EFFECTS OF GENOTYPE AND ENVIRONMENT ON THE ABUNDANCE OF A SPECIALIST APHID IN *SOLIDAGO ALTISSIMA*

A Thesis
by
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Abstract

EFFECTS OF GENOTYPE AND ENVIRONMENT ON THE ABUNDANCE OF A SPECIALIST APHID IN *SOLIDAGO ALTISSIMA*

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It is known that populations of herbivorous insects may be influenced by genetic variation within dominant plant species. The phytochemical composition of dominant plants has been demonstrated to vary by genotype (G) and impact the colonization of herbivorous insects. Terpenes are one of the largest classes of secondary metabolites and represent a plant trait known to vary by genotype. These chemicals play a role in both deterring and attracting herbivorous insects and, as such, provide a potential mechanism explaining host plant choice by insects. Though variation among genotypes in phytochemicals has been shown, less well known is how environment (E; e.g., soil nutrients) interacts with trait variation in plants (G X E). I investigated how genotype and environment might affect the chemical concentration and subsequent colonization and abundance of a numerically dominant aphid species in a widespread old-field plant species. Five genotypes of *Solidago altissima* were grown in a common garden experiment in three different soil nutrient conditions; nitrogen fertilized, phosphorus fertilized, and control (no added nutrients). I assessed how the colonization responses of the aphid *Uroleucon nigrotuberculatum*, and plant terpene and nutrient

chemistry varied in response to different plant genotypes and environments. My hypothesis was that higher levels of soil nutrients would result in a higher abundance of aphids on *S. altissima* due to increases in certain aphid-attracting terpenes and that differences among genotypes in the chemical constituents I measured would result in G, E and G X E effects. My data demonstrated that phytochemistry was affected by both environment and genotype. The abundance of *U. nigrotuberculatum* was not different between genotypes of *S. altissima*, but a significant G X E interaction showed that genotypes were not colonized equally across nutrient treatments, demonstrating that some plant attributes were affecting aphid colonization in certain genotypes but not others. I found that much of the variation in aphid abundance was due to differences in nutrient treatments, notably the nitrogen treatment, with a higher overall abundance of aphids. Plant terpene and nitrogen concentration was positively related to aphid abundance, with the amount of variation dependent on soil nutrients.

In conclusion, my study found that the soil nutrient environment of *S. altissima* is important in governing the populations of *U. nigrotuberculatum* and that environment affects the abundance of this insect on certain genotypes. My data demonstrated that phytochemistry may serve as a mechanism explaining G, E and G X E effects. This study sheds further light on how plant genotype and environment relate to one another in determining populations of dependent herbivorous insects, aiding in answering questions within the field of community genetics.

Acknowledgments

I owe many thanks to the members of my lab that worked with me to make this research possible, to my advisor Ray S. Williams, and to my lab mates Julie Ragsdale, Marae Lindquist, Jacob Pawlik and Bryan Taylor. I also greatly appreciated the help of my committee members Howie Neufeld and Mike Madritch along the way, for instructing me in research and statistical techniques that proved invaluable. For assistance in my research I thank Jerry Meyer, Quinn Griffin, Kate Lis, and Delaney Trimble for caring for our study plants. Special thanks to Matt Estep and his laboratory for conducting genetic analyses that were crucial for my study. Lastly I would like to thank the Appalachian State University Office of Student Research for its support in funding this investigation.

Dedication

This thesis is dedicated to my parents, Ken and Susan Bonville for always supporting and encouraging my love and interest in plants and the insects that live on them. And also to my wife, Quinn for always tagging along as I run off the trail to look at something.

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Introduction

Intraspecific Genetic Variation

Understanding how intraspecific genetic variation in plant species affects associated organisms, such as the insects that depend on them, is important for considering the processes that drive ecosystem structure and function. The field of community genetics strives to understand the importance that intraspecific genetic variation plays in impacting communities (Antonovics 1992, Whitham et al. 2008) and to elucidate how the concept of extended genotypes can explain genetic effects beyond the population level (Whitham et al. 2003, Bailey et al. 2005). Examining effects of genetic variation in plant species that serve as a foundation for ecosystems provides a framework for investigating interactions between herbivorous insects that feed on them (Crutsinger et al. 2006). When combined with effects of the environment (e.g., soil nutrients), one can more fully separate genetic effects from those imposed by the environment (Johnson and Agrawal 2005, Hersch-Green et al. 2011). The aim of my study was to examine genotype (G), environment (E), and possible genotype X environment interactions (G X E) in an important plant-herbivore system.

Intraspecific genetic diversity in plants could have a major impact on dependent insects, especially within foundational or dominant plant species. Dominant plant species substantially contribute to community biomass and if intraspecific genetic variation exists,

may exhibit a great deal of chemical and physical diversity that is subject to G X E interactions (Whitham et al. 2003). Due to their functional impacts within ecosystems, and the genetic and phenotypic variation that foundational plant species exhibit, studies examining these species largely support the importance of intraspecific genetic diversity in determining ecosystem processes and/or structure (Wimp et al. 2004, Johnson and Agrawal 2005, Lojewski et al. 2009, Genung et al. 2012a). The importance of intraspecific genetic diversity within populations of plants has been shown to impact communities of organisms at multiple trophic levels (Whitham et al. 2003, Bailey et al. 2006, Hughes et al. 2008). In addition, the effects of plant genetic variation may impact some insects and ecosystem processes more strongly than others, typically decreasing with ascending trophic levels. This was seen in a study conducted by Genung et al. 2012a, where aphids were impacted by host plant genotype, but their ladybugs predators responded only to differences in aphid populations, that varied in part due to these genetic variations. At the ecosystem level, plant interspecific variation can extend to impact primary productivity (Crutsinger et al. 2006), as well as nutrient fluxes and decomposition rates (Madritch et al. 2006, Hughes et al. 2008, Genung et al. 2012a), both of which have the potential to affect associated species.

Prior studies have demonstrated how genetic diversity can be a large influence in structuring arthropod communities. For example, Tovar-Sanchez et al. (2015) demonstrated an increase in arthropod community diversity in relation to an increase in genetic diversity among populations of oak tree species in Central Mexico. The structure of associated arthropod communities was also demonstrated to vary by host tree genotype in willow trees (Barbour et al. 2015). Crutsinger et al. (2009) demonstrated that genetic variation determined

the structure of associated arthropod communities in *Solidago altissima* by impacting the plant's susceptibility to gall formation and the shape and size of galls. Insect communities are important to consider with respect to foundational plants such as *S. altissima*, as they are arguably the most diverse and abundant associates of these plants. Many insect species depend upon plants as a primary energy and nutrient source and are possibly sensitive to phenotypic variation between host plants (Wimp et al. 2004). Studies have shown the importance of plant genetic diversity on insect abundance (Williams and Avakian 2015) and species diversity (Crutsinger et al. 2006), and also the potential for environment (spatial effects; Tack et al. 2010) and soil nutrients (Burkle et al. 2013) to impact insect communities as well.

Study Organisms

Tall or late goldenrod, *Solidago altissima* L., is a good model organism for my study. I asked how trait variation among genotypes in this widespread old-field plant differed by the soil nutrient environment, resulting in effects on phytochemistry and the abundance of a specialist aphid species. Previous investigations demonstrate the importance of intraspecific genetic variation on associated insects in this species (Crutsinger et al. 2006, 2009, Genung et al. 2012a, Burkle et al. 2013, Williams and Avakian 2015). This goldenrod species is often the dominant flowering plant in disturbed open sites throughout the Eastern US and is common throughout its range (Uesugi et al. 2013). There are at least 136 species of associated insect pollinators, predators, and herbivores found on *S. altissima* (Crutsinger et al. 2006). Maddox and Root (1990) demonstrated that different genotypes of *S. altissima* either attracted or repelled different suites of insect herbivores; therefore it provides a good

model for addressing my questions. Studies using *S. altissima* in a common garden approach have demonstrated that both genetic identity and genetic diversity impacts arthropod community structure (Crutsinger et al. 2006). Genung et al. (2012a) observed that the effects of genetic identity impacted the composition of visiting pollinators, as well as the pollinators on neighboring plants. Such studies delineate the importance of both genetic identity and genetic diversity, though specific plant traits responsible for shaping the arthropod communities on *S. altissima* are less well understood. It is possible that phytochemicals such as terpenes, that can vary among genotypes (Williams and Avakian 2015), may play a mechanistic role in determining insect responses to plant genotypes.

In order to gain greater understanding of how host plant genetic variation may impact associated insects, it is useful to examine the responses of the most prevalent herbivores in the insect community. Wise et al. (2009) examined the interactions between the often abundant goldenrod gall-fly (*Eurosta solidaginis*) and *S. altissima* populations, and described a genetically linked trait that conferred resistance to this herbivore. This study demonstrated that the genetically linked candy-cane stem trait in *S. altissima* plants conferred resistance to the plants. Another gall-forming insect, rosette leaf gall (*Rhopalomyia solidaginis*) was investigated by Crutsinger et al. (2009), who asked how the genetic susceptibility of *S. altissima* plants to form galls impacted the arthropod communities associated with these galls. The authors demonstrated a multi-trophic arthropod response based on host plant genotype and susceptibility to this herbivore. For an herbivore/predator system it was found that host plant genotypic identity could impact the first trophic level in a study that examined goldenrod aphids and ladybeetles, but that the genotypic identity effect did not extend to impact the ladybug predators at a higher trophic level (Genung et al. 2012c). In a study

conducted by Mooney and Agrawal (2008) it was suggested that plant genotype was associated with phloem sap quality in milkweed plants, which in turn impacted the feeding behavior of ants present in the system. Variation in ant populations was tied to the abundance of aphids, which was also associated with milkweed genotype. The mechanism suggested by Mooney and Agrawal (2008) is possibly the quality of the plant sap (whether based on carbohydrate content or secondary metabolite content), that would influence the presence and feeding behavior of the associated ants. Similarly, a study investigating the effects of genotype in evening primrose on aphid herbivores and aphid-tending ants demonstrated that plant phenotypic variation associated with genotype affected both ant and aphid populations (Johnson 2008). Studies such as these suggest that genetically variable traits within plants can impact common herbivores such as aphids.

The specialist aphid, *Uroleucon nigrotuberculatum* is an especially useful insect for the examination of herbivore colonization of *S. altissima*. This aphid occurs throughout the Eastern US and colonizes plants via winged individuals called alates, which then reproduce via parthenogenesis, forming clumped colonies on goldenrod plants (Cappuccino 1988). Genung et al. (2012b) demonstrated that *U. nigrotuberculatum* populations were affected by host plant genetic identity, but did not evaluate genetically driven mechanisms behind these different population levels. Investigations have examined how genetic variation and heritability impacted herbivore resistance, where it was found that heritability had a large role in governing resistance against *U. nigrotuberculatum* (Maddox and Root 1987). Specific genotypes of *S. altissima* exhibit differing levels of susceptibility to aphid colonization (Utsumi et al. 2011, Williams and Avakian 2015), though the mechanism behind this result is unknown. Though studies on this aphid demonstrate the role of intraspecific variation, prior

to my experiment it was largely unknown whether the effects of environment, genetic variation, or their potential interaction, were the factors that most governed the colonization of this aphid species on *S. altissima*.

Environment and Plant-Insect Relationships

The impact of environmental heterogeneity on plant-insect community structure is important to consider and understanding the contribution of the environment in relation to the effects of intraspecific genetic variation is important (Hersch-Green et al. 2011). The soil nitrogen environment has been demonstrated to impact insect communities, with higher soil nitrogen increasing herbivore populations (Throop and Lerdau 2004). In plants, the availability of essential soil macronutrients such as nitrogen and phosphorus are some of the environmental attributes that are likely to have a profound effect on plant physiological traits, and they may vary within the environment (Güsewell 2004). Nitrogen is essential to the production of enzymes such as RUBISCO, amino acids, nucleic acids, pigments like chlorophyll and secondary metabolites, whereas phosphorous is a component of cellular membranes, nucleic acids, energy storage and enzyme function (Maathuis 2009). Generally, elevated soil nitrogen is associated with increases in insect abundance (Herms 2002, Altieri and Nicholls 2003), while the effects of elevated phosphorus on insect abundance seem to vary case by case but have been shown to reduce insect abundance (Annan et al. 1997, Sun et al. 2000). An understanding of how differences in soil nitrogen and phosphorus impact foundational plant species and the insects that depend on them is important for determining the role that environmental heterogeneity may play in relation to genetic variation.

Because aphid species are often common and highly abundant herbivores associated with a variety of plants, they provide a good framework to address the effects of the soil environment. Aphids growing on milkweed plants experienced more rapid population growth rates and higher abundance as plant biomass and foliar nitrogen content increased (Zehnder and Hunter 2008). Another study found that aphids feeding on cucumber experienced higher reproduction rates and faster growth rates on plants growing in nitrogen enriched soil (Hosseini et al. 2010). Peach aphid growth exhibited a parabolic relationship, with the aphid populations increasing under moderate N fertilization but decreasing under high fertilization (Sauge et al. 2010). Annan et al. (1997) demonstrated that nitrogen fertilized cowpea fields hosted higher levels of aphid infestation, whereas fields fertilized with phosphorous had decreased aphid populations. This is result is interesting, as comparatively few studies have looked at the effects of elevated phosphorus and insect herbivores. For woody species, Nantucket pine tip moths were significantly more prevalent on nitrogen fertilized pines and marginally less prevalent on phosphorous fertilized pines as compared to a control (Sun et al. 2000). The authors attributed this to possible chemical differences in the tree's sap. Surprisingly few studies have incorporated plant genetic variation and insect responses to variation in soil nutrient levels.

