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# Effects of Chemical Variation on Competition and Insect Communities Across Solidago altissima Genotypes

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EFFECTS OF CHEMICAL VARIATION ON COMPETITION AND INSECT COMMUNITIES ACROSS *SOLIDAGO ALTISSIMA* GENOTYPES

by

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**ABSTRACT**

Individuals within a plant species can differ greatly from one another, especially regarding the range of chemical compounds produced. However, the functions of many of these chemicals are unknown, but likely include defenses against herbivores, attractants for pollinators and seed dispersers, as well as mechanisms for resource competition. For allelopathic plants, the costs of chemical production may create tradeoffs with investment in competition versus other ecological functions. To assess the effects of foliar chemical composition on interspecific plant competition and insect communities, I conducted a common garden and greenhouse experiment using 24 genotypes of the allelopathic species *Solidago altissima* for which the foliar chemistry had been characterized. Within the common garden, I measured a variety of above-ground plant performance measurements on each genotype of *Solidago altissima* as a measure of competitive ability, as well as assessed the foliar and floral insect communities. Using these data and chemical profiles of *S. altissima,* I linked foliar chemistry to plant performance and the foliar/floral insect communities. To assess the effects of chemical variation on interspecific plant competition, I conducted competitive trials in a greenhouse setting using the same 24 genotypes of *S. altissima* with the known chemical profiles. Clones of each genotype competed with four common target species: *Schizachyrium scoparium, Melilotus officinalis, Silphium integrifolium,* and *Abutilon theophrasti.*

The common garden experiment showed there was great variation in foliar chemistry between the genotypes. Ecological patterns existed between foliar chemistry and plant performance, as foliar chemistry was strongly related to most measures of plant

performance across genotypes. Pollinator communities were found to relate with total aboveground biomass, proportion of flower mass, and % light transmittance as well as plant chemistry. In contrast, foliar insect composition was independent of foliar chemical composition. The greenhouse experiment showed marked variation in both rhizome and above-ground biomass growth for *S. altissima*. The above-ground biomass of *Abutilon theophrasti*, *S. scoparium*, and *S. integrifolium* had their biomass significantly reduced via competition with *S. altissima*. Chemistry significantly affected the biomass of both *A. theophrasti* and *S. scoparium*, suggesting that chemistry is a critical driver of competition for *S. altissima*. Foliar chemistry of *S. altissima* also affected its own biomass, where different axes of chemistry affected different aspects of biomass growth.

These results from both experiments illustrate the multidimensionality and variation of the *S. altissima* chemical landscape. Chemistry affected the pollinator community, various plant performance measures, and the biomass of other competitors. Among genotypes, variation in chemical composition seems to be facilitating many of the ecological functions, with independent axes of foliar chemistry affecting different components of the system, creating various tradeoffs between competitive ability, biomass, insect associations, and other plant performance measures.

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## INTRODUCTION

*Solidago altissima* is a clonal perennial that is common in old fields and other open habitats across its native range of Eastern North America (Yip et al. 2019). *Solidago altissima* is very diverse in its allelochemical composition, allowing it to be a successful invader across Europe, Japan, and Australia (Abhilashaet al. 2019; Webber, 1999; Uesugi et al. 2019). The chemical properties of this species allows these plants to compete for space and resources via allelopathy, by reducing germination and/or the growth of neighboring species, increasing access to resources by directly suppressing neighbor growth or indirectly by affecting microbial mutualists or nutrient availability (Meiners et al. 2012; Uesugi et al. 2019). This sort of chemical production can also affect the associated insect communities, generating strong influences on multitrophic plant-insect interactions (Zytynska et al. 2019; Wetzel and Whitehead, 2020). Thus, intraspecific variation in chemical composition could impact plant performance and insect communities through anti-herbivore defenses and altered allocation to reproduction (Hale and Kalisz, 2012). Chemistry plays a very vital role in this species ecological functioning, however, the direct effects of these plant chemicals are unknown, as these chemical affect a variety of plant functions ranging from biomass growth to defense against herbivory (Uesugi and Kessler, 2013).

Here, I relate the chemical compositions of 24 genotypes of *Solidago altissima* to their foliar and pollinator insect communities and competitive abilities. The following chapters document the results of multiple studies done in both a field and greenhouse setting. In the first chapter I focus on the chemical composition of 24 genotypes of *S. altissima* and how it affects the natural insect communities and factors indicative of plant

performance to assess patterns between chemistry, competitive ability, and insects in a common garden site located in Clark County, IL. In the second chapter I focus on chemistry and direct competition with other plant species experimentally, to assess patterns between interspecific competition and chemistry in a greenhouse setting. Together, this work will form a more holistic view of intraspecific chemical variation in the ecology of this dominant plant species.

# CHAPTER 1

## EFFECTS OF CHEMICAL VARIATION ON PLANT PERFROMANCE AND INSECT COMMUNITIES


## ABSTRACT

Individuals within a plant species can differ greatly from one another, leading to variation in the outcome of interactions with other species, with one of the more diverse characteristics of plant species being the range of chemical compounds produced. However, the functions of many of these chemicals are unknown, but likely include defenses against herbivores, attractants for pollinators and seed dispersers, as well as mechanisms for resource competition at both the inter- and intraspecific scales.

To assess the effects of intraspecific chemical variation on plant competition and insect communities, I used a common garden of 24 genotypes of the allelopathic species *Solidago altissima*. I measured a variety of above-ground plant performance measurements on each genotype of *Solidago altissima* as a measure of competitive ability, as well as the foliar and pollinator insect communities. By using the chemical profiles of each *S. altissima* genotype, I explicitly link foliar chemistry to plant performance and the foliar/floral insect communities.

Although there was great variation in foliar chemistry between the 24 genotypes of S. *altissima*, not much variation was observed among the genotypes in terms of performance measures. Ecological patterns existed between foliar chemistry and plant performance, as foliar chemistry was strongly related to most measures of plant performance across genotypes. Pollinator communities were associated with total aboveground biomass, proportion of flower mass, and % light transmittance as well as

plant chemistry. In contrast, foliar insect composition was independent of foliar chemical composition.

These results demonstrate the multidimensionality and variation of the *S. altissima* chemical landscape. Chemistry not only affected the pollinator community directly, but also indirectly via plant performance and physiology. Among genotypes, variation in chemical composition seems to be facilitating many of the ecological functions, with independent axes of foliar chemistry affecting different components of the system, either directly or indirectly.

## INTRODUCTION

Individuals within a plant species can differ greatly from one another, leading to variation in the outcome of interactions with other species (Siefert et al. 2015), with one of the more diverse characteristics of plant species being the range of chemical compounds produced (Wetzel and Whitehead, 2020; Zytynska et al. 2019). While some plant species produce a few major phytochemicals, many more have the ability to produce thousands of unique compounds (Tasin et al. 2007). Thus, functional variation in plant chemistry may range from the abundance of a single chemical to a complex mixture of chemical compounds (Zytynska et al. 2019).

Plant species produce and release chemical compounds in response to changing biotic and abiotic factors in their environment, such as herbivory, pathogens, photodamage, or drought stress (Uesugi et al. 2019; Holopainen, 2004). Plant responses to local conditions may increase chemical variation as many species exhibit plasticity (Kong et al. 2018). However, the functions of many of these chemicals are unknown,

since plants produce a variety of secondary compounds for many different functions (Holopainen, 2004; Bardgett et al. 1998). Many of these chemicals mediate interactions with herbivores and competitors at both the inter- and intraspecific scales (Lankau, 2008; Beran et al. 2019). Many plant species exhibit chemical multifunctionality, where these chemical compounds are used as defenses against herbivores, attractants for pollinators and seed dispersers, as well as mechanisms for resource competition (Beran et al. 2019; Inderjit et al. 2011).

Allelopathic plant species alter resource competition by releasing chemical compounds into their environment (Rice, 1979; Duke 2010; Meiners et al. 2012). Allelopathic chemicals reduce germination and/or the growth of neighboring species, increasing access to resources by directly suppressing neighbor growth or indirectly by affecting microbial mutualists or nutrient availability (Meiners et al. 2012; Uesugi et al. 2019). Allelopathic compounds are released as either root exudates, volatile organic compounds, leaf leachate, or leaf litter (Uesugi et al. 2019, Inderjit et al 2011). However, it is still unclear whether allelochemicals are released as an adaptation or in response to direct competition (plant-specific chemical cues) or to changes in environmental conditions such as shading, drought, or nutrient stress (Uesugi et al. 2019; Inderjit and Del Moral, 1997; Kong et al. 2018). Allelopathic interactions between plant species may play a large role in determining species distribution, abundance, and community composition, especially in species invasions where resident species have not evolved any tolerances to these allelochemicals (Uesugi et al. 2019; Hierro, 2005; Abhilasha et al. 2008; Halligan, 1973; Hunter and Menges 2002).

However, the production of allelopathic chemicals is thought to be energetically costly, where the benefits and costs of allelochemical production may vary across time and space due to variation in competition (Kong et al. 2018). Natural selection may favor plant genotypes within heterogeneous competitive environments that employ induced production of allelochemicals, rather than genotypes that constitutively produce high levels of these chemicals (Uesugi et al. 2019; Novoplansky 2009; Kegge and Pierik 2009). If the variation of allelochemicals production is a cost-saving strategy, we should expect some sort of ecological trade-off between allelopathic chemical production and plant performance (Uesugi et al. 2019).

Plant chemical production can also affect the associated insect communities, generating strong influences on multitrophic plant-insect interactions (Zytynska et al. 2019; Wetzel and Whitehead, 2020). Even specialist gall-forming insect species are affected by chemical composition, influencing the selection of egg laying sites (Thompson et al. 2019; Abrahamson et al. 1991). Plants within a species may differ in chemical composition, flowering phenology, and seed set due to the variety of insect species they associate with, as well as inter- and intraspecific competition for pollinators (Gross and Werner, 1983; Beran et al. 2019). Floral and defensive traits are connected through physiological mechanisms, thus, linking selection on pollination and herbivory (Ramos and Schiestl, 2019). For example, plant investment in herbivore defense may negatively affect floral traits that attract pollinators, imposing an ecological trade-off (Ramos and Schiestl, 2019; Adler et al. 2006; Lucas-Barbosa, 2016; Knauer and Schiestl, 2017). Trade-offs between allelochemical production and herbivory defense can also occur, where plants may allocate their resources to defense against herbivory, rather than

resistance to competition (Strauss and Agrawal 1999; Stamp 2003). Thus, intraspecific variation in chemical composition could impact associated insect communities through anti-herbivore defenses and altered allocation to reproduction (Hale and Kalisz, 2012).

