THE EFFECTS OF GENOTYPE AND SPATIAL SCALE ON THE ASSOCIATED POLLINATOR COMMUNITY OF *SOLIDAGO ALTISSIMA*

A Thesis
by
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Abstract

THE EFFECTS OF GENOTYPE AND SPATIAL SCALE ON THE ASSOCIATED POLLINATOR COMMUNITY OF *SOLIDAGO ALTISSIMA*. (August 2016)

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Plant-pollinator interactions are among the most important mutualisms, as pollination is a necessary ecological service that contributes to the maintenance of biodiversity and ecosystem functioning. The plant *Solidago altissima* has been used to observe the effects of intraspecific genetic variation on arthropods, though pollinators have largely been ignored. My thesis research examines the relationships between phytochemistry, spatial scale and the pollinator community in *S. altissima*. I was interested in the trait variation within and between fields so that the role of genetic variation within patches (genetic identity effect) could be compared to effects of spatial scale (environment effect). I expected to find differences in both terpenes and the pollinator community between genotypes, and that there would be a greater impact of plant genotypic variation on the associated insect pollinator community of *S. altissima* partly due to terpenes.

I used four established populations of *S. altissima* as my sites and marked four patches within each site to observe during my pollinator surveys. Surveys lasted 5

minutes each and were conducted three times per patch throughout the blooming period. At the conclusion of the pollinator surveys, I collected inflorescences from all plants within each patch. Terpenes were extracted from flowers and analyzed using gas chromatography. Additionally, a small number of samples were analyzed to observe differences in terpenes between the flower and calyx. I collected soil cores from each patch to analyze soil nitrogen and the nitrogen to carbon ratio. I used one-way ANOVA to partition the effects of site from genotype in a general linear model. I used simple linear regression to find any potentially meaningful relationships between the pollinator community and terpenes. I also used partial least squares regression (PLSR) to model the effects of phytochemistry on pollinators.

Pollinator abundance and diversity were influenced more by statistical differences between patches, suggesting that in part genotypic variation played a larger role than the spatial separation of sites. Additionally, the concentrations and proportions of individual terpenes varied among patches. I found several significant relationships between certain pollinator taxa and terpenes. Overall, my results suggest that pollinators of *S. altissima* use terpenes when choosing host-plants. Though some of my relationships were relatively weak (low r^2), these data lend evidence that terpenes could play a role in genotype choice in my study. I also found that suites of compounds extracted from flowers related to pollinator abundance and diversity as groups of terpenes accounted for much of the variation observed in pollinator abundance and community measures. Though my data support the potential role of terpenes in the choice of genotypes by pollinators, my experimental design does not allow a definitive explanation for differences among patches in pollinator abundance, richness, and community evenness. Even so my study is strongly suggestive that further studies, including experiments designed to examine pollinator species preferences for terpenes, are warranted.

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Dedication

I dedicate this thesis to my family and friends, who have offered me so much support during this endeavor. In particular, to my mother Gaye Groot Johnson who has always been there for me with smile on her face. I am increasingly fortunate to have the never ending support and encouragement from my best friend and love of my life David Joel Kale. While I absolutely love the research that I have poured myself into, I would have had a far less enjoyable experience without you by my side.

You've a place in my heart no one else could have.

- F. Scott Fitzgerald

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INTRODUCTION

Community Genetics

The field of community genetics examines the interplay between ecology and evolution and how this interaction influences the diversity of communities and ecosystems (Antonovics 1992; Fritz and Price 1988; Whitham et al. 2003, 2006, 2008; Hughes and Stachowitcz 2004; Johnson and Agrawal 2005; Wimp et al. 2005; Johnson and Stinchcombe 2007). Antonovics (1992) first presented community genetics when she brought to light how little understood the effect that genetic variation within a species might have on the processes involved in species interactions, recognizing that this has evolutionary implications. Indirect genetic interactions take place when the genotype of a dominant foundation species influences the relative fitness of other species in a community (Whitham et al. 2008). Genetic variation lays the foundation for biodiversity, and thus evolution (Hughes et al. 2008).

Since the emergence of community genetics as a field of study, research has shown that plant intraspecific variation and diversity may affect community and ecosystem functioning. Such variation influences ecosystem processes such as leaf litter decomposition (Madritch et al. 2006; Lecerf and Chauvet 2008), nutrient cycling (Madritch et al. 2006), microbial respiration (Seliskar et al. 2002), and plant growth and subsequent resource availability (Seliskar et al. 2002). These impacts have been demonstrated in multiple tree genera, such as *Populus* (Whitham et al. 2003), *Eucalyptus* (Dungey et al. 2000), and *Pinus* (Brown et al. 2001; Whitham et al. 2003). For herbaceous species, in an experiment using eelgrass (*Zostera marina*), Hughes and Stachowicz (2004) found that the long-term damage

to plant density caused by grazing animals is significantly reduced by increased genotypic diversity. Further investigation manipulating the biomass of *Z. marina* confirms that genetic diversity can influence a population's response to disturbance (Hughes and Stachowicz 2011). The results from these experiments suggest that genetic diversity provides stability for ecosystems.

Arthropod Connection

Some studies in community genetics have focused on the influence of plant genetic variation on associated faunal communities. Much of the relevant research attempts to understand how intraspecific genetic variation in host plants affects associated arthropod communities (Fritz and Price 1988; Whitham et al. 2003, 2006, 2008; Hochwender and Fritz 2004; Wimp et al. 2004, 2005; Johnson and Agrawal 2005; Crutsinger et al. 2006; Johnson and Stinchcombe 2007; Genung et al. 2010; Hersch-Green et al. 2011; Burkle et al. 2013). Using cottonwoods, a dominant riparian tree, Wimp et al. (2004) found that the majority of the variation in arthropod diversity was due to genetic variation among the cottonwood stands. In a subsequent study, Wimp et al. (2005) found a strong correlation between arthropod species composition and a particular allele in the trees. Varying genotype and spatial scale, Johnson and Agrawal (2005) found that differences among *Oenothera biennis* (evening primrose) genotypes accounted for much of the variation in arthropod diversity, as well as richness, evenness, and abundance. Additionally, they found heritable variation in these arthropod community measures as well as choosiness in the herbivore community in selecting a host plant, suggesting that evolution in *O. biennis* can lead to changes in the arthropod community. Arthropod species richness and community structure are determined

in part by genetic diversity and variation (Crutsinger et al. 2006, 2008a, b, 2009). Crutsinger et al (2006, 2008a) manipulated *Solidago altissima* at the plot level and demonstrated that increasing genotypic diversity increases arthropod species richness, explained in part by the effects on plant productivity. Additionally, they found this effect to be non-additive, suggesting some genotypes contribute more than others to the observed difference the arthropod community. However, the impact that genotypic diversity has on associated communities varies between foliage-based and litter-based arthropod communities, demonstrating that the importance of these effects varies (Crutsinger et al. 2008b). The genetic variation of *S. altissima* also influences the diversity of associated arthropod communities across trophic levels and spatial scales (Crutsinger et al. 2009). While extremely relevant, the research Crutsinger and colleagues conducted did not address the pollinator community. Beyond the Solidago model using a willow (*Salix* sp.) and sawflies system, Fritz and Price (1988) demonstrated that genetic differences among willow clones affected sawfly density and oviposition, suggesting that the genetic variation of host plants strongly influences the community structure of some phytophagous insects. Studies exploring the relationship between a dominant riparian tree (Salicaceae: *Populus*) and its associated arthropod community showed that plant genetic diversity played a role in structuring arthropod diversity (Wimp et al. 2004) and that the associated arthropod community was influenced by genetic differences among cottonwood trees (Wimp et al. 2005). It seems clear that intraspecific genetic variation is important for structuring arthropod communities, especially those associated with foundation plant systems (Fritz and Price 1988; Whitham et al. 2003, 2006, 2008; Hochwender and Fritz 2004; Wimp et al. 2004, 2005; Johnson and Agrawal 2005; Crutsinger et al. 2006; Johnson and Stinchcombe 2007; Hersch-Green et al.

2011). This is relevant for my study, as a widespread and often numerically dominant plant species (S. *altissima*) was used as my plant model system. My focus on the contribution of spatial scale, genetic identity and phytochemistry fills a necessary gap in our understanding of the importance of plant intraspecific genetic variation to pollinator communities.

As presented above, though research has focused on the influence that intraspecific variation and genotype identity in plant species such as *S. altissima* have on arthropod communities (Crutsinger et al. 2006; Genung et al. 2012a, b), less attention has been given to pollinators. Studies investigating floral visitors have found connections between the pollinator community and host-plant intraspecific diversity and variation. Genung et al. (2010) found that the genetic diversity of *S. altissima* indirectly influences pollinator abundance and richness through its effects on floral abundance. Burkle et al. (2013) found variation in the floral visitor community among genotypes of *S. altissima*, concluding that host plant genetic variation is a critical component in structuring the diversity and composition of floral visitors. Though not investigated in that study, it is possible that floral phytochemicals serve as signals, indicating the presence of a reward, for these floral visitors. While some research has focused on the terpene variation in *S. altissima* (Williams and Avakian 2015), floral terpenes and their relationship with pollinators have been largely unexplored.

Insect Pollinators and Genetic Variation

Plant-pollinator interactions are among the most important mutualisms, as pollination is a necessary ecological service that contributes to the maintenance of biodiversity and ecosystem functioning (Costanza et al. 1997; Balvanera et al. 2005). The majority of

angiosperms rely on animal-mediated pollination for reproduction (Tepedino 1979; Buchmann and Nabhan 1996; Kearns et al. 1998). Insects are the most abundant animal pollinators, and many represent the most effective pollinators (Encinas-Viso et al. 2014). The European honeybee *Apis mellifera* is well-known for its importance in effectively pollinating commercially significant crops in the Americas (Free 1970; McGregor 1976; Canto-Aguilar and Parra-Tabla 2000; Ribeiro et al. 2015). Native pollinators are equally important to agricultural success and ecosystem functioning. Research comparing the pollination efficiency of native bees to that of *A. mellifera*, demonstrates that some native bees are as effective, if not more effective at pollinating certain crops (O'Toole 1993; Richards 1996). While hymenopterans, particularly bees, are often the most efficient pollinators, insects of other orders serve important roles as pollinators and can influence ecological processes related to pollination. Members of the orders Lepidoptera, Diptera, and Coleoptera are well known pollinators (Gross and Werner 1983; Huth and Pellmyr 2000; Ramirez 2004; Li et al. 2011; Pohl et al. 2011; Chen et al. 2014). Many taxa within Order Diptera, such as syrphid flies, blow flies, muscoid flies, and bee flies, are commonly found foraging among flowers (Kastinger and Weber 2001). Some beetles, such as *Megacyllene robiniae*, *Chauliognathus pennsylvanicus*, and *Epicauta pennsylvanica* feed on the pollen of herbaceous plants (Robertson 1928; Blackwell and Powell 1981; Gross and Werner 1983; Buchele et al. 1992).

Insect pollinators perceive various signals from angiosperms, and understanding these processes is critical in our understanding host-plant selection. Some of the more apparent mechanisms for pollinator choice of host plants include tactile cues, visual cues, and chemical cues emitted by plants. Because the surface of flower petals is flat by default, there must be some sort of evolutionary advantage to developing textured surfaces (Whitney et al.

2009). Bees have demonstrated the capacity for detecting differences in the microtexture of flower petals, the pattern of which changes from one end of a petal to the other and varies among species. Some pollinators to use this change in microtexture as a nectar guide as well as a way to discriminate between flowers of different plant species (Kevan and Lane 1985). The majority of pollinating insects heavily rely upon visual cues from flowers, such as floral traits like color, corolla tube length, and petal shape and size and movement. The vast spectrum of color that flowers can take on makes floral color an incredibly diverse trait, varying between species, within populations, and among individuals (He et al. 2011; Tang and Huang 2012; Sobral et al. 2015). The diversification of this trait is likely due to co-evolution with pollinating animals (Fenster et al. 2004; Gegear and Laverty 2005) and the selective pressures these pollinators place on plants due to preferences in host-plants (Fenster et al. 2004; Frey 2004; Sobral et al. 2015). Floral color acts as a cue for potential pollinators, signaling the presence of a reward (i.e., pollen or nectar) or tricking the pollinator into thinking there is a reward (Waser and Price 1981; Waser and Price 1983; Campbell et al. 2010; Campbell et al. 2012; Sobral et al. 2015).

Research demonstrates that the quality and quantity of phytochemicals are meaningful to potential floral visitors (Najar-Rodriguez et al. 2010). More studies are emerging that focus on combinations of stimuli, for example visual and tactile cues or visual and olfactory cues (Alcorn et al. 2012, Song et al. 2015). While visual cues attract more approaches from potential pollinators, olfactory cues elicit more landings (Song et al. 2015). A possible explanation for this is that visual cues are stronger for long-distance foraging, while olfactory cues are better suited to short-distance foraging (Galizia et al. 2005; Song et al. 2015). It seems plausible that with the wide variety of potential traits assessed by

pollinators when choosing a plant that trait variation between individuals (or even more broadly genotypes) is important to consider.

Angiosperms display tremendous interspecific and intraspecific genetic variation in floral traits (Ollerton et al. 2011). This variation is largely attributed to pollinator preference, as the majority of flowering plants rely on animal-mediated pollination for sexual reproduction (Ollerton et al. 2011). Insect pollinators perceive various signals that flowering plants emit, and understanding the way pollinators perceive these signals is critical in our understanding of the importance of biodiversity and plant intraspecific variation. Many host-plant traits important for pollinator choice, such as floral structure, scent, and pollen quantity and quality, may vary among individuals since they are genetically-based (Raguso et al. 2007; Johnson et al. 2009; Chen et al. 2014; Yeamans et al. 2014). Because host plant genetic variation influences associated pollinator communities, evolutionary processes in plants should have consequences for both pollinators and the ecosystem services they support (i.e., pollination, climate regulation, and nutrient cycling) (Genung et al. 2010). Plant intraspecific variation in floral odor may come about through a variety of methods, yet determining the basis of this variation in natural populations of particular species remains a challenge (Ackerman et al. 1997; Azuma et al. 2001; Knudsen 2002; Schlumpberger and Raguso 2008). Since as mentioned olfaction is an important mechanism by which insects choose host plants (Hossaert-Mckey et al. 1994; Couty et al. 2006; Du and Wu 2007; Mazzoni 2009; Karpati et al. 2013), phytochemical variation among host plant genotypes likely plays an important role in structuring associated insect communities (Lindroth and Hwang 1996; Hwang and Lindroth 1998; Wimp et al. 2007).

Phytochemistry

Plants synthesize and emit a wide range of volatile chemicals that play important roles in ecosystem processes such as pollination. Many of these phytochemicals are secondary metabolites, including terpenes, alkaloids, and phenols, as well as fatty-acid derivatives, benzenoids, and other aromatics (Knudsen et al. 1993). Plants exhibit remarkable interspecific and intraspecific variation in their phytochemistry, even though similar compounds undergo synthesis through the same biological pathways despite plant species (Schwab et al. 2008). This genetic variation may affect plant visitors in different ways (Berenbaum and Zangerl 1992; Lankau 2007; Macel and Klinkhamer 2010). Plant volatiles vary among populations and genotypes (Gouinguene et al. 2001; Hare 2007; Wimp et al. 2007; Delphia et al. 2009; Whitehead and Peakall 2009). This intraspecific genetic variation results in particular genotypes producing varying amounts of different compounds (Holopainen et al. 1987; Grayer et al. 1996; Egerton-Warburton et al. 1998; Kleine and Muller 2011). Species of the genera *Oenothera* and *Solidago* also display great intraspecific variation in their secondary chemistry, and numerous studies have demonstrated the effects this has on associated insect communities (Raguso et al. 2007; Johnson et al. 2009; Smith 2015). Because insects rely heavily on their perception of scents (Chittka and Raine 2006; Milet-Pinheiro et al. 2015), it is likely that these genotype-unique compositions of terpenes (and possibly other volatile compounds) influence floral visitor choice in host plant. Because plants produce different compounds and in different amounts, insects have likely evolved to detect certain scents or scent bouquets while searching for host plants. Floral phytochemistry displays significant intraspecific variation in *Buddleja davidii* (butterfly bush) and certain compounds attract pollinating butterflies to host plants (Chen et al. 2014).

Terpenes make up the largest and most diverse class of plant secondary metabolites (Langenheim 1994; Trapp and Croteau 2001) and serve as both attractants and deterrents for different types of insects (Langenheim 1994; Goncalves et al. 2015; Zeilinger et al. 2015). Williams and Avakian (2015) found that terpene variation among genotypes of *S. altissima* explained substantial variation in the abundance of a specialist aphid, *Uroleucon nigrotuberculatum*. Previous research conducted in the Williams laboratory at Appalachian State University support the notion that terpenes vary among genotypes of *S. altissima*, and that this variation affects associated insects (Howells 2012; Smith 2015). While terpenes can influence insect use of host-plants, much of this research has focused on antagonistic relationships (i.e., phytophagy and herbivory; Raffa et al. 1985; Werner 1995; Barnola et al. 1997; Huber and Bohlmann 2004; Kleine and Mulleur 2011) rather than mutualistic relationships such as pollination. Insect floral visitors might be attracted to strong, complex terpene emissions, as the intensity and complexity of volatile floral emissions have been shown to influence biotic pollination (Farre-Armengol et al. 2015). Of course, floral compounds that exist in low concentrations or proportions should not be discounted, as they may also be important to pollinators (McCormick et al. 2014). Two sulfur-containing volatile compounds in some *Euconis* species have been shown to influence the attractiveness of host plants to pollinating fly species, despite their low abundance (Stensmyr et al. 2002; Shuttleworth and Johnson 2010; Jurgens et al. 2013). Yet, it is probable that mixtures of scents, rather than individual compounds, are necessary in attracting pollinators (McCormick et al. 2014).

Spatial Scale

Spatial scale is important to consider in community genetics research because the effects of plant genotypic variation may be scale-dependent (Stratton and Benington 1998; Johnson and Agrawal 2005; Crutsinger et al. 2009; Genung et al. 2011; Genung et al. 2012b; Burkle et al. 2013**)**. For example, observed differences between genotype traits could be due to differences at the gene level or because of environmental influences, or both. Thus spatial scale is an important component to include in studies that look at possible effects of plant genetic variation on associated insect communities – particularly for plant species with wide distributions. The importance of genetic variation is relative to the size of a given experiment, and the interpretation of results from community genetics studies may depend on the spatial scale observed (Hersch-Green et al. 2008; Tack et al. 2010). The Scale-Dependent Hypothesis (Menge and Olson 1990; Jackson et al. 2001) assumes that biotic factors are more important in structuring communities over small spatial scales (representative of genetic effects), while abiotic factors are more important for larger spatial scales (representative of environmental effects). Johnson and Agrawal (2005) examined spatial scale in a study that explored how an arthropod community was shaped by host-plant genotype. Their results support the idea that at a small spatial scale such as habitat, plant genotype is more important than environmental variation in structuring the arthropod community, but at larger scales it becomes less important. Spatial connectivity may also be an important factor pertaining to spatial scale and was found to be a greater determinant of insect communities than were genetic factors (Tack et al. 2010).

