EFFECTS OF PRIOR HERBIVORY ON APHID COLONIZATION OF *SOLIDAGO ALTISSIMA* CLONES

A Thesis
by
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ABSTRACT

EFFECTS OF PRIOR HERBIVORY ON APHID

COLONIZATION OF *SOLIDAGO ALTISSIMA* CLONES. (August 2012)

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There is a growing body of evidence that intraspecific genetic variation in a foundation plant species is important for structuring the associated the arthropod community. There are few studies examining potential mechanisms, including how genetic variation in plant resistance and induction of allelochemicals influences key herbivores. I hypothesized that different clones of tall goldenrod (*Solidago altissima*) would respond to prior herbivory by producing different quantities of volatile terpenes, and these compounds would affect choice by *Uroleucon nigrotubercularum*.

To determine the effects of genetic variation and initial herbivore damage on tall goldenrod (*Solidago altissima*) and subsequent aphid colonization, I conducted a common garden experiment using 4 clones of *S. altissima* grown in insect cages in the Appalachian State University (ASU) greenhouse, Boone, NC. Plants of each clone were either damaged with leaf beetle (*Trirhabda virgata*) larvae or left undamaged, then planted in randomly chosen single-treatment plots in a common garden design at the ASU Gilley Research Station. Damaged and undamaged greenhouse plants were analyzed for foliar terpenes to

determine if larvae had induced the plants. Seventy-five days after planting in the common garden, I visually determined abundance of the specialist aphid *U. nigrotuberculatum*, nondestructively measured plant biomass, and collected leaves for chemical analysis. I analyzed water content, nitrogen, carbon, and terpenes.

Five foliar terpenes, α-pinene, limonene, β-elemene, azulene, and ledene oxide (II), were significantly higher in greenhouse-grown *S. altissima* damaged by *T. virgata* larvae. There were no significant differences in greenhouse plants among clones. In common garden plants I found significant differences among clones for bornyl acetate, β-elemene, azulene, ledene oxide (II), and bicyclo[4.4.0]dec-5-ene (bicyclo[4.4.0]), and significant interaction effects between clone and damage for α-pinene, limonene, caryophyllene, and γ-elemene. Some clones, but not others, produced higher quantities of terpenes in plants that had not been previously damaged. Aphid measures were significantly different among clones but were not significantly different between damage. Though foliar water, SLW, N and C:N were significantly different among clones, and SLW, N and C:N between damage, aphid performance only correlated with foliar terpenes. Aphid abundance was most closely positively related to the sesquiterpenes azulene, ledene oxide (II), and bicyclo [4.4.0].

I conclude from my research that colonization of *S. altissima* by *U. nigrotuberculatum* is largely due to genetic differences in terpene production among clones, and that differences in terpene production were affected in part by prior damage and other environmental factors. This indicates that terpenes are a potential mechanism by which specialist herbivores choose their host and suggests a role for plant genetic differences in terpene production in structuring natural ecosystem diversity.

DEDICATION

This thesis is dedicated to my husband, David R. Howells, for his love and support, and to my parents, the late Jesse Monroe Moss, who taught me to look at the stars, and Elizabeth Siler Moss, who taught me to appreciate and love the little things.

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INTRODUCTION

Plant Genetic Biodiversity

Biodiversity enables ecosystems to adapt to environmental change and challenges. However, as species loss has accelerated in the 20th century and human land use practices have fragmented ecosystems and created plant monocultures, biodiversity has declined worldwide (Thomas et al. 2004). Species diversity is extremely important in most ecosystems because the interactions between species via mutualism, competition, decomposition, top-down controls in trophic dynamics, and other interactions contribute to many ecosystem processes (Chapin et al. 1998, 2002). For plants, studies have demonstrated that species loss decreases the diversity of the associated arthropod community, further impacting trophic levels in an ecosystem (Haddad et al. 2009). Plant diversity affects ecosystem productivity, and increased plant diversity positively impacts ecosystem productivity regardless of nutrient availability (Hooper et al. 2005, Kotowska et al. 2010). In addition, a ten-year study of ecosystem diversity in grasslands found that increased plant diversity was related to greater long-term ecosystem stability (Tilman et al. 2006).

Primary producer interspecific genetic diversity impacts the associated insect community and other ecosystem services as "bottom-up" effects, and plant intraspecific genetic variation impacts the community as well. Many ecosystem properties, including stability and increased arthropod diversity, are influenced by intraspecific genetic diversity (Whitham et al. 2012). In a study of demographic data stretching 18 years on eleven forests in three areas of the southeastern United States, plant intraspecific genetic diversity was the

largest contributing factor pertaining to the promotion of coexistence and ecosystem structure (Clark 2010). Therefore, genotypic diversity of primary producers may impact the diversity and stability of the entire trophic system. While there is a greater understanding that genotypic diversity is important, many uncertainties remain about the mechanism by which variation within dominant plant species affects arthropod community structure. Key gaps in population and community ecology research include mechanisms that drive the relationship between plant intraspecific genetic diversity and diversity in the associated arthropod community (Agrawal et al. 2007).

Phenotypic expression of genetic differences may impact factors such as plant nutrient availability, stem or leaf toughness, or constitutive defensive chemicals, potentially eliciting an insect herbivore response. Research has demonstrated various *S. altissima* genotypes attract or repel different suites of herbivores (Maddox and Root 1990). Plant genotypic diversity can influence multiple trophic levels. Schädler et al. (2010) found that grass genotypic diversity influenced associated aphids. Foundation species, often primary producers that structure communities by creating stable conditions for other species and modulating ecosystem processes, can have individual genetic effects with large-scale effects on the community and ecosystem (Bangert et al. 2008). Trophic interactions, community diversity and stability, nutrient cycling, primary production, and associated arthropod communities are impacted by differences between foundation species genotypes (Wymore et al. 2011). Genotype can be more important that weather. For instance, in an eight-year study examining a population of the bud-galling mite *Aceria parapopuli* on cottonwood trees (*Populus* spp.) that encompassed record drought and wet years, variation among genotypes was 130 times greater than variation among years (Evans et al. 2012). In relocated *Erodium*

cicutarium that had increased genetic diversity there was an overall stronger adaptive plastic response (Baythavong and Stanton 2010). Therefore, plant genotypic diversity can and does impact the ecosystem on a much larger scale than the local plant/herbivore relationship, and plants can express this diversity through both phenotypic differences or variability of phenotypic responses.

Plant Defensive Strategies

Plant genetic variation that would impact insect herbivory include many defensive strategies. Herbivorous insects have coexisted and evolved with plants for at least 350 million years, and the most common relationship between plants and insects is antagonistic; many insects eat plants, and all plants have at least one insect predator (Gatehouse 2002). In their study of butterflies and their host plants, Ehrlich and Raven (1964) were two of the first researchers to propose that insect herbivory and the evolution of plant defenses in plants were driving forces behind species diversity for both plants and insects. The complex defense mechanisms that plants have evolved against herbivory vary widely. Mechanical plant defenses reduce herbivory by physical means such as leaf thickness or cuticle toughness and thorns or trichomes. Quantitative defenses involve impacting herbivores by the production of indigestible compounds such as lignin, cellulose, and tannins, while qualitative defenses include plant allelochemicals that act as toxins to herbivores, such as alkaloids and terpenoids (Takahashi and Yamauchi 2010). Plants employ multiple strategies simultaneously. For instance, a study of 24 species of milkweed (*Asclepias* spp.) identified three plant defense syndromes were utilized against insects involving (a) low nutritional quality, (b) high physical defenses, or (c) high chemical defenses (Agrawal and Fishbein 2006). Quantitative

and qualitative defenses are continuously produced in plants as constitutive defense, or may be induced in plants resulting in greater quantities in response to herbivory (Karban and Myers 1989). Studies utilizing the saliva from mandibulate insects and piercing/sucking insects indicate that the chemical defenses induced due to insect herbivory are distinctly different from a wound response, and that substances in insect saliva elicit different responses for different insect herbivores (Kigathi et al. 2009, Ma et al. 2010). An induced response can directly impact plant predators by increasing defensive chemicals that affect feeding or settling of other insects, or indirectly impact folivores by signaling parasitoids that prey on the attackers (Turlings et al. 1990). By utilizing an array of defensive strategies, plants are able to potentially impact a wide variety of herbivores.

Physical deterrents employed by plants can be effective against herbivory. Increased leaf toughness due to cuticle thickness and increased lignin have been found to deter chewing insects but not piercing, sucking insects (Peeters et al. 2007). An estimate of leaf toughness and thickness is specific leaf weight (SLW), which is a measure of mass per unit area. SLW varies within and between species due to environmental factors and genetic differences (Jurik 1986, Steinbauer 2000). A study of mulberries (*Morus* spp.) found that plant traits such as specific leaf weight were under genetic control (Ghosh et al. 2009). Other plant attributes such as specific leaf area and leaf weight ratio in alfalfa genotypes were shown to exhibit plasticity under drought conditions, which may help them maintain relative water content (Erice et al. 2010). Nitrogen availability is also known to affect the plasticity of SLW (Jullien et al. 2009), and, because leaf water content is inversely related to SLW, can impact plant quality and palatability to herbivores, thereby affecting resource dynamics in an ecosystem (Schädler et al. 2003).

Plant nutritive quality can vary between and within species. This variation affects insect abundance and herbivory, with foliar nitrogen concentrations serving as the best predictor of host plant quality for arthropod herbivores (White 1984, Throop and Lerdau 2004). Various species of plants take up and accumulate nitrogen differently (Throop and Lerdau 2004), and intraspecific genetic differences in nitrogen uptake and assimilation have been reported for numerous crops (Kant et al. 2011). Fundamental differences in translocation of nitrogen exist in different cultivars of the same species of sorghum, causing levels of nitrogen in stalks and leaves of different genotypes of the plant to vary (Crawford et al. 2009). A great deal of plasticity in nitrogen uptake and assimilation was due to genotypic variation in poplar trees (Novaes et al. 2009). Differences in plant nutrient quality exhibited by different foliar nitrogen levels can greatly impact associated herbivores. For instance, when *Pieris rapae* larvae were fed artificial diets, nitrogen availability was a key factor in growth and development of the butterfly larvae, with lower relative growth rates and longer development times on diets with reduced nitrogen (Morehouse and Rutowski 2010). In an aphid (*Aphis nerii*) host plant (*Asclepias tuberosa*) system, nitrogen added to the soil increased plant foliar nitrogen and plant biomass and increased associated aphid per capita population growth (Zehnder and Hunter 2008). Generalist herbivores often switch hosts in natural ecosystems, providing variation in their diets. For instance, grasshoppers shift their feeding choices to balance carbon, nitrogen, and potassium intake in an old-field system (Jonas and Joern 2008). Specialist herbivores also exhibit higher fecundity and growth when switching between host plant genotypes. Caterpillars of *Chrysopsyche imparilis* showed no preference for leaves due to size or toughness but were more attracted to leaves exhibiting

prior herbivory (Mody et al. 2007). Genotypic differences in nutrient assimilation and allocation very likely affect associated herbivore populations in many ways.

Plants produce secondary chemicals that have no readily apparent role in growth or metabolism and are purportedly used for defense. These allelochemicals are often classified based upon their chemical structure and the metabolic pathways from which they derive (Wink 2003). Chemical defenses are employed in greater or lesser amounts for younger vs. older leaves and at different stages of a plant's life cycle (McCall and Fordyce 2010, Takahashi and Yamauchi 2010). Different species of plants, and different genotypes within species, maintain varying amounts of thousands of different defensive chemicals (Wink 2003). Allelochemicals can negatively impact herbivores in numerous ways, including interfering with metabolic processes and poisoning the insect (Reigosa et al. 2006), reducing the efficiency of herbivore digestion of the plant material, inhibiting feeding and reducing food intake (Feng et al. 2009), and indirectly as volatiles that act as signals to predators or parasitoids (Agrawal and Fishbein 2006). Insects have evolved numerous methods of compensating or neutralizing plant defenses, and often several mechanisms are utilized. Strategies employed by insects against plant defenses include avoidance, suppression, excretion, sequestration, and metabolic resistance, with plasticity in an insect's response to plant chemical defenses evident (Deprés et al. 2007).

Plant Terpenes

Terpenoids (terpenes) are the largest group of plant defensive chemicals (Langenheim 1994). They are carbon-based compounds that are often volatile and are biosynthesized either through the deoxy-D-xylulose pathway in the plastids of cells or the mevalonate pathway in

the cytosol. Many are toxic to insects and have been used as pesticides against aphids (Sampson et al. 2005). Terpenoids may act as feeding deterrents to insect pests, though some can act as attractants, particularly for pollinators (Langenheim 1994). Some sesquiterpenes, which are composed of three isoprene subunits, mimic aphid alarm pheromones and may inhibit settling (Nishino et al. 1976, Gutiérrez et al. 1997) or may indirectly defend plants by attracting aphid predators (Kunert et al. 2010).

