EXPLORING CONSUMPTION BY TWO GENERALIST PREDATORS IN POTATOES
USING MOLECULAR GUT CONTENT ANALYSIS AND BEHAVIORAL STUDIES

By
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To the Faculty of Washington State University

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EXPLORING CONSUMPTION BY TWO GENERALIST PREDATORS IN POTATOES
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Abstract

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Generalist insect predators may search out prey species that are nutritious even when they are uncommon in the environment, such that rates of predation will not necessarily track prey abundance. This study examined patterns of predation and behavior for two species of insect predators, *Nabis alternatus* Parshley and *Geocoris bullatus* Stål, in laboratory, greenhouse and field experiments. The laboratory experiment detected the presence of DNA from *Leptinotarsa decemlineata* (Say) (Colorado potato beetle) and *Myzus persicae* (Sulzer) (green peach aphid) in both predator species at 0, 1, 2, 4, 6, 8, 16, and 24 hours after feeding, and *Nabis alternatus* was also tested using the same method for consumption of *G. bullatus* nymphs. Prey DNA was present up to 24 hours after consumption, and the digestion rate differed by predator species and prey item. An open field experiment manipulated densities of green peach aphid and Colorado potato beetle using pest specific insecticides to determine if changes in prey community structure were reflecting by changing predator feeding patterns. Both predator species consumed Colorado potato beetles and green peach aphids in this study, and *N. alternatus* also consumed *G. bullatus*. However, these feeding relationships were not significantly altered by changes in aphid or potato beetle densities. This suggests either that the predators’ diets were relatively unaffected by prey availability, or that predators mixed their diets by moving freely among our plots. A third study
in the greenhouse manipulated green peach aphid densities (10, 25, or 50 aphids) and predator community structure (8 *G. bullatus*, 8 *N. alternatus*, or 4 of each species) to determine if aphid consumption changed with pest density or predator species composition. Consumption increased as a higher number of green peach aphids were available to predators. Foraging activity was highest when single predator species were present, and it was reduced when both predator species were present. Altogether, this series of studies suggests that these generalist predators have remarkably rigid feeding patterns, and that predation of one predator species by another can be reduced through predator-avoidance behavior.
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Dedication

This dissertation is dedicated to my parents and husband for all of their support and encouragement throughout my two graduate degrees in entomology.
INTRODUCTION: Spud Web: Species Interactions and Biodiversity in Potatoes

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ABSTRACT
Agroecologists often see greater biodiversity as the key to reducing pest problems on farms. Others have suggested, however, that increasing species number only increases the risk of negative interactions among species, such as predation of one predator by another, which could disrupt biological control. Such multi-species interactions have long been a topic of interest among entomologists working in potato crops, and here we review the basic ecological knowledge that has come from work in this important model cropping system. We examine the effects of increasing biodiversity on potato-insect species interactions at multiple trophic levels: among plants, herbivores, and natural enemies. Increasing plant diversity at both local and landscape scales can help build predator populations and increase suppression of potato pests. However, planting flowering plants near potato crops sometimes provides supplemental food for pests, making pest problems worse. Effects of herbivore diversity are equally complex. Feeding by early-season herbivores sometimes makes plants resistant to late-arriving herbivores, harming those pests most active later in the growing season. On the other hand, multiple herbivore species
can “distract” predators from feeding on particular target pests, providing less-preferred herbivore species with protection from predation. Predator diversity likewise exerts varying effects. In most cases predator species appear to complement one another by foraging in different locations in the crop or by attacking different pest stages, thus improving biological control. In some cases, however, predator species feed on one another, disrupting biological control. In summary, although increasing biodiversity within potato crops sometimes worsens pest problems, it most often makes pest outbreaks less likely. Thus, work in potatoes largely supports the view of early agroecologists that increasing biodiversity restores natural balance among plants, herbivores, and natural enemies.

Key words: predation; competition; intraguild predation; facilitation; complementarity

Introduction

Much early work in biological control focused on interactions between particular natural enemy species and their pestiferous prey (DeBach and Rosen 1991). This approach likely reflected the many successes of classical biological control, where natural enemies of an introduced pest were released into the invasive range and dramatically reduced pest densities. Classical biological control agents were sought that had a high degree of prey specificity and a reproductive rate as high as the target pest (DeBach and Rosen 1991). Such highly-specialized natural enemies are tightly linked to particular herbivores, and have the clear ability to exert density-dependent regulation of pests (Hawkins et al. 1997, 1999). However, recent years have seen growing interest in the community ecology of biological control where interactions among more than two
species are considered (e.g., Vandermeer 1995, Matson et al. 1997). First, classical biological introductions have become more difficult and expensive because of growing societal concerns about potential unintended, negative impacts of biocontrol agents on native species (Howarth 1991). Furthermore, this approach is only possible when pests are non-native exotics. Instead, there is increasing interest in the conservation of native natural enemies, including generalist predators, which often interact with many other species in addition to any single target pest (Landis et al. 2000, Symondson et al. 2002). Second, consumer concerns about possible negative human- and environmental-health effects of pesticides have increased adoption of organic agriculture and other pesticide-reduction schemes (Crowder et al. 2010). With a reduction in broad-spectrum pesticide applications by growers, the abundance and diversity of natural enemies generally increases (Bengtsson et al. 2005, Hole et al. 2005, Straub et al. 2008). This inevitably increases the complexity of agricultural food webs (e.g., Tylianakis et al. 2007).

The relationship between biodiversity and biocontrol has long been a topic of interest to basic and applied ecologists. Early agroecologists thought that the relative ecological simplicity of agricultural monocultures rendered them more prone to herbivore outbreaks than natural systems (Pimentel 1961, Root 1973, van Emden & Williams 1974). If so, then the restoration of more natural levels of biodiversity would improve natural pest control (Straub et al. 2008). This viewpoint is consistent with the emerging sub-discipline of “biodiversity-ecosystem function” research, which suggests that ecosystem health is maximized only within species-rich communities (Ives et al. 2005, Hooper et al. 2006). Other viewpoints also exist. Simplest is the “green-world hypothesis” of Hairston et al. (1960), who proposed that the world’s ecosystems break down into three simple trophic levels: plants, herbivores, and predators. Predators generally regulate herbivore densities, so that plants grow and proliferate largely unscathed.
Biodiversity is never explicitly discussed in the 1960 paper, but it can be inferred that predators (and other natural enemies) form a cohesive third trophic level that acts consistently regardless of the number of species present (Hairston and Hairston 1993, 1997). In contrast, the “trophic-level omnivory hypothesis” suggests that complex feeding relationships among species blur the formation of distinct trophic levels; without trophic levels, cascading predator effects on herbivores and then plants cannot occur (Strong 1992, Polis and Strong 1996). For example, ecological communities typically include many species of generalist predators, which often feed on plants, detritivores and other predators in addition to herbivores (Polis 1991). Data from particular studies in agricultural crops variously supports each of these hypotheses (Straub et al. 2008).

Potato crops often contain diverse communities of herbivores and their natural enemies (Walsh and Riley 1868, Hough-Goldstein et al. 1993, Hilbeck and Kennedy 1996, Hilbeck et al. 1997, Koss et al. 2005), providing an ideal model system to study the ecological issues discussed above. Traditionally, potatoes have been heavily treated with insecticides, due to the high value of the crop and the severe damage caused by some insect and other pests (Hare 1990). However, recent years have seen growing pressure to reduce insecticide use on this food crop, leading to a growing organic sector and the adoption of pesticide-reduction schemes in conventionally-managed fields (Koss et al. 2005, Werling and Gratton 2010). Here, we explore species interactions among herbivores and natural enemies occurring in potato crops, and how these interactions are influenced by increasing biodiversity. We separately consider how biodiversity effects might operate at the plant, herbivore and predator trophic levels.

**Plant Biodiversity**
Root (1973) presented two hypotheses for how increasing plant diversity in agricultural fields might decrease pest problems. First, specialist herbivores might have a more difficult time finding their host plant against a background of other plant species. Second, diverse plantings might support a more diverse community of non-pest prey and other foods, increasing the abundance and diversity of natural enemies. Indeed, diversified plantings often house fewer pests than do agricultural monocultures (Russell 1989, Andow 1991). However, differentiating between the two above-described hypotheses as the root cause of this diversity effect has been problematic (Bommarco and Banks 2003). Increasingly, agroecologists have examined crop diversification plans as a means to conserve natural enemies (Landis et al. 2000). Plant diversification can be accomplished either at local scales, with resource-providing plants installed at field edges or within the crop (Landis et al. 2000), or at the scale of landscapes, where regions including more crop species and more non-crop habitats might provide diverse resources for highly-mobile natural enemies (Tscharntke et al. 2005).

**In-Field Plant Diversity.** Perhaps the most common approach to diversifying the in-field habitat of potato crops has been the application of straw mulch. Straw mulch can benefit predators by establishing more-benign environmental conditions relative to bare ground fields, by providing protection from the predators’ own natural enemies (including intraguild predators), and/or by providing food for detritivores that act as supplemental, non-pest prey (Settle et al. 1996, Langellotto and Denno 2004, Halaj and Wise 2002). Brust (1994) reported that potato plantings receiving straw mulch housed larger populations of generalist predators, and also lower densities of Colorado potato beetles. With fewer potato beetles, potato plants suffered less feeding damage. He also found no difference in potato beetle movement between mulched and
un-mulched plants, suggesting that the benefits of mulch were not due to potato beetles avoiding the colonization of mulched potatoes (Brust 1994). Several other studies confirmed that potato beetle densities decrease in straw-mulched plots (Zehnder and Hough-Goldstein 1990, Stoner 1993, Johnson et al. 2004). Often, the application of straw mulch has been associated with higher potato yields (Zehnder and Hough-Goldstein 1990, Stoner 1993), although it is not always clear whether this effect is due to reduced potato beetle densities, better moisture retention in the soil, or both (Stoner et al. 1996). Nonetheless, increased structural complexity is not universally beneficial. Szendrei and Weber (2009) found that potato crops planted into rye stubble attracted higher densities of predatory *C. maculata* lady beetles but lower densities of the predatory carabid beetle *Lebia grandis*. Mulching decreased overall beetle suppression by predators because the carabid was the most voracious predator of potato beetles (Szendrei and Weber 2009). Indeed, molecular gut-content analysis revealed that predators were more likely to have fed on potato beetles in un-mulched than in mulched plots (Szendrei et al. 2010).

Predators of aphid and potato beetle in potatoes, such as the lady beetle *Coleomegilla maculata*, are often highly mobile, suggesting they may be affected by the mixture of potato and other plant habitats within and around farms. For example, corn crops seem to be most attractive to predatory lady beetles, such that lady beetle densities in potato fields planted near corn can be too low to exert significant impacts on potato pests (Groden et al. 1990, Nault and Kennedy 2000). However, if the attractiveness of potato crops could be increased by, for example, planting flowering plants at field edges or between crop rows, potatoes might be more attractive to lady beetles and other natural enemies (e.g., Patt et al. 1997). This could increase biological control impacts in potatoes. For example, Groden et al. (1990) suggested timed cutting of adjacent alfalfa crops as a strategy to encourage *C. maculata* to move from alfalfa to potatoes as
potato-pest densities grow. Idoine and Ferro (1990) indicated that adult females of the Colorado potato beetle egg parasitoid *Edovum putterleri* benefited from feeding on aphid honeydew, suggesting that providing a sugar source for the wasps could benefit the control of potato beetles. Such approaches to predator conservation are fraught with risk, however. For example, Baggen and Gurr (1998) found that planting flowering plants near potatoes provided food for adults of the parasitoid *Copidosoma koehleri*, an egg parasitoid of the pestiferous potato moth *Phthorimaea operculella*. These “floral resources” extended parasitoid longevity and increased fecundity, both of which would benefit potato moth biocontrol. However, in field trials, providing flowers actually increased pest densities in potatoes and resulting crop damage; this occurred because potato moths also benefitted from the use of floral resources (Baggen and Gurr 1998). Subsequent work showed that flower species could be selected which benefitted only the parasitoid and not the moth (Baggen et al. 1999), such that the unintended benefits to the pest could be eliminated and the parasitoid selectively promoted (Baggen et al. 1999).

**Landscape-Scale Plant Diversity.** Plant diversity can also be beneficial at scales larger than single potato fields. Werling and Gratton (2010) examined how landscape structure and diversity impacted predation of green peach aphid (*Myzus persicae*) and Colorado potato beetle (*Leptinotarsa decemlineata*) pests, and found that control of the two pests was impacted at different spatial scales. Green peach aphid predation was highest in field margins adjacent to potato fields that were imbedded within landscapes containing diverse non-crop habitats within 1.5 km; however, this effect of landscape diversity did not alter predation of green peach aphids within the potato fields themselves (Werling and Gratton 2010). In contrast, predation of Colorado potato beetle eggs was unaffected by landscape diversity at larger scales, but instead increased as the ratio of field margin to crop increased. Thus, potato beetle predation was
greatest in smaller potato fields where relatively diverse edge habitats were always close by (Werling and Gratton 2010). Ground beetle predation of weed seeds was greater in field margins compared to adjacent potato crops (Gaines and Gratton 2010), again suggesting that greater plant diversity in field margins increased predation relative to what was seen in the plant-species-poor potato crop. In conclusion, the presence and size of adjacent non-crop habitat can have important implications for predation of herbivores in potato fields, but there is limited evidence for the role of landscape complexity at larger scales.

Herbivore Biodiversity

While pest management decisions in potatoes may often focus on just one or two pests perceived to be of the greatest economic importance, these crops typically house a diverse community of many herbivore species (Lynch et al. 2006). Multiple species of herbivores have the potential to indirectly impact one another’s densities, with these interactions generally transmitted indirectly by the host plant (Karban and Baldwin 1997, Lill and Marquis 2001, Ohgushi 2005, Rodriguez-Saona and Thaler 2005). These multi-herbivore effects can be either harmful or beneficial to particular key potato pests. Likewise, the presence of multiple herbivore species can either heighten or weaken the impact of natural enemies on a particular species of pest (e.g., Eubanks and Denno 2000). Below, we review research literature examining how herbivore diversity impacts interactions among herbivore species, and among predators and their herbivore prey, on potatoes.

Herbivore-Herbivore Interactions Mediated by Plants. Plant-mediated indirect interactions among herbivores generally fall into three categories: (1) those due to resource
depletion, where one herbivore species consumes plant resources that are then unavailable to a second herbivore species, (2) those due to induced plant defenses, where feeding by one herbivore species triggers the plant to deploy defensive strategies that harm a second, often later-arriving, herbivore species, and (3) those due to alterations in plant chemistry following feeding by one herbivore species that render a plant more susceptible and/or attractive to a second herbivore species. Interactions of all types have been described in potato crops, or are likely to occur.

The Colorado potato beetle is often on the losing end of plant-mediated competition with other herbivore species (e.g., Tomlin and Sears 1992a,b; Wise 2002, Lynch et al. 2006, Kaplan et al. 2007). This might be because the Colorado potato beetle generally does its greatest damage relatively late in the growing season, such that their feeding is influenced by changes in plant chemistry/quality that have been induced by earlier-arriving herbivores. For example, early-season feeding by both flea beetles (Wise and Weinberg 2002) and potato leafhoppers (Lynch et al. 2007) causes female potato beetles to avoid ovipositing on affected potato plants, and slows development of any larvae that do hatch. Slower larval development not only makes it more difficult for Colorado potato beetles to complete multiple generations each year, but also heightens the risk of beetle larvae falling victim to predators; predation risk is heightened because the beetles are forced to spend more time in vulnerable smaller stages (Kaplan et al. 2007).

Although the precise mechanism through which earlier feeding by flea beetles or leafhoppers harms potato beetles has not been demonstrated, potato plants defend themselves through some combination of altered nutritional quality, allelochemical defenses, and morphological alterations (Tomlin and Sears 1992 a,b; Hlywka et al. 1994, Bolter and Jongsma 1995, Pelletier et al. 1999).
It is likely that one or more of these defenses are involved in induced resistance to Colorado potato beetle. The indirect effect of leafhoppers on potato beetles depends on the density of the leafhoppers, unlike chemical defenses that can be triggered to high levels by relatively little herbivore feeding (Heil and Kost 2006), and leafhoppers are known to alter the amino acid profile of potato foliage (Tomlin and Sears 1992a). This implies that leafhoppers harm potato beetles, at least in part, by reducing the nutritional value of potato plants (Lynch et al. 2006).

It is worth noting that previous feeding by other herbivore species does not invariably deter subsequent potato beetle attack. For example, in one study potato plants that had cabbage looper regurgitant applied to wounds in the foliage, simulating caterpillar attack, attracted more potato beetle adults than did un-damaged plants (Landolt et al. 1999). Similarly, potato plants attacked by beet armyworm larvae were more attractive to colonizing potato beetles (Bolter et al. 1997). In the Bolter et al. (1997) study, previous feeding by Colorado potato beetles also heightened attractiveness of damaged plants to later-arriving potato beetles, suggesting that plant damage by any chewing herbivore rendered potato plants more attractive.

