ENHANCING INSECT-PEST MANAGEMENT IN ORGANIC SYSTEMS USING GENOTYPICALLY DIVERSE CULTIVAR MIXTURES.

A final report to the
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1. Project Summary

Organic growers rely on natural processes to help manage their crops. For pest management, growers rely on host-plant resistance and naturally occurring arthropod predators and parasitoids to control populations of plant-feeding insect pests. Using the former tactic can be challenging because resistance to various pests is often not well developed or advertised, but meaningful levels of resistance are more widespread than most people realize. Using the latter tactic is typically feasible on organic farms but often under developed. Unlike conventional farms, organic farms often harbor a diversity of crop and non-crop plant species, which can support diverse natural-enemy communities that can kill plant-feeding insects. Our research aims to better harness these two mechanisms of pest control to improve management of arthropod population in organic grain production.

Recent research with native-plant species has demonstrated that genotypic diversity can be nearly as valuable as plant-species diversity for structuring insect communities and driving ecological interactions. Field crop growers in Europe and Asia are aware of the utility of genotypically diverse cultivar mixtures and routinely use them to manage plant diseases. Surprisingly, this previous research has not been translated for use against insect pests, which have many similarities to disease pests. Moreover, genotypic diversity does not appear to have been promoted much in organic systems despite providing diversification benefits often embraced by the organic community. Our research hypothesizes that by simply planting numerous agronomically similar, yet genetically distinct cultivars in a field, growers can improve resistance of their grain crops to insect herbivores while simultaneously diversifying arthropod populations, including predators and parasitoids in the crop fields and reduce the incidence of outbreaks of insect pests. Our OFRF-supported work with soybean and wheat production in the Northeast Region supported the hypothesis that genotypic diversity can help moderate insect-pest populations while maintaining or even enhancing yield, providing strong evidence that genotypically diverse cultivar mixtures can help improve pest control in grain production.

2. Introduction to Topic

Organic crop production relies heavily on natural processes to control pests. Chief among these processes are host-plant resistance and natural-enemy mediated control, which relies on arthropod predators and parasitoids to kill plant-feeding insect pests and eat weed seeds. The former approach to pest control relies on genetic differences between varieties that are often found via traditional plant breeding research. The latter approach relies on plant-species diversity to harbor healthy populations of beneficial insect species. Fortunately, most organic-production systems rely on less-heavily selected crop varieties that tend to maintain some of the defensive capabilities of their wild progenitors. Also, organic farms tend to provide far more plant species diversity in space (local on-farm diversity) and time (i.e., crop rotations) than conventional farms, and this plant-species diversity can substantially improve local populations of natural enemies that can help contribute to improved pest control. Organic growers however face the challenge of finding crop varieties with higher levels of resistance and encouraging natural enemies to venture far enough into cropping fields to have a significant influence on the local pest populations.

An alternative, yet under studied, tactic to increase resistance to crop pests while improving populations of beneficial-insect species in crop fields is to plant genotypically diverse cultivar mixtures in crop fields rather than planting traditional genetically uniform monocultures. Two distinct bodies of research provide evidence that genetically diverse cultivar mixtures hold promise for increasing natural enemy diversity and managing insect pests in crop fields.

First, genotypically diverse cultivar mixtures have been widely adopted in field crops in Europe and Asia for controlling plant diseases. In fact, close to fifty percent of wheat fields in Europe and thousands of acres of rice in China have been sown as cultivar mixtures which range in their susceptibility to a few key diseases (Zhu et al. 2000, Mundt 2002).

Second, ecological work with native plant species has shown that increases in plant genotypic diversity can lead to greater arthropod species diversity and increases in natural-enemy abundance, both of which can have cascading effects that improve plant growth (Crutsinger et al. 2006, Johnson et al. 2006).

