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THE INFLUENCE OF INCREASED CROP GENOTYPIC DIVERSITY ON ARTHROPODS IN AGROECOSYSTEMS

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ABSTRACT

Higher levels of plant diversity within fields, farms, and landscapes helps counteract the vulnerability of crop fields to insect pests by promoting both plant-mediated (bottom-up) and natural enemy-mediated (top-down) control of insect herbivores. Cultivar mixtures increase crop genotypic diversity and present a tactic to diversify agricultural systems that is more feasible than increasing plant species diversity in fields. Encouragingly, mixtures of plant genotypes can influence arthropod communities and increase plant productivity in both natural and agricultural systems.

I examined the potential use of cultivar mixtures to suppress insect pests in field crops using a combination of field, greenhouse, and laboratory experiments with two model systems: soybean aphid (*Aphis glycines* Matsumura) and soybean, and bird cherry-oat aphid (*Rhopalosiphum padi* L.) and wheat. I also characterized the natural enemy community available to suppress soybean aphids in PA as well as ants that may serve as mutualists of the aphids. I further tested determined if the arthropod community sampled in the foliage at night was distinct from the community captured during the day. Soybean fields in central Pennsylvania hosted a diverse arthropod community that contained many taxa identified in previous surveys across the U.S., but also a number that were previously missed, omitted, or identified at a coarser level. The community of natural enemies collected at night was distinct from the day-collected community. Specifically, a number of predators and ant taxa were more abundant in the foliage at night and have been under-represented in previous work. Under low pest pressure, soybean cultivar mixtures did not host smaller aphid populations than monocultures, but did produce intermediate aphid populations and influenced natural enemies and predation. Similarly, genotypic diversity *per se* did not reduce *R. padi* populations in the greenhouse on spring wheat. Populations on individual mixtures rarely differed from those on the individual components of the mixtures

grown in monocultures. However, aphid populations on mixtures were intermediate in mixtures compared to the monocultures, and on individual plants of certain varieties, genotypic diversity influenced aphid populations. Interactions between plants or source-sink population dynamics likely increased or decreased aphid populations on some varieties in mixtures compared to monocultures. The spotted lady beetle (*Coleomegilla maculata* De Geer), an aphid predator, was influenced by plant genotypic diversity. It was more abundant on or attracted to aphid-infested plants in mixtures than in monocultures. In winter wheat, plant-plant interactions in diverse neighborhoods reduced aphid offspring production across tested varieties. However, the specific effect of neighborhood diversity depended upon variety identity. In addition, the influence of neighborhood diversity was most pronounced when plants were adequately watered and not drought-stressed.

My results indicate that while genotypic diversity *per se* does not always suppress pests and reduce pest populations, cultivar mixtures can significantly affect both herbivores and their natural enemies and the "correct" mixtures could play a role in suppressing pests. Both bottom-up and top-down forces can mediate effects of crop genotypic diversity on herbivores. Simple mixtures are now widely used for managing pest resistance to transgenic *Bt* corn, providing an opportunity to consider moving beyond these simple mixtures and genotypic monocultures in other crops. My findings indicate that producing effective mixtures will require careful development and will likely benefit from cooperation between entomologists and plant breeders to produce mixtures that are acceptable to growers and contain relevant diversity to suppress pests.

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Chapter 1

Introduction

Modern agriculture must effectively manage insect pests and minimize the often significant economic and environmental consequences of pesticide use (Pimentel et al. 1992). In intensive agricultural production, growers typically plant crop fields with genetically uniform monocultures, facilitating efficient crop production, processing, and marketing (Matson et al. 1997, Wolfe 2000). However, monoculture crop fields typically require external inputs to guarantee plant productivity (Altieri 1999). Additionally, crop fields are often susceptible to perturbations or stressors, such as pest outbreaks, in part because low levels of diversity likely diminish potentially beneficial ecological interactions (Altieri 1999, Tilman et al. 2002). Increasing plant diversity at the within-field, farm, and landscape levels can help counteract these vulnerabilities by fostering beneficial plant-plant interactions and by promoting both plant-mediated (bottom-up) and natural enemy mediated (top-down) control of insect pests (Andow 1991, Altieri 1999, Thies and Tscharntke. 1999, Altieri and Nicholls 2004, Gardiner et al. 2009b).

Growers can increase on-farm plant diversity in several ways, which can in turn help suppress insect pests. One method is to incorporate more plant species into the cropping system, for instance by planting mixtures of crop species within the same field (e.g., intercrops, polycultures, or trap crops), or by planting non-crop plant species within fields (e.g., refuge habitat strips, flowering strips, cover crops; Andow 1991, Altieri and Nicholls 2004, Gardiner et al. 2009a). Additionally, growers can increase plant diversity in areas outside the field (e.g., flowering plants in field borders, hedgerows; Landis et al. 2000). However, growers frequently do not adopt tactics that increase plant species diversity, especially for purposes of pest management,

because they are too expensive, labor-intensive, or logistically complex, and any potential benefits of plant species diversity are never realized (Lin 2011).

An alternative pest management tactic that holds promise, increasing plant genotypic (i.e., intraspecific) diversity by planting cultivar mixtures, would be compatible with modern crop production (Mundt 2002, Tooker and Frank 2012). This approach has received little attention for insect pests, but it promises to provide some of the benefits of plant species mixtures while allowing growers to maintain the crop uniformity necessary for economies of scale. Several lines of evidence suggest that cultivar mixtures could play a role in pest management. For instance, recent work in natural systems has demonstrated that genotypic diversity can influence herbivores and that genotypic diversity can produce effects on arthropods rivaling those of plant species diversity (Crutsinger et al. 2006, Johnson et al. 2006, Barton et al. 2014). In agricultural systems, genotypic diversity in the form of cultivar mixtures that contain cultivars varying in their resistance to key pathogen species can manage plant pathogens and reduce disease pressure (Wolfe 1985, Mundt 2002). Synthesizing this evidence suggests that genotypic diversity may help manage pest populations in crop fields. The relatively few studies addressing cultivar mixtures and insect pests have suggested that genotypically diverse mixtures of crop species hold promise for managing insect pests (Power 1988, 1991, Shoffner and Tooker 2013). In addition, the homogeneity of current production practices increases susceptibility of crop fields to pest outbreaks and climatic variability or extremes, which are predicted consequences of climate change for many regions (Salinger et al. 2000, Rosenzweig et al. 2001). Predicting the optimal variety for a given year may also prove more difficult with a changing climate. Cultivar mixtures therefore hold promise to improve crop resistance and response to the abiotic and biotic stressors (e.g., drought and pest insects) that will increasingly challenge crop fields in some regions as a result of climate change.

Plant species diversity for pest management in agricultural systems

The value of plant species mixtures in agroecosystems for pest management is well-supported (Andow 1991, Tonhasca and Byrne 1994, Letourneau et al. 2011). However, the deployment of species mixtures in large-scale, non-subsistence agriculture poses challenges, and effects can be variable with not all species mixtures reducing pests (Andow 1991, Letourneau et al. 2011). Understanding the mechanisms by which species mixtures can suppress pests and the challenges species mixtures create helps elucidate the potential role of genotypic diversity in agroecosystems. First, species mixtures can negatively affect pests through direct bottom-up forces that disrupt the ability of pest insects, especially highly specialized species, to find their hosts (resource concentration and associational resistance hypotheses; Tahvanainen and Root 1972, Root 1973, Vandermeer 1989, Finch and Collier 2000). Second, increasing species diversity can support stronger top-down control of pests by natural enemies by providing natural enemies with alternative prey, floral resources, greater food availability through time, and more favorable microclimates (i.e., natural enemies hypothesis; Russell 1989; Andow 1991; Landis, Wratten, and Gurr 2000). Knowledge of pest and natural enemy ecology is crucial to ensure the success of employing plant species diversity as a pest management tactic, as it is clear that suppressive effects of plant species diversity on herbivores are variable and context dependent (Poveda et al. 2008, Letourneau et al. 2011). Plant species diversity, including mixtures of crops species, can manage pests, but are difficult to plant, manage and harvest with modern agricultural machinery. Species mixtures can also lead to an overall decrease in yield and profit if they create logistical challenges or reduce the area of land in production (Letourneau et al. 2011). Most growers of large-scale field crops do not diversify their fields at the level of plant species within the same year because of these difficulties, which means crop diversification tactics for pest management must be more easily integrated into current agricultural practices (Letourneau et al. 2011, Lin 2011).

Effects of plant genotypic diversity in natural systems

Increasing crop genotypic diversity presents a more feasible strategy than species diversity to diversify agricultural systems. Evidence from natural systems has demonstrated that diversity within a single species, while qualitatively different than diversity among species, can regulate ecosystem processes and control community structure when genetically different individuals interact, frequently in unpredictable ways (Hughes et al. 2008). Of relevance to agricultural applications, many studies have manipulated genotypic diversity of primary producers. For instance, two-genotype mixtures of the algae *Chlamydomonas reinhardtii* Dang were about 10% more productive than genetic monocultures (Bell 1991). Genotypic diversity has improved system productivity, resiliency, and stability in bacteria, algae, plants (e.g., common evening primrose, *Oenothera biennis* L.; tall goldenrod, *Solidago altissima* L.; eelgrass, *Zostera marina* L.), invertebrates (e.g., honeybees, *Apis mellifera* L. ; *Daphnia;* bryozoans, *Bugula neritina* L.), and vertebrates (e.g., frogs (Bell 1991, Pearman and Garner 2005, Crutsinger et al. 2006, Johnson et al. 2006, Mattila and Seeley 2007, Mattila et al. 2008, Aguirre and Marshall 2012). Similarly, increased genotypic diversity increased plant biomass for small pots or plots of tall goldenrod, *Arabidopsis thaliana* (L.) Heynh, and evening primrose (Crutsinger et al. 2006, Kotowska et al. 2010, Cook-Patton et al. 2011). The increase in productivity likely resulted from complementary interactions between genotypes (i.e., niche partitioning or facilitation; Crutsinger et al. 2006). In contrast to yielding more than the corresponding monocultures, mixtures can also perform equivalently and the performance of individual mixtures can be predicted by their components in monoculture (Münzbergová et al. 2009, Vellend et al. 2010, Fridley and Grime 2010).

Similar to the influence of species diversity, a growing body of evidence has also demonstrated that genotypic diversity of primary producers can influence higher trophic levels,

shape arthropod communities, and affect herbivory, which can in turn have cascading effects on plant productivity (Hughes and Stachowicz 2004, Crutsinger et al. 2006, Johnson et al. 2006, Cook-Patton et al. 2011). Plant genotype alone can influence abundance, richness, and evenness of the associated arthropod community (Johnson and Agrawal 2005, Wimp et al. 2005). Combined, different plant genotypes can drive ecological interactions at higher trophic levels and produce non-additive effects that lead to unpredictable influences on arthropod communities (Crutsinger et al. 2006, Johnson et al. 2006, Underwood 2009, Cook-Patton et al. 2011, Ohgushi et al. 2011, Utsumi et al. 2011). It is clear that the effects of plant genotypic diversity on abundance and diversity of herbivore and natural enemy species are not unidirectional, but a number of studies have revealed negative effects on herbivores and herbivory and positive effects on natural enemies. For example, damage by Japanese beetles was lower in genotypic polycultures of evening primrose than in monocultures (McArt and Thaler 2013). In this case, herbivory was not reduced because plant phenotypic traits and constitutive defenses were different when a plant was grown in a diverse community compared to a monoculture. Instead, beetles reduced their rate of consumption when they sequentially fed on leaves from different genotypes. Higher levels of genotypic diversity in stands of silver birch (*Betula pendula* Roth) produced negative, non-additive effects on chewing herbivores and gall formers (Barton et al. 2014). Within diverse stands, birch genotype influenced the direction of the diversity effect, highlighting the importance of genotype identity within mixtures for diversity effects at the individual plant level. Additionally, herbivory by vertebrate herbivores can be reduced in genotypically diverse stands of plants. Higher levels of diversity in eelgrass increased resistance to geese (Hughes and Stachowicz 2004), while genotypic diversity of evening primrose reduced attack by voles (Parker et al. 2010). Plant genotypic diversity can also influence of natural enemies of herbivores and has increased the abundance and diversity of predators (Crutsinger et al. 2006, Johnson et al. 2006).

Conversely, genotypically diverse stands of plants can increase herbivore fitness and abundance. For example, in a microcosm experiment using *Arabidopsis*, caterpillars gained more weight in genotypic mixtures, perhaps due to benefits associated with dietary mixing (Kotowska et al. 2010). In a field experiment with goldenrod, a growth of aphid populations on susceptible host genotypes and subsequent movement of herbivores onto more resistant genotypes led to greater herbivore populations and overall population growth rate in mixtures (Utsumi et al. 2011). Taken with the previous cases, these studies show that the effect of mixtures can be situation-specific and dependent on complex interactions among and between trophic levels, as is also the case for mixtures of plant species (Andow 1991). Nevertheless, the potential for strong effects of genotypic diversity on plant productivity and the arthropod community suggests that it may be advantageous to deploy genotypic diversity in agricultural systems and that plant genotypic diversity as a pest management strategy warrants further attention.

Cultivar mixtures for disease management in agriculture

An exception to the general rule of monocultures in modern agriculture is the planting of mixtures of varieties for the purpose of disease management (Wolfe 1985, Mundt 2002). Cultivar mixtures are a well-established management tactic in small grains and rice that help overcome the ability of pathogens to sweep through fields planted with a single, highly susceptible cultivar (Wolfe 2000). Hundreds of thousands of acres of rice in China and large percentages of barley fields in Europe have been planted at times with mixtures of varieties to manage disease (Gacek 1997, Merz and Valenghi 1997, Munk 1997, Zhu et al. 2000, Mundt 2002). Within the U.S., fewer farmers have adopted variety mixtures and only 18% of soft winter wheat acreage in Washington in 2000 and 7% of wheat in Kansas in 2001 were planted as mixtures, although reasons for the limited use of mixtures is not clear (Bowden et al. 2001, Mundt 2002). Mixtures for disease management consist of cultivar mixtures (analogous to variety mixtures), which are

simple mixtures of agronomically compatible cultivars, or multiline cultivars, which consist of mixtures of cultivars bred for phenotypic uniformity of agronomically important traits or that differ solely for a resistance gene (Mundt 2002). Mixtures can reduce field susceptibility to disease, compensate for disease damage, lessen the need for fungicides, and increase yields (Finckh and Mundt 1992, Smithson and Lenné 1996, Garrett and Mundt 1999, Mundt 2002). For disease control, one of the most important mechanisms often appears to be the dilution of inoculum via greater distance between identical genotypes in mixtures compared to monocultures (Wolfe 1985). Yield advantages can also be gained by mixing a highly susceptible, high-yielding cultivar with a resistant, but lower yielding cultivar (Mundt 2002). Critically, this approach to reducing biotic stress in agroecosystems has proven to be adoptable by famers without substantially hindering the logistics of planting, harvesting and marketing the crop (Mundt 2002).

Genotypic diversity in crop plants and arthropods

While most research investigating effects of plant genotypic diversity on arthropods has occurred in natural settings, some studies have shown that this level of diversification can influence arthropod abundance and behavior in crops, leading to improved pest control (Cantelo and Sanford 1984, Power 1988, 1991). Mixtures of five corn varieties hosted lower densities of leafhoppers than the monocultures of the components, although the effect was only evident at high pest populations (Power 1988). The mechanism for the difference was likely altered pest behavior and increased movement and emigration of the leafhoppers, with the net result of reduced establishment. Notably, the mixture had lower leafhopper populations than the best-performing variety in monoculture. Incidence of corn stunt, which is transmitted by the leafhopper, did not differ between treatments, potentially because of an imprecise relationship between pathogen incidence and vector abundance or because of increased pest movement, which could have increased disease transmission (Power 1988). In a multi-year experiment with small

grain aphids, a 50:50 mixture of a variety resistant to barley yellow dwarf virus (aphid-transmitted) and a susceptible variety of oats led to a reduction in bird cherry-oat aphid (*Rhopalosiphum padi* L.) populations when aphid abundance was high (Power 1991). Populations of English grain aphid (*Sitobion avenae* Fabricius) were not affected by stand diversity, while disease prevalence was significantly lower in mixtures than would be predicted from prevalence in the monocultures and appeared to be nearly as low as in monotypic stands of the resistant variety (Power 1991). Lower aphid populations in mixtures could potentially explain lower disease levels for only one of the three years of the study. Instead, it is likely that the increased movement seen in the mixtures lowered disease transmission (Power 1991). In a growth chamber experiment with wheat and free-ranging bird cherry-oat aphids, mixtures of varieties also hosted lower populations (Shoffner and Tooker 2013).

Further evidence shows that increased genotypic diversity can promote plant-plant interactions that influence herbivores and natural enemies in a manner congruent with improved pest control. For certain combinations of barley varieties, aphids were less attracted to and settled less on plants that had been exposed to the volatile organic compounds (VOCs) of a different variety as compared to a plant of the same variety (Pettersson et al. 1999, Ninkovic et al. 2002, 2011, Ninkovic and Åhman 2009). Comparable effects were seen when aphid settling was measured in the field on plants that were grown in monoculture or in a two-variety mixture (Ninkovic et al. 2002). Genotypically diverse plantings can be more attractive to natural enemies, which could translate to increased predation (Glinwood et al. 2009, Jones et al. 2011, Ninkovic et al. 2011). Similar to the experiments with aphids, exposing plants to VOCs from different varieties increased their attractiveness to natural enemies, as did mixing VOCs from different varieties and planting mixtures in the field (Glinwood et al. 2009, Ninkovic et al. 2011). When compared to monoculture, mixtures of four ryegrass cultivars (*Lolium perenne* L.) increased parasitoid abundance by 56% and diversity by 88% and the effect was independent of herbivore

populations (Jones et al. 2011). These promising results suggest that we may be able to foster beneficial plant-herbivore and natural enemy-herbivore interactions by manipulating crop genotypic diversity.

Plant genotypic diversity and the abiotic and biotic stressors of climate change.

In part because of its effects on arthropods, genotypic diversity holds promise for maintaining crop yield in the face of intensifying biotic and abiotic stress associated with climate change in many regions. As climate change progresses, some crops will be challenged by increasing abiotic stress from variable weather conditions as well as intensifying biotic stress from pest populations likely to be fostered by crops suffering from stresses such as drought (Solomon 2007). Coupled with increased temperature, changes in the hydrologic cycle may directly increase plant stress through increased frequency and severity of drought and reduce yields for some crops (Rosenzweig et al. 1994, Solomon 2007, Gregory et al. 2009). In conjunction with the direct effects of climate on crops, climate change can also reduce yield via increased pest pressure. Importantly, these indirect effects of pests and biotic stressors have largely not been included when modeling future effects of climate change on agriculture (Adams et al. 1998, Gregory et al. 2009). With rising temperatures, insect pests such as aphids will undergo more generations in a season and populations will grow faster as they become active earlier and develop faster, causing greater damage and disease transmittance and further yield reductions (Cammell and Knight 1992, Bale et al. 2002).

In addition to independently stressing crops, there is strong evidence that extreme weather and insect pests will synergize to cause even greater damage to crops. Populations of herbivorous insects can spike when feeding on already stressed host plants due to effects on plant physiology, plant defenses, herbivore development and natural enemies (Mattson and Haack 1987, Gutbrodt et al. 2012). The direction of these effects can depend on the severity and duration

of drought, but intermittent water stress typically benefits phloem-feeders such as aphids and spider mites, and insect feeding will exacerbate losses of already suffering crop plants (Riedell 1989, Oswald and Brewer 1997, Huberty et al. 2004).

As described above, plant genotypic diversity may help manage pests and promote natural enemy populations in agriculture. Furthermore, plant genotypic diversity can help maintain productivity and diminish the negative influence of climate change's abiotic stressors on ecosystems (Peltonen-Sainio and Karjalainen 1991, Smithson and Lenné 1996, Reusch et al. 2005). For instance, greater levels of genotypic diversity in the eelgrass *Z. marina* led to greater biomass production, shoot density and faunal abundance in the field. These effects were explained by varietal complementarity, which could indicate facilitation between genotypes or better use of resources by mixtures of genotypes (Reusch et al. 2005). Importantly, genotypic diversity enhanced this species' recovery after experiencing abiotic stress. A factorial mesocosm experiment crossing genotypic diversity with temperature (ambient vs. heated) found consistent positive effects of genotypic diversity on *Z. marina* productivity (Ehlers et al. 2008). Notably, despite limited experimentation in crop systems, genotypic diversity has proven valuable in maintaining yield in the face of water stress; cultivar mixtures of oats yielded as much as 9% more than monocultures during a drought (Peltonen-Sainio and Karjalainen 1991). In both examples, higher levels of diversity led to greater "insurance" against stress because different genotypes perform best under different environmental conditions, stabilizing productivity (Siemann et al. 1998, Hooper et al. 2005, Garrett and Cox 2008, Hector et al. 2010, Haddad et al. 2011). These results suggest that genotypic diversity may help agriculture adapt to the abiotic and biotic stressors that will likely intensify with climate change.

Potential natural enemies of the soybean aphid

Many diversification strategies in agriculture seek to enhance natural enemy populations to improve pest control (Landis et al. 2000). Knowledge of the natural enemy community is needed to properly target habitat enhancement schemes or management efforts and to understand how any changes are affecting the natural enemy community. Soybeans provide a model system with which to test the effects of crop genotypic diversity on the associated arthropod community and insect pest management. Natural enemies play a critical role in suppressing aphid populations in both its native range of China and in its introduced range in North America (Rutledge et al. 2004, Costamagna and Landis 2006, Desneux et al. 2006, Costamagna et al. 2007, Miao et al. 2007, Ragsdale et al. 2011). Crop genotypic diversity has the potential to enhance the top-down control of soybean aphid, but knowing which predators are even present in the system is crucial to this tactic.

While Midwestern states are the primary soybean-producing region in the U.S. and Northeastern states such as Pennsylvania produce approximately 1/20 of the soybeans of grain belt states like Iowa, soybeans are still an important crop in the region (NASS 2013). However, the majority of the natural enemy surveys and assessments of predation services have occurred in the Midwest (e.g. (Rutledge et al. 2004, Costamagna and Landis 2006, Desneux et al. 2006) . It is potentially problematic to generalize results from other areas to the Northeast, which is also affected by this soybean pest.

Even the comprehensive predator surveys conducted in Midwestern states are missing information about the daily temporal dynamics of the predator community available to suppress soybean aphid. Because of the inconvenience and difficulties of night sampling, researchers often ignore night-active predators or their predation services are combined with day-active predators when sentinel aphid prey or exclusion cage studies span multiple day cycles. Foliar sampling for soybean aphid predators, including direct, counts, sweep sampling and vacuum sampling, has

typically occurred during the day, potentially missing predators that may move into the foliage at night to feed (e.g., Vickerman and Sunderland 1975). It has become increasingly evident that nocturnal predators provide a significant portion of the total predation service and that night-active predators can be completely different from those found during the day (Luff 1978, Pfannenstiel and Yeargan 2002, Weber et al. 2008, Pfannenstiel et al. 2008).

Outline

This dissertation comprises three major sections. The first (Chapter 2) describes the natural enemy community available to suppress the soybean aphid (*Aphis glycines* Matsumura) on soybeans (*Glycine max* L.) in the Northeast. In addition, this chapter identifies aphid-tending ant species that could contribute to aphid population dynamics and if the arthropod community captured at is distinct from that captured during the day, which helps clarify the importance of night-active arthropods for aphid populations. This chapter complements the first chapter in the second section (Chapter 3).

Chapters in the second section (Chapters 3-5) explore the potential pest management and production benefits of increasing crop genotypic diversity through variety mixtures. Chapter 3 uses soybeans and soybean aphid as a model system to measure the top-down and bottom-up ecological consequences of increasing plant genotypic diversity in crop fields. Chapter 4 makes use of a model system of spring wheat (*Triticum aestivum* L.), bird cherry-oat aphid (*R. padi*), and a lady beetle (*Coleomegilla maculata* DeGreer) to explore the influence of crop genotypic diversity on herbivore populations in stands of plants and on individual plants, on herbivore behavior, and on predator behavior. Chapter 5 explores how the genotypic diversity of a plant's neighborhood (winter wheat, *T. aestivum*) influences performance and fitness of individual herbivores (*R. padi*) confined on a focal plant and how drought stress alters the influence of neighborhood diversity.

The final section consists of Chapter 6, which discusses the seemingly inadvertent convergence of *Bt* seed mixtures, or "refuge-in-the-bag" products, on cultivar mixtures for insect resistance management to *Bt* traits, without consideration of the broader benefits of crop genotypic diversity (Grettenberger and Tooker 2015). The chapter explores how the narrow use of genotypic diversity in *Bt* crops fails to fully consider other potential ecological benefits of diversifying crop fields and discusses ways in which this may occur.

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Chapter 2

The natural enemy community in PA associated with soybean aphid, and the role of nocturnal predators and ant mutualists in aphid population dynamics

Abstract

Natural enemies often suppress key arthropod pests in crops and help maintain populations below levels that require management, which often consists of applications of insecticides. First detected in 2000, the soybean aphid is a problematic pest of soybeans in the U.S. and can substantially reduce soybean yields. Predator communities help suppress soybean aphid across soybean growing regions of the U.S. and are a crucial component of soybean aphid management. Continued outbreaks of soybean aphids that require insecticidal management suggest that suppression by natural enemies could still be improved. The community has been well-characterized only in the Midwest, although not always at a fine taxonomic resolution. However, no recent information is available for Pennsylvania, where aphid population dynamics and landscape structure are different than in the Midwest and where soybeans are a primary crop. An additional component of the arthropod community that could influence aphid populations, aphid-tending ants, has not been well-described. Previous work has largely not focused on night-active arthropods, which may underestimate predator abundance and predation or miss major predators. I comprehensively sampled potential predators and aphid-tending ants, as well as aphid populations, in eight conventional soybean fields over two years. I used a range of sampling methods, including direct counts, sweep netting during the day and night, pan traps, and pitfall traps to comprehensively sample the arthropod community. Aphid populations were consistently low and only surpassed the economic threshold for soybean aphid in one year and in one field. I collected a diverse assemblage of predators that included members of nine orders and at least 29

families. The predator community overlapped with, but was also distinct from previously described communities in other regions. For instance, the community was not as dominated by lady beetles or minute pirate bugs than in Midwestern studies. I identified two ant taxa that were commonly found in the foliage and were observed tending soybean aphids. Multivariate analyses indicated that the arthropod community captured at night with sweep net sampling was distinct from the community captured during the day. Taxa found more often at night that differentiated night- and day-sampled communities included sac spiders, an earwig species, harvestmen, immature/adult *Nabis roseipennis*, and the aphid-tending ant, *Prenolepis imparis*. Additional taxa collected at night were identified within years and fields. My results indicate the importance of regionally specific assessments of natural enemy communities and also show the night-sampled arthropod community is distinct from the day community. I also identified aphid-tending ants, which were more active at night. Moreover, my results provide information on potential soybean aphid predators, especially for the Northeast, which will be necessary if biological control of the soybean aphid is to be improved through changes in crop management.

Introduction

In many cropping systems, natural enemies can contribute substantially to pest control, which decreases the need for insecticides (Settle et al., 1996). For farmers to maximize the benefits of control by natural enemies, it is useful to know the suite of natural enemies that contribute to biological control and their daily and/or seasonal dynamics. This information may help minimize the impact from insecticide applications on natural enemies, and may also allow researchers to incorporate natural enemy abundance into economic thresholds (Hallett et al., 2014; Stern et al., 1959).

The soybean aphid (*Aphis glycines* Matsumura) is an invasive pest first documented in North America in 2000. Since its introduction, it has become apparent that natural enemies can prevent outbreaks of this pest species and produce a trophic cascade protecting soybean fields from substantial yield loss (Costamagna et al., 2008; Ragsdale et al., 2011; Rutledge et al., 2004). Research on the natural enemy community attacking soybean aphid has sought to improve biological control by identifying the relevant predators and quantifying their influence on aphid populations. Experiments using exclusion cages that protect aphids from predators, as well as direct observation of predation, have repeatedly revealed that generalist and primarily aphidophagous predators suppress aphid populations, and that parasitoids, including introduced species, play a minor role in regulating aphid populations (Brewer and Noma, 2010; Costamagna et al., 2008; Gariepy et al., 2015; Noma and Brewer, 2008). The most important predator taxa can vary regionally, emphasizing the benefits of region-specific, natural-enemy surveys (Costamagna and Landis, 2006; Desneux et al., 2006). Thus far, research in North America has shown that soybean aphid predators provide substantial economic benefits (Zhang and Swinton, 2012); however, soybean aphid outbreaks still frequently occur, which makes necessary intensive scouting and chemical management, and indicates that biological control could be improved (Ragsdale et al., 2011).

Most research characterizing the natural enemy community of soybean aphid has occurred in the Midwest where the majority of soybeans in the U.S. are grown (Ragsdale et al., 2011), but some research has described natural enemies of soybean aphid in New York (Hajek et al., 2007) and Quebec (Firlej et al., 2012; Mignault et al., 2006). Intensive field crop production and relatively low landscape diversity dominate the Midwestern landscapes where most of this research has been conducted. The natural-enemy communities, particularly day-active predators, have been well described in this region (e.g., Costamagna and Landis, 2007; Fox et al., 2005; Rutledge et al., 2004), but similar data, including assessments of nocturnal predators, are limited for regions where soybeans are less common, such as the Northeastern U.S. Landscape diversity in the Northeastern states, for example, Pennsylvania, is higher than in many Midwestern states. The landscape of Pennsylvania is diverse (Egan and Mortensen, 2012) and is characterized by smaller fields and larger non-crop area compared to other soybean-growing states. For examples, in Pennsylvania 27% of the land area is in farms, compared to 51, 85, and 76% in Michigan, Illinois, and Iowa USDA - NASS, 2012), and this different landscape could support different predators and influence patterns of pest suppression. The predators attacking northeastern populations of soybean aphid may also be distinct because of differences in regional aphid population dynamics compared to the Midwest. In the Northeast, aphid populations tend to develop later in the season from long-distance colonizers (Bachmann, 2012), and these populations do not tend to grow as large as those in the Midwest. I hypothesized that these differences would generate distinct natural enemy communities that are more diverse than those typical of the Midwest, and that primarily aphidophagous predators would not be as dominant. The predator community of Pennsylvanian soybeans was described previously, but this information predated arrival of the soybean aphid to the U.S. (Wheeler and Stimmel, 1983). More comprehensive information on the natural-enemy community will facilitate tests of more complex questions, such as how in- or near-field management affects natural enemies and how the broader

landscape influences biological control (Gardiner et al., 2009a; Koch et al., 2012; Woltz et al., 2012).

Across soybean growing regions, previous research has occasionally not provided enough taxonomic resolution to leverage knowledge at the family, genus, or species levels to interpret results and target future studies, or has overlooked some arthropods that may consume soybean aphids, such as nocturnal predators. For instance, lumping predators at the family or even order level can make it difficult to compare between predator surveys and to predict how management tactics influence predators and predation of aphids. Furthermore, it has been suggested that predatory arthropod activity may vary across the diel cycle (Rutledge et al., 2004) and night predation was identified as a research gap (Costamagna and Landis, 2007). The contribution of nocturnal predators has nevertheless been largely overlooked (Ragsdale et al., 2011, but see Petersen and Woltz, 2014; Woltz and Landis, 2014). Sampling or observing predators only during the day can be misleading and may underestimate predator abundance and predation or may even fail to identify key predators (Pfannenstiel, 2004; Pfannenstiel et al., 2008; Vickerman and Sunderland, 1975). Moreover, studies excluding predators of the soybean aphid with cages have eliminated both day- and night-active predators, conflating the relative contribution of each predator community (e.g., Fox et al., 2004; Gardiner et al., 2009a; Meihls et al., 2010). Some predators may climb into soybean foliage at night to feed on soybean aphids (Hajek et al., 2007; Hannam et al., 2008; Rutledge et al., 2004), although the importance of these predators is unclear. Surveys of ground-dwelling predators with pitfalls can detect natural enemies that will eat aphids traps (Firlej et al., 2012; Hajek et al., 2007; Lundgren et al., 2013), but even if predation is confirmed through molecular techniques, describing where predation occurs can be difficult without complementary sampling or observations (Firlej et al., 2013). Importantly, recent work in Michigan has used direct observation, video observation, and vacuum sampling across the diel cycle for potential predators of the soybean aphid (Petersen and Woltz, 2014; Woltz and Landis,

2014). Different predators were captured or observed foraging in the foliage at night, including spiders and harvestmen, clearly showing night predators of the soybean aphid are poorly understood.

Another arthropod group that could strongly affect aphid population dynamics is ants, but this taxon has been poorly described in the soybean aphid system. While ants are important predators in crop fields, some form mutualistic relationships with aphids and protect aphid population to gain access to sugary honeydew, which can substantially alter their ecological influence and exacerbate pest problems (Styrsky and Eubanks, 2007; Way and Khoo, 1992). At least four ant species tend soybean aphids (Herbert and Horn, 2008; Schwartzberg et al., 2010), but their occurrence and abundance is variable (Ragsdale et al., 2011; Wyckhuys et al., 2009). When present, ants can increase soybean-aphid performance and population growth directly and also indirectly by protecting aphids from predators (Herbert and Horn, 2008; Schwartzberg et al., 2010), although excluding ants does not necessarily change aphid population levels (Hesler, 2014). Identifying ants to genus or species may provide insight into the degree to which certain ant species mediate soybean aphid population growth. Finally, as with the overall predator community, sampling ants at night may provide better insight into soybean aphid populations dynamics, particularly because two ant species that tend soybean aphid, *Lasius neoniger* and *Prenolepis imparis*, are more active at night (Talbot, 1946; Wheeler, 1943).

To characterize the natural-enemy fauna associated with soybean aphid in the Northeast U.S., I sampled soybean fields in central Pennsylvania over two growing seasons. I sought to complement and build upon prior surveys by using a variety of sampling methods, comprehensively identifying potential predators, and increasing the taxonomic resolution when possible. Furthermore, included night-collected samples to clarify the temporal dynamics of the predator community. I hypothesized that composition of the natural enemy community in Pennsylvania would be generally similar to communities in other regions, but the taxa would

differ from previous studies in their relative abundance. I also expected that the community would contain taxa rarely found previously associated with soybean aphid or overlooked by coarse taxonomic groupings or day-only sampling.

Methods

Sampling locations

I surveyed commercial soybean fields in Centre County, Pennsylvania for eight *site* x *year* combinations. In 2010, I sampled five fields, four of which were conventionally managed and one of which was managed organically. In 2011, sampled three fields, two conventional and one organic. I selected sites representative of typical crop fields in the region and included fields primarily bordered by other fields, fencerows, and wooded areas. Sites were separated by an average of 15.4 km in 2010 (range 1.7-29.6 km) and 4.7 km in 2011 (range 2.3-7.7 km). As is typical for our region, field size was small, with an average size of 6.1 ha in 2010 (range 0.8-19.9) and 3.4 ha in 2011 (range 2.0-4.2), which are smaller than fields in previous studies on natural enemies in commercial Midwestern soybean fields (e.g., Gardiner et al., 2009b; Schmidt et al., 2011). In 2011, I sampled fields on farms that I had sampled in 2010, although fields were different between years and were separated by 0.2-1.3 km. Both conventional and organic fields were managed with standard commercial practices and had been planted with corn the previous year. All conventional fields were planted with glyphosate-resistant soybeans, were not tilled, and were treated twice with glyphosate to manage weeds: once before planting and once post-emergence. In each year, the organic field was tilled for weed management through the first two sampling dates each year. All fields were planted in mid- to late May. No fields were sprayed with insecticides to manage aphids, including the one case where aphid populations exceeded the established economic threshold of 250 aphids/plant (Ragsdale et al., 2007).

Arthropod sampling

Arthropod sampling within each year was the same across farms. In both years, sampling consisted of counts for aphids and for natural enemies and ants, direct counts, pitfall traps, pan traps, and sweep sampling (day and night). Each sampling method targeted different groups of the arthropod community. I initiated sampling after the last herbicide treatment, which corresponded to the arrival of soybean aphid (V4-R1 growth stage in 2010 and V3-V5 in 2011). Sites were sampled approximately every two weeks in 2010 for a total of five sampling periods and four sampling periods in 2011 (Table 1). I ceased sampling when all fields had reached, or would surpass by the next sampling date, the growth stage R6, at which point the current economic threshold for soybean aphid would no longer be valid (Ragsdale et al., 2007). All sampling occurred >30 m from the nearest edge, reducing the likelihood of strong edge effects (Caballero-López et al., 2012). I sampled potential predators and ants using the same sampling methods. I transferred all arthropods from pitfall traps, pan traps, and sweep samples into 80% ethanol before they were identified.

Aphids and parasitoids

I assessed aphid populations via whole plant counts. In 2010, I counted aphids on 10 plants in two parallel transects. In 2011, I counted aphids on 20 randomly selected plants in two parallel transects. Plants were separated by 10 m and all aphids were counted on each plant. Alate aphids were seldom found, with < 1 per 10 plants for all fields except for field 6 in 2011, which averaged 0.4 alates per plant on the final sampling date (8/11). On the same plants, I counted all aphid mummies. A small number of mummies each year were collected in gel capsules and incubated in the laboratory at room temperature and identified to genus. While I did not assess prevalence of fungal disease, pathogen-killed aphids (entomopathogenic fungi; Nielsen and

Hajek, 2005) were seldom observed and infection rates appeared to be low and not likely to affect aphid populations.

Direct counts of predators and ants

Direct counts describe components of the natural enemy community not captured by my other sampling techniques (Bannerman et al., 2015). In 2010, I visually inspected plants for fly larvae (Cecidomyiidae, Chamaemyiidae, Syrphidae) and eggs of lacewings and lady beetles (Noma and Brewer, 2008). In 2010, I searched the same 10 plants on which I counted aphids, beginning with mobile predators to avoid disturbing them before they were counted. In 2011, the same taxa and life stages were counted along with any other predators. All 20 plants used for aphid counts were visually inspected in 2011 and another 20 plants in two additional, parallel transects were visually inspected for predators and ants, totaling 40 plants per field and date.

Ground-dwelling arthropods: Pitfalls

I sampled ground-dwelling arthropods and measured their activity density with 10 pitfall traps at each field. I arranged pitfall traps in two transects of five paralleling the rows with 10 m separating transects and traps in each transect. Each pitfall trap consisted of a 16 oz plastic deli container (Reynolds Consumer Products, Lake Forest, Illinois) sunk into the ground with the edge flush with the soil surface. Another container with the top one inch removed was used as an insert to facilitate sample removal without disturbing the surrounding soil. A green plastic plate supported by nails served as a cover. I used propylene glycol as a killing agent with several drops per gallon of unscented dish detergent to break surface tension. Pitfall traps were left open for 72 hours during each sampling period. Some pitfall traps were disturbed by mammals (late season, 2010), decreasing sampling intensity for several field × date combinations.

Mobile predators: Pan traps

To capture mobile predators moving along the top of the foliage, such as adult syrphid flies (Diptera: Syrphidae) or long-legged flies (Diptera: Dolichopodidae), and to allow me to more easily identify specimens, I used two colors of pan traps. Pan traps were paired together at three locations within each field. Individual pan traps consisted of blue and yellow plastic bowls ("Caribbean blue" and "sunshine yellow," Amscan, Inc, Los Angeles, CA) filled with water with several drops per gallon of unscented dish detergent and 60 mL of propylene glycol to prevent total evaporation of the liquid. One bowl of each color was affixed with glue to the top of either end of a wooden board (50 × 7.5 × 1.5 cm). Another bowl of each color was placed in the glued bowl and served as the trap. I used screws or a bolt to attach the board to a wooden (2010) or rebar (2011) stake, and I adjusted the height of the pan traps at the start of each sampling period so they were flush with the top of the soybean canopy. Pan traps were used for 72 hours during each sampling period. I strained pan trap samples in the field using a mesh kitchen strainer (1 mm mesh) and then transferred arthropods via a funnel into an alcohol-filled vial. A mammal destroyed several traps at one field in 2010 (8/22).

Foliar predators: Sweep samples

I collected sweep net samples to capture arthropods in the foliage. Relative abundances of arthropods obtained by sweep-netting may differ from those obtained using other foliage-sampling techniques (e.g., transects, vacuum, or destructive whole-plant counts; Bannerman et al., 2015; Schmidt et al., 2008). However, this method still captures most taxa and allowed me to sample both day and night and to capture a large number of specimens that could be later identified in the laboratory, which improved the taxonomic resolution of my identifications. I collected sweep samples between 9:30 and 15:00 for day samples and 21:00 and 24:00 for night samples. Day and night sweep samples were collected within the same 24-hour period in 2011,

but in 2010, weather and logistical constraints prevented this, although samples were collected close enough in time that they are still comparable (Table 1). In 2011, field 6 was not sampled on the earliest date because the plants in this field were planted later and were small. Each time, I collected five sweep samples from locations separated by at least 20 m using a 15" diameter sweep net (Bioquip products, Rancho Dominguez, CA). Four of the samples consisted of 20 figure-eight sweeps and the fifth sample was 80 figure-eight sweeps, which could capture more individuals. For data presentation and analysis, I standardized sampling intensity across all five sweep samples and converted the fifth sample to number of individuals per 20 sweeps.

Statistical analysis

Statistics were conducted in CRAN software R 3.2.0 (R Development Core Team 2015). To compare community composition between day and night sweep samples across the season, I performed a permutational multivariate analysis of variance using distance matrices for each year with the *adonis* command in the R package vegan with 9999 permutations (Version 2.30; Oksanen et al., 2015). This is a distribution-free method that partitions the dissimilarity matrix among sources of variation and uses a permutation test with pseudo-F ratios. I used a distance matrix based on Bray-Curtis dissimilarities without standardization (Bray and Curtis, 1957). Because my objective was to compare the day and night sampling methods within each field, sampling time was used as a factor and I used the *strata* argument to constrain permutations within fields (Oksanen et al., 2015). Both ants and predators were included in the analysis. Before analysis, I summed samples for each field across dates, producing five replicate samples per field (25 for day, 25 for night). Extremely rare taxa were removed from the analysis to emphasize taxa more likely to contribute to predation or mutualistic interactions. In 2010, only taxa with > 20 total individuals were included in the analysis and in 2011, only taxa with > 5 were included (> 0.5 and 0.4% of total individuals, respectively). When necessary and possible, taxa were

combined into biologically relevant groupings at the genus or family level to reach this threshold. To visualize patterns in the arthropod assemblage for each sampling time, I performed non-metric multidimensional scaling (NMDS), implemented with the function *metaMDS* (vegan package) and using a distance matrix with Bray-Curtis dissimilarities. To help explain which taxa contributed to differences between day and night sweep samples and which taxa were highly associated with night samples, I performed two complementary analyses for each year, SIMPER (SIMilarity PERcentage, Clarke, 1993) and IndVal (Indicator Value, Dufrêne and Legendre, 1997). SIMPER makes pairwise comparisons among groups, computes the average contribution of each taxa to the average overall Bray-Curtis dissimilarity matrix, and lists taxa in order of importance for distance among groups. SIMPER was performed with the *simper* function of the vegan package. I also used IndVal analysis, which calculates the indicator value of different taxa by combining the mean abundance and frequencies of occurrence in groups of taxa (Roberts, 2015). High indicator values combine high abundances within a group and high fidelity to that group (maximum = 1). For the IndVal analysis, I used the function *indval* in the labdsv package, and restricted permutations (n = 9999) to within fields. I performed analyses both across fields and within individual fields to ignore and include variation between sites. For the different sampling methods for predators, I present means taken first across replicates within a farm and then across farms for each sampling date. To simplify presentation of data, I use the midpoint of dates. If only one or two individuals of a given taxon were collected across years for a given sampling method, I do not present their abundance.

Results

Populations of aphids and parasitism

Aphid populations were low during both years of the study and only exceeded the economic threshold in one field (field 3 in 2010). In this field, aphid populations peaked at 605

aphids per plant before decreasing on the final sampling date, although the biweekly sampling period meant the true peak was likely higher (Fig. 1). On the final sampling date in 2010, only one out of the four other fields exceeded 100 aphids per plant. Aphid populations in all fields were extremely low in 2011. While aphids were present during the second half of the sampling period, populations never reached 10 aphids per plant and were highest on the final sampling date. In both years, I found mummies parasitized by aphelinid wasps (Hymenoptera: Aphelinidae), but rates of parasitism rates were low and I found only two mummies in 2011. Parasitism rates in 2010, as determined by counting mummies, were below 6% over the whole season and were greatest on the final sampling dates. All mummified aphids were bluish-black, which is characteristic of parasitoids in the family Aphelinidae, and I did not encounter the tan mummified aphids that are characteristic of parasitoids in the families Braconidae or Aphidiidae. Parasitoid wasps reared to adulthood belonged to the genus *Aphelinus*.

Natural enemies and ants associated with soybean aphid

I collected or observed 12,917 arthropods across the two years, which provides the basis for a comprehensive description of both potential predators and ants that likely functioned as mutualists in soybean fields in central PA (Tables 2-5). Of the total arthropods, I identified 12,086 potential predators associated with the soybean aphid. Predators included members of nine orders, and at least 29 families. Arthropods were included in the survey that were potential predators at some life stage based on their natural history; in addition, many collected taxa have previously been found to consume soybean aphids (Ragsdale et al., 2011; Rutledge et al., 2004). Distinct communities of predators and ant mutualists inhabited each field within each year, but a comparison of individual fields is beyond the scope of the design of this study and I instead primarily focus on patterns across farms and seasonal patterns.

