VEGETATION STRUCTURE AND INTERSPECIFIC INTERACTIONS PREDICT
DISTRIBUTION AND ABUNDANCE OF *SOLIDAGO* SPECIALIST APHIDS

A Thesis
by
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Abstract

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Host specific insect colonization and feeding stimulants are key aspects of plant-insect interactions. Volatile compounds such as terpenes are often used by plants as insect herbivory deterrents, though terpenes broadly act as semiochemicals, both priming nearby plants and providing host recognition for insects. Insects that use terpene signals for host identification are often specialists on those plants. Furthermore, specialist phytophagous insects may have the ability to avoid inducing host defenses. In this study, I focused on the interactions of tall goldenrod, *Solidago altissima*, the goldenrod galling insects *Eurosta solidaginis* and *Gnorimoschema gallaesolidaginis*, and specialist aphids in the genus *Uroleucon*. Prior research demonstrated that *E. solidaginis* induces a volatile terpene response in *Solidago* and that *Solidago* specialist aphids preferentially colonize ramets with high foliar terpene concentrations. In this system, the ramet's chemical response to the gall insects may function as an allomone, deterring the inducing species, while simultaneously also functioning as a kairomone, promoting aphid colonization. Using spatial mapping techniques, I constructed a biologically-based model of interspecific induction in three

patches of *S. altissima* driven by insect gall formation to predict areas of aphid colonization. Terpenes were analyzed via gas chromatography in plants sampled at varying distances from *E. solidaginis*. Results of terpene sampling were used to develop a spatial model of terpene induction within a patch. To map relative aphid abundance within each patch, ramets were sampled on a grid transect and aphid counts were recorded per ramet at each sample point. My analysis revealed that terpenes in the field varied significantly by distance and in a similar way in all patches tested. The spatial models demonstrate the kairomone function of the gall insect-induced terpene response, and when combined with models of vegetation structure, predict relative aphid abundance with moderate accuracy across all three sampled patches. Laboratory experiments in sealed gas chambers confirmed that indirect semiochemical induction of terpenes in ungalled ramets by insect galls does occur. Although the precise mechanism of indirect induction is still unclear, leaf terpene concentrations significantly increased when galls were present. This study shows that chemical induction of terpenes is affected by distance from a gall and that specialist aphid abundance can be predicted using a spatial modeling technique. The spatial model I have described here may have applications in further research relating to community level dynamics of insect interactions in old field ecosystems.

Acknowledgments

I owe many thanks to my advisor Ray S. Williams for his patience, support, and wisdom. I also greatly appreciated the help of my committee members Howie Neufeld and Mike Madritch along the way, for instructing me in research and statistical techniques. I would like to thank Jerry Meyer for caring for my chamber study plants, Monica Winebarger for her assistance with field work, and Jeff Colby for help with my spatial model. I would like to thank Matt Estep as well as the members of his lab, Logan Clark and Byron Burrell, for their indispensable assistance with my genetic analysis. I would like to thank Bob Swarthout and Martin Fenn for assistance with my chamber experiments and the chemistry analysis so crucial to my study. A very special thanks to Jordan Willett and to my parents, Jan and Bill Thomas for all the love and support they have provided. Lastly, I would like to thank the Sigma Xi Foundation and the Appalachian State University Office of Student Research for funding in support of this investigation.

Dedication

I dedicate this thesis to my parents, Jan and Bill Thomas, for fostering my love of science and nature from a very young age and for helping me achieve all of my dreams.

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Introduction

Foundation plant species and associated insects

Insects are the most important group of herbivores globally, being responsible for up to 75% of all herbivory in some ecosystems (Grimaldi & Engel 2005). Phytophagous insects account for nearly one-half (43%) of all insect biomass and 75% of insect species (Grimaldi & Engel 2005). A multitude of factors drive variation in herbivore abundance, diversity, and distribution. The strongest contributing factors vary by scale. Of particular relevance may be the role of interspecific interactions among insect herbivores, where one species, or group of species, affects the distribution and abundance of others in communities such as old fields (Schmitz 1998). This may be especially relevant in the Eastern US, where the herbaceous canopy layers of old field habitats are often dominated primarily by goldenrod (i.e., *Solidago*) species (Root & Cappuccino 1992).

Tall goldenrod, *Solidago altissima* (L.), serves as an excellent model system for investigating insect herbivore interactions due to its extensive distribution, abundance in old field habitats, and the large insect diversity associated with this species (Maddox & Root 1990). *Solidago altissima* sprouts genetically identical ramets from rhizomes, producing clonal patches (i.e., genotypes) that suppress the growth of competing plant species (Butcko & Jensen 2002). Because *S. altissima* genotypes may represent a naturally occurring monoculture (Cain 1990), herbivorous insect density may be high but insect distributions

within the genotype remain heterogeneous. Mechanisms driving insect distributions in the natural environment include vegetation structure (Lawton 1978; Araujo et al. 1996), interspecific interactions between insect species (Denno et al. 1995), and local effects of individual host plant defensive response (Denno & McClure 1983; Conrath et al. 2015).   In this study, I focused on the interactions of *S. altissima*, two goldenrod stem galling specialists, *Eurosta solidaginis* (Fitch) and *Gnorimoschema gallaesolidaginis* (Riley), and two goldenrod specialist aphids, *Uroleucon nigrotuberculatum* (Olive) and *U. luteolum* (Williams, T.A.). These species are common and widely distributed throughout eastern North America. Both *Uroleucon* aphids' ranges encompass the entirety of tall goldenrod's range, although some authors treat *U. luteolum* as two species: *U. calligatum* in the northern portion of tall goldenrod's range and *U. tissotti* in the southern portion (Cappuccino 1988; Blackman & Eastop 2008). The distribution of *Eurosta solidaginis* also encompasses the entirety of the host's range (Abrahamson et al. 2003) and has been extensively studied by Abrahmson and Weis (1997).

Much is known about the effects of induced plant defenses on individual herbivore performance, yet few studies have addressed its population level effects and, in particular, whether effects on individual herbivore performance and behavior can mediate changes in spatial population distributions (Underwood, Anderson & Inouye 2005; McNutt & Underwood 2016). The gall making herbivores in my study system induce chemical defenses that *Uroleucon* aphids may respond to. The goal of my study was to predict relative aphid abundance and distribution within patches of *S. altissima* using a spatial model based on the influential factors of both vegetation structure and interspecific interactions of gall-making herbivores.

Vegetation structure and herbivores

At a landscape scale, vegetation structure is a significant driver of herbivore abundance and distribution (Price et al. 2011). Vegetation structure is comprised of two features: 1) vegetation texture, which includes patch size, plant density, and vegetation diversity, and 2) architectural complexity, which includes plant size and plant part diversity (Obermaier et al. 2008). Taken together, these factors describe the physical habitats of phytophagous insects. Two hypotheses relating to the relationship of vegetation structure and insect abundance have been proposed. First, the *resource concentration hypothesis*, proposes that herbivores are more likely to survive in higher densities in pure stands or monocultures than in mixed vegetation (Root 1973). The second hypothesis, *associational resistance*, proposes that plants in mixed landscapes are more resistant to herbivore attack and therefore herbivores are present in lower densities (Tahvanainen & Root 1972; Root 1973; Barbosa et al. 2009). Mechanisms driving associational resistance include increased predation in some mixed vegetation habitats and the effects of the *Janzen-Connell hypothesis/escape hypothes*is (Janzen 1970). The Janzen-Connell hypothesis proposes that mixed vegetation occurs frequently because of the intense herbivory pressures created by a monoculture, or at a minimum, by the aggregation of a single species of plant (Janzen 1970). Originally devised to describe tree distributions in tropical rainforests, recent research suggests the Janzen-Connell hypothesis also explains mixed vegetation in temperate habitats (Becerra 2015).

Within clonal patches of *S. altissima*, intraspecific variations in stem morphology have been found to drive patterns of *associational resistance* and *associational susceptibility* to attack by *E. solidaginis* galls (Wise, Yi & Abrahamson, 2009). Because patterns of *associational resistance* were already described in the *S. altissima* system, it was clear measurements of vegetation structure must be included in my spatial model used to predict *Uroleucon* aphid distribution and abundance.

Insect host-specificity

It is important to consider diet breadth and host specificity, and not just vegetation structure, when modeling the abundance and distribution of herbivorous insects. Herbivores may be host specialists or generalists. The *preference-performance hypothesis* states that female insects select oviposition sites that optimize the fitness of their offspring (Trivers 1974). The resulting distribution and fitness of offspring may then have cumulative effects on population distribution patterns. In the case of host specialist herbivores, these distributions may be highly aggregated on the host plant. Aggregation may be most common in largely sessile specialist herbivores such as aphids (Price et al. 2011). It is important to note that there is a continuous gradient of diet breadth among insect herbivores (Forister et al. 2015). Therefore, one can expect a gradient of aggregation across species. In my study system, all of the insect herbivores I investigated are highly specialized, feeding only on *S. altissima* or closely related species (Blackman & Eastop 2008; Tooker & De Moraes 2008). However, even highly specialized insect herbivores still demonstrate intraspecific host preference. A classic example of host preference and performance correlation was demonstrated by Craig, Itami and Price (1989) in the willow specialist shoot-galling sawfly,

Euura lasiolepis. Female sawflies strongly preferred oviposition on willow ramets with long shoot lengths and larva on those shoots had significantly higher survival rates.

Intraspecific preference and performance correlations with host genotype have been investigated in *E. solidaginis* and *S. altissima* (Cronin & Abrahamson 1999). Gall larva survival, a measurement of performance, does not appear to be correlated with female gall fly oviposition preference. Instead, preference appeared to be most closely related to the avoidance of oviposition on *S. altissima* ramets infested with a non-native spittlebug larva, *Philaenus spumarius*. This highlights the influence of interspecific interactions between insect herbivores on host choice and spatial distributions in the *S. altissima* system.

Phytochemical induction and signaling

Plant semiochemical communication increases heterogeneity in plant phenotypes, both chemically and physically, and influences herbivore feeding choice (Karban 2017). Although it is well established that herbivory may induce a systemic (plant-wide), phytochemical and plant volatile release (War et al. 2011), the importance of induction on interspecific relationships between herbivores is not as well understood. Potentially indirect relationships, where chemical signaling from one species affects the relationship with a host plant of another species, are of ecological and evolutionary significance. These chemical defenses, repellents, and attractants are important factors shaping species interactions (Friberg et al. 2014). Yip et al. (2017) recently found evidence of such interactions in the *S. altissima* system, where male *E. solidaginis* pheromone signaling prime terpenes in nearby *S. altissima* ramets and deters subsequent herbivory.

Plant-insect semiochemical interactions are complex, multitrophic, and occur in heterogeneous chemical environments. Phytochemistry can be affected by both biotic and abiotic factors in the surrounding environment (Meiners 2015). Insect response to vegetation odors and phytochemistry can vary greatly from species to species (Meiners 2015). Often plant defensive compounds will deter one insect species, while acting as colonization and feeding triggers for others (Pare´ & Tumlinson 1999). Multifactor studies taking into account multiple trophic levels can elucidate such chemical complexities. Morrell and Kessler (2017) have recently demonstrated such chemical complexities in *S. altissima*, where feeding by a specialist leaf beetle, *Trirhabda virgata*, causes *S. altissima* volatile semiochemical releases. These semiochemical releases then induce an indirect terpene response in nearby ramets that were not directly damaged by the beetle, which in turn deters subsequent herbivory (Morrell & Kessler 2017).

Herbaceous tissues normally release small amounts of volatile compounds involved in plant defense, but when a plant is damaged by herbivorous insects, larger quantities of volatiles are released (Pare´ & Tumlinson 1999; Conrath et al. 2015). The Jasmonic Acid (JA) response is a common response to insect herbivory in plants, often inducing volatile monoterpenes and sesquiterpenes within the plant (Pare´ & Tumlinson 1999). The methylated form of the compound, methyl jasmonate (MeJA), is volatile and may act as a semiochemical indirectly inducing a defensive response in exposed plants (Rodriguez-Saona et al. 2001). The JA response and associated increase in released volatile terpenes has been found in *S. altissima* in reaction to herbivory by some insects (Tooker et al. 2008), including *T. virgata* (Morrell & Kessler 2017). However, *S. altissima* ramets supporting *E. solidaginis* galls do not systemically express JA or comparatively high levels of foliar terpenes, even in

response to subsequent herbivory by other insects (Tooker et al. 2008). The lack of systemic JA expression suggests that *E. solidaginis* wields active control over the chemical defenses of the *S. altissima* host ramet (Tooker & Moraes 2008). It is possible that immediately adjacent ramets connected by a rhizome to the galled plant may be similarly suppressed. In contrast, nearby ramets (presumably those without rhizome connections) do systemically express JA and have higher levels of terpenes (Tooker et al. 2008). A mechanism of indirect induction or defensive priming by a galled ramet may be occurring. If indirect induction is taking place, plants in proximity to a galled ramet may be responding to a volatile semiochemical that had not been detected by Tooker et al. (2008). That study tested only the JA and Salicylic acid (SA) content in gall tissues infected in a lab, which were presumed to be necessary precursors for a terpene response. It is possible that other mechanisms of terpene induction without JA signaling could be at work. Chemical induction without JA has been described in several plant species (Geu-Flores et al. 2012). Tooker et al. (2008) did note that plants infected by *E. solidaginis* in the field expressed increased levels of terpenes although these increases were not significant ($\alpha \leq 0.05$). Though explanations of indirect induction are yet unclear, Tooker et al. (2008) also concluded that the extensive control *E. solidaginis* apparently exerts over *S. altissima* chemical defense responses could influence community-level dynamics. Tooker et al. (2008) believed the distribution of herbivorous insect species associated with *S. altissima* may be affected by *E. solidiginis*, but they did not consider the possibility of indirect terpene induction by host semiochemical signaling. Helms et al. (2014) found that *E. solidaginis* male-induced terpenes in *S. altissima* deterred female gall fly oviposition. Therefore, it is likely advantageous for the host plant to also respond to developing *E. solidaginis* galls in order to discourage additional oviposition and gall

development. Based on these discrepancies, I hypothesized indirect induction of ungalled

ramets by galled ramets may occur in *S. altissima*.