These two bodies of evidence, one illustrating the utility of this tactic in field crops and the other addressing its potential for controlling plant-feeding insects, suggest that plant genotypic diversity holds promise for pest control in organic production systems. Our research has begun the process of evaluating the utility of plant genotypic diversity for managing pests in organic field crops. Our research has the opportunity to provide organic growers another pest control tool that they can use to diversify their farms, and this tool may also generate other benefits. Several studies have demonstrated that genotypically diverse plantings can actually yield better even in the absence of pest pressure because of inter-varietal competition and/or outcrossing that occurs between genetically distinct cultivars (Schutz and Brim 1967, Brim and Schutz 1968, Schweitzer et al. 1986, Smithson and Lenne 1996).

3. Objectives Statement

The main objectives of our project were to assess the potential of using genotypic diversity cultivar mixtures to:

1. Decrease abundance of herbivorous insect pests in crops fields;
2. Increase natural-enemy diversity and abundance in crop fields;
3. Improve crop yields.

Each of these three objectives had corresponding measurable outcomes that verified successful or unsuccessful achievement. Relative to monoculture controls, genotypically diverse plots were expected to:

1. Have lower numbers of plant-feeding insect pests;
2. Harbor higher numbers of natural enemies and a greater diversity of natural-enemy species;
3. Generate higher per acre yield.

Our experimental work pursued each of these three objectives and relied on natural colonization of our fields by insect herbivores and their natural enemies. This approach worked well for our soybean experiment, but our wheat plots failed to generate significant numbers of herbivores. This was out of our control and somewhat problematic because natural enemy populations typically respond to herbivore populations and colonize plots with potential prey.

4. Materials and Methods

To explore the role of genotypic diversity in soybean production, in Spring 2010 we established a replicated field experiment in a randomized complete block design in a 1.7-acre certified organic field (certifier: Pennsylvania Certified Organic, Spring Mills, PA) at Penn State's Russell E. Larson Agricultural Research Center in Rock Springs, PA. This field was tilled and prepared using typical organic field crop production techniques and planted on 3 June 2010. Within each of five blocks, five treatments (Table 1) were randomly applied to a total of twenty-five plots. Individual plots were 100 ft long and 10 ft wide established with 30-in row spacing (each plot had four rows of soybeans). Thirty-inch soybean rows are common in the Mid-Atlantic region and they also make in-season arthropod sampling easier.

Plots were planted with a single variety of commercially available soybean seed or mixtures of three or six varieties (Table 1); five certified organic seed varieties and one untreated conventional variety were acquired from Albert Lea Seed (Albert Lee, MN) or Blue River Hybrids Organic Seed (Kelley, IA). One of the Blue River varieties, 29AR9, contained a native trait (the *Rag1* [Resistance against *Aphis glycies*] gene) conferring resistance against the soybean aphid (*Aphis glycies*); therefore this variety would be expected to have the lowest aphid populations of all the varieties. We generated cultivar mixtures by combining equal volumes of seeds from each variety in a garbage can and stirring them together. Prior to planting, seeds were inoculated with N-Dure (OMRI Certified; INTX Microbials, Kentland, IN) to ensure nodule development. Weeds were controlled via between-row cultivation three times during the season with a rotary hoe. Plots were harvested on 24 October 2010.

Table 1. Soybean varieties used for field experiment. Mixtures comprised equal volumes of all the varieties in the mixture.

Treatment	Soybean variety planted
Monoculture 1	Viking O-2265
Monoculture 2	Blue River 29AR9 (aphid resistant)
Monoculture 3	Blue River 35A0cnv
Three-line mixture	Viking O-2265, Blue River 29AR9 & 35A0cnv
Six-line mixture	Three-line mix + Viking O-2402, Blue River 27A9 & Blue River 30A7