Direct counts

Whole-plant counts for natural enemies did not yield many individuals, with 83 predators (egg, immature, adult) counted in 2010 and 89 in 2011. Cecidomyiid larvae were the most common predator in 2010 (50% of total) and were most common in the field with the greatest aphid population, and were most common when aphid populations were high (Fig. 1 & Table 2). I did not count ants in 2010, although both *Lasius* sp. and *Prenolepis imparis* were observed tending aphids. In 2011, I counted 17 ants of these same two species in the foliage, although this comprised only three separate groups of ants.

Ground-dwelling arthropods: Pitfalls

A greater total number of arthropods were captured in 2010 than in 2011 (3518 vs. 2816), but on a per-sampling time basis, the total was virtually identical at ≈ 704 total predators per date (Table 3). Activity-density in 2010 decreased over the course of the season, while it peaked in the middle of the season in 2011. In both years, the most commonly captured taxa with pitfalls were ground beetles (Coleoptera: Carabidae; 26.1 and 52.7%), wolf spiders (Araneae: Lycosidae; 19.7 and 15.7%), and ants (Hymenoptera: Formicidae; 17.7 and 10.7%). The total activity-density of ground beetles was dominated in both years by the omnivorous *Harpalus pensylvanicus* (34.3 and 68.4% of total ground beetles). However, this pattern was primarily driven by extremely high activity-density of omnivorous *H. pensylvanicus* in fields on one farm (fields 1 and 6). The accidentally introduced species *Pterostichus melanarius* was the next most prevalent species. The activity-density of harvestmen (Opiliones) was also high (2010: 19.4% of total, 2011: 12.6%), with most individuals appearing to be the introduced species *Phalangium opilio* (Phalangiidae), although the prevalence of immature individuals made identification difficult. Wolf spiders in the genus *Pardosa*, or thinlegged wolf spiders, were by far the predominant wolf spider and genus (55.8% of wolf spiders in 2010, and 71.3% in 2011), although members of the genus *Trochosa*

were also frequently captured. These wolf spiders were captured more often in the early or early-mid part of the season. Of the eight genera of ants caught in pitfall traps in 2010, *Lasius* sp. made up 26.1% of total individuals, while *P. imparis* made up only 3.5%. In 2011, *Lasius* sp. made up 17.3%, while *P. imparis* made up 8.3%.

Mobile predators: Pan traps

Dipteran predators were the taxa most commonly captured in the pan traps and consisted of long-legged flies (Diptera: Dolichopodidae) and hoverflies (Diptera: Syrphidae, predatory as larvae; Table 4). Hoverflies were captured more frequently early in the season and were much more abundant in 2010 than in 2011 (283 vs. 62). Diversity of other predators was fairly low, and the other most commonly collected taxa were minute pirate bugs (*Orius insidiosus*, rove beetles (Staphylinidae), and lady beetles (Coccinellidae; 17.5, 8.6, and 5.2% of total, respectively). Spiders (> 3 families) and harvestman were captured in pan traps, but pan trapping is not usually the best approach for sampling arachnid populations.

Foliar predators: Sweep samples

Sweep net samples captured a very diverse assemblage of predators, including those observed in direct counts and captured in pan traps (Table 5). I captured many fewer individuals overall in the second year of the study (3690 vs. 1319), and the magnitude of this difference was not corrected by adjusting for the smaller number of sampling periods in the second year. In addition, sweep netting caught ant species that were potentially in the foliage tending aphids, with *Lasius* sp. and *P. imparis* constituting 11.8% of the total arthropod capture in 2010 and 9.0% in 2011. These two ant genera made up the overwhelming majority of the total ants collected. Spiders as a whole were the most abundant group in 2010 (22.2% of total) and included eight identified families, with crab spiders (Araneae: Thomisidae) being the most common. However,

spiders only made up 8.4% of total capture in 2011. Sheet web spiders (Araneae: Linyphiidae) and wolf spiders, primarily *Pardosa* sp., were two taxa of spiders caught in both pitfall and sweep samples. *O. insidiosus* was very common during both years and tended to be more abundant later in the season. Six species of lady beetles were found in the foliage, although two non-native species, *Harmonia axyridis* and *Propylea quatuordecimpunctata* were the dominant species each year (combined, 75.6% in 2010, 51.1% in 2011), with *H. axyridis* more abundant towards the end of the season and *P. quatuordecimpunctata* in the beginning. Two species of damsel bugs (Hemiptera: Nabidae), *Nabis americoferus* and *N. roseipennis,* were also common, both as adults and immatures. Six species of ground beetles were captured in the foliage, two of which are not included in the table because they were single individuals; none were very abundant, and only *Chlaenius tricolor* was found in both pitfall traps and sweep samples.

Comparison of day and night sweep samples

For each year, permutational multivariate analysis of variance using distance matrices indicated the composition of the community sampled during the day and the night was different (2010: pseudo-$F_{1,28}$ = 7.69, R^2 = 0.33, P < 0.001; 2011: pseudo-$F_{1,28}$ = 13.75, R^2 = 0.33, P < 0.001). Based on NMDS, the two communities could be easily separated for both years and for all fields (Fig. 2). The SIMPER and IndVal analyses helped identify which taxa discriminated between the day and night communities and which taxa were indicative of specific communities. In 2010, 11 taxa were responsible for ≈ 60% of the difference between communities, with the night active *P. imparis* and adults of the day-active *O. insidiosus* contributing the most to the differences between groups, while three predators found much more often in night samples, immature *N. roseipennis*¸ immature Syrphidae, and the earwig *Forficula auricularia* also contributed (Fig. 3). In 2011, 6 taxa contributed to ≈ 60% of the difference, with *O. insidiosus*, contributing the most, followed, by *F. auricularia* and *P. imparis*. IndVal analysis selected seven

taxa as indicators of the night community in 2010 (immature Cantharidae, Clubionidae, immature Syrphidae, *F auricularia*, immature *N. roseipennis,* Staphylinidae, *P. imparis,* Carabidae) and one taxa, small unidentified Araneae, as an indicator of the day community. In 2011, the distribution of indicator taxa was more balanced, with three indicators of the night community (Clubionidae, *F. auricularia,* adult *N. roseipennis*) and four of the day community (unidentified Lycosidae, small unidentified Araneae, Salticidae, Oxyopidae), with three taxa shared between years.

SIMPER and IndVal analyses performed separately for each field selected many similar taxa as contributing strongly to the differences between day and night communities within each farm or as indicators of the different communities (Tables 6 & 7). For instance, the SIMPER analyses indicated that adult *O. insidiosus* contributed to differences between communities, as did adult *P. quatuordecimpunctata*, *F. auricularia*, *P. imparis*, and immature *N. roseipennis* (Table 6). The IndVal analyses for each farm, suggested adult *O. insidiosus* was indicative of the day community, while *F. auricularia*, sac spiders (Araneae: Clubionidae), *Pardosa* sp., and both aphid-tending ants (*Lasius* spp. and *P. imparis*) were indicators of the night community (Table 7).

Discussion

I documented a diverse community of predators associated with soybean aphid in Pennsylvania and identified the suite of natural enemies available to suppress soybean aphids in this area. Of particular significance, I sampled the arthropod community during the night and revealed temporal details of the community that is active after dark. Efforts like mine are necessary because predator communities in soybeans differ regionally, even within a relatively small region, that can alter the biological control service provided by natural enemies (Gardiner et al., 2010; Ragsdale et al., 2011). The arthropod community that I characterized was generally similar to those in previous studies (e.g., Bannerman et al., 2015; Costamagna and Landis, 2006; Ragsdale et al., 2011; Schmidt et al., 2008) , although there were notable differences for a number

of taxa. The range of sampling techniques I used captured a large number of individuals, providing a comprehensive inventory of predators that can interact with soybean aphids across the diel cycle, including ant species that tend aphids. While the different sampling methods captured overlapping arthropod communities, each sampling methods described a distinct community of predators. While all taxa were not identified to species, the taxonomic resolution of my identifications is finer than many previous studies and helps establish which specific taxa may be important for regulating soybean aphid populations.

Populations of soybean aphid were low during the experiment, exceeding the economic threshold in only one of eight fields. After low populations in the first year, I expected higher aphid populations in the second year of the study given the pattern in Pennsylvania of alternating low and high aphid years that had generally characterized soybean aphid population dynamics (Rhainds et al., 2010), but populations were again low. Field cage experiments that I ran concurrent with this study indicated that climatic conditions in our region did not restrict growth potential of soybean aphids (Chapter 3), and when protected from predators, aphid populations flourished. This evidence strongly suggests that natural enemies prevented immigrating aphids from producing damaging populations, consistent with previous studies (Costamagna et al., 2008; Ragsdale et al., 2011; Rutledge et al., 2004).

In central Pennsylvania, parasitoids do not appear to strongly regulate aphid populations. I did find aphids parasitized by aphelinid wasps, but they were uncommon. While parasitoids can control aphid populations in other cropping systems (e.g., wheat: Schmidt et al., 2003) or in the native range of soybean aphid (Miao et al., 2007), this does not appear to be the case in North America. Most studies that have assessed parasitism have found extremely low rates (Brewer and Noma, 2010; Brosius et al., 2007; Costamagna et al., 2008; Desneux et al., 2006). Currently, predators appear to be much more important than parasitoids for soybean aphid management and any strategies aiming to improve biological control should therefore focus on predators.

The predator community in our region was similar to others in North America and comprised a mixture of aphidophagous and more generalist natural enemies. Nevertheless, I documented notable differences in the community I described. The foliar community, for example, was not dominated by *H. axyridis* and *O. insidiosus*, as has been found in other areas (Costamagna and Landis, 2006; Desneux et al., 2006). In Indiana for instance, *H. axyridis* and *O. insidiosus* accounted for >85% of the foliar predators (Rutledge et al., 2004). Similarly, nearly 90% of natural enemies in another Indiana study were *O. insidiosus* (Desneux et al., 2006), and they were also the most common foliar natural enemy in Nebraska (Brosius et al., 2007). As a group, coccinellids have often been dominant predators in soybeans (Lundgren et al., 2013; Ragsdale et al., 2011). In Quebec, 57% of predators were coccinellids, most of which were *P. quattuordecimpunctata* (Mignault et al., 2006), and in Michigan, *C. septempunctata* and *H. axyridis* were the most abundant predators and were most effective at suppressing aphid populations (Costamagna and Landis, 2007, 2006). I found *P. quattuordecimpunctata* to be slightly more abundant than *H. axyridis*, but this species is not common in the Midwest (Gardiner et al., 2009b). Critically, *P. quattuordecimpunctata* was most abundant in the fields that I sampled early in the season when aphid populations were still low and predators could have had a strong influence on colonizing populations, and subsequent population growth (Desneux et al., 2006; Ragsdale et al., 2011). That lady beetles and *Orius* comprised a smaller percentage of the total predator community may be partially explained by my comprehensive effort to identify predatory arthropods, which likely resulted in a lower percentage of these important predatory species relative to the entire community, but also may have been due to lower numbers of lady beetles and *Orius* resulting from low aphid populations or the diverse landscape in the area. Damsel bugs (*Nabis* spp.) are common predators in soybean, but they are infrequently identified beyond genus (e.g., Rutledge et al., 2004, but see Seagraves and Lundgren, 2012). I identified two species, *N. americoferus* and *N. roseipennis*, and the distinction is important because they

differ in their natural history. These two species occupy different parts of the soybean plant (upper and lower, respectively; Braman and Yeargan, 1989). This influenced suppression of other pests (Braman and Yeargan, 1989) and could influence suppression of soybean aphid, which exhibits distinct within-plant distributions, partially due to predation (Costamagna and Landis, 2011; Mccornack et al., 2008). Using pan traps, I found that *Toxomerus marginatus* was the most abundant hoverfly, consistent with other areas (Cox et al., 2014; Eckberg et al., 2014, but see(Kaiser et al., 2007; Noma and Brewer, 2008). *T. marginatus* kills many aphids as a developing larvae (Hopper et al., 2011) and can help suppress aphid population (Smith et al., 2008). Low larval densities of hoverflies across years and fields suggests predation by hoverflies was low in my fields, although high aphid populations would likely prompt adults to oviposit in fields and increase the importance of this predator. Many of the taxa captured with pitfall traps on the soil surface were generally similar to previously described communities, although relative abundances and composition differed (Firlej et al., 2012; Gardiner et al., 2010; Hajek et al., 2007; Lundgren et al., 2013). *Pardosa* was one of the two most abundant genera in Michigan (Gardiner et al., 2010), and it was the most abundant wolf spider in my study. *Pardosa* was found on the ground, as well as in the foliage, and *Pardosa* can be an important component of predator communities that suppress other species of aphids (Birkhofer et al., 2008; Schmidt et al., 2003).

Taken together, my various sampling methods provided insight into the role different taxa may play in aphid populations dynamics, including predators that spend time both on the ground and in the foliage. Ground beetles, for example, are primarily ground-dwelling predators, but they will feed upon aphids (Lövei and Sunderland, 1996; Scheller, 1984) and it has been suggested they may move into soybean foliage at night (Firlej et al., 2013; Hajek et al., 2007; Rutledge et al., 2004). I found ground beetles were most associated with night samples, but I did not find many ground beetles in sweep samples (26 total across fields and years), and they were not species described in the foliage in association with soybean aphids. The ground beetle

Agonum muelleri has been observed climbing soybeans and feeding on soybean aphid in the laboratory, but was not captured in limited field sampling at night (Hannam et al., 2008). Video recordings have captured ground beetles in the foliage, primarily at night (Woltz and Landis, 2014), and similar observations found ground beetles in the foliage during the day (Petersen and Woltz, 2014). I did not catch many *A. muelleri* in pitfall traps, but I did capture many other ground beetles, including many individuals of *P. melanarius*, a species that may move into the foliage. Gut content analyses have shown this species will feed extensively on soybean aphid (Firlej et al., 2013), but my results suggest that they don't eat aphids or that they encounter aphids on the ground because I did not capture *P. melanarius* in the foliage. Moreover, ground beetles can benefit from aphids falling to the ground (Losey and Denno, 1998), although soybean aphid does not readily drop to evade predators like other aphid species (Rutledge et al., 2004). I also captured eight ant genera in pitfall traps, but, aside from the two genera of ant-tending aphids, I did not capture many ants in the foliage, suggesting ants in my fields primarily foraged on the soil surface.

Night sampling captured a distinct arthropod community in soybeans, which was partly due to lower abundances of some taxa, but night sweep sampling also caught more of other taxa, or revealed taxa not captured during the day. Across both years, sac spiders (Araneae: Clubionidae), *F. auricularia*, immature *N. roseipennis*, harvestmen (Phalangiidae), and *P. imparis* were all collected more often at night and were strong indicators of night samples. When compared within farms, I identified a number of other night taxa, including the other aphid-tending ant genera, *Lasius*. The lack of attention to nocturnal predation for soybean aphids is somewhat surprising given prior work in soybeans showing night predation could be greater than during the day and that a predator often associated with soybean aphid, *Nabis* spp., was responsible for this predation (Pfannenstiel and Yeargan, 2002). As another example, an initial study assessing the potential influence of different predators on soybean aphid through feeding

trials in the lab indicated *F. auricularia* consumed large numbers of aphids, likely in part because of its large body size compared to many aphid predators. In tree fruit systems, earwigs can be highly influential aphid predators (Carroll and Hoyt, 1984); however, until recently they had not been described as associated with soybean aphid, possibly because they are primarily captured in foliage at night, and only infrequently in pitfall traps. In my study, only 4.3% of earwigs were captured during the day with sweep sampling and very few in pitfall traps. Recent video observations during day and night indicate that predators are similarly abundant and active during both time periods, and these observations have including earwigs (Petersen and Woltz, 2014). Furthermore, my analysis indicates that the aphid-tending ants *P. imparis* and *Lasius* spp. are a large component of the nocturnal arthropod community. This daily cycle was expected; these species are more active at night (Lynch et al., 1980; Talbot, 1946; Wheeler, 1943). Day-only sampling, as has been typical for predators and ants associated with soybean aphid (but see Petersen and Woltz, 2014; Woltz and Landis, 2014), might capture the most influential predators in this pest system, but ignoring the nocturnal community may overlook taxa that influence soybean aphid populations.

I identified a suite of ant species in my pitfall and sweep samples, but I only witnessed two taxa, *P. imparis* and *Lasius* spp., tending aphids. Other ant genera were not abundant in sweep samples, suggesting they were not moving into the foliage to tend aphids. I did not quantify aphid-tending activity of ants, but observations of these ants tending aphids coupled with the observation that the abdomen of many specimens was distended, presumably from honeydew, suggests tending occurred for these species. Importantly, only three studies have tested if tending by ants influences soybean aphid populations and predation on soybean aphids, and none of them focused on my dominant species, *P. imparis*. Two studies focused on ant communities dominated by *L. neoniger* (Hesler, 2014; Schwartzberg et al., 2010), while one focused on *Monomorium minimum* (Herbert and Horn, 2008), a species not found in my survey. Experiments with *L.*

neoniger did not produce strong effects on aphid population levels, although tending increased aphid biomass (Schwartzberg et al., 2010). *M. minimum* harassed or killed predators, increasing aphid populations (Herbert and Horn, 2008). None of the studies manipulated ant presence at the plot or field level, as has been done in other ant-hemipteran studies (Kaplan and Eubanks, 2005). *P. imparis* could be more aggressive towards natural enemies than *L. neoniger*, which would increase the likelihood of population-level consequences for soybean aphids. I found *P. imparis* and *Lasius* more often at night, with 79 and 72% of *P. imparis* and *Lasius* captured at night, respectively, possibly accounting for why mutualisms between ants and soybean aphid have received little attention. More research needs to examine how ants affect aphid and predator populations and whether presence of ants changes the likelihood soybeans aphids become established in field or reach damaging levels.

My results suggest that a diverse community of predators may be important in regulating soybean aphid populations in Pennsylvania and, more broadly, the Northeast. Many of these predaceous species are shared across soybean growing regions, but my sampling revealed some unique features of Northeastern predator communities, which may be due to differences in aphid population dynamics, climate, or habitat characteristics at the farm or landscape level. I found that while common, lady beetles and minute pirate bugs were not as dominant as in Midwest predator communities, and the lady beetle community I found contained species in different relative abundances than in Midwest surveys. I also found a number of predator taxa in the foliage at night that are not often captured in surveys of soybean aphid predators. In addition, ants, which were more abundant in the soybean canopy at night, may exacerbate soybean aphid populations by tending and protecting aphids. My results demonstrate how important predators or ant mutualists may be missed and under-appreciated if temporal dynamics of the community are not considered and I identified a number of taxa that were caught much more often in the foliage at night. A firm understanding of arthropod communities associated with soybean aphid, as well

as their regional and temporal variability, will be helpful when trying to manage agroecosystems to improve biological control services.

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Tables

Table 2-1. **Characteristics of fields used in the study and dates of the methods used to sample soybean aphids and natural enemies.** I sampled five fields in 2010 and three fields in 2011. Soybean aphids were counted on whole plants, while natural enemies were sampled with direct counts on whole plants, pitfall traps, pan traps, and day and night sweep samples.

Year	Field #	Farm #	Management	Row spacing	Aphid count dates	Direct count of predators and parasitoids	Pan trap dates*	Pitfall trap dates*	Sweep sample day	Sweep sample night
2010	1	1	Conventional no-till	7-7.5"	6/28, 7/12, 7/25, 8/11, 8/23	6/28, 7/12, 7/25, 8/11, 8/23	6/28, 7/12, 7/25, 8/10, 8/23	6/28, 7/12, 7/25, 8/10, 8/23	6/28, 7/12, 7/25, 8/8, 8/23	6/28, 7/14, 7/27, 8/11, 8/25
2010	2	2	Organic	30"	6/28, 7/12, 7/27, 8/11, 8/23	6/28, 7/12, 7/27, 8/11, 8/23	6/28, 7/12, 7/25, 8/10, 8/23	6/28, 7/12, 7/25, 8/10, 8/23	6/28, 7/12, 7/27, 8/8, 8/23	6/28, 7/14, 7/27, 8/11, 8/25
2010	3	3	Conventional no-till	7-7.5"	6/28, 7/12, 7/27, 8/11, 8/23	6/28, 7/12, 7/27, 8/11, 8/23	6/28, 7/12, 7/25, 8/10, 8/23	6/28, 7/12, 7/25, 8/10, 8/23	6/28, 7/12, 7/27, 8/8, 8/23	6/28, 7/14, 7/27, 8/11, 8/25
2010	4	4	Conventional no-till	7-7.5"	6/28, 7/12, 7/27, 8/11, 8/23	6/28, 7/12, 7/27, 8/11, 8/23	6/28, 7/12, 7/25, 8/10, 8/23	6/28, 7/12, 7/25, 8/10, 8/23	6/28, 7/12, 7/27, 8/8, 8/23	6/28, 7/14, 7/27, 8/11, 8/25
2010	5	5	Conventional no-till	7-7.5"	6/28, 7/12, 7/25, 8/11, 8/23	6/28, 7/12, 7/25, 8/11, 8/23	6/28, 7/12, 7/25, 8/10, 8/23	6/28, 7/12, 7/25, 8/10, 8/23	6/28, 7/12, 7/25, 8/8, 8/23	6/28, 7/14, 7/27, 8/11, 8/25
2011	6	2	Organic	30"	6/28, 7/14, 7/28, 8/11	6/28, 7/14, 7/28, 8/11	7/1, 7/17, 7/31, 8/14	7/1, 7/17, 7/31, 8/14	7/14, 7/31, 8/11	7/14, 7/31, 8/11
2011	7	3	Conventional no-till	30"	6/28, 7/14, 7/28, 8/11	6/28, 7/14, 7/28, 8/11	7/1, 7/17, 7/31, 8/14	7/1, 7/17, 7/31, 8/14	6/29, 7/14, 7/31, 8/11	6/29, 7/13, 7/31, 8/11
2011	8	5	Conventional no-till	7-7.5"	6/28, 7/14, 7/28, 8/11	6/28, 7/14, 7/28, 8/11	7/1, 7/17, 7/31, 8/14	7/1, 7/17, 7/31, 8/14	6/29, 7/14, 7/31, 8/11	6/29, 7/13, 7/31, 8/11

*Dates for pitfall and pan trapping indicate the end of the 72-hour period during which I used the sampling method.

Table 2-2. **Direct counts of natural enemies across five fields in 2010 and three fields in 2011.**

		2010							2011					
Order	Family/species	**6/28**	**7/12**	**7/25**	**8/11**	**8/23**	**Total[2]**	**%[3]**	**6/28**	**7/14**	**7/28**	**8/11**	**Total[2]**	**%[3]**
Coleoptera	**Coccinelidae**													
	Coleomegilla maculata Ad.[1]	-	-	-	-	-	-	-	-	-	1	-	**1**	**1.1**
	Coccinellid Imm.	0	0	1	11	0	**12**	**14.5**	-	-	-	-	**0**	**0.0**
	Coccinellid Egg cluster	0	0	2	1	0	**3**	**3.6**	-	-	-	-	**0**	**0.0**
Hemiptera	**Anthocoridae**													**0.0**
	Orius insidiosus Ad.	-	-	-	-	-	-	-	1	8	22	13	**44**	**49.4**
	Orius insidiosus Imm.	-	-	-	-	-	-	-	-	8	8	4	**20**	**22.5**
	Nabidae													**0.0**
	Nabis Ad.	-	-	-	-	-	-	-	-	-	-	1	**1**	**1.1**
	Nabis Imm.	-	-	-	-	-	-	-	-	-	-	1	**1**	**1.1**
	Reduviidae	-	-	-	-	-	-	-	-	-	-	1	**1**	**1.1**
Neuroptera	**Hemerobiidae:** Imm.	0	0	0	2	1	**3**	**3.6**	-	-	-	-	**0**	**0.0**
	Chrysopidae/ Hemerobiidae: Egg	0	2	0	2	4	**8**	**9.6**	-	-	-	-	**0**	**0.0**
Diptera	**Cecidomyiidae:** Imm.	0	2	21	20	7	**50**	**60.2**	-	-	-	-	**0**	**0.0**
	Syrphidae: Imm.	0	0	2	3	2	**7**	**8.4**	-	-	-	-	**0**	**0.0**
Araneae	**Salticidae**	-	-	-	-	-	-	-	-	-	1	-	**1**	**1.1**
	Thomisidae	-	-	-	-	-	-	-	-	-	-	1	**1**	**1.1**
	Araneae UNID	-	-	-	-	-	-	-	-	-	2	-	**2**	**2.2**
Hymenotpera	**Formicidae**	-	-	-	-	-	-	-	-	-	1	16	**17**	**19.1**
	Total	0	4	26	39	14	**83**		1	16	35	37	**89**	

[1]Life stage of taxa is indicated with "Ad." for adult and "Imm." for immature. If not indicated, the collected life stage was an adult, except for Opiliones and Araneae, for which identified stages were combined.

[2]Total number of individuals captured across fields and dates for each year.

[3]Percent of total number of individuals (or egg/egg clusters) within each year.

Values for each date are totals for each field and then across fields. In 2010, each value is the summed total across all fields for 10 whole-plant counts in each field. In 2011, whole-plant counts I conducted whole-plant counts for 40 plants per field. I only counted larval Cecidomyiidae and Syrphidae plus eggs and larvae of Neuroptera and Coccinellidae in 2010. A "0" in the total column vs. a dash indicates a taxon was counted that year.

Table 2-3. **Predators and ants captured in pitfall traps across five fields in 2010 and three fields in 2011.**

		2010							2011					
Class/Order	**Family/Species[1]**	**6/29**	**7/12**	**7/25**	**8/10**	**8/22**	**Total[2]**	**%[3]**	**7/1**	**7/17**	**7/31**	**8/14**	**Total[2]**	**% [3]**
Coleoptera	**Carabidae**													
	Agonum cupripenne	-	-	0.07	-	-	**3**	**0.09**	-	-	0.03	-	**1**	**0.04**
	Agonum muelleri	-	0.04	0.04	-	-	**4**	**0.11**	-	-	-	-	-	-
	Anisodactylus sanctaecrucis	0.10	0.14	0.07	-	-	**15**	**0.43**	0.30	0.33	0.10	-	**22**	**0.78**
	Bembidion quadrimaculatum	1.58	0.48	0.43	0.04	0.02	**127**	**3.61**	0.43	-	0.07	0.03	**16**	**0.57**
	Chlaenius tricolor	0.75	0.50	0.02	0.08	-	**66**	**1.88**	0.13	0.07	0.07	-	**8**	**0.28**
	Cicindela punctulata	-	-	-	-	-	-	-	-	0.13	0.03	-	**5**	**0.18**
	Cyclotrachelus furtivus	-	0.02	0.04	0.02	-	**4**	**0.11**	-	-	-	-	-	-
	Dicaelus elongatus	0.26	0.06	0.13	0.02	0.02	**24**	**0.68**	-	-	0.07	-	**2**	**0.07**
	Diplocheila obtusa	0.12	0.04	-	-	-	**8**	**0.23**	0.03	-	-	0.03	**2**	**0.07**
	Harpalus affinis	0.02	0.02	-	-	-	**2**	**0.06**	0.03	0.03	-	0.03	**3**	**0.11**
	Harpalus pensylvanicus	0.58	0.84	0.89	1.82	2.26	**315**	**8.95**	10.2	2.83	16.8	3.93	**1015**	**36.0**
	Harpalus rubripes	0.06	-	-	-	-	**3**	**0.09**	-	-	-	-	-	-
	Poecilus chalcites	0.56	0.38	0.31	0.07	0.06	**97**	**2.76**	0.10	0.20	0.10	-	**12**	**0.43**
	Poecilus lucublandus	0.18	0.42	0.79	0.10	0.02	**74**	**2.10**	0.30	0.30	0.17	0.07	**25**	**0.89**
	Pterostichus melanarius	0.36	0.18	0.64	0.96	1.02	**147**	**4.18**	1.53	2.67	1.97	5.63	**354**	**12.6**
	Pterostichus spp.	-	0.02	0.02	0.02	-	**3**	**0.09**	-	-	-	-	-	-
	Carabid other Ad.	0.02	0.04	0.02	0.04	0.04	**11**	**0.31**	0.27	0.13	0.03	-	**13**	**0.46**
	Carabidae Imm.	0.16	0.02	0.04	0.06	0.03	**15**	**0.43**	0.07	0.10	0.07	-	**7**	**0.25**
	Coccinellidae: Imm	0.02	0.02	-	0.02	-	**3**	**0.09**	-	0.03	-	0.03	**2**	**0.07**
	Staphylinidae	1.75	0.90	1.44	0.93	0.25	**251**	**7.13**	0.70	1.20	1.43	0.40	**112**	**3.98**
Hemiptera	**Nabidae**													
	Nabis roseipennis Ad.	-	-	0.02	-	-	**1**	**0.03**	-	-	0.03	-	**1**	**0.04**
	Nabis roseipennis Imm.	-	-	0.02	0.04	-	**3**	**0.09**	-	-	-	-	-	-
	Nabis roseipennis Ad. + Imm.	-	-	0.04	0.04	-	**4**	**0.11**	-	-	-	-	**1**	**0.04**
Dermaptera	**Forficulidae:** *Forficula auricularia*	0.06	-	0.02	-	-	**4**	**0.11**	-	0.03	-	-	**1**	**0.04**
Opiliones	**Phalangiidae**	2.07	2.50	3.27	4.79	1.90	**677**	**19.2**	0.37	1.37	6.13	3.93	**354**	**12.6**
	Sclerosomatidae	-	-	-	-	0.13	**6**	**0.17**	-	-	-	-	-	-
Araneae	**Gnaphosidae**	0.06	-	-	0.02	-	**4**	**0.11**	-	-	-	-	-	-
	Linyphiidae	1.13	0.60	1.39	0.46	0.06	**170**	**4.83**	0.23	0.43	0.70	0.53	**57**	**2.02**
	Lycosidae													
	Allocosa spp.	-	0.04	0.02	-	-	**3**	**0.09**	0.43	-	0.13	-	**17**	**0.60**
	Pardosa spp.	1.54	3.46	2.14	0.61	0.45	**387**	**11.0**	0.87	6.00	3.17	0.47	**315**	**11.2**
	Pirata/Piratula spp.	0.10	-	-	0.02	-	**6**	**0.17**	-	-	0.07	-	**2**	**0.07**
	Schizocosa spp.	0.04	-	-	0.02	-	**3**	**0.09**	-	-	-	-	-	-
	Trochosa spp.	2.01	1.22	0.86	0.55	0.49	**250**	**7.11**	1.03	0.90	0.77	0.40	**93**	**3.30**
	UNID lycosid	0.12	0.24	0.24	0.22	0.11	**45**	**1.28**	0.13	0.03	0.20	0.13	**15**	**0.53**
	Oxyopidae	0.02	0.02	-	-	-	**2**	**0.06**	0.03	-	-	-	**1**	**0.04**
	Salticidae	0.04	-	-	0.04	-	**4**	**0.11**	0.03	-	-	-	**1**	**0.04**
	Thomisidae	0.02	-	-	0.04	-	**3**	**0.09**	0.07	-	-	-	-	-
	UNID Araneae	0.02	0.04	0.04	0.04	-	**7**	**0.20**	-	0.10	0.10	0.17	**11**	**0.39**
Chilopoda		2.10	0.18	0.22	0.21	0.06	**135**	**3.84**	0.50	0.60	0.20	0.27	**47**	**1.67**
Hymenoptera	**Formicidae**													
	Formica spp.	0.38	0.18	0.02	0.08	0.02	**34**	**0.97**	0.03	0.13	-	0.03	**6**	**0.21**
	Lasius spp.	1.96	0.50	0.53	0.36	-	**162**	**4.60**	0.27	0.93	0.33	0.20	**52**	**1.85**
	Myrmica spp.	-	0.06	0.02	0.02	0.02	**6**	**0.17**	0.03	-	0.03	0.03	**3**	**0.11**
	Ponera spp.	0.11	0.12	0.14	0.04	0.04	**20**	**0.57**	-	-	0.03	0.03	**2**	**0.07**
	Prenolepis imparis	0.14	-	0.13	0.10	0.12	**22**	**0.63**	0.03	0.10	0.07	0.63	**25**	**0.89**
	Solenopsis molesta	0.04	0.02	-	0.04	-	**5**	**0.14**	0.07	0.03	0.13	0.07	**9**	**0.32**
	Stennama spp.	0.59	0.60	0.63	0.12	-	**93**	**2.64**	0.93	0.37	0.33	0.13	**53**	**1.88**
	Tetramorium species-e	1.94	1.18	1.12	1.19	0.36	**279**	**7.93**	1.40	1.30	1.50	0.80	**150**	**5.33**
	Total arthropod	21.1	15.2	15.9	13.3	7.5	**3518**		20.6	20.4	34.9	18.0	**2816**	

[1]Life stage of taxa is indicated with "Ad." for adult and "Imm." for immature. If not indicated, the collected life stage was an adult, except for Opiliones and Araneae, for which identified stages were combined.

[2]Total number of individuals captured across fields and dates for each year.

[3]Percent of total number of individuals within each year.

Values for each date are means calculated first within each field and then across fields. Each value is number of individuals per trap per 72-hour sampling period. $N = 10$ for each field and sampling period except when traps were damaged.

Table 2-4. **Predators captured in blue and yellow pan traps across five fields in 2010 and three fields in 2011.**

		2010												2011									
		7/1		7/12		7/25		8/10		8/23				7/1		7/17		7/31		8/14			
Order	Family/species	B[4]	Y[4]	B	Y	B	Y	B	Y	B	Y	Total[2]	%[3]	B	Y	B	Y	B	Y	B	Y	Total[2]	%[3]
Coleoptera	**Coccinellidae**																						
	Coleomegilla maculata Ad.[1]	-	0.07	0.07	0.07	-	0.07	-	0.07	0.07	0.07	**7**	**0.7**	-	-	-	-	-	-	-	-	-	-
	Harmonia axyridis Ad.	0.07	0.13	-	0.13	0.33	0.40	-	0.47	0.20	0.40	**32**	**3.4**	-	-	-	-	-	-	-	0.33	**3**	**0.6**
	Hippodamia variegata Ad.	-	-	-	-	-	-	-	0.07	0.07	0.07	**3**	**0.3**	0.17	-	-	-	-	-	-	-	**1**	**0.2**
	Cycloneda munda Ad.	-	-	-	0.07	-	-	-	-	-	-	**1**	**0.1**	-	-	-	-	-	-	-	-	-	-
	Propylea quatuor-decimpunctata Ad.	-	0.47	-	0.20	-	0.20	0.27	0.27	-	0.13	**23**	**2.5**	-	-	-	0.50	-	-	-	-	**3**	**0.6**
	Staphylinidae	0.27	0.27	0.13	0.13	0.47	0.53	1.93	2.27	0.13	0.33	**97**	**10.4**	-	0.22	1.17	1.17	0.44	0.22	0.11	-	**23**	**4.9**
Hemiptera	**Anthocoridae:** *Orius insidiosus* Ad.	0.13	0.13	0.53	0.27	1.47	1.47	0.60	0.87	1.07	1.73	**124**	**13.2**	0.33	0.22	1.83	2.17	2.22	3.00	1.89	3.22	**122**	**26.2**
	Nabidae: *Nabis roseipennis* Ad.	-	-	-	-	-	-	-	0.13	-	-	**2**	**0.2**	-	-	-	-	-	-	-	-	**2**	**0.4**
Diptera	**Dolichopodidae**	0.40	4.00	0.20	2.27	0.07	1.53	0.20	2.67	0.40	8.60	**305**	**32.6**	0.11	3.56	0.33	2.00	0.11	7.89	0.11	8.00	**189**	**40.6**
	Syrphidae																						
	Toxomerus germinatus	0.13	0.20	1.27	1.80	0.27	0.40	0.13	0.07	-	0.07	**65**	**6.9**	-	-	-	0.50	-	0.22	-	0.11	**6**	**1.3**
	Toxomerus marginatus	2.40	3.33	2.13	3.07	0.33	0.87	0.20	0.33	-	0.40	**196**	**20.9**	1.06	1.39	0.33	2.17	0.33	0.44	-	0.11	**42**	**9.0**
	Toxomerus politus	-	-	0.07	0.07	-	-	0.13	0.33	-	0.13	**11**	**1.2**	-	-	-	-	-	-	-	-	-	-
	Sphaerophoria sp.	0.07	0.27	0.07	-	-	-	0.07	-	-	-	**7**	**0.7**	-	-	-	0.17	-	-	-	-	**1**	**0.2**
	Other Syrphidae	-	-	-	0.07	0.07	0.07	-	0.07	-	-	**4**	**0.4**	-	0.22	-	-	-	0.22	0.22	0.78	**13**	**2.8**
Araneae	**Phalangiidae**	-	-	-	-	0.07	0.07	0.07	0.13	-	0.07	**6**	**0.6**	-	-	-	-	0.11	0.22	-	-	**6**	**1.3**
	Salticidae	-	0.07	-	0.13	0.13	0.27	0.33	0.33	0.20	0.13	**24**	**2.6**	-	-	0.17	0.33	0.11	-	0.11	0.22	**24**	**5.2**
	Thomisidae	-	-	0.07	0.07	-	0.07	0.13	0.07	0.53	0.07	**15**	**1.6**	-	-	-	-	-	-	-	-	**15**	**3.2**
	Other Araneae	-	0.07	0.07	0.07	-	0.13	0.13	0.07	-	-	**8**	**0.9**	-	0.11	-	0.50	0.44	-	0.56	0.33	**8**	**1.7**
Neuroptera	**Chrysopidae:** *Chrysoperla* spp.	-	-	0.13	-	0.07	-	0.13	0.07	-	-	**6**	**0.6**	-	-	-	-	-	0.11	-	-	**6**	**1.3**
	Hemerobiidae: *Micromus posticus*	-	-	-	-	-	0.07	-	-	-	-	**1**	**0.1**	-	-	-	-	-	-	-	-	**1**	**0.2**
	Total arthropod	3.47	9.0	4.73	8.4	3.27	6.07	4.33	8.27	2.67	12.2	**937**		0.00	0.33	0.17	0.83	0.67	0.56	0.89	1.33	**465**	

[1]Life stage of taxa is indicated with "Ad." for adult and "Imm." for immature. If not indicated, the collected life stage was an adult, except for Opiliones and Araneae, for which identifiable stages were combined.

[2]Total number of individuals captured across fields and dates for each year.

[3]Percent of total number of individuals within each year.

[4]Pan traps consisted of either blue (B) or yellow (Y) bowls.

Values for each date are means calculated first within each field and then across fields, which only affects calculations when traps were damaged. Each value is number of individuals per trap per 72-hour sampling period. $N = 3$ for each field, color of pan trap, and sampling period.

Table 2-5. **Predators and ants captured with day and night sweep net sampling across five fields in 2010 and three fields in 2011.**

		2010												2011									
		6/29		7/13		7/26		8/9		8/24				6/29		7/13		7/31		8/11			
Order	Family/genus/species	D[5]	N[5]	D	N	D	N	D	N	D	N	Total[2]	%[3]	D	N	D	N	D	N	D	N	Total[2]	%[3]
Coleoptera	**Carabidae**																						
	Chlaenius tricolor	-	0.05	-	-	-	-	0.01	-	-	-	**3**	**0.1**	-	-	-	-	-	-	-	-	-	-
	Colliuris pensylvanica	-	0.05	-	0.33	-	-	-	0.01	-	-	**12**	**0.3**	-	-	-	-	-	0.02	-	-	**1**	**0.1**
	Lebia atriventris	-	-	-	0.04	-	-	-	-	-	-	**7**	**0.2**	-	-	-	-	-	-	-	-	-	-
	Lebia viridis	-	-	0.01	-	0.06	0.05	0.04	-	-	-	**1**	**0**	-	-	-	-	0.13	-	-	-	**2**	**0.2**
	Cantharidae: Imm.	-	-	-	-	-	0.01	-	0.35	-	0.06	**21**	**0.6**	-	-	-	-	-	-	-	-	-	-
	Coccinellidae																						
	Coccinella septempunctata Ad.	0.05	-	-	0.01	-	-	-	-	-	-	**3**	**0.1**	0.20	-	-	-	-	-	-	-	**2**	**0.2**
	Coleomegilla maculata Ad.	0.13	0.04	0.02	-	0.08	0.05	0.37	0.30	0.60	0.05	**65**	**1.8**	0.13	-	0.37	0.73	0.28	0.08	0.42	0.02	**38**	**2.9**
	C. maculata Imm.	-	0.04	-	-	0.04	-	0.14	0.90	0.92	-	**81**	**2.2**	-	-	-	-	-	-	-	-	-	-
	Cycloneda munda Ad.	-	-	-	-	-	0.04	0.01	0.09	0.04	0.01	**7**	**0.2**	0.10	-	-	-	0.07	-	-	-	**2**	**0.2**
	Harmonia axyridis Ad.	0.21	0.11	0.35	0.33	0.48	0.71	0.75	0.56	0.37	0.18	**171**	**4.6**	0.10	-	-	-	-	0.02	0.10	-	**5**	**0.4**
	Harmonia axyridis Imm.	-	-	-	-	0.01	0.05	0.76	1.65	2.10	0.79	**194**	**5.3**	-	-	-	-	-	-	-	-	-	-
	Hippodamia parenthesis Ad.	-	-	-	-	0.04	-	0.08	-	-	-	**3**	**0.1**	0.10	-	-	-	-	-	-	-	**1**	**0.1**
	Hippodamia variegata Ad.	-	-	-	-	-	-	-	-	0.11	0.05	**7**	**0.2**	-	-	-	-	-	-	-	-	-	-
	Propylea quatuordecimpunctata Ad.	0.77	0.30	1.00	0.36	0.58	0.23	0.52	0.35	0.58	0.31	**188**	**5.1**	0.23	0.40	0.35	0.13	0.23	0.20	0.12	0.17	**34**	**2.6**
	Propylea quatuordecimpunctata Imm.	-	-	-	-	0.08	0.04	0.16	0.10	0.16	-	**18**	**0.5**	-	-	-	0.07	-	-	0.08	-	**6**	**0.5**
	UNID coccinellid Imm	-	-	-	-	0.01	0.01	0.26	0.09	0.13	0.04	**18**	**0.5**	-	-	-	-	-	-	-	-	-	-
	Melyridae: *Collops*	0.03	-	-	-	-	-	-	-	-	-	**3**	**0.1**	-	-	-	-	-	-	-	-	-	-
	Staphylinidae	0.09	0.04	-	0.04	-	0.18	0.08	0.33	0.04	0.18	**32**	**0.9**	0.10	0.10	0.13	-	-	0.12	-	-	**8**	**0.6**
Hemiptera	**Anthocoridae**																						
	Orius insidiosus Ad.	0.66	0.17	1.43	0.28	2.90	0.37	2.81	1.34	1.25	-	**422**	**11**	3.43	0.60	4.58	0.63	8.72	1.40	3.15	1.40	**481**	**36**
	Orius insidiosus Imm.	-	-	-	-	-	-	0.19	0.18	0.29	0.13	**28**	**0.8**	-	-	0.73	0.08	0.22	-	0.17	0.20	**33**	**2.5**
	Berytidae: *Jalysus* spp.	-	-	-	-	-	-	0.08	0.08	-	-	**4**	**0.1**	-	-	-	-	-	-	-	-	-	-
	Geocoridae *Geocoris*	-	-	-	0.04	-	-	-	-	-	-	**5**	**0.1**	-	-	-	-	-	-	-	-	-	-
	Nabidae																						
	Nabis americoferus Ad.	0.15	0.25	0.33	0.36	0.32	0.40	0.60	0.67	0.20	0.44	**138**	**3.7**	0.03	-	0.87	0.58	0.32	0.50	0.22	0.30	**60**	**4.5**
	Nabis americoferus Imm.	-	0.02	0.35	0.35	0.49	0.32	0.53	0.89	0.24	0.19	**119**	**3.2**	-	-	-	-	0.02	0.02	0.07	0.37	**10**	**0.8**
	Nabis roseipennis Ad.	0.07	0.06	0.34	0.31	0.13	0.44	0.20	0.49	0.03	0.19	**85**	**2.3**	0.43	0.03	0.07	0.02	0.20	-	0.02	0.08	**14**	**1.1**
	Nabis roseipennis Imm.	-	0.04	-	-	-	0.11	-	1.53	0.60	2.23	**142**	**3.8**	-	-	0.08	-	-	0.08	0.22	0.20	**11**	**0.8**
	Pentatomidae: *Podisus sp.*	-	-	-	-	-	0.04	-	0.01	0.01	-	**3**	**0.1**	-	-	-	0.10	0.07	-	-	0.07	**8**	**0.6**
	Reduviidae	-	-	-	-	-	-	-	0.04	-	-	**6**	**0.2**	-	-	-	-	-	-	-	-	-	-
Neuroptera	**Chrysopidae**																						
	Chyrysopa oculata Ad.	-	-	-	-	0.04	-	-	-	-	0.08	**3**	**0.1**	-	-	0.02	-	-	-	-	-	**1**	**0.1**
	Chrysoperla spp.	0.04	-	-	-	-	-	0.04	0.05	-	-	**4**	**0.1**	-	-	-	-	-	-	-	-	-	-
	Chrysopidae Imm.	-	-	-	-	0.01	-	0.05	-	-	-	**4**	**0.1**	-	-	-	-	-	-	-	-	-	-
	Hemerobiidae																						
	Micromus posticus	-	-	-	-	-	0.04	-	0.04	-	0.04	**3**	**0.1**	-	-	-	-	-	0.02	-	-	**1**	**0.1**
	Micromus subanticus	-	-	-	0.04	-	0.01	-	0.05	0.01	0.01	**6**	**0.2**	-	-	-	-	0.08	0.03	-	0.02	**5**	**0.4**
	Hemerobiidae Imm.	-	-	-	0.01	0.01	0.02	0.09	0.43	0.20	-	**28**	**0.8**	-	-	-	-	-	-	-	0.07	**1**	**0.1**
Dermpatera	**Forficulidae:** *Forficula auricularia*	-	0.41	0.04	0.07	-	0.73	-	0.42	0.04	0.91	**82**	**2.2**	0.40	0.53	-	0.93	-	0.87	0.07	1.27	**78**	**5.9**

(Continued on next page)

Table 2-5. **cont.**

		2010												2011									
		6/29		7/13		7/26		8/9		1/0				0		0.06667		0.01667		0			
Order	Family/genus/species	D[5]	N[5]	D	N	D	N	D	N	D	N	Total[2]	%[3]	D	N	D	N	D	N	D	N	Total[2]	%[3]
Diptera	**Dolichopodidae**	0.38	1.44	0.34	0.04	0.53	0.41	0.37	0.67	0.11	0.24	**156**	**4.2**	-	-	0.03	0.02	0.07	0.02	0.08	0.08	**9**	**0.7**
	Syrphidae																						
	Allograpta obliqua	-	-	-	-	-	-	0.04	-	-	-	**1**	**0**	-	-	0.07	0.07	0.02	-	-	-	**9**	**0.7**
	Toxomerus germinatus	0.05	-	-	-	0.04	-	-	-	-	-	**3**	**0.1**	-	-	0.07	0.07	0.02	-	-	-	**3**	**0.2**
	Toxomerus marginatus	0.28	0.14	-	0.04	0.04	0.08	0.04	0.05	-	-	**22**	**0.6**	0.10	0.78	-	-	-	0.02	-	-	**15**	**1.1**
	Syrphidae other Ad.	-	-		-	-		-	-	0.1	-	**3**	**0.1**	-	-	-	-	0.02	-	-	-	1	0.1
	Syrphidae Imm.	-	-	-	-	0.04	-	0.04	3.33	-	0.01	**93**	**2.5**	-	-	-	-	-	-	-	-	-	-
Opiliones	**Phalangiidae**	0.03	0.30	0.81	0.92	0.30	1.26	0.33	0.62	0.40	0.23	**181**	**4.9**	1.33	0.25	0.13	0.23	0.17	0.25	-	0.82	**48**	**3.6**
	Sclerosomatidae	0.05	0.04	-	-	0.21	0.01	0.46	0.05	-	-	**28**	**0.8**	-	-	-	-	-	-	0.02	-	**1**	**0.1**
Araneae	**Araneidae**	0.06	0.12	0.16	-	0.04	0.19	0.10	0.05	0.12	0.01	**31**	**0.8**	-	-	0.27	0.02	0.15	0.13	-	0.17	**14**	**1.1**
	Clubionidae	-	0.17	-	0.12	-	0.41	-	0.27	-	0.11	**36**	**1**	-	0.43	-	0.07	0.02	0.57	-	0.40	**23**	**1.7**
	Linyphiidae	0.36	0.09	-	0.04	0.20	0.08	-	0.08	0.02	0.04	**31**	**0.8**	1.60	0.23	0.55	0.13	0.05	0.13	0.02	-	**42**	**3.2**
	Lycosidae																						
	Pardosa spp.	0.02	0.11	-	0.12	0.33	0.47	0.23	0.23	0.32	0.57	**84**	**2.3**	-	0.03	0.25	0.20	0.32	0.92	0.23	0.83	**55**	**4.2**
	Other Lycosidae	-	0.08	-	-	-	0.04	-	0.1	0.08	-	**14**	**0.4**	-	-	0.28	-	0.35	0.07	0.08	-	**14**	**1.1**
	Oxyopidae	-	-	-	0.02	0.30	0.22	0.13	0.35	0.09	0.12	**48**	**1.3**	-	-	-	-	0.13	-	0.23	-	**7**	**0.5**
	Salticidae	0.28	0.04	0.26	0.04	0.41	0.25	0.44	0.21	0.19	0.15	**83**	**2.2**	-	-	0.10	-	0.58	0.13	0.45	0.13	**33**	**2.5**
	Tetragnathidae	0.48	0.18	0.48	0.35	0.29	0.31	0.47	0.22	0.19	0.15	**126**	**3.4**	-	-	0.02	-	0.15	0.13	0.30	0.48	**48**	**3.6**
	Thomisidae	0.14	0.20	0.21	-	0.38	0.39	1.37	1.57	1.48	1.56	**262**	**7.1**	0.03	0.03	0.02	-	0.48	0.20	0.43	1.05	**40**	**3**
	UNID small Araneae	0.49	0.20	0.98	0.05	0.36	0.06	0.14	0.06	0.04	0.12	**94**	**2.5**	-	-	0.17	0.02	0.58	0.15	0.25	0.08	**27**	**2**
	Other Araneae	-	-	0.05	0.04	-	0.01	-	0.09	0.12	0.05	**12**	**0.3**	-	-	0.15	0.07	0.07	-	-	0.07	**6**	**0.5**
Hymentoper	**Formicidae**																						
	Brachymyrmex	-	-	0.04	-	0.04	-	-	0.09	-	-	**5**	**0.1**	-	-	-	-	-	-	-	-	-	-
	Formica	0.16	0.08	-	-	0.06	-	-	-	0.04	0.04	**11**	**0.3**	-	-	-	-	-	-	-	-	-	-
	Lasius sp.	0.09	0.17	0.23	0.01	0.02	0.54	0.22	1.13	0.13	0.12	**98**	**2.7**	-	-	-	-	-	0.33	0.10	-	**8**	**0.6**
	Prenolepis imparis	0.38	0.99	0.25	1.16	0.46	0.91	0.10	1.74	0.84	2.83	**336**	**9.1**	0.23	0.10	0.02	0.62	0.07	0.62	0.80	2.87	**111**	**8.4**
	Solenopsis molesta	-	0.01	0.04	-	-	-	-	0.15	-	-	**8**	**0.2**	-	-	-	-	-	-	-	-	-	-
	Tetramorium species-e	0.01	0.04	-	-	0.04	0.05	-	-	-	0.08	**8**	**0.2**	0.10	-	-	-	-	0.02	0.07	-	**3**	**0.2**
	Total arthropod	5.7	6.4	8.7	6.6	10.4	10.9	13.6	25.6	13.2	15.4	**3690**	**100**	9.4	3.7	9.5	4.9	13.9	7.0	7.5	10.7	**1319**	

[1]Life stage of taxa is indicated with "Ad." for adult and "Imm." for immature. If not indicated, the collected life stage was an adult, except for Opiliones and Araneae, for which stages were combined if possible.

