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DEPARTMENT OF ENTOMOLOGY

The Role of Root-Mediated Kin Recognition in a Genotypically Diverse "Neighborhood" in *Triticum aestivum* Defense Against *Rhopalosiphum padi* Herbivory

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ABSTRACT

Many studies have focused on the effects of local or "neighborhood" plant species diversity on herbivory, but few studies have considered the effects of population genotypic diversity within a major agricultural crop species, such as wheat. The herbivorous insect of interest in this study is the bird cherry-oat aphid, *Rhopalosiphum padi*. Based on previous research, we know that local genotypic diversity in *Triticum aestivum* wheat plants decreases the reproductive performance of bird cherry-oat aphids (Grettenberger and Tooker, 2016). However, the effect on herbivory of root exudates, as a form of plant-to-plant communication, is unknown.

Although little is known about root communication's influence on herbivory, researchers have shown that in the presence of roots from close relatives, plants tend to reduce root growth, hence minimizing competition with kin. In contrast, in the presence of unrelated plants from the same population, some plant species have been found to increase root growth, thereby minimizing shared access to resources. The hypothesis of this study is that if root-mediated plant kin recognition is prevented, then there will be no significant difference between aphid performance in the more genotypically diverse wheat "neighborhoods" and the minimally genotypically diverse wheat neighborhoods. I compared wheat biomass data, aphid offspring mass, and aphid tibia length in the presence and absence of aphids for different wheat varieties, neighborhood diversity, and with roots isolated or not. I did not find support for my original hypothesis, but I found that both the root treatment and the interaction of the root treatment and the neighborhood diversity resulted in significantly different mean wheat biomass. This result supports root interaction as a significant factor in plant growth, and that more research is needed

to understand specifically the interaction between root communication and neighborhood diversity.

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ACKNOWLEDGEMENTS

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Chapter 1

Literature Review

Charles Darwin said, “variation is the grist for the evolutionary mill.” Intraspecific variation is what makes adaption possible, and individuals can gain an advantage by selecting different strategies depending on certain conditions, namely, the genotypic diversity and density of a population. The effects of kin recognition among plants on plant-insect interactions is a relatively new avenue of research, with many recent notable discoveries. This literature review will cover the main body of literature on this topic, first addressing plant kin recognition generally, and then exploring the published results, specifically relating the influence of kin recognition on insect herbivores and pollinators. The significance of this work is broad, with potential applications in conservation, agriculture, and land management in general. A better understanding of plant kin recognition and, the associated role of plant genotypic diversity in plant-plant and plant-insect interactions will benefit future strategies for controlling invasive plants, for integrated pest management, and many other potential applications.

Chapter 1.1 Kin Recognition in Plants

Kin recognition is a phenomenon that has only recently been studied in plants. Much is still unknown, but large strides have been made in the field in the last couple of decades. Kin

recognition in plants is mediated through chemical signals. Chemical communication between plants, for example allelopathy between plants of different species, is well understood.

Kin recognition essentially means the capability of an organism to differentiate between a member of the same species and members of different species, or even between closely- and distantly-related members of the same species. Much of the early kin recognition studies focused on self-recognition, for example self-recognition in pea plants. Different root patterns of growth were observed when two pea plants grown together were detached clones versus separate individuals. An interesting result of this particular study was that the root pattern when the two plants were attached non-clones was intermediate to the other two patterns, which pointed to the existence of kin recognition mechanisms in plants. In *Arabidopsis thaliana*, root growth differed when individuals were switched into growth media that had previously held a plant of the same species, compared to a plant of a different species. Individuals that were kept in their own growth media exhibited a third phenotype, indicating that self-recognition produced different phenotypes. When researchers added a chemical that inhibited root secretion, the differences in phenotypes between the different treatments disappeared showing that root secretions are part of kin recognition (Biedrzycki and Bais, 2010). Root exudates are produced in large quantities by plants. The structure of these chemicals can be altered based on environmental cues. The rhizosphere, including the roots of neighboring plants and the soil microbiome, receives information in the form of these chemical signals (Sharifi and Ryu, 2021).

The chemicals (-)-loliolide and jasmonic acid have been detected in root secretions from numerous plant species and have been found to stimulate allelopathy in neighboring wheat, a result of kin non-recognition (Kong et al. 2018). In the presence of roots from close relatives, plants tend to reduce root growth, hence minimizing competition with related plants (i.e. kin). In

contrast, in the presence of unrelated plants from the same population, some plant species have been found to increase root growth, thereby minimizing shared access to resources. Economically significant crops in which kin recognition has been observed include rice, corn, and soybean. Kin recognition in cereal crops usually manifests in greater cooperation between individuals, resulting in better performance and yield. However, the relationship between the degree of kinship and the level of cooperation is not always straightforward. Wang N. et al. (2020) found allantoin, which stimulates root growth, to be actively secreted by the roots of rice plants only when neighbors were distantly related. When neighbors were closely related, plants released less allantoin. Again, a root secretion inhibitor removed this effect (Wang N. et al. 2020).

Triphysaria versicolor is a parasitic plant that is found on the roots of a diverse range of hosts. When *Triphysaria* roots encounter the roots of a non-kin (host) plant, haustorium development occurs. Haustoria are round root swellings with localized trichomes. Physical contact is not necessary, suggesting that root secretions by the host plant stimulate this response. Kin recognition was observed, in that when *Triphysaria* roots encounter the roots of another *Tryphysaria* plant, haustorium development rarely occurs. Through further experimentation, the authors found that this kin recognition is directly associated with a lack of active haustorium inducing factors in the root secretions of *Tryphysaria* (Wang Y. et al. 2020).

Chapter 1.2 Plant Kin Recognition and Insects

The full extent to which plant kin recognition affects insects still remains to be explored. However, it seems clear that kin recognition should be considered in plant-insect

interactions, most notably for specialist insect species (Glinwood et al. 2020). Dr. Richard Karban has published a great deal of work pioneering this field. Two notable discoveries made by his research group are that sagebrush plants exposed to airborne chemical cues from genetically identical cuttings had greater resistance to herbivory, measured by extent of insect feeding, compared to plants that received airborne cues from non-genetically identical cuttings. No physical contact of roots (or plant parts) was necessary to induce this effect. This research was the first observation of kin recognition influencing plant defense against herbivory (Karban and Shiojiri, 2009). Further work with sagebrush found that the more closely related two individuals were, the more effective the kin recognition-triggered response was at deterring herbivores. (Karban et al., 2013).

In a two-year study looking at common evening primrose, insect attack was quantified as a function of genotypic diversity and density. The authors predicted that herbivory would increase with plant density, but decrease with genotypic diversity, and their results confirmed this hypothesis (Cook-Patton et al., 2016). Similarly, genotypic diversity in wheat plants decreased the performance of aphids, and this effect was exaggerated in the presence of drought stress (Grettenberger and Tooker, 2016). The current literature supports the conclusion that increased genotypic diversity in a plant community increases resistance to herbivory, but also that plants are more resistant to herbivory when exposed to volatiles from a closely related plant compared to a distantly related plant. Further research is needed to understand these seemingly contradictory results.

Research has also explored kin recognition and its effect on herbivory in the context of two wholly different species. Greenhouse work found that *Centaurea maculosa,* an invasive weed, responded differently to experimentally applied methyl jasmonate depending on the

identity of its neighboring plant species. When paired with an individual of the same species, the production of phenolic compounds increased, while when paired with an individual of *Festuca idahoensis* more resources were allocated towards growth. Both methyl jasmonate and phenolic compounds are secondary metabolites related to plant defense. These results were confirmed in field studies of naturally occurring homogeneous and heterogeneous patches of *C. maculosa* and *Festuca idahoensis*. Further field work found a positive relationship between root herbivory from specialist insects and density of *C. maculosa* (Broz et al., 2010). This demonstrated that the relationship between kin-recognition and herbivory must be explored on two different levels, both intraspecific and interspecific.

More recent work has started to explore the effect of kin recognition on plant-pollinator interactions. Torices et al. found that *M. moricandioides*, a flowering herb, produced larger floral displays when grown with genetically related kin versus random unrelated individuals. This effect was even stronger with high plant density. These larger floral displays are more likely to attract pollinating insects, leading to an increase in plant fitness (Torices et al., 2018).

While much important research in the area of plant kin recognition has been done, a great deal more work is needed to understand how this phenomenon will affect higher trophic levels, beginning with insects. Plant kin recognition affects herbivory, pollination, and most likely, many other plant-insect interactions. Important next steps include further determining the paths of information flow for plant communication and recognition, specifically whether root secretions or volatile compounds are more important for these interactions. Characterization of the specific and possibly different roles that these chemicals serve will clarify understanding of results observed thus far.

Chapter 2

Methods

Chapter 2.1 Study Population and Growing Methods

The methods I followed were based on previous work (Grettenberger & Tooker 2016), but different in small ways. I, for example, only used three of the five *Triticum aestivus* varieties used in prior work. I used these three varieties based on availability of seed and because the varieties represented three different levels of aphid resistance (Grettenberger & Tooker 2016). The wheat varieties that I used were Freedom (susceptible to aphids), GR962 (moderately susceptible to aphids), and Patton (resistant to aphids; Grettenberger & Tooker 2016). I grew these varieties in plastic pots (15-cm diameter) with potting soil (Pro-Mix® BX Mycorrhizae, Premier Horticulture Inc., Quakertown, PA). The two treatments I established were neighborhood diversity, and isolation of the focal plant's roots. Figure 1 shows the factorial experimental design, with two levels for each of two factors, neighborhood diversity and root isolation treatment. I then repeated this design three times, for each of the three differently aphid-susceptible wheat varieties, for a total of twelve different combinations. I added aphids to the focal plants of each of these pots for the experimental batches. The control experiment followed the same 2x2x3 design with no aphid exposure.