**Herbivore-Herbivore Interactions Mediated by Shared Predators.** Herbivore species can also indirectly impact one another by changing the behavior of shared predators. For example, “apparent competition” occurs when the presence of one herbivore species draws in larger numbers of natural enemies than might otherwise be found. When these enemies switch to feeding also on a second herbivore species, the second herbivore species is harmed (Holt 1977, Harmon and Andow 2004). Thus, one herbivore species harms another by increasing the second species’ risk of predation. Apparent competition among potato herbivores has not been directly demonstrated, but there is good circumstantial evidence to suggest it might occur. For example, green peach aphid and other aphid pests of potato can attract large numbers of aphid-associated
lady beetles and other generalist predators (Koss et al. 2005), which likely opportunistically feed on potato beetle eggs and other vulnerable herbivore species (e.g., Chang and Snyder 2004). Similarly, potato plants damaged by Colorado potato beetles are more attractive to the predatory pentatomid *Perillus bioculatus* than are un-damaged plants (Weissbecker et al. 1999, 2000); presumably, once these generalist predators are drawn to a crop they would feed also on prey other than potato beetles.

However, the presence of one herbivore species will not necessarily lead to higher predator attack rates on a second, co-occurring herbivore species. In fact, the presence of a preferred prey might draw predator attacks away from a second, unpalatable or less-preferred herbivore species (Harmon and Andow 2004). For example, in laboratory arenas *Coleomegilla maculata* lady beetles eat fewer Colorado potato beetle eggs when aphids are available as alternative prey (Groden et al. 1990, Hazzard and Ferro 1991, Mallampalli et al. 2005). Similarly, in field cages where predators cannot aggregate at aphid infestations, the presence of green peach aphids protects Colorado potato beetles from attack by a diverse guild of spider and predatory bug generalist predators (Koss and Snyder 2005). Disruption of potato beetle predation in the presence of aphids apparently occurs because aphids are more attractive prey for most predator species. Consistent with this interpretation, the presence of Colorado potato beetles as prey has no impact on these same predators’ likelihood of attacking aphids (Koss et al. 2004).

**Natural Enemy Biodiversity**

Potato crops often house a remarkably high diversity of predator, pathogen and parasitoid natural enemies (Walsh and Riley 1868, Hough-Goldstein et al. 1993, Alyokhin and Sewell
For example, in North America, Colorado potato beetles are attacked by a diverse guild of generalist egg and larval predators, egg and larval parasitoids, and entomopathogenic nematodes and fungi (Lopez et al. 1993, Hilbeck and Kennedy 1996, Berry et al. 1997, Koss et al. 2005, Crowder et al. 2010). Indeed, both observational studies and predator surveys using molecular gut content analysis have found a vast array of predators feeding on Colorado potato beetles under entirely natural, open-field conditions (Chang and Snyder 2004, Greenstone et al. 2010, Szendrei et al. 2010).

As we have seen for plants and herbivores, increasing biodiversity among natural enemies can have either positive or negative consequences for particular potato pests. On the one hand, as more enemy species are added to a community this increases the risk that one enemy will feed on another. Such “intraguild” predation has the potential to greatly disrupt biological control (Polis et al. 1989, Rosenheim et al. 1995, Ives et al. 2005; Fig. 1). On the other hand, combining natural enemies that fill different ecological niches can lead to complementary impacts on a pest species, where different enemy species eliminate spatial or temporal refuges from predation that the pest might otherwise enjoy (Wilby and Thomas 2002, Casula et al. 2006, Straub et al. 2008; Fig. 2-3). Furthermore, natural enemies sometimes facilitate one another’s prey capture. For example, a predator may chase a prey species from one habitat to another second predator species located somewhere else in the environment (e.g., Losey and Denno 1998). In these cases, pest control is strongest where several natural enemy species co-occur. We next discuss examples of negative, and then positive, predator-predator interactions that have been found to impact biological control in potatoes.

**Negative Predator-Predator Interactions.** Natural enemies often feed upon one another, and this has the potential to greatly limit biological control (Rosenheim et al. 1995). Some
evidence for this comes from potato crops. For example, Mallampali et al. (2002) showed that the spined soldier bug (*Podisus maculiventris*) fed heavily on larvae of the twelve-spotted lady beetle (*Coleomegilla maculata*), which disrupted predation of Colorado potato beetle eggs by that lady beetle. Similarly, in laboratory feeding trials the predatory bug *Anthocoris nemorum* was as likely to feed on green peach aphids parasitized by the wasp *Aphidius colemani* as on unparasitized aphids (Meyling et al. 2004), and *Beauveria bassiana* fungi pathogenic to green peach aphids and Colorado potato beetles also attacked *C. maculata* lady beetles (Todorova et al. 2000). If these interactions also occur in the field, they could disrupt biological control.

One of the more detailed series of studies on intraguild predation among aphid predators examined interactions among predatory midges (*Aphidoletes aphidomyza*), lacewings (*Chrysoperla rufilabris*), and lady beetles (*C. maculate*) attacking potato aphids (*Macrosiphum euphorbiae*) (Fig. 1). The midges are highly susceptible to intraguild predation by the lacewing and lady beetle; lacewing and lady beetle larvae also attack one another (Lucas et al. 1998). Apparently in response to its high risk of falling victim to intraguild predation, the midge has developed several strategies to reduce this danger. First, midge larvae will “hide” at the center of aphid aggregations, so that aphids at the colony periphery will absorb most predator attacks (Lucas and Brodeur 2001). Second, midge eggs are deposited on plants with high trichome density, which makes them relatively immune from predation by *C. maculata* predators (Lucas and Brodeur 1999).

The parasitoid wasp *Aphidius nigripes* attacks green peach aphids (*Myzus persicae*) in potato crops in North America, where it faces attack by hyperparasitoids (Brodeur and McNeil 1992). As the parasitoid larva nears the end of its development, it somehow alters the host aphid’s behavior, causing the doomed aphid to walk to the top of the potato plant before being killed by
the parasitoid. The wasp then pupates beneath its former host’s exoskeleton. Brodeur and McNeil (1992) found that parasitoid pupae located at the tips of plants were unlikely to fall victim to hyperparasitoids. In alfalfa crops, this placement of parasitoid pupae was found to make the wasps less accessible for ground-foraging, predatory carabid beetles that occasionally climb onto plants to forage (Snyder and Ives 2001). Altogether, this suggests that the selective pressures exerted by intraguild predators have led the parasitoid to develop its ability to change host behavior, in order to be delivered to a pupation site largely out of reach of the parasitoid’s own natural enemies (Brodeur and McNeil 1992). Similarly, the lady beetle *C. maculata* leaves aphid-infested potato plants to pupate elsewhere, apparently in order to avoid intraguild predation by the many predators that aphids attract (Lucas et al. 2000).

**Positive Predator-Predator Interactions.** There is growing evidence that species that occupy different niches consume more resources than any single species can consume (Hooper et al. 2006, Cardinale et al. 2006). The same may hold true when herbivorous agricultural pests are the “resource”, and predator, parasitoid and pathogen natural enemies are the “consumers” (Straub et al. 2008). After all, natural enemy species differ from one another in where they hunt in the environment, the time of day or year that they are active, and in the particular hunting style they use, such that pests are likely to face a broad-based attack only when several natural enemy species co-occur (Wilby and Thomas 2002, Snyder 2009). In this sense, natural enemy species are likely to complement one another, and some of the best evidence of this comes from work in potato crops.

Working with potato plants enclosed in large field cages, Straub and Snyder (2006, 2008) compared the effects of single natural enemy species on green peach aphid prey to those of diverse mixes of 3 or 4 enemy species (Fig. 2). The biocontrol agents considered in this work
were a diverse group composed of *Nabis* and *Geocoris* true bugs and parasitoid wasps. All experiments manipulated predators following a substitutive design, in which total predator density (or, in some experiments, predator biomass) was held constant across species richness levels. Such designs isolate the impacts of changes in species number by eliminating differences among treatments in species abundance (Loreau and Hector 2001). Initial experiments showed that the different predator species strongly differed in how effective they were at killing aphids, but that diverse mixes of predator species killed only slightly more aphids than did single enemy species (Straub and Snyder 2006). This suggested that predator species did not strongly complement one another. However, subsequent work painted a more complex picture. When predator diversity was manipulated simultaneously on both collard (*Brassica oleracea*) and potato plants colonized by the green peach aphids – in that experiment, plants of the two species were present in different cages – diverse predator communities killed more aphids than any single enemy species on plants of both species (Straub and Snyder 2008). Predators complemented one another quite strongly on collard plants, where diverse predator communities killed 200 more aphids than single species, but quite weakly on potatoes, where diverse predator communities killed just 6 more aphids than single predator species (Straub and Snyder 2008). Behavioral observations indicated that lady beetles foraged largely at the edges of leaves, while predatory bugs and parasitoids foraged at leaf centers (Fig. 2). Because collard leaves are larger, these space-use niche differences, and thus predator-predator complementarity, were greater on collards than potatoes (Straub and Snyder 2008).

Natural enemy facilitation occurs when the presence of one natural enemy species indirectly heightens prey capture by a second predator species. In one well-known example of this phenomenon, lady beetles foraging in alfalfa foliage cause aphids to drop to the ground; once on
the ground, aphids are readily eaten by ground beetles on the soil surface (Losey and Denno 1998). Something analogous has been reported for Colorado potato beetle prey, where predators facilitate prey infection by pathogens (Ramirez and Snyder 2009; Fig. 3). During their development, the beetles move from the foliage where they feed to the soil where they pupate, and as they do so they transition between two quite distinct communities of natural enemies (Fig. 3). Aboveground, the beetles are attacked by lady beetles, ground beetles, and Nabis true bugs. Belowground, they face infection by entomopathogenic nematodes (Heterorhabditis spp. and Steinernema spp.), and Beauveria bassiana fungi. Ramirez and Snyder (2009) manipulated species number among these predator and pathogen natural enemies, and then measured potato beetle survival from egg to adult in large field cages. Enemy species number was again manipulated following a substitutive design, so that natural enemy densities did not differ as species richness was changed. They found that potato beetle mortality increased as predator/pathogen biodiversity increased. This happened because predators and pathogens were increasingly likely to co-occur at higher species-richness levels, and predator-pathogen pairings were particularly lethal to potato beetles. The authors suggest that energetically costly anti-predator defenses of the potato beetle larvae, deployed to escape from predators early in potato beetle development, weakened the beetles’ later ability to fight off pathogen infection (Ramirez and Snyder 2009, Ramirez et al. 2010). Thus, exposure to predators indirectly weakened beetle immune function. A similar type of facilitation appears to occur even within entomopathogen communities, as exposure to entomopathogenic fungi increases potato beetle susceptibility to entomopathogenic nematodes (Jabbour et al. 2011).

Getting Even With Pests: Natural Balance and Biocontrol
The term “biodiversity” is often used as if it were synonymous with the number of species present, known as “species richness”. However, ecologists have long suspected that ecosystem function might also improve when species’ relative abundances are evenly matched, known as greater “evenness” (Hillebrand et al. 2008). Indeed, most common biodiversity indices include some measure of evenness, in addition to richness, in their calculation. It is thought that communities with just a few very common species, typical for example of highly-disturbed areas dominated by weedy and/or invasive species, are less healthy than communities where many species are similarly-common. Unfortunately, ecologists’ intuition has, until recently, been backed by relatively little empirical evidence that more-even communities are in fact more stable and productive than their uneven counterparts (Hillebrand et al. 2008).

Interestingly, some of the best evidence for the importance of greater evenness comes from potato crops. Crowder et al. (2010) examined how the transition from conventional farming practices, dominated by intense insecticide use, to organic farming with its greater reliance on natural processes, influenced richness and evenness among natural enemies of insects in Washington potato fields. As in the Ramirez and Snyder (2009) study discussed previously, natural enemies of insects in Washington potatoes are dominated by insect generalist predators and nematode and fungus pathogens. In a regional survey of predators and pathogens spanning a broad geographic area and several years, Crowder et al. (2010) found, surprisingly, no increase in predator/pathogen species richness in fields using organic practices. However, natural enemy evenness was significantly higher in organic than conventional potato fields. This meant that just one or two natural enemies were abundant in conventional fields, but many enemies were similarly common in organic fields. A meta-analysis of predator surveys for crops worldwide,
not just potatoes, showed that greater evenness among natural enemy communities was generally
greater in organic than conventional fields across these many crops and world regions. When the
authors constructed natural enemy communities that ranged from very even to very uneven, they
found that control of potato beetle pests was significantly stronger when predators and/or
pathogens were evenly abundant. Indeed, greater enemy evenness translated into significantly
larger potato plants, and thus presumably higher potato yields (although yields were not
measured). Thus, balance among natural enemies may be as important for strong pest
suppression as having a large number of natural enemy species (Crowder et al. 2010).

Summary & Future Directions

Agroecologists often suggest that greater biodiversity is the key to reducing pest problems.
Work in potato crops, however, paints a more nuanced picture. Increasing plant structural
diversity in the crop by mulching often appears to augment natural enemy populations and
increase enemy impacts on pests (Brust 1994). Likewise, more diverse landscapes can foster
gerater impacts of natural enemies (Werling and Gratton 2010), as can the addition of flowering
plants that provide resources to natural enemies (Baggen et al. 1999). However, poorly-chosen
flowering plants can feed potato pests in addition to their predators and parasitoids, worsening
pest problems (Baggen and Gurr 1998).

Increasing herbivore diversity has similarly complex effects. Herbivores such as flea beetles
and leafhoppers that attack potato plants early in the growing season trigger induced resistance in
potato plants that renders plants less attractive and/or nutritious to later-occurring herbivores like
Colorado potato beetles (e.g., Lynch et al. 2007). As a result, early-season herbivory can dampen
later herbivory by other species. However, the presence of highly-attractive prey like aphids might draw predator attacks away from less-desirable prey like potato beetles, such that aphids indirectly protect potato beetles (e.g., Koss and Snyder 2005). Thus, increasing herbivore diversity can either harm or benefit particular pest species.

The impacts of predator diversity are equally multifaceted. In a few cases, adding predators that mostly eat other predators disrupts overall biological control (e.g., Mallampalli et al. 2002; see also Fig. 1). More often, however, predators, parasitoids and pathogens complement one another, attacking pests in different habitats and/or during different life stages. As a result, biological control is most effective when several enemy species are present (Straub and Snyder 2008, Ramirez and Snyder 2009; Fig. 2-3). In summary, while greater diversity sometimes makes pest problems worse, it appears that increasing biodiversity and species evenness within potato crops is more likely to make pest problems less frequent.

While entomologists have made great progress in delineating interactions among potato arthropods, several topics, in our opinion, are still worthy of further exploration:

1. **The Role of Behavioral Interactions among Species.** Species interact not only by killing one another (or by outcompeting one another for food), but also by changing one another’s behavior (Lima and Dill 1990). For example, predators that chase herbivores from preferred feeding sites can protect plants from damage even when the pest is not killed (Schmitz et al. 1997, 2004, Werner and Peacor 2003, Preisser et al. 2005). Such behavior-mediated or “nontrophic” interactions have been shown to be important in other cropping systems, but relatively little attention has been paid to them in potatoes. Similarly, interference among predator species occurs not only when predators actually eat one another, but also when a predator flees a
particular habitat to avoid being eaten by another predator (Moran and Hurd 1994); whether a predator is truly killed or simply retreats, herbivore suppression can be equally disrupted (Schmitz 2008, Steffan and Snyder 2010). The obvious anti-predator behaviors of potato-pests such as the Colorado potato beetle (e.g., Ramirez et al. 2010), and existing good evidence for natural enemies in potato behaviorally avoiding one another (e.g., Lucas and Brodeur 2001), together suggest that nontrophic interactions might be an important way that species interact in potatoes.

2. Biodiversity Aspects Other Than Species Richness. Ecologists interested in biodiversity’s effects most often manipulate the number of species present, or species richness (Hooper et al. 2006, Cardinale et al. 2006). However, biodiversity includes a second component, species’ relative abundances or evenness (Hillebran et al. 2008). Communities with more-evenly-abundant species are often thought to be more stable than those with highly skewed relative abundances, although this assumption has rarely been tested. Recently, Crowder et al. (2010) found that communities of predator and pathogen natural enemies were more evenly-balanced in organic than conventionally-managed potato fields, and this greater enemy evenness translated into significantly improved biocontrol of Colorado potato beetles. More work is needed, however, to see if these benefits of greater evenness are widespread in potato and other crops. Furthermore, the impacts of in-field and landscape factors on promoting or reducing evenness warrants further consideration.