Genotype, Environment and Interactions

An important consideration of community genetics investigations is to understand the role of genotype (G), environment (E) and the potential for G X E interactions (Johnson and Agrawal 2005, Hersch-Green et al. 2011). Understanding whether genotype or environment

primarily structures associated communities in foundational plant species may likely be based on the overall genetic diversity and phenotypic plasticity of the plant (Hughes et al. 2008). In some studies genetic diversity has been attributed to having the largest impact on associated insects (Wimp et al. 2004, Clark 2010, Evans et al. 2012), while in another the role of nutrient availability (environment effect) was stronger than the effects of genetic identity or diversity (Madritch et al. 2006). A genotype-environment (G X E) interaction demonstrates that trait differences among genotypes are due to environmental conditions, something that may result in forming a unique community structure associated with each genotype (Zuberi and Gale 1976). Johnson and Agrawal (2005) demonstrated this in their study that examined the insect communities associated with different genotypes of evening primrose. A G X E effect was observed that led to unique communities being formed on the plants between environments and genotypes. In the cases where a G X E effect is present, genetically distinct populations of plants may have their own characteristic associated communities of insects as a result of both genetic variation and variable responses to the environment.

Solidago altissima has an expansive range, from Northeastern and Central Canada to North Florida and Southern Texas and, as such, occurs in many different environments throughout its range (Halverson et al. 2008). Between different climates and habitats it is likely that *S. altissima* experiences different soil nutrients. In combination with extensive intraspecific genetic variation present in this species it is plausible that G X E effects could exist throughout its range. This interaction could lead to different genotypes impacting their herbivorous insect fauna in a unique habitat-dependent manner. Burkle et al. (2013) demonstrated G, E and G X E effects on flowering phenology, with a marginally significant

effect on floral visitor evenness between plants. Different nutrient treatments had variable effects on the flowering times of different genotypes. For the gall-forming *Eurosta solidaginis* oviposition on *S. altissima* differed significantly between genotypes and the relationship changed direction at different nutrient levels Horner and Abrahamson (1991). Studies such as these demonstrate that for *S. altissima* the examination of G, E, and their interaction is timely with respect to associated insects.

Plant Chemistry

Plant chemical composition is well known to impact plant herbivores (Langenheim 1994, Gershenzon and Dudareva 2007), including both nutrients and defensive chemicals. For example, plant nitrogen content is important for herbivorous insects as nitrogen is an essential macronutrient (Mattson 1980, Sauge et al. 2010). Similarly, the ratio of carbon to nitrogen within plants is regarded as an indicator of plant nutritive quality and has been associated with an increase in herbivorous insects as the ratio of carbon to nitrogen decreases (Herms and Mattson 1992, Haddad et al. 2001). Secondary metabolites represent a highly diverse group of phytochemicals, many of which have been demonstrated to act as both deterrents to insect feeding or as signaling molecules (Wink 2010, Nishida 2014). Though many are identified the functional roles of many compounds remain largely unknown, although it is suggested their functions lie in primarily defensive roles (Wink 2010) Terpenes are the most diverse class of secondary metabolites in higher plants (Langenheim 1994, Wink 2010), where they may attract pollinators, deter herbivores, or exhibit allelopathic suppression of other plants in an ecosystem (Langenheim 1994, Cheng et al. 2007). The effect of host plant terpene content on aphid abundance has been previously demonstrated,

but this likely varies based on the species of aphid as well as the host plant species (Klein and Müller 2011). Plant terpene content has been shown to vary under different nutritional regimes and soil types, though responses seem to vary case by case (Ormeño 2008, Ormeño and Fernandez 2012). With nitrogen and phosphorus fertilization there appears to be a species-dependent trend in responses, with some species responding positively in terms of terpene content, while others respond negatively or demonstrate no change in terpenes whatsoever (Ormeño and Fernandez 2012). Overall, the role of environment in governing plant terpene chemicals appears to situational, based on the species and soil nutrients in question. As such it merits further investigation to gain more insight into how terpenes may vary between environments.

Solidago altissima contains numerous mono-, sesqui- and diterpene compounds that may have roles as defensive or allelopathic compounds (Johnson et al. 2010). It is also known that tall goldenrod may vary in the production of terpenes among genotypes (Uesugi et al. 2013) and that this variation may affect the aphid *U. nigrotuberculatum* (Williams and Avakian 2015). In a previous study it was found that this specialist aphid had a marginally significant positive relationship with foliar β-pinene in *S. altissima* (Williams and Avakian 2015), and that overall 49% of variation in aphid colonization was explained by foliar terpene concentration. These results suggest a role of this secondary metabolite class for observed differences in abundance of this aphid species among genotypes of *S. altissima*. Other research has demonstrated that aphids may be deterred by certain volatile terpene chemicals released from host plants (Unsicker et al. 2009). There is still a great deal to be learned about the role of terpenes in plants, especially how genetics and environment influence the chemical profile of a plant and how associated insects respond to these

differences. In my investigation terpenes serve as a potential mechanism to explain the variation in aphid colonization of different genotypes grown under differing soil nutrient conditions.

The Williams laboratory reported that *S. altissima* exhibits considerable phenotypic plasticity with respect to traits important for insects and that *U. nigrotuberculatum* abundance was more affected by differences among genotypes than the environment from which plants were collected (Williams and Avakian 2015). Though insightful, that investigation was unable to separate effects of genotype from environment in this plant-insect system. My study addressed the effects of genotype (G) and environment (E) and a possible G X E interaction by manipulating the soil nutrient environment. I was especially interested in how plant attributes such as foliar terpenes, plant biomass, and leaf nutrients could affect aphid abundance. This study addresses a need in community genetics research as the independent role of genotype and environment, as well as their possible interaction, is not well understood. Additionally, there is still much to learn despite previous suggestions about the role of terpenes (especially) in shaping the relationship between *U. nigrotuberculatum* and *S. altissima*.

My study asked three main questions:

- How do soil nutrients (E) affect *U. nigrotuberculatum* abundance on different genotypes (G) of *S. altissima* and is there a G X E interaction?
- How do soil nutrients affect plant biomass, leaf terpenes and nutrients on different genotypes of *S. altissima* and is there a G X E interaction?
- Do terpenes and nutrients in *S. altissima* relate to *U. nigrotuberculatum* abundance and what is the role of genotype relative to environment?

I hypothesized that higher levels of soil nutrients would result in a higher abundance of aphids on *S. altissima* due to increases in certain aphid-attracting terpenes and that differences among genotypes in the chemical constituents I measured would result in G, E and G X E effects.

Materials and Methods

Study Species

Solidago altissima L. is a widespread and common plant species encountered in old-fields in our region that produces an underground rhizome sprouting multiple ramets to produce clonal patches (Maddox et al. 1989) and often occurs with multiple genotypes growing in proximity to each other (Maddox et al. 1989). Previous studies have examined effects of intraspecific genetic variation in this species (Crutsinger et al. 2006, Genung et al. 2012b), demonstrating the plant to be a good model system for our questions. The goldenrod specialist aphid *U. nigrotuberculatum* has previously been shown to differentially colonize various genotypes of *S. altissima* (Maddox and Root 1987, Utsumi et al. 2011, Williams and Avakian 2015) and as a specialist insect provides an especially appropriate model for addressing questions about the possible role of secondary metabolites for insect choice.

Plant Cultivation

Rhizomes from ramets of *S. altissima* were previously collected throughout Watauga County more than one year prior to the experiment and grown in 7.5 L pots (8.5 x 8.5x 10 cm) at the Appalachian State University (ASU) greenhouse. In April 2014, 3 cm rhizome

cuttings of six genotypes were grown in individual pots containing Metromix® 360 growing medium. Samples of plant tissue were used by Dr. Matt Estep at Appalachian State University for microsatellite analysis, which determined that six genotypes of *S. altissima* were distinct (see Appendix 1). In order to account for effects on biomass due to genotypic variations in rhizome size at the beginning of the experiment, a subset of rhizomes was measured for diameter and length in order to calculate their volume. Each plant had 50 mL of a 100: 1 dilution of Roots2® stimulator applied. Plants were watered as needed under greenhouse conditions until emergence and transplantation.

Experimental design

The experiment was set up using a common garden design in the courtyard of the ASU Biology greenhouse. Once the rhizome cuttings developed into individual plants approximately 10 cm in height these were planted in 19 L pots consisting of 50% Metromix© 360 and 50% sand. Five grams of soil inoculate obtained from a field of *S. altissima* was added to the mixture in order to ensure the presence of mycorrhizal spores, as there is evidence that mycorrhizal fungi could play an important role in plant terpene synthesis (Shrivastava et al. 2015). Genotypes were assigned to one of three nutrient treatments; nitrogen addition (8.2 g/m^2 of Espoma® Urea), phosphorus addition (14.4 g/m^2 of Bonide® Triple Super Phosphate), and control (no added nutrients). Each nutrient/genotype combination (i.e. treatment combination) was replicated three times. One replicate per treatment was randomly placed within a block consisting of 18 pots, resulting in a design with three blocks for a total of 54 pots that were spaced 0.33 m apart (See Fig. 1.).

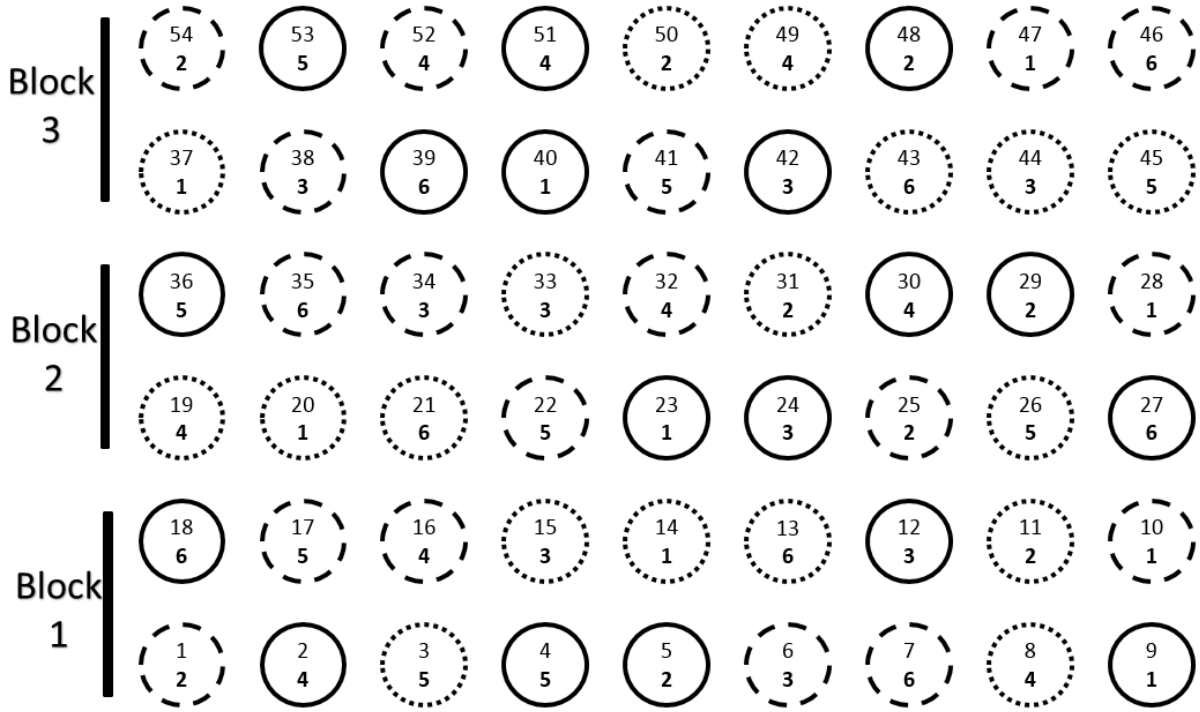

Fig. 1. Common garden layout. The upper number in each circle denotes plot number and the lower number the genotype. Nutrient treatment is shown by a dotted outline (phosphorus addition), dashed outline (nitrogen addition) or solid outline (control-no nutrients added).

Plant Chemistry and Insect Data Collection

Aphid abundance was recorded every three days after *U. nigrotuberculatum* were initially observed on the plants (June 18, 2014). The abundance of *U. nigrotuberculatum* peaked on July 9, 2014 across all pots. Aphid abundance measurements continued with weekly measurements until a second peak abundance was identified on September 3, 2014. At these times of peak abundance time leaf samples were taken and a non-destructive biomass estimate was conducted. The estimation followed a protocol previously described by Williams and Avakian (2015) and Howells (2012), where grams of above-ground biomass

was determined by measuring plant stem diameter (D) and height (H) according to this formula: Biomass (g) = (D^2H*0.0022)+6.3667 (R^2=0.70, P<0.0001). The aphid population was continually monitored until the growing season terminated, though no more chemical samples were taken. At the end of the growing season (October 3) plant above-ground biomass was harvested and quantified (dry mass).

