ECOLOGICAL ENGINEERING FOR PEST MANAGEMENT OF *OSTRINIA NUBILALIS* HÜBNER (LEPIDOPTERA: CRAMBIDAE) IN PEPPERS

By

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ABSTRACT OF THE DISSERTATION

Ecological Engineering for Pest Management of *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) in Peppers

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Ecological engineering of agricultural ecosystems for enhancement of biological control services provided by natural enemies of target pests holds promise to potentially reduce or replace pesticide use to control pest populations below economic thresholds. The European corn borer *Ostrinia nubilalis* Hübner is a major insect pest of New Jersey peppers, around which integrated pest management programs are designed. Provision of nectar and pollen resources from intercrops to natural enemies can enhance their biological control of pest species. I tested the effect of intercrop species planted with New Jersey peppers on abundance of anthocorid natural enemies, anthocorid predation on sentinel European corn borer egg masses and crop injury. Three intercrops were studied: coriander, *Coriandrum sativum* L., dill, *Anethum graveolens* L., and fennel, *Foeniculum vulgare* Miller.

The proximity and species composition of the intercrops to NJ peppers was evaluated. No significant results were found for proximity of intercrops to the peppers, apart from one non-intercropped predation treatment, testing within-row plants, planted interspersed among the peppers, one-row and three-row distant from peppers. There were

significant results for abundance of *Orius insidiosus* Say in the inter-row experiment, where peppers were planted in different rows from the intercrop. In 2015, coriander intercrops showed a higher anthocorid abundance than dill, fennel and control, non-intercropped, treatments. In 2016, in one field fennel had a higher anthocorid abundance than dill, mixed and control; whereas, in the other field, dill had a higher anthocorid abundance than fennel and control. There was a trend for higher crop damage in 2015 in the non-intercropped control intra-row treatment and in 2016 in the inter-row treatments.

Quantitative PCR of molecular gut contents of *O. insidiosus* was conducted to determine dietary preference. There were no significant results for the molecular gut content assays. The results indicated that *O. insidiosus* forages both in adjacent intercrops and in farther intercrops before entering peppers.

Pollen preference trials were investigated for *O. insidiosus* to determine whether there is a preference for one pollen over another among coriander, dill and fennel. There was significantly more coriander chosen over fennel. Other tests were non-significant, though there were slight trends shown for non-pollen treatment over coriander and dill, as well as slightly greater choice for dill over coriander.

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INTRODUCTION

Identification

The European corn borer, *Ostrinia nubilalis* Hübner, is in the family Crambidae, a group of stem boring moths, with dimorphic adults. Females have a wingspan of approximately 1 inch, with pale- to dark-yellow coloration, while males are light- to dark-brown and smaller in size. Longitudinal wavy lines occur on the outer 3rd of the wings, and when the wings are held naturally at rest these lines appear lateral to the body (Hazzard et al. 2001, Ghidiu 2006). Eggs have an average size of 0.97 by 0.74 mm (Caffrey & Worthley 1927) and are typically oviposited in irregular masses of overlapping rows on the underside of leaves in numbers of 15-30 (Capinera 2005). The five larval stages are characterized by dark coloration of the head with yellow to pink body coloration; young larvae are dull white and have dark spots on each segment (Ghidiu 2006, Hazzard et al. 2001, O'Day et al. 1998). The final fifth instar is approximately 1 inch in length (Hazzard et al. 2001, O'Day et al. 1998). The pupa is reddish brown and approximately 3/4 inch in length (Hazzard et al. 2001).

The European corn borer since its introduction into the U.S. was referred to as *Pyrausta nubilalis* (Hübner), until redescribed under the genus *Ostrinia* (Marion, 1957 as cited in Brindley & Dicke 1963).

History and Distribution

The European corn borer was introduced to the United States before 1917 in an area of about 50 square-miles north and northeast of Boston, Massachusetts (Vinal 1917). The moth was most likely brought into the U.S. as larvae on broomcorn shipments from part of its native range in Italy and Hungary (Caffrey & Worthley 1927). By 1925 it was found in eastern New York, the southeastern region of the Canadian province of Ontario, and in parts of Michigan, New York, Pennsylvania and Ohio (Caffrey & Worthley 1927). The European corn borer had spread to New Jersey by the early 1930s, having spread across the state by 1935 and caused economic injury to corn in Middlesex, Ocean and Monmouth counties (Pepper 1936). By 1950 the original infestations in Massachusetts around Boston and in New York had spread across the Corn Belt to the Rocky Mountains and south to Mississippi, Alabama and Georgia (Brindley & Dicke 1963). The current geographic range of the European corn borer in North America spans the eastern continental United States from northern Florida to Texas and New Mexico northward to the Canadian provinces of Ontario, Manitoba, Quebec and Saskatchewan (Mason et al. 1996).

Monitoring Methods

European corn borer population abundances can be monitored through the use of blacklight traps, pheromone traps and visual inspection. In the Northeastern US, the European corn borer is typically monitored for adult presence using pheromone or blacklight traps (Hazzard et al. 2001). Recommendations for insecticide treatment in peppers occur when an average of four adults are caught in blacklight traps for three consecutive nights in the midwest (Mason et al. 1996) or upon detection of the first moth if the fruit is ½ inch diameter or larger in the northeast (Hazzard et al. 2001). Visual sampling to monitor European corn borer populations in a crop involves scouting for egg masses. Scouting for egg masses in corn fields for integrated pest management (IPM) can be timed according to first detection of European corn borer flight activity (O'Day et al. 1998).

Blacklight traps utilize ultraviolet light emitted at night to attract nocturnally active insects that are collected in a funnel below fins adjacent to a typically 15-W ultraviolet bulb (Hazzard et al. 2001). In New Jersey an extensive blacklight network of ~81 traps maintained by the New Jersey Agricultural Experiment Station Cooperative Extension as part of IPM programs in vegetable crops are used to monitor European corn borer adult populations and other pests (Holmstrom et al. 2001).

Pheromone traps typically use a wire or nylon mesh in a *Heliothis* type model that utilizes a cone and inverted funnel whereby male moths attracted to a sex pheromone blend enter through a bottom opening and are caught in a bag. Two pheromone traps need to be placed beside the target field at the same time, one for each strain of European corn borer. This pheromone trap design provides the greatest catch numbers with less non-target catch than other pheromone trapping methods (Laurent and Frérot 2007).

Blacklight traps for European corn borer have been shown to differ in catch numbers from pheromone traps in Minnesota (Bartels et al. 1997). Blacklight traps are more reliable than pheromone traps. In Maine, corn damage was discovered before egg mass detection by visual sampling or by pheromone trap monitoring and trap placement was shown to be important temporally throughout the season (Ngollo et al. 2000).

Host Range

The European corn borer is a major pest of field corn and vegetable crops in the United States including potatoes, snap bean, winter wheat and pepper (Orton et al. 2014, Mason et al. 1996). The larval stages of European corn borer have been found on over 200 potential host plants in North America (Caffrey & Worthley 1927, Hodgson 1928).

Biology

Development

Eggs are typically laid on the underside of leaves in peppers and corn (Barlow & Kuhar 2004, Mason et al. 1996). Egg hatch from time of oviposition is typically 3-14 days under temperate spring or summer conditions (Hazzard et al. 2001); eggs develop a dark coloration in the head prior to eclosion from the chorion (O'Day et al. 1998). First instar larvae on bell peppers typically enter the fruit at the calyx, leaving sawdust-like frass at the entrance to the burrow (Hazzard et al. 2001, Mason et al. 1996). There are 5 instars, and from the 3rd instar on, larvae may leave the fruit or stem and enter another, in particular if conditions become unsuitable e.g. from bacterial soft rot (Caffrey & Worthley 1927; Mason et al.1996; Hazzard et al. 2001). Overwintering is passed as a fifth instar within stems of host plants, and the timing of pupation in spring is determined by exposure to decreasing night lengths, and increasing moisture and temperature (Gelman & Hayes 1980), with the duration of pupation lasting approximately two weeks (Hazzard et al. 2001). Prior to pupation a thin silken partition is made to cover the tunnel opening (Caffrey & Worthley 1927). Univoltine and bivoltine strains are common in northern regions, while 3-4 generations are possible in warmer climates (Mason et al. 1996, Sorenson et al. 1992).

Reproductive Behavior

Mating typically occurs in tall grassy vegetation areas, where adults also alight during the day, within or adjacent to cornfields or other host plants (Mason et al. 1996, Showers et al. 1976). Flight activity begins immediately after sunset, and initiation of pheromone emission and sexual activity is dependent on sufficient moisture as dew or rain drops and relative humidity (DeRozari et al. 1977; Mason et al.1996). Adults often travel distances of 0.5 miles or more and are able to fly 14 km in 100 minutes (Showers et al. 2001). Grassy vegetation of approximately 0.6-1.2 m height is the preferred mating habitat (Showers et al. 1976). European corn borer females show no oviposition preference between sweet and hot peppers (Larue & Welty 2010) or leaf height preference for oviposition in peppers (Barlow & Kuhar 2004).

There are two known strains to occur in the United States that differ by the sex pheromone females emit to attract mates: the E- and the Z-strains. They differ principally in the proportion of (*Z*)-11- and (*E*)-11-tetradecenyl acetate (4:96 blend for the E strain, 97:3 ratio for the Z strain) (Klun et al. 1973, Kochansky et al. 1975). Mating periodicity may differ between E and Z type strains as evidenced by laboratory colony comparison of the two types, revealing copulation of the Z strain occurring throughout the scotophase while the E strain colony mated only in the latter half of the scotophase (Liebherr & Roelofs 1975).

North American Structure

The Z strain is widespread in Canada and the Midwest, while the E strain occurs in eastern regions, often along with the Z strain, particularly in New York, Pennsylvania and mid-Atlantic states (Klun et al. 1975, Sorenson et al. 1992). Coates et al. (2013) showed through molecular assay that 3 sites in New Jersey and other parts of the Northeast contain mixed populations of both pheromone types of European corn borer. These two strains were found not to interbreed at a high frequency through allozyme allele frequency analysis in areas where they occur sympatrically in Pennsylvania (Cardé et al. 1978, Klun et al. 1975). Populations of European corn borer from New York and Pennsylvania have been shown to be significantly genetically different through the use of eight microsatellite markers from populations from Ohio to Colorado (Kim et al. 2011).

Gas chromatography analysis of female pheromone glands of European corn borer collected as larvae from pepper (*Capsicum frutescens* L.) and cocklebur (*Xanthium* L. sp.) in Europe were determined to be the Z strain, and these populations grouped together genetically based on six allozyme loci with European populations from maize (*Zea mays* L.), sorghum (*Sorghum* Moench sp.) and sunflower (*Helianthus annuus* L.), though not with hop (*Humulus lupulus* L.) (Leniaud et al. 2006).

Population Dynamics and Corn

Population abundances of the European corn borer as determined by blacklight captures have been shown to vary from year to year by up to ten fold and local population variation in peppers may be influenced by proximity to nearby corn fields (Welty 1995). Hutchison et al. (2010) demonstrates area-wide population reductions resulting from increased *Bacillus thuringiensis* Berliner (Bt) transgenic corn adoption across large areas, suggesting regional population abundances are dependent on availability of the preferred host crop. In recent years in New Jersey, European corn borer populations as determined by blacklight catches reveal this trend of lower numbers since the widespread adoption of Bt transgenic corn farming practice across the state (personal communication, K. Holmstrom; Dively et al. 2018).

Damage Caused

First-generation egg masses of European corn borer in bivoltine regions are often oviposited prior to fruit set in peppers and development of these larvae does not typically cause economic crop damage in peppers (Hazzard et al. 2001). Economic damage to peppers occurs from direct damage to the fruit. Pepper fruit infested early often will rot and drop off, and late-infested fruit may exhibit premature reddening (Mason et al. 1996). Peppers have been shown to have differential susceptibility to European corn borer in the field, with larger size pods and less pungent varieties more susceptible to damage (Jarvis & Guthrie 1972).

Pest Management

With the simplification of agricultural ecosystems there is increasing probability of pest buildup (Altieri 1991). Two hypotheses explain this general trend, the ‘natural enemies’ hypothesis and the ‘resource concentration’ hypothesis (Root 1973). The first proposes that more complex environments provide more microhabitats for shelter and food resources for natural enemies. The second proposes that, as food and shelter that are suitable for and/ or preferred by a particular pest species are increased in one place, pests can more easily flourish in this habitat, compared to a natural diverse environment.

Monocrop annual cropping systems rely on the presence of natural enemies to re-invade the crop each year from the surrounding landscape environment. The provision of permanent 'island' habitats such as the provision of certain grasses in raised beds within wheat crops can provide increases in populations of predatory carabid beetles and spiders that feed on pests in cereal crops and are normally encountered only on the edge of fields (Thomas et al. 1992).

Floral resources are usually provided to target parasitoids for enhanced management of pest populations. Flowering buckwheat (*Fagopyrum esculentum* (Moench)) was shown to improve parasitism of grape leafhopper (*Erythroneura elagantula* Osborn) eggs by Myrmarid parasitoids (*Anagrus* spp. Haliday), when compared to buckwheat without flowers (English-Loeb et al. 2003).

Abundance of adult parasitoids does not strictly relate to effectiveness of parasitoids in pest management. Grape leafhopper (*E. elagantula*) sentinel egg masses were parasitized by Myrmarid parasitoids in greater numbers in grapes planted adjacent to flowering buckwheat while adult abundance did not increase compared to control plots, while in another year greater numbers of adults were found in grapes planted adjacent to flowering buckwheat, with a non-significant positive correlation with parasitized grape leafhopper eggs (English-Loeb et al. 2003).

Additional plants can also provide alternative prey or food resources from flowers to increase predator populations. The importance of community assessment of the addition of plants is revealed in intraguild predation in the system of ornamental pepper in the provision of *Orius insidiosus* (Say) in greenhouses to control thrips populations, where spiders were found to inhabit the flower microhabitat as well and compete directly for this resource or consume the introduced predator, thereby reducing the effect of the predator on the pest population (Wong & Frank 2012). Alternatively, the mechanisms of natural enemy interactions within a community may be independent and fairly non-competitive or even synergistic. For example, *Coccinella septempunctata* L. has been shown to cause its prey pest the pea aphid to dislodge and fall from the host plant, becoming an easier target for predation by a second natural enemy once fallen to the ground, the carabid ground beetle *Harpalus pennsylvanicus* Dej. (Losey & Denno 1998) that provides a second carabid natural enemy with greater opportunity to consume this prey.

Alternatively, given accessible flower morphology pest lepidopterans may also utilize flower resources such as nectar from particular intercrops or other companion plants, thereby increasing longevity and/or fecundity of both the pest and its natural enemy. For example, buckwheat and coriander were shown to increase fecundity of the pest potato moth *Phthorimaea operculella* (Zeller) in laboratory assays, and in potato fields intercropped with faba bean and coriander pest numbers and crop damage increased along with parasitism rate in proximity to the strip of flowers (Baggen & Gurr 1998). Buckwheat was also shown to provide similar longevity to the pest tortricid *Acleris comariana* Zeller of strawberry as well as its natural enemy parasitoid *Copidosoma aretas* Walker (Sisgaard et al. 2013).

Plant species for habitat modification should be carefully selected to improve biological control (Gurr et al. 1998). The use of particular plants or varieties in buffer strips to specifically attract beneficial insects, compared to an overall increase in diversity for example for prairie restoration, has been shown to increase populations of natural enemies (Gill et al. 2014). Native weeds allowed to grow within plots by delayed herbicide treatment in field corn did not consistently affect *O. insidiosus* populations of adults or nymphs (Wilson et al. 2004).

Controlled laboratory experiments are often combined with field studies to isolate the effect and understand the role of individual or specific combinations of beneficial plants on natural enemy and pest populations. The effect of simple environments such as Petri dish choice preference tests or Y-tube olfactometer analysis can provide basic behavioral information in the absence of more complex stimuli. *Orius insidiosus* has been shown to not be attracted to particular weedy host plants through an olfactometer test compared to bare soil, though the predator population has been shown to increase in field studies on soybean when planted adjacent to these plants, suggesting non-volatile and perhaps visual or tactile sensory mechanisms of plant preference (Lundgren et al. 2009). Total number of eggs oviposited was found to be similar in soybean and the mixed-plant plots (Lundgren et al. 2009), suggesting *O. insidiosus* females may choose oviposition sites based on proximity, perhaps through suitable connected microhabitat. If this is true, within-row presence of the secondary plant may strongly influence success of intercrop or secondary plant use for biological control using *O. insidiosus*.

Biological control strategies have been proposed to increase natural enemy populations by provision of habitat that provides food, water or shelter before the appearance of the target pest to provide a quicker and larger response once the pest appears. Harwood et al. (2007) through molecular analysis of gut contents of *O. insidiosus* showed significant consumption of aphids for 3 weeks prior to their detection using standard whole-plant count sampling in field populations on Indiana soybean. Similar results were reported from molecular gut analysis of earwig predators of aphids in citrus, detecting positive results one month earlier than standard sampling of aphid populations (Romeu-Dalmou et al. 2012). Other predators such as linyphiid spiders have been shown to prefer scarce prey and may also prevent or delay early outbreaks of aphid populations by preferential predation as they immigrate into the crop ecosystem (Chapman et al. 2013).

Anthocorid Predation

The response of *O. insidiosus* populations to different components of an environment encountered in the field can improve biological control. *Orius insidiosus* can complete development on a diet of only lepidopteran eggs and on this diet it has the highest fecundity, longevity and shortest development time, compared to diets containing pollen or thrips (Kiman & Yeargan 1985, Calixto et al. 2013). In the laboratory, the presence of thrips, pollen or a combination of the two in addition to a lepidopteran egg diet did not reduce predation rate or alter the longevity or fecundity of *O. insidiosus* females (Calixto et al. 2013). *Orius insidiosus* adults consume approximately 1-2 European corn borer eggs per day, and in the presence of alternative prey or pollen, egg predation rates have been shown to decrease (Musser & Shelton 2003).