Terpenes exist in most plants in constitutive quantities but also can be induced in greater quantities when plants are attacked by herbivores. Induction is a much less costly means of protection than is maintaining constitutive levels of secondary chemicals, and higher induced resistance than constitutive resistance has been demonstrated in more competitive plant species (Kempel et al. 2011). Via the process known as *direct induction* plants can increase production of defensive volatile chemicals in response to herbivory; these repel predators such as aphids and egg-laying butterflies (Unsicker et al. 2009). Many conifers can be directly induced to increase production of terpenoids. For example, herbivory was demonstrated to increase the production of monoterpene cyclases in pine, spruce, and white fir (Litvak and Monson 1998). Monoterpenes and sesquiterpenes have been induced in the needles of Norway spruce with the elicitor methyl jasmonate (Martin et al. 2003). Terpene induction has also been examined in deciduous trees. One study of the aphid *Myzus persicae* on resistant and susceptible peach tree cultivars found resistant cultivars had significantly increased emissions of the terpenoids farnesene, (*E*)-β-ocimene, and (*E*)-4,8-dimethyl-1,3,7-nonatriene upon aphid attack, though in the absence of aphid attack volatile emission from all peach plants barely exceeded trace levels and aphid-susceptable cultivars were not induced to produce terpenes (Staudt et al. 2010).

Direct induction of terpenes has also been documented in forbs and herbaceous plants. Research on induction of the terpene 17-Hydroxygeranyllinalool diterpene glycoside (HGL-DTG) in coyote tobacco (*Nicotiana attenuata*) was facilitated by the use of genetically altered plants. Stably transformed tobacco plants were silenced in jasmonate production and perception and a oligophagous herbivore, tobacco hornworm (*Manduca sexta*) larvae, were allowed to feed on these and wild-type plants. A 50 – 75% reduction in HGL-DTGs resulted in *M. sexta* larvae that grew as much as 10 times larger than those on the wild-type plants, demonstrating that when terpene induction is silenced, insects are able to grow faster and gain reproductive maturity quicker (Heiling et al. 2010). Induction of the volatile terpenes α-pinene, β-pinene, camphene, myrcene, limonene, (*E*)-β-ocimene, germacrene D, and α-farnescene were documented after herbivory by a generalist caterpillar (*Heliothis virescens*) in *Solidago altissima* (Tooker et al. 2008). Interestingly, gall-inducing species introduced to the plants suppressed this strong induced response.

Volatile chemicals can signal predators and parasitoids of the herbivores that are feeding on the plants in a process known as *indirect induction* (Litvak and Monson 1998, Huang et al. 2003, Kigathi et al. 2009). Egg parasitoids favorably respond to volatile chemicals produced when plants are attacked by hosts such as the brown stink bug (Moraes et al. 2005). Female parasitic wasps sense volatile chemicals released by corn plants in response to feeding caterpillars and utilize these cues to find hosts on which to lay their eggs (Turlings et al. 1990). Research of aphid infestations on such diverse plant species as potatoes, fava beans, and lesser knapweed have demonstrated that terpenoids produced were powerful attractants to aphid predators (Harmel et al. 2007, Pareja et al. 2007, Verheggen et al. 2008).

Insect Elicitors

Insects with different feeding habits generate different volatile chemical responses in plants, with plants producing a different combination of volatile terpenoids when attacked by sucking insects, such as thrips or aphids, versus chewing caterpillars (Delphia et al. 2007). Aphid infestations induce trees to release greater quantities of volatile terpenes (Blande et al. 2010). In a study of volatile terpenoid emissions from silver birch and black alder trees, the chemical cocktail emitted was significantly different for aphid feeding than it was for feeding by leaf beetles. Significantly more limonene, (*E*)-carophyllene, methyl-salicylate, and β-farnesene were emitted from trees with aphids than from controls, while emissions from plants induced by leaf-chewing beetles had greater amounts of sesquiterpenes (Blande et al. 2010). Aphid saliva hormones appear to trigger methyl salicylate mediated defense responses, while mandibulate insect feeding triggers jasmonate mediated defense responses for volatile organic carbon induction (Staudt et al. 2010). Polyphenol oxidase was found to be a major contributing factor in aphid saliva-induced expression of the genes *aos* and *fps*, which encode key enzymes in terpene signal pathways and trigger a defense response that includes production of methyl salicylate and unique volatile terpenes (Ma et al. 2010). Significant differences in polyphenol oxidase activity have been observed when five *Lupinus* spp. varieties with known aphid resistance were exposed to aphids, indicating genotypic differences in terpene induction response in that plant species (Cardoza et al. 2005). Volatile terpenes such as (*E*)-β-farnesene are induced in many plants. This terpenoid is a constituent of an aphid alarm pheromone that causes aphids to drop off plants and walk away (Nishino et al. 1976). However, studies have not found that this compound provides a direct defense

against aphids, as they seem to be able to distinguish between the (*E*)-β-farnesene produced by plants and that produced by aphids (Qiao et al. 2009). Therefore aphids often elicit different chemical responses in the plants than mandibulate insects, and these responses can differ among different plant species or among genotypes within a plant species.

Aphid Selection of Host Plant

Most species of aphids specialize on certain host plants and are able to resist or detoxify defensive chemicals produced by plants. This ability is genetically determined, therefore plant genetic heterogeneity may play a role in the evolution of the insects' abilities (Pompon et al. 2011). In addition to allelochemicals, plant genetic variation has also been demonstrated to have a strong bottom-up effect on aphid population growth based upon the plant traits of nitrogen concentration, leaf water content, and trichome density (Johnson 2008). In a study of the strawberry aphid *Chaetosiphon fragaefolii*, both alate and apterous aphids moved away from certain plants, responding to the genotype on which they settled and choosing among genotypes based upon plant quality (Underwood et al. 2011). The genetic basis of plant quality in aphid host-plant selection is an important consideration, as plant appearance and secondary chemical production, in addition to plant nutritional quality and other defensive mechanisms, may be genetically determined. The major factors that influence aphid host-plant preference come into play after the aphid alights and begins to probe with its stylet. Though it is difficult to determine the definitive cue that an aphid uses to select its host, the selection is done before plant phloem is tapped. It is likely that aphids choose their hosts based upon intracellular metabolites in the plants' epidermis (Powell et al.

2006). Therefore, aphid host-plant preference may actually depend more on unacceptable cues than on searching and finding acceptable ones.

Insect Response to Plant Induction

There are feedback mechanisms with respect to the response of plants to herbivores and herbivores to plants. A study of the grain aphid *Sitobion avenae* found a great deal of plasticity in P450s detoxification enzymes when the aphids were raised on host grains with differing levels of hydroxamic acid (Castañeda et al. 2010). Higher levels of this compound in the plant meant higher levels of P450s in the aphids, indicating that these enzymes were induced in the aphids to detoxify the host's defenses. Reproductive performance of the aphids was highest on the plants with the highest levels of hydroxamic acid, indicating that aphids have been selected to be tolerant of high hydroxamic acid hosts (Castañeda et al. 2010). In addition, direct herbivore/plant interactions can alter plant response and affect host plant choice by subsequent colonizers. Some parasitic species of insects can dramatically alter host defenses. For instance, the gall-inducing tephritid fly, *Eurosta solidaginis*, suppressed volatile chemical induction when infested *S. altissima* test plants were subsequently attacked by a generalist caterpillar (Tooker et al. 2008). In a study of induced responses of bittersweet nightshade to two chrysomelid beetles, it was found that plant responses induced by the initial herbivore differed and, depending upon the initial herbivore, altered the occurrence of conspecifics (Viswanathan et al. 2007). Therefore, there is a great deal of plasticity in the response of plants to arthropod herbivores, as well as in the response of the herbivores to the host plant's defenses.

There has been a large amount of research on induced responses in the past thirty years, from initiation at the molecular level, looking at the expression of genes that code for various steps on the metabolic pathways that produce these chemical defenses, to community level responses in the field. For example, induction of wild radish (*Raphanus sativus*) early in the growing season by caterpillar larvae of *Pieris rapae* induced defensive glycosides, and those induced plants experienced significantly less herbivory from mandibulate insects and aphids than plants that had not been induced (Agrawal 1998). Induction of common milkweed by early season stem feeding weevils reduced subsequent growth of monarch larvae and leaf beetle larvae and altered herbivore response in the community for two years (Van Zandt and Agrawal 2004). Induction of plants by *P. rapae* caterpillars in a common garden experiment caused generalist insect herbivores to avoid induced plants, but caused specialist herbivores from both leaf-chewing and sap-sucking guilds to preferentially colonize induced plants, (Poelman et al. 2010) possibly because specialist insects used induced allelochemicals to locate or choose their host, and they had the means to detoxify or sequester these chemicals. Therefore, both direct and indirect induction of plant defensive chemicals may alter the associated herbivore community in ecosystems such as old fields, though the community-wide response is more complex due to the influence of so many factors.

Tall Goldenrod in an Old-field Ecosystem

Solidago altissima (tall goldenrod) is native throughout eastern North America and represents a dominant clonal old-field species that reproduces both sexually and clonally via rhizomes (Crutsinger et al. 2008). This species is host to more than 100 species of insect

herbivores (Maddox and Root 1987). Many species of aphids colonize *S. altissima*, with *Uroleucon nigrotuberculatum* a common specialist aphid herbivore in the eastern United States. Genetic variation for resistance in the host plant, along with other factors such as host plant morphology and local host density, has been found to significantly affect the aggregation of these aphids in populations of *S. altissima* (Pilson and Rausher 1995, Ohgushi et al. 2011). In a study of the distribution patterns of *U. nigrotuberculatum* on *S. altissima*, it was found that genetic variation in local host plant density, morphology, and resistance to herbivory accounted for the degree of aggregation in specialist aphids (Pilson and Rausher 1995). Research conducted on *S. altissima* in a common garden experiment in Minnesota found that plants in mixed genotype plots supported a greater abundance of *U. nigrotuberculatum* (Ohgushi et al. 2011). Studies have demonstrated that genotypic diversity of a clonal plant species such as *S. altissima* can affect the susceptibility of a community to invasive plants (Crutsinger et al. 2008), impact the nutrient concentrations of leaf litter (Crutsinger et al. 2009), and impact the diversity of the arthropod community that is associated with this species (Crutsinger et al. 2006).

Resistance to herbivory in plants such as *S. altissima* consists of complex interactions between insects, plant defenses, soil conditions, and nutrient uptake and availability (Pilson 1992). For example, studies have shown that *S. altissima* has an arbuscular mycorrhizal association (Antoninka et al. 2009), and that this association can affect defensive sesquiterpene production (Rapparini et al. 2008). Gall insects have been found to inhibit the production of volatile terpenes in *S. altissima* (Tooker et al. 2008). In addition, some insects, such as goldenrod bunch gall midges, function as ecosystem engineers as they alter plant structure and provide habitat for larger communities of arthropod species. Abundance of the

midges is significantly and positively influenced by genotypic diversity in the *S. altissima* host plant (Crawford et al. 2007). Though there are many complicating factors in an old-field ecosystem, additional studies on intraspecific variation of a dominant old-field species such as tall goldenrod would assist in determining the contributions of direct induction to community diversity and the role this plays in an old-field ecosystem.

My thesis research is based upon a preliminary study in which we assessed *U. nigrotuberculatum* abundance on different clones of *S. altissima*. My observations of native *S. altissima* communities at the Appalachian State University (ASU) Biology Department's Gilley Field Station site found that plants were first colonized by a mandibulate leaf beetle, *Trirhadba virgata*, with beetle larvae consuming approximately 20% of leaf material, then later colonized by the specialist aphid *U. nigrotuberculatum*, a phloem feeder. This was an interesting model for the effects of genetic differences on induced responses of *S. altissima*.

In my thesis research I examined possible induction of foliar terpenes in different *S. altissima* clones by introducing *T. virgata* larvae to plants in a field-based common garden design. Though as yet unverified, due to the distance between collected ramets and the broad knowledge of genetic variation in this clonal species (Crutsinger et al. 2008), I presume these to represent different genotypes. For the sake of this study, until verified, ramets represent clones. By determining the effects of genetic variation on leaf chemistry and colonization by the specialist aphid *U. nigrotuberculatum* in a community garden experiment, my study examines a potential mechanism for host plant choice.

My study had two primary objectives:

- To examine the impact of plant genotypic diversity and prior herbivory on secondary chemical production, biomass, foliar water content, specific leaf weight, and foliar carbon and nitrogen in *S. altissima*
- To relate the differences in plant chemistry to plant colonization by a specialist aphid

I hypothesize that different genotypes of *S. altissima* respond to prior herbivory by producing different quantities of volatile terpenoid compounds and that these compounds affect choice of a key herbivorous aphid. In addition, other key plant constituents important to aphids are predicted to vary by genotype. My study may provide insight into mechanisms by which herbivores choose based on genetic variation in host plants.