Spatial context of herbivore distribution

Yip et al. (2017) found male *E. solidaginis* induction correlated spatially with

herbivory by leaf chewing insects. Specifically, indirect induction by male pheromones

primed a terpene defensive response. My study takes into account male *E. solidaginis*

pheromone induction but focuses, in particular, on its effects on specialist aphids rather than

leaf-chewing insects and further takes into account gall driven induction. Yip et al. (2017)

found ramets with an indirectly induced terpene response by male *E. solidaginis* emissions in

the field received less subsequent foliar herbivory. I expected to observe the opposite effect

in my study: increased aphid abundance due to the apparent correlation of the *Uroleucon*

aphids with increased host terpenes as observed by Williams and Avakian (2015).

Considering aphid distribution's relationship to vegetation structure, I identified three

components I hypothesized may be important to *Uroleucon* aphid distribution and abundance

based on existing literature. Components of vegetation structure that might affect herbivore

abundance can include stem height, stem diameter, and patch edge proximity. Richardson

and Hanks (2011) established that *S. altissima* ramet height positively correlated with higher

Uroleucon aphid abundance in the field, and a positive correlation with host plant stem

diameter has also been established (Stoeckli et al. 2008). Although a link between

intraspecific aphid distribution and abundance with patch edge has not been directly

investigated, Kareiva (1987) demonstrated that *U. nigrotuberculatum* aphid abundance

increased in fragmented habitats due to decreased predation. Furthermore, Cappuccino and

Root (1992) found significant aggregation and colonization preference of *Solidago* patch edges by another specialist insect, *Corythucha marmorata*. Taken together, these findings hint that patch edge responses may be important to *Uroleucon* aphid colonization choice and survival, therefore influencing aphid distribution and abundance.

Study approach and hypothesis

Prior research has demonstrated that *E. solidaginis* can induce an indirect terpene response in *S. altissima* (Helms et al. 2014) and *Solidago* specialist aphids appear to preferentially colonize genotypes with increased amounts of specific terpenes (Williams & Avakian 2015). I hypothesized that, in addition to vegetation structure, an *S. altissima* ramet's indirectly-induced terpene response to the galling insects *E. solidaginis* and *G. gallaesolidaginis*, functioning as a kairomone, signals *Uroleucon* aphid colonization (Figure 1). Furthermore, I hypothesized I could predict the spatial pattern of aphid colonization and abundance with a spatial model. In order to test this hypothesis, I used gas chromatography for terpene analysis of *S. altissima* tissue samples collected in the field and combined these findings with measurements of vegetation structure to predict areas of aphid colonization in the spatial model. The model data were collected from three patches of *S. altissima*. *Eurosta solidaginis* gall indirect induction of terpenes was also explored (and confirmed) in a laboratory gas chamber experiment. Finally, in an effort to better understand the chemistry of *E. solidaginis* gall tissue, gas chromatography and mass spectrometry were used to investigate terpene and green leaf volatile (GLV) compounds that may be responsible for the indirect induction of terpenes observed in the field and chamber experiments.
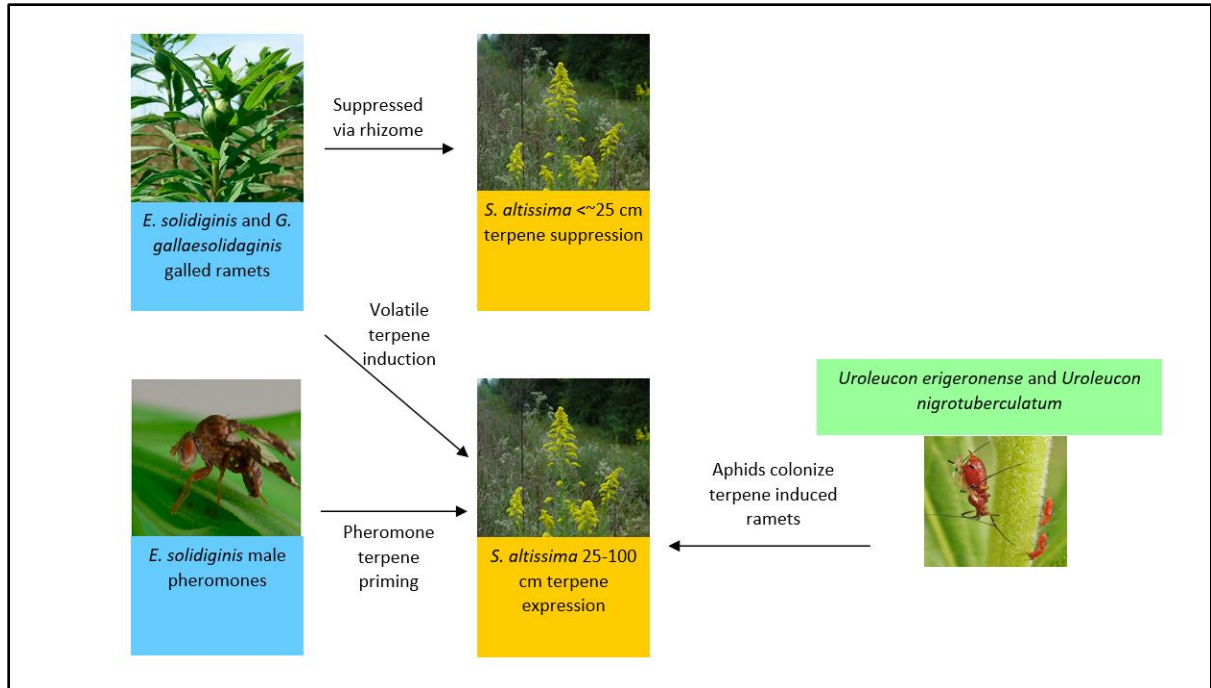
Figure 1   A conceptual overview of hypothesized interactions in the study system. *Eurosta solidaginis* male pheromones and galled ramet semiochemical signaling indirectly induce terpene responses in nearby ungalled *Solidago altissima* ramets. Galled ramets and ramets immediately adjacent to galls do not have an induced terpene response because it is suppressed by the gall. *Uroleucon nigrotuberculatum* and *U. luteolum* aphids selectively colonize ramets with induced terpenes.

Objective

- Use spatial models to predict *Uroleucon* aphid distribution in the field based on vegetation structure as well as spatial patterns of indirect terpene induction.

- Assess the accuracy of the spatial models.

- Assess the relative contribution of vegetation structure and indirect terpene induction to aphid distribution and abundance.

- Determine whether or not *E. solidaginis* galls are capable of indirectly inducing a terpene response via semiochemicals.

- Identify chemical compounds present in *E. solidaginis* gall tissue and determine if these compounds are more or less abundant in gall stem tissue than in ungalled stem tissue.

Materials and Methods

Overview of study system

Solidago altissima produces an underground rhizome that sprouts multiple ramets, forming dense patches of clonal stems (Maddox et al. 1989). Gall forming specialist insects, including *Eurosta solidaginis* and *Gnorimoschema gallaesolidaginis*, feed primarily on *S. altissima*, with emissions of adult male *E. solidaginis* flies priming a defensive terpene response (Helms et al. 2013; Helms et al. 2014; Helms et al. 2017; Yip et al. 2017). The common stem-feeding aphids *U. nigrotuberculatum* and *U. luteolum* produce multiple generations per year and feed primarily on *S. altissima* (Cappuccino 1988). These aphids have been shown to preferentially colonize genotypes with higher foliar concentrations of terpene compounds (Williams & Avakian 2015). Therefore, I expected induction of these compounds would result in higher aphid abundance.

Field study

To collect insect abundance and leaf terpene samples, I randomly chose three discrete *S. altissima* patches (denoted A, B, and C) in an old field in Watauga County, NC (Lat: 36° 15' 58.13" N, 81° 36' 53.5" S; 994 m). A semi-permanent survey grid was established in each patch in the early spring of 2016, with grid intersect points every 0.5 m (Figure 2). Grid

based field surveys are the most appropriate sampling method for spatial interpolation (Fischer & Getis 2009), a critical component of my model construction (see below).

Figure 2   A semi-permanent grid set up at patch B in the early spring of 2016. Each grid cell is 0.5 m by 0.5 m and ramets were sampled at each intersection point.

Genotypic effects on foliar terpene content (Cronin & Abrahamson 1999; Heath et al. 2014; Williams & Avakian 2015) and *E. solidaginis* preference are well described in *S. altissima* (Halverson et al. 2008). In order to determine the genetic identity of each patch, nine foliar tissue samples were collected from patches A and B, and six from the smaller patch C. Total genomic DNA was extracted using a modified CTAB protocol (Doyle & Doyle 1987). A set of four neutral, non-coding, and co-dominant microsatellite markers were used in the analysis (Beck et al. 2014), Sg_1, Sg_2, Sg_8, and Sg_10. Primers were labeled with GeneScan 6FAM™, VIC™, NED™, PET™ dyes and pseudo-multiplexed with

GeneScan LIZ 500 dye size standard (Thermo Fisher Scientific, Waltham, MA). Fragment analysis was conducted at the Georgia Genomics facility (Athens, GA) on an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA).

Based on previous investigations, male gall fly emergence and chemical signaling takes place over a two-week period in mid-May (Yang 2003). Three observations of males were made per patch for a one-hour period each in mid to late May. In total, only seven males were observed, three in Patch A, three in Patch B, and one in patch C. Because males tend to stay on the ramet from which they emerged (Craig et al. 1993), observed male locations were considered single points for the purposes of statistical and spatial modelling. Surveys of *U. nigrotuberculatum* and *U. luteolum*, and quantification of plant biomass were made in each patch four times throughout the growing season of 2016. During each survey, aphids were counted on the ramet nearest each survey grid point, and both the stem height and diameter of the ramet used to estimate plant biomass. Plant biomass was estimated using the formula developed for *S. altissima* and reported by Williams and Avakian (2015): Biomass (g) = $(D^2H \times 0.0022) + 6.3667$ ($p < 0.001$, $r^2 = 0.70$), where D = stem diameter (mm) at 3 cm above ground and H = height (cm). Determining wet above-ground biomass allows aphid abundance to be calculated per gram. The first aphid survey was done during the first week of June, approximately two weeks after male gall fly observation. Exact locations of *E. solidaginis* and *G. gallaesolidaginis* galls were recorded and, when combined with locations of male displays, were used to create a map of terpene induction for spatial modelling. Tooker and De Moraes (2008) suggest similar terpene suppressing and inducing mechanisms may be at work in both *E. solidaginis* and *G. gallaesolidaginis*. For the purposes of my spatial model (see below), predicted terpene induction as a function of

distance from both gall species was fitted to foliar terpene concentrations observed in spatial

relation to only *E. solidaginis* galls.

Foliage samples were made at 10 cm intervals out to a distance of 150 cm along two

straight lines while surveying plants for aphids within each of the three patches in June 2016.

Both lines originated at a ramet where an adult male *E. solidaginis* fly was previously

observed (total of 90 samples = 15 samples/line X 2 lines per patch X 3 patches; Figure 3).

These samples were later used in GC terpene analysis. Three to six leaves were collected

from each ramet at the midpoint of the stem to obtain approximately 1.5 g of tissue. Leaves

were collected because they are the primary source of non-floral volatiles released by plants

(Karban & Baldwin 1997).
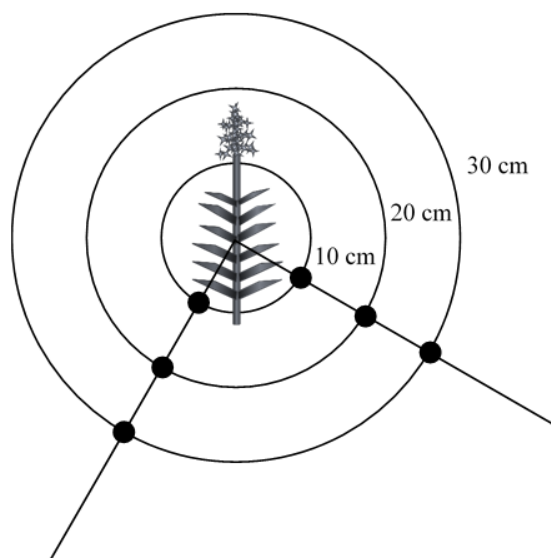
Figure 3 Diagram of foliar terpene sampling in each patch, where each dot corresponds to a 10 cm interval

foliage sample. Terpene sampling extended out to a distance of 150 cm along two straight lines, for a total of

15 samples per line. The center plant represents a ramet on which a male *E. solidaginis* fly was previously

observed displaying in June terpene sampling. In the August samplings, the plant corresponds to a ramet with a

maturing *E. solidaginis* gall.

Subsequent aphid counts and plant biomass measurements were made in July, August, and September of 2016. Using a similar protocol to that used in June terpene sampling, leaf collections for terpene quantification were made in each patch in August 2016. Sample lines in August originated at ramets with developing galls rather than observed male locations. August was chosen for terpene sampling in relation to developing galls as it appeared to correspond with peak aphid colonization based on observations made in the previous year, 2015. Therefore, *Uroleucon* alates should have been responding to gall-induced terpenes within the sampling timeframe.

