

SPATIAL ANALYSIS OF TERPENES AND APHID ABUNDANCE IN  
*SOLIDAGO ALTISSIMA*

by

Beatrice Ellen Smith

Honors Thesis

Appalachian State University

Submitted to the Department of Biology  
and The Honors College  
in partial fulfillment of the requirements for the degree of

Bachelor of Science

May, 2015

Approved by:

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Ray S. Williams, Ph.D., Thesis Director

---

Kristan M. Cockerill, Ph.D., Second Reader

---

Lynn M. Siefferman, Ph.D., Biology Honors Director

---

Leslie Sargent Jones, Ph.D., Director, The Honors College

## ABSTRACT

This study explores the connection between plant chemistry, plant herbivores, and the environment in which plants are grown. Understanding interspecific, community-level interactions is an essential part of understanding ecosystems as a whole. As native ranges shift with climate change, understanding which spatial and environmental factors may impact host plants and their associated herbivores is essential in order to best prepare for and manage these changes.

Ramets from 5 *Solidago altissima* genotypes were collected, as part of a larger study, from five locations in Watauga County, North Carolina (USA) in 2013. Clonal replicates from each plant were grown in a greenhouse under common soil conditions, and *Uroleucon nigrotuberculatum*, a specialist aphid, was allowed to colonize the plants. Aphid abundance was recorded during the 2014 growing season (June - October). Leaf samples were taken from 3 individuals of each genotype and were tested using gas chromatography (GC) to find the amounts of volatile terpenes. Results were statistically analyzed using SAS version 9.3 and Excel 2010 to explore the relationships among genotype, terpene content, and aphid abundance. Spatial factors from the locations the plants were originally harvested included proximity to streams, land cover type, mean annual precipitation, elevation, and soil type. These data were compiled using ArcGIS 10.2 and were statistically analyzed using Excel 2010.

A statistically significant relationship was found between plant genotype and aphid abundance ( $p=0.046$ ), with aphids strongly preferring genotype number six. One terpene,  $\beta$ -pinene, was found to vary significantly by genotype ( $p=0.03$ ), but the others did not. Although results were not statistically significant, genotype 20 had the highest terpene

content, and genotypes 3 had the lowest terpene content. Environmental factors of the location from which the original ramet was harvested were regressed against both terpene content and aphid abundance. These factors included precipitation, elevation, slope, land cover, hillshade, and proximity to nearest stream or water body. None of the environmental factors were found to significantly effect aphid colonization or terpene content.

This research was part of a larger study in the laboratory of Ray Williams. This data joins a broad body of research on community genetics, community ecology, and plant-insect interactions. Information about population and community dynamics is crucial to the understanding of ecosystems, land management. Understanding how environmental attributes impact species and species interactions may help with predicting and mitigating the effects of climate change and range shifts of organisms.

## DEDICATION

This thesis is dedicated to my parents, who have been my first, and best, teachers. To Scott Smith, who read to me every day and showed me how to be a hard worker, and to Cinda Payne-Smith, who spent every day making sure I had endless opportunities to learn, that I was challenged, and that I believed in myself.

## ACKNOWLEDGMENTS

There are so many people I would like to thank for their part in the formation of my thesis. To Dr. Ray Williams, who let me join his lab as a freshman, I cannot thank you enough for everything I have learned during my four years in your lab. I appreciate your patience, kindness, and calm advice when things go wrong. Thank you for the opportunity to work in your lab and to do my own project has taught me a great deal about biology, the scientific method, and my own abilities.

To Dr. Kristan Cockerill, my second reader, thank you for your enthusiasm, both in this endeavor and as my honors seminar teacher. Your eagerness to learn about my project, your dedication to using the sciences to make our community better, and your ability to challenge your students appropriately have all enriched my college experience.

To Dr. Matt Estep, thank you for your countless hours of patience, even when I made mistakes, for your enthusiasm for teaching and research, and for including me in your lab. Knowing that, even when you were busy and had a dozen other things to do, you would always find time to explain a concept or help me when I got stuck gave me the confidence to work independently, ask questions, and try my hardest on this project.

I would like to thank the Office of Student Research and the Honors College for providing the funding that made my project possible. I would also like to thank the Appalachian State University Alumni Memorial Scholarship, the New River Light and Power Scholarship, and the Lowe's Carl Buchan Scholarship for their generous financial support over the last four years. This funding has made it possible for me to pursue my interests and passions in college, including this independent research.

I have had a wonderful, challenging, and strong advisor for the last four years. Dr. Leslie Seargent- Jones, thank you for encouraging me to pursue “the major of Bess” and for not only answering my questions but for also asking me tough questions.

Additionally, I would like to thank my teachers, both professors and peers, but I learned a great deal from each of you, and I thank you for sharing your subject, your passion, and your time with me. Jessica Howells, Megan Avakian, and Brian Bonville, thank you all for paving the way with your projects, for your kindness, and for your enthusiasm about my project. Brian, thank you again for generously sharing your aphid data with me.

I would especially like to thank Lee Payne, Cinda Payne-Smith, Zoe Liu, Carrie Streeter, and Kelly White for showing me that it is possible to finish a project like this. I saw the process as each of you worked on your theses or dissertations, and I will always look up to you.

To Flip Coulling and the past and present Nature Camp Staff, thank you for your unconditional love and support every summer. You have been there with me every step of the way over the past nine years as I became a scientist, a teacher, and a naturalist.

I have many friends who I would like to thank for studying with me, brainstorming, watching movies, having adventures, and making me take breaks. You have given richness to my college experience, from living in East Hall to playing games to going on ASEs, and I appreciate each one of you.

To my roommate of four years, Miana Breed: getting a random roommate was one of the first choices I made in college, and it was also one of the best. I cannot believe how fast four years has gone! Thank you for putting up with me for so long. Madi, Diane, Meredith,

Katie, and Sarah: I am privileged to have lived with each of you and will miss seeing you every day.

To my family: thank you for your love and support, not only in the last four years, but also in the last 22 years. I love you and hope that you feel you can take some credit in the completion of this thesis and in my graduation. It would not have been possible without you.

To my boyfriend, Chris Denis: thank you for supporting me always, for understanding the biology terminology, and for distracting me when it was time to take breaks. I love you more than words.

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## INTRODUCTION

### *Community Genetics*

Community genetics investigations provide a way to understand both ecosystem processes and evolutionary relationships. Community genetics can be defined as “the study of the dynamic interplay between ecology and evolution among multiple interacting populations” (Hersch-Green et al. 2011) and provides an important direction of research for understanding how ecosystems may react to climate change, invasive species, and landscape changes. Because plants make up the lowest trophic level in many terrestrial ecosystems, changes in plant populations can affect higher trophic levels at the community level (Whitham et al. 2012). For example, an increase in plant species richness has been found to increase the number of insect species in the community (Haddad et al. 2009). This may occur because many herbivorous insect species are specialist feeders; therefore, the higher the host plant diversity, the more insect species are able to survive (Bernays and Graham 1988). It is also likely that an increase in above-ground net primary production will support more insect individuals and a higher number of species: the “more individuals hypothesis” (Srivastava and Lawton 1998). Recent studies have shown that factors aside from plant species diversity, such as intraspecific genetic variation, are important in shaping arthropod communities (Crutsinger et al. 2006).

### *Community Genetics and Solidago altissima*

*Solidago altissima* is a well-known model plant system in community genetics research (Crutsinger et al. 2006, 2008; Genung et al. 2010, 2011b). Known as tall goldenrod, *S. altissima* is found throughout the eastern United States, primarily in old-field habitats (Howells 2012). This plant species can reproduce sexually or clonally (Crutsinger et al.

2008), and its rhizomes are often used in community genetics studies to create clonal replicates. More than 100 insect species have been found to colonize *S. altissima*, making it useful for understanding the effects of intraspecific genetic variation on plant-insect dynamics (Maddox and Root 1987).

#### *Community Interactions between Solidago altissima and Insects*

Community-based connections between plants and insects are both ecologically and evolutionarily important. Many specialist insect species have co-evolved with their plant hosts (such as *S. altissima* and *Uroleucon nigrotuberculatum*), so environmental impacts on one species will indirectly impact the other (Howells 2012), as well as impacting all other trophic levels in an ecosystem (Haddad et al. 2009). More than 130 insect species have been found to associate with *S. altissima* (Root and Cappuccino 1992). Ecosystem processes and functioning may be compromised if a foundation population such as *S. altissima* becomes scarce.

Genotypic differences between *S. altissima* individuals have been found to have effects on phenotype, such as resistance to galling insects. Therefore, genotype may have effects on the type and number of insects feeding on the plants (Crutsinger et al. 2006; Maddox and Root 1987). One recently identified relationship is between terpene content and herbivorous aphids (Williams and Avakian 2015). Communities can be affected by both top-down and bottom-up forcing (caused by changes in either the way insects consume plants or in the way plants affect the insect communities); therefore, a trait such as secondary chemical production (e.g., terpenes) could play an important role in determining community structure (Avakian 2012).

### *Terpenes, Solidago altissima, and Insects*

Because plant chemicals are important to insects (Takahashi and Yamauchi 2010), allelochemicals, such as terpenes, that vary among *S. altissima* genotypes may have broad effects on the insects feeding on them (Williams and Avakian 2015). Terpenes are widely considered to be plant defensive chemicals, and can be used to repel herbivory by generalists (Takahashi and Yamauchi 2010), but they may also be used to attract specialist feeders (Poelman et al. 2010). Many insects, such as *U. nigrotuberculatum* - the red goldenrod aphid, are specialist feeders and can withstand the defensive chemicals found in their associated host plants (Howells 2012). Terpenes may also be used to attract pollinators and to inhibit growth of other nearby plants (Langenheim 1994). Howells (2012) found that terpene content in plants varied based on prior herbivory, and that specialist insects may choose *S. altissima* genotypes based partially on terpene content in leaves.

#### *The Specialist Aphid: Uroleucon nigrotuberculatum*

*U. nigrotuberculatum* is important because it exclusively colonizes plants in Family Asteracea, including *S. altissima* (Cappuccino 1988). This insect has been used to understand diverse aspects of *S. altissima* ecology (Avakian 2012; Howells 2012; Williams and Avakian 2015). This aphid is a phloem-feeding insect that lives on the stems of *S. altissima* in the eastern United States (Cappuccino 1988). Cappuccino (1988) studied the life cycle of *U. nigrotuberculatum* and found that the alates (winged individuals) colonize goldenrod stems and stay relatively stationary throughout the apterate generation, even when exposed to predation or fungal pathogens. These clumped colonies allow better access to mates and herd protection from predation (Hamilton 1971) but also have the adverse effects of encouraging the spread of disease and fungal infection (Cappuccino 1988). Cappuccino (1988) also found

that *U. nigrotuberculatum* exhibits similar behaviors throughout its range, and that the aphid's red color does not seem to deter predators.

Past studies have shown a positive correlation between *S. altissima* terpene concentration and abundance of *U. nigrotuberculatum* (Howells 2012; Williams and Avakian 2015). Population-level effects in communities with *S. altissima* may also impact aphids. Genung et al. (2011a) found that spatial orientation impacted *U. nigrotuberculatum* colonization even more strongly than did differences in plant genotype, although some of that spatial effect seemed to come from genotypic diversity at the study plot level. The relationship between ecological diversity of a given taxa having positive effects on diversity and success of other associated taxa is well known in ecology, and *S. altissima* and *U. nigrotuberculatum* seem to follow this trend (Crutsinger et al. 2008; Genung et al. 2011a; Hersch-Green et al. 2011; Howells 2012; Williams and Avakian 2015). Genetic diversity has been shown across a variety of ecosystems to be protective against both invasive and non-native species (Crutsinger et al. 2008) and pathogens (Schmid 1994). In ecosystems with high diversity of primary producers, higher trophic levels also exhibit greater diversity (Crutsinger et al. 2006; Haddad et al. 2009).

### *Spatial Analysis*

Although *S. altissima* is a widely used species in the fields of community genetics and plant ecology, few studies focus on the environmental factors that best predict its chemical composition or success. It is understood that nutrient dynamics, genotypic diversity in the plant population, and herbivory can all impact goldenrod individuals (Avakian 2012; Genung et al. 2012; Williams and Avakian 2015). It is also possible that genetic distance may be correlated with spatial distance, and that genetic distance may relate to differences in

phenotype, such as terpene content (Azizi et al. 2012). A literature review did not find any studies with a focus on the spatial and environmental predictors of environmental characteristics such as topography, elevation, land cover, or soil type. Perhaps this is because *S. altissima* is such a widely distributed species and can survive in many environments. Understanding how environmental conditions can impact plant chemistry and insect colonization is likely to be useful to future research.

