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THE POTENTIAL OF GENOTYPICALLY DIVERSE CULTIVAR MIXTURES FOR
CONTROLLING APHID POPULATIONS IN WHEAT

ALEXANDRA V. SHOFFNER
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Reviewed and approved* by the following:

John F. Tooker
Assistant Professor of Entomology
Thesis Supervisor

S. Blair Hedges
Professor of Biology
Honors Adviser

Michael Saunders
Professor of Entomology
Honors Adviser

*Signatures are on file in the Schreyer Honors College.

Abstract

1. Traditionally, plant species diversity was thought to be the primary driver of arthropod species diversity; however, recent research has shown that genotypic diversity can be at least as important as species diversity in structuring arthropod communities. Genotypic diversity has already been exploited in some agricultural systems to improve disease control, and has promise for managing a wide range of insect species as well.
2. I investigated the effect of wheat genotypic diversity on aphid population growth (*Rhopalosiphum padi* L.) to explore the potential for genotypic diversity in a crop species to manage an insect pest. Increasingly genotypically diverse mixtures of wheat had lower aphid populations, and genotypic mixtures grown in the absence of aphids were more productive than monocultures.
3. Volatiles emitted by non-infested genotypic mixtures were analyzed to determine a possible mechanism influencing aphid choice. Mixtures of wheat emitted more volatiles than monocultures, but the component volatiles emitted were not different.
4. These results suggest that genotypic mixtures could be an effective method for managing insect pests in crop fields. Volatiles may influence aphid choice in such a way that causes mixtures to be less desirable than monocultures.

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Introduction

A goal of integrated pest management (IPM) is to increase the sustainability and resilience of agriculture while reducing use of pesticides (Altieri 1999, Lin 2011). Fostering biodiversity in agroecosystems has been proposed as a tactic that could facilitate IPM because outbreaks of pest populations rarely occur in diverse natural systems, such as tall grass prairie or rainforests; therefore, this mimicking of natural systems may provide some benefits (Benyus 1997, Altieri 1999). Increased plant species diversity within agricultural fields (i.e., polyculture) has been demonstrated to decrease the incidence of pest problems by a few mechanisms, including associational resistance and increased abundance and diversity of natural enemies (Tahvanainen and Root 1972; Andow 1991; Siemann et al. 1998; Landis, Wratten, and Gurr 2000). However, logistical and financial constraints associated with most polycultures have limited their adoption on large scales, particularly in grain production (Andow 1991).

Another approach for creating cropping systems that bear a greater resemblance to natural systems is to increase the genetic diversity they contain. The majority of crop fields worldwide are monocultures, containing only a single genetic variety. Because crop fields planted with single genetic varieties have uniform susceptibility to biotic stress, pests can spread rapidly through these fields and pesticides are often the only recourse for protecting crop yields. In contrast, genotypically diverse cultivar mixtures can prevent pests from invading and spreading across fields. Years of research have demonstrated that cultivar mixtures can be effective for managing plant pathogens. Genotypic mixtures reduce the speed of pathogen spread by increasing the distance between plants of the same genotype, and the effectiveness of

this process is related to the resistance of specific genotypes chosen and the spatial scale of deployment (Mundt 2002).

Genotypic diversity has already been exploited in some agricultural systems around the world to improve pest control. In China, cultivar mixtures of genotypically diverse varieties of rice have been adopted across tens of thousands of hectares, decreasing the severity of rice blast disease by 94% (Zhu et al. 2000). Moreover, approximately 90% of Germany's barley fields in 1990 (150,000 hectares) were planted as mixtures as a management tactic against powdery mildews (Mundt 2002). In the U.S., there has been less interest in cultivar mixtures, for reasons that are not clear. Nevertheless, at least 18% of wheat acreage planted in Washington in 2000 was planted as genotypically diverse cultivar mixtures meant to control key plant pathogens (Mundt 2002). Increased genetic diversity can also provide additional benefits, such as a 30% larger yield than monocultures when disease is present (Zhu et al. 2000). Unlike plant species diversity, genotypic diversity is already proving to be helpful in agricultural systems, and because it has been adopted on a large scale worldwide, there do not appear to be strong barriers to implementation.