Leaf Chemistry Analysis

Approximately 7-8 leaves totaling 4 g wet weight were taken from the mid-stem of each plant, chilled and later frozen at -20 °C for terpene analysis. Two leaves were collected and later freeze dried for carbon and nitrogen analysis. Leaf samples and biomass measurements followed the same protocol as above.

Terpenes were analyzed using a gas chromatography protocol modified from Johnson et al. (2010) and previously used in the Williams laboratory (Williams and Avakian 2015). In order to prepare samples for analysis, previously weighed frozen leaf samples (approximately 2 g) were allowed to thaw 5 minutes, cut into 1.5 cm sections and placed into a 50 mL culture tube along with 15 mL pentane. Each sample was then thoroughly ground using a Polytron© PT 10/35, Kinematica Inc., Luzern, Switzerland tissue homogenizer for 1.5 minutes. Each sample was then poured through filter paper and into a glass collection tube. The filtrate was evaporated to 0.5 mL by bubbling N_2 gas through the sample. A 1.0 µL sample was injected into a GC-14A gas chromatograph, Shimadzu Corporation, Kyoto, Japan. The program for the GC was as follows; injector temperature 250 ^{o}C; detector temperature 275 ^{o}C; initial column temperature 80 ^{o}C, increased 10 ^{o}C per minute to 280 ^{o}C

and a final hold for 2 minutes. The total run time was 24 minutes. Individual terpenes were identified by comparison to the retention times of known standards, and quantified using the internal standard tridecane. In rare cases when analytical standards were not available, a compound was identified using previous information from GC-Mass spec analysis (Howells 2012). Freeze- dried leaf samples for carbon and nitrogen analysis and determination of C:N ratios were analyzed using the Thermo Fisher EA 1112 Elemental Analyzer, Thermo Fisher Scientific Inc., Waltham, MA, USA and were carried out by Dr. Mike Madritch in his laboratory at Appalachian State University.

Statistical Analyses

A general linear model (Proc GLM, SAS ver. 9.3, SAS Institute Inc., Cary, NC, USA) was used to analyze the effects of genotype (G), nutrient (E) and G X E interaction on aphid abundance, phytochemistry and plant biomass. Data were analyzed in three ways; first by averaging the two sampling dates and second by comparing dates using a repeated measures ANOVA, followed by each date being independently analyzed (hereafter generation 1 (G1) and generation 2 (G2). The independent variables were genotype and nutrient treatments, and the dependent variables were aphid abundance, rhizome volume, plant dry weight, leaf N, C:N, and terpene concentration. Root volume was found to differ among genotypes so my general linear model included the initial rhizome volume as a covariate to account for potential effects on plant final biomass between treatments. A post-hoc analysis was conducted by running a Tukey's test on the means of fertilization treatments and genotypes. Marginally significant relationship were defined by $0.05< P <0.10$.

To examine the potential that plant proximity could affect aphid abundance (nearest neighbor or autocorrelation effects), a linear regression model was developed using the mean aphid abundance of all plots surrounding a focal pot following Haddad et al. (2001). I found no significant nearest neighbor effects ($P<0.05$) for the first (G1:F=3.21, df =1,43, $R^2=0.069$, $P=0.080$) or second (G2: $F=0.24$, df =1,43, $R^2=0.006$, $P=0.625$) sample dates.

Relationships between phytochemistry and aphid abundance were analyzed using both simple linear regression (Proc Reg, SAS ver 9.3 SAS Institute Inc., Cary, NC, USA) and Partial Least Squares Regression (PLSR; JMP Pro 10, SAS Institute Inc., Cary, NC, USA). This is an appropriate multivariate technique for modeling the effects of phytochemistry on aphid abundance if collinearity exists between variables (Wold 1984; Wold et al. 2001). Partial least squares regression has been used for ecological investigations to examine the relationship between phytochemicals and insect performance (Couture et al. 2012) and to relate terpenes to aphid abundance (Williams and Avakian 2015). A critical consideration of the model is using the appropriate number of latent variables so as not to "over fit" the data. Therefore, a cross-validation technique described as "leave one out" was used (Cox and Gaudard 2013). A Variable Importance Projection (VIP) determined the predictor variables (i.e., plant chemical constituents) in the model that showed the best response between both predictor and response matrices. Regressing observed versus predicted values provides the relationship between plant chemistry and aphid abundance. Linear regressions were run on treatment-level means generated by averaging the three replicates of each treatment.

Results

Average Data

Aphid Abundance

A G X E interaction effect was observed on aphid abundance (F= 2.34, df= 8,30, P= 0.0434). Tukey's test demonstrated more aphids on nitrogen fertilized plants (Fig. 2A), followed by phosphorus fertilized plants, while the fewest were found on the control plants. An exception to this was genotype 2, which had the fewest aphids on nitrogen fertilized plants and the most aphids on the control plants (Fig. 2A). These variations in abundance among nutrient treatments and genotypes likely explain the G X E interaction effect that was observed.

Plant Biomass

Plant biomass varied by both environment (F= 21.14, df= 2, 30, P<0.001) and genotype (F= 38.43, df= 4,30, P<0.001). There was no observed interaction effect (P>0.05). Nitrogen fertilized plants had the highest biomass, with phosphorus fertilized and control plants having similar biomass (Fig. 2B).

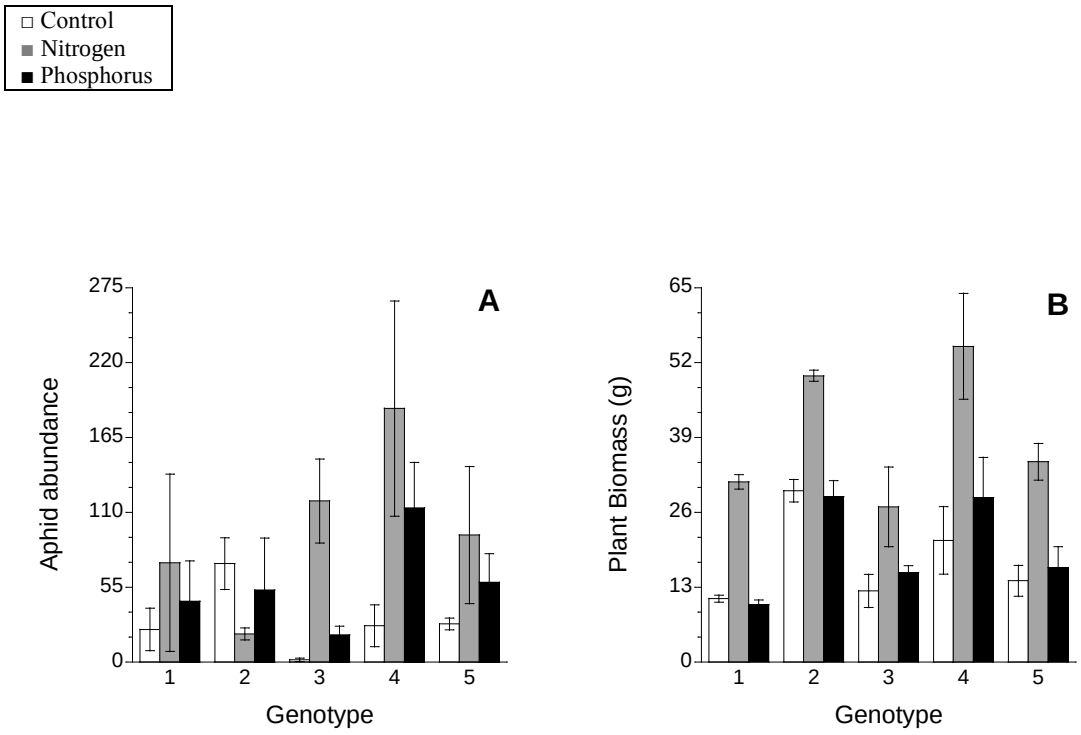

Fig. 2. (A) Aphid abundance averaged between G1 and G2 and (B) final plant biomass by treatment.

Phytochemistry

Foliar nitrogen content was significantly affected by environment and genotype, but there was no interaction effect (Table 1). While phosphorus fertilized and control plants had similar leaf nitrogen contents, the average foliar nitrogen content was higher among the nitrogen fertilized plants (Fig. 3A). Foliar C:N varied in a similar fashion to leaf N, differing between environments, with a marginally significant difference between genotypes and no G X E interaction (Table 1).

Terpenes were largely affected by both environment and genotype and a significant G X E interaction was found for the terpenes limonene, γ-elemene and caryophyllene (marginal) (Table 1). These GXE interactions were largely driven by the higher concentration of limonene and γ-elemene in genotype 1 and in the higher caryophyllene concentration in genotype 4, as demonstrated by Tukey's test. Though considerable variation was observed, overall terpene concentration was highest in control and phosphorus fertilized plants, and differed among genotypes (Figs. 4 and 5). Plant genotype was the only significant factor by which terpene allocation varied (Table 2).

Table 1. Two-way ANOVA for phytochemicals using averaged data. E = environment, G = genotype, G X E = genotype x environment interaction. Values of P <0.05 are presented in **bold** text.

	E		***G***		***G X E***	
Phytochemistry	*F*	*P*	*F*	*P*	*F*	*P*
Nitrogen (mg/g)	**30.12**	**<.0001**	**7.17**	**0.0004**	0.86	0.5625
C:N (mg/mg)	**4.89**	**0.0145**	2.40	0.0718	0.45	0.8816
Terpenes (mg/g)						
α-pinene	**4.50**	**0.0196**	**19.79**	**<.0001**	1.00	0.4586
β-pinene	**3.56**	**0.0409**	**14.91**	**<.0001**	0.83	0.5821
p-cymene	**4.15**	**0.0257**	**12.58**	**<.0001**	0.85	0.5685
Limonene	**4.11**	**0.0264**	**56.76**	**<.0001**	1.98	0.0848
caryophyllene	**5.98**	**0.0065**	**102.94**	**<.0001**	**3.82**	**0.0034**
Germacrene	**3.58**	**0.0405**	**7.13**	**0.0004**	0.53	0.8257
Azulene	1.99	0.1548	**6.14**	**0.0010**	0.44	0.8888
γ-elemene	**6.45**	**0.0047**	**28.34**	**<.0001**	**2.75**	**0.0211**

df: environment= 2,30; genotype= 4,30; environment X genotype= 8, 30

Table 2. Two-way ANOVA for allocation of terpenes using average data. . E = environment, G = Genotype, G X E = genotype x environment interaction. Values of *P* <0.05 are presented in **bold** text.

	E		***G***		***G X E***	
Terpenes (mg/g)	*F*	*P*	*F*	*P*	*F*	*P*
α-pinene	0.34	0.7174	**28.61**	**<.0001**	0.93	0.5071
β-pinene	2.09	0.1414	**15.08**	**<.0001**	0.87	0.5542
p-cymene	1.16	0.3281	**42.37**	**<.0001**	1.21	0.3261
Limonene	1.60	0.2193	**85.69**	**<.0001**	0.65	0.7289
Caryophyllene	2.48	0.1008	**676.19**	**<.0001**	1.42	0.2276
Germacrene	0.38	0.6884	**62.84**	**<.0001**	1.17	0.3479
Azulene	0.54	0.5877	**11.65**	**<.0001**	0.41	0.9071
γ-elemene	0.83	0.4475	**31.93**	**<.0001**	1.54	0.1869

df: environment= 2,30; genotype= 4,30; environment X genotype= 8, 30

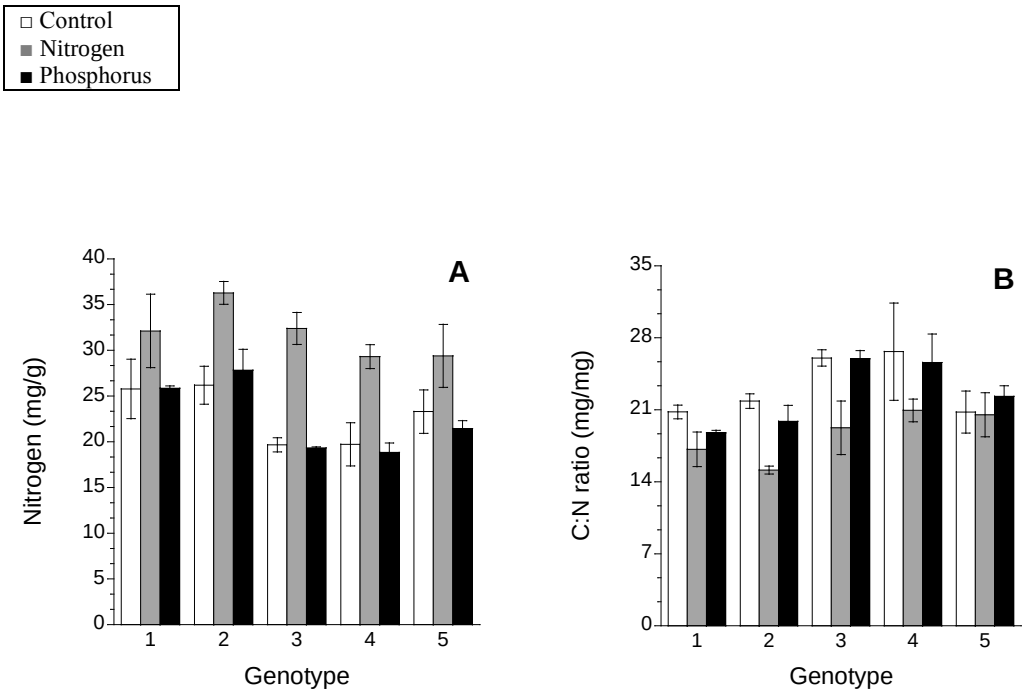

Fig. 3. (A) Plant nitrogen content and (B) carbon-nitrogen ratio by treatment averaged between G1 and G2.