Solidago altissima as a Model System

Foundation species such as goldenrod support a large diversity of associated organisms (Ellison et al. 2005). The genus *Solidago* (goldenrod) is a well-understood plant system that has been used to address questions rooted in community genetics (see Crutsinger et al. 2006, Genung et al. 2012a). Certain characteristics of this genus, such as its wide distribution and reproductive attributes, make it particularly useful in answering questions concerning inter- and intraspecific genetic variation (Hakes and Cronin 2006). The aster *S. altissima* (tall goldenrod) is a perennial herb that may dominate old field habitats (Root 1996). The species is native to much of North America (Halverson et al. 2008; Fenesi et al. 2015; Zhao et al. 2015) and has a conspicuous architecture with yellow flowers. One of the earliest studies that examined goldenrod genetic variation found that genotypes of *S. altissima* varied in resistance to herbivorous insects, demonstrating heritable resistance (Maddox and Root 1987). *Solidago altissima* hosts over 100 insect species from a variety of feeding guilds (Maddox and Root 1987; Root and Cappuccino 1992; Root 1996). This goldenrod species may reach a height of 50-200 cm and has one or more free-standing stems that possess trichomes (FNA). Leaves are simple lanceolate, sometimes have serrate margins, and grow in an alternating pattern along the stem. Leaves have three notable main veins that can be observed on the underside (Mackenzie 1927). Inflorescences are terminal pyramidal panicles consisting of many branches and flower heads. A capitulum can contain 10 to 15 pistillate ray flowers surrounding 3 to 7 hermaphroditic disc flowers (Abrahamson and Weis 1997). Each flower (ray and disc) produces a single seed (Wise et al. 2008). Because the pollen of *S. altissima* is too heavy and sticky to be dispersed by wind, the genus largely relies on insect-mediated pollination for sexual reproduction (Gross and Werner 1983). Species

such as *S. altissima* are self-incompatible and exhibit obligatory outcrossing. The relationship with pollinators allows for the seed production that is necessary for colonizing new patches and increasing or maintaining intraspecific genetic diversity (Meyer and Schmid 1999; Burkle et al. 2013). *Solidago altissima* is particularly important to late season floral visitors since the species blooms in September to October. That is, these plants are typically some of the last sources of pollen and nectar for floral visitors before overwintering (Mader et al. 2011). Because this species is known to exhibit considerable intraspecific genetic variation and rely on pollinators exclusively it provides an excellent model system to address my research questions.

Objectives

My thesis research examines the relationships between phytochemistry, spatial scale and the pollinator community in *S. altissima*. I was interested in the trait variation within and between fields so that the role of genetic variation within patches (genetic identity effect) could be compared to effects of spatial scale (environment effect). My study had four primary objectives, to determine in *S. altissima if*:

- Genotype and site influenced the associated insect pollinator community.
- Genotypic (small-scale) and site (large-scale) influenced flower phytochemistry.
- Phytochemical variation and pollinator abundance and diversity within sites (genetic identity effect) was greater or lesser than that between sites (environment effect).
- The pollinator community was related to volatile terpenes in flowers.

I expected that genotype would have a greater effect on the abundance and diversity of the associated pollinator community, and that phytochemistry would vary among genotypes.

Additionally, I expected to find a meaningful relationship between the pollinator community and terpene variation.

My conclusions regarding effects of genotype were made with caution due to my experimental design, though analyzing differences between my study sites allowed for a clear interpretation of site effects and estimates as to where the major contributions to phytochemistry and the pollinator community arose.

MATERIALS AND METHODS

Experimental Design

Solidago altissima reproduces vegetatively by rhizomes, resulting in discrete patches composed of a single genotype (Gross and Werner 1983; Halverson et al. 2008; Burkle et al. 2013). Clonal reproduction provides a useful system to investigate intraspecific genetic diversity in this foundation plant species that often achieves high abundance in old-field ecosystems. *Solidago altissima* has a minimum threshold size required for sexual reproduction to occur, and there is an increasing probability of flowering with increasing size (Schmid et al. 1995). Plants will put resources into clonal growth, but if they possess sufficient resources to reproduce sexually, they will do so at a cost in vegetative reproduction (Ashmun and Pitelka 1985; Pitelka et al. 1985; Schmid et al. 1995). Previous investigations have demonstrated that *S. altissima* patches of 35 meters or more apart represent distinct genotypes (Crutsinger et al. 2006).

I used four sites in Watauga County, North Carolina, each containing four patches of *S. altissima*. Patches were assigned SF (State Farm, Site 1), TJ (Tom Jackson, Site 2), PKY (Parkway, Site 3), and 421 (U.S. 421, Site 4). GPS coordinates and elevation of patches within sites are found in Appendix 1. The site SF was located along the Kennedy Trail at the ASU State Farm greenway; TJ is an abandoned field located at the intersection of Castle Ford Road and Tom Jackson Road; PKY is located along the Blue Ridge Parkway off of Day Drive (Boone, NC), and 421 is an abandoned field located along the US 421 Highway in Vilas, NC. I chose sites that were similar in light availability, slope, elevation, and

surrounding environment (each site had a road along one side). In 2014 at each site I chose discrete patches of *S. altissima* that were a minimum of 35m apart, allowing for strong confidence in choosing different genets. Within each patch I chose a $1m^2$ area to sample plants and observe pollinators. Five individual stems within the $1m^2$ patch were chosen at random and subsequently marked using orange ribbon. These five individuals were my genotype replicates and were observed to characterize the pollinator community, as well as to collect flower samples for volatile terpene analysis.

Pollinator Surveys

I characterized the associated pollinator community by conducting three sets of surveys during the blooming period of September through October 2014 (4 sites X 4 patches X 3 visits = 48 total visits). Surveys were carried out on relatively warm days (12.8°C to 21.1°C) between 11:00-16:00 with either full or partial sun. I attempted to account for differences in flower phenology among the sites and patches by only surveying pollinators when approximately 75% or more of the flowers in a given patch were in bloom. Surveys were five minutes each. During this time any individual that landed on an inflorescence of a marked stem was counted. Insects were initially identified in the field and for some furtherer identified in laboratory using the supplemental information from Burkle et al. (2013) as a guide to the pollinators associated with *S. altissima*. This guide, in addition to field guides and dichotomous keys, allowed me to identify many of the pollinators to the species level. Insects I was unable to speciate were assigned to a morphospecies.

Terpene Samples and Analysis

At the conclusion of the pollinator surveys, inflorescences were collected from all plants observed in each patch to analyze flowers for terpenes. There were five samples collected per patch, for a total of 80 inflorescence samples in my experiment. Inflorescences were placed into plastic bags, stored in a cooler for transportation to the laboratory, and placed in a refrigerator for no more than 4 days. Processing the inflorescences entailed separating approximately 2 g of florets with the calyx from the peduncle. Samples were placed in 20 ml HPLC-grade pentane in glass culture tubes and stored at 4°C for a minimum of 6 weeks to extract terpenes from the flowers. Analysis of flower terpenes followed a modified procedure of protocols previously established in the Williams laboratory (see Williams and Avakian 2015). Each sample was ground for 60 seconds using a Polytron homogenizer and immediately filtered into 125x50 mm glass tube. Nitrogen gas was used to gently bubble the samples, concentrating them to 0.5 ml. A 1 µl sample was injected into a Shimadzu 14-A gas chromatograph with a flame ionization detector. The injector temperature was set to 250°C with the detector temperature set to 275°C. The starting temperature was held at 80°C for 2 minutes. The column temperature increased 10°C/minute until it reached 280°C, and was then held at this temperature for 2 minutes. The total run time was 24 minutes. The retention times of analytical standards were used to identify terpenes. Unknown compounds were labeled (Unknown 0 to Unknown *n*). Some compounds (example germacrene D) could be identified with high confidence based on previous studies in the Williams laboratory. Such compounds could be referred to as "tentatively identified" since no analytical standard was available. The internal standard (IS) tri-decane (Sigma-Aldrich) was

used to calculate compound concentration. Note that these samples including the calyx were used for any linear regressions with pollinator and other data.

In addition to the analysis of flower terpenes, in order to analyze the contribution of the calyx, which could contain terpenes, three samples were collected in September 2015 from each of two sites (SF and TJ). These samples were prepared the same way as previously described. Tweezers were used to carefully pull the calyx from the floret in each sample. Calyces from a given sample were placed into a separate tube from the rest of the florets for the same sample. The procedure for analyzing terpenes followed that described above. The concentration of each known terpene in each sample was calculated, and the mean of each terpene was used to find the percentage difference in concentrations between the flower and calyx. For 12 of the 14 terpenes present, the flower contained a higher percentage (6-92%) of the terpenes than did the calyx. The compounds (-)trans-caryophyllene and azulene were found in higher concentrations in the calyx than the flower (see Appendix 3).

Soil Nutrient Analysis

Soil cores were collected after the conclusion of pollinator surveys to examine potential variation in soil carbon, nitrogen, and their ratio among patches and sites, as this could affect flower terpene production. Four 15 cm samples were collected from each patch (4 sites X 4 patches X 4 samples = 64 total samples) using a 2.5cm X 15cm soil corer. Upon collection, individual samples were placed into a plastic bag and stored in a cooler for transportation to the laboratory. Soil samples were individually sieved using a 2 mm mesh sieve to remove rocks and plant matter. Samples were then placed into scintillation vials and freeze-dried. After drying, three bb pellets were placed into each scintillation vial, and

samples were ground for 5 minutes using a Pacer Industrial mixer. Once freezedried and ground to a very fine powder, each sample was measured between 25-30 mg and placed into a Costech Analytical Technologies, Inc. 5x9 mm tin capsules. Atropine was used as an analytical standard. The carbon and nitrogen concentration and their calculated ratio for soil samples were analyzed alongside the atropine standards and soil controls using a Flash EA112 (ThermoFisher) elemental analyzer.

Statistical Analysis

I calculated the coefficient of determination (r^2) in a general linear model (One-way ANOVA, JMP 12.1.0) to partition the effects of site (model SS) from genotype (part of error SS) for my dependent variables. In my analysis the site contribution is the coefficient of determination (r^2). The calculation $1\text{-}r^2$, which takes into account the error terms in my ANOVA model, in theory represents the genotype contribution. I recognize that other factors could have contributed to the experimental error in my model and that without replication of genotypes (not possible in my field observational experiment), definitive conclusions on a genotype contribution are tenuous. Nevertheless, the robustness of my model allows for clear conclusions as to the effect of site variation and conclusions about a genotype contribution, as least in part. Values of $P \leq 0.05$ are reported as significant, while values of $0.05 \leq P \leq 0.10$ are reported as marginally significant. For pollinators, four measures were analyzed: abundance, richness, adjusted richness, and evenness. Abundance was calculated as both total abundance for the three visits and mean abundance, using the mean of three visits. For the terpene analysis, while there were over 100 compounds found in some flower samples, many of these were unknown and could not be confirmed as terpenes. For this study only

compounds either positively identified using analytical standards, or compounds known with a high degree of confidence (see previous), are reported. Data were log transformed (log_e) to increase normality. A linear regression was used (JMP 12.1.0) to regress individual terpenes with insect and soil nutrient data measures. Cook's D Influence was used (JMP 12.1.0) to determine whether any outliers were significant enough to be excluded, and any such outliers were removed prior to the regressions (Cook 1979). Terpenes were analyzed in two ways: concentration (mg compound/g flower) and the allocation of individual terpenes in a sample. Data were arcsine transformed to increase normality.

The relationships between flower terpenes and pollinator measures were analyzed using Partial Least Squares Regression (PLSR; JMP Pro 10). This is an appropriate multivariate technique for modeling the effects of phytochemistry if collinearity exist between variables (see Wold 1984). Partial Least Squares Regression has been used for ecological investigations to examine the relationship between phytochemicals and insect performance (Couture et al. 2012) and in the Williams laboratory to relate terpenes to aphid abundance (Williams and Avakian 2015). For this analysis a two-factor model was used (see Cox and Gaudard 2013). In order to fit the most appropriate model to the data, the Score Scatterplot Matrix was examined and any points outside of the confidence circle were omitted as outliers. A Variable Importance Projection (VIP) determined the predictor variables (i.e., terpenes) in the model that showed the strongest response between both predictor and response matrices. Regressing observed versus predicted values provides the relationship between flower terpenes and pollinator measures.

RESULTS

Pollinator Community

Using *1- r^2* from my ANOVA model as previously described, I found that variation most likely due to *S. altissima* genotypes for total pollinator abundance contributed much more to my model than did site (r^2), where all but two taxa had a non-significant site effect (Table 1; Figs 1-4). Differences between sites explained generally less than 30% of the variation, with exceptions being for order Coleoptera and the eastern carpenter bee *Xylocopa virginica* (Table 1). Similar results were found when mean pollinator abundance was calculated, with exceptions again for order Coleoptera and *X. virginica* (Table 2; Figs 5-8).

Table 1. The *P* value, *F* ratio, and *df* (One-way ANOVA, JMP 12) of total pollinator abundance by taxa.

	p	*F*	r^2	$1\text{-}r^2$
All Pollinators	0.672	0.527	0.116	0.884
Hymenoptera	0.573	0.694	0.148	0.852
Diptera	0.365	1.161	0.225	0.775
Coleoptera	**0.073**	2.996	0.428	0.572
Lepidoptera	0.677	0.520	0.115	0.885
Apis mellifera	0.218	1.710	0.299	0.701
Bombus impatiens	0.569	0.701	0.149	0.851
Dominant Pollinators	0.447	0.952	0.192	0.808
Xylocopa virginica	**0.040**	3.803	0.487	0.513
Bee 1	0.626	0.602	0.131	0.869
Ichneumonidae	0.726	0.444	0.100	0.900
Braconidae	0.493	0.851	0.175	0.825
All Parasitoid Wasps	0.759	0.395	0.090	0.910
All Wasps	0.201	1.799	0.310	0.690
Syrphidae	0.185	1.890	0.321	0.679
Tachinidae	0.115	2.436	0.378	0.622
Sarcophagidae	0.734	0.432	0.097	0.903
Fly 1	0.394	1.081	0.213	0.787
Megacyllene robiniae	0.363	1.168	0.226	0.774
Chauliognathus pennsylvanicus	0.426	1.000	0.200	0.800
Cisseps fulvicollis	0.426	1.000	0.200	0.800
Atteva aurea	0.397	1.073	0.212	0.788

Note: $p \leq 0.05$ (significant) and $p \leq 0.10$ (marginally significant) presented in **bold** text. dominant pollinators is comprised of *A. mellifera* and *B. impatiens*, as they were the most abundant pollinators.
df = 3, 12.

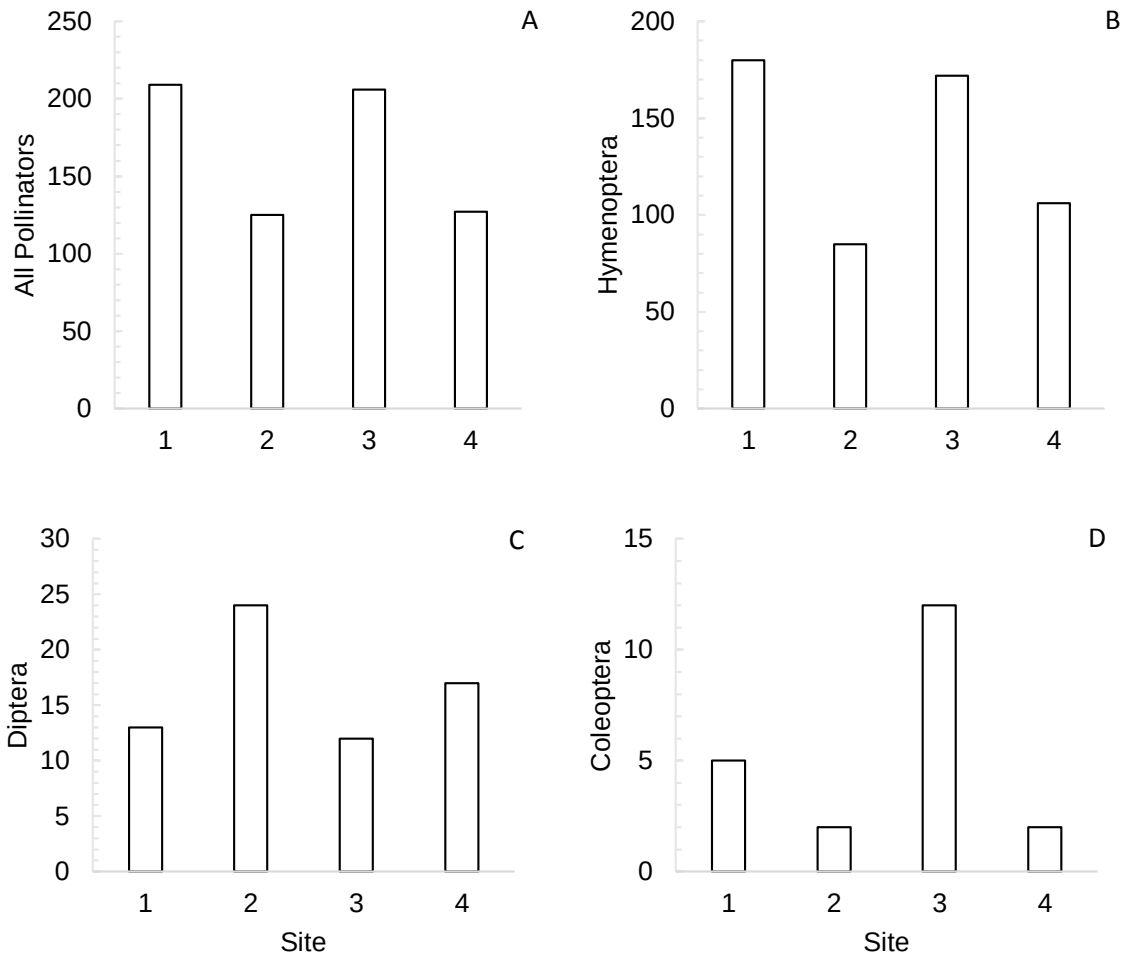

Fig 1. Total insect abundance by site for: all pollinators (A), Order Hymenoptera (B), Order Diptera (C), and Order Coleoptera (D).