A growing body of literature is providing evidence that intraspecific genetic diversity (i.e., genotypic diversity) can strongly influence insect communities in natural systems, decreasing herbivore abundance while improving plant productivity, and that the influence of genotypic diversity may rival that of interspecific plant species diversity (Crutsinger et al. 2006, Johnson, Lajeunesse, and Agrawal 2006; Cook-Patton et al. 2011). Because genotypic diversity has already been shown to influence herbivore populations in natural ecosystems and has been

used to manage diseases in field crops, using genotypic diversity to manage herbivore populations in agroecosystems could be a viable IPM tactic.

There has, however, been only limited research on the influence of cultivar mixtures of crop species on herbivorous pest populations, though many of such findings appear promising. Mixing preferred and nonpreferred cultivars of potato and cabbage led to reduced populations of potato leafhopper *Empoasca fabae* and imported cabbageworm *Pieris rapae*, respectively, than when cultivars were grown in monocultures (Cantelo and Sanford 1984). Similarly, population densities of the corn leafhopper *Dalbulus maidis* were significantly lower in mixed stands of maize varieties than would be expected from averaging the leafhopper population densities in monoculture stands of each component variety (Power 1988). These studies and others also demonstrate that genotypic diversity as a management tool can be effective across several taxa. In addition to its potential for pest suppression, increased genotypic diversity has the benefit of slightly but significantly improving yield over monocultures (Trenbath 1974, Mundt 2002, Cowger and Weisz 2008). For example, mixture yields of wheat are greater than their component means by 5.4% on average (Smithson and Lenne 1996).

To explore the potential of genotypic diversity to contribute to insect pest control, I chose for several reasons to study an aphid-wheat (*Triticum aestivum* L.) interaction. First, genotypic diversity has already been used in wheat to control disease (Mundt 2002), and wheat is an important crop in Pennsylvania. Second, aphids can be considered disease-like in that they trigger disease-associated plant defense pathways (Walling 2000) and can spread like diseases, first to adjacent plants, then more distant plants (Ragsdale, Voegtlin, and O'Neil 2004). Though I chose to use aphids as a model pest, it is important to note that several other herbivore species

may be suitable candidates for studying the potential of genotypic diversity for insect pest control (Cantelo and Sanford 1984, Power 1988). Moreover, because wheat is similar to other small grains, my work is relevant to pest control in other grain crop species and likely in other field and forage crops.

To study how genotypic diversity of wheat helps suppress populations of insect herbivores, I worked with one of the more common aphid pest species that attacks wheat in Pennsylvania, the bird cherry-oat aphid *Rhopalosiphum padi* L. My research objectives were to evaluate levels of aphid-resistance of a few wheat varieties, and then assess the influence of cultivar mixtures on aphid populations. Using *R. padi* and wheat as a model system, I hypothesized that different cultivars of wheat would exhibit variable resistance to aphids, and that genotypically diverse plantings of wheat would moderate aphid populations and increase the above-ground biomass of the wheat. A potential mechanism influencing the distribution of aphids on monocultures or cultivar mixtures are volatile cues released by the plants that aphid can use as foraging cues; therefore, I analyzed volatile blends released by groups of plants of varying genetic diversity, hypothesizing that abundance and complexity of volatile blends would increase with higher levels of genotypic diversity. My results provide insight into the potential for using genotypic diversity to improve management of pest populations in grain crops.

Materials and methods

To understand some of the mechanistic influences underlying the potential benefits of genotypic diversity, I conducted a series of relatively simple experiments testing the growth of three wheat varieties in the presence and absence of aphids and/or plant-plant competition. These

experiments culminated in a test of the pest-suppressive potential of genotypic diversity and a glimpse of plant chemistry as a potential mechanism for driving the patterns I found.

Plants and insects

For all experiments, common wheat (*Triticum aestivum* L. cultivars Tribute, Patterson, FS627, Truman, Freedom, and Patton) was grown from seeds in a climate-controlled growth chamber (16:8 h L:D; 22:20 °C day:night; 65% RH) that was insect-free. Seeds were planted in square-shaped pots (10 x 10 cm, 9 cm tall) in a peat-based, general-purpose potting soil (Pro-Mix BX, Premier Horticulture Inc., Quakertown, Pennsylvania) and watered as needed. For all experiments using aphids, I obtained bird cherry-oat aphids from a laboratory colony maintained in the Department of Plant Pathology at Penn State University. Statistical tests were conducted with Minitab 16 (Minitab Inc., State College, PA) unless stated otherwise.