[2]Total number of individuals captured across fields and dates for each year.

[3]Percent of total number of individuals within each year.

[4]Sweep samples were collected during the day (D) or at night (N), (9:30-5:00 for day and 21:00-24:00 for night) samples)

Values for each date are means calculated first within each field and then across fields. Each value is number of individuals per 20 sweeps. $N = 5$ for each field, sampling method, and sampling period combination. One of the five sweep samples consisted of 80 sweeps, which I standardized to individuals per 20 sweeps for calculations of means, but not for total number of individuals.

Table 2-6. **Taxa within each farm most responsible for differentiating predator communities by sampling time based on SIMPER analyses.**

Year	Farm	Taxon[1]	%Contrib.[2]	Day/Night[3]
2010	1	*Prenolepis imparis*	15.0	Night
		Orius insidiosus Ad.	11.2	Day
		Nabis roseipennis Imm.	8.9	Night
		Propylea quatuordecimpunctata Ad.	7.4	Day
		Clubionidae	7.4	Night
	2	*Orius insidiosus* Ad.	19.1	Day
		Propylea quatuordecimpunctata Ad.	7.7	Day
		Thomisidae	7.4	Night
		Coleomegilla maculata Ad.	6.0	Day
		Salticidae	5.3	Day
	3	**Syrphidae:** Imm.	20.7	Night
		Orius insidiosus Ad.	12.3	Day
		Dolichopodidae	5.7	Night
		Thomisidae	5.4	Day
		Prenolepis imparis	5.3	Night
2011	4	**Phalangiidae**	12.4	Night
		Nabis americoferus Imm.	11.7	Day
		Orius insidiosus Ad.	9.0	Day
		Nabis roseipennis Imm.	8.8	Night
		Tetragnathidae	8.0	Day
	5	*Propylea quatuordecimpunctata* Ad.	22.0	Night
		Orius insidiosus Ad.	13.3	Day
		Forficulidae: *Forficula auricularia*	12.2	Night
		Nabis roseipennis Imm.	8.6	Night
		Lasius spp.	5.2	Night
	6	*Orius insidiosus* Ad.	28.5	Day
		Prenolepis imparis	18.6	Night
		Coleomegilla maculata Ad.	8.0	Day
		Thomisidae	4.6	Day
		Nabis americoferus Ad.	4.0	Night
	7	*Orius insidiosus* Ad.	44.5	Day
		Pardosa spp.	6.8	Night
		Forficulidae: *Forficula auricularia*	5.0	Night
		Prenolepis imparis	4.9	Night
		Toxomerus marginatus	4.4	Night
	8	*Orius insidiosus* Ad.	34.9	Day
		Forficulidae: *Forficula auricularia*	14.6	Night
		Linyphiidae	4.7	Day
		Phalangiidae	4.7	Night
		Tetragnathidae	4.6	Day

[1]Taxa are ranked by decreasing order of percent contribution to community difference between sampling time until their percent contribution to community difference reached at least 60%. $N = 10$ for each field, divided between sampling times. The five most important taxa are listed for each field.

[2]Percent contribution of each taxa to community differences between sampling times within each field.

[3]Time of sampling (see text for sampling methods).

Table 2-7. **Taxa selected within each field as characteristic of the natural enemy community sampled via either day or night sweep netting through IndVal analysis, with their respective indicator values.**

Day/Night	Taxon	Field[1]	IndVal[2]
Day	*Orius insidiosus* Ad.	1, 5	0.92*, 0.97**
	Sclerosomatidae	3	0.94**
	Linyphiidae	7, 8	0.86*, 0.93*
	Salticidae	7	0.93*
	Araneae UNID small	2, 8	0.89*, 0.86*
	Oxyopidae	1	0.89*
	Lycosidae UNID	8	0.89*
	Orius insidiosus Imm.	7	0.88*
Night	*Forficula auricularia*	3, 5, 8	1.00**, 0.99**, 0.96**
	Clubionidae	1, 7, 8	1.00**, 0.89*, 0.89*
	Pardosa spp.	5	1.00**
	Syrphidae: Imm.	3	0.99**
	Prenolepis imparis	1, 3	0.97**, 0.93*
	Nabis roseipennis Imm.	1, 4, 5	0.93*, 0.94*, 0.97*
	Lasius spp.	2, 3	0.89*, 0.94*
	Cantharidae: Imm.	2	0.89*
	Toxomerus marginatus Ad.	7	0.89*

[1]Listing of multiple fields indicate the taxa was identified as an indicator taxa for multiple fields. Fields 1-5 were sampled in 2010, 6-8 were sampled in 2011. $N = 10$ for each field, divided between sampling times.

[2]Indicator values of multiple fields indicate the taxa was identified as an indicator taxa for multiple fields via separate analyses. Fields 1-5 were sampled in 2010, 6-8 were sampled in 2011.

Figures

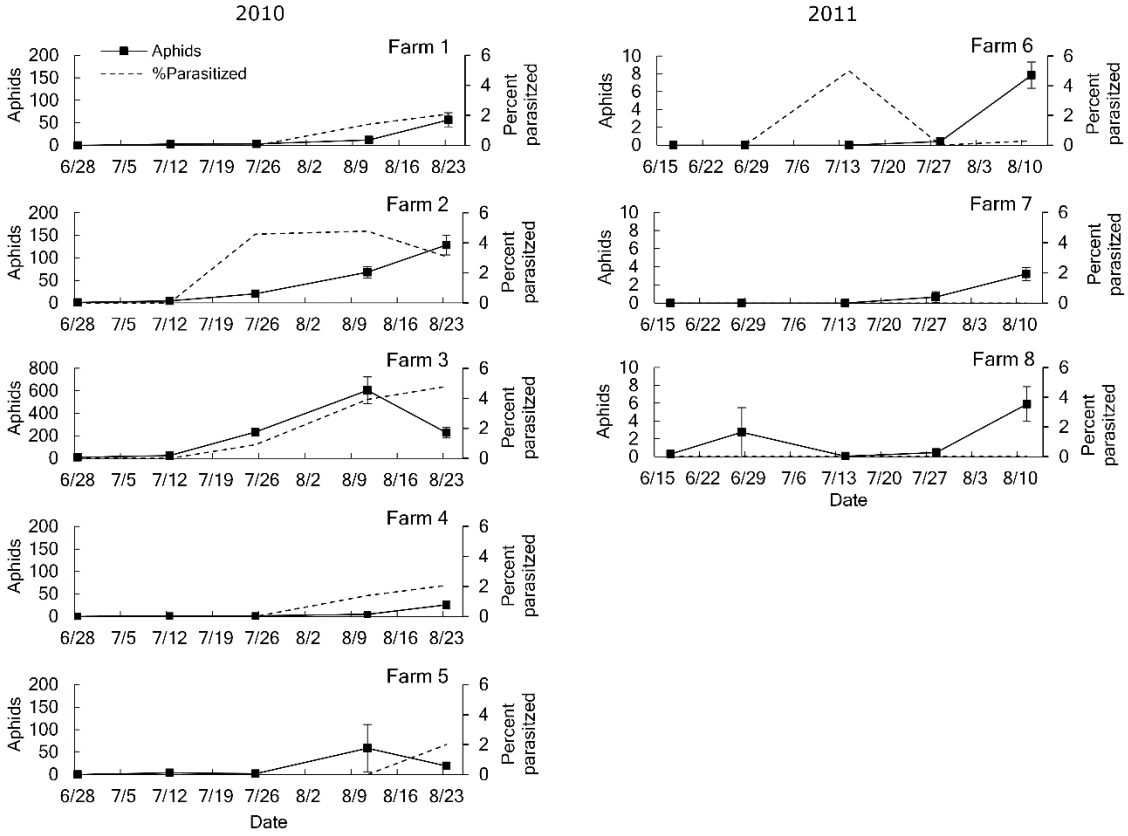

Figure 2-1. **Soybean aphid populations and percent parasitism in five fields in 2010 and in three fields in 2011.** I assessed aphid populations with whole plant counts. *N* = 10 plants per field in 2010 and *N* = 20 in 2011. Percent parasitism was measured by field counts of mummified aphids, all of which were to due parasitism by aphelinid parasitoids (Hymenoptera: Aphelinidae). Farm 3 in 2010 hosted much higher aphid populations, so the axis for aphid abundance is scaled differently. Error bars for aphids represent ± 1 SE and are not shown for parasitism rates, which were overall low and highly variable among samples.

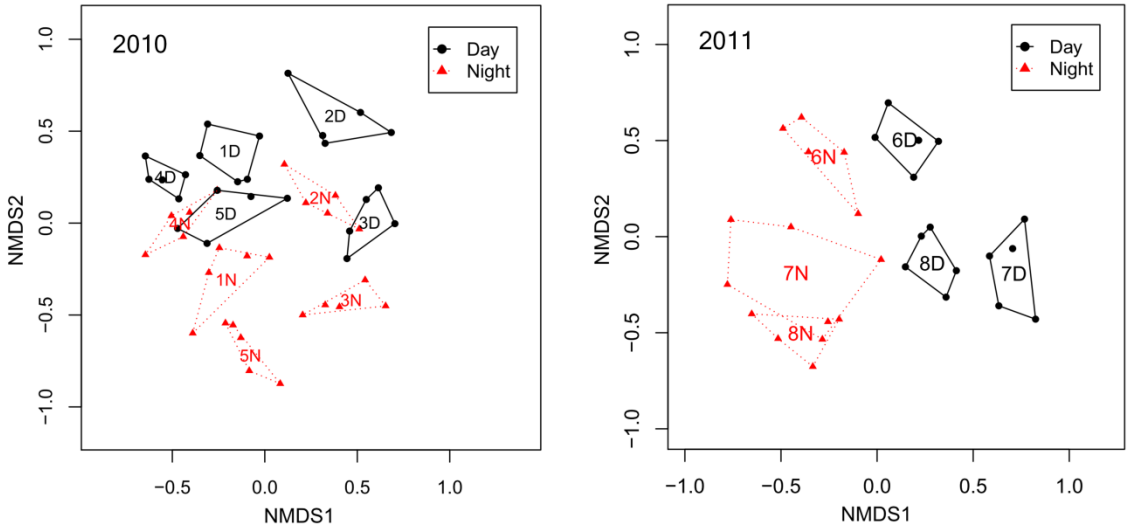

Figure 2-2. **NMDS ordinations of the predator communities sampled in different fields with day and night sweep netting**. Numbers identify the field number and the accompanying letter corresponds to either day (D) or night (N) samples. Hulls connect replicates within a field and sampling time. The Bray-Curtis distance was used as the distance measure (k=4 each year). Predator abundance was summed across sampling time points (N = 5 for each combination of field and sampling time).

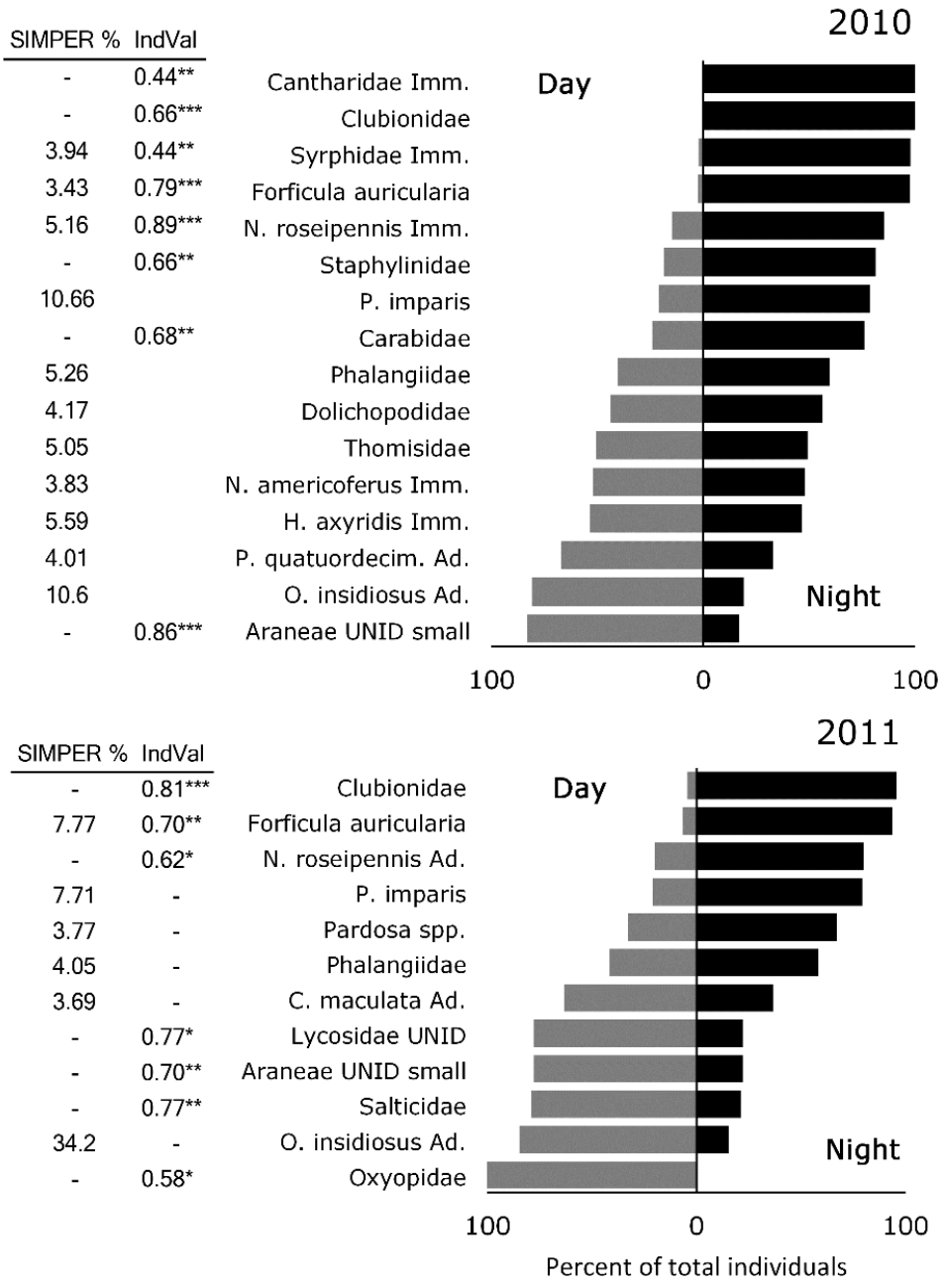

Figure 2-3. **Percentage of different taxa caught during the day and night with a sweep net and their percent contribution to the overall dissimilarity (%SIMPER) between communities based on a SIMPER analysis, as well as their indicator value from an IndVal analysis (IndVal).** Indicator values had a maximum of 1 (complete indicator). Taxa presented are all taxa for a given year that contributed to the first 60% of the differences between sampling times based on the SIMPER analysis across farms or which the IndVal statistics was significant in an IndVal analysis across farms. Abundances for taxa were summed across dates. $P < 0.05$ = *, < 0.01 = **, < 0.001 = ***.

Chapter 3

Effects of soybean variety mixtures on yield, herbivore and natural enemy populations, and predation.

Abstract

The soybean aphid is an invasive pest species that has forced growers to increase use of insecticides across the Midwestern U.S. Therefore, there is a clear need for alternative and more sustainable control methods. Mounting evidence suggests that increasing within-species (or genotypic) crop diversity by planting mixtures of varieties is a promising tactic for managing herbivorous pests. I conducted a two-year field experiment with soybeans and soybean aphid as a model system to measure the top-down and bottom-up ecological consequences of increasing plant genotypic diversity in crop fields. Soybean aphid populations are often suppressed by both diurnal and nocturnal predators, but aphid populations can also be influenced by aphid-tending ants. Specifically, I evaluated if variety mixtures influence soybean aphid populations, the associated arthropod community, predation, and crop yield. I compared monocultures of six soybean varieties to all six possible five-variety mixtures using 30 × 30 ft plots. I evaluated aphid, potato leafhopper, predator, and ant populations, measured predation services, and assessed yield. Sampling for non-aphid arthropods, as well as measurements of predation services were divided into day and night components. Over the course of the study, aphid populations remained low, and I found that genotypic diversity did not alter season-long aphid pressure or yield, but did produce intermediate yield and aphid populations. Crop genotypic diversity increased leafhopper populations, likely because of non-additive interactions involving one highly susceptible or attractive variety, but populations did not reach economically damaging levels. Genotypic diversity did not influence ant-tending aphids. In both years, predators helped suppress aphid

populations, and predator abundance and predation was influenced by genotypic diversity during specific parts of the season and for some sampling times, but results were mixed and inconsistent. Of the most abundant taxa, genotypic diversity most strongly influenced abundance of *H. axyridis* and total lady beetles in different years of the study, with distinct patterns evident during day and night. Overall, results from this study demonstrated that variety mixtures do not produce clear benefits under low pest pressure. Mixtures did appear to produce intermediate aphid populations and yield, and also influenced other herbivore species which could influence natural-enemy control of soybean aphids. Effects on herbivores and predators could become more important under high pest pressure. My study also provides some evidence that plant genotypic diversity in crop fields can influence higher trophic levels, paralleling work in natural systems that has demonstrated system-wide consequences of diversity.

Introduction

Prior to 2000, insect pest populations in soybeans in the northern U.S. rarely required control with insecticides. Introduction of soybean aphid (*Aphis glycines* Matsumura) in 2000, however, drastically altered insect pest management (Ragsdale et al. 2011). Because of the soybean aphid, the proportion of land treated with foliar insecticides, primarily organophosphate and pyrethroid insecticides, rose to 13% of total acreage by 2006 (Ragsdale et al. 2011). Between 2004 and 2007, growers from Michigan, Minnesota, Iowa, and Wisconsin treated 13 to 89% of their fields for aphids, and those treating their fields applied insecticides to 43-87% of their acreage (Olson et al. 2008). This chemical management was necessary because unmanaged soybean aphids can decrease yield by up to 40% (Ragsdale et al. 2007)

Management strategies founded on principles of integrated pest management (IPM) can reduce insecticide use and management costs (Ragsdale et al. 2007, Johnson et al. 2009); however, insecticides remain the primary tool for managing soybean aphids. Therefore, developing alternative tactics that suppress aphid populations would help reduce reliance on insecticides.

Across the U.S., a diverse community of arthropod predators consume soybean aphids and can significantly affect their populations (Fox et al. 2004, 2005, Costamagna and Landis 2006, Desneux et al. 2006). Predators can drive down aphid populations (Costamagna and Landis 2006), whereas ants that form mutualistic associations with soybean aphids may protect aphid populations from predators, although their influence is not well-understood (Herbert and Horn 2008, Schwartzberg et al. 2010, Ragsdale et al. 2011). At the landscape level, higher levels of landscape diversity increase regional natural enemy populations, improving biological control of soybean aphid and yield (Landis et al. 2008, Gardiner et al. 2009a, 2009b). Individual growers, however, cannot usually manage this type of diversity because it is driven by broad, landscape-level changes (Landis et al. 2008). Within fields, host plant resistance may help manage soybean

aphids and growers can readily take advantage of this management tactic. Aphid-resistance genes, or *Rag* (Resistance to *Aphis glycines*) genes can suppress soybean aphid populations (Hill et al. 2012, Wiarda et al. 2012), but presence of virulent aphids biotypes that can feed upon resistant varieties already threaten sustainability of these resistant varieties (Hesler et al. 2013, Crossley and Hogg 2015); therefore, opportunities remain to develop new tools for soybean aphid control.

By increasing crop genotypic diversity, variety mixtures hold promise for improving insect pest management (Tooker and Frank 2012), and may provide another option for managing soybean aphid. A large body of evidence has demonstrated that crop genotypic diversity can effectively limit damage caused by plant pathogens (Finckh et al. 2000, Zhu et al. 2000, Mundt 2002), and more recent research with non-crop plant species has established that genotypic diversity can help suppress herbivore populations and reduce herbivory (Parker et al. 2010, McArt and Thaler 2013, Barton et al. 2014). A more limited body of evidence suggests that crop genotypic diversity may have similar effects in production fields (Cantelo & Sanford 1984; Power 1988, 1991; Glinwood, Ninkovic & Pettersson 2011) reducing reliance on insecticides without disrupting conventional crop production practices (Grettenberger & Tooker 2015 [Chap 6]). How plant genotypic diversity influences arthropod communities in agroecosystems is not well understood (Wilhoit 1992), but several lines of evidence suggest that genotypic diversity improves bottom-up and top-down control of herbivore populations (Andow 1991, Letourneau et al. 2011, Tooker and Frank 2012). With relatively few studies having explored the value of cultivar mixtures, there is still a need to better explore the potential role of crop genotypic diversity for insect pest management, especially for large-acreage crops such as soybeans.

I conducted a field study across two seasons to determine if variety mixtures of soybeans can: 1) reduce populations of soybean aphid or other herbivores, 2) affect potential aphid predators and the predation services they provide, and 3) improve soybean productivity. In addition, I conducted a separate small-scale field experiment to evaluate if increasing crop

genotypic diversity attracts predators and improves predation compared to monocultures. I hypothesized that compared to genotypic monocultures, genotypically diverse variety mixtures would decrease herbivore populations, increase predator populations, and improve predation and soybean yield. .

Methods

Varieties and diversity treatments

To test the pest management benefits of soybean variety mixtures, I first chose six varieties to use in the experiment (Table 1). Aphid resistance information was not available for commercially available soybean varieties, so I chose varieties based on information available on their phenotypic differences (height, maturity, and resistance to disease). No varieties chosen contained known aphid resistance genes because Rag genes were not yet widely available when varieties were selected in 2010. All varieties I selected were in maturity group III, which encompassed maturities of soybeans typically planted in PA. I also chose varieties from five seed companies, and one publicly developed variety (Variety A), to broaden the underlying genetic diversity in the mixtures. All varieties were glyphosate-resistant to facilitate conventional weed management with herbicides. Low diversity treatments consisted of monocultures of each of the chosen varieties. High diversity treatments consisted of all possible five-variety mixtures formed from the pool of six varieties (Table 1). The size of the variety pool and number of varieties in the mixture provided a moderately high level of diversity in the mixtures compared to other studies addressing how crop genotypic diversity affects herbivores (Power 1988, 1991, Ninkovic et al. 2011, Shoffner and Tooker 2013). Seeds were hand-mixed in equal volumes before planting. This produced approximately a 1:1:1:1:1 ratio of the five varieties in each mixture.

Site, field, and plot descriptions

The study was conducted across two years in adjacent fields at Penn State's Russell E. Larson Agricultural Research Center. Corn grain preceded soybeans in these fields, which were not tilled for >3 years prior to use. Fields were approximately 1.5 ha (2010: 1.66 ha; 2011: 1.40 ha). Fields were longer than wide (2010: 262 × 62 m; 2011: 264 × 59 m; Fig. 1). The fields were planted with oats (*Avena sativa* L.) in mid-spring, and soybean plots were planted into the matrix

of oats (5 June, 2010 and 31 May, 2011). The oats matrix provided separation between plots, allowing herbivore and predator populations to develop more independently within each plot than if they were not separated. In 2010, several plots were damaged by groundhogs (*Marmota monax* L.). Four that were significantly damaged were mechanically replanted two weeks after the initial planting, while two more had small sections of rows replanted by hand. All replanted sections were hand-watered to facilitate establishment. Plots were arranged in a randomized complete block design with five blocks. Each block contained one replicate of each monoculture (six monocultures) and one replicate of each of the six mixtures for a total of 30 low diversity and 30 high diversity plots (total $N = 60$). Treatments were randomized within blocks. Plots measured 9.1 × 9.1 m and were separated from each other by 4.6 m. All plots were separated from the area surrounding the field (corn field or grass strip) by at least 4.6 m. To kill the oats, each plot was sprayed with glyphosate 1-2 weeks before planting. Each plot comprised 12 rows, which were planted 0.76 m apart. I performed all experimental activities in the middle eight rows of the plots to avoid strong edge effects. The surrounding oats were allowed to mature and dry down, at which point they were harvested (Jul. 23, 2010 and Aug. 2, 2011), leaving stubble between plots. All plots were sprayed with glyphosate at the end of July to control weeds.

Herbivore populations

I counted aphids in the middle four rows of each plot to assess aphid populations. I performed nondestructive whole-plant aphid counts on five plants located a minimum of 2 m from the edge of the plot in 2010 and on ten plants in 2011 when aphid populations were lower. I counted parasitized aphids, or aphid mummies, when counting aphids. I began counting aphids when plants reached the V1 growth stage (23 June 2010, 22 June 2011), although populations remained near zero for several weeks. I ceased aphid sampling when all plots reached the R6 growth stage (8 September 2010, 30 August 2011), the point when the current economic

threshold for soybean aphid is no longer relevant (Ragsdale et al. 2007). Aphid levels were converted to cumulative aphid-days (CAD), which is a measure of seasonal aphid abundance that represents the area under the aphid population curve and provides a single value for aphid populations over the course of the season (Ragsdale et al. 2007).

To determine how genotypic diversity influenced another herbivore species, I also assessed adult potato leafhopper (*Empoasca fabae*, Homoptera: Cicadellidae) populations when plots were sampled for natural enemies (see below). Briefly, I collected sweep net samples of arthropods that contained *E. fabae* and stored them in 80% ethanol for identification. Potato leafhoppers are a common herbivore in soybeans, although they rarely reach economically damaging levels.

Predator and ant sampling

To determine if crop genotypic diversity affected predators or ant mutualists of the soybean aphid, I sampled plots with a sweep net, a commonly used and effective method for sampling predators of the soybean aphid in the foliage (Schmidt et al. 2008, Bannerman et al. 2015). I collected samples during both day and night to capture diurnal and nocturnal predators, expecting that predators that feed upon soybean aphids at different times of the day may respond differently to increased diversity within crop fields. Day-only sampling or measuring predation services across the diel cycle would miss this distinction between different components of the predator community. Night predators of the soybean aphid are not well understood, but night-active predators do feed on soybean aphids (Petersen and Woltz 2014).

To avoid interfering with plants that I used for aphid counting, I sampled the third and fourth rows in on each side of the plots. The south side was used for day sampling while the north side was used for night sampling. I collected day sweep samples between 9:30 and 13:00. I only collected sweep samples after dew on leaves had dried and on days when it did not rain. I

collected night sweep samples between 21:00 and 24:00. For each sample, I walked in the middle of the two rows and swept the middle 7.1 m (1 m from the ends of the plot) of the rows using 15 figure-eight pendulum sweeps. I repeated this twice (30 figure-eight sweeps total) and sampled the same portion of the rows as the first 15 sweeps. Samples for each plot were inverted into a bag constructed from organdy (Quick Service Textile Industries, Chicago, IL). I collected five samples in 2010 (6/8 July, 20/21 July, August 3/4, August 17 and August 29) and four samples in 2011 (7 July, 21 July, 4 August 4, and 24 August). Day and night sampling at times were not conducted on the same date because of weather or logistical constraints. All potential natural enemies were identified to the lowest taxonomic level possible and counted (family to species for predators, genus or species for ants).

To better estimate minute pirate bug (*Orius insidiosus* Say, Hemiptera: Anthocoridae) populations in 2011, I performed timed beat-tray samples in each plot. I sampled on 4 August, which was close to when *Orius* populations were at their highest levels. Sampling techniques that involve beating sections of plants are effective ways to sample adult *Orius* (Bechinski and Pedigo 1981). I collected four samples within each plot and within the middle four rows (rows not used for sweep sampling). To collect a sample, I held a 52 × 40 × 8 cm plastic pan on one side of a soybean row and 0.4 m off the ground. Another person then gently bent over 50-55 cm of the row of soybeans and beat this portion of the row with the end of a measuring stick for 1 minute. I counted all adult *Orius* that fell into the tray and then summed across beat samples to calculate total adults per plot.

Predation: Exclusion cages

To measure predation services within each plot, I added aphids to focal plants in each plot and exposed aphids to different components of the predator community. Each trial lasted two weeks and I conducted one trial during each year during the middle of the season when the added

populations of aphids would approximate field levels. The trial in 2010 began on 26 July and on 27 July in 2011. In each year, there were five treatments: total predator exclusion (cage), day predators (day), night predators (night), sham cage (sham), and uncaged control (uncaged). The five treatments were randomly assigned to the following plants in each plot: two plants each to the rows that were five rows in from each side of the plot and separated by 4 m and to one plant at one end of a middle row, 2 m from the end of the row. Because cages were in the middle four rows, they did not impede predator sampling. At each of the five locations, I randomly chose a plant of average height for the plot to serve as the focal plant. Plants in the same row within 20 cm of the focal plant were cut at ground level two to three days before the start of the trial. All treatments except the uncaged control were enclosed by a wire tomato cage. I placed organdy sleeves over the tomato cage to prevent predators from accessing the focal plant. To press the bases of the sleeves to the ground, I constructed long and thin sandbags from 30 gallon black plastic garbage bags filled with soil and closed with duct tape. I wrapped these sandbags around the base of the tomato cage to hold down the mesh and prevent natural enemies from moving onto the foliage. "Full exclusion cages" were permanently covered by the mesh sleeves. The "day cage" gave predators access to the focal plant during the day. Every morning at 7:30, I lifted, rolled, and secured the mesh at the top of the cage. At 19:30, I lowered the mesh and secured it with the sand bag. The "night cage" gave predators access to focal plants during the evening, so I raised their mesh sleeves at 19:30 and lowered them at 7:30. Sham cages comprised a wire cage with the mesh permanently secured at the top of the cage allowing full predator access, controlling for the effect of the wire cage and mesh. A focal plant without any cage in both years comprised an "uncaged" control and was only used in three of the five blocks (n = 36 each year) to decrease an already large sampling effort.

To start the trial, I added ten aphids to the top trifoliate of the focal plant with a fine, camel hair paintbrush. Aphids came from a colony maintained at Penn State (24^{o}C, 16:8 [L:D])

on a variety not used in this study. I transferred three late-instar nymphs or adult aphids and seven medium nymphs to each plant in 2010 and five late-instar nymphs or adult aphids and five medium nymphs in 2011. I counted aphids 7 and 14 days after initially adding aphids. In 2011, I added aphids to the uncaged control five days after the other treatments because of logistical constraints. Any mobile predators (predators other than fly larvae) found on the plants when raising and lower the mesh sleeves or when counting aphids were removed if the plant was not in a sham cage or was not enclosed.

Predation: Sentinel aphids

As another measure of the predation service provided by predators within each plot, in 2011 I placed sentinel aphids in each plot. I performed one trial in early July (7-8 July) when plants were small (growth stage V3-V4, 11-18 cm) and one trial when plants were larger (26-27 July; R2, 25-35 cm). Each trial consisted of a 12-hour period during the day (8:00-20:00) followed by a 12-hour period at night (20:00-8:00). Sentinel aphids consisted of pea aphids (*Acyrthosiphon pisum* Harris) glued to a 1.5 × 3 cm piece of white cardstock with non-toxic tacky glue (Gaunt Industries, Inc., Franklin, IL). Pea aphids came from lab maintained colony. I glued three aphids to each card and deployed two cards per plot. Cards were placed in the middle two rows, were separated by 4 m, and were attached with a paper clip to the bottom of the third trifoliate from the top of a plant. I checked cards every hour for the first three hours to identify predators feeding on the aphids and then again after twelve hours. I checked the cards carefully, attempting to not disturb any predators. If a predator was found feeding on an aphid, or if an aphid had clearly been chewed or sucked dry, the aphid was counted as predated. A few aphids molted while in the field, escaping from the card. These were counted as not predated, unless a chewing predator (e.g., a lady beetle) had eaten the rest of the aphids, in which case it was

counted as predated because these predators typically ate all the aphids on a card. I summed predated aphids after twelve hours across cards for each plot prior to analysis.

Plant measurements: Yield

I assessed yield in the plots by harvesting a portion of the middle two rows. The middle 5.3 m of these rows were harvested with a plot combine at the beginning of November each year. No yield loss from shattering was apparent. I dried the shelled seeds at 93ºC for 10 days and then determined their dry mass.

Separate predation experiment: Potted plants

In an experiment separate from the main field experiment with large plots (and in 2012), I tested if predators were more attracted in the field to pots of soybean monocultures or mixtures, both of which were infested with soybean aphids, and if this translated to greater predation services. I used the same low and high diversity treatments as in the earlier experiment (six monocultures and six mixtures) and planted five soybeans per pot. I grew plants in 15-cm diameter pots filled with a peat-based general-purpose potting mix (Premier Promix BX, Premier Horticulture Inc., Quakertown, PA) and in a semi-climate controlled greenhouse with natural light supplemented with artificial light (24ºC, 16:8 [L:D]). I watered plants as needed and fertilized all pots once with Miracle Gro All Purpose fertilizer (The Scotts Company, LLC, Marysville, OH). To infest plants with aphids, I transferred 5 large and 15 small nymphs to the top trifoliate of each plant in the pot (R1 growth stage). Six days after adding aphids, I counted all aphids on the plants in each pot to determine if initial aphid populations at the pot level differed between diversity treatments. The following day (22 August), I placed pots in a conventionally managed, late-planted soybean field (R1-R2 growth stage) at the Russell E. Larson Agricultural Research Center. Pots were exposed to predators for 48 hours. I placed pots 2.5 m into the field

along one edge. Pots were separated by 5 m and I arranged the pots in three linear blocks moving down the field. Each block contained each monoculture and mixture (12 pots per block) and the individual treatments were randomized within blocks (total $N = 36$). Plants in the pots touched neighboring plants, providing a bridge for foraging natural enemies.

Pots were placed in the field at 9:00. Several times, I counted predators and parasitoids (mainly larger, non-aphid parasitoids belonging to the families Braconidae, Chalcididae, Ichneumonidae) that were foraging on the potted plants. I did not count ants because they were species that tend rather than kill aphids. I checked plants at 11:00 and 15:00 on the first and second day, and again at 9:00, 48 hours after putting the pots in the field (five time points). After 48 hours, I counted all aphids on plants in each pot to determine how aphid populations had changed after exposure to predators. After the final aphid count, I removed all large predators from the pots and transferred the pots to an outdoor field cage to allow any predatory fly larvae eggs that had been laid (hoverflies, Diptera: Syrphidae, and gall midges, Diptera: Cecidomyiidae) to hatch and to prevent intraguild predation by larger predators. Six days after the final aphid count, I checked plants for immature predators, which consisted almost exclusively of cecidomyiid larvae.

Statistical analysis

I conducted all statistical analyses using SPSS (IBM, Version 22.0.0.0). I analyzed each year separately because of differences in timing of experimental activities, growing conditions, and distributions of data. I treated block as a fixed factor throughout because I had few levels for all analyses (3-5) and they did not represent a random sample of a larger population. I report results based on type III sum of squares. I present raw means or transformed means graphically.

Each year, CAD were analyzed using ANOVA with fixed factors for diversity, treatment, and block. Because each individual "treatment" was an individual monoculture or mixture and

was therefore only contained within one of the diversity treatments, the treatment factor was nested within diversity. Treatment was nested within diversity throughout analyses. In 2011, CAD was square-root transformed to satisfy assumptions of ANOVA. I used post hoc independent contrasts to test if individual mixtures out- or under-performed compared to their component varieties in monoculture. Four plots in 2010 were outliers because of groundhog damage (two monocultures, two mixtures) and were not included in the analysis. I tested if potato leafhopper populations differed between diversity treatments over the course of the season by fitting a mixed effects ANOVA model to account for repeated measurements for each plot across dates with fixed factors for diversity, treatment, block, and date. I performed separate analyses for each sampling time (day/night) within each year. I chose among candidate covariance structures using Akaike information criteria (AIC) in all analyses and chose a first-order autoregressive covariance structure for all leafhopper analyses. To satisfy ANOVA assumptions leafhopper abundance was transformed $\log_{10}(x+1)$. Interactions of diversity and treatment with date were excluded from the model when nonsignificant. One variety (variety A) had very high leafhopper populations, so I used independent contrasts to compare this treatment to the rest of the monocultures combined as well as to all other monocultures and mixtures. In 2010, day samples for one date (4 August) were lost before leafhoppers could be identified, so this date was excluded from the analyses. When the diversity × date interaction was significant, I performed simple tests of main effects to test for the effect of diversity on each date.

For predators, I analyzed early and late season dates separately. I summed all numbers of potential aphid predators within each sampling date and sampling time. The first sample in 2010 at the beginning of July was collected when plants were still very small and had not grown much because of hot, dry weather. Very few predators were caught on this date, so it was excluded from early- and late-season analyses. Dates were divided into July (early) and August (late) dates for each year and analyzed with mixed model ANOVA to account for repeated measurements with

fixed factors for diversity, treatment, block, and date. Interactions of diversity and treatment with date were excluded from the model when nonsignificant. Day and night samples were analyzed separately to better capture small differences in experimental factors within each sampling time. I selected a compound symmetry covariance structure for all predator analyses. Predator abundance was $\log_{10}(x+1)$ transformed. To determine if specific predators were influenced by genotypic diversity, I conducted factorial ANOVA with fixed factors for diversity, treatment, and block on abundances summed across dates of several of the most abundant predatory taxa within each year. In 2010, the tested taxa were the multicolored Asian lady beetle (*Harmonia axyridis* Pallas), *O. insidiosus* (square-root transformed), and total spiders (Araneae). In 2011, I tested *O. insidiosus*, and total spiders again and total adult lady beetles (square-root transformed) because *H. axyridis* was much less abundant. I analyzed *O. insidiosus* abundance from the beat-pan sampling with ANOVA with fixed factors of diversity, treatment, and block.

Abundance of total aphid-tending ants (*Lasius* spp. and *Prenolepis imparis*) did not meet normality assumptions of ANOVA even with transformation and were analyzed with nonparametric tests. Each year, I performed separate Mann–Whitney *U* tests for each date within each sampling time testing for the effect of diversity on ant abundance.

Data from the predation trials using exclusion cages were analyzed differently each year because of different distributions of data. In 2010, aphid counts were analyzed separately for each of the two weeks. I fit a mixed model ANOVA to account for repeated measurements of the different cage type within each plot. For each week, aphid counts were $\log_{10}(x+1)$ transformed, and I used an unstructured covariance structure. I specified fixed factors of cage type, diversity, treatment, and block with post hoc multiple comparisons for cage type with a Dunn–Šidák correction. Higher-order interactions were nonsignificant and were excluded from the model. In 2011, I analyzed data from the cage experiment with nonparametric methods because of the distribution of the data. A number of predators were trapped in the total exclusion cages during

both weeks, so these data were not included in the analysis. Within each week, I conducted Mann–Whitney *U* tests for each cage type to test for the effect of diversity. In addition, I used Mann–Whitney *U* tests to compare night and day cages, ignoring the diversity level of the plot, which had a nonsignificant effect. I also compared sham, day, and uncaged treatments with a Kruskal-Wallis test because these cage types had low aphid numbers and had been equivalent across dates in 2010.

I analyzed sentinel aphid data from the first date (6-7 July) using ANOVA with fixed factors for time (day/night), diversity, treatment, and block and with the $\log_{10}(x+1)$ transformed number of aphids eaten as the response variable. On the second date (25-26 July), predation was lower and based on the data distribution, I tested for an effect of diversity on predation using Mann–Whitney *U* tests for both the day and night time points.