Monocrops provide a simple environment, and under similar herbivory pressure/ pest abundance increased populations of *O. insidiosus* can be provided by the addition of secondary plants in soybean, with greater oviposition on the secondary plants (Lundgren et al. 2009). An increase in anthocorid population was shown by the use of diverse strips including fennel, buckwheat and coriander adjacent to organically-grown tomato (Balzan et al. 2014). Floral availability in California perennial hedgerows was shown to provide a more suitable habitat for anthocorids, though this was with a several-species mixture of vegetation, and factors other than floral availability such as microhabitat provided by structure or availability of alternative prey may have strong influences on natural enemy populations and activity (Gareau et al. 2013).

Increased numbers of anthocorids were found in a mixture of secondary plants adjacent to pumpkin that included dill and coriander on clay soil, and a strong negative effect of bare ground was found on the presence of anthocorids in pumpkin plots, an effect also seen for another major predator of European corn borer egg masses, chrysopids (Grasswitz 2013). Anthocorids have also been found in greater numbers in weedy plots of *Brassica* L. crops and may have contributed to greater predation of *Brevycoryne brassicae* L. aphids and reduction in pest damage on brussel sprouts (Smith 1976).

Peppers provide low reproductive capacity for *O. insidiosus*, though they have a high preference for pepper, neonate nymphs do not survive well on pepper, as they do not on corn (Coll 1996). *Orius insidiosus* lay similar total numbers of eggs given suitable vegetation, though greater numbers of eggs will be oviposited on preferred plants, for example over corn and soybean (Coll 1996; Lundgren et al. 2009).

Integrated pest management in New Jersey peppers involves scouting for several pests and diseases, including the European corn borer (Boucher & Ashley 2001). Accurate incorporation of minimum natural enemy population density into economic thresholds that can confer biological control capacity would reduce costs by avoidance of unnecessary insecticide sprays if natural enemies could provide the same level of control (Musser et al. 2006). Insecticide sprays are recommended when pest numbers or crop injury levels reach a certain density as determined by sampling the pest population (Table 1). Though ~95% control of European corn borer populations is possible with spinosad insecticide use, nymphal *O. insidiosus*, *C. maculata* and *H. axyridis* predator populations can be reduced by 30-50% as well (Bazok et al. 2009, Musser et al. 2006). The sublethal impacts of particular insecticide sprays on natural enemies may also impact their effectiveness, though evaluation of the effectiveness of natural enemies in agroecosystems can assist in overall improvements through their incorporation in IPM strategies (Roubos et al. 2014). In peppers, once above the action threshold, insecticide applications are recommended until harvest every 7 to 10 days (Mason et al. 1996), or depending on residual activity of the particular insecticide (e.g. weekly for spinosad) and weather conditions that affect degradation of the insecticide (Hazzard et al. 2001).

Table 1. Pepper crop chemical control in New Jersey (Boucher & Ashley 2001, Kline & Walker 2005).

Chemical Control	Insecticide (Company)	Maximum Applications Season^{-1}
Acephate	Orthene 75S (Valent)	7 applications
Bacillus thuringiensis subsp. *kurstaki*	Mattch, Crymax, Javelin (Mycogen, Ecogen, Abbott)	Application every 3-4 days
Bifenthrin		1-2 applications
Carbaryl	Sevin 50W, 80S, XLR Plus, 4F (various)	7 applications
Cyfluthrin	Barythroid 2E (Bayer)	6-10 applications
Endosulfan	Thiodan, Endosulfan 3EC, 50W (FMC, MicroFlo, Setre)	2 applications

Esfenvalerate	Asana XL (DuPont)	7-11 applications
Indoxacarb		1-2 applications
Lambdacyhalothrin		1-2 applications
Methomyl	Lannate (DuPont)	5-10 applications
Methoxyfenozide		1-2 applications
Permethrin	Ambush 2E, 25W, Pounce 3.2EC, 25WP (Zeneca, FMC)	Application every 5-7 days (8 applications)
Spinosad	SpinTor 25C (Dow Agrosciences)	3-7 applications
Tebufenozide	Confirm 2F	1-2 applications
Zeta-cypermethrin		1-2 applications

Biological Control in Corn

Several parasitoids have been imported and released in the United States for biological control attempts of European corn borer. Prior to 1963, 24 species of exotic parasitoids were released into the US to control the European corn borer, of which six established (Brindley & Dicke 1963). Generalist predators have been shown to provide the greatest level of control of larval European corn borer in sweet corn (Coll & Bottrell 1991), though the presence of corn pollen may cause predation levels to be reduced as it has been noted that during corn pollen shed plants are most susceptible to European corn borer infestation (Wright & Witkowski 1998).

The influence of broad-leafed weeds and grassy weeds in corn fields was determined on parasitization of European corn borer with no difference found, while parasitization varied from 2-6% in one year to 20-29% the following year (Pavuk & Stinner 1992).

Biological Control in Peppers

Trichogramma ostriniae Pang and Chen parasitoids have been shown to be effective against the European corn borer in an inundative release program in Virginia peppers (Kuhar et al. 2004). However, in later tests in peppers no difference in marketable fruit or on fruit damage was detected between inundative releases of the same parasitoid species, insecticide application methods using economic thresholds, regular selective or broad-spectrum insecticide applications, and untreated controls (Chapman et al. 2009); this may be due to lower overall European corn borer damage in the latter control plots (~10% compared to 27%, whereas damage in biological control plots was ~7% compared to 9%). Significant biological control as measured by reduced percentage of loss of marketable peppers adjacent to sweet corn was shown in Kentucky using both inundative releases of *T. ostriniae* and intercropping with buckwheat (Russell & Bessin 2009), though natural fruit infestation by European corn borer was low (3-4% compared to 2-3% with biological control). Russell & Bessin (2009) showed that abundance of predators of European corn borer did not differ between buckwheat intercropped and non-intercropped pepper plots.

The effect of alternative prey abundance, abundance of predator, and provision of food resources from plants on predation of a target pest can be complex. For carabids presence of alternative prey can reduce predation of slug pests, and that the predators with the most access to alternative prey diversity reduced pest populations the least (Symondson et al. 2006).

Orius laevigatus (Fieber) was shown to prefer host plants with two types of prey, thrips or mites, over clean plants (Venzon et al. 1999). Wild and lab-reared *O. insidiosus* both showed a significant host preference based on Y-tube olfactometer choice tests for sweet pepper and cotton infested with thrips over clean plants (Carvalho et al. 2011). Sunflower plants were shown to increase thrips populations when used as companion plants to peppers, though *Orius* populations were not shown to be affected by these increases (Tyler-Julian et al. 2014).

In New Jersey peppers, a conservation biological control approach using dill, coriander and buckwheat intercrops showed increased predation by *O. insidiosus* in four out of five fields of pepper (Bickerton & Hamilton 2012).

Habitat Modification

Pepper plants may provide a less suitable microhabitat for *O. insidiosus* development than does bean or corn as greater numbers of *O. insidiosus* were encountered on the latter (Coll & Ridgway 1995). Also corn pollen due to its attractiveness as a component of diet prevents predation of European corn borer by *O. insidiosus* in corn. *Orius insidiosus* adult populations were found to fall in soybean adjacent to corn during pollen shed and silking in corn and then rise again during peak flower bloom in soybean, with greatest amount of adults captured in rotary traps between corn and soybean between these times and by abundances sampled within fields (Isenhour & Marston 1981).

Generalist Predators

Orius insidiosus is a known generalist predator that attacks a wide variety of prey, including thrips, lepidopteran eggs and larvae, aphids, midges, mites and springtails; it is also an omnivore and population peaks have been correlated with pollen shed in corn

(Dicke & Jarvis 1962). *Orius insidiosus* populations have also been shown to increase through the vegetative growth stages on soybean with population peaks coinciding with flowering (Isenhour & Marston 1981).

Andow (1990) characterized predation sources of European corn borer egg masses by visual inspection to several classes: chewing predation (mostly by *Coleomegilla maculata* DeGeer), and sucking predation by *O. insidiosus*, and *Chrysopa* spp. predation, as compared to hatched eggs. *Orius insidiosus* on Iowa corn has been reported under cage mesh studies to provide low predation, whereas *C. maculata* was shown to provide ~50% reduction in egg mass density (Phoofolo et al. 2001). *Orius insidiosus* can be found on peppers but likely does not reproduce or in the absence of prey, nymphal anthocorids (*O. insidiosus*) may not survive well on peppers (Coll & Ridgway 1995). Distance from companion plantings of tomato in corn fields has been shown to increase oviposition and larval densities of *C. maculata* in the corn crop up to 5 meters (Seagraves & Yeargan 2006). Both pest abundance of the potato moth *Phthorimaea operculella* (Zeller) and parasitism rates by *Copidosoma koehleri* Blanchard increased with proximity of potato plots to a perpendicular row of flowering coriander and faba bean plants, along with foliar and tuber damage to the crop (Baggen & Gurr 1998). *Orius insidiosus* populations were found to decline on soybean when adjacent corn enters silking and pollen-shed stages and return to seasonal peaks when the corn silks dried (Isenhour & Marston 1981).

Predatory mirids have been shown to be effective in biological control of greenhouse sweet peppers, in combination with another predator, *Amblyseius swirskii* (Athias-Henriot) and shown to result in lower pest levels when given supplemental food resources (Brenard et al. 2018; Bouagga et al. 2018a). *Orius* in greenhouse peppers was shown to reduce crop yield loss by 24% and control the pest mite target approximately ten-fold more than chemical pesticide application (El Arnaouty et al. 2018).

Conservation Biological Control

Biological control of European corn borer is challenging in peppers due to the neonate's behavior of boring into the stems and fruit of host plants (Larue & Welty 2010), where they are protected from predators and parasitoids. Several parasitoids were imported from its native range in Europe, with several establishing, at least initially, and some biological control of target pest populations to reduce crop injury (Brindley & Dicke 1963). However, economic losses generally continue to occur in most regions; such damage including costs of control measures have been estimated to cost growers across the US one billion dollars annually (Mason et al. 1996). In corn, incorporation of transgenic modifications to include bacterial toxin(s) from *Bacillus thuringiensis* (Bt) has resulted in effective control, which is used in conjunction with mandated areas of refugia to manage resistance development to this strategy (Hutchison et al. 2010). However Bt transgenic modifications in other crop systems such as peppers have not been developed and European corn borer continues to be one of the most widespread and damaging pest insects of peppers, as additionally evidenced by Integrated Pest Management (IPM) programs in peppers in the Northeastern U.S. designed around its control (Boucher & Ashley 2001).

Conservation Biological Control is an approach to pest management, first proposed in the sense of habitat modification, i.e. alteration of the environment, to preserve or enhance natural enemies to decrease or maintain low levels of crop injury by the pest (Barbosa 1998, DeBach 1964). The use of secondary plants such as insectary plants, that are planted adjacent to the main crop to provide food resources from flowers for natural enemies, can enhance biological control of a target pest in adjacent crops. Flowers may provide food resources directly as pollen or nectar, or indirectly through alternative prey attracted to the flowers. Furthermore, preferred sites for oviposition may be provided also by vegetation adjacent to the primary crop. Intercropping, the practice of planting secondary crops, and timing transplants to the field has the potential to enhance biological control of European corn borer eggs and neonate larvae prior to tunneling into pepper fruits so as to prevent economic damage. Timing transplants to the field is done so flowering coincides to provide food resources of nectar or pollen, microhabitat or oviposition sites for optimal development or microhabitat that provides protection of natural enemies and their movement into the adjacent primary crop. Bickerton and Hamilton (2012) demonstrated that intercropping of three flowering insectary plants, dill (*Anethum graveolens* L.), coriander (*Coriandrum sativum* L.) and buckwheat (*Fagopyrum esculentum* Moench) can provide increased predation of European corn borer eggs in peppers.

Floral Resources

Flowering plants can provide nectar and pollen resources for natural enemies of target pests. Baggen et al. (1999) show that the potato moth does not increase its longevity or fecundity with flowers of phacelia (*Phacelia tanacetifolia* Benth), while its parasitoid *C. koehleri* shows increased longevity compared to shoot-only control. Nectar and pollen provided in flowers may be consumed by insects. Floral morphology, however, may exclude or allow access for specific insects present in the plant's environment (Patt et al. 1997a).

Predator and parasitoid populations of particular pest insects or invertebrates may be enhanced by the provision of flowering plants. Plants incorporated in agro-ecosystems for this purpose are referred to as insectary plants (Parolin et al. 2012). Flowering sweet alyssum, *Lobularia maritima* (L.), planted in apple orchards was shown to reduce pest aphid populations (Gontijo et al. 2013). For example, use of dill or coriander as intercrops in eggplant was found to increase predator abundance of *C. maculata* by approximately 10 X and parasitization of sentinel egg masses of the Colorado potato beetle (*Leptinotarsa decemlineata* Say) by approximately 30% compared to control plots without flowering intercrops (Patt et al. 1997b).

Certain predators such as linyphiid spiders may prefer particular types of prey that are non-flying, jumping or slow-moving including collembolans and aphids even in abundance of other similar-sized prey (Chapman et al. 2013).

Molecular Gut-Content Analysis

Population abundance of *O. insidiosus* does not necessarily reflect the rate of predation on European corn borer eggs due to prey availability and diet preference. Comparative analyses of environmental conditions on rates of predation would be useful to determine those that favor biological control of European corn borer eggs or other key pests. Characterization of predator type can be determined from analysis of natural or sentinel egg masses in the field (Andow 1990). Alternatively, analysis of *O. insidiosus* gut contents using molecular methods can be used to determine recent prey items in the diet and rates of predation given calibratory feeding trials for DNA detection limits before digestion of the target genomic fragment is too degraded to amplify (Greenstone et al. 2014a, Harwood et al. 2007, Greenstone & Hunt 1993). Microscopic or morphological analysis of dissected gut contents is also possible (e.g. Reynaga et al. 2014, etc), though DNA-based methods are especially useful for Hemiptera and other insect orders with piercing-sucking mouthparts, such as *O. insidiosus*. DNA detection half-lives for particular assays can be used to approximate number of items consumed in the diet over a period of time (Harwood et al. 2007, Chen et al. 2000). A typical half-life is determined by groups of individuals, for example 10 adults, after a period of starvation, given a particular food item and at certain time intervals post-consumption are tested; the proportion at which half of these provide a positive result is the half-life (Greenstone et al. 2014a, Harwood et al. 2007). This measurement provides an estimate for the absolute numbers of prey or food item consumed. DNA analysis to monitor predator diet and test for consumed animal or plant material is more challenging than typical genetic analysis; the DNA is digested into short DNA fragments, typically 80 to 450 bp in size (Traugott et al. 2013).

Weber & Lundgren (2011) found the half-life for detection of molecular gut contents of *C. maculata* fed on maize pollen was 56 minutes and for Colorado potato beetle eggs 46 minutes; however this increased to over 8 hours for pollen if adults had been reared prior to analysis for 7 days on eggs, though this was not found for the reverse (individuals previously fed on a diet of pollen for 7 days). Post-consumption DNA detection half-lives were found for the carabid beetle predator *Pterostichus melanarius* Illiger fed on aphid prey *Sitobion avenae* F. to be approximately 24 hours with starvation after prey consumption (Sheppard et al. 2005). Schmidt et al. (2014) found DNA detection half-lives to be between 9-48 hours for *C. maculata*, Geocorid, Nabid and two spider predators for consumption of 1st-2nd instar squash bug (*Anasa tristis* DeGeer), a major hemipteran prey pest of squash and pumpkin in the United States.

Coleomegilla maculata had undetectable DNA from prey when held for 4 hours at room temperature, and starvation after consumption lengthened DNA detection times of 16 and 31 minutes for aphid and *C. maculata* chaser diet to 59 minutes (Weber & Lundgren 2009). It has been observed for *L. decemlineata* egg predation that dissection and extraction of the gut compared to whole *C. maculata* gDNA did not improve DNA detection (Weber & Lundgren 2009). Time since consumption and temperature can affect the ability to detect prey DNA from gut contents of particular predators (Hoogendoorn & Heimpel 2001). rDNA was used to design primers to detect pollen (Wilson et al. 2010), and pollen may not contain a large number of chloroplasts since the main function of these cells is for reproduction, not photosynthesis.

CHAPTER 1: FLOWERING PLANT LAYOUT INTERCROPPING FOR CONSERVATION BIOLOGICAL CONTROL BY NATIVE GENERALIST PREDATOR *ORIUS INSIDIOSUS* OF EUROPEAN CORN BORER EGGS IN PEPPERS

Abstract

The European corn borer, *Ostrinia nubilalis* Hübner, is a major pest of peppers, *Capsicum annuum* L. Intercropping with flowering plants can improve biological control of pests in peppers by generalist predator incentives and enhancement. Studies were designed to evaluate the impact of species composition of coriander, *Coriandrum sativum* L., dill, *Anethum graveolens* L., and fennel *Foeniculum vulgare* L. on biological control over two years in intercropped bell pepper fields in New Jersey. *Orius insidiosus* (Say) numbers and sentinel European corn borer egg masses were sampled weekly during the experiment and crop injury was evaluated at harvest. Species composition results were significant for abundance of *O. insidiosus* in the inter-row treatments in 2016, and not significant for crop injury or predation. In 2015, there was a trend for higher crop damage in the intra-row non-intercropped treatment and also in the inter-row treatments in 2016. It is possible that *O. insidiosus* was attracted to other nearby treatments; future experiments should increase the distance between treatments.

Introduction

The European corn borer, *Ostrinia nubilalis* Hübner, is a major insect pest of peppers, *Capsicum annuum* L., and can cause severe damage to pepper crops in the Northeastern United States and around which integrated pest management (IPM) practices in peppers are developed (Boucher, 2001). It was introduced into the United States from Europe around the Massachusetts area in the 1910's (Vinal, 1917). Caffrey & Worthley (1927) found that the source of this introduction was likely broomcorn from a region in Italy and Hungary. Since then, the European corn borer has spread to Canada, across the corn belt in the United States, and south to Florida and New Mexico (Mason et al. 1996). It was known immediately that the corn borer would be an economically important pest of corn and other vegetables, including bell peppers (Vinal 1917; Caffrey & Worthley 1927).