To set up the root isolation treatments, I glued a clear plastic tube (4-cm diameter, 12-cm tall) to the base of the pot, filled this tube with soil to the same height as the rest of the soil in the pot, and planted the focal plant inside this tube. To ensure drainage of tubes, I drilled a small

hole in the base of the pots. Treatments without root isolation lacked this barrier to root communication.

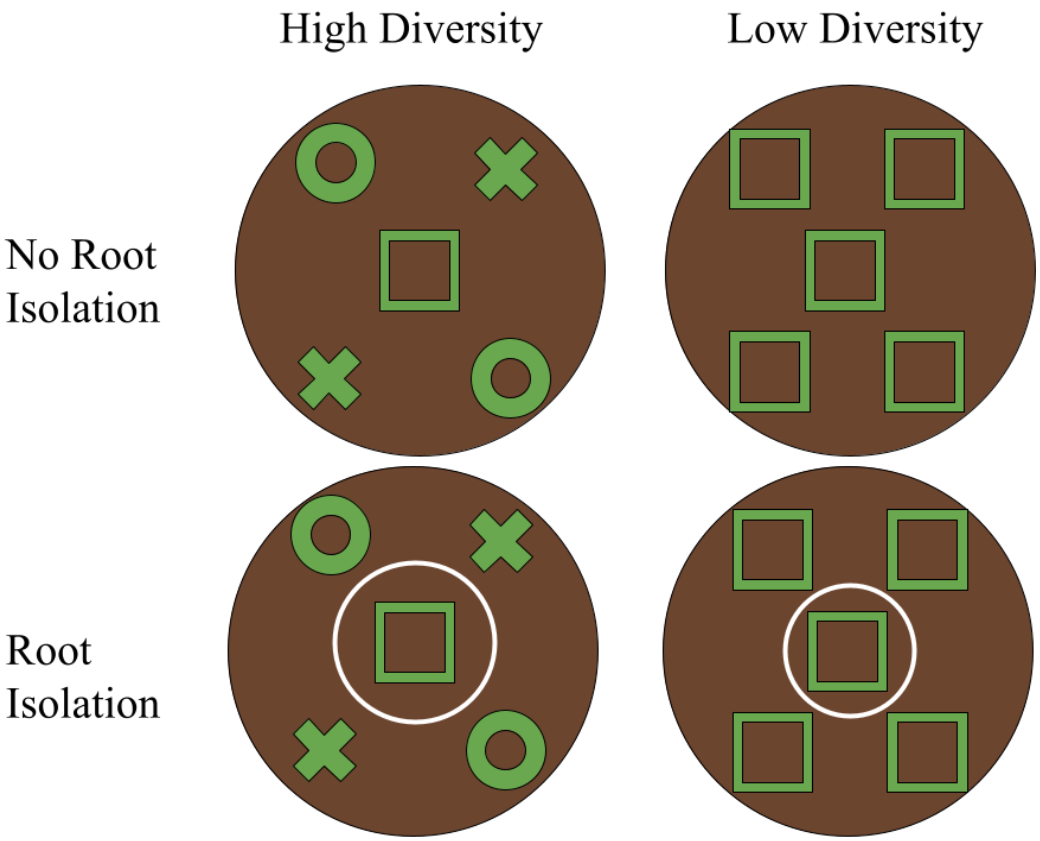

Figure 1. Visualization of Experimental Design. The different symbols represent different wheat varieties, and the white circle represents the root isolation tube. This setup was repeated for each of the three possible focal plant varieties for a total of twelve combinations.

Chapter 2.2 Experimental Design and Procedure

In my experiment, I established all twelve possible combinations of the focal plant variety, the local diversity, and root isolation treatments. For the aphid (experimental) treatments, I performed twelve replicates with six replicates for the no aphid (control) treatments. I made this decision due to time and space limitations and the assumption that there would be less variation in the control groups. The total number of pots in the entire experiment was 216, with 72 pots per focal plant wheat variety.

All pots grew in a greenhouse inside of mesh cages (90 x 60 x 60 cm “Bug dorms”; “Popup Cage with Vinyl Window” from raisingbutterflies.org) to prevent interference from pests, like mice, thrips, and mites. Due to limited space, the pots grew in three batches of 72 pots each. The first batch included all of the aphid-free plants, and the second two batches included all of the aphid-treated plants. I made a small change in procedure after the first (aphid-free) batch. In this first batch, I filled the focal plant tubes with soil up to the level of the surrounding soil. However, this may have created a local warming effect at the top of the soil in the tube. To prevent this in future batches, I filled all focal plant tubes up to the top with soil.

Aphid colony growth, selection, and application on the focal plant followed exactly the procedure of Grettenberger & Tooker (2016). I maintained the aphid colony in a separate greenhouse to the greenhouse where the experiment took place, to avoid potential contamination. I cultivated the aphids on a wheat variety that I did not use in the experiment (SW60). To collect aphids for the experiment, I clipped leaves of the aphid colony wheat plant near the base, and left them in a sealed petri dish overnight at room temperature. To select aphids for the focal plants of Batch 2 and 3, I used a microscope and a paintbrush to find the smallest aphids.

I transferred and confined bird cherry-oat aphids, *Rhopalosiphum padi*, to plants using the clip cages, which were made from 5-cm diameter petri dishes, organdy, popsicle sticks, hot glue, and binder clips (Figure 2). I added aphids to the experimental focal plants two weeks after planting. Experiment followed schedule shown in Table 1. For the wheat planted without aphids, I collected the focal plant 22 days after planting. For Batch 2 and Batch 3, I added an aphid to the focal plant after 14 days and collected the focal plant 27 days after planting. I decreased the length of the control experiment for logistical reasons, as I was working under a limited time frame dictated by the shortening daylengths in October and early November. I increased the length of the Batch 2 and Batch 3 experiments to ideally have the aphid confined on the plant for 13-14 days. The more time the aphid is confined on the plant, the clearer and more significant any effects on the plant will be.

Table 1. Experiment Schedule

	Batch 1	Batch 2	Batch 3
Planting date	Sept. 20	Sept. 26	Oct. 13
Prepping date	N/A	Oct. 9	Oct. 26
Aphid placement date	N/A	Oct. 10	Oct. 27
Sample collection date	Oct. 12	Oct. 27	Nov. 9

Figure 2. Clip Cage Setup on Focal Plant, which emerges from soil inside the plastic tube that isolates its roots from surrounding plants.

At the end of each experiment, I collected all aphids inside each clip cage. I identified the mother aphid by size, and placed her into a 1.5mL Eppendorf tube to be stored in a freezer. I took tibia measurements using a microscope with a micrometer as an estimate of body size. I then placed any aphid offspring into a separate pre-weighed 1.5mL Eppendorf tube. I recorded the number of offspring and the mass of the 1.5mL Eppendorf tube with the aphids. The total mass of the offspring is a measure of aphid productivity and acceptability of their host plants. After taking these measurements, I froze these tubes for storage. At the end of the batch period, I uprooted focal plants and washed soil from their roots and placed the plants in coin envelopes to dry. I left the envelopes in the greenhouse to air-dry. After drying, I weighed plants whole.

Chapter 2.3 Methodology of Data Analysis

I conducted statistical analyses using R Markdown. I analyzed the control and the experimental batches separately due to differences in methodology and data (the experimental batches included aphids). I also analyzed Batch 2 and Batch 3 separately due to differences in overall data distribution. I tested the effects of neighborhood diversity (low or high), root treatment, and focal variety identity on offspring mass, tibia length, and focal plant mass using analysis of variance (ANOVA). I set the threshold for statistical significance at $\alpha = 0.05$. For aphid data, I omitted replicates in which the aphid escaped or died. Percent difference between two means was calculated for diversity as $\frac{\text{Mean}_{\text{High}} - \text{Mean}_{\text{Low}}}{\text{Mean}_{\text{Low}}} \times 100$ and for root treatment as $\frac{\text{Mean}_{\text{T}} - \text{Mean}_{\text{NT}}}{\text{Mean}_{\text{NT}}} \times 100$ (Grettenberger & Tooker 2016).

Chapter 3

Data and Analysis

Chapter 3.1 Results

The main hypothesis of this study was that if root-mediated plant kin recognition is prevented, then there will be no significant difference between aphid performance in the more genotypically diverse wheat "neighborhoods" and the minimally genotypically diverse wheat neighborhoods. Plants in the control group were not exposed to aphids, while plants in Batch 2

and Batch 3 received aphids. In the control group, the mean wheat biomass (Figures 3 and 4 and Table 2) also was changed by -50.86% in the tubed treatment versus the not tubed treatment for all strains ($F = 22.4$, $P < 0.001$). This decrease in biomass suggests that growing the focal plants in a tube limited their growth. Diversity alone did not have a significant effect on the mean focal plant biomass. However, the diversity x root interaction was statistically significant for wheat biomass in the absence of aphids ($F = 11.0$, $P = 0.002$). This means that within the different root treatments, the different diversity treatments had significantly different mean focal plant biomasses. In the control experiment, the difference in mean biomass between high and low diversity was -42.20% for the no tube group versus 70.65% for the tubed group (Figure 5). In the no tube treatment, the high diversity neighborhood decreased focal plant biomass, while in the tubed treatment, the high diversity neighborhood increased biomass.