Foliar samples were ground for 90 seconds in 15 ml pentane using a Brinkmann (Kinematica) Polytron PT10/35 tissue homogenizer (Kinematica, Luzern, Switzerland). The homogenized material was then filtered and evaporated to 0.5 ml using nitrogen gas. A 1 µl sample was injected into a Shimadzu GC-14A Gas Chromatographer (Shimadzu Corporation, Kyoto, Japan) with a flame ionization detector (FID) and HP-5 column (30 m length with 0.25 mm i.d. and 0.25 mm film thickness) (Hewlett-Packard, Palo Alto, California). The GC program was modified from Johnson Hull-Sanders, and Meyer (2007), and followed the protocol established previously for *S. altissima* (Williams & Avakian 2015). The GC program was as follows: initial oven temperature of 80 °C, held 2 min, increasing the oven temperature 10 °C/min, final temperature of 280 °C. The injector temperature was maintained at 250 °C and the detector at 275 °C. Analytical standards for the terpenes: α-pinene, β-pinene, camphene, myrcene, α-phellandrene, p-cymene, limonene, ocimene (mixed isomers), sabinene, R(+) camphor, methyl salicylate, bornyl acetate, β-elemene, γ-elemene, (-)-trans caryophyllene, α-humulene, cis caryophyllene, farnesene (mixed isomers), azulene, methyl jasmonate (mixed isomers), were purchased from Sigma-Aldrich (St. Louis, MO) and

run in triplicate to establish retention times. The compounds germacrene D, ledene oxide, bicyclo[4.4.0]des-5-ene, 5-dimethyl-3-hydroxy-8-(1-methylene-2-hydroxyethyl-1) were identified by Agilent 6890 GC with an Agilent 5973 Mass Selective Detector (MSD) (Agilent, Santa Clara, CA). Agilent's MSD ChemStation ver. E.02.02.1431 and NISTMS Search 2.0 (National Institute of Standards and Technology, Gaithersburg, MD) were used for peak identification. I was unable to obtain analytical standards for these compounds and therefore they are reported as tentatively identified.

Chamber experiment

In order to determine if a defensive terpene response in ungalled ramets was induced by semiochemical signaling by the galled ramets, I conducted a controlled experiment in a sealed gas chamber. This chamber experiment complimented my field studies by determining whether or not a *S. altissima* ramet harboring an *E. solidaginis* larva can induce a terpene response in other nearby *S. altissima* plants via semiochemical signaling. It did not, however, seek to identify what particular compound or compounds may be responsible for this indirect induction.

The chamber experiment followed a simple pretest-posttest design, with an untreated control group and a gall exposed treatment group. Individuals from two genotypes (represented henceforth by A and B) were selected from populations of *S. altissma* previously collected in Watauga County, NC, and grown at the Appalachian State University (ASU) greenhouse. A total of 10 plants per genotype were propagated by rhizome cuttings from parental stock in Metro-mix 360 (Sungrow Horticulture, Agawam, MS) soil medium and grown in 3.8 L pots outside. Plants were treated with Marathon (OHP Incorporated,

Mainland, PA) systemic insecticide to ensure a defensive response would not be induced by herbivorous insects. Previous work with this pesticide found no evidence of chemical induction (Williams & Garrido, unpublished data). For each genotype, 10 ungalled control plants were individually placed in a sealed polytetrafluoroethylene (PTFE) gas chamber with dimensions of 60 cm X 60 cm X 90 cm. The chamber was illuminated by a Hortilux Blue 57816 400 W - T17 - Metal Halide Grow Light (EYE Lighting International of North America, Inc., Mentor, OH) in a Hydrofarm Sunburst Digital Series Convertible ballast HID Fixture (Hydrofarm, Petaluma, CA). Temperature within the chamber was maintained at 24 ± 1 °C, relative humidity at $50 \pm 5\%$, and light exposure at 0.065 ± 0.005 kW/m^2. These parameters were monitored via a PC200W datalogger (Campbell Scientific, Logan, Utah). Plants were exposed to the chamber for an acclimation period of one hour before the chamber was briefly opened for initial foliar terpene sampling. Approximately 1.5 g of leaf tissue was taken. Five plants of each genotype, designated as the control group, remained in the chamber for an additional period of two hours before a second round of sampling occurred. A second set of five plants per genotype, designated the treatment group, also remained in the chamber for an additional two hours after initial sampling. However, a galled plant collected from my field site was also placed in the chamber at a distance of 35 cm from the ungalled plant before the chamber was resealed. Foliar samples were collected from ungalled treatment group plants a second time after the two-hour gall exposure period. Particular care was taken to ensure plants did not touch the sides of the chamber and, in the case of the gall exposed treatment group, that the ungalled and galled plants did not touch.

Stem and gall terpene and GLV analysis

Tooker et al. (2008) found that jasmonic acid and salicylic acid was either suppressed or not induced in stem tissues by galling insects. To test if an alternate inducing pathway for downstream defensive terpene and green leaf volatile (GLV) responses was possibly occurring within *E solidigins* gall tissue, I carried out a series of solvent extractions and GC analyses.

In order to determine terpene content of galled and ungalled stems, I collected 12 spatially separated *S. altissima* galled and ungalled stems from two genotypes collected at my field sites in Watauga County, NC. These 24 samples were analyzed using the same terpene analysis protocols used in the field and chamber experiments. To analyze GLVs, I utilized GC-time-of-flight mass spectrometry (TOF). Seven *S. altissima* galls and seven ungalled stems were collected from one genotype at my field site in Watauga County, NC. Samples were ground under liquid nitrogen with a cryogenic mortar and pestle and suspended in 20 ml dichloromethane. The suspension was kept at -20 °C for a 72-hour period, after which 5 ml of the extract was filtered through a glass fiber filter into a small glass vial and 1 ml pipetted into a 1.5 ml autosampler vial. Gall and stem GC-TOF identification and quantitative analysis of GLVs was carried out on an Agilent 7890 GC with Leco TOF-MS. The GC was equipped with an HP-5 column (30 m length with 0.25 mm i.d. and 0.25 mm film thickness). The temperature program was adapted from Ruther (2000). The GC program was as follows: initial oven temperature 40 °C, held 3 min, increasing the oven temperature 3 °C/min, final temperature of 280 °C. The injector temperature was maintained at 250 °C. Leco ChromaTOF ver. 1.81 software was used for peak identification. I was unable to obtain analytical standards for these compounds.

Spatial model construction

A model of terpene induction by distance was based on a B-spline fit to standardized total identified foliar terpenes against ramet distance from inducer (either *E. solidaginis* adult males or galls) using the PROC GLIMMIX function in SAS (knots = 8). Using this spline fit inducer distance model, as well as the vegetation structure components of edge proximity, ramet height, and diameter as measured in field surveys, a spatial model of induction and predicted aphid abundance was constructed in ArcGIS ver. 10.3.1 (ESRI, Redlands, California). ArcGIS Model Builder was used to construct an automated model that was then applied to each of the three spatial maps created, one per patch. Seasonal average stem height and stem diameter data were interpolated using ordinary kriging with a spherical semivariogram to produce a raster map. Edge distance and inducer distance were calculated using the Euclidean distance tool to create raster maps. The inducer distance raster was then reclassified by values based on the spline fit inducer distance model, rounded, and rescaled on a scale of 3 to 10. Edge distance, seasonal average stem height, and seasonal average stem diameter raster maps were reclassified into 10 equal area categories using the slice tool. All four reclassified raster maps were averaged using the weighted overlay tool with equal weights. The resulting model map was then smoothed using focal statistics and finally sliced into five equal area quantile categories. An overview of the spatial model construction is provided in Figure 4.
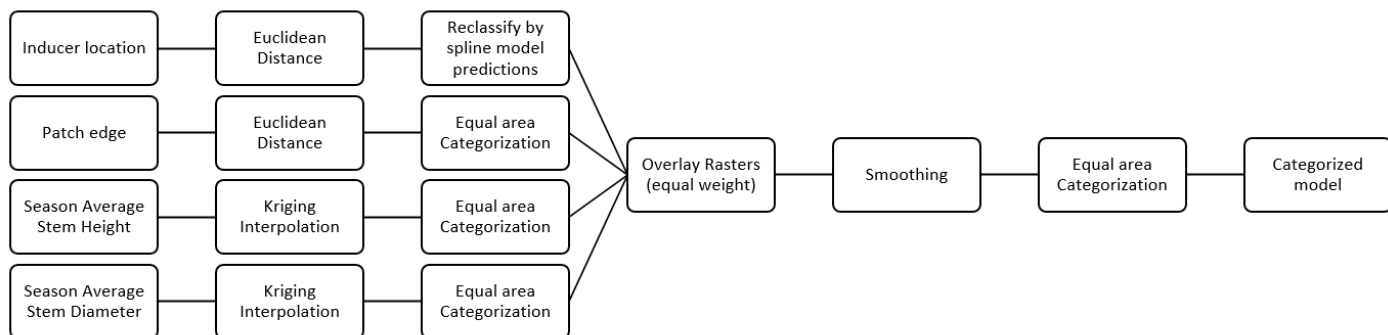
Figure 4   The spatial model overview. Inputs are on the left, and processing steps continue to the right. The completed model is on the far right.

In order to assess the accuracy of the spatial models, three aphid seasonal count maps were created by kriging for model validation. Kriging is an established method of creating insect abundance heat maps (Sciarretta & Trematerra 2014). *Uroleucon* aphid counts per sample period were interpolated, reclassified, smoothed via focal statistics, and sliced into 10 equal area categories to estimate relative aphid abundance. These maps were then overlaid with equal weights, smoothed via focal statistics, and sliced again into five equal area quantiles. This best represents the seasonal average of relative aphid distribution and abundance.

Statistical analysis

To analyze the main effects of distance, variation among patches, and interactions between these factors on foliar terpene concentrations, a two-way unbalanced factorial ANOVA was run on total identified terpenes by distance from inducers (male *E. solidaginis* flies and galls of the two galling species) in each patch using SAS (ver. 9.4, SAS Institute

Inc., Cary, NC). Significance for all analyses are reported at $\alpha \leq 0.05$. The data were not normally distributed. Because a non-parametric equivalent does not exist for a two-way unbalanced model, the data set was Johnson S_U normalized, with distance groups based on 10 cm sampling intervals (Johnson 1949). Bartlett's test found all factors to be homoscedastic at $\alpha = 0.05$ (Distance: $p = 0.53$, Group: $p = 0.17$, Interaction: p=0.13). A two-way factorial ANOVA was also run on methyl jasmonate isomers (MeJA), both by distance from inducing factors, and by which inducer type (either adult *E. solidaginis* males or developing galls). Groups were Johnson S_b normalized and based on 10 cm sampling intervals (Johnson 1949). Tukey post-hoc analysis was applied to MeJA by patch. Because distance from inducer is continuous, PROC GLIMMIX was also used to fit B-Spline models (knots = 8) to total identified terpenes after being standardized by patch, with spline model similarity assessed at 5 cm intervals via the Holmes-simulated Method described by Westfall (1997) in SAS (ver. 9.4).

To analyze the effect of patch on average aphid abundance, a Wilcoxon signed-rank test was run in SAS (ver. 9.4). A non-parametric test was chosen because aphid totals could not be normalized. A Steel-Dwass post-hoc test was used to identify significantly different patches.

For the chamber experiments, a paired Wilcoxon signed-rank test (SAS, ver. 9.4) was used to compare foliar concentrations of total terpenes and individual terpene compounds in pre- and post-exposure samples. A Wilcoxon signed-rank test was used to compare the terpene concentrations, as well as the GLV concentrations in field collected galled and ungalled stems.

The spatially modeled aphid abundance was analyzed using The Map Comparison Package developed and described by Visser and De Nijs (2005). A linear weighted Fuzzy Kappa comparison (Hagen-Zanker, Straatman and Uljee 2005, Hagen-Zanker 2009) was conducted to determine concordance of observed *Uroleucon* abundance with the model projections. Cohen's Kappa and its Fuzzy derivative are useful metrics for determining spatial model agreement as the Kappa statistic corrects for chance agreement (Landis & Koch 1977, Hagen-Zanker 2009). The Fuzzy Kappa variant has greater statistical power when compared to the traditional Cohen's Kappa (Hagen-Zanker 2009). The weighted Kappa coefficient is applied to the comparison of thematic maps. Weighted Kappa is a useful measure of accuracy when map classes are ordered, or when the relative seriousness of the different possible errors may vary (Næsset 1996). Linear weighting is the most conservative weighting scheme widely used and was deemed most appropriate for my map comparison. Following Landis and Koch (1977), any positive Kappa indicates some spatial concordance, with Kappa values greater than 0.20 indicating fair agreement, while Kappa values greater than 0.40 indicating moderate agreement. "Moderate" agreement is generally regarded as the standard metric for strong biological significance (Landis & Koch 1977). Spatial model comparison via the Kappa statistic has been widely used in disease and conservation ecology to assess species distribution models (Manel et al. 2001; Atkinson et al. 2012; 2014)

To assess the relative contribution of the vegetation structure model, the spatial model was also run for each patch using only edge distance, seasonal average stem height, and seasonal average stem diameter. These vegetation structure only models were again compared to average seasonal aphid distribution maps via the weighted Fuzzy Kappa

statistic. Similarly, an inducer distance model was compared to average seasonal aphid distribution via the weighted Fuzzy Kappa statistic. In order to better visualize the relationship of aphid abundance with model parameters, *Uroleucon* aphids per g (aphids adjusted for biomass) were plotted against distance to inducer (adult *E. solidaginis* males and galls of both gall species) and distance from edge. Means were smoothed via PROC LOESS in SAS (ver. 9.4). Total observed *Uroleucon* aphids were also plotted on a graph against height and stem diameter, again, with means smoothed via PROC LOESS. $\lambda=100$ for all plots.

Results

Field studies terpene analysis:

    Solidago altissima is capable of producing large clonal genets (i.e., genotypes) that suppress the growth of competing plant species (Butcko & Jensen 2002). Williams and Avakian (2015) found that *S. altissima* terpene expression correlated with genotype. Although I attempted to analyze effects of genotypes at the level of patch, large contiguous patches of *S. altissima* often comprise of several clonal genets (Maddox et al. 1989; Cain 1990). Microsatellite analysis confirmed that all three patches were genetically different. However, each patch consisted of a mixture of genotypes, nine genotypes in patch A, eight genotypes in patch B, and two genotypes in patch C (Appendix A). Because of these patch mixtures, effects of individual genotypes on foliar terpenes was not possible. Therefore, I refer to genetic variation in my study at the level of patch and not genotype alone.