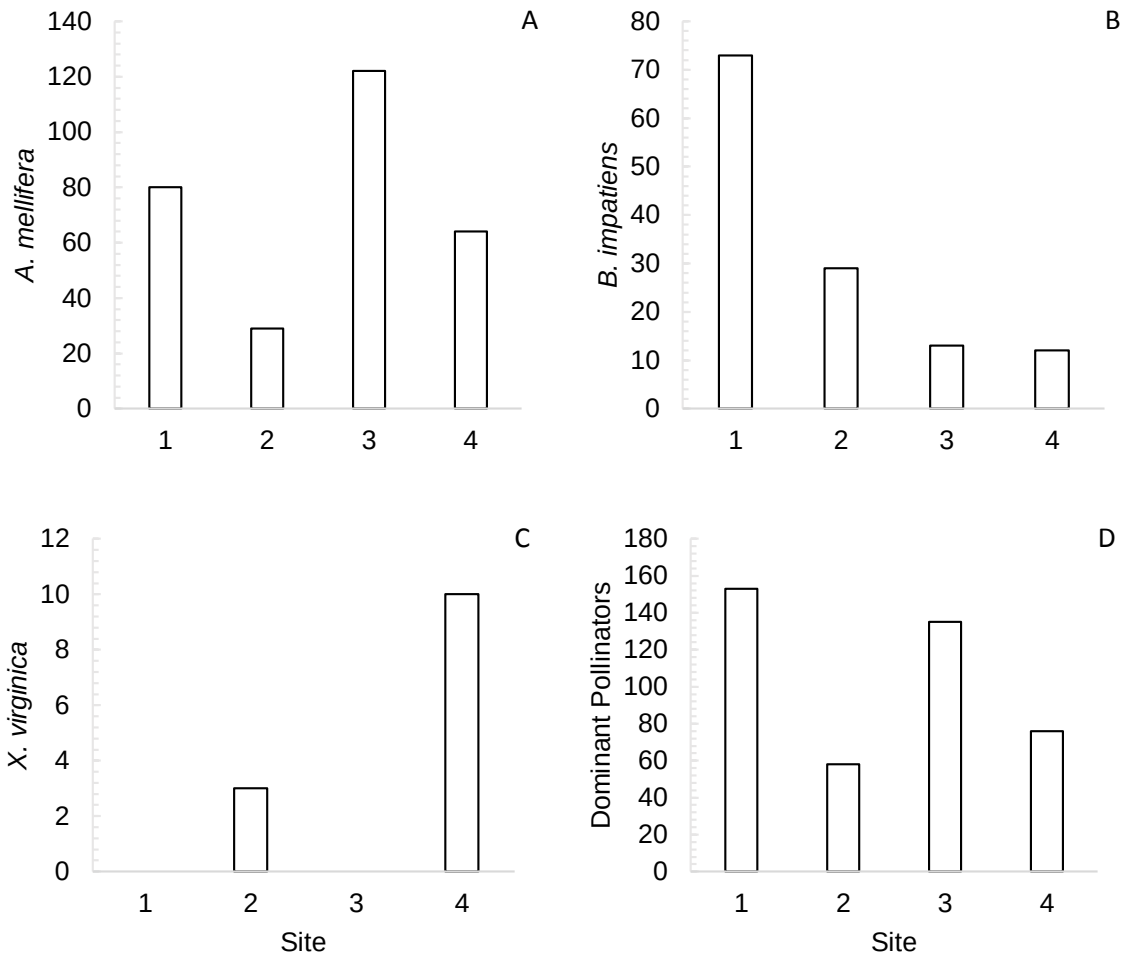

Fig 2. Total insect abundance by site for: *Apis mellifera* (A), *Bombus impatiens* (B), *Xylocopa virginica* (C), and dominant pollinators (D).

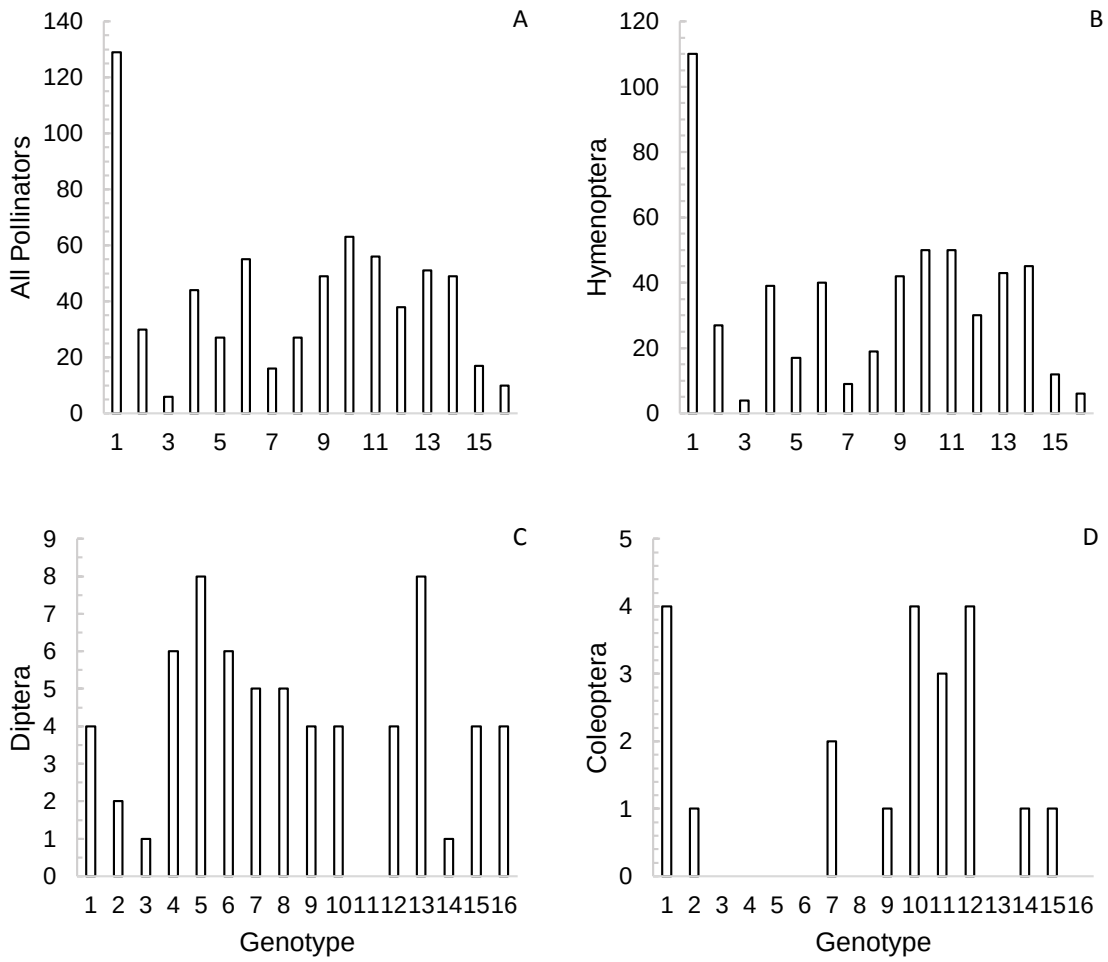

Fig 3. Total insect abundance by genotype for: all pollinators (A), Order Hymenoptera (B), Order Diptera (C), and Order Coleoptera (D).

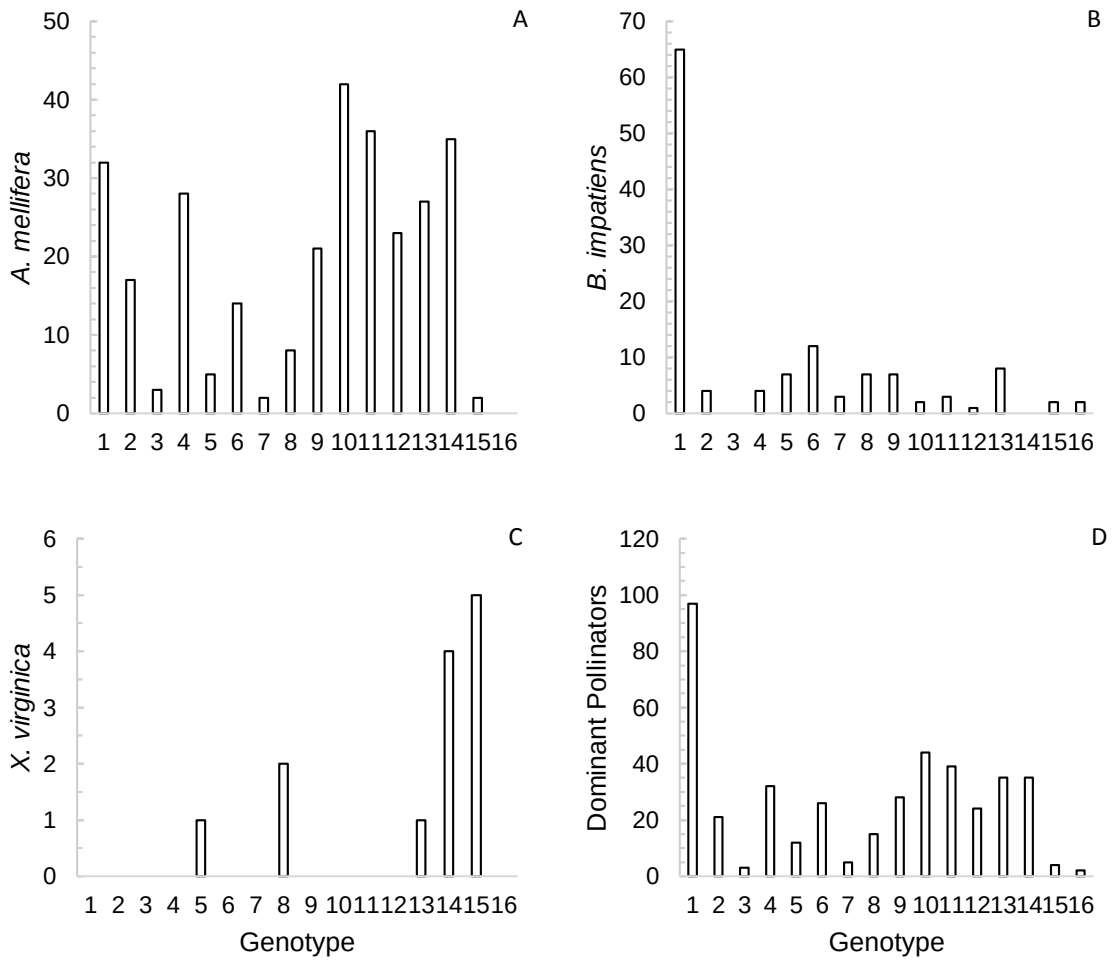

Fig 4. Total insect abundance by genotype for: *Apis mellifera* (A), *Bombus impatiens* (B), *Xylocopa virginica* (C), and dominant pollinators (D).

Table 2. The *P* value, *F* ratio, and *df* (One-way ANOVA, JMP 12) of mean pollinator abundance by taxa.

	p	*F*	r^2	$1\text{-}r^2$
All Pollinators	0.669	0.532	0.117	0.883
Hymenoptera	0.561	0.717	0.152	0.848
Diptera	0.293	1.393	0.258	0.742
Coleoptera	**0.070**	3.052	0.433	0.567
Lepidoptera	0.627	0.600	0.130	0.870
Apis mellifera	0.188	1.876	0.319	0.681
Bombus impatiens	0.574	0.693	0.148	0.852
Dominant Pollinators	0.455	0.933	0.189	0.811
Xylocopa virginica	**0.053**	3.422	0.461	0.539
Bee 1	0.649	0.565	0.124	0.876
Ichneumonidae	0.668	0.534	0.118	0.882
Braconidae	0.484	0.868	0.178	0.822
All parasitoid wasps	0.776	0.370	0.085	0.915
All wasps	0.275	1.460	0.267	0.733
Syrphidae	0.217	1.714	0.300	0.700
Tachinidae	0.126	2.326	0.368	0.632
Sarcophagidae	0.640	0.578	0.126	0.874
Fly 1	0.383	1.112	0.218	0.782
Megacyllene robiniae	0.361	1.171	0.226	0.774
Chauliognathus pennsylvanicus	0.426	1.000	0.200	0.800
Cisseps fulvicollis	0.426	1.000	0.200	0.800
Atteva aurea	0.385	1.107	0.217	0.783

Note: $p \leq 0.05$ (significant) and $p \leq 0.10$ (marginally significant) presented in **bold** text. $df = 3, 12$.

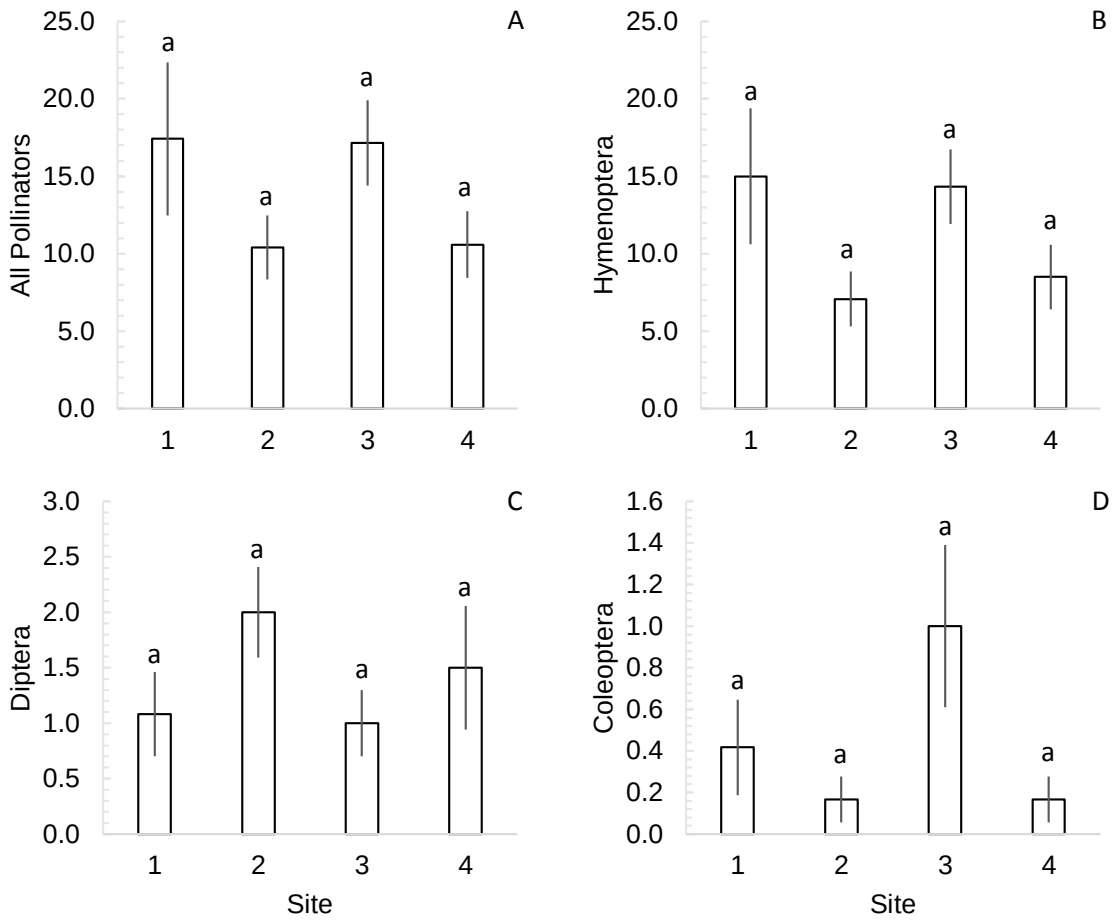

Fig 5. Mean insect abundance by site for: all pollinators (A), Order Hymenoptera (B), Order Diptera (C), and Order Coleoptera (D).

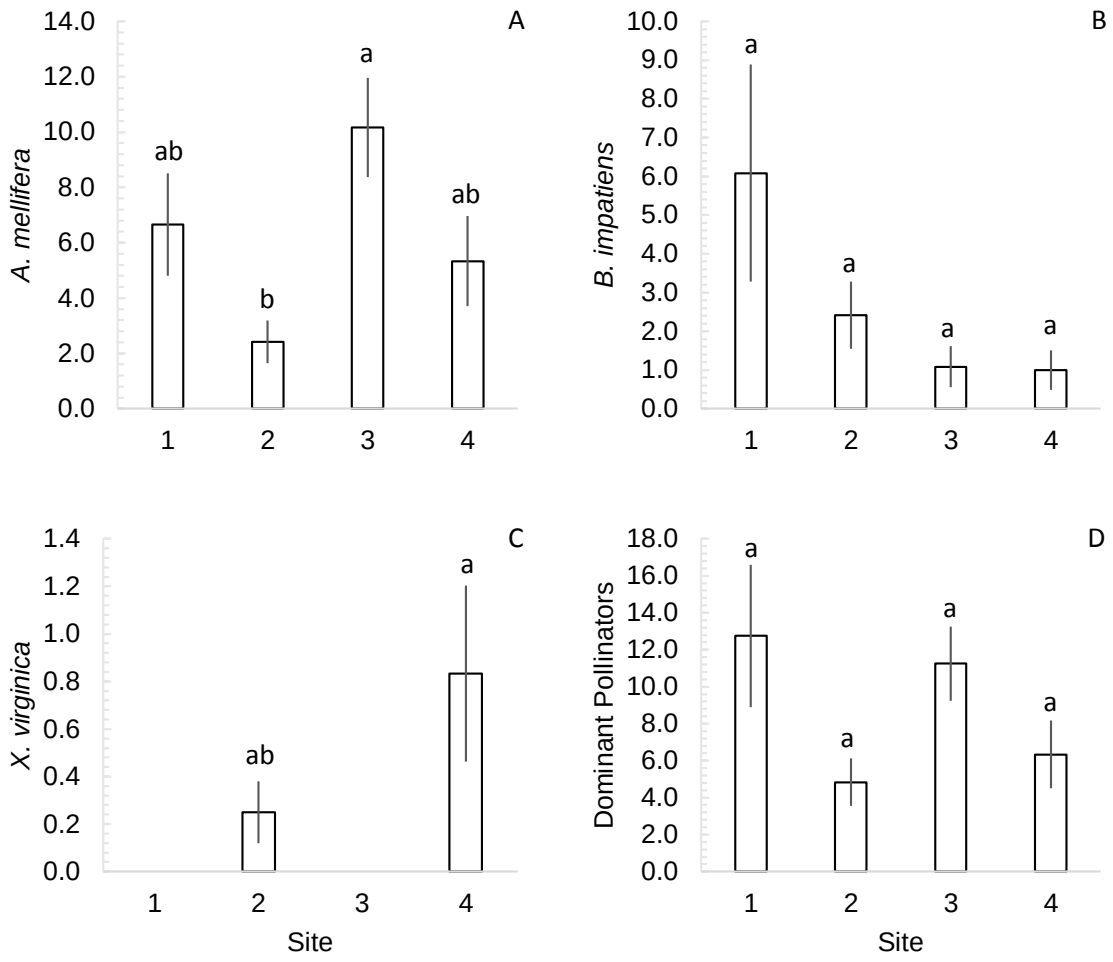

Fig 6. Mean insect abundance by site for: *Apis mellifera* (A), *Bombus impatiens* (B), *Xylocopa virginica* (C), and dominant pollinators (D).