Intra- and inter-varietal plant competition

To evaluate the effects of intra-varietal competition on plant growth, I planted seven pots of wheat in six combinations: Tribute versus Tribute, Patterson versus Patterson, FS627 versus FS627, Tribute versus Patterson, Tribute versus FS627, and Patterson versus FS627 (seven pots by six combinations for 42 pots total.) I planted each pot with four seeds and later thinned each pot to two plants. After eight weeks of growth, plants were harvested at soil level, dried for 48 hr, and weighed.

I compared aboveground biomass of plants from all six treatments using a one-way ANOVA, then separated the groups into intra-varietal and inter-varietal combinations. I re-

grouped plants from the three inter-varietal combinations to make comparisons among Tribute versus the other varieties, Patterson versus others, and FS627 versus others. I performed a one-way ANOVA to compare the intra-varietal treatments and another one-way ANOVA to compare the three inter-varietal combinations.

Aphid resistance of individual varieties

To evaluate the resistance of individual varieties to aphid population growth, I planted 24 pots of wheat: eight pots each of Tribute, Patterson, and FS627. I planted each pot with two seeds and thinned each pot to one plant after germination. After four weeks of plant growth, I placed one adult aphid per plant and beginning in the fifth week I counted the number of aphids in each pot once a week for six weeks.

I compared aphid counts over time from each of the three varieties using a General Linear Model ANOVA, with aphids as the response and variety, date, and the variety-date interaction as predictors.

Inter-varietal competition in the presence of aphids

To determine if the outcome of plant-plant competition among varieties changed in the presence of aphids, I planted individual pots with two plants each in three different combinations: Tribute versus Patterson, Tribute versus FS627, and Patterson versus FS627. Fifty pots were planted per combination (i.e. three competition treatments with 50 pots each for 150 pots total) and all were allowed to grow for four weeks. In the fifth week, I infested half of the plants with one adult aphid. In weeks six through ten I counted aphids on infested plants of five pots from each of the

six combinations, and then sacrificed the plants by cutting each plant at the base. I then dried the plants in an oven for 48 hr, removed any aphids, and weighed aboveground biomass.

For the analysis, I used only plants that had been infested in the fifth week since planting (N = 150). I re-grouped plants from the three inter-varietal combinations to again make comparisons among Tribute versus others, Patterson versus others, and FS627 versus others. I compared the number of aphids over time and across diversity treatments using General Linear Model ANOVA. I also compared the masses of plants by diversity treatment, week, and aphid counts. Finally, I performed a regression of biomass against aphids on the three wheat varieties, both collectively and individually, using SigmaPlot (Systat Software, San José, CA).

The influence of genotypic diversity on aphid populations

To determine the influence of genotypic diversity on aphid population growth, individual pots were planted as monocultures, three-line mixes, or six-line mixes, with six pots per diversity treatment (i.e., five diversity treatments of six pots each, for thirty pots total). Only the Tribute, Patterson, and FS627 cultivars were used for the monocultures and three-line mixes, and all six cultivars were used for the six-line mixes. Six sets of two seeds were planted in each pot; if both seeds germinated, one of the plants was thinned to leave six plants per pot. There were six pots of each of the three monocultures (Tribute, Patterson, and FS627), six pots of three-line mixtures (within each pot two plants each of Tribute, Patterson, and FS627 randomly positioned, but the identity of each plant was recorded), and six pots of six-line mixtures (within each pot one plant each of the six cultivars, randomly positioned and identity recorded).

I conducted this experiment twice: once without aphids, which I used for volatile collections (see below), and once in the presence of aphids. To infest pots with aphids, after four weeks of growth, I placed one adult on each plant (six aphids per pot). Beginning one week after infestation, the number of aphids on each plant was counted once a week for four weeks. After the fourth count, I harvested the plants at the base and froze them. I manually removed the aphids from each plant, then dried the plants in an oven for 48 hr and weighed the dried plants.

I averaged plant biomass and aphid numbers of the three monocultures to compare monocultures with three- and six-line mixtures. I also compared the number of aphids over time across the three diversity treatments using a Repeated Measures ANOVA.