For Batch 2, the same trends in mean wheat biomass for diversity and root treatment were evident. The mean wheat biomass (Figure 6 and 7 and Table 3) also was changed by -37.63% for the tubed treatment versus the not tubed treatment for all strains in the Batch 2 group ($F = 6.14$, $P = 0.016$). The diversity x root interaction also had a statistically significant effect on wheat biomass in the presence of aphids ($F = 4.73$, $P = 0.034$). In Batch 2, the difference in mean biomass between high and low diversity was 47.35% for the no tube group versus -36.40% for the tubed group (Figure 8). In the no tube treatment, the high diversity neighborhood increased focal plant biomass, while in the tubed treatment, the high diversity neighborhood decreased biomass. This is the opposite result to the control experiment for these variables.

For Batch 3, the majority of individual plant biomasses (roots and shoots) were less than 0.2g, so Batch 3 focal wheat biomass was excluded from my analysis. The loss of wheat

biomass from the control group and Batch 2 to Batch 3 likely can be attributed to the shortening day lengths in October and early November. The greenhouse used for the experiment relies on sunlight. The lamps used to supplement the amount of light on the plants per day do not appear to have been sufficient to completely ameliorate the effect of decreasing daylight as autumn progressed. For this reason, Batch 3 was not included in my analysis of focal wheat plant biomass.

In Batch 2, for the aphid-susceptible Freedom strain, the mean mass of aphid offspring was greater in high diversity and tubed trials. For the moderately susceptible GR962, I observed the opposite; mean offspring mass was greater in low diversity and no tube trials (Figure 9 and 10). For the aphid-resistant Patton strain, the mean offspring mass stayed roughly equal across high and low diversity and was greater in no tube trials. Mean tibia length was higher in the low diversity treatment and the tubed treatment for Freedom and Patton (Figures 13 and 14).

However, statistical significance was not found for either of treatments (roots and diversity) or their interaction, and not all of these results were replicated in Batch 3. In Batch 3, the mean offspring mass was higher in low diversity trials for only the Freedom strain (Figure 11 and 12). For the other two strains, mean offspring mass was lower in the low diversity treatment. The mean offspring mass was higher in the non-tubed Freedom plants and lower in the non-tubed GR962 and Patton plants. Offspring mass (Table 4) was also significantly different between Batch 2 and Batch 3 ($F = 14.4$, $P < 0.001$). For tibia length measurements, no response variable had a significant effect (Table 5 and Figures 15 and 16).

Table 2. ANOVA (analysis of variance) results listing effects of neighborhood diversity, root treatment, and focal variety identity on focal plant mass for the control aphid-free experiment

Response and effect	df	Sum of Squares	Mean Sum of Squares	F	P*
strain	2	0.061	0.030	1.969	0.149
diversity	1	0.030	0.030	1.944	0.169
roots	1	0.344	0.344	22.381	**<0.001**
strain × diversity	2	0.048	0.024	1.561	0.219
strain × roots	2	0.007	0.004	0.228	0.797
diversity × roots	1	0.169	0.169	10.993	**0.002**
strain × diversity × roots	2	0.051	0.025	1.655	0.200
Residuals		0.893	0.015		

* Bold values are significant at α = 0.05

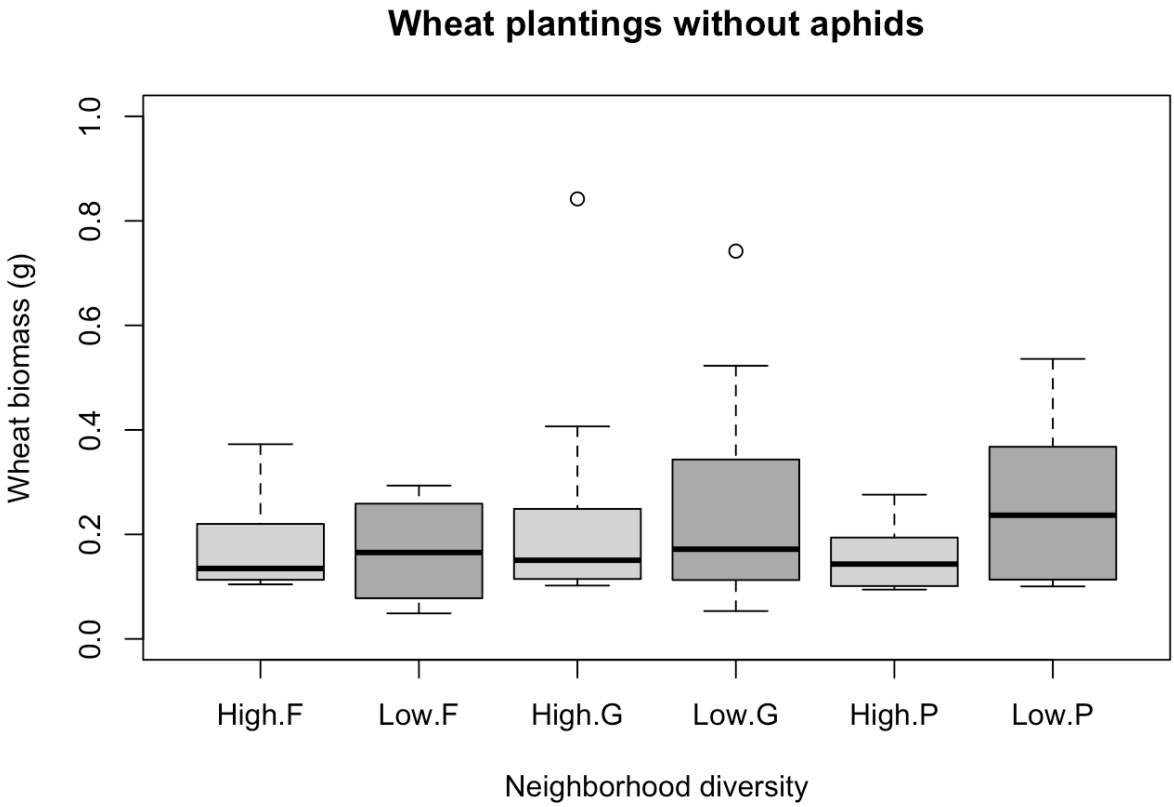

Figure 3. Boxplot of focal wheat plant (*Triticum aestivum*) biomass in grams, for the control (aphid-free) experiment. The x axis is organized by focal wheat variety (F=Freedom, G=GR962, P=Patton) and differentiated by high vs. low diversity treatment. Asterisk indicates significance at $\alpha = 0.05$.

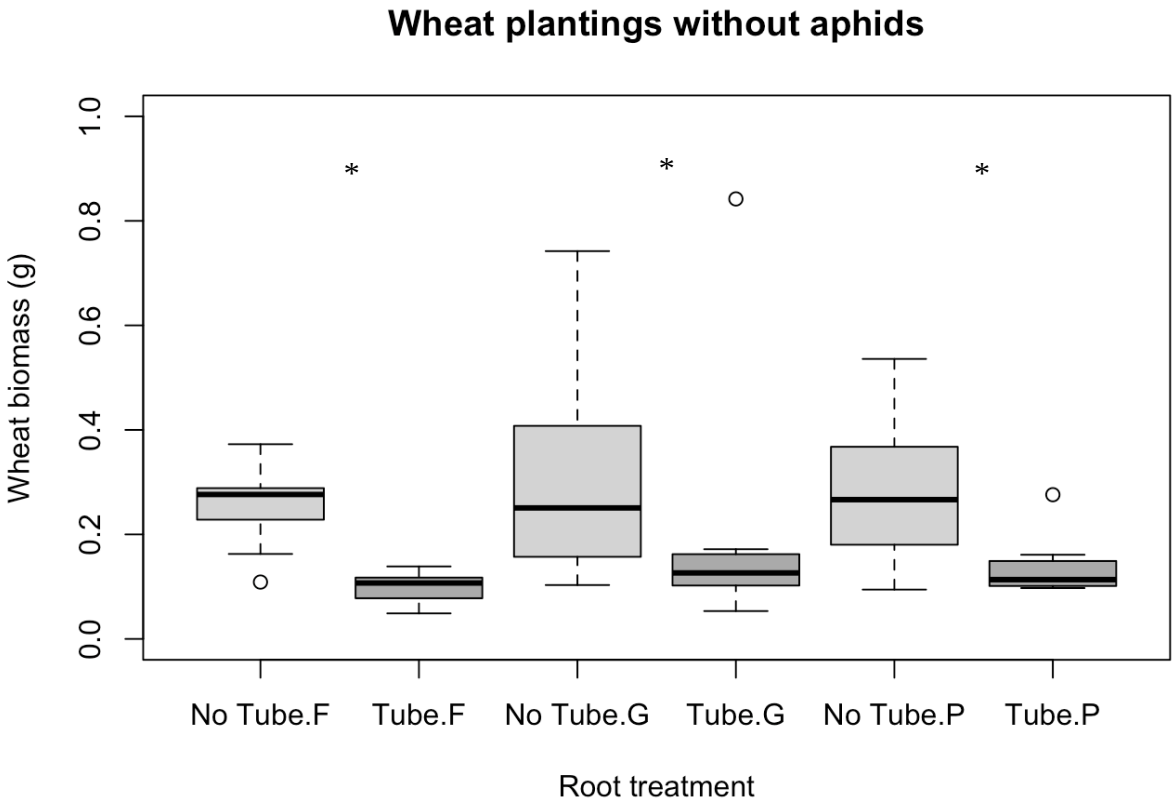

Figure 4. Boxplot of focal wheat plant (*Triticum aestivum*) biomass in grams, for the control (aphid-free) experiment. The x axis is organized by focal wheat variety (F=Freedom, G=GR962, P=Patton) and differentiated by root treatment of the focal plant. Asterisk indicates significance at $\alpha = 0.05$.