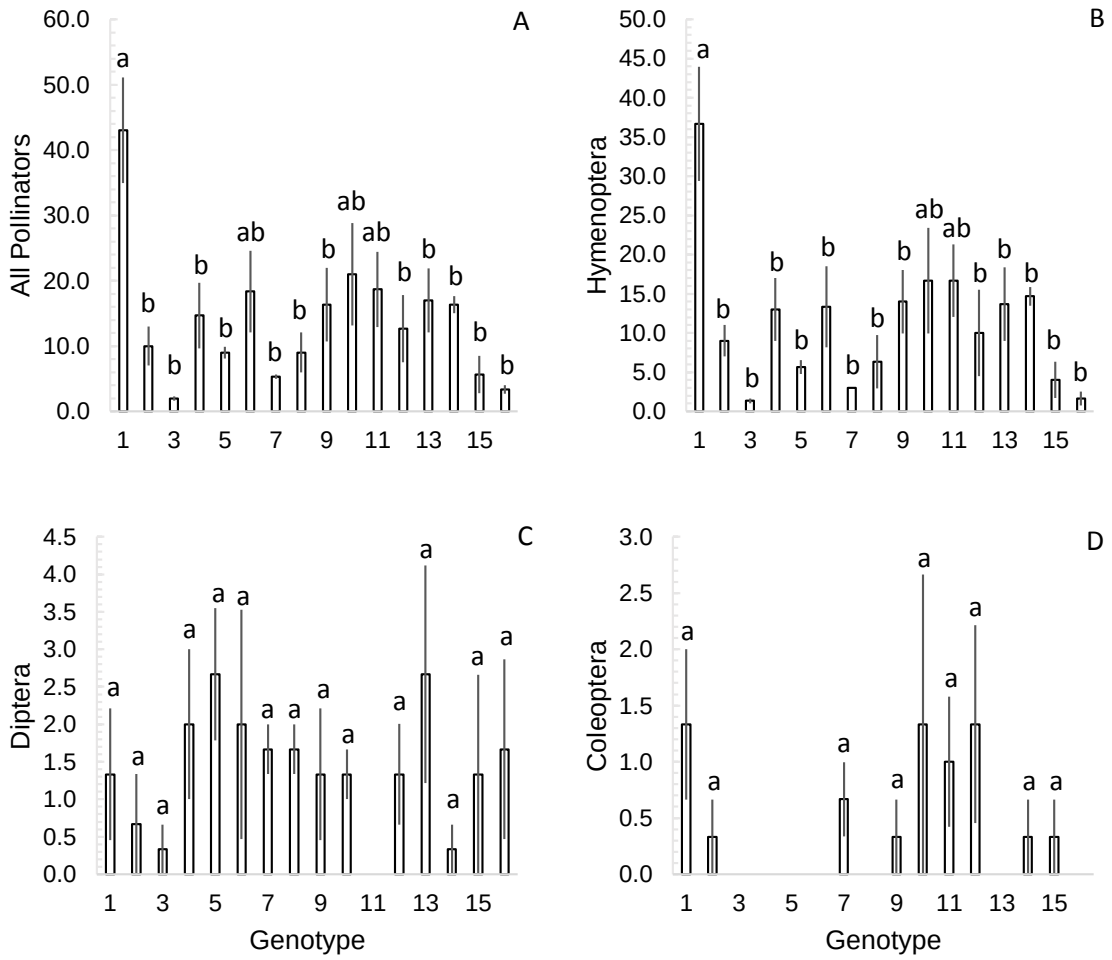

Fig 7. Mean insect abundance by genotype for: all pollinators (A), Order Hymenoptera (B), Order Diptera (C), and Order Coleoptera (D).

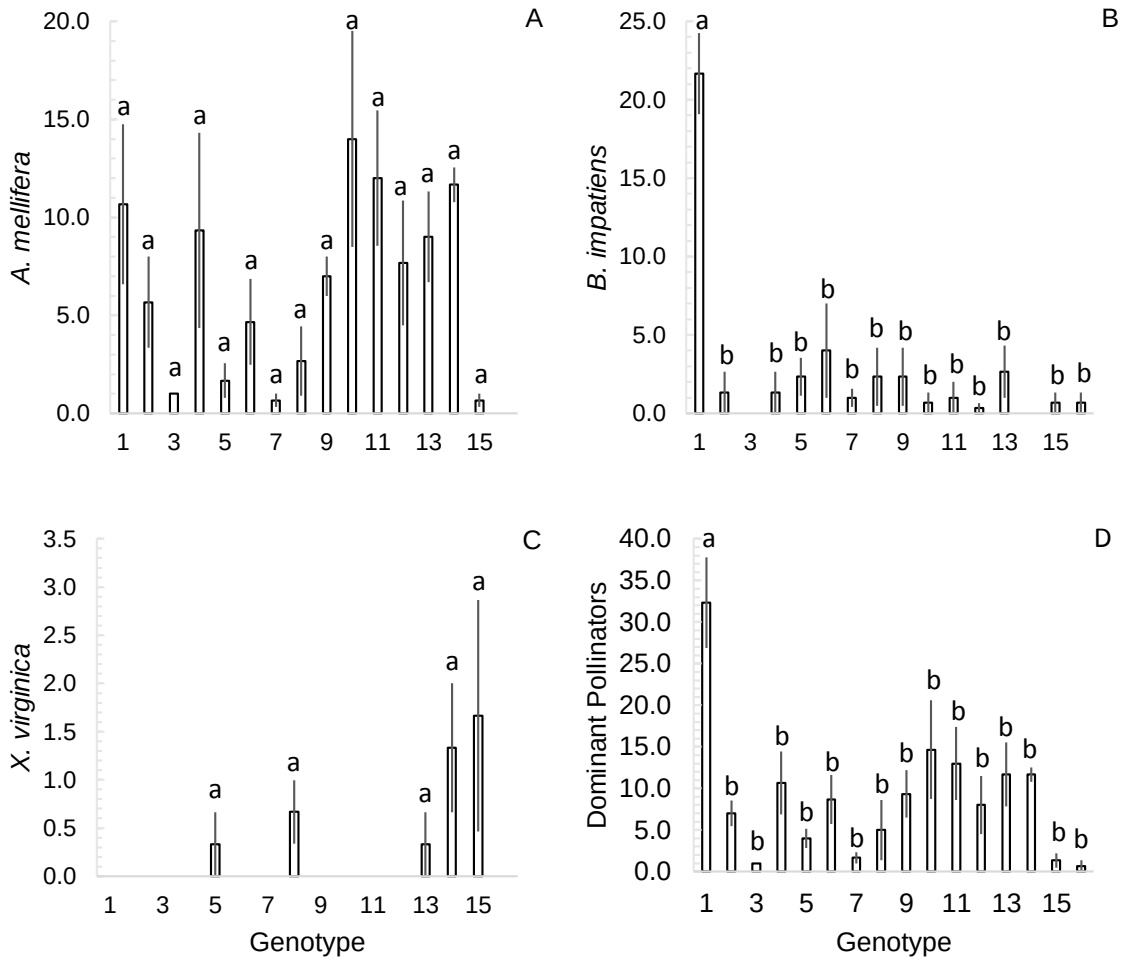

Fig 8. Mean insect abundance by genotype for: *Apis mellifera* (A), *Bombus impatiens* (B), *Xylocopa virginica* (C), and dominant pollinators (D).

Using the same interpretation of my analysis as for pollinator abundance, variation most likely due to *S. altissima* genotypes ($1\text{-}r^2$) for mean pollinator richness and evenness contributed much more to my model than did spatial separation of sites (p value, r^2) (Table 3; Figs 9-10). The mean adjusted richness for pollinators was found to be marginally related to *S. altissima* intraspecific variation (Table 3). For mean richness, mean adjusted richness, and mean evenness differences between sites explained 17%, 41%, and 28% of the variation, respectively (Table 3).

Table 3. The *P* value, *F* ratio, and *df* (One-way ANOVA, JMP 12) for pollinator diversity measures.

	p	F	r^2	$1\text{-}r^2$
Mean Richness	0.508	0.818	0.170	0.830
Mean Adjusted Richness	**0.088**	2.757	0.408	0.592
Mean Evenness	0.255	1.541	0.278	0.722

Note: $p \leq 0.05$ (significant) and $p \leq 0.10$ (marginally significant) presented in **bold** text. $df = 3, 12$.

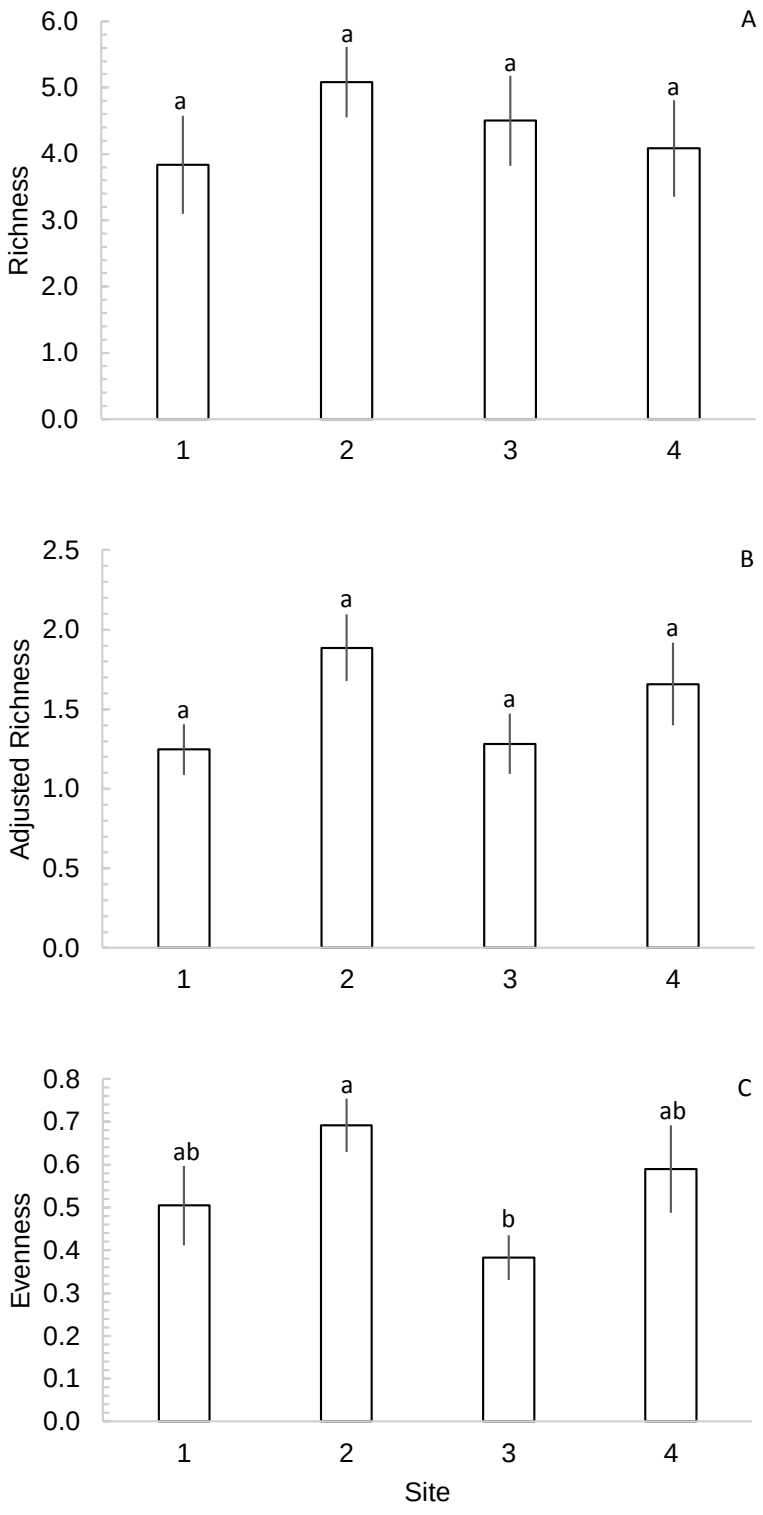

Fig. 9. Mean pollinator richness (A), adjusted richness (B), and evenness (C) by site.

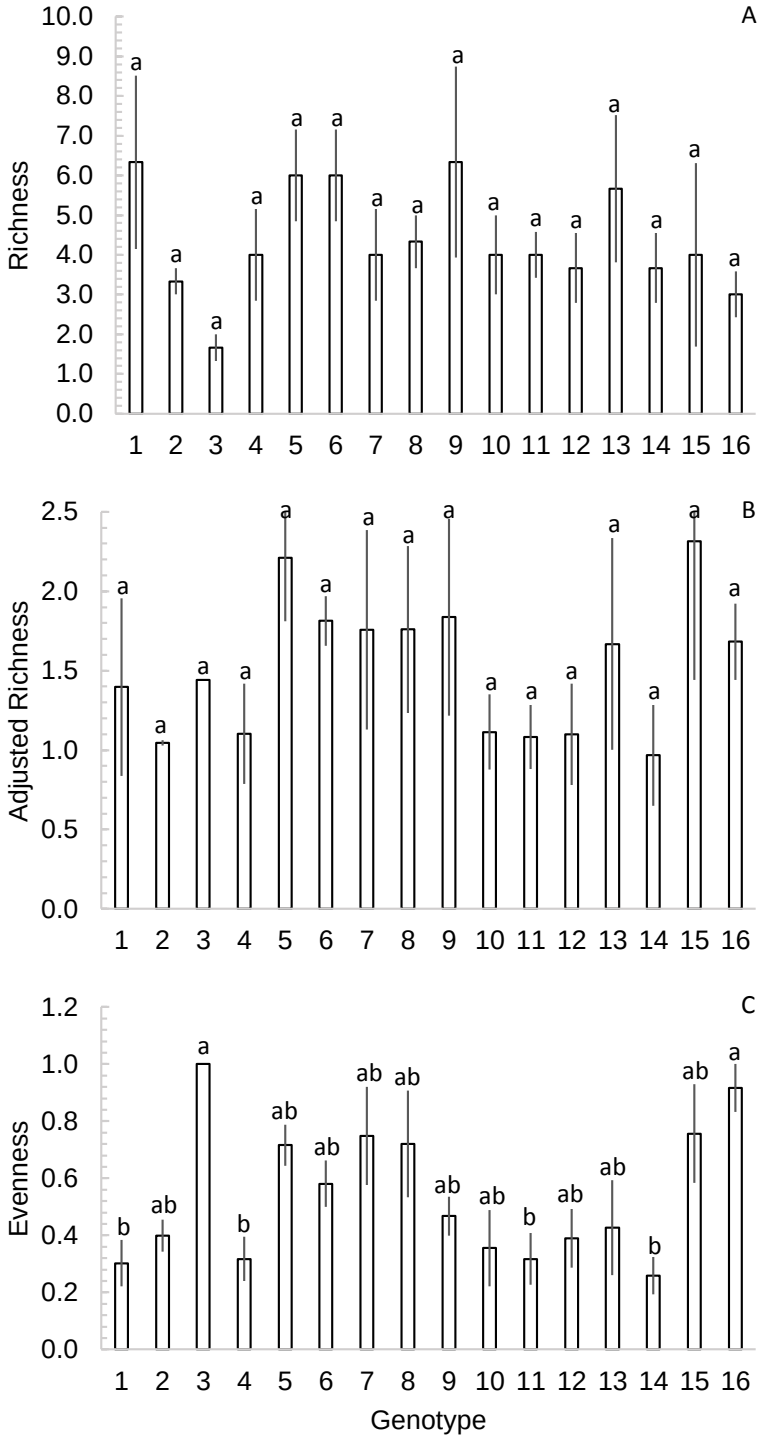

Fig. 10. Mean pollinator richness (A), adjusted richness (B), and evenness (C) by genotype.

Terpene Concentrations and Proportions

Floral terpenes in *S. altissima* were primarily affected by differences among patches and not by spatial separation of fields (Table 4; Figs 11-14). Of the twelve terpenes I report, only one (α-phellandrene) differed between sites, and only marginally. The error contribution ranged from 57.7-96.3 % (Table 4). The proportions of three terpenes, α-phellandrene, p-cymene and germacrene-D were affected by site (Table 5) though as with terpene concentration, the genotype contribution was by and large the biggest contribution to the ANOVA model for most compounds.

Table 4. The *P* value, *F* ratio, and *df* (One-way ANOVA, JMP 12) for concentrations of individual terpenes.

Terpene (mg/g)	*p*	*F*	r^2	$1\text{-}r^2$
α-pinene	0.574	0.693	0.148	0.852
camphene	0.551	0.073	0.155	0.845
β-pinene	0.191	1.856	0.317	0.683
α-phellandrene	**0.077**	2.929	0.423	0.577
p-cymene	0.220	1.701	0.298	0.702
β-elemene	0.869	0.237	0.056	0.944
caryophyllene	0.431	0.989	0.198	0.802
germacrene-D	0.318	1.306	0.246	0.754
azulene	0.564	0.710	0.151	0.849
γ-elemene	0.925	0.154	0.037	0.963
ledene oxide	0.647	0.567	0.124	0.876
bicyclo (4.4.0) dec-5	0.335	1.251	0.238	0.762

Note: $p \leq 0.05$ (significant) and $p \leq 0.10$ (marginally significant) presented in **bold** text. $df = 3, 12$.

Table 5. The *P* value, *F* ratio, and *df* (One-way ANOVA, JMP 12) for proportions of individual terpenes.

Terpene	*p*	*F*	r^2	$1\text{-}r^2$
α-pinene	0.124	2.345	0.370	0.630
camphene	0.151	2.121	0.347	0.654
β-pinene	0.658	0.549	0.121	0.879
α-phellandrene	**0.036**	3.937	0.496	0.504
p-cymene	**0.007**	6.625	0.624	0.377
β-elemene	0.832	0.290	0.068	0.932
caryophyllene	0.189	1.866	0.318	0.682
germacrene-D	**0.050**	3.501	0.467	0.533
azulene	0.468	0.904	0.184	0.816
γ-elemene	0.948	0.117	0.029	0.972
ledene oxide	0.453	0.936	0.190	0.810
bicyclo (4.4.0) dec-5	0.482	0.873	0.179	0.821

Note: $p \leq 0.05$ (significant) and $p \leq 0.10$ (marginally significant) presented in **bold** text. $df = 3, 12$.