Bell peppers, *Capsicum annuum* L. are the most commonly grown pepper in the United States. They are typically non-pungent with little 'heat', or capsaicins, and are used in sandwiches, pizza topping and salads (Carter 2001). IPM programs for peppers in the Northeastern United States target two major insect pests, European corn borer and aphids. Of these two, European corn borer is the only one that can cause potentially severe damage to the pepper fruit (Boucher 2001). Adult female European corn borer moths oviposit masses of typically between 5 and 60 eggs on the underside of pepper leaves. Neonate larvae upon emerging from the chorion generally immediately burrow into the calyx of the pepper fruit when present (Hazzard et al. 2001). Before fruit set, eggs may be laid and then the neonate would enter the stem. However, in peppers this rarely damages the plant, i.e. effecting crop yield or quality of fruit (Hazzard et al. 2001).

Once inside the pepper plant, European corn borer larvae are completely protected from predation and insecticide sprays. Larvae expose the inside of pepper to bacteria that cause fruit rot, and larvae can leave one fruit and enter another (Hazzard et al. 2001). Holes created by European corn borer can also make fruit unmarketable.

European corn borer show no ovipositional preference for different types of pepper, though larval infestation was significantly higher on bell among five varieties and lowest on pepper with high capsaicin levels (Larue and Welty 2010). Oviposition of European corn borer egg masses in “Paladin” bell peppers show no vertical preference for the undersides of leaves (Barlow and Kuhar 2004). Breeding-line selections in pepper show that various traits are associated with reduced infestation of European corn borer among non-pungent peppers, including smaller leaf size, greater fruit set and spreading growth habit (Abdul-Aziz et al. 1983). Therefore, potentially by choosing the correct pepper genotype, European corn borer risk can be mitigated.

European corn borer can be controlled using IPM practices in the Northeastern U.S. through a series of steps. Monitoring via a blacklight trap once fruit set has begun or with pheromone-baited Scentry Heliothis traps (Gempler’s, Madison, WI) for 2nd and/or 3rd generation flights of European corn borer adults is an important step to time insecticide applications. Timing is five to seven days post-detection of moths in a blacklight trap or one week after a combined trap capture of two pheromone traps (Boucher 2001). Alternation of insecticides is important to prevent resistance, and beneficial insects or predators can be preserved by using selective insecticides (Boucher 2001). Infestation of European corn borer can be further reduced in bell peppers by sprayer choice (air-curtain sprayer or conventional boom sprayer) for certain insecticides, perhaps by increased spray deposition on fruit and/or the undersides of leaves (Grafius et al. 1990) where European corn borer egg masses are laid. Monitoring directly via scouting for European corn borer egg masses on the underside of leaves has been proposed for IPM programs (Barlow and Kuhar 2004). Monitoring of post-mated age of European corn borer females caught in traps adjacent to pepper plots was found to vary on average from approximately three to five days and was not shown to be useful in forecasting fruit injury in green peppers (Elliott et al. 1982). One alternative to insecticide application in pepper IPM programs is the use of augmentation biological control with *Trichogramma nubilale* Ertle and Davis which has been shown to provide 80% parasitism in peppers with mature commercial leaf surface areas of 5500 cm^2 (Burbutis and Koepke 1981).

Conservation biological control enhances natural enemy effect on target pests through various means, but increased diversity or abundance does not necessarily translate to reduced crop damage for a variety of reasons. Often herbivores also increase or can also be attracted to conservation biological control plantings such as use of intercrops or increased variety to provide additional shelter or pollen and/ or nectar from flowers. For example, Fielder and Landis (2007) describe specific plant traits including increasing floral area, maximum flower height and period of peak bloom that are associated with both significant increases in herbivore as well as natural enemy abundance, however with a weaker correlation in herbivores. Altieri (1991) discusses the theory that agriculture with monocrops increases the risk of pest population buildup and resulting damage. With increasing diversity in the agricultural system in general, this risk is reduced. There are many types of plants with specific functions to provide benefit in an agricultural setting, in addition to the main crop (Parolin 2012). Arguably, the best kind of additional plant is an intercrop, i.e. another crop that can be sold in addition to the main crop. However, wildflower plantings have been shown to increase natural enemy abundance with larger size plots without a corresponding increase in herbivore populations (Blaauw and Isaacs 2012).

Orius insidiosus (Say) (insidious flower bug) has been shown to consume eggs of the European corn borer (Froeschner 1950). It has also been shown that rates of predation of the insidious flower bug in peppers is increased significantly with a combination intercrop of coriander *Coriandrum sativum* L., dill, *Anethum graveolens* L., and buckwheat *Fagopyrum escuelentum* Moench (Bickerton and Hamilton, 2012). *Orius insidiosus* populations have been shown to increase with pollen-shed in corn and with flowering in soybeans (Isenhour and Marston 1981). Methods to enhance the abundance of the insidious flower bug or its rate of predation, to reduce crop yield damage/ losses from the European corn borer would be beneficial.

In this study we examined the effect of the insidious flower bug on crop yield damage caused by European corn borers to peppers. To evaluate the effect we measured the rate of predation of European corn borer eggs and abundance of the insidious flower bug. The intention was to enhance biological control through habitat modification.

We examined the effect of three intercrops, coriander, dill, and fennel, individually and in combination in peppers. These intercrops are potential sources of natural enemies and the insidious flower bug (Bickerton and Hamilton 2012; Grasswitz 2013; Patt et al. 1997a). We also tested the effect of distance between the intercrops to peppers.

As part of an integrated pest management program, the goal of this work is to enhance the efficiency of the insidious flower bug reducing European corn borer damage in pepper crops.

Materials & Methods

Study Sites

In 2015 and 2016, two bell pepper "Paladin" plots were established in May, approximately 400 m apart on the Rutgers Snyder Research and Extension Farm in Pittstown, NJ. Both plots consisted of a randomized complete block design replicated four times per plot of three rows of peppers in 2015 with one plot containing companion flower intercropping treatments (coriander "Santo", dill "Bouquet", and fennel, *Foeniculum vulgare* L. "Florence") within the same row with peppers on black plastic mulch (Fig. 1, intra-row); the other plot in 2015 was a standard (inter-row) planting of the same intercrop variations as above however in a 4th and 5th row on white plastic mulch (Fig. 2). In 2016, the randomized complete block design was replicated sixteen times (eight replicates per plot), half intra-row and the other half inter-row plantings (Fig. 3 and 4). Each set of three rows of peppers was separated by 3.7 m of bare ground in 2015 or 4.3 m of bare ground in 2016. Intra-row treatments contained companion flower plantings between pepper plantings within each row of each treatment, where present. All peppers and herbs received drip irrigation.

Plot Establishment

All seeds were purchased or produced at Stokes Seeds, Thorold, ON, Canada. Pepper transplanting was done with a mechanical water wheel at 45.7 cm on-center single rows or double rows and 4.6 m between treatments in each block of three rows in 2015 or 2.3 m between treatments in each block of three rows in 2016. Treatments consisted of coriander, dill, fennel, a mixture of all three herbs and a non-intercropped control. Within each block, treatments were assigned randomly. Mixture treatments had three sections, coriander, dill then fennel, each companion flower grouped together along each mixture treatment. A mechanical 30 cm spacing double water wheel was additionally used in the first planting of herbs-only rows.

In 2015, both plots were transplanted with pepper seedlings and herb seedlings on June 11; the second planting of herb seedlings was on July 3. Each treatment consisted of single-row peppers, and when present, double-row herbs. The intra-row plot was 41.1 m by 32.9 m and consisted of 12 evenly spaced peppers per treatment per block with 24 herbs per row, or eight herbs between each of the four pepper plants per row. Each replicate sub-plot was 4.6 m by 5.5 m. The inter-row plot was 41.1 m by 47.5 m and consisted of 30 peppers per treatment per block with 60 herbs per row of intercrop. Each replicate sub-plot was 4.6 m by 9.1 m. Herbs were planted in double-rows at 15 cm between rows and 15 cm between plants.

In 2016, pepper seedlings were transplanted on June 9; first and second herb seedlings were transplanted on June 16 and July 8, respectively. Peppers were planted in double rows. The intra-row treatments consisted of 12 peppers and 12 herbs per treatment with four herbs per row between the four pepper plants per row. The inter-row treatments consisted of 30 peppers per treatment with 15 herbs per row of intercrop. Both plots were 45.7 m by 42.1 m. Sub-plot replicates of intra-row treatments were 2.3 m by 5.5 m. Sub-plot replicates of inter-row treatments were 2.3 m by 9.1 m. Replicates were separated by at least 2.3 m within the same block. Herbs were planted as in 2015 except in the inter-row plots the double-rows were at 15 cm between rows and 30 cm between plants. The reason for the difference between 2015 and 2016 was to plant the same ratio of herbs to peppers in the intra-row and inter-row treatments and to increase the number of replicates.

Sticky Card Sampling for *O. insidiosus*

Populations of *O. insidiosus* were monitored both years using clear sticky cards, made by brushing TangleTrap® Insect Trap Coating on both sides of 10.8 cm x 14.0 cm write-on transparency film (School Smart, Appleton, WI). These were placed weekly for 48-hour for six weeks on July 6, 13, 20, 29 and August 3 and 10, 2015 and July 9, 13, 20, 27 and August 3 and 10, 2016, after which these were collected then frozen until analyzed. In 2015, four clear sticky cards per row were deployed per treatment within the first and third row of peppers and four additional clear sticky cards were placed per treatment within the companion flower plantings (or where they would have been planted for the non-intercropped treatment), in the first and third rows for the intra-row experiment and in the 4^{th} and 5^{th} rows for the inter-row experiment. In 2016, two sticky cards apiece were placed in the first row and third row of the peppers. For the intra-row treatments this was repeated for the intercrop-placed sticky cards. Two sticky cards were placed in the 4^{th} row and two in the 5^{th} row for inter-row treatments.

Evaluation of Egg Mass Predation

The fourth and seventh plant in 2015 in the outside rows (rows 1 and 3) of each pepper treatment/rep were used to evaluate egg mass predation of European corn borer masses. Two egg masses per plant (French Agricultural Research, Inc., Lamberton, MN) were placed in the field with paper clips on the underside of pepper plant leaves on July 6, 13, 20, 29 and August 3 and 10, 2015. In the intra-row plot, the 2nd and 3rd pepper plant were utilized. After 48 hours, exposed egg masses were collected in 37-ml soufflè cups (Solo Cup Company, Lake Forest, IL) and frozen until analyzed. Methods of analysis followed Andow (1990) to determine sucking predation of *O. insidiosus* compared to other forms of predation. Egg masses showing other forms of predation or egg masses that had fallen from the plants were excluded from the analysis of *O. insidiosus* predation rates. In 2016, evaluation and analysis was as in 2015 except only one egg mass was deployed per plant on July 9, 13, 20, 27 and August 3 and 10, 2016. Also, the second and fourth plant were utilized in the inside row (facing the middle row) of the double-rows of the first and third rows inter-row treatments, whereas both inside rows of the double-rows of the first and third rows was utilized for intra-row analysis. The proportion of egg masses damaged was calculated as the number of egg masses attacked divided by the total number of egg masses retrieved.

Evaluation of Injury to Peppers

In 2015 all intra-row peppers were harvested for damage evaluation by European corn borer larvae: entrance/ exit hole(s), tunnels and/or presence of European corn borer larva or pupa within the pepper. All peppers from the 2nd (middle) row were harvested and analyzed in 2015 as well as 3 plants from the outside rows of peppers daily Monday through Friday from August 17 to 25. A knife was used to cut open and slice all harvested peppers to look for evidence of European corn borer infestation (injury) to peppers. In 2016, all peppers in the study were harvested and analyzed daily Monday through Friday from August 15 to 29.

Statistical Analysis

All statistical tests were performed using SAS software (SAS Institute 2018). The effect of field was tested using analysis of variance (randomized block design) with interactions (Cody 2011). The data was analyzed for normal distribution by Proc Univariate and because the data was not normally distributed the effect of intercrop was determined using mixed model, Proc Mixed, with independent variables of treatment and the interaction, with the random variable as field, for each of the following dependent variables, anthocorid abundance, pepper injury, and European corn borer egg mass predation. Significant differences were determined by Mann-Whitney U Test for non-normality and small sample sizes. Each year was analyzed separately.

Results

All field and interaction effects were not significant except for inter-row abundance of *O. insidiosus* in 2016. In 2015, anthocorid abundance was not significant for the intra-row field and for the inter-row field (Fig. 5 and 6; $F=1.42$, $df=4$, $p=0.28$, $F=2.29$, $df=4$, $p=0.11$, respectively). In some cases lack of data collection for anthocorids was due to lost or misplaced sticky cards. Abundance of anthocorids in 2016 was not significant for intra-row treatments (Fig. 7; F=1.24, df=4, p=0.31) but was significant for inter-row treatments, in the flowers (F= 10.00, df=4, p=0.0004, mixed model); in the flowers, dill had significantly greater abundance than the control treatment (Fig. 8, S=26.0, df=1, p=0.0143, Mann-Whitney U Test).

Predation of sentinel European corn borer egg masses by anthocorids showed no significant differences between intercrop treatments in 2015 (Fig. 9; F=0.92, df=4, p=0.48) and 2016 (Fig. 10; F=0.63, df=4, p=0.64).

No significant differences were found in injury to fruit due to European corn borer feeding (Fig. 11 and 12; F=0.44, df=4, p=0.78). Although there was no significant difference in 2015, there was a trend for greater fruit injury in the non-intercropped treatment of the intra-row experiment. In 2016, also despite no significant differences between treatments, fennel had a trend for less fruit injury and in the inter-row treatments, non-intercropped had slightly more fruit damage than the other treatments, in particular fennel.

Discussion

Generally, the difference between fields was not significant in 2016 and so analyses were combined from both fields. While no significant differences were found in terms of damage from European corn borer populations to the pepper fruit at harvest, there were differences in abundance of *O. insidiosus* in the inter-row treatments in 2016.

The non-significant difference in damage to peppers from European corn borer may be in part due to low incidence of damage overall, in particular in 2016, as higher numbers may reveal subtler differences. In 2015, fruit damage from European corn borer was 4.2% in the intra-row treatments and 2.0% in the inter-row experiment, and in 2016 fruit damage from European corn borer was 0.2% in the intra-row experiment and 0.6% in the inter-row treatments. Damage to bell peppers from European corn borer can vary from exceptionally high, where for example 64% of the crop can be lost without insecticide sprays to moderate or low, and years with such variation in damage can occur subsequently (Welty 1995). This may, at least in part, explain the greater damage to the pepper harvest overall in 2015 than that seen in 2016. Low incidence of infestation and crop damage may be in part due to possible area-wide suppression of European corn borer from general wide adoption of Bt corn in New Jersey. Cost savings from such a result to non-Bt corn growers and possibly other crops attacked by European corn borer has been shown and suggested on a large scale (Hutchison et al. 2010; Hutchison and Burkness 2008).

Abundance of *O. insidiosus* in 2015 had a trend to be greater in coriander than in dill, fennel and non-intercropped treatments. Coriander generally had larger growth and vegetative mass, likely providing greater numbers of blooming flowers than dill and fennel throughout the study. This may have contributed to the greater abundance of *O. insidiosus*. The insidious flower bug has been shown to increase in abundance with pollen-shed in corn and with flowering in soybeans (Isenhour and Marston 1981). Larger amounts of pollen or flower masses have been shown to increase natural enemy abundance and biological control in wildflowers (Blaauw and Isaacs 2012). A mixture of flowering plants that included dill and coriander as intercrops of pumpkin were shown to significantly increase anthocorid abundance in New Mexico (Grasswitz 2013). Flowering strips alongside organically-grown tomato that included coriander and fennel were shown to have increased anthocorid abundance throughout the season when buckwheat and white mustard *Sinapsis alba* L. were added (Balzan et al. 2014). In 2016, *O. insidiosus* abundance in dill was greater than the control plot in the flowers of the inter-row treatments. This result was not found to be correlated as well with predation rates, as these were found to be not significantly different among treatments. Increase in abundance of natural enemies does not always translate to increased biological control. Musser and Shelton (2003) found that presence of pollen, alternative prey, and aphids, more than offset the associated increase in abundance of generalist predators of European corn borer with these greater food resources, resulting in reduced rates of predation of European corn borer in sweet corn in New York. Nonsignificant differences in abundance of natural enemies, combined with an augmentation biological control program and habitat modification, separately, has resulted in significantly reduced European corn borer crop damage in bell peppers (Russell and Bessin 2009).

Orius insidiosus consumption of sentinel European corn borer egg masses was found not to differ among treatments. The furthest row from the companion plantings is the most isolated spot in the field from pollen or nectar resources. Predators may have a typical distance for searching and may consume less prey as distance increases from shelter or originating food sources. Perhaps anthocorid predators attracted to and present near the intra-row along the same row search for prey at least the equivalent of 3 rows distance (approx. 4 meters) that separated treatments and blocks. Should this be a valid reason, treatments should be larger and separated by greater distances to investigate best interval of intercrop among peppers for enhanced conservation biological control by natural predators. At least, the minimum distance possible for intercropping, of intercrops grown next to and within the same row as peppers, does not provide sufficient benefit to warrant change in practices from standard parallel or adjacent row intercrop planting. Should minimum intercrop distance not have affected this study, results suggest that single intercrops compared to mixed intercrops as well as non-intercropped do not influence at least predation rates by natural anthocorids or damage by target pests of NJ peppers. Predation rates have been shown in prior work in peppers to increase *O. insidiosus* predation of sentinel European corn borer egg masses (Bickerton and Hamilton 2012). Since this has been the case in prior years, the minimum distance must be the issue in the present study.

Anthocorids were attracted with companion plants, however the effect was variable. The mixture of all three intercrops did not appear as attractive as the intercrops on their own. The reason for this may be that the anthocorids may not prefer to feed on all of the three intercrops. See results in Chapter 3 that *Orius insidiosus* significantly prefers dill alone and shows a trend to prefer coriander alone and fennel alone, though these trends are non-significant. Other studies have found similar results, where results varied depending on offering plants in combination or alone. For example, Pumariño et al. (2012) found fava bean to be the least preferred plant alone however in combination it was preferred for oviposition. There was no significant difference in terms of predation. It is possible the anthocorids were spending more time feeding on the pollen than on European corn borer eggs. It is also possible the anthocorids were feeding on some other host, for example aphids, that were in the peppers as host-switching depending on the density of prey is known to occur in *O. insidiosus* (Bickerton, 2011). Fennel has been shown as an intercrop in cotton to reduce pest abundance and increase generalist predator populations (Ramalho et al. 2012b).

