010 (Table 3). In contrast to the observation results, in 2011 a more diverse community of insect herbivores was collected in fields adjacent to the wildflower plantings (Table 3).

3.4. Biological control services

In the four day period of deployment, we found 20% of the eggs were removed on average in the control fields and 36% were removed in the fields adjacent to wildflower plantings. In 2011, significantly higher levels of biological control (BSI) of corn earworm eggs were measured within the wildflower plantings and along the crop edge adjacent to the wildflower plantings than those in the corresponding positions in the grass control perimeter (Figure 2; $U = 273.5$, $P = 0.012$ and $U = 237$, $P = 0.001$, respectively). Although BSI values were higher adjacent to flower treatments, at the field interior position there was no significant difference (Figure 2; $U = 386.5$, $P = 0.34$). The same trend was also found in 2012, with significantly greater BSI values within the wildflower plantings and along the crop edge adjacent to the plantings (Figure 2; $U = 192.5$, $P = 0.048$ and $U = 128$, $p = 0.001$, respectively), but not within the interior positions (Figure 2; $U = 208.5$, $P = 0.10$). Egg removal as measured through BSI for the covered egg mass was low in 2011 (Control: 0.007 ± 0.005 ; Flower: 0.006 ± 0.004) and 2012 (Control: 0.015 ± 0.009 ; Flower: 0.009 ± 0.005) for both treatments with no significant difference in either year (2011: $U = 111.5$, $P = 0.97$; 2012: $U = 110.5$, $P = 0.94$)

4. Discussion

This study demonstrates that establishing wildflower plantings in areas adjacent to blueberry fields positively affects the density and diversity of some natural enemy groups and enhances biological control of sentinel pest eggs along the crop edge. Importantly, insect herbivore densities, and blueberry pests specifically, were not enhanced by the provision of wildflower habitat. Some of the groups were neutral while others declined, suggesting that local-scale habitat manipulation through the establishment of these plantings can selectively enhance beneficial insects and support regulation of pest populations.

The research outlined here was performed at commercial highbush blueberry farms that were chemically managed for common blueberry pests, such as cranberry fruitworm, blueberry maggot, and blueberry aphids. Due to the intensive management programs, it was difficult to measure differences in abundances of these pests or the subsequent impact due to natural enemies. Despite this, we focused sampling on natural enemy groups that are likely to have the biggest impact on blueberry pest control. For example, parasitoids are important natural enemies of cranberry fruitworm (Murray et al., 1996) and blueberry maggot (Stelinski et al., 2004). Additionally, carabids may be important biological control agents of overwintering cranberry fruitworm, blueberry maggot, and Japanese beetles (O'Neal et al., 2005b). blueberry aphid, *Illinoia pepperi* (MacGillivray) (Hemiptera: Aphidae), are suppressed by insect natural enemies such as syrphids, coccinellids, chrysopids, and *Orius* spp. (Whalon and Elsner, 1982).

Sampling of insects in the wildflower plantings and the blueberry crop fields revealed that two to three years after wildflower establishment, natural enemy populations were generally enhanced. However, the results for individual natural enemy groups varied depending on the sampling method used. Vacuum samples from the crop perimeters detected a lower abundance of natural enemies within the flower treatments in 2010, but there was no significant difference between the two treatments in 2011. This contrasts with the observational and sticky trap data from samples collected within the adjacent crops where in 2010 and 2011 significantly greater densities of natural enemies were measured in crop fields adjacent to wildflower plantings (Figure 1). This inconsistency may be due to the increased efficiency of vacuum sampling of shorter vegetation (Hossain et al., 1999), such as was found in the mown control field perimeters compared to the stands of wildflowers and grasses.