To assess the effects of intraspecific chemical variation on plant competition and insect communities, I used a common garden of 24 genotypes of the allelopathic species *Solidago altissima*. I measured a variety of above-ground plant performance measurements on each genotype of *Solidago altissima* as a measure of competitive ability, as well as assessed the foliar and pollinator insect communities associated with each genotype. Using these data, I addressed the following two questions: 1). Does genotype chemical composition alter plant performance, and if so, what sort of patterns arise? And 2). What patterns do we see between chemical composition and the associated foliar and floral insect communities?

## METHODS

### *Background and study species*

*Solidago altissima* is a model system for studying allelopathy in response to competition under experimental and natural environments (Uesugi et al. 2019). *Solidago altissima* is a clonal perennial that is common in old fields and other open habitats across its native range of Eastern North America (Yip et al. 2019). *Solidago altissima* is very diverse in its allelochemical composition, allowing it to be a successful invader across Europe, Japan, and Australia (Abhilashaet al. 2019; Webber, 1999; Uesugi et al. 2019). Newly established populations of *S. altissima* have a large number of genetic individuals. However, as *S. altissima* densities increase via clonal expansion, inferior genotypes are

displaced, leading to fewer genotypes within older populations (Hartnett and Bazzaz, 1985). Since *S. altissima* populations are expected to be more variable before genotype sorting, I would expect the greatest phytochemical diversity in younger populations. *Solidago altissima* is also self –incompatible, supporting a diverse array of insect herbivores and pollinators (Root, 1996; Hafdahl and Craig, 2013; Abrahamson and Weis, 1997), making it a useful system to explore relationships between phytochemistry and insect community composition.

*Study site and establishment of common garden*

In the spring of 2014, five ramets of each genotype of *S. altissima* were collected as rhizome/stem segments from Douglas-Hart Nature Center (Mattoon, IL; 39° 29’ N; 88° 17’ W) in a recently restored prairie. The area had been in row crop agriculture three years prior and *S. altissima* was not a part of the initial seeding. Therefore, all *S. altissima* genotypes represented colonists from the surrounding area. The young site age represents the phase before the sorting of genotypes, potentially retaining high genetic and chemical diversity within these *S. altissima* clones (Hartnett and Bazzaz, 1985). Rhizome/stem segments were collected from distinct patches (within 0.5 m) and isolated (>5 m) from other such patches to ensure the collection of genetically distinct genotypes.

The common garden site was a level section of land in Clark County, IL (39° 19’ N; 87° 55’ W) that was used to grow corn in the previous year. Five ramets from each genotype were planted in a regular pattern (center and in each corner) into individual 1.6 × 1.6 m plot with aluminum flashing buried 15 cm deep to prevent rhizome spreading. Plots were separated by 2 m and the spaces between each plot were maintained by

mowing. After the initial planting of *S. altissima*, other plant species were allowed to colonize the plots naturally. During the first two years of growth, all *S. altissima* flowering heads were removed prior to seed set in order to prevent the colonization of the plots by new *S. altissima* genotypes.

*Chemical analysis*

HPLC analysis was done to characterize leaf chemistry across *S. altissima* genotypes following the protocol of Meiners et al. (2017). Briefly, in the summer of 2016, fully expanded leaves were collected from several stems of each genotype of *S. altissima*. Metabolites were extracted using 1 mL of HPLC-grade methanol from 100 mg of dried leaf tissue that was ground after freezing with liquid nitrogen. After centrifugation, the supernatant was filtered through a 0.22 µm filter and analyzed using a Hitachi Chromaster HPLC with a 5430 Diode Array detector. The mobile phase was a mixture of acetonitrile:water (v/v) at 20:80 from 0-5 minutes, a linear gradient of 20:80 to 95:5 from 5-45 minutes, 95:5 from 45-55 minutes, a linear gradient of 95:5 to 20:80 for 55-60 minutes, and 20:80 for 60-70 minutes. The flow rate was held constant at 0.7 mL/min and the sample loading volume was 10 µL. Chemical constituents were separated by time of emergence and the area of the peak used an estimate of the amount present. Only peaks that were discernable from the baseline (>75 µV * s) were retained for analysis.

Chemical variation for all genotypes was described with non-metric multidimensional scaling (NMDS) to generate independent axes of chemical variation. Peaks that occurred in 5 or fewer genotypes were omitted from the analysis as

uninformative. The optimum number of dimensions for the NMDS was determined by comparison to randomized data in PC-ord (McCune and Grace, 2002). The three axes resulting from this analysis were used to relate chemical composition to plant competition as well as foliar and pollinator insect communities.

*Plant performance measures*

As a measure of competitive ability, stem density was recorded for each genotype of *S. altissima*. In July of 2018, five 0.25 × 0.25 m quadrats (center and 15 cm from each corner) were placed into each plot of *S. altissima*. The number of stems within each quadrat were counted and averaged for each *S. altissima* genotype. Similarly, light transmittance was recorded as measure of resource uptake ability. A Line Quantum Sensor (Li-Cor®, model LI-250A) was inserted 30 cm above the ground of each plot in September of 2018. Two measurements were taken diagonally across the plot and standardized to a measurement above each plot to calculate light transmittance and averaged.

The final measurements of plant performance came from a biomass harvest. In early October of 2018, before the *S. altissima* started to shed seed, a single 0.5 × 0.5 m quadrat was placed in the center of each plot, approximately 0.55 m from each side. All plant vegetation within the quadrat was cut 0.5 cm from the ground for each genotype. For each plot, flowers, stems, and leaves of *S. altissima* were separated (flower heads cut 0.5 cm below from the lowest point of flowering) and dried. For all non-*S. altissima* species, their biomass was pooled for each plot, regardless of species, and dried at 60 C°

for 48 h. Proportion of flower mass was calculated for each genotype by dividing the biomass of the flowers by the total biomass harvested.

*Foliar insects*

To relate foliar insect communities to *S. altissima* genotypes, I used yellow insect sticky cards (Alpha Scents, Inc., Linn, OR, USA). Three times, between late August to mid-September of 2018, with a week between each sampling, sticky cards were placed in the center of each plot on a metal rod. One side of each sticky card was exposed for 24 h, wrapped in plastic film, and stored frozen to preserve the specimens. Insects that fell within the grid lines on the cards were identified down to their taxonomic order and pooled across all 3 foliar insect assessments. Data from these foliar insect assessments resulted in 3,831 individuals spanning 9 insect orders (Table 1.1).This foliar insect data was then analyzed with NMDS ordinations as described above. The optimum number of dimensions for these data was two, which were then used to relate to plant performance and chemistry.

*Pollinator insects*

From early September to late-September of 2018, I conducted three rounds of floral visitor observations, with each census occurring approximately a week apart on a clear day. Each census was done by placing a 0.5 × 0.5 m quadrat into the center of each plot. All *S. altissima* flower heads within this quadrat were observed for 4 minutes during solar noon (10:00 a.m. – 12:00 p.m.) All insects within this area that made physical

contact with an open *S. altissima* flower was recorded. All insects that were observed were then identified down to their taxonomic order and pooled across censuses. These pollinator data were divided into two segments: potential pollinators and floral visitors. Insect predator species such as ambush bugs and arachnids were identified as floral visitors and would not serve as pollinators, and were omitted from the analyses. Data from these pollinator insect assessments resulted in 887 individuals spanning 8 insect orders (Table 1.2). Potential pollinator data was then analyzed with NMDS ordination as above. The three resulting axes were then used to relate pollinator insects to plant competition as well as plant chemistry.

*Data analysis: Variation among Genotypes*

Variation in plant performance measures of proportion of flower mass, light transmittance, and stem density were compared across genotypes of *S. altissima* with a series of one-way ANOVAs. A scatterplot was then made comparing NMDS chemical axis 1 and NMDS chemical axis 3 to help visualize plant chemical variation across the two most significant chemical NMDS axes.

*Data analysis: Plant performance vs. Chemistry*

Using the plant performance measurements of light transmittance, stem density, and proportion of flower mass, a series of multiple regression analyses were conducted to relate plant performance to plant chemistry using the three chemical NMDS axes as predictors.

*Data analysis: Insect communities*

A series of Pearson correlations were conducted in order to relate plant performance measures to the foliar and pollinator insect communities. These correlation analyses were conducted in order to determine any correlation between all five insect NMDS axes (2 foliar and 3 pollinator) and the performance measurements of total *S. altissima* aboveground biomass, light transmittance, flower proportion, and stem density. A series of Multiple Regression analyses were also conducted in order to relate the pollinator and foliar insect communities to plant chemistry. These multiple regression analyses were conducted in order to predict the influence of chemistry on all five insect axes (2 foliar and 3 pollinator). All statistical analyses were done using R version 3.1.2 (R Foundation for Statistical Computing).

Table 1.1. Foliar insect totals pooled across all three sticky card assessments. All insects are organized by taxonomic order.

| | Pooled Insect Totals | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Genotype** | **Coleoptera** | **Lepidoptera** | **Diptera** | **Hymenoptera** | **Hemiptera** | **Aranea** | **Orthoptera** | **Thysanoptera** | **Ioxida** |
| **1** | 44 | 18 | 195 | 10 | 26 | 0 | 0 | 3 | 2 |
| **2** | 62 | 10 | 216 | 6 | 36 | 0 | 0 | 5 | 0 |
| **3** | 18 | 8 | 156 | 2 | 19 | 2 | 1 | 4 | 2 |
| **4** | 46 | 16 | 250 | 20 | 37 | 5 | 0 | 4 | 0 |
| **5** | 14 | 13 | 133 | 10 | 13 | 9 | 0 | 5 | 0 |
| **6** | 16 | 9 | 98 | 6 | 27 | 2 | 0 | 2 | 0 |
| **7** | 71 | 4 | 97 | 8 | 15 | 4 | 0 | 11 | 0 |
| **8** | 114 | 4 | 360 | 17 | 41 | 1 | 0 | 6 | 0 |
| **9** | 20 | 6 | 247 | 22 | 27 | 2 | 0 | 2 | 1 |
| **10** | 36 | 5 | 131 | 10 | 16 | 4 | 2 | 0 | 0 |
| **11** | 102 | 7 | 250 | 10 | 27 | 5 | 0 | 8 | 0 |
| **12** | 14 | 2 | 147 | 7 | 26 | 8 | 6 | 3 | 0 |
| **13** | 111 | 18 | 306 | 47 | 42 | 0 | 2 | 7 | 0 |
| **14** | 6 | 5 | 179 | 11 | 14 | 7 | 2 | 6 | 4 |
| **15** | 20 | 4 | 172 | 14 | 26 | 8 | 2 | 5 | 0 |
| **16** | 14 | 4 | 260 | 16 | 18 | 2 | 0 | 7 | 0 |
| **17** | 24 | 12 | 185 | 16 | 33 | 4 | 0 | 5 | 0 |
| **18** | 10 | 7 | 240 | 16 | 34 | 3 | 0 | 3 | 0 |
| **19** | 12 | 6 | 203 | 16 | 21 | 0 | 0 | 11 | 2 |
| **20** | 7 | 7 | 150 | 17 | 22 | 2 | 2 | 5 | 3 |
| **21** | 33 | 9 | 162 | 16 | 18 | 0 | 0 | 14 | 0 |
| **22** | 9 | 3 | 130 | 8 | 36 | 2 | 0 | 9 | 0 |
| **23** | 18 | 10 | 240 | 11 | 21 | 5 | 0 | 5 | 0 |
| **24** | 14 | 19 | 221 | 11 | 33 | 5 | 0 | 9 | 0 |