In summation, there was no significant effect of the insidious flower bug on crop yield damage caused by European corn borer. In 2015, in the intra-row experiment, there was a trend for higher damage in the non-intercropped treatment. This was found also in 2016 in the inter-row experiment, and overall in 2016 there was less damage in the fennel treatment. These result trends tend to agree with the general theory that flowering intercrops can reduce crop damage. It is possible that the insidious flower bug was attracted to other nearby treatments; future experiments should increase the distance between treatments.

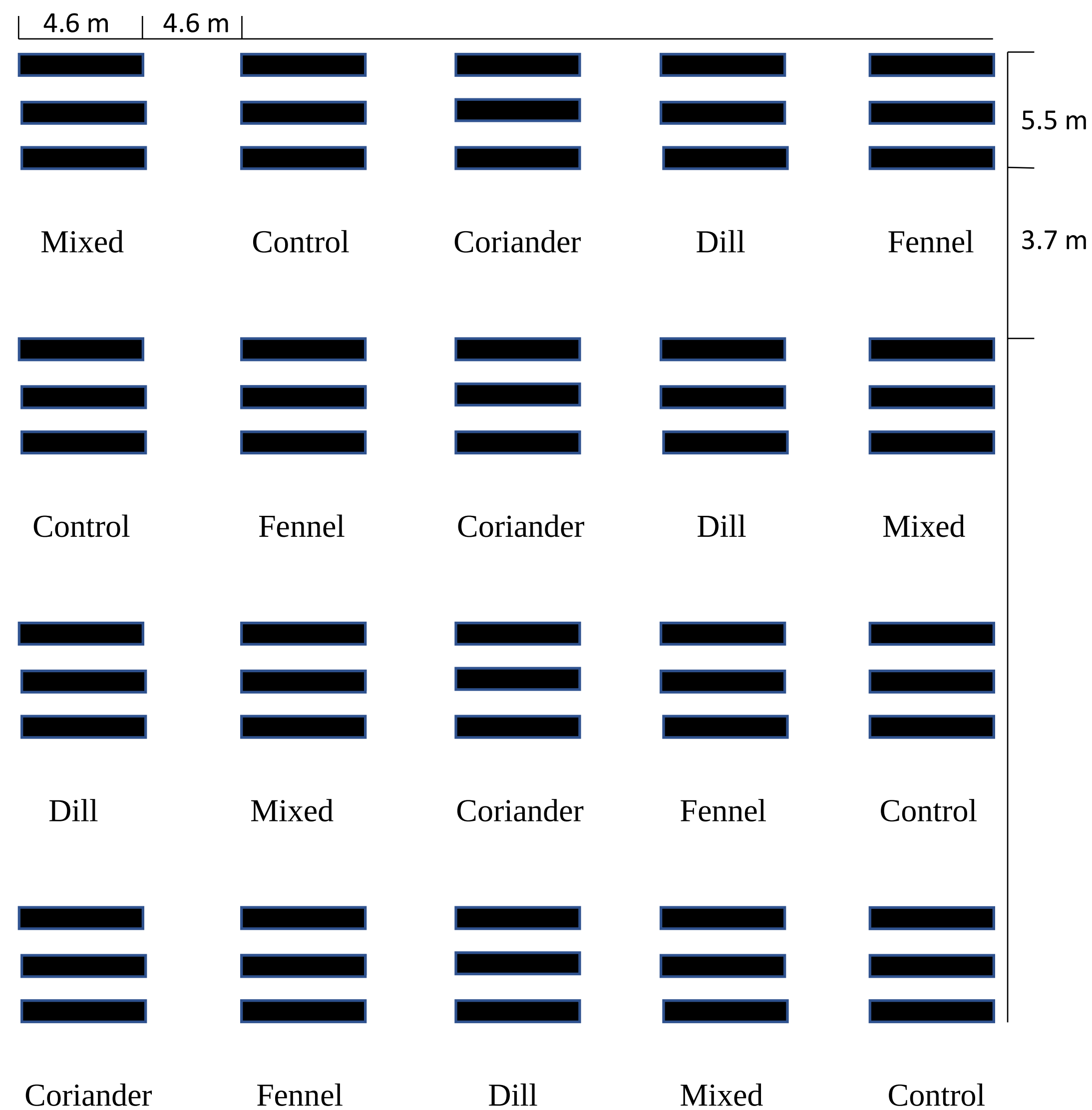


Fig. 1. Plot layout of intra-row field 2015. The companion flowers are planted in 3 patches between evenly-spaced 4 peppers along each bar, except in the control where no companion flowers were planted. Measurements in meters are repeated along the line for each bar or set of bars and gap between bars.

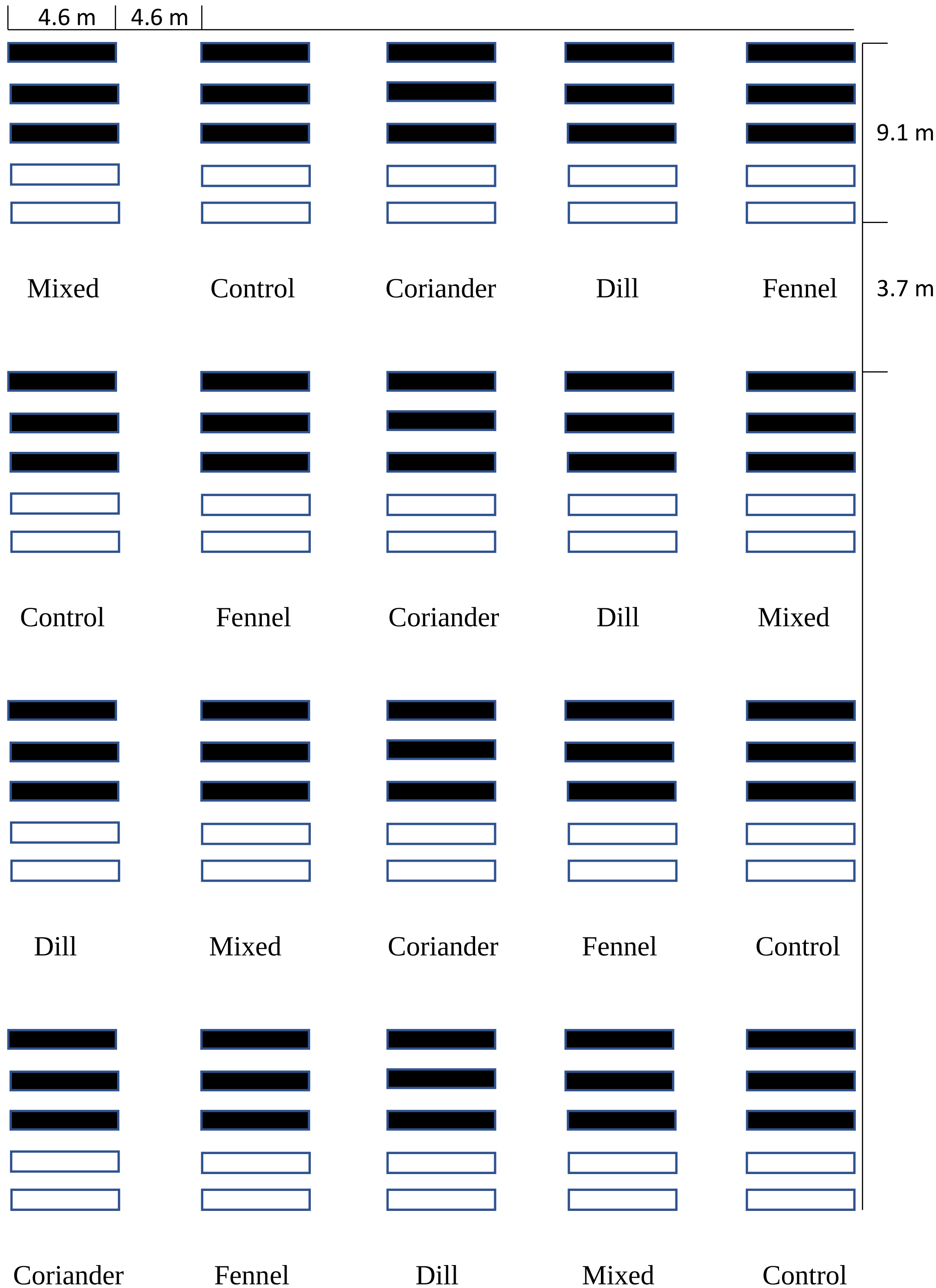


Fig. 2. Plot layout of inter-row field 2015. Measurements are repeated along each line.

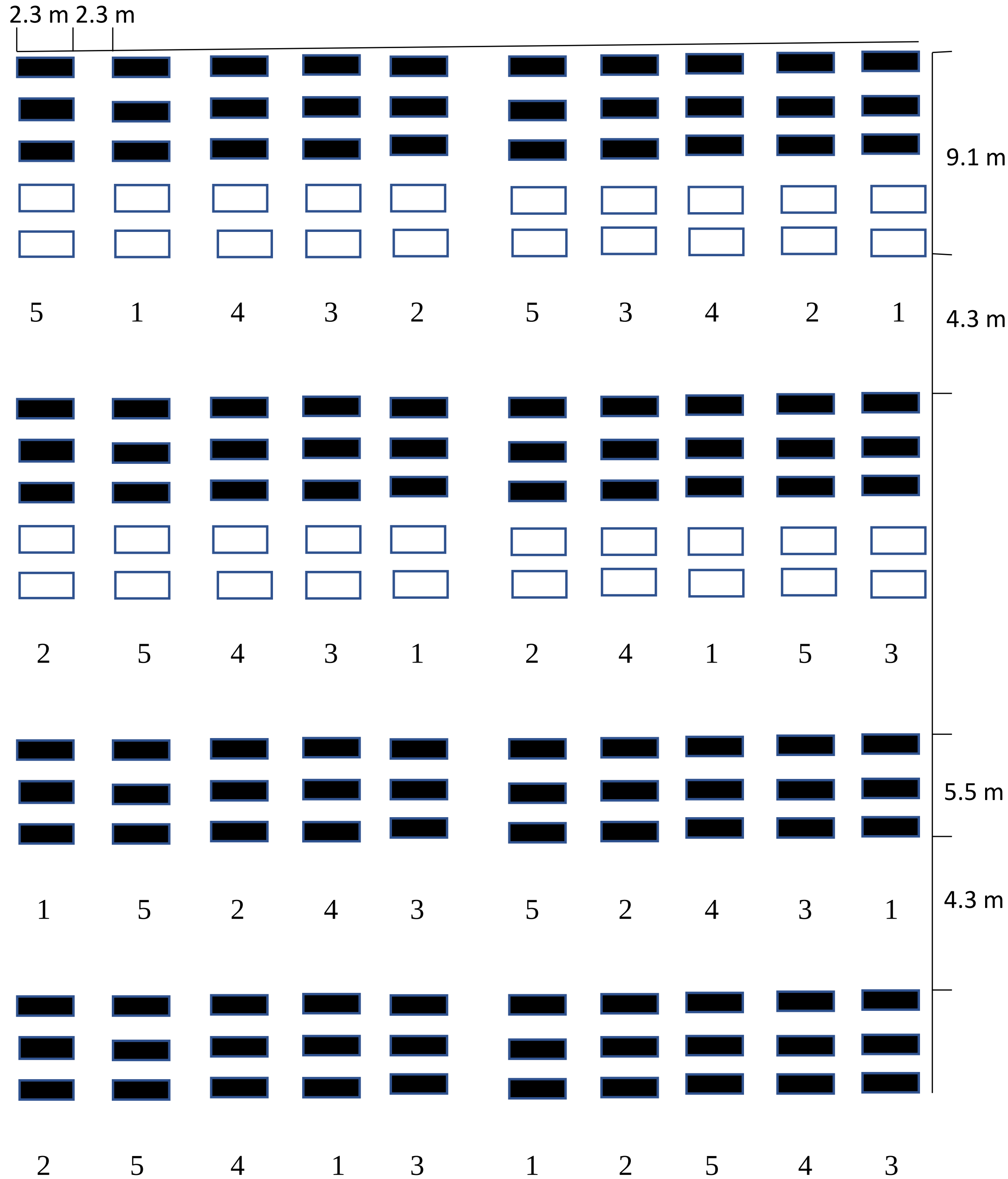


Fig. 3. Plot layout of field 1 2016. Treatments were 1: Mixed, 2: Control, 3: Coriander, 4: Dill, 5: Fennel. The inter-row study has blocks with open boxes that are white plastic intercrop rows. The blocks without open boxes are the intra-row study. Measurements of bars or sets of bars and gaps between bars are repeated along the line.

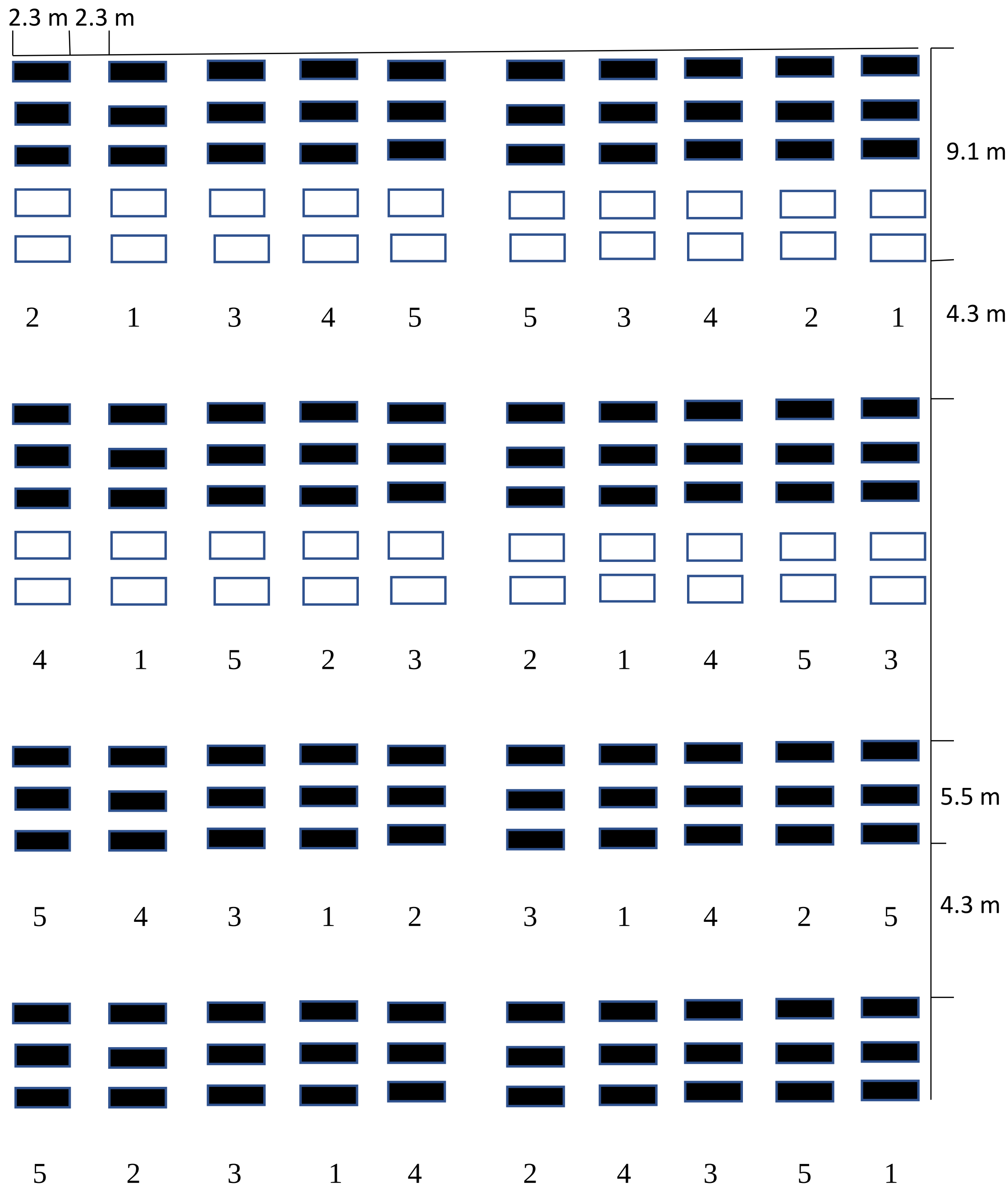


Fig. 4. Plot layout of field 2 2016. Treatments were 1: Mixed, 2: Control, 3: Coriander, 4: Dill, 5: Fennel. The inter-row study has blocks with open boxes that are white plastic intercrop rows. The blocks without open boxes are the intra-row study. Measurements of bars or sets of bars and gaps between bars are repeated along the line.

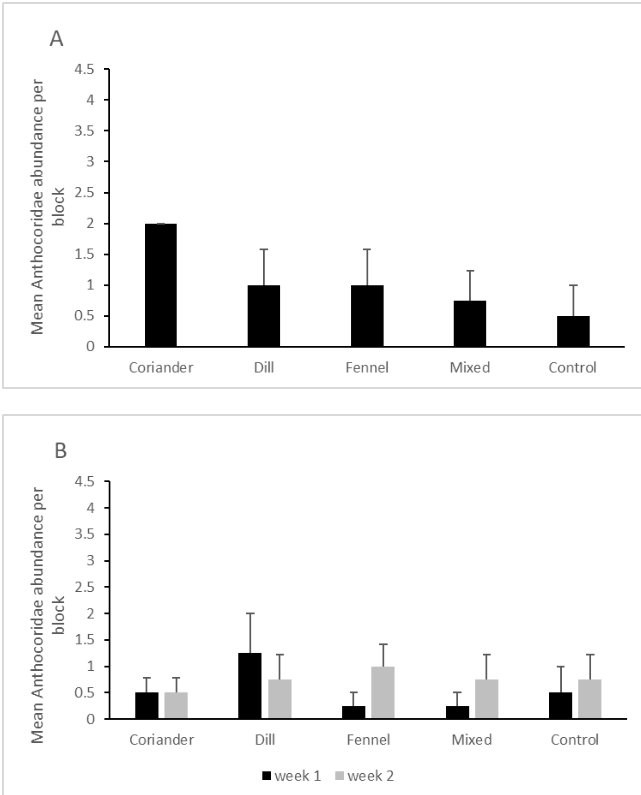


Fig. 5. Seasonal mean anthocorid abundance in 2015 intra-row within the peppers, week 2 (A) and within the flowers (B) intercropped NJ peppers. All treatments are shown with standard error.