MATERIALS AND METHODS

Ramets of four clones of *S. altissima* were collected from spatially separated sites in east Tennessee. Plants for my experiment were propagated from parent stock ramets at the ASU Biology Department Greenhouse in summer 2010 and watered daily. In March 2011 three cm sections of rhizome from selected clones were planted in 7.5 cm diameter pots in Fafard 4M potting soil mix. To each flat was added 50 ml of a 100:1 (water:concentrate) root stimulator mixture (Roots, Hummert International). After reaching approximately 10 cm in height, plants were fed with a commercial fertilizer (Scotts Miracle-Gro), prepared according to package directions. Plants were assigned to one of two treatments, damaged by *T. virgata* larvae or undamaged (controls). For each clone, enough plants were produced for three replicate plots of four plants each per treatment (96 plants total). Additional plants were propagated to determine if induction occurred after *T. virgata* damage treatment.

To prevent insect damage in the greenhouse, plants were contained in 61 cm x 33 cm x 102 cm insect cages made from 1.3 cm diameter pvc pipe and organza fabric (Y. J. Cardoza, personal communication). Twenty-four plants of each clone were assigned to two flats, 12 to a flat, for damaged and undamaged treatments. Each of the eight flats was contained in a separate cage.

I used herbivores from the chewing feeding guild to induce production of terpenes in test plants. On May 20, 2011, I collected *T. virgata* larvae from native *S. altissima* and *S. rugosa* plants at the Gilley Research Station. In the greenhouse cages six beetle larvae were placed on each plant to be damaged and the larvae allowed four days to feed. After this

period I removed the larvae and visually estimated the amount of herbivory. To standardize amount of leaf material lost across all damaged plants, amount of foliage lost was visually estimated, then additional leaf material was removed from each plant by cutting intact leaves in half across the mid-vein with scissors so that approximately 20% foliar removal was attained. This amount of foliage loss was consistent with the maximum amount of loss seen in the greenhouse or in the field.

On May 25, 2011, 24 hours after removing the larvae, plants were taken from the greenhouse and planted at the Gilley Research Station in a common garden design. The garden area had been previously prepared by clearing surrounding trees and shrubs, pulling stumps, and applying Roundup, a systemic broad-spectrum herbicide, one month prior to planting to remove native plants. The area was tilled and raked prior to planting. There were three replicate 0.25 m^2 plots per clone with four plants each (total 12 plants/clone) for damaged (i.e. induced) and undamaged plants (Figure 1). Plots were randomly assigned and planted 0.61 m apart. This provided 24 plots total for damaged and undamaged plants with a total of 96 plants in the experiment.

To determine if terpene induction occurred in my clones after damage in the greenhouse, I repeated the treatment on plants not planted in the common garden, though there were only enough replicates left to treat three of the clones. I placed six *T. virgata* larvae on each of four individuals of three clones. Four individuals of each clone were undamaged and the damaged and undamaged plants were maintained in separate insect cages. Insect larvae were again allowed to feed for four days. The amount of herbivory was assessed and leaf material removed from damaged plants to simulate 20% foliar removal as before for standardization. One day after the *T. virgata* larvae were removed, approximately

1.5 g samples of leaves were collected from each plant and placed in the freezer for later terpenoid analysis.

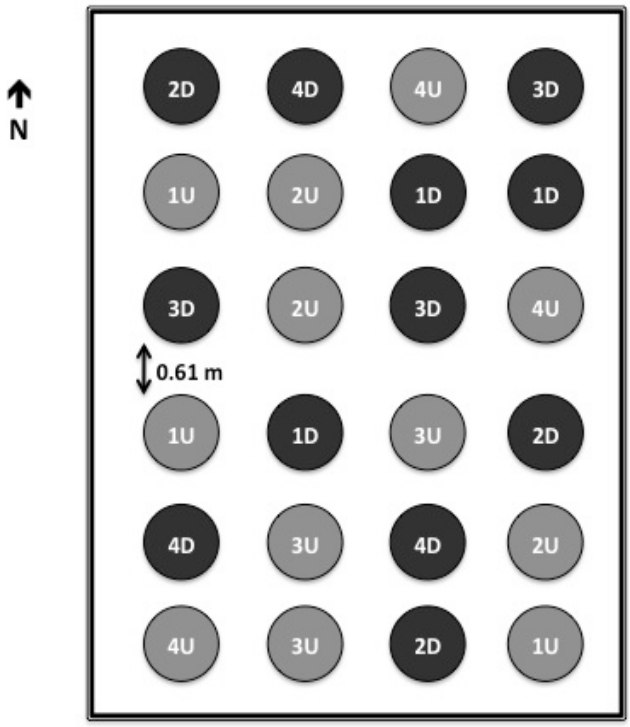

Figure 1. Common garden design at Gilley Research Station with location and identification of randomly assigned plots. Numbers = clone, D = damaged plants, and U = undamaged. Damage plots shown in darker shade.

After 76 days of growth in the common garden, *U. nigrotuberculatum* abundance was assessed using visual techniques. At the same time biomass in each plot was related to stem diameter measured three cm above ground surface and total plant height using the allometric equation (Proc Reg, SAS 9.3): Biomass (g) = (D^2H*0.0022) + 6.3667 (R^2 = 0.70, $p < 0.0001$). To account for differences in plant size on aphid abundance, total aphid abundance/total biomass, total aphid abundance/mean plot biomass, and total aphid abundance/mean clone biomass were calculated for each plot.

At the time of aphid quantification, leaf samples were taken from randomly selected plants. Three samples per plot were weighed and stored in a -20°C freezer for terpene analyses. Four samples per plot were weighed and leaf areas measured using a Li-Cor Model 3100 Leaf Area Meter (Li-Cor, Inc., Lincoln, NE). Samples were stored in paper envelopes in an Econotherm 60^{o}C drying oven for 3 days, then reweighed to determine water content. Specific Leaf Weight (SLW) was calculated as g dry leaf weight/cm^2. Dried leaf samples were ground in an amalgamator (Darby Dental Co., East Lansing, MI). Aliquots of the ground, mixed dried leaf material were weighed for Micro-Dumas carbon and nitrogen analysis utilizing a NC Soil Analyzer (Thermo Scientific, Pittsburgh, PA).

To quantify foliar terpenes, I processed leaf samples by adding 15 ml pentane to each sample and grinding it in a 50 ml culture tube with a Polytron tissue homogenizer. Each sample was filtered and reduced to 0.5 ml by gently bubbling with nitrogen gas. I injected 1 μl of each sample into a Schimadzu GC-14A gas chromatograph with a flame ionization detector (FID) and a HP-5 cross-linked 5% PH ME Siloxane column (30 m x 0.25 mm id, 0.25 μm film thickness). The GC program was as follows: injector temperature 250°C; detector temperature 275°C. The start temperature was 80°C, held for 2 minutes. Column temperature was increased 10°/minute to 280°C then held for 2 minutes for a total run time of 24 minutes (program adapted from Johnson et al. 2007). A standard curve was determined using five dilutions of the hydrocarbon tridecane in pentane. Consistently scoreable terpenes were identified and quantified using gas chromatography/mass spectroscopy (GC/MS) (Cardoza et al. 2005, Moraes et al. 2005, Sampson et al. 2005, Johnson et al. 2007). The GC/MS used was an Agilent 6890 GC with an Agilent 5973 Mass Selective Detector. The column for the GC/MS was an Agilent HP-5 PH ME Siloxane column (30m length, 0.25mm

diameter, 0.25um film thickness). The GC program was the same as previously described. Software used to process the GC/MS data was Agilent's MSD ChemStation version E.02.02.1431 and the software used to analyze the MS data was NIST MS Search 2.0.

To determine whether group means were from the same or different populations, data was analyzed with SAS 9.3 using a combination of two-way and one-way Analysis of Variance (PROC GLM). Correlations (PROC CORR) were run on all parameters, and regression analyses (PROC REG) performed on parameters correlating with aphid measures. P values < 0.05 were deemed significant, while p values $> 0.05 < 1.0$ were deemed marginally significant. To model the covariance structure and test causal relationships of nutrients (nitrogen and C:N) and terpenes, JMP 9 was used to analyze Partial Least Squares regression analysis of terpene data. Multi-response permutation procedure (MRPP) analyses of terpenes for herbivory, clones and interaction effects using dummy variables were conducted with PC-ORD, Version 6 (MJM Software Design, Gleneden Beach, Oregon). Dummy variables were variables assigned to each clone and level of herbivory (1=1U, 2=1D, 3=2U, 4=2D, 5=3U, 6=3D, 7=4U, 8=4D) for the purpose of looking at possible interactions between damage and clone. Sørensen (Bray-Curtis) distance measures were used for MRPP; p values < 0.05 were deemed significant.

RESULTS

Aphid Measures

Solidago altissima clones, but not *T. virgata* damage, influenced aphid abundance (Table 1, Figure 2). Numbers of aphids per g total biomass, numbers of aphids per g mean biomass, and numbers of aphids per g mean clone biomass in each plot differed significantly between different clones, but was unaffected by damage or the interaction between variables (Figure 2). Aphids preferred clone 3 over all others, and had the lowest abundance on clone 2.

Table 1. *F* ratio, *p* value and *df*[a] for the effects of clone (CLONE), damage (DAM), and CLONE * DAM interaction on the aphid *Uroleucon nigrotuberculatum* colonization of *Solidago altissima* biomass (Proc GLM).

	Aphid abundance		**Aphids/g total biomass**		**Aphids/g plot biomass**		**Aphids/g clone biomass**	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*
CLONE	6.93	**0.003**	11.20	**0.000**	11.32	**0.000**	13.93	**<0.001**
DAM	0.29	0.595	2.80	0.114	2.74	0.118	2.02	0.175
CLONE*DAM	0.88	0.471	0.57	0.640	0.60	0.625	0.16	0.924

Note: $p \leq 0.05$ presented in **bold** text.
[a]Aphid abundance, aphids/g total biomass, aphids/g plot biomass, and aphids/g population biomass: *df* = 3, 23 for CLONE and CLONE * DAM, *df* = 1, 23 for DAM. N = 24.

Plant Nutrient and Biomass Measures

Biomass of *S. altissima* plants was not affected by either clone or prior herbivory though there was considerable variation between clones (Table 2). Foliar water content was significantly different among different *S. altissima* clones, though was not different for plants damaged by leaf beetle feeding (Table 3). The specific leaf weight (SLW) was significantly different for clone and damage treatments, with plants that had been subjected to herbivory prior to planting having higher SLW than plants that had not been eaten by *T. virgata* (Table

2). Nitrogen (%) was significantly different among clones and between damage treatments, with undamaged plants 6% higher in nitrogen than damaged. C:N was also significantly different among clones and between damage treatments, with undamaged plants 7% lower in C:N than damaged plants (Table 2).