3. The Mechanisms of Induced Plant Defenses in Potatoes. Robert Denno and his colleagues have clearly shown that early-season feeding by leafhopper herbivores renders potato plants less-
susceptible to Colorado potato beetles later in the growing season (Lynch et al. 2006, Kaplan et al. 2007). Potatoes are known to have many possible defenses against these and other herbivores, but it is not always clear precisely which defenses are activated against which herbivore species, and whether chewing and sucking herbivores are battled in the same ways. Increasing knowledge about the molecular bases of defenses within closely related plant species, such as tomato, should facilitate our ability to learn about the specific operations of anti-herbivore defenses in potato (Mueller et al. 2005). Such information would increase our understanding of tradeoffs for the plant in defending against one herbivore species versus another (e.g., Kaplan et al. 2009).

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Figures

Fig. 1. Lucas et al. (1998) found that lacewing larvae fed not only on potato aphids, but also on larvae of predatory *Aphidoletes* midges. “Intraguild” predation of this type has the potential to disrupt aphid control, as lacewing larvae consume midge larvae that otherwise might have eaten many aphids. Arrows denote energy flow and so point from prey to predator; black arrows indicate predation of the herbivore by predators, while the red arrow indicates predation of one predator by another.

Fig. 2. In potato foliage, *Nabis alternatus* bugs and *Hippodamia convergens* beetles exert complementary impacts on *Myzus persicae* aphids (Straub and Snyder 2008). The lady beetles forage primarily at leaf edges, whereas the predatory bugs also forage at leaf centers. Because of these differences in space use, aphids face heavy predation pressure everywhere on the plant only when both predator species occur together.

Fig. 3. In Washington potato crops, predators and pathogens exert complementary impacts on Colorado potato beetles (Ramirez and Snyder 2009). Potato beetle eggs and larvae in the foliage are eaten by a diverse group of predatory *Hippodamia convergens* (Hc) and *Pterostichus melanarius* (Pmel) beetles, and *Nabis alternatus* (Nabis) bugs. Once the beetles enter the soil to pupate they are infected by entomopathogenic *Steinernema carpocapsae* (Scarp) and *Heterorhabditis marelatus* (Hmar) nematodes, and *Beauveria bassiana* (Bbass) fungi. Because of the spatiotemporal separation of predators and pathogens, potato beetles face attack throughout their life cycle only when both classes of natural enemy are present.
Fig. 2
Fig. 3

Aboveground predators

Herbivore Stage

Belowground pathogens
CHAPTER 1: Detecting *Leptinotarsa decemlineata* (Colorado potato beetle) in two generalist predators, *Geocoris bullatus* and *Nabis alternatus*, and intraguild predation of *Geocoris bullatus* in *Nabis alternatus* over time

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ABSTRACT

Molecular gut-content analysis is an established and increasingly important approach for tracking arthropod predation in the field. It is difficult to know what hemipteran generalist predators consume because there are no identifiable prey pieces in their gut. In order to relate the proportion of predator individuals found to contain pest DNA to the number of pests eaten over a given time period, it is necessary to determine how long pest DNA can be detected after predation occurred. This study examined digestion rates for two species of insect predators, adult *Nabis alternatus* and *Geocoris bullatus*, using Colorado potato beetle larvae, and *G. bullatus* nymphs as prey items. After feeding was observed on a prey item, predators were then killed 0, 1, 2, 4, 6, 8, 16, and 24 hours later by being placed in 95% ethanol and freezing immediately. For each predator-prey combination, 10 females and 10 males were used at each time point for a total of 80 females and 80 males. Prey-species-specific PCR primers were used to test for prey DNA in predators’ guts. Detection of Colorado potato beetle and *G. bullatus* nymphs occurred for both
G. bullatus and N. alternatus over time, and a small percentage were positive for prey items up to 24 hours later. This study helps confirm results of molecular gut-content analysis that is used for studying predator-prey interactions among these species in potato fields; successful detection of prey DNA indicates that predation was most likely to have occurred within the previous 24 hours.

Keywords: Generalist predators; Damsel bug; Big-eyed bug; Colorado potato beetle, DNA, molecular gut content analysis

1. Introduction

The available prey species, prey handling times, and changing field microhabitats could change the species of prey eaten and the predator’s ability to capture prey items over time, since generalist predators feed on multiple prey species (Symondson et al. 2002). Therefore, predators’ densities are not tied to those of any particular prey (Harmon and Andow 2004). Generalists can impact many different pest species because they switch feeding to different pest species as each pest becomes abundant (Symondson et al. 2002). For example, many predators also receive nutrients from plant tissues that include pollen, seeds, floral nectars, extrafloral nectars and honeydew (Lundgren 2009). Insect pests are often attacked by a variety of natural enemies, and some natural enemies will be more effective at pest suppression than other species (Loreau et al 2001, Straub and Snyder 2006). Determining which natural enemies to conserve is a difficult question to answer, when there are multiple promising candidates in an agroecosystem with many species (Cardinale et al 2003, Greenstone and Sunderland 1999, Greenstone et al 2010). The detection and quantification of predation patterns is multifaceted
and is important to the expansion and improvement of conservation biological control (Symondson et al. 2002). Direct observation of predation events, controlled manipulation of predator and prey densities to determine predation mechanisms, and the detection of prey markers in predators are methods used to determine the occurrence, frequency and impact of predators on prey populations (Weber and Lundgren 2009).

Molecular gut-content analysis is an established and increasingly important approach for tracking arthropod predation in the field (Hagler et al. 1992, Hagler & Naranjo 1994, Symondson 2002, Harwood et al. 2007, Juen & Traugott 2007, Kuusk et al. 2008, Lundgren et al. 2009). Predation is a difficult interspecific interaction to study in the field (Sunderland 1988, Greenstone and Morgan 1989), and gut analysis is considered the least disruptive approach for ecosystem processes (Greenstone et al. 2010). This is because gut-content analysis can be conducted using predators freshly collected from the field, where they have been allowed to forage entirely naturally (Greenstone et al. 2010). Monoclonal antibody based assays (Greenstone 1996, Harwood et al. 2004) and polymerase chain reaction (PCR) amplification of prey deoxyribonucleic acid (DNA) sequences (Symondson 2002, Sheppard & Harwood 2005, Gariepy et al. 2007) are the two most common molecular gut-content analysis methods (Greenstone et al. 2010). Remains detected in the gut of a particular predator may not show predation on a live prey item, but it may actually be scavenging on a dead animal or reflects secondary predation of one predator eating another that consumed a prey item (Harwood et al. 2001, Calder et al. 2005, Foltan et al. 2005, Juen & Traugott 2005, Sheppard et al. 2005). However, studies have repeatedly shown these biases to be extremely minor with nearly all “positive” tests reflecting predation of live prey (Harwood et al. 2001). Predator species with low digestion rates could house detectable prey DNA much longer than predators that digest
quickly, with slow-digesters giving the impression of being much more important predators of a
given prey even though the feeding histories were identical to those of fast digesters
(Greenstone et al. 2010). For this reason, it is important to compare among predator species the
length of time that prey DNA can be detected.

We are interested in determining digestion curves for the two main omnivorous generalist
predator species in potatoes, which are the big-eyed bug *Geocoris bullatus* Stål and the damsel
bug *Nabis alternatus* Parshley (Koss et al. 2004; Tamaki and Weeks 1972a, b). Both of these
predators consume the major pests species in Washington, which are *Myzus persicae* (Sulzer),
the green peach aphid, and *Leptinotarsa decemlineata* (Say), the Colorado potato beetle (Biever
and Chauvin 1992, Mowry 2001). Intraguild predation may also be important in this predator
community: in simple laboratory arenas, *Nabis alternatus* (damsel bug) and *Geocoris bullatus*
(big-eyed bug) will feed on each other depending on which predator is in the larger
developmental stage (Raymond 2000). We investigated the detection limits of target prey DNA
using *G. bullatus* and *N. alternatus* predators with *L. decemlineata* (Colorado potato beetles or
CPB) and .*G bullatus* nymphs (for *N. alternatus* only) as prey items. This study will help us
know possibly how long field collected predators would test positive for a prey item.

2. Materials and methods

2.1. Field collection of generalist predators

Predators, *Nabis alternatus* and *Geocoris bullatus*, were collected live using a D-vac
suction sampler (B&S Model 24 Ventura, CA). An alfalfa field at the WSU Research Farm near
Othello, WA was used to collect predators until the potato field had grown tall enough to support
an insect community. Mesh D-vac bags were brought back in coolers with ice packs from the field and were transferred to a cool incubator (10 °C) back at the laboratory. Predators were later sorted individually into 60 mm x 15 mm sterile polystyrene Petri dishes (Fisher Scientific) with damp cotton dental wicks and were fed *Acyrthosiphon pisum* (Harris), pea aphids, if they were not going to be used immediately for an experiment. Predators used in experiments were held without aphids for 48 hours before use.

2.2. Rearing potato plants, green peach aphids, and Colorado potato beetles

Potato plants for the greenhouse microcosm experiments were grown in the greenhouse at ambient day length and 22–25 °C. Whole certified organic Ranger russet potato seed (Basin Gold Cooperative, Warden, WA) were planted in decagonal planting containers (McConkey and Co; Sumner, WA) filled with Sunshine Professional growing mix potting soil (Sungro Horticulture; Seba Beach, AB Canada). The containers were put in the greenhouse and were watered. Containers were watered once a week, and potato plants were broken off of the main tuber around 10.16 cm tall and were transplanted into separate 15.24 cm square pots with WIL-GRO professional products pro balance slow release granular fertilizer (16-16-16-7S, Wilbur-Ellis Co.; Halsey, OR) sprinkled on top of the Sunshine Professional growing mix potting soil (Sungro Horticulture; Seba Beach, AB Canada). Plants were watered as needed (three times a week) before use in insect colonies, when they were about 15.24 cm tall or taller.

A pea aphid, *Acyrthosiphon pisum* (Harris), colony was originally started from pea aphids collected on alfalfa at the WSU Research Farm near Othello, WA, in 2011 was used to feed predators being held until use in feeding trials; pea aphids were reared on pea plants, *Pisum*
* sativum, in 60 x 60 x 60 cm fine mesh BugDorms (Megaview Science; Taiwan, China). This colony was maintained under the same conditions and in the same greenhouse as the potato plants but in a separate room as the potato plants. The same square pots, soil, and fertilizer as the potato plants were used to start pea plants from seeds (4 seeds in each pot). Potato and pea plants in the aphid colonies were watered 3 times a week.

Adult Colorado potato beetles (CPB) collected in eastern Washington potato fields produced the eggs and larvae used in our feeding trials. Beetles in the colony were fed live potato plants and were housed in the same greenhouse but different room as the aphid colony in 60 x 60 x 60 cm fine mesh BugDorms (Megaview Science; Taiwan, China). The greenhouse was maintained at 22–25°C with ambient day length. Eggs were harvested daily and placed in a refrigerator (5°C) to retard egg development.

2.3. Feeding trials

Adult *N. alternatus* and *G. bullatus* were the life stage of the predators used, and *G. bullatus* nymphs and Colorado potato beetle first instar larvae were the life stages of the prey used. After field collection and cool storage, *N. alternatus* and *G. bullatus* were sorted in the lab into clean individual petri dishes with moistened dental wicks, and they were fed pea aphids for about seven days to eliminate current prey DNA in their guts. After predators were fed pea aphids, they were moved to a clean petri dish, and a new damp cotton dental wick was added after each predator was sexed and dish labeled with sex. Then the predators were starved for 48 hours before starting the feeding trials. Next, each predator was fed one individual of focal prey (CPB or *G. bullatus*). Once a predator initiated feeding, the petri dish was marked
with a dot, and the predator was allowed to feed for at least 5-10 minutes. If a predator just probed a prey item or fed for less time, it was not used in the experiment.

After the predator fed on the focal prey item for 5 to 10 minutes, then the prey item was removed and was replaced with one pea aphid. Predators that did not feed on the pea aphid “chaser” were also not used in the experiment. The time was marked on the dish after a predator fed on both prey items, and a moistened dental wick was added to the petri dish. Predators were either killed right away for time zero or one of the other seven time points (0, 1, 2, 4, 6, 8, 16, and 24 hours), and each predator was killed by being placed into an Eppendorf 1.5 ml microcentrifuge tube containing 95% ethanol and freezing them immediately. For each predator-prey combination, 10 females and 10 males were used at each time point for a total of 80 females and 80 males. There were two predators and three prey items. If not enough predators fed on the prey items to complete the entire experiment, then it was broken into blocks with the same number of males and females for each time point (e.g. 2 males and 2 females all at times 0, 1, 2, 4, 6, 8, 16, and 24 hours).

Each predator was crushed using a 0.5 ml mortar-and-pestle microcentrifuge tube with a buffer (eg, Harwood et al. 2009), and total DNA was extracted from each crushed specimen using QIAGEN DNaseasy Tissue kits (QIAGEN Inc., Chatsworth, CA, USA). The manufacturer’s animal tissue protocol was used for all of the DNA extractions (Chapman et al. 2010). Specific primers for each prey item were used to detect CPB (Greenstone et al 2010), or *G. bullatus* in each predator gut (Table 1). PCR (50 µL) consisted of 1U Takara buffer (Takara Bio Inc., Shiga, Japan), 0.2 mM of each dNTP, 0.2 mM of each primer, 1U Takara Ex Taq and template DNA (1–5 µL of total DNA). PCR were carried out in Bio-Rad PTC-200 and C1000 thermal cyclers (Bio-Rad Laboratories, Hercules, CA, USA). The PCR cycling protocol for the
Colorado potato beetle primers were 94.5 °C for 3 minutes, followed by 40 cycles 94.5 °C at 45 seconds, 37 °C for 1 minute, 72° C for 2 minutes, and a final extension of 72 °C for 5 minutes (Greenstone et al. 2007). The PCR cycling protocol for the *G. bullatus* primers were 94 °C for 1 minute followed by 50 cycles of 94 °C for 45 seconds, 61 °C for 45 seconds, 72 °C for 30 seconds. Reaction success was determined by electrophoresis of 10 µL of PCR product in 3% SeaKem agarose (Lonza, Rockland, ME, USA) stained with ethidium bromide (0.1 mg / µL). The results of each predator were recorded after viewing the results of the gel.

2.4. Statistics

We examined the relationship between time and probability that prey DNA was detected using nonlinear logistic regression. We investigated the detection limits of target prey DNA using *G. bullatus* and *N. alternatus* predators with CPB as prey items for both species, and *G. bullatus* nymphs as a prey item for *N. alternatus*. The regressions for all five feeding trials were fitted in SigmaPlot (Systat Software, San Jose, CA). Durbin-Watson, Normality test, and Constant variance test were also done for each curve, and all of data sets passed these tests.

3. Results

3.1. Feeding trials Colorado potato beetles

Both *G. bullatus* and *N. alternatus* were fed a single Colorado potato beetle and a chaser pea aphid for each predator tested. The chaser pea aphid was used as a control to make sure that the predator was consuming each prey item. Detection of prey DNA decreased over time for both
predators between 0 and 24 hours. The digestion curve decreased significantly over time for *Geocoris bullatus* (Fig. 1) feeding on CPB ($F_{3,8} = 9.5328, P = 0.0165$). There was 100 % detection of CPB at time zero and this slowly decreased to 60 % detection around 8 hours after feeding and about 50 % detection by 24 hours. The digestion curve decreased significantly over time for *N. alternatus* (Fig. 2) feeding on CPB ($F_{3,8} = 43.6457, P = 0.0005$). There was 100 % detection of CPB at time zero and this slowly decreased to 70 % detection around 8 hours after feeding and about 15 % detection by 24 hours.

3.2. Feeding trials *Geocoris bullatus* nymphs

The predators *N. alternatus* were fed a single *G. bullatus* nymph and a chaser pea aphid for each predator tested. The chaser pea aphid was used as a control to make sure that the predator was consuming each prey item. Detection of prey DNA decreased over time for *N. alternatus* between 0 and 24 hours. The digestion curve decreased significantly over time for *N. alternatus* (Fig. 3) feeding on *G. bullatus* nymphs ($F_{3,8} = 159.6697, P < 0.0001$). There was 95 % detection of GPA at time zero and this gradually decreased to 50 % detection around 8 hours after feeding and about 5 % detection by 24 hours.