    Numerous aphids were counted throughout the field season. Across all three patches 5,978 aphids were counted on 496 ramets. *Eurosta solidaginis* galls were far more abundant at my field site than *G. gallaesolidaginis* galls, with 154 and 27 galls counted respectively across the three patches.

    Terpenes varied significantly in relation to distance from inducing gall flies and by patch (Distance: $F = 3.56$, $p < 0.01$; Patch: $F = 28.54$, $p < 0.01$). However, here was no Distance X Patch interaction ($F = 1.10$, $p = 0.35$), Figure 5. In addition to total terpenes, a

significant difference in MeJA concentrations between patches (F = 125.82, p < 0.01), Figure

6. Both the distance from inducer and the interaction of distance and patch were insignificant

(Distance: F = 1.08, p = 0.38; Interaction: F = 1.04, p = 0.42). Tukey post-hoc analysis

revealed all foliar terpene concentrations in each patch were significantly different (p < 0.01),

with Patch C exhibiting the highest MeJA concentrations and Patch B the lowest MeJA

concentrations. Although individual terpenes were analyzed, variability was very

high. Because it is unclear precisely which terpenes are relevant to my study system only

total identified terpenes were analyzed. A summary of select identified terpenes grouped by

distance from the inducer is found in Appendix B.
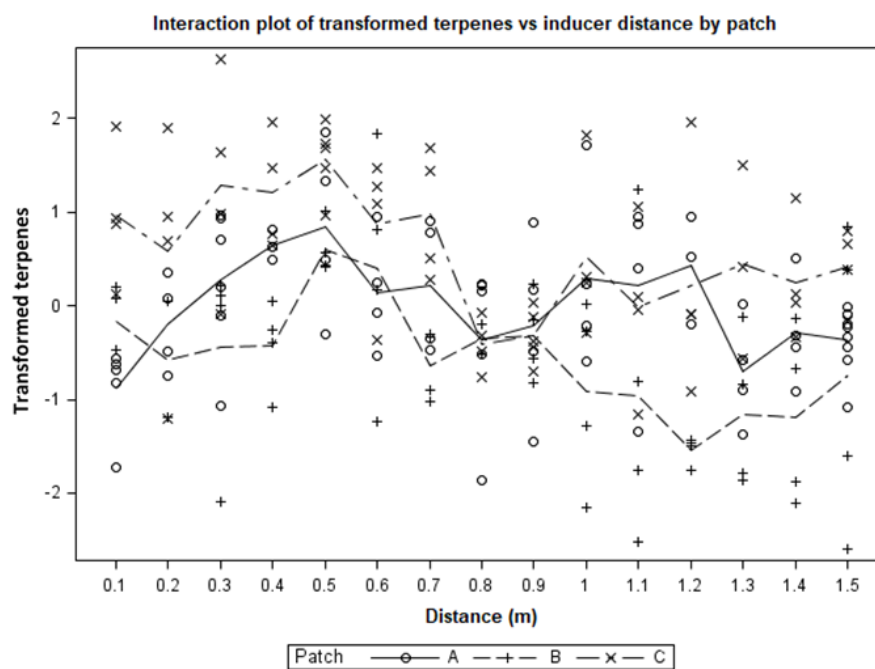
Figure 5 Interaction plot showing Johnson's S_U normalized foliar terpene concentrations vs distance from inducer by patch. Trendlines connect the means of each distance group.
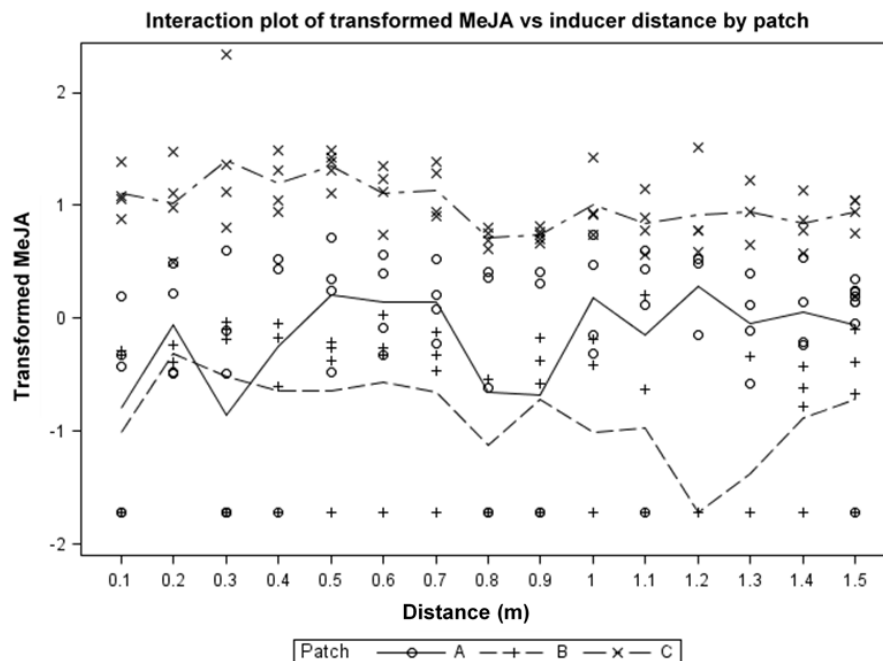
Figure 6 Interaction plot showing Johnson's S_b normalized foliar MeJA concentrations vs distance from inducer by patch. Trendlines connect the means of each distance group.

Standardized patch B-spline models (knots = 8) were fit to Patch A (p = < 0.01, r² = 0.36), B (p = 0.02, r² = 0.40), and C (p < 0.01, r² = 0.36), Figure 7. Models were compared via Holmes-simulated multiplicity corrected p-values at five cm intervals (Adj p), yielding p-values ranging from 0.21 to > 0.99, Table 1. As all corrected p-values (i.e., Adj p) are insignificant, the spline model comparison indicates distance from inducer effects on foliar terpene content are consistent between the three patches.
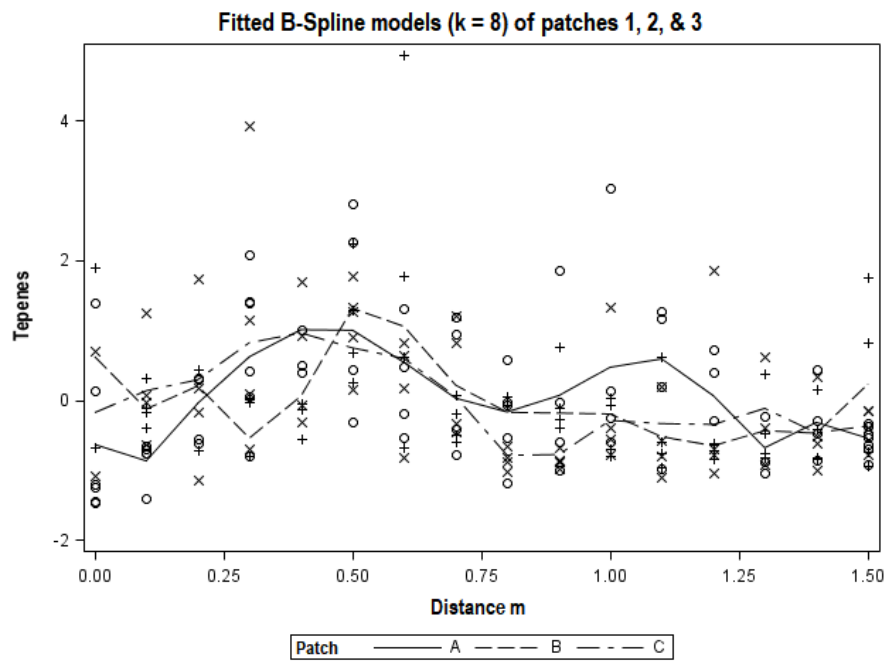
Figure 7   Fitted B-Spline model (knots = 8) of field collected standardized foliar terpenes vs distance (m) by patch.

Table 1   A listing of all multiplicity adjusted p-values comparing B-spline fit models of patch differences.

| | Estimates | | | | | |
| | Adjustment for Multiplicity: Holm-Simulated | | | | | |
Label	Estimate	SE	DF	t Value	Pr > |t|	Adj p
Diff at x= .05	-0.7888	0.7839	157	-1.01	0.3159	0.9531
Diff at x= .10	-0.742	0.6051	157	-1.23	0.2219	0.8997
Diff at x= .15	-0.6903	0.616	157	-1.12	0.2641	0.9268
Diff at x= .20	-0.2477	0.7305	157	-0.34	0.735	0.9962
Diff at x= .25	0.5003	0.5593	157	0.89	0.3725	0.9645
Diff at x= .30	1.1635	0.4889	157	2.38	0.0185	0.2414
Diff at x= .35	1.3516	0.5532	157	2.44	0.0157	0.2153
Diff at x= .40	0.9512	0.4902	157	1.94	0.0541	0.5078
Diff at x= .45	0.2787	0.4885	157	0.57	0.5691	0.9945
Diff at x= .50	-0.3128	0.5489	157	-0.57	0.5695	0.9945
Diff at x= .55	-0.5544	0.4859	157	-1.14	0.2556	0.9215
Diff at x= .60	-0.514	0.4579	157	-1.12	0.2633	0.9268
Diff at x= .65	-0.3443	0.5366	157	-0.64	0.522	0.9909
Diff at x= .70	-0.1885	0.5155	157	-0.37	0.7151	0.9962
Diff at x= .75	-0.0829	0.4704	157	-0.18	0.8603	0.9962
Diff at x= .80	0.004905	0.5535	157	0.01	0.9929	0.9962
Diff at x= .85	0.1084	0.574	157	0.19	0.8504	0.9962
Diff at x= .90	0.2502	0.4743	157	0.53	0.5986	0.9945
Diff at x= .95	0.4359	0.4885	157	0.89	0.3736	0.9645
Diff at x= 1.00	0.6697	0.5505	157	1.22	0.2256	0.8997
Diff at x= 1.05	0.9329	0.482	157	1.94	0.0548	0.5078
Diff at x= 1.10	0.7884	0.5274	157	1.49	0.137	0.801
Diff at x= 1.15	1.0795	0.5859	157	1.84	0.0673	0.5604
Diff at x= 1.20	0.7167	0.534	157	1.34	0.1814	0.8671
Diff at x= 1.25	0.1714	0.5125	157	0.33	0.7385	0.9962
Diff at x= 1.30	-0.245	0.6138	157	-0.4	0.6903	0.9962
Diff at x= 1.35	1.0795	0.5859	157	1.84	0.0673	0.5604
Diff at x= 1.40	0.7167	0.534	157	1.34	0.1814	0.867
Diff at x= 1.45	0.1714	0.5125	157	0.33	0.7385	0.9962

Aphid abundance between patches was significantly different ($p < 0.01$; Figure 8).

Steel-Dwass post hoc analysis found all patches were significantly different from one another

(Patch A: mean = 2.87, Z = 5.37, $p < 0.01$; Patch B: mean = 4.79, Z = -3.10, $p < 0.01$; Patch

C: mean = 1.62, Z = -6.62, $p < 0.01$).
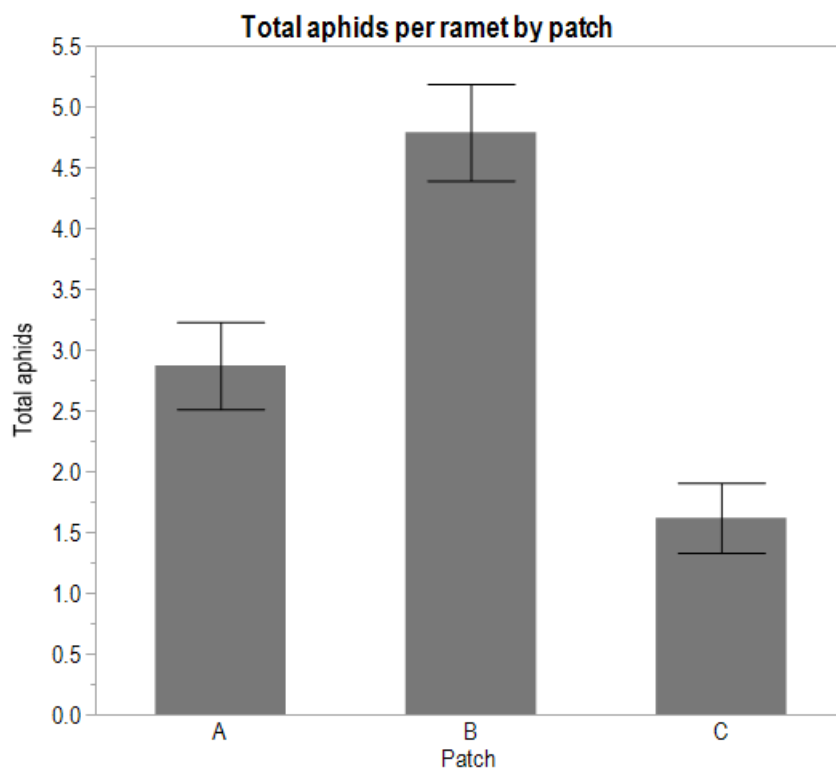
Figure 8   Means and standard errors of total aphids per ramet by patch.