### *Summary*

Despite the large body of research on many of these topics (community genetics, plant-insect interactions, genetic distance, and terpenes), there is very little research connecting genetic distance to terpene content in *Solidago*. Hersch-Green and colleagues (2011) listed understanding the “relative importance of intraspecific genetic variation compared with other ecological factors in affecting the structure and dynamics of communities” as one of the top three areas for future research in community genetics. Originally, this study was designed to explore the question: how does genetic distance between *S. altissima* genotypes affect terpene concentration? This could have important implications for future work with insect-plant interactions studies in old-field ecosystems. However, I was unsuccessful in my exploration of genetic distance using Amplified Fragment Length Polymorphism (AFLP) (see Appendix A), so I shifted my focus to examining the relationships between terpenes, aphid abundance, and the spatial relationships between “parent” plants. As molecular techniques are increasingly incorporated into all aspects of ecology, considering genetic distance alongside other ecological factors is likely to become more useful, user-friendly, and common. In the revised study I asked these questions:

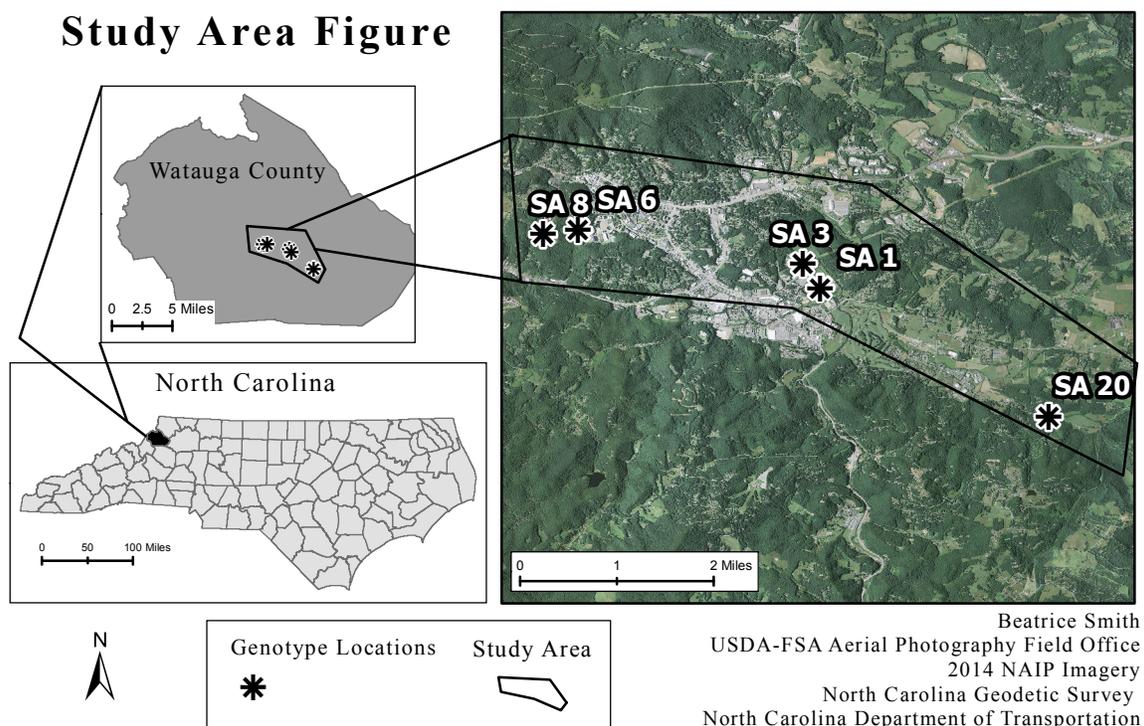
- (1) Are there differences in terpene concentrations among five genotypes of *S. altissima*?
- (2) Are there differences in aphid abundance among the genotypes?
- (3) Are aphid abundance and terpene concentrations related?
- (4) Is there a spatial or environmental pattern associated with aphid abundance and/ or terpene content using the location from which the ramet was originally harvested?

The information gained here joins a large body of research on community ecology, plant-insect interactions, and community genetics.

## MATERIALS AND METHODS

As part of larger investigations in the laboratory of Dr. Ray Williams in the Department of Biology, ramets of *S. altissima* were collected throughout Watauga County, North Carolina in 2013 (Figure 1).

**Figure 1.** Study area showing location of five *S. altissima* collection sites within Watauga County, North Carolina.



Plants provided rhizomes for propagation of individual clones for each of five spatially separated genotypes. Using previously developed techniques in the Williams Laboratory, 3cm of rhizomes were removed from each “parent plant” and grown in a standard soil medium in the Appalachian State University greenhouse in April 2014. The terpene investigation used 3 replicates per genotype for a total of 15 plants. This study used 5 randomly selected samples (genotype numbers 1, 3, 6, 8, and 20) from the larger experiment’s collection of approximately 18 genotypes. In July 2014, 5-6 leaves were

obtained from each plant for both terpene analysis and DNA isolation. For terpenes, leaves were weighed and frozen at  $-20^{\circ}\text{C}$  for later analysis.

As part of a larger study in the Williams laboratory, another set of plants from the same genotypes (three replicates per genotype) were planted outdoors at the ASU Biology Greenhouse in the summer of 2014 in a common garden experiment. Plants were grown in 19L pots in a soil/ sand medium. An abundance of *U. nigrotuberculatum* was observed throughout the growing season (June-October). Total aphid abundance was determined for each plant every three days.

Using modified Johnson et al. (2007) and Williams laboratory protocols, I analyzed each plant sample to quantify the amount of volatile terpenes per gram of leaf mass. Leaf samples were ground in 15 mL of pentane using a Polytron tissue homogenizer. Gravity filtration using filter paper was performed and the resulting liquid evaporated to 0.5 mL using gaseous  $\text{N}_2$ . To analyze the terpenes, I used gas chromatography (GC). A  $1\mu\text{L}$  sample was injected into a Shimadzu 14-A gas chromatograph containing a flame ionization detector and an HP-5 cross-linked 5% PH ME Siloxane column (dimensions: 30 m x 0.25 mm with a  $0.25\mu\text{m}$  film). The following 24 minute GC program was used with an injector temperature of  $250^{\circ}\text{C}$  and a detector temperature of  $250^{\circ}\text{C}$ : start temperature  $80^{\circ}\text{C}$  with a two minute hold, followed by column temperature increase of  $10^{\circ}\text{C}$  per minute to  $280^{\circ}\text{C}$ , then held for 2 minutes (Howells 2012).

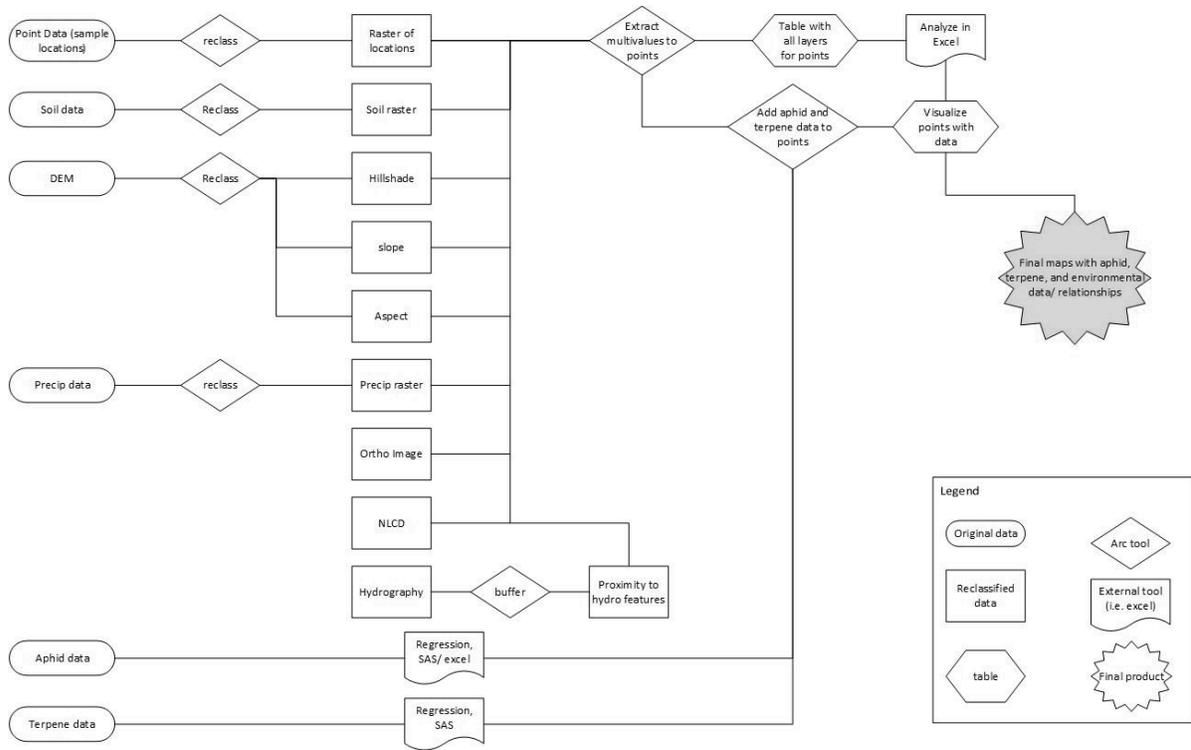
Data were analyzed by calculating the terpene concentration of unknowns using the area of a known quantity of the hydrocarbon tri-decane, following protocols from previous investigations. Unknowns were compared to known terpene retention times using analytical standards and data from GC/mass spectrometry. Differences in aphid abundance and leaf

terpene content among genotypes were analyzed using a general linear model (SAS Institute, version 9.3, Cary, North Carolina). A linear regression was performed to compare aphid abundance and terpene content.

As seen in Figure 1, Genotypes 1 and 3 were collected from the same area, a greenway in Boone, NC with walking trails and managed or mowed fields. Genotypes 6 and 8 were collected from the campus of Appalachian State University from areas that are also managed, but perhaps less intensively. Genotype 20 was collected from an area towards the edge of the city limits, in a more rural, unmanaged area. Terpene and aphid data were analyzed spatially using ArcGIS 10.2 (ESRI) software (Figure 2). The following environmental factors were used: proximity to nearest water body, elevation, precipitation, watershed, land cover, soil type, aspect, hillshade, and slope. After reclassifying vector layers to raster, resulting raster data were compiled using the extract multivalued to points tool. This technique was used to determine environmental factors for each sampling location. Proximity to water features using the buffer tool was also performed. Results were then regressed against terpene content and aphid abundance to explore whether the environment had any effect on plant phenotype (Tables 5 and 6).

Genetic Distance methods can be seen in Appendix A.

**Figure 2.** Spatial analysis flowchart using ArcMap 10.1 showing spatial analysis techniques and layers used.



## RESULTS

### *Terpenes*

Terpene analysis identified six compounds that were consistently scoreable for all samples. Only one terpene,  $\beta$ -pinene, showed statistically significant differences in concentration between genotypes (Table 1). Figures 3-8 show the mean concentrations of the six most common terpenes for each genotype.

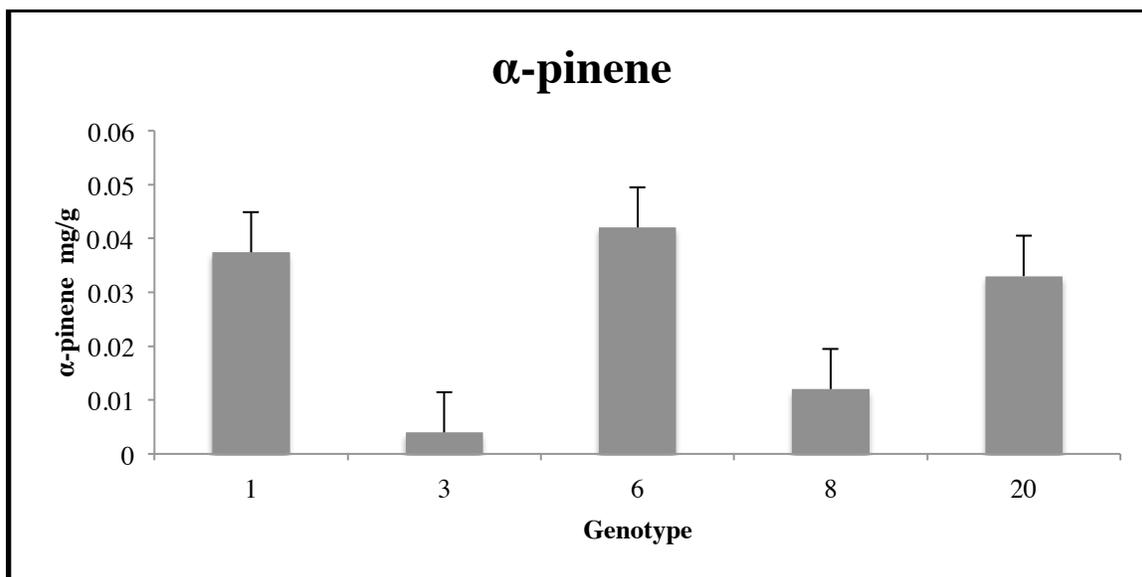
**Table 1.** Analysis of terpenes using Proc GLM SAS, version 9.3.