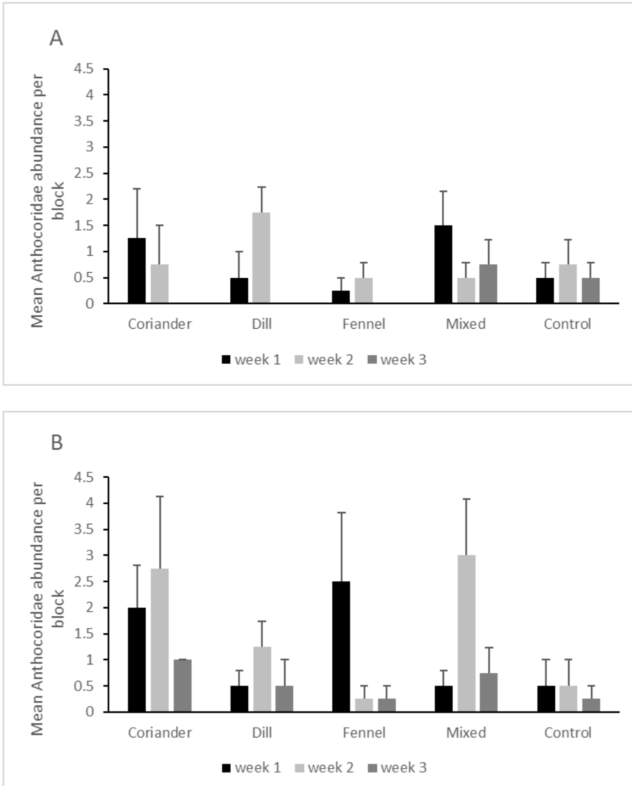


Fig. 6. Seasonal mean anthocorid abundance in 2015 inter-row within the peppers (A) and within the flowers (B) intercropped NJ peppers. All treatments are shown with standard error.

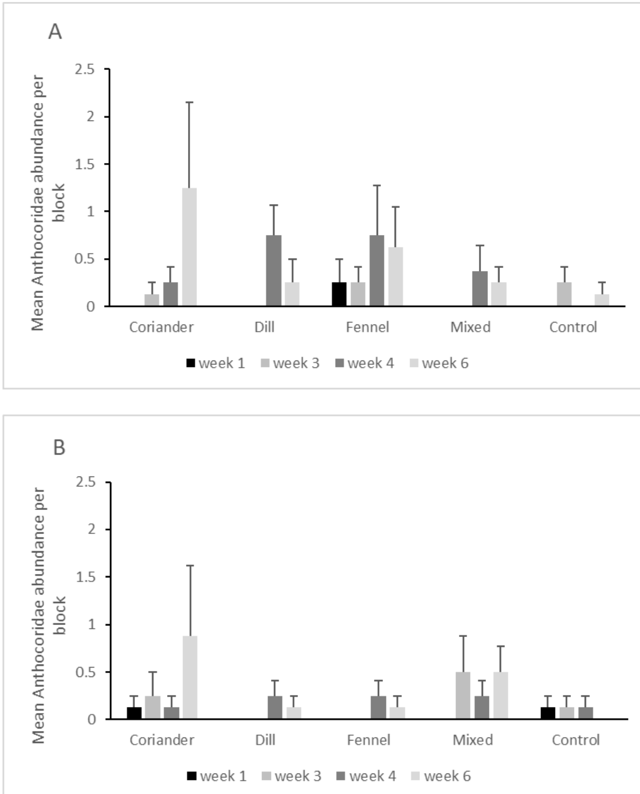


Fig. 7. Seasonal mean anthocorid abundance in NJ peppers 2016 intra-row intercropped, A) within the peppers and B) within the flowers. All treatments are shown with standard error.

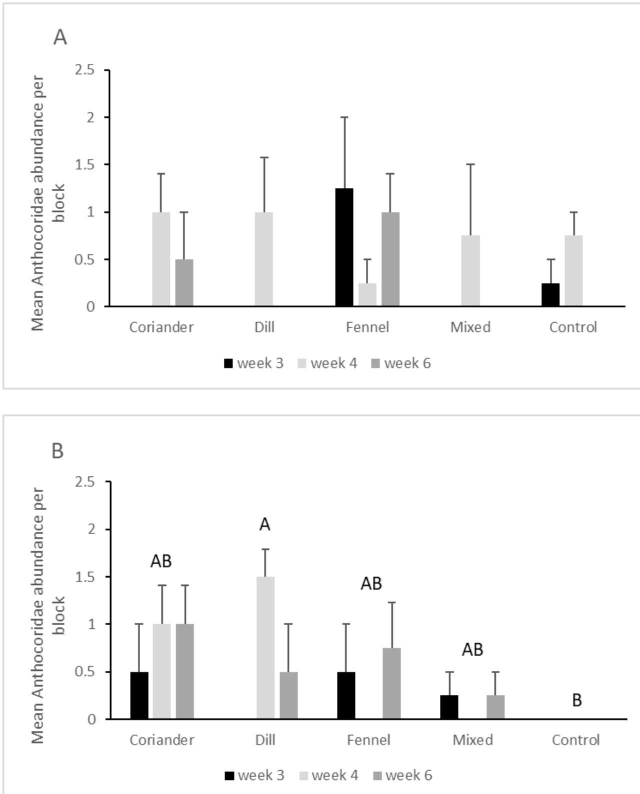
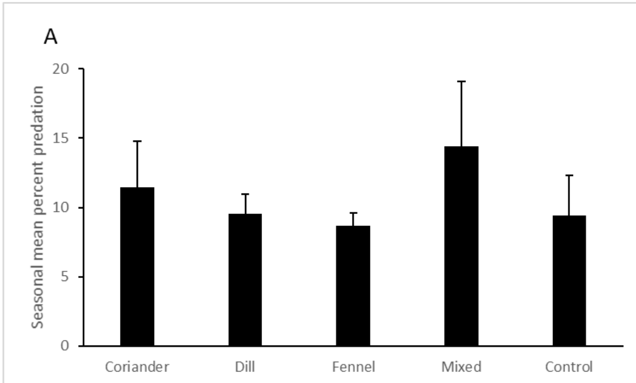


Fig. 8. Seasonal mean anthocorid abundance in NJ peppers 2016 inter-row intercropped, A) within the peppers and B) within the flowers. All treatments are shown with standard error. Means with the same letter are not significantly different ($p<0.05$) Mann-Whitney U Test.



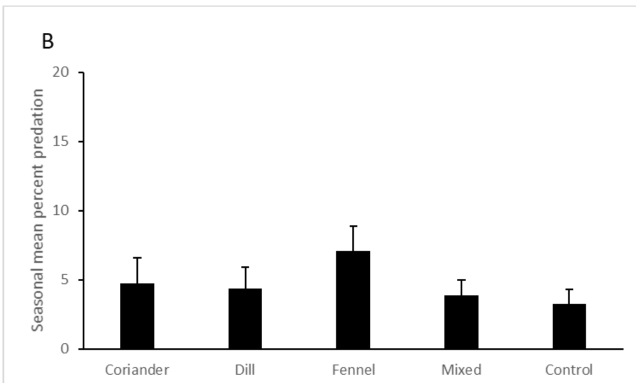


Fig. 9. Seasonal mean percent anthocorid predation of sentinel European corn borer egg masses in NJ peppers 2015. Intercrop treatment, coriander, dill, fennel, all intercrops mixed and non-intercropped control, effect on predation by natural populations of Anthocoridae in A) intra-row, and B) inter-row bell pepper in New Jersey in 2015. Standard error is shown as error bars.

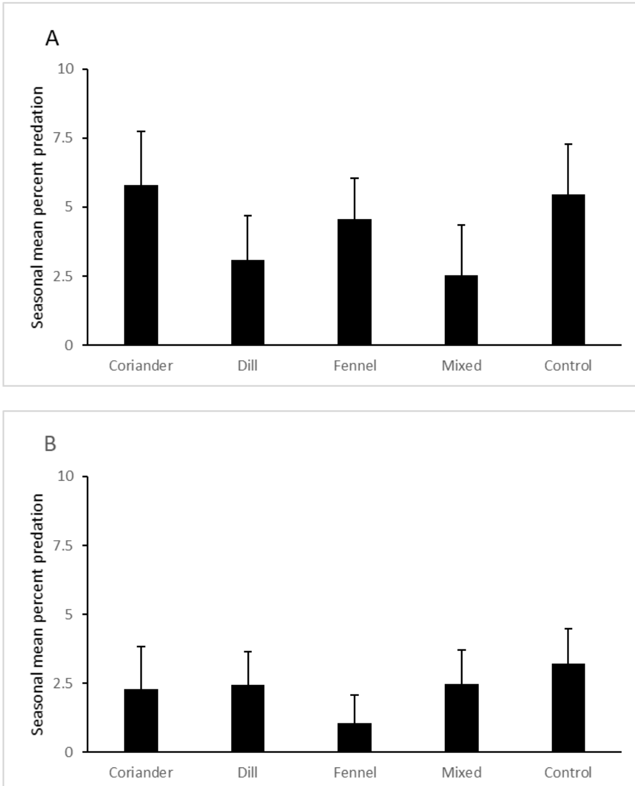


Fig. 10. Intercrop species composition effect on seasonal mean percent predation by natural Anthocoridae populations on sentinel European corn borer egg masses in intra-row and inter-row New Jersey Paladin peppers 2016. Error bars show standard error.

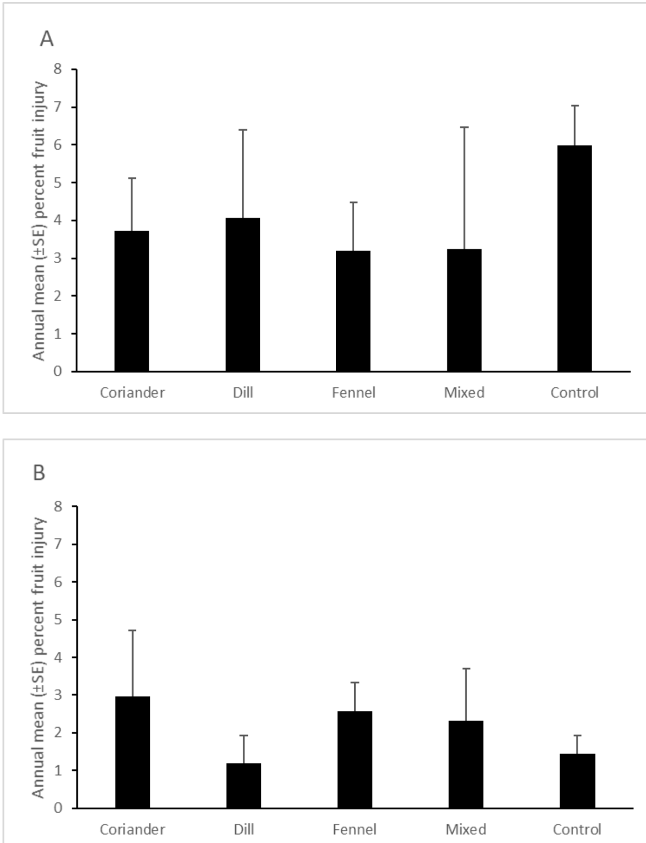


Fig. 11. Effect of intercrop treatments coriander, dill, fennel, a mixed composition of all and non-intercropped on annual mean percent pepper damage from European corn borer with standard error for intra-row and inter-row intercrops in New Jersey Paladin pepper 2015. A) Intra-row and B) inter-row. Total peppers damaged by European corn borer (A: N=69, B: N=45) were found from total harvest from each field: N=1649 (A) and N=2223 (B).

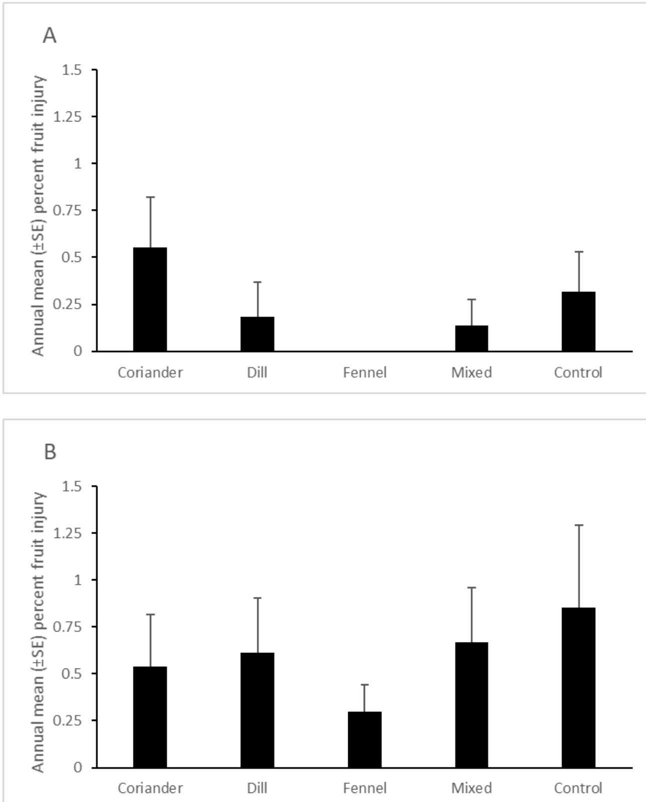


Fig. 12. Harvest damage from European corn borer in intra-row and inter-row 2016. A) Intra-row and B) inter-row percent annual injury from European corn borer to all harvested peppers with bars of standard error for four blocks per field of treatment; all peppers were harvested in August 2016. Peppers injured by European corn borer (intra-row: N=7; inter-row: N=41) are shown as percentages over total peppers harvested (intra-row: N=2856; inter-row: N=6879).

CHAPTER 2: MOLECULAR GUT CONTENT ANALYSIS OF *ORIUS INSIDIOSUS* IN INTERCROPPED NEW JERSEY PEPPERS

Abstract

Molecular gut content analysis can detect predation at low levels that are difficult or impossible to observe in the field through standard scouting techniques. At low predation rates, this technique may be required to observe small differences in prey preference of predators in the field. Quantitative PCR was utilized to test for differences in the predation of European corn borer and the consumption of pollen from coriander, *Coriandrum sativum* L., dill, *Anethum graveolens* L., and fennel *Foeniculum vulgare* Miller by *Orius insidiosus* (Say) in a field study in 2016 to determine dietary preference of *O. insidiosus*. Positive molecular gut contents were found for European corn borer, coriander, dill and fennel. No significant differences were found between positive molecular gut contents samples. These results indicate that *O. insidiosus* forages within adjacent intercrops as well as further away intercrops before entering peppers.

Introduction

Companion planting can enhance the biological control effectiveness of natural enemies of pests. Food resources can be provided by companion plants through nectar and/ or pollen for generalist omnivorous predators. Wildflower plantings have been shown to increase the abundance of natural enemies and the effectiveness of biological control in Michigan blueberry *Vaccinium corymbosum* L. fields (Blaauw and Isaacs 2015). Larger wildflower plots showed greater abundance of natural enemies and in one year, greater biological control as measured by predation of aphids (Blaauw and Issacs 2012). In a literature review on biodiversity in agroecosystems, of 198 herbivore species, 53% were reduced in population in more diverse agricultural systems, suggesting benefits of companion crops that would increase plant diversity (Risch et al. 1983).

Molecular gut-content analyses can reveal dietary preferences such as the finding that linyphiid and tetragnathid spider predators in winter wheat *Triticum aestivum* L. prefer collembolans and aphid prey over Brachycera and Platygastridae prey (Chapman et al. 2013). Molecular methods to detect gut contents can also confirm that the presence of alternative prey does not necessarily reduce biological control by natural enemy predators, as seen in the consumption of two-spotted spider mites *Tetranychus urticae* Koch by *Geocoris* by *Nabis* predators in potato *Solanum tuberosum* L. (Krey et al. 2017).

Molecular gut-content analyses can also focus on one pest to determine trophic relationships at different times of the season. For example, Schmidt et al. (2014) found that hunting spiders were the most important natural enemy of the squash bug *Anasa* *tristis* (De Geer) early in the season, while coccinellids and geocorids were important natural enemies later in the season when abundance was high. Greenstone et al. (2014b) show through molecular gut-content analysis that conservation biological control of the kudzu bug *Megacopta cribraria* (F.) is possible in soybean *Glycine max* (L.) Merrill from predators enhanced in adjacent cotton *Gossypium hirsutum* L. field. Four spider predator species were ranked by quantitative PCR as having molecular gut-content detectability half-lives of the pest *Empopasca vitis* (Göthe) (Yang et al. 2017). Biological control of the pest by these predator species was also tested in field tea plantations (Yang et al. 2017).

Qualitative positive and negative molecular data on the gut content of predators is useful, in particular in combination with a detectability half-life (Greenstone & Hunt 1993). Quantitative PCR provides the opportunity for precise amounts of relative molecular signals to be determined resulting in a high sensitivity test of predation, and shorter PCR-amplified DNA fragments can be detected for longer times in molecular gut-content analyses (Hoogendooorn & Heimpel 2001). For example, European corn borer has been shown to be present and detectable at 150-bp amplified fragments in the guts of *Coleomegilla maculata* De Geer adults for up to 12 hours (Hoogendoorn & Heimpel 2001).