I compared plot yield among experimental treatments using ANOVA with fixed factors for diversity, treatment, and block. I used post hoc independent contrasts to test if individual mixtures out-or under-performed compared to their component varieties in monoculture. Six plots in 2010 (four monocultures and two mixtures) were outliers because of groundhog damage and were excluded from the analysis.

For the separate predation experiment using potted plants, I first compared initial aphid counts before plants were placed in the field using ANOVA with fixed effects for diversity, treatment, and block. I compared final aphid counts after exposure to predators ($\log_{10}[x+1]$ transformed) using the same model as initial counts. One pot was not counted on the final day and was excluded. I also calculated both the absolute difference in aphid numbers between time points and the percent change. These values were analyzed using ANOVA with fixed effects for diversity, treatment, and block and both with and without initial aphid counts as a covariate.

Results

Herbivore populations

Aphid populations were below the economic threshold in all plots in 2010, with numbers peaking at 71 aphids per plant across plots at the end of the season. In 2011, populations were lower, reaching an average of 16 aphids per plant late in the season. For both 2010 and 2011, season-long aphid pressure based on CAD were not different between mixtures and monoculture plots (2010: $F_{1,40} = 0.251$, $P = 0.619$; 2011: $F_{1,44} = 0.247$, $P = 0.622$; Fig. 2). Cumulative aphid days differed among individual monoculture and mixture treatments during 2010 ($F_{1,40} = 2.23$, $P = 0.030$), but not during 2011 ($F_{1,40} = 1.69$, $P = 0.11$). In both years, mixtures did not out-or under-perform compared to their component varieties in monoculture (during each year, $P > 0.05$ for all individual contrasts). In addition, aphid pressure in the mixtures was intermediate to the range covered by the monocultures each year (Fig. 2). During aphid counts, mummies were infrequently encountered during both years, consistent with previous studies that have found low parasitism rates of soybean aphid (Noma and Brewer 2008, Ragsdale et al. 2011).

During the day, genotypic diversity generally increased potato leafhopper abundance across dates and years (Fig. 3, Table 2). In 2010, diversity consistently increased potato leafhopper abundance across dates, and populations varied with date (Fig. 3, Table 2). During the day in 2011, leafhopper populations were not different between low and high diversity plots on 7 July ($F_{1,214.1} = 1.18$, $P = 0.28$), but were lower in mixtures on 21 July ($F_{1,214.1} = 3.71$, $P = 0.056$), and greater in mixtures on the last two dates (4 August: $F_{1,214.1} = 7.00$, $P = 0.009$; 24 August: $F_{1,214.1} = 3.47$, $P = 0.064$).

In both years, treatment significantly influenced leafhopper populations during the day and night (Table 2). Leafhoppers were significantly more abundant in variety A than in other monocultures or mixtures ($P < 0.05$ for all contrasts). No other herbivores reached high population levels during the course of the season. Some of the other common herbivores both

years included various lepidopteran species such as green cloverworm (*Hypena scabra* Fabricius, Lepidoptera: Noctuidae), and other leafhopper species, including *Agallia* sp.

Predators and ant abundance

During both years, predators were influenced by crop genotypic diversity, although effects were not consistent across dates and sampling times. Effects of diversity were greatest during the early season and for night samples. In 2010, diversity did not influence early- or late-season predators sampled during the day (Fig. 4, Table 3). However, night-sampled predators were 33% less abundant in mixture plots during the early season, but not different later in the season (Fig. 4, Table 3). In 2011, abundance of day-sampled predators was only affected by diversity on the final sampling date, when predators were 25.7% more abundant in diverse plots (*diversity* × *date* interaction). Night-sampled predators were 22.8% more abundant in mixture plots early in the season, but were not affected by diversity later in the season (Fig. 4, Table 3). Treatment had mixed effects on predator diversity across years, sampling times, and dates (Table 3).

Of the most common predators, only lady beetles were affected by crop genotypic diversity. In 2010, the influence of diversity on *H. axyridis* depended on sampling time (*diversity* × *time* interaction: $F_{1, 102} = 6.85$, $P = 0.01$). *H. axyridis* was 46.2% more abundant in diverse plots when summed across dates during the day ($F_{1, 102} = 6.50$, $P = 0.012$), but was not more abundant in either type of plot during the night ($F_{1, 102} = 1.33$, $P = 0.25$). In 2011, the effect of diversity on all lady beetle species (11 species) likewise depended on sampling time (*diversity* × *time* interaction: $F_{1, 102} = 4.52$, $P = 0.036$). Lady beetles were more abundant in diverse plots at night ($F_{1,102} = 4.64$, $P = 0.034$), but diversity did not influence their abundance during the day ($F_{1,102} = 0.73$, $P = 0.39$). In neither year did diversity affect the abundance of *O. insidiosus* (2010: $F_{1,103} =$

0.03, $P = 0.87$; 2011: $F_{1,103} = 0.53$, $P = 0.47$) or spiders (2010: $F_{1,103} = 2.28$, $P = 0.13$; 2011: $F_{1,92} = 1.89$, $P = 0.17$).

Peak *Orius* populations in 2011, collected with beat pans, did not differ between low and high diversity plots ($F_{1,44} = 0.35$, $P = 0.56$) and were not influenced by treatment ($F_{1,44} = 0.80$, $P = 0.63$).

Aphid-tending ants were generally not more abundant in low or high diversity plots (Table 4). Ant abundance in the foliage was highest later in the season (Table 4). In 2010, the median number of ants was marginally greater in diverse plots in night samples during the late part of the season (Median = 2 vs. 1).

Predation: Exclusion cages

In the 2010 exclusion cage trial, diversity did not affect aphid populations across cage treatments either one ($F_{1,41.3} = 0.35$, $P = 0.56$) or two weeks ($F_{1,42.1} = 1.38$, $P = 0.25$) following infestation (Fig.). Cage treatment strongly influenced aphid populations after one week ($F_{1,49.4} = 49.40$, $P < 0.001$) and two weeks ($F_{1,52.6} = 52.61$, $P < 0.001$; Fig.). Plants with aphids completely protected from predators (full exclusion cages) had the greatest aphid populations both weeks, while day, sham, and uncaged plants had the smallest populations; caged plants that were only accessed by night-active predators had intermediate numbers of aphids (Fig.). In 2011, aphid populations were compared between diversity treatments within each week and cage treatment. After one week, aphid populations in low diversity plots were not different than those in high diversity plots for any cage treatment ($P > 0.10$ for all comparisons, Fig.). Populations on plants available only to night predators were much higher than populations on plants accessed by day-active predators ($H = 3.88$, $df = 2$, $P = 0.14$, $N = 156$). Day-predator access, sham, and uncaged treatment plants all had similar numbers of aphids ($H = 3.02$, $P = 0.003$, $N = 36$, $r =$

0.50). After two weeks, differences between diversity treatments were evident for certain cage treatments. Aphid populations in diverse plots reached higher levels in high diversity plots when only night predators were allowed access ($Mean_{High}$ = 40.0, $Mean_{Low}$ = 13.6; U = 2.02, P = 0.044, N = 60, r = 0.26). Conversely, aphid populations on uncaged plants in diverse plots were significantly lower than in monoculture plots ($Mean_{High}$ = 4.5, $Mean_{Low}$ = 1.7; U = 4.60, P < 0.001, N = 60, r = 0.42).

Predation: Sentinel aphids

Predation was measured in the plots during two 24-hour periods in 2011. On 7-8 July, genotypic diversity did not affect the number of aphids killed by predators ($F_{1, 103}$ = 0.03, P = 0.85) and this was consistent for both day and night predation (nonsignificant *diversity* × *time* interaction; Fig.). Night predation was higher than day predation and more aphids were removed during the night ($F_{1,103}$ = 4.02, 0.048). Predatory mites, lady beetles (larvae and adults), earwigs, and ants were observed eating the sentinel aphids, although the ants were not the dominant aphid-tending taxa *Lasius* spp. and *P. imparis*. On 25-26 July, overall predation was lower. Plot diversity did not affect the number of aphids killed during the day (U = 0.59, P = 0.55) or the night (U = 1.54, P = 0.12). Ignoring diversity treatments, predation was similar during the day and night (U = 0.21, P = 0.83). Lady beetle adults, lacewing larvae, harvestman, earwigs, damsel bugs, and mites were observed eating the sentinel aphids.

Yield

In both 2010 and 2011, yield (dry seed biomass) was not different between mixtures and monoculture plots (2010: $F_{1,38}$ = 0.64, P = 0.43; 2011 $F_{1,38}$ = 3.00, P = 0.091; Fig.). Yield did not differ among individual monoculture and mixture treatments either year (2010: $F_{1,38}$ = 0.90, P = 0.48; 2011: $F_{1,44}$ = 1.68, P = 0.12). In both years, monocultures did not out-or under-perform for

yield compared to their component varieties in monoculture ($P > 0.05$ for all individual contrasts during each year) and aphid pressure in the mixtures was intermediate to the range covered by the monocultures each year (Fig.).

Predation experiment: Potted plants

Aphid populations on potted plants used to measure predation services in the field did not differ at the start of the experiment (six days after adding aphids) before pots were placed in the field ($F_{1,22} = 0.007$, $P = 0.94$). Populations did differ between individual treatments ($F_{1,22} = 2.08$, $P = 0.011$). After 48 hours of exposure to predators, aphid populations were not different between low and high diversity pots ($F_{1,21} = 0.13$, $P = 0.87$). The difference in number of aphids between placement of pots in the field and after predator exposure also was not influenced by genotypic diversity ($F_{1,21} = 0.82$, $P = 0.38$). Including the number of aphids at the start as a covariate (significant at $P < 0.01$) did not change the conclusions. Similarly, the percent difference in aphid populations between the start and end of the predator exposure period did not differ between diversity treatments ($F_{1,22} = 0.58$, $P = 0.46$). Initial aphid population was not significant as a covariate and did not change the conclusions. The total number of predators observed on the potted plants was not influenced by genotypic diversity ($Mean_{Low} = 5.44$, $Mean_{High} = 5.89$; $F_{1,22} = 0.47$, $P = 0.50$), nor was the number of parasitoids ($Mean_{Low} = 5.61$, $Mean_{High} = 6.72$; $F_{1,22} = 0.47$, $P = 0.50$). A number of different predators were observed on the potted plants, including various species of adult lady beetles (primarily *Cycloneda munda* Say), mites, several families of spiders, damsel bugs, and *Orius*. The number of cecidomyiid larvae that developed on the plants after they were removed from the field was highly variable (0-37 larvae per pot) and did not differ between low and high diversity pots ($U = 1.11$, $P = 0.28$).

Discussion

I found that increasing crop genotypic diversity in soybeans had mixed effects across trophic levels. While genotypic diversity did influence certain components of the soybean arthropod community during certain years, parts of the growing season, or times of the day, effects varied and were inconsistent (e.g., predator abundance: Fig. 4, Table 3), or genotypic diversity had no effect (e.g., soybean aphid populations: Fig. 2). While studies examining the ecological consequences of plant genotypic diversity have not uniformly found that increasing diversity influences ecosystem processes, the effects in my study were more muted than many (Mundt 2002, Crutsinger et al. 2006, Johnson et al. 2006, Hughes et al. 2008). Results from my study, however, still indicate genotypic diversity in crop fields can influence arthropod communities and that variety mixtures may provide benefits for soybean production.

From the viewpoint of a grower or a pest manager, the most important responses I measured were aphid populations and crop yield. In the northern U.S., most other herbivores do not cause substantial damage to soybeans and are therefore of much less concern. I found that both CAD and yield were unaffected by plot diversity (Figs. 2 and 8). The effect of diversity on CAD was similar to the effect on aphid populations when they were analyzed with individual time points across the season. In mixtures, CAD and yield fell within the range of values for the monocultures, and the monocultures made up the most extreme values. Thus, these results provide evidence that mixtures can produce intermediate pest populations by reducing variation that is seen among the constituent varieties in monocultures (the 'portfolio effect'; Haddad *et al.* 2011; Thibaut & Connolly 2013).

Soybean aphid population remained low over the two years of my study. Whether variety mixtures influence aphids under low pest pressure would likely be of minimal interest to a grower. Populations of soybean aphid in the U.S. had followed a two-year oscillating cycle of a low aphid year followed by a high aphid year (Rhainds et al. 2010). Populations of aphids were

extremely high in 2001, 2003, 2005, 2007, and 2009, exceeding the economic threshold in many fields. Low populations were expected in 2010, but the even lower populations in 2011 were not. These limited populations did not allow me to measure the benefits of enhanced crop diversity when aphid populations reached levels that were high enough to enough to threaten yield, in which case there would be an opportunity for diversity effects on aphids to produce cascading effects on plant productivity. Under low pest pressure, there were no downsides to planting variety mixtures in terms of yield averaged across varieties. Mixtures did not produce yield benefits, but yield benefits of soybean mixtures tend to be lower than other crop species (Smithson and Lenné 1996). Variety mixtures of soybeans still need to be evaluated in the field under pest populations that become economically significant. This will better evaluate their benefits for dampening high pest populations in certain monocultures and producing emergent, non-additive effects (Hughes et al. 2008).

For most sampling dates, crop genotypic diversity increased abundance of another herbivore, potato leafhopper, when it was sampled during the day (populations were not different at night, Fig. 3, Table 2). In 2010, the effect was consistent across dates, but in 2011, populations were higher in mixtures on two dates, but lower on one. Notably, leafhoppers did not achieve populations that would affect yield in mixtures (Ogunlana and Pedigo 1974); thus, again, the mixtures were not challenged by significant populations of pests. Plots of variety A hosted many more leafhoppers than any other monoculture or any of the mixtures. Instead of increasing populations averaged across monocultures, this likely contributed to the higher leafhopper populations in mixtures. Higher herbivore population in mixtures can occur if spill-over effects and associational susceptibility occurs (Barbosa et al. 2009). Populations can build on a susceptible or preferred variety, increasing abundance on all neighboring varieties, which may have happened with leafhopper populations. Alternatively, this variety could have driven non-additive effects of diversity in mixtures if it was highly attractive to leafhoppers moving through

the field. From an aphid management perspective, mixtures could actually improve aphid suppression, particularly at low aphid populations, by supporting higher populations of herbivores that do not achieve pest status and serve as alternative prey for predators. If these herbivores do not divert predators away from aphids (e.g., Harwood & Obrycki 2005), they can help predators establish populations early in the season, arrest mobile predators in fields, or support larger predator populations that are available to suppress burgeoning aphid populations (Settle et al. 1996, Evans 2003, Yoo and O'Neil 2009).

Predators helped suppress aphid populations in this study and patterns of predation varied temporally. Aphid populations rapidly increased when they were protected from predators in full exclusion cages, but did not increase or increased more slowly when exposed to predators (Fig. 6). Predators also consumed aphids on sentinel aphid cards or on infested potted plants, consistent with previous work that showed predators suppress soybean aphid populations (Costamagna and Landis 2006, 2007, Desneux et al. 2006). Day and night predation rates were not equivalent on all dates during the season. Predation was greater during the night when measured with sentinel aphid cards (2011) and when plants were small (7-8 July, Fig. 7). Predation was not different between times of the day at the end of July. When measured with exclusion cages during late-July to early-August (2010 and 2011), day predators suppressed aphid populations to levels comparable to those exposed to the full predator community on sham-caged or uncaged plants (Fig. 6), whereas, night predators provided less predation than day predators as evidenced by greater populations of aphids exposed only to night predators.

The differing results produced by these two methods of evaluating predation may be explained by the different types of aphid populations subjected to predators with each technique. Sentinel aphid cards contained only three aphids, while cage populations contained at least ten aphids. Aphids were also actively feeding on plants only in the exclusion cage trials. Day-active predators like lady beetles that actively seek out aggregations of soybean aphid (Nakamuta 1987,

Megan Woltz and Landis 2013) and are attracted to aphid-induced plant volatiles (Zhu and Park 2005) may have been most attracted to the populations presented in the cage trial. This would result in the pattern seen in this study, with the greatest amount of predation occurring during the day. In contrast, predators may not have been attracted to aphids on cards, but rather simply discovered them as they foraged in the soybean canopy, producing a different pattern of predation than the cage trial. Both methods suggest, however, that day and night communities provide different patterns of predation services. This observation helps clarify the role these temporally distinct predator communities play in aphid suppression, which has not been well understood for soybean aphid (but see Petersen & Woltz 2014; Woltz & Landis 2014)).

The overall arthropod community that could influence aphid populations, predators and aphid-tending ants, was not consistently influenced by diversity across years, parts of the season, or sampling times (Figs 4 and 5, Tables 3 and 4). For example, genotypic diversity affected the total number of predators caught at night early in the season in both years of the study, but not during the day. Predators, however, were less abundant in mixture plots at night in 2010 and were more abundant in mixtures in 2011 (Fig. 4). Later in the season, predators were more abundant in mixtures only on the final sampling date in 2011 in day samples. Of the predators tested whose abundance was summed across the season, only lady beetles were affected by plant diversity (Fig. 5). *H. axyridis* was more abundant in diverse plots during the day in 2010, while total lady beetles were more abundant in diverse plots at night in 2011. The effect during the day on *H. axyridis* is likely more significant for aphid control because lady beetles are primarily diurnal (Nakamuta 1987). In 2010, *H. axyridis* may have preferentially moved into mixtures to forage during the day but then randomly distributed among plots at night. It is unclear why lady beetles were not influenced by diversity during the day in 2011. Abundance of ants, was influenced little, if at all, by crop genotypic diversity. Ant colonies are not mobile and most were likely established in the

field prior to my study. I suggest that single colonies or individual ants would therefore not be strongly influenced by a single-year manipulation of crop genotypic diversity.

Overall, results from this work indicate that variety mixtures may hold potential to serve as a component of an IPM program for soybean aphid and may help manage soybean aphids, in part by influencing their predators, although I did not find strong evidence to support this tact. Taken as a whole, results from this study indicate that under low pest populations, increasing crop genotypic negligibly influences aphid populations. Nevertheless, growers may appreciate the intermediate aphid populations in mixtures. Further work is necessary to assess if any differences found in this study in predator abundance or predation persist with high herbivore populations. Day predation appears to be comparatively more influential than nighttime predation on aphid populations, so positive effects of genotypic diversity on day-active predators, such as those I found on *H. axyridis*, are likely to be important. Still, effects on night predators early in the season when the relative contribution of night predators appears to be larger may be meaningful. Both predator communities help suppress soybean aphids and could together improve aphid control in mixtures. Given the importance of predators for soybean aphid control, effects of genotypic diversity on predators will be key to improving aphid suppression with variety mixtures. Any potential improvements in top-down control of aphid populations in mixtures could complement other IPM practices for soybean aphids, such as cover crops or insect-resistant varieties. My findings begin to clarify the role genotypically diverse mixtures of crop could play in modern agriculture, specifically for pest management. Even if benefits are small, the cost for growers could also be minimal in terms of planting and management. Furthermore, these findings contribute to an expanding collection of research that has demonstrated ecological consequences of plant genotypic diversity in human-managed systems like crop fields and in natural systems.

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Tables

Table 3-1. **Varieties used in the study and mixtures (high diversity treatments) formed from these varieties.**

Variety	Variety code	Mixture	Varieties in mixtures
KS3406RR	A	Mix1	BCDEF
NK S35-T9	B	Mix2	ACDEF
AG3039	C	Mix3	ABDEF
HS 32A90	D	Mix4	ABCEF
TS3989RS	E	Mix5	ABCDF
92M91	F	Mix6	ABCDE

Table 3-2. **Abundance of potato leafhoppers and the effects of diversity, treatment, date and their interactions.** Leafhoppers were sampled with a sweep net during both the day and night. Nonsignificant interactions were not included in the model. Effects significant at $\alpha = 0.05$ are shown in bold.

Year	Time	Factor	*DF*	*F*	*P*
2010	Day				
		Diversity	1	9.41	**0.003**
		Treatment	10	22.77	< **0.001**
		Date	2	9.54	< **0.001**
		Treatment × date	22	1.62	0.056
	Night				
		Diversity	1	0.41	0.52
		Treatment	10	6.85	< **0.001**
		Date	3	1.66	< **0.001**
		Treatment × date	33	0.00	**0.024**
2011	Day				
		Diversity	1	3.16	0.079
		Treatment	10	15.45	< **0.001**
		Date	3	61.27	< **0.001**
		Diversity × date	3	4.11	**0.008**
	Night				
		Diversity	1	1.74	< **0.001**
		Treatment	10	4.82	< **0.001**
		Date	3	88.97	0.088
		Diversity × date	3	2.23	**0.002**
		Treatment × date	30	2.15	2.15

Table 3-3. **Effects of diversity, treatment, date and their interactions on abundance of potential soybean aphid predators.** Predators were sampled with a sweep net during both the day and night. In both years dates were divided into early and late season. Effects significant at $\alpha = 0.05$ are shown in bold.

Year	Time	Season	Factor	*DF*	*F*	*P*
2010	Day	Early	Diversity	1	0.01	0.94
			Treatment	10	1.06	0.41
			Date	1	43.67	< **0.001**
			Treatment × date	11	2.52	**0.013**
		Late	Diversity	1	0.10	0.75
			Treatment	10	2.05	0.051
			Date	1	0.87	0.36
	Night	Early	Diversity	1	6.65	**0.013**
			Treatment	10	0.59	0.81
			Date	1	100.16	< **0.001**
		Late	Diversity	1	0.00	< **0.001**
			Treatment	4	7.47	0.95
			Date	1	20.90	< **0.001**
			Treatment × date	11	2.84	< **0.001**
2011	Day	Early	Diversity	1	0.33	0.57
			Treatment	10	3.24	**0.003**
			Date	1	205.09	< **0.001**
		Late	Diversity	1	0.18	0.68
			Treatment	10	0.89	0.55
			Date	1	254.63	< **0.001**
			Diversity × date	1	4.58	**0.037**
	Night	Early	Diversity	1	4.19	**0.047**
			Treatment	10	0.25	0.99
			Date	1	3.98	0.051
		Late	Diversity	1	0.09	0.76
			Treatment	10	1.31	0.26
			Date	1	77.06	< **0.001**

Table 3-4. **Effect of diversity on ant abundance as determined through sweep net sampling.** The effect of genotypic diversity on ant abundance was tested within each year, time of the season (early vs. late), and sampling time with nonparametric Mann-Whitney U tests.

Year	Time	Season	*N*	*U*	*P*
2010	Day	Early	120	1.68	0.094
		Late	120	0.03	0.97
	Night	Early	120	0.42	0.67
		Late	120	1.80	0.072
2011	Day	Early	120	0.94	0.35
		Late	120	0.33	0.75
	Night	Early	120	0.62	0.53
		Late	120	0.05	0.96

Figures

Figure 3-1. **Overhead view of field and plot layout in 2011.** Plots were laid out similarly in 2010. Accessed from Bing Maps.

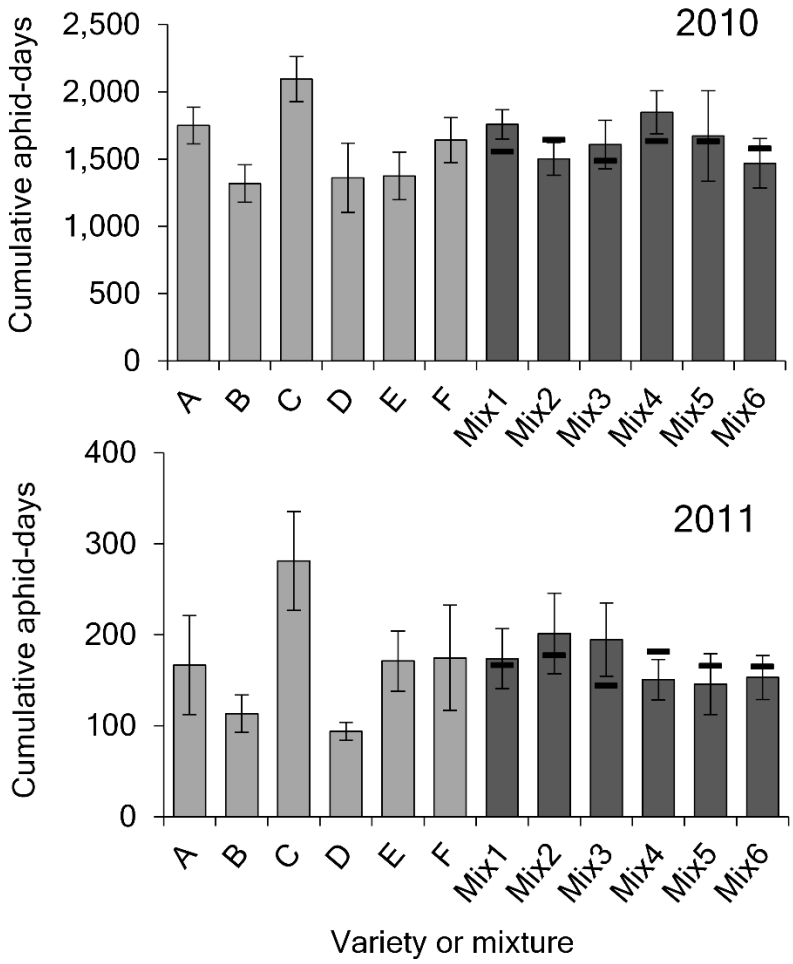

Figure 3-2. **Cumulative aphid-days for the six individual monocultures and six mixtures during each year.** Cumulative aphid-days did not differ between low (light grey bars) and high diversity plots (dark grey bars), but did among individual monocultures and mixtures. Black bars represent expected CAD based on the mean CAD in monocultures of the varieties that compose the mixture. See Table 1 for a summary of varieties included in the individual mixtures. Cumulative aphid-days were square root transformed prior to analysis in 2011. Error bars represent ±1 SE.

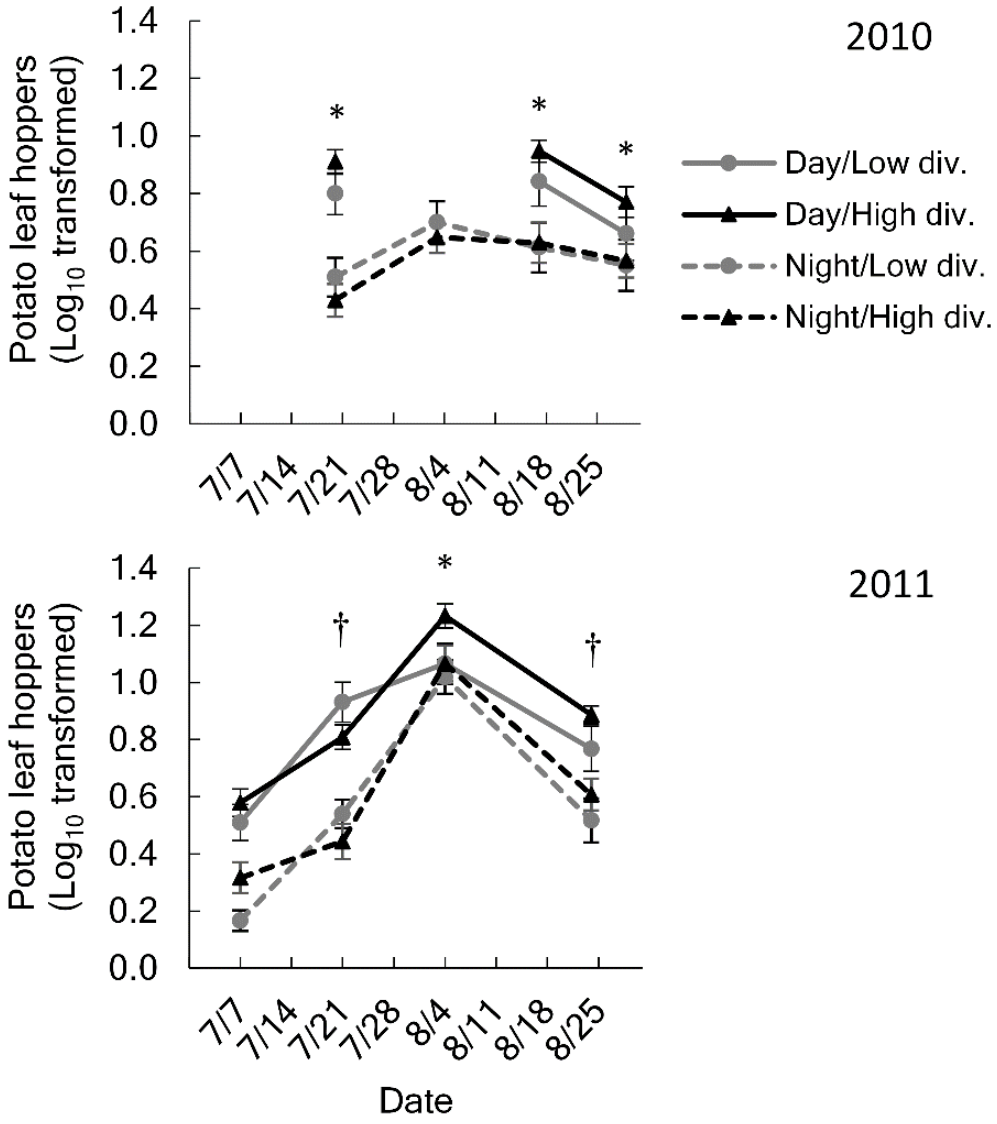

Figure 3-3. **Potato leafhopper abundance (log_{10}[x+1] transformed) in low (monoculture) and high (mixture) diversity plots.** Populations were assessed with sweep net sampling during day and night. Monoculture differed from mixtures in day samples but not in night. No data were available for 4 August, 2010 during the day. If day and night samples were collected on different dates, the mid-point or the later of the two dates is presented. Error bars represent ±1 SE.

* Monocultures differ from mixtures in the day samples at $\alpha = 0.05$

† Monocultures differ from mixtures in the day samples at $\alpha = 0.10$

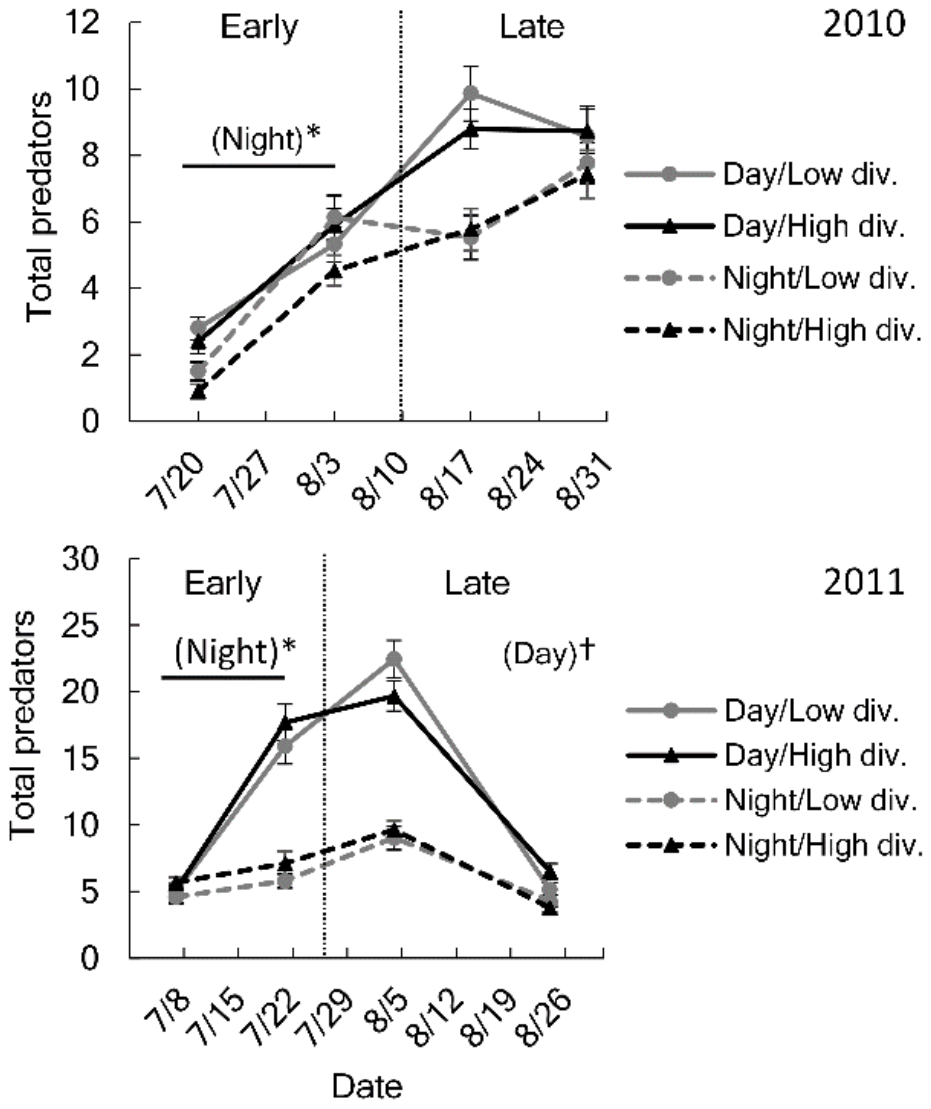

Figure 3-4. **Abundance of potential soybean aphid predators during both years, divided into early and late season samples.** Sampling times were separated for analyses, and significant effects of diversity on predator abundance within sampling times during the early or late part of the season or on single dates are indicated. Horizontal bars indicate significant effects of diversity during that portion of the season, while only presentation of sampling time indicates a significant effect of diversity on that date. The time of day in parentheses indicates for which sampling time the effect was significant. Predator abundance was $\log_{10}(x+1)$ transformed each year prior to analyses. Error bars represent ±1 SE.

* Monocultures differ from mixtures at $\alpha = 0.05$

† Monocultures differ from mixtures at $\alpha = 0.10$

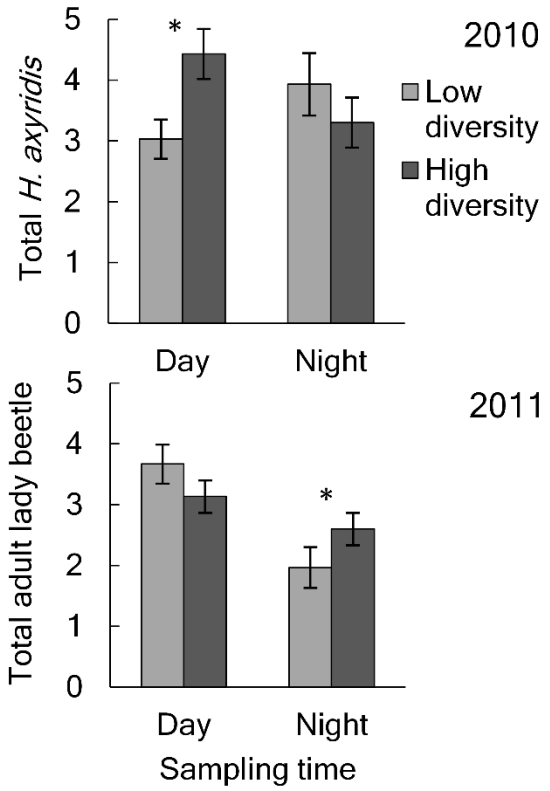

Figure 3-5. **Lady beetle abundance (*H. axyridis* only in 2010 and total lady beetles in 2011) summed across the growing season in low and high diversity plots.** Lady beetle abundance was measured with sweep net sampling, which was conducted during both day and night. Asterisks indicate significant simple effects of diversity within sampling times. Error bars represent ±1 SE.

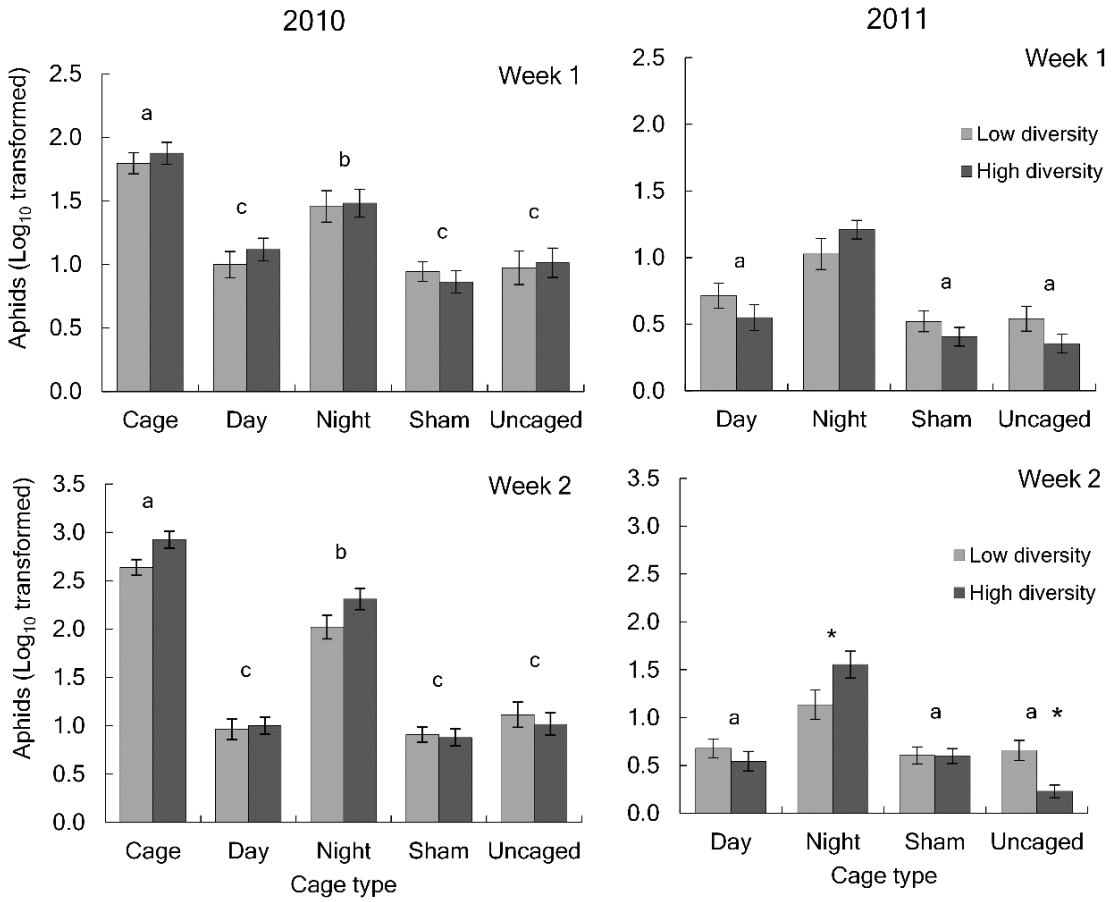

Figure 3-6. **Aphid populations in different exclusion cage types and uncaged controls in low and high diversity plots**. Means for $\log_{10}(x+1)$ transformed are presented. Plants were infested with 10 aphids (transformed = 1.0), and then aphid populations were assessed one and two weeks later. The trial in 2010 began on 26 July and on 27 July in 2011. In 2011, the timing of infestation and counting for the uncaged plants was staggered 5 days after the rest of the plants. Asterisks indicate significant effects of diversity within a cage type as determined by Mann-Whiteny *U* tests (see text for details on statistical analyses). Cage types with different letters are statistically different. If bars do not have a letter, they were not included in the comparison. Error bars represent ±1 SE.

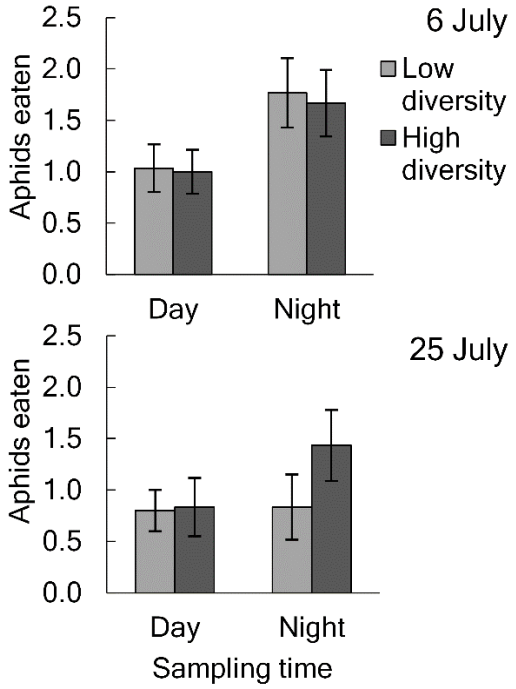

Figure 3-7. **. Sentinel aphids killed after 12 hours during the day and during the night in low and high diversity plots.** Two trials were conducted in 2011, one starting 6 July and one 25 July. Two cards were placed in each pot with three aphids per card. Values represent the total aphids eaten (out of six) in each plot. Error bars represent ±1 SE.

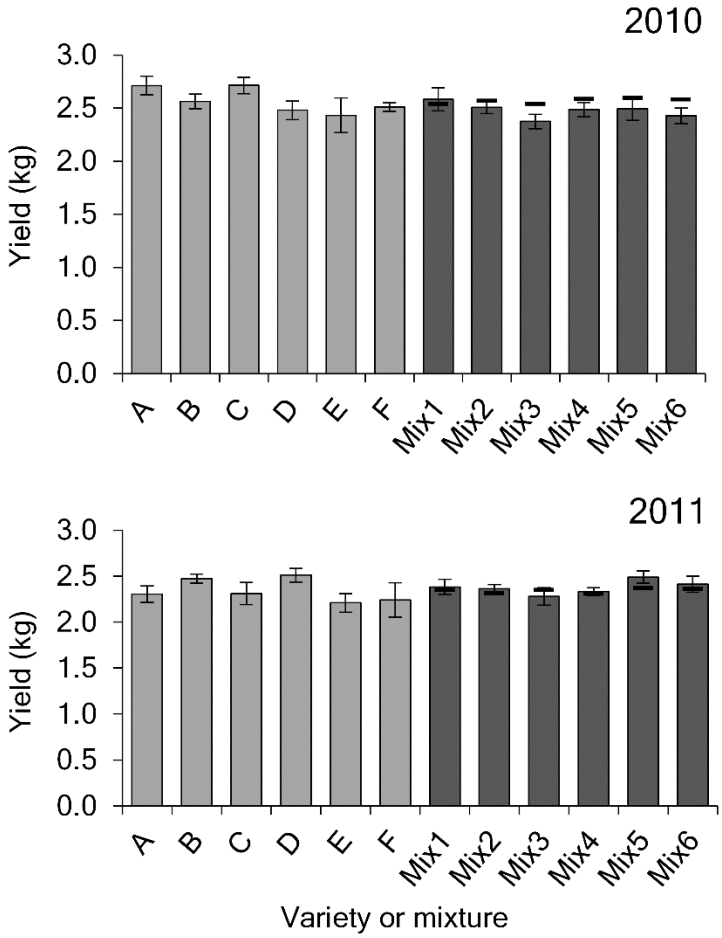

Figure 3-8. **Yield (dry seed mass) for the six individual monocultures and six mixtures during each year.** Yield did not differ between low (light grey bars) and high diversity plots (dark grey bars), or among individual monocultures and mixtures. Black bars represent expected yield based on the mean yield in monocultures of the varieties that compose the mixtures. Error bars represent ±1 SE.

Chapter 4

The influence of variety mixtures of wheat on aphid populations and an aphid predator

Abstract

Increasing plant diversity in crop fields can help manage insect pests. Diverse stands of plants can reduce herbivore abundance and plant damage by influencing the ability of herbivores to colonize, establish, and reproduce in crop fields. Plant diversity can also increase predator abundance and predation on herbivores. Mixtures of plant species can provide clear benefits, but are challenging to employ in modern agriculture. Mixing crop varieties and increasing crop within-species (genotypic) diversity within field is a promising alternative to increasing plant species diversity. To explore the potential of crop genotypic diversity for managing insect pests, I used a model system of wheat (*Triticum aestivum* L.), bird cherry-oat aphid (*Rhopalosiphum padi* L.), and a lady beetle (*Coleomegilla maculata* DeGreer). I conducted greenhouse and laboratory experiments comparing monocultures of five varieties and the fifteen possible four-variety mixtures formed from the pool of five varieties. I found that genotypic diversity did not strongly influence aphid populations at the stand level, but produced intermediate aphid populations. At the individual plant level, aphid populations on certain varieties differed in mixtures from what would be expected based on populations on individual plants of the same variety in monocultures, including consistently lower populations on one variety when it was grown in mixtures. When I tested five of what had appeared to be the most promising mixtures from the first part of the study, I found that none decreased aphid populations compared to their constituent varieties in monoculture, while aphid populations were higher for only one mixture at one time point. Diversity of the surrounding neighborhood influenced aphid populations on individual plants of

certain varieties. Similar to the effects on aphid populations, when comparing measures of biomass to all monocultures, overyielding of mixtures above the monoculture mean did not occur, but total plant biomass was also not different than the most productive monocultures and all measures of productivity were stabilized. In separate experiments, aphids showed no preference for mixtures or monocultures, but lady beetles were more attracted to aphid-infested mixtures than to monocultures. Combining the bottom-up effects of plant-plant interactions on aphids with effects on natural enemies could create pest management benefits in the field. Solely increasing diversity *per se* may not provide benefits for aphid management, aside from producing intermediate aphid populations. Rather, specific types of diversity and influential varieties are crucial for producing beneficial interactions in crop fields to control pests, so future research will need to identify these varieties and develop beneficial combinations of varieties in mixtures.