Chamber experiments:

There were no significant differences between terpenes in control plants after the exposure period in both genotype A (Z = -0.10, p = 0.92) and genotype B (Z = 0.94, p = 0.35; Figure 9). Analysis of gall exposed treatment groups revealed that in genotype A total terpene concentration significantly increased by a factor of 1.5 ± 0.5 (Z = -2.19, p = 0.03), while in genotype B total terpene concentration significantly increased by a factor of 2.5 ± 1.2 (Z = -2.19, p = 0.03) (Figure 9).
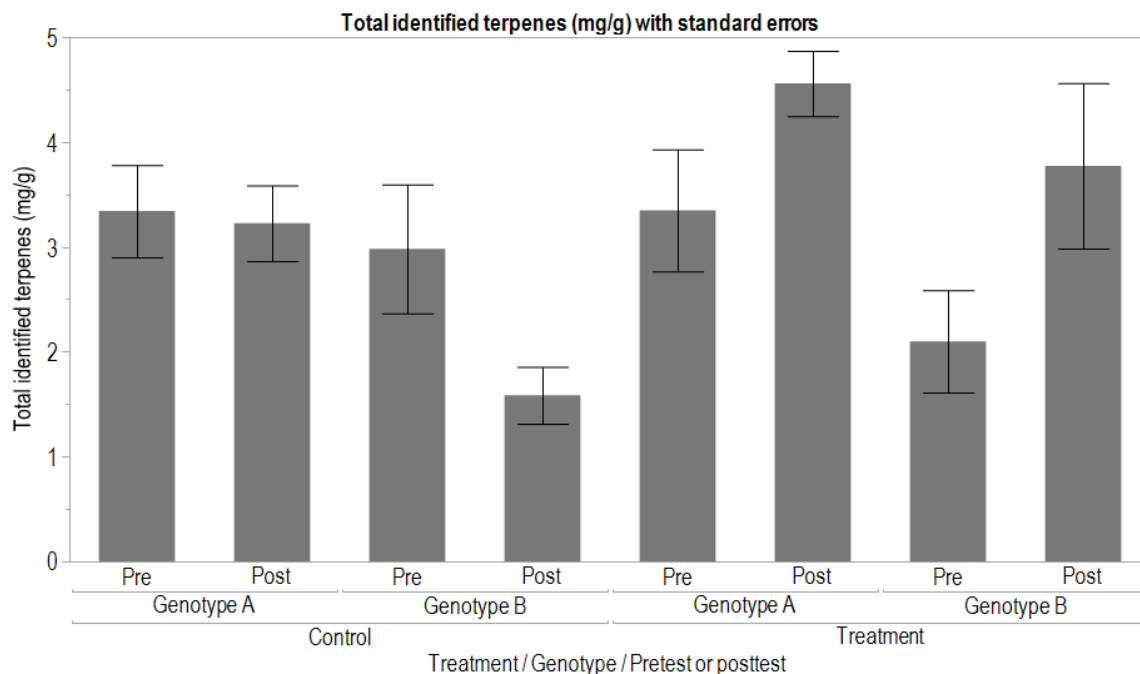
Figure 9   Means and standard errors of total identified terpenes by genotype and treatments pretest and posttest. No significant differences were observed in control pretest and posttest. Significant induction of total terpenes was observed posttest in both genotypes.

Of the 24 terpenes identified in my analysis, three had significant increases in foliage concentration after the gall exposure treatment in both genotypes A and B (Table 2). These include bornyl acetate (A: $Z = -2.40$, $p = 0.02$; B: $Z = -2.40$, $p = 0.02$), β-farnesene (A: $Z = -2.19$, $p = 0.03$; B: $Z = -2.19$, $p = 0.03$), and β-elemene (A: $Z = -2.19$, $p = 0.03$; B: $Z = -2.61$, $p = 0.01$). All other compounds either did not significantly increase after exposure or only increased in one of the two genotypes.

Stem and gall terpene and GLV analysis:

A significant increase in *E. solidaginis* gall terpenes was noted when compared to ungalled stem terpenes ($p < 0.01$). The mean concentration of total identified terpenes in

galled stem tissue was 8.26 times greater than that of ungalled stem tissue (galled = 1.38

mg/g, ungalled = 0.17 mg/g). Inverse results were found in GLVs. A significant decrease of

the only consistently identifiable GLV, hexanal, was found in ball gall tissues when

compared to the ungalled stem tissues tested ($p < 0.01$). A summary of identified terpenes

and GLVs is provided in Table 3.

Table 2   Summary of chamber sampled foliar terpenes (mg/g) grouped by genotype, control/treatment, and pretest/posttest. Mean ± standard error of the mean.

Group:	Summary of foliar terpenes by chamber group (mg/g)					
	Genotype A treatment Pre-test	Genotype A treatment Posttest	Genotype A Significance	Genotype B treatment Pre-test	Genotype B treatment Posttest	Genotype A Significance
α-pinene	0.136 ± 0.035	0.253 ± 0.029	0.095	0.153 ± 0.038	0.256 ± 0.027	0.095
camphene	0.016 ± 0.007	0.037 ± 0.003	0.047*	0.018 ± 0.006	0.033 ± 0.004	0.141
β-pinene	0.05 ± 0.015	0.091 ± 0.013	0.047*	0.019 ± 0.007	0.047 ± 0.016	0.210
p-cymene	0.089 ± 0.028	0.167 ± 0.021	0.095	0.084 ± 0.023	0.131 ± 0.021	0.210
ocemene isomers	0	0.002 ± 0.002	0.424	0.017 ± 0.005	0.022 ± 0.008	0.753
bornyl acetate	0.13 ± 0.025	0.221 ± 0.01	0.022*	0.075 ± 0.016	0.168 ± 0.032	0.022*
β-elemene	0.101 ± 0.023	0.164 ± 0.012	0.037*	0.021 ± 0.002	0.098 ± 0.042	0.003*
(-)-trans caryophyllene	0.091 ± 0.011	0.12 ± 0.008	0.095	0.063 ± 0.01	0.134 ± 0.023	0.060
α-humulene	0.035 ± 0.004	0.046 ± 0.003	0.095	0.02 ± 0.003	0.046 ± 0.009	0.095
cis carophyllene	0.014 ± 0.001	0.019 ± 0.002	0.116	0	0.007 ± 0.007	0.424
germacrene D	2.373 ± 0.282	3.188 ± 0.201	0.095	1.028 ± 0.192	2.622 ± 0.619	0.095
β-farnesen	0.096 ± 0.01	0.131 ± 0.008	0.037*	0.021 ± 0.003	0.072 ± 0.031	0.037*
azulene	0.031 ± 0.007	0.046 ± 0.018	0.676	0.016 ± 0.003	0.035 ± 0.007	0.146
α-farnesene	0.022 ± 0.002	0.026 ± 0.001	0.095	0	0.007 ± 0.007	0.424
γ-elemene	0	0.001 ± 0.001	0.424	0	0.01 ± 0.008	0.180
MeJA Isomers	0.041 ± 0.011	0.047 ± 0.006	0.404	0.052 ± 0.006	0.086 ± 0.018	0.300

Table 3   Summary of field sampled stem and gall terpenes and GLVs (mg/g). Mean ± standard error of the mean. Hexenal concentrations significantly decreased in gall tissue, all other significant differences correspond to an increased concentration in gall tissue.

Summary of stem and gall terpenes and GLVs (mg/g)			
	Gall	**Stem**	**Significance**
α-pinene	0.144 ± 0.159	0.019 ± 0.018	0.007*
camphene	0.021 ± 0.044	0.001 ± 0.001	0.106
β-pinene	0.065 ± 0.033	0.034 ± 0.03	0.026*
myrcene	0.025 ± 0.027	0.003 ± 0.004	0.010*
α-phellandrene	0.019 ± 0.038	0 ± 0	0.037*
p-cymene	0.148 ± 0.305	0.016 ± 0.023	0.244
limonene	0.017 ± 0.03	0.003 ± 0.007	0.306
ocimene isomers	0.038 ± 0.096	0.003 ± 0.011	0.286
sabinene	0 ± 0.001	0 ± 0	0.359
methyl salicylate	0 ± 0.001	0 ± 0	0.359
bornyl acetate	0.002 ± 0.003	0.005 ± 0.006	0.209
β-elemene	0.039 ± 0.048	0.004 ± 0.004	0.009*
(-)-trans caryophyllene	0.031 ± 0.036	0.004 ± 0.005	0.008*
α-humulene	0.011 ± 0.014	0 ± 0	0.003*
cis caryophyllene	0.117 ± 0.251	0.002 ± 0.005	0.008*
germacrene D	0.384 ± 0.589	0.043 ± 0.026	0.061
β-farnesene	0.218 ± 0.53	0.009 ± 0.022	0.114
azulene	0.012 ± 0.014	0.001 ± 0.002	0.004*
α-farnesene	0.031 ± 0.076	0 ± 0	0.079
γ-elemene	0.008 ± 0.012	0 ± 0.001	0.024*
MeJA Isomers	0.049 ± 0.089	0.005 ± 0.009	0.031*
ledene oxide	0.001 ± 0.005	0.001 ± 0.003	1.000
bicyclo[4.4.0] dec-5 -ene	0.003 ± 0.007	0 ± 0	0.037*
hexanal	0.204 ± 0.095	1.186 ± 0.199	0.002**

Spatial model:

A B-spline (knots = 8) was fit to total identified terpene data obtained in field studies which was standardized by patch ($p < 0.01$, $r^2 = 0.22$; Figure 10). This combined patch terpene spline model was used in the spatial model as a measure of induced terpenes. A combined patch model was deemed appropriate because there was no divergence between terpene spline models between patches. The weighted Fuzzy Kappa showed moderate

concordance of modeled seasonal average aphid distribution across all three patches, Kappa

> 0.40 (Figure 11). The weighted Fuzzy Kappa of the vegetation structure-only model

revealed decreased concordance across all three patches (Table 5). The Fuzzy Kappa of all

three inducer-only model values were < 0.01. Terpene induction by inducers is partly

influencing *Uroleucon* aphid distribution and abundance as indicated by the significant

Fuzzy Kappa values in the combined model. However, alone, inducer distance is not an

accurate predictor of aphid distribution.
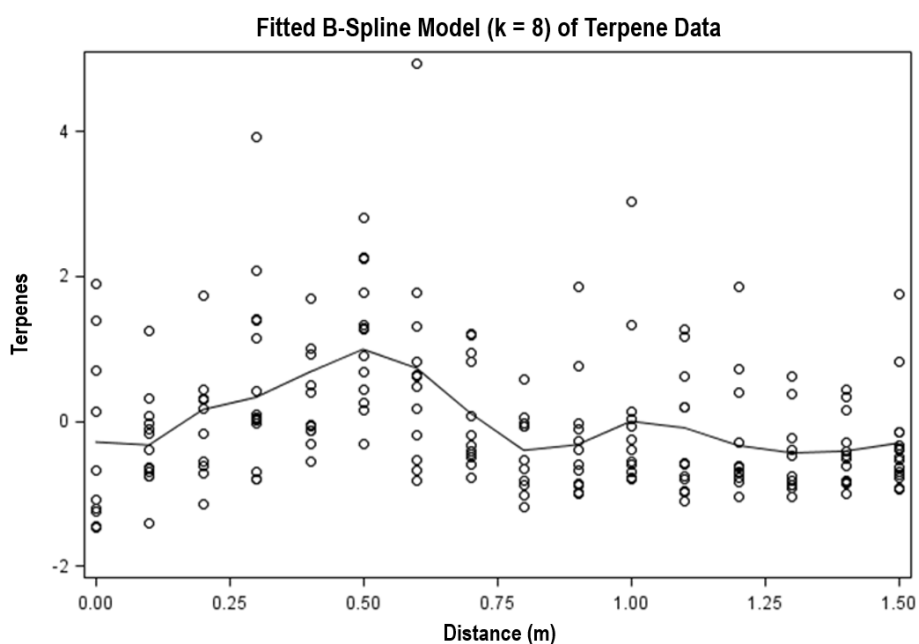
Figure 10   Fitted B-Spline model (knots = 8) of all field collected standardized foliar terpenes vs distance

(m).

Patch	Model Projected Aphids	Observed Aphids	Kappa Map
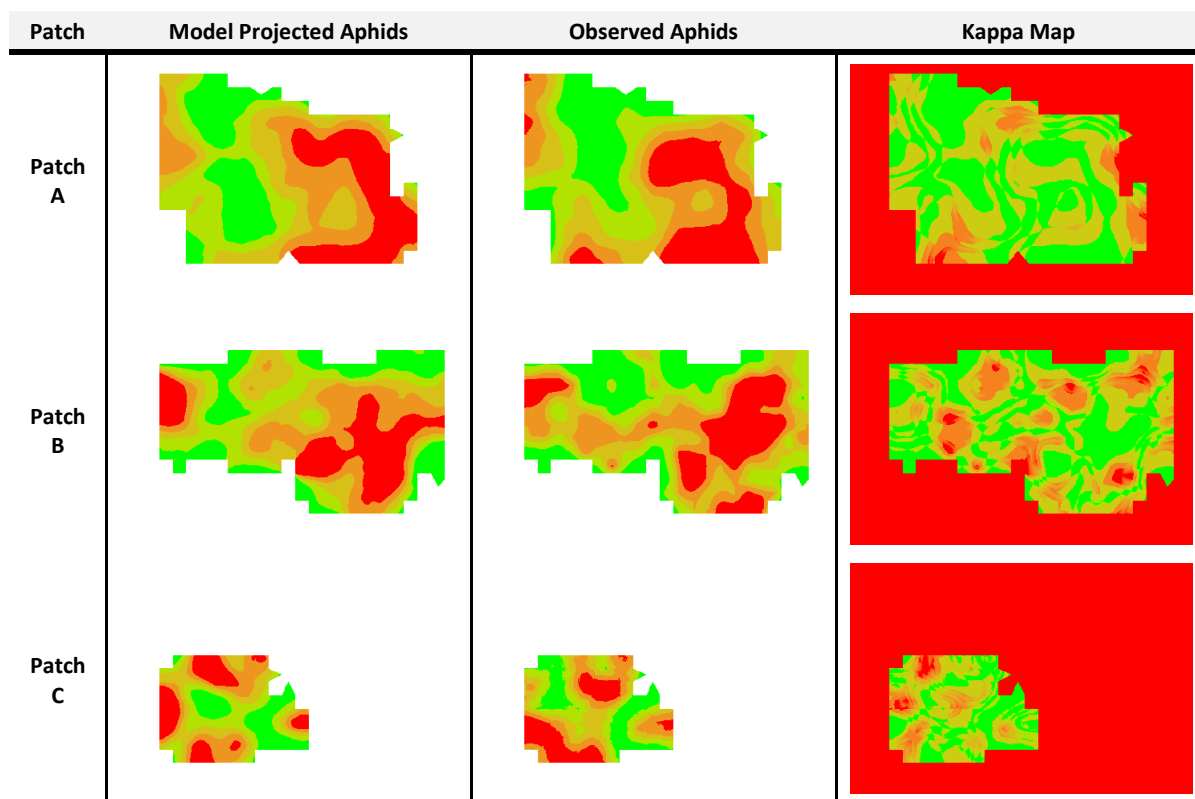
Figure 11   An Array of raster maps depicting model projected relative aphid abundance, observed relative aphid abundance, and a fuzzy weighted Kappa comparison map for patches A, B, and C. In model maps and observed aphid maps, green values indicate lower relative aphid abundance and red values indicate higher relative aphid abundances. In Kappa maps, red values indicate lower agreement between maps while green values indicate higher agreement between maps. All maps are scaled by quantile (equal area) from 1 to 5.