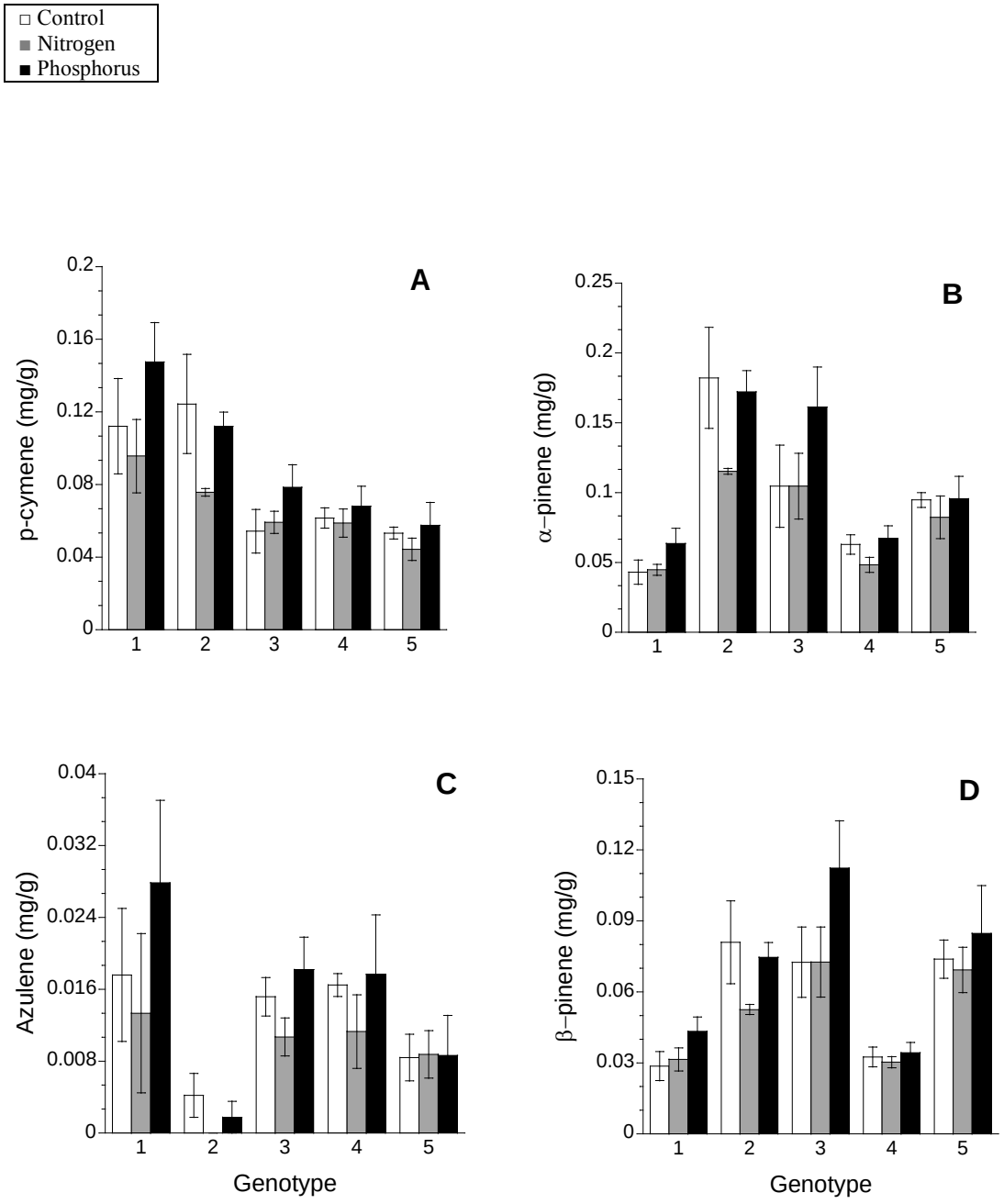

Fig. 4. Plant terpene concentration for (A) α-pinene, (B) p-cyemene, (C) azulene and (D) β-pinene by treatment, averaged between G1 and G2.

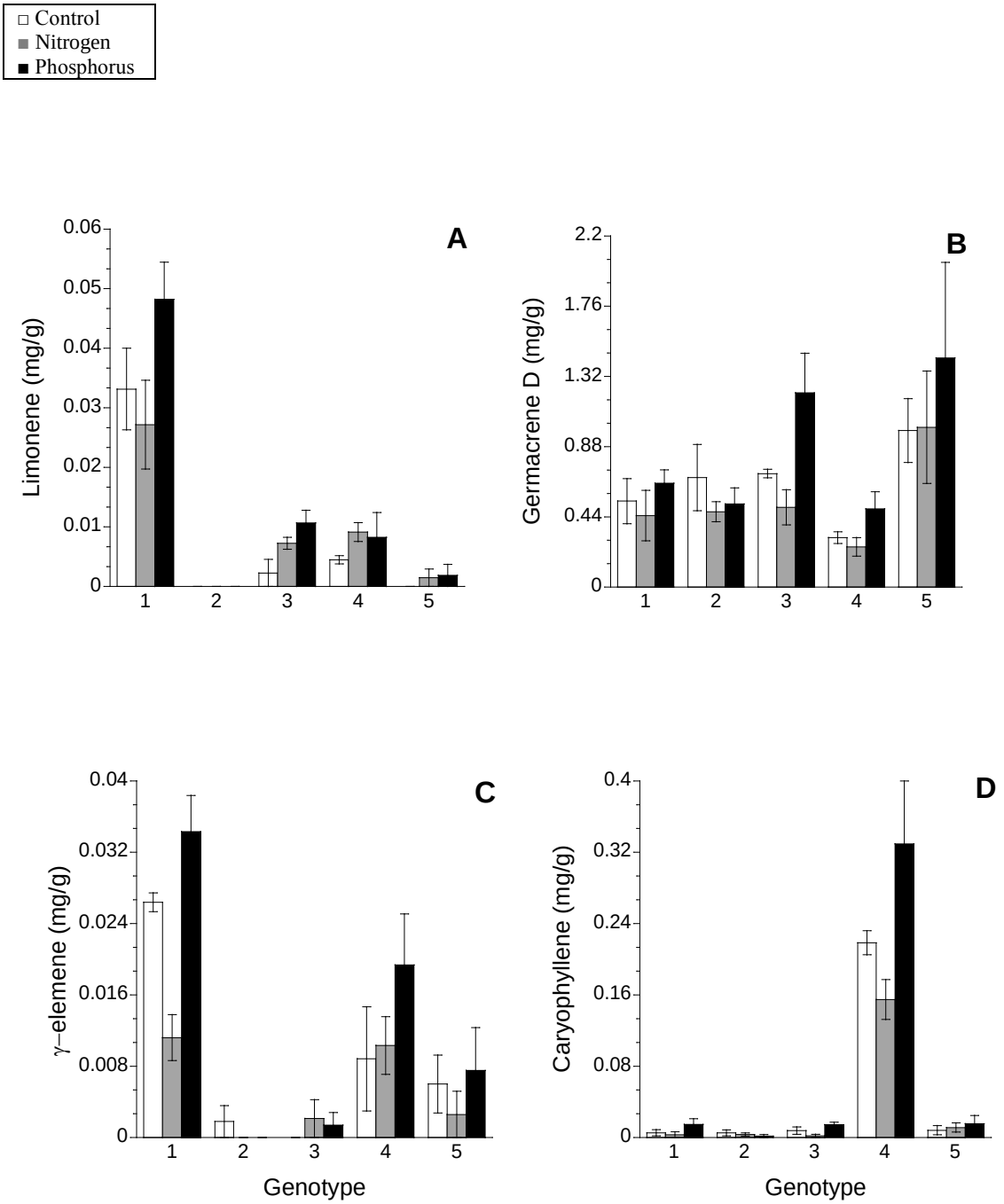

Fig. 5. Plant terpene concentration for (A) limonene, (B) germacrene D, (C) caryophyllene and (D) γ-elemene by treatment, averaged between G1 and G2.

Linear Regression Analysis

A statistically significant positive relationship between the natural log of aphid abundance and final plant biomass was observed (Fig. 6). It is notable that when the control treatment of genotype 3 plants is removed the relationship became marginally significant (P= 0.060, R^2=0.265). Significant and marginally significant relationships between phytochemicals and nitrogen-fertilized plants were observed for azulene (marginally significant), nitrogen and C:N ratio (Fig. 7). Aphid abundance declined with increasing leaf nitrogen.

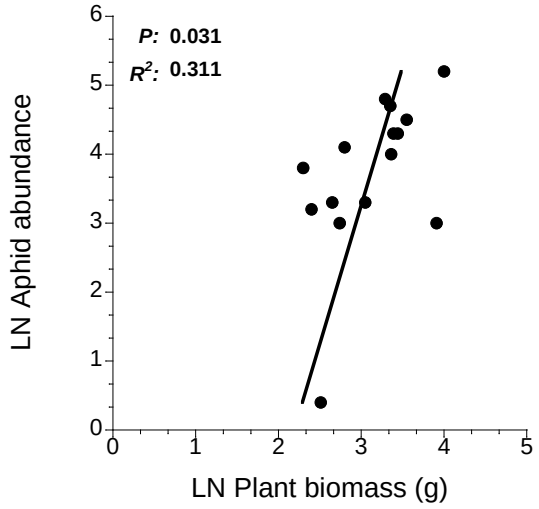

Fig. 6. The relationship between aphid abundance averaged between G1 and G2 and final plant biomass

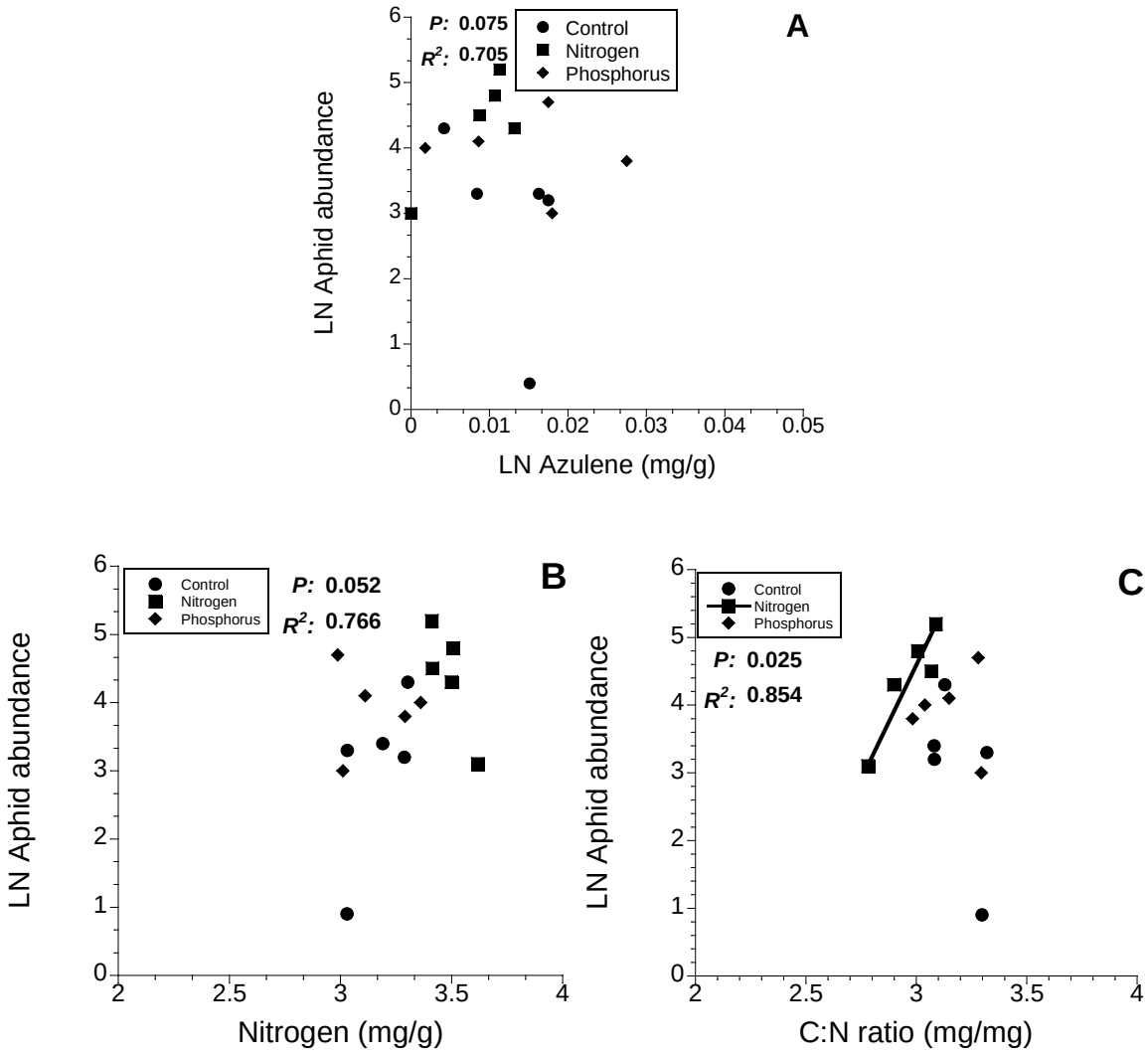

Fig. 7. The relationship between aphid abundance and (A) azulene, (B) nitrogen and (C) C:N ratio averaged between G1 and G2.