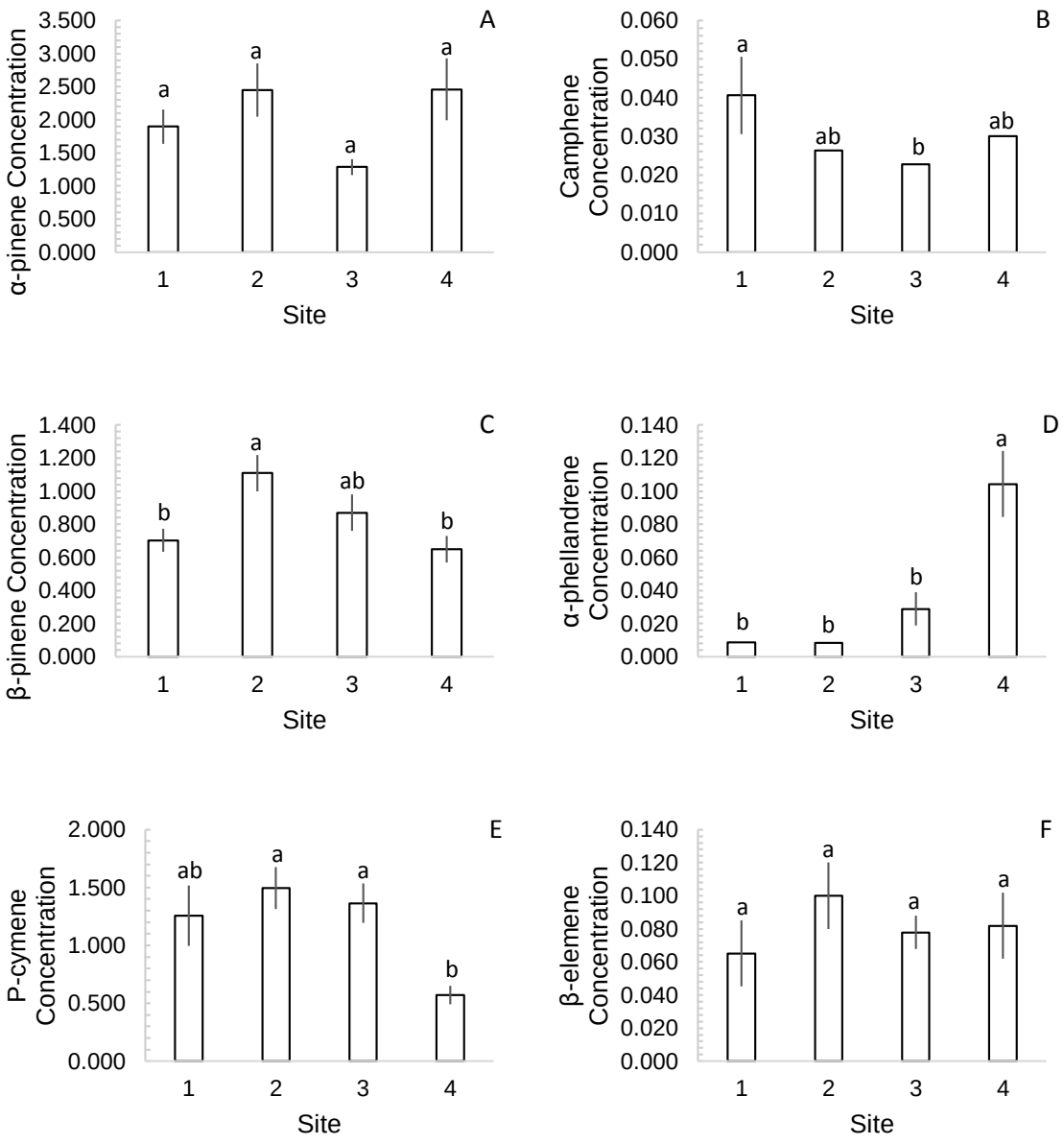

Fig. 11. Concentrations of individual terpenes by site: α-pinene (A), Camphene (B), β-pinene (C), α-phellandrene (D), P-cymene (E), and β-elemene (F).

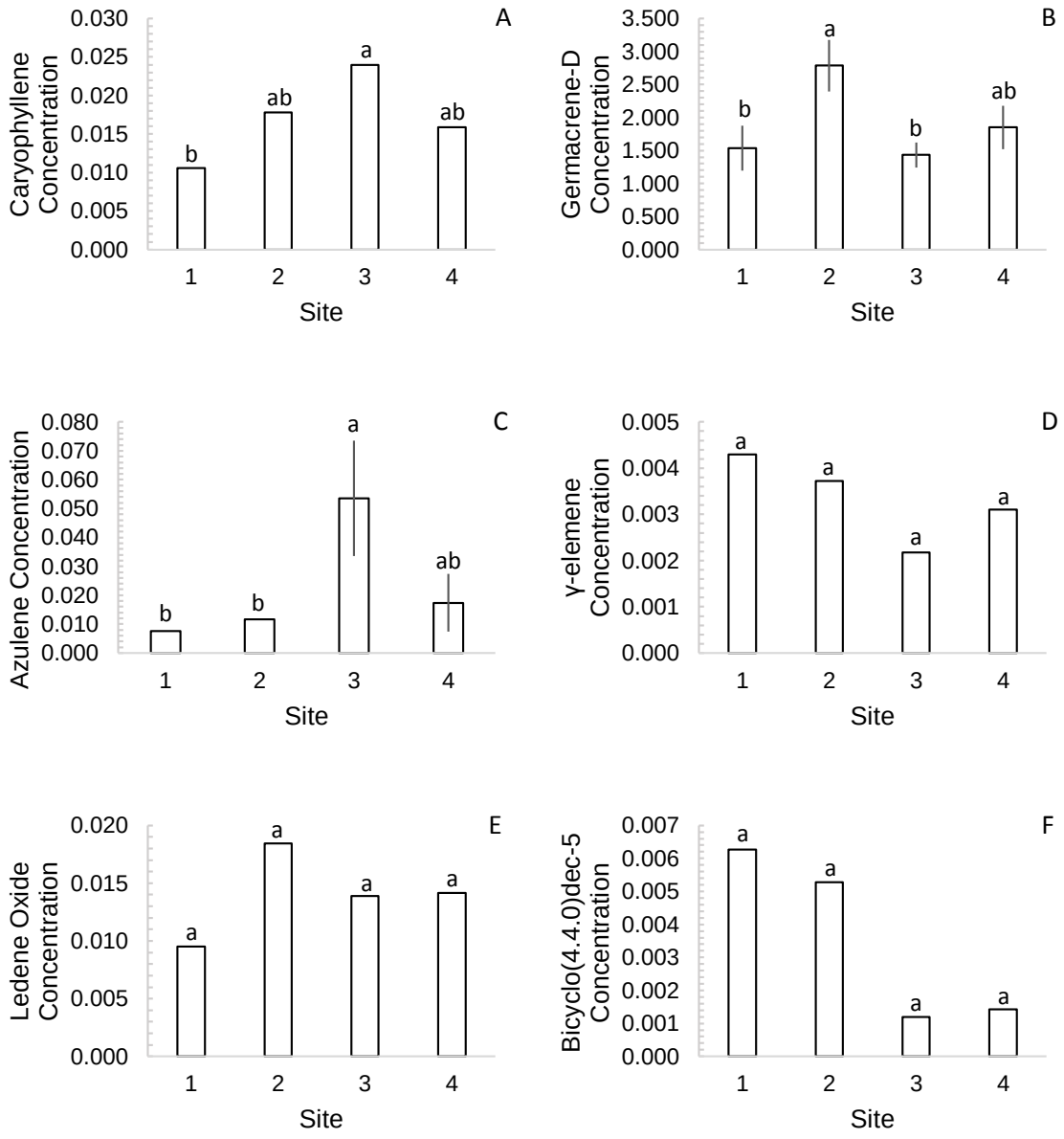

Fig. 12. Concentrations of individual terpenes by site: Caryophyllene (A), Germacrene-D (B), Azulene (C), γ-elemene (D), Ledene Oxide (E), and Bicyclo(4.4.0)dec-5 (F).

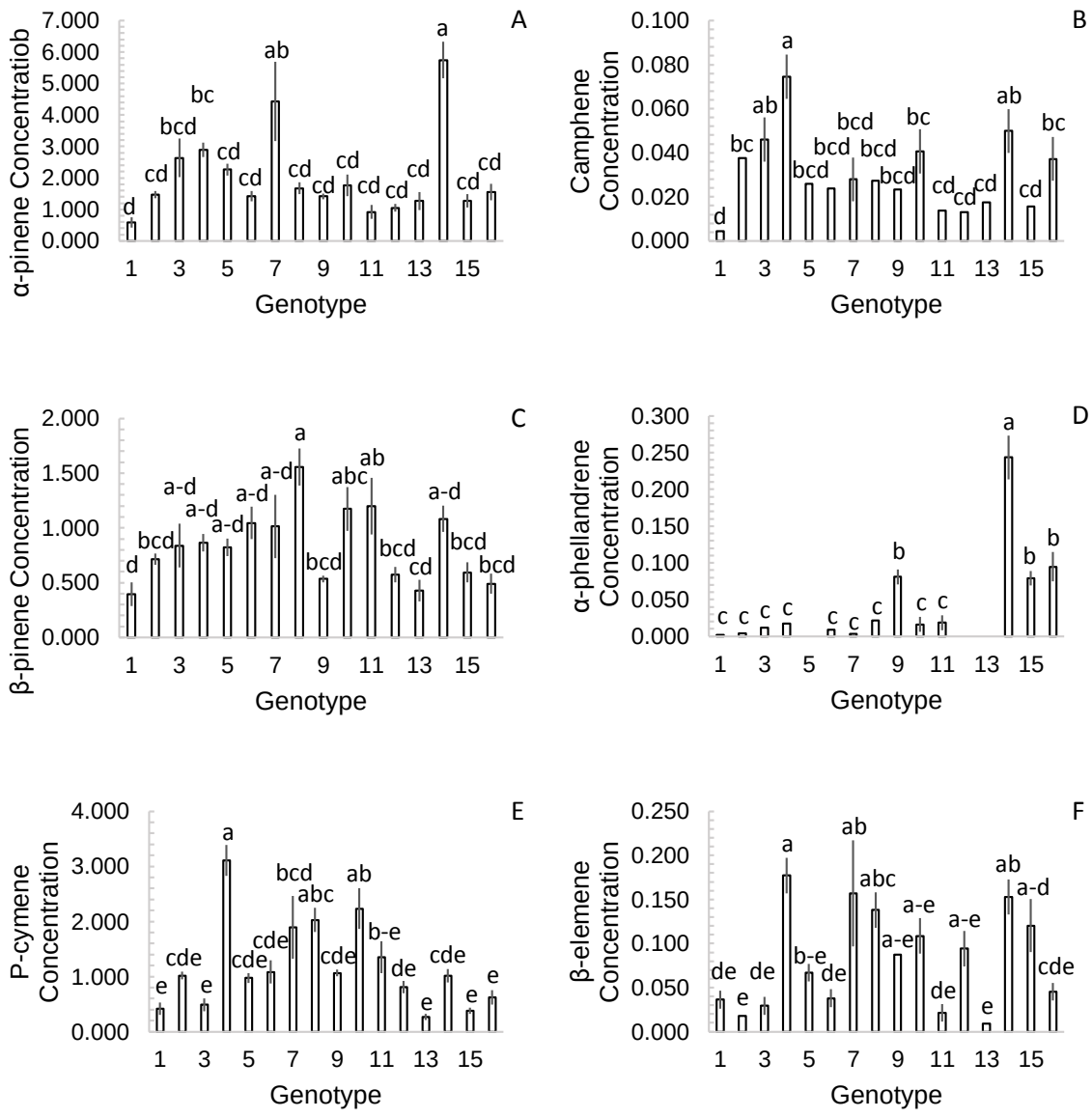

Fig. 13. Concentrations of individual terpenes by genotype: α-pinene (A), Camphene (B), β-pinene (C), α-phellandrene (D), P-cymene (E), and β-elemene (F).

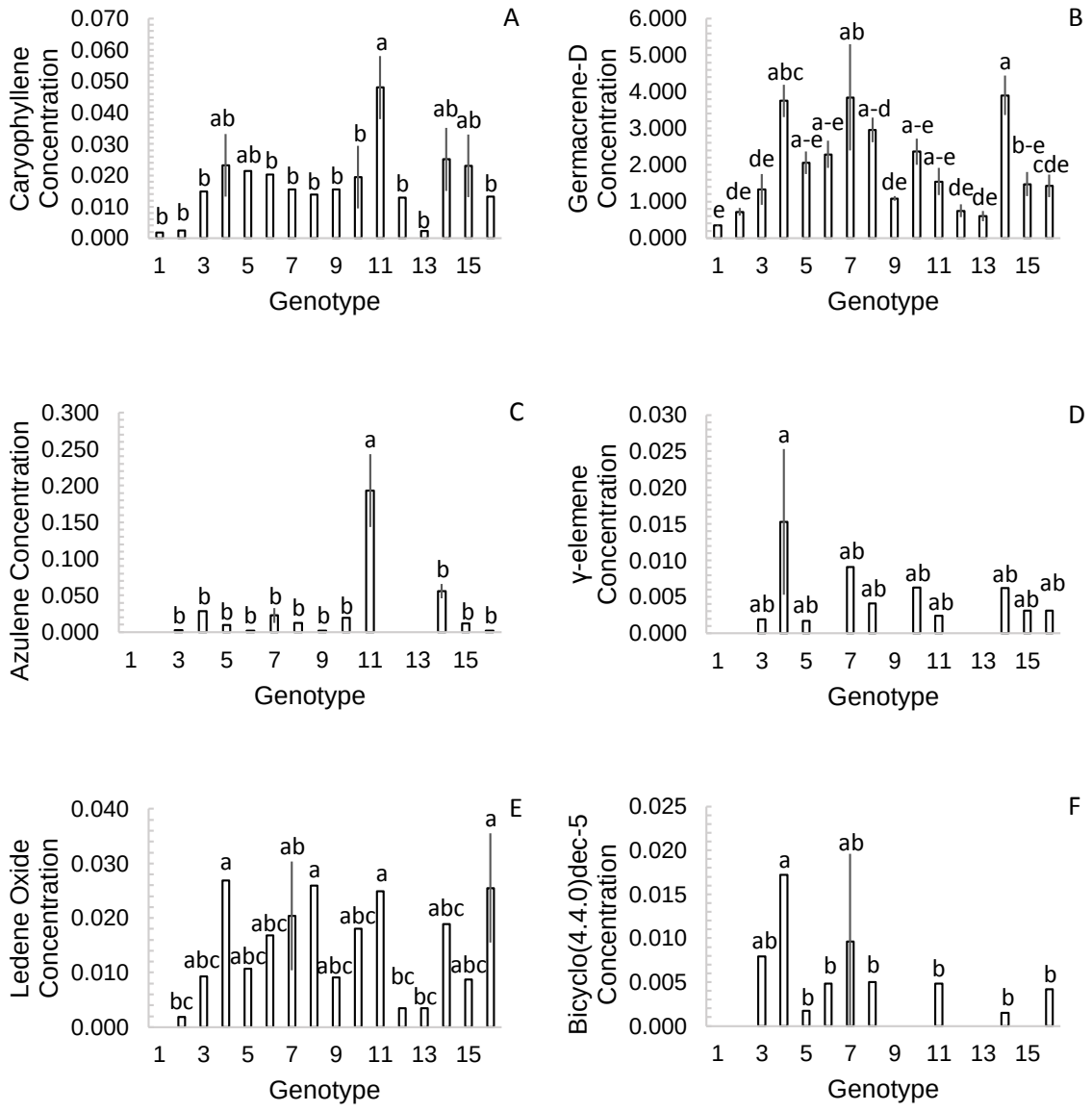

Fig. 14. Concentrations of individual terpenes by genotype: Caryophyllene (A), Germacrene-D (B), Azulene (C), γ-elemene (D), Ledene Oxide (E), and Bicyclo(4.4.0)dec-5 (F).

Soil Nutrients

There were differences in soil nitrogen, carbon, and C:N among sites (Table 6, Fig 15). The C:N ratio was largely driven by differences in carbon content between sites. Overall the contribution of patches was less than 20% of the observed variation in my model.

Table 6. The *P* value, *F* ratio and *df* (One-way ANOVA, JMP 12) for soil nutrient means.

	p	*F*	r^2	$1\text{-}r^2$
Nitrogen	**0.0003**	14.648	0.786	0.215
Carbon	**0.0001**	18.795	0.825	0.175
C:N	**0.0001**	23.794	0.856	0.144

Note: $p \leq 0.05$ presented with bold text.
df = 3, 12.

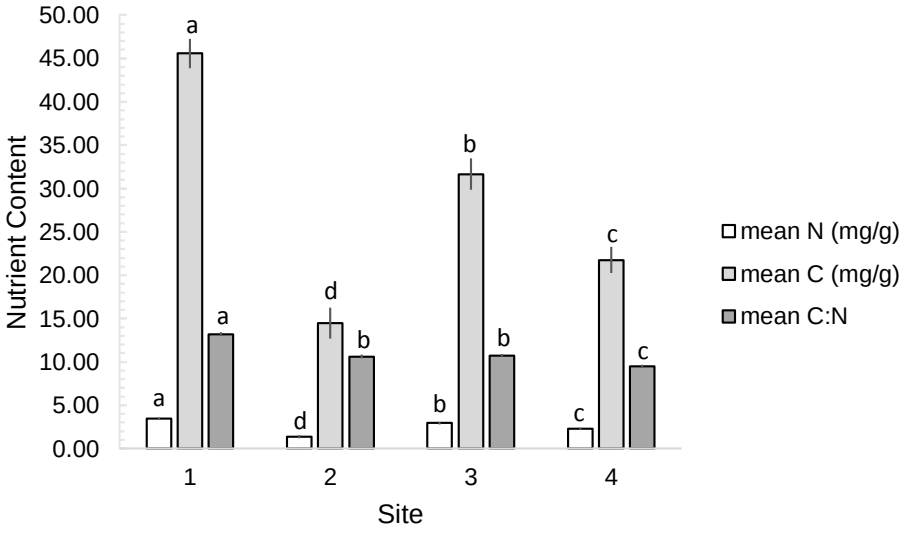

Fig. 15. Mean nitrogen content (mg nitrogen/g soil), carbon content (mg carbon/g soil), and C:N ratio among sites.

Linear Regression

The total abundance of all pollinators was significantly related to α-pinene and bicyclo(4.4.0)dec-5 concentrations, with a marginally significant relationship with camphene observed (Table 7, Figure 16A, 16B, 16F). Noteworthy is the decline in pollinator abundance as α-pinene and bicyclo(4.4.0)dec-5 and camphene concentration increased. I found no relationship between all pollinators and the concentrations of other terpenes (Table 7). Concentrations of α-pinene and bicyclo(4.4.0)dec-5 were also related to the total abundance of Order Hymenoptera (Table 7, Figure 17A, 17D), again with a decline in insects as terpenes increased. Total abundance for Order Diptera has a significant relationship with the concentration of one terpene, azulene (Table 7, Figure 17C). No significant relationships were found between total abundances for Orders Coleoptera or Lepidoptera and any individual terpene concentrations (Table 7). The total abundance of *A. mellifera* was marginally related to α-phellandrene and significantly related to bicyclo(4.4.0)dec-5 concentrations (Table 7, Figure 18C, 18F). The total abundance of *B. impatiens* was marginally related to both α-phellandrene and caryophyllene concentrations, with significant relationships found with both α-pinene and camphene concentrations (Table 7, Figure 18A-D). Dominant pollinators, consisting of *A. mellifera* and *B. impatiens*, were related to α-pinene and bicyclo(4.4.0)dec-5 concentrations (Table 7, Figure 18A, 18F). The total abundance of Family Syrphidae was related to β-elemene concentration (Table 7, data not shown).

