THE EFFECT OF *SOLIDAGO ALTISSIMA* CYTOTYPES AND SOIL NUTRIENTS ON TERPENE PRODUCTION, LEAF NUTRIENTS AND UROLEUCON NIGROTUBERCULATUM ABUNDANCE

A Thesis
by
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ABSTRACT

THE EFFECT OF *SOLIDAGO ALTISSIMA* CYTOTYPES AND SOIL NUTRIENTS ON TERPENE PRODUCTION, LEAF NUTRIENTS AND *UROLEUCON NIGROTUBERCULATUM* ABUNDANCE

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The specific factors that drive plant genotype choice by insects remain uncertain. Though trait variation among genotypes in phytochemistry can be important, the function of plant polyploidy (i.e. cytotype) on secondary chemistry and insect associations has not be thoroughly studied. I investigated the connection between tall goldenrod, *Solidagoaltissima*, cytotypes (diploid and hexaploid), phytochemistry (C, CN and terpenes) and soil nutrient level on the abundance of a specialist aphid *Uroleuconnigrotuberculatum*. Terpenes are one of the largest classes of secondary metabolites and can vary among plant genotypes. I hypothesized that chromosome number and available nutrients would affect leaf nutrientsterpenes concentration in *S.altissima*and affect the abundance of *U.nigrotuberculatum*. A randomized common-garden design used sixty-four plants (32 diploid and 32 hexaploid representing four genotypes (cytotype), with eight plants per

genotype. I added soil nutrients to four plants per treatment, while four plants had no nutrient addition. After allowing aphids to naturally colonize our plants I quantified aphid abundance throughout a growing season. During peak aphid abundance I took estimates of plant biomass and leaf samples for phytochemical analyses. I found that soil nutrients had a significant effect on aphid abundance ($p=0.0178$) and no effects of cytotypes or cytotypes x nutrient interaction. The biomass of 2n plants were significantly larger than 6n plants (Cytotypes effect=0.045) and high nutrient plants were significantly larger than ambient nutrient plants (nutrient effect, $p>0.001$). This study found nutrient and cytotypeinteractions with CN ($p=0.0009$), suggesting that soil fertility and chromosome number may have considerable effects on carbon- nitrogen usage in plants. Soil nutrients only marginally affected leaf nitrogen on plant. Chromosome number(cytotypes) had significant effects on three foliar terpenes: α-pinene ($p=0.004$), β-pinene ($p=0.003$) and Bornyl acetate ($p=0.027$) of hexaploid plant with nutrient addition.Terpenesconcentrations were not related to soil nutrients addition. Because terpene concentrations were not related to soil nutrients addition, both 2n and 6n plants apparently used nitrogen more likely for growth purposes than secondary metabolite production. There were no relationship between terpenes concentrations and aphid abundance as a result; this plant trait variation cannot account for my observed plant-insect associations.

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DEDICATION

This thesis is dedicated to my parents, Late Mr. Pius Akhiwu, JustinaAkhiwu and my siblings for their supports and encouragements.

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INTRODUCTION

Plant Genetic Variation and Associated Insects

An important issue in community genetics research is determining the relevance of intraspecific genetic variation and genetic differentiation (i.e. divergence) on ecological and evolutionary processes at the community and ecosystem level (Whitham et al., 2012). Intraspecific genetic variation in a plant species not only affects the composition of associated insect communities (Crutsinger et al., 2006) but can cause community members to evolve in response to genetic differentiation across the focal species' range (Genung et al., 2010). Such findings provide a strong argument for the necessity of considering intraspecific genetic variation in ecological studies. The diversity of plant species has been positively linked to the diversity of associated animals through the provision of different types of food and habitat resources (Hutchinson., 1959). For example, it is well established that plant species diversity positively affects the diversity of aboveground arthropods through increased primary production and the presence of preferred host plants (Crutsinger et al., 2008). But in addition to diversity, genetic variation provides great resources for arthropod communities (Wimpet al., 2004), who reported that genetic diversity plays a major role in structuring arthropod diversity. Such studies demonstrate that genetic variation among plant exert a strong influence on arthropod communities and can reorganize and restructure arthropod colonization and abundance (Wimp et al., 2004). Previous investigations have identified linkages between host plant genetics and associated communities, and reported that such linkages influenced arthropod communities by providing insects with greater choices (Utsumi et al., 2011). The movements of insect herbivores amongst plants have also been

attributed to genetic variation (War et al., 2018). Plant genotypic variation can affect different species in an ecological community (Root., 1973). For example, genetic diversity among plants may provide resistance to outbreaks of herbivores in diverse communities, while also encourage specialist insect feeders (King et al., 2012). Understanding the consequences of intraspecific genetic variation and genotypic diversity within plant communities on the local diversity and abundance of insect communities represents a critical research direction to explore.

Importance of Plant Phytochemistry for Insects

The genetic makeup of a plant is important to consider for understanding secondary metabolite production because of the role these compounds play in shaping plant-insect associations (Bourgaud et al., 2001), and because allelochemicals may affect their resistance to herbivores (Orians et al., 2003). Plant secondary metabolites are well-known for their role in plant defense against insect herbivory (Bidart-Bouzat et al., 2008). These chemicals, although not needed for primary plant metabolic processes, such as growth, respiration or reproduction, have been reported to have important roles in a plants defense against herbivore attack, biotic defense and in some cases for attracting pollinators (Kliebenstein ., 2004). They are as such important "mediators" of plant-insect interactions (Cornell and Hawkins., 2003). Allelochemicals such as terpenes are known to affect insects (Islam et al., 2017) and play important defensive roles in the plant kingdom (Gershenzon et al., 1992). Compounds such monoterpene esters (pyrethroids) found in leaves and flowers of *Chrysanthemum* species has been proven to have insecticidal activity and are widely used in manufacturing of insecticides (Trapp and Croteau., 2001). Plants such as *Azadiractin* (neem tree) deters herbivore insects by emitting terpenes known as limonoids (Aerts and Mordue.,

1997).These terpenes are also defensive mechanism during herbivory (Gershenzon and Dudareva., 2007).Though terpenes are a large chemical class (reviewed in Langenheim 1994), their diverse chemistry creates difficulties in defining them as purely defensive (Pichersky and Raguso, 2016).

The importance of soil nutrients to plant physiology and phytochemistry cannot be over emphasized. Nutrients such as Nitrogen, potassium and phosphorus (Johnson et al., 1996) play important roles in plant growths and development. A study has demonstrated that fertilization with NPK increases plants biomass, nitrogen content of plant (wheat, maize, etc), and that this influences herbivore colonization (Schutz et al., 2008). There are positive relationships between the concentrations of nitrogen and extractable phosphorus in many plants (Ormeño et al., 2008). Studies reported that aphid choices are influenced by soil fertility. For example, in two species of aphids: *Metapolophiumdirhodium* and *Rhopalosiphu. Metapolophium*, *M.dirhodium* prefers plant with fertilizer, while *Rhopalosiphum* prefers plants with no fertilizer (Garratt et al., 2010). The author concluded that these insect choices where based on the color, size, biomass and growth of the host plant (Garratt et al., 2010). Plant nutritional qualities can interact to either increase or decrease insect abundance (Karley et al., 2004), where it is reported that soil nutrients resulted in aphid abundance whilenutrients attracted the insects natural enemies such as lady birds and ants. Nutrients in soil have implications for phytochemistry. Plants may allocate nutrients either for growth or production of secondary metabolites (Tuomi et al., 1991) such that increases in soil nutrients would result in the production of more secondary metabolites, while low soil nutrients result in a plant allocating nutrients for growth (Tuomi et al., 1991). For aphids, plants with more nutrients (Nitrogen) would likely have more amino acid metabolism, possibly attracting these

insects (Guo et al., 2013). Specific nutrients may influence the leaf chemistry of a plant. Noma et al., (2010), in a study on soybean aphids, reported that the abundance was mostly associated with more Potassium, whereas plants with Potassium deficiency had less aphid abundance. The ratio or percentage of each nutrient in the soil plays an important role in determining insect herbivore abundance (Noma et al., 2010). The body tissues of insects contain higher concentrations of nitrogen and phosphorus than their host plants (Zehnder and Hunter., 2009) so it follows low nutrient availability could limit herbivore growth and reproduction. However, there is a certain nutrient threshold herbivores need for growth, development and reproduction. Increases in foliar nitrogen has been demonstrated to increase insect (aphid) population growth (Sauge et al., 2010, whereas decreases in foliar nitrogen would result in decreased aphid populations (Zehnder and Hunter., 2008). Since alterations in allelochemicals influence herbivore composition by either attracting or deterring herbivore insects (Segraves., 2017), how soil nutrients affect their production is important to understand. Some studies have reported that soil nutrients may have no effect on plant secondary chemistry but instead influence plant biomass and amino acid content (Bethke et al 1998; Casey and Raupp., 1999; Aber et al., 2003; Stevens et al 2004; Throop and Lerdau., 2004; Muller et al., 2005). Soil nutrients may not on its own alone affect plant secondary chemistry or insect's abundance but rather interact with other factors to influence plant secondary chemistry and thereby alter herbivore compositions (Guo et al., 2013).