Table 1.2. Pollinator insect totals pooled across all three floral observations. All insects are organized by visitor type and taxonomic order.

| | Pollination Visitation: Pooled total | | | | | | Floral visitors: Pooled total | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Genotype** | **Coleoptera** | **Lepidoptera** | **Diptera** | **Hymenoptera** | **Hemiptera** | **Thysanoptera** | **Coleoptera** | **Aranea** | **Hemiptera** | **Orthoptera** |
| **1** | 20 | 0 | 7 | 1 | 0 | 0 | 1 | 0 | 0 | 0 |
| **2** | 25 | 2 | 2 | 12 | 1 | 0 | 0 | 0 | 0 | 0 |
| **3** | 15 | 3 | 9 | 5 | 1 | 0 | 0 | 0 | 0 | 0 |
| **4** | 18 | 1 | 17 | 4 | 0 | 1 | 0 | 0 | 0 | 0 |
| **5** | 6 | 0 | 7 | 6 | 3 | 4 | 1 | 3 | 0 | 0 |
| **6** | 13 | 2 | 3 | 0 | 3 | 0 | 1 | 2 | 0 | 0 |
| **7** | 22 | 3 | 5 | 10 | 1 | 2 | 0 | 1 | 0 | 0 |
| **8** | 34 | 2 | 7 | 15 | 2 | 0 | 0 | 2 | 0 | 1 |
| **9** | 4 | 0 | 5 | 18 | 1 | 0 | 1 | 3 | 0 | 0 |
| **10** | 16 | 2 | 2 | 9 | 5 | 0 | 0 | 4 | 0 | 0 |
| **11** | 5 | 2 | 4 | 11 | 4 | 1 | 0 | 4 | 1 | 0 |
| **12** | 25 | 3 | 3 | 2 | 2 | 2 | 1 | 1 | 0 | 1 |
| **13** | 14 | 2 | 7 | 7 | 2 | 3 | 0 | 2 | 1 | 0 |
| **14** | 9 | 1 | 4 | 14 | 4 | 4 | 0 | 4 | 0 | 0 |
| **15** | 16 | 3 | 4 | 15 | 2 | 1 | 0 | 1 | 0 | 0 |
| **16** | 4 | 1 | 2 | 14 | 6 | 2 | 0 | 2 | 1 | 0 |
| **17** | 25 | 1 | 6 | 6 | 1 | 1 | 0 | 0 | 0 | 0 |
| **18** | 20 | 2 | 7 | 2 | 2 | 1 | 0 | 1 | 1 | 0 |
| **19** | 14 | 0 | 4 | 7 | 2 | 3 | 2 | 5 | 0 | 0 |
| **20** | 26 | 0 | 2 | 2 | 3 | 1 | 0 | 0 | 2 | 0 |
| **21** | 14 | 5 | 1 | 13 | 2 | 0 | 0 | 7 | 0 | 0 |
| **22** | 27 | 0 | 3 | 2 | 2 | 0 | 0 | 6 | 1 | 0 |
| **23** | 7 | 2 | 3 | 11 | 1 | 0 | 0 | 3 | 0 | 0 |
| **24** | 15 | 2 | 5 | 3 | 1 | 0 | 0 | 1 | 1 | 0 |

## RESULTS

### *Variation among Genotypes*

Of the plant performance measures, proportion of flower mass, and stem density did not vary across genotypes. Only light transmittance was found to vary significantly across genotypes (ANOVA: $F_{1, 22} = 10.314$, $P = 0.004$, $R^2 = 0.2882$; Fig. 1.1). Despite the limited variation in physical traits among genotypes, there was marked variation in their chemical composition as visualized with NMDS chemical axis 1 and axis 3 (Fig 1.2).

### *Plant performance vs. Chemistry*

Of the three plant performance measures, only the proportion of flower mass was unrelated to any of the chemical NMDS axes (Table 1.3). Of these, only NMDS chemical axis 3 had any relationship to plant performance. Both stem density (Regression analysis: $F_{3,20} = 2.087$, $p = 0.0329$, $R^2 = 0.1242$) and % light transmittance (Regression analysis: $F_{3,20} = 8.306$, $p = <0.0001$, $R^2 = 0.4879$) were significantly influenced by NMDS chemical axis 3, resulting in an overall significant model for % light transmittance. NMDS chemical axis 3 was positively associated with light transmittance (Fig. 1.3) and negatively associated with stem density (Fig. 1.4), reflecting the inverse relationship between these two variables.

### *Insect communities*

To relate plant performance to foliar and pollinator insect communities, a series of Pearson pairwise correlations were conducted. The plant performance measures included in these analyses were: stem density, % light transmittance, flower proportion, and total

*S. altissima* aboveground biomass. Though none of the foliar insect axes correlated with any of the plant performance measures, the foliar insect NMDS axis 1 correlated with pollinator NMDS axis 3 (Table 1.4). Aspects of the pollinator community also lined up with a few of the plant performance measures. Pollinator NMDS axis 1 correlated heavily with both proportion of flower mass and total *S. altissima* above-ground biomass, while pollinator NMDS axis 2 correlated with % light transmittance, all with negative associations. Though stem density did not correlate with any of the insect axis, we were still able to relate multiple performance measures to multiple axes of the pollinator insect community.

To determine the influence of NMDS chemical axes on the foliar and pollinator insect communities, I conducted a series of multiple regression analyses. For the foliar insect communities, neither of the two NMDS axes lined up with any of the three chemical NMDS axes, indicating no influence of chemistry on this insect community (Table 1.5). However, for pollinator insects, NMDS axis 1 correlated significantly with chemical NMDS axis 1 ($r = 3.5066$, $p = 0.001992$; Fig. 1.5). This indicates that some of the pollinator insects in the community are responding positively to some of the plant chemistry exuded by *S. altissima* genotypes.

Table 1.3. Multiple Regression analyses of the relationship between NMDS axes of plant chemistry on plant performance measures. Significant values are indicated in bold.

| Source | *t* | *p* | β | F | *df* | *p* | Adj. $R^2$ |
|---|---|---|---|---|---|---|---|
| <u>Stem Density</u> | | | | | | | |
| Overall Model | | | | 2.087 | 3, 20 | 0.134 | 0.1242 |
| NMDS 1 | -0.191 | 0.8508 | 0.723 | | | | |
| NMDS 2 | 0.561 | 0.5811 | 0.9950 | | | | |
| NMDS 3 | -2.292 | **0.0329** | 1.0193 | | | | |
| <u>% light transmittance</u> | | | | | | | |
| Overall Model | | | | 8.306 | 3, 20 | **0.0009** | 0.4879 |
| NMDS 1 | -1.144 | 0.266 | 5.467 | | | | |
| NMDS 2 | -0.325 | 0.748 | 7.524 | | | | |
| NMDS 3 | 4.901 | **<0.0001** | 7.708 | | | | |
| <u>Proportion of flower mass</u> | | | | | | | |
| Overall Model | | | | 1.401 | 3, 20 | 0.2718 | 0.04967 |
| NMDS 1 | -1.148 | 0.2647 | 0.0104 | | | | |
| NMDS 2 | -0.507 | 0.6176 | 0.0144 | | | | |
| NMDS 3 | 1.745 | 0.0963 | 0.0147 | | | | |

Table 1.4. Pearson Pairwise correlations comparing pollinator and foliar insect NMDS axes to plant performance measures. Significant values are indicated in bold.

| Variables | Pollinator NMDS 1 | Pollinator NMDS 2 | Pollinator NMDS 3 | Foliar NMDS 1 | Foliar NMDS 2 |
|---|---|---|---|---|---|
| Pollinator NMDS 2 | -0.001 | --- | | | |
| Pollinator NMDS 3 | -0.001 | -0.001 | --- | | |
| Foliar NMDS 1 | -0.234 | -0.049 | **0.440** | --- | |
| Foliar NMDS 2 | -0.049 | 0.087 | 0.072 | -0.001 | --- |
| Stem Density | -0.161 | -0.179 | 0.130 | 0.310 | -0.299 |
| Light Attenuation | -0.024 | **-0.422** | 0.017 | 0.124 | 0.307 |
| Prop. of Flower Mass | **-0.404** | 0.164 | 0.151 | -0.119 | 0.320 |
| Above-Ground Biomass | **-0.465** | -0.044 | -0.088 | 0.078 | -0.192 |

Table 1.5. Multiple Regression analyses of the relationship between NMDS axes of plant chemistry on NMDS axes of foliar and pollinator insect communities. Significant values are indicated in bold.