Terpene	<i>F</i> - statistic	<i>p</i>
$\alpha$ -pinene	2.38	0.13
$\beta$ -pinene	4.45	0.03*
p-cymene	1.66	0.24
bornyl acetate	2.23	0.15
germacrene D	1.15	0.40
sabinene	1.91	0.19
Total	1.13	0.40

*df* = 4,13 \*= $p < 0.05$

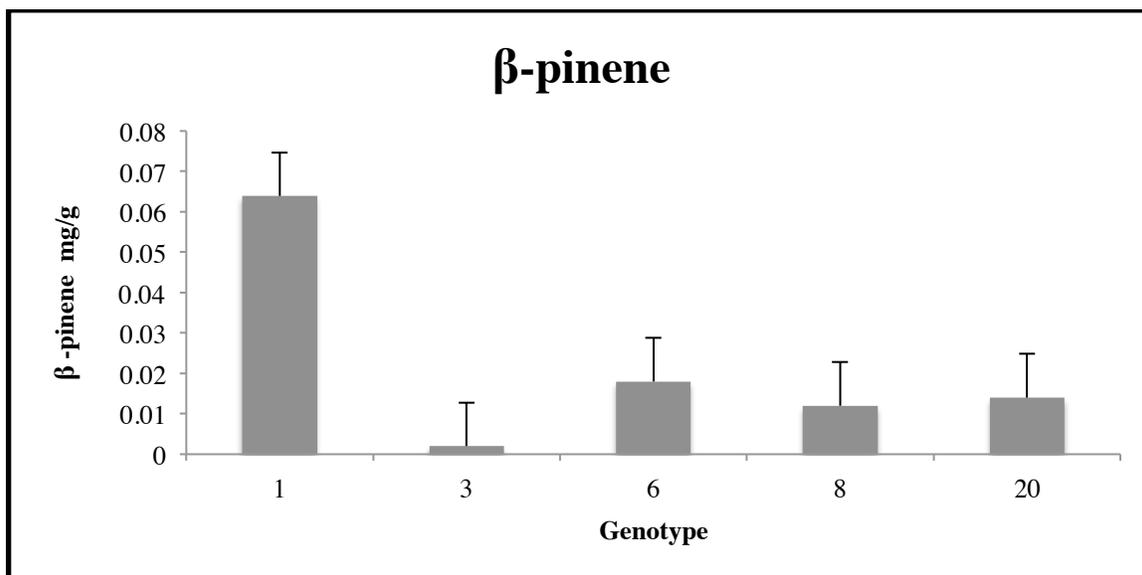
Genotype 3 had the least  $\alpha$ -pinene of all five samples, with genotypes 1, 6, and 20 all having a much larger amount (Figure 3). Genotype and  $\alpha$ -pinene were not significantly related.

**Figure 3.** Mean concentration of  $\alpha$ -pinene for each genotype.



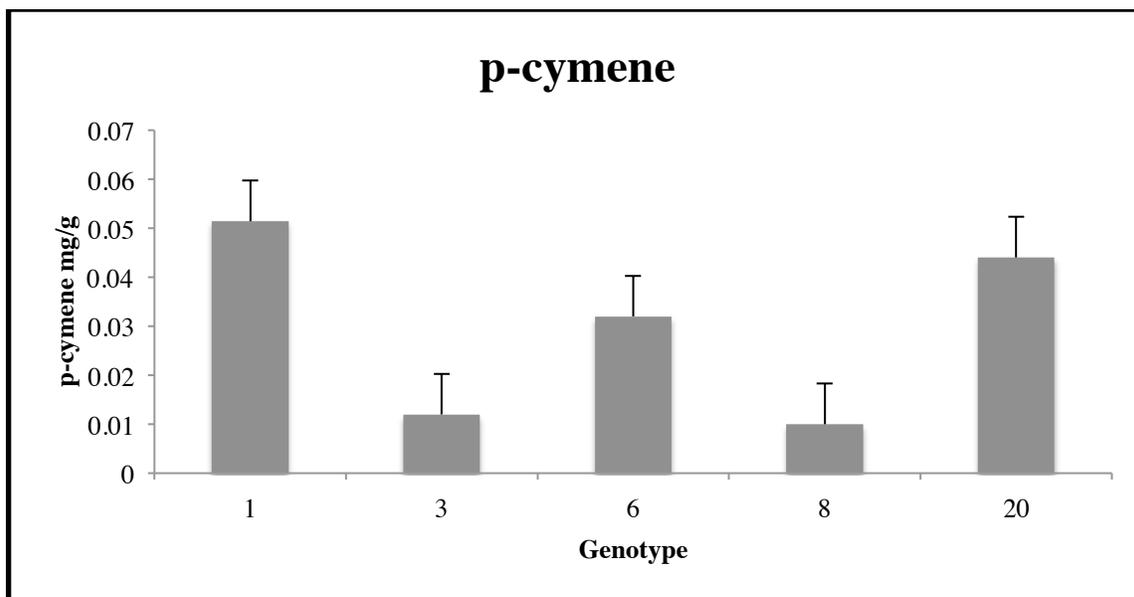
The samples showed a statistically significant difference between  $\beta$ -pinene concentrations between genotypes (Figure 4). Genotype 1 has far more  $\beta$ -pinene than the other 4 samples.

**Figure 4.** Mean concentration of  $\beta$ -pinene for each genotype.



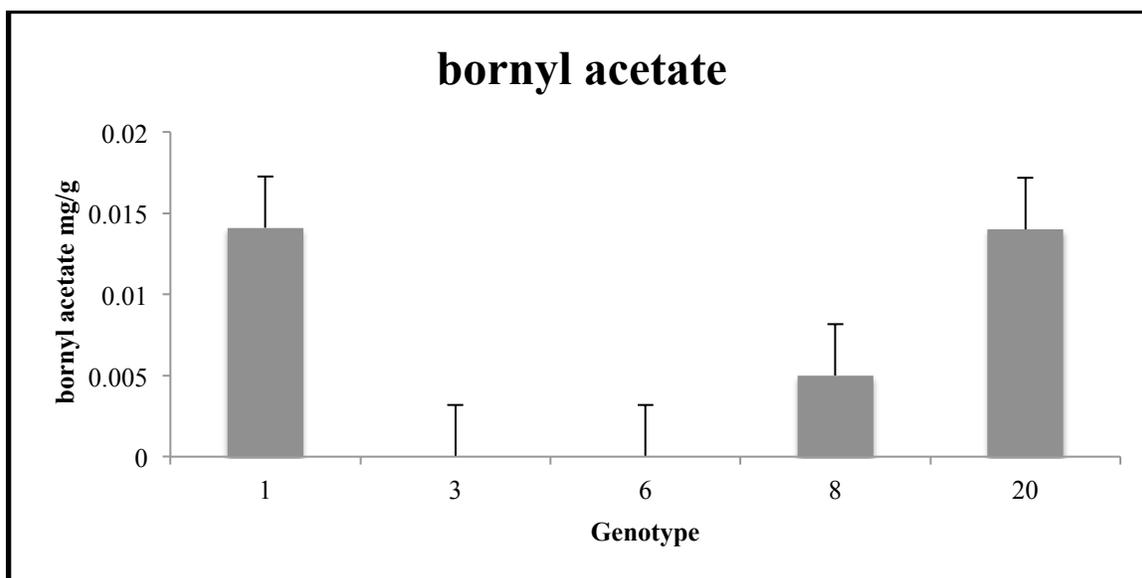
The graph of p-cymene, like  $\alpha$ -pinene, show that genotypes 1, 6, and 20 have much greater terpene concentrations compared to the other two genotypes (Figure 5). P-cymene and genotype were not significantly related.

**Figure 5.** Mean concentration of p-cymene for each genotype.



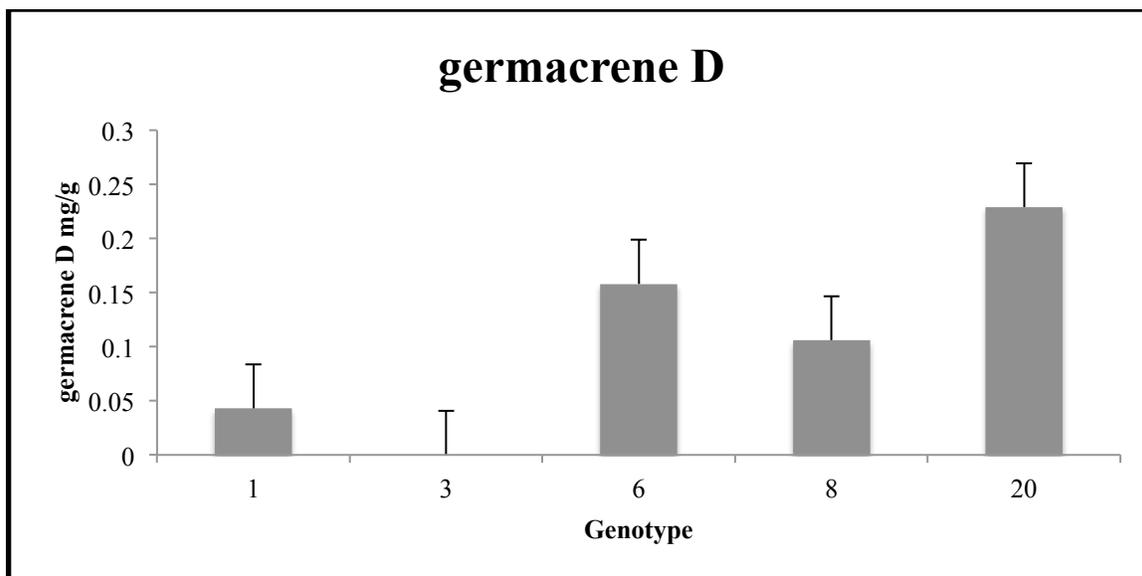
Bornyl acetate was present in only three out of the five genotypes, with genotypes 3 and 6 producing none (Figure 6). Bornyl acetate and genotype were not significantly related.

**Figure 6.** Mean concentration of bornyl acetate for each genotype.



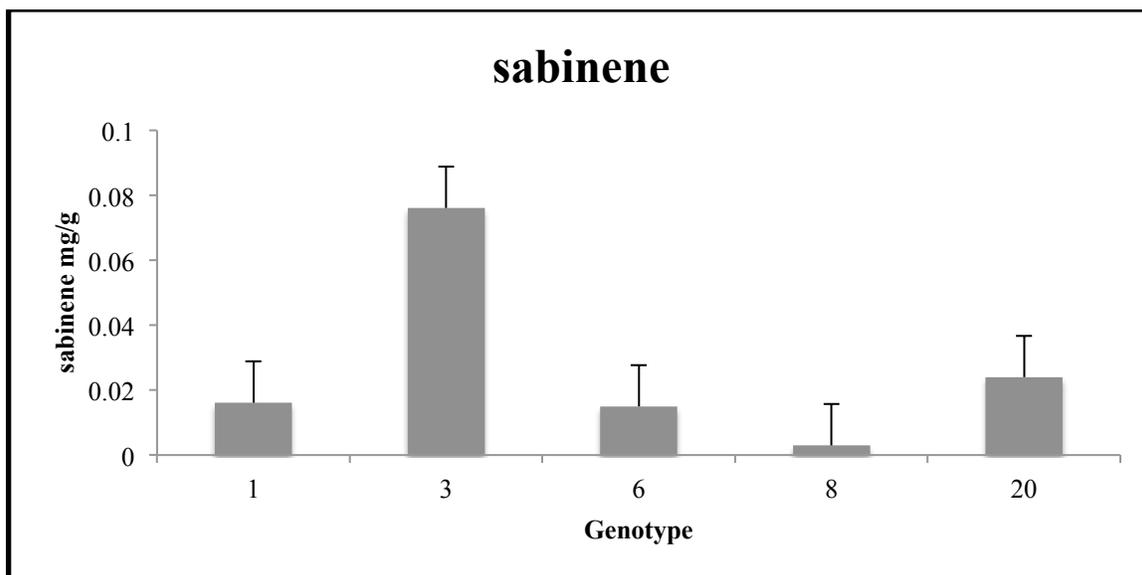
Germacrene D was found in all of the genotypes except for number 3, with genotype 20 having the highest concentration (Figure 7). Germacrene D and genotype were not significantly related.

**Figure 7.** Mean concentration of germacrene D for each genotype.



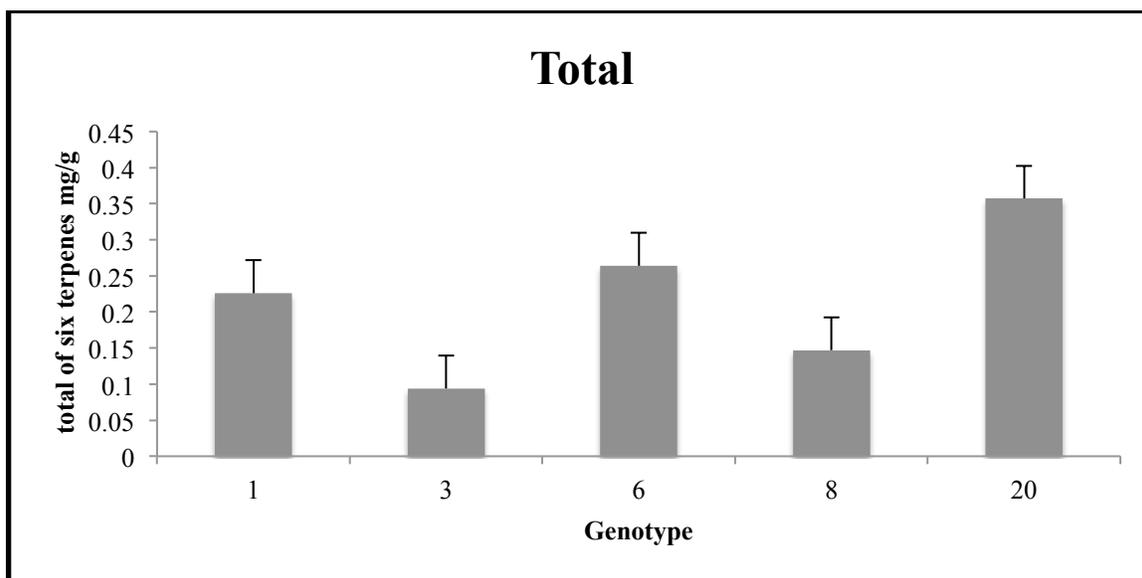
In contrast, genotype 3 had the highest concentration of sabinene, while the other genotypes had far less (Figure 8). Sabinene and genotype were not significantly related.