Importance of Plant Genetic Variation on Plant Chemistry

Previous studies have found effects of intraspecific genetic variation on phytochemistry. A qualitative difference in terpene production in *Austrailiamyrtaceae* has been attributed to variation in terpene syntheses and terpene-modifying enzymes as a result of genetic variation (Keszei et al., 2007). Plant genetic variation serves as a primary factor that affects plant chemistry as proposed by Wimp et al., 2007. A study by Vourch et al., (2000) on cedar trees reported that genetic variation influenced the amount of monoterpene produced within two cedar trees (yellow and red cedar). The study reported that red cedar trees had higher monoterpene concentrations than yellow cedar trees because of genetic differences between them.

A study on *Eucalyptus amygdalina, E.risdonii*, and their F1 hybrids by Dungey et al., (2007), documented that there was a connection between leaf terpenoids of hybrids to the parent secondary metabolites. This study also demonstrated that there were significant interactions between genetic variation and terpenoids. Genotypes may vary in a number of carbon-based defensive compounds (for example phenolics- Madritch et al., 2006). In *S.altissima*differences among genotypes in the allelochemicals class terpenes are well documented (Williams and Megan., 2015), where they play a role in genotype choice of a specialist aphid. Plant genetic variation can affect the amount of phytochemicals either by increasing or decrease the concentration (Orians et al., 2003).Similarly, the composition of the mixture of chemicals can vary within and among individuals (Moore et al., 2013), affecting the diversity of specific phytochemical interactions between plants and their enemies (Moore et al., 2013).

Polyploidy in Plants

Polyploid plants (represented as cytotypes) have three or more complete sets of chromosomes in their nuclei as compared to the two found in a diploid (Leitch and Bennett., 1997). Polyploidy has been reported to cause an array of phenotypic changes in plants and affects interactions with other species (Segraves and Anneberg., 2016). For example, increases in flower size have been reported to have a direct effecton pollinator species (Segraves and Anneberg., 2016). Polyploidy in plants provides a major source of genetic diversity among plants (Halverson et al., 2008). There are two general ways in which polyploidy can occur: multiplication of one chromosome set and merger of structurally different chromosome set (Tate et al., 2005). Autopolyploidy plants are formed by chromosomes of same species, and are fertile products, whereas allopolyploidy plants are formed by different species (for example when specie A X specie B to form a polyploid), resulting in non-fertile individuals (Avraham and Moshe.,2004). Polyploidy has been reported to facilitate survival and adaptation (Estep et al., 2014; Soltis et al., 2014; Van da peer et al., 2017) and these attributes of polyploidy have been recognized as a major process that facilitates formation of new species (Mable., 2013). Polyploidy alters regulatory interactions, rapid genetic and epigenetic changes in flowering plants (Song and chen., 2015) and can result in genetic isolation, ecological differences among cytotypes and speciation, which can influence herbivore choices and community compositions (Richardson et al., 2011). Some herbivore insects, such as leaf galling flies and aphids, have a preference for plants with a higher chromosome number (for example hexaploid (6n) compared to diploid (2n) and tetraploid (4n; Richardson and Hanks., 2011). Cytotypes can show large differences in the composition of insect herbivore communities. One classic example of such a

difference was reported by Münzbergová et al., 2015, where the seeds damaged by the herbivorous insects studied were higher in frequency in a tetraploid than diploid. This study shows also found chromosome number affects the secondary metabolites of a plant and can influence herbivore insects' preference (Münzbergová al., 2015). Polyploidy may provide diversification for insect choices (Nuismer et al., 2007; Thompson and Janz., 2002). Since insect herbivores may have a preference for certain cytotypes (Nuismer et al., 2007) it follows that cytotypes may also vary in phytochemicals, thereby making insects sensitive and selective to genetic structure of a plant (Hull-Sanders et al., 2009a; Nghiem et al., 2011). Chromosome doubling may have an indirect effect on phytochemistry and herbivore compositions by causing increase in plant's growth or decrease in growth (Segraves.,2017). Plant defensive chemistry is known to be affected by polyploidy and can cause a reduction in generalists' herbivores attack while increases the frequency of specialists with counter defenses (Segraves., 2017). Cytotypes may differ in the overall suite of pollinator they attract due to differences in secondary metabolites (Thompson et al., 2004). Studies suggest that insect pollinator visits a plant or prefers certain cytotypes over other due to differences in phytochemical concentration (Münzbergová et al., 2015; Roccaforte et al., 2015). For example, two bees were studied (*Andrenaerythronii* bees and *Andrenacarlinii*), where *Andrenaerythronii*bees prefers diploids, while *A. carlinii* prefers a tetraploid. Their choices were due to differences in secondary chemistry of the cytotypes (Roccaforte et al., 2015). Since an insect herbivore may have certain maximum tolerance level for phytochemicals during each developmental stage (i.e. larvae stage) it follows they may prefer a certain cytotype based on age and preference may change as they develop or grow into adult stage (Koniget al., 2014).

Polyploidy results in complex interactions between plants and associated insects. This complexity is either by increasing or decreasing the likelihood of two herbivores sharing a host plant ramet or even no effect of ploidy at all (Halverson et al., 2007). Chromosome doubling affects the morphology of the resulting plant and thereby affects the communities of herbivores (Richardson and andHanks., 2011). In *S. altissima*hexaploids are known to have taller ramets and larger leaves than other cytotypes, which results in specialist insects making a preference for hexaploids (Richardson and Hanks., 2011). With respect to phytochemistry, cytotypes have been reported to vary in terpene production in *Solidago* (Hull-Sanders et al., 2009a), where diploid and tetraploid plants have lower concentrations of monoterpene, diterpenes and sesquiterpenes than hexaploid plants, resulting one effect on herbivores (Hull-Sanders et al., 2009b).In this study the generalist *Spodopteraexigua*and specialist *Trirhabdavirgata* were influenced by chemical compositions of cytotypes, suggesting cytotypes exert strong influence on the insects growth and development (Hull-Sanders et al., 2009b).

Solidagoaltissima **as a Study System**

Solidagoaltissima L. (Asteraceae), tall goldenrod, is a rhizomatous perennial plant species with a native distribution over much of temperate North America (Semple and Cook 2006). Its original habitats likely included prairies and forest openings but since European colonization *S. altissima* has become an abundant plant of roadsides, old fields, and other disturbed or succession areas (Abrahamson et al., 2005). This speciesis known to be autopolyploid and there are three known cytotypes; diploid (2n=18), tetraploid (4n=36), and hexaploid (6n=54) (Halverson et al., 2008). This goldenrod species is predominantly diploid in the west (treated as *ssp.gilvocanescens* by Semple and Cook, 2006) and hexaploid in the

east *(ssp.altissima*), and these cytotypes may co-occur in some local populations (Halverson et al., 2007). Because of the existence of cytotypes,*S. altissima* provides an excellent model to examine the effects of cytotype on phytochemistry and associated insects due to the numerous previous investigations showing the importance of intraspecific genetic variation in this species (Crutsinger et al., 2006; Genung et al. 2012). Also, the colonization of *S.altissima* by the aphid specialist *Uroleuconnigrotuberculatum* has provided key insights into *S. altissima*-insect interactions. Previous studies in the Williams laboratory at Appalachian State University include examining the colonization *of S.altissima*collected from different elevations (Williams and Megan., 2015), effects of nutrient treatments and genotype (Williams and Bonville., unpublished data), effects of spatial scale and genotype on the associated pollinator community (Williams and Ragsdale., unpublished data), effects of prior herbivory (Williams and Howells., unpublished data), and terpene production *in S. altissima* in response to aphid herbivory (Garrido, unpublished data). Though previous studies found differences in compounds such as terpenes among cytotypes in the related *S. giganteum* (Hull-Sanders et al., 2009a), and differences in insect herbivore responses (Hull-Sanders et al.,2009b), there have been no investigations relating secondary defense chemistry and cytotype for the widespread *S. altissima*.

Uroleuconnigrotuberculatum

The specialist aphid *U.nigrotuberculatum (*Hemiptera; Aphidae) is native to North American (Adachi et al., 2016). These aphids are primary associated with *S. altissima*, and their colonization and population are regulated by different factors, such as temperature, predators and fungal disease (Cappuccino, 1987; Alyokhinet al., 2011). Previous work

supports that this aphid feeds on numerous genotypes of *S.altissima* but clearly chooses some over others (Maddox and Root, 1987; Williams and Megan, 2015). Utsumiet al., (2011) compared *U. nigrotuberculatum* densities in single and mixed genotype plots and found that differences between treatments likely resulted from movement of aphids to resistant genotypes. This species has been reported to be distributed across clumps of *S. altissima*, where the distribution was characterized by differences in genetic structure (Pilson and Rausher, 1995). The abundance of this aphid is affected by host-plant genotype identity (Genung et al., 2012) and phytochemicals, especially terpenes, are known to contribute to observed responses (Williams and Megan, 2015). *Uroleuconnigrotuberculatum*has the ability to synthesize cholesterol as their primary sterol and as a result they prefer to feed on certain cytotypes that contain a lot of cholesterol (Janson et al., 2009). Therefore, nutritionaldifferences between cytotypes in tall goldenrod could be an important consideration. Though the preference for hexaploidcytotypes of *S. altissima*over diploid and tetraploid plants was found (Richardson and Hanks., 2011), reasons at the phytochemistry level are unclear.