Given time for flower establishment, natural enemies were more abundant in wildflower habitats and significantly more abundant in crop fields adjacent to wildflowers, when compared

to fields adjacent to mown grass field margins. This supports the established pattern of naturalized non-cropped habitats supporting greater abundance and diversity of beneficial insects compared with simple agricultural landscapes (Thomas et al., 1991; Lee et al., 2001). Another important aspect of this study was to determine whether floral habitats can in turn increase specific natural enemies within the adjacent crop fields. The finding that Parasitica and Syrphidae were at significantly greater densities in 2010 and 2011 within crop fields adjacent to the wildflower plantings than in fields adjacent to the control perimeters supports the hypothesis that adding flowering plant habitat back into intensively managed cropland can increase agriculturally-important natural enemies. The mechanism of this enhancement is likely to be the increased availability of nutritional resources, since parasitoid wasps (Winkler et al., 2009) and hoverflies (White et al., 1995) depend on and are enhanced by pollen and nectar. Creating a floral habitat that increases the carrying capacity of insect natural enemies may enhance the degree of predator spillover and their impact on pest insects within the adjacent crop field (Holt and Hochberg, 2001).

Coccinellidae, which also benefit from access to pollen and nectar (Lundgren, 2009), were sampled in significantly greater abundance in 2010 from fields adjacent to wildflower plantings, but this trend did not continue into 2011. Carabids are another notable natural enemy group that can respond positively to non-cropped land (Lee et al., 2001). These beetles often go unnoticed because they are generally active at night and hunt near the ground (Lövei and Sunderland, 1996), but the sticky trap sampling revealed significantly greater ground beetle captures in blueberry fields adjacent to wildflower plantings. This supports the results of O'Neal et al. (2005a) who showed that other conservation practices such as increased ground cover and reduced-risk insecticides can enhance ground beetle abundance in blueberry fields.

One might expect that if greater densities of natural enemies were found in crop fields adjacent to wildflower plantings then there would also be enhancement within the nearby plantings. The lack of such a finding suggests that the vacuum sampling method biased our results. This method can be more efficient at sampling in shorter vegetation (Hossain et al., 1999), such as was found in the mown control field perimeters. Sanders and Entling (2011) demonstrated that vacuum sampling can underestimate species richness in cross-habitat comparisons and misses many ground dwelling arthropods. Because of this, it is possible that vacuum samples underestimated the overall abundance of arthropods in the wildflower plantings compared to the control samples that were taken from the shorter vegetation of the mown grass control perimeters. Additionally, some natural enemies remain mostly within the cropping system (Duelli et al., 1990), while others utilize resources in the adjacent natural habitats (Landis et al., 2000), so it is possible that the lower natural enemy abundance in the wildflower plantings was partially due to the insects leaving the plantings to redistribute into the adjacent crop fields. Despite the potential under-estimation in density and diversity, in 2011 greater numbers of Araneae, Cantharidae, Staphylinidae, and Syrphidae were collected within the wildflower plantings than the mown grass controls (Table 2).

Wildflower plantings established to help conserve natural enemy populations may provide resources and habitat for insect (Lavandero et al., 2006; Wäckers et al., 2007) and vertebrate herbivores (Briner, 2005). In this study, insect herbivore density was never significantly different between the two treatments using vacuum, observational, or sticky trap sampling. Notwithstanding, the concerns described above about the efficiency of vacuum sampling in tall vegetation, the result is consistent across methods. In 2011, insect herbivores were more diverse in crop fields adjacent to the control perimeters than in fields adjacent to the wildflower plantings. Only Cercopidae and Chrysomelidae captures were influenced by the

addition of floral resources. Previous research has shown that these groups are attracted to floral resources (Fiedler and Landis, 2007a). Although these families are not considered important pests of highbush blueberries, they may be pests of other crops (Evans, 1972; Stenberg, 2012), highlighting the need to consider the crop- and region-specific benefits and costs of the establishment of wildflower resources within the agro-ecosystem.