A single egg of Colorado potato beetle, *Leptinotarsa decemlineata* (Say), can have a half-life detectability in the guts of predator species varying from 7 hours to 84 hours with an amplified PCR fragment of 214 bp (Greenstone et al. 2010). Half-life detectability was shown to be 2.3 hours for *O. insidiosus* consuming a single corn earworm *Helicoverpa zea* (Boddie) egg with a PCR-amplified fragment of 201 bp

(Peterson et al. 2018). Consumption of a single *L. decemlineata* egg by *O. insidiosus* has a detectability half-life of 21.8 hours with an amplified PCR product of 214 bp (Simmons et al. 2015). *Orius insidiosus* was shown to have a molecular gut-content detectability half-life around 4 hours for a single *Harmonia axyridis* (Pallas) egg, and greater length of detectability half-lives for a consumed adult *Neohydatothrips variabilis* (Beach) or a *Aphis glycines* Matsumura nymph with amplified PCR fragment sizes of 261, 160 and 255 bp, respectively (Harwood et al. 2007). Predated olive fruit fly, *Bactrocera oleae* (Rossi), have been shown to have a molecular gut-content detectability half-life of between 48 and 78 hours in the guts of the carabid beetle *Orthomus barbarus* (Dejean) with amplicons of 81 and 106 bp (Lantero et al. 2017). A 4.4 hour molecular gut-content detectability half-life was found for the second-instar of the pest mirid bug *Apolygus lucorum* (Meyer-Dür) in the guts of the predator *Harmonia axyridis* with a 323 bp amplified PCR fragment (Li et al. 2016).

It is hypopthesized that a greater proportion of anthocorids collected from peppers, *Capsicum annuum* L., adjacent to the flowering herbs, coriander, *Coriandrum sativum* L., dill, *Anethum graveolens* L., fennel, *Foeniculum vulgare* L., or all three in combination, will have more gut contents belonging to the adjacent plantings than the control non-intercropped treatment. It is further hypothesized that greater consumption of European corn borer will occur in the most diverse planting of all three herbs, compared to the control.

Materials & Methods

Sticky card samples

Samples from 2016 were collected from pepper fields intercropped with coriander, dill and fennel located on the Rutgers University Snyder Research and Extension Farm in Pittstown, NJ. Five treatments were created, one of each of the intercrops, a mixture of all three intercrops and a non-intercropped control. Each treatment consisted of three rows of double-row peppers, consisting of 30 plants, and a fourth and fifth row of double-row intercrop, consisting of 30 plants or non-intercrop control. Each treatment was replicated eight times in two isolated fields and four sticky cards were placed per treatment per day. Clear sticky cards were utilized for sampling and were made by brushing TangleTrap® Insect Trap Coating on both sides of 10.8 cm x 14.0 cm write-on transparency film (School Smart, Appleton, WI). The sticky cards were placed in 2016 within the first and third pepper rows on July 9, 13, 20, 27 and August 3 and 10 for 48 hours and then collected and frozen at -20ºC until analysis. *Orius insidiosus* were removed from the sticky cards with forceps and placed individually in 1.5-ml microfuge tubes and again stored at -20ºC until molecular gut content analysis (N = 106).

Gut content analysis

Orius insidiosus individuals were partially homogenized using sterile plastic pestles in 100 µl of high salt extraction buffer (Aljanabi & Martinez, 1997). Ten microliters of 20% SDS and 2 µl of proteinase K (20 mg/ml) were added and thoroughly mixed. Samples were then centrifuged for one minute at 10,000 *g*, and incubated overnight at 65ºC. A volume of 75 µl of 6M sodium chloride was then added, and samples were vortexed for 30 seconds at maximum, followed by a centrifuge step for ten minutes at 14,000 *g*. The supernatant was then transferred to a new microcentrifuge tube. The DNA was precipitated by adding 185 µl of isopropanol with 30 µg of glycogen. Samples were then incubated for one hour at -20º C, followed by a centrifugation step at 14,000 *g* for 10 minutes. The supernatant was then discarded and pellets were dried on a heat block at 37ºC. Pellets were resuspended in 15 µl double-distilled H_2O.

Primers were designed using PrimerExpress (Applied Biosystems Inc., Woolston, UK) (Table 1). The elongation factor 1 (EF1) gene of (GenBank accession # KU176086) *O. insidiosus* was chosen as the endogenous control gene to prevent false-negatives and was used to calibrate quantitative PCR to account for differences in starting DNA amounts to determine a relative gut content amount of target sequences. These primers were also used to confirm successful extraction of genomic DNA from each anthocorid sample. The other primers were specifically chosen to be from the ITS gene because it is highly divergent among closely-related species, allowing separate amplification of each species.

Reactions for quantitative PCR were made up to 10 µl with 1 µl of the forward primer and 1 µl of the reverse primer, each at a concentration of 5 picomoles per microliter. Five microliters of 2× PowerSYBR® Green PCR Master Mix (Applied Biosystems, Woolston, UK) was used with 1 µl of genomic DNA (gDNA) and 2 µl of double-distilled H_2O. Each quantitative PCR reaction was performed in duplicate at the same time. Quantitative PCR runs were under a thermocycling program at 95ºC for 10 minutes followed by 40 cycles of 95ºC for 15 seconds then 60ºC for 1 minute. Standard curves were generated for all primer pairs, and curves were as expected with R^2 values between 0.98 – 0.99, indicating the efficient amplification of all gene targets.

All PCR reactions included a control of an *O. insidiosus* adult that was not exposed to coriander, dill, fennel or European corn borer and a negative control (sterile water instead of DNA). A PCR was considered positive when both replicates of a sample had a threshold cycle of two cycles lower or less than any of the control replicates. All six sets of primers for each sample were used in the same run for accurate comparison. Any samples without positive results for both *O. insidiosus* PCRs were discarded from further analysis.

Feeding trials

Detection limits were determined for *O. insidiosus* for target DNA (*A. graveolens*, *F. vulgare*) through feeding trials. *Orius insidiosus* adult individuals purchased from Plant Products (Leamington, ON, Canada) were starved for 24 hours, then transferred individually to a clear gelatin capsule (size 000; Capsuline) with approximately 20 micrograms of dill or fennel pollen for 2 hours. After the feeding period, individual adults were frozen or transferred into clean plastic pots with a moistened cotton wick and kept at room temperature. Those transferred into pots were later frozen at intervals of 1, 2, 4, 8, 24, 48, 72 and 96 hours. Ten individuals were frozen for each time, except 9 individuals for 48 hours for both treatments and 9 individuals for fennel at 24 hours.

Statistical Analysis

Kruskal-Wallace tests were performed to analyze the potential difference in positive molecular gut contents between the treatment tests of European corn borer, coriander, dill and fennel for each field.

Results

European corn borer PCR showed positive molecular gut contents in anthocorids from coriander and fennel treatments for field 1 (Fig. 1). The positive molecular gut contents result for coriander was 10% and 6% for fennel. Coriander PCR for field 1 had high positive molecular gut contents at 30% from the coriander treatment and also positive results for fennel (Fig. 2); the molecular gut contents result from fennel was 6%. Results from dill PCR for field 1 revealed positive molecular gut contents from the dill, mixed and coriander treatments (Fig. 3). The positive molecular gut contents for dill was 14%, while the mixed treatment had positive molecular gut contents of 11% and the coriander had positive molecular gut contents at 20%. Fennel PCR results for field 1 were positive for anthocorid molecular gut contents only from the mixed treatment (Fig. 4); the positive molecular gut contents from the mixed treatment was 11%. There was no statistical difference between the tests to amplify anthocorid molecular gut contents in field 1 (χ^2=2.3084, df=3, p=0.51), i.e. none of the diets were preferred over other diets given equivalent efficiency of detection of the molecular gut contents tests.

For field 2, positive European corn borer PCR results from anthocorid gut contents were found for the non-intercropped control (8%) and dill (17%) (Fig. 5). Positive coriander PCR results were found in the mixed treatment (9%) (Fig. 6). While positive dill PCR results were found for coriander (8%), dill (17%), fennel (17%) and the non-intercropped control (8%) (Fig. 7). Dill was the only positive result for the fennel PCR (Fig. 8). The positive molecular gut contents from the dill treatment were 8%. There were no statistical differences between the means found for the positive PCR results for field 2 (χ^2=4.9843, df=3, p=0.17).

Ten of the 22 positive gut content samples were from *O. insidiosus* individuals that were positive for multiple species. Two of the five individuals amplified both dill and fennel, one from the mixed treatment and the other from the dill treatment. Two individuals from the coriander treatment amplified two positive gut contents, one amplified coriander and dill, and the other amplified coriander and European corn borer. The final individual from the dill treatment amplified dill and European corn borer.

Feeding trials for both dill and fennel revealed 100% positive molecular gut contents for all times tested (0-96 hours (Table 2)), showing that the pollen is detectable for at least 96 hours. The threshold cycle (CT) value means are shown in Table 3. The detection of positive gut contents of the dill and fennel pollen at 72 and 96 hours is close to the outer limit of detection because the CT value is approaching the typical cut-off of 35 cycles that is considered reliable for a positive result.

Discussion

The use of molecular gut content analyses can reveal important dietary components that sustain generalist predators in the field when pest populations are low, as was discovered in the consumption of earthworms by carabid beetles in woodlands in the United Kingdom and Croatia (Šerić Jelaska and Symondson 2016). *Orius insidiosus* has been found to be a major predator of pests, including the stink bug *Nezara viridula* (L.) in cotton, having 91.6% showing positive molecular gut contents (Tillman et al. 2015).

In field 1, presence of gut contents from other treatments, i.e. fennel in coriander and coriander in dill confirms that anthocorids feed farther than immediately adjacent intercrops to forage for prey in peppers. The coriander and dill PCR tests confirmed that anthocorids also feed immediately adjacent to peppers, showing positive tests in field 1 at 30% and 14% respectively from coriander and dill. Positive results were found from the mixed treatment of all three herbs in fennel and dill in field 1 and could indicate immediately adjacent foraging for pollen. European corn borer was positive for molecular gut contents from coriander and fennel treatments in field 1, suggesting that anthocorids perhaps attracted by coriander or fennel also feed on European corn borer eggs in these treatments. In field 2, European corn borer PCR was positive for anthocorid molecular gut contents from dill, suggesting also that anthocorids perhaps attracted by dill also feed on European corn borer.

In field 2, positive molecular gut contents results were found for dill in treatments other than dill, including coriander, fennel and non-intercropped control, showing that anthocorids forage more widely than immediately adjacent flowers. This was also the case for fennel where the only positive result was from the dill treatment. Molecular gut contents results from dill were also positive from the dill treatment, suggesting that anthocorids also forage near peppers before entering to forage among the peppers. Coriander-positive results of the molecular gut content analysis were found from the mixed treatment with all three herbs so again this result could be either adjacent feeding from the mixture of herbs or from a farther coriander treatment then foraging of the anthocorid in the peppers of the mixed treatment.

Previous work on molecular gut contents analysis of intercropped NJ peppers with coriander, dill and buckwheat *Fagopyrum escuelentum* Moench revealed overall positive results for coriander of 27% and for dill 36.4% (Bickerton, 2011). Here, overall positive molecular gut contents for coriander and dill were 5.1% and 9.4% between both fields, with fennel at 1.9% and European corn borer at 4.7%. Such variation may occur from year to year as Bickerton (2011) only analyzed one year as well.

Molecular gut content analysis of two grasshopper species *Oedaleus asiaticus* B. Bienko and *Dasyhippus barbipes* (Fischer-Waldheim) revealed that one species adapts its diet of grasses and the other does not, suggesting a cause for distribution differences between the species (Huang et al. 2016). Future analyses could further incorporate possible changes in diet of anthocorid generalist predators depending on their environment and associated available pollen resources and alternative prey. Corn pollen was found to be a major component of the diet of *O. insidiosus*, while European corn borer eggs were found to be consumed at low levels in corn (Corey et al. 1998). This work could be expanded to determine optimized layout for intercrops in corn to maximize biological control from anthocorids.

The generalist predator anthocorid *Anthocoris nemorum* L. was shown to consume two-spotted spider mite by molecular gut content analysis in strawberry *Fragaria* × *ananassa* Duchesne (Jacobsen et al. 2019). Similarly, Peterson et al. (2018) showed *O. insidiosus* to consume eggs of *H. zea* in sweet corn. Here we show the generalist predator anthocorid *O. insidiosus* to consume European corn borer eggs in pepper in the field, as well as consumption of coriander, dill and fennel pollen. This confirms anthocorid predation of European corn borer eggs as noted by Froeschner (1950), though not found previously in molecular gut content analysis of *O. insidiosus* in the field in pepper (Bickerton, 2011). Trophic links have been established for *Orius majusculus* (Reuter) on lettuce *Lactuca sativa* L. using molecular gut content analysis (Gomez-Polo et al. 2016). While these analyses showed importance of alternative prey, intraguild predation of anthocorids can occur by hoverfly species in lettuce (Gomez-Polo et al. 2015). Future analyses should incorporate such possible intraguild predation and abundance of intraguild predators in each treatment.

Further analysis may determine the potential differential role of *O. insidiosus* adults and nymphs in controlling European corn borer populations, as determined in soybean where *O. insidiosus* nymphs consume proportionally greater amounts of the target aphid pest *Aphis glycines* Matsumura (Harwood et al. 2009).

There is the possibility of error using molecular gut content analyses if there is variation in the target tested gene(s), in particular where the primers are located to affect amplification. Intraspecific variation has been shown in the internal transcribed transgenic spacer ITS1 gene in Diptera (Wesson et al. 1992, Tang et al. 1996). However, this work with ITS1 genes has been successfully shown before with different primers

(Bickerton, 2011), therefore such concerns are minimized and only higher estimations of predation would be predicted.

In summary, positive molecular gut contents results were found for *O. insidiosus* in the consumption of European corn borer, as well as coriander, dill and fennel pollen. While there was positive gut contents detected, there was no significant pattern between the molecular tests to determine preference of pollen or European corn borer.

Table 1. Primer sequences and lengths of amplified fragments from quantitative PCR of coriander, dill, fennel, the insidious flower bug *Orius insidiosus* and the European corn borer *Ostrinia nubilalis*.

Target gene	Species	Amplified fragment length (bp)	Primer pair sequences
CsITS1	*Coriandrum sativum*	60	F 5’ GGGGGGCTTTTGTCCCTTG 3’ R 5’ AGCGGCCACCCAGGAGGG 3’
AgITS1	*Anethum graveolens*	78	F 5’ CACATTGGGCAAGCTTCAG 3’ R 5’ GGTGACCACCATAGAGGGG 3’
FvITS1	*Foeniculum vulgare*	77	F 5’ CACATCGGGCAAGCGTCAG 3’ R 5’ GGTGGCCACCATAGAGGG 3’
OiITS1	*Orius insidiosus*	108	F 5’ GGTGGCTTACCCTCCAGA 3’ R 5’ CCCCTTTATGGCTTTCGTGG 3’
OnITS1	*Ostrinia nubilalis*	132	F 5’ GATACACAAGTGTCATGTGG 3’ R 5’ CTGTACACGTAGAATATCG 3’
OiEF1	*Orius insidiosus*	70	F 5’ ACTCAGGCTCCCGCTCCAAG 3’ R 5’ TTCAACTCTTCCGACCGGG 3’

Table 2. Feeding trial positive molecular gut contents of *Orius insidiosus* per time interval.

Pollen source	0 h	1 h	2 h	4 h	8 h	24 h	48 h	72 h	96 h
A. graveolens	10/10	10/10	10/10	10/10	10/10	10/10	9/9	10/10	10/10
F. vulgare	10/10	10/10	10/10	10/10	10/10	9/9	9/9	10/10	10/10

Table 3. Threshold cycle (CT) means for feeding trials of dill and fennel of *O. insidiosus*.

Pollen source	0 h	1 h	2 h	4 h	8 h	24 h	48 h	72 h	96 h
A. graveolens	17.45	20.53	21.26	23.33	20.85	19.89	19.48	28.72	26.80
F. vulgare	19.58	22.52	22.19	22.83	21.35	23.20	23.73	31.00	31.50

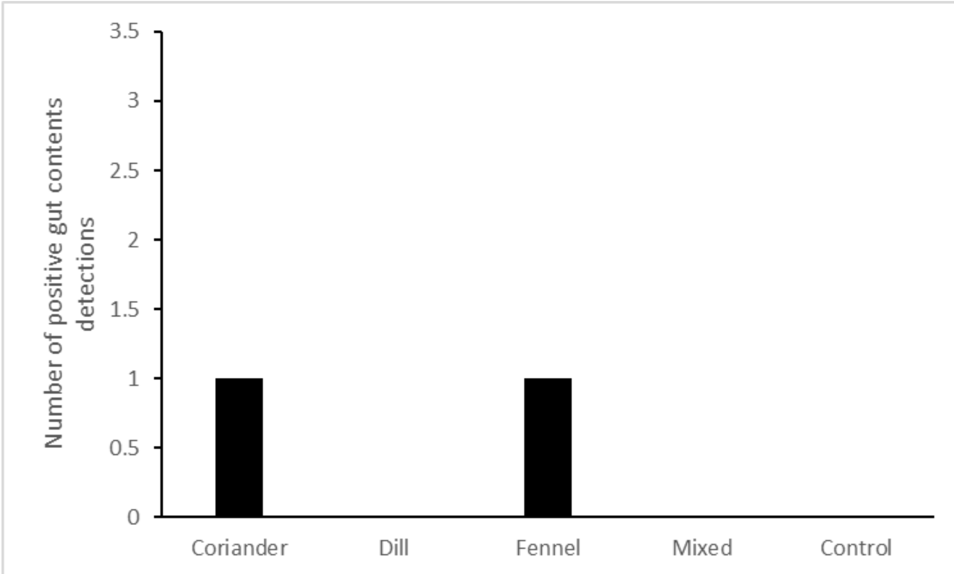


Figure 1. Anthocorid molecular gut contents samples positive for European corn borer in field 1 inter-row peppers.