Partial Least Squares Regression Analysis

For aphid abundance in all plants the partial least squares regression (PLSR) model found that phytochemicals explained a significant amount of the variation in aphid abundance (R^2= 0.287, P<0.0001; Fig. 8). When fertilization treatments were separated, the strongest relationship in the model was seen in nitrogen-fertilized plants (R^2= 0.789, P<0.0001; Fig. 9B). PLSR demonstrated that phytochemistry explained 52% of the variation in phosphorus fertilized (R^2= 0.520, P=0.0024; Fig. 9C) and 70.6% of the variation in control plants (R^2= 0.706, P<0.0001; Fig. 9A).

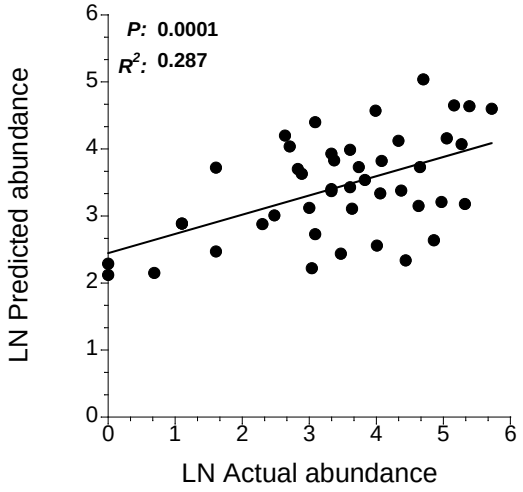

Fig. 8. Partial least squares regression of the relationship between phytochemical data and aphid abundance for all plants.

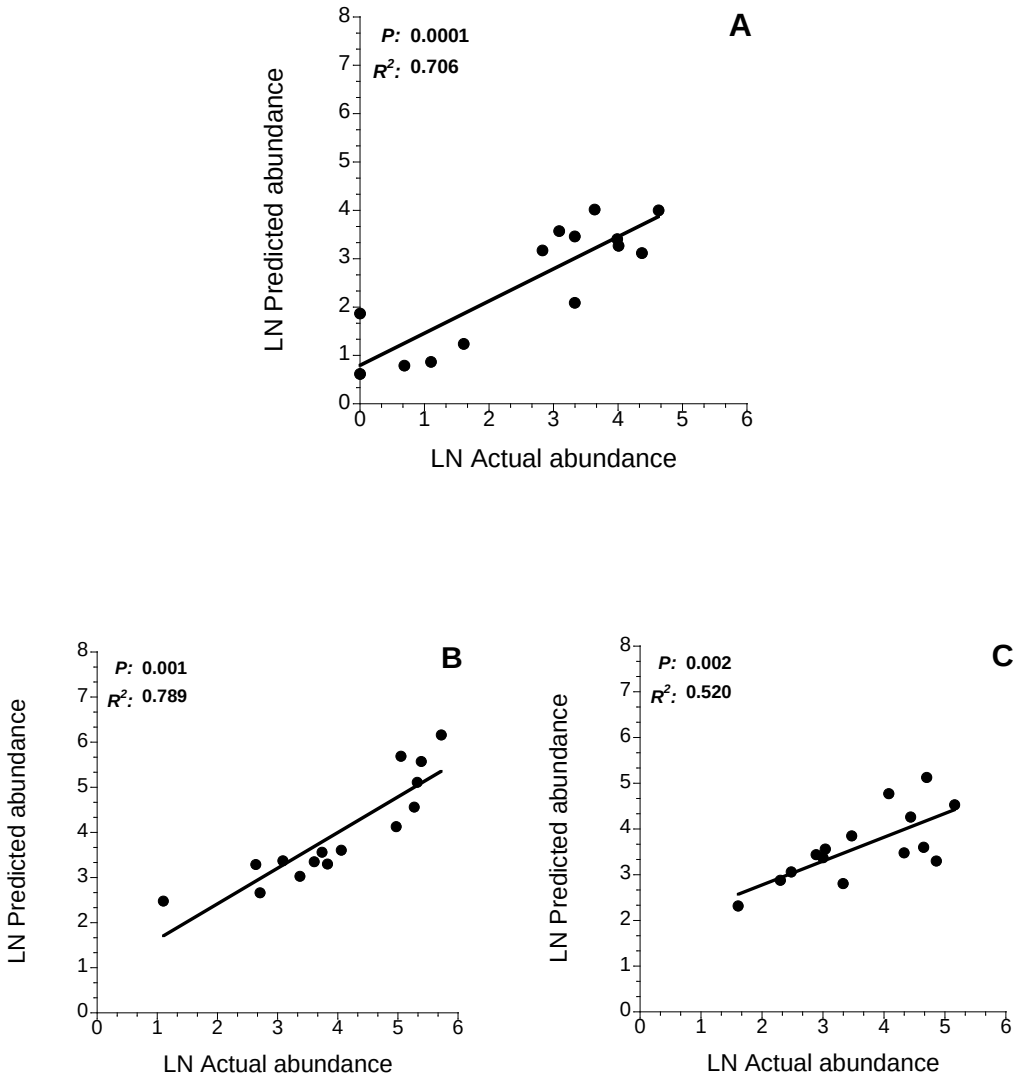

Fig. 9. Partial least squares regression of the relationship between phytochemical data and aphid abundance in (A) control, (B) nitrogen fertilized and (C) phosphorus fertilized plants.

Generation Data

Aphid Abundance

The repeated measures analysis of the two aphid generations on July 9 (G1) and September 3 (G2) found there was no statistical effect of environment, genotype, while there was a marginal G X E interaction (Table 3). For G1 there was no significant effect of G (F=1.45, df= 4,30, P=0.2418) or E (F= 1.76, df= 2,30, P=0.190), with a significant G X E effect observed (F= 2.60, df= 8,30, P= 0.0272). This can be seen by how aphid abundance varies between fertilizations and genotypes in G1, and was supported by Tukey's tests (Fig. 10A). For G2 there was an effect of E on aphid abundance (F= 6.55, df= 2,30, P= 0.0044) but no effect of G (F= 0.98, df= 4,30, P=0.4352). A marginal G X E effect (F= 1.89, df= 8,30, P= 0.0989) was observed. The environmental effect was largely driven by a much higher aphid abundance in nitrogen fertilized plants, demonstrated by Tukey's test, which generally had greater aphid abundance (Fig. 10B). The interaction effect comes from the difference between nutrient treatments in some genotypes (e.g., genotype 2) and not others (Fig. 10B).

Table 3. Repeated measures two-way ANOVA (Proc Glm) for terpenes in generations 1 and 2. . E = environment, G = Genotype, G X E = genotype x environment interaction. Values of *P* <0.05 are presented in **bold** text.

	E		***G***		***G X E***	
	F	*P*	*F*	*P*	*F*	*P*
Aphid abundance	2.43	0.1055	1.97	0.1252	2.14	0.0631
Estimated biomass	**19.10**	**<.0001**	**12.61**	**<.0001**	0.57	0.7939
Phytochemistry						
Nitrogen (mg/g)	**30.12**	**<.0001**	**7.17**	**0.0004**	0.86	0.5625
C:N (mg/mg)	**4.89**	**0.0145**	2.40	0.0718	0.45	0.8816
Terpenes (mg/g)						
α-pinene	**4.58**	**0.0184**	**19.82**	**<.0001**	1.03	0.4333
β-pinene	**3.59**	**0.0401**	**14.90**	**<.0001**	0.85	0.5689
p-cymene	**4.10**	**0.0267**	**12.33**	**<.0001**	0.85	0.5699
Limonene	**4.07**	**0.0274**	**56.22**	**<.0001**	1.96	0.0875
Caryophyllene	**7.39**	**0.0025**	**142.39**	**<.0001**	**4.55**	**0.0010**
Germacrene	**3.71**	**0.0365**	**7.68**	**0.0002**	0.56	0.8046
Azulene	1.99	0.1541	**6.14**	**0.0010**	0.44	0.8902
γ-elemene	**6.39**	**0.0049**	**28.30**	**<.0001**	**2.71**	**0.0223**
Terpene allocation						
α-pinene	0.22	0.8021	**32.35**	**<.0001**	0.69	0.6937
β-pinene	1.90	0.1671	**15.13**	**<.0001**	1.00	0.4573
p-cymene	2.20	0.1281	**13.86**	**<.0001**	1.51	0.1956
Limonene	3.27	0.0520	**90.01**	**<.0001**	0.78	0.6249
Caryophyllene	2.63	0.0888	**738.11**	**<.0001**	1.33	0.2663
Germacrene	0.85	0.4393	**83.41**	**<.0001**	1.30	0.2833
Azulene	0.72	0.4973	**11.67**	**<.0001**	0.32	0.9510
γ-elemene	0.03	0.9750	**31.49**	**<.0001**	1.10	0.3907

df: environment= 2,30; genotype= 4,30; environment X genotype= 8, 30

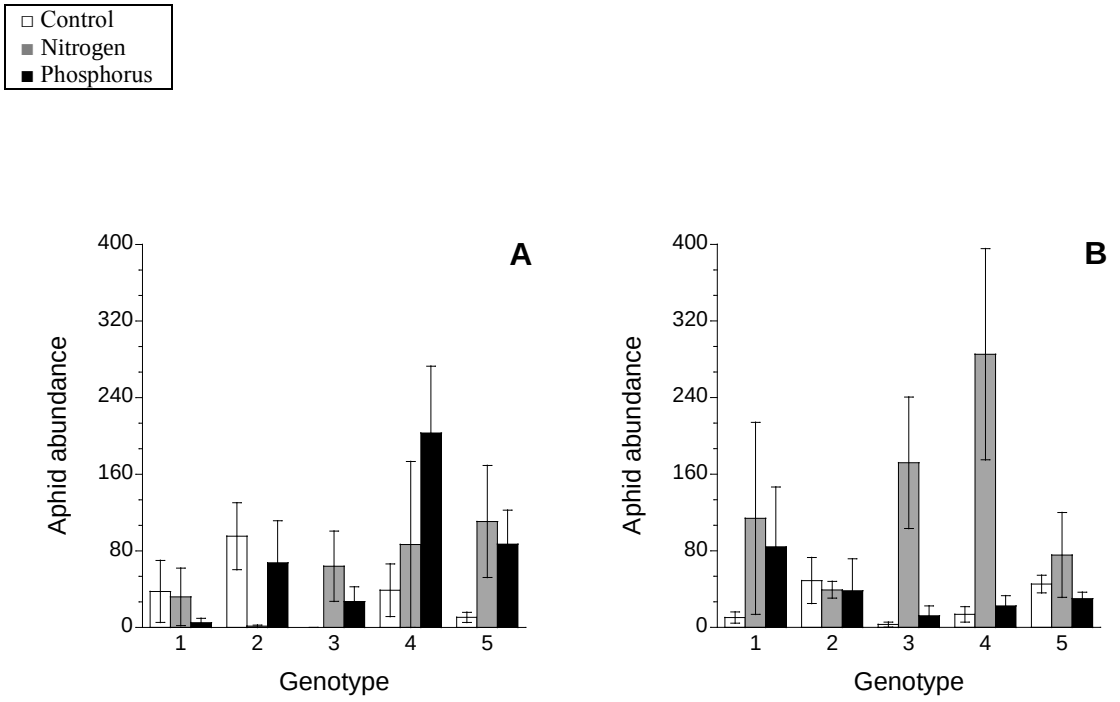

Fig. 10. Aphid populations by treatment at (A) G1 and (B) G2.

Repeated measures analysis of phytochemistry between the two generations demonstrated that nearly all phytochemicals varied between G1 and G2 for environment and genotype (Table 3). A significant G X E between G1 and G2 was observed in γ-elemene and a marginal G X E interaction in limonene (Table 3). Tukey's test shows that these were driven by higher concentrations of γ-elemene and limonene in genotype 1. A G X E interaction was also seen in caryophyllene, with Tukey's test showing this relationship to be driven by higher concentrations of caryophyllene in genotype 4 (Table 3). At G1 foliar nitrogen and C:N ratio significantly differed by both environment and genotype (Table 4). During G2 plants were only significantly different in foliar nitrogen content among genotypes (Table 5). Nitrogen content decreased in all plants from G1 to G2, however,

during both periods nitrogen was generally higher among nitrogen-fertilized plants (Fig. 12). Between the two generations mean foliar C:N ratios were generally lower in nitrogen fertilized plants, but the strength of this effect decreased during G2 (Fig. 11).

Terpene concentration at G1 was largely different among genotypes, with few E or G X E effects observed (Table 4). The terpene p-cymene had a marginally significant effect of G, with a significant G X E interaction demonstrated for the monoterpene α-pinene (Table 4). Terpene concentration at G2 largely differed between genotypes and environments (Table 5). A G X E effect was seen in γ-elemene at G2 (Table 5). The allocation of terpenes at G1 and 2 were mostly similar in terms of the effects of G, E and G X E, with only G contributing to significant variation in terpene allocation in *S. altissima* plants (Tables 6 and 7).