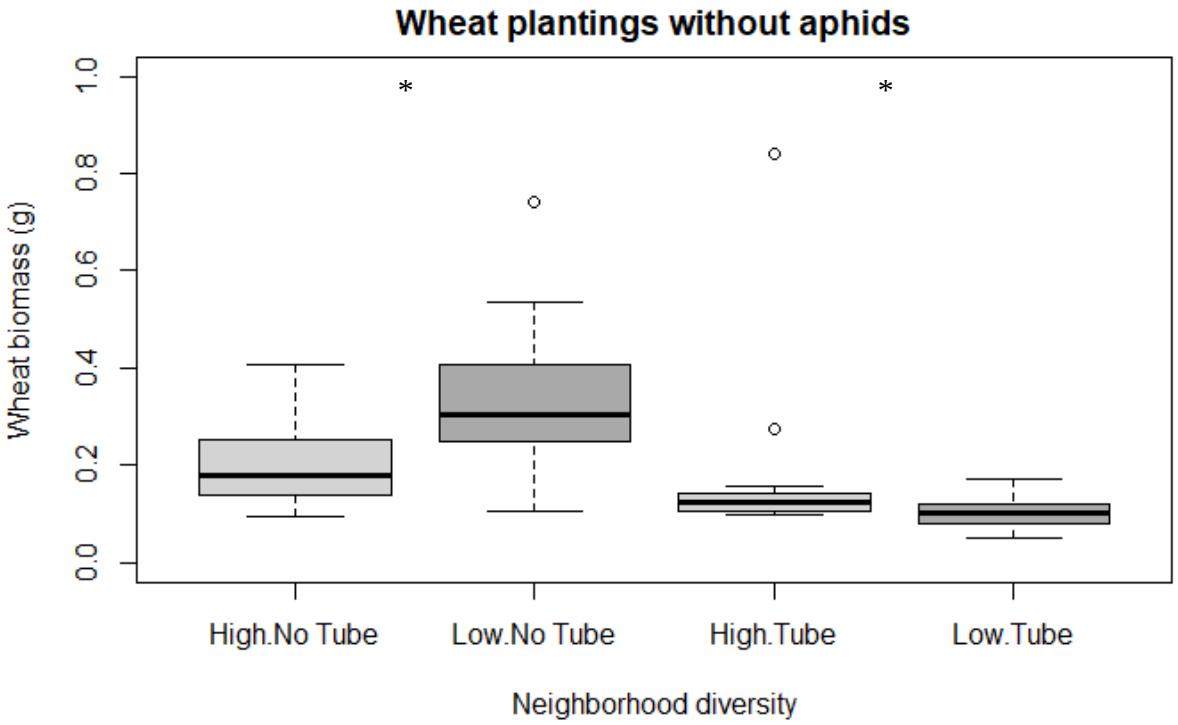

Figure 5. Boxplot of focal wheat plant (Triticum aestivum) biomass in grams, for the control (aphid-free) experiment. The x axis is organized by root treatment and differentiated by diversity treatment. Asterisk indicates significance at α = 0.05.

Table 3. ANOVA (analysis of variance) results listing effects of neighborhood diversity, root treatment, and focal variety identity on focal plant mass for batch 2, the first sequential experiment

Response and effect	df	Sum of Squares	Mean Sum of Squares	F	P*
strain	2	0.140	0.070	2.066	0.136
diversity	1	0.004	0.004	0.120	0.730
roots	1	0.208	0.208	6.137	**0.016**
strain × diversity	2	0.037	0.019	0.550	0.580
strain × roots	2	0.078	0.039	1.152	0.323
diversity × roots	1	0.160	0.160	4.726	**0.034**
strain × diversity × roots	2	0.027	0.014	0.398	0.673
Residuals	60	2.036	0.034		

* Bold values are significant at α = 0.05

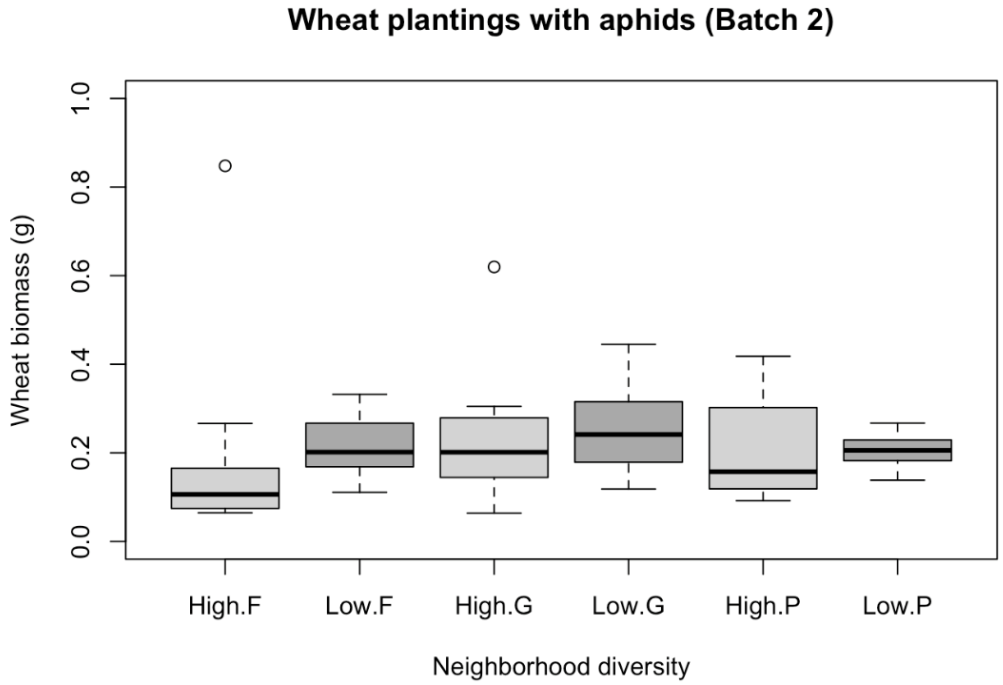

Figure 6. Boxplot of focal wheat plant (*Triticum aestivum*) biomass in grams, for batch 2, the first sequential experiment including aphids. The x axis is organized by focal wheat variety (F=Freedom, G=GR962, P=Patton) and differentiated by high vs. low diversity. Asterisk indicates significance at α = 0.05.

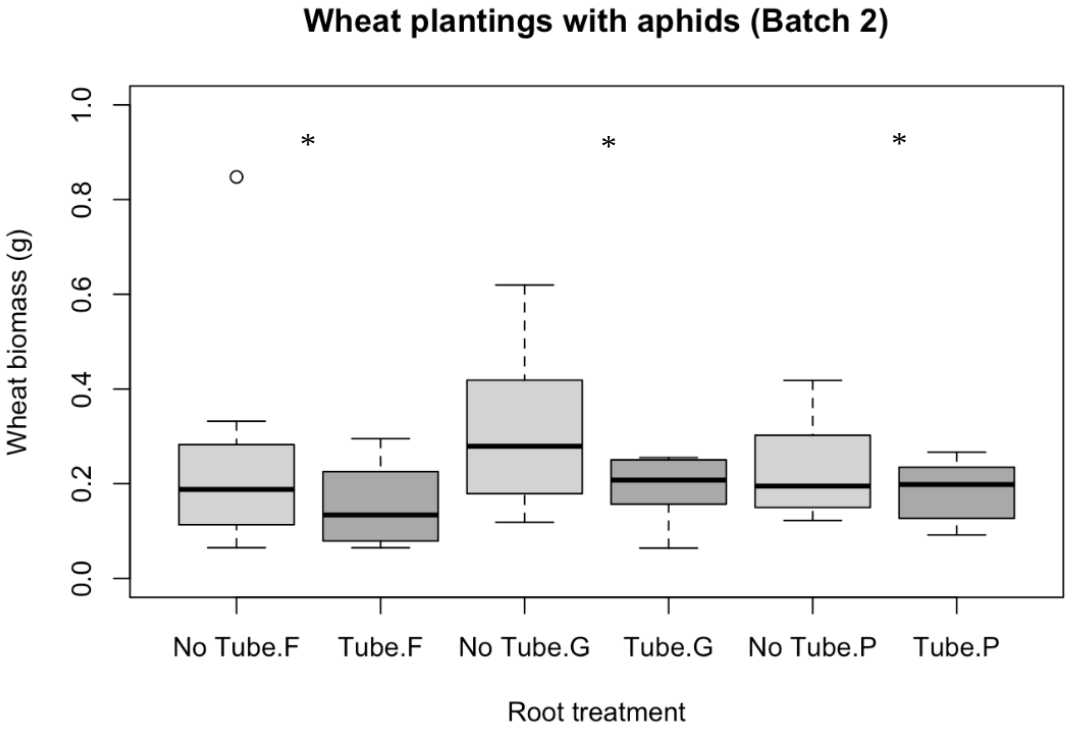

Figure 7. **Boxplot of focal wheat plant (*Triticum aestivum*) biomass in grams, for batch 2, the first sequential experiment including aphids. The x axis is organized by focal wheat variety (F=Freedom, G=GR962, P=Patton) and differentiated by root treatment of the focal plant. Asterisk indicates significance at α = 0.05.**