Table 7. The *P* value*, r^2, and *df* (Linear Regression, JMP 12) for total pollinator abundance and individual terpene concentrations.

	All Pollinators		Hymenoptera		Diptera		Coleoptera		*A. mellifera*		*B. impatiens*		Dominant		Syrphidae	
Terpene	*p*	r^2	*p*	r^2	*p*	r^2	*p*	r^2	*p*	r^2	*p*	r^2	*p*	r^2	*p*	r^2
α-pinene	**0.034**	0.301	**0.024**	0.334	0.805	0.005	0.321	0.070	0.112	0.183	**0.025**	0.311	**0.059**	0.248	0.596	0.021
Camphene	**0.067**	0.235	0.108	0.186	0.836	0.003	0.110	0.173	0.955	0.000	**0.008**	0.434	0.566	0.024	0.785	0.006
β-pinene	0.927	0.001	0.915	0.001	0.302	0.076	0.860	0.002	0.652	0.015	0.310	0.073	0.862	0.002	0.921	0.001
α-phellandrene	0.265	0.095	0.254	0.099	0.217	0.107	0.806	0.005	**0.071**	0.229	**0.085**	0.198	0.733	0.009	0.663	0.014
P-cymene	0.523	0.030	0.574	0.023	0.831	0.003	0.839	0.003	0.370	0.058	0.766	0.007	0.452	0.041	0.342	0.065
β-elemene	0.963	0.000	0.875	0.002	0.442	0.043	0.826	0.004	0.933	0.001	0.290	0.080	0.831	0.003	**0.068**	0.219
Caryophyllene	0.922	0.001	0.905	0.001	0.884	0.002	0.516	0.033	0.730	0.009	**0.074**	0.225	0.993	0.000	0.719	0.010
Germacrene-D	0.580	0.022	0.498	0.033	0.920	0.001	0.363	0.059	0.659	0.014	0.772	0.007	0.534	0.028	0.322	0.070
Azulene	0.433	0.045	0.381	0.055	**0.002**	0.499	0.252	0.093	0.255	0.091	0.106	0.188	0.394	0.052	0.645	0.017
γ-elemene	0.775	0.006	0.295	0.084	0.768	0.006	0.749	0.008	0.374	0.061	0.114	0.181	0.301	0.082	0.386	0.054
Ledene oxide	0.550	0.026	0.472	0.038	0.624	0.018	0.349	0.063	0.489	0.035	0.317	0.072	0.437	0.044	0.686	0.012
Bicyclo(4.4.0)d	**0.025**	0.331	**0.017**	0.368	0.942	0.000	0.156	0.138	**0.049**	0.266	0.586	0.022	**0.039**	0.288	0.940	0.000

Note: $p \leq 0.05$ (significant) and $p \leq 0.10$ (marginally significant) presented in **bold** text.

df = 1, 14 for individual terpene concentrations.

df = 1, 13 for all pollinators (α-pinene, camphene, α-phellandrene, bicyclo(4.4.0)dec-5), Hymenoptera (α-pinene, camphene, α-phellandrene, γ-elemene, bicyclo(4.4.0)dec-5), Diptera (caryophyllene), Coleoptera (caryophyllene), Lepidoptera (azulene), *A. mellifera* (α-pinene, α-phellandrene, γ-elemene, bicyclo(4.4.0)dec-5), *B. impatiens* (camphene, caryophyllene, germacrene-D, azulene, γ-elemene), dominant pollinators (α-pinene, γ-elemene, bicyclo(4.4.0)dec-5), and Syrphidae (caryophyllene, azulene).

*A Bonferroni correction would set significance of *P* to 0.0042.

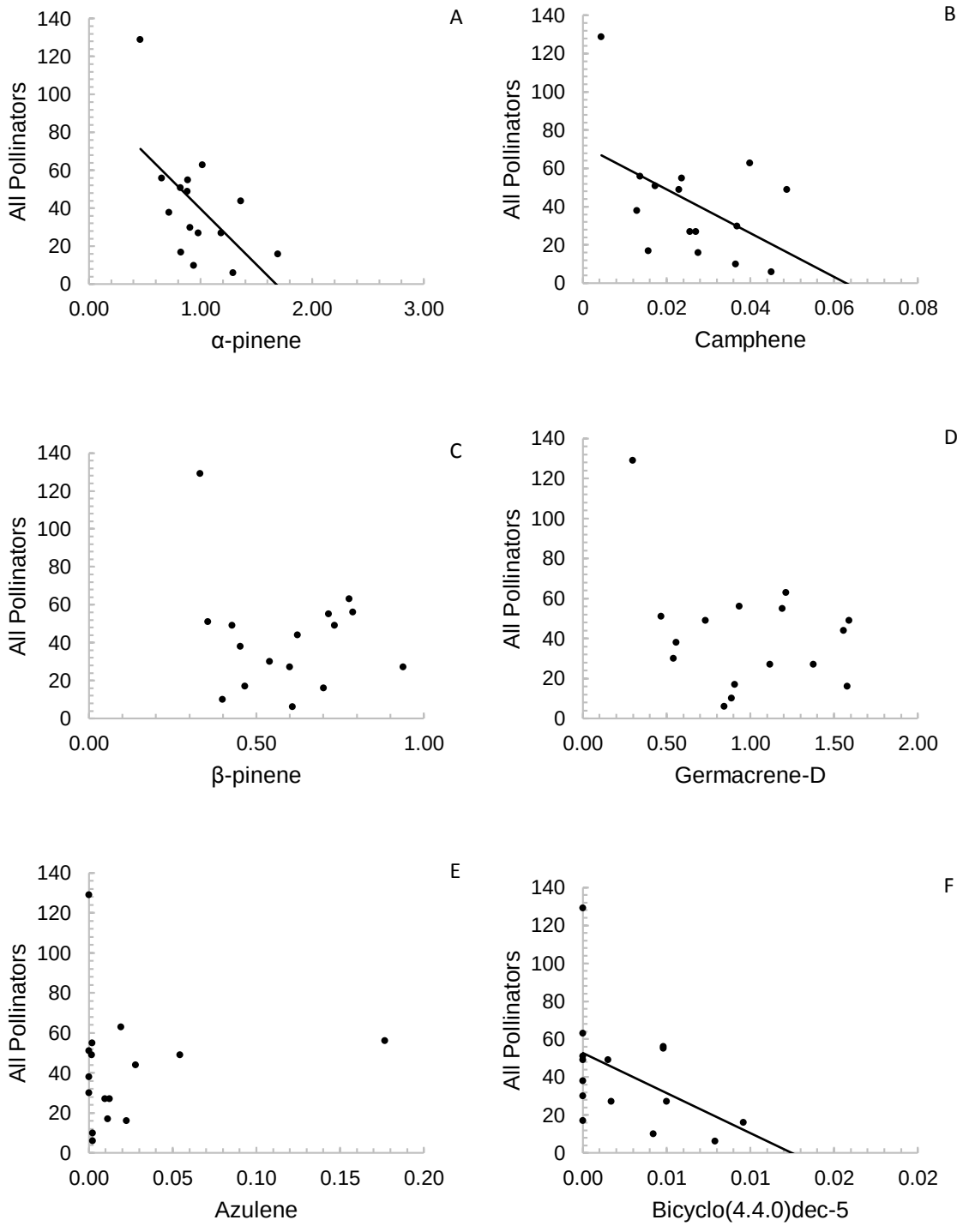

Fig. 16. Linear regressions between total abundance for all pollinators and individual terpene concentrations.

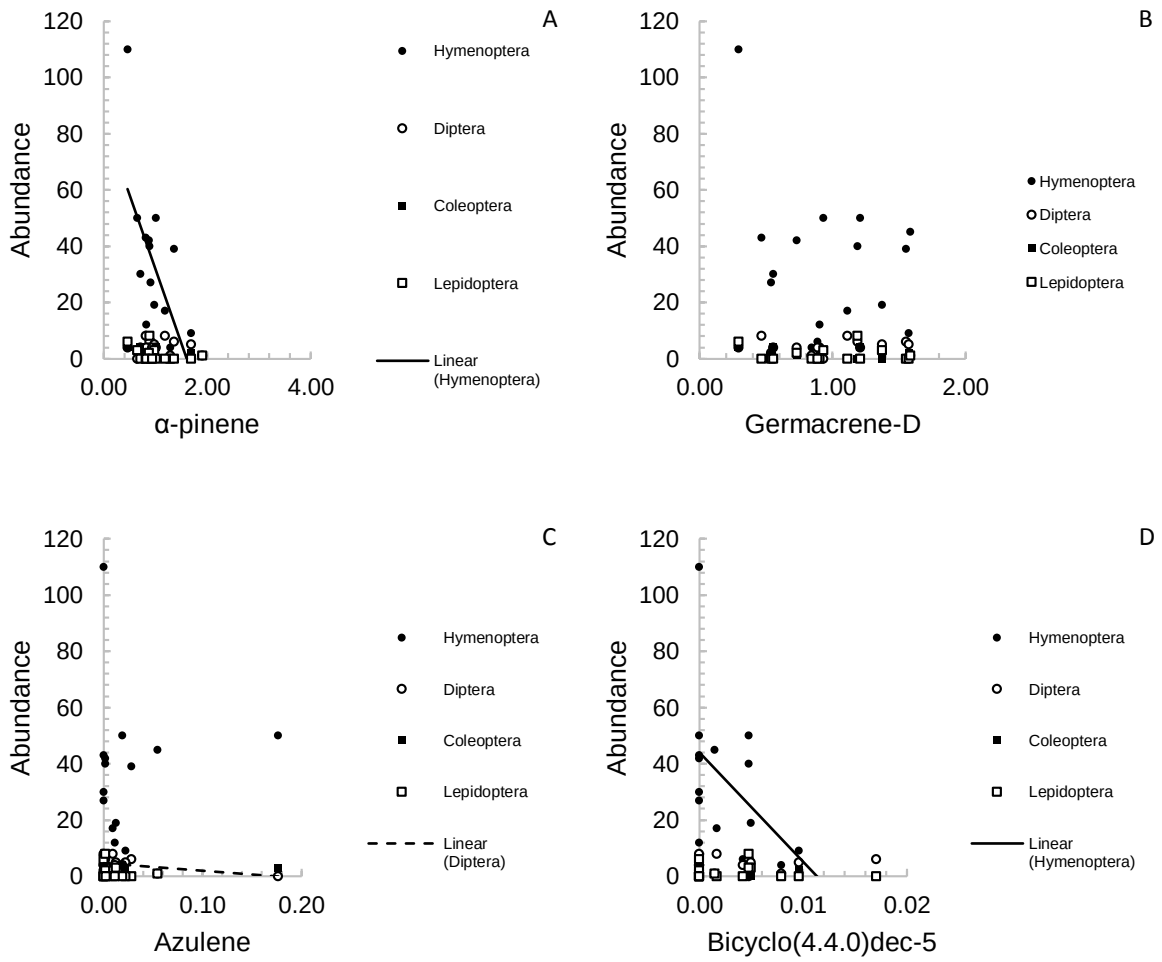

Fig. 17. Linear regressions between total abundance for Orders Hymenoptera (solid line), Diptera (dashed line), Lepidoptera, and Coleoptera and individual terpene concentrations.

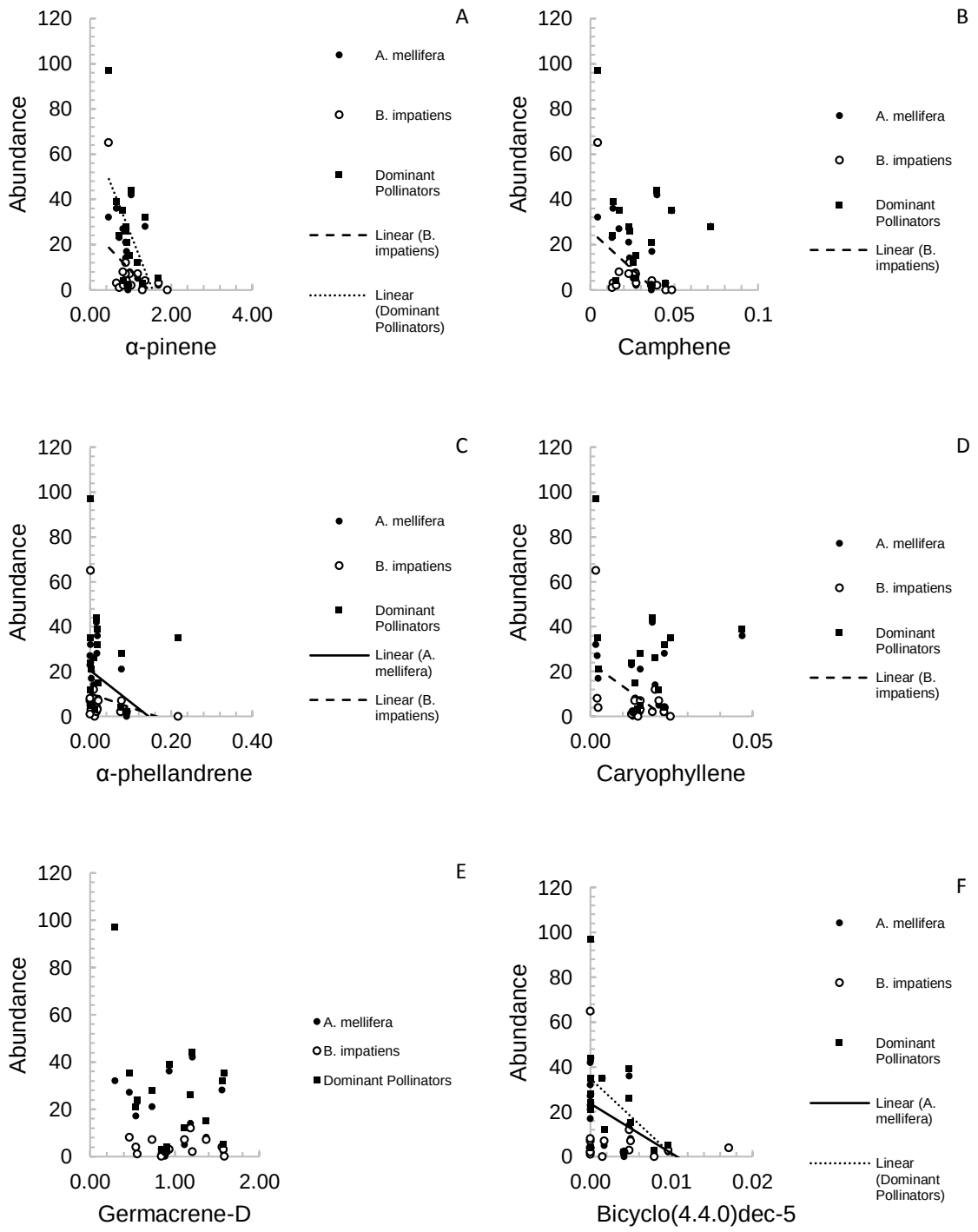

Fig. 18. Linear regressions between total abundance for *A. mellifera* (solid line), *B. impatiens* (dashed line), and dominant pollinators (dotted line) and individual terpene concentrations.

The total abundance of all pollinators was marginally related to the proportion of camphene and bicyclo(4.4.0)dec-5, with a significant relationship with p-cymene proportion (Table 8, Figure 19B, 19D, 19F). As pollinator abundance declined, floral allocation of these compounds increased. The total abundance of Order Hymenoptera was marginally related to γ-elemene and bicyclo(4.4.0)dec-5 proportions, with a significant relationship with p-cymene proportions (Table 8, Figure 20D-F). The total abundance of Order Diptera was not related to individual terpene proportions (Table 8). The total abundance of Order Coleoptera demonstrated marginally significant relationships with camphene, p-cymene, and bicyclo(4.4.0)dec-5 proportions (Table 8, Figure 20B, 20D, 20F). The total abundance of Order Lepidoptera was marginally related to α-pinene proportions and significantly related to both camphene and β-pinene proportions (Table 8, Figure 20A-C). The total abundance of *A. mellifera* was marginally related to bicyclo(4.4.0)dec-5 and significantly related to p-cymene (Table 8, Figure 21C, 21F). The total abundance of *B. impatiens* was marginally related to both β-pinene and azulene proportions (Table 8, Figure 21B, 21D). The total abundance of dominant pollinators was significantly related to both p-cymene and γ-elemene proportions (Table 8, Figure 21C, 21E). The total abundance of family Syrphidae had no significant relationships with individual terpene proportions (Table 8).

Table 8. The *P* value*, r^2 and *df* (Linear Regression, JMP 12) for total pollinator abundance and individual terpene proportions.

	All Pollinators		Hymenoptera		Diptera		Coleoptera		Lepidoptera		*A. mellifera*		*B. impatiens*		Dominant	
Terpene	*p*	r^2	*p*	r^2	*p*	r^2	*p*	r^2	*p*	r^2	*p*	r^2	*p*	r^2	*p*	r^2
α-pinene	0.134	0.153	0.182	0.124	0.118	0.177	0.316	0.072	**0.074**	0.210	0.387	0.054	0.251	0.093	0.261	0.089
camphene	**0.072**	0.213	0.137	0.151	0.918	0.001	**0.066**	0.221	**0.042**	0.264	0.408	0.049	0.235	0.099	0.196	0.116
β-pinene	0.202	0.114	0.177	0.126	0.847	0.003	0.197	0.116	**0.039**	0.271	0.243	0.096	**0.085**	0.197	0.156	0.139
α-phellandrene	0.423	0.046	0.450	0.041	0.486	0.035	0.683	0.012	0.872	0.002	0.214	0.108	0.227	0.103	0.188	0.120
p-cymene	**0.041**	0.267	**0.042**	0.263	0.913	0.001	**0.097**	0.185	0.397	0.052	**0.046**	0.255	0.192	0.119	**0.032**	0.289
β-elemene	0.692	0.012	0.776	0.006	0.379	0.073	0.133	0.154	0.840	0.003	0.849	0.003	0.909	0.001	0.965	0.000
caryophyllene	0.336	0.071	0.303	0.081	0.828	0.004	0.411	0.049	0.556	0.025	0.202	0.122	0.397	0.052	0.143	0.158
germacrene-D	0.449	0.042	0.303	0.075	0.379	0.056	0.144	0.146	0.766	0.007	0.137	0.151	0.498	0.036	0.173	0.128
azulene	0.486	0.035	0.437	0.044	0.441	0.046	0.244	0.096	0.482	0.039	0.337	0.066	**0.093**	0.202	0.934	0.001
γ-elemene	0.326	0.069	**0.078**	0.220	0.948	0.000	0.690	0.012	0.133	0.154	**0.070**	0.230	0.175	0.127	**0.047**	0.271
ledene oxide	0.266	0.087	0.217	0.107	0.536	0.030	0.307	0.074	0.803	0.005	0.977	0.000	0.405	0.050	0.785	0.006
bicyclo(4.4.0)d	**0.062**	0.227	**0.068**	0.218	0.519	0.030	**0.075**	0.209	0.924	0.000	0.196	0.117	0.380	0.055	0.121	0.163

Note: $p \leq 0.05$ (significant) and $p \leq 0.10$ (marginally significant) presented in **bold** text.

df = 1, 14 for individual terpene proportions.

df = 1, 13 for all pollinators (caryophyllene), Hymenoptera (caryophyllene, γ-elemene), Diptera (α-pinene, β-pinene, caryophyllene, azulene, ledene oxide), Lepidoptera (azulene), *A. mellifera* (caryophyllene, γ-elemene, ledene oxide), B. impatiens (germacrene-D, azulene), dominant pollinators (caryophyllene, azulene, γ-elemene, ledene oxide), and Syrphidae (azulene).