There is relatively little information on the magnitude of effect for wildflower plantings supporting natural enemies, but these floral habitats clearly provide resources that help support natural enemies in adjacent crop fields. In a review by Bianchi et al. (2006), the increase in natural enemy populations with natural habitat translated directly into enhanced pest suppression in adjacent crop fields in 45% of studies evaluated. Here, with the potential enhancement of generalist predators of corn earworm eggs such as coccinellids (Pfannenstiel and Yeargan, 2002), spiders (Pfannenstiel, 2008), and carabids (Lesiewicz et al., 1982), egg predation of sentinel corn earworm eggs was significantly greater within wildflower plantings, as well as along the adjacent crop edge compared to those from the control treatments (Figure 1). Although greater numbers of eggs were removed from the crop interior in fields adjacent to the wildflower plantings, the BSI values for this position were not significantly greater than the control. Similarly, Hassan et al. (2012) found greater abundance of carabids in cereal fields surrounded by more natural grassland, but their abundance in the crop decreased rapidly with distance from the strip. Eilers and Klein (2009) found that parasitoid densities were greatest along margins of almond orchards with significantly fewer parasitoids detected within the orchard interior. Although dependent on foraging range, preferences, and cues of natural enemies, these results suggest that the edges of crop fields adjacent to floral resources will experience the greatest benefit from natural enemies compared to the crop interior (Walton and Isaacs, 2011). However, for many pests and in the

particular case of cranberry fruitworm (*A. vaccinii*) in highbush blueberry (Mallampalli and Isaacs, 2002) pest populations often have the greatest need for control at the field edges.

The lower densities of Japanese beetle (*P. japonica*) and blueberry maggot (*R. mendax*) observed and/or collected via sticky traps in blueberry fields adjacent to the flower plantings suggests there is increased control of these two pests when floral resources are available. This may be a response to the vegetation structure, since Japanese beetle females oviposit in areas with short, sparsely distributed vegetation (Potter and Held, 2002), similar to the vegetation found within the control field perimeters. Thus, wildflower plantings may reduce populations of some pests by mechanisms other than the enhancement of biological control, such as by the removal of suitable oviposition habitat. It will be important for future research to explore the mechanisms underlying how these plantings affect pest and natural enemy populations, as well as pest regulation.

Pest management is often studied at the field scale, but processes critical to natural enemy conservation and pest control also occur at larger scales (Thies and Tscharntke, 1999; Gardiner et al., 2009; Meehan et al., 2011). Larger plantings of floral resources are expected to have more resources, and hence higher capacity to support populations of beneficial insects (Slobodkin, 1980; Kruess and Tscharntke, 2000), as well as positively affecting the services provided by beneficial insects (Blaauw and Isaacs, 2012; Blaauw and Isaacs, 2014b). The complexity of the surrounding landscape may also affect local insect abundance and diversity in agricultural systems (Tscharntke et al., 2002), while small-scale habitat manipulation may only attract and concentrate natural enemies that are already present in the surrounding landscape (Gurr et al., 1998). A more thorough investigation of how landscape complexity, timing of resource provision, planting size, and configuration affect the response of natural enemies to the

addition of floral resources would be needed to support recommendations for placement of these habitats into farm landscapes (Brosi et al., 2008).

The costs associated with establishment of wildflower plantings are not trivial, but government programs (EU, 2005; NRCS, 2010) and crop yield enhancement (Blaauw and Isaacs, 2014a) can compensate for those initial costs, making this strategy more economically appealing for grower adoption. This study demonstrates that modification of resource-poor non-cropped areas within farms by adding native flowering plants can enhance biodiversity and pest control, similar to the enhancement of crop yield through pollination by wild bees measured at the same sites (Blaauw and Isaacs, 2014a). Understanding how to support insects that provide multiple services to crop production including yield increases and biologically-based pest control, and the return on investment of these practices, should encourage their adoption in situations where land owners will experience positive benefits. This is expected to stimulate further adoption of these practices that will provide additional benefits (Wratten et al., 2012) that can further enhance sustainability of intensive farming systems.