To explore the value of genotypic diversity in wheat production, we similarly established wheat plots in the same 1.7 acres field as above. Again, we tilled and prepared the field using typical organic field crop production techniques and planted our winter wheat on 1 Nov 2010. In each of six blocks, treatments (Table 2) were randomly applied to plots. Individual plots were 100 ft long and were established with a 10-ft wide grain drill. Plots were planted with a single variety of seed or mixtures of three or six varieties (Table 2); varieties of certified organic seed (one bag each) were purchased from Great Harvest Organics (Atlanta, Indiana) and Welter Seed & Honey Co. (Onslow, Iowa). The remaining four varieties were conventionally produced but untreated and were purchased from American Hybrids and Organics (Warren, Illinois) and the Virginia Crop Improvement Association (USG-3770). We generated cultivar mixtures by combining equal volumes of seeds from each variety in a garbage can and stirring them together. Wheat plots were fertilized once on 12 May 2011 using bull manure applied at a rate of 20 tons per acre. Plots were harvested on 16 July 2011. Some plots were heavily infested with Canada thistle (*Cirsium arvense*).

Table 2. Wheat varieties used for field experiment. Mixtures comprised equal volumes of all the varieties in the mixture.

Treatment	Wheat variety planted
Monoculture 1	American Hybrid 9121
Monoculture 2	Great Harvest Organic GH4532SR
Monoculture 3	Welter WS44
Three-line mixture	American Hybrid 9121, Great Harvest Organic GH4532SR, Welter WS44
Six-line mixture	Three-line mix plus USG-3770, American Hybrid 9915 & 9643

During the growing season of each crop, we regularly sampled arthropod populations in the individual plots. In soybean, which is reliably infested annually with soybean aphid, we counted the numbers of aphids per plant weekly. Other herbivore species did not colonize our plots in large enough numbers to be significant so our weekly aphid counts are our only assessment of herbivore density. To assess the natural-enemy community in each plot, two or three times during the season we sampled the resident arthropod community using vacuum samplers, which is a modified leaf blower. Arthropods were subsampled from five plants per plot and collected into fine mesh bags by vacuuming each plot in a random pattern for thirty seconds. Collected arthropods were preserved in buckets with closable lids using ethyl acetate and then returned to the lab for sorting and identification to species or morphospecies. In wheat plots, herbivore populations did not accumulate, so we were unable to visually assess their populations and relied solely on vacuum sampling to characterize the community of arthropods in each plot.

5. Project Results

Our results demonstrated that genotypically diverse cultivar mixtures can significantly alter arthropod communities, including densities of yield-sapping herbivores and some groups of herbivore-killing natural enemies. Our experiments, however, failed to detect expected yield increases in genotypically diverse plots. Importantly, yield was maintained and did not suffer in mixed plots. We would expect that experiments conducted over multiple seasons would have a better chance to detect the yield benefit, particularly given the reductions in herbivores we documented (see below).

Soybeans

1. Decrease abundance of herbivorous insect pests in crop fields

Genotypic diversity significantly reduced aphid populations compared with the conventional soy variety. Over the course of the season, plots containing three- or six-line mixtures harbored significantly fewer aphids than the monoculture variety that generated the highest aphid populations despite this variety being included in the mixtures (Fig. 1, ANOVA $F_{4,249} = 4.3$, $P = 0.011$). Importantly, when aphid populations increased toward the end of the season, the genotypically diverse plots maintained the lowest aphid populations and these populations were as low as the aphid populations in the aphid-resistant monoculture (Blue River 29AR9), which would be expected to harbor the lowest aphid populations (Fig. 2, ANOVA $F_{4,24} = 3.7$, $P = 0.03$). Cultivar mixtures even better suppressed aphid populations than monocultures, with three-line mixtures harboring significantly lower aphid populations at the end of the season compared to monocultures whereas six-line plots were intermediate (Fig. 3a, ANOVA $F_{2,24} = 3.8$, $P = 0.05$). When varietal mixtures were compared with single variety plots, cultivar mixtures again significantly decreased aphid numbers relative to monoculture plots (Fig. 3b, ANOVA $F_{1,24} = 8.0$, $P = 0.013$). All these results combine to strongly support the conclusion that genotypic diversity can help maintain lower herbivore populations than genotypically uniform monocultures.