Table 5   Kappa values and percent agreement between observed relative aphid abundance maps and modeled relative aphid abundance maps by patch. Values are given for models that are only vegetation structure based (no inducer), only inducer based (no vegetation structure), as well as models that include predictions based on both vegetation structure and inducers.

	Fuzzy weighted Kappa comparison					
	Inducer Kappa	Inducer Agreement	Vegetation structure Kappa	Vegetation structure Agreement	Vegetation structure + Inducer Kappa	Vegetation structure + Inducer Agreement
Patch A	0.006	0.611	0.587	0.845	0.588	0.846
Patch B	-0.075	0.675	0.364	0.773	0.417	0.796
Patch C	-0.160	0.663	0.398	0.806	0.407	0.810

In order to better visualize aphid abundance in relation to each spatial model factor, graphs of kernel smoothed means of aphid abundance across all three patches are provided and plotted against inducer distance and edge distance in Figure 12, against stem height in Figure 13, and against stem diameter in Figure 14.
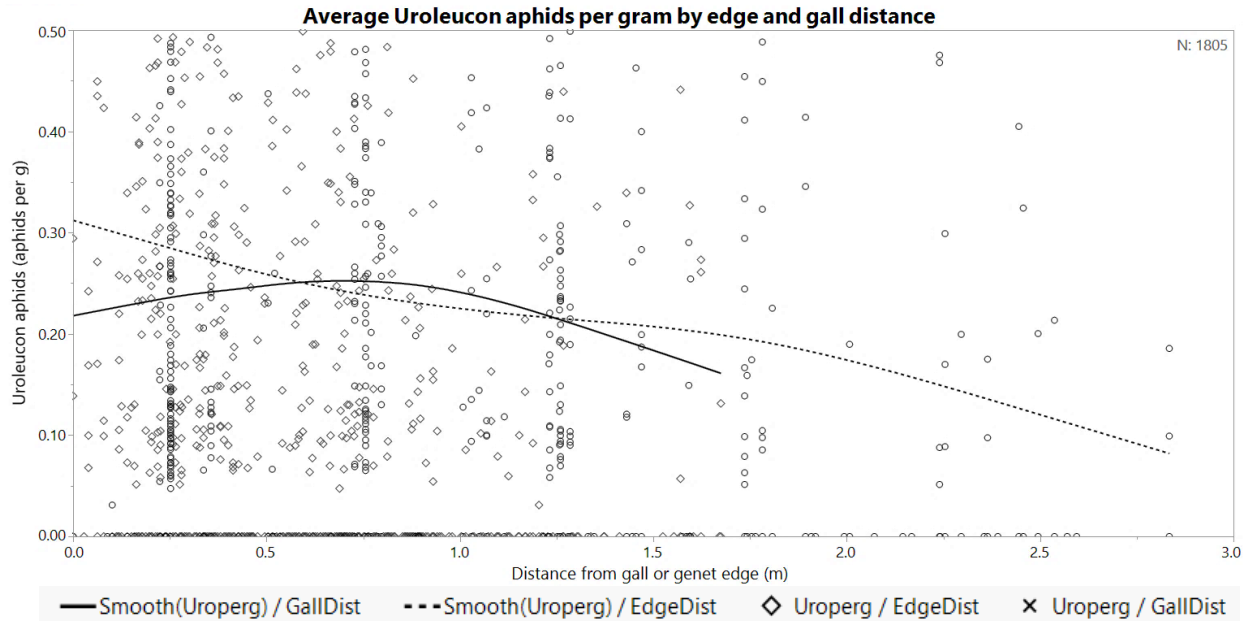
Figure 12   Average *Uroleucon* aphids adjusted for biomass (aphids per g) vs edge distance and gall distance (m). Means are LOESS kernel smoothed ($\lambda = 100$).
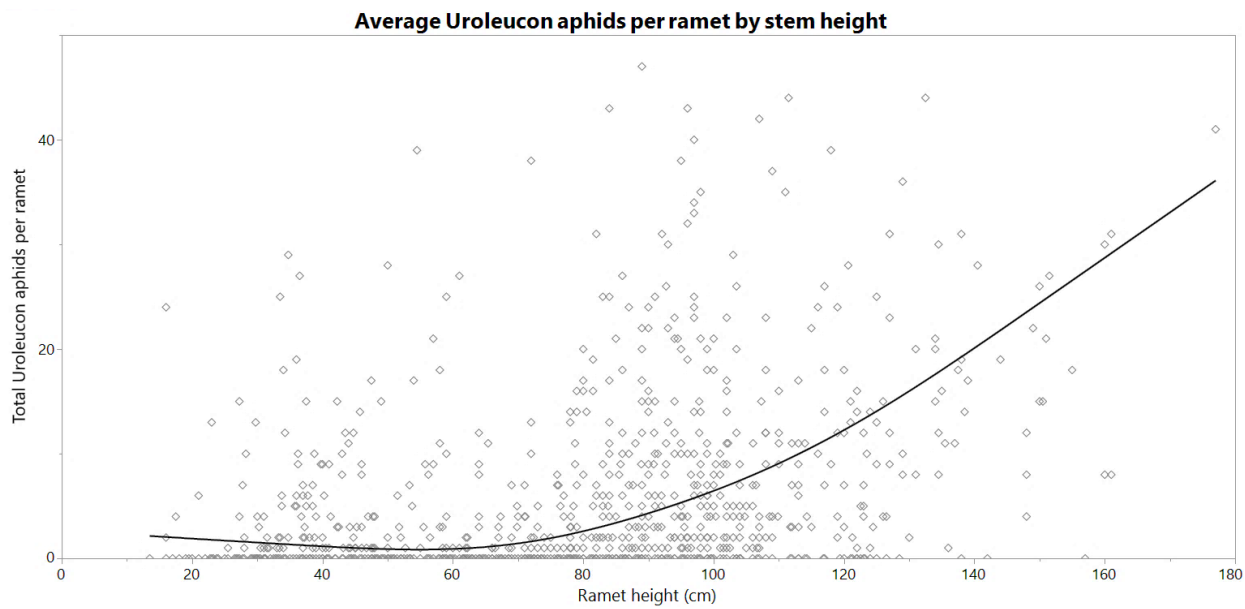
Figure 13   Average *Uroleucon* aphids vs stem height (cm). Means are LOESS kernel smoothed ($\lambda = 100$).
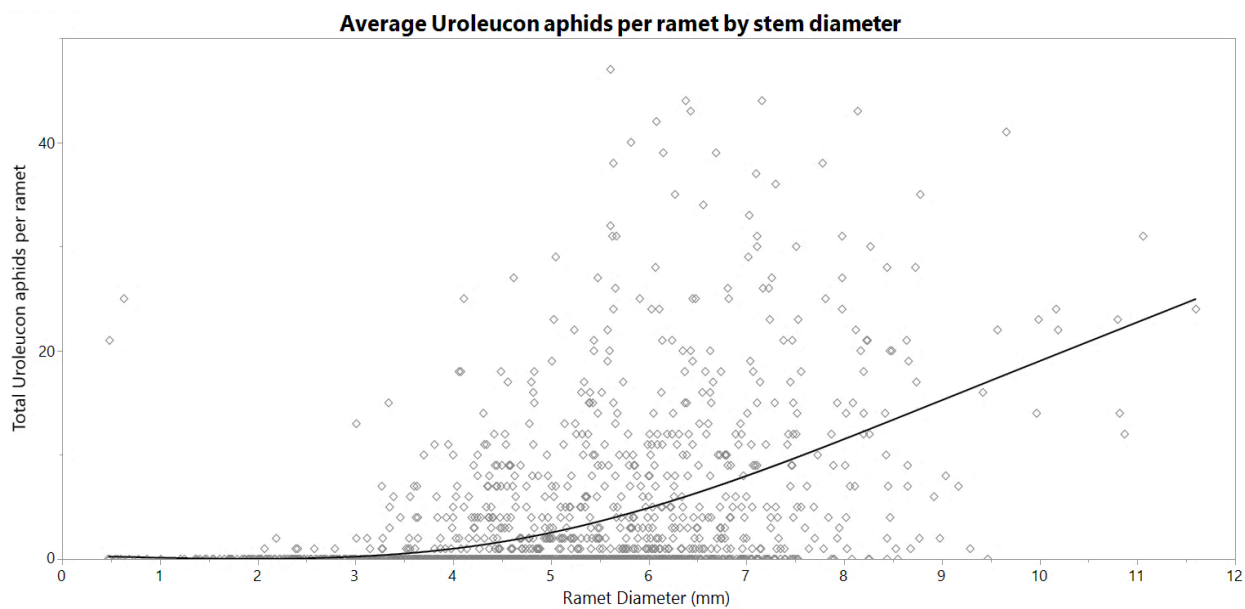
Figure 14   Average *Uroleucon* aphids vs stem diameter (mm). Means are LOESS kernel smoothed ($\lambda = 100$).

Discussion

In this study I focused on the interactions between tall goldenrod, *Solidago altissima*, the goldenrod galling insects *Eurosta solidaginis* and *Gnorimoschema gallaesolidaginis*, and goldenrod specialist aphids in the genus *Uroleucon*. Prior research has demonstrated that *E. solidaginis* males prime a volatile terpene response in *S. altissima* (Helms et al. 2014). Another study found a specialist aphid in the genus *Uroleucon* preferentially colonizes *S. altissima* ramets with higher foliar terpene concentrations (Williams & Avakian 2015). I hypothesized that a ramet's chemical response to gall insects may function as an allomone, deterring oviposition by the inducing species, while simultaneously functioning as a kairomone by attracting aphids to plants in proximity. I also expected that by spatially modeling the terpene induction caused by gall insects, along with field vegetation structure, I could predict relative aphid distribution in an old-field. This unique model approach provides a valuable illustration of aphid spatial distribution. Furthermore, the model addresses spatial interactions of terpene induction and vegetation structure that are otherwise difficult to assess.

Semiochemical induction by E. solidaginis galls

To better understand the underlying mechanisms of gall insect induction, I tested the ability of *E. solidaginis* galls to indirectly induce a terpene response via semiochemical

signaling in a controlled chamber experiment. This experiment revealed significant

induction of three foliar terpenes in ungalled *S. altissima* plants exposed to the volatile

emissions of galled plants (Figure 9). Total foliar terpenes also increased. This indirect

induction has not been previously described in the *S. altissima* and *E. solidaginis* system.

This finding was not unexpected as gall-making insects are known to induce terpenes in their

hosts (Rostás et al. 2013). Furthermore, herbivore-induced plant volatiles have been shown

to induce an indirect defense in neighboring plants (Kost & Heil 2006).

Chemical analysis of *S. altissima* stem tissue and *E. solidaginis* gall tissue was done

in the hopes of identifying semiochemicals responsible for indirect induction. The analysis

of terpenes in galls via gas chromatography revealed significantly higher concentrations of

total terpenes in *E. solidaginis* galls in comparison with ungalled stem tissue (Table 3). This

induction occurred in spite of a suppressed chemical response in leaf tissues collected in the

field. Additionally, three green leaf volatiles (GLVs) were present, although only hexanal

was consistently identified in both galled and ungalled stems. Several GLVs, including

hexanal, are known to act as semiochemicals priming defensive responses of nearby

conspecifics (Engelberth et al. 2004; Scala et al. 2013), or to cause indirect induction.

Linoleic acid is known to increase in galls and larva of both *E. solidaginis* and *G.*

gallaesolidaginis (Joanisse & Storey 1996; Bennett, Pruitt, & Lee 1997; Tooker & De

Moraes 2009). Furthermore, α-linolenic acid is a known precursor of many GLVs (Scala et

al. 2013). Because high concentrations of α-linolenic acid were found in gall tissues by

previous authors, I suspected GLVs in gall tissues would also be more abundant than in

ungalled stems. However, the opposite was found. Hexanal concentrations in galls were

significantly lower than in ungalled stems, and therefore seems unlikely to be the compound

responsible for the indirect induction of terpenes in nearby ramets. It is likely that compounds outside the focus of this research contribute to the indirect gall induction observed in my chamber experiments. Gall phenolics are another potential source of volatile semiochemicals, as phenolics are known to significantly increase in viable gall tissues (Abrahamson et al. 1991; Abrahamson & Weis 1997). I was unable to consistently identify phenolic compounds in my GC-TOF analysis of stem and gall tissue. Although non-polar solvents such as pentane and dichloromethane can be used to extract some phenolic compounds from plant tissues (Khoddami et al. 2013), positive identification and quantification of phenolics via GC-TOF may require a polar solvent. Besides phenolics, Mapes and Davies (2001) found significant increases of indole-3-acetic acid concentration in *E. solidaginis* gall tissue. Indole-3-acetic acid is derived from indole which has recently been identified in maize as another long distance volatile priming signal (Erb et al. 2015; Li et al. 2016). Its effect on plants other than maize has yet to be established, but indole should be a compound of interest for future research on *E. solidaginis* galls.