The influence of genotypic diversity and presence absence of aphids on biomass

I also compared the aboveground biomass of plants that were grown in the presence and absence of aphids (see below). To do this, I combined the masses of the six individual plants per pot to obtain masses per pot, and then used ANOVA to compare the masses of pots from the three diversity treatments grown with and without aphids. Finally, I performed a regression of biomass as a function of the number of aphids per pot.

Volatile collections and analysis

Because volatile plant compounds are known to influence aphid distributions, I measured the influence of genotypic diversity on volatiles released by monocultures and three-line and six-line mixtures grown in the absence of aphids. I collected volatiles from the pots eight weeks after planting. I collected volatiles from four pots of each monoculture, and six pots each from the

three-line and six-line mixtures (24 pots total). Plant volatiles were collected from wheat using a closed push/pull system (Analytical Research Systems, Inc., Gainesville, FL) in a climate-controlled growth chamber (conditions as above). Filtered air was pushed ($1.5 \text{ liter min}^{-1}$) through Teflon® tubing into a glass dome (46.5 cm tall, 8 cm diameter), which enclosed each pot of wheat. The dome rested on a two-piece Teflon® base, which lay on the rim of the plant pot and had a hole for the group of plant stems to pass through to the dome. Plant stems were wrapped in cotton where they passed through the hole to plug the gap between the stems and base. Air was pulled from the cylinder through side ports ($0.8 \text{ liter min}^{-1}$) across 25 mg beds of the polymeric adsorbent SuperQ (Alltech Associates, Deerfield, IL). Plant volatiles from each pot were collected continuously for 48 hr with daytime volatiles being entrained on one SuperQ® filter (06.00-22.00 hours for 2 days; 32 hr total) and night-time volatiles being entrained on another (22.00-06.00 hours for 2 days; 16 hr total). After I completed the volatile collection I harvested plants at the base, dried the plants in a drying oven for 48 hr, and weighed the dried plants.

I eluted SuperQ® traps with 150 μl of dichloromethane spiked with 400 ng nonyl acetate, and I added 200 ng *n*-octane to each sample as an internal standard. Samples were injected in 1- μl aliquots into an Agilent model 5890 gas chromatograph fitted with a flame ionization detector, using a splitless injector held at 220°C. The column (HP-1, 15 m x 0.25 mm x 0.25 μm film thickness; J&W Scientific, Folsom, CA) was maintained at 35 °C for 30 s, then ramped 2°C min^{-1} to 130°C, and ramped again at 20°C min^{-1} to 220°C. I used ChemStation software (Agilent Technologies, Wilmington, DE) to make quantifications of compounds relative to the nonyl acetate standard.

I compared the total volatiles released by pots of plants across diversity treatments using one-way ANOVA. I also compared the total volatiles collected during the day and night, as well as the quantities of (Z)-3-hexenyl acetate and monoterpenes released by the three diversity treatments, both at day and at night.

Results

Intra- and inter-varietal plant competition

When plants were grown in intra-varietal competition, the dry masses of plants of the three varieties were not statistically different (Fig. 1A; $F_{2,41} = 0.03$, $P = 0.968$). Similarly, when plants were grown in inter-varietal competition, the dry masses of plants of the three varieties were not different (Fig. 1B; $F_{2,41} = 0.69$, $P = 0.509$); therefore, it appears that the three varieties compete equally well with each other.

Aphid resistance of individual varieties

In contrast to their equal competitive ability, the three wheat varieties varied in their resistance to aphids. For all varieties, aphid populations were lowest in the first week after infestation, highest in the second week, and intermediate for the following four weeks ($F_{5,143} = 33.90$, $P < 0.0001$). Patterson plants supported a significantly higher mean aphid population than Tribute and FS627 plants over the six weeks (Fig. 1C; $F_{2,143} = 13.22$, $P < 0.0001$). When compared across weeks and varieties, aphid populations were the highest on Patterson plants in the second week after infestation, intermediate for all varieties and dates from week three to week six, and lowest on all three varieties in the first week ($F_{10,143} = 3.60$, $P < 0.0001$).