Fig. 1. Number of aphids on soybean monocultures, three- and six-line mixtures during the entire growing season. Mixtures moderated aphid populations despite containing the constituent variety with significantly higher numbers of aphids. Variety BR 29AR9 is an aphid-resistant soybean variety and is expected to have the lowest numbers of aphids, but three- and six-line mixes were comparable. Treatments labeled with different letters are significantly different from each other ($P < 0.05$). See text for statistical details.

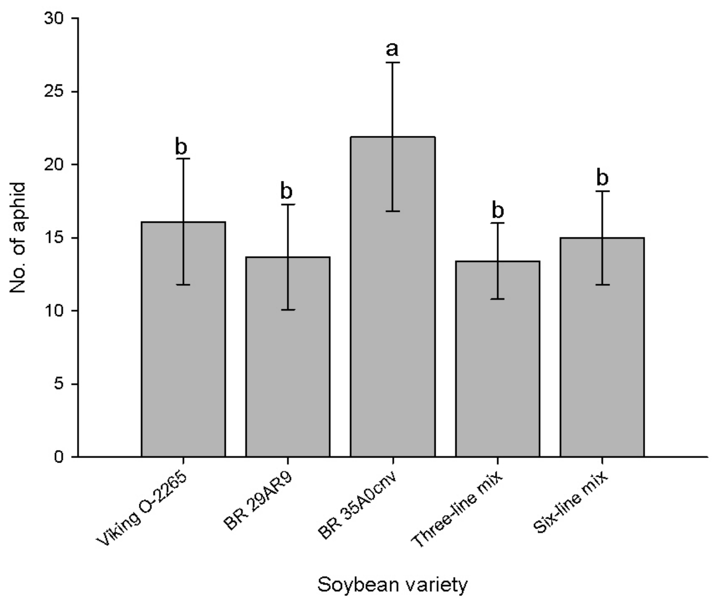


Fig. 2. Number of aphids on soybean monocultures, three- and six-line mixtures at the end of the growing season. Mixtures moderated aphid populations despite containing the constituent variety with significantly higher numbers of aphids. Variety BR 29AR9 is an aphid-resistant soybean variety and is expected to have the lowest numbers of aphids, but three- and six-line mixes appeared to be comparable and, in the case of the three-line mix, appeared to perform even better despite containing two soybean varieties not resistant to aphids. Treatments labeled with different letters are significantly different from each other ($P < 0.05$). See text for statistical details.