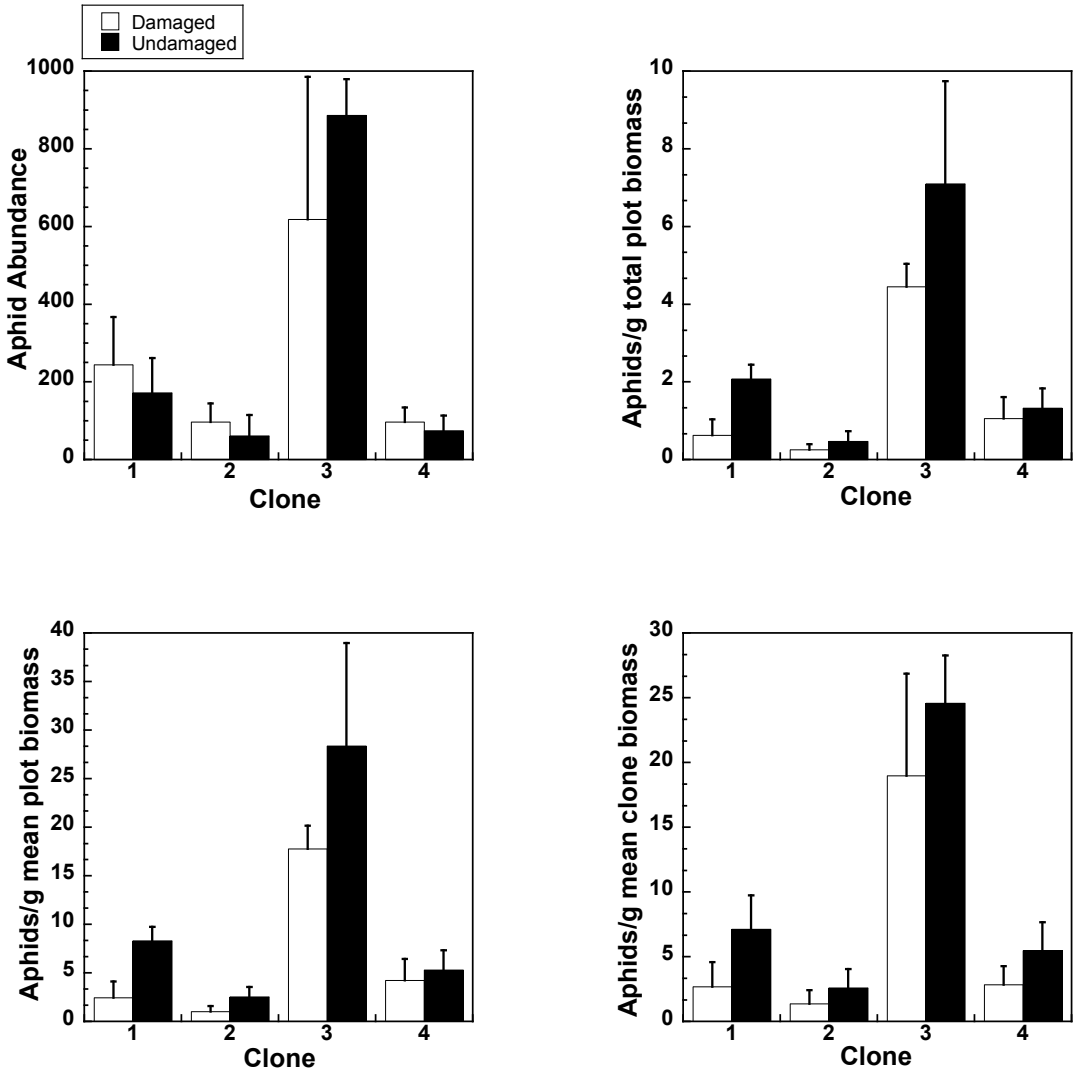

Figure 2. Aphid measures ± SE for plant clones and prior *Trirhabda virgata* damage on *Solidago altissima* clones at the Gilley Research Station.

Table 2. Effects of clone and damage level on *Solidago altissima* biomass and leaf chemistry means and standard errors (± SE) of plants from the common garden at Gilley Research Station, ASU. F ratio, p values and *df*[a] (Proc GLM) for clone (CLONE), insect damage (DAM), and CLONE*DAM interactions.

S. altissima	Clone	*T. virgata* damage (Mean ± SE)		CLONE		DAM		CLONE*DAM	
		Undamaged	Damaged	*F*	*p*	*F*	*p*	*F*	*p*
Biomass (g)									
	1	237.7 ± 39	137.2 ± 68	2.2	0.127	2.6	0.129	0.76	0.533
	2	179.3 ± 70	199.1 ± 22						
	3	186.8 ± 53	100.1 ± 19						
	4	104.4 ± 46	71.0 ± 4						
Foliar water (%)									
	1	65.4 ± 1.4	64.1 ± 1.2	25.8	**<0.001**	0.24	0.628	0.54	0.656
	2	59.7 ± 0.4	60.6 ± 0.5						
	3	63.1 ± 1.1	64.2 ± 0.6						
	4	69.2 ± 1.4	69.9 ± 1.3						
SLW (mg/cm^2)									
	1	7.2 ± 0.4	8.11 ± 0.4	8.20	**<0.001**	5.96	**0.017**	0.65	0.582
	2	8.0 ± 0.1	8.58 ± 0.4						
	3	7.1 ± 0.2	7.65 ± 0.2						
	4	6.8 ± 0.2	6.85 ± 0.3						
N (%)									
	1	2.9 ± 0.1	2.53 ± 0.1	43.0	**<0.001**	**5.35**	**0.023**	1.10	0.354
	2	2.3 ± 0.1	2.32 ± 0.1						
	3	3.1 ± 0.1	2.84 ± 0.1						
	4	3.6 ± 0.2	3.51 ± 0.2						
C:N									
	1	16.9 ± 0.7	19.3 ± 0.9	**51.3**	**<0.001**	**7.06**	**0.009**	1.31	0.278
	2	21.0 ± 0.3	21.1 ± 0.7						
	3	15.6 ± 0.4	17.1 ± 0.6						
	4	13.3 ± 0.6	13.9 ± 0.7						

Note: $p \leq 0.05$ (significant) and $p \leq 0.10$ (marginally significant) presented in **bold** text.
[a]Foliar water and SLW: *df* = 3, 95 for CLONE and CLONE*DAM, *df* = 1, 95 for DAM. Biomass: *df* = 3, 23 for CLONE and CLONE*DAM, *df* = 1, 23 for DAM.

Terpene Analyses for Greenhouse Plants

Five foliar terpenes, α-pinene, limonene, β-elemene, azulene, and ledene oxide (II), were significantly higher in *S. altissima* damaged by *T. virgata* larvae (Table 3). Averaged across genotypes, α-pinene was 15% higher, limonene 17% higher, β-elemene 14% higher, azulene 11% higher, germacrene D 23% higher, and ledene oxide (II) 20% higher in damaged plants compared to undamaged plants. Foliar bornyl acetate, caryophyllene,

γ-elemene and bicyclo[4.4.0]dec-5-ene, 1,5-dimethyl-3-hydroxy-8-(1-methylene-2-hydroxyethyl-1)- were not significantly different between damaged and undamaged plants. No significant differences were observed between clones, and no interaction effects were evident, though ledene oxide (II) showed a marginally significant increase in clones (Table 3). When all measured terpenes were combined, there was a marginally significant increase in terpenes in plants that had been fed upon by *T. virgata* larvae.

Terpene Analyses for Common Garden Plants

For plants growing in the field, treatments had varying effects on foliar terpenes (Table 4). There were interaction effects between clones and *T. virgata* damage for α-pinene and limonene. When analyzed using one-way ANOVA, both terpenes were significantly different between damage treatments, though not among clones (Table 4). Interestingly, *S. altissima* plants that had been defoliated by *T. virgata* prior to planting had significantly lower levels of α-pinene and limonene after 76 days in the common garden than those plants that had not been defoliated by *T. virgata* (Figure 3). Bornyl acetate (Figure 3) and β-elemene (Figure 4) were significantly different among clones, but unaffected by damage level (Table 4). Caryophyllene exhibited a significant interaction effect between prior herbivore damage and clone. When calculated using one-way ANOVA, differences among clones were significant, with no significant difference between damage observed (Table 4).

Table 3. Effects of clone and damage level on foliar terpenes produced by *Solidago altissima* plants from the Appalachian State University greenhouse after damage by *Trirhabda virgata* larvae. Means and standard errors (± SE), F ratio, p values and *df*[a] (Proc GLM) for clone (CLONE), insect damage (DAM), and CLONE*DAM interactions.

Terpene	Clone	*T. virgata* Damage (Mean ± SE)		CLONE		DAM		CLONE*DAM	
		Undam(mg/g)	Dam(mg/g)	*F*	*p*	*F*	*p*	*F*	*p*
α-Pinene									
	1	0.0305 ± 0.03	0.0871 ± 0.03	0.45	0.6448	4.78	**0.0439**	0.06	0.9402
	2	0.0587 ± 0.03	0.1061 ± 0.02						
	3	0.0602 ± 0.02	0.0984 ± 0.02						
Limonene									
	1	0.0419 ± 0.03	0.1406 ± 0.02	0.40	0.5638	6.18	**0.0243**	0.19	0.8273
	2	0.0801 ± 0.06	0.1380 ± 0.03						
	3	0.0988 ± 0.04	0.1606 ± 0.04						
Bornyl acetate									
	1	0.0882 ± 0.05	0.0103 ± 0.01	0.90	0.4253	2.83	0.1120	3.23	0.0655
	2	0.0339 ± 0.02	0.0115 ± 0.00						
	3	0.0290 ± 0.01	0.0490 ± 0.01						
β-Elemene									
	1	0.0392 ± 0.03	0.0942 ± 0.02	0.24	0.7916	6.37	**0.0226**	0.19	0.8282
	2	0.0372 ± 0.04	0.0665 ± 0.01						
	3	0.0374 ± 0.02	0.0878 ± 0.01						
Carophyllene									
	1	0.0112 ± 0.01	0.0133 ± 0.01	0.78	0.4769	2.71	0.1193	1.23	0.3184
	2	0.0154 ± 0.01	0.0204 ± 0.01						
	3	0.0098 ± 0.01	0.0334 ± 0.01						
Azulene									
	1	0.2843 ± 0.17	0.6803 ± 0.21	0.62	0.5491	5.72	**0.0294**	0.31	0.7353
	2	0.2392 ± 0.17	0.4137 ± 0.06						
	3	0.3094 ± 0.10	0.5562 ± 0.09						
Germacrene D									
	1	0.9814 ± 0.45	2.0009 ± 0.58	2.12	0.1520	3.27	**0.0894**	0.51	0.6096
	2	0.9612 ± 0.66	1.4092 ± 0.19						
	3	0.5760 ± 0.18	0.8385 ± 0.10						
γ-Elemene									
	1	0.0064 ± 0.01	0.0088 ± 0.00	1.34	0.2888	1.28	0.2749	1.46	0.2613
	2	0.1439 ± 0.01	0.0084 ± 0.00						
	3	0.0090 ± 0.01	0.0135 ± 0.00						
Ledene Oxide (II)									
	1	0.0220 ± 0.02	0.0896 ± 0.04	3.02	**0.0771**	5.21	**0.0365**	1.60	0.2318
	2	0.0081 ± 0.01	0.0052 ± 0.00						
	3	0.0136 ± 0.01	0.0588 ± 0.01						
Bicyclo [4.4.0]									
	1	0.0885 ± 0.06	0.1878 ± 0.06	1.25	0.3135	3.02	0.1012	0.49	0.6217
	2	0.0774 ± 0.07	0.0908 ± 0.02						
	3	0.0384 ± 0.03	0.1102 ± 0.02						
Total Terpenes									
	1	1.5936 ± 0.79	3.3129 ± 0.86	0.86	0.4406	3.78	**0.0695**	0.38	0.6915
	2	1.6553 ± 1.19	2.2699 ± 0.33						
	3	1.1817 ± 0.38	2.0063 ± 0.20						

Note: $p \leq 0.05$ (significant) and $p \leq 0.10$ (marginally significant) presented in **bold** text.
[a]All terpenes: *df* = 2, 21 for CLONE and CLONE*DAM, *df* = 1, 21 for DAM. N = 22.

Azulene, ledene oxide (II), and bicyclo [4.4.0] dec-5-ene, 1,5-dimethyl-3-hydroxy-8-(1-methylene-2-hydroxyethyl-1)- were significantly different among clones but were not affected by damage (Table 4, Figure 4). There was a significant interaction effect between clone and damage for γ-elemene, with this compound significantly different between clones and for plants damaged by herbivory prior to planting in the field (Table 4). Again, the levels of γ-elemene were lower in plants exposed to damage by *Trirhabda virgata* than in those unexposed plants (Figure 5). The total terpenes were significantly different among clones and between plants previously damaged by *T. virgata* larvae prior to planting in the common garden (Table 4, Figure 6). Total terpene quantities were lower in those plants subjected to damage in the greenhouse than in the plants that had no leaf beetle larvae prior to putting them in the field.

Table 4. *F* ratio, *p* value and *df*[a] for the effects of clone (CLONE), damage (DAM), and CLONE*DAM interaction on foliar terpenes identified in *Solidago altissima* from Gilley Research Station common garden (Proc GLM).

Terpene	2-Way ANOVA						1-Way ANOVA			
	CLONE		DAM		CLONE*DAM		CLONE		DAM	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
α-Pinene	0.6	0.651	6.3	0.014	2.9	**0.044**	0.6	0.646	5.9	**0.018**
Limonene	1.1	0.345	7.2	0.009	3.7	**0.017**	1.0	0.414	6.6	**0.013**
Bornyl acetate	9.4	**<0.001**	2.9	**0.093**	1.1	0.340				
β-Elemene	6.2	**0.001**	1.6	0.211	2.6	**0.060**				
Caryophyllene	4.4	0.007	3.3	0.076	2.8	**0.045**	4.0	**0.011**	2.9	**0.094**
Azulene	19.1	**<0.001**	2.4	0.123	2.1	0.115				
Germacrene D	0.5	0.666	5.3	**0.025**	2.6	**0.060**				
γ-Elemene	6.4	0.001	6.4	0.014	6.2	**0.001**	4.8	**0.005**	4.8	**0.032**
Ledene oxide (II)	17.4	**<0.001**	0.0	0.956	0.4	0.752				
Bicyclo[4.4.0]	18.9	**<0.001**	0.2	0.689	0.2	0.916				
Total Terpenes	3.7	**0.017**	4.5	**0.038**	2.6	**0.057**	3.3	**0.025**	4.2	**0.045**

Note: $p \leq 0.05$ (significant) and $p \leq 0.10$ (marginally significant) presented in **bold** text. One-way ANOVA values for CLONE and DAM reported if significant interaction effect observed.
[a]All terpenes: *df* = 3, 23 for CLONE and CLONE*DAM, *df* = 1, 23 for DAM. N = 24.