5. Conclusions

Planting a mix of native flowering plants can provide season-long resources that once established will increase the abundance of generalist natural enemies at the edge of adjacent crop fields. This enhancement also has the potential to augment biological control within the adjacent crop fields as well as other valuable ecosystem services, such as pollination. Having multiple benefits from this one technique will make it more appealing to growers and increase the chance for widespread adoption.

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Figures

Fig. 1

Fig. 2

Tables

Table 1.

Common Name	Scientific Name	Bloom Period (month)						Seeding Rate	
		M	J	J	A	S	O	kg/ha	seeds/m ²
Flowers									
Golden Alexanders	<i>Zizia aurea</i>	X	X					0.07	10.88
Foxglove beard-tongue	<i>Penstemon digitalis</i>		X	X				0.14	64.24
Sand coreopsis	<i>Coreopsis lanceolata</i>		X	X				0.28	19.76
Black-eyed Susan	<i>Rudbeckia hirta</i>		X	X	X	X		0.14	90.94
Swamp milkweed	<i>Asclepias incarnata</i>			X	X			0.28	4.74
Butterfly milkweed	<i>Asclepias tuberosa</i>			X	X			0.14	3.78
Wild bergamot	<i>Monarda fistulosa</i>			X	X			0.07	69.18
Joe Pye weed	<i>Eupatorium maculatum</i>			X	X	X		0.03	48.94
Boneset	<i>Eupatorium perfoliatum</i>			X	X	X		0.28	39.54
Blue lobelia	<i>Lobelia siphilitica</i>			X	X	X		0.14	61.78
Yellow coneflower	<i>Ratibida pinnata</i>			X	X	X		0.14	14.82
Cup plant	<i>Silphium perfoliatum</i>			X	X	X		0.28	1.38
Stiff goldenrod	<i>Solidago rigida</i>				X	X	X	0.28	20.26
New England aster	<i>Symphyotrichum novae-angliae</i>				X	X	X	0.14	32.62
Smooth aster	<i>Symphyotrichum laevis</i>					X	X	0.28	13.58
Grasses									
Canada wild-rye	<i>Elymus canadensis</i>		X	X	X			0.28	22.61
Indiangrass	<i>Sorghastrum nutans</i>			X	X	X		0.28	11.86
Big bluestem	<i>Andropogon gerardii</i>			X	X	X	X	1.23	9.88

Table 2.