4. Discussion

Molecular gut-content analysis has transformed the way that researchers determine the role of predators in suppressing insect populations, and using the incidence of a pest in a predator’s gut to rank predators importance is a logical first step in ranking them for
conservation biological control (Greenstone et al. 2010). Detection of CPB and G. bullatus nymphs occurred for both G. bullatus and N. alternatus over time, although the length of DNA detection in predator guts differed by prey item and predator species. By 24 hours, only a small percentage of predators showed a low detection rate for prey items in both predator species and most prey items. For Colorado potato beetle, G. bullatus (Fig. 1) and N. alternatus (Fig. 2) percent positive for CPB was similar at 8 hours but dropped much lower for N. alternatus at 24 hours. The intraguild predation feeding trial with Nabis alternatus consuming G. bullatus nymphs (Fig. 3) showed the lowest percent positive predators for the N. alternatus at 8 hours and 24 hours, so G. bullatus fluids were digested quickly by N. alternatus. This is similar to a study with Orius insidiosus, a minute pirate bug, consuming soybean aphids, thrips and eggs/larvae of Asian multi-colored ladybeetles where this predator showed a low detection of prey DNA for the ladybeetle and thrips between 10 and 24 hours (Harwood et al. 2007). This species is also a hemipteran predator, so it is not surprising the digestion of prey would be similar. Digestion in Coleomegilla maculata, spotted lady beetle, was very rapid in starved predators being fed Colorado potato beetle eggs and slower with predators that were fed potato aphids, and this predator’s digestive system aggressively degraded the prey (Weber and Lundgren 2009), which has been shown in other systems (eg; Zhang et al. 2007, Nejstgaard et al. 2008)

Variables that affect the detectability of samples positive for prey DNA include the number and size of prey eaten, stage of prey eaten, time since prey consumption, temperature from consumption to collection, and the consumption of alternate prey during feeding (Sopp & Wratten 1986, Sopp et al. 1992, Hagler & Naranjo 1997, Weber and Lundgren 2009). For conventional Polymerase chain reaction, the target DNA decay rates are not available because the data is presence or absence. Logistic models can be used to characterize the decline in
detectability (Weber and Lundgren 2009), which includes the detectability half-life for PCR (Chen et al. 2000, Greenstone et al. 2007). The detectability half-life is defined as the time after which only half of the target meals can be detected in a cohort of predators as estimated by probit analysis, and it is an index of the detectability interval (Chen et al. 2000). We used a logistic model because it fit our data, and either logit or probit models are acceptable to use for the data (Dey and Astin 1993, Trexler and Travis 1993). Chaser prey (e.g., Hagler and Naranjo 1997, Symondson et al. 1999, Greenstone et al. 2007, Harwood et al. 2007) and starvation (e.g., Hagler 1998, Chen et al. 2000, Harper et al. 2006) are commonly used in studies to determine the rate of target prey marker disappearance (Weber and Lundgren 2009). Non-target chaser prey is used to simulate normal feeding rates and eliminate the adverse effects of starvation on digestion rate and DNA detectability (Greenstone & Hunt 1993, Chen et al. 2000, Harwood et al. 2007).

There are limitations to using feeding trials and using PCR for molecular gut content analysis. Predators were not used for a feeding trial if they did not consume both the target prey item and the chaser prey. A least three times more predators were used for each feeding trial, which means that a large number of live predators must be collected to complete a feeding trial. Smaller predators like *G. bullatus* often did not feed on both the target CPB larvae and chaser aphid prey, so more insects were used to have enough to run each trial. PCR only detects the presence or absence of the prey DNA, but it does not tell you how many prey items or prey stages have been consumed (Greenstone et al. 2010).

This study helps us know possibly how long field collected predators would test positive for a prey item. For both species of predators, prey DNA is low around 24 hours, so most predators collected from fields and tested positive for prey most likely ate the prey less than 24 hours before collection. It would also be possible to map networks of intraguild predators and
alternate prey by using multiple DNA primers for predators and prey present in more complex systems (Agustý´ et al. 2003, Harwood et al. 2007, Saccaggi et al. 2008). Results of a large field study would provide more insight into the mechanism of biological control, and it would help managers enhance biological control (Greenstone et al. 2010).

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community of a subterranean herbivorous insect based on polymerase chain reaction.


SigmaPlot, Version 10. Systat Software, San Jose, CA.


Figure legends

**Fig. 1.** Detection of *Leptinotarsa decemlineata* larvae DNA in *Geocoris bullatus* from time 0 to 24 hours later ($r^2 = 0.8512$).

**Fig. 2.** Detection of *Leptinotarsa decemlineata* larvae DNA in *Nabis alternatus* from time 0 to 24 hours later ($r^2 = 0.9632$).

**Fig. 3.** Detection of *Geocoris bullatus* nymph DNA in *Nabis alternatus* from time 0 to 24 hours later ($r^2 = 0.9897$).
Fig. 1
Fig. 2
Table 1. Primers used to detect the two different prey species in predators guts for the feeding trials.

<table>
<thead>
<tr>
<th>Prey species tested</th>
<th>Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Leptinotarsa decemlineata</em></td>
<td>Cpb5s-F: 5’- CTTTTCTCTTGGGCAGTTAT-3’</td>
</tr>
<tr>
<td></td>
<td>Cpb6A-R: 5’- TTATCCAAATCCAGGGTAGAAT-3’</td>
</tr>
<tr>
<td><em>Geocoris bullatus</em></td>
<td>Geo-294-F: 5’- TATCAAGAAGTATAGTAGAAATAGGCT-3’</td>
</tr>
<tr>
<td></td>
<td>Geo-449-R: 5’- AAATAAAAATTAATAGCTCCAGAATAGAAC-3’</td>
</tr>
</tbody>
</table>
CHAPTER 2: Manipulation of *Myzus persicae*, green peach aphid, and *Leptinotarsa decemlineata*, Colorado potato beetle, to determine predator feeding patterns in potatoes

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ABSTRACT

Generalist predators, by definition, feed on many different prey species. This can make it difficult to predict how changes in overall prey abundance or diversity will alter these predators’ impacts on particular target pests. We independently manipulated densities of two herbivores common on potatoes, the green peach aphid (*Myzus persicae*) and the Colorado potato beetle (*Leptinotarsa decemlineata*), using flonicamid to target aphids and *Bacillus thuringiensis* Tenebrionis to target potato beetles. We then used molecular gut-content analysis to track the effects of these manipulations on patterns of predation by the polyphagous predators *Geocoris bullatus* and *Nabis alternatus* on the two pests; to measure intraguild predation we also examined predation of the smaller *G. bullatus* by the larger *N. alternatus*. Aphids and potato beetles were reduced by the insecticides that targeted them, and both *G. bullatus* and *N. alternatus* reached lower densities in plots where aphids were reduced. However, these changes in predator and prey community structure did not translate into significant effects on either predator’s likelihood of
having eaten aphids or potato beetles, or of *N. alternatus* eating *G. bullatus*. This suggests either that the predators’ diets were relatively unaffected by prey availability, or that predators mixed their diets by moving freely among our plots. Our study provides evidence that, through movement or innate preferences, the diets of generalist predators may remain surprisingly rigid in the face of a mosaic of prey availability.

*Keywords:* Prey diversity; Arthropod generalist predators; Intraguild predation: Colorado potato beetle; Green peach aphid;

1. Introduction

By definition, generalist predators feed on multiple prey species (Symondson et al. 2002). Therefore, their densities are not tied to those of any particular prey (Harmon and Andow 2004). Some authors have suggested that this lack of a tight dynamical linkage with particular pests might limit the ability of generalists to tightly regulate pest densities (Snyder and Ives 2008). Rather, the available prey species, prey handling times, and changing field microhabitats might change the species of prey eaten (Symondson et al. 2002). In contrast, other authors have argued that, at least in some circumstances, the polyphagy of generalists might actually improve their biocontrol impact. For example, generalists may already be present in a field feeding on non-pest prey when the pests first invade; in this way, generalists can form a “first line of defense” (e.g., Settle et al. 1996). Likewise, by switching among feeding on different pests as each pest becomes abundant, generalists can impact many different pest species active at different times of the year (Symondson et al. 2002). Indeed, the use of non-pest prey can be exploited to improve
biological control. For example, many predators also feed on plant tissues that include pollen, seeds, floral nectars, extrafloral nectars and honeydew (Lundgren 2009). By managing agroecosystems to increase the availability of these resources, it may be possible to build predator densities, and thus impacts on pests, across a farm (Landis et al. 2000).

However, increasing availability of alternative prey does not reliably improve biological control. For example, Prasad and Snyder (2006) showed that alternative prey disrupted biological control by ground and rove beetles because the predators preferred to feed on aphids in *Brassica* fields rather than the cabbage root maggots that were the main pest. Omnivory is perhaps most disruptive to biological control when one predator species feeds on another (Polis et al. 1989, Rosenheim 1998). For example, generalist carabid ground beetles indirectly disrupted biological control of aphids by eating a specialist parasitoid of the aphids; predation of the parasitoid eventually led to higher aphid densities over time even though aphid predation by the beetles was initially intense (Snyder and Ives 2001). Likewise, Mallampalli and colleagues (2002) showed that intraguild predation between the spined soldier bug, *Podisus maculiventris* Say (Heteroptera: Pentatomidae), and the twelve-spotted ladybeetle, *Coleomegilla maculata* Lengi (Coleoptera: Coccinellidae) reduced overall predation on Colorado potato beetle eggs. Some studies have shown that the presence of extraguild prey can reduce intraguild predation, with the frequency of intraguild predation decreasing as extraguild prey density increases (e.g.; Bailey and Polis 1987, Lucas et al. 1998).

Two pests affecting potatoes in Washington are *Myzus persicae* (Sulzer), the green peach aphid (GPA), and *Leptinotarsa decemlineata* (Say), the Colorado potato beetle (CPB) (Biever and Chauvin 1992, Mowry 2001). Aphids harm potatoes primarily by transmitting plant viruses (Radcliffe 1982, Blackman and Estop 2000), while potato beetles harm potatoes by consuming...
plant foliage (Boiteau 2001). The predator guild on plant foliage in Washington State potato fields is dominated by omnivorous generalist hemipteran predators, primarily the big-eyed bug *Geocoris bullatus* Stål and the damsel bug *Nabis alternatus* Parshley (Koss et al. 2004; Tamaki and Weeks 1972a, b). Previous feeding trials in simple laboratory arenas have shown that while the presence of aphids reduced these predators’ predation rate on potato beetles, when potato beetle eggs fill the role of “alternative prey” they do not reduce the predators’ predation rate on aphids (Koss et al. 2004, Koss and Snyder 2005). Likewise, a field cage study showed that potato beetle suppression by a complex of generalist predators was reduced when aphids were also present (e.g., Koss and Snyder 2005). Intraguild predation may also be important in this predator community: in simple laboratory arenas, *Nabis alternatus* (damsel bug) and *Geocoris bullatus* (big-eyed bug) will feed on each other depending on which predator is in the larger developmental stage (Raymond 2000). In field-cage studies in Washington potato crops, results sometimes suggested the occurrence of intraguild predation between *Nabis* and *Geocoris* (e.g., Koss and Snyder 2005, Straub and Snyder 2006) whereas other studies found no evidence for predation among these predators (e.g., Straub and Snyder 2008).

Here, we report the results of two open-field experiments wherein we experimentally manipulated densities of green peach aphids (GPA) and Colorado potato beetles (CPB) to determine if these prey manipulations altered patterns of predation by *Nabis alternatus* and *Geocoris bullatus*. Densities of these pests were reduced using specific insecticides, or left unaltered, to form a fully-factorial manipulation of both pest species (Figure 1). Predation of CPB and GPA by both predator species and of *G. bullatus* by *N. alternatus* was measured by molecular gut content analysis using prey-specific PCR primers. The use of molecular gut
content analysis allowed us to track predation without the need to use field cages or to directly observe cryptic predator behavior.

2. Materials and methods

2.1. Field site and planting methods

This open field experiment was conducted at the Washington State University Research Farm in Othello, WA during 2010 and 2011. The field was first hilled, and then the cut potato seed was planted using a potato planting machine that planted potatoes 0.03 meters apart in rows that were the full length of the field. The potato seed was planted all in one day (April 20, 2010 and April 29, 2011), and the field contained furrows between each row without dammer dike. The length of the field was 43.89 meters, and the width across each row was 109.97 meters. The field was 1.19 acres (0.48 hectares) with twenty 5 by 10 meter plots that contained approximately 160 potato plants and were marked with flags within the full rows of Ranger russet potatoes. The rest of the field was broken down into 5 by 10 meter buffer plots around all four edges of the field and between the rows of 20 plots. Each treatment plot was completely surrounded by buffer plots of the same size (Fig. 1). The potato plants were spaced 0.03 meters apart with 0.86 meters in between rows, and there were 52 rows of potatoes. A random number generator was used to determine the order of each different treatment for all of the plots in the field. The field was sampled 4 times (every two weeks starting mid June) for herbivores and predators in 2010 and was sampled 3 times in 2011. The first sample of the predators in the field was taken in June each year before any pesticide was sprayed.
Using selective insecticides that specifically targeted each pest, we conducted a fully-factorial manipulation (i.e., Sih et al. 1998) of GPA and CPB within an open-field experiment that was conducted in each of 2 years (2010 and 2011). Each year, there were five replicates of each of the four pest-manipulation combinations, which were no spray for GPA or CPB, spray to suppress CPB only, spray to suppress GPA only, and sprays to suppress both pest species (Figure 1). Novodor (*Bacillus thuringiensis* Tenebrionis) was applied to reduce densities of Colorado potato beetles, while Beleaf (Flonicamid) was sprayed to reduce densities of green peach aphids. The control plots were sprayed with water, and a separate Chapin® 15.14 liter (4 gallon) backpack sprayer with piston pump (Model 61800 Batavia, NY) was used for each insecticide or water application. All of the treatments in the field were sprayed three times throughout the experiment on 1 July, 21 July, and 11 August in 2010; and on 11 July, 22 July, and 9 August in 2011 (in both year plots were first sprayed one week after the introduction of green peach aphids, as described below). Each plot was completely sprayed and an extra meter-wide area on each side of each plot was also sprayed for a total spray area of 7 meters by 12 meters. All 20 plots were sprayed by two different people on the same day.

CPB naturally established in our plots as overwintering adults emerged in the spring. For GPA, in addition to natural colonization by overwintering alates, we also artificially infested those plots designated to house robust populations of GPA (Figure 1) by adding aphids from a long-term greenhouse colony of aphids not infected with any potato virus; this was done because GPA densities can vary greatly from year-to-year (Koss and Snyder 2005). Aphids in our colony were originally collected on potatoes near Prosser, WA USA and reared on potato (organically-produced Russet and Yukon) plants in the greenhouse (16:8 light-dark cycle and temperatures of 22–25 °C). We first established field-hardy aphid colonies by transferring GPA from our
greenhouse colony to four 2 x 2 x 2-m field cages covered on all sides, except for the bottom, with a 32 x 32 Lumite mesh screen (BioQuip, Gardena, California, USA). Each of these field cages contained 6 Ranger russet potato plants from the greenhouse that were watered three times a week. After allowing six weeks for aphids to adjust to field conditions and reproduce, we attached leaflets containing 10 aphids each to 10 different plants using a stick-pin in each aphid+ plot. GPA were added to plots with aphids or control plots on 25 June, 6 July, and 28 July in 2010, and on 1 July, 14 July, and 1 August in 2011.

Potato plants for aphid field cages were started on whole certified organic Ranger russet potato seed (Basin Gold Cooperative, Warden, WA) and were planted in decagonal planting containers (McConkey and Co; Sumner, WA) filled with Sunshine Professional growing mix potting soil (Sungro Horticulture; Seba Beach, AB Canada). The containers were put in the greenhouse and were watered. Containers were watered once a week, and potato plants were broken off of the main tuber around 10.16 cm tall and were transplanted into separate 15.24 cm square pots with WIL-GRO professional products pro balance slow release granular fertilizer (16-16-16-7S, Wilbur-Ellis Co.; Halsey, OR) sprinkled on top of the Sunshine Professional growing mix potting soil (Sungro Horticulture; Seba Beach, AB Canada).

2.2. Sampling

The field was sampled for the arthropod community and for predators. A sample of the predators in the field was taken one week after the pesticides were first sprayed (on 15 June in 2010 and on 30 June 2011), and then again periodically thereafter throughout the growing season (on 15 July, 5 August and 16 August in 2010, and on 28 July and 18 August in 2011).
Predators and herbivores were sampled using a D-vac suction sampler (B&S Model 24 Ventura, CA). Two sets of D-vac samples were collected with one set for identification (predators and herbivores) and one for molecular analysis for each sample date (only predators). The survey samples of herbivores and predators were stored at 0 °C. The predators from the second sample were processed using molecular gut content analysis to determine what they were feeding on in the field. The arthropod community from the first sample were sorted, identified and placed into labeled vials with 95% EtOH in the lab. Concurrent with these D-vac collections, but on different plants than those sampled using the D-vac, visual counts of GPA and CPB were recorded for 10 plants per plot. During these visual surveys, on each plant we spent 30 seconds each searching the ground around the base of each plant, the lower half of the foliage, and the upper half of the foliage. There were three sampling dates for each year (2010 and 2011).