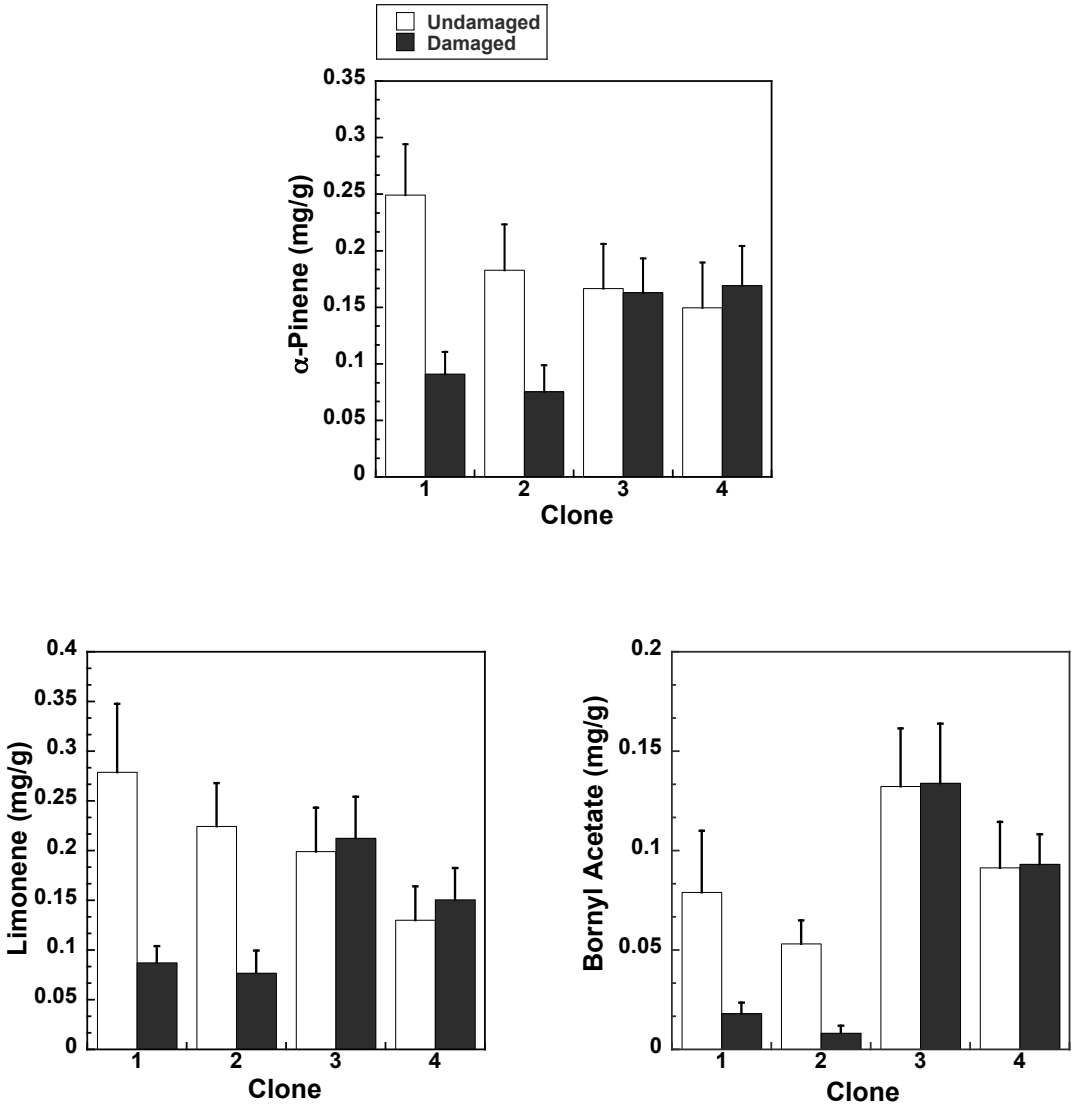

Figure 3. Mean foliar monoterpenes α-pinene, limonene, and bornyl acetate ± SE for clones and prior *T. virgata* damage on *S. altissima* at Gilley Research Station.

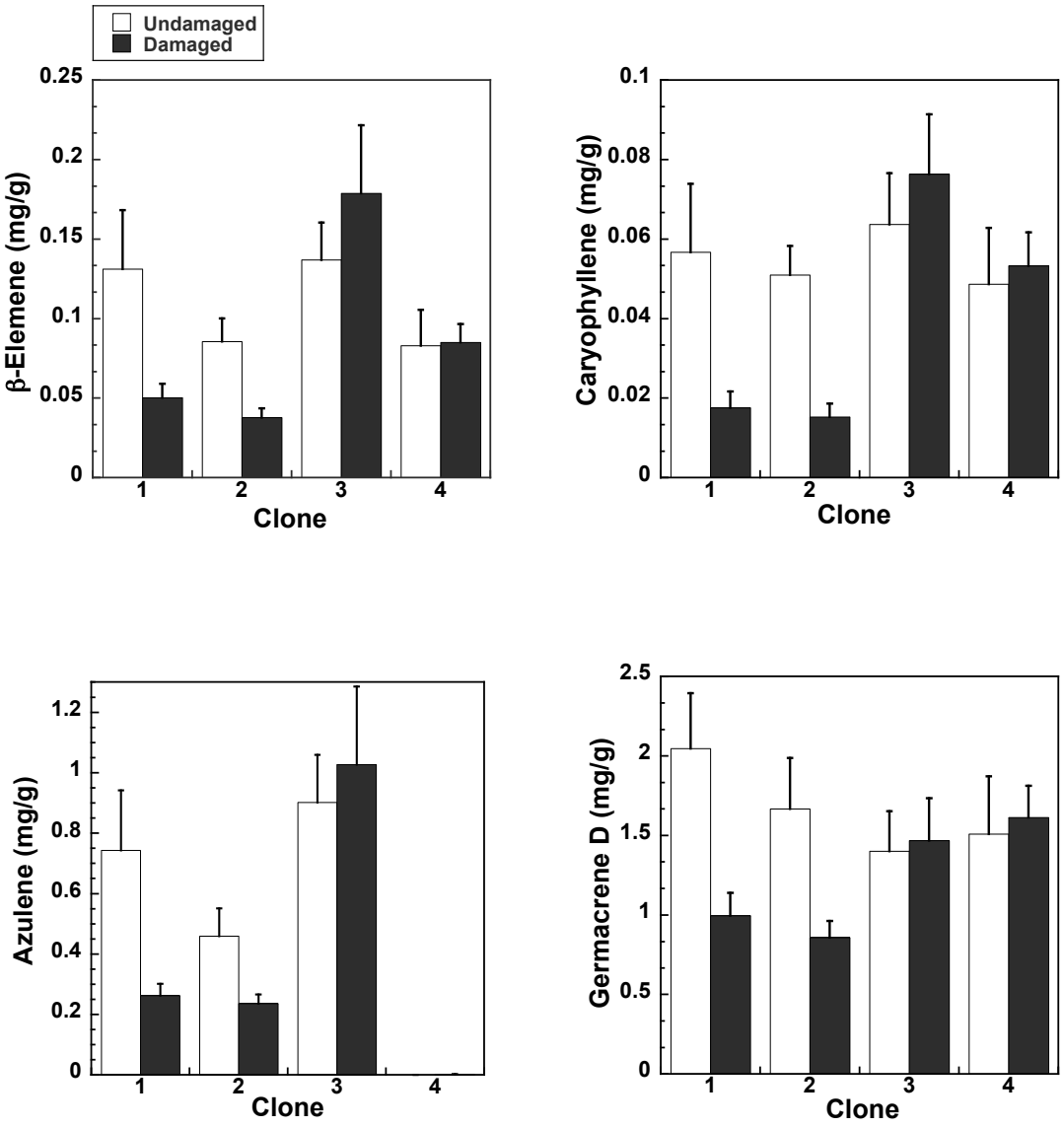

Figure 4. Mean foliar sesquiterpenes β-elemene, caryophyllene, azulene, and germacrene D levels ± SE for clones and prior *T. virgata* damage on *S. altissima* at Gilley Research Station.

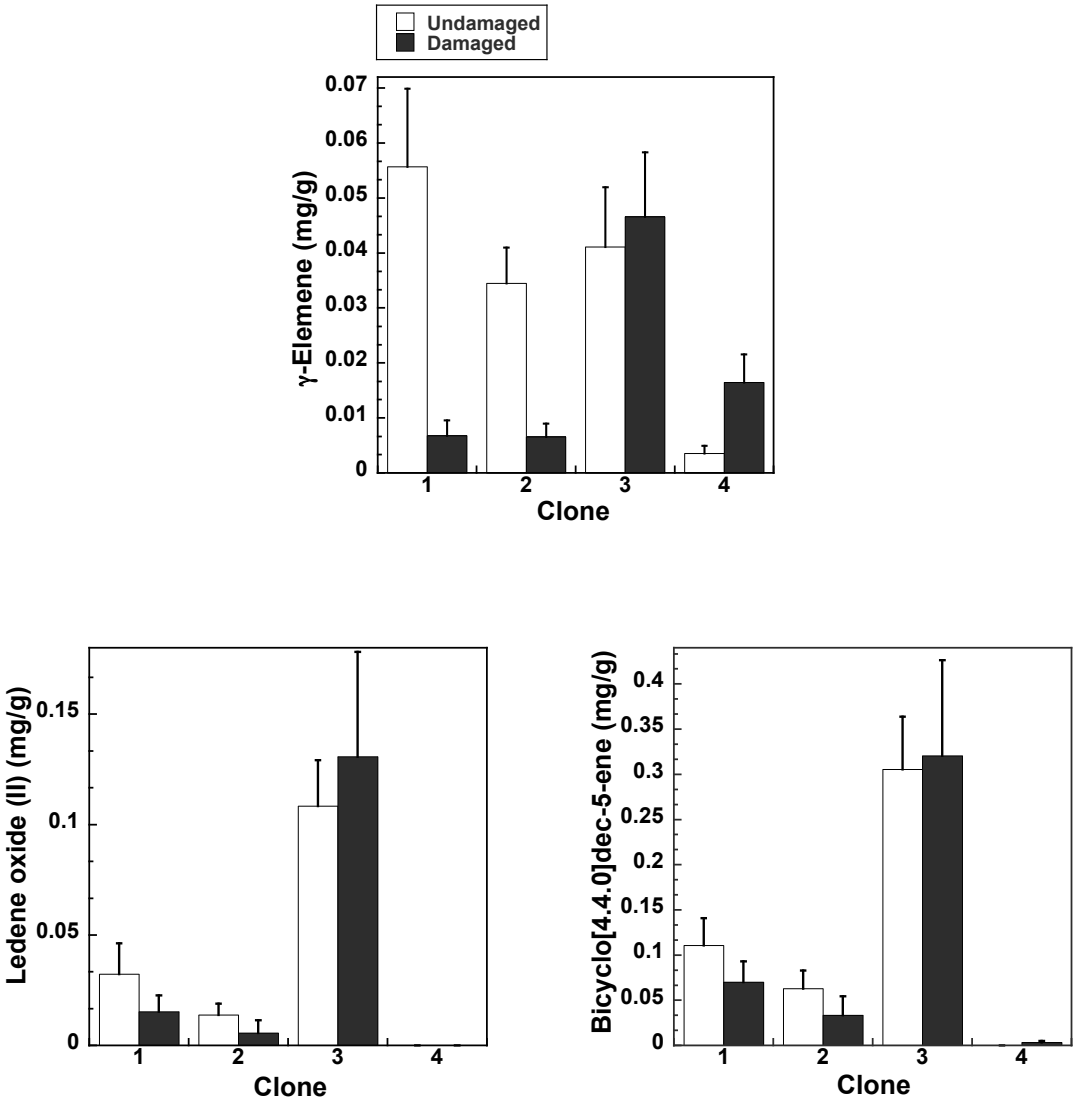

Figure 5. Mean foliar sesquiterpenes γ-elemene, ledene oxide (II), and bicyclo[4.4.0]dec-5-ene levels ± SE for clones and prior *T. virgata* damage on *S. altissima* at Gilley Research Station.