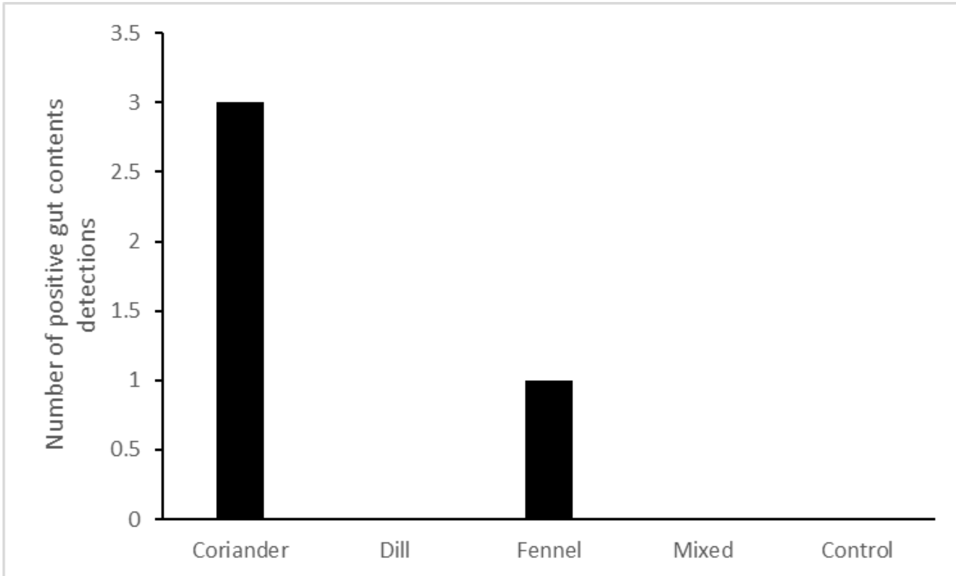


Figure 2. Anthocorid molecular gut contents samples positive for coriander in field 1 inter-row peppers.

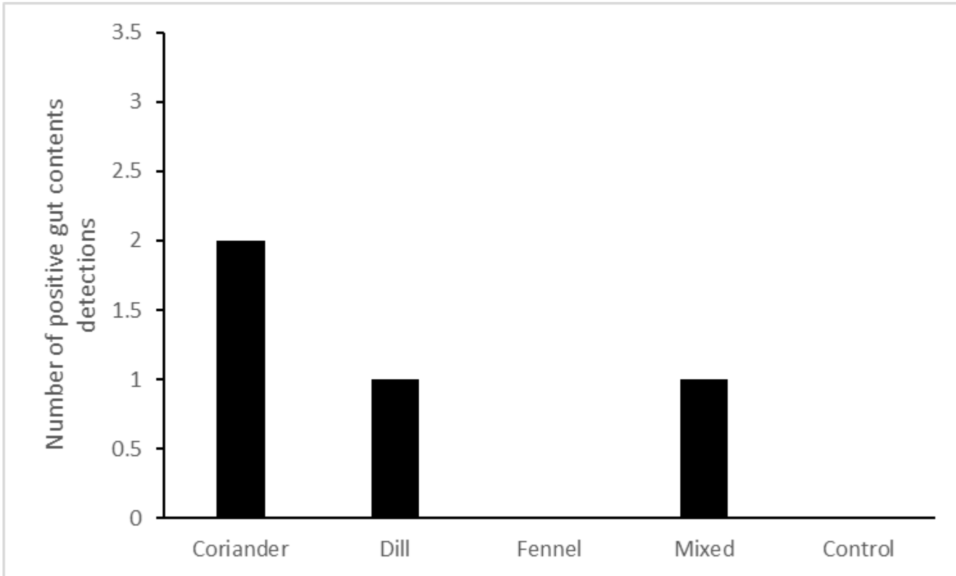


Figure 3. Anthocorid molecular gut contents samples positive for dill in field 1 inter-row peppers.

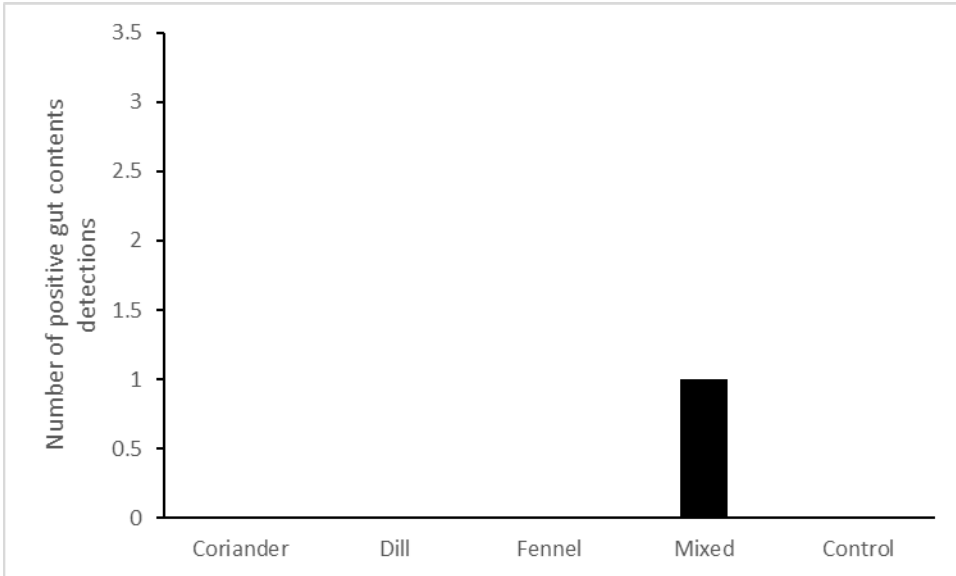


Figure 4. Anthocorid molecular gut contents samples positive for fennel in field 1 inter-row peppers.

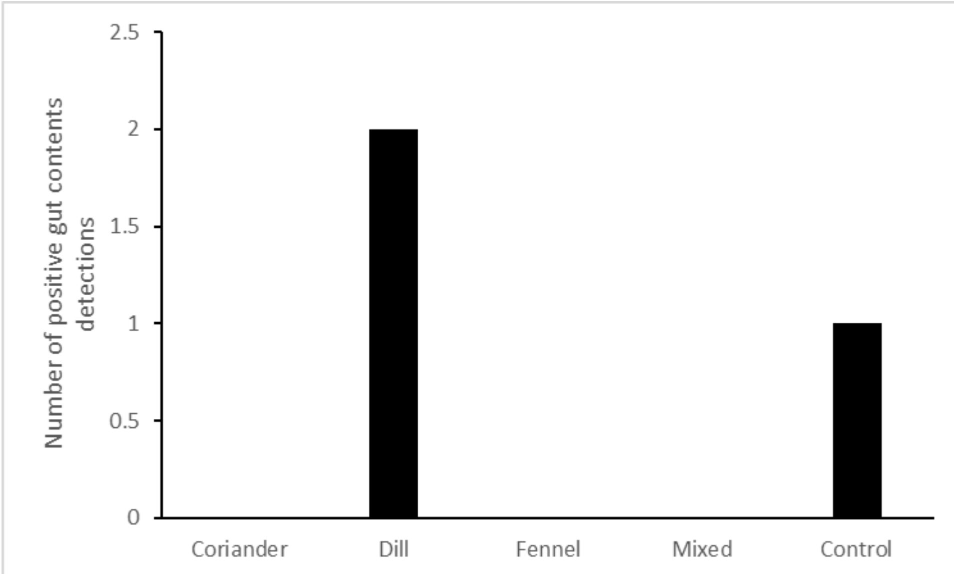


Figure 5. Anthocorid molecular gut contents samples positive for European corn borer in field 2 inter-row peppers.

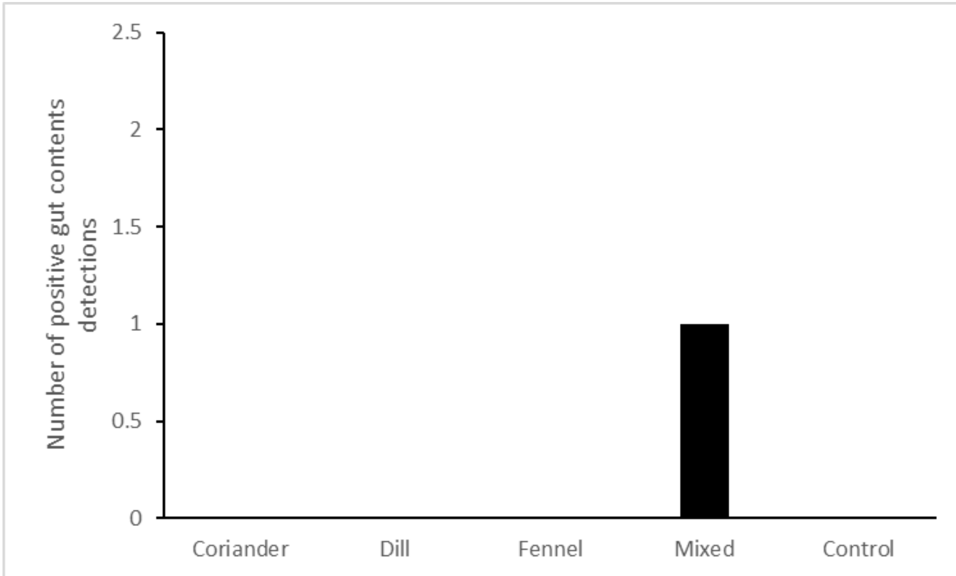


Figure 6. Anthocorid molecular gut contents samples positive for coriander in field 2 inter-row peppers.

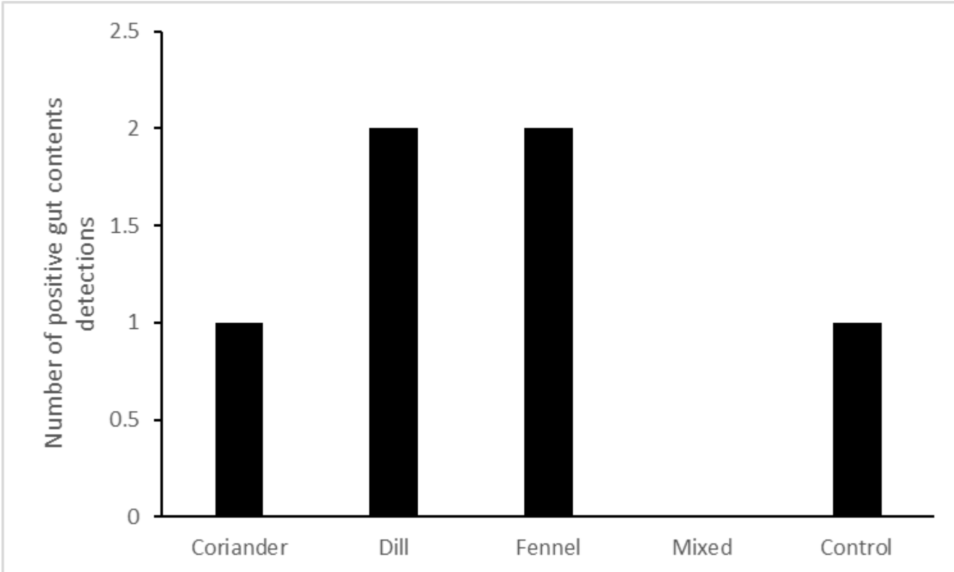


Figure 7. Anthocorid molecular gut contents samples positive for dill in field 2 inter-row peppers.

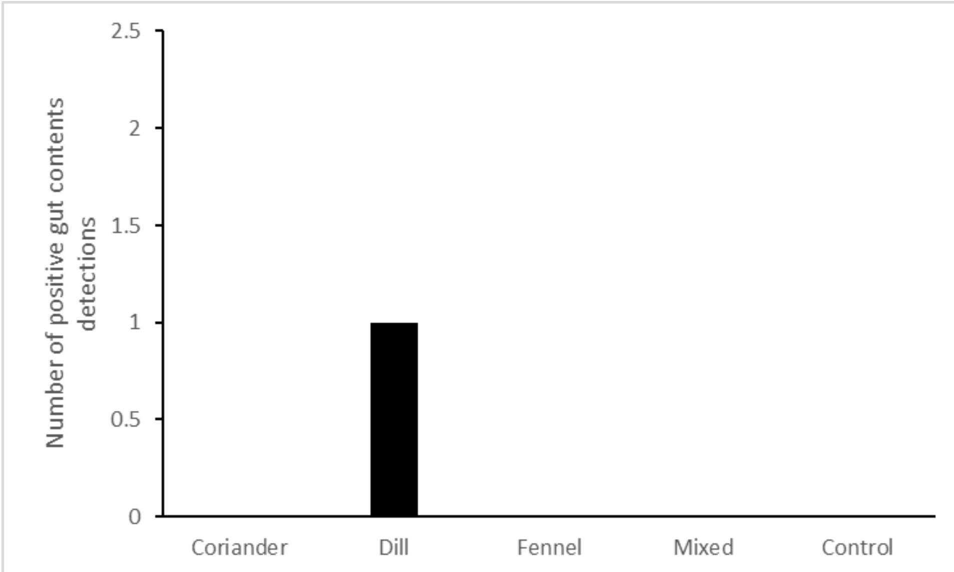


Figure 8. Anthocorid molecular gut contents samples positive for fennel in field 2 inter-row peppers.

CHAPTER 3: POLLEN PREFERENCE TRIALS OF *ORIUS INSIDIOSUS*

Abstract

The use of intercrops to enhance biological control of specific pests in agricultural ecosystems has the potential to replace or reduce pesticide use to maintain pests below economic thresholds in integrated pest management programs. Intercrops can provide nectar and pollen food resources to omnivorous natural enemies of pests. Understanding preferences of key natural enemies for one food source over another can have clear advantages to enhance biological control by providing intercrops that are preferred. I examined the preference of *Orius insidiosus* Say for pollen from coriander, *Coriandrum sativum* L., dill, *Anethum graveolens* L., and fennel, *Foeniculum vulgare* Miller. Arenas consisted of a 12-cm 4-arm olfactometer with pollen distributed in opposite arms for no-choice or choice tests. Ten-minute observations determined preference by time spent by adult *O. insidiosus* in an arm. Significantly more *O. insidiosus* chose dill over the no-pollen treatment, however other tests including choice tests with dill compared to coriander or fennel revealed non-significant results. Dill is suggested as a preferred pollen source and is recommended to be planted alongside peppers to enhance biological control of the European corn borer, *Ostrinia nubilalis* Hübner.

Introduction

Conservation biological control involves the maintainance and enhancement of natural enemies of target pest(s) in agroecosystems to reduce crop damage. Habitat modification of natural enemy shelters that provide non-prey food and suitable microclimates include flower strips and intercroppings (Gontijo 2019). Intercropping in wheat systems has been shown to significantly reduce pest abundance, however significant increases in natural enemy abundance and biological control was not shown in parallel (Lopes et al. 2016). Intercropping canola with alfalfa has been shown to enhance the yield of canola, reduce pest abundance and increase species diversity of predators of diamondback moth, *Plutella xylostella* (L.), compared to a monoculture (Tajmiri et al. 2017). Companion plant use in intercropping to reduce pest aphid abundance and crop damage can not only provide natural enemy shelter and food resources, but can attract aphids away from the target plant or react chemically and physiologically with the host plant to make the crop less suitable for aphid attack (Ben-Issa et al. 2017). Coriander, *Coriandrum sativum* L., and fennel, *Foeniculum vulgare* Miller, were assessed as intercrops with chickpea, *Cicer arietinum* L.. In this system fennel was found to have a higher monetary advantage index with less competition between chickpea and fennel than the other intercropping systems (Poddar et al. 2017). Garlic, *Allium sativum* L., was found to reduce pest populations of twospotted spider mite *Tetranychus urticae* Koch when intercropped with strawberry *Fragaria ananassa* Duch (Hata et al. 2016). Fennel intercropped with cotton *Gossypium hirsutum* L. was found to increase the abundance of generalist predators and decreased fennel aphid populations *Hyadaphis foeniculi*

(Passerini) compared to a monoculture of fennel (Ramalho et al. 2012a). Cotton intercropped with fennel was also shown to significantly reduce cotton aphid *Aphis gossypii* Glover populations and significantly increase generalist predator populations in the three rows of cotton next to two rows of fennel than in other intercropping systems using less rows of cotton. The losses of cotton seed yield were also significantly lower in this intercropping system (Ramalho et al. 2012b).

The use of plants other than the crop in agroecosystems can have microclimate effects that can benefit natural enemies. Diehl et al. (2012) shows how light intensity and surface temperature are lower under weeds and artificial weed-like structures benefited predator carabid beetle activity and species richness. Dispersal, foraging and oviposition of *Orius minutus* (L.) can be affected by temperature and density dependence of prey, and conspecifics and leaf surface area in agroecosystems (Tuda and Shima 2002). Contrarily, earlier closed canopies in soybean *Glycine max* (L.), although a known cultural control practice to prevent outbreaks of *Helicoverpa zea* (Boddie) populations, did not alter generalist predator abundance or biological control of *H. zea* (Anderson and Yeargan 1998).

The beneficial predator *Chrysoperla externa* (Hagen) was shown to feed and reproduce solely on floral resources provided by coriander, dill *Anethum graveolens* L. and fennel, with adults consuming hundreds of grains of pollen (Resende et al. 2017). *Orius* species were shown to increase in abundance in one year with buckwheat, *Fagopyrum esculentum* Moench, and in interplanted fields compared to the monocrop control in sweet corn (Manandhar and Wright 2016). Ribeiro and Gontijo (2017) found that alyssum *Lobularia maritima* (L.) planted in collard greens *Brassica oleracea* (L.)

increased the abundance of generalist predators including *Orius* species, as well as reduced pest populations. Zarei et al. (2019) found intercropping tomato *Lycopersicon esculentum* (Mill.) with sainfoin *Onobrychis viciifolia* Scop. increased diversity of generalist predators and significantly reduced pest egg and larvae abundance.