For molecular gut content analyses, *G. bullatus* and *N. alternatus* predators were put on dry ice in the field and individually placed into a 1.5 mL microcentrifuge tube containing 100% EtOH. All samples were stored at -12 °C until analysis. To prepare for DNA extraction, each predator was crushed using a 0.5 ml mortar-and-pestle microcentrifuge tubes with a buffer (e.g., Harwood et al. 2009). Total DNA was extracted from each crushed specimen using QIAGEN DNeasy Tissue kits (QIAGEN Inc., Chatsworth, CA, USA). The manufacturer’s animal tissue protocol was used for all of the DNA extractions (Chapman et al. 2010). Specific primers for each prey item were used to detect aphids (Chapman et al. 2010), CPB (Greenstone et al 2010), or *G. bullatus* in each predator gut (Table 1). PCR (50 µL) consisted of 1U Takara buffer (Takara Bio Inc., Shiga, Japan), 0.2 mM of each dNTP, 0.2 m M of each primer, 1U Takara Ex Taq and template DNA (1–5 µL of total DNA). PCR were carried out in Bio-Rad PTC-200 and C1000 thermal cyclers (Bio-Rad Laboratories, Hercules, CA, USA). The PCR cycling protocol
for the aphid primers were 94 °C for 1 minute followed by 50 cycles of 94 °C for 50 seconds, 40 °C for 45 seconds, 72 °C for 45 seconds and a final extension of 72 °C for 5 min. The PCR cycling protocol for the CPB primers were 94.5 °C for 3 minutes, followed by 40 cycles 94.5 °C at 45 seconds, 37 °C for 1 minute, 72 °C for 2 minutes, and a final extension of 72 °C for 5 minutes (Greenstone et al. 2007). The PCR cycling protocol for the G. bullatus primers were 94 °C for 1 minute followed by 50 cycles of 94 °C for 45 seconds, 61 °C for 45 seconds, 72 °C for 30 seconds. Reaction success was determined by electrophoresis of 10 µL of PCR product in 3% SeaKem agarose (Lonza, Rockland, ME, USA) stained with ethidium bromide (0.1 mg / µL). The results for each predator were recorded after viewing the results of the gel.

2.3. Statistics

The experimental design was a 2 x 2 factorial (Sih, et al. 1998) with the two years as temporal blocks and five replicates of each treatment. We compared the seasonal trends in predator and prey densities along with the proportion of predators testing positive for having eaten each prey item using repeated measures MANOVA in JMP (JMP, Cary, North Carolina, USA). Predator consumption data from molecular gut content analysis were arcsin square root-transformed before analysis to improve normality and homogeneity of variance. For the arthropod community samples, the different arthropod species were summed into functional groups that included herbivores (other than aphids or potato beetles), predators (other than G. bullatus or N. alternatus), detritivores, pollinators, arachnids, parasitoids, pollinators, and miscellaneous insects by plot.
3. Results

3.1. Arthropod community sampling

For the arthropod community sampling using a D-vac suction sampler, 10 different insect orders were found along with three other arthropod orders, i.e., Order Araneae, Opiliones, and Collembola (Table 2). Eleven different families of pests or possible prey items were found including aphids and CPB, and more than twelve families of predators were also found in these plots including *G. bullatus* and *N. alternatus*. All of arthropods were divided into functional groups that included herbivores, detritivores, predators, pollinators, arachnids, parasitoids, and miscellaneous insects for those species with an unknown diet or not fitting into the other categories. First, the functional group data were summed for all of the sampling dates for each year. Next, the functional data were broken down into treatments by sampling date for each year. Arthropods that consume other arthropods (predators, parasitoids, arachnids) were displayed together (Fig. 2) and herbivorous groups (herbivores, aphids, and Colorado potato beetles) were displayed together (Fig. 3). The predator group did not include *G. bullatus* or *N. alternatus*, and those two species were analyzed separately.

The predator population changed between sampling months (Fig. 2 a, b) and had the highest increase in July (F7, 32 = 1.8709, P < 0.0001, all between main effect). Predators were more commonly collected in 2011 (Fig. 2b) than 2010 (Fig. 2a) (F1, 32 = 2.3836, P < 0.0001, year main effect). Predator densities increased in plots that were not sprayed for aphids (F1, 32 = 0.1705, P = 0.0259, main effect aphids). As predator density increased in plots not sprayed for aphids, it decreased in plots not sprayed for CPB in July (F1, 32 = 0.1628, P = 0.0292, CPB x
aphid interaction). The predator population changed over the field season especially in July where the highest population occurred ($F_{2,31} = 1.6380, P < 0.0001$, time main effect). Predator densities changed in plots not sprayed for aphids with the peak population in July ($F_{2,31} = 0.8930, P < 0.0001$, time x aphid interaction).

The parasitoid population changed between sampling months (Fig. 2 c, d) especially in July ($F_{7,32} = 2.2329, P < 0.0001$, all between main effect). Parasitoids were more commonly collected in 2011(Fig. 2d) than 2010 (Fig. 2c) ($F_{1,32} = 1.3073, P < 0.0001$, year main effect). Parasitoid densities increased in plots that were not sprayed for aphids with a population peak in July ($F_{1,32} = 0.5170, P = 0.0003$, main effect aphids). Parasitoid densities changed by experiment year with the more found in 2011 than 2010 in plots that were not sprayed for aphids ($F_{1,32} = 0.2545, P = 0.0075$, year x aphid interaction). Parasitoid densities changed over the field season with an increase in July and decrease in August ($F_{2,31} = 5.9917, P < 0.0001$, time main effect). The parasitoid population increased in July for both years with a higher population in 2011 ($F_{2,31} = 1.8050, P < 0.0001$, time x year interaction). Parasitoid densities increased in July and decreased in August in plots not sprayed for aphids ($F_{2,31} = 0.6074, P = 0.0006$, time x aphid interaction). Parasitoid densities changes by year over time and in plots not sprayed for aphids, increased by year from 2010 to 2011 and highest in July for both years ($F_{2,31} = 0.4908, P = 0.0021$, time x year x aphid interaction).

Arachnid population changed between sampling months (Fig. 2 e, f) with increases in July and August ($F_{7,32} = 0.6401, P = 0.0174$, all between main effect). Arachnids were more commonly collected in 2011 (Fig. 2f) than 2010 (Fig. 2e) ($F_{1,32} = 0.5152, P = 0.0003$, year main effect). The arachnid population increased over the field season, higher densities in July and August ($F_{2,31} = 2.1010, P < 0.0001$, time main effect). The arachnid population increased
over the field season and by year ($F_{2, 31} = 0.3088, P = 0.0154$, time x year interaction). Arachnid densities increased in plots not sprayed for CPB over time for each year ($F_{2, 31} = 0.2301, P = 0.0403$, time x year x CPB interaction).

The herbivore population changed between sampling months with an increase in July for both years (Fig. 3a, b) and a decrease in August for 2011 ($F_{7, 32} = 1.3481, P = 0.0001$, all between main effect). Herbivores were more commonly collected in 2011 (Fig. 3b) than 2010 (Fig. 3a) ($F_{1, 32} = 0.9063, P < 0.0001$, year main effect). Herbivore densities increased in plots that were not sprayed for aphids ($F_{1, 32} = 0.2222, P = 0.0119$, main effect aphids). The herbivore population changed over the field season with high densities in July decreasing in August ($F_{2, 31} = 2.2151, P < 0.0001$, time main effect). The herbivores collected changed by sampling date with an increase in July and differences between the two years ($F_{2, 31} = 0.3582, P = 0.0087$, time x year interaction). Herbivore densities changed in plots not sprayed for aphids by sampling date ($F_{2, 31} = 0.2269, P = 0.0420$, time x aphid interaction).

Aphid population changed (Fig. 3c, d) between sampling months with an increase in July ($F_{7, 32} = 1.7997, P < 0.0001$, all between main effect). Aphids were more commonly collected in 2011 (Fig. 3d) than 2010 (Fig. 3c) ($F_{1, 32} = 0.6049, P < 0.0001$, year main effect). Aphid densities increased in plots that were not sprayed for aphids ($F_{1, 32} = 0.8380, P < 0.0001$, main effect aphids). Aphid densities changed by year with more found in 2011 than 2010 in plots not sprayed for aphids ($F_{1, 32} = 0.3668, P = 0.0017$, year x aphid interaction). Aphid densities increased in July and then decreased in August for 2010 and 2011 ($F_{2, 31} = 1.7441, P < 0.0001$, time main effect). The aphids collected changed by sampling date and increased by year ($F_{2, 31} = 0.2903, P = 0.0193$, time x year interaction). Aphid densities increased in plots not sprayed for aphids in July and decreased in August ($F_{2, 31} = 0.8930, P < 0.0001$, time x aphid interaction).
Aphid densities generally increased by year, over time and in plots not sprayed for aphids ($F_{2,31} = 0.4958$, $P = 0.0024$, time x year x aphid interaction).

Colorado potato beetle population changed (Fig. 3 e, f) between sampling months ($F_{7,32} = 0.5890$, $P = 0.0260$, all between main effect). Colorado potato beetles were more commonly collected in 2010 (Fig. 3e) than 2011(Fig. 3f) ($F_{1,32} = 0.3650$, $P = 0.0017$, year main effect). Colorado potato beetle density increased over the sampling season especially in August ($F_{1,32} = 0.5796$, $P = 0.0008$, main effect time). Colorado potato beetle density increased by sampling date and decreased in 2011 ($F_{1,32} = 0.3603$, $P = 0.0085$, time x year interaction).

The two most abundant predators (*G. bullatus* and *N. alternatus*) were in separate categories from the other predators in order to compare their densities in different treatments (Fig. 4). The *G. bullatus* population increased between sampling months ($F_{7,32} = 1.1451$, $P = 0.0005$, all between main effect). *G. bullatus* were more commonly collected in 2011(Fig. 4b) than 2010 (Fig. 4a) ($F_{1,32} = 0.6230$, $P < 0.0001$, year main effect). *Geocoris bullatus* densities increased in plots that were not sprayed for aphids ($F_{1,32} = 0.4393$, $P = 0.0007$, main effect aphids). *Geocoris bullatus* densities generally increased over the field season ($F_{2,31} = 3.3993$, $P < 0.0001$, time main effect). *Geocoris bullatus* densities increased by sampling date and year ($F_{2,31} = 0.4489$, $P = 0.0032$, time x year interaction). ($F_{2,31} = 0.3803$, $P = 0.0068$, time x aphid interaction).

The *N. alternatus* population increased between sampling months ($F_{7,32} = 2.5492$, $P < 0.0001$, all between main effect). *Nabis alternatus* were more commonly collected in 2011 (Fig. 4d) than 2010 (Fig.4c) ($F_{1,32} = 1.3111$, $P < 0.0001$, year main effect). *Nabis alternatus* densities increased more in plots that were not sprayed for aphids ($F_{1,32} = 0.1511$, $P = 0.0015$, main effect aphids) but did also increase in plots not sprayed for CPB ($F_{1,32} = 0.1511$, $P = 0.0352$, main
effect CPB). *Nabis alternatus* densities changed by experiment year with more found in 2011 than 2010 in plots not sprayed for aphids ($F_{1,32} = 0.1298$, $P = 0.0499$, year x aphid interaction). The *N. alternatus* population increased in all treatments with higher numbers in 2011, but more *N. alternatus* were found in plots not sprayed for aphids ($F_{1,32} = 0.1821$, $P = 0.0217$, year x CPB x aphid interaction). *Nabis alternatus* densities increased over the field season ($F_{2,31} = 1.4406$, $P < 0.0001$, time main effect). *Nabis alternatus* densities changed by sampling date and year ($F_{2,31} = 0.2910$, $P = 0.0191$, time x year interaction). Location of *N. alternatus* in plots not sprayed for aphids increased over the season ($F_{2,31} = 0.8588$, $P < 0.0001$, time x aphid interaction). *Nabis alternatus* were found in plots not sprayed for aphids more often over time and in 2011 ($F_{2,31} = 0.5006$, $P = 0.0019$, time x year x aphid interaction).

Insects with other diets included detritivores, pollinators and unknown feeding habits (miscellaneous insects) were displayed together (Fig. 5). Detritivores were more commonly collected in 2011 (Fig. 5b) than 2010 (Fig. 5a) ($F_{1,32} = 0.3681$, $P = 0.0017$, year main effect). The pollinator population changed between sampling months ($F_{7,32} = 1.3526$, $P = 0.0001$, all between main effect). Pollinators were more commonly collected in 2011 (Fig. 5d) than 2010 (Fig. 5c) ($F_{1,32} = 0.8706$, $P < 0.0001$, year main effect). More pollinators were collected in plots not sprayed for CPB in 2011 than 2010 ($F_{1,32} = 0.1338$, $P = 0.0467$, year x CPB interaction). The pollinator population increased over the sampling season for 2011 and peaked in July for 2010 ($F_{1,32} = 0.6607$, $P = 0.0004$, main effect time).

Insects in the miscellaneous group had an unknown diet or varied diet. The miscellaneous insect population changed between sampling months peak in July for 2011 and August for 2010 ($F_{7,32} = 1.0002$, $P = 0.0012$, all between main effect). Miscellaneous insects were more commonly collected in 2011 (Fig. 5f) than 2011(Fig. 5e) ($F_{1,32} = 0.8986$, $P < 0.0001$, year main
effect). The miscellaneous insect density changed over the sampling season increased in July and
decreased in August for 2011 and increased over time for 2010 ($F_{1,32} = 0.5921, P = 0.0007$, main
effect time). The miscellaneous insect group changed by sampling date and year with an increase
in August for 2010 and increase in July for 2011 ($F_{1,32} = 0.8724, P < 0.0001$, time x year
interaction).

3.2. Visual observations of aphids and CPB

Both aphids and CPB were counted in plots to see where more of each target pest was
found. The aphid population changed between sampling months (Fig. 6) with an increase in July
and decrease in August ($F_{7,32} = 2.9291, P < 0.0001$, all between main effect). Aphids were more
commonly observed in 2011(Fig. 6b) than 2010 (Fig. 6a) ($F_{1,32} = 1.4311, P < 0.0001$, year main
effect). Aphid densities increased in plots that were not sprayed for aphids ($F_{1,32} = 0.9625, P <
0.0001$, main effect aphids). Aphid densities changed by experiment year with more observed in
2011 than 2010 in plots not sprayed for aphids ($F_{1,32} = 0.5116, P = 0.0003$, year x aphid
interaction). Aphid densities changed over the field season with an increase in July and decrease
in August ($F_{2,31} = 1.7506, P < 0.0001$, time main effect). The aphids observed changed by
sampling date and year with increase in July and higher number in 2011 than 2010 ($F_{2,31} =
1.3106, P < 0.0001$, time x year interaction). Aphid densities changed in plots not sprayed for
aphids by sampling date with increase in July and decrease in August ($F_{2,31} = 0.7677, P =
0.0001$, time x aphid interaction). Colorado potato beetle population decreased in July and
increased in August ($F_{7,32} = 1.1929, P = 0.0003$, all between main effect). Colorado potato
beetles were more commonly observed in 2010 (Fig. 7a) than 2011(Fig. 7b) ($F_{1,32} = 0.4109, P =
Colorado potato beetle densities increased in plots that were not sprayed for CPB \((F_{1,32} = 0.2343, P = 0.0100, \text{main effect CPB})\). Colorado potato beetles density was higher in plots not sprayed for CPB and lower in plots sprayed for CPB for both years, and the overall CPB population was much higher in 2010 than 2011 \((F_{1,32} = 0.1592, P = 0.0310, \text{year x CPB x aphid interaction})\).

### 3.3. Molecular gut content analysis of two generalist predators

The number of *G. bullatus* found positive for aphids changed by sampling date and year with a decrease over time for 2010 (Fig. 8a) and 2011 (Fig. 8b) \((F_{2,31} = 0.3334, P = 0.0116, \text{time x year interaction})\). *Geocoris bullatus* were detected for aphids in plots not sprayed for aphids with a decrease slightly over time and slight increase by year \((F_{2,31} = 0.2282, P = 0.0414, \text{time x year x aphid interaction})\). The *G. bullatus* detectability of CPB DNA changed between sampling months \((F_{7,32} = 1.1105, P < 0.0006, \text{all between main effect})\). More *G. bullatus* were detected for CPB in 2010 (Fig. 8c) than 2011 (Fig. 8d) \((F_{1,32} = 0.9978, P < 0.0001, \text{year main effect})\). Colorado potato beetle DNA in *G. bullatus* was higher in June and August \((F_{2,31} = 2.0938, P < 0.0001, \text{time main effect})\). *Geocoris bullatus* were found in plots not sprayed for CPB and increased over time and by year \((F_{2,31} = 0.3259, P = 0.0126, \text{time x year x CPB interaction})\). *Geocoris bullatus* were positive for CPB in plots not sprayed for aphids and increased over time and by year, increased as aphid population increased over time and for 2010 \((F_{2,31} = 0.2152, P = 0.0487, \text{time x year x aphid interaction})\).