| Source | *t* | *p* | β | F | *df* | *p* | Adj. $R^2$ |
|---|---|---|---|---|---|---|---|
| Pollinator NMDS axis 1 | | | | | | | |
| Overall Model | | | | 3.810 | 3, 20 | **0.026** | 0.268 |
| Chemical NMDS 1 | 2.997 | **0.007** | 0.284 | | | | |
| Chemical NMDS 2 | -0.005 | 0.996 | 0.391 | | | | |
| Chemical NMDS 3 | 0.400 | 0.693 | 0.400 | | | | |
| Pollinator NMDS axis 2 | | | | | | | |
| Overall Model | | | | 0.369 | 3, 20 | 0.776 | 0.052 |
| Chemical NMDS 1 | 0.043 | 0.966 | 0.199 | | | | |
| Chemical NMDS 2 | -0.224 | 0.825 | 0.274 | | | | |
| Chemical NMDS 3 | -1.040 | 0.311 | 0.281 | | | | |
| Pollinator NMDS axis 3 | | | | | | | |
| Overall Model | | | | 1.459 | 3, 20 | 0.256 | 0.057 |
| Chemical NMDS 1 | -0.305 | 0.763 | 0.159 | | | | |
| Chemical NMDS 2 | 1.667 | 0.109 | 0.219 | | | | |
| Chemical NMDS 3 | -0.345 | 0.734 | 0.224 | | | | |
| Foliar NMDS axis 1 | | | | | | | |
| Overall Model | | | | 0.665 | 3, 20 | 0.584 | 0.091 |
| Chemical NMDS 1 | -0.469 | 0.644 | 0.371 | | | | |
| Chemical NMDS 2 | 0.966 | 0.346 | 0.511 | | | | |
| Chemical NMDS 3 | -0.201 | 0.843 | 0.524 | | | | |
| Foliar NMDS axis 2 | | | | | | | |
| Overall Model | | | | 0.734 | 3, 20 | 0.544 | 0.099 |
| Chemical NMDS 1 | -0.767 | 0.452 | 0.208 | | | | |
| Chemical NMDS 2 | -1.41 | 0.173 | 0.286 | | | | |
| Chemical NMDS 3 | 0.323 | 0.750 | 0.293 | | | | |

Figure 1.1. Variation in light transmittance for field plots of 24 genotypes of *S. altissima*. Data plotted are means with SE

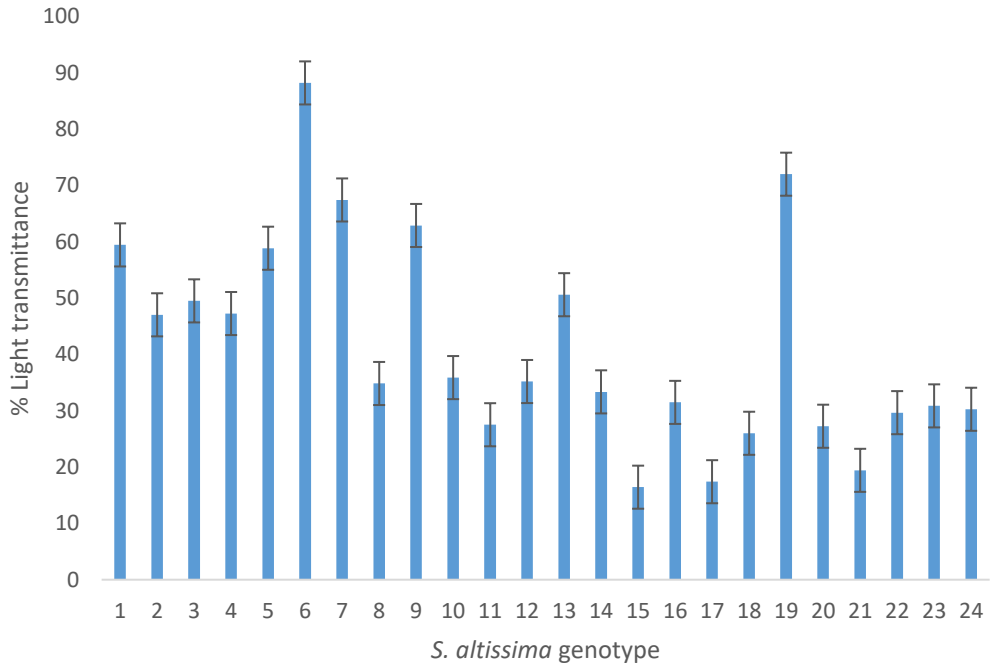


Figure 1.2. Scatterplot of chemical NMDS values illustrating the diversity of chemical expression across *S. altissima* genotypes.

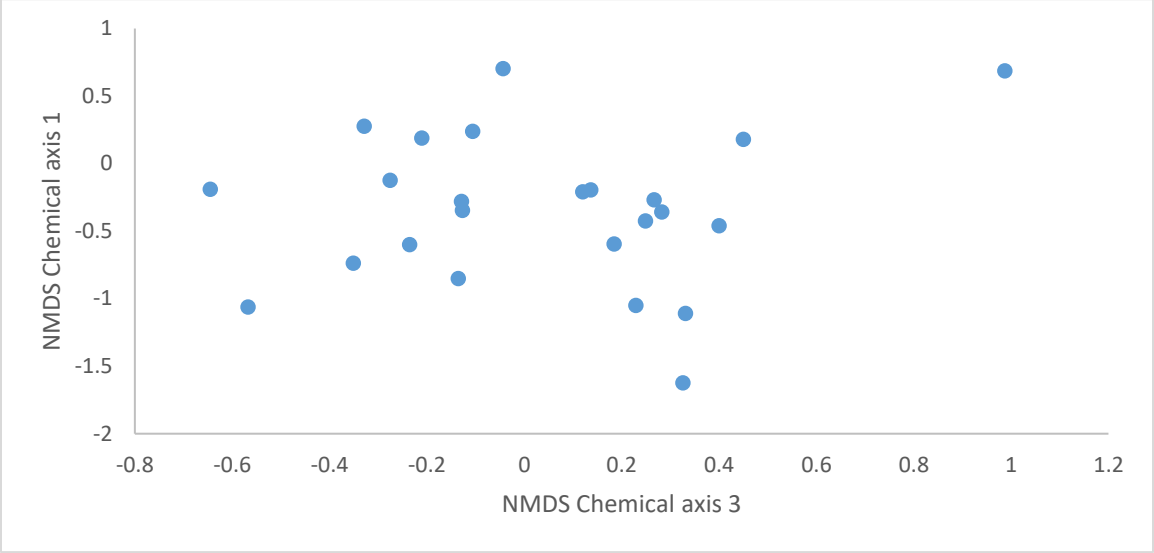


Figure 1.3. Relationship between % light transmittance and NMDS chemical axis 3. Data plotted are means for light transmittance for each genotype with trend line.

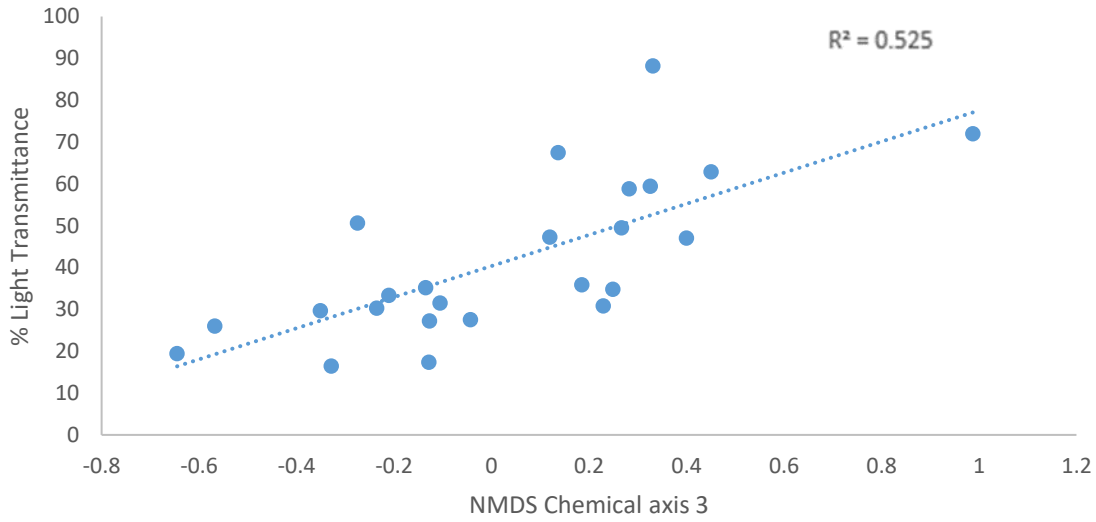


Figure 1.4. Relationship between stem density and NMDS chemical axis 3. Data plotted are mean stem densities for each genotype with trend line.

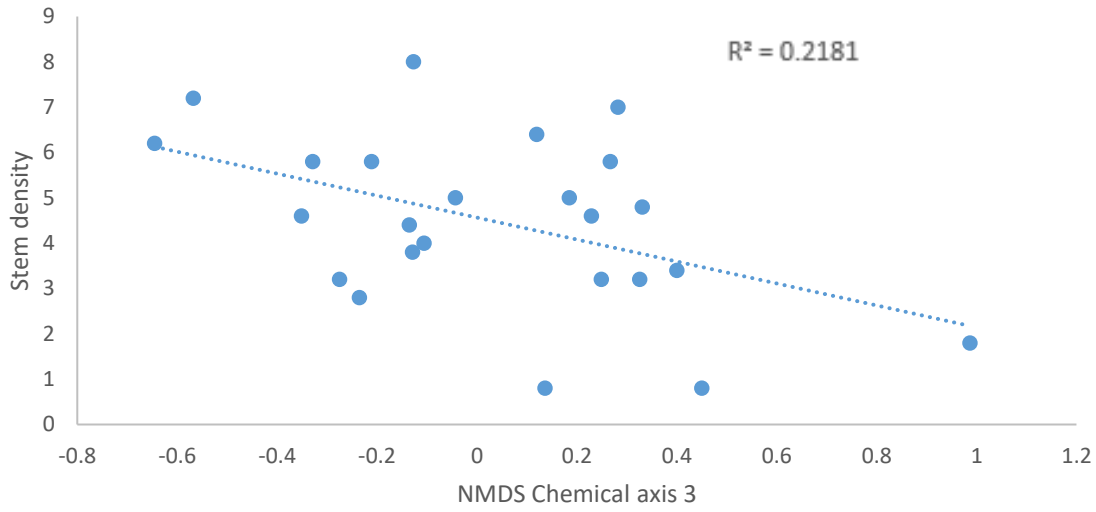
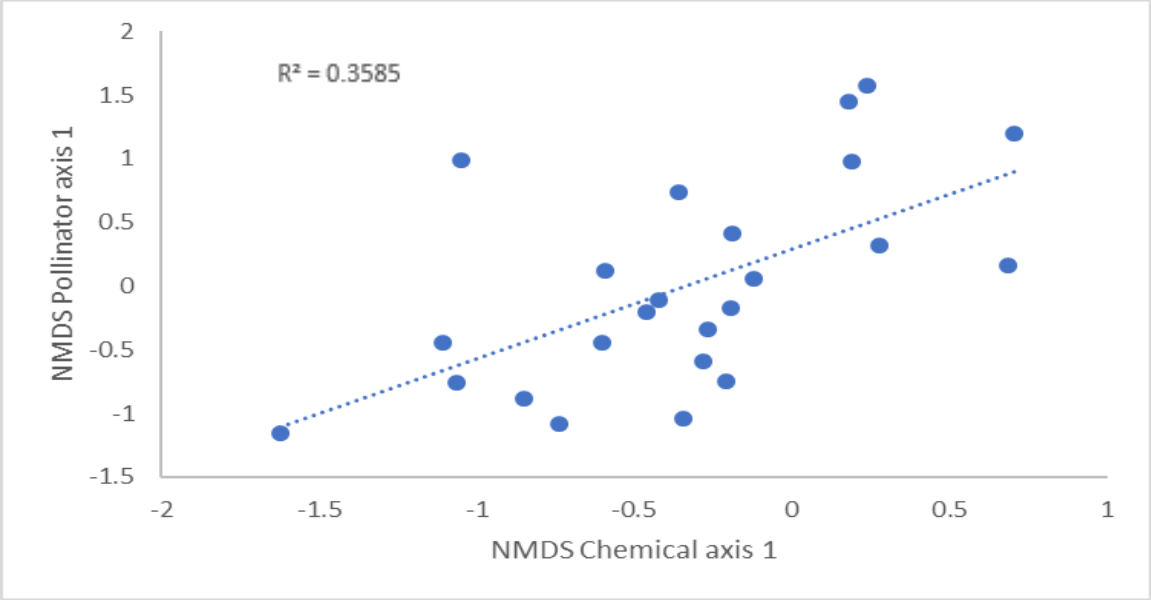


Figure 1.5. Relationship between NMDS chemical axis 1 and pollinator NMDS axis 1 with trend line.