Introduction

Plant diversity in a variety of forms can benefit agroecosystems by improving a range of functions, including nutrient retention, plant biomass and yield, pollination, disease management, and insect pest control (Andow 1991, Thomas et al. 1991, Liebman et al. 2013, Schipanski et al. 2014). Farm managers can increase plant species diversity within the confines of fields (cover crops, intercrops, ground covers, strips of non-crop plants, polycultures) or in areas immediately adjacent to fields (field margins, insectary plantings) to provide beneficial services (Altieri 1999, Landis et al. 2000). Many types of diversification strategies increase yields, decrease some management costs, or reduce environmental pollution. For example, increased plant species diversity can help suppress insect pest populations directly by making host plants more difficult to find and indirectly by enhancing populations of natural enemies that can kill feeding herbivores (Andow 1991, Finch and Collier 2000, Letourneau et al. 2011). Unfortunately, species mixtures are often challenging to implement in large-scale agriculture (Tooker and Frank 2012) and can alter typical crop production in many systems, reducing yields even when pests are suppressed (Letourneau et al. 2011, Brennan 2013).

Planting mixtures of genotypic varieties is a simpler method to increase within-field plant diversity than by using species mixtures and can provide plant-mediated (bottom-up) and natural enemy-mediated (top-down) benefits, although only limited research has explored the value of genotypic diversity for insect management (Tooker & Frank 2012; Grettenberger & Tooker 2015 [Chap 6]). Nevertheless, the influence of genotypic diversity in ecosystems can rival that of species diversity (Crutsinger et al. 2006, Cook-Patton et al. 2011). Moreover, theoretical and empirical evidence suggests blends of varieties may improve pest suppression compared to genetically uniform monocultures by influencing both herbivores and natural enemies (Tooker and Frank 2012). Much of the work with genotypic mixtures has investigated the ecological effects of increasing plant genotypic diversity in stands of wild plants. While some results have

found that higher levels of genotypic diversity can increase populations of insect herbivores (Crutsinger et al. 2008, Utsumi et al. 2011), other results indicate that genotypic diversity can decrease herbivore populations and plant damage and increase abundance of predators (e.g., Johnson, Lajeunesse & Agrawal 2006; McArt & Thaler 2013; Barton *et al.* 2014).

A more limited number of agricultural studies have shown, encouragingly, that intraspecific mixtures of crop varieties can also affect arthropod populations, including economically important herbivorous insect pests and their natural enemies (Tooker & Frank 2012; Grettenberger & Tooker 2015 [Chap 6]). Effects of higher levels of diversity could improve pest suppression. Lady beetles were more attracted to mixed genotype stands of barley and odors from mixed plantings or plants that had interacted with other genotypes (Glinwood et al. 2009, Ninkovic et al. 2011). Variety mixtures of corn, lima beans, oats, potatoes and wheat, among other crops, have been shown to host fewer insect pests compared to crop monocultures (Cantelo and Sanford 1984, Power 1988, 1991, Shoffner and Tooker 2013). The body of research from both natural and agricultural systems on the influence of plant genotypic diversity on herbivores suggests that genotypic mixtures need to be further investigated to clarify how mixtures affect herbivore populations and to identify any potential pest-management benefits.

While variety mixtures are largely untested for managing insect pests, they have already provided benefits for disease management (Wolfe 1985, Mundt et al. 2002). In particular, variety mixtures, typically of rice or small grains, reduce the overall incidence of disease by decreasing genotypic uniformity in fields. This strategy has been extensively used to manage key pathogens in China and Europe (Zhu et al. 2000, Mundt 2002), and variety mixtures have even seen limited use the United States (Bowden et al. 2001). The use of variety mixtures for disease management importantly shows that higher levels of genotypic diversity can be successfully incorporated into production crop fields and that this diversity can help regulate a biotic stressor and improve yields (Mundt 2002).

To investigate if mixtures of crop varieties can also reduce biotic stress induced by herbivores and manage insect pests via bottom-up and top-down forces, I used wheat (*Triticum aestivum* L.), bird cherry-oat aphid (*Rhopalosiphum padi* L.), and an aphid predator, the spotted lady beetle (*Coleomegilla maculata* DeGeer), as a model system. Aphids, including *R. padi,* are problematic pests in small grains and decrease yields by removing phloem from plants and transmitting barley yellow dwarf virus (Kieckhefer and Kantack 1988, Leather et al. 1989). I conducted a series of experiments in a semi-climate-controlled greenhouse and a laboratory space. I first examined if aphid populations were influenced by genotypic diversity (*per se*) by comparing aphid populations in mixtures of wheat genotypes to monocultures. I then conducted complementary experiments that tested if specific mixtures suppress aphid populations and if aphids and lady beetles were more strongly attracted to mixtures than monocultures. My primary hypotheses for these experiments were: (i) variety mixtures would decrease aphid populations, in part because populations on constituent varieties differ when they are grown in mixtures and monocultures; and (ii) specific mixtures would be especially influential and would reduce aphid populations. I also hypothesized that (iii) increased genotypic diversity would influence plant biomass at levels of the pot and on individual plants. Finally, I hypothesized that plant genotypic diversity would influence attraction of (iv) aphids to uninfested plants and (v) lady beetles to plants infested by aphids. The results I present here help describe the effect of plant genotypic diversity on herbivores and clarify the role of variety mixtures for insect pest management.

Methods

Plants and insects

To test if variety mixtures influence aphids and their predators, I used varieties of spring wheat, even though winter wheat is more commonly grown in the U.S. (USDA-NASS 2014). Unlike winter wheat, spring wheat does not require vernalization to undergo heading and produce seed and will therefore generate seeds under warm greenhouse conditions, allowing me to examine both leaf/stem and seed biomass. I chose six different varieties of hard red spring wheat with a variety of agronomic characteristics (Table 1), but assessments of aphid resistance were not available for these varieties.

Plants were grown in square plastic pots (10 × 10 × 9 cm) filled with a peat-based general-purpose potting mix (Premier Promix BX, Premier Horticulture Inc., Quakertown, PA). Each pot contained four plants arranged in a square. Low diversity monocultures comprised four plants of the same variety. From the pool of six varieties, there were fifteen possible four-variety mixtures and these formed the high diversity mixtures. Diverse variety mixtures consisted of four plants, each of a different variety. Each variety appeared in 10 of the mixtures. The size of the variety pool and number of varieties in the mixture permitted sufficient replication of monocultures and a moderately high level of diversity in the mixtures compared to other studies on the influence of genotypic diversity on herbivores (Ninkovic et al. 2011, Shoffner and Tooker 2013). I sowed two seeds per position, and thinned seedlings to one plant per position as necessary. I watered plants to maintain sufficient moisture levels and sprayed once with fungicide (Prosaro 421 SC: prothioconazole and tebuconazole) to prevent disease. I obtained *R. padi* from a pathogen-free colony housed in the Department of Plant Pathology at Penn State. I maintained aphid colonies at room temperature (21^{o}C) under a 16:8 (L:D) photoperiod on a variety of winter wheat not used in the experiment (SW60). Lady beetles for the lady beetle choice experiment were hand-collected from a field of corn (*Zea mays* L.) at the Penn State's Russell E. Larson

Research Center. I transferred them to a mesh rearing cage and maintained them at room temperature (21ºC) under a 16:8 (L:D) photoperiod. Lady beetles were fed live and freeze-killed pea aphids before being used in the experiment.

Aphid populations and plants

To determine the benefits of genotypic diversity for pest suppression and plant productivity in spring wheat, I conducted a two-part greenhouse experiment with potted wheat plants. The primary focus in Part I was to compare aphid populations and plant biomass in monocultures to all possible mixtures formed from the six varieties. I replicated each monoculture ten times (monoculture $N = 60$), while each mixture was replicated twice (mixture $N = 30$). This replication allowed me to test the influence of genotypic diversity *per se* by comparing the mean performance of mixtures and monocultures, while also providing insight on performance of specific monocultures relative to mixtures. Pots were randomly arranged in five blocks. Pots were fertilized once when they had begun to tiller with 0.39 g of Miracle Gro All Purpose fertilizer (The Scotts Company, LLC, Marysville, OH) dissolved in 150 ml water. Overhead halogen lights supplemented natural light (16:8 photoperiod [L:D]). Temperatures averaged 27ºC. To infest plants, I transferred three mid-instar *R. padi* nymphs to each of the four plants with an ultrafine-hair paintbrush when plants had 2-3 tillers (Zadok's scale 2.2-2.3; Zadok et al. 1974). I counted aphids on each plant in the pot at 7, 14, and 21 days after first adding aphids. Two to three days after the final aphid count, all pots were sprayed with Decathlon 20 WP insecticide (OHP, Inc, Mainland, PA) to kill all aphids. Plants were then allowed to mature and dry naturally, at which point I harvested and individually bagged each plant's stem and leaves (vegetative biomass), and seed head (reproductive biomass). These were dried for 14 days at 93ºC. I then determined mass of leaf/stem material and the seed head.

In Part II of this experiment, I examined aphid suppression by promising mixtures identified from Part I to determine if these mixtures did indeed control aphid population growth relative to monocultures. Using data from the second week of Part I, I chose five mixtures that appeared to host lower aphid populations and had low variance after two weeks of population growth (see Table 1 for selected mixtures). Because all six constituent varieties were included within the chosen mixtures, all monocultures were again grown in Part II. Each monoculture and mixture was replicated ten times (monoculture $N = 60$, mixture $N = 50$). I randomized pots within ten blocks and fertilized them once, as in Part I. Lighting, greenhouse temperatures, and procedures infesting plants with aphids did not deviate from Part I. I counted aphids 7 and 14 days following establishment. I ended the experiment after 14 days because another week of population growth would have created extreme overcrowding. The populations after 2 weeks in Part II were similar to the three-week populations of Part I. All pots were sprayed with Decathlon 20 WP to kill all aphids 3-4 days after the final aphid count. Leaves/stems and seed heads were dried and massed after maturation as in Part I.

Aphid choice assay

To test if alate aphids were more attracted to mixtures or monocultures, I used a static-air choice arena and allowed aphids to choose between a monoculture and a mixture. In the paired choice test, aphids chose between plants in a variety mixture and a monoculture of one of the varieties contained within the mixture. Each of the fifteen mixtures was compared to monocultures twice and equal numbers of monoculture pots for each variety (five), were randomly paired with the mixtures ($N = 30$ for paired tests). The arena consisted of an overturned 53 L Sterilite polypropylene plastic storage box (Sterilite Corp., Townsend, MA) attached to particle board base (Fig. 1). To facilitate airflow through the container and prevent buildup of plant volatiles, I drilled a large number of 1.2 cm holes in the sides and top of the arena and

covered them with mesh fabric. Pots containing plants were placed into two square holes in the base at one end of the arena. Plants used for the choice test had 2-3 tillers (Zadok's 2.3-2.4). The edges of the pots were separated by 10 cm. I used bamboo skewers and wire to support the plants and prevent leaves from touching the bottom board or the plastic sides of the arena. This ensured aphids accessed plants only by walking completely into the pot and climbing up the stems or by flying onto a plant. A rectangular hatch on the opposite end of the arena provided access to the arena once the plants had been added and the arena locked to the base with bolts. For each choice test, I used ten alate aphids. I gathered alates that were attempting to emigrate from a colony plant that had been temporarily drought-stressed to promote emigration. I placed the ten aphids into a transfer container composed of three centrifuge tubes glued into a diet cup. Aphids were chilled on ice before adding them to the arena, which prevented them from immediately dispersing at the start of the trial. Trials began at 9:00 and lasted for 24 hours, at which point pots were removed from the arena and all aphids on each pot were counted. The length of the trial allowed the aphids sufficient time to choose a pot. The trials were performed over three separate days. Between each trial, the entire arena was wiped with ethanol. Monocultures and mixtures were randomly assigned to each side of the arena. Lighting consisted of overhead fluorescent lights (16:8 photoperiod [L:D]) and temperature was 21^{o}C.

Lady beetle choice assay

To test if lady beetles (*C. maculata*) were more attracted to mixtures than monocultures, I used the same static-air choice arena and treatment randomization scheme as for aphids (N = 30). Forty-eight hours before the start of the assay, I infested each of the four plants in the pot with five aphids to give them time to induce production of volatile organic compounds (VOCs). For 24 h prior to choice tests, I starved lady beetles by placing them in diet cups (30 ml). At the start of the test, I gently transferred beetles from the cup to the floor of the arena. For the first hour when

beetles were most mobile, I recorded their location every 20 min, and then continued to track them hourly for the next 5 h (6 h total, eight observations). At each time point, I noted whether beetles were on the mixture or monoculture pot, or off the plants. Conditions and other methods were the same as for the aphid assay.

Statistical analysis

Aphid populations and plants

Part I

All statistical analyses were performed using SPSS V22.0.0.0 (IBM, Armonk, NY). To test for effects of genotypic diversity on aphid populations, I used univariate analysis of variance (ANOVA) with fixed factors for diversity (monoculture vs. mixture), "treatment" nested within diversity, and block. For the factor *treatment*, levels for low diversity pots were the individual varieties in monoculture and were simply "mixture" for all mixtures, creating seven possible levels for treatment in Part I. Individual mixtures were not specified within the treatment factor because there were many and replication was low ($n = 2$). Including each mixture identity in the analysis did not change the results. The factor *diversity* tested for the influence of diversity *per se* by comparing all varieties in monoculture to all possible mixtures. When treatment was significant, I used contrasts to compare the individual monocultures to all mixtures. Aphid values were square-root transformed. Four replicates were excluded from all aphid analyses because spiders were found in these pots seven days after adding aphids, and aphid populations were extremely low (one replicate each for Glenn and Jenna and two mixture replicates). I performed similar analyses using ANOVA and post hoc independent contrasts to compare vegetative biomass, seed head mass, and total aboveground biomass among individual monocultures and the mixtures. Seed head mass was highly correlated with mass of seeds separated from the rest of the seed head ($r = 0.958$, $P < 0.001$, $N = 90$), so I analyzed seed head mass. Seed weight can be

interpreted as the most important yield component (Voss et al. 1997). All pots were included in the analysis for plant biomass data because within treatments, there was no clear relationship between components of plant mass and number of aphids during each week. I used Pearson product-moment correlation coefficient to test if there was a relationship between aphid populations and plant biomass.

To compare how varieties performed for aphid resistance plant biomass in mixtures compared to when they were grown in monocultures, I computed an index value for each response variable. For example, I calculated the Relative Aphid index by dividing the aphid count for each plant in a mixture (within each week), by the mean value for that variety when it was grown in monoculture. Thus, values > 1 indicated that for that particular plant, populations were greater than when that variety was in monoculture. Conversely, values < 1 indicated the variety hosted fewer aphids in mixtures. I also computed index values for measures of plant biomass. Prior to analysis, I square-root transformed index values to reduce the influence of extreme values. I analyzed index values with one-sample t-tests for each variety to determine if mean values differed from one.

Part II

In Part II, I tested for effects on aphid populations for the five mixtures I tested (of 15), with separate analyses for each of the two dates on which I assessed populations. I used univariate ANOVA with fixed factors for diversity, treatment (nested within diversity), and block. The factor *treatment* comprised eleven levels: one for each of the varieties in monoculture and one for each of the five tested mixtures. However, I focused on the contrasts between each individual mixture and its component varieties rather than on the effect of genotypic diversity *per se* because not all potential variety mixtures were tested in this portion of the study and individual varieties were not evenly distributed among mixtures. Index values were calculated and analyzed

as in Part I. Four pots (one replicate for Mixture 10, and one each for Faller, Glenn, and SY Soren) were excluded from aphid and plant analyses because a plant did not germinate correctly. I again used Pearson product-moment correlation coefficient to test if there was a relationship between aphid populations and plant biomass.

Aphid choice assay

To test whether aphids were more attracted to monoculture or mixtures, I performed a paired t-test. I performed another paired t-test to test if plant mass of the mixtures was different from monocultures within each choice test. If plant mass differed, aphids may simply respond to greater production of volatiles by larger plants.

Lady beetle choice assay

I performed two separate analyses comparing the attraction of ladybeetles to monocultures and mixtures. First, I used a chi-squared test to test if the first choice of lady beetles was affected by diversity. Next, I summed the number of observations across time points for each mixture and monoculture pot and then tested if lady beetles spent more time (i.e., more observations) on one type of pot using a Wilcoxon signed rank test. In addition, I compared plant mass between diversity levels with a paired t-test.

Results

Aphid populations and plants

Part I

One week after infesting plants, aphid populations in Part I averaged 18 aphids per pot across treatments, and there was no effect of diversity ($F_{1,75} = 2.10$, $P = 0.15$) or of treatment ($F_{5,75} = 0.45$, $P = 0.82$) on aphid populations (Fig. 2). Levels of "treatment" in Part I consisted of the individual monocultures and all mixtures combined (Fig. 2). After two weeks, aphid populations had increased to an average of 202 per pot and remained similar between monocultures and mixtures ($F_{1,75} = 135$, $P = 0.25$); however, at this time point, aphid populations differed among treatments ($F_{5,75} = 6.16$, $P < 0.001$). Populations on the mixture pots were 37.2% lower than on the Faller monoculture ($t_{75} = 3.78$, $P < 0.001$) and 24.3% lower than on Jenna ($t_{75} = 2.27$, $P = 0.026$), but aphid populations were 55.2% higher on mixtures than on Brennan ($t_{75} = 2.72$, $P = 0.008$; Fig. 2). Other contrasts between mixtures and the monocultures were not significant ($P > 0.05$). Three weeks after adding aphids, populations averaged 546.6 aphids, and there was again no overall effect of diversity ($F_{1,75} = 0.064$, $P = 0.80$; Fig. 2), but treatment continued to influence aphid populations ($F_{5,75} = 5.80$, $P < 0.001$). Populations in mixtures were only different from two of the varieties in monoculture: 16.6% lower than on Faller ($t_{75} = 2.06$, $P = 0.043$), and 49.8% higher than on Brennan ($t_{75} = 3.90$, $P < 0.001$).

At the level of an individual plant, only one variety during each week hosted a statistically different number of aphids than would be expected based on aphid populations on plants in monoculture. The Relative Aphid Index for all varieties was less than 1, although only significantly so for Rollag (Fig. 3; $t_{17} = 2.70$, $P = 0.015$). After two weeks, the pattern was similar, with the index lower than 1 for all varieties, but only Rollag had lower aphid populations than in monocultures when it was grown in mixtures (Fig. 3; $t_{17} = 2.70$, $P < 0.001$). After three

weeks, the index was only significantly different than 1 for Brennan, which hosted more aphids in mixtures than in monocultures (Fig. 3; $t_{17} = 3.06$, $P = 0.006$).

Overall, genotypic diversity did not affect any measures of plant biomass in Part I (Fig. 4). Vegetative biomass of all monocultures combined did not differ from biomass of the mixtures ($F_{1,79} = 2.87$, $P = 0.094$), but did differ among individual treatments (Fig. 4A; $F_{1,79} = 8.24$, $P < 0.001$). Vegetative biomass of mixtures was 19.8% higher than monocultures of Rollag (Fig. 4A; $t_{79} = 2.55$, $P = 0.013$). Similarly vegetative biomass of mixtures was 12.2% higher than monocultures of Faller and 11.9% higher than monocultures of Glenn (Faller: $t_{79} = 2.60$, $P = 0.011$; Glenn: $t_{79} = 2.54$, $P = 0.013$). Compared to monocultures of Jenna, mixture vegetative biomass was 9.6% lower ($t_{79} = 2.55$, $P = 0.013$). Seed head mass did not differ between monocultures and mixtures ($F_{1,79} = 1.02$, $P = 0.32$), and did differ among treatments (Fig. 4B; $F_{1,79} = 6.42$, $P < 0.001$). Head mass of the mixture was very similar to four of the varieties in monoculture, but was 59.0% greater than monocultures of Faller ($t_{79} = 4.03$, $P < 0.001$) and 21.0% less than of Brennan ($t_{79} = 2.90$, $P = 0.005$). Total plant biomass (leaf + seedhead) was also not different between monocultures and mixtures (Fig. 4C; $F_{1,79} = 2.19$, $P = 0.14$), but treatment significantly affected total biomass ($F_{1,79} = 6.35$, $P < 0.001$). Total biomass of mixtures was equivalent to four of the monocultures, but was significantly greater than monocultures of Faller and Rollag (Fig. 4C; Faller: $t_{79} = 3.93$, $P < 0.001$; Rollag: $t_{79} = 2.63$, $P = 0.010$;). Aphid populations at three weeks were highly negatively correlated with head mass ($r = -0.51$, $P < 0.001$) and total biomass ($r = -0.31$, $P = 0.003$), but not with vegetative biomass ($r = 0.094$, $P = 0.38$).

The Relative Plant Index values for vegetative biomass, seed head mass, and total biomass were each different than 1 for at least one variety (Fig. 5). Two varieties, Brennan and Falller, had more vegetative biomass when they were grown in mixtures than in monocultures (respectively, $t_{19} = 3.57$, $P = 0.002$; $t_{19} = 32.58$, $P = 0.018$). Jenna produced less stem and leaf

mass in mixture than in monoculture ($t_{19} = 2.77$, $P = 0.012$). For head mass, the index was only significantly different than 1 for Faller, which had larger seed heads when growing in mixtures ($t_{19} = 2.31$, $P = 0.032$). Only Faller had greater overall biomass when growing in mixtures ($t_{19} = 3.30$, $P = 0.004$), and no varieties had significantly less, although index values for Brennan, Glenn, and Jenna were all close to different than 1.

Part II

Aphid populations in mixtures in Part II (five of the original fifteen mixtures) largely did not differ from those that developed in monocultures of their constituent varieties across both weeks (Fig. 6). Further, the model tested in Part II accounted for much less of the total variation in aphid populations than in Part I (Part I model R^2: 7 days: 0.13; 14 days: 0.44; 21 days: 0.43; Part II model R^2: 7 days: 0.28; 14 days: 0.27). Treatment did not significantly affect aphid populations ($F_{9,86} = 1.01$, $P = 0.44$), and only aphid populations on Mix 11 were different than monocultures ($t_{86} = 2.07$, $P = 0.042$; Fig. 6). After two weeks, aphid populations averaged 377.7 per plant and while numerical differences were evident among monocultures and mixtures, treatment did not affect aphid populations, although the effect was close to significant ($F_{9,86} = 1.80$, $P = 0.080$). The most resistant variety in monoculture, Brennan, did harbor less aphids than the most susceptible variety, Rollag ($t_{86} = 2.67$, $P = 0.009$). However, none of the mixtures differed from their constituent varieties in monoculture ($P > 0.05$ for all contrasts; Fig. 6).

At the individual plant level and after seven days, only one variety, Rollag, differed for aphid populations when it was grown in mixtures compared to when grown in monocultures based on the Relative Aphid Index (Fig. 7). Aphid populations were much lower on Rollag in mixtures than when this variety grew in monoculture ($t_{38} = 4.48$, $P < 0.001$). This pattern persisted into the second week of the experiment, and Rollag again hosted significantly fewer aphids in mixtures than in monoculture ($t_{38} = 7.74$, $P < 0.001$; Fig. 7). Populations of aphids in

mixtures were significantly higher for Faller (t_{28} = 2.82, P = 0.009) and values for other varieties were very close to 1.

Diversity and mixture treatments influenced plant biomass more than aphid populations in Part II (Fig. 8). First, at the pot level, treatment significantly influenced both vegetative biomass (Fig. 8A; $F_{9,86}$ = 3.41, P = 0.001) and seed head mass (Fig. 8B; $F_{9,86}$ = 3.94, P < 0.001), but not total biomass (Fig 8C; $F_{9,86}$ = 1.80, P = 0.080). For the individual mixtures, vegetative biomass was not different for three of the mixtures compared to their constituent monocultures (Mixture 11, 13, and 15), while both Mixture 10 and 12 produced less vegetative biomass (Mix 10: t_{28} = 3.94, P < 0.001; Mix 12: t_{28} = 2.51, P = 0.014). For seed head mass, mass for only one mixture, Mixture 10, was lower in mixtures than in monocultures (t_{28} = 2.52, P = 0.013). The differences in vegetative biomass and head mass for Mixture 10 between mixtures and monocultures translated to lower total biomass for this variety in mixtures (t_{28} = 3.22, P = 0.002). Total biomass for all other mixtures was not influenced by diversity (P > 0.05). Populations of aphids at two weeks were negatively correlated with all measures of biomass (vegetative: r = -0.30, P < 0.001; head: r = -0.49, P < 0.001; total: r = -0.451, P < 0.001).

Based on the Relative Plant Index values for vegetative biomass, seed head mass, and total biomass, there was a tendency across varieties to have lower mass for all plant mass measures in mixtures compared to the monocultures, with values for four varieties falling below 1 for all plant measurements (Fig. 9). Values for the other two varieties were greater than 1, but not significantly so. Three of the varieties produced significantly less vegetative biomass in mixtures (Brennan: t_{48} = 2.62, P = 0.012; Rollag: t_{48} = 10.16, P < 0.001; SY Soren: t_{48} = 3.40, P = 0.002), four produced smaller seed heads in mixtures (Brennan: t_{48} = 2.74, P = 0.009; Jenna: t_{48} = 3.26, P = 0.003; Rollag: t_{48} = 4.10, P < 0.001; SY Soren: t_{48} = 2.06, P = 0.048), and the same four produced lower total biomass in mixtures (Brennan: t_{48} = 2.76, P = 0.008; Jenna: t_{48} = 2.67, P =

0.012; Rollag: $t_{48} = 6.35$, $P < 0.001$; SY Soren: $t_{48} = 2.68$, $P = 0.012$). The variety with the largest deviation from 1 for all measures of biomass was Rollag (Fig. 9).

Aphid attraction

Aphids did not show a preference for either monocultures or mixtures ($t_{29} = 0.59$, $P = 0.56$). On average, 6.6 aphids were recovered on plants, and the rest did not make a choice by the end of the 24-hour trial or died. There were no differences in plant mass between monocultures and mixtures within each choice test ($t_{29} = 0.97$, $P = 0.34$)

Lady beetle choice

Of the 30 lady beetles tested, significantly more of them (21 vs. 9) chose to first forage on the mixtures of wheat rather than the monocultures ($\chi^2 = 4.80$, $df = 1$, p=0.029). In some instances, lady beetles were observed leaving plants and switching between mixtures and monocultures. Across the six hours of the trial, however, lady beetles were more consistently observed on mixtures than on monocultures ($Z = 2.18$, $P = 0.029$, $r = 0.40$). There were no differences in plant mass between monocultures and mixtures within each choice test ($t_{29} = 1.14$, $P = 0.26$).

Discussion

My results indicate that genotypic diversity inconsistently influenced herbivore populations. From my first experiment (Part I), genotypic diversity *per se* did not suppress aphid populations any better than the constituent monocultures. Only specific genotypic diversity mattered; only one variety (Rollag) consistently hosted fewer aphids when it was grown in mixtures than in monocultures. This finding may be explained by plant phenotypic changes induced by plant-plant interactions (Grettenberger and Tooker, submitted; Chapter 5), and could

be harnessed for improved pest control if there was a better understanding of how herbivores fare on a range of wheat varieties and how aphid resistance changes in varieties when they are grown in mixtures, but this information will have be accumulated with further research. From Part II, I found that while several specific mixtures had appeared promising in Part I, they did not host lower numbers of aphids compared to their constituent varieties in monoculture. Furthermore, one mixture resulted in higher aphid populations compared to its constituent varieties in monoculture, but only when aphid populations were low. Rollag consistently harbored much lower aphid populations when grown in mixtures than when in monocultures. Aphids were not preferentially attracted to either monocultures or mixtures, whereas lady beetles were more attracted to mixtures. Diverse plantings of wheat affected all three trophic levels, plants, herbivores, and predators in some manner, but the implications for pest management and crop production is not straightforward. Diversity *per se* did not provide aphid suppressive benefits (Part I), nor did the five specific mixtures I tested in Part II, although some of the untested mixtures may have. At the individual plant level, Rollag hosted fewer aphids across parts of the experiment and testing many more varieties could identify enough promising varieties to form aphid-suppressive mixtures. Moreover, combining bottom-up effects of plant-plant interactions on aphids with effects on natural enemies could create pest management benefits in the field.

The population-level experiment from Part I showed that variety mixtures did not affect aphid populations compared to the average of monocultures on any of the dates. The lack of an overall effect of diversity at the population level was consistent with the absence of a preference by alate aphids for either diversity level. While plant diversity can both decrease (Shoffner and Tooker 2013) and increase (Utsumi et al. 2011) herbivore populations, it can also have no effect on herbivore abundance (Genung et al. 2012), which is consistent with the overall effect I documented. Nevertheless, 14 and 21 days post-infestation, aphid numbers on individual monocultures were the most extreme, either numerically higher or lower than all mixtures, and

some significantly so (e.g., Brennan and Faller; Fig. 2). Therefore, while mixtures as a whole did not decrease aphid populations, they did have an effect on aphid populations by dampening the extremes, similar to their stabilizing influence on yield, which is one of their touted benefits (Mundt 2002).

By examining aphid populations on individual plants, I identified effects I may have missed if I had focused only on pot-level populations. Aphid populations after 7 days were fairly low and not different on mixtures than on any of the monocultures (Fig. 2). At the level of the individual plant, however, genotypic diversity altered the abundance of aphids for one of the varieties (Rollag), which hosted lower numbers of aphids in mixtures than would be predicted from populations on monoculture (Fig. 3). Relative to the other varieties at the pot-level, Rollag in monoculture had about average resistance to aphids across dates (Fig. 2) and there was no other reason to expect that the relative resistance of this variety would improve in mixtures.

During weeks 2 and 3 of the experiment in Part I, populations on individual plants in mixtures deviated from what was expected (Fig. 3). Distribution of herbivores within plant populations can be influenced by the genotypic diversity of the planting. In an example from strawberries, aphid populations on the lowest quality, or most resistant, genotypes were at different times lower and higher than would be expected based on their populations in single-genotype plantings (Underwood 2009). Greater aphid movement in mixed genotype plantings would lead to high levels of redistribution of aphids among plant genotypes in a mixture (Underwood et al. 2011). In my study, genotypic diversity may not have influenced aphid populations across varieties, but the analyses involving individual plants indicated that mixing varieties produced non-additive effects, potentially via source-sink population dynamics (Utsumi et al. 2011), or changes in plant-plant interactions that directly influence herbivores (Ninkovic et al. 2002, Glinwood et al. 2009). These non-additive effects are ultimately necessary to produce

population-level differences between monocultures and mixtures and suppress herbivores (Hughes et al. 2008, Underwood 2009).

Testing five of the original fifteen four-variety mixtures in Part II of the population-level experiment that had appeared most promising did not identify mixtures that consistently reduced aphid populations as I had expected. I did find, however, that mixtures again influenced aphid populations on individual plants (Fig. 7). In fact, while aphid populations in individual monocultures and in the mixtures appeared to differ, these differences were generally not significant and the treatment factor did not affect aphid populations. In only one case, Mixture 11 at the first time point, did any of the mixtures significantly differ from their constituent monocultures. Based on patterns in Part II of the study, it seems probable that further experimentation with greater replication (more than the 10 per treatment I used) would identify that at least one of the five mixtures will host lower aphid populations, and likely to a degree that would matter to pest managers, although variation within treatments was large. I may also have simply chosen the "wrong" mixtures to more robustly test and some of the untested ten could have proven beneficial. These mixtures were chosen based on their low aphid populations in Part I, but this choice was based on only the two replicates that were included in Part I for each mixture. As mentioned above, Rollag hosted lower aphid populations when grown in mixtures, and Mixture 12 tended to host fewer aphids than monocultures, a trend driven by non-additive effects of diversity on two varieties, Jenna and Rollag. This type of detail is often not seen in plant genotypic diversity studies in natural systems, where the overall effect of diversity is at the forefront. However, in agriculture, the goal is to use diversity to decrease pest populations, and if diversity *per se* does not provide benefits, identifying particular mixtures or specific varieties that produce benefits can still be useful, especially if the best variety for a given year is not easily predicted (Mundt 2002).

Plant biomass was also not strongly influenced by plant genotypic diversity, although pot-level and plant-level effects were evident in Part I and also when the subset of mixtures were tested in Part II. Diversity *per se* did not affect any measure of plant biomass in Part I, and vegetative biomass and seed head mass were intermediate compared to the monocultures and different than monocultures at the extremes for each measure (Fig. 4A,B). The mean total biomass of the mixtures, promisingly, was very similar and not significantly different from the three most productive monocultures, while it was significantly greater than two of the monocultures (Fig. 4C). I did not see a productivity advantage of mixtures above the mean of monocultures ("overyielding"; Tilman 1999)), which has been found in previous research (Smithson and Lenné 1996, Cowger and Weisz 2008, Kiær et al. 2009). My results on plant biomass are nonetheless encouraging, because when I tested all potential mixtures in Part I, I did not detect a cost to productivity of using mixtures; mixtures produced intermediate levels productivity (in addition to aphid populations) and if anything, enhanced productivity when viewed as individual comparisons to individual monocultures. Results with the five specific mixtures in Part II were somewhat similar to these results. Only one mixture consistently differed from its constitutive monocultures and measures of biomass were lower (Fig. 8). Given the results from Part I that showed no effect of diversity *per se*, some of the other ten untested mixtures would likely counteract this effect if effects on biomass were taken across mixtures. Plant genotypic diversity also influenced the mass of individual plants and index values were significantly different than 1 for a number of varieties in both Parts I and II. Plants therefore competed with or facilitated neighboring plants differently in mixtures with unrelated neighbors than with neighbors of the same variety in monocultures, consistent with a growing body of literature on plant kin recognition (Dudley and File 2007, File et al. 2012).

The enemies hypothesis states that greater plant diversity will support greater biological control (Root 1973, Russell 1989), and my results support this hypothesis for increased diversity

via variety mixtures. Lady beetles were more attracted to aphid-infested mixtures than monocultures and then spent more overall time in the mixtures. More diverse plantings can increase the diversity and abundance of natural enemies (Crutsinger et al. 2006, Johnson et al. 2006, Jones et al. 2011) and can alter predator behavior and increase predator attraction (Glinwood et al. 2009, Ninkovic et al. 2011). Even when overall direct effects of diversity on herbivores are minimal, enhanced biological control could reduce pest populations. Furthermore, by altering herbivore behavior, such as increasing movement among plants, mixtures could expose herbivores to greater levels of predation (Power 1991, Straub et al. 2013). Lady beetles, as generalist predators, forage in many types of crop fields and non-crop habitats (Hodek et al. 2012), so any type of increased attraction that induces natural enemies to move into fields and remain there would be beneficial for suppressing pests.

In summary, the findings presented here demonstrate that plant genotypic diversity can influence multiple trophic levels. For wheat and *R. padi*, I did not find that genotypic diversity alone reduces herbivore populations, but it may help stabilize aphid populations at moderate levels and similarly stabilize plant productivity, and even increase certain measures of productivity to the level of the most productive monocultures. I also discovered that genotypic diversity can alter aphid populations on, and productivity of, some wheat varieties depending on the identity of the surrounding plants. To continue to evaluate the value of crop genotypic diversity for insect control, researchers will need to identify those varieties that respond well to growing in mixtures and then combine them to produce mixtures that should prove beneficial, but this will still need to be tested. Blindly increasing genotypic diversity with random mixtures may occasionally have value, but well-constructed genotypic mixtures hold the real potential to improve sustainability of pest management.

Acknowledgments

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Tables

Table 4-1 **Varieties of hard red spring wheat used in the study, and the composition of the 15 possible four-variety mixtures formed from the pool of six varieties.**

Individual varieties	Mixture*	Varieties in Mixture
Brennan	Mix 1	Brennan, Faller, Glenn, Jenna
Faller	Mix 2	Brennan, Faller, Glenn, Rollag
Glenn	Mix 3	Brennan, Faller, Glenn, SY Soren
Jenna	**Mix 4**	**Brennan, Faller, Jenna, Rollag**
Rollag	**Mix 5**	**Brennan, Faller, Jenna, SY Soren**
SySoren	**Mix 6**	**Brennan, Faller, Rollag, SY Soren**
	Mix 7	**Brennan, Glenn, Jenna, Rollag**
	Mix 8	Brennan, Glenn, Jenna, SY Soren
	Mix 9	**Brennan, Glenn, Rollag, SY Soren**
	Mix 10	Brennan, Jenna, Rollag, SY Soren
	Mix 11	Faller, Glenn, Jenna, Rollag
	Mix 12	Faller, Glenn, Jenna, SY Soren
	Mix 13	Faller, Glenn, Rollag, SY Soren
	Mix 14	Faller, Jenna, Rollag, SY Soren
	Mix 15	Glenn, Jenna, Rollag, SY Soren

*Mixtures shown in bold were tested in Part II. All mixtures were included in Part I.

Figures

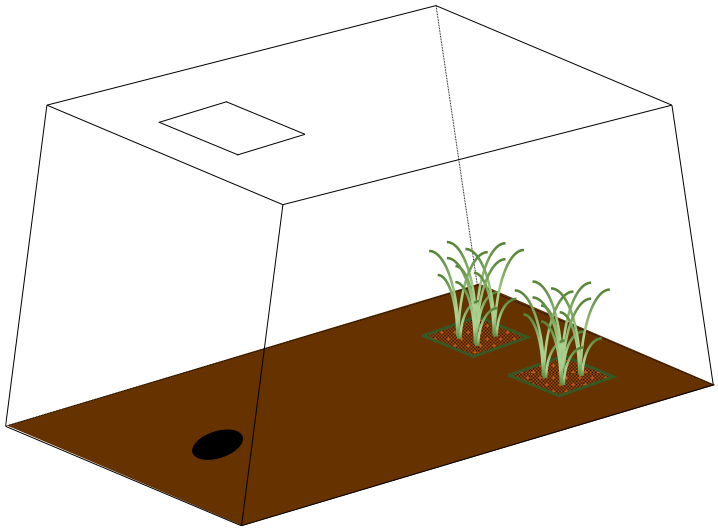

Figure 4-1. **Choice arena used for the aphid and lady beetle choice assays.** Ventilation holes are not shown. One pot contained a mixture of four plants while the other pot contained a monoculture of four plants of one of the varieties that were contained in the mixture. The black circle indicates the release point for aphids and lady beetles at the start of each choice test, and the rectangle at the top is the access hatch.

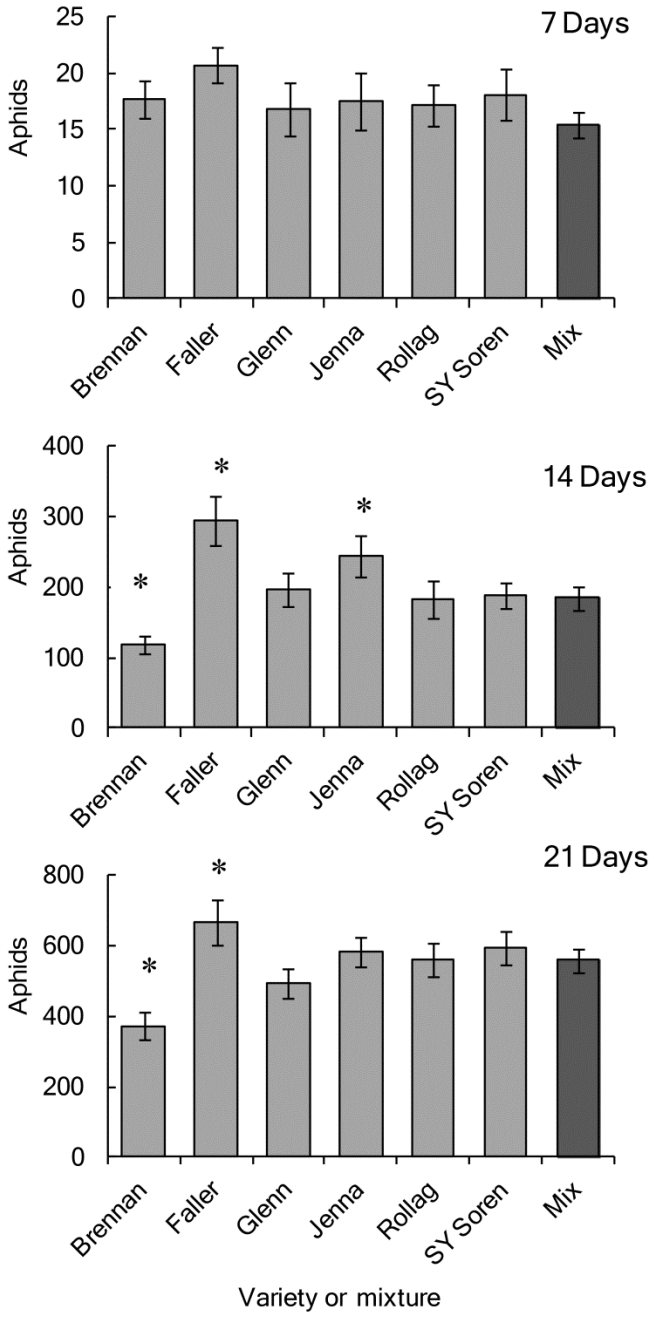

Figure 4-2. **Mean aphid populations 7, 14, and 21 days after initial infestation on six varieties in monoculture (light grey bars) and in four-variety mixtures (dark grey bars) in Part I.** Error bars represent ±1 SE. Asterisks indicate individual monocultures that were significantly different than the mixtures.

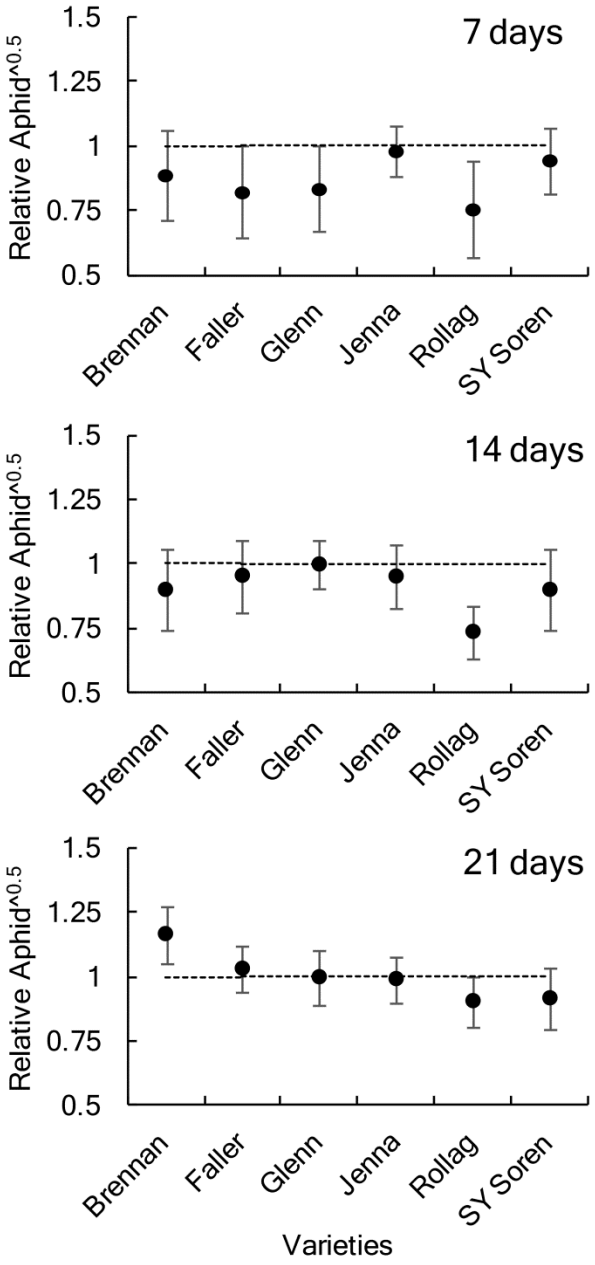

Figure 4-3. **Relative Aphid Index values for each of the six varieties 7, 14, and 21 days after infesting plants with aphids in Part I.** The index compares the number of aphids on an individual variety grown in mixtures to when grown in monocultures. Values > 1 signify more aphids on the variety when it grows in mixtures, while values < 1 signify fewer aphids in mixtures. Error bars represent 95% confidence intervals and varieties whose error confidence intervals do not cross 1 are significantly different than 1 at $\alpha = 0.05$.

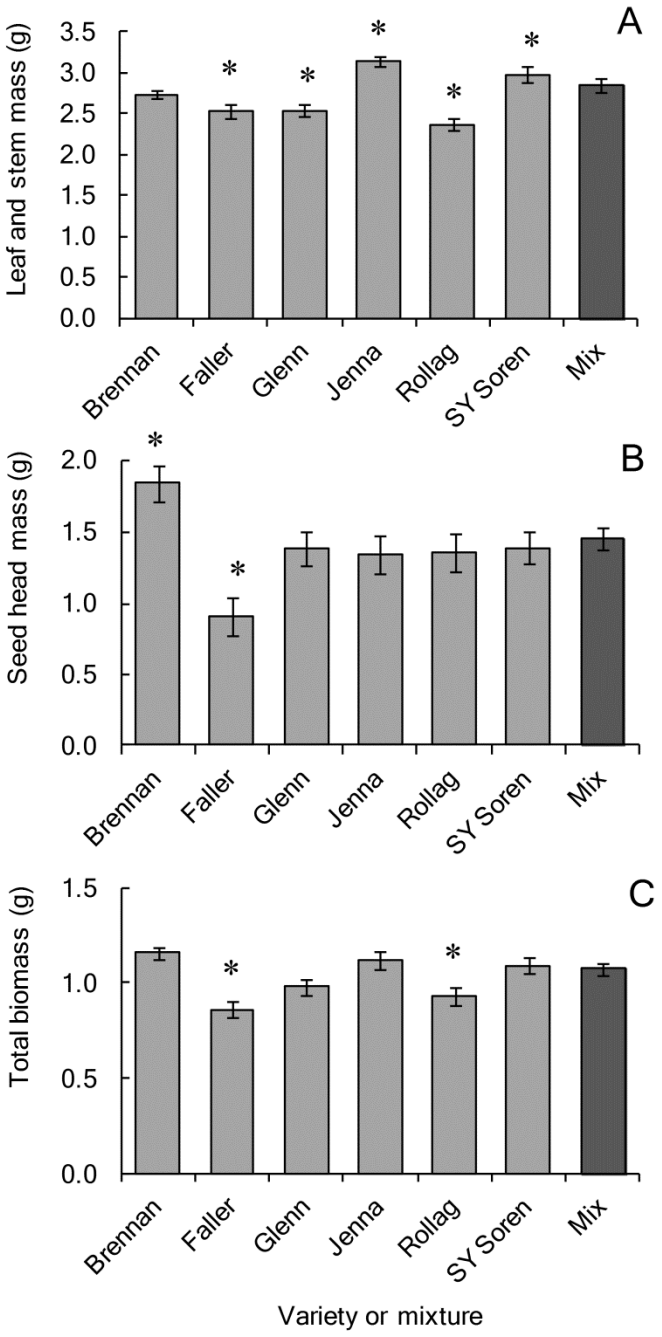

Figure 4-4. **Total leaf and mass (A), seed head mass (B), and total aboveground biomass (C) for six varieties in monoculture (light grey bars) and for four-variety mixtures (dark grey bars) in Part I.** Error bars represent ±1 SE. Asterisks indicate individual monocultures that were significantly different than the mixtures.