*Nabis alternatus* detectable for aphid DNA decreased over time in 2011 (Fig. 9b) and peaked in July for 2010 (Fig. 9a) \((F_{2,31} = 0.4455, P = 0.0033, \text{time main effect})\). *Nabis alternatus*
detectable for aphids increased by sampling date for 2010 and decreased for 2011 \((F_{2, 31} = 0.6506, P = 0.0004, \text{time \times year interaction})\). The \textit{N. alternatus} detected for CPB increased between sampling months \((F_{7, 32} = 0.6977, P < 0.0112, \text{all between main effect})\). \textit{Nabis alternatus} were more often detected for CPB DNA in 2010 (Fig. 9c) than 2011 (Fig. 9d), which follows the higher CPB population in 2010 \((F_{1, 32} = 0.5905, P < 0.0001, \text{year main effect})\). \textit{Nabis alternatus} detectable for CPB DNA increased over the field season \((F_{2, 31} = 0.8851, P < 0.0001, \text{time main effect})\). \textit{Nabis alternatus} detectable for CPB increased by sampling date for both years and decreased in 2011 compared to 2010 \((F_{2, 31} = 0.3238, P = 0.0129, \text{time \times year interaction})\). In plots not sprayed for aphids, \textit{N. alternatus} was detected for \textit{G. bullatus} DNA decreased over time for 2010 (Fig. 10a) and slightly increased over time for 2011 (Fig. 10b) \((F_{2, 31} = 0.2641, P = 0.0265, \text{time \times year \times aphid})\).

4. Discussion

Aphids and potato beetles were reduced by the insecticides that targeted them, and both \textit{G. bullatus} and \textit{N. alternatus} reached lower densities in plots where aphids were reduced. The systemic insecticide flonicamid inhibits the feeding activities of piercing-sucking insects like aphids and whiteflies (Morita et al. 2007, Cloyd 2012). After they insert their stylets into plant tissue, the chemical interferes with the neural regulation of fluid intake through the mouthparts causing the insects to starve (Cloyd 2012). Indeed, we saw significantly lower aphid densities in plots sprayed with flonicamid (Fig. 6). The insecticidal activity of \textit{Bacillus thuringiensis} is mainly due to the Cry and Cyt proteins, and the Cry protein strain tenebrionis is toxic only to Coleoptera including the Colorado potato beetle (Federici and Bauer 1998). The Cry protein or
endotoxin proteins of the spore-forming bacterium of *B. thuringiensis* must be ingested, and the activated toxin molecule binds to glycoprotein receptors on the midgut epithelium membrane forming pores or lesions that lead to cell lysis and damage the midgut-hemocoel barrier causing insect death (Federici and Bauer 1998). As expected, CPB densities were significantly reduced in plots treated with our Bt sprays (Fig. 7). We found no evidence that potato beetle densities were reduced in plots sprayed with flonicamid, or that aphids were reduced in plots sprayed with Bt, further supporting the selectivity of these two chemicals.

In a laboratory study testing both flonicamid and pymetrozine, development time, fertility, and parasitism were not negatively affected for a variety of natural enemies including the hoverfly *Episyrphus balteatus*, the carabid beetle *Bembidion lampros*, the parasitoid *Aphidius rhopalosiphi*, the ladybird beetle *Adalia bipunctata* or the rove beetle *Aleochara bilineata* (Cloyd 2012). Therefore, we did not anticipate any negative effects of the chemicals on any insects beyond those that feed on plants using piercing-sucking mouthparts. Of course, both of our focal predators, *G. bullatus* and *N. alternatus*, while primarily predatory, also do some plant feeding using their piercing-sucking mouthparts (Koss et al. 2004, Tamaki and Weeks 1972a, b). Thus it was perhaps not surprising that densities of both of these predators were reduced in plots sprayed with flonicamid (Figure 4). The predator, parasitoid, herbivore, and pollinator groups were also possibly impacted by flonicamid and had larger populations plots not sprayed aphids and control plots not sprayed for aphids or CPB. Neither insecticide had an effect on arachnids, detritivores, and miscellaneous insect groups for either year sampled. The insecticide we used to reduce potato beetle densities is more selective than flonicamid, impacting only beetles within a narrow taxonomic range (Federici and Bauer 1998). This is consistent with the lack of non-target
effects across any group of arthropods other than potato beetles (Figure 2, 3, 5), in plots receiving Bt sprays.

Despite the clear manipulation of prey by both selective insecticides, there were no significant changes in predation patterns for *G. bullatus* (Fig. 8) or *N. alternatus* (Fig. 9, 10). One possible explanation for this negative result could be the consumption of alternative (non-target) prey items by predators instead of the target pest insect, and the densities of prey other than aphids or Colorado potato beetle were driving the two predators’ hunting behavior.

Another possibility is that the buffer plots in between each experimental plot allowed the predators to freely move among plots, such that individuals were harvesting prey across plots differing in pest-species densities. In an earlier study, we found that aphid and potato beetle prey are generally detectable within predators for less than 24 hours, and frequent movement would be needed to cause no distinct feeding patterns among plots. Predators may maintain relatively inflexible feeding preferences even when background densities of particular prey vary. Indeed, predators sometimes seek nutritional balance through prey mixing (Symondson et al. 2002), and there may be benefits to sticking to a particular, balanced mix of prey. The results presented here in our open-field study contrast sharply with the results of two previous studies where predator movement and prey community composition was constrained within laboratory or field cages (Koss et al. 2004, Koss and Snyder 2005). Those earlier feeding trials with *Nabis* spp. and *Geocoris* spp. in simple laboratory arenas showed that the presence of aphids reduced their predation rate on Colorado potato beetles, and predators do no reduce their predation rate on aphids when potato beetle eggs fill the role of “alternative prey” (Koss et al. 2004, Koss and Snyder 2005).
Intraguild predation did occur with *N. alternatus* consuming *G. bullatus*, and intraguild predation was highest in June for both years. The insect population was much lower in June than the rest of the summer, so predators may have encountered each other more frequently than prey items. In July and August, a wide variety of prey choices were available to predators in our study including aphids, leafhoppers, Collembola, thrips, CPB, aphids, fungus gnats, vinegar flies, stink bugs, psyllids, whiteflies, seed bugs and stilt bugs. This diversity of prey items may have changed the predators’ diet because generalist predators will sometimes switch to attacking more abundant prey items (Symondson et al. 2002). If we had tested predators for more prey species, we might have seen an effect of prey other than aphids and Colorado potato beetles on intraguild predation patterns. Intraguild predation may also be important in this predator community: in simple laboratory arenas, *N. alternatus* (damsel bug) and *G. bullatus* (big-eyed bug) will feed on each other depending on which predator is in the larger developmental stage (Raymond 2000). In field-cage studies in Washington potato crops, results sometimes suggest the common occurrence of intraguild predation between *Nabis* and *Geocoris* (e.g., Koss and Snyder 2005, Straub and Snyder 2006) whereas others find no evidence for predation among these predators (e.g., Straub and Snyder 2008). Intraguild predation sometimes reduces a predator’s effectiveness in suppressing pest insects. For example, Mallampalli and colleagues (2002) showed that intraguild predation between the spined soldier bug, *Podisus maculiventris* Say (Heteroptera: Pentatomidae), and the twelve-spotted ladybeetle, *Coleomegilla maculata* Lengi (Coleoptera: Coccinellidae) reduced overall predation on Colorado potato beetle eggs. A study with Cecidomyiidae, Chrysopidae, and Coccinellidae predators and potato aphids as prey showed that the presence of extraguild prey (aphids) reduced intraguild predation, and intraguild predation decreased as extraguild prey density increased (Lucas et al. 1998). This effect was only
strong at high or low aphid densities (Lucas et al. 1998), and our experiment also had more *N. alternatus* detected positive for *G. bullatus* at the beginning of the season when the prey population was low.

For many of the functional groups, the highest arthropod population was in the aphid only plots, which were sprayed with *Bacillus thuringiensis* tenebrionis. The results showed that this insecticide was specific to CPB and did not reduce the population of other natural enemies or pest species. Our study found similar predatory insect and spider species as those reported by Koss and colleagues (2004), who worked previously in the same region of Washington State. The dominant predatory hemipteran species were *G. bullatus* and *N. alternatus* in our arthropod-community collection. Other generalist predators found in our experimental field were ground beetles, assassin bugs, minute pirate bugs, green and brown lacewings, ladybeetles, dance flies, and spiders. Most of these arthropods were also recorded in a previous study (Koss et al. 2004). Regardless of the mechanism, the use of flonicamid reduced the generalist predator population and possible biological control of our target pest insects. Flonicamid is a systemic insecticide that causes inhibition of salivation and sap feeding after the insect starts feeding (Morita et al. 2007), and both of the predator species used in this study will feed on plant fluids, when other prey items are unavailable and could be killed from feeding on the plants sprayed with flonicamid. Bacterial insecticides like *B. thuringiensis* tenebrionis are considered by many entomologist and growers to be selective and can be used to combine chemical and biological control (Federici and Bauer 1998). Many selective insecticides including insect growth regulators, selective feeding blockers, and microbials are often considered safe for natural enemies, but even these insecticides can have long term indirect effects on natural enemies (Cloyd 2012).
Despite the change in pest community structure by manipulation of CPB and GPA, there was no distinct predation pattern or large change in predation rates for consumption of the target prey items. A field cage study showed that potato beetle suppression by a complex of generalist predators was reduced when aphids were also present (e.g., Koss and Snyder 2005), but this did not occur in our study most likely because of other available prey species. The higher insect population did slightly decrease intraguild predation from June to later in the season, but we expected to see a greater drop in intraguild predation as the prey population increased. *Geocoris bullatus* may be easy for *N. alternatus* to catch and consume or may be more nutritious than other prey, which is why they were found detectable for *G. bullatus* throughout the field season for both years of the study. Predators found in plots with fewer Colorado potato beetles and green peach aphids nonetheless commonly consumed these prey items. This suggests that the predators search out both species of prey even when rare, perhaps because they provide a unique nutritional contribution to the predators’ diets. However, these changes in predator and prey community structure did not translate into significant effects on either predator’s likelihood of having eaten aphids or potato beetles, or of *N. alternatus* eating *G. bullatus*. This suggests either that the predators’ diets were relatively unaffected by prey availability, or that predators mixed their diets by moving freely among our plots. Our study suggests that, through movement or innate preferences, the diets of generalist predators may remain surprisingly rigid in the face of a mosaic of prey densities.

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References


Feeding mode and prey detectability half-lives in molecular gut-content analysis: an example with two predators of Colorado potato beetle. Bulletin Entomological Research. 97: 201-209.


Figure legends

**Fig. 1.** maps of field plot experimental design arrangement for 4 treatments (A = aphids present, ACPB = aphids and Colorado potato beetle, CPB = CPB present, and O = neither species) in (a) 2010 and (b) 2011. Plots were randomized each year using a random number generator.

**Fig. 2.** Average number of three different types of natural enemies. Predatory insects (a, b), arachnids (c, d), and parasitoids (e, f) found during Dvac suction sampling in the four treatments (A = aphids present, ACPB = aphids and Colorado potato beetle, CPB = CPB present, and O = neither species) in 2010 (a, c, e) and 2011 (b, d, f).

**Fig. 3.** Average number of herbivores other than aphids and Colorado potato beetles (a, b), aphids (c, d), and Colorado potato beetles (e, f) found during Dvac suction sampling in the four treatments (A = aphids present, ACPB = aphids and Colorado potato beetle, CPB = CPB present, and O = neither species) in 2010 (a, c, e) and 2011 (b, d, f).

**Fig. 4.** Average number of the predators: *Geocoris bullatus* (a, b) and *Nabis alternatus* (c, d) found during Dvac suction sampling in the four treatments (A = aphids present, ACPB = aphids and Colorado potato beetle, CPB = CPB present, and O = neither species) in 2010 (a, c) and 2011 (b, d).

**Fig. 5.** Average number of detritivores (a, b), pollinators (c, d), and miscellaneous insects (e, f) found during Dvac suction sampling in the four treatments (A = aphids present, ACPB = aphids and Colorado potato beetle, CPB = CPB present, and O = neither species) in 2010 (a, c, e) and 2011 (b, d, f).
**Fig. 6.** Average number of aphids found during visual counts in the four treatments (A = aphids present, ACPB = aphids and Colorado potato beetle, CPB = CPB present, and O = neither species) for (a) 2010 and (b) 2011.

**Fig. 7.** Average number of *Leptinotarsa decemlineata* (Colorado potato beetle or CPB) found during visual counts in the four treatments (A = aphids present, ACPB = aphids and Colorado potato beetle, CPB = CPB present, and O = neither species) for (a) 2010 and (b) 2011.

**Fig. 8.** Proportion of *Geocoris bullatus* positive for consumption of (a, c) aphids and (b, d) Colorado potato beetles in the four treatments (A = aphids present, ACPB = aphids and Colorado potato beetle, CPB = CPB present, and O = neither species) in (a, b) 2010 and (c, d) 2011.

**Fig. 9.** Proportion of *Nabis alternatus* positive for consumption of (a, c) aphids and (b, d) Colorado potato beetles in the four treatments (A = aphids present, ACPB = aphids and Colorado potato beetle, CPB = CPB present, and O = neither species) in (a, b) 2010 and (c, d) 2011.

**Fig. 10.** Proportion of *Nabis alternatus* positive for consumption of *Geocoris bullatus* in the four treatments (A = aphids present, ACPB = aphids and Colorado potato beetle, CPB = CPB present, and O = neither species) in (a) 2010 and (b) 2011.
Fig. 1.

a) 2010

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A = only aphids, ACPB = aphids and CPB, CPB = only CPB, and O = neither CPB or aphids

b) 2011

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A = only aphids, ACPB = aphids and CPB, CPB = only CPB, and O = neither CPB or aphids
Fig. 2

a) Predators 2010

b) Predators 2011

c) Parasitoids 2010

d) Parasitoids 2011

e) Arachnids 2010

f) Arachnids 2011

Average # of predators found

Average # of parasitoids found

Average # of arachnids found

Sampling month

June July August
Fig. 3

a) Herbivores 2010

b) Herbivores 2011

c) Aphids 2010

d) Aphids 2011

e) CPB 2010

f) CPB 2011

Average # of herbivores found

Average # of aphids found

Average # of CPB found

Sampling month

June July August
Fig. 4

a) Geocoris spp. 2010

b) Geocoris spp. 2011

c) Nabis spp. 2010

d) Nabis spp. 2011
Fig. 6

a) Aphid visual counts 2010

Average # of aphids found

b) Aphid visual counts 2011

Average # of aphids found

Sampling month

June July August
Fig. 7

(a) CPB visual counts 2010

(b) CPB visual counts 2011
Fig. 8

a) Geocoris positive for aphids 2010

b) Geocoris positive for aphids 2011

c) Geocoris positive for CPB 2010

d) Geocoris positive for CPB 2011
Fig. 9

a) Nabis positive for aphids 2010

b) Nabis positive for aphids 2011

c) Nabis positive for CPB 2010

d) Nabis positive for CPB 2011
Fig. 10

a) Geocoris consumption 2010

b) Geocoris consumption 2011
Table 1. Primers used to detect the three different prey species in predators guts for the field experiment.

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<th>Prey species tested</th>
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<td><em>Myzus persicae</em></td>
<td>Aphid-414-F: 5’- GGAATTTCATCAATTTTAGGAGCAA-3’</td>
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<tr>
<td></td>
<td>Aphid-565-R: 5’- ACCAGCTAGAACTGGTAGAGATAAAAT-3’</td>
</tr>
<tr>
<td><em>Leptinotarsa decemlineata</em></td>
<td>Cpb5s-F: 5’- CCTTTTCTCTTGGGCAGTTAT-3’</td>
</tr>
<tr>
<td></td>
<td>Cpb6A-R: 5’- TTATCCCAAATCCAGGTAAGAAT-3’</td>
</tr>
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<td><em>Geocoris</em> spp.</td>
<td>Geo-294-F: 5’- TATCAAGAAGTATAGTAGAAATAGGAGCT-3’</td>
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<td>Geo-449-R: 5’- AAATAAAATTAATAGCTCCAAGAATAGAAC-3’</td>
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Table 2. Total number of insect and spider species collected from insect community sampling for all twenty plots for 2010 and 2011.