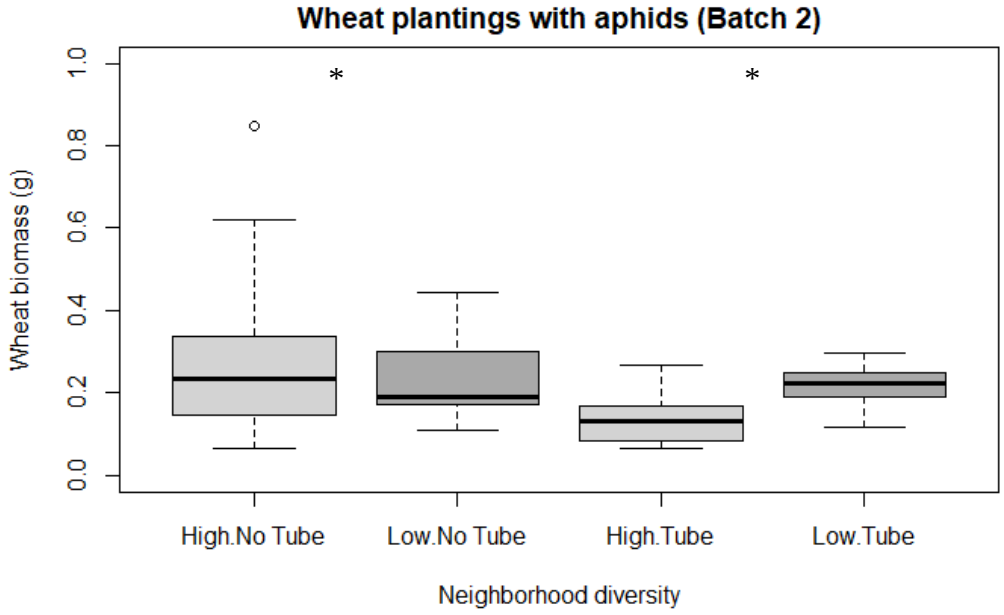

Figure 8. **Boxplot of focal wheat plant (*Triticum aestivum*) biomass in grams, for batch 2, the first sequential experiment including aphids. The x axis is organized by root treatment and differentiated by diversity treatment. Asterisk indicates significance at α = 0.05.**

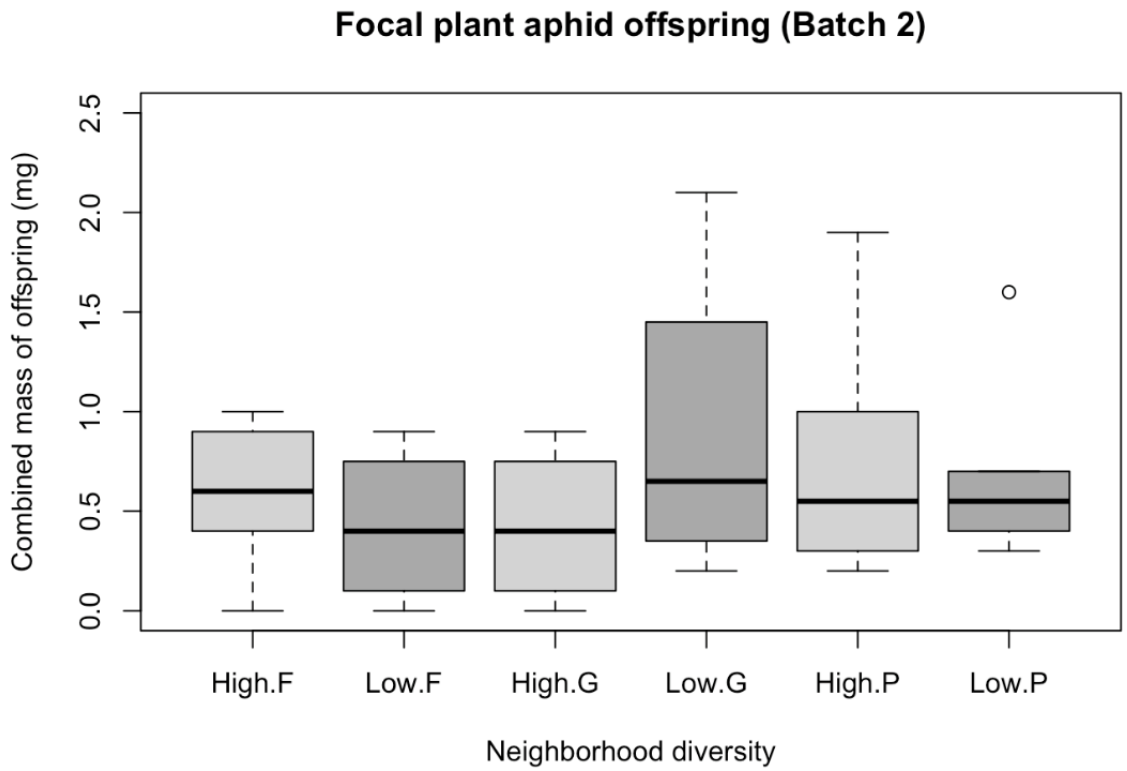

Figure 9. Boxplot of offspring mass of *R. padi* in milligrams for batch 2, the first sequential experiment including aphids. The x axis is organized by focal wheat variety (F=Freedom, G=GR962, P=Patton) and differentiated by high vs. low diversity. Asterisk indicates significance at α = 0.05.

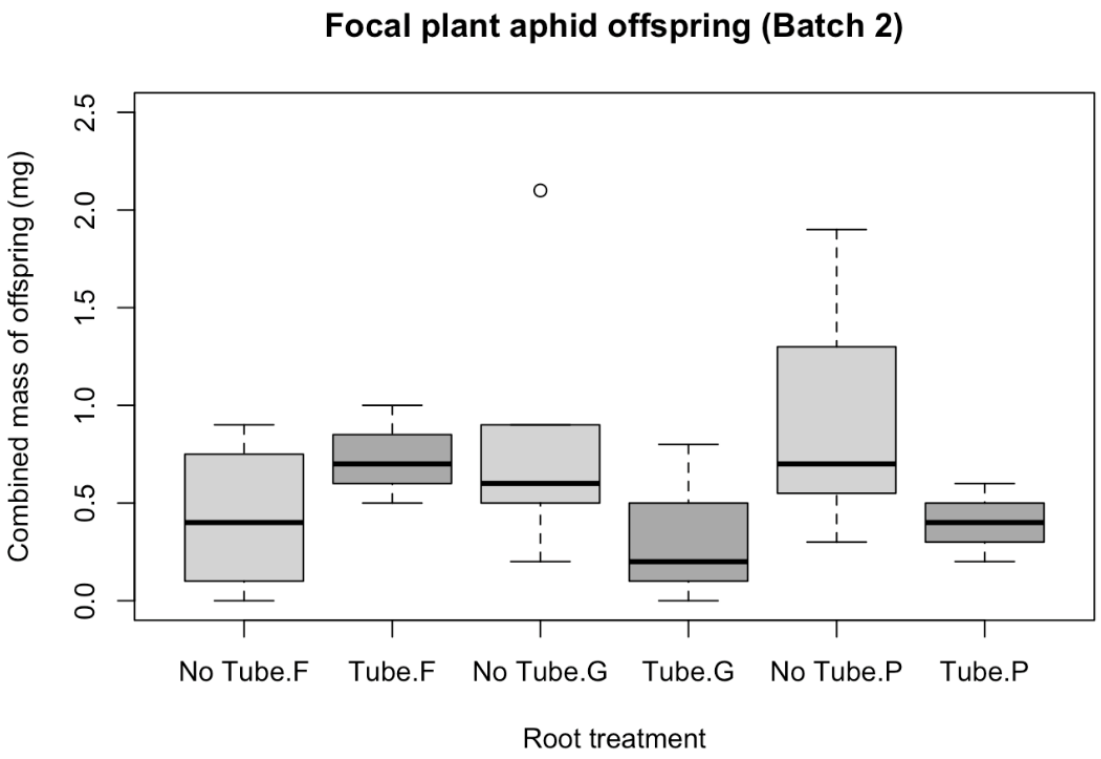

Figure 10. Boxplot of offspring mass of *R. padi* in milligrams for batch 2, the first sequential experiment including aphids. The x axis is organized by focal wheat variety (F=Freedom, G=GR962, P=Patton) and differentiated by root treatment of the focal plant. Asterisk indicates significance at α = 0.05.

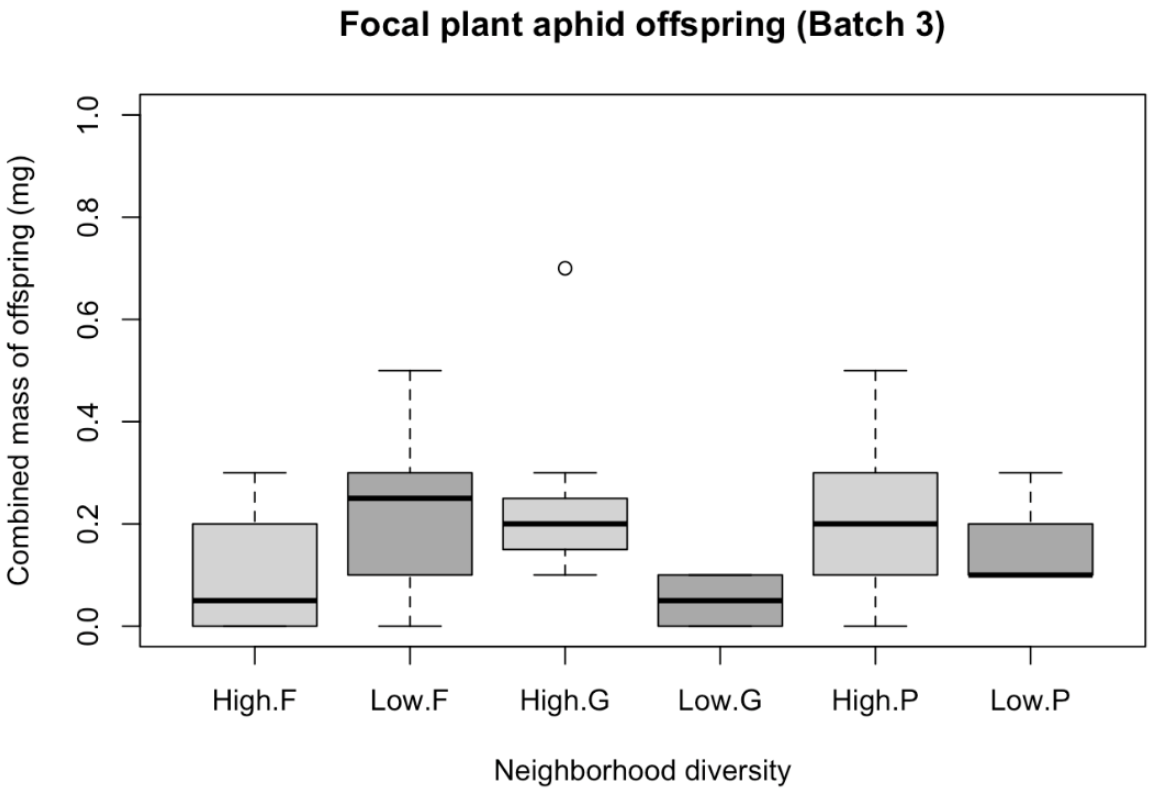

Figure 11. Boxplot of offspring mass of *R. padi* in milligrams for batch 3, the second sequential experiment including aphids. The x axis is organized by focal wheat variety (F=Freedom, G=GR962, P=Patton) and differentiated by high vs. low diversity. Asterisk indicates significance at α = 0.05.