*A Bonferroni correction would set significance of *P* to 0.0042.

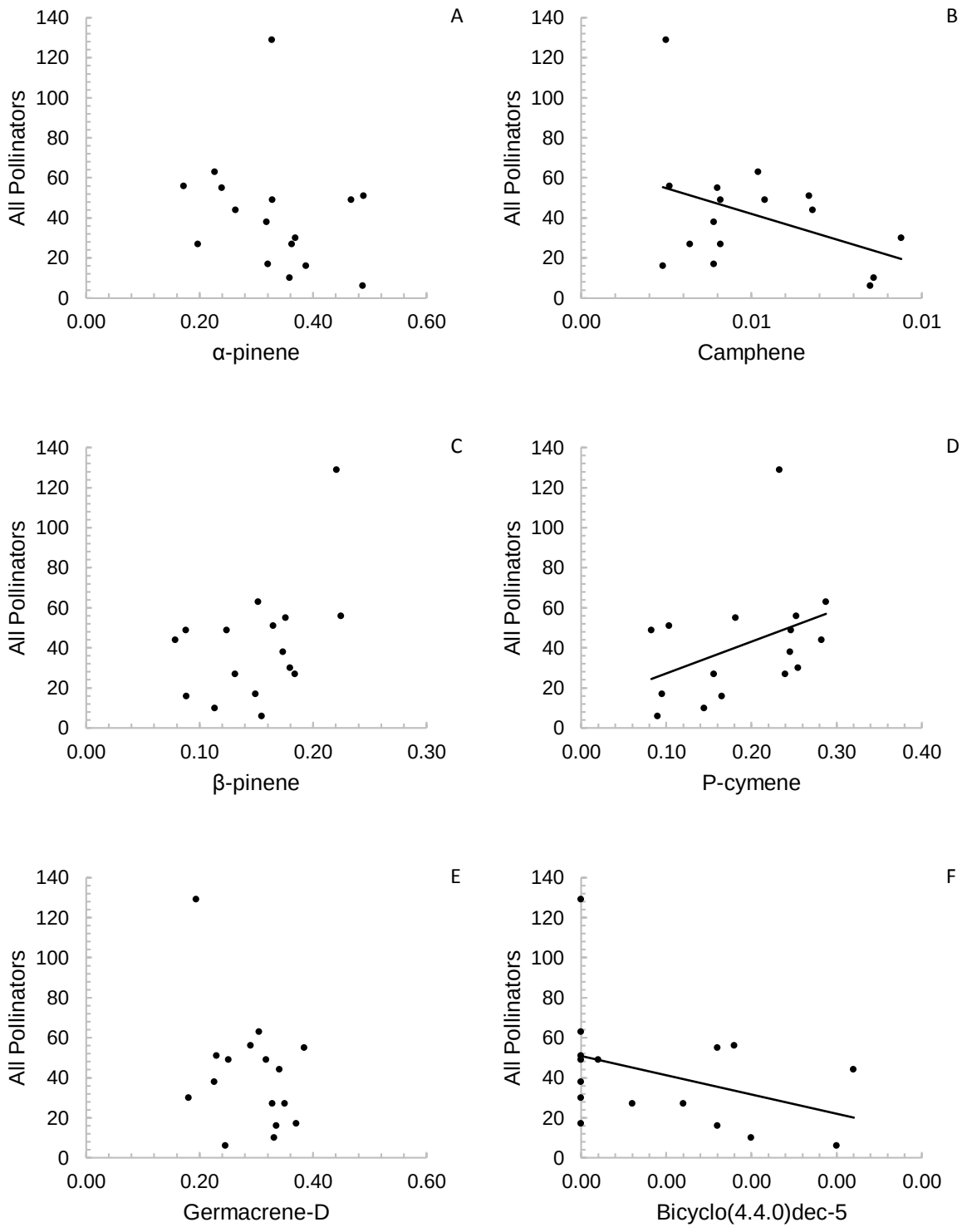

Fig. 19. Linear regressions between total abundance for all pollinators and individual terpene proportions.

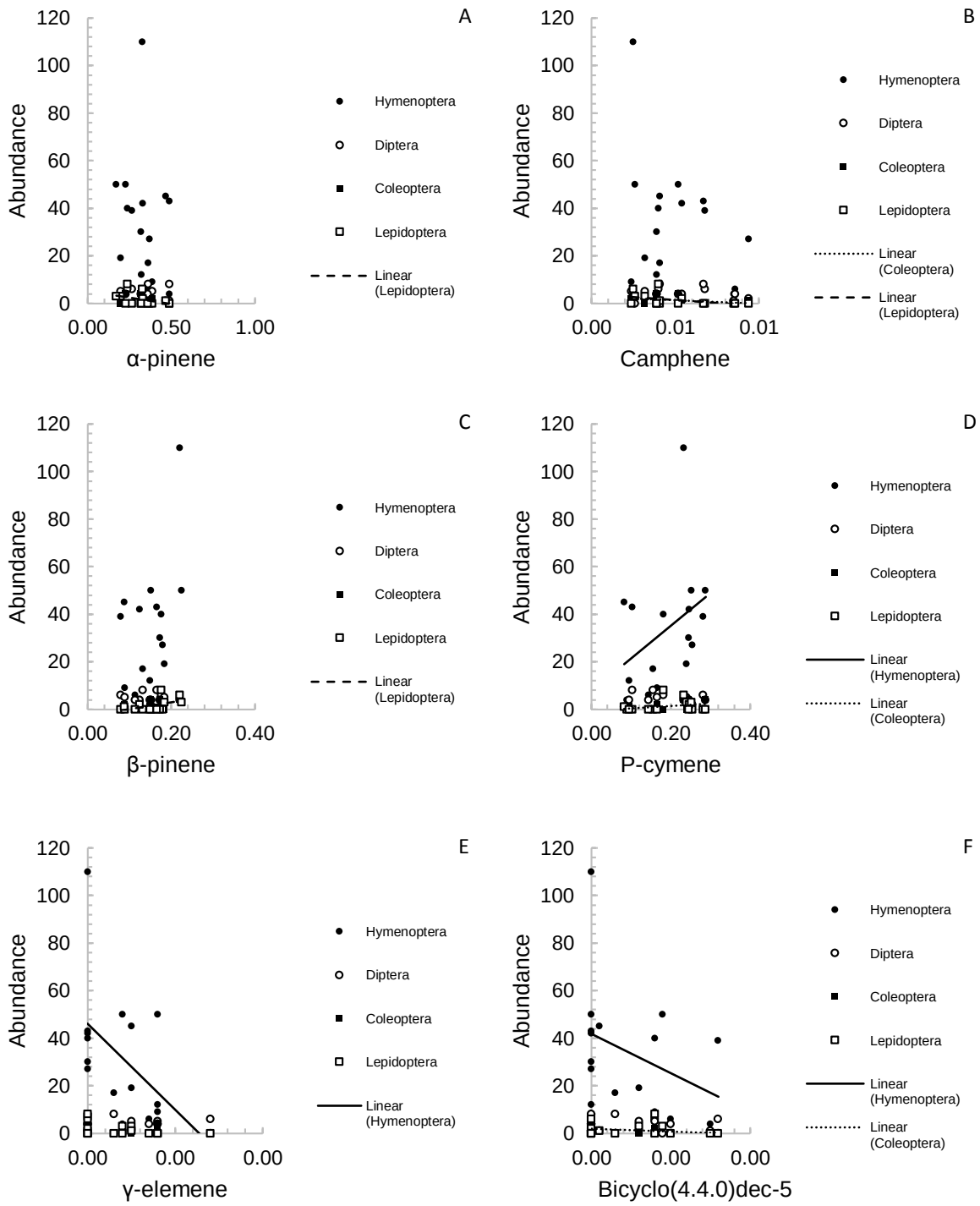

Fig. 20. Linear regressions between total abundance and Orders Hymenoptera (solid line), Diptera, Lepidoptera (broken line), and Coleoptera (dotted line) and individual terpene proportions.

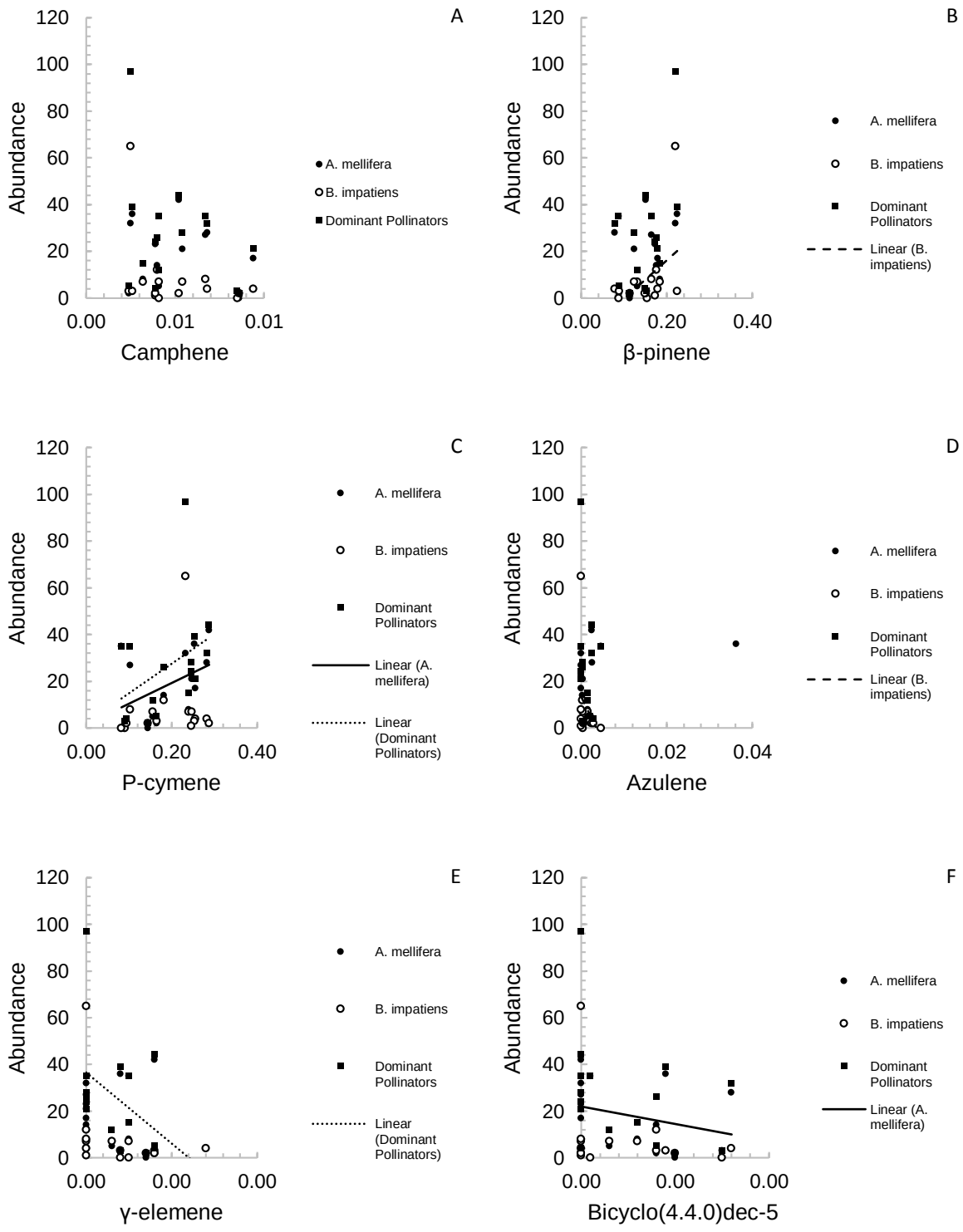

Fig. 21. Linear regressions of total abundance for *A. mellifera* (solid line), *B. impatiens* (broken line), and dominant pollinators (dotted line) and individual terpene proportions.

The mean abundance of all pollinators was marginally related to camphene concentration and significantly related to both α-pinene and bicyclo(4.4.0)dec-5 concentrations (Table 9, Figure 22A, 22B, 22F). Noteworthy is the negative relationship between abundance and these three compounds, indicating that as these compounds increase in concentration, pollinator abundance declines. The mean abundance of Order Hymenoptera was related to both α-pinene and bicyclo(4.4.0)dec-5 concentrations (Table 9, Figure 23A, 23D). The mean abundance of Order Diptera was significantly related to azulene concentration (Table 9, Figure 23C), while the mean abundances of Orders Coleoptera and Lepidoptera were unrelated to individual terpene concentrations (Table 9). The mean abundance of *A. mellifera* was marginally related to α-pinene concentration, with a significant relationship with bicyclo(4.4.0)dec-5 concentration observed (Table 9, Figure 24A, 24F). The mean abundance of *B. impatiens* was marginally related to camphene concentration (Table 9, Figure 24B). For dominant pollinators the mean abundance was marginally related to both α-pinene and α-phellandrene concentrations, with a significant relationship with bicyclo(4.4.0)dec-5 concentration (Table 9, Figure 24A, 24C, 24F). The mean abundance of family Syrphidae was marginally related to β-elemene concentration (Table 9, data not shown).

Table 9. The *P* value*, r^2, and *df* (Linear Regression, JMP 12) for mean pollinator abundance and individual terpene concentrations.

	All Pollinators		Hymenoptera		Diptera		Coleoptera		*A. mellifera*		*B. impatiens*		Dominant		Syrphidae	
Terpene	*p*	r^2	*p*	r^2	*p*	r^2	*p*	r^2	*p*	r^2	*p*	r^2	*p*	r^2	*p*	r^2
a-pinene	**0.034**	0.303	**0.028**	0.321	0.448	0.045	0.266	0.088	**0.086**	0.210	0.164	0.144	**0.056**	0.253	0.604	0.020
Camphene	**0.067**	0.236	0.104	0.190	0.800	0.005	0.103	0.178	0.989	0.000	**0.095**	0.215	0.556	0.025	0.818	0.004
b-pinene	0.929	0.001	0.948	0.000	0.319	0.071	0.892	0.001	0.735	0.009	0.960	0.000	0.873	0.002	0.806	0.004
a-phellandrene	0.261	0.096	0.230	0.109	0.191	0.119	0.605	0.020	0.110	0.184	0.145	0.146	**0.084**	0.212	0.478	0.037
P-cymene	0.527	0.029	0.558	0.025	0.831	0.003	0.793	0.005	0.385	0.054	0.555	0.026	0.459	0.040	0.283	0.082
belemene	0.959	0.000	0.900	0.001	0.580	0.022	0.891	0.001	0.907	0.001	0.257	0.091	0.832	0.003	**0.093**	0.188
Caryophyllene	0.919	0.001	0.902	0.001	0.860	0.003	0.472	0.041	0.707	0.010	0.472	0.044	0.993	0.000	0.345	0.064
Germacrene-D	0.575	0.023	0.507	0.032	0.902	0.001	0.354	0.062	0.635	0.017	0.811	0.005	0.529	0.029	0.350	0.063
Azulene	0.431	0.045	0.380	0.056	**0.009**	0.393	0.262	0.089	0.221	0.105	0.141	0.159	0.393	0.053	0.746	0.008
g-elemene	0.771	0.006	0.302	0.082	0.769	0.006	0.771	0.006	0.410	0.053	0.115	0.180	0.304	0.081	0.397	0.052
Ledene Oxide	0.543	0.027	0.451	0.041	0.824	0.004	0.395	0.052	0.569	0.024	0.993	0.000	0.437	0.044	0.701	0.011
Bicyclo(4.4.0)d	**0.025**	0.331	**0.018**	0.360	0.848	0.003	0.203	0.113	**0.041**	0.284	0.524	0.030	**0.037**	0.293	0.873	0.002

Note: $p \leq 0.05$ (significant) and $p \leq 0.10$ (marginally significant) presented in **bold** text.

df = 1, 14 for individual terpene concentrations

df = 1, 13 for all pollinators (α-pinene, camphene, α-phellandrene, bicyclo(4.4.0)dec-5), Hymenoptera (α-pinene, camphene, α-phellandrene, γ-elemene, bicyclo(4.4.0)dec-5), Diptera (α-pinene, caryophyllene), Coleoptera (caryophyllene), Lepidoptera (azulene), *A. mellifera* (α-pinene, α-phellandrene, γ-elemene, bicyclo(4.4.0)dec-5), B. impatiens (α-pinene, camphene, β-pinene, caryophyllene, germacrene-D, azulene, γ-elemene, ledene oxide, bicyclo(4.4.0)dec-5), dominant pollinators (α-pinene, α-phellandrene, γ-elemene, bicyclo(4.4.0)dec-5), and Syrphidae (azulene), and *df* = 1, 12 for *B. impatiens* (camphene, caryophyllene).

*A Bonferroni correction would set significance of *P* to 0.0042.