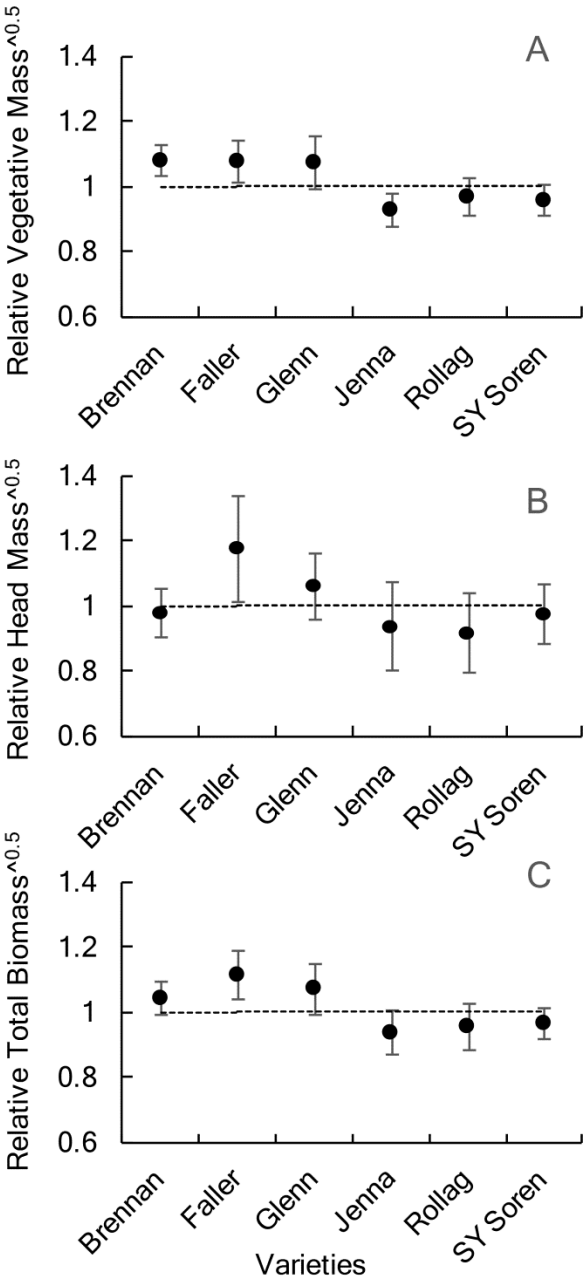

Figure 4-5. **Relative Plant Index values for vegetative biomass (A), seed head mass (B), and total biomass (C) for each of the six varieties in Part I.** The index compares dried plant mass for an individual variety grown in mixtures to when grown in monocultures. Values > 1 signify larger mass for the variety when it grows in mixtures, while values < 1 signify less mass in mixtures. Error bars represent 95% confidence intervals, and varieties whose error confidence intervals do not cross 1 are significantly different than 1 at $\alpha = 0.05$.

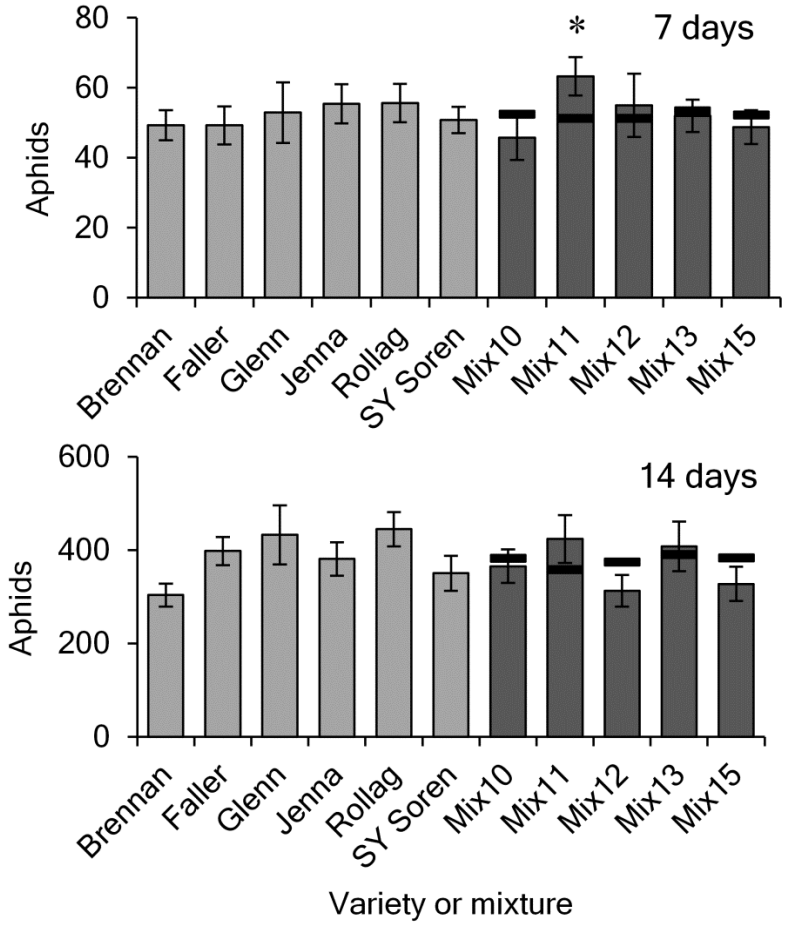

Figure 4-6. **Mean aphid populations 7 and 14 days after initial infestation on six varieties in monoculture (light grey bars) and for five of the fifteen possible four-variety mixtures (dark grey bars) in Part II.** Error bars represent ±1 SE. Black horizontal bars represent expected aphid populations based on the mean populations in monocultures of the varieties that compose the mixture. In Part II, the emphasis was on comparing each mixture to this mean. Asterisks indicate mixtures that were significantly different than their constituent varieties in monoculture.

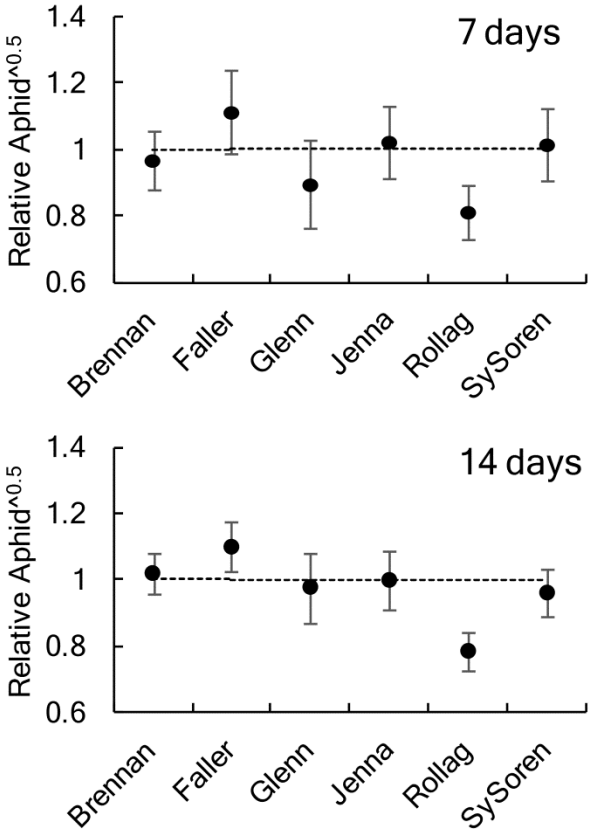

Figure 4-7. **Relative Aphid Index values for each of the six varieties 7 and 14 days after infesting plants with aphids in Part II.** The index compares the number of aphids on an individual variety grown in mixtures to when grown in monocultures. Values > 1 signify more aphids on the variety when it grows in mixtures, while values < 1 signify fewer aphids in mixtures. Error bars represent 95% confidence intervals, and varieties whose error confidence intervals do not cross 1 are significantly different than 1 at $\alpha = 0.05$.

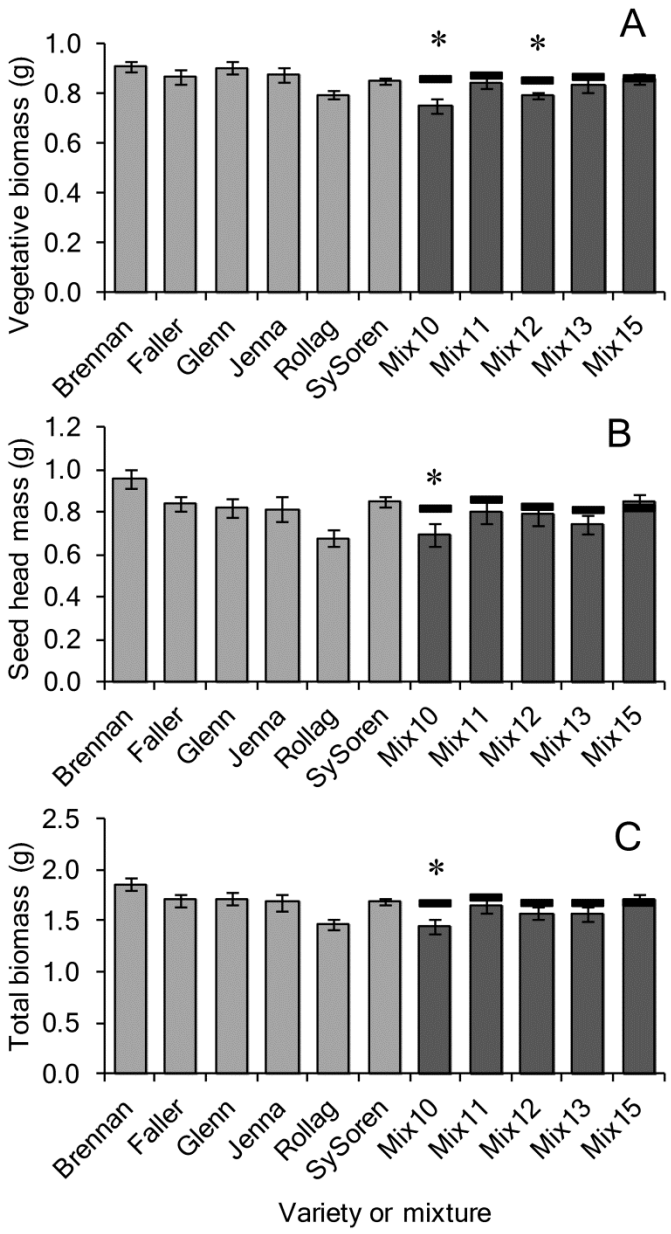

Figure 4-8. **Total vegetative biomass (A), seed head mass (B), and total aboveground biomass (C) for six varieties in monoculture (light grey bars) and for five of the fifteen possible four-variety mixtures (dark grey bars) in Part II.** Error bars represent ±1 SE. Black bars represent expected mass based on the mean mass in monocultures of the varieties that compose the mixture. In Part II, the emphasis was on comparing each mixture to this mean. Asterisks indicate mixtures that were significantly different than their constituent varieties in monoculture.

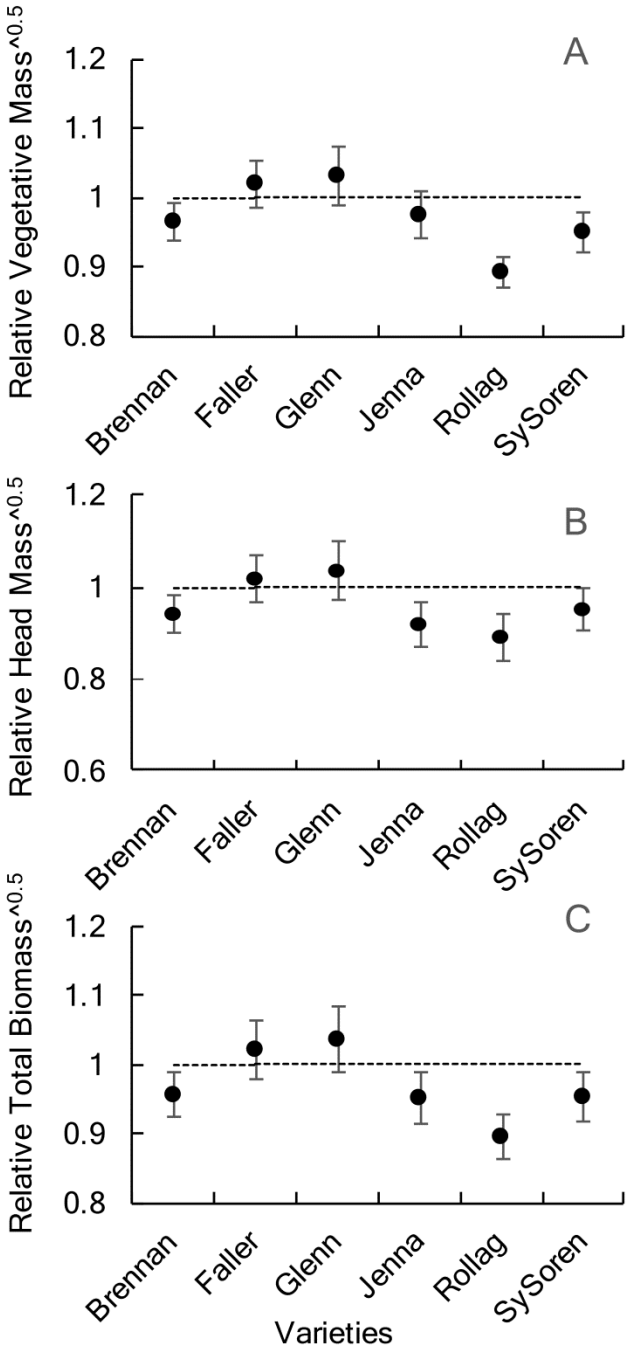

Figure 4-9. **Relative Plant Index values for vegetative biomass (A), seed head mass (B), and total biomass (C) for each of the six varieties in Part II..** Values are for comparisons involving five of the fifteen possible mixtures. The index compares dried plant mass for an individual variety grown in mixtures to when grown in monocultures. Values > 1 signify larger mass for the variety when it grows in mixtures, while values < 1 signify less mass in mixtures. Error bars represent 95% confidence intervals, and varieties whose error confidence intervals do not cross 1 are significantly different than 1 at $\alpha = 0.05$.

Chapter 5

Intra-varietal interactions between plants in variety mixtures tend to decrease herbivore performance

Abstract

Both diversity among and within plant species can influence resistance of plant communities to biotic and abiotic stress in natural and agricultural ecosystems. Much research has explored the effects of plant species diversity on herbivore populations, but far less has considered how the genotypic diversity of a plant community can influence resistance to herbivores, or how this resistance can be modified by abiotic stress, like drought. In previous research, genotypically diverse plantings have inconsistently influenced herbivore populations, mostly decreasing populations, but occasionally increasing them. The mechanisms by which plant genotypic diversity affects herbivore populations, including agricultural pests, remain largely unresolved. I used greenhouse studies with wheat (*Triticum aestivum* L.) and bird cherry-oat aphid (*Rhopalosiphum padi* L.) to explore how the genotypic diversity of a plant's neighborhood influences performance of individual herbivores confined on a focal plant, measured through number of offspring produced, as well as how drought stress changes the influence of neighborhood diversity. Across five varieties of wheat, plant-plant interactions in more diverse neighborhoods reduced aphid performance and generated associational resistance, although the specific response of aphid performance to neighborhood diversity depended on variety identity. Drought stress greatly diminished the overall reduction in aphid performance in diverse mixtures. Neighborhood diversity influenced the size of mother aphids, and appeared to partially explain how plant-plant interactions reduced the number of offspring produced in mixtures. Plant size did not mediate effects on aphid performance, although neighborhood diversity reduced plant mass

across varieties and watering treatments. My results suggest that the plant-plant interactions in genotypic mixtures can affect herbivores even in the absence of herbivore movement and that abiotic stress may minimize the strength of the effect. Accounting for how neighborhood diversity influences resistance of an individual plant to herbivores will help clarify the role of plant genotypic diversity in ecosystems and aid development of mixtures of varieties that effectively reduce insect pests.

Introduction

To predict how ecosystems will respond to diversity loss, climate change, and biotic invasions, it is necessary to describe the ecological consequences of plant diversity. Higher levels of diversity within plant communities can improve resistance and resilience when they are confronted with abiotic and biotic stressors (Díaz and Cabido 2001, Hughes et al. 2008). For instance, diverse assemblages of plant species can resist drought and maintain biomass better than monocultures (Mulder et al. 2001, Steudel et al. 2011, Prieto et al. 2015). Improved resistance provided by diverse communities is vital because climate change will increase abiotic stress in many regions (Porter et al. 2014), and has already reduced crop yields in some areas (Moore and Lobell 2015). Climate change is also expected to influence herbivory (Bale et al. 2002), one of the primary biotic stressors in plant communities. Clarifying how plant diversity influences ecosystem processes in agricultural systems will help determine how we can manipulate diversity to improve ecosystem services like pest control. Increased plant species diversity can help alleviate stress on crops by influencing herbivore abundance and diversity, often improving control of insect pest populations in crop fields (Andow 1991, Tonhasca and Byrne 1994, Schlapfer and Schmid 1999, Letourneau et al. 2011).

Several hypotheses have been proposed to explain the influence of diversity within species on herbivores in natural and agricultural system, and they incorporate both natural enemy- and plant-mediated effects (i.e., top-down and bottom-up effects; Finch & Collier 2000; Poveda, Gómez & Martínez 2008; Letourneau *et al.* 2011). The enemies hypothesis suggests that natural-enemy communities provide better top-down control of herbivores in species mixtures than in monocultures (Root 1973, Russell 1989). Hypotheses describing bottom-up effects of diversity on herbivores suggest diversity disrupts host-finding by masking, repelling or otherwise obscuring hosts, changing the likelihood of herbivores finding appropriate hosts, or some combination of these effects (Poveda et al. 2008). The associational resistance hypothesis combines many of the

previous mechanisms and states that herbivore pressure is lower when plants are in diverse communities (Tahvanainen and Root 1972).

Diversity within plant species (i.e., genotypic or intraspecific diversity) can also influence arthropod behavior and populations (Power 1988, Utsumi et al. 2011), and this influence can be as strong as plant species diversity (Crutsinger et al. 2006, Cook-Patton et al. 2011). Intraspecific versions of associational resistance and the enemies hypothesis likely mediate some of these effects, but it is unclear how interactions among different genotypes influence herbivore populations. Relative to genetic monocultures, plantings with increased genotypic diversity can alter herbivore behavior and the diversity and structure of herbivore and/or predator communities, at times decreasing herbivore populations (Power 1988, 1991, Shoffner and Tooker 2013). For example, increasingly diverse plantings of wheat reduced the growth rate and size of aphid populations, which is promising for pest management (Shoffner and Tooker 2013). Conversely, plant genotypic diversity can benefit herbivores, leading to higher populations (Crutsinger et al. 2008, Utsumi et al. 2011), or may simply not influence herbivores (Genung et al. 2012). These seemingly inconsistent responses of herbivores to genotypic diversity are generally not well understood or explained within a mechanistic framework.

Identifying the mechanisms behind the influence of plant genotypic diversity on herbivores could help explain these inconsistent effects of plant genotypic diversity on herbivory and how interactions among plant genotypes alter resistance to herbivores. This knowledge will be essential if variety mixtures are to be developed as an effective management tactic for pest insects (Tooker and Frank 2012, Grettenberger and Tooker 2015). Many studies have focused on population-level responses of herbivores to genotypically diverse plantings relative to genetic monocultures. This type of research often explores the influence of diversity by assessing herbivore abundance or herbivory across a population of plants. While clearly important for describing whether genotypic diversity affects herbivores, this approach may not address how

diversity influences herbivores and may not untangle plant- and insect-based effects. To clarify the mechanistic influence of diversity, studies can evaluate individual plants and compare the response of herbivores to genotypes grown in mixtures and monocultures (Johnson et al. 2006). However, these studies often conflate changes in herbivore performance, fitness, movement, and predation (e.g., Kotowska et al., 2010; Shoffner and Tooker, 2013; Utsumi et al., 2011). Alternatively, studies can focus on how individual herbivores respond to higher levels of genotypic diversity. Such studies have shown herbivores may cause less damage as they feed in more genetically diverse settings (McArt and Thaler 2013), but also that they can increase in size and survive better in mixed plantings (Kotowska et al. 2010). In these cases, herbivores feed upon multiple plants or sequentially feed on foliage from different genotypes, obscuring how the associational resistance/susceptibility of an individual genotype is influenced by diversity of the plant neighborhood.

What needs to be understood is how the genotypic diversity of a plant's neighborhood influences performance of herbivores, which would help clarify the mechanisms by which plant intraspecific diversity affects herbivore populations. To address this question, I conducted greenhouse studies using wheat (*Triticum aestivum* L.) and bird cherry-oat aphid (*Rhopalosiphum padi* L., Fig. 1), a pest of small grains, as a model system. By restricting aphid movement, I isolated how genotypic diversity of a focal plant's neighborhood influences reproductive performance of herbivores. Moreover, I tested if the influence of genotypic diversity was similar when the plant community faces an abiotic stressor, such as drought. Like species-diverse plant communities, genotypically diverse communities can be more resistant and resilient to abiotic stressors (Peltonen-Sainio and Karjalainen 1991, Reusch et al. 2005). Consequently, how genotypic diversity influences herbivores may depend on environmental context and effects may only emerge when plants are stressed (Steudel et al. 2011). I hypothesized that: 1) aphid performance would depend on whether the host plant is surrounded by neighbors of the same or

different genotypes; 2) the effect of diversity will depend on which variety is the focal plant; and 3) the influence of neighborhood diversity will be greatest in the presence of drought. I assessed aphid performance by the number of offspring produced. To identify specific mechanisms by which genotypic diversity could influence aphid performance, I measured mother aphid developmental time and size. I assessed changes in plant mass and phytohormone levels in the focal plant to provide insight on whether plant defenses mediate how neighborhood diversity affects aphid resistance.

Methods

Plants and insects

To choose wheat varieties, I first conducted a variety trial. In the trial, I measured the independent resistance to aphids and drought stress of eighteen varieties of soft red winter wheat (for more details, see Appendix A1). From these, I chose five to use in the main experiment based on their various combinations of screened, independent resistances to drought and aphids. The trial and subsequent main experiment were conducted in a semi-climate-controlled greenhouse, where plants grew in square pots (10 × 10 × 9 cm) filled with a peat-based potting mix (Premier Promix BX, Premier Horticulture Inc., Quakertown, PA). Unless noted otherwise, I watered plants as needed to maintain sufficient soil moisture. I obtained *R. padi* from a pathogen-free colony housed in the Department of Plant Pathology at Penn State. I maintained the colony at room temperature (21ºC) under a 15:9 h (L:D) photoperiod on a winter wheat variety I did not use experimentally (SW60).

Experiment: Overview and treatments

To determine how neighborhood diversity influenced herbivore performance, I used the pool of five varieties selected in the variety trial (see Appendix A1, Table A1-2) to create "minimally diverse" and "more diverse" neighborhoods surrounding a focal plant. Each variety served as the focal plant, facilitating more robust conclusions about the influence of diversity *per se* compared to the response of specific varieties to diversity or of particular types of diversity. In the minimally diverse neighborhood, the focal plant was surrounded by four plants of the same variety, creating a monoculture of one variety with plants arranged like the five dots on a die (see Appendix A2 for more details on general growing conditions). In the higher diversity neighborhood, two different varieties (two plants of each) surrounded the focal plant, creating a three-variety mixture. During the experiment, all six possible pairwise combinations of the

remaining four varieties surrounded each focal variety (Fig. 2), controlling the influence of particular mixtures and surrounding varieties. I will refer to the two diversity treatments as "low" and "high" diversity neighborhoods for simplicity. Due to logistical constraints, the experiment was conducted over two years. In Year I (2013), I tested two of the varieties, USG3770 and Patton, as focal varieties. I tested the other three varieties as the focal variety in Year II (2014), and included a previously tested variety (USG3770) as an internal control. Within the two diversity treatments, I subdivided pots equally between watering treatments (watered or drought-stressed).

In Year I, I replicated the low diversity (monoculture) treatment 16 times for each of the two watering treatments for each focal variety, and I replicated the individual high diversity treatments four times (6 possible combinations per variety; $n = 24$ diverse for drought, $n = 24$ for watered). Year I was divided between four spatial blocks (total n per variety = 80). In Year II, I reduced replication because of the greater number of focal varieties, but actual replication was similar because of small differences in experimental methods that reduced loss of replicates. For each focal variety, I replicated the low diversity treatment was 10 times for each watering treatment. I replicated individual mixture treatments twice each (6 possible per variety; drought: $n = 12$, watered: $n = 12$). I also divided the experiment in Year II among four spatial blocks (total per variety: $n = 44$).

In both years, I standardized moisture levels in pots before initiating pulsed drought cycles by withholding water from pots in the drought stress treatment. I subjected pots in the drought treatment to three cycles of drought in Year I, and two in Year II, which was based on timing of experimental events. I assessed moisture levels to determine if the diversity of the pot affected moisture levels and, potentially, drought stress. See Appendix A3 for a more detailed description of drought stress methods and moisture levels.

Experiment: Aphids

I added aphids to the focal plant when plants had several tillers (four weeks post planting, Zadok's scale 2.2-2.3; Zadok et al. 1974). I placed individual aphids in clip cages constructed from a 4.5-cm diameter Petri dish and lid, craft foam, mesh fabric, and a hair clip. I placed a 6-12 h-old, first instar *R. padi* nymph within the clip cage on the penultimate leaf of the main stem (4 cm from the leaf base).

I collected data for the focal mother aphid and her offspring. I checked cages daily to measure time-to-adulthood of the focal aphid, which would affect the number of offspring she produced. The nymphs developed into alates, although their large size indicated it was not because of excessive stress on the experimental plants (a very small percentage grew into apterous adults and were excluded from analyses). I counted the number of offspring eleven (Year II) or thirteen (Year I) days post-infestation, and before a third generation, I measured the combined mass of all offspring to provide another measure of aphid performance. Compared to a population of larger offspring, smaller offspring would impose less stress on the plant and would likely produce lower population growth rates. However, the mass of offspring feeding on the same leaf as the mother would combine effects on the aphid mother with similar effects on the offspring, so would likely accentuate any treatment effects. These measurements of the offspring were used to assess aphid "performance." I collected and froze the mother in Year II to determine if experimental factors affected mother aphid's body size, which could in turn alter how many offspring she produced (Dixon 1976). I estimated her body size by measuring the length of her right-hind tibia using a calibrated dissection microscope fitted with an eyepiece graticule (Nicol and Mackauer 1999).

Plant measurements: Plant biomass and plant defenses

To assess treatment effects on plant biomass, I cut focal plants at soil level, dried them for five days at 93°C, and weighed them. To provide a measure of plant defensive response, in Year II, I extracted and measured levels of salicylic acid (SA) and jasmonic acid (JA) in the focal plant 12 days after initial infestation. SA and JA are defense-related phytohormones that mediate plant resistance to herbivores (Walling 2000). I used a previously described method for this analysis (Schmelz et al. 2003, 2004). Briefly, at the end of the experiment, I cut the leaf section contained by the clip cage (≈ 0.1 g fresh weight), transferred it to a micro-centrifuge tube, and froze it in liquid nitrogen. I stored leaf tissues at -80°C until later analysis. I derivatized carboxylic acids to methyl esters and then isolated them using vapor-phase extraction and then analyzed phytohormone levels via coupled GC-MS with isobutane chemical ionization using selected-ion monitoring. Peaks were quantified and compared to internal standards. Phytohormone levels were calculated per gram of fresh leaf weight. JA levels were very low and obscured by background noise and were therefore not quantified.

Statistical analysis

I conducted statistical analyses using SPSS (IBM, Version 22.0.0.0). I analyzed Years I & II separately because of small differences in methodological details and data distributions. I tested with factorial analysis of variance (ANOVA) the effects of neighborhood diversity (low or high), watering treatment, and focal-variety identity on offspring number, offspring mass, tibia length, focal plant mass, and SA content. Values were transformed as needed. When applicable, I tested the significance of "simple main effects," adjusted for multiple pairwise comparisons with a Dunn-Šidák correction. In all ANOVA analyses, I excluded the three-way interaction (*diversity* × *watering* × *variety*) when non-significant. For aphid data, I omitted replicates in which the aphid escaped, died, or was apterous (44 replicates in Year I, 2 in Year II). Due to errors in

processing, I removed six samples for SA from the analysis and two extreme values were winsorized (Sokal and Rohlf 1995), although this did not change the overall conclusions. Two replicates for plant mass were lost while drying in Year I. Development time was 5 or 6 days in Year I and 6 or 7 days in Year II. I therefore tested for treatment effects on development time using generalized linear models and Wald chi-square statistics, assuming a binomial distribution with the logit link function to link development time to the linear scale, and a maximum likelihood scale parameter estimate. Following a significant main effect or interaction, I tested pairwise differences with Wald tests and a Dunn-Šidák correction. I treated block as a fixed factor throughout because of relatively few levels that were not a representative random sample of a larger population. Unless noted otherwise, means reported in-text are marginal means and I calculated percent differences with these values. I back transformed marginal means when raw data were transformed for analysis.

Generalizing across years of the experiment and focal varieties: Effect of diversity *per se*

To determine how neighborhood diversity *per se* affects offspring number when plants were watered or drought-stressed, I generalized across the experiment and combined summary data from Years I & II for all varieties. Within each watering treatment and for each variety, I first calculated the difference between monoculture and diverse neighborhoods for each response variable. I then summed these differences across the five varieties for each watering treatment. This estimated the average number of offspring mother aphids would produce, on each of the five varieties in a diverse neighborhood compared to the same varieties in monocultures. The variety USG3770 was tested in both years, so I averaged the number of offspring produced in Years I & II before calculating differences. The overall mean number of offspring was similar for each year for this variety, so I weighted them equally.

I treated focal plant mass similarly to offspring number, again allowing me to estimate the effect of neighborhood diversity *per se*. The overall average masses varied between years, including for the variety used as an internal control, USG3770. However, the overall patterns were the same for plant mass in both years for USG3770 and plant mass responded consistently to treatments across years. To account for the inter-year variation, I used a weighting factor to up-weight means for each treatment from Year I before I averaged across years and calculated differences between diversity treatments. I determined the weighting factor by calculating the ratio of average plant mass for USG3770 from Year I to Year II. I used this weighting factor to weight data from Year I equally to Year II when I calculated the predicted biomass in a mixture of all five varieties.

Results

Generalizing across years of the experiment and focal varieties: Effect of diversity per se

When I generalized across the experiment and focal varieties to examine the influence of diversity *per se* on offspring production, neighborhood genotypic diversity tended to reduce offspring production in both watering treatments, but the neighborhood influence was strongest when plants were watered (Table 1). Diversity appeared to reduce plant mass more when plants were drought-stressed, although the differences combined across varieties were similar for both watering treatments (Table 1).

Offspring number and mass

Neighborhood diversity influenced reproductive output of mother aphids. For the two varieties tested in Year I (USG3770 and Patton), increased diversity reduced the number of offspring the mother aphid produced by 14.0% (N=116, Table 2, Fig. 3). The effect was similar across watering treatments and varieties (Table 2). However, there was an almost twofold greater reduction in the number of aphids in diverse neighborhoods when plants were watered compared to when plants were drought-stressed (18.6% vs. 9.5% reduction; Table 2, Fig. 3). Focal variety did not influence offspring production in Year I (Table 2, Fig. 3). For all results, see Appendix A4 for additional details.

In Year II, aphids produced similar numbers of offspring as in Year I (Year I: *Mean* = 22.8; Year II: *Mean* = 20.86, *N* = 174,). In particular, aphids on USG3770 produced equivalent numbers of offspring across treatments in Years I & II (*Mean* = 22.6 and 22.8, respectively). For Year II, neighborhood diversity influenced offspring production, but the effect depended on which variety was the focal plant (Table 2, Fig. 3). In addition, there was a significant *diversity* × *watering* interaction for number of offspring (Table 2, Fig. 3), which was driven by two patterns. First, when plants were adequately watered, neighborhood diversity reduced offspring production

across varieties by 10.5% ($F_{1,158} = 6.54$, $P = 0.011$), but this influence disappeared when plants were drought-stressed ($F_{1,158} = 0.11$, $P = 0.74$). Second, when focal plants were in diverse neighborhoods, drought decreased offspring production ($F_{1,158} = 3.79$, $P = 0.053$), but not in monocultures ($F_{1,158} = 0.83$, $P = 0.36$).

As expected, general patterns for total mass of offspring between treatments and varieties were similar to patterns for number of offspring (Table 2, Fig. 4). Furthermore, offspring number was positively related to their summed mass (mass was square-root transformed; Year I & II: $r = 0.90$, $P < 0.001$). In Year I, high neighborhood diversity reduced offspring mass relative to monocultures (21.9% decrease in offspring mass), while the *diversity* × *variety* and *diversity* × *watering* interactions were not significant (Table 2). Though the *diversity* × *watering* interaction was not significant (Table 2), neighborhood diversity reduced offspring mass more when plants were adequately watered (32.6% reduction) than when plants were drought-stressed (14.4% reduction; Fig. 4).

Diversity influenced offspring mass in Year II, but with these varieties the effect of diversity again depended on the focal variety (Table 2, Fig. 4). The *diversity* × *watering* interaction was nonsignificant (Table 2), although the pattern was similar to that of offspring number (i.e., lower offspring mass in diverse neighborhoods, but only when plants were adequately watered; Fig. 4). Offspring mass differed between focal varieties, but the diversity of the plant neighborhood determined ordering and significance of differences between varieties (*diversity* × *variety* interaction, Table 2, Fig. 4).

Mother aphid size and development time

Mother aphid size correlated strongly with the number of offspring she produced (untransformed data, $r = 0.69$, $P < 0.001$, $N = 174$; Year II). A substantial portion of the variation (52.4%) remained unshared between offspring number and mother size. Size of the mother aphid

therefore mirrored some, but not all, of the patterns exhibited by offspring. Strength of treatment effects on mother size were generally smaller than for measurements of offspring. Neighborhood diversity influenced mother size, but the effect depended on focal variety and watering treatment (Table 2). Across varieties, neighborhood diversity did not influence mother aphid size when plants were adequately watered ($F_{1,158} = 0.66$, $P = 0.42$), but significantly increased mother aphid size when plants were drought-stressed (3.11% larger; $F_{1,158} = 7.78$, $P = 0.006$; Fig. 5). This indicates a greater influence of neighborhood diversity on mother size when plants were drought-stressed, which was opposite of the pattern seen for offspring number. Identity of the focal variety did not affect mother size in diverse neighborhoods ($F_{3,158} = 1.24$, $P = 0.30$), but did in monocultures $F_{3,158} = 13.98$, $P < 0.001$; Fig. 5).

Neighborhood diversity and watering treatment influenced the time it took mother aphids to develop during both years of the experiment (Table 3, Fig. 6). In Year I, the *diversity* × *watering* interaction was significant (Table 3). In watered pots, mother aphids were more likely to develop slowly in diverse neighborhoods than in monoculture neighborhoods. However, when plants were drought-stressed, aphids in diverse neighborhoods were less likely to develop slower (Fig. 6). Focal variety did not influence development time (Table 3). In Year II, focal variety was important in moderating the interacting effects of neighborhood diversity and watering treatment (*variety* × *diversity* × *watering* interaction, Table 3). Generally, neighborhood diversity influenced the time it took the mother aphid to develop in only one of the watering treatments, but the direction of the effect as well as the watering treatment in which diversity affected development time depended on focal variety (Fig. 6).

Focal plant mass

Mass of the focal plant was influenced in both years by the interaction between neighborhood diversity and variety (Table 2, Fig. 7). For both years, the influence of diversity on

focal plant mass was unaffected by watering treatment (Table 2). Drought stress decreased plant mass by nearly 20% during each year (Year I: 18.9% decrease, Year II: 17.7%), an effect that did not depend on focal variety or neighborhood diversity (Table 2, Fig. 7).

Phytohormones: Salicylic acid

Diversity of the focal plant's neighborhood did not consistently affect the levels of SA across watering treatments or focal varieties. For SA, the interaction between diversity, variety, and watering treatment was significant (data only for Year II; Table 2; Fig. S1). I focused on the simple two-way interactions involving neighborhood diversity. The simple *diversity* × *watering* interaction was only significant for Freedom ($F_{1,148} = 7.49$, $P = 0.007$), and plants in diverse neighborhoods produced greater concentrations of SA than monocultures when drought-stressed ($F_{1,148} = 4.47$, $P = 0.036$) and smaller concentrations when plants were watered ($F_{1,148} = 3.27$, $P = 0.073$; Fig. S1). The simple *diversity* × *variety* interaction was not significant for either watering treatment (watered: $F_{1,148} = 2.87$, $P = 0.093$; drought: $F_{1,148} = 2.37$, $P = 0.13$). Across treatments, SA concentrations were not correlated with number of offspring ($r = 0.131$, $N = 167$, $P = 0.093$) or weight of offspring ($r = -0.028$, $N = 167$, $P = 0.72$). Similar to plant mass, the patterns did not correspond well to patterns for measures of offspring performance (Table 2, Figs 3, 4, and 7).

Discussion

My results provide evidence that genotypic diversity of plant neighborhoods can influence herbivores via host-plant mediated interactions. By restricting movement, I found that genotypic diversity shapes herbivore populations by altering aphid performance, as measured by offspring production and offspring mass, even though the mother aphid did not directly interact with neighboring plants. Furthermore, the results provide the novel insight that genotypic diversity can influence associational resistance/susceptibility, and I expect that this altered host

preference emerges from a combination of above- and below-ground interactions that alter plant phenotype (Barbosa et al. 2009). Consistent with my original hypothesis, I found that the genotypic diversity of plant neighborhood strongly influenced aphid performance. Moreover, the interactive influence of variety identity and neighborhood diversity on aphid performance supported my hypothesis that the effect of neighborhood diversity would depend on identity of the focal variety. For each variety, neighborhood diversity affected offspring mass and offspring number similarly (Tables 1, Figs 3 and 4). However, contrary to my expectations, stressing plants with drought dampened the effect of neighborhood diversity on aphid performance. How drought moderates the influence of neighborhood diversity on offspring production appears to depend on focal variety and the direction of the diversity effect. The mixed effects of different genotypes on aphid performance may provide insight on conflicting results that have indicated that genotypic diversity can decrease (Power 1988, Shoffner and Tooker 2013) or increase herbivore populations (Utsumi et al. 2011, Genung et al. 2012, Hambäck et al. 2014), especially in cases where the experimental design encompassed limited genotypic diversity.

My results generally suggest that neighborhood genotypic diversity, and diversity *per se*, can reduce herbivore performance. By combining results across experimental years and varieties, I found that diversity *per se* produced emergent effects on herbivores. Differences for USG3770 between the two years of the experiment may reflect methodological differences (e.g., number of drought events, differences in environmental conditions), but how neighborhood diversity affected aphid performance depended on the watering treatment. When plants were adequately watered, aphids produced 12% fewer offspring in mixtures than in monocultures. Periodic drought stress dampened the magnitude of this influence of genotypic diversity to about 1.5%. It should be noted, however, that aphids could not move and choose preferred varieties, which would likely occur in natural populations. Nevertheless, my results demonstrate that variety mixtures may reduce aphid populations and that effects of neighborhood diversity on aphid

performance, which are mediated by host-plant effects, could drive this reduction. If a similar overall effect also occurred in the field, it may help explain why genotypically diverse mixtures can maintain lower herbivore populations (Power 1991, Shoffner and Tooker 2013).

My experimental design did not allow me to determine the respective roles of above- and belowground interactions, but previous work suggests they likely combined to alter herbivore performance (Cahill 2002, Glinwood et al. 2009). Belowground, roots of the focal plants intertwined with those of its neighbors, and plant roots can detect the presence and relatedness of neighbors (Chen et al. 2012). Root exudates appear to mediate these belowground interactions by altering plant phenotypes, which can then influence interactions with herbivores (Glinwood et al. 2003, Biedrzycki and Bais 2010). Aboveground, volatile organic compounds (VOCs) can mediate interactions among plants (Glinwood et al. 2009, Ninkovic et al. 2009, Karban et al. 2014); thus, focal plants may have responded to airborne VOCs produced by genotypically different neighbors, altering their interactions with herbivores (e.g., Kellner et al., 2010). Alternatively, mother aphids may have responded directly to exposure to VOCs, which tend to be more diverse for genotypically diverse mixtures (Shoffner and Tooker 2013). Both types of interactions mediate how plant phenotype and resistance to herbivores change as genotypic diversity of neighborhoods shift. Plant phenotypes in mixtures may differ from those in monocultures because of kin cooperation, niche partitioning, or competitive hierarchies (Dudley and File 2007, File et al. 2012). Further research is needed to distinguish the effects of above- and belowground plant-plant interactions, the underlying ecological causes of these effects, and importantly, how these interactions affect higher trophic levels.

While the precise mechanism is not well-defined, my results clearly indicate that plant-plant interactions altered offspring number and mass on the focal wheat variety and provide some indication of how diversity influenced aphid performance. Aphids were not allowed to move, so effects of neighborhood diversity on the mother aphid resulted from interactions between the

focal plant and its neighbors. Plant defenses are one mechanism by which neighborhood diversity may have affected aphid performance. Levels of SA that I measured did not appear to explain the treatment effects, but this outcome may stem from the timing of the assay (two weeks after infestation), rather than earlier in the interaction between plant and aphid. Plant defenses are likely to play a role in determining how neighborhood diversity influenced aphids, but these interactions among plants could also affect nutritional quality of the plants for herbivores (Schädler et al. 2007). For aphids, changes in nutritional quality typically involve altered amino acid profiles of the phloem (Karley et al. 2002). Neighborhood diversity may have also simultaneously altered both plant defenses and nutrition, which together can interactively affect herbivore performance (Simpson and Raubenheimer 2001, Behmer et al. 2014).

Regardless of the specific mechanism, plant-plant interactions significantly influenced the size of the aphid mother at maturity, and mother size appeared to account for much of the influence of neighborhood diversity on offspring production. Across treatment combinations, patterns in mother size largely matched those for offspring number (Figs 3, 4, and 5) and total mass and mother size correlated with offspring production. Tibia length does not change much after the final molt into adulthood, suggesting treatments altered growth of the mother aphid and therefore her size at maturity. This in turn changed offspring production because larger aphids produced more offspring (Foster and Benton 1992). Nonetheless, the relationship between mother size and offspring production was imperfect, indicating neighborhood diversity influenced offspring production via additional routes. Altered development time does not appear to account for the overall influence of neighborhood diversity, but the interacting effects of variety, diversity, and watering treatments on development time may help explain why the influence of diversity changed when plants were drought-stressed (Table 3, Fig. 6). In a field setting, increased development time could further accentuate population-level effects by exposing aphids to natural enemies for a longer period.

When generalized across the experiments, focal plant biomass was reduced ~4% in genotypically diverse neighborhoods under both watering treatments. This overall reduction contrasts with results from field studies that showed a yield increase in genotypic mixtures (Smithson and Lenné 1996, Kiær et al. 2006, Cowger and Weisz 2008) and may suggest that aphid suppression comes at a small cost of plant productivity. It should be noted, however, that the individual varieties behaved variably, including a variety that consistently exhibited increases in plant biomass (USG3770, Fig. 7). Moreover, my experiment did not include herbivore-free control plants to control for the influence of the herbivore relative to the cost/benefit of genotypic diversity. *R. padi* can reduce yield considerably in the field (Kieckhefer and Kantack 1988), but aphids in my experiment had little opportunity to significantly influence biomass in because they were only on plants for 2 weeks and "large" aphid populations (~20 aphids on the focal plant) only occurred at the end of the experiment. Nevertheless, plant genotypic diversity still reduced herbivore population size and associated feeding damage and, as has been found for pathogens, resulting in net positive effects on productivity (Mundt 2002). Notably, while neighborhood diversity significantly affected plant mass, it did not consistently influence aphid growth or development similarly; thus, reductions in plant mass did not appear to mediate the effect of neighborhood diversity on herbivore performance.