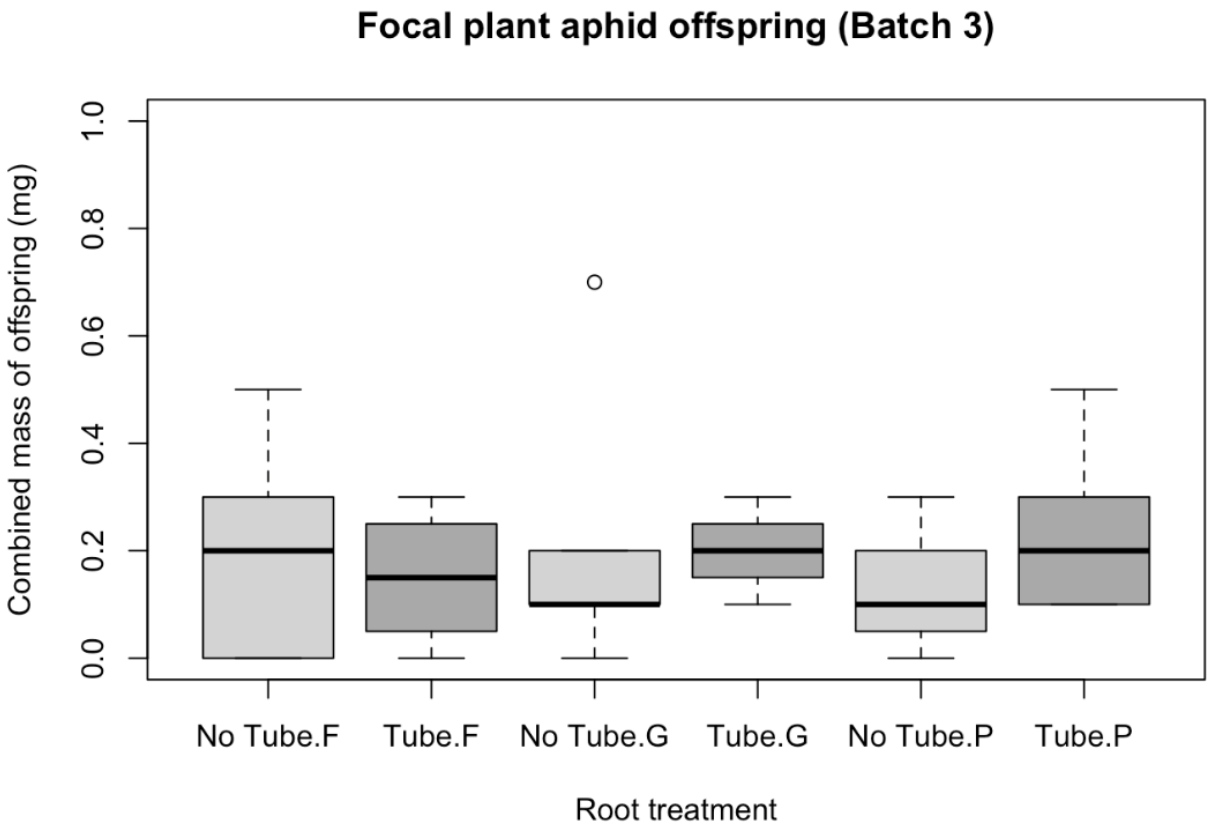

Figure 12. Boxplot of offspring mass of *R. padi* in milligrams for batch 3, the second sequential experiment including aphids. The x axis is organized by focal wheat variety (F=Freedom, G=GR962, P=Patton) and differentiated by root treatment of the focal plant. Asterisk indicates significance at α = 0.05.

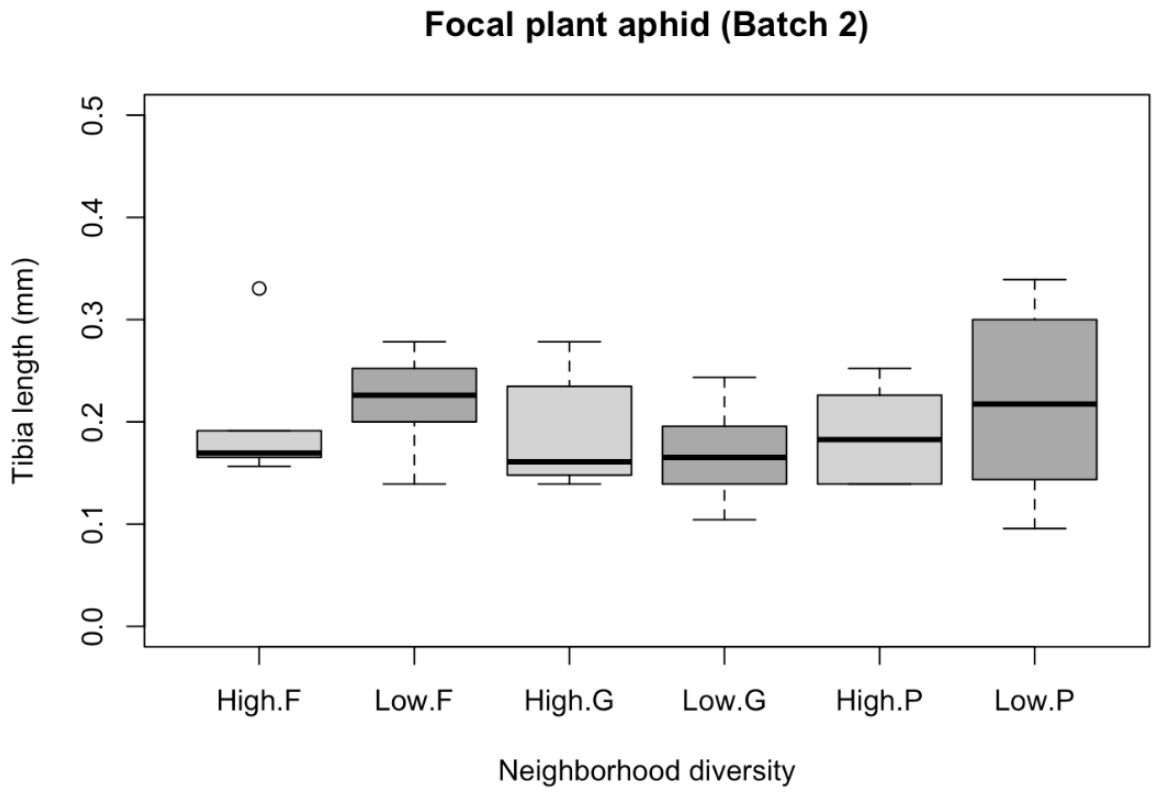

Figure 13. Boxplot of tibia length of *R. padi* in millimeters for batch 2, the first sequential experiment including aphids. The x axis is organized by focal wheat variety (F=Freedom, G=GR962, P=Patton) and differentiated by high vs. low diversity. Asterisk indicates significance at α = 0.05.