Though studies with*Uroleuconnigrotuberculatum*demonstrate the role of intraspecific variation in *S. altissima* on their abundance, prior to my experiment it was largely unknown what potential effects cytotype and soil nutrients may play on the production of terpenes and leaf nutrients. Since these plant constituents contribute to aphid abundance this study expands our understanding of how genetic variation in this foundation old-field plant species affects associated insects.

Objectives

My study had three primary objectives, to examine in *S. altissima:*

(1) Cytotypes effects on phytochemistry (N, CN and terpenes)

(2) Soil nutrients effects on phytochemistry

(3) Insect abundance relationships between cytotypes and soil nutrients

I proposed three hypotheses:

H_1: Increased chromosome number will increase terpene concentration in *S. altissima* such that 6n>2n.

H_2: Soil nutrients level will affect terpene concentration, where plants with nutrients supplied will have more amount of terpene than plants without nutrients supplied.

H_3: Insect abundance will relate to cytotypes and soil nutrient phytochemistry, where 6n plants will have highest insect abundance due to higher terpene production and higher leaf N concentrations.

MATERIALS AND METHODS

Study Species

Solidagoaltissima L., tall goldenrod, is a rhizomatous perennial plant species with a native distribution over much of temperate North America (Semple and Cook 2006). Its original habitats likely included prairies and forest openings but, since European colonization, *S. altissima* has become an abundant plant of roadsides, old fields, and other disturbed or succession areas. There are three known cytotypes; diploid (2n=18), tetraploid (4n=36), and hexaploid (6n=54) (Halverson et al., 2008).This species is predominantly diploid in the western part of its range (treated as *ssp.gilvocanescens* by Semple and Cook 2006) and hexaploid in the eastern part*(ssp.altissima*), with a broad zone of overlap in the Midwest, where tetraploids are also found. For the sake of my research I refer to the species as simply *S. altissima*. In the overlap zone, all three ploidies co-occur on very fine spatial scales in some local populations (Halverson et al., 2007). *S. altissima* provides an excellent model to examine the effects of cytotype on phytochemistry and associated insects due to the numerous previous investigations showing the importance of intraspecific genetic variationon associated insects (Crutsinger et al., 2006 ;Genung et al., 2012) and phytochemistry (Williams and Megan, 2015). The colonization of *S.altissima* by the aphid specialist *Uroleuconnigrotuberculatum*has provided key insights into *S. altissima*-insect interactions (Maddox and Root ,1987;Utsumi et al., 2011; Williams and Avakian, 2015) and as a specialist insect this aphid species provides an excellent model for addressing questions about the role of secondary metabolites for insect choice.

Experimental Design

I used two cytotypes (2n and 6n) for this study. Genotypes of *S. altissima* were previously collected from Watauga County NC (6n) and the Midwestern US(2016), with2n and 6n collected across a range spanning western Illinois to eastern Nebraska. For plants used in my experiment, those from the Midwest were grown at the ASU greenhouse for a minimum of one year, while local plants had been grown since 2015. For both cytotypes and regions plants were randomly chosen and transplanted in 2017 into a common garden at the Gilley Research Station, with four plants per plot. An example plot is found in Figure.1. The currently maintained garden consists of seven genotypes that are 2n, four genotypes that are 4n and twelve genotypes that are 6n. For 6n plants, two genotypes are from Watauga County and the rest are part of the Midwest collection from 2016.

For my experiment, eight genotypes (four 2n and four 6n) of *S. altissima* were chosen from the Gilley common garden to set up a randomized common garden design at the ASU Biology greenhouse on June 14, 2018 (Figure 2). Plants were grown individually in 5.7L pots in a Metro Mix 360 soil medium combined with 50% sand to reduce nutrient effects from a commercial mix. There were 64 plants in the garden; eight plants for each genotype, for a total of 32 diploid and 32-hexaploid plants (i.e. 8 individuals X 4 genotypes/cytotype= 32). For each genotype four plants received a N.P.K (18-6-12) nutrient addition (Osmocote™-applied according to manufactures guidelines) and four received no nutrients (control). Each pot had 90 grams of native (natural) topsoil added to assure the presence of mycorrhizae possibly important for allelochemical production. The distance between each plant was

45cm, with the exception of a center "isle" to facilitate watering (90 cm wide).The initial height and diameter of each plant was measured to allow for a non-destructive biomass determination based on previous studies with this plant species (Williams and Megan, 2015).

Figure 1: Plot of *S.altissima* at Gilley Research Station

Figure 2. Plot Layout. The first number for a pot is the genotype and the second the replicate for that genotype. Even numbered plants within a genotype received the nutrient treatment, while odd numbered plants did not.

Insect Abundance

Quantification of *U. nigrotuberculatum*abundance began on June 27, 2018 and ended on September 8. Abundance was determined by visual observation and tracked weekly in order to estimate the time of peak abundance, as *U. nigrotuberculatum*populations are known to vary across growing season (Root and Cappuccino, 1992).Peak abundance was estimated at the time maximum abundance began to decline in the majority of the plots. Within two days of peak aphid abundance leaf samples were taken and non-destructive biomass estimate was conducted. The estimation followed a protocol previously described by Williams and Avakian (2015), where the 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) +6.3668 (R^2=0.70, P<0.0001).Using the non-destructive estimate of plant biomass, aphid abundance could be expressed as the actual number or aphids/g to account for treatment effects on plant biomass. I monitored the aphid population continually until the growing season ended, though no more leaf samples or chemical analysis was conducted.

Leaf Chemistry Analyses

Leaves were collected for phytochemistry (nitrogen (N), carbon (C) and terpenes) at the peak of insect abundance on August 10.A total of 8-10 leaves were taken from each plant; two samples for N and C, and two samples for terpenes. Therefore, for each cytotype/nutrient treatment a total of 16 samples for N, C and terpenes was analyzed, for a total of 128 samples (4 cytotypes X 2 nutrient treatments X 8 genotypes X 2 samples = 128). Leaves were placed in a cooler and transported to the laboratory. Leaves for N and C analysis were weighed and dried to constant weight at 65ºC, while those for terpene analysis were weighed and frozen at -20ºC.

Carbon and nitrogen was quantified using the micro-Dumas method. Oven-dried leaves were finely ground in a scintillation vial using a metal ball and paint shaker. A ground sample was placed onto foil paper, weighed to5-10mg on a Mettler microbalance and placed into a tin cup for combustion. Caron and nitrogen were quantified (and later C: N ratio determined) with aThermoFisher FlashEA1112 NC Elemental Analyzer.

Terpenes were analyzed using a gas chromatography protocol previously established in the Williams laboratory (see Williams and Megan, 2015). Previously weighed frozen leaf samples (approximately 2g) were allowed to thaw 5 minutes, cut into small pieces and placed into a 50ml culture tube along with 15ml pentane. Each sample was then thoroughly ground

using a polytron tissue homogenizer (Kinematic Inc.) for 1.5 minutes. The sample was poured through filter paper and into a glass collection tube and the filtrate evaporated to 0.5ml by slowly bubbling N_2 gas through the sample. A 1.0µl sample was injected into a GC-14A gas chromatograph (Shimadzu Scientific). The program for the GC was as follows; injector temperature 250°C**;** detector temperature 275°C; initial column temperature 80°C, increased 10°C per minute to 280°C, with a final hold of 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. Standards were available for all compounds identified except Germacrene D, where no analytical standard is commercially available. We identified this compound based on similar retention times to previous studies (Johnson et al., 2007) and the fact this is one of the most common and most abundant terpenes found in *Solidago*.

Statistical Analyses

Effects of cytotype, nutrient andcytotype x nutrient interaction were analyzed using a general linear model (Proc GLM, SAS ver. 9.4). Dependent variables included aphid abundance, aphid abundance/g plant biomass, leaf N, CN and volatile terpenes. The non-destructive measure of plant biomass taken at the time of peak aphid abundance allowed for the separate effects of total aphid abundance from that corrected for plant size, as larger plants may be expected to support more aphids than smaller plants. Data for terpenes was log transformed to increase normality. The relationship between aphid abundance and abundance/g with individual terpenes was examined using linear regression (JMP Pro 13).

RESULTS

Aphid Abundance

Soil nutrients had a significant effect (Table 1, Figure 1) on aphid abundance (P=0.017) but not when plant biomass was accounted for (Figure 2). Abundance was much higher in both 2n and 6n plants supplied with nutrients compared to those with no nutrient addition. With plant biomass considered (i.e. abundance/g) 6n plants had the highest abundance when nutrients were supplied, while 2n and 6n plants had similar abundance/g at both nutrient levels (Figure 2). There were no effects of cytotype or cytotype x nutrient interaction on aphid abundance measures (Table 1).