**Figure 8.** Mean concentration of sabinene for each genotype.



Despite genotype 3 having by far the most sabinene, it had the lowest terpene concentration overall (Figure 9). Genotype 20 had the most, and the overall pattern mirrored what was seen in some of the previous figures, with genotypes 1, 6, and 20 having the highest concentrations. There was no significant relationship between genotype and total terpene concentration.

**Figure 9.** Mean concentration of all terpenes for each genotype.

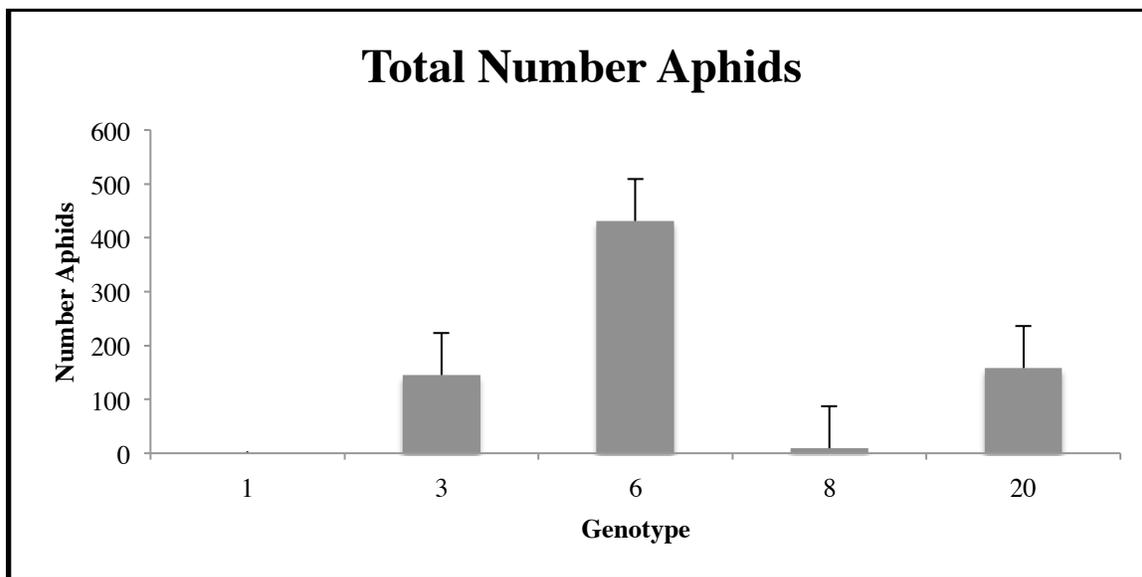


### *Aphid abundance*

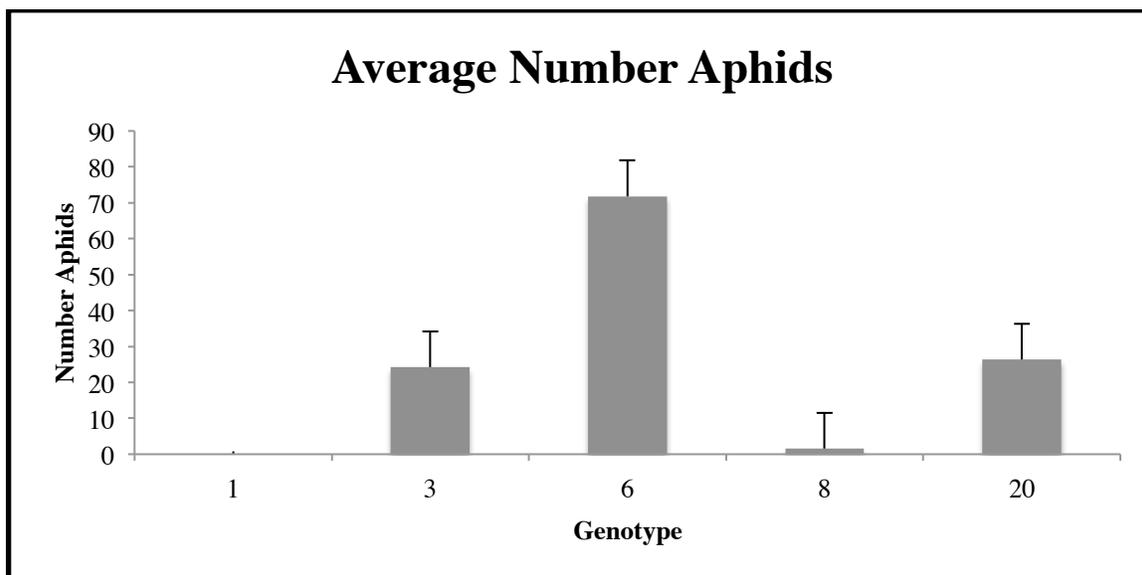
Throughout the growing season (June to October), two distinct peaks in aphid abundance were observed, one in July and one in September, possibly representing two distinct generations. For the sake of this study, aphid abundance was analyzed in two ways: (1) abundance at each peak was combined to give total aphid abundance, and (2) abundance was averaged across the entire growing season giving average number of aphids. Genotype 1 showed no aphid colonization and was not included in subsequent statistical analyses (Figures 10, 11). Both total and average aphid abundance were found to vary statistically by genotype (Table 2).

Genotypes 3, 6, and 20 all showed high aphid numbers. Genotype 8 showed low aphid numbers.

**Figure 10.** Total aphid abundance for each genotype over the growing season.



**Figure 11.** Average aphid abundance for each genotype at each sampling period during the growing season.



**Table 2.** Analysis of total aphid abundance per genotype (excluding genotype 1) using Proc GLM SAS, version 9.3 and Excel 2010.

<b>Aphid Data</b>	<b><i>f</i>-statistic</b>	<b><i>P</i></b>
Total Abundance	4.22	0.046*
Average Abundance	4.22	0.046*

$df = 3, 11$  \*= $p < 0.05$

*Aphids and terpenes- linear regression*

I found no statistical relationship between total aphid abundance and individual terpene content (Table 3). Regression graphs may be found in Figures 11-22.

**Table 3.** Analysis of terpenes and total aphid abundance regression using Proc GLM SAS, version 9.3 and Excel 2010.

<b>Terpene</b>	<b><i>r</i><sup>2</sup></b>	<b><i>p</i></b>
$\alpha$ -pinene	0.08	0.38
$\beta$ -pinene	0.02	0.69
p-cymene	0.04	0.55
bornyl acetate	0.06	0.46
germacrene D	0.01	0.96
sabinene	0.01	0.77
Total	0.01	0.88

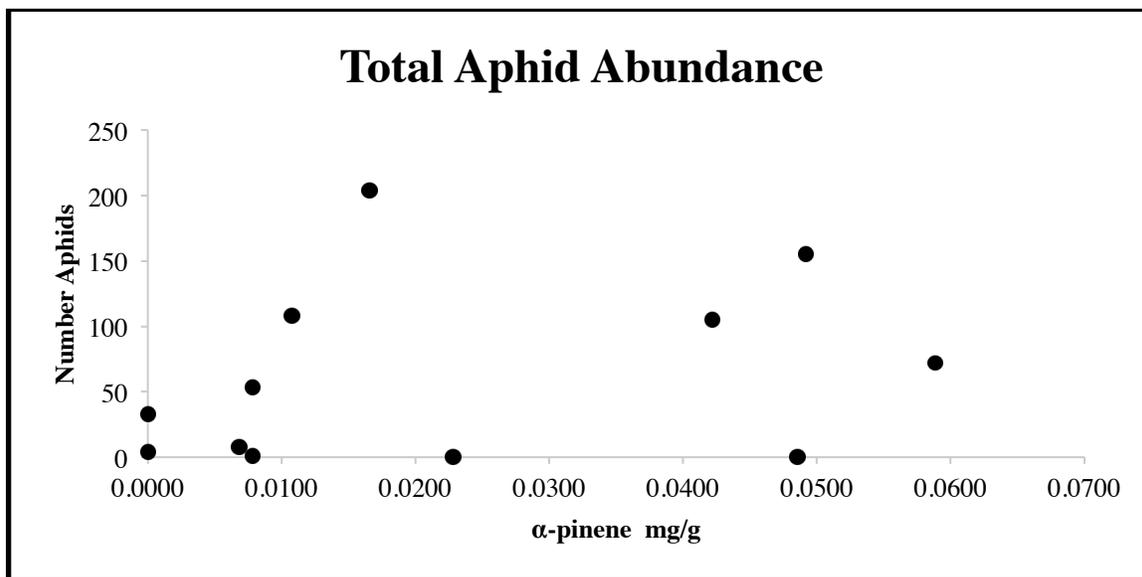
No relationship was found between average aphid abundance and individual terpene content (Table 4). Regression graphs may be found in Figures 11-22.

**Table 4.** Analysis of terpenes and average aphid abundance regression using Proc GLM SAS version 9.3 and Excel 2010.

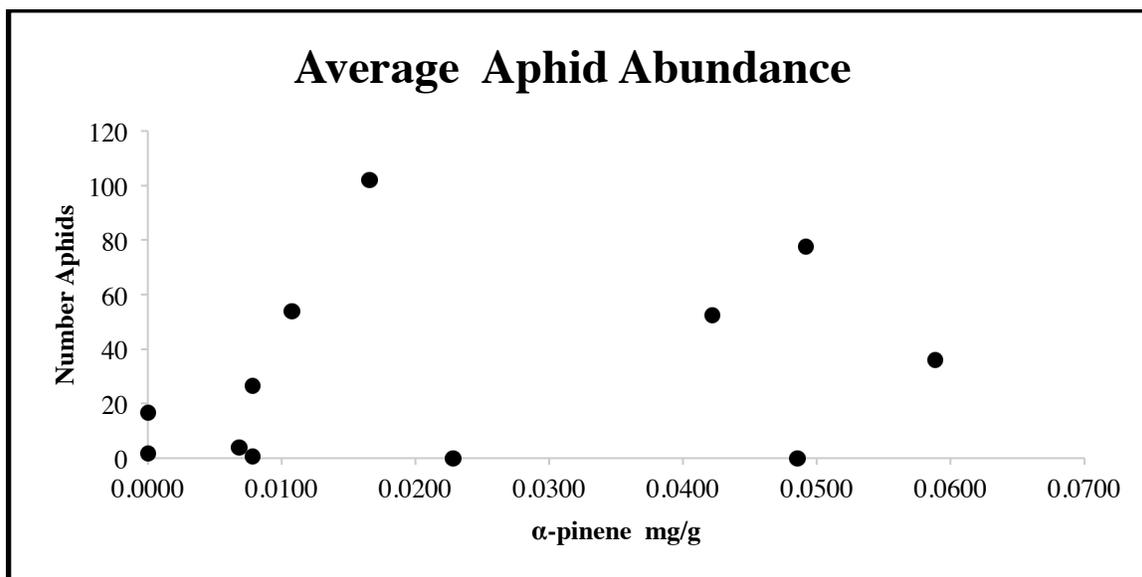
<b>Terpene</b>	<b><math>r^2</math></b>	<b><math>p</math></b>
$\alpha$ -pinene	0.08	0.38
$\beta$ -pinene	0.02	0.69
p-cymene	0.04	0.55
bornyl acetate	0.06	0.46
germacrene D	0.01	0.96
sabinene	0.01	0.77
Total	0.01	0.88

Total (Figure 12) and average (Figure 13) aphid abundance were not significantly impacted by  $\alpha$ -pinene concentration.