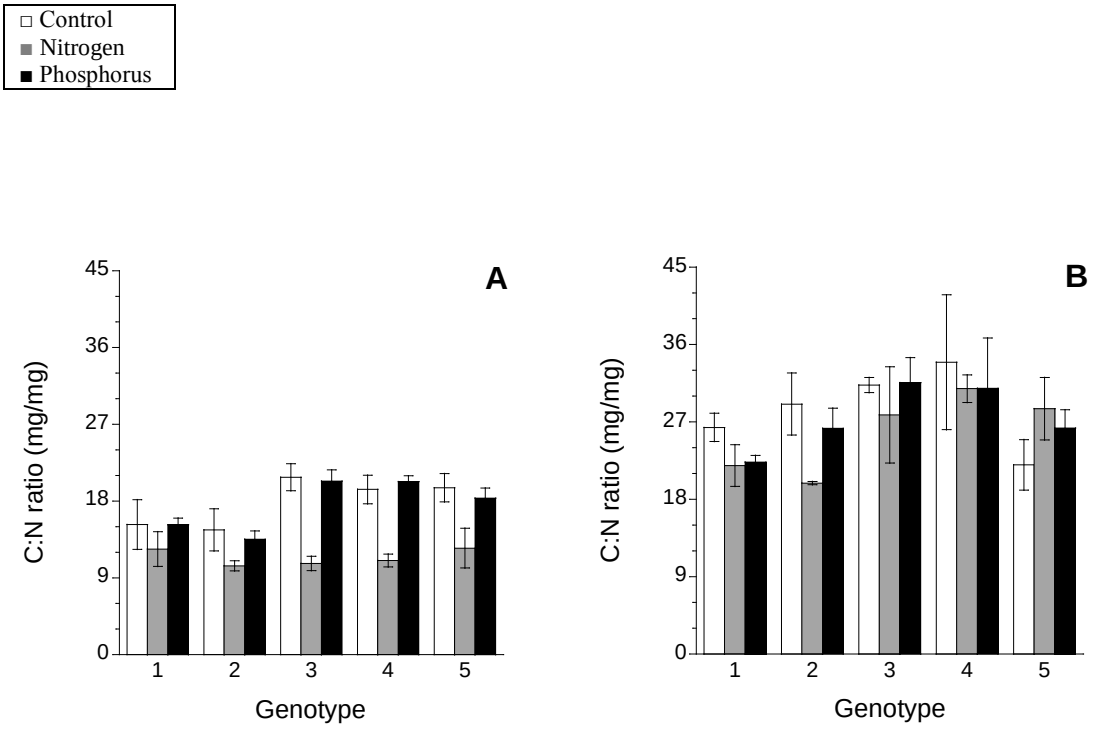

Fig. 11. C:N ratio by treatment at (A) G1 and (B) G2.

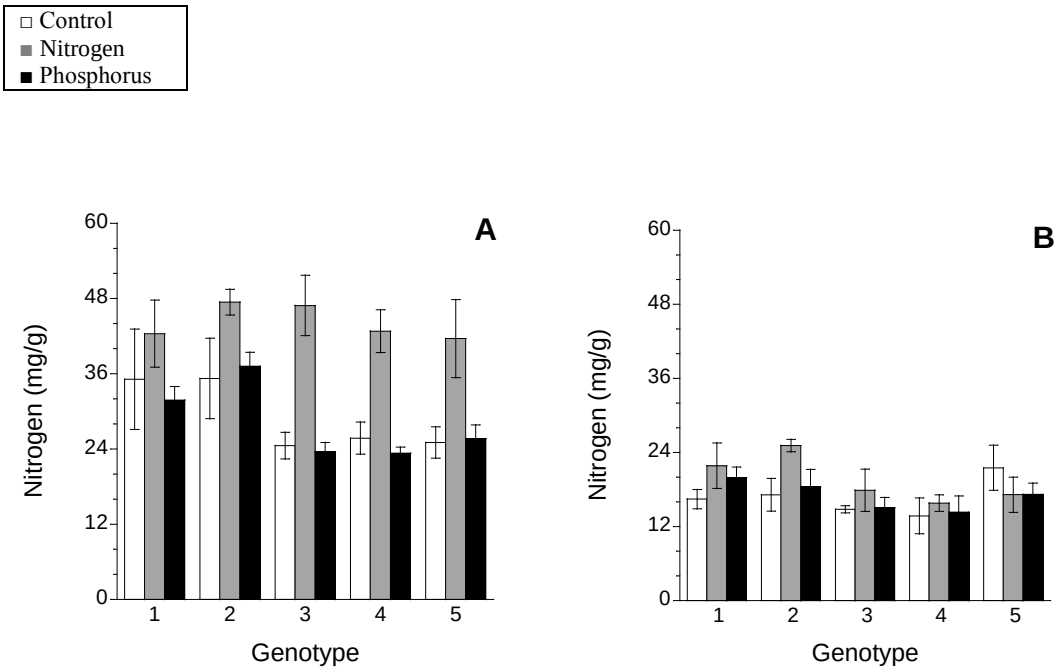

Fig. 12. Nitrogen content by treatment at (A) G1 and (B) G2

Table 4. Two-way ANOVA for phytochemicals for G1. E = environment, G = Genotype, G X E = genotype x environment interaction. Values of *P* <0.05 are presented in **bold** text.

	E		***G***		***G X E***	
Phytochemistry	*F*	*P*	*F*	*P*	*F*	*P*
Nitrogen (mg/g)	**26.13**	**<.0001**	**4.06**	**0.0096**	0.91	0.5196
C:N (mg/mg)	**25.54**	**<.0001**	**3.55**	**0.0174**	1.02	0.4412
Terpenes (mg/g)						
α-pinene	2.73	0.0814	**6.28**	**0.0008**	2.79	0.0196
β-pinene	1.60	0.2188	**6.62**	**0.0006**	1.73	0.1313
p-cymene	0.94	0.4018	1.99	0.1214	0.66	0.7187
Limonene	0.81	0.4542	**12.13**	**<.0001**	1.00	0.4594
Caryophyllene	1.27	0.2964	**40.86**	**<.0001**	0.63	0.7472
Germacrene	2.23	0.1251	**6.95**	**0.0004**	1.05	0.4198
Azulene	1.30	0.2874	**3.71**	**0.0143**	0.46	0.8756
γ-elemene	0.58	0.5646	**4.09**	**0.0092**	0.35	0.9361

df: environment= 2,30; genotype= 4,30; environment X genotype= 8, 30

Table 5. Two-way ANOVA for phytochemicals for G2. E = environment, G = Genotype, G X E = genotype x environment interaction. Values of *P* <0.05 are presented in **bold** text.

	E		***G***		***G X E***	
Phytochemistry	*F*	*P*	*F*	*P*	*F*	*P*
Nitrogen (mg/g)	1.62	0.2155	**2.74**	**0.0468**	0.72	0.6735
C:N (mg/mg)	0.94	0.4026	**3.36**	**0.0218**	1.11	0.3869
Terpenes (mg/g)						
α-pinene	**4.44**	**0.0204**	**18.06**	**<.0001**	0.77	0.6326
β-pinene	**4.19**	**0.0249**	**13.12**	**<.0001**	0.73	0.6641
p-cymene	**4.49**	**0.0196**	**14.80**	**<.0001**	0.81	0.5960
Limonene	**4.83**	**0.0151**	**63.66**	**<.0001**	1.60	0.1666
Caryophyllene	2.08	0.1427	**26.16**	**<.0001**	1.64	0.1542
Germacrene	2.90	0.0708	**4.22**	**0.0080**	0.30	0.9600
Azulene	1.13	0.3361	**4.88**	**0.0038**	0.54	0.8180
γ-elemene	8.16	0.0015	**29.71**	**<.0001**	**3.71**	**0.0041**

df: environment= 2,30; genotype= 4,30; environment X genotype= 8, 30

Table 6. Two-way ANOVA for allocation of terpenes for G1. E = environment, G = Genotype, G X E = genotype x environment interaction. Values of *P* <0.05 are presented in **bold** text.

	E		***G***		***G X E***	
Terpenes (mg/g)	*F*	*P*	*F*	*P*	*F*	*P*
α-pinene	1.47	0.2468	**34.53**	**<.0001**	1.43	0.2234
β-pinene	1.58	0.2235	**8.54**	**0.0001**	1.35	0.2594
p-cymene	2.20	0.1281	**13.86**	**<.0001**	1.51	0.1956
Limonene	0.53	0.5930	**15.39**	**<.0001**	0.65	0.7317
Caryophyllene	2.71	0.0828	**557.25**	**<.0001**	1.17	0.3504
Germacrene	1.83	0.1779	**81.06**	**<.0001**	2.02	0.0787
Azulene	0.51	0.6079	**8.42**	**0.0001**	0.53	0.8241
γ-elemene	0.23	0.7942	**8.49**	**0.0001**	0.66	0.7250

df: environment= 2, 30; genotype= 4, 30; environment X genotype= 8, 30

Table 7. Two-way ANOVA for allocation of terpenes for G2. E = environment, G = Genotype, G X E = genotype x environment interaction. Values of *P* <0.05 are presented in **bold** text.

	E		***G***		***G X E***	
Terpenes (mg/g)	*F*	*P*	*F*	*P*	*F*	*P*
α-pinene	0.03	0.9673	**17.33**	**<.0001**	0.41	0.9049
β-pinene	1.25	0.3023	**12.34**	**<.0001**	0.53	0.8228
p-cymene	0.87	0.4294	**36.27**	**<.0001**	1.03	0.4356
Limonene	3.52	0.0425	**90.84**	**<.0001**	1.01	0.4506
Caryophyllene	1.40	0.2628	**572.93**	**<.0001**	1.01	0.4522
Germacrene	0.07	0.9345	**31.05**	**<.0001**	0.45	0.8803
Azulene	0.47	0.6283	**7.99**	**0.0002**	0.39	0.9189
γ-elemene	2.09	0.1411	**51.36**	**<.0001**	1.12	0.3799

df: environment= 2, 30; genotype= 4, 30; environment X genotype= 8, 30

Linear Regression Analysis

At G1, aphid abundance was positively correlated with the terpene azulene in the nitrogen fertilized plants (Fig. 13A). Among the phosphorus fertilized plants there was a statistically significant negative relationship between aphid abundance, p-cymene and limonene (Fig. 13B and 13C). The phosphorus-fertilized plants in G2 demonstrated a marginally positive relationship between aphid abundance and foliar p-cymene (marginal), and nitrogen content and a negative correlation between aphid abundance and C:N ratio (Fig. 14).

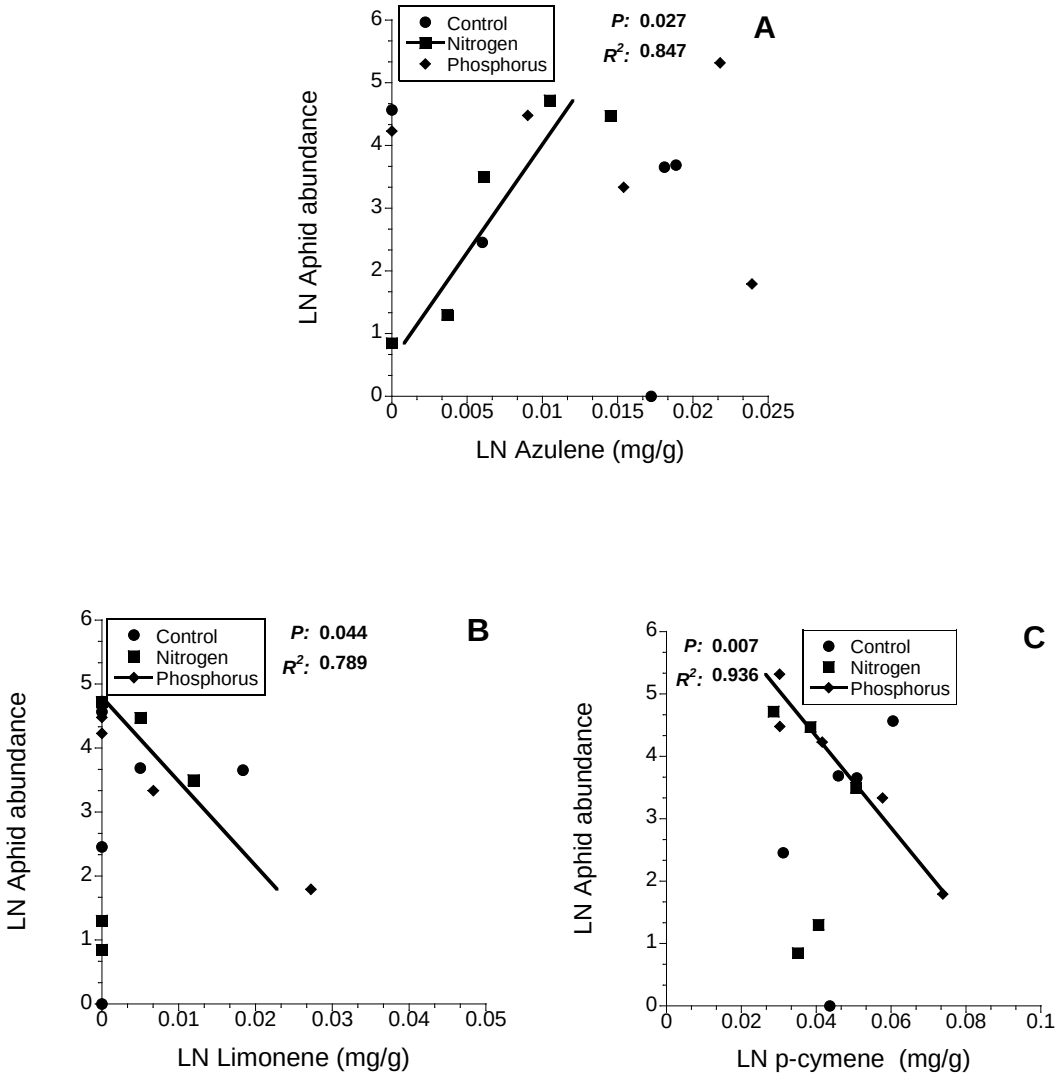

Fig. 13. The relationship between aphid abundance and (A) azulene, (B) limonene and (C) p-cymene at G1.