My results emphasize that modeling or empirical efforts investigating the influence of genotypic diversity on herbivore populations should consider host-plant mediated effects on herbivore performance. This applies to both research seeking to understand the ecological consequences of genotypic diversity, and to research whose aim is to develop variety mixtures for pest control. For instance, manipulative experiments and mechanistic models have shown that plant neighborhoods can affect herbivore movement to, within, and from mixtures compared to monocultures of the same genotypes and that these processes can alter herbivore populations (Power 1991, Underwood et al. 2011, Hambäck et al. 2014). My results indicate that the

neighborhood in which an herbivore develops can influence its production of offspring and its fitness as an adult. Incorporating the fitness consequences for herbivores of this neighborhood diversity would improve the predictive power of models and help to partition effects of diversity in experiments. Furthermore, determining how genotypic diversity of plant neighborhoods affects herbivores will be imperative to develop effective crop variety mixtures. When creating variety mixtures for disease management, plant pathologists have accounted for how variety mixtures actually reduce the spread and severity of disease (Wolfe 1985, Mundt 2002). Research developing variety mixtures for insect pest management should similarly delineate how mixtures of varieties affect crop pests and how variety choice influences pest populations at field levels in mixtures. My results demonstrate that the specific components of variety mixtures matter because varieties respond differently to neighborhood diversity and can determine whether mixtures are more or less effective at suppressing pest populations than monocultures. Consequently, researchers need to identify varieties that lower herbivore performance when surrounded by diverse neighbors and that possess desirable agronomic traits. By accounting for the direct effects of plant genotypic diversity on herbivores, variety mixtures will be more effective for pest management and we can better understand the role of plant genotypic diversity in structuring arthropod communities and ecosystems.

In summary, my findings provide evidence that neighborhood genotypic diversity can significantly influence herbivore performance via associational resistance and susceptibility, which would likely produce cascading effects on herbivore abundance and population dynamics. Across varieties, diverse plant neighborhoods reduced aphid reproductive output, although the direction and magnitude of the effect varied between varieties and depended on whether an abiotic stressor also challenged the plants. These results provide mechanistic evidence that mixtures of crop varieties may prove useful in managing pests and suggest genotype identity can determine the response of herbivores to plants grown in mixtures. However, the presence of an

abiotic stress, such as drought, may reduce the pest-management benefits of mixtures, although further research is needed to explore this issue, particularly at field scales. Nevertheless, my findings suggest that genotypically diverse mixtures generally reduced herbivore performance via altered host-plant physiology (e.g., increased defenses and/or lower nutritional quality) that altered herbivore population growth. These findings contribute to a growing body of work demonstrating the ecological significance of genotypic diversity for plant communities and higher trophic levels in both agricultural fields and natural ecosystems (Johnson et al. 2006, Hughes et al. 2008, Glinwood et al. 2011). Furthermore, these findings suggest that harnessing crop genotypic diversity via mixtures of varieties holds potential as a sustainable pest management strategy that could reduce herbivore populations and reduce pesticide inputs and costs of pest management (Tooker and Frank 2012, Grettenberger and Tooker 2015).

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Tables

Table 5-1. **Difference (numerical and percent) in offspring number and dried focal plant mass between high and low diversity neighborhoods for each watering treatment within each variety (low diversity as reference) and combined across Years I & II.**

Variety	Watering treatment	Offspring number		Focal plant mass	
		Difference	%Difference	Difference	%Difference
USG3770‡	Watered	-2.86	-12.21	0.43	21.59
	Drought	-0.69	-2.94	0.42	26.88
Patton	Watered	-5.18*	-20.54*	-0.13	-6.76
	Drought	-3.10*	-12.43*	-0.12┼	-7.54┼
Freedom	Watered	-6.29*	-25.62*	-0.47*	-19.68*
	Drought	-3.82*	-16.47*	-0.50*	-22.65*
GR962	Watered	-5.32*	-22.43*	-0.21	-7.71
	Drought	-2.74*	-12.14*	-0.24	-11.11
SW27	Watered	5.88*	43.01*	0.03	1.13
	Drought	8.84*	70.61*	-0.01	-0.40
Combined†	Watered	-13.77	-12.42	-0.36	-3.16
	Drought	-1.51	-1.42	-0.46	-4.85

Difference = $\text{Mean}_{\text{High}} - \text{Mean}_{\text{Low}}$

%Difference = $(\text{Mean}_{\text{High}} - \text{Mean}_{\text{Low}})/\text{Mean}_{\text{Low}} \cdot 100$.

*These differences are significant at $\alpha = 0.05$, based on a factorial ANOVA (see Tab. 1 and text). The *diversity* × *variety* × *watering* interaction was nonsignificant in both years, so when the effect of diversity was significant, the effect was significant for both watering treatments.

┼Difference is marginally significant at $\alpha = 0.10$.

†Combined values consist of sums of the individual varieties.

‡Differences for USG3770 were averaged across Years I & II and statistical significance is therefore not presented.

Means used to calculate these values can be found in Figs. 2 and 6.

Table 5-2. **The effects of neighborhood diversity, drought stress, and focal variety identify on offspring number, offspring mass, mother size, focal plant mass, and salicylic acid concentration.**

Response and effect	*df*	*F*	*P*	Partial *Eta*^2
Offspring number				
Year I				
Diversity	1	17.94	< **0.001**	0.14
Watering	1	6.87	**0.010**	0.06
Variety	1	0.29	0.59	0.00
Diversity × Variety	1	0.79	0.38	0.01
Diversity × Watering	1	1.62	0.21	0.02
Watering × Variety	1	2.64	0.11	0.02
Year II				
Diversity	1	2.52	0.11	0.02
Watering	1	0.68	0.41	0.00
Variety	3	13.90	< **0.001**	0.21
Diversity × Variety	3	16.19	< **0.001**	0.24
Diversity × Watering	1	4.21	**0.042**	0.03
Watering × Variety	3	0.86	0.46	0.02
Offspring mass				
Year I				
Diversity	1	14.22	< **0.001**	0.12
Watering	1	13.78	< **0.001**	0.12
Variety	1	1.09	0.30	0.01
Diversity × Variety	1	1.16	0.28	0.01
Diversity × Watering	1	2.00	0.16	0.02
Watering × Variety	1	2.29	0.13	0.02
Year II				
Diversity	1	0.78	0.38	0.00
Watering	1	0.06	0.81	0.00
Variety	3	18.20	< **0.001**	0.26
Diversity × Variety	3	23.36	< **0.001**	0.31
Diversity × Watering	1	2.39	0.12	0.01
Watering × Variety	3	2.45	0.065	0.04
Mother tibia length				
Year II				
Diversity	1	1.91	0.17	0.01
Watering	1	6.47	**0.012**	0.04
Variety	1	7.19	< **0.001**	0.12
Diversity × Variety	1	9.24	< **0.001**	0.15
Diversity × Watering	1	6.44	**0.012**	0.04
Watering × Variety	1	0.32	0.81	0.01

Table 5-2 (cont.)

Focal plant mass				
Year I				
Diversity	1	2.12	0.15	0.01
Watering	1	55.11	< **0.001**	0.27
Variety	1	21.41	< **0.001**	0.13
Diversity × Variety	1	15.29	< **0.001**	15.29
Diversity × Watering	1	0.01	0.91	0.01
Watering × Variety	1	0.38	0.54	0.38
Year II				
Diversity	1	0.25	0.62	0.00
Watering	1	34.63	< **0.001**	0.18
Variety	3	4.06	**0.008**	0.07
Diversity × Variety	3	10.20	< **0.001**	0.16
Diversity × Watering	1	0.06	0.81	0.00
Watering × Variety	3	1.30	0.28	0.02
Salicylic acid				
Year II				
Diversity	1	0.15	0.70	0.00
Watering	1	16.59	< **0.001**	0.10
Variety	3	21.54	< **0.001**	0.30
Diversity × Variety	3	2.51	0.061	0.05
Diversity × Watering	1	2.02	0.16	0.01
Watering × Variety	3	0.79	0.50	0.02
Diversity × Variety × Watering	3	2.91	**0.037**	0.06

Three-way interaction between neighborhood diversity, watering treatment, and focal variety was excluded when nonsignificant. Significance of treatment effects was determined using an *F*-statistic with the following denominator degrees of freedom for each response variable: Offspring number, Year I: 106; Year II: 158; Offspring mass, Year I: 106, Year II: 158; Tibia length: 158; Plant mass, Year I: 149, Year II: 160; Salicylic acid: 166.

*Offspring number was square root transformed in Year I and transformed using square root of $(y_{max}+1)-y_i$ in Year II. Offspring mass was square root transformed in Years I & II. Salicylic acid levels were using $\log_{10}$ transformed.

*Bold values are significant at $\alpha = 0.05$.

Table 5-3. **Effects of neighborhood diversity, focal variety identity, and drought stress on development time of the mother aphid.**

Response and effect	*df*	*Wald Chi-square*	*P*
Year II			
Diversity	1	0.22	0.64
Watering	1	2.54	0.11
Variety	1	1.80	0.18
Diversity × Variety	1	0.06	0.81
Diversity × Watering	1	4.99	**0.025**
Watering × Variety	1	2.68	0.10
Year II			
Diversity	1	0.11	0.73
Watering	1	2.01	0.16
Variety	3	2.96	0.40
Diversity × Variety	3	3.88	0.27
Diversity × Watering	1	0.43	0.51
Watering × Variety	3	3.67	0.30
Diversity × Variety × Watering	3	8.21	**0.042**

Results from a generalized linear model testing effect of treatments on development time. Development time could take one of two values for each year, separated by 1 day (i.e., 5 or 6 days in Year I, 6, or 7 days in Year II

*Bold values are significant at $\alpha = 0.05$

Figures

Figure 5-1. **Alate bird cherry-oat aphid (*Rhopalosiphum padi*) and her offspring.**

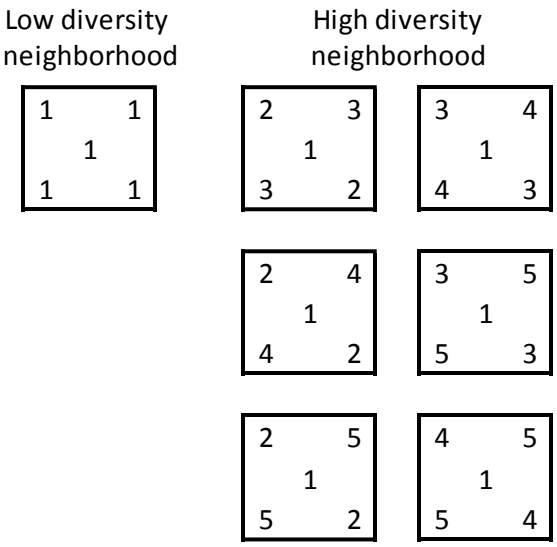

Figure 5-2. **Schematic of how I manipulated neighborhood diversity.** In this example, variety 1 serves as the focal plant. The focal plant is surrounded by the same variety in the low diversity neighborhood. In the high diversity neighborhood, the focal plant is surrounded by all six possible pairwise comparisons of varieties 2, 3, 4, and 5.

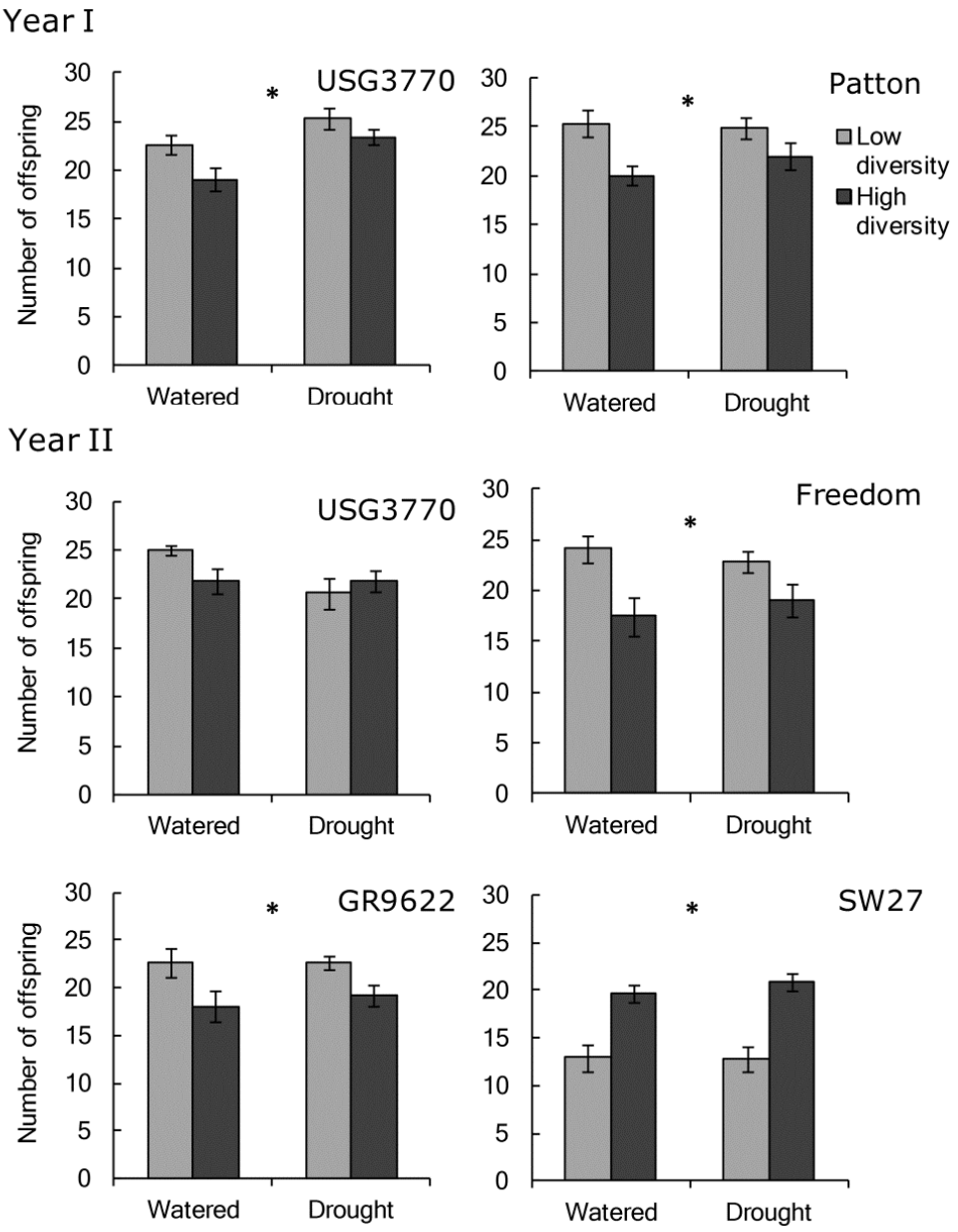

Figure 5-3. **The influence of neighborhood genotypic diversity, focal variety identity, and watering treatment on offspring number.** For neighborhood diversity treatments, low diversity treatments were monocultures of the focal variety, and high diversity treatments were the focal variety surrounded by two different varieties (three-variety mixtures). Watering treatment were either adequately watered or drought-stressed. Asterisks denote significance of the simple diversity effect across watering treatments at $\alpha = 0.05$. The three-way *diversity* × *variety* × *watering* interaction was nonsignificant.

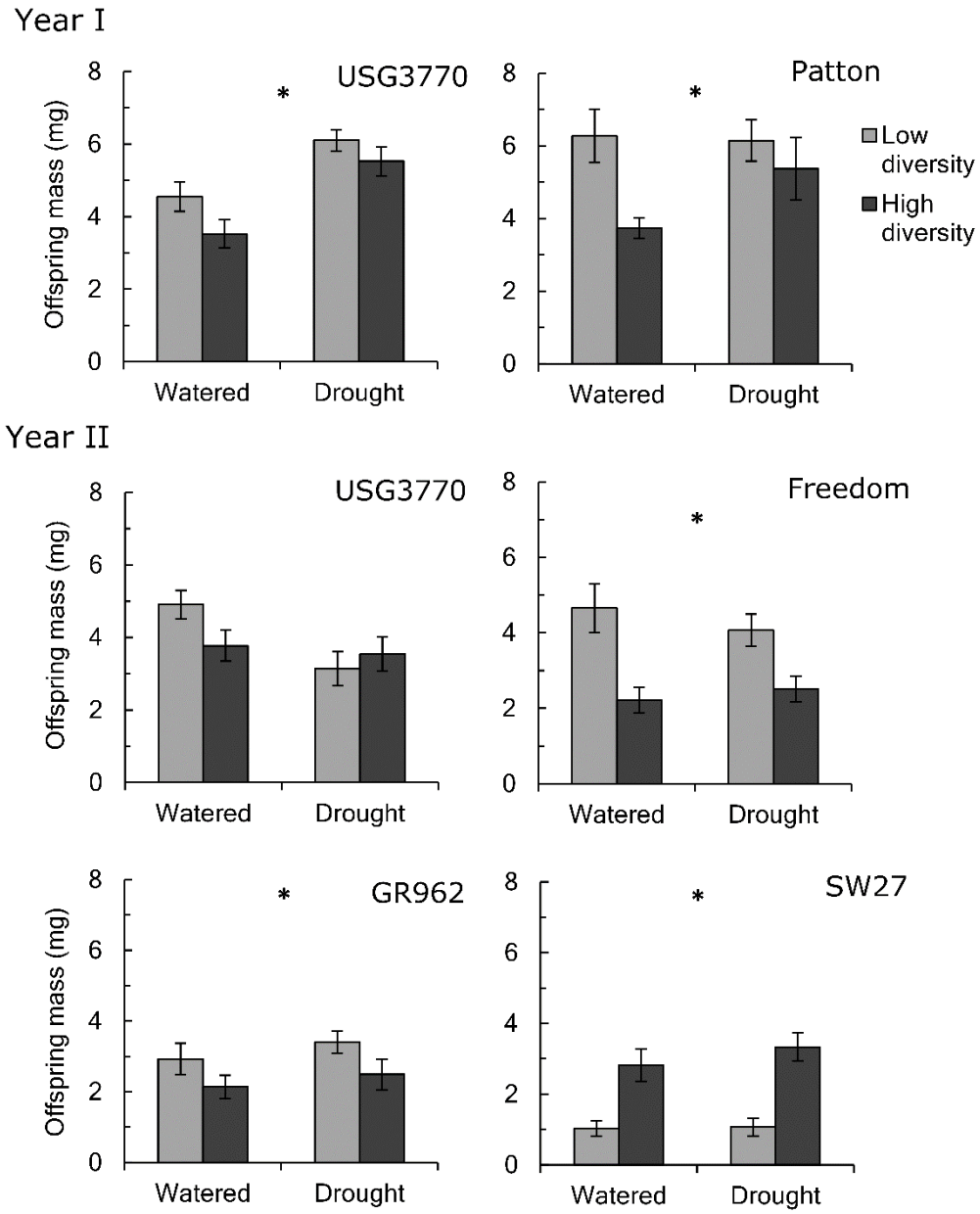

Figure 5-4. **The influence of neighborhood genotypic diversity, focal variety identity, and watering treatment on offspring mass.** For neighborhood diversity treatments, low diversity treatments were monocultures of the focal variety, and high diversity treatments were the focal variety surrounded by two different varieties (three-variety mixtures). Watering treatment were either adequately watered or drought-stressed. Asterisks denote significance of the simple diversity effect across watering treatments at $\alpha = 0.05$. The three-way *diversity* × *variety* × *watering* interaction was nonsignificant.

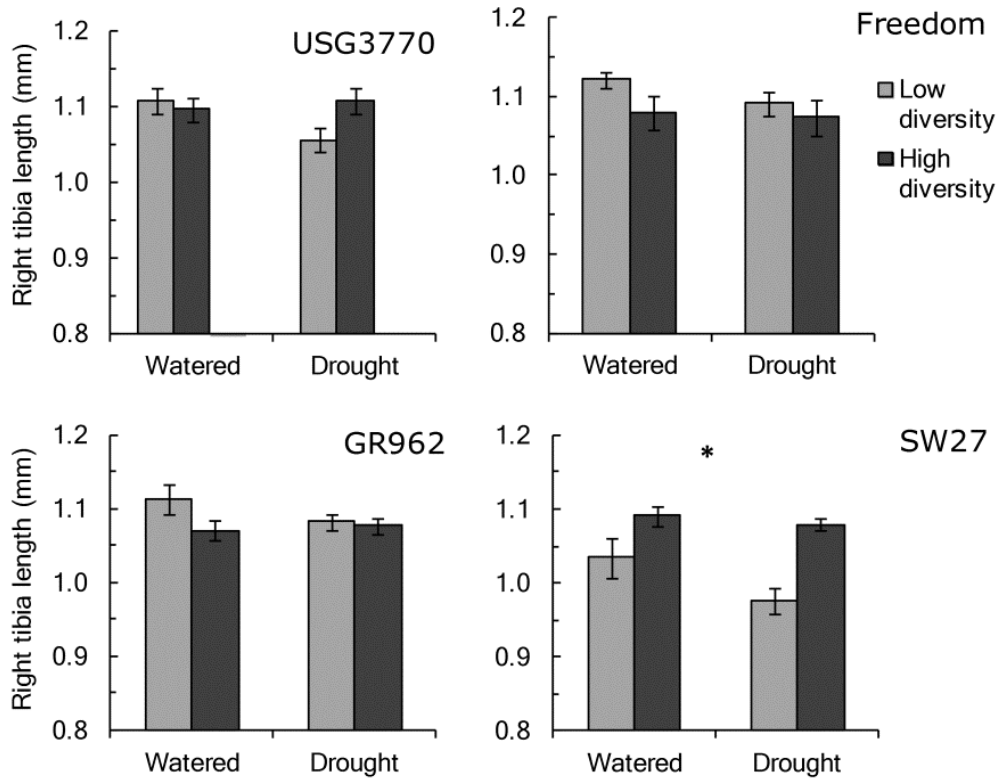

Figure 5-5. **The influence of neighborhood genotypic diversity, focal variety identity, and watering treatment on mother size, as estimated by right hind tibia length.** For neighborhood diversity treatments, low diversity treatments were monocultures of the focal variety, and high diversity treatments were the focal variety surrounded by two different varieties (three-variety mixtures). Watering treatment were either adequately watered or drought-stressed. Asterisks denote significance of the simple diversity effect across watering treatments at $\alpha = 0.05$. The three-way *diversity* × *variety* × *watering* interaction was nonsignificant.

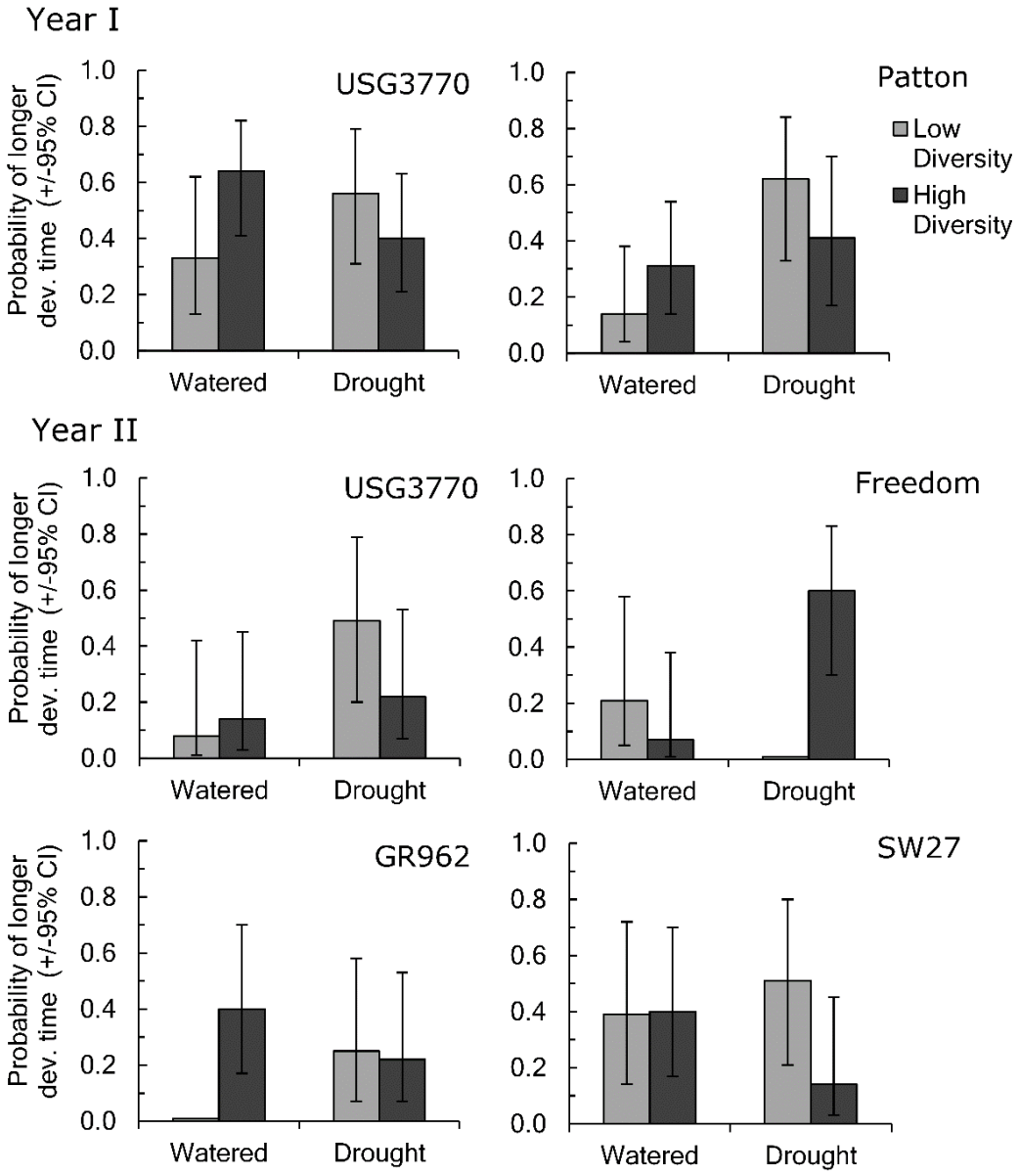

Figure 5-6. **The influence of neighborhood genotypic diversity, focal variety identity, and watering treatment (watered or drought-stressed) on mother development time.** Development time could take one of two values for each year, separated by 1 day. Values represent the estimated probability of a later development time (six days in Year I, seven days in Year II) for each treatment combination. In Year II, for the combination of variety 10, low diversity, and drought as well as the combination of variety 11, low diversity, and watered, all replicates took the same time to develop (6 days). For neighborhood diversity treatments, low diversity treatments were monocultures of the focal variety, and high diversity treatments were the focal variety surrounded by two different varieties (three-variety mixtures). Error bars represent 95% Wald confidence intervals (CI).

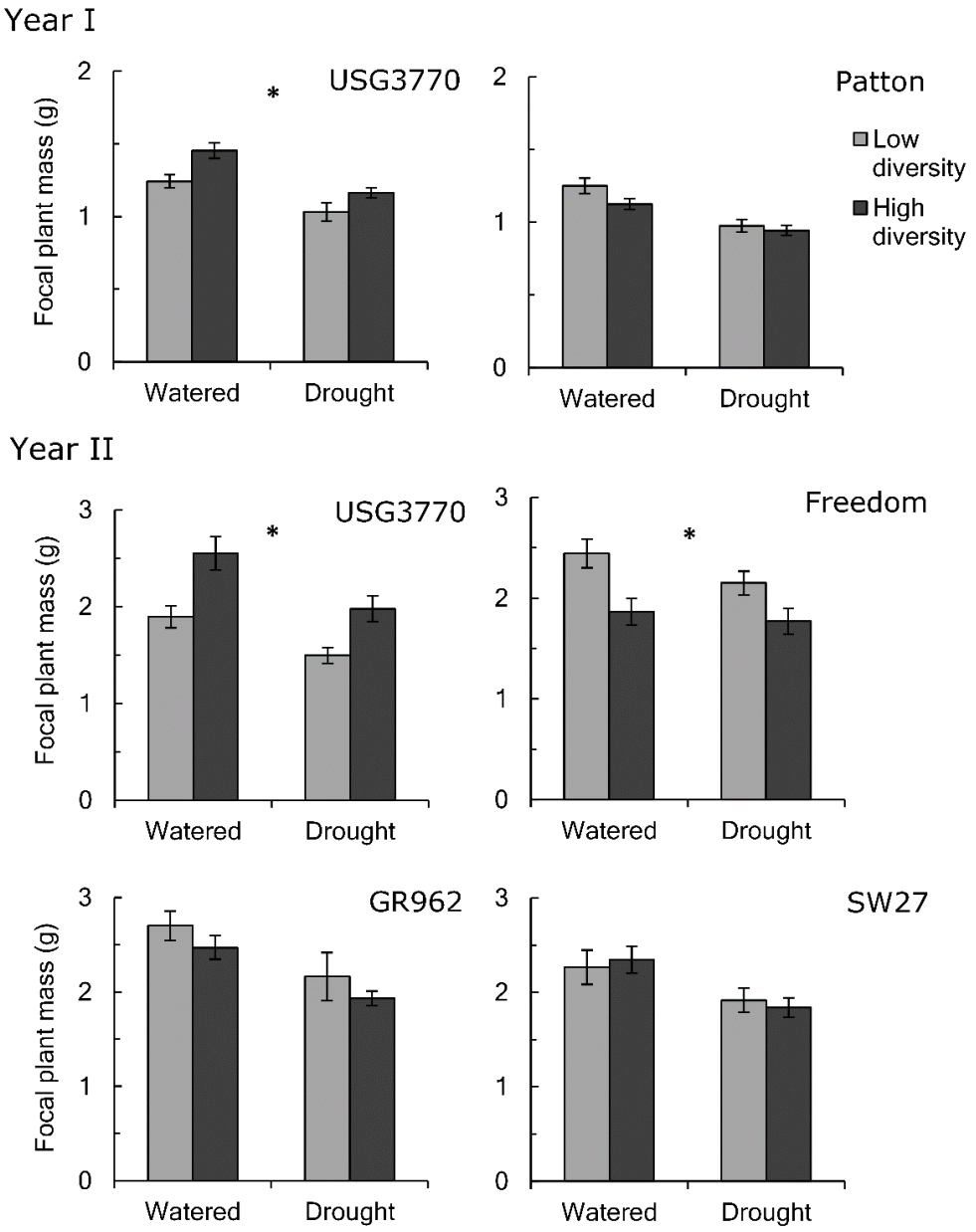

Figure 5-7. **The influence of neighborhood genotypic diversity, focal variety identity, and watering treatment on focal plant mass.** For neighborhood diversity treatments, low diversity treatments were monocultures of the focal variety, and high diversity treatments were the focal variety surrounded by two different varieties. Watering treatment were either adequately watered or drought-stressed. Asterisks denote significance of the simple diversity effect across watering treatments at $\alpha = 0.05$. The three-way *diversity* × *variety* × *watering* interaction was nonsignificant.

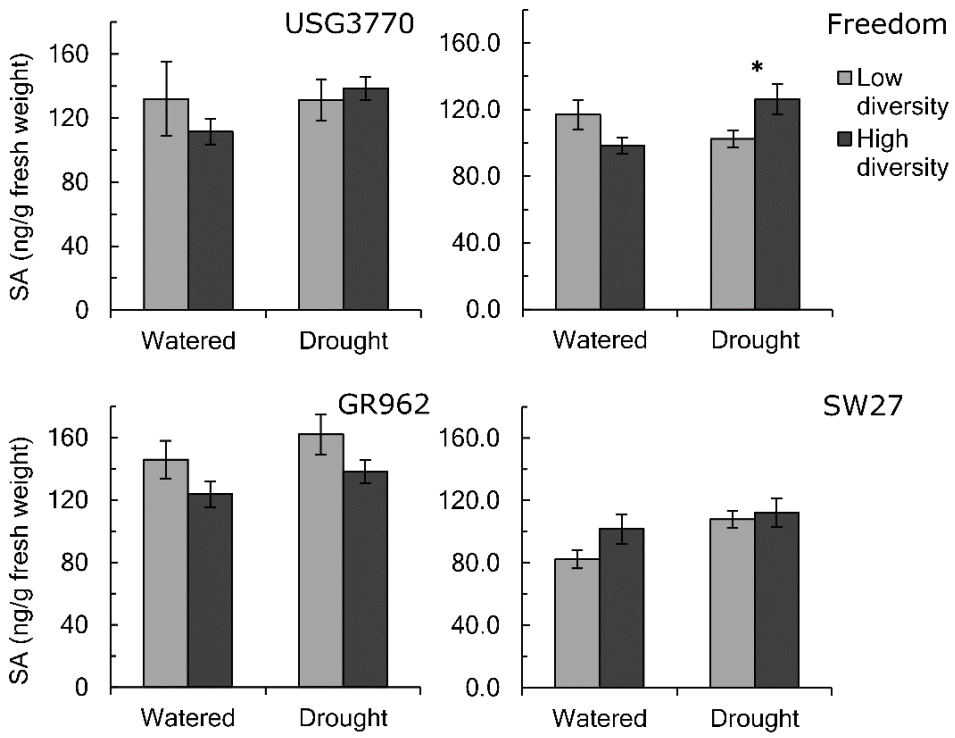

Figure 5-8. **The influence of neighborhood genotypic diversity, focal variety identity, and watering treatment on salicylic acid concentration in the 4.5 cm section of leaf contained within the clip cage.** For neighborhood diversity treatments, low diversity treatments were monocultures of the focal variety, and high diversity treatments were the focal variety surrounded by two different varieties. Watering treatment were either adequately watered or drought-stressed. Asterisks denote significance of the simple diversity effect across watering treatments at $\alpha = 0.05$. The three-way *diversity* × *variety* × *watering* interaction was nonsignificant.

Chapter 6

Moving beyond resistance management toward an expanded role for seed mixtures in agriculture

Abstract

Insect resistance management for *Bt* traits in corn has recently moved toward "refuge-in-the-bag," which consists of simple cultivar mixtures of susceptible and resistant hybrids. The purpose of these seed mixtures has thus far been solely to maintain susceptible pest insects, which will help prevent the development of resistance and preserve utility of the technology. It appears that this narrow focus on resistance management overlooks broader production benefits that may be achieved by planting genotypically diverse cultivar mixtures. While not yet widely, cultivar mixtures have been successfully used for disease management, demonstrating that the strategy is logistically feasible for intensified agriculture. Evidence from both natural and agricultural systems demonstrates that more genotypically diverse plantings can increase yield or productivity through a variety of mechanisms. These effects are in part attributable to improved response to both abiotic and biotic stressors, such as drought, temperature stress, competitors, herbivores, and disease. Similar to transgenic traits, cultivar mixtures also hold promise for resistance management for traditionally bred, or native, traits.

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Introduction

Across the U.S., many farmers are now growing simple "cultivar mixtures" when they plant their transgenic, insect-resistant corn containing proteins from the soil bacterium *Bacillus thuringiensis* (*Bt*). Cultivar mixtures are blends of crop varieties that are agronomically similar enough to be grown together, but possess key phenotypic differences, such as differences in disease or insect resistance (Wolfe 1985). Farmers in the U.S. are planting corn seed that is a mixture of *Bt* and non-*Bt* hybrids, commonly known as "refuge-in-the-bag" (RIB). The purpose of these seed mixtures has thus far been to prevent pest insects from developing resistance against *Bt* toxins by using non-*Bt* seeds as a refuge. This is a change from the previous sole reliance on non-*Bt* refuges that are spatially separate from *Bt* plants. By mixing different types of seeds together to create a ready-to-plant integrated refuge, seed companies have generated simple cultivar mixtures. These are recognized as effective tools for managing plants pathogens to increase yield (Wolfe 1985, Mundt 2002). Current corn seed mixtures intentionally contain limited diversity relative to typical cultivar mixtures (Mundt 2002), but it may be possible for seed companies to develop cultivar mixtures that capitalize on other ecological benefits of intraspecific diversity (aka genotypic diversity), such as managing pathogens, herbivores, and abiotic stressors (Hajjar et al. 2008, Tooker and Frank 2012).

The objective of this paper is to link the current insect resistance management (IRM) strategy of seed mixtures to the broader discussion on the role of genotypic diversity in ecosystems. Subsistence farmers around the world often grow cultivar mixtures for pest management, risk avoidance, and overall yield benefits (Smithson and Lenné 1996), but in industrial agriculture, cultivar mixtures remain poorly explored. I seek to bridge the gap between reviews that have focused on benefits of cultivar mixtures for specific goals (e.g., disease or insect management, insect resistance management; Mundt, 2002; Onstad et al., 2011; Tooker and Frank, 2012) and current use of RIB seed mixtures to emphasize potential benefits of cultivar

mixtures. First, I will highlight empirical evidence from natural and agricultural systems to illustrate some of the ecological benefits that may be derived from cultivar mixtures. I will then describe the current rise of cultivar mixtures for managing resistance to transgenic *Bt* traits to contextualize how cultivar mixtures are currently employed in *Bt* crops. I will also describe the role cultivar mixtures can serve for managing resistance for native, or non-transgenic, traits. Finally, I will consider a broader future role for cultivar mixtures in pest management and agriculture, with an emphasis on their use in intensified production of annual crops and primarily in the U.S., though the discussion is pertinent to many types of agriculture.

Ecological benefits of genotypic diversity in ecosystems

Typical modern agricultural fields are planted with a single variety of a single crop species and therefore tend to have limited species and genetic diversity (Bonneuil et al. 2012). A diverse body of research from both crop and natural systems, however, provides evidence that increasing crop genotypic diversity via cultivar mixtures may help improve plant productivity by buffering agroecosystems against a range of stressors. Much of this evidence comes from non-crop systems, but the underlying ecological principles and mechanisms are likely to be similar in both types of systems.

Crop systems differ from natural systems in ways key to understanding the potential for applying information on within-species diversity from natural systems to crop fields. Natural communities self-assemble and usually persist across multiple seasons and generations, allowing changes in diversity through natural selection to accumulate with time (Bell 1991, Stuefer et al. 2009). Growers of annual species (e.g., corn, wheat) largely determine the composition of their crop when they plant their fields, setting levels of genotypic diversity at the beginning of the season each year and permitting manipulation of genotypic diversity for specific production goals. If growers do not save and replant seed from the mixture, selection generally does not

change the relative abundance of genotypes, unless an extreme event occurs (e.g., weather, pests). Some forage, biofuel (e.g., switchgrass, willow), or fruit systems are perennial, and in these fields the relative abundance of genotypes can shift.

Yield

Because yield is one of the key factors influencing farmer adoption of a new practice, it is promising that higher levels of genotypic diversity can increase plant productivity or reproductive output (i.e., yield) in a diverse array of crop and non-crop plant species (Smithson and Lenné 1996, Crutsinger et al. 2006, Johnson et al. 2006, Kiær et al. 2009, Cook-Patton et al. 2011). For non-crop plant species, increases in plant productivity or reproductive output from higher intraspecific diversity (measured by aboveground biomass or seed production) have varied from 3 to 35% (Crutsinger et al. 2006, Johnson et al. 2006, Cook-Patton et al. 2011). This range is somewhat similar to yield increases for cultivar mixtures of crop plants, which tend to be around 5-10%, but have reached 30% for mixtures that effectively suppress disease outbreaks (Wolfe 1985, Smithson and Lenné 1996). Notably, these increases are comparable to increases in productivity and reproductive fitness that have been observed from increasing interspecific diversity in a number of studies (Crutsinger et al. 2006, Cook-Patton et al. 2011).

Intraspecific diversity can increase productivity relative to monocultures when genotypes benefit from presence of another genotype and/or divide up the available niche space and resources (complementarity), or when genotypic diversity leads to a higher probability of including in the mixture a productive or well-adapted genotype (sampling effect; Hughes et al., 2008; Loreau and Hector, 2001). One argument against cultivar mixtures has been growers could just plant a well-adapted genotype rather than complicate matters with a mixture, but it should be recognized that identifying the variety that will do well in the coming growing season can be challenging, and a mixture increases the chances of having that variety in the field. In crop fields,

effects driven by complementarity, or instances in which certain genotypes facilitate or support other genotypes, are likely more influential than selection effects because the frequency of genotypes tends to stay consistent. Moreover, recent research has found plants can recognize relatedness of neighbors of the same species and then express different phenotypes and strategies of growth or biomass allocation when they are surrounded by kin or non-kin plants; these phenotypes have potential to produce cascading effects that influence herbivore populations (Dudley and File 2007, File et al. 2012). Changes in phenotypic expression other than productivity and yield, such as defensive chemistry or drought resistance, affect responses of the plant community to its abiotic and biotic environment and could alter relationships between diversity and productivity (Agrawal et al. 2006).

Abiotic stressors

Abiotic stressors, including extremes in soil pH, temperature, salinity, and water availability, constrain productivity in many ecological systems, including agricultural fields, and plantings of genotypically diverse mixtures can be more resilient to abiotic stress than monocultures (Peltonen-Sainio and Karjalainen 1991, Ehlers et al. 2008). In natural systems for example, increased genotypic diversity in eelgrass stands enhanced biomass production and shoot density following extreme, naturally occurring heat stress, in part due to niche partitioning or complementarity among genotypes (Reusch et al. 2005). In the same system, genetic diversity improved stem density during winter when stands were subjected to a variety of abiotic stressors (e.g., lower light, desiccation, and lower salinity; Hughes and Stachowicz, 2009).

Similar to its role in natural systems, genotypic diversity can also buffer agricultural fields and crop yield against abiotic stressors by both maintaining and reducing variability in yields (Dawson and Goldringer 2012). Yield compensation occurs in cultivar mixtures when individual genotypes respond variably to stressors and moderate the overall negative influence of

the stressor (Bowden et al. 2001). For example, when faced with drought, cultivar mixtures of oats yielded as much as 9% more than monocultures because some constituents of the mixtures performed relatively well when temperatures were high and water availability was low (Peltonen-Sainio and Karjalainen 1991). Importantly, genotypic diversity did not decrease yield under low-stress growing conditions, indicating that genetic diversification was valuable when plants were stressed, but did not impose a cost in absence of drought (Peltonen-Sainio and Karjalainen 1991). The best chance for genotypic diversity to benefit yield may occur when abiotic stress develops early enough in the season to allow more resilient varieties to compensate with vegetative and/or reproductive growth (Bowden et al. 2001). For wheat, variety mixtures can better resist lodging, which often results from effects of weather, and its accompanying yield loss (Finckh et al. 2000). By better withstanding abiotic stress, diverse mixtures can also reduce yield variability relative to monocultures, resulting in yield stability (Cowger and Weisz 2008). Yield stability can result from compensatory growth by genotypes less influenced by the stressor, or from statistical averaging (i.e., the portfolio effect; Hooper et al., 2005; Tilman et al., 1998).

Biotic stressors

In addition to moderating abiotic stress, research suggests that cultivar mixtures can increase resiliency of fields challenged by biotic stressors, such as pathogens, insect pests, and weeds (Mundt 2002, Hughes et al. 2008, Tooker and Frank 2012). This resiliency at the field level emerges from features of the constituent cultivars in the mixture, including differing competitive abilities and variable susceptibilities to key pathogens and insect species. As mentioned above, inter-varietal interactions among plants of different genotypes appear to influence plant phenotype, including plant vigor and chemistry, with cascading effects that can affect pathogens and insect pests and ultimately benefit productivity (Tooker and Frank 2012).

Potential gains associated with cultivar mixtures are likely greater for biotic than abiotic stressors because cultivar mixtures can only directly influence biotic stressors (Wolfe, 1985)

Higher levels of genotypic diversity within a stand of plants can enhance competitive ability and prevent competing seedlings from establishing (Crutsinger et al. 2008). Crop varieties vary in their ability to compete with weeds and researchers have attempted to enhance weed suppression in crop fields by combining varieties with complementary competitive abilities against weeds (Kiær et al., 2006; Rodríguez, 2006). In a meta-analysis of small grain cultivar mixtures, winter wheat trials that created mixtures with varieties that varied in their ability to suppress weeds produced higher yields in mixtures than in monocultures (Kiær et al. 2009). Certain variety combinations can reduce weed pressure compared to monocultures, but diversity *per se* does not necessarily suppress weeds, and proper variety selection is necessary to improve weed suppression (Kiær et al. 2006, Rodríguez 2006). Experiments with non-agricultural plants have also shown that plant populations containing multiple genotypes can better compete with neighboring plants of other species and thereby competitively exclude individuals of other species (Crutsinger et al. 2008, Wang et al. 2012).

Intraspecific diversity within populations can moderate the spread and intensity of disease (Wolfe 1985, Keesing et al. 2010). Decades of research and relatively widespread adoption of cultivar mixtures in Europe and Asia have demonstrated genotypically diverse mixtures of crops can help manage diseases (Wolfe 1985, Zhu et al. 2000, Mundt 2002). Mixtures can reduce disease, especially for airborne pathogens, by diluting inoculum through increased distance between susceptible plants, providing physical barriers for the spread of spores between susceptible plants, inducing resistance in certain varieties, or modifying the crop microclimate (for comprehensive reviews, see Finckh et al., 2000; Garrett and Mundt, 1999; Mundt, 2002). Besides reducing disease, mixtures may also help increase durability of resistance genes (Mundt et al. 2002), which is critical to disease management because pathogens often rapidly evolve

virulence against resistance, nullifying its value (McDowell and Woffenden 2003). Nevertheless, while mixtures have been used in the U.S. in commercial production, in part for disease control, they have not been widely adopted, with only about 16% of winter wheat in Washington in 2011 and 9% in Kansas in 2013 planted with mixtures (NASS 2013, 2011).