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CHAPTER 3: Density of green peach aphid, *Myzus persicae*, alters behavior of the predators *Geocoris bullatus* and *Nabis alternatus*

Christine A. Lynch and William E. Snyder

*Department of Entomology, Washington State University, Pullman, WA 99164, USA*

ABSTRACT

Generalist predators may more efficiently hunt prey species that are in greater abundance. Competition from predators of the same or different species may interfere with location and consumption of prey. This study examined patterns of predation and behavior for two species of insect predator, *Nabis alternatus* and *Geocoris bullatus*, when *Myzus persicae* (GPA) density was manipulated on potato, *Solanum tuberosum*, plants in greenhouse arenas. We examined three different GPA densities (10, 25, or 50 aphids) and three predator combinations (8 *G. bullatus*, 8 *N. alternatus*, or 4 of each species). Both species of predators consumed more GPA at when more were available. Foraging activity was highest when single predator species were present, and it was reduced when both predator species were present together. When foraging in the same arenas, *G. bullatus* and *N. alternatus* more frequently observed at the middle of the potato plant. More predator observations occurred at the top of the plant in single species treatments. Cannibalism was observed with *N. alternatus* consuming *N. alternatus* as was
intraguild predation with *N. alternatus* consuming *G. bullatus*. Predator behavior and consumption was affected by predator species composition and aphid density. The risk of predation presumably made these predators spread out more and stay away from each other when they occurred in the same arena, and predators could forage together without reducing the overall aphid consumption.

*Keywords*: Generalist predators; Green peach aphid; Damsel bug; Big-eyed bug: GPA

1. Introduction

Generalist predators feed on multiple prey species (Symondson et al. 2002), and their densities are not tied to a particular prey species (Harmon and Andow 2004). The available prey species, prey handling times, and changing field microhabitats might change the species of prey eaten by any individual predator through the season (Symondson et al. 2002). For example, generalists may already be present in a field feeding on non-pest prey when the pests first invade; in this way, generalists can form a “first line of defense” (e.g., Settle et al. 1996). Likewise, by switching among feeding on different pests as each pest becomes abundant, generalists can impact many different pest species active at different times of the year (Symondson et al. 2002). Many predators (including generalist predators) also receive nutrients from plant tissues that include pollen, seeds, floral nectars, extrafloral nectars and honeydew (Lundgren 2009). There is a limited understanding of how regulation of pests is affected by intraguild competition, behavioral interference, predation and cannibalism among predators, interactions between generalists and specialists, and disruption of parasitoid biological control by generalist predators (Symondson et al. 2002).
Omnivory is perhaps most disruptive to biological control when one predator species feeds on another (Polis et al. 1989, Rosenheim 1998). For example, Mallampalli and colleagues (2002) showed that intraguild predation between the spined soldier bug, *Podisus maculiventris* Say (Heteroptera: Pentatomidae), and the twelve-spotted ladybeetle, *Coleomegilla maculata* Lengi (Coleoptera: Coccinellidae) reduced overall predation on Colorado potato beetle eggs. The outcome of intraguild predation is influenced by the size of the predators and feeding specificity of the predators (Lucas et al. 1998). This mainly occurs with generalist predators including predation within the same species (Polis 1981, Polis et al. 1989), and the smaller individual is more likely to be eaten by larger individual (Werner and Gilliam 1984). Predators sometimes retreat during a predator-predator confrontation (Edmunds 1974, Sih 1987), and more mobile predators have a better chance of escaping (New 1991). *Nabis* spp. (damsel bug) and *Geocoris* spp. (big-eyed bug) will feed on each other depending on which predator is in the larger developmental stage (Raymond 2000). Some studies have shown that the presence of extraguild prey can reduce intraguild predation, and intraguild predation decreased as extraguild prey density increased (Bailey and Polis 1987, Lucas et al. 1998). For example, under controlled conditions intraguild predation was higher for most combinations of *Aphidoletes aphidmyza*, *Chrysoperla rufilabris*, and *Coleomegilla maculata lengi* at high levels with no prey present, and intraguild predation was reduced for the same predator combinations with the addition of aphids (Lucas et al. 1998).

*Myzus persicae* (Sulzer), the green peach aphid or GPA, is one of the major pests affecting potatoes in Washington (Biever and Chauvin 1992a, b), and this aphid species is a vector for the transmission of potato viruses (Radcliffe 1982). Green peach aphids are capable of vectoring over 100 plant viruses (Blackman and Eastop 2000) including the potato leafroll virus.
(Radcliffe 1982) and potato virus Y (Novy et al 2002). The predator guild on plant foliage in Washington State potato fields is dominated by omnivorous generalist hemipteran predators, primarily the big-eyed bug *Geocoris bullatus* Stål and the damsel bug *Nabis alternatus* Parshley (Koss et al. 2004; Tamaki and Weeks 1972a, b). Previous feeding trials in simple laboratory arenas have shown that the presence of aphids reduced these predators’ predation rate on Colorado potato beetles (Koss and Snyder 2005). Likewise, a field cage study showed that potato beetle suppression by a complex of generalist predators was reduced when aphids were also present (e.g., Koss and Snyder 2005). Intraguild predation may also be important in this predator community: in simple laboratory arenas, *N. alternatus* and *G. bullatus* will feed on each other depending on which predator is in the larger developmental stage (Raymond 2000). In field-cage studies in Washington potato crops, results sometimes suggest the common occurrence of intraguild predation between *Nabis* and *Geocoris* (e.g., Koss and Snyder 2005, Straub and Snyder 2006) whereas others find no evidence for predation among these predators (e.g., Straub and Snyder 2008).

Prior studies have shown that green peach aphids (GPA) are more attractive to *Nabis alternatus* and *Geocoris bullatus* than Colorado potato beetles in different stages (Koss and Snyder 2005, Koss et al 2004). Therefore, we wanted to investigate possible mechanisms of this conclusion in a greenhouse experiment. Here we investigated the consumption and behavior of two generalist predators, *G. bullatus* and *N. alternatus*, in mixed and single species predator assemblages at varying densities of GPA. We conducted regularly-timed observations of predator foraging behavior, and then recorded changes in aphid density at the end of the experiment.
2. Materials and methods

2.1. Field site and planting methods

Predators, *Nabis alternatus* and *Geocoris bullatus*, were collected live using a D-vac suction sampler (B&S Model 24 Ventura, CA), from fields of alfalfa, *Medicago sativa*, and potatoes, *Solanum tuberosum*, (Ranger and Burbank russet varieties) planted at the Washington State University Research Farm near Othello, WA, during the 2012 growing season. Mesh D-vac bags were brought back in coolers with ice packs from the field and were transferred to a cool incubator (12 °C) back at the laboratory. Predators were later sorted individually into 50 mm x 9 mm BD Falcon Tight-fit lid Petri dishes (Fisher Scientific) with damp cotton dental wicks and were fed *Acyrthosiphon pisum* (Harris), pea aphids, if the predators were not going to be used immediately for an experiment. Predators used in experiments were held without aphids for 24-48 hours before use.

2.3. Rearing potato plants and aphids

Potato plants for the greenhouse microcosm experiments were grown in the greenhouse at ambient day length and 22–25 °C. Whole certified organic Ranger russet potato seed (Basin Gold Cooperative, Warden, WA) was planted in decagonal planting containers (McConkey and Co; Sumner, WA) filled with Sunshine Professional growing mix potting soil (Sungro Horticulture; Seba Beach, AB Canada). The containers were put in the greenhouse and were watered. Containers were watered once a week, and potato plants were broken off of the main
tuber around 10.16 cm tall and were transplanted into separate 15.24 cm square pots with WIL-GRO professional products pro balance slow release granular fertilizer (16-16-16-7S, Wilbur-Ellis Co.; Halsey, OR) sprinkled on top of the Sunshine Professional growing mix potting soil (Sungro Horticulture; Seba Beach, AB Canada). Plants were watered as needed (three times a week) before use in experiments at ca. 3 weeks of age or about 15.24 cm tall.

Green peach aphids for both experiments were from a long-term laboratory colony originally started from GPA collected on potatoes near Prosser, WA USA. This colony was maintained under the same conditions and in the same greenhouse as the potato plants, but the aphid colonies were kept in 60 x 60 x 60 cm fine mesh BugDorms (Megaview Science; Taiwan, China) in a separate room from the potato plants. A pea aphid, *Acyrthosiphon pisum* (Harris), colony was originally started from pea aphids collected on alfalfa at the WSU Research Farm near Othello, WA, in 2011 was used to feed predators being held until use in feeding trials; pea aphids were reared on pea plants, *Pisum sativum*. Potato and pea plants in the aphid colonies were watered 3 times a week.

2.4. Manipulation of aphid density and predator behavior in greenhouse

*Nabis alternatus* and *G. bullatus* feeding behavior was observed in greenhouse microcosms using three densities of green peach aphid and three predator combinations. Twenty-seven 37.85 liter (10 gallon) glass aquariums, covered with a wire-mesh lid, served as our experimental units; one potato plant in a 15.24 cm tall square pot was placed inside each aquarium. In between the top of the aquarium and the wire-mesh lid was a piece of cotton fabric, which decreased the predators’ escape from the aquarium. The experimental design included
three aphid densities (10, 25, or 50 aphids per plant) and three predator species-compositions (8 *G. bullatus*, 8 *N. alternatus*, or 4 *G. bullatus* and 4 *N. alternatus*). These factors were manipulated within a fully-crossed design, leading to 9 unique treatment combinations. There were 3 replicates of each combination within each experimental block, and four blocks of this experiment were run over the summer of 2012 on 25-29 June, 1-6 July, 1-6, 16-20 July, and 13-17 August. In total across the four blocks, this yielded 12 replicates of each treatment for a total of 108 experimental units (3 aphid densities x 3 predator communities = 9 predator-aphid combinations x 3 replicates of each per block x 4 blocks = 108 experimental units). A random number generator was used to determine the location within the greenhouse for each replicate for each replicate within a block.

*Nabis alternatus* and *G. bullatus* were collected using a D-vac suction sampler the week before starting each block. The 27 potato plants were watered before being added to each tank the day before the experiment started, and the fabric and lid was placed on top of each tank after the single potato plant was placed inside each tank. Thirty-six hours before predator release into the arenas, leaves (or pieces of leaves) housing aphids from the aphid rearing colony were selected that contained 10, 25 or 50 aphids, and the leaves were removed with scissors, each leaf placed in a separate 59.15 milliliter (2 oz) Souffle cup with lid (Dixie; CA USA), were moved to the experimental greenhouse, and were placed on top of each potato plant in each microcosm as appropriate by treatment. Fabric was placed over the top opening of the aquarium and the lid was placed on top of the fabric immediately after releasing the predators in each tank. Two sterile specimen cups (VWR; Radnor, PA USA) full of water were placed on both horizontal sides of each cage lid to prevent predators from escaping. Predators were released, and they were observed at 0, 2, 4, 6, and 8 hours after release into aquariums. Each observation per tank was 2
minutes long, and we recorded predators’ location on plants and behavior. (feeding, moving, mating, and location of plant or tank). A final aphid count was done 24 hours after predators were removed to quantify the change in aphid density from the beginning to end of the experiment. Predators were placed individually into 1.5 ml Eppendorf tubes filled with 95% EtOH.

2.5. Statistics

We examined the relationship between two generalist predators and aphid prey with three different predator combinations and three different aphid densities. This relationship was assessed using Standard Least Squares ANOVA for aphid counts or predator observations followed by a LS means Tukey HSD post hoc test (P < 0.05) to determine differences between treatments in JMP (JMP, Cary, North Carolina, USA). This method was used to analyze the final aphid density counts, predators observed over time, predator location on potato plant, observed predator behaviors (feeding and mating), and observed intraguild predation. The sum of the predator observations for each species was divided by the number of predators of each species present in each treatment by block, which is represented as per capita in the figures.

3. Results

3.1. Predator aphid consumption in greenhouse
Green peach aphids (GPA) were consumed by both species of predators for all four blocks and at all three aphid density levels (Fig. 1). Consumption of GPA differed by block ($F_{3,107} = 3.3938, P = 0.0224$, block main effect) for block 2 (Fig. 1b) had the lowest overall aphid consumption and block 3 (Fig. 1c) had the highest overall aphid consumption. More aphids were consumed when more aphids were present ($F_{2,107} = 286.3282, P < 0.0001$, main effect aphid density), which is expected because of different aphid density levels.

3.2. Predators observed and location on plants

Predators were observed for two minutes immediately after placement in each cage then every other hour over an eight hour period. The number of predators observed at each time point changed by species and treatment (Fig. 2). The sum of the predator observations by each time point for each species was divided by the number of predators of each species present in each treatment, which is represented as per capita in the figures. For *G. bullatus* (Fig. 2a) predators, the number of predators observed changed between observations ($F_{35, 72} = 9.9511, P < 0.0001$, between observations main effect) and for predator species, the single species treatment G was higher than treatments with both species (GN) ($F_{2, 72} = 9.4540, P < 0.0001$, predator species main effect). The number of predators observed changed over time, decreasing for *G. bullatus* in the two species treatment and increasing for single species ($F_{4, 69} = 0.8881, P < 0.0001$, time main effect). Over time *G. bullatus* observed in the treatment with both predator species (GN) decreased and more *G. bullatus* were observed in the single species (G) treatment ($F_{8, 138} = 0.6346, P < 0.0001$, time x predator species interaction).
For *N. alternatus* (Fig. 2b) predators, the number of predators observed changed between observations ($F_{35, 72} = 16.8304, P < 0.0001$, between observations main effect) and for predator species with the single species treatment showing more observations than two-species treatment ($F_{2, 72} = 16.5172, P < 0.0001$, predator species main effect). The number of predators observed changed over time, decreasing for *N. alternatus* in (GN) both species treatment and increasing for single species ($F_{4, 69} = 0.2357, P = 0.0051$, time main effect). Over time, the number of *N. alternatus* observed changed by block ($F_{12, 183} = 0.7059, P = 0.0162$, time x block interaction). Over time, *N. alternatus* observed in treatment with both predator species (GN) decreased and *N. alternatus* observed in the single species (G) treatment increased ($F_{8, 138} = 0.6346, P < 0.0001$, time x predator species interaction). Over time, *N. alternatus* observed in the treatment with both predator species (GN) decreased and more *N. alternatus* were observed in the single species (G) treatments ($F_{8, 138} = 0.7433, P = 0.0074$, time x predator species interaction). ($F_{16, 211} = 0.6341, P = 0.0074$, time x aphid density x predator species interaction).

3.3. Predator behavior in greenhouse

Predator location (Fig. 3, Fig. 4) and behavior (Fig. 5, Fig. 6) on each potato plant were also recorded during the observations over time. The sum of the predator observations by block and for each species was divided by the number of predators of each species present in each treatment, which is represented as per capita in the figures. The number of *G. bullatus* observed on the top of potato plants (Fig. 3a, c, e, and g) changed by block, and block 1 (Fig. 3a) had more *G. bullatus* observed than the rest of the blocks ($F_{3, 107} = 9.7857, P < 0.0001$, block main effect). More *G. bullatus* were observed in the treatment with both species (GN) for block 1 (Fig. 3a)
than the other three blocks for either the single species treatment (G) or treatment with both species (GN) (Fig. 3a) \( (F_{6, 107} = 4.3487, P = 0.0008, \text{block x predator treatment interaction}) \). The number of *N. alternatus* observed on the top of potato plants (Fig. 3b, d, f, and f) changed by block, and block 1 (Fig. 3b) had more *N. alternatus* observed than the rest of the blocks \( (F_{3, 107} = 3.2272, P = 0.0274, \text{block main effect}) \).

The number of *G. bullatus* observed on the middle of potato plants (Fig. 4a, c, e, and g) changed by predator treatment, and more *G. bullatus* were in treatments with both species (GN) than in single species treatments (G) \( (F_{2, 107} = 37.9088, P < 0.0001, \text{predator treatment main effect}) \). The number of *N. alternatus* observed on the middle of potato plants (Fig. 4b, d, f, and f) changed by predator treatment, and higher numbers of *N. alternatus* were observed in both species treatments (GN) treatments than single species (N) treatments \( (F_{3, 107} = 20.0023, P < 0.0001, \text{predator treatment main effect}) \). Both predators were found to feed on both GPA and plant tissue, but there were no significant effects of treatment or predator species.

*Geocoris bullatus* and *N. alternatus* were both observed mating in the tanks (Fig. 5). Mating *G. bullatus* pairs (Fig. 5a, c, e, g) occurred much more frequently in tanks with only *G. bullatus* (G) than in tanks with both species (GN) \( (F_{8, 99} = 24.2436, P < 0.0001, \text{predator treatment main effect}) \). More *G. bullatus* were observed mating in the single species treatments (G) than the treatments with both species (GN), and block 2 had the highest observed mating out of all four blocks (Fig. 5c) \( (F_{6, 107} = 2.6026, P = 0.0244, \text{block x predator treatment interaction}) \).