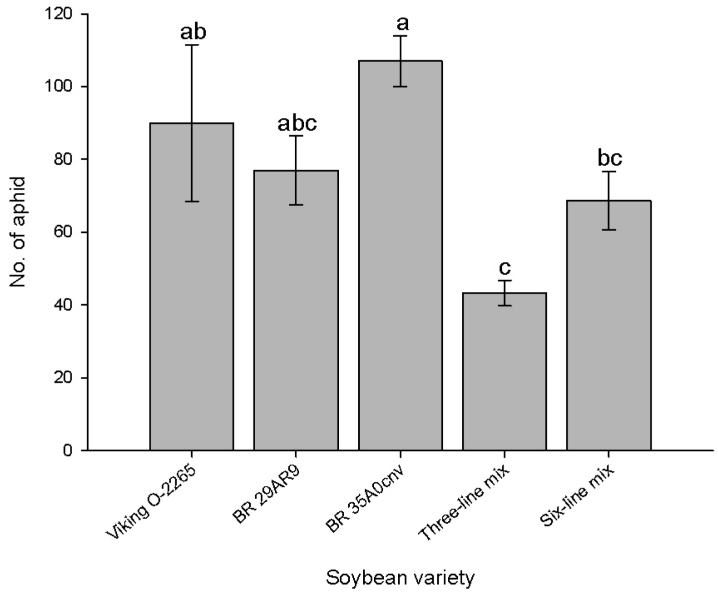
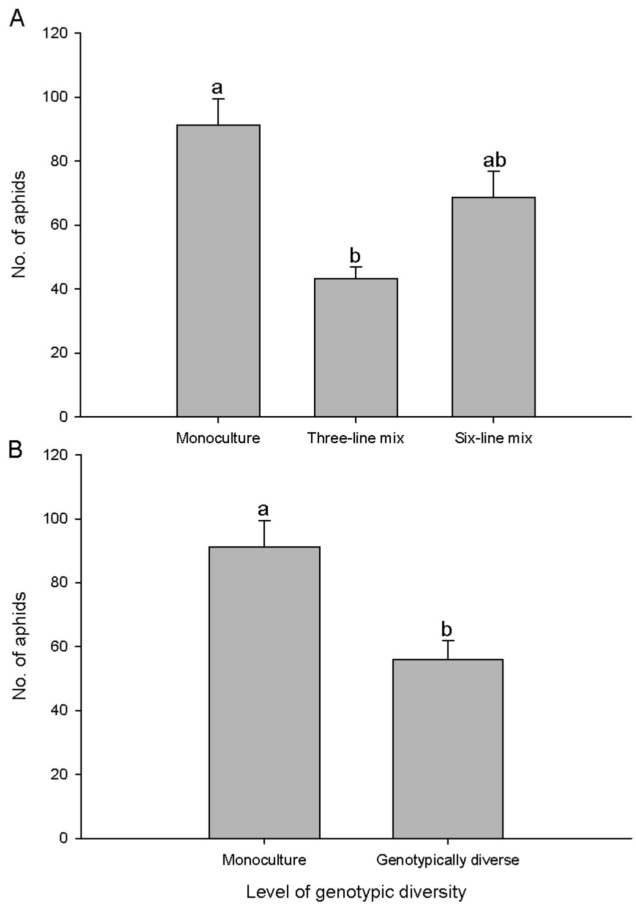


Fig. 3. Number of aphids on soybean varieties at the end of the growing season as considered by the level of genotypic diversity. Data represented here are also in Fig. 2 but here are depicted as averages of A) monocultures, three-line and six-line mixture or B) monocultures or cultivar mixtures. In both representations, genotypic diversity moderated aphid populations. Treatments labeled with different letters are significantly different from each other ($P < 0.05$). See text for statistical details.



2. Increase natural-enemy diversity and abundance in crop fields

   Our vacuum sampling revealed that genotypic diversity did not significantly alter the total number of arthropods nor the abundance of natural enemies found over the course of the season across the three different monoculture varieties, the three-line mix, or the six-line mix (Total arthropods: ANOVA $F_{4,74} = 0.7$, $P = 0.61$; Natural enemies: ANOVA $F_{4,74} = 0.2$, $P = 0.93$). However, variety did appear to influence some individual taxa of natural enemies. For example, spiders were significantly influenced across the season by variety (ANOVA $F_{4,74} = 4.4$, $P = 0.01$) with the variety Viking O-2265 harboring twice as many spiders as the other monocultures or the three- and six-line mixtures. Microhymenoptera (i.e., small parasitic wasps) were marginally influenced by the treatments with microhymenoptera being slightly more than two times as abundant in plots with Blue River 35A0cnv than the six-line mixtures with the other treatments being statistically intermediate (ANOVA $F_{4,74} = 2.0$, $P = 0.10$). Damsel bugs (bugs in the family Nabidae) were also marginally influenced by variety with plots planted with

Viking O-2265 having five times more small parasitic wasps than Blue River 35A0cnv with the other treatments being intermediate (ANOVA $F_{4,74} = 2.0$, $P = 0.10$).