French bean *Phaseolus vulgaris* L. intercropped with baby corn *Zea mays* L. was found to have a higher abundance of *Orius* generalist predators as well as lower marketable damage of the overall crop by approximately 30% compared to a French bean monocrop (Nyasani et al. 2012). *Orius tantillus* (Motschulsky) was found to feed on eggs of *Helicoverpa armigera* (Hübner) and intercropping pigeonpea (*Cajanus cajan* L.) with sorghum (*Sorghum bicolor* L.) may facilitate biological control of *H. armigera* (Sigsgaard and Esbjerg 1997).

Orius niger Wolff was found to have greater populations in warmer years and it was found to be related to semi-natural areas such as forest plantations, intensive degraded grasslands, and young forests (Veres et al. 2012). *Orius* spp. were found to be conserved when insecticides were chosen as part of integrated pest management, as opposed to conventional treatment, in the field against European corn borer in maize (Vasileiadis et al. 2017).

Anthocorids are often considered in integrated pest management programs due to their potential for biological control of pest species in agroecosystems (Lattin 1999). Despite annual crops' high disturbance due to habitat destruction and requirements for colonization each season, anthocorid species play important roles in biological control of pest species in major crops including potato, strawberry, cotton and pepper (Perdikis et al. 2011). Musser and Shelton (2003) found biological control by *Orius insidiosus* Say of

European corn borer was reduced in the presence of the alternative food resources corn pollen and aphids.

In this study *O. insidiosus* adults were evaluated for their preference of three pollen types: coriander, *Coriandrum sativum* L., dill, *Anethum graveolens* L., and fennel, *Foeniculum vulgare* Miller.

These tests were conducted to determine if insidious flower bugs prefer one pollen over another. These results were then compared to the results in Chapters 1 and 2. The recommendation would be to plant preferred flowers with peppers.

Materials & Methods

To conduct the choice and no-choice tests, 210 *O. insidiosus* adults purchased from Plant Products (Leamington, ON, Canada). They were starved for 24 hours and then individually placed in a 12-cm 4-arm olfactometer (Volatile Assay Systems; Rensselaer, NY) arena for 10 minutes at a channel pressure of 10 kPa with two pollen choices of pollen treatment and monitored for their behavioral preference. Pollen was purchased from Pollen Ranch (Lemoncove, CA) for dill or fennel. Coriander pollen was hand-collected from mature coriander plants grown in a greenhouse. Individual *O. insidiosus* were transferred using a clear gelatin capsule (size 000, Capsuline) or a paintbrush. The arena was split so that the end of opposite arms contained 10 micrograms of pollen, while the other two arms did not contain pollen. A glass plate lid covering the 4-arm olfactometer arena prevented escape during each trial. Individual anthocorids were placed in the center of the 4-arm olfactometer arena. When the anthocorid stepped from its original position, the 10-minute observation began. A stop watch was utilized to measure time. Choice was determined by time spent during the 10 minutes. Treatments in the olfactometer were replaced, and their position changed, every five insects. Where time was spent entirely on the center part of the arena without arms during the ten minutes, the result was not considered in statistics because no choice was made.

These trials were replicated 35 times each. The treatments tested were coriander pollen alone, dill pollen alone, fennel pollen alone, coriander vs. dill pollen, coriander vs. fennel pollen, and dill vs. fennel pollen (Table 1).

Time spent by *O. insidiosus* adults were compared using a t-test.

Results

Although not significant, a slight trend toward the choice of pollen was observed when *O. insidiosus* adults were given a choice between fennel or coriander and no treatment (Fig. 1; t=1.19, df=1, p=0.24; t=1.34, df=1, p=0.19, respectively). When offered a choice between dill and fennel, a slight trend to choose dill was seen, though this was non-significant (Fig. 2; t=0.73, df=1, p=0.47). There was a slight trend to choose coriander over dill when given the choice between these two pollen types, however the trend was not significant (Fig. 2; t=0.89, df=1, p=0.38), and a slight trend to choose fennel over coriander was observed when given a choice between these two pollens, however the trend was not significant (Fig. 2; t=-0.77, df=1, p=0.45).

A significant preference of *O. insidiosus* adults for dill over no-pollen treatment was observed ($t=2.13$, $df=1$, $p=0.0379$) (Fig. 1).

Discussion

There are not many studies on pollen attraction. Pumariño et al. (2012) found the least preferred plant, fava bean *Vicia faba* L., under no-choice tests became the most-preferred plant for oviposition of *O. insidiosus* when presented in combination with other plants. In my experiments, coriander compared to dill pollen showed only a slight non-significant trend towards *O. insidiosus* choosing coriander pollen (Fig. 2). Seagraves and Lundgren (2010) found ovipositional preference of *O. insidiosus* for plants with suitable prey and also for green bean *Phaseolus vulgaris* L., on which nymphs survive better under circumstances without prey associated with plants. Even with suitable prey on less suitable host plants, greater amounts of oviposition were found for green bean that were prey-free. When tested for ovipositional preference, among tomato, strawberry, bell pepper, eggplant and the wild South-American poppy *Bidens pilosa* L., *O. insidiosus* preferred *Bidens pilosa* (Pascua et al. 2019). *Orius sauteri* (Poppius) was found to most suitably develop on a diet of thrips, but also completed development on two-spotted spider mite and four aphid species (Wang et al. 2014). *Orius sauteri* was shown to prefer thrips over two-spotted spider mite (Xu and Enkegaard 2009). *Orius minutus* was shown to have significantly higher fecundity on Savalan cultivar of potato than on Agria, Morene, Kondor and Diamant potato cultivars (Fathi 2014). *Orius laevigatus* preferred prey, western flower thrips larvae, compared to pollen (Hulshof and Jurchenko 2000).

Orius insidiosus was shown to have faster juvenile development on diet including *Ephestia kuehniella* (Zeller) or *Tyrophagus putrescentiae* Schr prey compared to *Ricinus* sp. pollen diets (Bernardo et al. 2017). Also, a mixture of pollen collected from honeybees that visited a variety of plants was not shown to improve fecundity of *O. insidiosus* compared to prey-only diets (Calixto et al. 2013). In contrast, pollen was found in a different study to increase female longevity, decrease juvenile development time, and yield larger females of *O. insidiosus* compared to prey-only diet of thrips (Wong and Frank 2013). Richards and Schmidt (1996) found for *O. insidiosus* the addition of pollen to a diet of *E. kuehniella* eggs and green bean slightly increased egg production. In my experiments there was a non-significant trend for *O. insidiosus* to choose dill over fennel pollen.

Orius spp. were found to be significantly higher in abundance in faba bean with flowering weeds than weed-free plots (Atakan 2010). Hedges and grasslands nearby agricultural ecosystems were shown to increase abundance of hoverfly predators (Alignier et al. 2014). In a pea field, hoverfly larvae were more abundant when adjacent to wildflower strips (Hatt et al. 2017). Higher parasitism rates of aphids in wheat and barley crops were found adjacent to mustard *Synapis alba* L. flowering cover crops (Damien et al. 2017). Dill was found to not increase pest *Delia radicum* L. cabbage root fly numbers in cabbage, and in one of three study years less pupae were found in the intercropped plot suggesting biological control improvements from either dill or buckwheat or both (Nilsson et al. 2012).

In my experiments there was a significant trend for *O. insidiosus* individuals to choose dill pollen over a no-pollen treatment. No significant trend was found for fennel pollen compared to a no-pollen treatment (Fig. 1). Greater abundance of Orius species in relation to prey availability was noted with sunn hemp intercrop compared to monocrop corn treatment (Manandhar and Wright 2015). *Anthocoris nemoralis* Fabricius was found to have a strong association with elevated levels of biological control in olive orchards and its abundance was influenced by natural habitat nearby (Paredes et al. 2019).

In my experiments with adult insidious flower bugs, there was a preference for dill when compared to a no-pollen treatment. However, there was no statistical preference for dill when presented alongside fennel or coriander, though there was a slight trend for dill compared to fennel.

Therefore, the recommendation would be to plant the preferred dill with peppers in order to increase insidious flower bug populations.

Table 1. Six treatments of pollen preference trials and number of anthocorid adults tested for each.

Number of anthocorids tested	Pollen Type(s)
35 adults	Coriander vs. No treatment
35 adults	Dill vs. No treatment
35 adults	Fennel vs. No treatment
35 adults	Coriander vs. Dill
35 adults	Coriander vs. Fennel
35 adults	Dill vs. Fennel

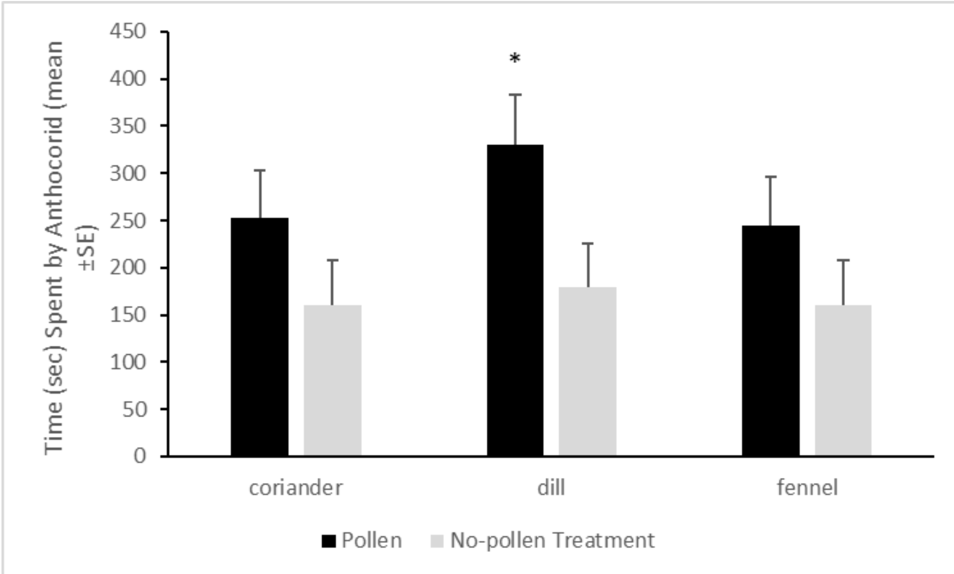


Figure 1. The number of *O. insidiosus* adult time spent in pollen vs. no pollen choice tests. Columns with an asterisk show a significant effect.

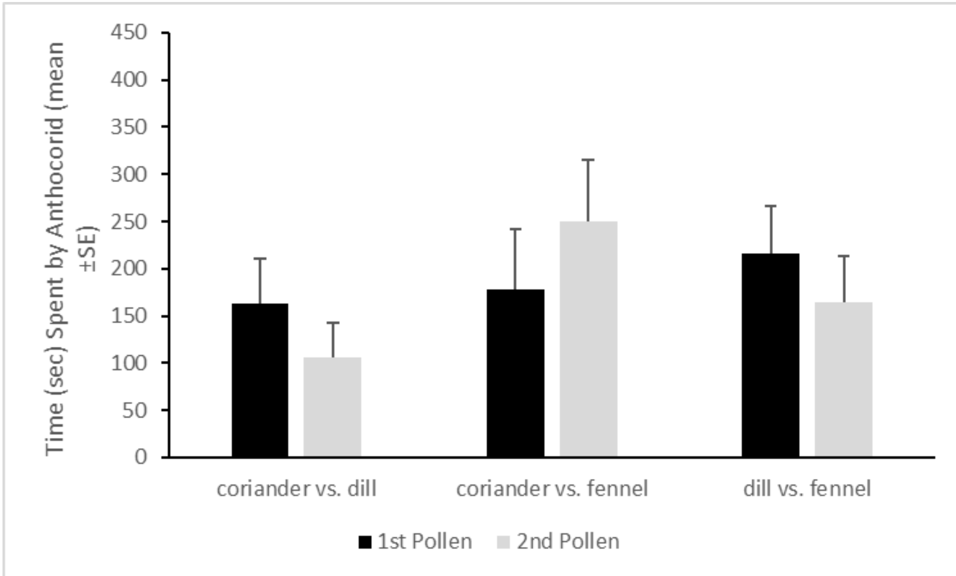


Figure 2. The number of *O. insidiosus* adult time spent in pollen choice tests. Columns with an asterisk show a significant effect.

CHAPTER 4: CONCLUSIONS

The goal of this work was to evaluate intercrop species composition of peppers to make recommendations for improved biological control. The intercrop species investigated were coriander, *Coriandrum sativum* L., dill, *Anethum graveolens* L., and fennel, *Foeniculum vulgare* Miller. The objective was to determine whether one of these intercrops increased abundance of anthocorids or increased predation by anthocorids of European corn borer egg masses more than the other intercrops and whether the effect could be enhanced by increased proximity of the intercrop to the peppers.

Field results were significant for inter-row abundance of anthocorids, and there was a trend in 2015 intra-row and 2016 inter-row treatments for the non-intercropped treatment to have more crop damage. In 2016 inter-row experiment, there was higher abundance of anthocorids in dill compared to the control treatment.

The molecular gut contents analyses revealed that anthocorids forage in both immediately adjacent intercrops as well as other intercrops before entering peppers. Coriander PCR from fields one and two revealed anthocorids feeding on coriander pollen 30% of the time in field 1, with some results from fennel and mixed treatments as well in fields 1 and 2 respectively. Dill PCR revealed positive results from coriander, dill, fennel, control and mixed treatments when considering both fields. Fennel PCR found positive gut contents from mixed and dill treatments for field 1 and 2 respectively.

The significant result from the pollen preference trials showed that *Orius insidiosus* Say chose coriander over fennel more often. There was no significant trend for coriander compared to dill nor coriander versus no-pollen treatment. The slight trends were for dill preference over coriander and no-pollen treatment over coriander.

These findings support the hypothesis that *O. insidiosus* forages and is attracted to flowers more broadly than the 3 – 4.6 m spacing used in this study. Future experiments should increase the distance between replicates and trials to not overlap results from one flower species with another in the same experiment. Mark, release, recapture experiments should also be done for *O. insidiosus* to determine typical distance traveled in intercropped pepper fields.

Orius insidiosus (Say) was shown to be a significant predator of immature *Drosophila suzukii* (Matsumura), reducing populations by 50% where this predator was included in strawberry and blueberry (Renkema and Cuthbertson 2018). *Frankliniella bispinosa* (Morgan) populations were shown to decrease with increases in *Orius insidiosus* and *Orius pumilio* (Champion) populations in bell pepper (Tyler-Julian et al. 2014). Chilli thrips *Scirtothrips dorsalis* Hood were controlled by *O. insidiosus* at 20 predators per infested pepper plant to prevent damage at less than or equal to 1% compared to >40% damage in control plants (Doğramaci et al. 2011). *Orius laevigatus* (Fieber) at 2 adults per meter squared in sweet pepper was shown to control western flower thrips *Frankliniella occidentalis* (Pergande) as well as 6 individuals per square meter (Weintraub et al. 2011). *Orius laevigatus* was shown to induce plant defenses that repel thrips *Frankliniella occidentalis* (Bouagga et al. 2018b). Spiders can reduce *Orius insidiosus* abundance by causing emigration from banker plants (Wong and Frank 2012). Fertility of *Orius majusculus* (Reuter) was increased by the addition of prey *Ephestia kuehniella* Zeller eggs to green bean *Phaseolus vulgaris* L. and alyssum *Lobularia* *maritima* L. plants (Pumariño and Alomar 2012). Abundance of *O. insidiosus* increased compared to unfed controls with diets of pollen, *E. kuehniella* eggs or a combination of both (Labbé et al. 2018).

Orius insidiosus was attracted to a *Halyomorpha halys* (Stål) brown marmorated stink bug-associated volatile (Fraga et al. 2017). Glandular trichomes may impede movement and predation by *O. insidiosus* (Nemec et al. 2016). *Orius insidiosus* showed high levels of preadaptation to consume novel aphid prey *Melanaphis sacchari* (Zehntner) compared to *Schizaphis graminum* Rondani (Colares et al. 2015).

In greenhouse cage experiments ornamental pepper cultivar Purple Flash showed the greatest population growth of *Orius insidiosus* among marigold, castor bean, ornamental pepper cultivar Black Pearl, gerbera daisy, feverfew and sunflower (Waite et al. 2014).

Blue sticky traps may be beneficial to use in future experiments as they were shown to capture high amounts of *Orius* adults without large nontarget capture (Furihata et al. 2019). *Orius insidiosus* adults spent the majority of their time in flowers in pepper fields (Hansen et al. 2003). When alternative food of pollen or aphids are present, *O. insidiosus* has reduced biological control of European corn borer eggs (Musser and Shelton 2003).

Commercial stock populations of *Orius majusculus* (Reuter) were shown to be fit in terms of predation rate, starvation tolerance, body size, locomotor activity and heat tolerance and were suggested not to have severe deleterious effects of inbreeding or bottlenecks through hybrid crossing (Rasmussen et al. 2018). *Orius insidiosus* commercial adults were shown to have a similar olfactory response to thrips-infested plants as wild individuals, however *Orius laevigatus* individuals made a choice less often and did not choose prey-infested plants as often either (Carvalho et al. 2011).

Orius majusculus Reuter did not show any difference in dispersal based on weed density in maize (Madeira and Pons 2015). The soybean aphid *Aphis glycines* Matsumura was shown to reach outbreak levels when *O. insidiosus* was not well-established in soybean fields in one year whereas *A. glycines* was at low levels when *O. insidiosus* populations were well-established in two years (Yoo and O'Neil 2009).

No adverse effects to survivorship, nymphal development time, adult weight, fecundity, preoviposition or postoviposition periods or adult longevity were detected in *Orius insidiosus* that had fed on *Thrips tabaci* Lindeman prey that had consumed *Bacillus thuringiensis* Berliner (Bt) cotton variety Bollgard-II plants (Kumar et al. 2014). Similarly, no negative effects on survival, development, fecundity, adult mass or fertility were found on exposure through prey to Cry proteins from Bt that would be found in Bt corn or Bt cotton using resistant prey (Tian et al. 2014).

Even though aphids were present throughout the season, pollen was consumed in over 90% of all individuals of the multicolored Asian lady beetle *Harmonia axyridis* (Pallas) collected from the field (Berkvens et al. 2010). Pollen was also an important diet component in *Coccinella septempunctata* L. (Triltsch 1999). Pollen similarly was shown to be an important diet component in *O. insidiosus* (Corey et al. 1998).

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