Given the greater than 8-fold increase in total identified terpene concentrations in galls when compared to ungalled stems, it seems likely that one or more of these terpenes are acting as the semiochemical(s) responsible for indirect induction of nearby ramets. Volatile terpene releases by plants have been found to prime or induce defensive responses in conspecifics. Recently, the terpene (*E*)-β-ocimene has been found to prime a defensive response in tobacco (Arimura, Muroi & Nishihara 2012). Other terpenes are not as well studied in plant-plant communication and may still be responsible for priming or induction in some plant species. In order for terpenes to be the agent responsible for induction in my study, however, the increased terpene concentration in the gall tissue must correspond to an

increase in volatile terpene release, which was not tested in my experiment. Tooker et al. (2008) found decreased active volatile release in *E. solidaginis* galled ramets in laboratory studies. Therefore, it seems unlikely terpenes are responsible for induction. However, Tooker et al. (2008) did note a non-significant increase of terpene volatiles in field-collected galled plants. Furthermore, Hogan (2007) found increased α-pinene volatilization by *S. altissima* plants attacked by *E. solidaginis*, contradicting the findings of Tooker et al. (2008). Further investigation of gall terpenes, indole, and phenolic volatiles should be the focus of future work in order to better understand the mechanisms behind gall indirect induction of terpenes.

Spatial pattern of terpene induction

My chamber experiment findings support the idea that gall-making insects are at least partially responsible for foliar terpene content observed in the field. However, these data do not explain the non-linear relationship of field observed terpene induction relative to inducer (gall insect) distance as illustrated in my terpene spline model (Figure 10). While significant differences were found in field sampled foliar terpenes by ANOVA along with what appears to be a continuous trend influenced by distance in the spline model, the r^2 fit of the spline model is low. Nevertheless, this spline model was used to predict terpene induction by ramet distance to the inducer in my spatial model. Although uncovering precise factors causing this non-linear relationship fell outside the scope of my study, previous research of *S. altissima* provides plausible explanations for the spatial pattern of induction observed. Foliar terpene concentrations were consistently suppressed at distances less than 20 cm from both adult male *E. solidaginis* flies and *E. solidaginis* galls in the field. Because *S. altissima*

propagates by rhizomes, individual ramets may be connected underground (Abrahamson & Weis 1997). Priming cues and nutritive molecules might pass through underground connections (Alpert 1996; Gómez & Stuefer 2006), thereby influencing defensive chemistry of nearby individuals. It is possible that immediately adjacent ramets connected by rhizome to the galled plant may be chemically suppressed in a similar manner to the galled plant (Tooker & DeMoraes 2008). Yip et al. (2017) found that very few *S. altissima* rhizomes extended beyond a 30 cm distance from the parent stem. Any terpene suppressing signal via rhizome was unlikely to occur at this distance or beyond from a gall. Apparent terpene suppression occurring in relation to adult male fly displays may have been due to residual effects of the prior year's gall. Males tend to stay on the galled ramet from which they emerged (Craig et al. 1993). Rhizome connections are significantly more likely to disintegrate following ball gall infestation (McCrea & Abrahamson 1985; How, Abrahamson & Zivitz 1994). It is possible that decreased nutrient sharing between ramets after rhizome disintegration energetically limits terpene production in subsequent years as terpenes are metabolically costly to the plant producing them (Gershenzon 1994). This lack of rhizome connection could result in the apparent suppression of terpenes in ramets immediately adjacent to male fly displays, but additional research is required to confirm this.

The induction observed in my field study occurred beyond the reach of potential gall suppression by *E. solidaginis* via rhizome, and extended out to distances of approximately 75 cm from the galled ramet (Figure 10). This induction is likely in response to the unidentified gaseous semiochemical responsible for foliar terpene induction in my chamber experiments. Plant-plant communication and subsequent priming or induction by volatile organic compounds (VOCs) is well known and may be due to terpenes, terpenoids, GLVs, phenolics,

methyl jasmonate, methyl salicylate, or ethylene (Ueda, Kikuta & Matsuda 2012). The drop

off of terpene concentration beyond 75 cm is likely due to the diffusion of the

semiochemicals released by the galled ramet to concentrations that no longer elicit a defense

response in the ungalled ramets. Others have observed or speculated on this diffusion of

semiochemicals in the field (Karban et al. 2000; Kost & Heil 2006; Heil & Walters 2009).

Given these possibilities, the expected result is an annulus pattern of induced ramets

clustered in a ring around galled ramets as seen in Figure 15.

Total aphid numbers and field measured terpene concentrations, when sampled at

similar distances from an inducer, did not correlate well without taking into consideration

vegetation structure. When aphid abundance was corrected for biomass, mean aphid

abundance plotted against inducer distance reveals a trend closely resembling that observed

in foliar terpene levels (see Figures 10 and 12). With this correction, mean observed aphid

abundance near an inducer was low, increased at intermediate distances, and then peaked at

approximately 75 cm. Beyond this distance aphid abundance dropped off, corresponding to

lower concentrations of foliar terpenes.
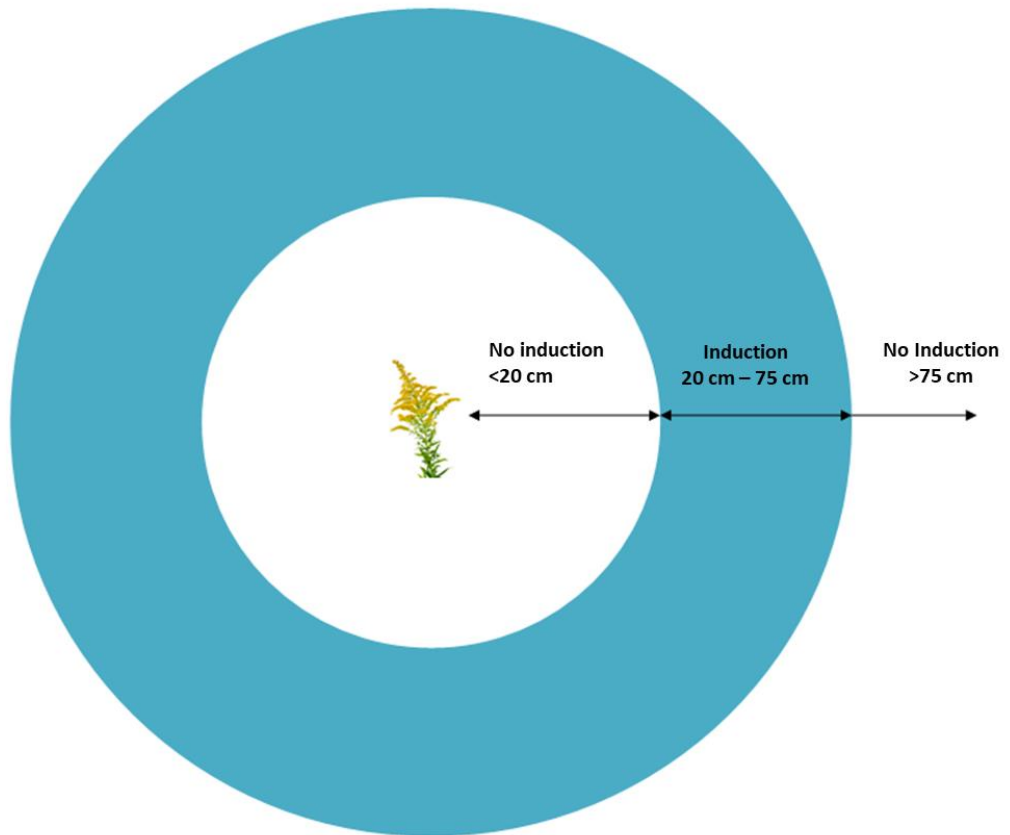
Figure 15   The spatial pattern of terpene induction suggested by the terpene spline model in a *S. altissima* patch. A galled ramet lies in the center and the blue region represents areas of terpene induction. Ramets in the patch falling within this region are expected to have elevated levels of foliar terpenes. Aphid abundance is expected to also increase in this region in response to the elevated terpenes.

The spatial model

Modeling and predicting the effects of multiple and indirect interactions between insect herbivores is difficult because environments tend to be structurally heterogeneous (Schowalter 2016). Spatial models have previously been used to describe distributions of the predatory ladybeetle, *Coccinella septempunctata*, in response to *Uroleucon* aphid distributions in patches of *S. altissima* (Grünbaum 1998). However, my model is unique in that it models both interspecific interactions between *S. altissima* specialist insect herbivores

as wells the effects of vegetation structure on insect distribution. Yip et al. (2017) and Helms et al. (2013; Helms et al. 2014; Helms et al. 2017) established the adult male gall fly's ability to prime a terpene response via volatile pheromones and that this priming drives a spatial pattern in insect herbivory. My chamber experiment revealed that developing ball galls also induce a foliar terpene response in ungalled ramets. Induction by both adult male fly and developing galls became important components of an inducer model. Combined with a model of vegetation structure, which took into account *S. altissima* stem height, diameter, and patch edge proximity, my model predicted the spatial relative abundance and distribution of aphids in tall goldenrod patches.

Across the three patches studied, my spatial models of predicted *U. nigrotuberculatum* and *U. luteolum* aphid abundance had satisfactory agreement with maps of measured aphid abundance. The Kappa agreements of these maps were all greater than 0.40, which is considered biologically significant (Landis and Koch 1977). This level of Kappa agreement was only achieved when the model was based on both vegetation structure and indirect terpene induction (i.e., the combined model). Models based only on vegetation did not have significant agreement in two of the three *S. altissima* patches tested, providing evidence that vegetation structure alone is not a sufficient predictor of relative aphid distribution and abundance in all cases. Although one patch did show significant agreement between the vegetation structure only model and observed aphid abundance, agreement of the combined model with observed aphid abundance was higher. The indirect induction only model did not have a >0.40 Kappa agreement in any of the three patches tested. Together, these results suggest that both vegetation structure and indirect terpene induction effect *Uroleucon* spatial distribution.

Kappa values of the combined model weighted all individual factors of vegetation structure (stem height, stem diameter, edge distance) and terpene induction equally. This means that the inducer only model (terpene induction by galls and adult male gall flies) is only making a 25% contribution to the combined model. Kappa values of the inducer only models are also quite low, with two patches having no agreement at all (Kappa <0). The higher Kappa value of the vegetation structure model may indicate that the effects of vegetation structure are more important predictors of aphid spatial distribution and abundance. This agrees with findings by Richardson and Hanks (2011), who found that *S. altissima* ramet height was the best predictor of *U. nigrotiburculatum* and *U. luteolum* abundance in the field. In that study, ramet height was an even greater predictor of aphid abundance than host genetics.

Induction by other insect herbivores

Though my data demonstrate vegetation structure and terpene signaling driven by gall-making insects is important, interspecific interactions other than those between aphids and gall-makers may also influence aphid distribution. Maddox and Root (1990) identified clusters of insect herbivores that co-occur on *S. altissima* as herbivore suites. Insects identified in the *Uroleucon* herbivore suite, such as *Phytomyza sp.* leaf miners, have not been well studied in the *S. altissima* system. It is unknown if these insects induce a terpene response in the host or if they are simply responding to the same host traits as the *Uroleucon* aphids. Although I collected data on occurrence of other herbivores and herbivore damage, I chose not to include these data in my spatial model parameters because the reason for co-occurrence was unclear. If other insects are found to induce terpenes important to *Uroleucon*

aphid colonization in future studies, the addition of these induction factors may further increase the accuracy of my spatial model.

Genetic differences at the level of patch

My microsatellite analysis attempted to account for possible differences among genotypes between patches (Appendix A). Genotype could influence factors important to my model. My analysis revealed that all patches were a mix of genotypes. Thus, it is not clear what role individual *S. altissima* genotypes contributed to *Uroleucon* aphid distribution and abundance in sampled patches. Aphid abundance per ramet did differ significantly between all three patches (Figure 8), and there appears to be a positive correlation between *S. altissima* genetic diversity within the patch and aphid abundance. Patch C contained two genotypes and had the lowest aphid abundance. Patch B contained nine genotypes and had the highest aphid abundance. This finding supports previous conclusions by Crutsinger et al. (2006) who's work demonstrated genotypic diversity in patches of *S. altissima* corresponded to increased arthropod richness and abundance.

More research will be needed to understand genotype effects on terpenes. Work by Williams and Avakian (2015) suggested *S. altissima* genotype did influence foliar terpene concentrations. Furthermore, their research found *U. nigrotiburculatum* abundance was associated with particular high terpene expressing genotypes, a relationship I was unable to explore due to genotype mixtures in my patches. Foliar MeJA levels (Figure 6) were significantly different between patches and correlated negatively with aphid abundance at patch level, but it is unlikely foliar sampling accounted for all genotypes present in each patch.

Synergistic effects of gall induction and vegetation structure

Kappa agreement between observed aphids and combined models in all three patches are greater than agreement of vegetation structure only or inducer only models (Table 5). Moreover, combined model Kappa is greater than the sum of the two individual model Kappa values in two patches: B and C. This suggests synergistic effects between induction and vegetation texture. Synergistic effects are ecological factors that, together, have greater influences than the sum of their parts (Didham et al. 2005). The apparent synergistic interactions of induction and vegetation structure may be temporal. First, terpenes act as a positive colonization signal to aphids. Aphids that colonize more robust plants located far away from the patch center where predator and parasite densities are highest are then more likely to survive and reproduce in large numbers (Kareiva 1987).

Terpenes have been found to act as colonization signals in a study of related aphid species by Clancy et al. (2016), although this relationship is complex with some terpenes actually deterring aphids. The role of particular terpenes identified in my study as either attractants or deterrents is not well known. Previous work in *S. altissima* found a positive, albeit weak relationship between foliar β-pinene concentrations and *U. nigrotuberculatum* abundance (Williams & Avakain 2015). Individual compounds may be of less value for explaining signaling. Host recognition and colonization signals are known to be dependent on mixtures of compounds in particular ratios (Bruce & Pickett 2011). The identity and particular ratio of terpenes responsible for signaling aphid colonization in *S. altissma* ultimately remains unclear.