**Figure 12.** Regression of  $\alpha$ -pinene and total aphid abundance.

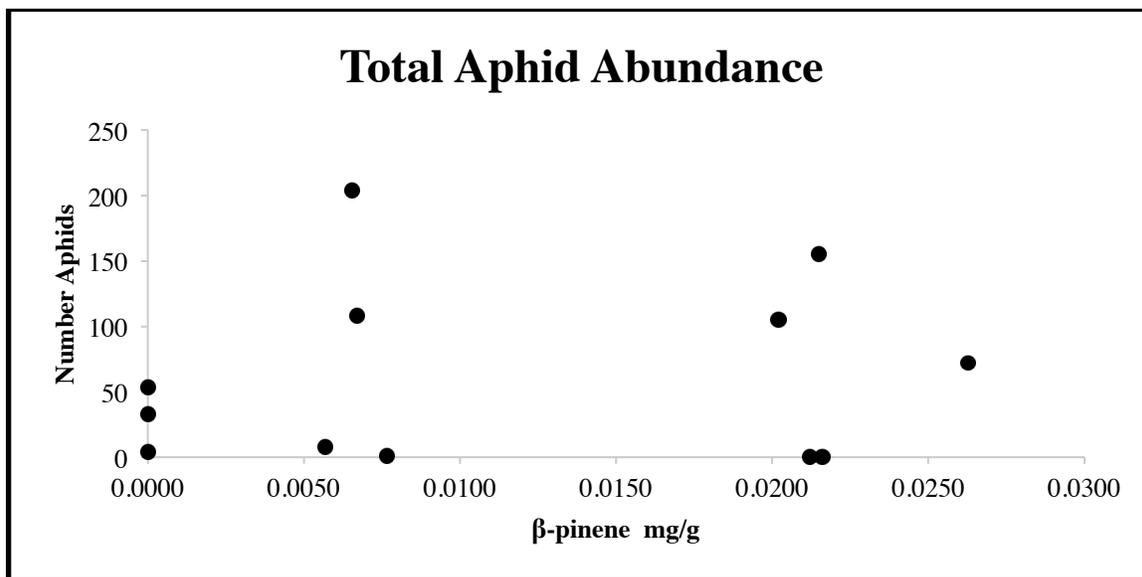


**Figure 13.** Regression of  $\alpha$ -pinene and average aphid abundance.

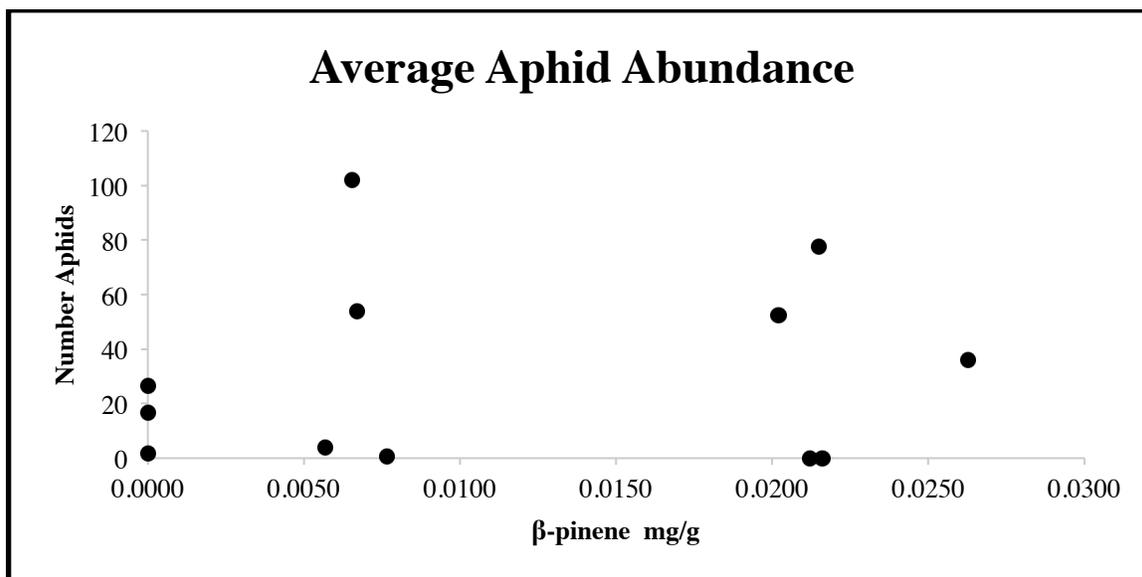


Total (Figure 14) and average (Figure 15) aphid abundance were not significantly impacted by  $\beta$ -pinene concentration.

**Figure 14.** Regression of  $\beta$ -pinene and total aphid abundance.

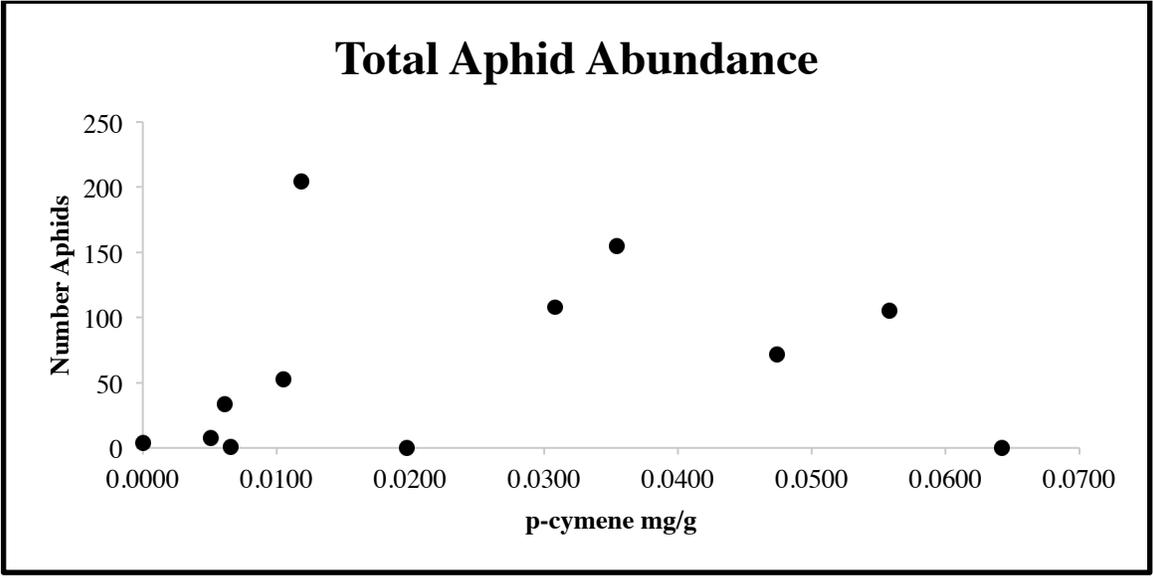


**Figure 15.** Regression of  $\beta$ -pinene and average aphid abundance.

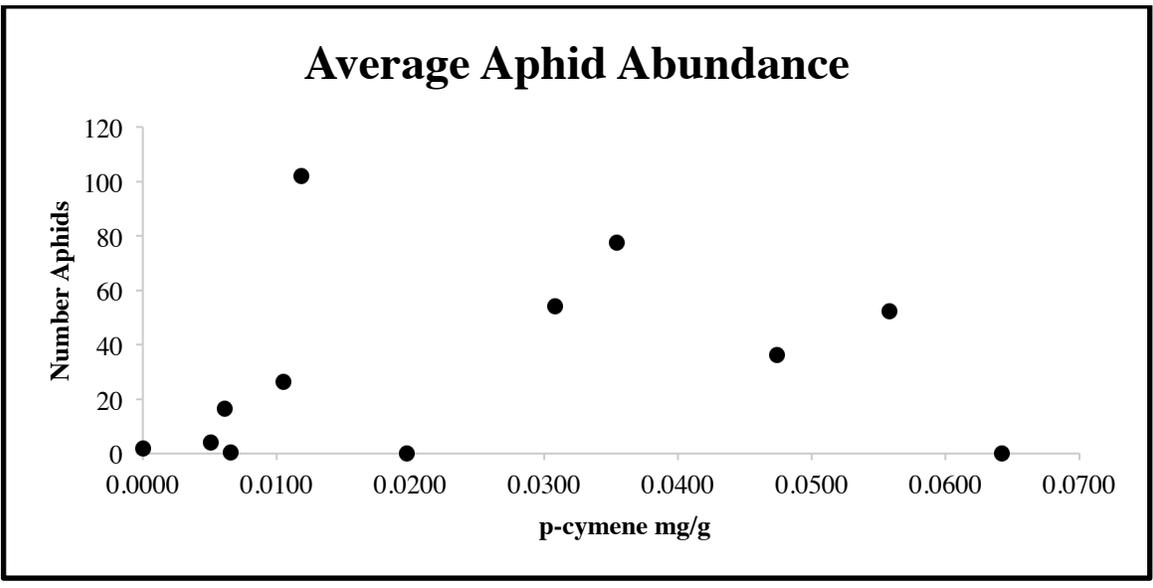


Total (Figure 16) and average (Figure 17) aphid abundance were not significantly impacted by p-cymene concentration.

**Figure 16.** Regression of p-cymene and total aphid abundance.

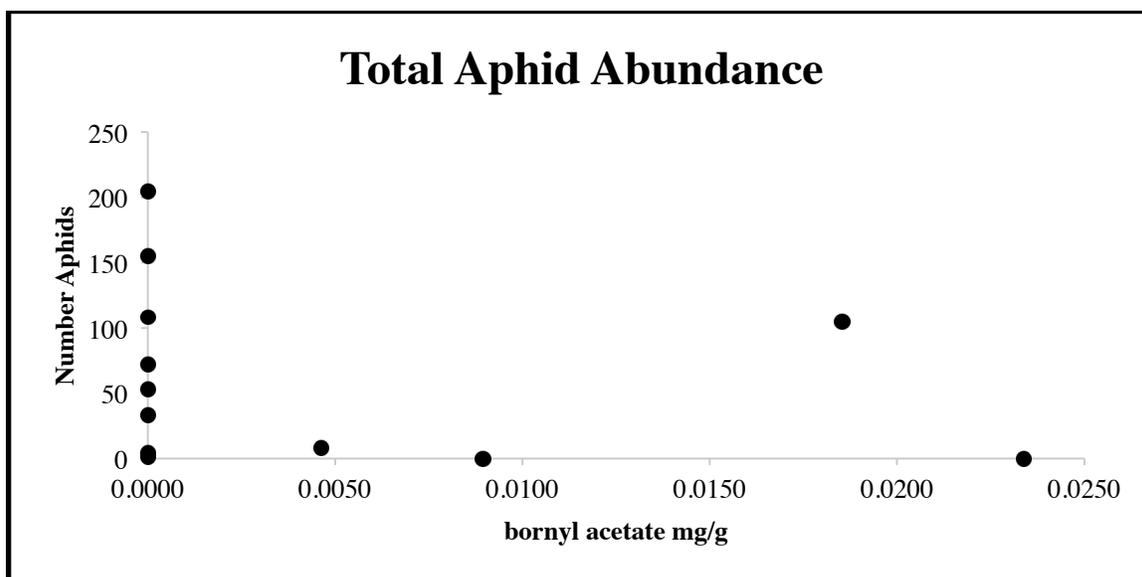


**Figure 17.** Regression of p-cymene and average aphid abundance.

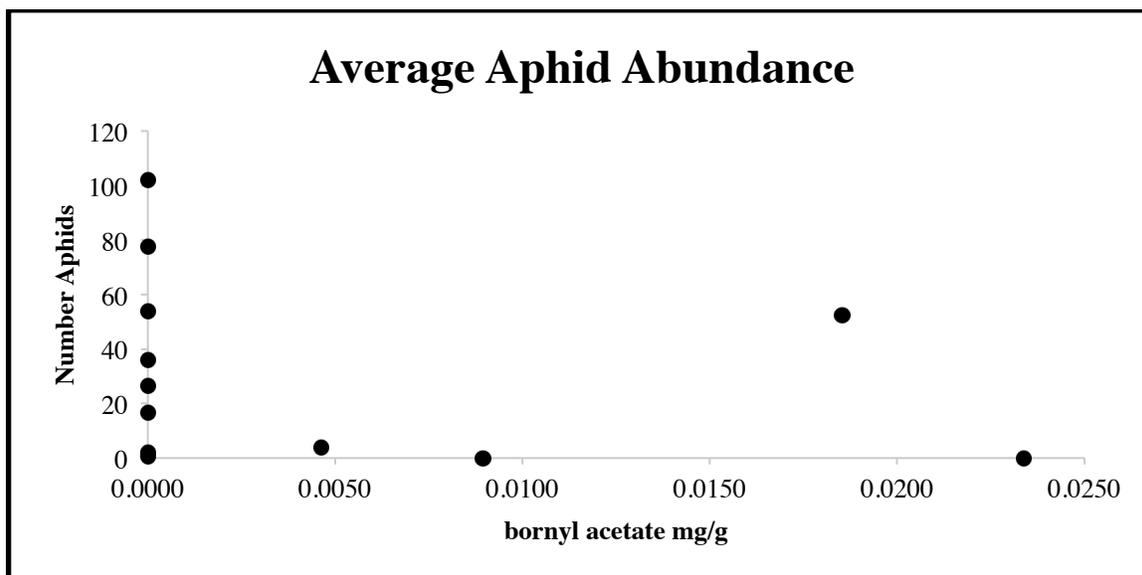


Total (Figure 18) and average (Figure 19) aphid abundance were not significantly impacted by bornyl acetate concentration.