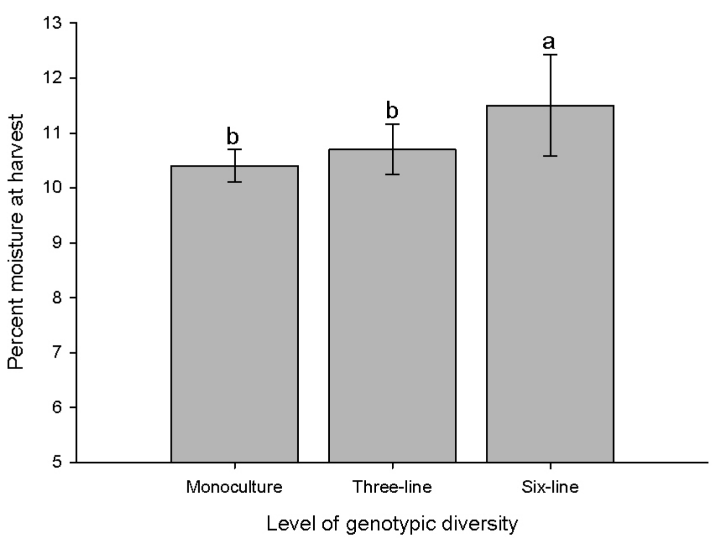
When we considered individual sampling dates, however, a signal of the influence of higher genotypic diversity emerged. Early in the season (June), nabids were significantly more abundant in genotypically diverse plots than in any of the monocultures (ANOVA $F_{4,24} = 4.2$, $P = 0.02$). Nabids, which are very effective aphid predators, were most abundant in six-line plots and Viking O-2265 plots, which were greater than the other three treatments. Late in the season (September), spiders were statistically most abundant in three-line plots and plots with the monoculture Viking O-2265 (ANOVA $F_{4,74} = 3.4$, $P = 0.035$).

3. Improve crop yields

During our one season growing soybean, we did not detect an influence of genotypic diversity on soybean yield whether considered across treatments (ANOVA $F_{4,24} = 0.4$, $P = 0.83$) or levels of diversity (monoculture vs. three-line vs. six-line: ANOVA $F_{2,24} = 1.1$, $P = 0.37$; monoculture vs. diverse mixtures: ANOVA $F_{1,24} = 0.38$, $P = 0.55$).

Genotypic diversity did, however, influence soybean moisture at harvest but not via the five treatments, which had approximately similar levels of moisture (ANOVA $F_{4,24} = 2.0$, $P = 0.15$). We found that six-line mixtures had significantly higher moisture content than three-line and single-line plots (Fig. 4; ANOVA $F_{2,24} = 8.3$, $P = 0.008$). Similarly, when diverse plots regardless of the number of lines were compared to monocultures, diverse plots had significantly higher levels of moisture at harvest (ANOVA $F_{1,24} = 5.9$, $P = 0.03$).

Fig. 4. Percent moisture in soybean monocultures, three-line and six-line mixtures. Six-line mixtures had significantly higher moisture content at harvest than the other two treatments. Treatments labeled with different letters are significantly different from each other ($P < 0.05$). See text for statistical details.



Wheat

1. Decrease abundance of herbivorous insect pests in crops fields

   As mentioned above, our wheat plots were very poorly colonized by insect pests or natural enemies. Not surprisingly, then, we found no detectable influence of treatment or levels of genotypic diversity on the abundance of arthropod pests.

2. Increase natural-enemy diversity and abundance in crop fields

   Similar to our results with soybeans, our vacuum sampling revealed that genotypic diversity did not significantly alter the total number of arthropods nor the abundance of natural enemies found over the course of the season across the three different monoculture varieties, the three-line mix, and the six-line mix (total arthropods: ANOVA $F_{4,59} = 0.16$, $P = 0.96$; natural enemies: ANOVA $F_{4,59} = 0.27$, $P = 0.89$).