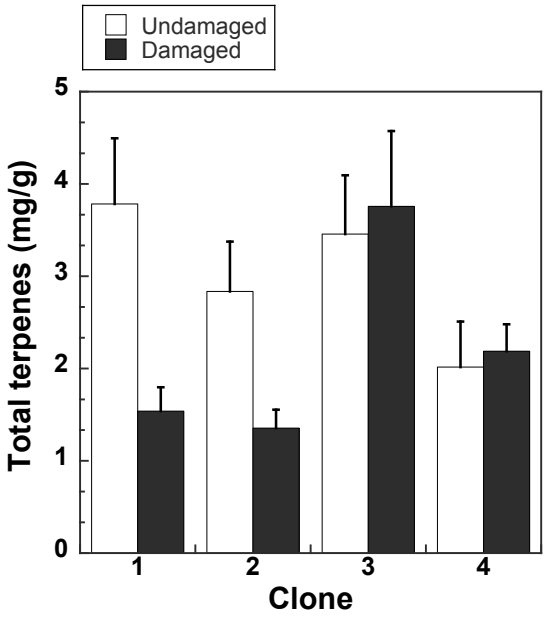

Figure 6. Mean foliar total terpene levels ± SE for clones and prior *T. virgata* damage on *S. altissima* at Gilley Research Station.

Regression Analysis

Correlation analysis (PROC CORR) was used to identify plant measures related to aphid responses at $p < 0.05$. Only terpenes were related to bornyl acetate, β-elemene, azulene, ledene oxide (II), and bicyclo [4.4.0] dec-5-ene, 1,5-dimethyl-3-hydroxy-8-(1-methylene-2-hydroxyethyl-1)- (Table 5), which were used to test relationships between terpenoid levels and aphid measures using linear regression.

Table 5. Correlation results (R^2 and p values) for *S. altissima* foliar terpenes related to aphid measures.

Terpenes related to aphid measures	Aphid Abundance		Aphids/total plot biomass		Aphids/mean plot biomass		Aphids/mean clone biomass	
	R^2	*p*	R^2	*p*	R^2	*p*	R^2	*p*
Bornyl acetate	0.126	0.089	0.126	0.089	0.128	0.086	0.154	0.058
β-Elemene	0.161	0.052	0.131	0.082	0.133	0.080	0.194	0.031
Azulene	0.243	0.014	0.171	0.045	0.170	0.045	0.234	0.016
Ledene Oxide	0.271	0.009	0.276	0.008	0.276	0.008	0.327	0.004
Bicyclo[4.4.0]…	0.311	0.005	0.265	0.010	0.265	0.010	0.335	0.003

Results of the linear regression analyses are found in Figures 4 – 11 using all four measures of aphid abundance, with terpenes the independent and aphid responses the

dependent variable. Bornyl acetate was marginally related to all aphid measures (Figure 7), and the positive relationship was somewhat weak (i.e. $R^2 = 0.126 – 0.154$).

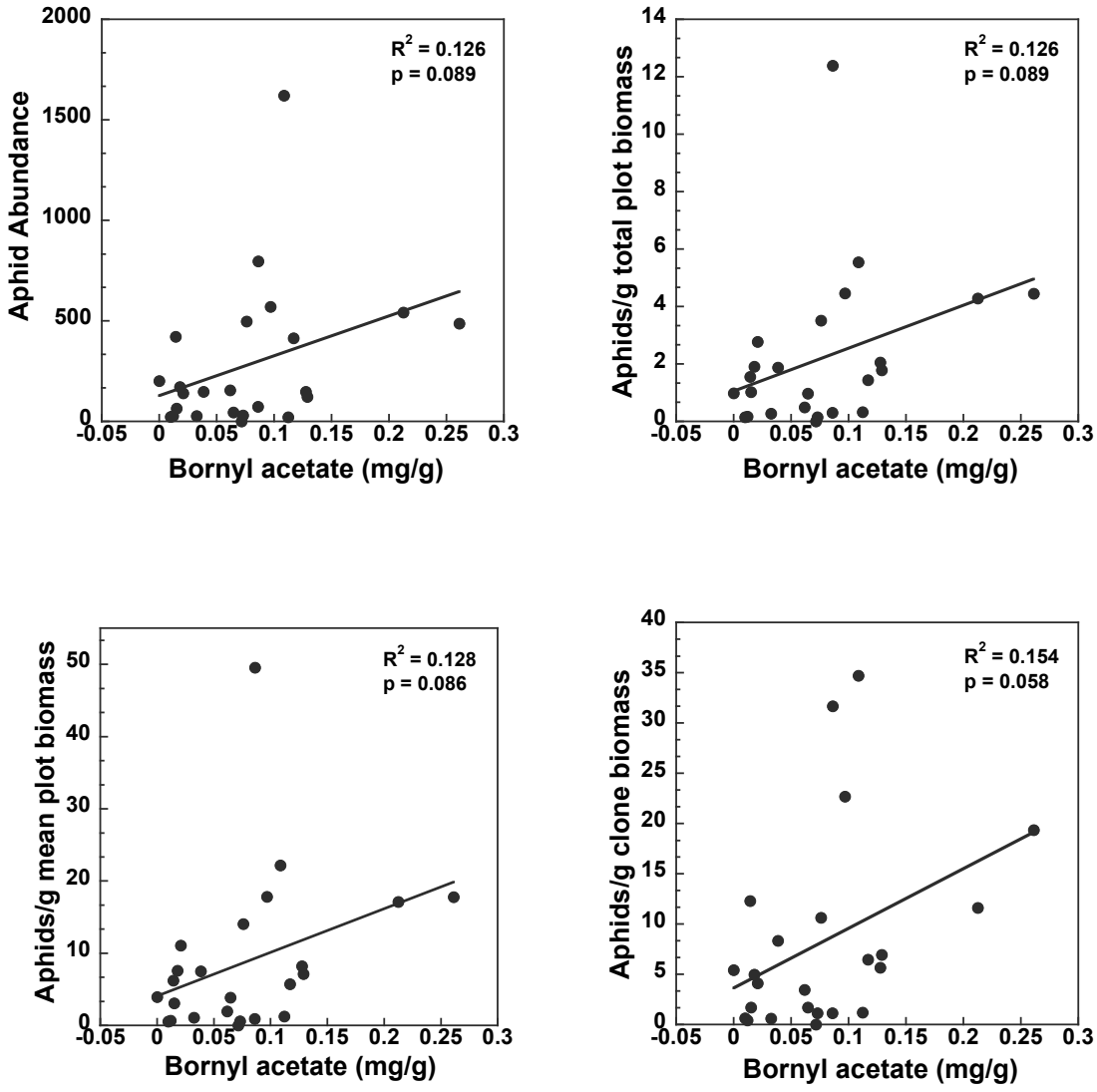

Figure 7. Relationship between aphid measures and foliar bornyl acetate in *S. altissima*.

The sesquiterpene β-elemene was also related to aphid measures (i.e. $R^2 = 0.131 – 0.194$), with levels more strongly associated with total aphid abundance in each plot and aphid abundance in each plot per mean clone biomass (Figure 8).

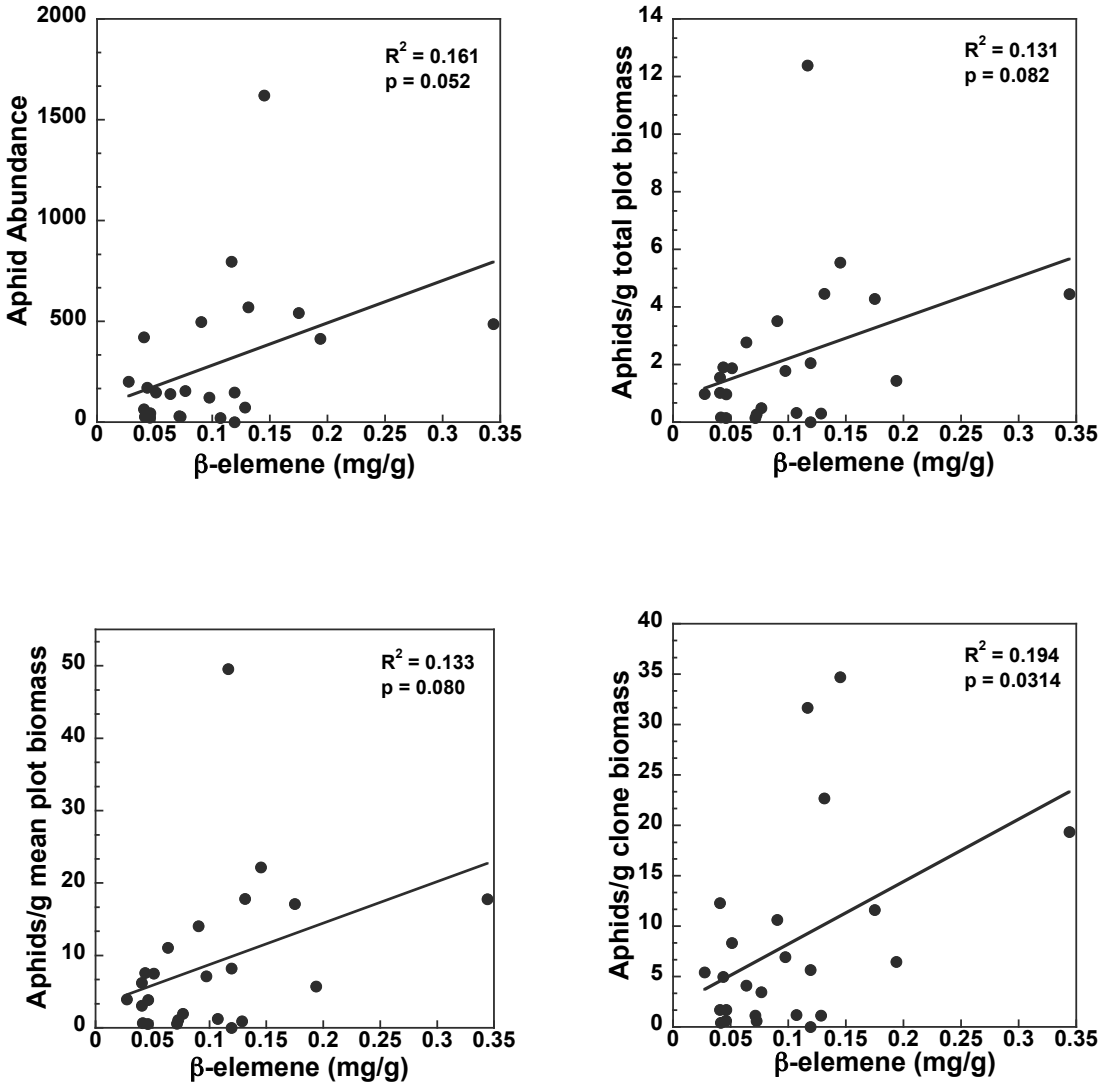

Figure 8. Relationship between aphid measures and foliar β-elemene in *S. altissma*.

Azulene was more strongly related to aphid measures than bornyl acetate and β-elemene, particularly aphid abundance and aphids per g mean clone biomass (Figure 9). Ledene oxide (II) (R^2 = 0.271 – 0.327) and bicyclo[4.4.0]dec-5-ene, 1,5-dimethyl-3-hydroxy-8-(1-methylene-2-hydroxyethyl-1)- (R^2 = 0.265 – 0.335) had the strongest correlation with aphid abundance (Figures 10 and 11).