Insect type	2010				2011			
	Control	Flower	χ^2	<i>P</i>	Control	Flower	χ^2	<i>P</i>
Natural enemies								
Araneae	5.7 ± 1.4	8.0 ± 1.7	2.89	0.09	5.3 ± 0.9	10.2 ± 2.0	5.43	0.02
Cantharidae	0.1 ± 0.1	2.3 ± 1.7	3.82	0.049	0.2 ± 0.1	2.2 ± 1.2	5.99	0.01
Carabidae	0.4 ± 0.2	0.6 ± 0.3	0.67	0.41	0.1 ± 0.07	0.3 ± 0.2	1.61	0.20
Chrysopidae	0.1 ± 0.1	0.3 ± 0.1	1.76	0.18	0.2 ± 0.1	0.4 ± 0.1	1.17	0.28
Coccinellidae	1.2 ± 0.4	0.2 ± 0.1	4.04	0.04	0.5 ± 0.1	0.7 ± 0.2	0.51	0.47
Formicidae	6.9 ± 2.3	3.2 ± 1.5	0.94	0.33	5.0 ± 1.2	5.1 ± 1.1	0.01	0.94
<i>Orius</i> spp.	2.5 ± 0.9	0.7 ± 0.4	2.60	0.11	1.8 ± 0.5	4.6 ± 1.8	2.84	0.09
Parasitica	59.6 ± 11.4	25.2 ± 4.5	4.66	0.03	30.1 ± 4.9	36.9 ± 4.5	1.05	0.31
Staphylinidae	1.2 ± 0.6	0.08 ± 0.05	6.22	0.01	0.8 ± 0.3	0.08 ± 0.05	10.52	0.001
Syrphidae	2.6 ± 1.2	3.5 ± 1.6	0.58	0.45	1.8 ± 0.5	4.0 ± 1.2	4.62	0.03
Herbivores								
Aphidae	5.9 ± 2.1	4.3 ± 1.2	0.05	0.82	19.1 ± 7.7	11.9 ± 2.9	0.85	0.36
Cercopidae	0	0.12 ± .07	9.07	0.003	0.8 ± 0.3	2.8 ± 0.9	5.43	0.02
Chrysomelidae	0.2 ± 0.1	1.4 ± 0.4	12.35	0.0004	2.8 ± 0.9	1.0 ± 0.3	4.97	0.03
Cicadellidae	18.6 ± 4.9	8.2 ± 2.4	2.08	0.15	91.3 ± 15.5	40.6 ± 6.7	10.70	0.001
Miridae	3.3 ± 0.7	2.0 ± 0.5	0.65	0.42	57.9 ± 11.6	74.2 ± 16.4	0.68	0.41
Cucurlionidae	0.05 ± 0.05	0.2 ± 0.1	2.18	0.14	5.7 ± 1.4	5.6 ± 2.0	0.00	0.97
<i>Popillia japonica</i>	0	0	-	-	0.4 ± 0.3	0.4 ± 0.2	0.05	0.81

Table 3.

Insect type	Sample location	2010				2011			
		Control	Flower	<i>U</i>	<i>P</i>	Control	Flower	<i>U</i>	<i>P</i>
Natural enemies									
Vacuum	Perimeter	1.07 ± 0.09	1.3 ± 0.07	134.0	0.07	1.2 ± 0.08	1.3 ± 0.08	272.5	0.44
Observations	Crop	0.9 ± 0.05	1.2 ± 0.05	2254.0	0.001	1.0 ± 0.05	1.2 ± 0.04	2525.0	0.021
<u>Sticky traps</u>	<u>Crop</u>	<u>0.8 ± 0.02</u>	<u>0.08 ± 0.03</u>	<u>18928.0</u>	<u>0.350</u>	<u>0.7 ± 0.02</u>	<u>0.8 ± 0.02</u>	<u>19364.5</u>	<u>0.58</u>
Herbivores									
Vacuum	Perimeter	1.3 ± 0.1	1.4 ± 0.06	162.0	0.30	1.3 ± 0.07	1.4 ± 0.1	276.5	0.48
Observations	Crop	0.3 ± 0.3	0.3 ± 0.04	2915.5	0.28	0.5 ± 0.05	0.3 ± 0.05	2637.0	0.045
Sticky traps	Crop	0.8 ± 0.02	0.8 ± 0.02	18267.5	0.13	0.8 ± 0.03	0.9 ± 0.02	17344.0	0.022

Table 4.