## DISCUSSION

Genotypes of *S. altissima* did not show much variation across plant performance measures. Measures of stem density and proportion of flower mass varied little across all genotypes, indicating these genotypes have overall similar stem, leaf, and flower growth. However, % light transmittance did vary significantly (Fig. 1.1), suggesting that certain genotypes are better able to capture sunlight. As light attenuation would also be a combination of stem density and the amount/arrangement of leaf tissue, it is interesting that only light interception varied. Light competition is strong in the early successional environments often occupied by *S. altissima* (Banta et al. 2008), suggesting that these genotypes may vary in their light competitive ability. This variation may then allow for resource-allocation trade-offs between competitive ability and other life history strategies such as plant defense, stem architecture, and leaf density (Hakes and Cronin, 2012; Banta et al. 2008). However, some of this variation in light interception may be due to natural disturbances that create gaps in the canopy that allow for more space and resources within the community (Carson and Pickett, 1990). Though the 24 genotypes of *S. altissima* did not vary much in the plant performance measures, there was great variation in foliar chemistry between the genotypes (Fig. 1.2), which may lead to potential intraspecific variation in ecological function of each genotype as some suggested (Heath et al. 2014; Bosio et al. 1990).

Ecological patterns existed between foliar chemistry and plant performance, as foliar chemistry was strongly related to most measures of plant performance across genotypes. Of the three plant performance measures in the analyses, only the proportion of flower mass was not related to any chemical NMDS axes (Table 1.3). Both stem

density and % light transmittance were significantly influenced by chemical NMDS axis 3. Stem density was negatively related to NMDS axis 3 (Fig. 1.4), while light transmittance was positively related (Fig. 1.3), reflecting their inverse relationship. Mechanistically, lower stem densities would allow for greater light transmittance to the ground, thus, these chemicals related to axis 3 are associated with plant competitiveness. Though chemicals associated with axis 3 may not directly contribute to larger overall biomass growth of stems and leaf tissues, other aspects of chemistry can still contribute to the success of these genotypes via allocation of resources to chemical defense and attractants for pollinators (Heath et al. 2014; Szymura and Szymura, 2015).

Foliar insect composition was not aligned with any of the plant performance measures (Table 1.4). Though foliar insects were independent from plant performance, there was some association between the foliar and floral insects, suggesting that these insects share a common pattern in response to some aspect of the *S. altissima* genotypes. Patterns also arose between plant performance and pollinator insects as pollinator communities were associated with total *S. altissima* aboveground biomass, proportion of flower mass, and % light transmittance (Table 1.4). Pollinator NMDS axis 1 was correlated with total *S. altissima* aboveground biomass and proportion of flower mass, while pollinator NMDS axis 2 was correlated with % light transmittance. The pollinator insects related to pollinator NMDS axis 1 were related to plant growth, where these pollinator insects decrease when there are higher proportions of flowers and above-ground biomass growth. Pollinator insects related to NMDS axis 2, however, are positively linked with plant growth as these insects associate with genotypes that have less light transmittance and more vegetative growth. Vegetative biomass was

significantly correlated with floral biomass (Pearson correlation: $r = 0.828$, $p < 0.0001$), indicating that these better performing genotypes disproportionally have more flowers, which may contribute to the attraction of these floral insects. This variation in insect community response may be a reflection of the multi-functionality of plant chemicals; these chemicals may affect the pollinators directly, as well as indirectly via plant performance (Hale and Kalisz, 2012, Abhilasha et al. 2008).

Associated insect communities were also related to foliar chemistry (Table 1.5). Foliar insect composition was independent of all chemical NMDS axes (Table 1.5), indicating that chemistry had no influence on foliar insects. This suggests that the foliar insect communities were generalists, feeding on or around the *S. altissima* genotypes indiscriminately. While anti-herbivore chemicals are known to be abundant in *Solidago* (Heath et al. 2014), this result was somewhat surprising. My approach did not focus in on individual anti-herbivore compounds, so the approach may have missed the role of individual chemical constituents that may affect these insects directly via repellents or feeding cues or indirectly by attracting predators (Williams and Avakian, 2015). Generalist herbivores may have been equally responsive to the defensive chemistry of these genotypes, generating equivalent communities. Similarly, the variation in anti-herbivore compounds may have been insufficient to alter the abundance of specialist insect species (Maddox and Root, 1987), since I was not able to find any variation in specialist gall forming insects across genotypes (unpublished data). As some foliage feeding insects on *Solidago* are episodic specialists (Carson and Root, 2000), the study may not have encompassed a critical expansion of specialist monophagous insects (Ali and Agrawal, 2012). The pollinator community, however, was significantly associated

with foliar chemical composition as pollinator NMDS axis 1 was positively correlated with chemical NMDS axis 1 (Fig. 1.5). This demonstrates that the pollinator community is responding positively to some of the chemicals in these *S. altissima* populations, generating patterns between the pollinator insect communities and chemistry. Here, chemistry may be facilitating these interactions, positively altering floral attractiveness but also adversely affecting other traits such as net photosynthesis and/or overall vigor (Hale and Kalisz, 2012).

These data outlined here demonstrates the multidimensionality and variation of the *S. altissima* chemical landscape. Foliar chemical composition varied across genotypes and aligned with both plant performance measures as well as composition of the insect communities; showing that phytochemical composition has multiple functions within *S. altissima*. Independent axes of chemistry were correlated to different functions, those functions being performance and plant-insect associations. Here, chemistry is not only affecting the pollinator community directly (Fig. 1.5), but they are also affecting them indirectly via plant performance and physiology, which then affects the pollinator community. Among genotypes, chemical composition seems to be facilitating many of the ecological functions, generating patterns between chemistry, plant performance, and insect communities; with independent axes of foliar chemistry affecting different components of the system, either directly or indirectly. The results reported here represent an important framework linking chemical composition to both plant performance and insect communities. These chemicals not only vary greatly and serve multiple ecological functions, but they are also very essential in the establishment and success of the *Solidago altissima* plant species.

# CHAPTER 2

EFFECTS OF CHEMICAL VARIATION ON INTERSPECIFIC PLANT COMPETITION


## ABSTRACT

In clonal plant species, both inter- and intraspecific competition can affect fitness via changes in growth and reproductive allocation. Competing demands for resources inevitably lead to trade-offs in investment between defense against herbivory, reproductive efficiency, and competition against other plant species. For allelopathic plants, the optimal balance of investment in competition versus other ecological functions may ultimately depend on chemical production and their associated costs.

To assess the effects of foliar chemical variation on interspecific plant competition, I conducted competitive trials in a greenhouse setting using 24 genotypes of *Solidago altissima* with known chemical profiles. Clones of each genotype competed with four common target species: *Schizachyrium scoparium, Melilotus officinalis, Silphium integrifolium,* and *Abutilon theophrasti.* After 60 days (37 for *Abutilon*), aboveground biomass was harvested, along with *S. altissima* biomass and the rhizome biomass.

Genotypes of *S. altissima* showed marked variation in both rhizome and above-ground biomass growth, demonstrating diverse resource allocation among genotypes. The above-ground biomass of target species varied drastically when compared to the non-competition pots. *Abutilon theophrasti*, *S. scoparium,* and *S. integrifolium* all had their biomass significantly reduced via competition with *S. altissima*. Regression analyses revealed that chemistry significantly affected the biomass of both *A. theophrasti* and *S.*

*scoparium*, suggesting chemistry is a critical driver of competition for *S. altissima* in at least some situations. Foliar chemistry of *S. altissima* also affected its own biomass, where chemical NMDS axis 2 was negatively associated with rhizome biomass and axis 3 is positively associated with aboveground biomass. This pattern may be due to possible tradeoffs between expensive classes of compounds and growth, which then may be offset by the benefits of the chemical production.

The results reported here illustrate the strong and diverse competitive ability of *S. altissima* and how its chemistry may be a critical component to its competitive success. These results are also consistent with my findings in the previous chapter, highlighting the importance of chemistry as the main driver for many *S. altissima* interactions, both at the intra- and interspecific scale; where chemical production is may create various tradeoffs between competitive ability, biomass, insect associations, and other plant performance measures, suggesting alternative strategies across genotypes.

## INTRODUCTION

In clonal plant species, both inter- and intraspecific competition can affect fitness via changes in growth and reproductive allocation (Van Kleunen et al. 2001). Competing demands for resources inevitably lead to trade-offs in investment between defense against herbivory, reproductive efficiency, and competition against other plant species (Uesugi et al. 2017; Van Kleunen et al. 2001). However, the optimal balance of investment in competition versus other ecological functions will ultimately depend on ecological conditions such as local density, diversity of herbivores, plant chemical composition, and

competitor plant communities, which are all likely to vary over time and space (Uesugi et al. 2017; Adomako et al. 2019).

One mechanism that allows for colonial plants to rapidly dominate large landscapes and compete for resources is allelopathy. Allelopathic compounds are released into the environment as root exudates, volatile organic compounds, leaf leachate, or leaf litter (Uesugi et al. 2019; Inderjit et al. 2011). Allelochemicals may reduce the germination and/or growth of neighboring species, increasing access to resources by directly suppressing neighbor growth or indirectly by affecting microbial mutualists or nutrient availability (Meiners et al. 2012; Uesugi et al. 2019). These allelochemicals may also exhibit multi-functionality, where they may simultaneously function to defend against herbivory, attract pollinators, and/or enhance competitive ability (Beran et al. 2019; Inderjit et al. 2011).