The number of *N. alternatus* observed mating (Fig. 5a, c, e, and g) changed by block, and block 4 (Fig. 5h) had the fewest *N. alternatus* observed mating out of all four blocks \( (F_{3, 107} = 2.8467, P = 0.0435, \text{block main effect}) \). There was less mating observed for *N. alternatus* in the treatments with 10 aphids than the treatments with 25 or 50 aphids \( (F_{2, 107} = 7.7600, P = 0.0009, \text{aphid density interaction}) \).
aphid density main effect). More *N. alternatus* pairs were observed mating in single species treatments (G) then in treatments with both species (GN) \((F_{2, 107} = 15.3800, P < 0.0001,\) predator treatment main effect). *Nabis alternatus* were observed mating more frequently in the treatments with only *N. alternatus* (N) in all blocks except for block 1 (Fig. 5b), and there was more mating observed in the treatments with both species (GN) than the single species treatments (G) \((F_{6, 107} = 2.4467, P = 0.0329,\) block x predator treatment interaction). Overall, there were fewer mating events observed at 10 aphid density than 25 or 50 densities, but block 3 was the exception because mating was more frequently seen in the 10 aphid treatment \((F_{4, 107} = 3.8000, P = 0.0074,\) block x aphid density interaction).

*Nabis alternatus* was observed to consume both *G. bullatus* and other *N. alternatus* (Fig. 6). Intraguild predation was not observed in block 1, which is why the figure only shows blocks 2, 3, and 4. More *G. bullatus* were consumed by *N. alternatus* (Fig. 6a, b) \((F_{2,107} = 5.3333, P = 0.0069,\) predator treatment main effect), and consumption of *N. alternatus* by another *N. alternatus* (Fig. 6b, c) happened less frequently \((F_{2,107} = 3.5714, P = 0.0332,\) predator treatment main effect) than consumption of *G. bullatus* by *N. alternatus*. Intraguild predation on *G. bullatus* was observed in blocks 2 (Fig. 6a) and 3 (Fig. 6b) with more predation observed in block 2 (Fig. 6a) \((F_{2,107} = 3.5714, P = 0.0332,\) block x aphid density x predator treatment interaction). Consumption of *N. alternatus* by another *N. alternatus* was observed in blocks 3 (Fig. 6b) and 4 (Fig. 6c) and was lower in block 4 (Fig. 6c) \((F_{2,107} = 2.1429, P = 0.0241,\) block x aphid density x predator treatment interaction). A small number of both predator species were found dead at the bottom of a tank, but there were no significant effects of treatment or predator species. Some of the dead predators were due to the observed intraguild predation.
4. Discussion

Both *G. bullatus* and *N. alternatus* consumed GPA at all three densities and consumed higher numbers of aphids when more were available. Previous feeding trials in simple laboratory arenas have shown that while the presence of aphids reduced these predators’ predation rate on potato beetles, when potato beetle eggs fill the role of “alternative prey” they do not reduce their predation rate on aphids (Koss et al. 2004, Koss and Snyder 2005). Likewise, a field cage study showed that potato beetle suppression by a complex of generalist predators was reduced when aphids were also present (e.g., Koss and Snyder 2005). However, increasing availability of alternative prey does not reliably improve biological control. For example, Prasad and Snyder (2006) showed that alternative prey disrupted biological control by ground and rove beetles, because the predators preferred to feed on aphids in *Brassica* fields rather than the cabbage root maggots that were the main pest. Likewise, generalist predator carabid ground beetles indirectly disrupted biological control of aphids by a specialist parasitoid by reducing the aphid population that could be parasitized, which led to a higher aphid population over time (Snyder and Ives 2001). Aphids may not be the most nutritious prey item for *Geocoris punctipes*, but it prefers mobile pea aphids to corn earworm eggs because it focuses its attack on mobile prey (Eubanks and Denno 2000).

Both species were also observed consuming potato plant tissue. It is not uncommon for predators to receive nutrients from plant tissues that include pollen, seeds, floral nectars, extrafloral nectars and honeydew (Lundgren 2009). Mated pairs were observed more frequently in single species treatments and in higher aphid density treatments. More food resources were available to the predators in treatments with higher aphid densities. Foraging activity was highest
when single predator species were present, and it was reduced when both predator species were present. Predators of different species also altered their foraging location on plants in the presence of other predator species. *Geocoris bullatus* and *N. alternatus* were more frequently observed in treatments with both species at the middle of the potato plant, but more predator observations occurred at the top of the plant and for mating in the single species treatments. Both species of predators moved throughout the cage over the 8 hour observation period, but they spent most of their time still or cleaning their mouthparts of antennae. The risk of predation made these predators spread out more and stay away from each other in the tanks, but it did not stop consumption of the green peach aphid. Predators sometimes retreat during a predator-predator confrontation (Edmunds 1974, Sih 1987), and more mobile predators have a better chance of escaping (New 1991). The outcome of intraguild predation is influenced by the size of the predators and feeding specificity of the predators (Lucas et al. 1998). This mainly occurs with generalist predators including predation within the same species (Polis 1981, Polis et al. 1989), and the smaller individual is more likely to be threatened by a larger predator (Werner and Gilliam 1984).

Some studies have shown that the presence of extraguild prey can reduce intraguild predation, and intraguild predation decreased as extraguild prey density increased (Bailey and Polis 1987, Lucas et al. 1998). For example, under controlled conditions intraguild predation was higher for most combinations of *Aphidoletes aphidmyza*, *Chrysoperla rufilabris*, and *Coleomegilla maculata lengi* at high levels with no prey present, and intraguild predation was reduced for the same predator combinations with the addition of aphids (Lucas et al. 1998). In our study, both predator species mainly consumed the extraguild prey (GPA) and were not observed consuming each other very frequently. Another indicator of intraguild predation is dead
predators in the aquarium, but dead predators were not seen often and some could have died from other causes like old age or parasitoids. *Geocoris bullatus* was not seen consuming other *G. bullatus* or *N. alternatus*, but *N. alternatus* was observed to consume other *N. alternatus* and *G. bullatus*. This is most likely because of size, since the *N. alternatus* is the larger species as an adult, which is the life stage for both species in the experiment. In simple laboratory arenas, *Nabis alternatus* (damsel bug) and *Geocoris bullatus* (big-eyed bug) will feed on each other depending on which predator is in the larger developmental stage (Raymond 2000). In field-cage studies in Washington potato crops, results sometimes suggest the common occurrence of intraguild predation between *Nabis* and *Geocoris* (e.g., Koss and Snyder 2005, Straub and Snyder 2006) whereas others find no evidence for predation among these predators (e.g., Straub and Snyder 2008). For example, Mallampalli and colleagues (2002) showed that intraguild predation between the spined soldier bug, *Podisus maculiventris* Say (Heteroptera: Pentatomidae), and the twelve-spotted ladybeetle, *Coleomegilla maculata* Lengi (Coleoptera: Coccinellidae) reduced overall predation on Colorado potato beetle eggs. The outcome of intraguild predation is influenced by the size of the predators and feeding specificity of the predators (Lucas et al. 1998). This mainly occurs with generalist predators including predation within the same species (Polis 1981, Polis et al. 1989), and the smaller individual is more likely to be threatened by a larger predator (Werner and Gilliam 1984). Predators sometimes retreat during a predator-predator confrontation (Edmunds 1974, Sih 1987), and more mobile predators have a better chance of escaping (New 1991).

Even though the results of this study are promising, there are limitations to this experiment. It was difficult to view all of the predators in each tank because the potato foliage was often blocking the view. A solution to this problem would be to remove some of the potato
leaflets from the plant before starting this experiment, but fewer refuges could increase intraguild predation. A stronger predator species effect may occur at very low aphid densities or very high densities, which were not part of this experiment. A field cage experiment with dispersed, even, or clumped aphid densities may help explain if predators are more attracted to plants with high or low aphid densities. Using molecular gut content analysis of aphid DNA inside the predators would tell us how many of the predators were consuming aphids or each other. Intraguild predation was not observed very often, but the molecular gut content analysis would give us a more accurate accounting of predation events.

This study showed that both species of predators consumed GPA at all three densities and consumed higher numbers as more were available. The predator species combinations did affect predator behavior, but they did not have a large affect on aphid consumption. Foraging activity was highest when single predator species were present, and it was reduced when both predator species were present. Predators of different species also altered their foraging location on plants in the presence of other predator species. Geocoris bullatus and N. alternatus were more frequently observed in treatments with both species at the middle of the potato plant, but more predator observations occurred at the top of the plant and for mating in the single species treatments. Cannibalism was observed with N. alternatus consuming N. alternatus as was intraguild predation with N. alternatus consuming G. bullatus. Intraguild predation did not have a strong effect on aphid predation, but it did occur because of close contact of these predators. The risk of predation presumably made these predators spread out more and stay away from each other in the tanks, but it did not stop consumption of the green peach aphid. Future studies would include a field experiment that shows if predator movement is changed by different aphid densities or predator combinations.
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Figure legends

Fig. 1. Number of aphids eaten by monocultures of *Geocoris bullatus* (G) and *Nabis alternatus* (N), or a mix of *G. bullatus* and *N. alternatus* (GN), when foraging among 10, 25, or 50 green peach aphids per potato plant. The experiment was conducted within four blocks: (a) block 1, (b) block 2, (c) block 3, and (d) block 4.

Fig. 2. Per capita number of times that predators were observed over 8 hours when *Geocoris bullatus* foraged among only conspecifics (G) or when this species foraged alongside *Nabis alternatus* (G with N) (panel a.), or when *N. alternatus* foraged among only conspecifics (N) or alongside *G. bullatus* (N with G) (panels b). Predators were presented with potato plants initially housing either 10, 25 or 50 green peach aphids.

Fig. 3. Per capita number of times that predators were observed on top of the potato plant when *Geocoris bullatus* foraged among only conspecifics (G) or when this species foraged alongside *Nabis alternatus* (G with N) (panels a, c, e and g), or when *N. alternatus* foraged among only conspecifics (N) or alongside *G. bullatus* (N with G) (panels b, d, f and h). Predators were presented with potato plants initially housing either 10, 25 or 50 green peach aphids, and the experiment was conducted within four blocks: block 1 (a, b), block 2 (c, d), block 3 (e, f) and block 4 (g, h).

Fig. 4. Per capita number of times that predators were observed on the middle of the potato plant when *Geocoris bullatus* foraged among only conspecifics (G) or when this species foraged
alongside *Nabis alternatus* (G with N) (panels a, c, e and g), or when *N. alternatus* foraged among only conspecifics (N) or alongside *G. bullatus* (N with G) (panels b, d, f and h). Predators were presented with potato plants initially housing either 10, 25 or 50 green peach aphids, and the experiment was conducted within four blocks: block 1 (a, b), block 2 (c, d), block 3 (e, f) and block 4 (g, h).

**Fig. 5.** Per capita number of times that predators were observed mating when *Geocoris bullatus* foraged among only conspecifics (G) or when this species foraged alongside *Nabis alternatus* (G with N) (panels a, c, e and g), or when *N. alternatus* foraged among only conspecifics (N) or alongside *G. bullatus* (N with G) (panels b, d, f and h). Predators were presented with potato plants initially housing either 10, 25 or 50 green peach aphids, and the experiment was conducted within four blocks: block 1 (a, b), block 2 (c, d), block 3 (e, f) and block 4 (g, h).

**Fig. 6.** Per capita number of times that *Nabis alternatus* were observed consuming *Geocoris bullatus* when *G. bullatus* was present with *N. alternatus* (G with N) (panels a and b), or when *N. alternatus* consumed other *N. alternatus* among only conspecifics (N) (panels b and c). Predators were presented with potato plants initially housing either 10, 25 or 50 green peach aphids, and the experiment was conducted within four blocks: block 2 (a), block 3 (b), and block 4 (c). There was no intraguild predation for block 1, so it is not shown in this figure.
Fig. 2.

(a) *Geocoris bullatus*

(b) *Nabis alternatus*
Fig. 3.

- a) Block 1 *Geocoris bullatus*
  - G
  - GN

- b) Block 1 *Nabis alternatus*
  - N
  - GN

- c) Block 2 *Geocoris bullatus*
  - G
  - GN

- d) Block 2 *Nabis alternatus*
  - N
  - GN

- e) Block 3 *Geocoris bullatus*
  - G
  - GN

- f) Block 3 *Nabis alternatus*
  - N
  - GN

- g) Block 4 *Geocoris bullatus*
  - G
  - GN

- h) Block 4 *Nabis alternatus*
  - N
  - GN
Fig. 4.

a) Block 1 *Geocoris bullatus*

b) Block 1 *Nabis alternatus*

c) Block 2 *Geocoris bullatus*

d) Block 2 *Nabis alternatus*

e) Block 3 *Geocoris bullatus*

f) Block 3 *Nabis alternatus*

g) Block 4 *Geocoris bullatus*

h) Block 4 *Nabis alternatus*
Fig. 5.

a) Block 1 *Geocoris bullatus*

b) Block 1 *Nabis alternatus*

c) Block 2 *Geocoris bullatus*

d) Block 2 *Nabis alternatus*

e) Block 3 *Geocoris bullatus*

f) Block 3 *Nabis alternatus*

- **Aphid density**
  - 10
  - 25
  - 50

- **Per capita # observed**
  - 10
  - 25
  - 50
Fig. 6.

a) Block 2 Intraguild predation by Nabis

b) Block 3 Intraguild predation by *Nabis alternatus*

c) Block 4 Intraguild predation by *Nabis*
CONCLUSIONS

Generalist insect predators may search out prey species that are nutritious even when they are uncommon in the environment, such that rates of predation will not necessarily track prey abundance. This study examined patterns of predation and behavior for two species of insect predators, *Nabis alternatus* Parshley and *Geocoris bullatus* Stål, in laboratory, greenhouse and field experiments. The laboratory experiment detected the presence of DNA from *Leptinotarsa decemlineata* (Say) (Colorado potato beetle) and *Myzus persicae* (Sulzer) (green peach aphid) in both predator species at 0, 1, 2, 4, 6, 8, 16, and 24 hours after feeding, and *Nabis alternatus* was also tested using the same method for consumption of *G. bullatus* nymphs. This study helps us know possibly how long field collected predators would test positive for a prey item. For both species of predators, prey DNA is low around 24 hours, so most predators collected from fields and tested positive for prey most likely ate the prey less than 24 hours before collection.

An open field experiment manipulated densities of green peach aphid and Colorado potato beetle using pest specific insecticides to determine if changes in prey community structure were reflected by changing predator feeding patterns. Both predator species consumed Colorado potato beetles and green peach aphids in this study, and *N. alternatus* also consumed *G. bullatus*. However, these feeding relationships were not significantly altered by changes in aphid or potato beetle densities. This suggests either that the predators’ diets were relatively unaffected by prey availability, or that predators mixed their diets by moving freely among our plots. Despite the change in pest community structure by manipulation of CPB and GPA, there was no distinct predation pattern or large change in predation rates for consumption of the target prey items. The higher insect population did slightly decrease intraguild predation from June to later in the
season, but we expected to see a greater drop in intraguild predation as the prey population increased. *Geocoris bullatus* may be easy for *N. alternatus* to catch and consume or may be more nutritious than other prey, which is why they were found detectable for *G. bullatus* throughout the field season for both years of the study. Predators found in plots with fewer Colorado potato beetles and green peach aphids nonetheless commonly consumed these prey items. This suggests that the predators search out both species of prey even when rare, perhaps because they provide a unique nutritional contribution to the predators’ diets. However, these changes in predator and prey community structure did not translate into significant effects on either predator’s likelihood of having eaten aphids or potato beetles, or of *N. alternatus* eating *G. bullatus*. Our study suggests that, through movement or innate preferences, the diets of generalist predators may remain surprisingly rigid in the face of a mosaic of prey densities.

A third study in the greenhouse manipulated green peach aphid densities (10, 25, or 50 aphids) and predator community structure (8 *G. bullatus*, 8 *N. alternatus*, or 4 of each species) to determine if aphid consumption changed with pest density or predator species composition. This study showed that both species of predators consumed GPA at all three densities and consumed higher numbers as more were available. The predator species combinations did affect predator behavior, but they did not have a large affect on aphid consumption. Foraging activity was highest when single predator species were present, and it was reduced when both predator species were present. Predators of different species also altered their foraging location on plants in the presence of other predator species. *Geocoris bullatus* and *N. alternatus* were more frequently observed in treatments with both species at the middle of the potato plant, but more predator observations occurred at the top of the plant and for mating in the single species treatments. Cannibalism was observed with *N. alternatus* consuming *N. alternatus* as was
intraguild predation with *N. alternatus* consuming *G. bullatus*. Intraguild predation did not have a strong effect on aphid predation, but it did occur because of close contact of these predators. The risk of predation presumably made these predators spread out more and stay away from each other in the tanks, but it did not stop consumption of the green peach aphid. Future studies would include a field experiment that shows if predator movement is changed by different aphid densities or predator combinations.
ATTRIBUTIONS

Chapter 1

Gretchen B. Snyder: helped run feeding trials

Eric Chapman and James Harwood: taught us feeding trials and ran molecular samples

William E. Snyder: provided grant funding for research and supplies

Chapter 2

Eric Chapman and James Harwood: ran molecular samples

William E. Snyder: provided grant funding for research and supplies