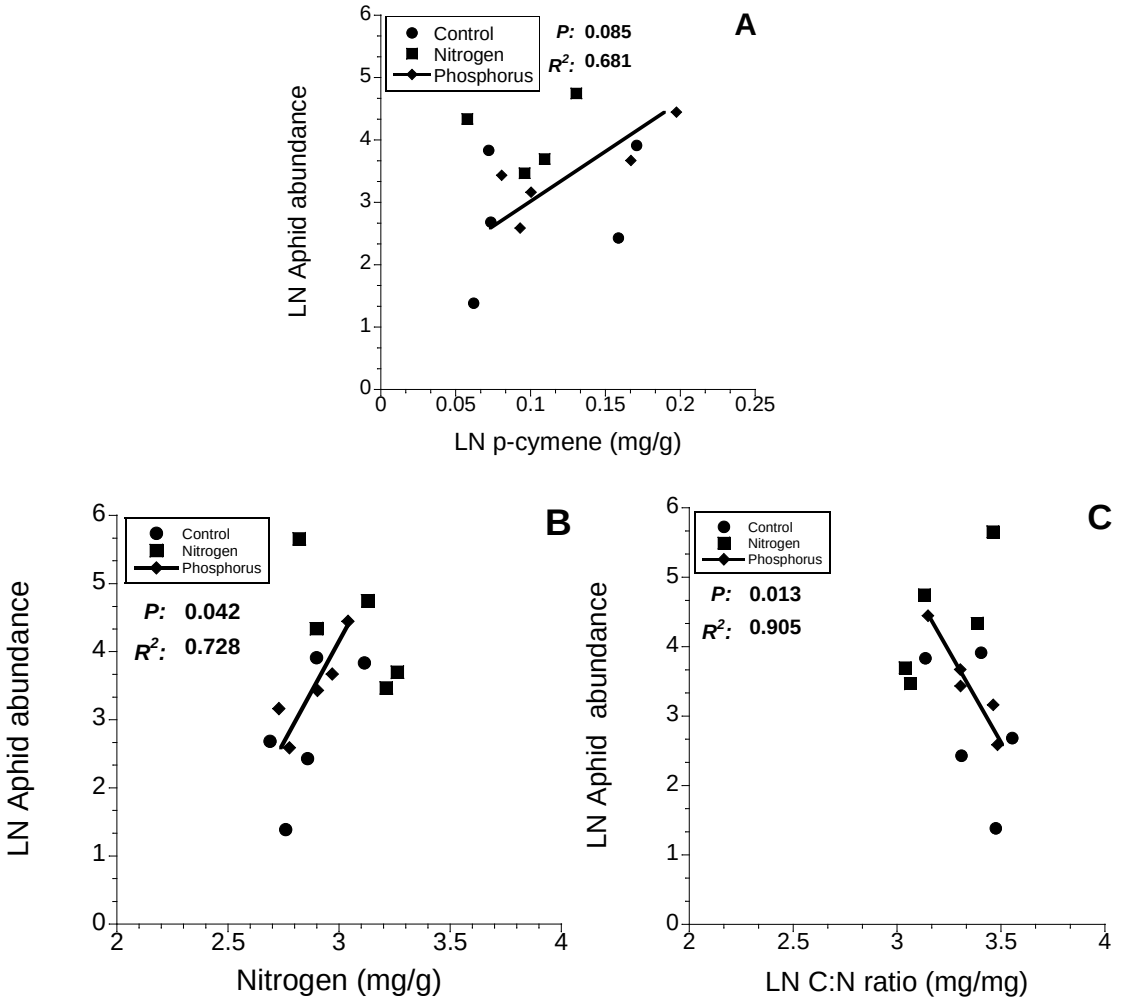

Fig. 14. The relationship between aphid abundance and (A) p-cymene, (B) nitrogen and (C) C:N ratio at G2.

Partial Least Squares Regression Analysis

For aphid abundance in G1, the partial least squares regression (PLSR) model of phytochemicals explained a significant amount of the variation in aphid abundance when nutrient treatments were analyzed separately (Fig. 15B,C,D), but was weakly significant when all treatments were analyzed together (Fig. 15A). In G2 PLSR of phytochemicals was not able to explain aphid abundance when all treatments were considered together (Fig. 16A) and was only able explain with marginal significance the relationship between aphids and phytochemicals in the phosphorus treatment (Fig. 16D). However, in G2 PLSR demonstrated that phytochemistry served as a significant predictor of aphid abundance in nitrogen and control treatments (Fig. 16B, C).

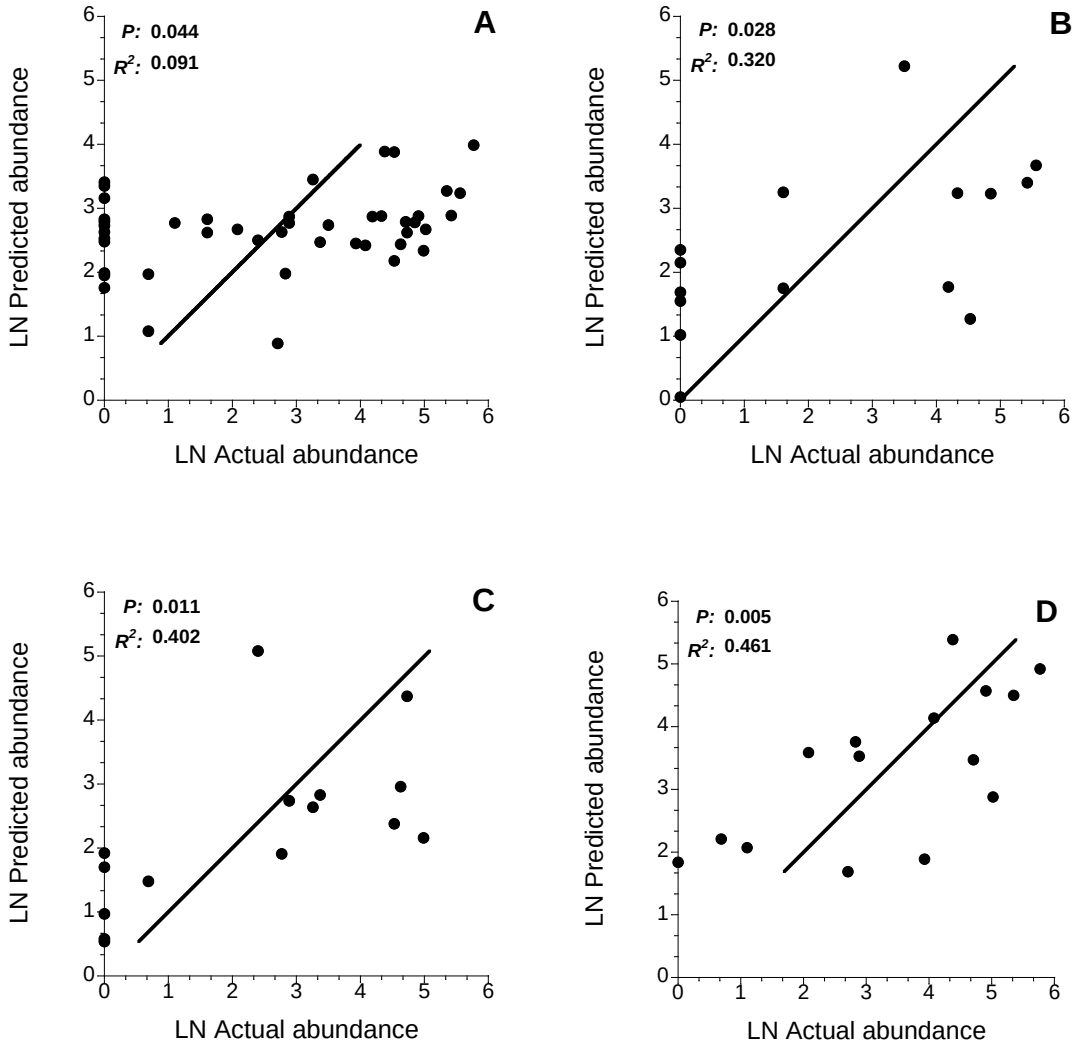

Fig. 15. Partial least squares regression of the relationship between phytochemical data and aphid abundance in (A) all plants, (B) control, (C) nitrogen fertilized and (D) phosphorus fertilized plants, at G1.

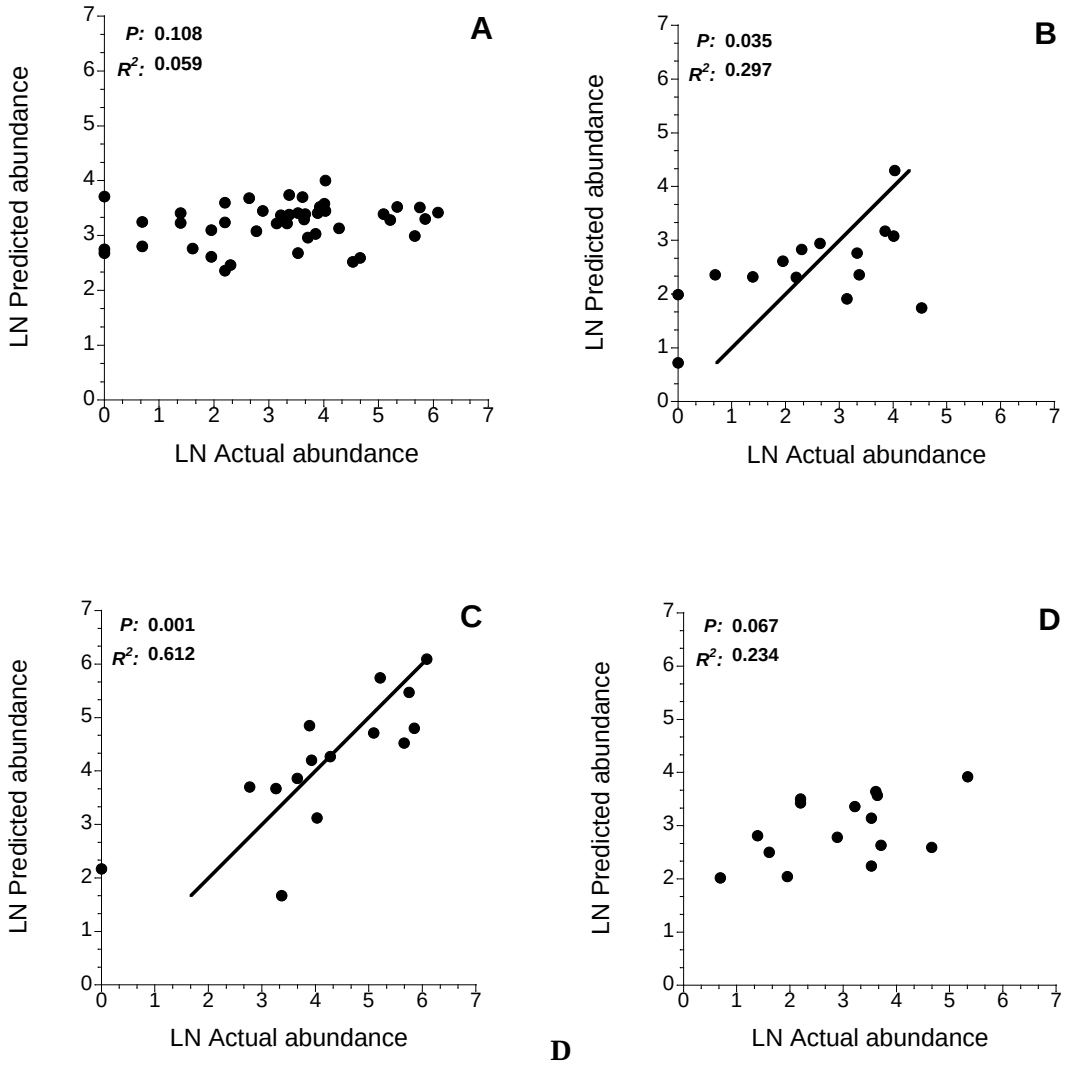

Fig. 16. Partial least squares regression of the relationship between phytochemical data and aphid abundance in (A) all plants, (B) control, (C) nitrogen fertilized, and (D) phosphorus fertilized plants, at G2.