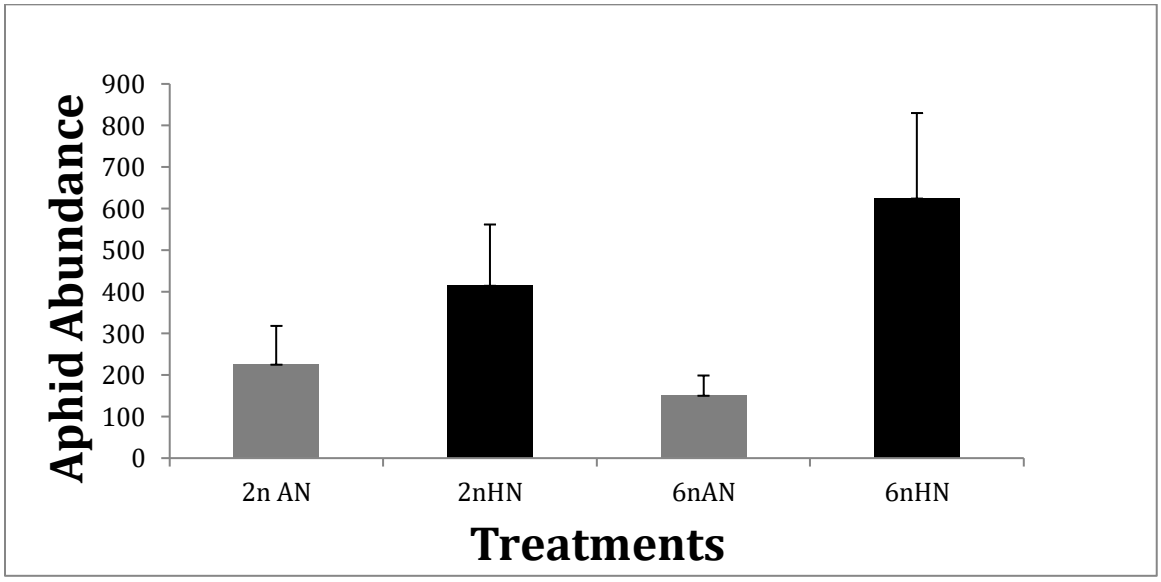

Figure 1.Aphid Abundance on diploid (2n) and hexaploid (6n) cytotypes of *S. altissima*.AN represents Ambient Nutrient and HN High Nutrient.

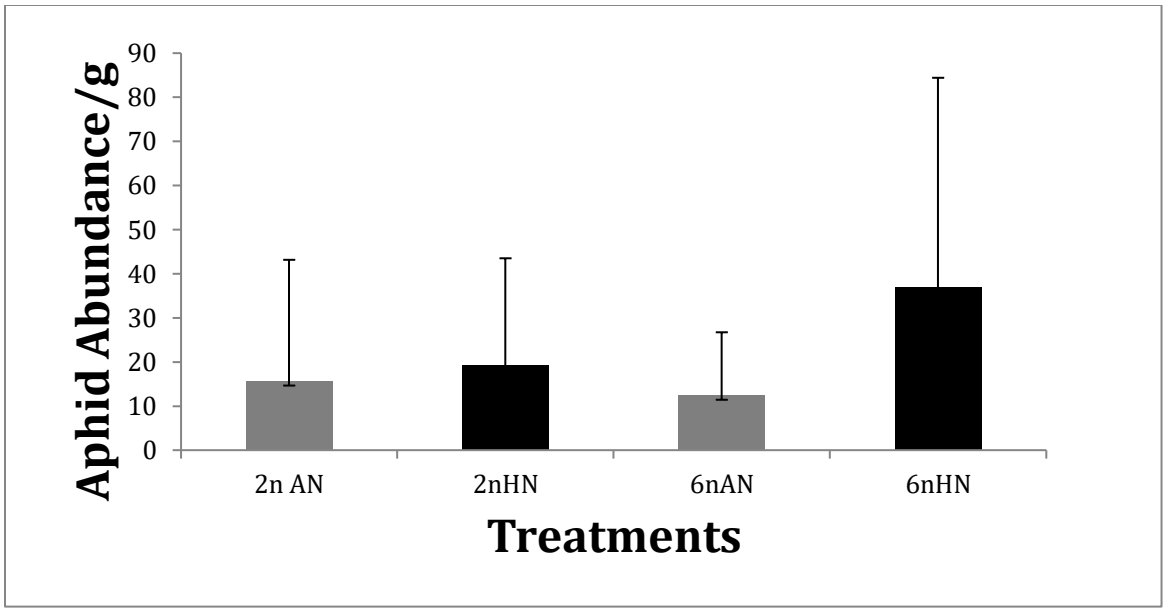

Figure 2.Aphid Abundance/g on diploid (2n) and hexaploid (6n) cytotypes of *S. altissima*.AN represents Ambient Nutrient and HN represents High Nutrient.

Table 1. F and P values for effects of Nutrient, Cytotype and Nutrient X Cytotype on Aphid

Terpenes	**Nutrients**		**Cytotype**		**Nutrient X Cytotype**	
	F	P	F	P	F	P
Aphid Abundance (g)	5.54	**0.017**	0.025	0.622	1.09	0.301
Aphid Abundance/g	3.32	0.0733	0.089	0.3497	1.85	0.179

$p \leqslant 0.05$ presented in **bold** text, df= 1, 60 for nutrient, cytotype and nutrient X cytotype.

Plant Nutrient and Biomass Measures

Plant biomass was significantly different among cytotypes (Figure 3, Table 2.), with 2n plants overall larger. Plants provided nutrients grew larger than those without nutrients at both cytotypes. Leaf nitrogen was higher (Figure 4, Table 2.) with nitrogen addition in 2n but not 6n plants (nutrient X cytotype interaction, P=0.0042).C: N was significantly different between cytotypes (Figure 5, Table 2.).

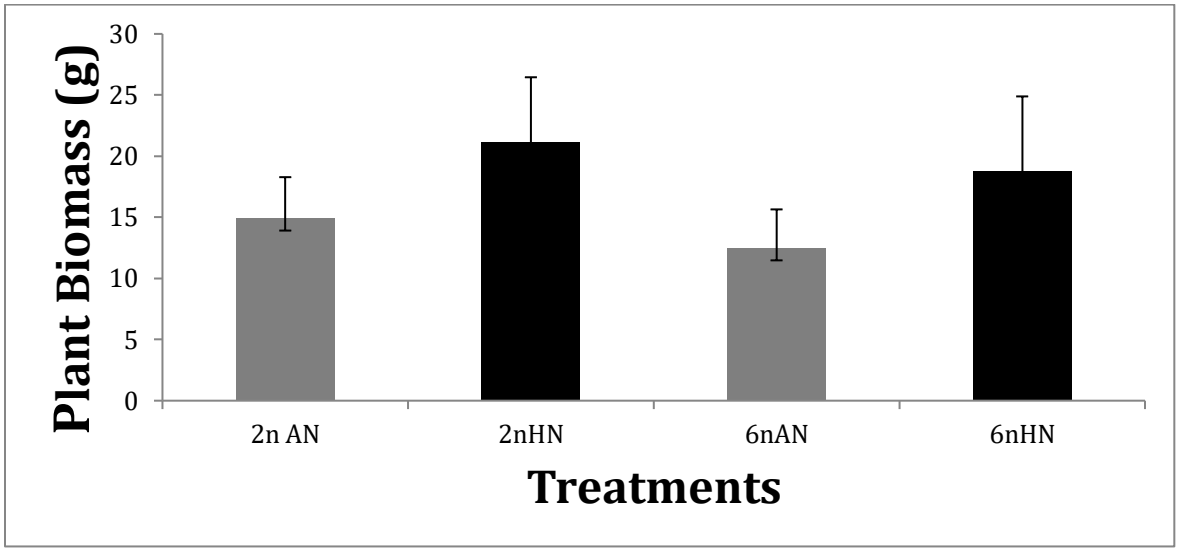

Figure 3. Plant Biomass of diploid (2n) and hexaploid (6n) cytotypes of *S. altissima.*AN represents ‘’Ambient Nutrient while HN represents ‘’High Nutrient’’.