**Figure 18.** Regression of bornyl acetate and total aphid abundance.

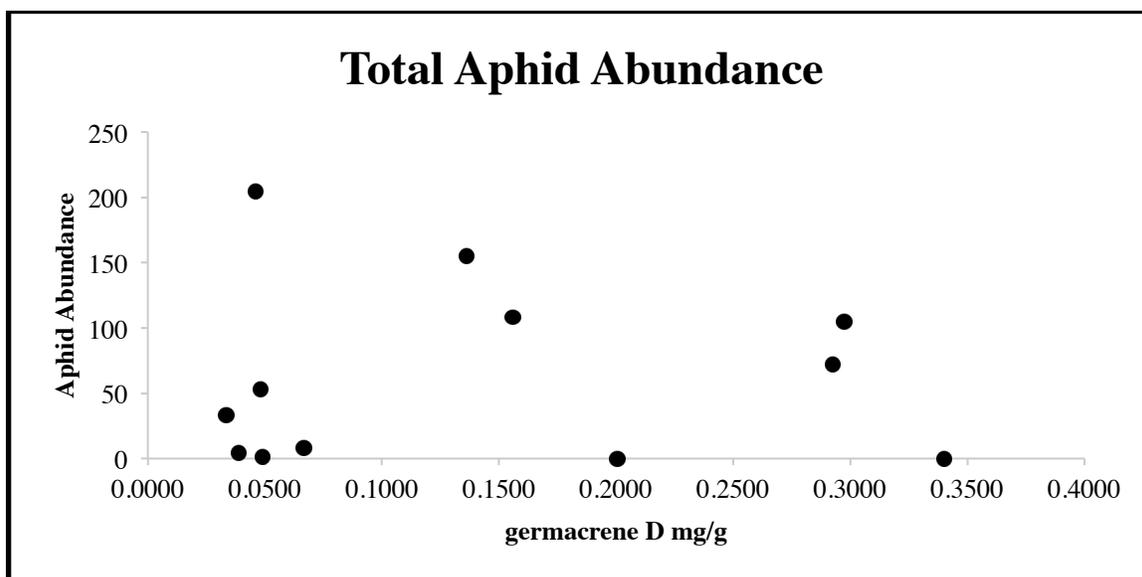


**Figure 19.** Regression of bornyl acetate and average aphid abundance.

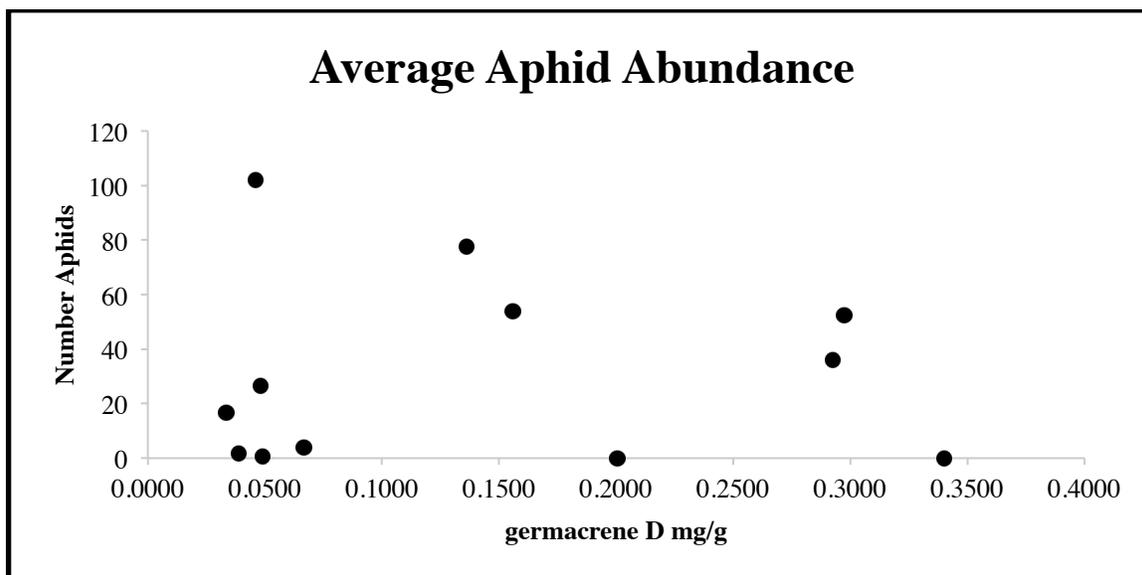


Total (Figure 20) and average (Figure 21) aphid abundance were not significantly impacted by germacrene D concentration.

**Figure 20.** Regression of germacrene D and total aphid abundance.

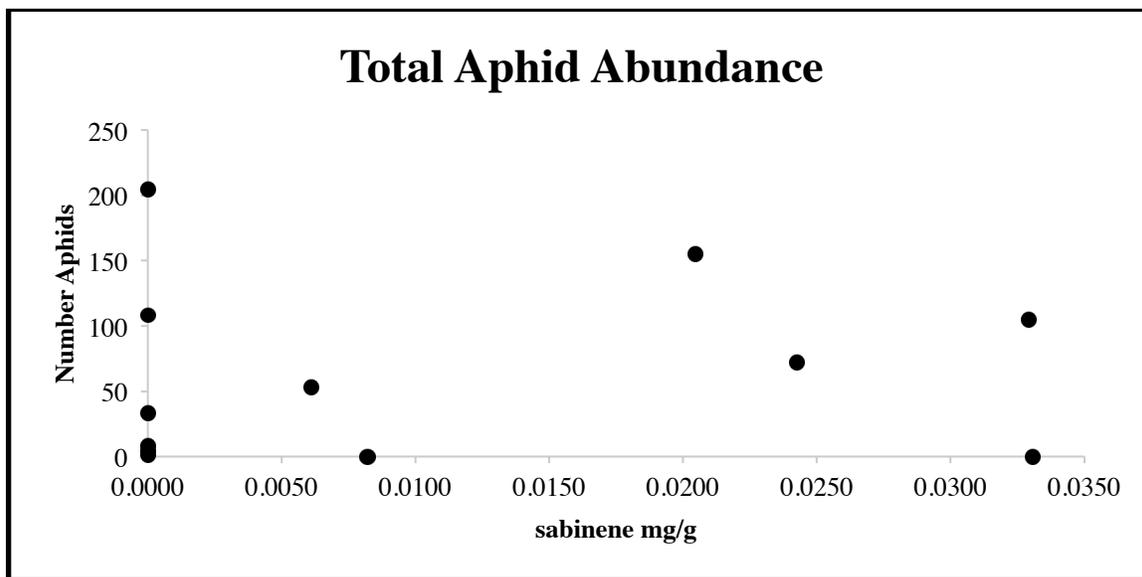


**Figure 21.** Regression of germacrene D and average aphid abundance.

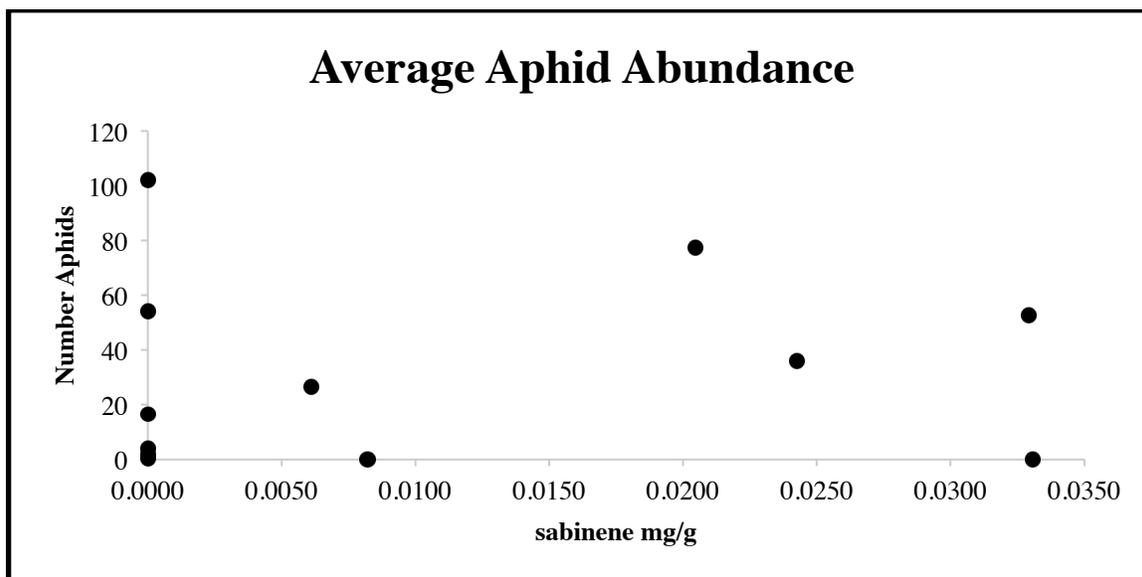


Total (Figure 22) and average (Figure 23) aphid abundance were not significantly impacted by sabinene concentration.

**Figure 22.** Regression of sabinene and total aphid abundance.



**Figure 23.** Regression of sabinene and average aphid abundance.



*Spatial Analysis*

No statistically significant relationship was found between spatial and environmental factors and either terpene content or aphid abundance (Tables 5 and 6).

**Table 5.** Analysis of environment and terpene content using Excel, 2010.

<b>Environmental Factor</b>	<b><math>r^2</math></b>	<b><math>P</math></b>
Proximity to stream	0.20	0.17
Elevation	0.01	0.85
Precipitation	0.01	0.88
Land cover type	0.18	0.19
Aspect	0.11	0.31
Slope	0.10	0.35
Hillshade	0.05	0.50

**Table 6.** Analysis of environment and total aphid abundance using Excel, 2010.

<b>Environmental Factor</b>	<b><math>r^2</math></b>	<b><math>P</math></b>
Proximity to stream	0.30	0.61
Elevation	0.01	0.93
Precipitation	0.08	0.41
Land cover type	0.08	0.41
Aspect	0.14	0.26
Slope	0.04	0.56
Hillshade	0.18	0.19

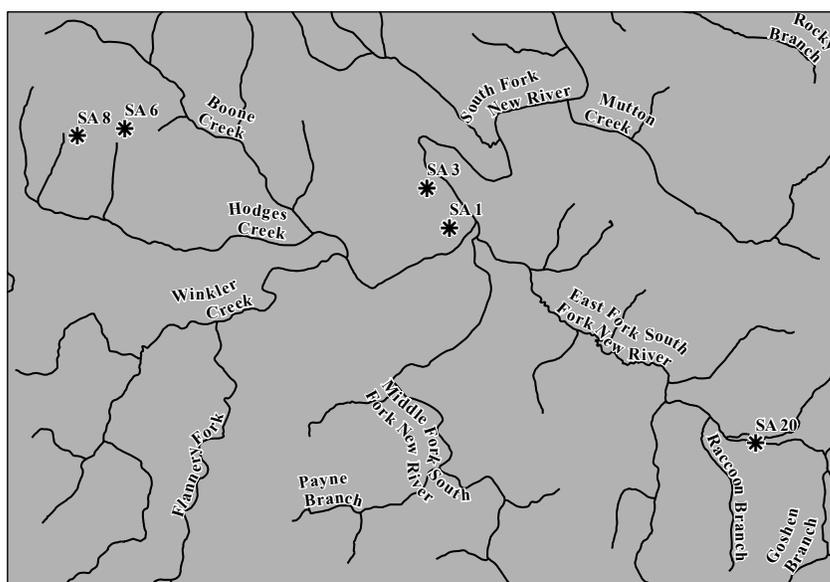
Stream proximity can be seen in Table 7 and Figure 24. Average distance to water body was 111 m. All streams are within the headwaters of the South Fork New River watershed (Figure 25).