Discussion

The primary objective of my study was to examine the effects of plant genotype (G), soil nutrient environment (E), and G X E interaction on *U. nigrotuberculatum* abundance in *S. altissima.* This study demonstrated the impacts that environment, genetic variation and their interaction have on a population of a specialist insect. When my data was averaged between two collection dates, G did not significantly explain variation in aphid abundance, which was likely due to the fact that E had a large impact on some of the plant attributes that were important for this aphid. Understanding the role of environment within this plant-insect system is important with respect to which phytochemical factors might influence aphid selection and colonization of *S. altissima.* Though other studies within the field of community genetics have examined the importance of the role environment might play (Johnson and Agrawal, 2005; Burkle et al. 2013), the need for further investigation into G, E and possible G X E effects is well recognized (Hersch-Green et al. 2011).

My study examined the data in two ways; averaging data from two collection dates and individually analyzing each date (i.e., seasonal analysis). It is notable that aphid abundance peaked twice during my study; July 9 (G1) and September 3 (G2). This is somewhat in agreement with a previous investigation of *U. nigrotuberculatum*, where multiple population peaks throughout the season were observed (Cappuccino, 1988). For the average data I found no main effect of genotype on aphid abundance ($P>0.05$). Previous studies examining differences among genotypes in insect abundance demonstrated that host

plant genotype plays an important role (Mooney and Agrawal, 2008, Pilson and Rausher 1995, Schädler et al. 2010, Genung et al. 2012a, 2012b). Especially noteworthy is an investigation that found the same aphid species in my study varied among genotypes of *S. altissima,* though no soil nutrient effect was examined (Williams and Avakian 2015). The lack a significant effect of host plant genotype within my experimental set up may be due to other factors that could have had a greater impact, obscuring any effect of plant genotype. The effect of environment was evident in the averaged data. A possible manifestation of this was a significant positive relationship between aphid abundance and plant biomass. The size of the host plant may affect aphids by impacting plant chemistry, or nutrition, or merely creating more habitat to support a greater number of individuals. Previous studies involving nitrogen addition demonstrated a strong positive relationship between nitrogen addition and aphid abundance (Annan et al. 1997, Zehnder and Hunter, 2008, Hosseini et al. 2010), as well as a parabolic response to nitrogen addition in a study conducted by Sauge et al. (2010). Annan et al. (2010) also examined aphid response to elevated soil phosphorus and demonstrated a negative relationship between aphid populations and increased soil phosphorus.

The fact that both genetics and environment have been demonstrated to influence aphid abundance tells us that our lack of a significant genotype effect was likely due to the strength of the environment effect outweighing that of genotype in within our system. Similar relationships between aphid populations and host plants have been observed before where aphid populations, plant foliar nitrogen content and plant biomass were all higher in nitrogen-fertilized plants, suggesting a possible relationship between these factors (Zehnder and Hunter, 2008; Hosseini et al. 2010). This relationship contributes to some of the late-season

environmental effect that was observed. It is notable that this correlation does not account for all of the variation in aphid abundance and that many other factors such as phytochemistry likely affected aphid colonization. These findings relating to plant biomass and aphid abundance are in support of the growth-differentiation balance hypothesis. This hypothesis proposes that there is a physiological trade-off between growth and the metabolism of secondary compounds that varies based on resource availability (Herms and Mattson, 1992). This trade-off is rooted in the idea that plants must grow fast enough to compete all the while using specialized, differentiated growth traits such as defensive secondary compounds, thickened cuticles, trichomes and other secondary growth processes to defend the nutrient resources gained through growth (Herms and Mattson, 1992). These trade-offs mean that certain plants may be more dependent of rapid growth rather than defensive differentiation, or vice versa, to remain competitive against other plants and herbivores depending on environment, nutrient availability, herbivore pressure, and other aspects of the natural history of a given plant (Herms and Mattson 1992). In the case of *S. altissima*, it may be that under optimal nutrient conditions (high nitrogen availability), the growth strategy of our plant is to grow rapidly to essentially outgrow other plants as well as herbivores, leading to plants with high numbers of herbivores, relatively low plant nutrient content and high biomass. Similarly, under sub optimal nutrient conditions (for example in my study possibly phosphorus fertilization or control treatments), there were relatively higher plant nutritional contents and relatively higher content of phytochemicals relative to plant biomass.

Other investigations have demonstrated G X E effects in plant associated insect communities (Johnson and Agrawal, 2005, Burkle et al. 2013). In my study there was a notable G X E interaction effect present in the averaged and seasonal data, with the strongest effect seen at

G1, suggesting that at this portion of the growing season G X E played a particularly important role in aphid colonization. Different levels of aphid abundance were observed on different fertilization treatments among the genotypes. Similarly, in an investigation that assessed the impact of plant soil nutrient environment and genotype, a G X E effect was shown to influence the frequency and diversity of floral visitation by insects (Burkle et al. 2013).

My findings are particularly exciting as this demonstrates that effects of plant genotype on herbivorous insects can be environment dependent. This is especially relevant in a plant species such as *S. altissima*, which you expect to experience a variety of soil nutrient conditions across its very large geographical distribution. Smith et al. (2011) also demonstrated a significant effect of host plant G X E on aphids, telling us that these impacts are known to occur. Similarly, Johnson and Agrawal (2005) demonstrated an effect of host plant G X E on the abundance of herbivorous insects where the independent environmental variable was microhabitat based on land usage. The ecological implications of my findings are significant as it highlights the importance of plant genetic diversity in leading to heterogeneous herbivore population responses within and between environments, and the idea that individual plant genotypes can have varying impacts on plant-dependent organisms. My averaged data showed that both G and E significantly affected phytochemistry (Table 1), where both leaf N and terpenes varied among genotypes and nutrient treatments. Terpene variation between genotypes has previously been demonstrated in tall goldenrod (Heath et al. 2014; Williams and Avakian, 2015), though the role of nitrogen is perhaps less evident. Plant genetic diversity has been shown to impact associated arthropod communities (Johnson and Agrawal 2005, Crutsinger et al. 2006), and it is possible that some of the effects that

genetic variation may have on plant chemistry may be due to genetically-governed differences in plant chemistry. The strong G X E relationships with terpenes that were observed in my investigation are driven by the fact that plant genotype influenced terpene concentration with some terpenes being entirely undetectable in certain genotypes, such as limonene in genotype 2 (Fig. 5). If terpenes play a repellant or attractant role with respect to insects, these large variations in terpene presence may have an important role in the varying G X E responses we observed in aphid colonization. The G X E effect seen with terpenes makes it plausible that these phytochemicals contributed to observed effects on *U. nigrotuberculatum*. Based on linear regression the majority of terpenes demonstrated positive relationships with aphid abundance, suggesting they were playing a role as aphid attractants or possibly feeding stimulants. I was able to show in my study that some of the terpenes found in *S. altissima* were present in the aphid body, providing strong evidence they are actively imbibed. Our lab had previously seen a positive relationship between *U. nigrotuberculatum* and the terpene β-pinene, as well as a positive relationship between aphids and all terpenes using the multivariate technique PLSR (Williams and Avakian, 2015). Another study that demonstrated a positive relationship between a specialist aphid and a terpene found that the aphid *Macrosiphoniella tanacetaria* was more abundant on *Tanacetum vulgare* plants that had higher concentrations of the monoterpene β-thujone (Kleine and Müller, 2011). Relationships between a given herbivorous insect and the phytochemistry of a plant are almost certainly dependent on the insect species and phytochemistry within the host plant. My identification of genotype and environment effects, and their interaction for some terpenes and aphid abundance is highly suggestive of phytochemistry playing a role in my plant-insect system.

Though environment played an important role in determining chemical composition, the magnitude of its effects at each sampling date was fairly different. These large differences in chemical compositions (i.e., amount and allocation of terpenes among genotypes especially) between times are likely driven by changes in soil nutrient effects between the two generations. For example, fertilizers were only applied at the onset of the growing season and it is possible that the nitrogen treatment was diminished in the soil as the season progressed. This could be possibly explained by plant nitrogen content becoming diminished by G2. There was also a notable increase in terpene concentrations in the phosphorus treated plants during G2, likely accounting for much of the E effect that emerged at this point in the season. The change in effects on aphid abundance between G1 and G2 also highlights this, during G1 the G X E interaction effect predominated, whereas in G2 the effect of environment was most significant, likely due to the effects of fertilization on plant physiology later in the season.

Examining how phytochemicals were impacted by genotype and environment allowed me to explore a possible mechanism to describe the observed abundance differences of *U. nigrotuberculatum* on *S. altissima*. In nitrogen fertilized treatments the averaged data shows a negative relationship between aphid abundance and nitrogen content and a positive relationship between C:N ratio and aphid abundance. Mabry et al. (1997) demonstrated a similar relationship between aphid abundance and foliar nitrogen concentration. My data indicates that nitrogen content is associated with other plant traits that may impact aphids differently in different environments. For example, we saw that nitrogen fertilized plants displayed higher overall nitrogen concentration, as well as higher biomass and lower terpene contents in general. These results also support the growth-differentiation balance hypothesis,

as when more growth-related nutrients are abundant, fixed carbon is allocated more towards growth and less to defense (i.e., terpenes) (Herms and Mattson 1992). In an investigation of the impact of soil fertility on carbon-based phenylpropanoid chemical concentrations in two willow species, there was a negative relationship between nutrient availability and carbon-based secondary metabolites (Glynn et al. 2007). Mabry et al. (1997) demonstrated a similar relationship between fertilization and foliar nitrogen content, as well as plant biomass. In terms of the reduction in terpenes in my experiment under nitrogen fertilization, Blanch et al. (2007) also saw a similar reduction in terpene content in *Pinus halepensis*. The increase in biomass and foliar nitrogen were both related to aphid abundance, so teasing out when each trait might play a role in determining aphid population is highly dependent on the individual environment and genotype of the plants.

Our partial least squares model was run using all phytochemicals and demonstrated that plant chemistry explained a significant amount of the variation in aphid abundance, especially when fertilization treatments were separated (Figures 7-10). This suggests that when considered together phytochemicals play an important role in aphid colonization and subsequent abundance. In a previous investigation conducted by Williams and Avakian (2015), terpenes were also implicated in playing an important role in determining aphid abundance, with PLSR demonstrating that 49% of aphid abundance could be explained by terpene chemistry. The VIP within our PLSR model removed the inputs p-cymene and azulene when examining all nutrient treatments in the averaged data, meaning that these two chemicals did not contribute significantly toward predicting aphid abundance. The only chemical not removed between the PLSR analyses run on different nutrient treatments was

caryophyllene, highlighting that this particular chemical contributes towards predicting aphid abundance regardless of the soil nutrient treatment.

Summary

My investigation demonstrated that G and E, and in some cases a G X E interaction was observed for the concentration of terpenes in *S. altissima*. I also demonstrated an effect of E and G X E on *U. nigrotuberculatum* abundance. Terpenes, foliar nitrogen content and C:N ratio were shown to be contributing factors for predicting aphid abundance. Therefore, my study provides support for the conclusion that plant phytochemistry, which is known to vary by genotype and environment, affects the abundance of this specialist aphid on tall goldenrod. This study addresses the role that G, E, and G X E play within this plant-insect system and provides compelling evidence that terpene chemistry, along with nutrients, represent a potential mechanism explaining aphid abundance among genotypes and environments. This study addresses the need within the field of community genetics to understand the role of environment along with effects of intraspecific genetic variation on associated insects in a foundation plant species. For my study the effect of environment was generally stronger than that of host plant genotype effects for determining aphid abundance on *S. altissima*. However, the significant G X E observed on phytochemistry and aphid abundance shows that differences among individual genotypes within this often dominant old-field plant species can be environment dependent. Therefore, both genetic variation and environment are important considerations for determining impacts on associated organisms.

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Appendix 1

Results of genetic analysis analyzing the locations of base pair repeats and specific markers in the five *S. altissima* genotypes assessed by my study. The numbers listed in the table below represent the number of repeats present at different loci in each marker analyzed.

Plant	Marker Sg_1		Marker Sg_8				Marker Sg_10				Marker Sg_2					
	1_1	1_2	8_1	8_2	8_3	8_4	10_1	10_2	10_3	10_4	2_1	2_2	2_3	2_4	2_5	2_6
SA2	148		148	152	172		298	302			185	193	206	218	222	226
SA3	148		148	168	172	190	298	302	306		200	204	211	212	218	225
SA4	148		152	168	172		298	302	306		200	202	204	207	211	223
SA5	148	164	148	152	156	172	290	298	302	322	189	195	207	211	231	232
SA6	148	164	148	156	168	172	298	302			199	213	214	218	219	221

Microsatellite primers used based on Beck et al. (2014)

Vita

Brian Kenneth Bonville was born to Kenneth and Susan Bonville of Pinehurst, North Carolina. He always demonstrated an interest in plants and insects, being involved in his home town of Pinehurst in running nature camps for children and attending other plant and insect related community events throughout his youth. He continued on to complete a Bachelor of Science Degree in Biology at Appalachian State University. Throughout his undergraduate career Brian pursued research in ecology and fermentation sciences. He continued on to complete a Master of Science Degree in Biology under the tutelage of Dr. Ray S. Williams at Appalachian State University in August 2016. He is currently planning to continue his research on plants and insects through working in industry and agriculture after relocating to Raleigh, North Carolina along with his wife, Quinn Griffin.