Inter-varietal competition in the presence of aphids

Over five weeks, the three varieties did not differ in the quantity of aphids they supported or in plant biomass (aphids: Fig. 1D; $F_{2,149} = 1.92$, $P = 0.150$; plant biomass: Fig. 1E: $F_{2,149} = 1.75$, $P = 0.180$). Time was a significant factor for aphid populations ($F_{4,149} = 4.21$, $P = 0.003$). For all varieties, aphid populations were lowest in the first week after infestation, intermediate in weeks two and three, highest in week four, and intermediate in week five. Biomasses across varieties differed significantly with time: the lowest plant biomass occurred in the fourth and fifth weeks, and the highest in the first and third weeks, whereas mass in the second week was intermediate ($F_{4,149} = 8.44$, $P < 0.0001$). When I related plant biomass to the number of aphids that developed per plant, I found statistically significant relationships. First, when all the data from each variety were combined, I found the relationship to be best described by a third order polynomial (Fig. 2A). Second, I detected significant first order linear relationships for each variety, when their biomass and aphid populations were plotted separately (Fig. 2B).

The influence of genotypic diversity on aphid populations

Genotypic diversity treatments significantly influenced aphid populations over time. Monocultures, three-line mixtures, and six-line mixtures had significantly different mean aphid populations over four weeks: six-line mixtures had the lowest aphid populations, whereas monocultures and three-line mixes generated higher aphid populations (Fig. 3; $F_{6,719} = 6.23$, $P < 0.0001$), supporting my main hypothesis that genotypic diversity can help reduce aphid populations. Aphid populations were also significantly different each week of the experiment: populations were lowest in the first week after infestation, highest in the second week, and then

decreased in the third and fourth weeks ($F_{3,719} = 74.32, P < 0.0001$). Individual plants of each of the six varieties supported the same number of aphids regardless of their diversity treatment ($F_{5,719} = 1.14, P = 0.340$) and did not have significantly different masses in the presence of aphids ($F_{5,170} = 0.48, P = 0.788$).

The six-line mixtures moderated aphid populations over four weeks. Monocultures had higher peak aphid populations (i.e., populations in the second and third weeks) than the six-line mixtures, and the three-line mixtures had significantly lower aphid populations than the monocultures in weeks one and two (significant diversity x time interaction: $F_{6,719} = 6.23, P < 0.0001$). Despite the lower aphid populations on six-line pots, biomass appeared similar across the three diversity treatments in the presence of aphids. There was a trend toward monocultures having slightly lower biomass than three- and six-line mixtures ($F_{2,170} = 2.73, P = 0.068$).

The influence of genotypic diversity and presence absence of aphids on biomass

When the three diversity treatments were grown in the absence of aphids, individual plants grown in six-line mixtures had statistically greater biomass than those grown in monocultures (Fig. 4: $F_{2,174} = 4.57, P = 0.012$). When biomasses of entire pots were compared I only detected a trend toward significance among diversity treatments, with the six-line mix appearing to have the greatest biomass ($F_{2,23} = 2.67, P = 0.092$). The biomasses of plants from all three diversity treatments were greater when plants were not infested with aphids (Fig. 4: t -test, $P < 0.0001$). Regression analysis detected a significant but weak positive relationship between the number of aphids per pot and biomass, though the biological significance of this finding is unclear ($F = 7.85, P = 0.0056, R^2 = 0.04$).

Volatile collections and analysis

The total volatile blend released by plants grown in the absence of aphids increased marginally with diversity, and the types of volatiles released showed quantitative but not qualitative differences (Fig. 5A: $F_{2,23} = 3.13$, $P = 0.065$). The positive influence of diversity on total volatiles was driven by (*Z*)-3-hexenyl acetate, a “green leafy” volatile emitted by most plant species, that accounted for 55.3% of the total volatiles emitted during daytime hours (Fig 5B; $F_{2,23} = 6.3$, $P = 0.007$). Monoterpenes showed a pattern opposite to that of (*Z*)-3-hexenyl acetate: significantly greater quantities of monoterpene volatiles were released by monocultures than either three- or six-line mixtures (Fig. 5C: $F_{2,23} = 5.8$, $P = 0.01$).

A significantly smaller quantity of volatile compounds were released by plants at night than by day ($F_{1,47} = 66.13$, $P < 0.0001$). There was no difference in the quantity of volatiles released among the three diversity treatments at night ($F_{2,23} = 66.13$, $P = 0.392$). Although the quantity of (*Z*)-3-hexenyl acetate released at night accounted for less than 5% of the total volatiles released at night, more (*Z*)-3-hexenyl acetate was still released with increasing levels of diversity ($F_{2,23} = 8.83$, $P = 0.002$).