**Table 7.** Proximity of genotypes to water bodies.

Genotype	Near Water Body	Distance (m)
1	Winkler Creek	164
3	South Fork New River	137
6	Small tributary to Hodges Creek	135
8	Small pond near tributary to Hodges Creek	105
20	East South Fork New River	14

**Figure 24.** Prominent streams of headwaters of the South Fork New River watershed.

### Streams of Watauga County, NC



#### Map Features

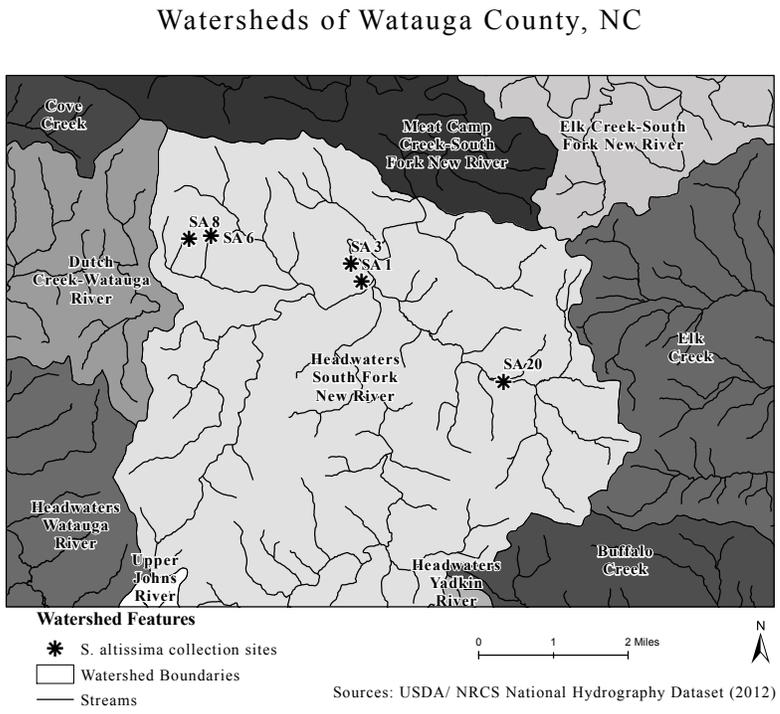
- \* *S. altissima* collection sites
- Streams

0 0.5 1 Miles



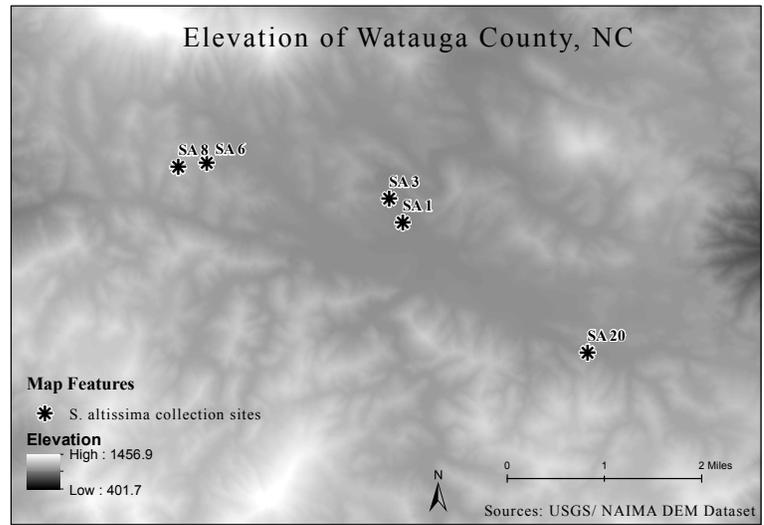
Sources: USDA/ NRCS National Hydrography Dataset (2012)

**Figure 25.** Watersheds of Watauga County, NC surrounding collection sites.



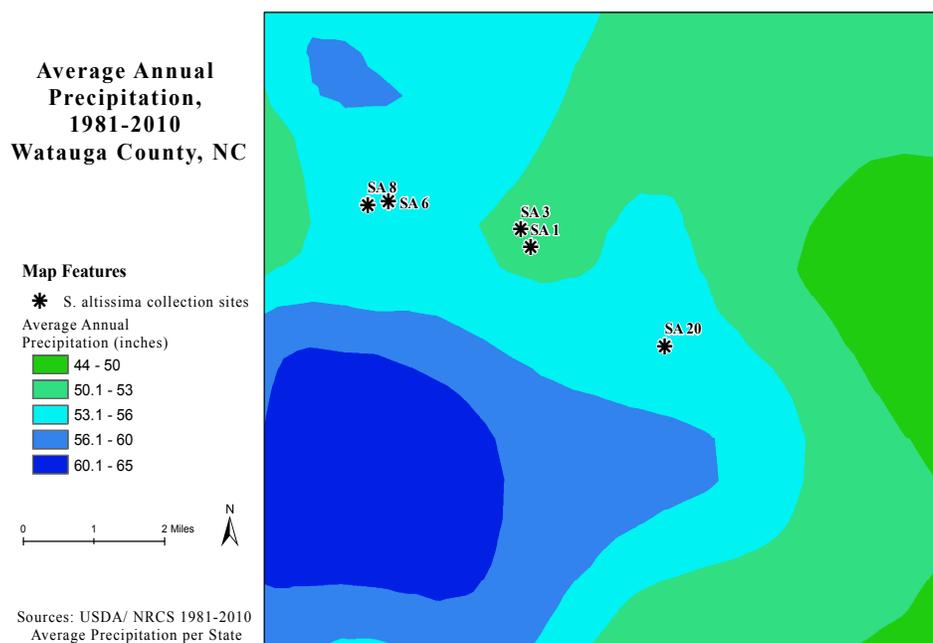
The average elevation of the collection sites was 987 m (Figure 26). Elevations ranged from 955 m to 1057 m.

**Figure 26.** Digital Elevation Model of Watauga County, NC showing collection sites.



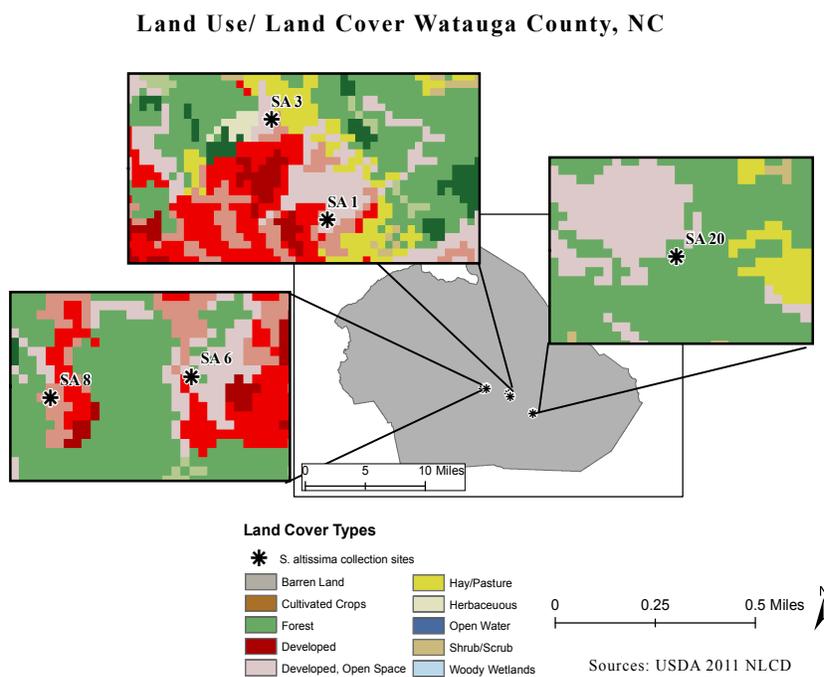
Precipitation for all sites was relatively homogenous, with values of either 53 in (134.6 cm) or 55 in (139.7 cm) per year (Figure 27).

**Figure 27.** Average annual precipitation 1981-2010 across Watauga County, NC.



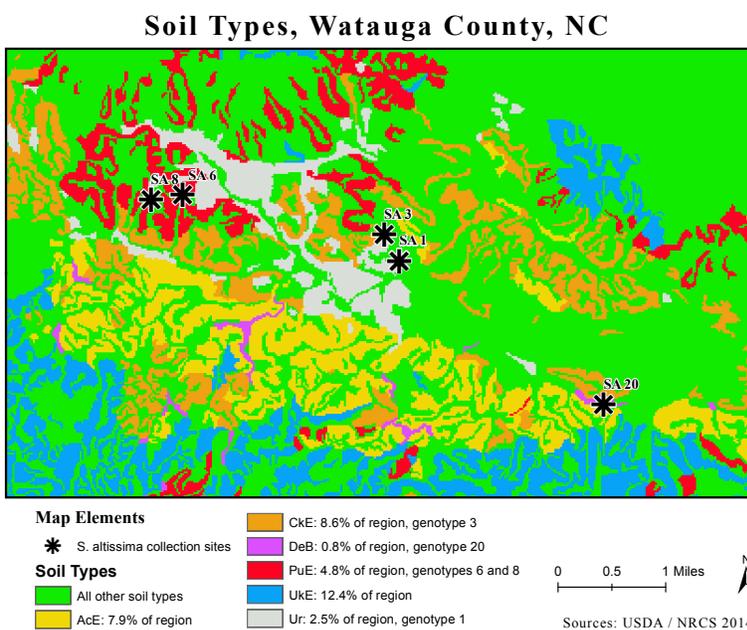
Land cover was organized into several categories, with genotypes 1, 3, and 6 being classified as developed- open space, genotype 8 being classified as developed- low intensity, and genotype 20 being classified as mixed forest (Figure 28). For the purposes of performing the regression (Tables 5 and 6), land cover type was reclassified by amount of disturbance, with mixed forest receiving a value of 1, developed- low intensity receiving a value of 2, and developed- high intensity receiving a value of 3.

**Figure 28.** Land cover in Watauga County, NC.



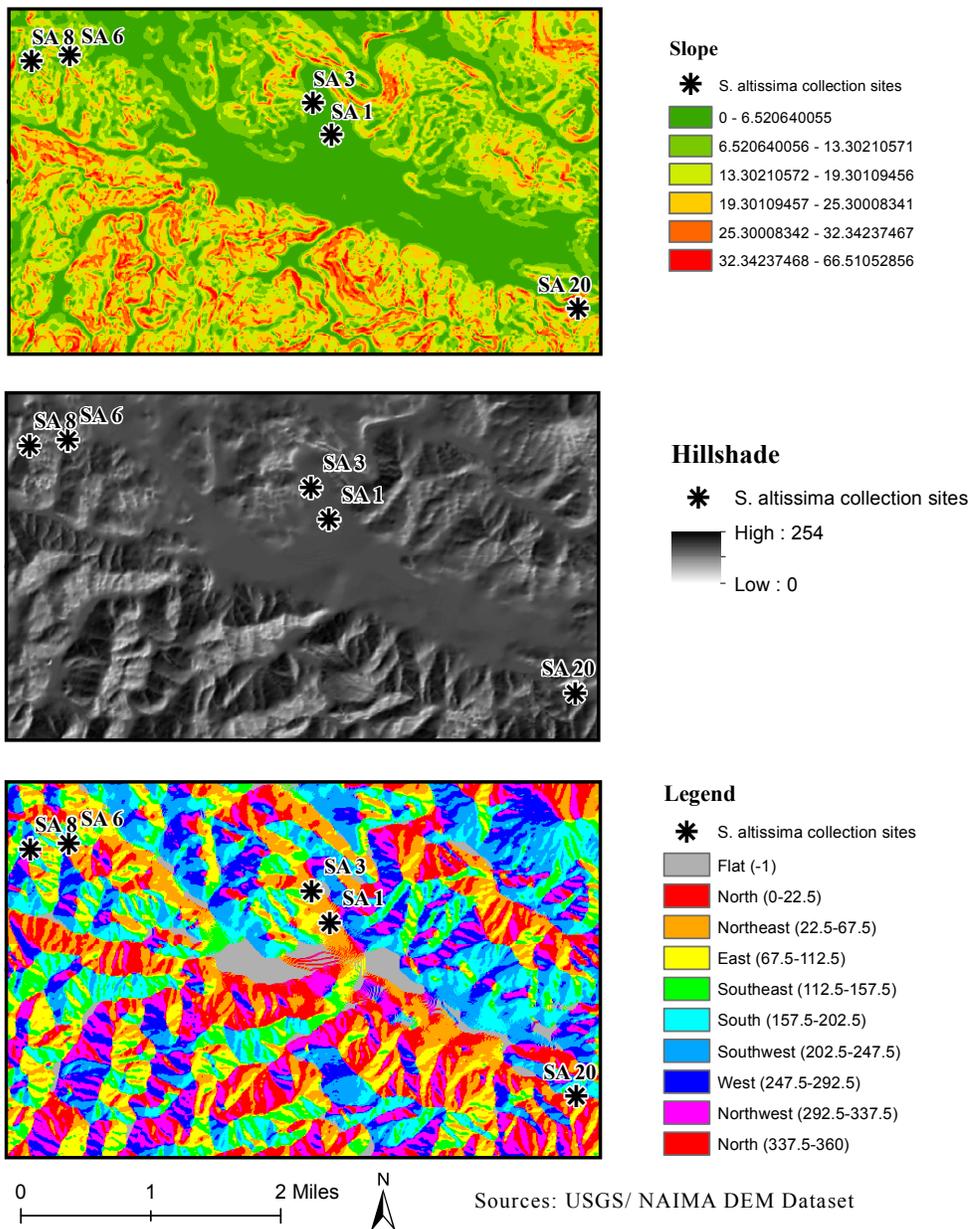
Soil types for collection sites, as well as the dominant soil types for the region were mapped (Figure 29).

**Figure 29.** Soil map of Watauga County, NC.



Terrain features (Figure 30) had no statistically significant effect on terpene content or total aphid abundance.

**Figure 30.** Topographic features from a Digital Elevation Model.  
**Topographic Features, Watauga County, NC**



## DISCUSSION

### *General Findings*

This study attempted to explore the relationship between terpene content in *S. altissima* and colonization of the specialist aphid *U. nigrotuberculatum*. Using a common garden experiment, replicates of 5 genotypes collected from several locations across Watauga County, NC were allowed to grow and be colonized by aphids. I found that aphid abundance does vary by plant genotype, but that only 1 terpene,  $\beta$ -pinene, varied by genotype. There was no clear statistical relationship between aphid content and terpene content. Spatial and environmental analyses found that no statistical relationship between environment plants were harvested from and characteristics of daughter plants.