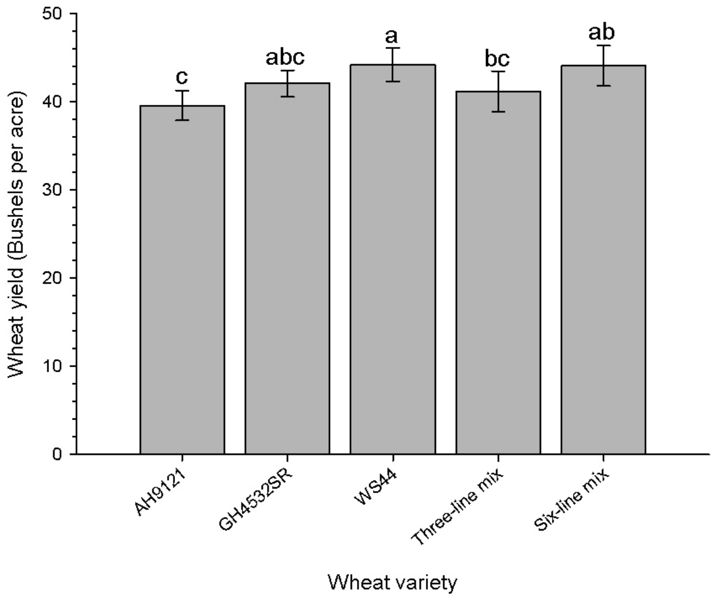
   Similarly, the two levels of genotypic diversity were statistically similar to the monoculture whether considered separately (monoculture vs. three-line vs. six-line: ANOVA $F_{2,59} = 0.27$, $P = 0.76$) or together (monoculture vs. diverse mixtures: ANOVA $F_{1,59} = 0.10$, $P = 0.76$). Even when considered separately, the different sampling dates failed to reveal any groups of natural enemies that were significantly influenced by genotypic diversity.

3. Improve crop yields

   From our one growing season with winter wheat, our five treatments influenced wheat yields (ANOVA $F_{4,29} = 4.0$ $P = 0.016$), but this treatment effect did not appear to be strictly driven by levels of genetic diversity. Monoculture WS44 and the six-line mixtures had equivalent yields, which were significantly higher than monoculture AH9121 (Fig. 5). Importantly, mixtures, particularly the six-line mix, yielded as well as the highest yielding monoculture, indicating that the mixture did not impose a yield cost and the lower yielding constituent varieties maintained yield in the mixtures. Yield was not influenced by level of genotypic diversity (monoculture vs. three-line vs. six-line: ANOVA $F_{2,29} = 1.8$, $P = 0.21$) or the presence or absence of genotypic diversity (monoculture vs. diverse mixtures: ANOVA $F_{1,29} = 0.33$, $P = 0.58$).

   Percent moisture was not significantly influenced by treatment (ANOVA $F_{4,29} = 0.69$ $P = 0.61$), level of diversity (monoculture vs. three-line vs. six-line: ANOVA $F_{2,29} = 0.49$, $P = 0.63$) or the presence or absence of genotypic diversity (monoculture vs. diverse mixtures: ANOVA $F_{1,29} = 0.71$, $P = 0.41$).

Fig. 5. Wheat yield for monocultures, three-line and six-line mixtures. Six-line mixtures had yields that were statistically equivalent to the highest yield varieties. Treatments labeled with different letters are significantly different from each other ($P < 0.05$). See text for statistical details.



6. Conclusions and Discussion

Our results indicate that genotypic diversity holds good potential to moderate insect populations in grain production. Our soybean experiments clearly show that genotypic diverse plots harbored lower aphid populations whether analyzed by treatment, level of genotypic diversity, or the presence or absence of genotypic diversity. Importantly, these moderated aphid populations appear to be driven largely by plant-mediated, or bottom-up, effects rather than natural-enemy mediated, or top-down, effects because our arthropod sampling detected only taxa-specific difference among treatments rather than substantial differences in total arthropods or natural-enemy populations across treatments or levels of diversity. And while higher populations of spiders and damsel bugs in some of the genotypically diverse plots may have contributed to lower aphid populations, it seems more likely that host-plant resistance contributed more significantly to our results. Had taxa of natural enemies played a large role in moderating aphid populations, the statistical signature of their influence would have been stronger and more consistent across the higher levels of genotypic diversity. Therefore, it appears that the genotypically diverse soybean plots were better able to defend themselves against the build-up of aphid population via enhanced host-plant resistance. Importantly, the genotypically diverse three-line and six-line mixtures had aphid populations that were as low or lower than the aphid-resistant monoculture, suggesting that a synergy among cultivars developed when they were mixed together within a plot, driving aphid populations lower than would be expected based only on the aphid populations that developed on the constituent varieties grown in monoculture. The mechanism behind this apparent synergy is unknown but research in our laboratory is exploring various possibilities including the influence of inter-varietal interactions on host-plant defenses.