Discussion

My results support the notion that genotypic diversity may help control populations of insect herbivores. When grown separately, the three varieties from the three-line mixture varied in the aphid populations they supported (Fig. 1C), but did not differ in their ability to grow in the presence of other varieties in the presence or absence of aphids (Fig. 1A, B, E). When the three lines were grown together, the mixture significantly slowed aphid population growth in the first

two weeks of my experiment relative to monocultures, but did not decrease the maximum aphid population (Fig. 3). When three additional lines were added to a make a six-line mixture, the aphid population was significantly depressed relative to both the three-line and six-line mixtures (Fig. 3). This result was somewhat unexpected given that differences in aphid resistance of the individual wheat varieties (Fig. 1C) were not apparent when varieties were grown in inter-varietal competition (Fig 1E). Nevertheless, my results appear promising for using genotypic diversity as a pest management tool in wheat production. If this effect translates to the field, growers may have a simple yet effective pest management tool that they can use to help mitigate insect problems. A notable limitation of my research is that I did not directly determine the genetics of the six wheat varieties; such an effort was beyond the scope of my thesis, but I tried to ensure via literature searches that the varieties I used came from different lineages (e.g., Griffey et al. 2005). Moreover, the varying resistance against aphids across the three monocultures I tested suggested they are genetically distinct at least for this trait.

The explicit test of the herbivore-control value of diversity clearly indicated that genotypically diverse mixtures of wheat moderated aphid populations (Fig. 3). Because there were no differences in aphid populations among the three main varieties grown in pairwise competition and no differences in aphid populations among the same three main varieties grown in monoculture, it is possible that the additional three varieties in the six-line mixtures were responsible for the diversity pattern. If these three varieties alone were driving my results, it would have been unlikely to see intermediate aphid populations in the three-line mixtures, which contained only the same three main varieties that did not show differences in monocultures or in pairwise competition. These results suggest that increased diversity, rather than just unknown

phenotypic benefits of the three additional varieties, contributed to controlling aphid populations. The beneficial influence of increased genotypic diversity against aphid populations revealed effects similar to other experiments which found that genotypic diversity influenced insect communities as strongly as plant species diversity (Crutsinger et al. 2006; Johnson, Lajeunesse, and Agrawal 2006; Cook-Patton et al. 2011) as well as agricultural studies showing genotypically diverse fields reduced severity of disease (Zhu et al. 2000, Mundt 2002), but mine is among just a few to show a clear benefit of genotypic diversity for managing herbivore populations in an agricultural system (Cantelo and Sanford 1984, Powers 1988, 1991).

In the absence of aphids, the most diverse wheat mixtures had greater productivity than monocultures (Fig. 4). In the presence of aphids, however, the biomass was similar across the three diversity treatments despite the lower aphid populations on six-line pots. Unexpectedly, I detected a positive relationship between plant biomass and the number of aphids per plant, and similar relationships were evident in my experiments investigating inter-varietal competition in the presence of aphids (Fig. 2). These results are counterintuitive because herbivores usually negatively influence plant production. The results are also different from similar studies, which found that slightly improved yield over monocultures even in the presence of pests was an additional benefit of using plant genotypic diversity to manage pest populations (Smithson and Lenne 1996, Mundt 2002, Cowger and Weisz 2008). However, because in these cases yields are typically only improved by one to five percent, it is possible that the five weeks of this experiment were not enough time for biomass differences among mixtures and monocultures to develop in the presence of aphids (Mundt 2002).

The total volatiles released by plants grown in the absence of aphids increased marginally with diversity, and the types of volatiles released showed quantitative but not qualitative differences (Fig. 5). These results are relevant because differences in the quantity and classes of volatiles emitted by monocultures and cultivar mixtures of host plants are a potential mechanism to explain the distribution of aphids on monocultures and varietal mixtures. Previous experiments have shown that *R. padi* prefers volatiles from monocultures over those of some varietal mixtures of barley; a similar field study showed that aphid settling was reduced in some plots of genotypic mixtures compared to pure stands (Pettersson, Ninkovic, and Ahmed 1999; Ninkovic, Olsson, and Pettersson 2002; Glinwood et al. 2009). Varietal mixtures also induced an increased attraction of ladybirds and parasitoids, suggesting volatile interactions can influence higher trophic levels (Glinwood et al. 2009). My results indicate that differences in the quantity of volatiles released by genotypically diverse groups of plants may influence the number of aphids that feed there.