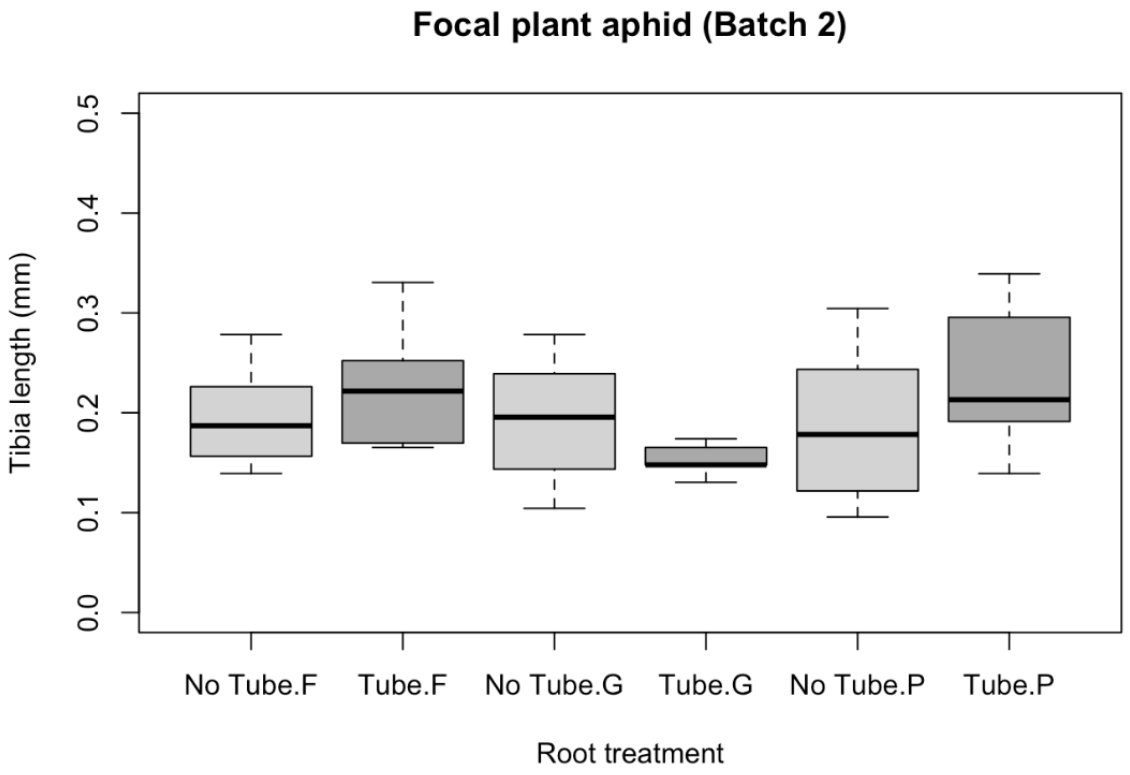

Figure 14. Boxplot of tibia length of *R. padi* in millimeters for batch 2, the first sequential experiment including aphids. The x axis is organized by focal wheat variety (F=Freedom, G=GR962, P=Patton) and differentiated by root treatment of the focal plant. Asterisk indicates significance at $\alpha = 0.05$.

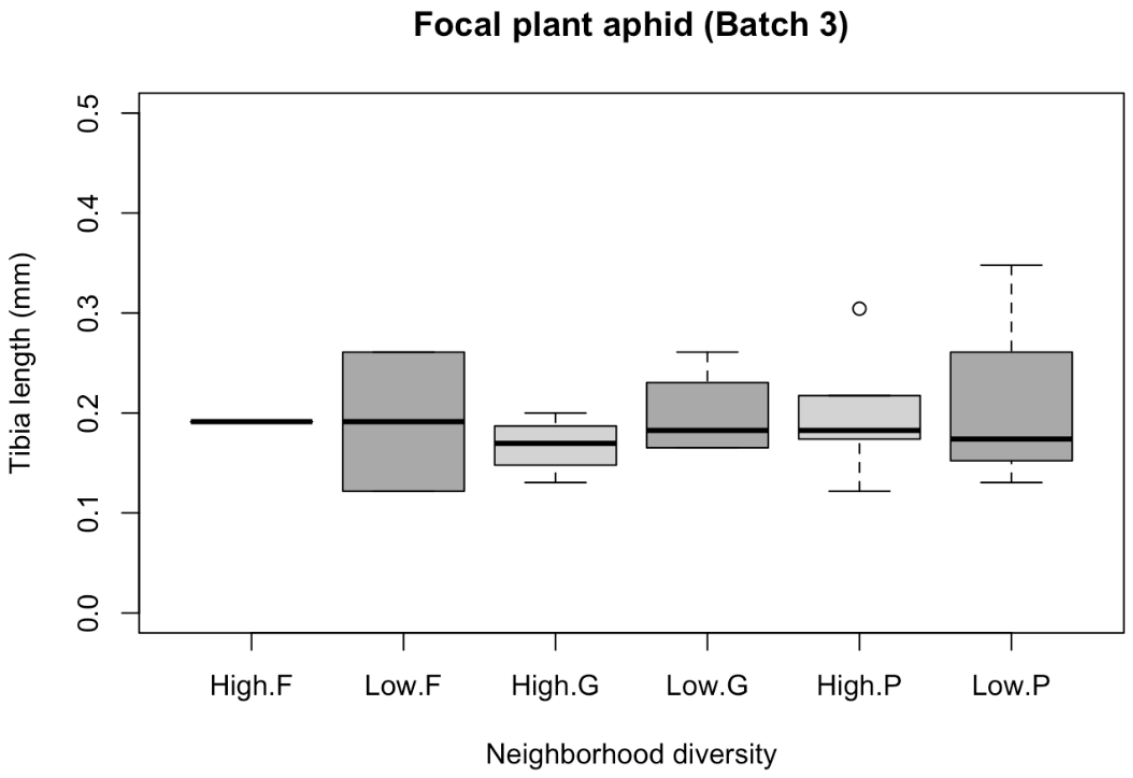

Figure 15. Boxplot of tibia length of *R. padi* in millimeters for batch 3, the second sequential experiment including aphids. The x axis is organized by focal wheat variety (F=Freedom, G=GR962, P=Patton) and differentiated by high vs. low diversity. Asterisk indicates significance at α = 0.05.

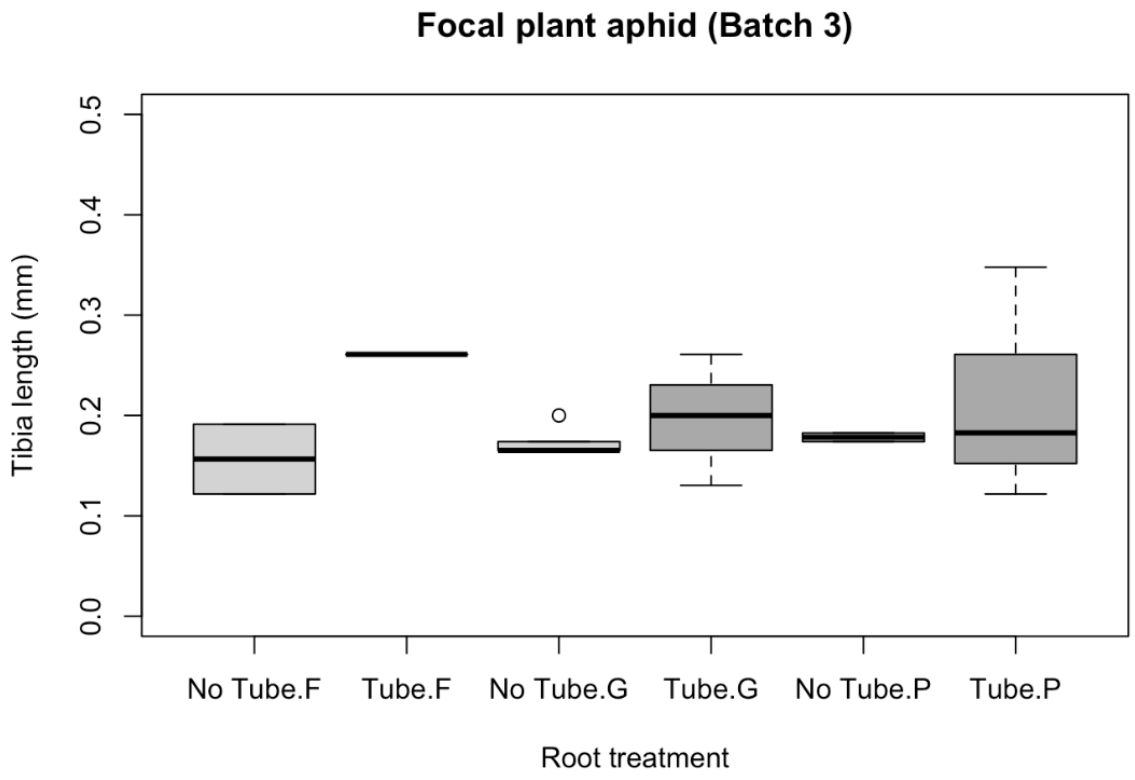

Figure 16. Boxplot of tibia length of *R. padi* in millimeters for batch 3, the second sequential experiment including aphids. The x axis is organized by focal wheat variety (F=Freedom, G=GR962, P=Patton) and differentiated by root treatment of the focal plant. Asterisk indicates significance at $\alpha = 0.05$.

Table 4. ANOVA (analysis of variance) results listing effects of neighborhood diversity, root treatment, and focal variety identity on R. padi offspring mass for batch 2 and batch 3, the experiments including aphids

Response and effect	df	Sum of Squares	Mean Sum of Squares	F	P*
diversity	1	0.035	0.035	0.210	0.649
tube	1	0.410	0.410	2.484	0.124
strain	2	0.357	0.178	1.079	0.351
batch	1	2.379	2.379	14.401	**< 0.001**
diversity × tube	1	0.024	0.024	0.146	0.704
diversity × strain	2	0.087	0.043	0.263	0.770
tube × strain	2	0.456	0.228	1.382	0.265
diversity × batch	1	0.037	0.037	0.225	0.638
tube × batch	1	0.185	0.185	1.117	0.298
strain × batch	2	0.068	0.034	0.205	0.816
diversity × tube × strain	2	0.460	0.230	1.393	0.262
diversity × tube × batch	1	0.000	0.000	0.000	0.991
diversity × strain × batch	2	0.409	0.205	1.239	0.302
tube × strain × batch	2	0.408	0.204	1.236	0.303
Residuals	35	5.781	0.165		

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

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Table 5. ANOVA (analysis of variance) results listing effects of neighborhood diversity, root treatment, and focal variety identity on R. padi tibia length for batch 2 and batch 3, the experiments including aphids