After aphids have colonized their host ramet, subsequent effects of vegetation structure then become important to survival of these aphid colonies. Generally, increased

plant height is associated with lower rates of insect herbivore mortality due to parasitism. The protective effects of increased plant height have been well described in the Tansy Leaf beetle, *Galeruca tanaceti*, a common insect in old fields (Obermaier et al. 2008). In *S. altissima*, *U. nigrotuberculatum* aphid abundance increased in fragmented patches due to decreased predation by the ladybeetle *Coccinella semipunctata* (Kareiva 1987). Significant aggregation along *S. altissima* patch edges by other specialist insects have been observed (Cappuccino & Root 1992), and particularly strong aggregation was noted in *U. nigrotuberculatum* (Edson 1985). These studies suggest larger ramets and proximity to patch edges may decrease aphid predation, increase aphid survival, and ultimately lead to higher aphid abundance.

Vegetation structure and plant chemistry are hypothesized to be the two primary factors influencing insect distribution in the field (Randlkofer et al. 2010). Together, these factors have been characterized as vegetation complexity. Randlkofer et al. (2010) noted that ecologists have scarce knowledge of the interaction between plant structural and chemical traits or how they affect insects in field habitats. My combined model, with its findings of potential synergistic effects between vegetation structure and plant phytochemistry in an *S. altissima* dominated old field habitat, provides new insights into this relatively unexplored hypothesis. My work provides a foundation for future research by identifying a unique dynamic between gall-making specialist insects and specialist aphids.

Conclusions

      Uroleucon aphid abundance is related to *S. altissima* foliar terpene concentrations as demonstrated by Williams and Avakian (2015). The results of my chamber experiments and field terpene analysis demonstrate significant induction of terpenes by *E. solidaginis* galls. When compared with my field observations of aphid abundance as a function of inducer distance, my work suggests an important role of terpenes and aphid distribution. Furthermore, aphid distribution appeared to be dependent on synergistic interactions of foliar terpene induction and vegetation structure. By modeling the combined effects of vegetation structure and terpene induction, I was able to predict aphid abundance and distribution in the field with a satisfactory degree of accuracy. Work by previous authors suggests this synergistic effect may be due to induced terpenes acting as a kairomone, signaling aphid colonization, and subsequent effects of vegetation structure protecting these aphid colonies. Future research should focus on better understanding the mechanism of indirect terpene induction by *E. solidaginis* galls and further explore the role of *S. altissima* genotype on aphid spatial distributions.

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Appendix A

Sample	Marker Sg_1		Marker Sg_2						Marker Sg_8						Marker Sg_10				
	1_1	1_2	2_1	2_2	2_3	2_4	2_5	2_6	8_1	8_2	8_3	8_4	8_5	8_6	10_1	10_2	10_3	10_4	10_5
A1	148		192	196	200	208			152	156	160	172	176		288	296	300	304	
A2	148		184	192	200	204	208	212	152	168	172	176			288	296	300	304	
A3	148		184	200	204	208	212		152	168	172	176			296	300			
A4	148		200	204	208	212	268		152	156	168	172	176		288	296	314		
A5	148		184	192	212	256			152	156	168	172	176		288	296	304		
A6	148		184	204	208	212	216	244	152	156	168	172			288	296	304		
A7	148		184	204	208	212	216	244	152	156	160	168	172		288	296	300	304	
A8	148		184	200	204	208	268		152	156	160	168	172	176	288	296	300	304	314
A9	148		192	212	220	240			152	156	172	188			288	296	300	310	
B1	148	164	184	192	196	200	208	232	144	148	164	168	172		288	296	300	304	
B2	148	164	200	204	212	220			160	184	168	172	184		292	296	300	304	
B3	148	164	192	196	208	232			156	168	172	180			292	296	300	304	
B4	148	164	184	192	196	200	208	232	144	148	164	168	172		288	292	296	300	304
B5	148	164	184	192	196	200	208	232	144	148	152	164	168	172	288	292	296	300	304
B6	148		184	192	196	200	208	232	144	148	152	168			288	296	300		
B7	148	164	196	208	216	220	232		144	148	152	164	168	172	288	292	296	300	304
B8	148		200	204	212	216			148	156	168	176			288	292	296	300	304
B9	148	164	184	200	204	212	220	224	148	168	172	184	188		296	300	310		
C1	148	164	200	204	212	216	220		152	156	160	176			288	296	300	314	
C2	148	164	200	204	212	216	220		152	156	160	176			288	296	300	314	
C3	148	164	200	204	212	216	220		152	156	160	176			288	296	300	314	
C4	160	164	220	224					144	172					288	296	314		
C5	148	164	200	204	212	216	220		152	156	160	176			288	296	300	314	
C6	148	164	200	204	212	216	220		152	156	160	176			288	296	300	314	

Results of genetic analysis analyzing the locations of base pair repeats and specific markers across leaf samples at patches A, B, and C. Samples are labeled by patch and replicate within the patch. The numbers listed in the table above represent the number of repeats present at different loci in each marker analyzed. Microsatellite primers used were based on Beck et al. (2014).

Appendix B

Summary of field sampled *S. altissima* foliar terpenes (mg/g) grouped by distance at 10 cm intervals. Mean ± standard error of the mean.

	Summary of foliar terpenes by distance from inducer (mg/g)															
Distance from Inducer (cm):	0	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150
α-pinene	0.097 ± 0.025	0.058 ± 0.013	0.045 ± 0.016	0.12 ± 0.031	0.058 ± 0.015	0.082 ± 0.026	0.085 ± 0.028	0.08 ± 0.022	0.076 ± 0.025	0.062 ± 0.027	0.06 ± 0.014	0.055 ± 0.021	0.05 ± 0.016	0.033 ± 0.009	0.046 ± 0.012	0.037 ± 0.01
camphene	0.006 ± 0.004	0.009 ± 0.004	0.009 ± 0.004	0.016 ± 0.005	0.003 ± 0.002	0.011 ± 0.004	0.005 ± 0.004	0.006 ± 0.004	0.011 ± 0.005	0.008 ± 0.004	0.004 ± 0.002	0.005 ± 0.003	0.006 ± 0.003	0.001 ± 0.001	0.006 ± 0.002	0.004 ± 0.002
β-pinene	0.05 ± 0.01	0.073 ± 0.017	0.089 ± 0.019	0.121 ± 0.033	0.14 ± 0.042	0.119 ± 0.02	0.111 ± 0.024	0.076 ± 0.019	0.052 ± 0.01	0.062 ± 0.017	0.08 ± 0.019	0.055 ± 0.014	0.062 ± 0.016	0.056 ± 0.019	0.09 ± 0.031	0.05 ± 0.014
myrcene	0.041 ± 0.015	0.049 ± 0.016	0.049 ± 0.022	0.094 ± 0.025	0.073 ± 0.022	0.073 ± 0.018	0.062 ± 0.016	0.059 ± 0.016	0.05 ± 0.013	0.056 ± 0.014	0.05 ± 0.014	0.026 ± 0.009	0.052 ± 0.017	0.044 ± 0.011	0.049 ± 0.015	0.042 ± 0.007
α-phellandrene	0	0	0	0.001 ± 0.001	0	0	0	0	0	0	0	0	0	0	0	0
p-cymene	0.078 ± 0.035	0.047 ± 0.016	0.067 ± 0.031	0.095 ± 0.03	0.093 ± 0.022	0.086 ± 0.025	0.081 ± 0.026	0.079 ± 0.024	0.065 ± 0.016	0.042 ± 0.011	0.056 ± 0.025	0.026 ± 0.012	0.086 ± 0.031	0.05 ± 0.019	0.053 ± 0.022	0.052 ± 0.014
limonene	0.001 ± 0.001	0	0	0.001 ± 0.001	0	0	0	0	0	0	0	0	0.007 ± 0.007	0	0	0
ocemene isomers	0.001 ± 0.001	0.012 ± 0.009	0.04 ± 0.025	0.035 ± 0.021	0.004 ± 0.003	0.052 ± 0.035	0.033 ± 0.023	0.041 ± 0.029	0.017 ± 0.013	0.007 ± 0.006	0.056 ± 0.033	0.063 ± 0.037	0.002 ± 0.002	0.009 ± 0.006	0.031 ± 0.015	0.015 ± 0.007
R(+) camphor	0	0	0	0.001 ± 0.001	0	0	0	0	0	0	0	0	0	0	0	0
methyl salicylate	0.001 ± 0.001	0	0	0.001 ± 0.001	0	0	0	0	0	0	0	0	0	0	0	0
bornyl acetate	0.053 ± 0.034	0.039 ± 0.012	0.032 ± 0.012	0.076 ± 0.018	0.027 ± 0.008	0.065 ± 0.018	0.061 ± 0.024	0.054 ± 0.013	0.045 ± 0.014	0.038 ± 0.011	0.033 ± 0.01	0.042 ± 0.017	0.034 ± 0.01	0.021 ± 0.008	0.03 ± 0.01	0.027 ± 0.01
β-elemene	0.051 ± 0.029	0.037 ± 0.008	0.059 ± 0.019	0.082 ± 0.018	0.068 ± 0.02	0.071 ± 0.021	0.075 ± 0.019	0.052 ± 0.01	0.047 ± 0.013	0.043 ± 0.011	0.047 ± 0.013	0.062 ± 0.023	0.044 ± 0.017	0.035 ± 0.008	0.04 ± 0.012	0.04 ± 0.008
(-)-trans caryophyllene	0.06 ± 0.015	0.043 ± 0.006	0.051 ± 0.01	0.074 ± 0.014	0.066 ± 0.011	0.096 ± 0.01	0.075 ± 0.01	0.052 ± 0.01	0.037 ± 0.007	0.038 ± 0.009	0.055 ± 0.015	0.048 ± 0.011	0.038 ± 0.011	0.033 ± 0.007	0.036 ± 0.006	0.042 ± 0.008
α-humulene	0.02 ± 0.006	0.017 ± 0.005	0.023 ± 0.008	0.034 ± 0.01	0.032 ± 0.01	0.044 ± 0.006	0.031 ± 0.006	0.025 ± 0.007	0.012 ± 0.003	0.012 ± 0.003	0.022 ± 0.007	0.019 ± 0.004	0.017 ± 0.008	0.013 ± 0.006	0.016 ± 0.004	0.015 ± 0.003
cis carophyllene	0.004 ± 0.003	0.001 ± 0.001	0.003 ± 0.002	0.006 ± 0.003	0.005 ± 0.003	0.007 ± 0.004	0.004 ± 0.003	0	0	0.002 ± 0.002	0.003 ± 0.003	0	0	0	0	0.001 ± 0.001
germacrene D	0.905 ± 0.387	0.947 ± 0.142	1.209 ± 0.235	1.625 ± 0.297	1.371 ± 0.187	2.346 ± 0.273	2.066 ± 0.573	1.207 ± 0.198	0.847 ± 0.148	0.966 ± 0.245	1.121 ± 0.271	0.942 ± 0.206	0.873 ± 0.231	0.76 ± 0.171	0.792 ± 0.148	1.016 ± 0.217
β-farnesen	0.022 ± 0.01	0.015 ± 0.002	0.026 ± 0.006	0.032 ± 0.007	0.032 ± 0.007	0.042 ± 0.009	0.042 ± 0.009	0.027 ± 0.007	0.013 ± 0.005	0.016 ± 0.006	0.02 ± 0.006	0.023 ± 0.009	0.02 ± 0.009	0.015 ± 0.005	0.019 ± 0.006	0.023 ± 0.004
azulene	0.04 ± 0.026	0.057 ± 0.026	0.085 ± 0.035	0.093 ± 0.043	0.079 ± 0.035	0.138 ± 0.039	0.046 ± 0.019	0.065 ± 0.031	0.016 ± 0.008	0.017 ± 0.008	0.056 ± 0.027	0.034 ± 0.016	0.053 ± 0.032	0.038 ± 0.023	0.04 ± 0.02	0.035 ± 0.013
α-farnesene	0.003 ± 0.002	0.003 ± 0.002	0.001 ± 0.001	0.006 ± 0.002	0.017 ± 0.012	0.015 ± 0.003	0.005 ± 0.002	0.006 ± 0.004	0.009 ± 0.006	0.008 ± 0.005	0.021 ± 0.01	0.021 ± 0.007	0.013 ± 0.007	0.007 ± 0.004	0.003 ± 0.003	0.004 ± 0.002
γ-elemene	0.018 ± 0.012	0.006 ± 0.003	0.012 ± 0.008	0.024 ± 0.009	0.03 ± 0.009	0.039 ± 0.016	0.024 ± 0.011	0.009 ± 0.006	0.001 ± 0.001	0.002 ± 0.002	0.005 ± 0.003	0.01 ± 0.007	0.001 ± 0.001	0.002 ± 0.002	0.004 ± 0.004	0.01 ± 0.004
MeJA Isomers	0.48 ± 0.344	0.774 ± 0.365	1.001 ± 0.499	1.19 ± 0.514	1.207 ± 0.653	1.624 ± 0.558	0.951 ± 0.403	0.957 ± 0.421	0.352 ± 0.12	0.378 ± 0.115	0.803 ± 0.37	0.547 ± 0.221	0.781 ± 0.455	0.527 ± 0.292	0.493 ± 0.216	0.477 ± 0.179
ledene oxide	0.002 ± 0.002	0.003 ± 0.002	0.002 ± 0.002	0.005 ± 0.003	0.006 ± 0.003	0.008 ± 0.003	0.004 ± 0.002	0.003 ± 0.002	0.001 ± 0.001	0	0.003 ± 0.003	0.01 ± 0.008	0.002 ± 0.002	0	0	0.004 ± 0.002
bicyclo[4.4.0] dec-5-ene	0	0	0	0.001 ± 0.001	0	0.002 ± 0.001	0	0	0	0	0	0	0	0	0	0

Vita

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