The association between genotypic diversity and total volatiles was driven by (Z)-3-hexenyl acetate, which composed 55.3% of the total volatiles emitted. (Z)-3-hexenyl acetate is a volatile emitted by many plants after herbivore or artificial mechanical damage and has been shown to attract some aphid natural enemies (e.g. *Stethorus punctum picipes*, the mite-eating ladybeetle, and *Aphidius ervi*, an aphid parasitoid) in both laboratory and field conditions (Du et al. 1998, James 2003). Because plants in this study were undamaged, the baseline levels of (Z)-3-hexenyl acetate that increase with diversity may indicate that more diverse groups of plants may be more attractive to natural enemies (Fig. 5B).

Monoterpenes showed a pattern opposite to that of (*Z*)-3-hexenyl acetate: significantly greater quantities of monoterpene volatiles were released by monocultures than either three- or six-line mixtures (Fig. 5). Many aphid species, including bird cherry-oat aphid, are attracted to some monoterpenes in addition to (*Z*)-3-hexenyl acetate, indicating that perhaps monocultures may be more attractive to aphids than more genotypically diverse mixtures (Quiroz and Niemeyer 1998).

Though these results were obtained using an aphid species, it is important to note the potential for similar results with a broad range of herbivores. It has previously been shown that populations of other insect pests of crops (potato leafhopper, corn leafhopper, and imported cabbageworm) decrease significantly in increasingly genotypically diverse mixtures of their respective host plants (Cantelo and Sanford 1984, Power 1988). Additionally, several studies have reported the effectiveness of plant species diversity against a variety of insect pests (Tahvanainen and Root 1972, Andow 1991). Those results coupled with the increasing number of studies showing that plant genotypic mixtures can provide the same level of resistance to pests that plant species mixtures can (Crutsinger et al. 2006; Johnson, Lajeunesse, and Agrawal 2006; Cook-Patton et al. 2011) indicate that there is much unexplored potential for genotypic diversity as an approach for moderating a variety of insect pests.

In conclusion, I have demonstrated that genotypically diverse mixtures of wheat effectively moderate aphid populations. Additionally, increasingly diverse mixtures of wheat grown in the absence of aphids had significantly greater productivity than monocultures. These are promising preliminary results for crop production. I also identified volatile compounds as a mechanism that may be driving the distribution of aphids in genotypically diverse plantings and

monocultures. Because I only conducted lab-based experiments, fieldwork will be necessary to validate my results regarding the effectiveness of plant genotypic diversity in managing aphid populations. Despite these limitations, my results indicate that genetically diverse mixtures have great potential to improve management of a variety of pests in field crops.

Appendix

Figure captions

Figure 1. Intra- and inter-varietal competitive abilities of three wheat varieties in the absence and presence of aphids. (A) Masses of three varieties grown in intra-varietal competition. (B) Masses of three varieties grown in inter-varietal competition. (C) Differential resistance of each variety to aphid population growth. (D) Resistance of inter-varietal pairs to aphid population growth. (E) Masses of plants of each variety when grown in an inter-varietal pair mixture in the presence of aphids. Letters above error bars indicate significant differences.

Figure 2. Regression analyses relating plant biomass to the number of aphids that developed per plant. (A) Third-order polynomial best describes all data: $M = 0.0514 + 0.00932N - 0.000148N^2 + 0.000000754N^3$ ($F = 47.5$, $P < 0.001$, $R^2 = 0.72$). (B) First-order polynomials best describe each of the three varieties. FS627: $M_F = 0.120 + 0.002N$ ($F = 15.6$, $P < 0.001$, $R^2 = 0.46$); Patterson: $M_P = 0.096 + 0.0021N$ ($F = 15.9$, $P < 0.001$, $R^2 = 0.47$); Tribute: $M_T = 0.031 + 0.0076N$ ($F = 55.1$, $P < 0.001$, $R^2 = 0.75$).

Figure 3. The influence of genotypic diversity on aphid populations over four weeks. Asterisk indicates aphid populations on six-line mixtures were significantly lower than monocultures and three-line mixtures in the second and third week after infestation.

Figure 4. Comparisons of biomass of monocultures, three-line mixtures, and six-line mixtures grown in the presence and absence of aphids. Letters indicate statistical differences.