However, the production of allelopathic chemicals is thought to be energetically costly, where the benefits and costs of allelochemical production vary across time and space with variation in competition (Kong et al. 2018). Natural selection may favor plant genotypes within heterogeneous competitive environments that employ induced production of allelochemicals, rather than genotypes that constitutively produce high levels of these chemicals (Uesugi et al. 2019; Novoplansky 2009; Kegge and Pierik 2009). If the variation of allelochemical production is a cost-saving strategy, we should expect some sort of ecological trade-off between allelopathic chemical production and plant performance/competitive ability (Uesugi et al. 2019).

To assess the effects of foliar chemical variation on interspecific plant competition, I conducted competitive trials in a greenhouse setting using 24 genotypes of

*Solidago altissima* with known chemical profiles. Clones of each genotype, competed with four common target species: *Schizachyrium scoparium, Melilotus officinalis, Silphium integrifolium,* and *Abutilon theophrasti*. Using these data, I attempt to explicitly link foliar chemical composition of *S. altissima* genotypes to interspecific plant-plant competition.

## METHODS

### *Background and study/target species*

*Solidago altissima* is a model system for studying allelopathy in response to competition under experimental and natural environments (Uesugi et al. 2019). *Solidago altissima* is a clonal perennial that is common in old fields and other open habitats across its native range of Eastern North America (Yip et al. 2019). *Solidago altissima* has a diverse allelochemical composition, allowing it to be a successful invader across Europe, Japan, and Australia (Abhilashaet al. 2019; Webber, 1999; Uesugi et al. 2019). Within its native range, newly established populations of *S. altissima* are composed of a large number of genetic individuals. However, as *S. altissima* densities increase via clonal expansion, inferior genotypes are displaced, leading to fewer genotypes within older populations (Hartnett and Bazzaz, 1985). Since *S. altissima* populations are expected to be more genetically variable before genotype sorting, I would expect the greatest phytochemical diversity to also occur in younger populations.

*Schizachyrium scoparium* (Little bluestem) is a $C_4$ grass which is a major component in mesic habitats across its native habitat of central North America (Van Auken and Bush, 1988). *Schizachyrium scoparium* is a very competitive species,

especially in less productive, nitrogen-poor soils; where its efficient nutrient consumption allows it to dominate (Tilman, 1989). *Melilotus officinalis* (Yellow sweet clover) is a biennial legume that is native to Eurasia (Klebesadel, 1992). Due to this species nitrogen fixing ability, it has been introduced and become naturalized throughout the world and has become a conservation problem across North America (Van Riper and Larson, 2009; Wolf et al. 2003). *Silphium integrifolium* (Rosinweed) is a deep-rooted, perennial forb endemic to tallgrass prairies of the Midwestern United Sates (Tooker and Hanks, 2006; Fay et al. 1993). *Silphium integrifolium* consists of a few to about 100 shoots which form tightly packed clumps, suggesting below-ground processes are critical to this plants survival (Fay et al. 1993). *Abutilon theophrasti* (Velvetleaf) is an introduced annual weed found across the Midwestern United States (Lee and Bazzaz, 1980). *Abutilon theophrasti* is a specialist within early successional communities, dominating areas with low competition where it grows rapidly (Sattin et al. 1992).

*Rhizome collection and transplanting*

In early May of 2019, 5 rhizomes from each genotype of *S. altissima* were collected from a common garden, a level section of land in Clark County, IL (39° 19’ N; 87° 55’ W). Rhizomes 6-10 cm in length were collected to standardize among the rhizomes. All rhizomes were washed with water and individually planted in 15.0 cm diameter x 14.5 cm tall standard round pots filled with all-purpose professional growing mix (Pro-Mix, Premier Tech., QC). Rhizomes were planted approximately 1.5 cm. deep and 2.5 cm. from the edge of the pots and watered consistently. Any rhizomes that died were replaced within the first two weeks of transplanting.

*Target species transplanting*

Seeds of *Schizachyrium scoparium* and *Silphium integrifolium* were procured from Prairie moon nursery (Winona, MN), *Melilotus officinalis* from Seed world USA (Tampa, FL) and *Abutilon theophrasti* were collected from our common garden site. In early May 2019, seeds of *Abutilon theophrasti* were treated with hot water (60° C) for 1 hour. Once dormancy was broken, seeds of all four target species were germinated in their own flats in the greenhouse for one week. Individual seedlings of each target species were then transplanted into the pots containing *S. altissima* rhizomes, with one seedling per species in each pot. All seedlings and rhizomes were planted in a circular fashion with even spacing, in the same exact order for every pot (Fig. 2.1). An additional 10 control pots that only contained the four target species were intermingled with the competition pots. Once all seedling were transplanted, pots were put into a randomized order on the greenhouse bench. During the first week after transplanting, any dead target species were replaced. All experimental pots were watered and weeded consistently and were allowed to grow for 60 days except for *Abutilon theophrasti* (37 days), because of its rapid growth. The growing time was set at 60 days due to the sizes of the pots; after 60 days, target species and *S. altissima* would grow too large and possibly become entangled.

*Biomass harvest*

After the growing period, all seedlings were harvested. For each pot, the above-ground biomass for the four target species and *S. altissima* were harvested by cutting all

stems 0.5 cm from the soil surface. The below-ground biomass of the *S. altissima* rhizomes were also collected from each pot. Tissues were dried at 60° C for 48 hours and weighed. For *S. altissima* rhizomes, all dirt and root hairs were removed after drying and prior to it being weighed. The biomass data for all 5 replicates per *S. altissima* genotype were then pooled to form a single average value per genotype to avoid issues of pseudo replication.

*Data analysis: Variation among Genotypes & Target species*

Variation in the above and below-ground biomass was compared across all genotypes of *S. altissima* using a series of one-way ANOVA's. Separate one-way ANOVA's were also conducted on the average biomass in each of the 24 competition and control pots to test whether direct competition with *S. altissima* significantly affected the biomass of all four target species. Separate bar graphs were then made to help illustrate the variation in biomass growth of both target species and *S. altissima*.

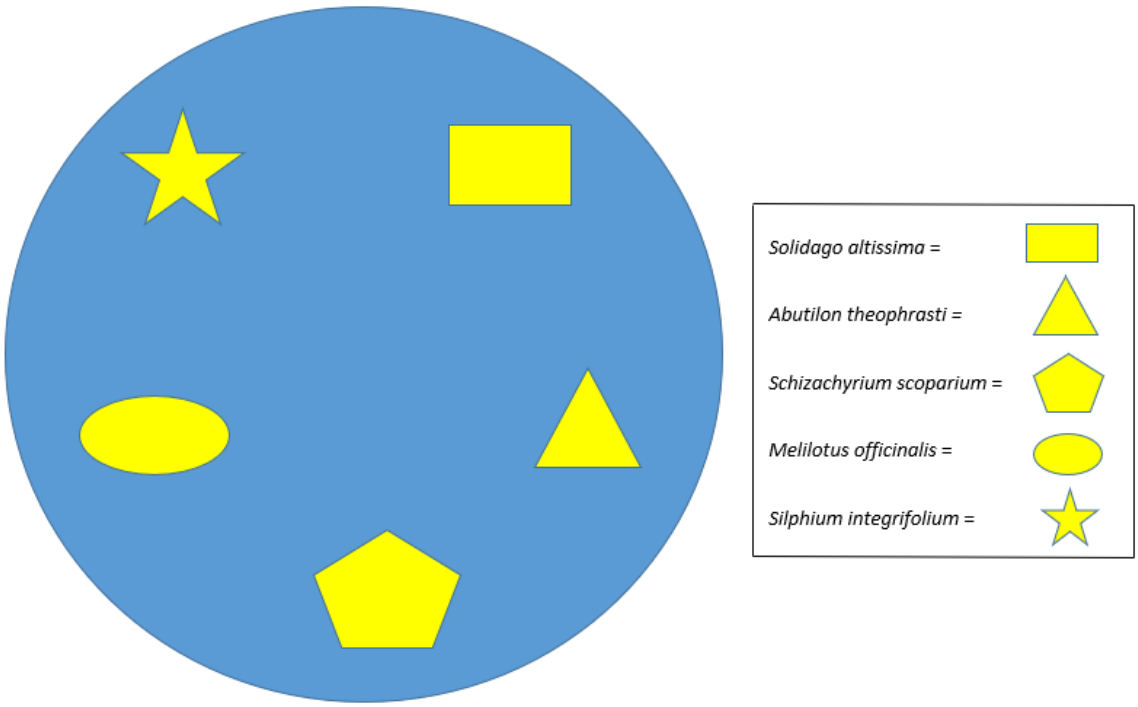
*Data analysis: Target species performance vs. S. altissima performance & chemistry*

A series of multiple regression analyses were conducted to relate performance of the target species to the above/belowground biomass as well as the foliar chemistry of *S. altissima*. Initially, *S. altissima* rhizome biomass was included as a predictor in these models, however, it was removed due to non-significance. These multiple regression analyses were used to predict the influence of *S. altissima* biomass and chemistry on the growth of all four target species. Separate scatterplots were then made to help visualize some of these significant associations.

*Data analysis: S. altissima above- and belowground biomass vs. chemistry*

To detect any effects of chemistry on the above- and belowground biomass of *S. altissima* genotypes a series of multiple regression analyses were conducted. The three chemical NMDS axis were used as predictors in these analyses. All statistical analyses were done using R version 3.1.2 (R Foundation for Statistical Computing).

Figure 2.1. Arrangement of focal *S. altissima* and target species seedlings in experimental pots.



## RESULTS

*Variation among Genotypes & Target species*

Rhizome biomass (ANOVA: $F_{23, 96} = 2.512$, $P = 0.001$, $R^2 = 0.2261$) as well as above-ground biomass (ANOVA: $F_{23, 96} = 3.226$, $P = 3.15e^{-5}$, $R^2 = 0.301$; Fig. 2.2) of *S. altissima* were found to vary significantly across the genotypes. Most target species also experienced marked variation in growth when grown in competition with *S. altissima* vs. the control pots. *Abutilon theophrastus* (ANOVA: $F_{1,32} = 18.55$, $P = 0.0001$, $R^2 = 0.347$), *S. scoparium* (ANOVA: $F_{1,32} = 7.911$, $P = 0.008$, $R^2 = 0.173$), and *S. integrifolium* (ANOVA: $F_{1,32} = 18.55$, $P = 0.0001$, $R^2 = 0.347$) all had significant reductions in biomass growth when in direct competition with *S. altissima* compared to the control pots (Fig. 2.3). However, the growth of *M. officinalis* was unaffected by *S. altissima* (ANOVA: $F_{1,32} = 0.693$, $P = 0.411$, $R^2 = -0.009$).