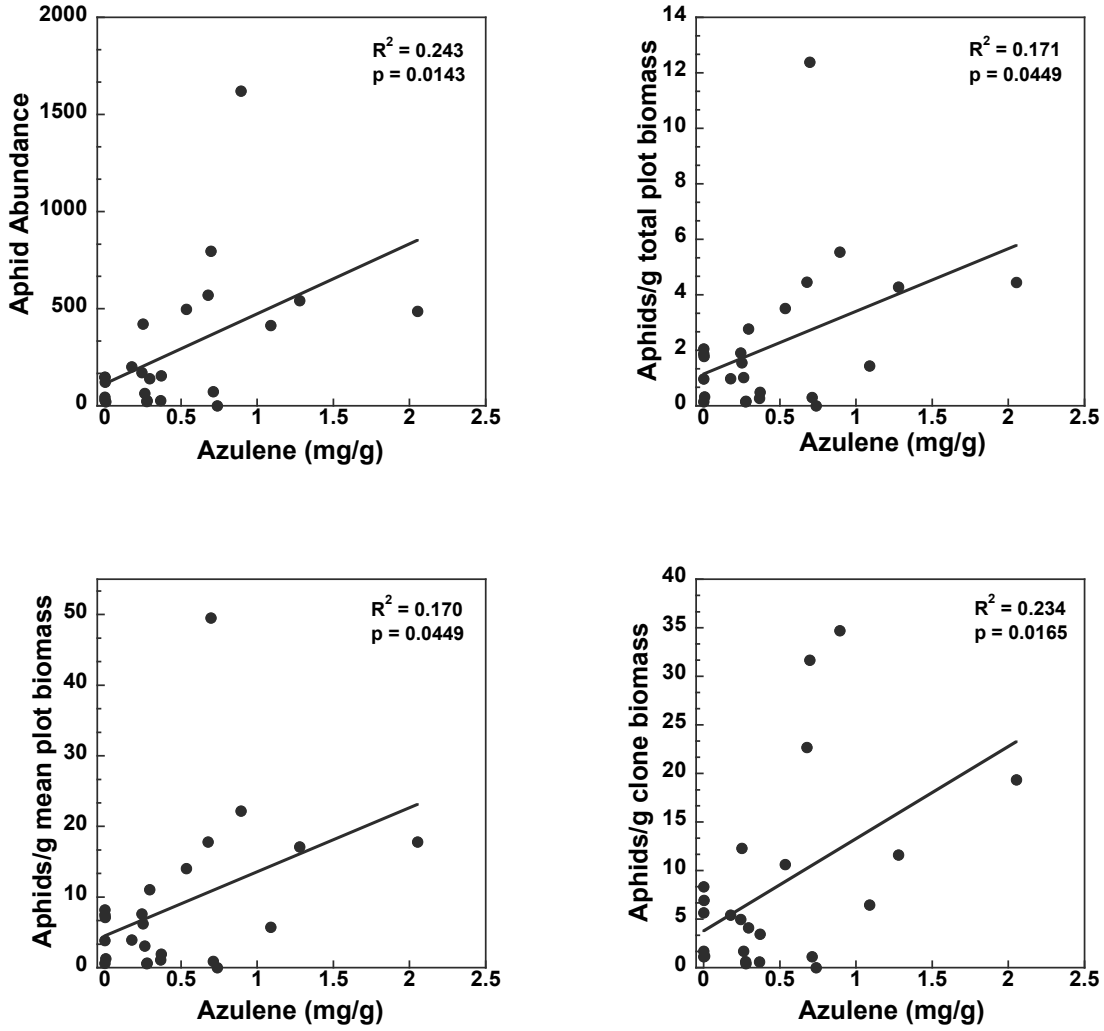

Figure 9. Relationship between aphid measures and foliar azulene in *S. altissma*.

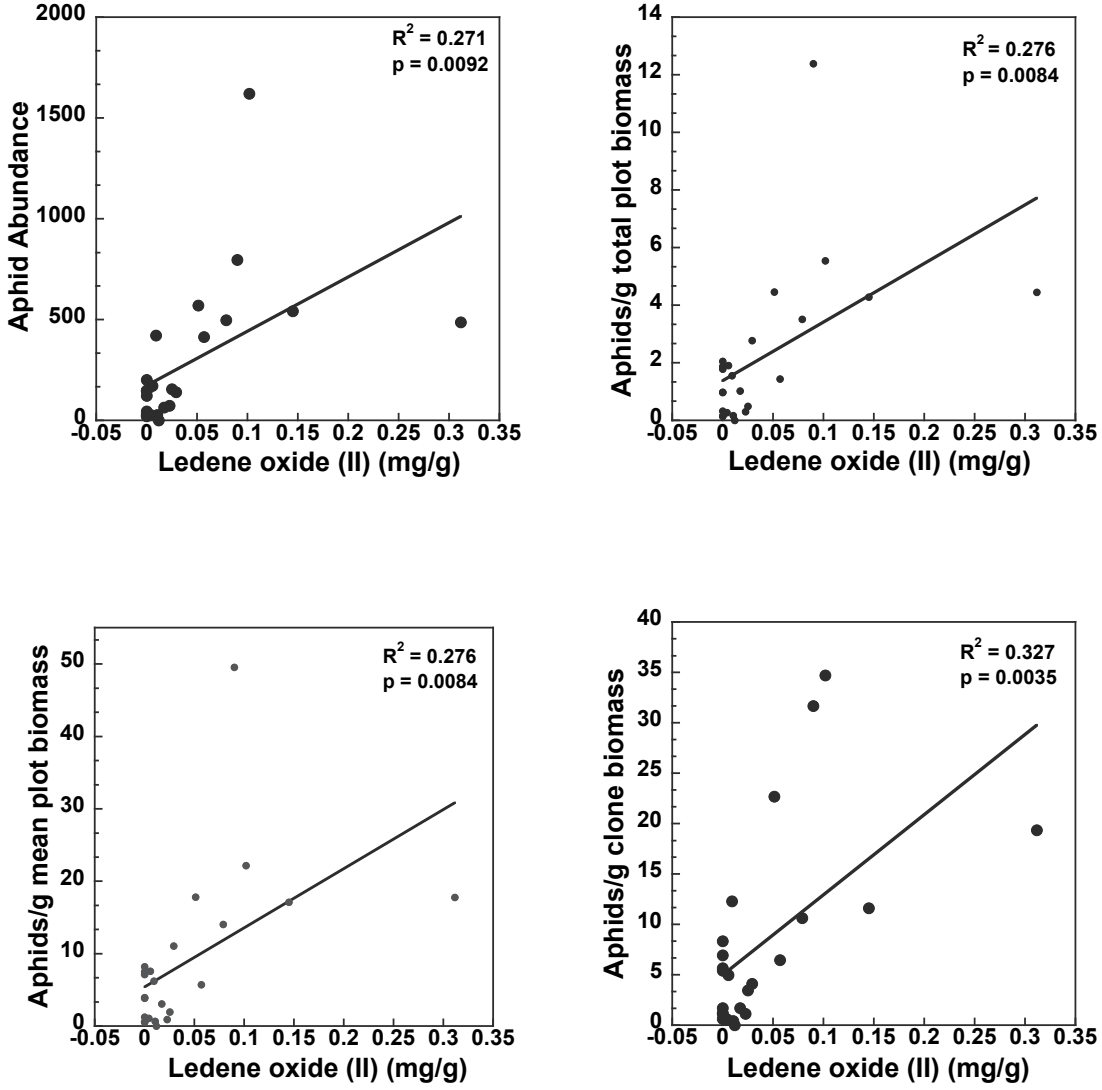

Figure 10. Relationship between aphid measures and foliar ledene oxide (II) in *S. altissma*.

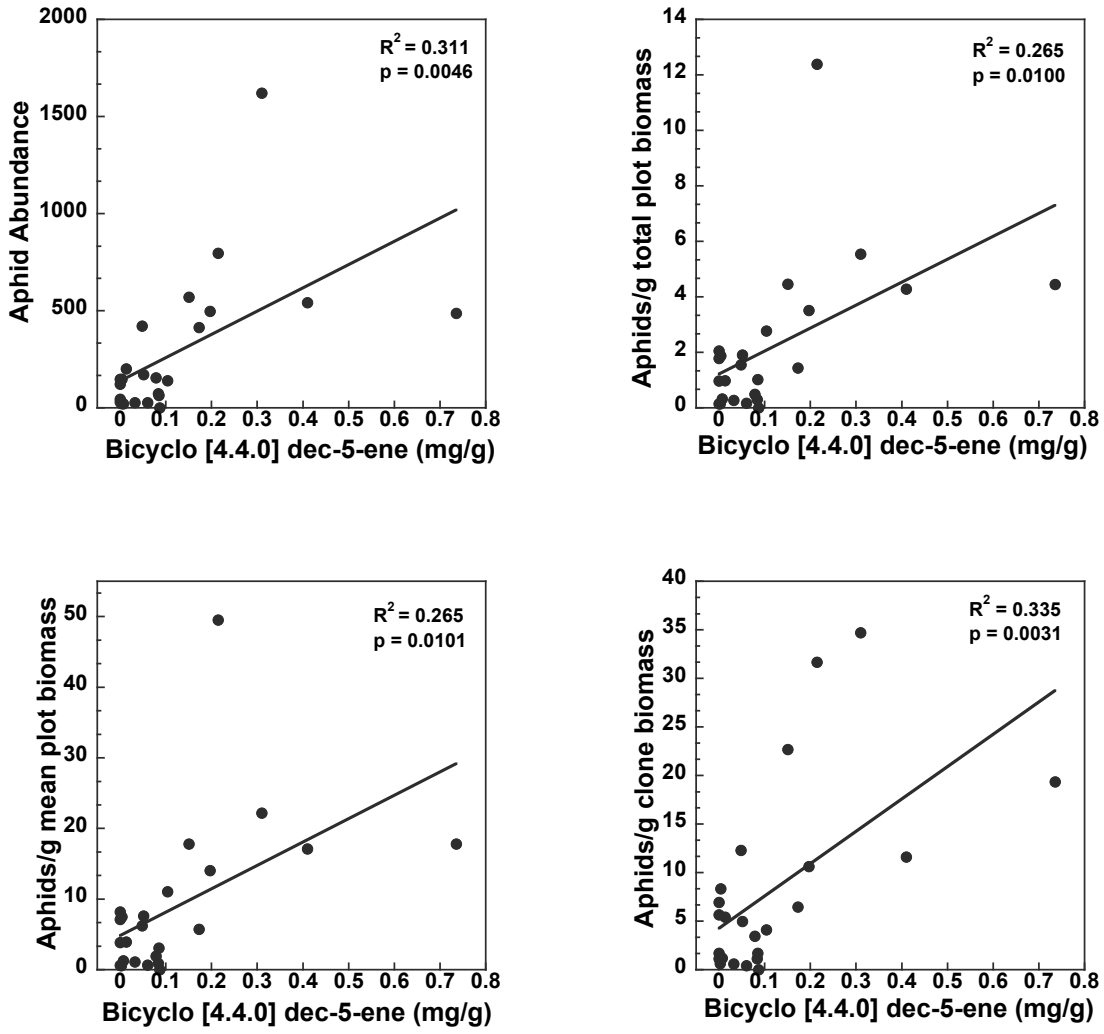

Figure 11. Relationship between aphid measures and foliar bicyclo [4.4.0] dec-5-ene, 1,5-dimethyl-3-hydroxy-8-(1-methylene-2-hydroxyethyl-1)- in *S. altissma*.

PLS Regression and MRPP Analysis

Partial least squares analysis indicated that for all terpenes, 60% of aphid abundance per g total plot biomass is explained by terpene values ($p < 0.001$). Carbon and nitrogen data did not predict aphid measures as accurately ($R^2 = 0.23$, $p = 0.018$). Therefore, terpenes more closely predicted aphid abundance per g total plot biomass than did foliar nutrient levels (Figure 12).

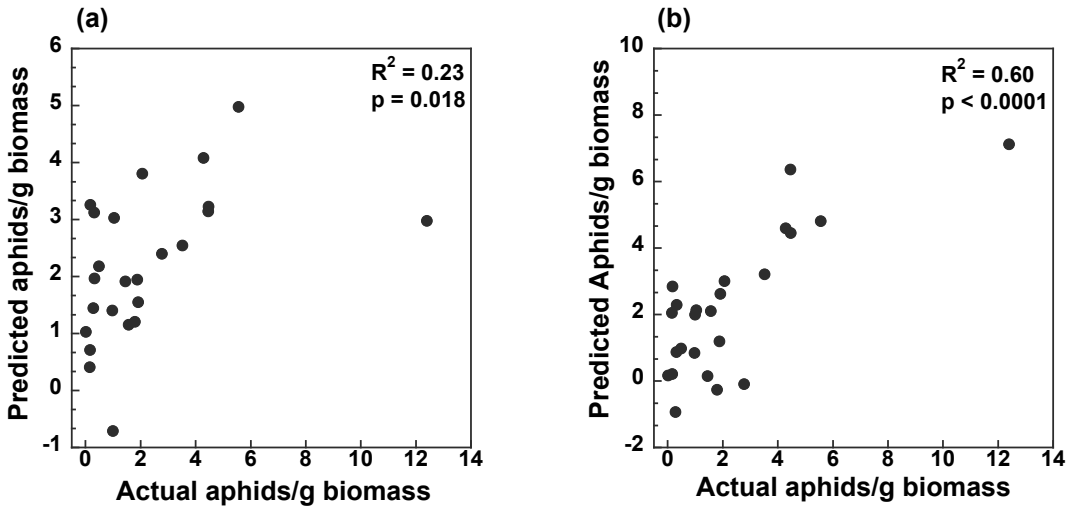

Figure 12. Comparison of predicted vs. measured aphids/g total plot biomass for (a) C and N data and (b) terpene data in *S. altissma*.

MRPP results indicated that terpenes among clones (A = 0.099, p = 0.045) and terpene interactions between clone and damage (A = 0.226, p = 0.006) were more similar to each other statistically than would be expected if they were put in other groups. Within damage terpenes were not significantly similar (A = 0.021, p = 0.166).