Figure 5. Analysis of volatiles emitted from monocultures, three-line mixtures, and six-line mixtures grown in the absence of aphids. (A) Total volatiles in ng released from three diversity treatments. (B) (*Z*)-3-hexenyl acetate emitted from the three diversity treatments. (C) Monoterpenes released from each of the three diversity treatments. Letters indicate significant differences.

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ACADEMIC VITA OF ALEXANDRA V. SHOFFNER

8500 River Walk Landing • Suwanee, GA, 30024 • allieshoff@gmail.com • (770) 633-0802

EDUCATION

B.S. degree in Biology, Penn State University, Spring 2012

B.S. degree in Wildlife and Fisheries Science, Penn State University, Spring 2012

Minor in Chemistry

Honors in Biology and Entomology

Thesis Title: The potential of genotypically diverse cultivar mixtures for controlling aphid populations in wheat

Thesis Supervisor: John F. Tooker

PROFESSIONAL EXPERIENCE

Animal Care Intern, Centre Wildlife Care Jan. 2012 – May 2012

As an animal care intern, I worked ten hours a week preparing food for and cleaning the enclosures of a variety of bird, mammal, and reptile species. I also assisted with all aspects of planning, hosting, and attending events such as conventions and fundraisers.

Honors Independent Study, School of Forest Resources Jan. 2012 – May 2012

Advised by Dr. Margaret Brittingham, I freeze-dried bird specimens to learn about freeze-drying as a technique for preserving specimens for teaching collections, and to improve the School of Forest Resources collection.

Bird Banding Volunteer, The Arboretum at Penn State Feb. 2011 – May 2012

As a bird banding volunteer, I gained over sixty hours of experience working with a team of several experienced banders and other students. I learned and practiced proper procedures for mist netting songbirds, and helped to educate visiting classes about birds and banding.

TimeTree Specialist, Hedges Evolutionary Biology Lab Jan. 2011 – May 2012

As a TimeTree Specialist, I managed and built upon a database of over 7,000 publications for the Time Tree of Life project. I conducted literature searches, read academic papers, assessed their suitability for inclusion in the database, and corresponded with authors to obtain data.

Resident Assistant, East Halls Residence Life Aug. 2010 – May 2011

As a Resident Assistant, I built community on a floor of forty first-year students by coordinating weekly floor social activities and monthly educational meetings. I also enforced laws and Residence Life policies in two residence halls.

Research Assistant, Tooker Insect Ecology Lab Dec. 2008 – Dec. 2010

As an undergraduate Research Assistant, I assisted the Principal Investigator and three graduate students in conducting ecological experiments based in laboratory, greenhouse, and field settings. I sampled insect and plant communities using various methods.

PRESENTATIONS

Poster presentation: **Freeze-drying as a means of preserving birds for teaching collections**

- Pennsylvania Chapter of the Wildlife Society Annual Conference, State College, PA
- March 2012

Poster presentation: **The potential of genotypically diverse wheat mixtures for controlling aphid populations**

- Undergraduate Poster Competition, The Pennsylvania State University, University Park, PA
- April 2011

Ten-minute talk: **The potential of genotypically diverse cultivar mixtures of wheat for controlling aphid populations**

- The Entomological Society of America Annual Meeting, San Diego, CA
- December 2010

HONORS AND AWARDS

- Schreyer Ambassador Travel Grant, 2011
Funding to study abroad in Costa Rica.
- Africa and Latin America Grant-in-Aid award, 2011
Funding to study abroad in Costa Rica.
- ESA Undergraduate Student Achievement Award, 2010
Funding to present my thesis research in a ten-minute talk at the Entomological Society of America's Annual Meeting in San Diego
- Pearson Prize for Higher Education Community Fellows Grant, 2011
- Robert T. Billin Scholarship from the College of Agriculture, 2011-2012
- Wheeler P. Davey Memorial Scholarship from the Biology Department, 2010-2012
- Schreyer Ambassador Travel Grant, 2010
- Pennsylvania Commonwealth Education Abroad Scholarship, 2010
- Honors Thesis Research Grant, 2010
- National Merit Scholar award, 2008-2012
- Schreyer Academic Excellence Scholarship, 2008-2012
- Shapiro Family Scholarship from the Eberly College of Science, 2008-2012
- Dean's List, 2008-2012