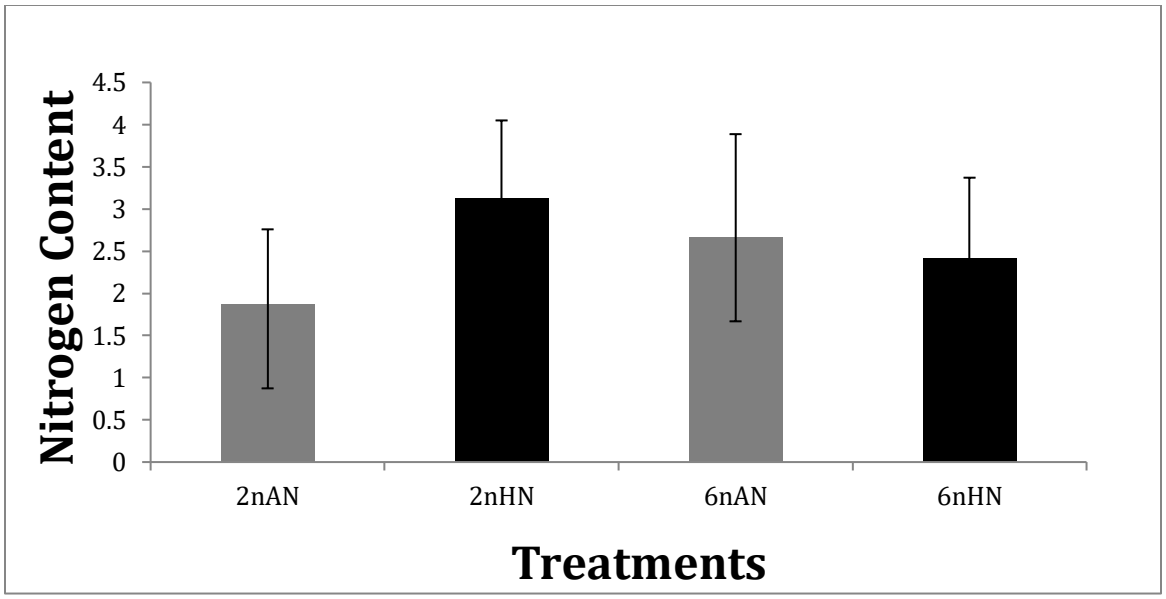

Figure 4.Nitrogen Content of diploid (2n) and hexaploid (6n) cytotypes of *S. altissima*.AN represents‘ ‘Ambient Nutrient while HN represents ‘’High Nutrient’’.

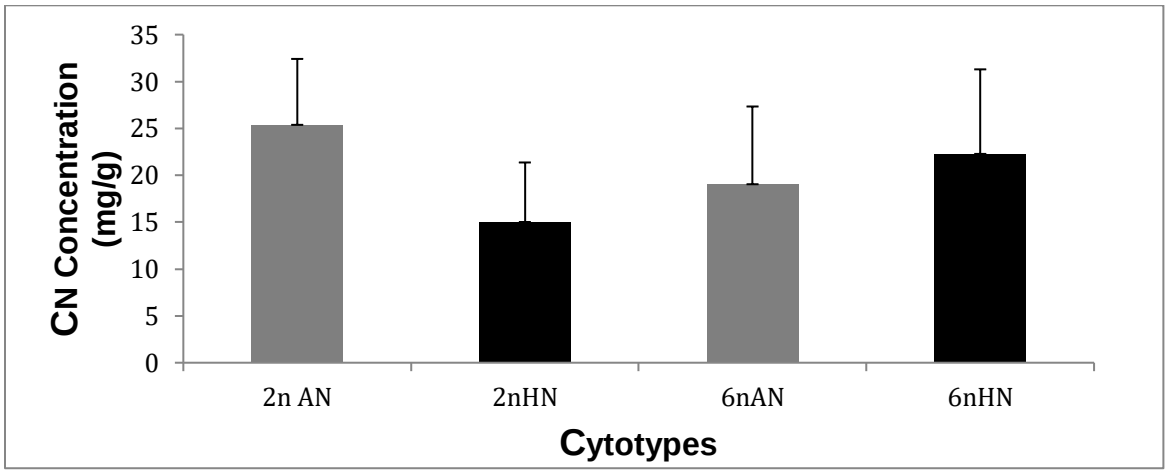

Figure 5. C: N Concentration of diploid (2n) and hexaploid (6n) cytotypes of *S. altissima.* AN represents ‘’Ambient Nutrient’’ while HN represents ‘’High Nutrient’’

Table 2.F and P values for Effects of Nutrient, Cytotype and Nutrient X Cytotype on Phytochemistry and Biomass.

Phytochemistry and Biomass	**Nutrients**		**Cytotype**		**Nutrient X Cytotype**	
	F	P	F	P	F	P
Nitrogen (mg/g)	3.93	**0.052**	0.03	0.843	8.88	0.0042
CN (mg/mg)	3.46	0.068	0.06	0.804	12.11	0.0009
Biomass (g)	28.5	0.0001	4.19	0.045	0.001	0.970

$p \leqslant 0.05$ presented in **bold** text, df= 1,60 for nutrient, cytotype and nutrient X cytotype.

Terpene Analyses

Three foliar terpenes, α-Pinene, β-pinene and Bornyl acetate, were significantly different among cytotypes (Figures 6-8, Table 3). All of these compounds were noticeably increased in hexaploid plants with nutrient addition. While some differences were seen in compounds grown under different nutrients, there were no nutrient or nutrient x cytotypes effects (Table 3).

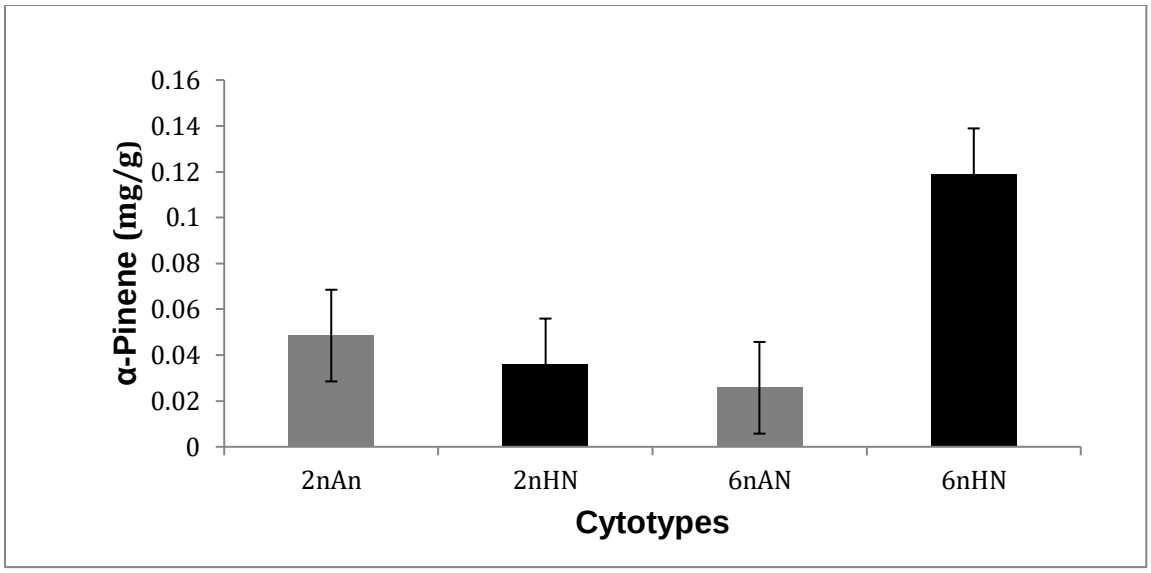

Figure 6. Foliar terpene concentration of α-Pineneon diploid (2n) and hexaploid (6n) cytotypes of *S. altissima.*AN represents ‘’Ambient Nutrient while HN represents ‘’High Nutrient’’

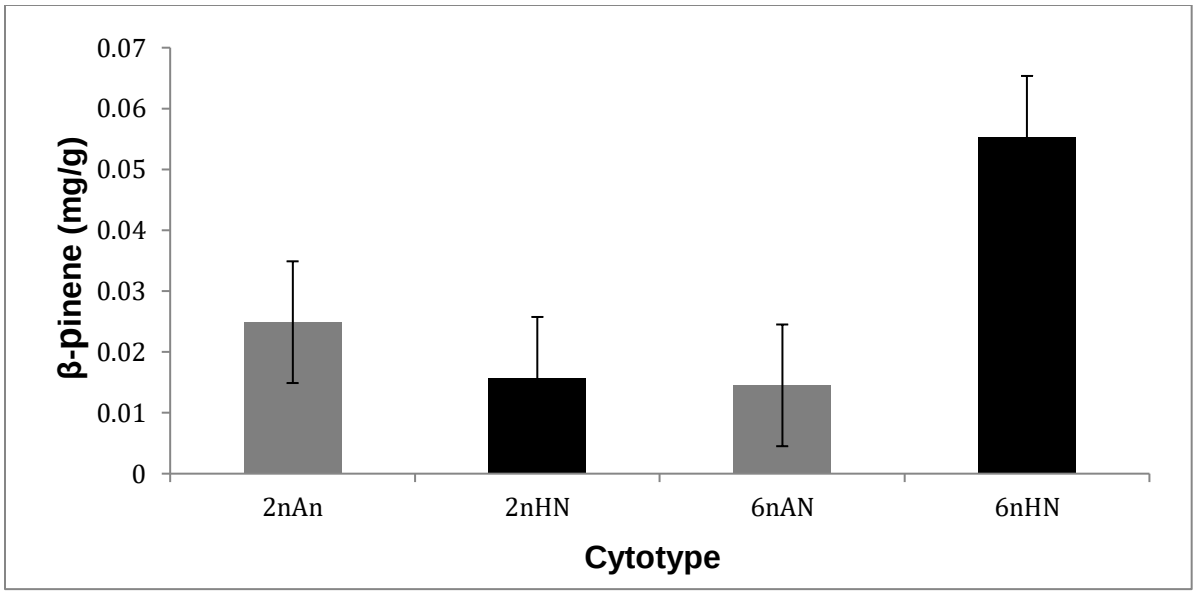

Figure 7.Foliar terpene concentration of β-Pinene, diploid (2n) and hexaploid (6n) cytotypes of *S. altissima.*AN represents ‘’Ambient Nutrient while HN represents ‘’High Nutrient’’.