Response and effect	df	Sum of Squares	Mean Sum of Squares	F	P*
diversity	1	0.004	0.004	0.942	0.338
tube	1	0.008	0.008	2.118	0.154
strain	2	0.007	0.004	0.920	0.407
batch	1	0.000	0.000	0.029	0.865
diversity × tube	1	0.007	0.007	1.746	0.194
diversity × strain	2	0.001	0.001	0.173	0.842
tube × strain	2	0.011	0.005	1.371	0.266
diversity × batch	1	0.000	0.000	0.024	0.877
tube × batch	1	0.003	0.003	0.643	0.428
strain × batch	2	0.002	0.001	0.284	0.755
diversity × tube × strain	2	0.002	0.001	0.236	0.791
diversity × tube × batch	1	0.001	0.001	0.243	0.625
diversity × strain × batch	2	0.014	0.007	1.794	0.180
tube × strain × batch	2	0.000	0.000	0.062	0.940
Residuals	39	0.156	0.004		

Chapter 3.2 Discussion

The original hypothesis of this study is that if root-mediated plant kin recognition is prevented, then there will be no significant difference between aphid performance in the more genotypically diverse wheat "neighborhoods" and the minimally genotypically diverse wheat neighborhoods. My results neither confirmed nor disproved this hypothesis, because aphid performance was not significantly affected by any of the variables I manipulated in this experiment. However, my results revealed two interesting findings about plant growth both in the absence and presence of herbivores. The first interesting finding is that introducing a physical barrier between the roots of a focal plant and its neighbors significantly decreased plant

growth, both when an aphid was confined to the focal plant, and also with no aphids. The second is that in both wheat plantings with and without herbivory, the introduction of the tube used in this experiment reversed the direction of the trend in wheat biomass caused by neighborhood diversity. With no tube present in wheat without aphids, the high diversity neighborhood effected a decrease in wheat biomass compared to the low diversity neighborhood. In wheat with aphids with no tube present, the high diversity neighborhood effected an increase in wheat biomass. In both experiments, isolation of the focal plant roots caused the opposite effect to that seen without isolation.

Regarding the first result, three possible explanations could be responsible for decreases in biomass caused by the plastic tubes meant to isolate roots. It is possible that the tubes limited the amount of space that roots had to grow, or possibly the limited amount of soil in the tubes imposed nutrient limitations on focal plants. Alternatively, it seems plausible that the barrier to root communication caused a decrease in biomass. The significant outcome of my experiment is most likely caused by some combination of these three factors. However, to limit the effect of abiotic factors, the tubes were selected to be as large as possible to accommodate focal plant growth without impinging on the other plants in the same pot. In addition, care was taken to keep the tubes filled appropriately with soil, watered properly, and to allow for proper drainage, to minimize the abiotic differences between the environments of the focal plants grown without tubes and the focal plants grown in tubes. Based on these precautionary measures, my results may suggest that if the focal plant's ability to communicate with its environment is limited, then plant growth will be inhibited, regardless of the presence or absence of herbivory. This appears to be true in both genotypically diverse and genotypically homogenous neighborhoods.

For the results regarding diversity and root treatment interaction, the outcome suggests that in the non-tubed and the tubed case, diversity has an effect on wheat biomass. Interestingly the direction of change is reversed in the no aphid experiment as compared to Batch 2 (for the non-tubed treatment, high diversity results in lower biomass in the no aphid case, but higher biomass with aphids). For the tubed treatment, the trend is reversed, since in the tubed case high diversity results in higher biomass in the no aphid case, but lower biomass in the experiment with aphids. More research is needed to understand and confirm these findings, but they suggest that cooperation versus competition strategies in plant kin and non-kin interactions cannot be considered without also considering herbivory.

The plastic barrier of the tube prevented interaction not only between the roots of the focal plant and the roots of its neighbors, but also between the rhizosphere inside the tube and the rhizosphere outside the tube. Not only are root exudates significant in plant-plant interactions, but the soil microbiome also plays a role. Recent work has found that plant-plant communication can be enhanced by introducing fungal networks into the soil between plants (Sharifi and Ryu, 2021).

Biomass was significantly lower in my experiment when roots were not able to interact in both an aphid free experiment (control) and in an experiment with aphids (Batch 2). Previous studies with *T. aestivum* did not find a significant change in root or shoot biomass when wheat was grown with heterospecific neighbors (multiple weed species) compared to wheat grown with only wheat (Kong *et al.* 2018). However Kong *et al.* 2018 only experimented with a single wheat variety. Neighborhood diversity was investigated in Grettenberger and Tooker (2016), but it did not have a significant effect on focal plant mass. Only the interaction between diversity and variety was significant. Grettenberger and Tooker (2016) also studied the varieties Freedom,

GR962, and Patton and did find that variety had a significant effect on focal plant mass. In my experiment with the same varieties, I did not find that variety had a significant effect on focal plant mass. However, the above study only measured shoot biomass while I measured both the shoot and the root biomass together.

The significance of plant population genotypic diversity to plant-insect interactions is an under-researched area. In common evening primrose, researchers found that herbivory increases with greater plant density, but decreases with higher genotypic diversity. (Cook-Patton et al., 2016). In sagebrush, plants exposed to chemical cues from genetically identical cuttings had greater resistance to herbivory, measured by extent of damage, compared to plants that received cues from non-genetically identical cuttings (Karban and Shiojiri, 2009). In allelopathic wheat (*Triticum aestivum*), the production of the allelopathic chemical DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) is density dependent, and present even with conspecific neighbors. This allelochemical production is triggered by the root exudates (-)-loliolide and jasmonic acid (Kong et al. 2018). While my results were inconclusive on this topic, this is an under-explored and important area of research.

The lack of significant results from my *R. padi* data may be due to a shortage of replicate data, so one suggestion for future work would be to repeat this experiment multiple times. I would also like to repeat this experiment in a field setting, as greenhouse results cannot always be replicated in a real-life situation. Wheat is typically grown as a monoculture. The main disadvantages of growing cultivar mixtures of multiple varieties are additional financial and time costs. However, genotypically diverse plant mixtures have been shown in many settings to be more resistant to numerous biotic and abiotic stressors, not only including herbivory, but also disease and drought (Grettenberger and Tooker, 2016). In my experiment, genotypically diverse

wheat mixtures had higher productivity (measured by biomass of the focal plant) than monocultures when an aphid was confined to the focal plant. If this result can be reproduced in the field, then this knowledge could potentially be used to increase grain output and therefore profit margin.

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ACADEMIC VITA

Education:

The Pennsylvania State University, University Park, PA May 2021

Schreyer Honors College

Bachelors of Science in Biology | Marine Sciences, Minor

Millennium Scholars Program, Cohort 5

Research/Teaching Experience:

Dartmouth University Online

Undergraduate Participant, 2020 vASURE Program (June 2020 - Aug. 2020)

- Participated in virtual Dartmouth faculty research talks and graduate student panels as well as casual networking events
- Prepared for the graduate school application process through weekly professional development workshops, assignments, and one-on-one mentoring
- Practiced and presented a lightning talk and a research talk

The Leadership Alliance Online

Undergraduate Participant, 2020 VPD Series (June 2020 - Aug. 2020)

- Attended virtual weekly professional development workshops and engaged with Leadership Alliance program alumni about research and social justice
- Visited Leadership Alliance member institutions at virtual recruitment events

The Pennsylvania State University University Park, PA

Undergraduate Researcher, Tooker Lab (Aug. 2020 - Present)

- Conducting independent research for honors thesis titled "The role of root-mediated plant kin recognition on aphid behavior and performance in genotypically diverse wheat mixtures"
- Becoming proficient in agricultural and entomological research techniques and gaining greenhouse experience

Undergraduate Research Assistant, Iglesias-Prieto Lab (Oct. 2017 - Dec. 2019)

- Developed scientific knowledge and research skills in coral photobiology working part-time during the school year and full-time during the summers
- Abstract for independent research titled "The awakening of a symbiotic coral" accepted for poster presentation at ABRCMS 2019 and SACNAS 2019

Assistant Teaching Assistant, Biology 110 (Aug. 2018 - May 2019)

- Facilitated communication between teaching assistant and students and helped students complete lab tasks in an organized and timely manner

The Penn State Millennium Scholars Program University Park, PA

Peer Tutor (Aug. 2018 – Dec. 2020)

- Help students better understand and retain information in STEM-related classes as well as practice learning and study strategies for success in their academic career
- Invited to help run a "How to be an MSP tutor" training session and serve as a panelist during a Q&A for other scholars in the Spring 2020 semester

Presentation/Outreach Experience:

SACNAS The National Diversity in STEM Conference 2019 Honolulu, Hawai'i

Student Presenter Oct. 31 - Nov. 2, 2019

- Selected to tour UH Manoa and the C-MORE Hale facility with Dr. David Karl

- Presented a scientific poster and developed public speaking and networking skills

Central Pennsylvania Festival of the Arts 2019 State College, PA

Student Presenter, Penn State's Art of Discovery Booth July 13, 2019

- Participated in "'Light Lovers': Animals that capture sunlight for food"

The Great Insect Fair 2019 University Park, PA

Student Presenter Sep. 28, 2019

- Helped present specimens at the "Crustaceans: Insects of the Sea" booth

Leadership Experience:

Undergraduate Research Society University Park, PA

President (Jan. 2020 – Dec. 2020)

- Schedule and run meetings, plan events, present workshops, manage executive board, and invite speakers

Secretary (Sep. 2019 - Dec. 2019)

- Prepared agendas, recorded meeting minutes, sent emails to faculty and club members, and kept a calendar for this entirely student-run organization

Skills/Certifications:

Proficient in Microsoft Office, R, Python, and image processing software (*ImageJ***)**

EPA Agricultural Worker Protection Standard Training Certificate

PADI Open Water Scuba Certification

American Red Cross Adult First Aid/CPR/AED Certification

Awards/Recognition:

Dean's List (*Fall 2017-present***)**

Phi Beta Kappa (*Fall 2020-present)*

DAAD RISE Germany 2020 Scholarship (*program cancelled due to COVID-19***)**

Languages:

German (*intermediate***)**, **Spanish (***beginner***), English (***native***)**