Insect type	2010				2011			
	Control	Flower	χ^2	<i>P</i>	Control	Flower	χ^2	<i>P</i>
Natural enemies								
Araneae	1.1 ± 0.1	1.3 ± 0.2	1.63	0.20	1.6 ± 0.1	1.8 ± 0.2	0.83	0.36
Cantharidae	0	0.01 ± 0.01	2.77	0.10	0	0.06 ± 0.05	4.10	0.04
Carabidae	0.01 ± 0.01	0	2.77	0.10	0	0.01 ± 0.01	2.77	0.10
Chrysopidae	0.3 ± 0.05	0.4 ± 0.06	0.03	0.86	0.5 ± 0.2	0.9 ± 0.2	2.28	0.13
Coccinellidae	0.2 ± 0.05	0.4 ± 0.07	4.15	0.04	0.06 ± 0.03	0.1 ± 0.04	0.94	0.33
Formicidae	2.9 ± 0.4	4.1 ± 0.6	2.23	0.14	3.7 ± 0.5	3.9 ± 0.5	0.05	0.82
<i>Orius</i> spp.	0.01 ± 0.01	10	2.77	0.10	0	0	0	0
Parasitica	0.2 ± 0.05	0.5 ± 0.1	9.77	0.002	0.3 ± 0.06	0.6 ± 0.1	8.58	0.003
Syrphidae	0.7 ± 0.1	1.9 ± 0.2	29.27	0.0001	0.9 ± 0.2	2.8 ± 0.4	19.21	0.0001
Vespidae	0.1 ± 0.05	0.03 ± 0.02	3.46	0.06	0.03 ± 0.02	0.03 ± 0.02	0.00	1.00
Herbivores								
<i>Acrobasis vaccinii</i>	0.8 ± 0.3	0.5 ± 0.2	0.71	0.40	0.7 ± 0.4	0.3 ± 0.2	1.25	0.26
Aphidae	0.8 ± 0.3	0.9 ± 0.3	0.03	0.86	0.3 ± 0.1	0.9 ± 0.4	3.16	0.08
Cercopidae	0	0	0.00	1.00	0.01 ± 0.01	0.1 ± 0.07	5.52	0.02
Chrysomelidae	0.01 ± 0.01	0	2.77	0.10	0.01 ± 0.01	0	2.77	0.10
Cicadellidae	0.3 ± 0.08	0.3 ± 0.09	0.10	0.76	0.5 ± 0.1	0.4 ± 0.1	3.16	0.08
Cucurionidae	0.03 ± 0.02	0.06 ± 0.03	1.37	0.24	0.01 ± 0.01	0.01 ± 0.01	0.00	1.00
<i>Grapholita packardi</i>	0.3 ± 0.09	0.3 ± 0.1	0.01	0.93	0.3 ± 0.1	0.3 ± 0.1	0.00	1.00
Miridae	0.4 ± 0.1	0.3 ± 0.06	0.25	0.62	0.3 ± 0.08	0.2 ± 0.08	0.18	0.67
<i>Popillia japonica</i>	1.8 ± 0.4	1.1 ± 0.3	1.76	0.18	5.0 ± 2.3	4.6 ± 2.1	0.02	0.89
<i>Rhagoletis mendax</i>	0	0	0	0	0.04 ± 0.03	0	5.04	0.02

Table 5.