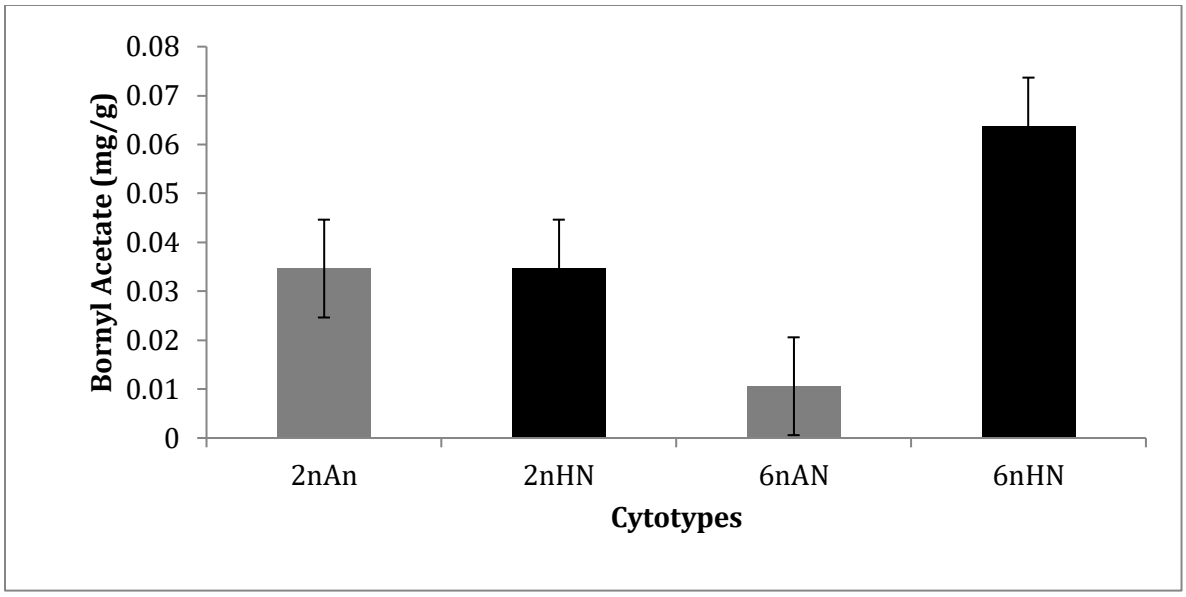

Figure 8. Foliar terpenes concentration of Bornyl acetate, diploid (2n) and hexaploid (6n)cytotypes of *S. altissima.*AN represents ‘’Ambient Nutrient” while HN represents ‘’High Nutrient”.

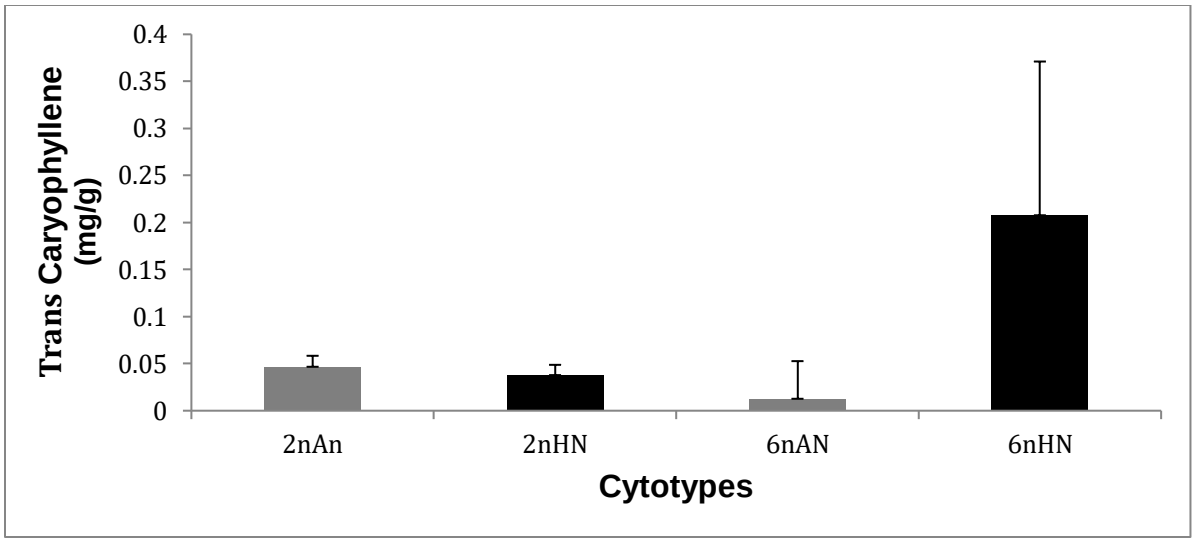

Figure 9. Foliar terpene concentration of Transcaryophyllene, diploid (2n) and hexaploid (6n) cytotypes of *S. altissima.* AN represents ‘’Ambient Nutrient” while HN represents ‘’High Nutrient”.

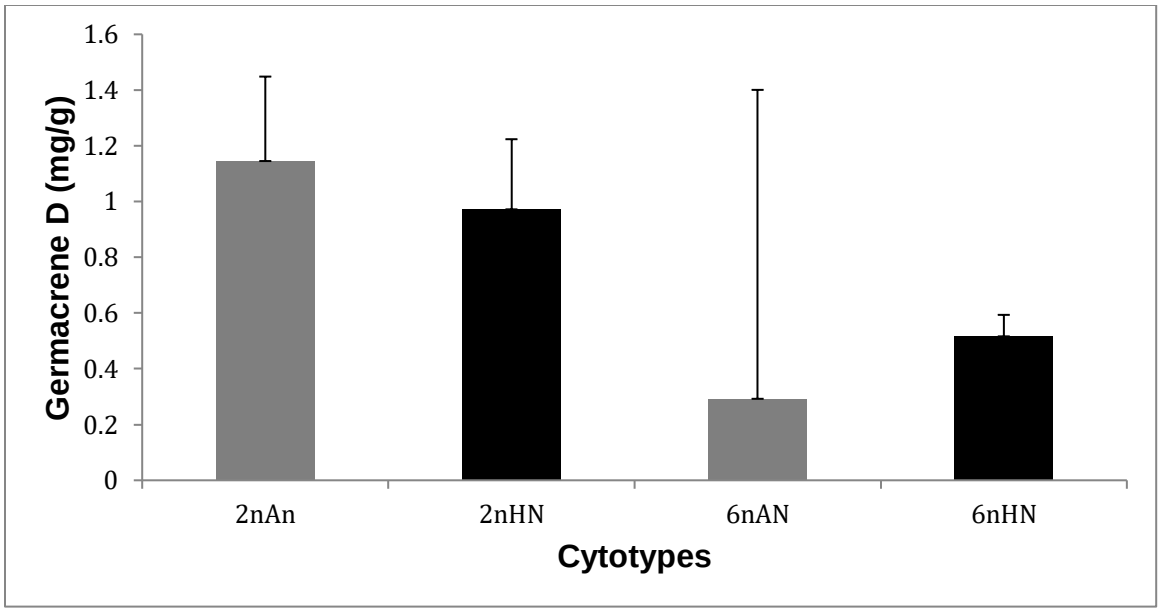

Figure 10.Foliar terpene concentration of Germacrene D, diploid (2n) and hexaploid (6n) cytotypes of *S.altissima.*AN represents ‘’Ambient Nutrient” while HN represent ‘’High Nutrient”.