*Target species performance vs. S. altissima performance & chemistry*

Among all four target species, none were influenced by the aboveground biomass of each *S. altissima* genotype (Table 2.1). This suggests that the biomass of *S. altissima* does not directly affect the performance of these target species. Though *S. altissima* biomass had no effect on the biomass of the target species, the foliar chemistry of *S. altissima* had a striking effect. For *M. officinalis* and *S. integrifolium*, foliar chemistry did not significantly influence plant growth, though, *M. officinalis* also did not respond to *S. altissima* when compared to non-competition controls (Table 2.1). However, for *A. theophrasti* and *S. scoparium*, their biomass correlated significantly with chemical NMDS axis 1 (Fig. 2.4; 2.5). These two correlations are also positive, indicating that

these two species' biomass growth are positively influenced by chemicals positively related to axis 1.

*S. altissima above- and belowground biomass vs. chemistry*

Plant chemistry was also found to significantly affect both the above- and belowground biomass of *S. altissima* genotypes (Table 2.2). NMDS axis 3 was positively correlated with aboveground biomass while NMDS axis 2 was negatively correlated with belowground biomass, suggesting tradeoffs within the chemical landscape in biomass resource allocation.

Table 2.1. Multiple Regression analyses of the relationship between NMDS axes of plant chemistry and *S. altissima* plant performance on target species plant performance. Significant values are indicated in bold.

| Source | *t* | *p* | β | Adj. $R^2$ |
|---|---|---|---|---|
| *A. theophrasti* | | | | |
| Overall Model | | | | 0.209 |
| Above-ground | 0.001 | 0.714 | 0.001 | |
| Chemical NMDS 1 | 6.849 | **0.050** | 3.317 | |
| Chemical NMDS 2 | 0.910 | 0.839 | 4.442 | |
| Chemical NMDS 3 | -0.237 | 0.967 | 5.654 | |
| *S. scoparium* | | | | |
| Overall Model | | | | 0.239 |
| Above-ground | -0.009 | 0.174 | 0.007 | |
| Chemical NMDS 1 | 36.09 | **0.039** | 16.33 | |
| Chemical NMDS 2 | -6.811 | 0.759 | 21.86 | |
| Chemical NMDS 3 | 27.38 | 0.338 | 27.82 | |
| *M. officinalis* | | | | |
| Overall Model | | | | 0.035 |
| Above-ground | $4.57e^{-5}$ | 0.979 | <0.001 | |
| Chemical NMDS 1 | 1.638 | 0.715 | 4.414 | |
| Chemical NMDS 2 | -2.884 | 0.631 | 5.911 | |
| Chemical NMDS 3 | $4.49e^{-1}$ | 0.953 | 7.524 | |
| *S. integrifolium* | | | | |
| Overall Model | | | | 0.177 |
| Above-ground | -0.004 | 0.092 | 0.002 | |
| Chemical NMDS 1 | -2.321 | 0.682 | 5.571 | |
| Chemical NMDS 2 | 2.214 | 0.769 | 7.459 | |
| Chemical NMDS 3 | 16.86 | 0.092 | 9.495 | |

Table 2.2. Multiple Regression analyses of the relationship between NMDS axes of plant chemistry on *S. altissima* above- and belowground biomass. Significant values are indicated in bold.

| Source | *t* | *p* | β | Adj. $R^2$ |
|---|---|---|---|---|
| <u>Above-ground biomass</u> | | | | |
| Overall Model | | | | 0.279 |
| Chemical NMDS 1 | -594.6 | 0.280 | 535.9 | |
| Chemical NMDS 2 | -230.4 | 0.758 | 737.5 | |
| Chemical NMDS 3 | 2509 | **0.003** | 755.5 | |
| <u>Below-ground biomass</u> | | | | |
| Overall Model | | | | 0.105 |
| Chemical NMDS 1 | -110.8 | 0.221 | 87.72 | |
| Chemical NMDS 2 | -262.6 | **0.042** | 120.7 | |
| Chemical NMDS 3 | 96.69 | 0.444 | 123.7 | |

Figure 2.2. Variation in above-ground biomass growth of the 24 genotypes of *S. altissima*. Data plotted are means with SE.

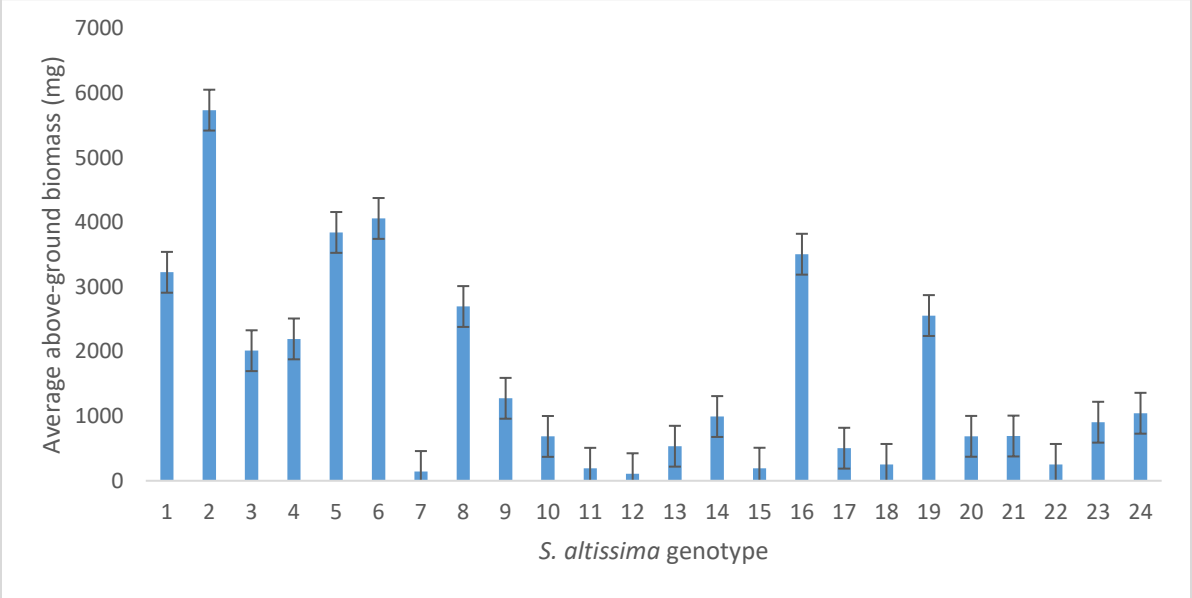


Figure 2.3. Biomass of target species when grown in control pots vs. competition with *S. altissima*. Data plotted are means with SE.

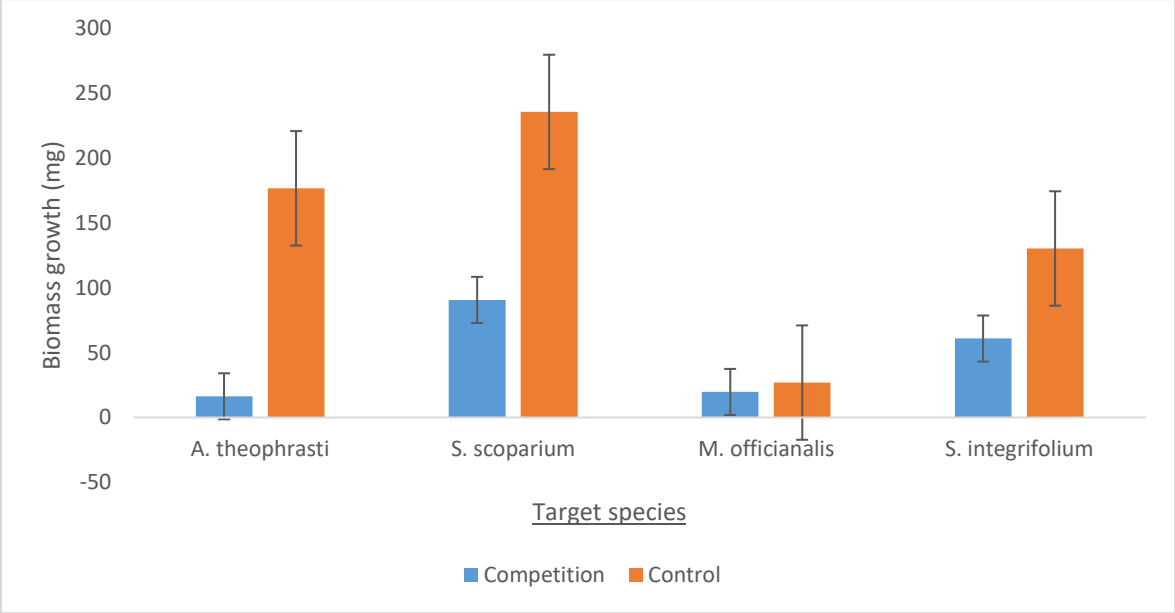


Figure 2.4. Relationship between *A. theophrasti* biomass growth and NMDS chemical axis 1. Data plotted are mean biomass of *A. theophrasti* with each clone with trend line.