Our results are somewhat unexpected because we detected a strong effect of genotypic diversity on herbivores (i.e., aphids) at low population levels. In contrast, some of the only previous research addressing genotypic diversity and its effects on populations of insect herbivores found that the influence of genotypic diversity was strongest when insect herbivore populations were high, but weak when pest populations were low (Power 1988, 1991). Therefore, our work with soybean provides evidence that genotypic diversity can influence herbivore populations over a range of population sizes.

While our research found support for the influence of genotypic diversity in moderating insect pest populations, it failed to detect a significant influence on yield in either soybeans or wheat. This was somewhat surprising because previous research had detected a 5-11% yield increase for cultivar mixtures of soybeans and wheat (Schutz and Brim 1967, Brim and Schutz 1968, Schweitzer et al. 1986, Smithson and Lenne 1996). This lack of difference likely reflects a limitation of conducting a single-year experiment. One of the benefits of cultivar mixtures is their stabilizing influence on yields; that is, large year to year swings in yield are dampened by having multiple varieties mixed together in a field. In some years, certain varieties perform well while in other years, other varieties perform well. Therefore, to truly test the influence of cultivar mixtures on yield, we would need to repeat our experiment with the same varieties over multiple years to expose treatments to different growing conditions. Our laboratory has plans for large-scale experiments to pursue just this sort of long-term research.

While our research did not detect a significant influence of genotypic diversity on yield, it did reveal that genotypically diverse soybean mixtures had significantly higher moisture content than the monoculture constituents. This finding is important because it suggests that cultivar mixtures must remain in the field longer than monocultures to allow the constituents a better chance to dry down further and generate a more uniform crop. While this detail might not be that important for dairymen that roast their soybeans prior to feeding them to livestock, it is still a detail that needs to be considered if growers are going to adopt this approach to grain production.

In contrast to our successful work with populations of insect herbivores in soybeans, our experiment with wheat provided equivocal results. It is difficult to draw substantial conclusions about the influence of genotypic diversity in wheat because arthropod pest, and corresponding natural enemy, populations simply did not develop. Such a lack of herbivorous insect pests further supports the notion that complicated ecological field experiments should be conducted over a series of years and conditions to truly evaluate their potential. (Along these lines, we have had similar experiments with wheat in progress to evaluate the value of genotypic diversity in small grain production. These other experiments, however, are not being conducted in certified organic ground because of space and funding limitations. Nevertheless, the generality of our findings will be applicable to organic grain production.)

A substantial body of research supports the efficacy and value of genotypic diversity in small grain production for managing plant pathogens and generally improving productivity (Smithson and Lenne 1996, Mundt 2002); therefore, we are encouraged that our future research efforts with cultivar mixtures of wheat will be productive.

7. Outreach

Our experiments with genotypic diversity were featured on three organic research tours sponsored by The Pennsylvania State University and held at the Russell E. Larson Agricultural Research Center in Rock Springs, PA that exposed the ideas to close to 70 organic growers and community members. Our ideas on the value of genotypic diversity in crop production were also a featured topic at a winter agronomy meeting in Adams County, Pennsylvania that was attended by approximately 100 growers. Lastly, our work with genotypic diversity will culminate in an invited 80-min workshop for attendees of the Annual Meeting of the Pennsylvania Association for Sustainable Agriculture (PASA) held in February in University Park, PA. This meeting annually attracts thousands of individuals interested in sustainable agriculture and organic production.

8. References

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