A growing body of work is demonstrating that intraspecific diversity can influence insect herbivory via a combination of bottom-up (plant-mediated) and top-down (natural enemy-mediated) effects (Crutsinger et al. 2006, Johnson et al. 2006, Tooker and Frank 2012). Intraspecific variation in phenotypic traits and interactions among genotypes in plant populations can alter the structure and composition of the associated animal community (Bailey et al. 2009, Kanaga et al. 2009). By altering trophic interactions, higher levels of genotypic diversity in plant systems can lead to greater resilience to herbivory (Hughes et al., 2008; Tooker and Frank, 2012; but see Utsumi et al., 2011), although much of this work has been conducted in natural systems. In contrast, relatively little research has explored managing arthropod pests with genotypic diversity in agriculture (Tooker and Frank 2012). Nevertheless, studies in agroecosystems suggest that cultivar mixtures can lead to lower insect pest populations and increase natural-enemy populations, both of which can combine to lower levels of crop damage (Cantelo and Sanford, 1984; Ninkovic et al., 2002; Shoffner and Tooker, 2013). Planting mixtures of varieties with different susceptibility to herbivores is a straightforward approach to experimentally increase genotypic diversity and reduce herbivore populations (Cantelo and Sanford 1984, Teetes et al. 1994). Mixtures can affect herbivores through diverse mechanisms, including reduced acceptability of plants and source-sink population dynamics (Pettersson et al. 1999, Underwood 2009). A broad mechanism by which diversity can affect herbivore populations is associational resistance, in which the resistance of one plant genotype alters herbivory on a neighboring genotype (Barbosa et al. 2009). In addition, by changing behavior of herbivores that vector pathogens, variety mixtures can reduce disease incidence (Power 1991). In addition to direct

effects on herbivores, plant genotypic diversity can indirectly affect herbivores by increasing abundance or diversity of natural enemies and their associated services through a variety of mechanisms, including greater abundance of herbivores and changes in inter-plant volatile signaling (Crutsinger et al. 2006, Johnson et al. 2006, Cook-Patton et al. 2011, Ninkovic et al. 2011).

Role of seed mixtures for genetically modified traits

With the current transition from structured, or block, refuges to integrated refuges, or seed mixtures, for transgenic corn (Onstad et al., 2011), it is appropriate to consider potential benefits of cultivar mixtures for crop production within the context of insect-resistant *Bt* crops, with a primary emphasis on corn. With the U.S. Environmental Protection Agency's (EPA) approval, seed mixtures are now used to prevent the emergence of insect resistance to *Bt* corn. Survey results indicate that seed mixtures of *Bt* and non-*Bt* corn have become increasingly common (2012: 50% of growers planted at least one seed mixture product, 5% planted 100% seed mixtures; 2014: 74% planted at least one seed mixture product, 47% planted 100% seed mixtures; NCGA, 2015; Smith and Smith, 2014), even though these integrated refuges can be less effective than structured refuges at managing resistance (Onstad et al. 2011). The possibility that seed mixtures would be less effective for IRM than block refuges was raised by EPA Scientific Advisory Panels on refuges (U.S. EPA, 2011, 2009). Nevertheless, the trajectory of refuges for IRM has decidedly shifted towards seed mixtures, establishing a new paradigm for IRM (Carroll et al., 2012). In contrast, while RIB has been discussed for the other primary *Bt* crop, cotton, significant efficacy issues remain (Agi et al. 2001), and cotton seed mixtures have yet to be deployed.

To understand how well seed mixtures prevent development of resistance and how they influence pest populations, one must consider the complexity of the *Bt* technology landscape and

differences in biology of each pest species (e.g., feeding habits, susceptibility to toxins, susceptibility to predation; Onstad et al., 2011; Tabashnik, 1994). Cultivar mixtures could include pyramided traits (multiple traits against a target pest complex in the same plant), stacked traits (multiple traits in the same plant, not all targeting the same pest complex), and susceptible components. The first *Bt* corn hybrids were released commercially in 1996 and expressed only the insecticidal protein Cry1Ab, which targeted European corn borer ("corn borer," *Ostrinia nubilalis* Hübner), one of the primary corn pests (Pilcher et al. 2002). Biotechnology companies have since introduced other traits (both crystalline [Cry] and vegetative insecticidal proteins [Vip]), expanding the diversity of traits and range of pest species that *Bt* traits target, and novel traits have been proposed (e.g., RNAi; Kim et al., 2015). Corn hybrids now include Lepidoptera-specific *Bt* traits that provide control of corn borer and a suite of other caterpillar species, and Coleoptera-specific traits that target corn rootworm species (*Diabrotica* spp., "rootworms"; Castagnola and Jurat-Fuentes, 2012). *Bt* traits have been most valuable for controlling corn borer and western corn rootworm (*Diabrotica virgifera virgifera* LeConte), the two primary target pests, and most studies examining seed mixtures in corn have focused on these two pest species (e.g., Davis and Onstad, 2000; Head et al., 2014; but see Carroll et al., 2013).

The EPA and non-U.S. regulating agencies required that IRM plans accompany commercial release of transgenic *Bt* crops to delay development of pest resistance to *Bt* traits. Although the IRM requirement has endured, the nature of the refuge requirement has evolved and the durability of *Bt* technology has been challenged by some pests. When planting *Bt* corn targeting corn borer, growers were initially required to plant a structured refuge (20% of their acreage in the Corn Belt, 50% in cotton-growing regions), and this approach has prevented evolution of resistance to *Bt* traits targeting corn borer (Tabashnik et al. 2013). In fact, *Bt* crops have controlled corn borer so well, corn borer populations have declined dramatically in some parts of the U.S., reducing pest pressure for growers and even benefitting growers planting non-*Bt*

corn (Hutchison et al. 2010, Bohnenblust et al. 2014). Refuges were likewise required for *Bt* traits targeting rootworm. More recently, with the introduction of hybrids containing pyramided traits with apparently different modes of action, some refuges have decreased to 5-10% (mostly integrated refuges), and appear to acceptably control target pest species while ensuring growers plant refuges for resistance management. The adequacy of a reduced refuge size has been questioned, especially for western corn rootworm (Tabashnik and Gould 2012), which has evolved field resistance to multiple *Bt* toxins (Gassmann et al. 2011, 2014).

Compared to block refuges, seed mixtures possess some advantages, although they may also suffer from weaknesses for IRM, such as larval movement between plants of different genotypes, which would likely raise the risk of resistance developing (Ives et al., 2011; Roush, 1997; Tabashnik, 1994). Despite possible weaknesses resulting from larval movement, seed mixtures could perform at least as well for IRM as structured refuges given the same percentage of non-*Bt* plants because of increased compliance with refuge requirements (Onstad et al. 2011, Carroll et al. 2012); the structured refuge is only effective if growers follow guidelines and plant a sufficient refuge, and grower compliance was declining (Jaffe 2009). Seed mixtures alleviate this problematic decline by guaranteeing 100% compliance of purchasers because the seed reaches the grower pre-mixed (Onstad et al. 2011). Seed mixtures are also advantageous in that they promise to provide greater mixing and mating of resistant and susceptible adult pests, although the true balance between strengths and weaknesses of RIB remains unknown. On top of issues of adult and larval movement and grower compliance, current seed mixtures will likely provide benefits and pose challenges at field, farm, and landscape scales for a range of pests (for a more complete discussion, see Onstad et al., 2011).

Resistance management with native traits against insects

Similar to transgenic traits, cultivar mixtures may help limit the evolution of pest insects against native traits. Multiple native resistance genes have often been discovered for important pests (e.g., Hesler et al., 2013; Stuart et al., 2012), so strategies for deploying multiple genes have been considered to manage pest resistance.

Just as mixing *Bt* and non-*Bt* corn is known to protect *Bt* traits, blending cultivars containing native resistance traits with susceptible plants can increase durability of native traits (Gould 1986a; Smith et al., 2007). This mixing strategy and creation of a refuge can preserve utility of traits while maintaining yield, despite the common belief that any susceptible plants decrease yields (Gould, 1998, 1986a; Smith et al., 2004). Susceptible plants in mixtures do not necessarily reduce yield because mixtures can slow spread of the insect pest across the field, and resistant plants sometimes compensate for damage to susceptible plants (Gould, 1986a; Tooker and Frank, 2012).

In simulation studies, different deployment strategies for multiple resistance genes produce variable estimates for the effective combined lifespan of the genes (Cox and Hatchett 1986, Gould 1986a, 1986b). When possible, pyramiding resistance genes, accompanied by a refuge strategy (i.e., a cultivar mixture), appears to provide the greatest durability for resistance (Zhao et al. 2003, Onstad and Meinke 2010), and under some conditions, including refuge plants can provide essentially indefinite durability (Gould 1986b). Without some type of refuge (natural or sown), pyramiding traits increases selection pressure and is less durable than other deployment strategies (Cox and Hatchett 1986, Gould 1986a). When multiple resistance genes are incorporated into separate plants and pest larvae are not very mobile, cultivar mixtures generally supply durability similar to sequential release of resistance genes (Cox and Hatchett, 1986; Gould, 1986a; Roush, 1997). Some theoretical and experimental evidence, however, has indicated that deploying multiple resistance genes in a mixture, i.e., mosaic, can compromise durability

(Roush, 1997; Tabashnik, 1994; Zhao et al., 2003). The use of this type of cultivar mixture has therefore generally been regarded as inferior to sequential deployment and discounted (Bates et al. 2005). Importantly, concurrent deployment of plants containing single genes and plants containing pyramided genes, even with a refuge, will likely result in failure of all resistance genes faster than if only the pyramid is deployed (Zhao et al. 2005, Onstad and Meinke 2010).

Brown planthopper (*Nilaparvata lugens* Stål) and Hessian fly (*Mayetiola destructor* Say) are example pest species for which various strategies for deploying resistance genes have been considered, but not employed (Foster and Ohm, 1991; Gould, 1986b; Khush, 1979). Thus far, resistance genes against both pests have been deployed sequentially (Jena and Kim 2010, Stuart et al. 2012), likely due to a combination of time and effort required to pyramid genes into high-yielding cultivars or hybrids, and the availability of cultural or chemical control options (Foster and Ohm 1991). Multiple resistance genes against brown planthopper have been pyramided in rice, although their potential benefits for IRM remain unexplored (Qiu et al. 2011). In wheat, the only commercialized two-gene pyramids have comprised already-defeated genes combined with new genes, and this approach does not appear to provide much IRM benefit (Foster and Ohm 1991). To date, there has been no refuge strategy against Hessian fly; however, pyramids of unreleased genes that provide effective control have been developed, and deployment with a refuge is under investigation (B. Schemerhorn, personal communication).

A cultivar mixture-based refuge strategy has been used for IRM in Canada against the orange wheat blossom midge or wheat midge, *Sitodiplosis mosellana* Géhin (Vera et al., 2013). An effective resistance gene, *Sm1*, confers antibiotic resistance against the wheat midge (McKenzie et al., 2002), and researchers proposed incorporating a refuge via cultivar mixture to improve durability of single-gene resistance (Smith et al. 2004). The seed mixture (10% susceptible seed) was first commercialized in 2010, with other mixtures subsequently made available (Vera et al. 2013). The mixture tactic has proven effective in producing susceptible

pests, and in mixtures under high pest pressure, the *Sm1* gene provided an 11% yield advantage over susceptible varieties (Vera et al. 2013). In addition, oviposition deterrence in the resistant varieties combined with *Sm1* has potential to increase the effective size of the refuge, which would improve resistance management (Gharalari et al., 2009).

Among the more recently discovered suites of native host-plant resistance traits are *Rag* (Resistance to *Aphis glycines* Matsumura) genes targeting soybean aphid, and seed mixtures may improve durability of these traits. At least eight *Rag* genes provide resistance against soybean aphid, with several genes requiring further genetic testing for formal designation (Hesler et al., 2013). Two genes (*Rag1* and *Rag2*) are currently commercially available, either as single traits or incorporated together into cultivars as a pyramid, which improves aphid control over single genes (Hesler et al., 2013; McCarville et al., 2014). Even before resistant cultivars were widely available, aphid biotypes virulent to *Rag* genes were discovered, including a biotype that could survive on both *Rag1* and *Rag2*, indicating that pre-existing variation could allow soybean aphid to quickly overcome resistance genes (Hill et al., 2012; Kim et al., 2008). Fortunately, because aphid-resistant varieties are not yet widely available, there is time to develop a resistance management strategy, perhaps via pyramiding and mixtures of varieties, while non-resistant varieties in the landscape serve as a refuge for susceptible aphids (Hesler et al. 2013).

Improving agriculture with seed mixtures

Agroecosystems could further benefit from ecological interactions fostered by diversity, as evidenced by known advantages of cultivar mixtures and recent research on genotypic diversity in natural and agricultural systems (Mundt 2002, Crutsinger et al. 2006, Johnson et al. 2006, Kiær et al. 2009). Mixtures can provide "non-additive" benefits beyond what would be predicted based on varieties or genotypes grown in monoculture (Hughes et al., 2008).

Previous research on biodiversity and ecosystem function can inform development of mixtures, although further research will likely need to build upon this work. For example, simply increasing diversity *per se* by manipulating the number of genotypes or cultivars is sometimes insufficient to affect ecosystem processes (Kiær et al. 2006, Hughes et al. 2008). If diversity *per se* does not provide benefits, mechanisms responsible for diversity effects will need to be identified. Identifying mechanisms will simplify including relevant variation in mixtures rather than random variation (for instance, race-specific resistance for disease suppression; Mundt, 2002). To capitalize on the opportunity crop genotypic diversity presents, it will also be necessary to identify functional diversity that can enhance crop productivity (Hajjar et al., 2008). This reliance on functional diversity, and mixtures of specific functional traits, has been well documented for management of plant pathogens (Wolfe 1985). To reduce disease, mixtures must consider host and pathogen genotypes and match plant resistance genes and avirulence/virulence genes in the pathogen population (Mundt 2002). Mechanisms are less well understood for other functions of interest, such as insect pest management. For some insect pests like aphids that respond to plant resistance genes and spread similar to pathogens, some, but not all of the mechanisms may be similar (Tooker and Frank 2012). Given the myriad ways in which diversity has affected herbivores, functional diversity for plant traits such as resistance to herbivores, defensive chemical compounds, physical defenses, production of volatile compounds, or plant architecture will likely contribute to emergent or non-additive effects of diversity (Barbosa et al. 2009, Tooker and Frank 2012). In addition, in typical cultivar mixtures, different genotypes are randomly mixed across the field. In some crops, however, growers employ planting strategies that change the spatial arrangement of diversity (e.g., clumped, rows) to achieve better disease control and higher yields (Zhu et al. 2000, Newton and Guy 2009). A logical step would therefore be to evaluate how spatial arrangement of genotypic diversity influences the response of a field to stressors and, ultimately, crop production.

We may be able to improve resistance management for insects and even field-level crop yield through targeted changes in genotypic diversity, especially where larval movement threatens effectiveness of seed mixtures (Onstad et al. 2011). By altering plant phenotypes and influencing insect movement, mixtures may generate directional movement among cultivars and create source-sink population dynamics, potentially ameliorating problems created by normal larval movement (Power, 1991; Underwood, 2009). Increasing functional diversity by manipulating specific traits (e.g., some level of tolerance of pest damage in refuge plants) could allow pest populations to persist for resistance management while maintaining yield. With rootworm damage, higher levels of compensatory growth and tolerance may be possible with certain hybrids and genetics and under certain environmental conditions (Gray and Steffey 1998), and having such features in non-*Bt*, refuge hybrids could be valuable.

Other types of diversity could simply be layered on top of *Bt* seed mixtures to achieve other production benefits. The insect-resistant component of a mixture could consist of multiple elite hybrids with the same *Bt* traits, height, and maturity, but that vary in phenotypes for some key trait. For example, hybrids that all contain the same *Bt* traits targeting insect pests could possess resistance genes that are effective against different races of a key pathogen. This could provide more effective disease control while also preventing resistance to the genes (Mundt 2002). Resistance to minor insect pests could also be added to the cultivar mixture. Even without manipulation of true resistance, variation in host quality can affect herbivore movement, which can then alter population dynamics, severity of damage, and/or susceptibility to predation (Power, 1988; Straub et al., 2013; Underwood, 2009). Additionally, increasing genotypic diversity may improve resilience to abiotic stressors such as drought; hybrids could vary in phenotypes for drought resistance or root architecture, improving crop resilience via variety complementarity whether or not the stressor appears (Peltonen-Sainio and Karjalainen 1991). A similar tactic may

be relevant for other abiotic stress, such as weather-induced lodging (Trenbath 1974, Smithson and Lenné 1996).

Forming and developing valuable cultivar mixtures will require a variety of tactics. Plant breeding will be crucial to the effort and will help create the heterogeneity that provides benefits in mixtures. Once native or transgenic traits of value are developed, some may be associated with cultivars that are not most preferred in terms of yield or product quality. These traits or cultivars might be best deployed as components of mixtures where they could still benefit production, for instance by compensating for lower performing varieties (Castilla et al. 2003). With or without information on functional traits, varieties can still be tested using mixing ability analyses, which help identify varieties and variety combinations that are better "mixers" (Lopez and Mundt, 2000). These analyses could identify promising varieties for mixtures as well as traits of interest for further breeding scrutiny. In addition to developing heterogeneity for traits of interest for use in mixtures, breeding efforts will also need to consider crop uniformity. Even with acceptance of some crop heterogeneity by growers, components in cultivar mixtures will need to be bred or matched for uniformity for traits where substantial variation could create logistical obstacles. Ecologically relevant diversity will need to be included in mixtures, but this diversity must not impinge on production. Specifically, features like maturity date, plant height, emergence, initiation of the reproductive stage, vigor, timing of maturation, and grain quality will need to be consistent across a mixture so that growers will not have to change production practices to accommodate mixtures, but the importance of uniformity will vary with crop (Wilhoit 1992, Mundt 2002).

One of the primary unknowns is how willing growers are to accept and adopt mixtures and incorporate diversity into their fields. The use of cultivar mixtures for disease management across the world provides evidence that growers can see the value provided by intraspecific diversity, especially if research effort and plant breeding expertise can be leveraged to generate

value-added mixtures. Still, growers may not choose varieties (or mixtures) to plant based on resistance to disease, and other factors, such as market forces or yield potential, may drive planting choices (Vanloqueren and Baret 2008). Further, growers may not prefer variety mixtures in cases where they provide partial resistance to a stressor rather than complete resistance, although unpredictability of stressors and production tradeoffs for different varieties may still make partial resistance valuable. Depending on the crop and production system, growers may only adopt mixtures with low levels of phenotypic heterogeneity and crop breeding will need to overcome this challenge. The rapidly expanding use of RIB technology does suggest growers may become accustomed to some level of crop heterogeneity within their fields, but the increased diversity will need to possess demonstrated benefits.

Agriculture can benefit from the increased genotypic diversity in cultivar mixtures. Plants in natural systems benefit from intraspecific diversity (e.g., Hughes et al., 2008), and ample evidence indicates that genotypic diversity can improve disease and insect pest management in crop fields (Mundt 2002, Tooker and Frank 2012). Mounting evidence supports the notion that genotypic diversity can also help manage abiotic stressors (Peltonen-Sainio and Karjalainen, 1991; Reusch et al., 2005), an increasing concern as climate change progresses. Cooperation between agronomists, pathologists, entomologists, and plant breeders during the development of cultivar mixtures will help develop multi-functional mixtures. Importantly, once effective mixtures have been developed, there appear to be relatively low barriers to farmer adoption because conventional equipment can easily handle properly developed mixtures, as evidenced by adoption of mixtures in Europe and Asia (Mundt 2002), and more recently, RIB in the U.S. (Onstad et al. 2011). More widely considering the value of genotypic diversity in agricultural systems therefore holds strong potential to provide incremental benefits that should add up to real value for crop production, increasing yield, crop resilience, and sustainability.

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Chapter 7

Conclusions

Decades of research has investigated the ecological consequences of plant diversity in both agricultural and natural systems with efforts first focusing on plant species diversity and, more recently, on genetic diversity within plant species. If we want to predict how biodiversity loss will influence ecosystems and how diversity may be employed in crop fields for production and pest management benefits, we must better understand relationships between plant diversity and ecosystem processes, as well as the effect of plant diversity on higher trophic levels. Because they pose fewer logistical challenges compared to species mixtures, cultivar mixtures are an appealing method for improving sustainability of farming (Tooker and Frank 2012). In crop fields, cultivar mixtures can provide production benefits by reducing disease pressure and better maintaining yield (Mundt 2002). Still, we do not have a good understanding of how much genetic diversity in crop fields is enough to matter or whether the influence of genotypic diversity is consistent over different years with variable growing conditions. It may be that the value of genotypic diversity emerges only in very specific situations, such as for managing pathogens or delaying evolution of resistance (Gould 1986).

While elementary, it is important to remember the direction of the diversity effect is vital in agricultural systems. For natural system research examining the response of arthropod communities to diversity (performance, herbivory, abundance, or diversity), the null hypothesis is that diversity does not influence arthropods. Any deviation from this null is interpreted as evidence that diversity "matters" and can influence other trophic levels. For instance, work in natural systems has produced many statistically significant effects of diversity on herbivore abundance, including cases in which genotypic diversity reduced herbivory or herbivore abundance (McArt and Thaler 2013, Barton et al. 2014), but also cases in which diversity

increased herbivore abundance (Utsumi et al. 2011). In crop fields, the desired response is only in one direction: fewer herbivores and less herbivore damage. Higher abundance of herbivores in diverse variety mixtures would only be desirable in certain cases, such as for a non-pest herbivore that serves as alternative prey for natural enemies which can in turn suppress a key herbivore. The same logic of a desired response in agroecosystems holds true for diversity-productivity relationships, although in this case, natural systems research typically hypothesizes that productivity will increase with diversity (e.g., Johnson, Lajeunesse & Agrawal 2006). Considering the desired outcomes in crop fields is important when comparing research in natural and agricultural systems and when determining the likelihood of beneficial effects.

In my studies, I found generally mixed effects of crop genotypic diversity on pests, their natural enemies, and yield across two broad systems (soybean and soybean aphid and spring/winter wheat and bird cherry-oat aphid). Diversity effects varied from positive to negative, and some response variables were not altered at all by diversity level. Across my studies with aphids, the weight of the evidence suggests that crop genotypic diversity does not provide strong pest management benefits. I identified the greatest pest management benefits when the winter wheat varieties I chose contained known and substantial variation for pest resistance (Chap. 5, Table 5). However, even in this case, the influence of diversity on aphid performance was inconsistent and depended on the abiotic stress environment. Genotypic diversity reduced pest performance when plants were adequately watered, but only slightly when plants were drought-stressed. Variety mixtures will be much less beneficial if the environmental context, a constantly changing factor, determines whether plant genotypic diversity influences herbivores.

Crop genotypic diversity, or diversity *per se*, may simply not be very useful for insect pest management. In many cases, I found no difference in herbivore populations between mixtures and monocultures. While specific mixtures may prove beneficial, or particular varieties may perform well when grown in mixtures, my results do not imply that diversity alone is

beneficial. For disease management, mixtures are often meticulously constructed. Mixtures usually comprise crop genotypes that closely match the genotypes of a focal pathogen to reduce the percentage of the crop that is susceptible to a particular pathogen race, and the mixture is often dynamic with new genotypes replacing old over time (Mundt 2002). In the case of managing disease in rice, particular mixtures, rather than random mixtures of varieties, helped reduce disease in China (Zhu et al. 2000). When only specific mixtures are useful, diversity is increased in crop fields, but stating that diversity is important and ecologically significant may be misleading. In addition, if only certain types of diversity produce pest management benefits, costs of development and implementation for variety mixtures would likely be higher.

If variety mixtures are to be developed for pest management, a number of considerations could increase the likelihood that successful mixtures are eventually deployed and could increase the efficiency and cost-effectiveness of their development. Mechanisms by which plant genotypic diversity could suppress insect pests are varied (Tooker and Frank 2012), and the best course of action for developing variety mixtures is ambiguous and likely dependent on circumstances of the individual pest. Still, some broad generalizations can be made. First, a crop and pest(s) will need to be chosen. Because variety mixtures would typically not completely suppress pest populations, pest species should be chosen that do not have population tolerances near zero. Furthermore, pests that are consistently problematic, i.e., not pests that are extremely damaging in only a subset of years, should be chosen to facilitate field trials. Pests that spread through fields like pathogens would also be good candidates. However, aphids are such a pest (Tooker and Frank 2012), and yet my results do not provide strong support of variety mixtures for aphid management. From the crop side, it would be easiest if crops are chosen for which a mixture of varieties that are not nearly identical would still be acceptable to growers and processors. In cases where sufficient pest resistance traits are not available or in cases where sufficiently similar varieties need to be developed so a mixtures is acceptable to growers, collaboration between entomologists and plant

breeders would be helpful. Breeders could help identify varieties and resistance traits for the pest of interest. In addition, breeders could help guarantee that varieties that contain useful variation for resistance traits are similar enough agronomically that they could be grown together. Next, varieties to test will need to be chosen. Varieties should consist of regionally competitive varieties that vary in their resistance to the targeted pest. A variety trial, either under greenhouse or field conditions could provide this information if it was not already available. Once a pool of varieties are chosen, a number of courses of action could be taken. For one, mixing ability analysis (Knott and Mundt 1990) using many pairs of varieties could help identify varieties that would perform well in mixtures. Mixtures containing more diversity (likely 3-6 varieties) could then be compared to monocultures in further tests, either solely in the field, or first in the greenhouse and then in a more limited fashion in the field with fewer varieties and mixtures. If expected mechanisms for diversity effects are unknown, field tests, while more difficult, would probably be better and could incorporate both bottom-up and top-down forces on pests. However the tests are performed, they should be streamlined and made efficient because a successful development program would ideally test many varieties and combinations of varieties. While these recommendations are very general and the best approach would depend on the crop and pest, they should help the development of mixtures as a pest management strategy if it is deemed worth of investment.

In agroecosystems, crop genotypic diversity, or even plant species diversity, may only prove beneficial to growers if the multidimensionality of diversity effects is considered. Crop genotypic diversity can influence a wide array of ecosystem services (Hajjar et al. 2008, Haddad et al. 2011), and net positive effects may accumulate from many small consistent changes or perhaps stronger changes that only appear under certain environmental conditions. Variety mixtures would contain variation for multiple traits that could in theory produce these positive effects. In most cases, variety mixtures would contain variation for many traits (e.g., resistance to

pests, competitive ability, and leaf and root traits). Even multilines, which are bred for phenotypic uniformity, would still contain variation for many traits and would contain underlying genetic variation (Mundt 2002). Considering the combined effects of diversity and using a holistic framework would not be simple and would likely require a multidisciplinary team, especially if the underlying mechanisms for any effects of diversity are to be identified (Hajjar et al. 2008).

The pest management benefits of variety mixtures, outside of resistance management, remain unclear, although there is empirical and theoretical evidence supporting use of the tactic. Thus far, no reviews have attempted to determine how plant genotypic diversity affects herbivores by using either vote-counting or meta-analysis. This may in part be because of the diverse response variables used, such as herbivory, performance, abundance, and diversity, or because of the wide range of experimental methods used. It is clear that in a variety of natural systems, effects can be extremely variable, while the paucity of data from agroecosystems makes any general conclusions more difficult. Nevertheless, there are many examples in which plant genotypic diversity negatively influences herbivores. It can be argued that crop genotypic diversity still holds potential for pest management, but given finite resources, we must determine if this tactic is more worth pursuing than other sustainable pest control tactics, and if so, carefully identify situations in which variety mixtures are most likely to be beneficial and most easily tested and developed.

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Appendix A

Supplementary information for Chapter 5

Appendix A1: Variety trial and variety selection for Chapter 5

To choose five varieties for my experiments, I performed a variety trial with 18 wheat varieties that were available at Pennsylvania State University's Russell E. Larson Agricultural Research Center. I screened all varieties for their independent resistance to aphids and drought relative to the other varieties in the trial. Testing 18 varieties from numerous sources of seed allowed me to include a range of presumed genetic variation and resistances for my experiments. I could then deliberately manipulate this diversity for the traits of interest, maximizing the probability of generating influential interactions in mixtures (Mundt 2002). For the variety trial, I planted seeds of each variety in the pattern of a square with two seeds at each corner. I thinned seedlings to one per position, for a total of four plants per pot. Pots were arranged in a randomized blocked design. Treatment combinations were replicated four times (each variety for aphid trial, each *variety* × *watering* combination for drought trial). The variety trial was conducted May-June 2013 under a 15:9 (L:D) photoperiod with natural light supplemented by overhead halogen lights and temperature averaging 23.5ºC. Pots were fertilized once (0.39 g fertilizer dissolved in 150 ml H_2O).

To measure resistance of each variety to aphids, I infested each plant with two late-instar *R. padi* nymphs when plants started tillering (Zadok's scale 2.4-2.6; Zadok et al. 1974). I transferred aphids with an ultrafine-hair paint brush. Two weeks post-infestation, I counted apterous aphids on each plant (I found very few alates).

To assess drought resistance of the varieties, I subjected plants to periodic drought stress and then contrasted plant biomass under drought with unstressed (i.e., "watered") conditions. I established plants as described above. When plants began tillering, I standardized soil water content by weighing pots with a scale and ensuring pot mass was equivalent across pots. After standardizing moisture levels, I withheld water from drought-stressed plants until nearly complete wilting, at which point they were re-watered. I repeated this cycle three times, and each cycle lasted 4-7 days. After each drought cycle, I re-watered the stressed plants several times, allowing them to recover from wilting and regain leaf turgor (about 24 hours). I then re-standardized moisture levels based on pot mass, ensuring pots were well- but not over-saturated. I watered non-stressed pots as needed to maintain soil moisture. On dates when moisture levels were at their lowest in the drought treatment, or when the drought-stressed pots had recovered from drought, I measured volumetric water content (m^3 H_20 per m^3 potting mix) for each pot in the drought treatment using a Decagon EC-5 moisture probe and a ProCheck readout device (Decagon Devices, Inc., Pullman, WA). While not absolutely representative of true moisture content, this technique still allowed me to compare among pots. I tested for an effect of variety on moisture levels on the three dates when drought-stressed plants were most dry using a generalized linear mixed model to account for covariance among dates. Within-subjects covariance structures were chosen using the Akaike Information Criterion (AIC). I specified a log link function with a gamma error distribution to account for the distribution of the data and chose a first order auto-regressive covariance structure. Importantly, variety identity did not influence moisture levels in the drought treatment when they were drought-stressed ($F_{17,195} = 0.73$, $P = 0.77$). I harvested aboveground biomass 33 days after planting, combined biomass at the pot level, and then dried it at 93ºC for five days.

I selected five varieties for the experiment based on their screened resistances to aphids and drought. For aphid resistance, I square-root transformed the average number of aphids per

plant at the pot level and then averaged across replicates for each variety. I ranked varieties by these measures of aphid populations as an assessment of aphid resistance (Underwood 2009). For resistance to drought stress, I ranked varieties by percent reduction in biomass, which I computed for each replicate as ($Mass_{Watered}$ - $Mass_{Drought}$)/ $Mass_{Watered}$* 100. I chose one variety that was susceptible to both drought and aphids (USG3770), and one that was resistant to both stressors (Patton). I also chose two that possessed crossed resistances (aphid resistant/drought susceptible and vice versa; SW27 and Freedom, respectively), and one that was somewhat susceptible to both aphids and drought (GR962, Table A1). These varieties formed a pool of five that came from different breeding programs and companies and presumably possessed underlying genotypic differences. This notion was supported by clear phenotypic differences between some of the varieties, which included the measured resistances and growth habit (upright vs. more prostrate).

Appendix A1: Table

Table A1-1. **Rankings for drought resistance and aphid resistance from the variety trial for the five soft red winter wheat varieties used in the main experiment.** Resistances were determined through independent variety trials for each stressor with 18 different varieties.

Variety	Drought resistance ranking (% reduction under drought)	Aphid resistance ranking (aphid populations)	Drought/Aphid resistance*
USG3770	17	15	S/S
Patton	1	3	R/R
Freedom	4	16	R/S
GR962	12	12	MS/MS
SW27	13	1	S/R
Total range	23-48%	222-416	

*S = Susceptible, R = Resistant, MS = moderately susceptible (relative to other tested varieties)

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Appendix A2: General growing conditions in the main experiment

The main experiment described below was conducted over two years. Photoperiod remained constant: 15:9 [L:D]). Overhead halogen lights supplemented natural light. The experiment in Year I ran from Sept.-Nov. 2013, and temperatures averaged 23.5^{o}C. The experiment in Year II ran from Jun.-July 2014 and temperatures averaged 27.5^{o}C. I planted two seeds per hole and, if necessary, thinned pots shortly after germination so only one plant remained at each position. To prevent disease, plants were sprayed once with fungicide (Prosaro 421 SC: prothioconazole and tebuconazole, Bayer Crop Science, Research Triangle, NC) when plants had produced tillers. In both years, all pots were fertilized twice with 0.39 g of Miracle Gro All Purpose fertilizer (The Scotts Company, LLC, Marysville, OH) dissolved in 150 ml water, once when plants had just begun to tiller and again 7 or 11 days later (dependent on watering regimes).

Appendix A3: Moisture measurements for main experiment in Chapter 5

Similar to the variety trial (Appendix 1) and during both years of the main experiment, I standardized water content before initiating drought stress for the drought treatment by withholding water. I used a scale to measure total pot mass and then added water to standardize mass and soil moisture content across treatments. The weight of the small plant, and any differences in plant mass between treatments, was minimal compared to the mass of water held by the potting soil. Any total weight differences between pots due to differences in plant weight would be negligible. Pots also received roughly equivalent quantities of potting soil and the mass of the potting soil was small when dry.

During both years of the experiment, I assessed pot moisture levels at various time points to test if moisture levels were different between treatments and varieties. As in the variety trial, pots in the watered treatment received enough water to maintain high levels of soil moisture throughout the experiment. In Year I, I measured mass of the pots to assess moisture levels. Because the primary focus was determining the effect of pot diversity on moisture levels in drought-stressed pots, I only weighed pots in the drought treatment and only when they were most water stressed (26, 31, and 35 days post-planting). In Year II, I measured volumetric water content ($m^3 H_2O$ per m^3 potting mix) for all pots with a Decagon GS3 probe and ProCheck readout device (Decagon Devices, Inc., Pullman, WA). This probe produced more accurate measurements than the probe used in the variety trial. I measured moisture levels on four dates in both watered and drought treatments. I assessed moisture levels one day after I had standardized them to begin each period of drought stress (26 and 30 days post-planting), when drought-stressed pots were most stressed (29 and 35 days post-planting), and on one other date for watered treatment pots (33 days post-planting). I did not measure moisture levels after pots were re-watered the final time because all pots were watered as necessary at this point.

For both years, I analyzed moisture levels across dates using linear mixed models to account for repeated measurements. In Year I, I included fixed factors of date, diversity, variety, block, and the two-way interactions between date, diversity, and variety and chose an unstructured structure. The *date* × *diversity* × *variety* interaction was nonsignificant throughout analyses and not included in the model. I examined simple main effects in the presence of significant interactions, with the family-wise error rates corrected for multiple comparisons with a Dunn-Šidák correction. In Year II, I performed two separate analyses. First, I performed an analysis using only data for drought treatments when they were most drought-stressed (two dates), which is when treatment differences for soil moisture would be most important. I included fixed factors of date, diversity, variety, block, and the two-way interactions between date, diversity, and variety and chose a compound symmetry covariance structure. Then, I performed an analysis for both watered and drought treatments using data from dates when pots had just been watered (one day after standardizing soil water content). I included fixed factors for date, diversity, variety, watering, block, and the two-way interactions between date, diversity, variety, and watering.

For drought-stressed pots in Year I, the effect of neighborhood diversity on moisture levels was consistent across dates (nonsignificant *date* × *diversity* interaction, Table A3*).* However, the influence of neighborhood diversity depended on which of the two varieties was the focal variety (significant *diversity* × *variety* interaction, Table A3*).* Neighborhood diversity did not influence moisture levels for USG3770 ($F_{1, 73.17} = 2.02$, $P = 0.16$), but did for Patton ($F_{1, 73.17} = 19.67$, $P < 0.001$). Across dates, diverse pots with Patton as the focal variety weighed approximately 3% less at the height of moisture stress. This represented about 2% of the total moisture change (based on mass) between when plants were recently watered and when they were at the peak of drought stress.

In Year II, when testing moisture levels for drought treatment pots during times they were drought-stressed, the effect of diversity depended on variety, but not date (Table A3). For the four varieties, moisture levels were only different between neighborhood diversity treatments for USG3770, for which moisture levels were higher in the diverse treatments ($Mean_{High}$ = 0.046 m^3/ m^3, $Mean_{High}$ = 0.038 m^3/ m^3; $F_{1,77}$ = 8.54, P = 0.005). When both watered and drought treatment pots were analyzed using dates when they had just been watered, the effect of neighborhood diversity on moisture levels depending on the watering treatment (significant *diversity* × *watering* interaction, Table A3). For drought-stressed pots, neighborhood diversity did not affect moisture levels ($F_{1,160}$ = 1.39, P = 0.24). For watered pots, diverse pots were drier ($F_{1,160}$ = 3.01, P = 0.085), although the magnitude of the difference was small (0.269 vs 0.274 m^3/ m^3).

Appendix A3: Table

Table A3-1. **The influence of experimental treatments on moisture levels in pots.** In both years, treatment effects were tested for drought treatments when they were most drought stressed. In Year II, treatment effects were also tested for both watered and drought treatment pots when all pots were well watered.

Response and effect	*df*	*F*	*P**
Year I			
Drought treatments: drought stressed†			
Diversity	1	4.52	**0.037**
Variety	1	0.95	0.33
Date	2	548.68	**< 0.001**
Diversity x Variety	1	17.21	**< 0.001**
Date x Diversity	2	1.83	0.17
Date x Variety	2	4.82	**0.011**
Year II			
Drought treatments: drought stressed†			
Diversity	1	1.02	0.32
Variety	3	2.73	0.050
Date	1	0.002	0.96
Diversity x Variety	3	2.96	**0.038**
Date x Diversity	1	0.02	0.90
Date x Variety	3	0.91	0.44
Watered and drought treatments: watered‡			
Diversity	1	0.16	0.69
Watering	1	2.05	0.15
Variety	3	4.65	**0.004**
Date	1	59.44	**< 0.001**
Diversity x Variety	3	1.23	0.30
Diversity x Watering	1	4.25	**0.041**
Watering x Variety	3	0.97	0.41
Date x Diversity	1	0.12	0.73
Date x Watering	1	2.002	0.16
Date x Variety	3	3.54	**0.016**

† In Year I, drought stressed plants were subjected to three rounds of drought stress and therefore three dates were used in the analysis. In Year II, plants were subjected to two rounds of drought stress and two dates were used.

‡ I assessed soil moisture twice when both watered and drought treatment pots had just been watered. These two dates were the day after moisture content was standardized to start the first drought treatment and the day after moisture content was standardized a second time following cessation of the first drought period

*Effects significant at $P < 0.05$ are shown in bold.

Appendix A4: Additional details from results in Chapter 5

Offspring number and mass

In Year I, diversity reduced offspring production across the two varieties tested by 4.5 nymphs when plants were watered and by 2.4 nymphs when plants were drought stressed (Fig. 3). In Year II, the *diversity* × *variety* interaction was significant. Compared to genotypic monocultures, diverse neighborhoods reduced offspring by 5.1 nymphs for Freedom (21.2% reduction; $F_{1,158} = 15.92$, $P < 0.001$) and by 4.0 nymphs for GR962 (17.4% reduction; $F_{1,158} = 10.28$, $P < 0.001$; Fig. 3). However, diverse neighborhoods increased the number of offspring produced on SW27 by 7.4 nymphs (56.3% increase; $F_{1,158} = 24.01$, $P < 0.001$), and did not alter offspring production on USG3770 ($F_{1,158} = 0.64$, $P = 0.43$). The effect of focal variety on offspring production depended on neighborhood diversity in Year II. Based on pairwise comparisons within each diversity treatment, aphids on SW27 produced the fewest offspring ($P < 0.001$ for comparisons with all other varieties) in low diversity neighborhoods, with aphids on the other varieties producing equivalent numbers of offspring ($P > 0.05$ for all other comparisons). In the high diversity neighborhoods, aphids on USG3770 produced the most offspring, while those on Freedom produced the fewest (difference was significant, $P = 0.032$). Aphids on GR962 and SW27 produced intermediate numbers of offspring.

In Year I, drought significantly increased offspring mass by 27.5% compared to the watered treatments (Table 1, Fig. 4). In Year II, overall means for offspring mass were lower compared to Year I (Year II: *Mean* = 2.75mg, Year I: *Mean* = 4.92mg, $N = 174$; Fig. 4). While mother aphids in Year II produced similar numbers of offspring as in Year I (Fig. 3), they produced them more quickly, so their offspring had less time to gain mass. As with offspring number, the *diversity* × *variety* interaction was significant (Table 1). Offspring weighed less in diverse neighborhoods for Freedom and GR962 (Freedom: $F_{1,158} = 21.31$, $P < 0.001$; GR962:

$F_{1,158} = 7.11$, $P = 0.008$), weighed more for SW27 ($F_{1,158} = 41.63$, $P < 0.001$), and were not different for USG3770 ($F_{1,158} = 0.69$, $P = 0.41$; Fig. 4). Based on pairwise comparisons within each diversity treatment, aphids on SW27 produced the fewest offspring ($P < 0.001$ for comparisons with all other varieties) in low diversity neighborhoods, and aphids on the other varieties produced similar numbers of offspring ($P > 0.05$ for all comparisons). In the high diversity neighborhoods, aphids on USG3770 produced the most offspring, which was significantly different than aphids on Freedom and GR962 ($P = 0.006$ and $P = 0.002$, respectively), where aphids produced the fewest offspring. Aphids on SW27 produced numbers of offspring intermediate to, but not different from, all other varieties ($P > 0.05$)

Mother aphid size and development time

The interactive effect of neighborhood diversity and variety on mother size was significant (data only for Year II). Ignoring watering treatments, mother aphids were significantly larger in diverse neighborhoods for SW27 (8.0% larger; $F_{3,158} = 22.49$, $P < 0.001$), smaller for Freedom (2.8% smaller; $F_{3,158} = 3.265$, $P = 0.073$), and of equal sizes in diverse and monoculture neighborhoods for GR962 ($F_{3,158} = 1.78$, $P = 0.18$) and USG3770 ($F_{3,158} = 1.42$, $P = 0.24$; Fig. 5). When comparing size of mother aphids on different varieties within each neighborhood type, mother aphids on SW27 were smaller than those on all other varieties ($P < 0.05$ for all comparisons), and mother aphids on all other varieties did not differ in size ($P > 0.05$). All pairwise comparisons between varieties were nonsignificant ($P > 0.05$) in low diversity neighborhoods.

For development time in Year II, the interaction between focal variety, neighborhood diversity, and watering treatment was significant (Table 2). Diversity tended to influence development time of the mother aphid for one of the watering treatments, but the pattern was not consistent across varieties (Fig. 6). For instance, neighborhood diversity increased development

time for aphids on Freedom and reduced development time for aphids on SW27 and USG3770 when plants were drought stressed, but did not influence development time when plants were well watered for these varieties. The difference between diversity treatments for aphids on GR962, slower development in diverse neighborhoods, was apparent only when plants were well watered. Only the largest difference between diversity treatments, for aphids on drought stressed plants of Freedom, was statistically significant after correcting for multiple comparisons. However this included the correction for cases where all aphids took the same time to develop, and aphids on GR962 in watered pots took longer to develop in diverse neighborhoods than aphids in monocultures, which all developed in the minimal amount of time, 11 days (Fig 6).

Focal plant mass

In Year I, USG3770 was larger when growing with unrelated neighbors (15% larger, $F_{1,149} = 14.26$, $P < 0.001$), while Patton was smaller (7.1% smaller, $F_{1,149} = 3.04$, $P = 0.083$; Fig. 6). In Year II, USG3770 was once more larger when growing with unrelated neighbors (33% larger, $F_{1,160} = 16.2$, $P < 0.001$), while Freedom was smaller (21.1% smaller, $F_{1,160} = 12.10$, $P = 0.001$; Fig. 7). Neighborhood diversity did not affect the mass of GR962 ($F_{1,160} = 2.56$, $P = 0.11$) or SW27 ($F_{1,160} = 0.004$, $P = 0.95$).

Phytohormones: Salicylic acid

The simple *diversity* × *watering* interaction for each variety was only significant for Freedom (3: $F_{1,148} = 1.12$, $P = 0.29$; GR962: $F_{1,148} = 0.004$, $P = 0.95$; SW27: $F_{1,148} = 1.61$, $P = 0.21$).

VITA

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EDUCATION

Pennsylvania State University, State College, PA (2009-2015)
Ph.D Candidate in Entomology, Department of Entomology
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PUBLICATIONS

Grettenberger, I.M. and J.F.T. Tooker. 2015. Moving beyond resistance management toward an expanded role for seed mixtures in agriculture. Agriculture, Ecosystems & Environment. 208: 29-36.

Smith, J., M. Douglas, I. Grettenberger, S. Sidhu, E.L. Niño, and T. Bentley. 2012. Global climate change will have substantial long-term negative effects on arthropod diversity. American Entomologist. 58 (2): 99-103.

GRANTS AND AWARDS

USDA AFRI NIFA Fellowship
Toshiba America Foundation Grant
CarbonEARTH Fellowship, NSF GK-12 program
USDA NESARE Graduate Student Grant
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TEACHING

Pennsylvania State University
Penn State, ENT 316: Teaching Assistant for Field Crop Entomology (2011)
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AWARDS

2nd place Student Oral Presentation Competition ESA National (2014)
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PROFESSIONAL ASSOCIATIONS

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