### *Terpenes*

My first research question was: Are there differences in terpene concentrations among 5 genotypes of *S. altissima*? Out of the 6 terpenes analyzed, only  $\beta$ -pinene, showed statistically significant concentration differences between genotypes (Table 1). This result is somewhat in contrast to previous investigations of terpenes in *S. altissima* (Avakian 2012; Howells 2012; Williams and Avakian 2015), where several monoterpenes and sesquiterpenes differed among genotypes. The other terpenes showed noticeable, although not statistically significant, differences between genotypes, with genotypes 3 and 6 having far lower terpene concentrations than genotypes 1, 8, and 20 (Figure 8). These differences could be because of environmental factors (the soil the original plants grew in, the insects colonizing the plants, disease or fungus, etc.), genetic differences, or perhaps experimental error.

### *Aphid Abundance*

My second research question asked: Are there differences in aphid abundance among the genotypes? Genotype 1 had stunted growth and never showed any aphid colonization. This was likely because of a disease or fungal infection, and therefore genotype 1 was not included in the statistical analysis. The other genotypes showed distinct patterns, with genotype 6 having the highest aphid abundance, genotypes 3 and 20 having similar abundances, and genotype 8 having very low abundance (Figures 9 and 10). Overall, my study was in agreement with others that *U. nigrotuberculatum* abundance does vary by genotype in *S. altissima* (Howells 2012; Williams and Avakian 2015)

### *Relationship between Terpenes and Aphids*

My third research question was: Are aphid abundance and terpene concentrations related? Although the analysis of aphid abundance and terpene concentration was not statistically significant for any of the terpenes, distinct patterns can be seen (Figures 12-23). Regressions of terpene concentration against total and average aphid abundance showed similar patterns but at different scales (Figures 12-23). Most plants showed large differences between the lowest aphid count and highest aphid count, leading to average abundance being much lower than total abundance. This could be because of the aphids' biphasic life cycle patterns through the growing season (Cappuccino 1988). Perhaps heavy colonization was noted during the apterate phase, and during the alate phase some individuals flew away to colonize other plants, giving lower aphid abundance at those times. The only terpene in this study that varied between genotypes,  $\beta$ -pinene, has been related to abundance of *U. nigrotuberculatum* on *S. altissima* in a past investigation (Williams and Avakian 2015), though this relationship was not found here.

### *Spatial and Environmental Factors*

My fourth and final question was: Is there a spatial or environmental pattern associated with aphid abundance and/ or terpene content using the location from which the ramet was harvested? Using ArcGIS and a variety of spatial data, I found no relationship between the environment the “parent” plant was grown in and the terpene content or aphid abundance of the individuals grown in the common garden. This phenomenon was explored by Hakes and Cronin (2011) and can be attributed to phenotypic plasticity. Individuals are able to adapt to their own environment (i.e., the common garden), rather than keeping residual adaptations to the environments of previous generations (i.e., the plants that were harvested from across Watauga County). Because *S. altissima* can live in a wide variety of habitats, it makes sense that it has a plastic, or flexible, set of traits. This plasticity was seen in my study in terpene content and propensity to be fed upon by aphids.

### *Future Research*

In the future, including genetic distance analyses could give useful insights into how genotype is impacted by environment, how aphids react to genetic differences, and how terpenes may vary based on genotype. Understanding how spatial distance and genetic distance are correlated would be helpful when designing future experiments (i.e., how far apart should samples be from one another?). Further refinement of the AFLP technique described in Appendix A has great potential to produce useful genetic information for the field of community genetics.

More extensive spatial and environmental analyses could also be useful; perhaps collecting samples from a wider variety of habitat types would show further relationships between environment, genotype, and plant characteristics. As climate change alters

ecosystems and the native range of many species, understanding the influence of environment on genotype, terpenes, and aphid host plant choice will prove crucial to landscape management. Understanding how organisms are impacted by their environment may also have broader conservation implications. *S. altissima* could be used as a model organism for understanding how the environment impacts other species of interest, perhaps even for at-risk species such as the critically endangered Short's goldenrod (*Solidago shortii*) found only in small ranges in Kentucky and Indiana (Williams et al. 2014).

### *Summary*

These data support the effect of genotype on important plant allelochemicals (terpenes) and on abundance of important insect herbivores (aphids). Past experiments in the Williams laboratory have shown a positive relationship between terpene concentration and aphid abundance (Howells 2012; Williams and Avakian 2015). While this study does not show the same positive correlations, it includes an analysis of spatial and environmental factors not previously studied in the Williams laboratory. This study was designed to include a genetic distance component. Although that analysis was inconclusive due to the time constraints associated with an undergraduate thesis, the methods and troubleshooting techniques established here will be used in future studies in the Williams laboratory (Appendix A).

This research joins a large body of work in the field of community genetics and population ecology. The information gained here may be used to inform future research on *S. altissima*, *U. nigrotuberculatum*, and other, broader fields of ecology. Understanding both interspecific and ecological interactions is crucial to the study of conservation, ecosystem interactions, nutrient dynamics, and the discipline of ecology as a whole. Although previous

studies found positive relationships between terpene content and aphid abundance, my findings challenge that idea. My study did find that genotype is related to aphid abundance and with  $\beta$ -pinene concentration. Further work involving plant- insect ecology will help researchers to understand these complex community- level relationships. This project has served not only to further the understanding of *S. altissima* community ecology but also as a valuable educational experience.

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## APPENDIX A

*Genetic Distance Background*

Genetic distance is an avenue of great potential for future community genetics research. Although genetic distance analyses were unsuccessful in this experiment, a better understanding of how genetic distance correlates with terpene chemistry will help us to understand how this measure affects herbivores and other associates. Genetic differences among plants of the same species have been found to correlate with chemical differences between individuals (Azizi et al. 2012). Genetic distance, or a measure of how similar or different genetic information is between two or more organisms, can be used to measure relatedness or potential phenotypic changes (Azizi et al. 2012). In the case of *S. altissima*, genetic distance may indicate phenotypic differences such as terpene content. From those phenotypic differences, community changes, such as insect abundance, may be noted (Williams and Avakian 2015).

AFLP uses Polymerase Chain Reactions (PCR) to acquire genetic information about an individual organism. AFLP is used in biological studies because of its potential for high-quality results and ease of use, and is often employed in systematics, population, and conservation genetics, and in determining kinship (Mueller and Wolfenbarger 1999). AFLP products are run on a gel so that bands of similarly- sized DNA fragments can be visualized. Genetic distance is then scored using an AFLP scoring software (Meudt and Clarke 2007). AFLP shows genetic distance based on the number of common bands between individuals and is considered to be a reliable technique for determining whether two individuals are related or are the same (Meudt and Clarke 2007).

GenAlEx software is a Microsoft Excel add-in program that can be used for a variety of population genetic analysis techniques, including AFLP and genetic distance and

relatedness (Peakall and Smouse 2012). One capability of GenAlEx is performing Mantel tests. Mantel tests use a matrix to do a pairwise comparison of genotypes and test whether genetic distance is correlated with geographic distance (Peakall and Smouse 2009). These data can be compared to terpene data to determine whether plant chemical similarities and differences may be caused by plant genotype.

### *Methods*

In the fall of 2014 and spring of 2015, I performed AFLP on frozen leaf samples from the five genotypes. Previously collected leaf samples were stored in a -80°C freezer. Samples were ground using liquid nitrogen and a mortar and pestle. Ground samples were stored in the -80°C freezer before extraction. A CTAB DNA extraction was performed (Table 8) using a protocol modified from Doyle and Doyle (1987). DNA was quantified using a Nanodrop (Nanodrop Technologies; Wilmington, DE, USA) spectrophotometer and run on a 1% agarose gel to determine DNA quality and quantity.

An AFLP protocol modified from Jonathan Wendel's laboratory (Hawkins et al. 2005) by the Estep laboratory at Appalachian State University was used (Table 9). The AFLP was unsuccessful, but had it worked quickly enough for this project, the AFLP products would have been run on a vertical polyacrylamide gel and imaged on a Typhoon imager. The image would have been analyzed using GenAlEx software to determine genetic distance. A Mantel test would be used to compare genotypes to terpene data.

**Table 8.** CTAB extraction of plant DNA from leaf tissue (modified from Doyle and Doyle 1987).

<b>Cell lyses</b>	<ol style="list-style-type: none"> <li>1. Add 2% CTAB buffer (100mM tris, 2% CTAB, 1.4M NaCl, 20mM EDTA) with BME (65°C) to ground leaf tissue (5ml buffer/ 1g tissue)</li> <li>2. Incubate for 1-2 hours at 65°C, mixing every 15 minutes</li> </ol>
<b>Removal of protein</b>	<ol style="list-style-type: none"> <li>1. Cool sample to room temperature or put on ice for 15 minutes.</li> <li>2. Add equal volume phenol/ chloroform/isoamyl (25:24:1)</li> <li>3. Mix gently by hand until phases incorporated</li> <li>4. Spin @3500 rpm for 15 minute (4°C)</li> <li>5. Move aqueous phase to new tube</li> <li>6. Add equal volume chloroform/ isoamyl (24:1)</li> <li>7. Mix gently by hand until phases incorporated</li> <li>8. Spin @3500 rpm for 15 minute (4°C)</li> <li>9. Move aqueous phase to new tube</li> </ol>
<b>Removal of RNA</b>	<ol style="list-style-type: none"> <li>1. Add RNase (1<math>\mu</math>g/mL final concentration)</li> <li>2. Incubate at 37°C for 1 hour (or overnight)</li> </ol>
<b>Precipitate DNA</b>	<ol style="list-style-type: none"> <li>1. Add equal volume isopropanol</li> <li>2. Mix gently</li> <li>3. Spin @3500 rpm for 15 minute (4°C)</li> <li>4. Discard supernatant (pellet may be loosely attached)</li> <li>5. Wash with 70% ethanol</li> <li>6. Spin @3500 rpm for 15 minute (4°C)</li> <li>7. Discard supernatant</li> <li>8. Allow to air dry for 10-15 minutes until ethanol smell gone</li> <li>9. Re-suspend in 100-200ul 1xTE buffer</li> </ol>

**Table 9.** AFLP protocol (modified from Hawkins et al. 2005).

<b>Step</b>	<b>Component</b>	<b>Amount (μL)</b>	
<b>Digestion</b>	DNA	200 ηg	
	10 RE buffer	2.0	
	RE #1	10 units (this study used MSE I)	
	RE #2	10 units (this study used Eco RI)	
	dH <sub>2</sub> O	xx	
	Total	20.0	
	Incubate at 37°C for 3 hours. Immediately proceed to ligation.		
<b>Preparing Adaptors</b>	Forward adaptor #1 (MSE I)	250 @ 100 μM	
	Reverse adaptor #1 (MSE I)	250 @ 100 μM	
	Total	500 @ 50 μM	
	Forward adaptor #2 (Eco RI)	250 @ 100 μM	
	Reverse adaptor #2 (Eco RI)	250 @ 100 μM	
	Total	500 @ 100 μM	
	Heat at 95°C for 5 minutes, than cool SLOWLY in a Styrofoam box (approx. 3 hours) to renature.		
	<b>Ligation</b>	10X ligase buffer	4.0 (1X)
		Adaptor 1 (50 μM)	1.5 (75 pmoles)
		Adaptor 2 (50 μM)	1.5 (75 pmoles)
T4 DNA ligase (2000 U/μL)		0.01 (20 units)	
dH <sub>2</sub> O		12.99	
Total		20	
Add 20 μL ligation mix to each tube and incubate overnight at 16°C. Add 160 μL dH <sub>2</sub> O and invert to mix.			

<b>Step</b>	<b>Component</b>	<b>Amount (μL)</b>
<b>+1 selective amplification</b>	MgCl <sub>2</sub> (50 μM)	1.5 (1.5 mM)
	dNTP (2.5 μM)	4.0 (200 μM)
	+1 primer #1 (5 μM)	8.0 (40 pmoles)
	_1 primer #2 (5 μM)	8.0 (40 pmoles)
	Taq polymerase (5U/ μL)	0.5 (2.5 U)
	dH <sub>2</sub> O	13.0
	Total	40.0
	<p>Add 10 μL dilute/ digestion mix to 40 μL +1 master mix.            Use to following reaction conditions: 1. 75°C 2 min, 2. 94°C 30 sec, 3. 56°C 30 sec, 4. 75°C 2 min, 5. go to #2 19x, 6. 60°C 30 min, 7. 4°C hold</p> <p>Dilute each reaction with 720 μL dH<sub>2</sub>O</p>	
<b>+3 selective amplification</b>	10x PCR buffer	2.5 (1X)
	MgCl <sub>2</sub> (50 mM)	0.75 (1.5 mM)
	dNTP (2.5 mM)	3.0 (300 μM)
	Primer #1	1
	Primer #2	1
	Taq polymerase (5U/ μl)	0.25 (1 U)
	dH <sub>2</sub> O	20.0
	<p>Add 5 μL dilute to 2 μL +3 master mix. Use the following reaction conditions: 1. 94°C 2 min, 2. 94°C 30 sec, 3. 65°C 30 sec (reduce by 1°C per cycle), 4. 72°C 2 min, 5. go to step #2 9x, 6. 94°C 30 sec, 7. 56°C 30 sec, 8. 72°C 2 min, 9. go to step #6 25x, 10. 60°C 5 min, 11. 4°C hold</p>	