Insect type	2010				2011			
	Control	Flower	χ^2	<i>P</i>	Control	Flower	χ^2	<i>P</i>
Natural enemies								
Araneae	1.1 ± 0.2	1.2 ± 0.2	0.63	0.43	0.8 ± 0.1	1.2 ± 0.2	2.81	0.09
Cantharidae	0.06 ± 0.02	0.08 ± 0.03	0.19	0.66	0.2 ± 0.04	0.3 ± 0.05	2.50	0.11
Carabidae	0.1 ± 0.02	0.2 ± 0.03	6.73	0.01	0.1 ± 0.02	0.2 ± 0.04	9.11	0.02
Chrysopidae	0.2 ± 0.03	0.1 ± 0.03	0.85	0.36	0.1 ± 0.03	0.2 ± 0.03	0.73	0.39
Coccinellidae	0.5 ± 0.1	1.2 ± 0.2	15.96	<0.0001	0.4 ± 0.06	0.5 ± 0.08	0.16	0.69
Formicidae	0.5 ± 0.07	0.7 ± 0.1	4.46	0.03	1.1 ± 0.1	1.1 ± 0.1	0.00	1.00
<i>Orius</i> spp.	0.6 ± 0.09	0.6 ± 0.08	0.20	0.65	0.6 ± 0.08	0.6 ± 0.08	0.01	0.93
Parasitica	27.2 ± 3.2	40.8 ± 3.1	8.94	0.003	33.9 ± 1.6	49.5 ± 2.9	22.70	<0.0001
Staphylinidae	0.2 ± 0.05	0.3 ± 0.04	0.70	0.40	0.2 ± 0.03	0.4 ± 0.06	13.36	0.003
Syrphidae	0.9 ± 0.1	1.3 ± 0.1	4.19	0.04	1.1 ± 0.1	1.5 ± 0.2	4.02	0.04
Vespidae	0.02 ± 0.01	0.01 ± 0.01	0.20	0.65	0.05 ± 0.02	0.04 ± 0.01	0.35	0.55
Herbivores								
Aphidae	30.1 ± 4.3	39.1 ± 5.6	1.62	0.20	37.4 ± 5.2	37.9 ± 5.4	0.00	0.94
Cercopidae	0.1 ± 0.03	0.2 ± 0.04	2.63	0.10	0.1 ± 0.03	0.2 ± 0.04	6.94	0.01
Chrysomelidae	0.2 ± 0.04	0.8 ± 0.2	16.90	<0.0001	0.2 ± 0.04	0.8 ± 0.2	15.13	0.001
Cicadellidae	15.5 ± 1.5	16.7 ± 1.1	0.41	0.52	14.7 ± 1.5	17.4 ± 1.2	1.96	0.16
Cucurlionidae	0.1 ± 0.03	0.2 ± 0.04	1.45	0.23	0.1 ± 0.02	0.2 ± 0.02	2.96	0.09
Miridae	0.7 ± 0.1	0.9 ± 0.1	3.09	0.08	0.7 ± 0.1	1.1 ± 0.1	4.44	0.04
<i>Popillia japonica</i>	0.01 ± 0.01	0	5.57	0.01	0.01 ± 0.007	0	5.57	0.02
<i>Rhagoletis mendax</i>	0.02 ± 0.01	0.02 ± 0.01	0.12	0.73	0.2 ± 0.07	0.02 ± 0.01	9.22	0.002

Figure captions

Figure 1. Comparison of total (A) natural enemy and (B) herbivore densities (mean $\pm$ SEM) at fields with or without wildflower plantings in field perimeters. Insect were collected via vacuum sampling (per 2.5 min) in field perimeters or by observations (per min) and yellow sticky traps (per trap) within crop fields adjacent to control or flower treatments. Asterisks indicate levels of significance (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) for difference between treatments.

Figure 2. Biological control services measured by corn earworm egg removal from sentinel egg masses (mean BSI $\pm$ SEM) located at three positions (control/flower plot, crop edge, and crop interior) and two treatments (control and flower). Asterisks indicate levels of significance (* $P < 0.05$, ** $P < 0.01$) of difference between treatments.

Table captions

Table 1. List of native Mid-Western perennial wildflowers and grasses used for the seed mix sown in the wildflower plantings, with their bloom periods and respective seeding rates.

Table 2. Comparison of specific insect groups collected via vacuum sampling (mean $\pm$ SEM per 2.5 min) from control field margins or within wildflower plantings over a two year period. Bolded values indicate significant difference between treatments ($P < 0.05$).

Table 3. Comparison of insect diversity (H , mean $\pm$ SEM) collected via observational and yellow sticky trap sampling methods within crop fields adjacent to control (mown) or flower planting perimeters, and insects collected via vacuum sampling from control field margins or within wildflower plantings over a two year period. Bolded values indicate significant difference between treatments ($P < 0.05$).

Table 4. Specific insect groups observed (mean $\pm$ SEM per 1 min) within crop fields adjacent to control or flower treatments over a two year period. Bolded values indicate significant difference between treatments ($P < 0.05$).

Table 5. Comparison of specific insect groups collected via yellow sticky traps (mean $\pm$ SEM per trap) within crop fields adjacent to control or flower treatments over a two year period. Bolded values indicate significant difference between treatments ($P < 0.05$).