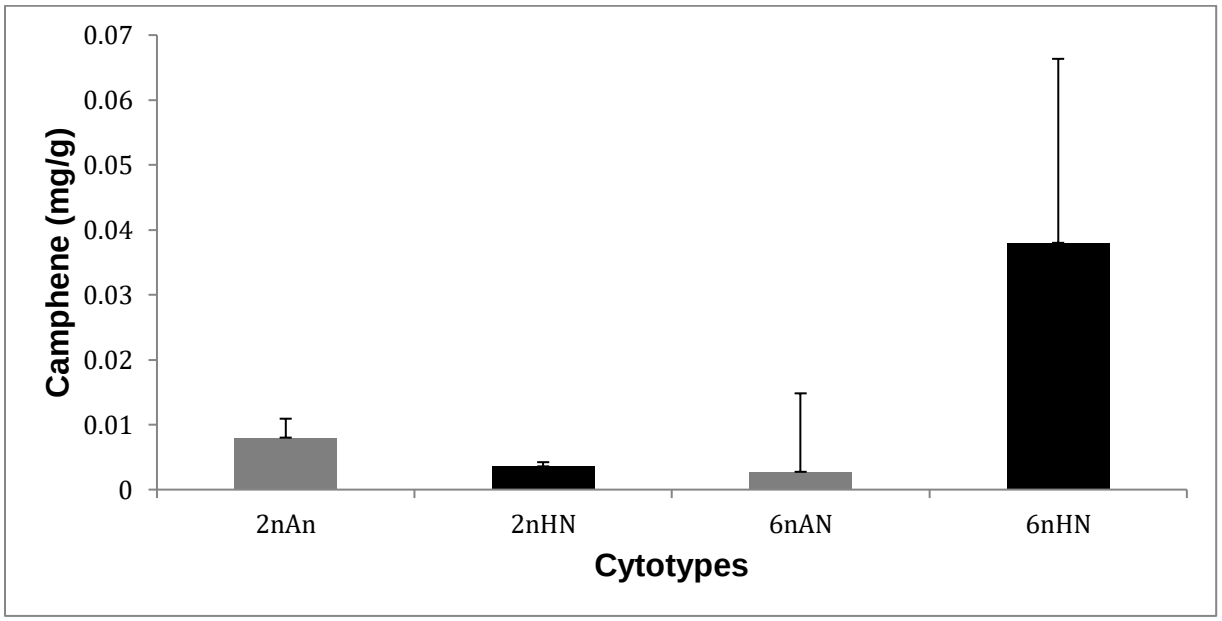

Figure 11.Foliar terpene concentration of Camphene of diploid (2n) and hexaploid (6n) cytotypes of *S. altissima.*AN represents ‘’Ambient Nutrient” while HN represents ‘’High”.

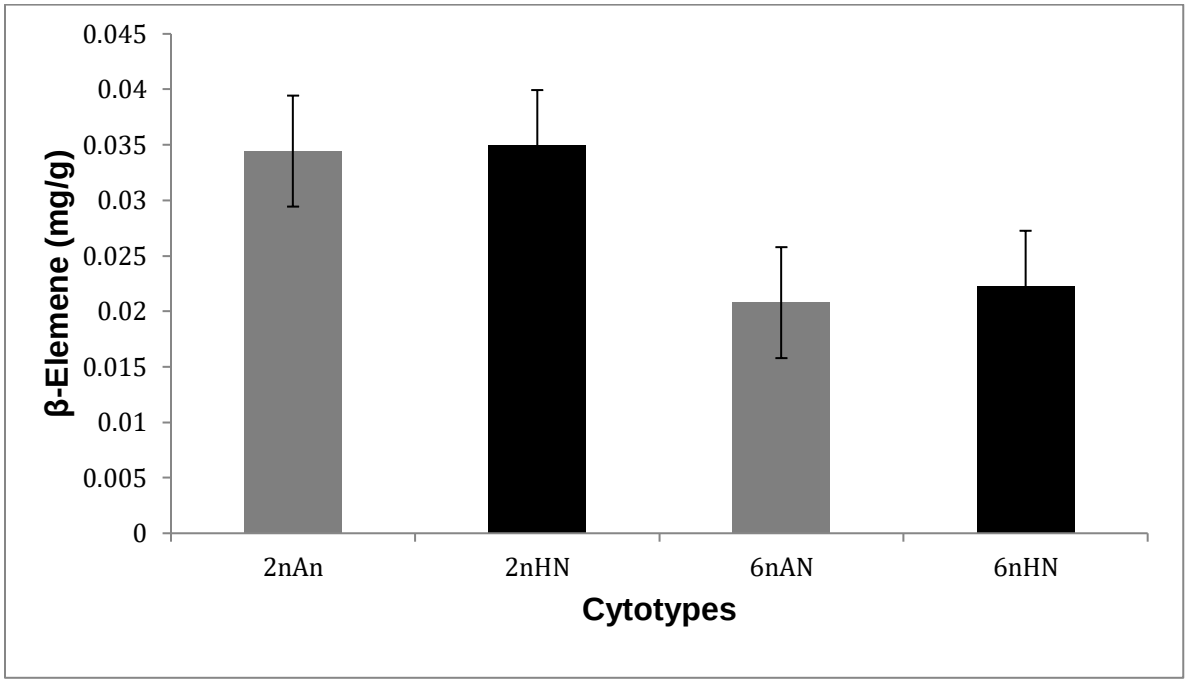

Figure 12. Foliar terpenes concentration of β-Elemene on diploid (2n) and hexaploid (6n) cytotypes of *S. altissima.*AN represents ‘’Ambient Nutrient” while HN represents ‘’High Nutrient”.

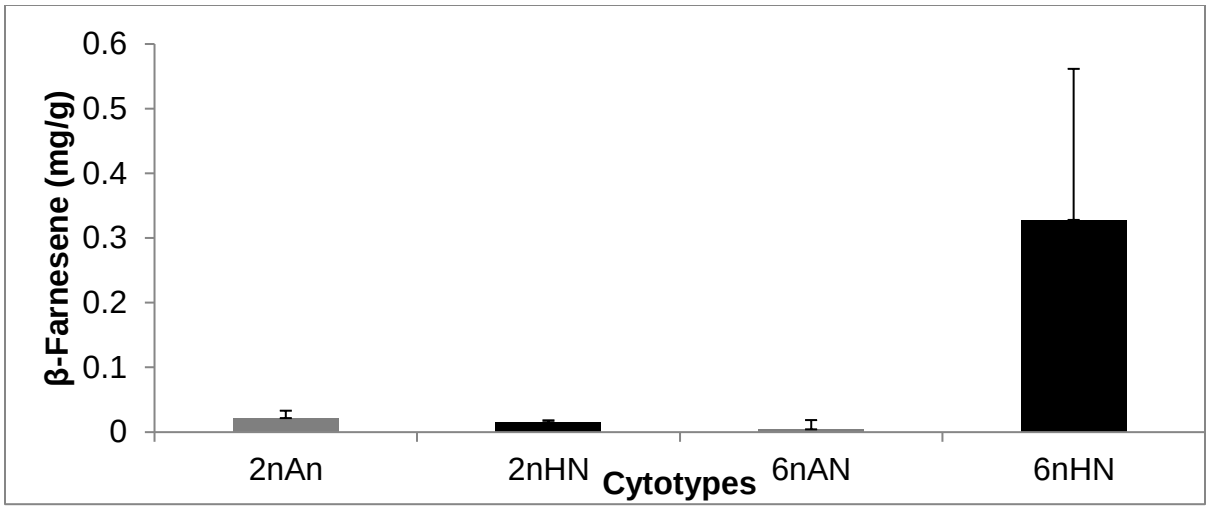

Figure 13. Foliar terpene concentration of β-Farnesene of diploid (2n) and hexaploid (6n) cytotypes of *S. altissima.*AN represents ‘’Ambient Nutrient” while HN represents ‘’High Nutrient”.

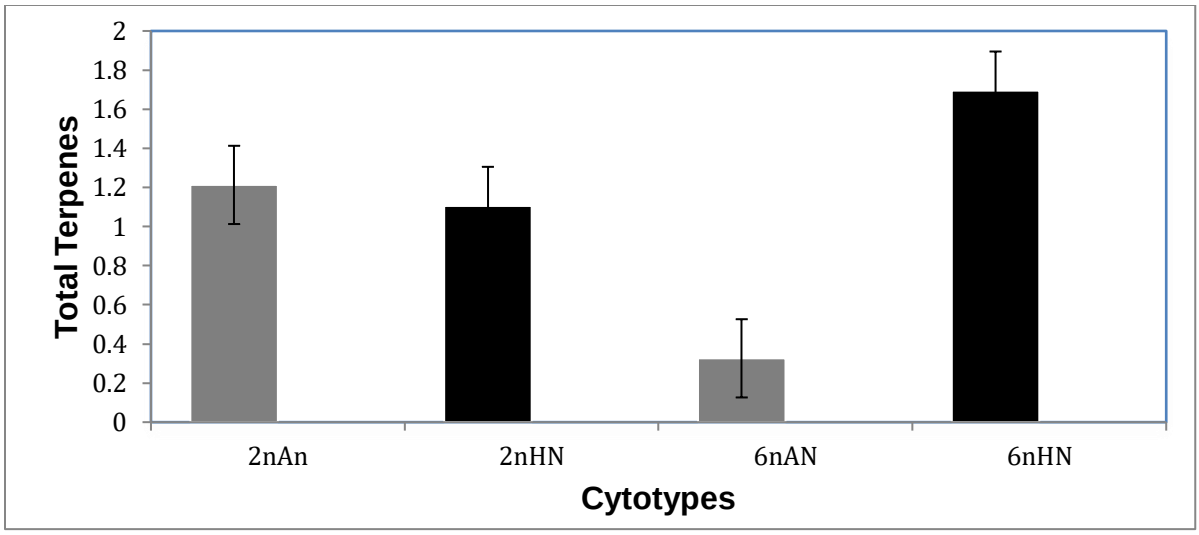

Figure 14.Foliar total terpenes concentration of diploid (2n) and hexaploid (6n) cytotypes of *S. altissima.*AN represents ‘’Ambient Nutrient” while HN represents ‘’High Nutrient”.

Table 3.F and P values for effects of Nutrient, Cytotype and Nutrient X Cytotype on terpenes.