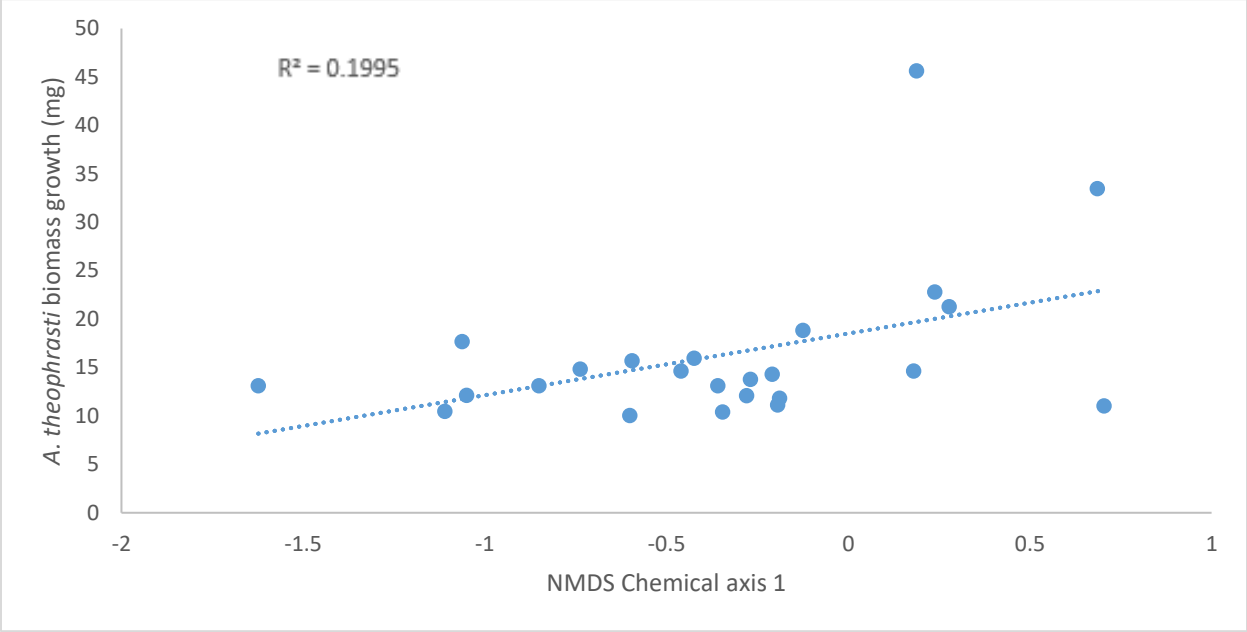
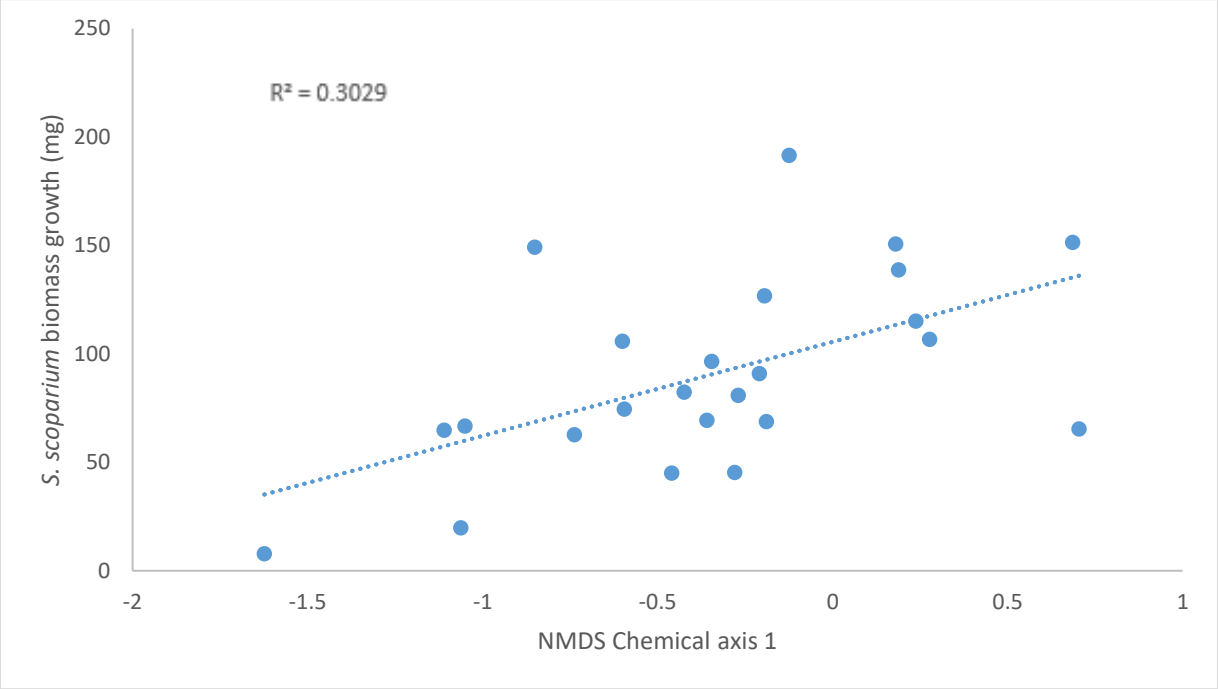


Figure 2.5. Relationship between *S. scoparium* biomass growth and NMDS chemical axis 1. Data plotted are means for *S. scoparium* biomass growth with trend line.



## DISCUSSION

Genotypes of *S. altissima* showed marked variation in both rhizome and above-ground biomass growth (Fig. 2.2), demonstrating diverse above and below-ground biomass resource allocation among genotypes. This variation in above and below-ground biomass may allow for resource-allocation tradeoffs between competitive ability and other ecological functions as these plants face a diverse array of ecological stressors that they must respond to (Montesinos-Navarro et al. 2020; Hakes and Cronin, 2012).

The above-ground biomass of target species also varied drastically when compared to the non-competition, control pots (Fig. 2.3). *Abutilon theophrasti*, *S. scoparium*, and *S. integrifolium* all had their biomass significantly reduced via competition with *S. altissima*, with *M. officianalis* being the only target species unaffected. Since *M. officianalis* is a nitrogen-fixing legume, this lack of impact is not surprising. This species is known to increase nitrogen levels and accelerate the rate of nitrogen cycling, allowing it to be very competitively dynamic and successful (Van Riper and Larson, 2009). The significant reduction in the above-ground biomass of the other three target species indicates that *S. altissima* was efficiently competing, either indirectly via allocation of resources or directly via allelopathy (plant chemistry). As the legume was the only target species unaffected by *S. altissima*, this suggests that competition for nitrogen may have been important.

In order to determine what altered target species biomasses, I conducted a multiple-regression analysis using *S. altissima* above-ground biomass, rhizome biomass and foliar chemical NMDS axes as predictors (Table 2.1). The above ground and rhizome biomasses of *S. altissima* did not show any correlation with target species biomass,

suggesting a functional role for chemistry within these competitive trials. NMDS chemical axis 1 did in fact significantly affect the biomass of two of the target species, signifying that chemistry is a critical driver of competition for *S. altissima*. Both *A. theophrasti* (Fig. 2.4) and *S. scoparium* (Fig. 2.5) had their biomass significantly affected by chemical NMDS axis 1, revealing that the chemicals related to this axis are the ones responsible for the reduction of biomass growth. The associations between these two target species and NMDS axis 1 were both positive, where target biomass increases with chemicals positively associated with this axis. Though we know the association between this chemical axis and target biomass, we still do not know whether or not this association is due to the presence or absence of certain chemicals. Due to the design of my analyses, the directionality of these associations cannot be determined, as my project did not encompass chemical concentration and identification protocols. These chemicals are playing a vital role in in mitigating interspecific competition within these competitive trials, consistent with findings from other similar competitive experiments involving *Solidago* (Abhilasha et al. 2008). Though these chemical traits are similar across experimental competitive trials, this may not be a general competitive response across both native and non-native ranges for *S. altissima*. Levels of secondary compounds have been found to vary between native and non-native populations of *Solidago*, with higher levels of some chemicals being found in native ranges. This suggests a lower investment into these chemicals as plant competitors in these invasive ranges are naturally more susceptible (Abhilasha et al. 2008).

Foliar chemistry of *S. altissima* also affected its own biomass, both above -ground and rhizome. Chemical NMDS axis 2 and 3 were both correlated with above- and

belowground biomass growth (Table 2.2), with axis 2 negatively associated with belowground biomass and axis 3 positively affecting aboveground biomass. This suggests that there are certain costs to producing these chemicals related to these two axes; possibly due to tradeoffs between expensive classes of compounds and cheap ones, which may be offset by the benefits of chemical production (Neilson et al. 2013; Poorter and De Jong, 1999). At whatever cost, foliar chemistry of *S. altisisma* seems to be performing a variety of functions, causing physiological changes to both itself and competitors in response to interspecific competition (Montesinos-Navarro et al. 2020).

These competition trials demonstrated that *S. altissima* competed at a high level, even in competition with seedlings of multiple plant species. The data outlined here shows a direct effect of biomass reduction when target plants are grown in competition with *S. altissima* compared to control pots. The above and below-ground biomass of *S. altissima* also varied greatly across genotypes. This suggests resource-allocation tradeoffs between biomass investment and chemical production, where genotypes allocating more resources to allelochemicals are expected to have reductions in growth and reproduction, which is then offset by the benefits from the chemicals (Meiners et al. 2012). The reduction in the biomass of target species may be a reflection of *S. altissima's* allelopathic capabilities, since chemistry was found to correlate with some of the target species biomass. Though directionality is unknown, we do know that the target species' biomass growth is influenced by chemicals related to axis 1. Foliar chemistry of *S. altissima* was also found to affect other ecological functions outside of interspecific competition. NMDS chemical axis 2 & 3 were found to correlate with *S. altissima* above- and belowground biomass, possibly aiding in the functions of light attenuation and

nutrient absorption as there was no need for or benefit to herbivore defense in the greenhouse.

The results reported here represent the diverse array of functions that are performed or affected by *S. altissima* chemistry. These results are also consistent with my findings in the previous chapter, highlighting the importance of chemistry as the main driver for *S. altissima*, where chemical production is creating various tradeoffs between competitive ability, biomass, insect associations, and other plant performance measures.

## CONCLUDING REMARKS

In both the common garden study and the greenhouse experiment, *S. altissima* showed marked variation in both above and below-ground biomass growth across genotypes. Coupled with their variation in foliar chemistry, these *S. altissima* genotypes showed great chemical and physiological diversity. The functional relationships of these chemicals were also found to vary greatly as all three NMDS axes were found to be associated with plant functions ranging from competitive ability, biomass resource allocation, and insect associations. This study highlights the fact that plant chemistry is playing a critical role in the ecological functioning of these plants, creating tradeoffs between these functions across genotypes. Chemistry is clearly involved in the success of *S. altissima* in its native range. However, we should not expect these chemical responses to stay consistent across native and invasive ranges since selection pressure and competitor community responses likely change with invasion.

The study of secondary metabolites and their roles in plants has been extensively covered in the scientific community, particularly with regards to herbivore defenses. However, the main focus on one or a few chemicals and their functions leaves many questions unanswered. Recommendations from this study would be to focus on separating allelochemical effects from others chemical roles as well as to include the interaction between plant chemicals and soil biota. In order to create a broader, more holistic view of the ecological importance of intraspecific chemical variation, the cost and maintenance of chemical production must be studied in parallel with their functions and underlying molecular mechanisms. This study is a good start in determining the importance of allelopathy, however, much more will be needed in order to build upon the

foundation of studies focusing on the role and importance of these secondary metabolites, as to build a broader ecological context.

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