DISCUSSION

To better understand how intraspecific plant diversity can affect an associated specialist herbivore, I examined the foliar constituents of *S. altissima* and potential induction of terpenes. Terpenes are known in *Solidago* (Kalemba et al. 2001, Johnson et al. 2007), but there are few, if any, studies of the effect of plant genotypic differences on terpene production and possible induction as a mechanism for bottom-up effects in an ecosystem. Data from greenhouse grown plants subjected to herbivory indicated that several terpenoids increased after foliar feeding by *T. virgata* larvae compared to plants that had not been fed upon, providing evidence of induction in damaged plants. After 75 days in the field, terpenes were more affected by plant genotype than by prior damage by *T. virgata* larvae, though there were some differences in response due to previous damage, indicating plasticity in how plants responded to field conditions is due to genetic variation. Other plant constituents such as N, C:N, foliar water, and SLW also differed by clone and damage treatments. However, my analyses suggest they provide a less likely mechanism for specialist aphid responses. Plants appeared to express an adaptive response to prior herbivory in numerous chemical measures, but only terpenes correlated with the differences observed between clones for aphid abundance measures.

A primary objective of my study was to examine the impact of plant genotypic variation and prior herbivory on a number of plant constituents. Interaction effects between clones and prior *T. virgata* damage provide interesting insight into how interspecific genetic variation affects subsequent herbivore colonization. My data supports the conclusion that

prior damage to plants in the form of leaf beetle feeding appears to affect clones differently. Two clones had much higher levels of terpenes in previously undamaged than in damaged plants. These two clones did not have significant differences in aphid abundance between damage, though there was a trend toward higher aphid abundance in previously damaged plants for these clones (Figure 2). A study of herbivore induction in domesticated *Brassica oleracea* plants found that early-season herbivory by the caterpillar *Pieris rapae* caused generalist insects to avoid induced plants, while specialist insects preferentially colonized induced plants (Poelman et al. 2010). The fact that previously undamaged plants, after 75 days in the field, had higher levels of terpenes than did damaged ones for some clones indicates that the damaged plants may be allocating carbon resources differently in response to previous herbivory. Other clones showed no significant differences between levels of damage, though, on average, previously damaged plants were slightly lower in terpenes than undamaged plants. Differences in clone and prior herbivore damage interaction in the field-grown plants indicate that one effect of genotype on colonization by specialist aphids would be differences in plasticity of response, particularly relating to the production of terpenes.

Since my terpene analyses of greenhouse-grown plants found no significant differences between clones (Table 3), yet terpene analyses of clones after 75 days in the field showed very significant differences between clones (Table 4), undamaged and damaged clones in my study reacted differently to conditions in the field. Studies of conspecific insect herbivores have demonstrated that the identity of the initial herbivore can greatly alter subsequent arthropod community structure (Van Zant and Agrawal 2004, Erb et al. 2011). In a study of bittersweet nightshade plants, initial feeding by flea beetles caused lowered colonization later in the season by conspecifics, while initial

feeding by tortoise beetles did not lower herbivory later in the season (Viswanathan et al. 2007). By the time the plants in my study were planted in the common garden, all native *T. virgata* larvae had pupated, and uninduced plants would not have been subject to herbivory by this leaf beetle larvae. These plants were likely attacked by other insect herbivores, which could have elicited a different defensive response. In addition, rhizomes were initially grown in sterilized potting soil then transplanted to a common garden. As *S. altissima* has an arbuscular mycorrhizal association (Antoninka et al. 2009), and this association affects sesquiterpene production (Rapparini et al. 2008), I would expect a different allelochemical profile in field plants than in plants grown only in a commercial potting mix. Mycorrhizal associations have been shown to vary between host plant genotypes of the same species (An et al. 2010) so below-ground effects could also be a contributing factor to the terpene variation seen between clones in my study.

When looking at terpenes, MRPP analysis demonstrated that interaction effects were much more similar within groups than either clones or damage. This indicates that in addition to reacting differently to conditions in the field, genetically differing clones varied in response to prior damage by *T. virgata* larvae by producing different quantities of terpenoids. A study of induction of native and invasive tallow trees (*Triadica sebifera*) found intraspecific genetic variation in the trees affected induction of flavenoids and tannins (Wang et al. 2012). Across plant taxa, the majority of phenotypic variation in the production of plant allelochemicals is due to plant genotype (Wimp et al. 2007). Volatile terpenoid blends produced by genetically varying *Arabidopsis thaliana* accessions differ in quantity and quality (Tholl and Lee 2011). My study also indicated that plant genetic differences governing induction response had long-term effects on volatile terpenoid production.

The sesquiterpenes germacrene D and bornyl acetate were significantly lower among plants that had been damaged by prior herbivory, and this effect was most evident in two of the clones. Studies with *S. altissima* have found compensatory mechanisms in response to herbivory that include increased leaf area to intercept more sunlight to increase biomass and increased photosynthetic rates (Meyer 2000). My study indicates that induction of terpenes by *T. virgata* larvae may have negatively affected the production of some terpenes in populations of tall goldenrod mid-season, as plants are allocating carbon-based resources to other needs. Differences in such allocation lend a possible reason for clonal variation in my study, though more targeted experiments are needed to fully explain the plant responses.

Other plant constituents, particularly foliar water content, SLW, foliar N, and C:N, were significantly different between clones, and SLW, foliar N, and C:N were significantly different between damage, though there were no interaction effects. These differences in important nutrients and plant quality indicators indicate differing responses by *S. altissima* to herbivore damage and differences between clones, but they did not especially relate to *U. nigrotuberculatum* colonization of plants. Plant intraspecific genetic variation has been demonstrated in other studies to affect SLW, leaf water content, and foliar nitrogen (Crawford et al. 2009, Ghosh et al. 2009, Kant et al. 2011). In a study of intraspecific genetic differences in *Quercus laevis*, foliar nutrient composition was found to vary significantly, affecting ecosystem nutrient cycling (Madritch and Hunter 2005). Herbivory has also been demonstrated to shift nitrogen allocation in plants (Alcoverro and Mariani 2005, Frost and Hunter 2008). My study also found nitrogen and C:N differed between clones and herbivore damage, but all clones responded similarly to damage by predominantly having lower foliar nitrogen and higher SLW in previously damaged plants (Table 2). Though these are very

important considerations for many insect herbivores, my study indicated that nitrogen, C:N, SLW, and foliar water were not significantly correlated to aphid colonization. However, clones with genetically determined increased terpenes had higher aphid abundance than clones with decreased terpenes.

A second objective of my study was to relate differences in plant chemistry to colonization by a specialist aphid. It has been demonstrated that increased genotypic diversity in *S. altissima* populations causes increased diversity in associated arthropod communities (Crutsinger et al. 2006). In a study of *S. altissima* and *U. nigrotuberculatum*, it was found that genetically diverse goldenrod populations supported greater abundance of this specialist aphid (Ohgushi et al. 2011). My study indicates that the highest quantities of aphids were associated with clones that produced the largest amounts of terpenoids, and the lowest aphid abundance occurred on those clones with lower levels of some terpenoids. PLSR analysis of the data found a strong positive relationship between terpenes an aphids/g plant biomass ($R^2 = 0.60$, $p < 0.0001$), demonstrating that the number of aphids was related to the amount of terpenes. This same analysis found weaker relationships for nutritional measures. In a study of white cabbage (*Brassica oleracea*) cultivars it was demonstrated that plants subjected to prior herbivore damage repelled generalist insects but attracted specialist insects (Poelman et al. 2010). However, it is unlikely that aphid colonization produced these compounds, as clones with moderate aphid abundance and aphids/g total plot biomass produced very little ledene oxide (II) and bicyclo [4.40] dec-5-ene, which were highly correlated with aphid measures. Also, those clones that had no prior damage and high levels of bornyl acetate, β-elemene, and azulene had similar aphid abundance and aphids/g total plot biomass compared the damaged plants of the same clones with lower levels of these

terpenes, though bornyl acetate, β-elemene, and azulene correlated with aphid measures. It seems likely that *U. nigrotuberculatum*, as a specialist insect herbivore, was attracted to the terpenes produced in greater quantities in one clone in particular, and the higher levels of terpenoids in this clone, which were not documented in the greenhouse plants, were expressed as a variable response to environmental conditions or herbivory in the field.

The foliar terpenoids that I identified in leaf samples of *S. altissima* were volatile monoterpenes and sequiterpenes. A previous study of the essential oils of the related *S. gigantean* found many of the same compounds that I identified in my samples; α-pinene, limonene, bornyl acetate, β-elemene, caryophyllene, and, in the largest quantity, germacrene D (Kalemba et al. 2001). There are many other unidentified terpenes present in *S. altissima*, in various amounts, and these compounds may also affect aphid colonization. The fact that my study identified a relationship between *U. nigrotuberculatum* abundance and plant terpene production and also identified effects of *S. altissima* clone and induction on terpenes indicates that these are important constituents in structuring specialist aphid communities and are potentially a mechanism behind how genotypic intraspecific plant diversity affects an associated specialist herbivore.

Linear regression found no relationship between aphid measures and other plant constituents, and though PLSR analysis of N, C:N, foliar water, and SLW indicates that these measures were related to aphids/g plant biomass, the relationship was weak ($R^2 = 0.23$, $p = 0.02$) compared to foliar terpenes ($R^2 = 0.60$, $p < 0.0001$). One clone had the lowest nitrogen levels, highest SLW, and lowest foliar water content and subsequent lowest aphid abundance. It is possible that these measures of lower clone quality may have affected aphid colonization regardless of terpene attraction, though a single clone response does not allow for strong

conclusions. My data does suggest nutritional quality may play a role in specialist aphid selection of host plants, and genetic variation in this quality would contribute toward variation in herbivore colonization. However, relationships measured by PLSR and regression analyses indicate that terpenes are a much better predictor of aphid colonization than nutritional levels.

Due to the fact that aphids responded primarily to terpenoids produced by the plants, and the production of these terpenes was largely due to the interaction between *S. altissima* genetic variation and prior herbivory, my study suggests that a likely mechanism for aphid colonization of *S. altissima* is genetic differences in production of terpenes as a response to natural growth conditions and potentially insect damage resulting in terpene induction. Only trace amounts of volatile terpenes are detected in *Arabidopsis thaliana* leaves under normal physiological growth conditions, but volatile blends are induced by insect herbivory, fungal infection, or the application of jasmonate, and these blends vary in quantity and quality in different accessions that differ genetically (Tholl and Lee 2011). The role of terpenes in my plant-insect system draws some similarities from others. For example, in a study of terpene production by *Pinus pinaster* varieties sensitive and resistant to the pine weevil *Hylobius abietis*, it was found that sensitive varieties had higher levels of foliar terpenoids, which acted as an attractant to the pine weevil (Blanch et al. 2012). Heavily browsed trees yellow-cedar trees have been found to have significantly lower levels of monoterpenes with significant variation between genets (Vourc'h et al. 2002). My study was somewhat limited by the small number of clones that I was able to propagate and study. Even so, my data supports the role of plant genetic variation in terpene production affecting colonization by a specialist aphid.

Conclusion

In conclusion, I found that colonization of *S. altissima* by *U. nigrotuberculatum* was related to genetic differences in terpene production between clones, and that clones produced terpenes differently in response to prior damage and other unmeasured environmental factors. Other plant constituents, N, C:N, foliar water, and SLW, also varied by genotype and prior damage, but were not as closely related to aphid colonization. This study indicates that terpenes are a potential mechanism by which specialist herbivores choose their hosts, and that terpenes produced vary with genetic variation of host plants and their response to initial herbivory. My study focused on the colonization of *S. altissima* by a specialist aphid. However, it has broader implications. Since increased interspecific plant diversity increases diversity in associated arthropod communities, terpene induction by an initial herbivore and variation in constitutive and induced levels of terpenes could be a principle mechanism behind community dynamics. Though further studies are needed to determine how genetic differences in terpene production impact other species in the insect community, the effect of genetic variation of plant terpene production on specialist herbivores, and parasitoids and predators of herbivores, indicates that these bottom-up effects are instrumental in structuring ecosystem diversity.

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VITA

Jessica Moss Howells was born Jessica Linn Moss to her parents Jesse Monroe and Elizabeth Siler Moss, a middle child of five daughters. Jessica attended North Carolina State University in Raleigh, North Carolina, graduating with a B.S. degree in Wildlife Biology in 1980. While an undergraduate, Jessica married and was employed by National Institute of Environmental Health Sciences, where she continued to work after graduation. Jessica was later employed at East Carolina University's Brody School of Medicine, and then with the North Carolina Department of Environment and Natural Resources in the Department of Water Quality. After Jessica had two children, she realized an interest in education and taught part-time at Western Piedmont Community College. In fall 2007, Jessica began work on a Master of Arts degree in Higher Education with a teaching specialty in Biology at Appalachian State University. While taking the required biology classes, Jessica became fascinated with the advances in biology made over the past 30 years and added a Master of Science degree in Biology under the tutelage of Dr. Ray Williams at Appalachian State University. Jessica completed all requirements for a Master of Arts degree in Higher Education and a Master of Science degree in Biology in August 2012. She is a member of Sigma Xi and is on the Board of Directors for the Exploring Joara Foundation, which promotes public archaeology and education. Jessica is currently pursuing a career teaching biology full-time.