Terpenes	**Nutrients**		**Cytotype**		**Nutrient X Cytotype**	
	F	P	F	P	F	P
α-Pinene (mg/g)	0.040	0.842	9.070	**0.004**	0.130	0.722
β-Pinene (mg/g)	0.330	0.567	9.900	**0.003**	0.040	0.835
Camphene (mg/g)	0.540	0.464	2.200	0.144	1.380	0.245
Bornyl Acetate (mg/g)	0.270	0.604	5.160	**0.027**	0.010	0.997
TransCaryophyllene (mg/g)	0.960	0.332	1.000	0.352	1.420	0.238
β -Elemene (mg/g)	2.700	0.106	0.890	0.349	3.070	0.085
Germacrene D (mg/g)	2.14	0.149	0.770	0.384	0.860	0.357
β -Farnesene (mg/g)	1.99	0.163	1.840	0.181	2.500	0.119
Total Terpenes (mg/g)	0.04	0.820	0.930	0.338	0.070	0.794

$p \leqslant 0.05$ presented in **bold** text, df= 1,60 for nutrient, cytotype and nutrient X cytotype

Simple linear regression found no relationship between any terpene compound and aphid abundance ($p>0.05$, data not shown).

DISCUSSION

The main purpose of my study was to examine the effects of chromosome number (polyploidy), soil nutrient, and their interaction on the phytochemistry (C, CN and terpenes) in the old-field plant *S. altissima*on the abundance of an associated specialist herbivore (*U.nigrotuberculatum*). By examining possible effects of cytotype, previous findings for the role of genetic variation and phytochemistry on a specialist aphid could be better understood. This study found that the concentration of some terpenes was significantly affected cytotype, but not leaf nutrients, and the abundance of *U.nigrotuberculatum*was related to soil nutrients. However, there was no nutrient or cytotype effect on insect abundance when plant biomass was accounted for.

Understanding the role of polyploidy within this plant-insect interaction is important with respect to phytochemical production; a plant trait which might influence aphid choice and abundance on *S.altissima.* In addition, because soil nutrients affect phytochemistry (Johnson et al., 1996) and biomass in plants (Schutz et al., 2008) the potential for an interaction between these exist. Though other studies within the field of community genetics have examined the impacts of soil nutrients on insect abundance (Schutz et al., 2008; Ormeno et al., 2008; Segraves., 2017) no study has investigated the impact of soil nutrients on cytotypes of *S. altissima* and the possible effects on terpene concentration and their influence on an associated specialist insect.

Quantification of *U.nigrotuberculatum*abundance used an estimate of peak abundance based on weekly observations. Populations of this species are known to vary across a growing season (Root and Cappuccino, 1992) and it was the time of greatest abundance that

provided the best evaluation of treatment effects. I found with plant biomass not considered, aphid abundance was significantly affected by soil nutrients, being higher in plants supplied with nutrients compared to those with no nutrient addiction for both cytotypes (Figure 1, Table 1). This result is in agreement with previous studies by Schutz et al, (2008) and Ormeno et al, (2008) who demonstrated that NPK (nitrogen, phosphorus and potassium) increases plant biomass, nitrogen content, and influences herbivore colonization. In my study soil nutrients only marginally affected leaf nitrogen but had a strong effect on plant biomass (Table 2, Figure 3-5). This is somewhat in agreement with Johnson and Decoteau, (1996), who indicated that plants use nitrogen for growth and development. Zehnder and Hunter, (2009) demonstrated that aphids prefer to feed on plants with high nitrogen content and larger biomass, somehow in agreement with my study. The significant interaction of nutrient and cytotype for leaf nitrogen occurred because unlike 2n plants, 6n plants were not higher in leaf N when soil nutrients were added to the soil (Figure 4). However, my data show that 2n leaf nitrogen contents differ from that of 6n plants. These differences may suggest that 6n plants have other factors such as nutrient allocations (Tuomi et al., 1991) affecting its nitrogen usage and 6n plants may have focused more on carbon-based allelochemicals (Tuomi et al., 1991). There were nutrient and cytotype interactions with CN (Table 2), suggesting that soil fertility and chromosome number may have considerable effects on carbon- nitrogen usage in plants (Tuomi et al., 1991).

I found no effect of cytotype or cytotype X nutrient interaction on aphid abundance, though abundance was overall highest on plants that received additional nutrients (Figure 1). When calculated as aphids per gram of plant biomass, neither nutrients nor cytotype affected abundance. This suggests that additional factors than chromosome number, such as genotype

differences (not accounted for in my study) may have played an influential role on the abundance of *U. nigrotuberculatum* abundance. Plant genotype plays an important role in determining aphid abundance in previous studies (Pilsonand Rausher., 1995), including in *S. altissima*Genung et al., 2012). Though my study did not investigate the effects of genotypes, previous studies by Williams and Megan, 2015 found genotype strongly affected *U. nigrotuberculatum*abundance. Perhaps differences between genotypes for the two cytotypes used in my experiment created enough variation that is making cytotype effects hard to discern, though a definite conclusion on this is not possible.

Both nutrients and cytotype significantly affected plant biomass in my experiment. Previous studies have shown that soil nutrient influence plant biomass (Bethke et al., 1998; Casey and Raupp., 1999; Aber et al., 2003; Stevens et al 2004; Throop and Lardau., 2004; Muller et al., 2005). The availability of nutrient such as nitrogen promotes growth of the leaves and stems and improves quality of foliage by increasing leaf nitrogen (Johnson and Dacoteau., 1996; Guo et at., 2013).In my study perhaps one of the largest effects of nutrient addition was on plant biomass, where in both cytotypes plants grew larger with added soil nutrients (Figure 3). Even though the abundance of aphids was significantly affected by soil nutrients and greatest on high nutrient plants (Figure 1, Table 1) when corrected plant size (i.e. abundance/g) these relationships are no longer seen. This finding is in agreement with other studies (Zehnder et al and Hunter., 2009; Guo et al., 2013; Karley et al., 2004).With respect to biomass there were differences between cytotypes, where 2n plants were overall larger than 6n plants (Figure 3), though unlike leaf nutrients cytotype effects on biomass did not contribute to aphid abundance (Table 1, 2).

My terpene analysis found no effects of soil nutrients, despite some compounds having higher production with nutrient addition (Table 3). There may be a possibility that soil nutrient does not directly affect terpene production (Guo et al., 2013) in my study. The increase of some foliar terpene (α-pinene, β-pinene and Bornyl acetate) concentration in 6n plants with nutrient addition mayindicate that 2n and 6n plants use nitrogen differently. Hexaploids may have indirectly allocated carbon and nitrogen to production and concentration of the three foliar terpenes, however further study is needed to confirm this. Three foliar terpenes (α-pinene, β-pinene and Bornyl acetate) increased in 6n plants with nutrient addition. This result is in agreement with previous studies (Ormeno et al., 2008), indicating that there is a positive relationship between the concentration of nitrogen and terpene content in some plants. Ormeno et al., (2008) found that terpene concentration responded to soil nutrients, suggesting that plants may respond to soil resources availability by allocating carbon resources to the synthesis of terpenes. However, my data showed that only cytotype had significant effect on foliar terpenes (Table 3), increasing on 6n plants for three compounds (Figure 6-8), which contrast with the results in a related *Solidago* species in where diploids had higher amounts of mono- and sesquiterpenes than hexaploids (Hull-Sanders et al., 2009).

To relate differences between cytotypes to the abundance of *U. nigrotuberculatum*in my study, previous investigations using *S. altissima* population provides insights. Halverson et al (2008) examined five gallmaking insect herbivores on diploid, tetraploid and hexaploidcytotypes of *S. altissima* in Midwestern US populations where cytotypes co-occur on large spatial scales. The authors show that plant ploidy variation appeared to have a major impact on insect community organization. Though not specifically addressing the aphid in

my study, it seems plausible that the choice of certain cytotypes by insect herbivores demonstrates that they possess important evolutionary traits such that the advantage associated with polyploidy may make these plants preferential to specialist insects.Despite previous investigations found a herbivore-cytotype relationship, the lack of significant effects of cytotype on insect a specialist aphid in my investigation is contrary to this (see especially Halverson et al. (2007), indicating that traits due to chromosome number did not explain my observed insect response. My study found that differences in aphid abundance due to nutrient addition and cytotype was through effects these factors had on plant biomass, where large plants support more aphids. In addition, the lack of relationships between particular phytochemical constituents and aphid abundance (i.e. linear regression) also supports the conclusion that phytochemistry effects on plant biomass and not aphid growth or development was most important in my study.

Conclusions

In conclusion, I found that the abundance of *U. nigrotuberculatum* was due primarily to soil nutrient effects on plant biomass, and while leaf nitrogen content did affect insect abundance this was not the case when plant biomass was accounted for. Because terpene concentrations were not related to soil nutrients addition, both 2n and 6n plants apparently used nitrogen more likely for growth purposes than secondary metabolite production. Since there were no significant relationships between terpene concentration and aphid abundance, this plant trait variation cannot account for my observed plant-insect associations. Further studies using *S. altissima*are needed to determine how chromosome number and soil nutrients influence terpene production and herbivore choice.

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