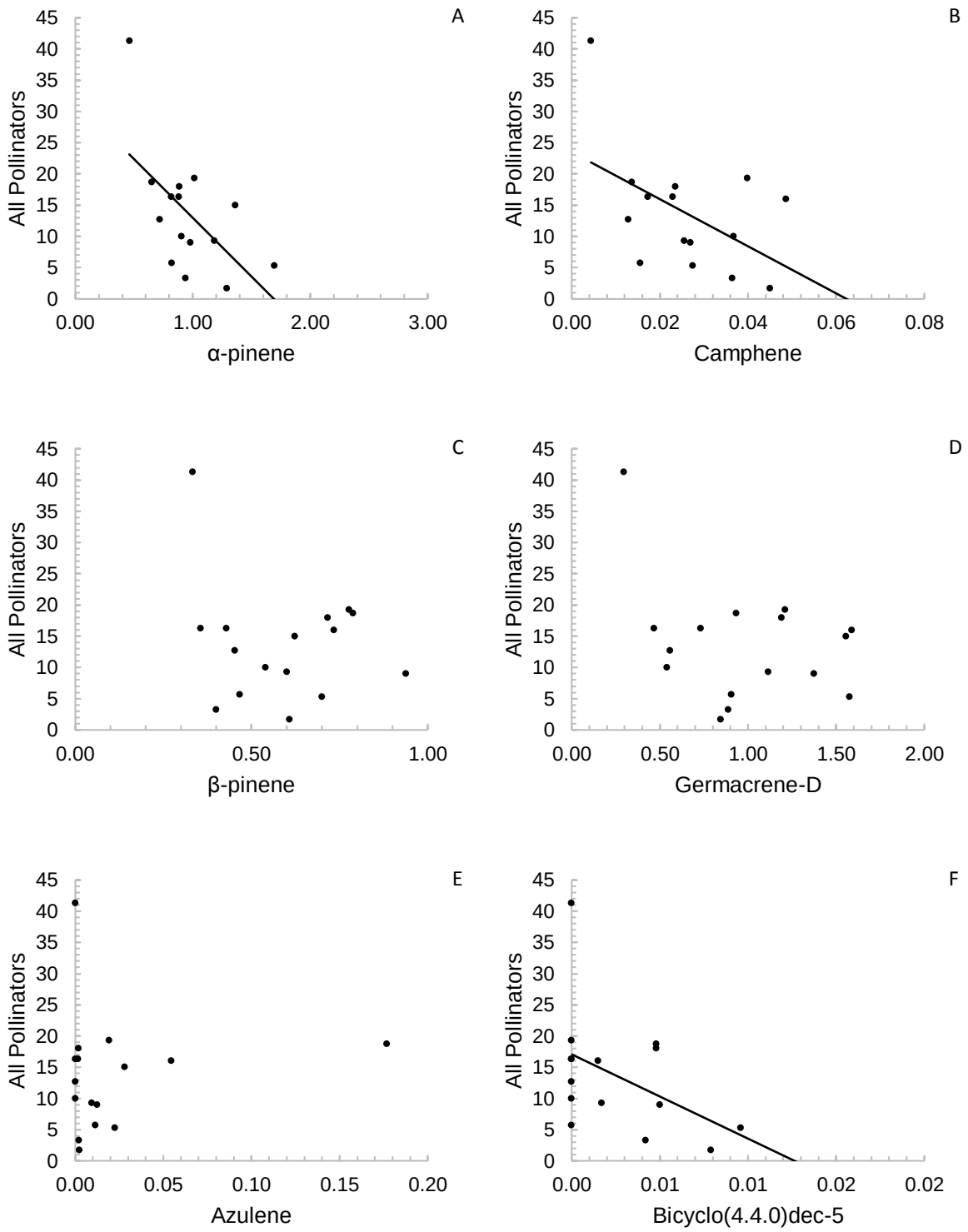

Fig. 22. Linear regressions of mean abundance for all pollinators and individual terpene concentrations.

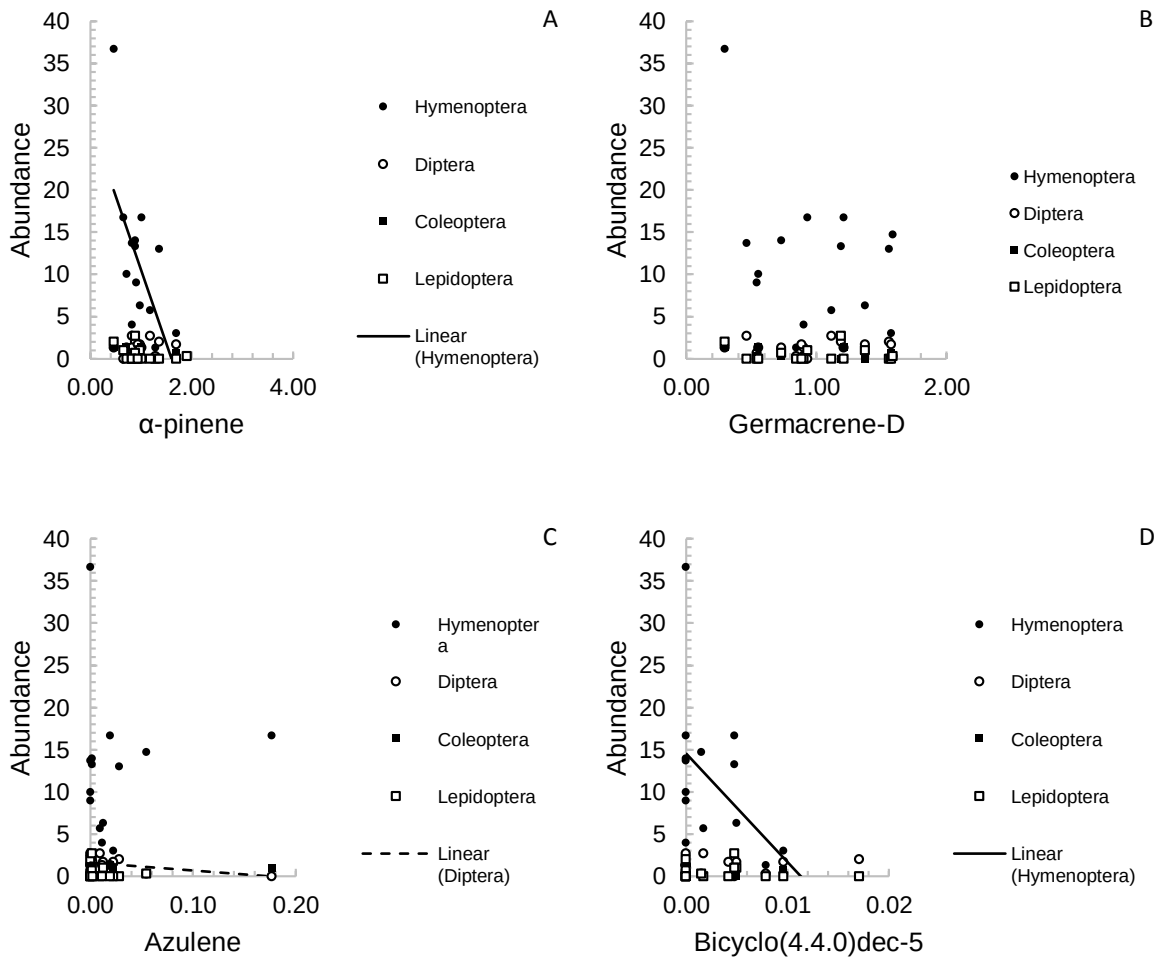

Fig. 23. Linear regressions of mean abundance for Orders Hymenoptera (solid line), Diptera (dashed line), Lepidoptera, and Coleoptera and individual terpene concentrations.

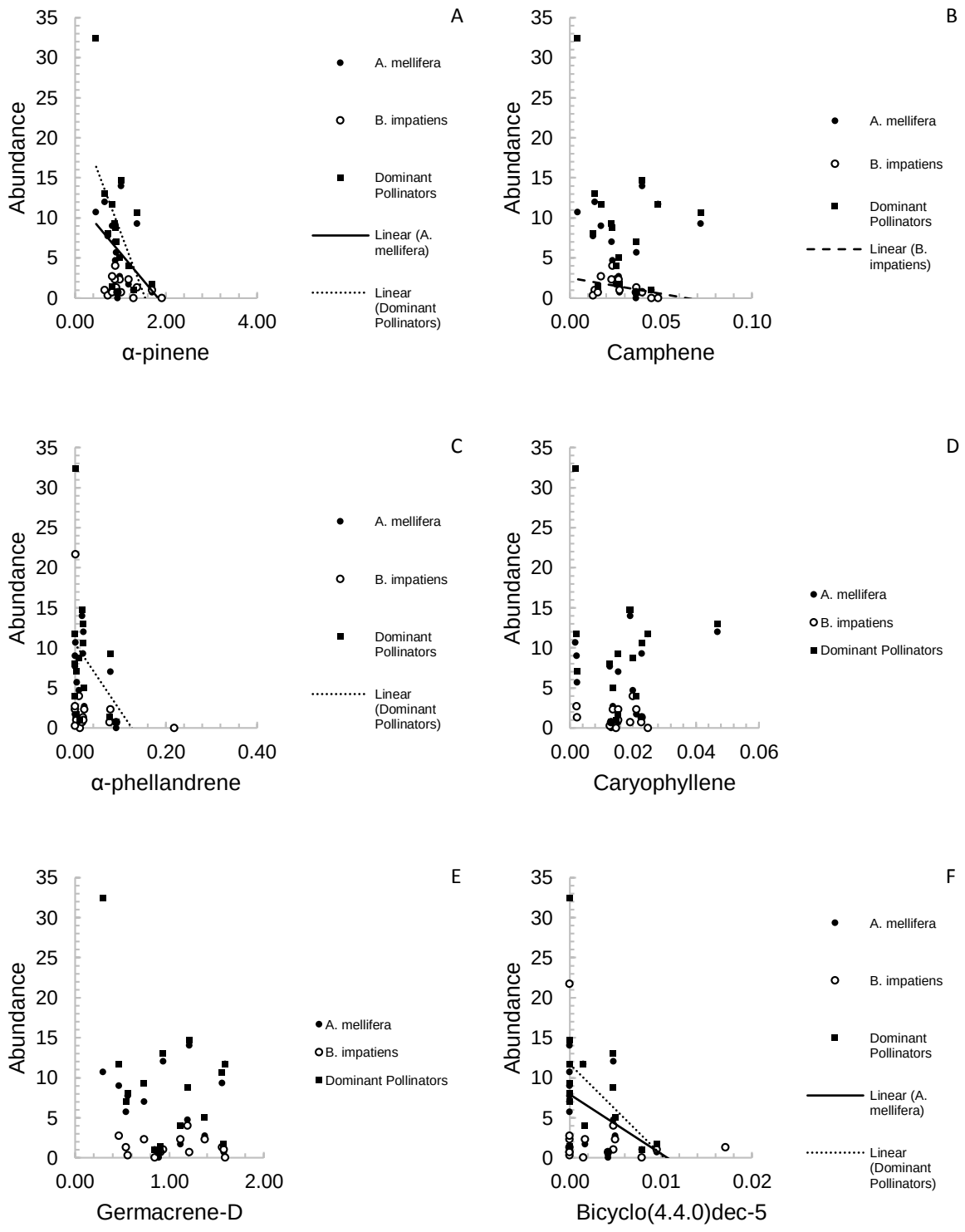

Fig. 24. Linear regressions of mean abundance for *A. mellifera* (solid line), *B. impatiens* (dashed line), and dominant pollinators (dotted line) and individual terpene concentrations.

The mean abundance of all pollinators was marginally related to both camphene and bicyclo(4.4.0)dec-5 proportions, with a significant relationship with p-cymene proportion (Table 10, Figure 25B, 25D, 25F). The mean abundance of order Hymenoptera was marginally related to both γ-elemene and bicyclo(4.4.0)dec-5 proportions, with a significant relationship with p-cymene proportion (Table 10, Figure 26D-F). The mean abundance of order Diptera was shown to have no statistically significant relationships with any individual terpene proportions (Table 10). The mean abundance of order Coleoptera was marginally related to both camphene and p-cymene proportions (Table 10, figure 26B, 26D). The mean abundance of order Lepidoptera was marginally related to both α-pinene and camphene proportions, with a significant relationship with β-pinene proportion (Table 10, Figure 26A-C). The mean abundance of *A. mellifera* was marginally related to γ-elemene proportion, with a significant relationship with p-cymene proportion (Table 10, Figure 27D-E). The mean abundance of *B. impatiens* was shown to have no significant relationships with any individual terpene proportions (Table 10). The mean abundance of dominant pollinators was significantly related to both p-cymene and γ-elemene proportions (Table 10, Figure 27D-E). The mean abundance of family Syrphidae was shown to have no significant relationships with individual terpene proportions (Table 10).

Table 10. The *P* value*, r^2, and *df* (Linear Regression, JMP 12) for mean pollinator abundance and individual terpene proportions.

	All Pollinators		Hymenoptera		Diptera		Coleoptera		Lepidoptera		*A. mellifera*		*B. impatiens*		Dominant	
Terpene	*p*	r^2	*p*	r^2	*p*	r^2	*p*	r^2	*p*	r^2	*p*	r^2	*p*	r^2	*p*	r^2
α-pinene	0.134	0.153	0.182	0.124	0.182	0.133	0.264	0.088	**0.072**	0.212	0.380	0.056	0.406	0.050	0.258	0.090
Camphene	**0.072**	0.213	0.128	0.158	0.942	0.000	**0.059**	0.232	**0.052**	0.243	0.459	0.040	0.217	0.107	0.193	0.118
β-pinene	0.197	0.116	0.171	0.129	0.343	0.064	0.155	0.139	**0.032**	0.287	0.268	0.087	0.422	0.050	0.155	0.139
α-phellandrene	0.419	0.047	0.414	0.048	0.481	0.036	0.492	0.034	0.733	0.009	0.298	0.077	0.263	0.089	0.196	0.117
P-cymene	**0.041**	0.266	**0.041**	0.265	0.943	0.000	**0.076**	0.207	0.397	0.052	**0.044**	0.260	0.307	0.074	**0.032**	0.288
β-elemene	0.691	0.012	0.760	0.007	0.492	0.034	0.157	0.138	0.820	0.004	0.837	0.003	0.881	0.002	0.977	0.000
Caryophyllene	0.338	0.071	0.303	0.081	0.892	0.002	0.429	0.045	0.613	0.019	0.192	0.127	0.329	0.068	0.146	0.155
Germacrene-D	0.444	0.043	0.299	0.077	0.308	0.074	0.151	0.141	0.749	0.008	0.135	0.153	0.464	0.042	0.177	0.126
Azulene	0.484	0.036	0.436	0.044	0.418	0.051	0.989	0.000	0.373	0.061	0.300	0.076	0.108	0.187	0.561	0.039
γ-elemene	0.323	0.070	**0.076**	0.222	0.893	0.001	0.712	0.010	0.122	0.162	**0.097**	0.198	0.136	0.152	**0.049**	0.267
Ledene Oxide	0.261	0.089	0.189	0.120	0.779	0.006	0.352	0.062	0.849	0.003	0.971	0.000	0.845	0.003	0.784	0.006
Bicyclo(4.4.0)d	**0.062**	0.227	**0.068**	0.219	0.668	0.014	0.114	0.168	0.971	0.000	0.200	0.115	0.373	0.057	0.117	0.167

Note: $p \leq 0.05$ (significant) and $p \leq 0.10$ (marginally significant) presented in **bold** text.

df = 1, 14 for individual terpene proportions.

df = 1, 13 for all pollinators (caryophyllene), Hymenoptera (caryophyllene, γ-elemene), Diptera (α-pinene, caryophyllene, azulene), Coleoptera (azulene), Lepidoptera (azulene), *A. mellifera* (caryophyllene, γ-elemene, ledene oxide), *B. impatiens* (β-pinene, germacrene-D, ledene oxide), dominant pollinators (caryophyllene, γ-elemene, ledene oxide), and Syrphidae (azulene).

*A Bonferroni correction would set significance of *P* to 0.0042.

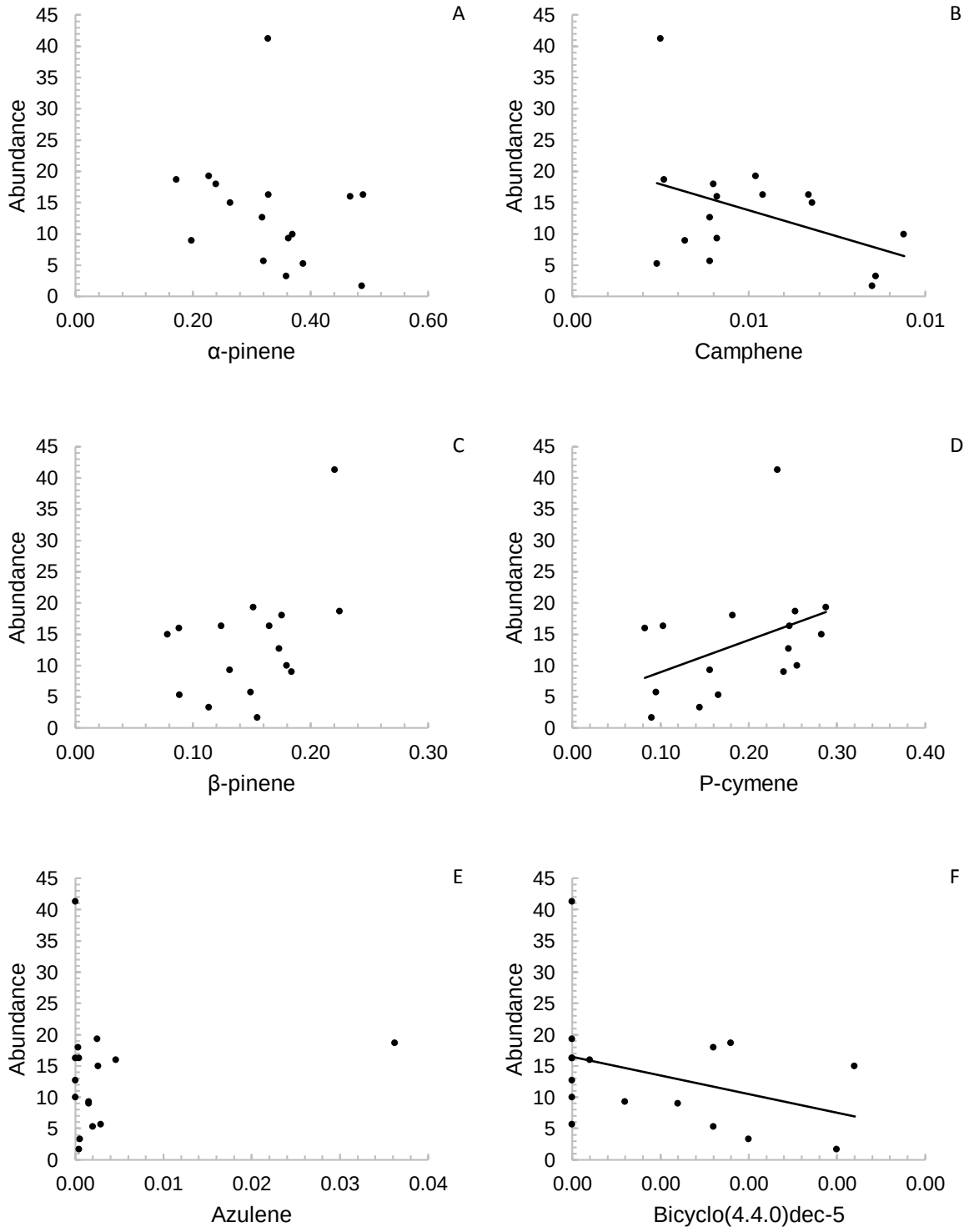

Fig. 25. Linear regressions of mean abundance for all pollinators and individual terpene proportions.

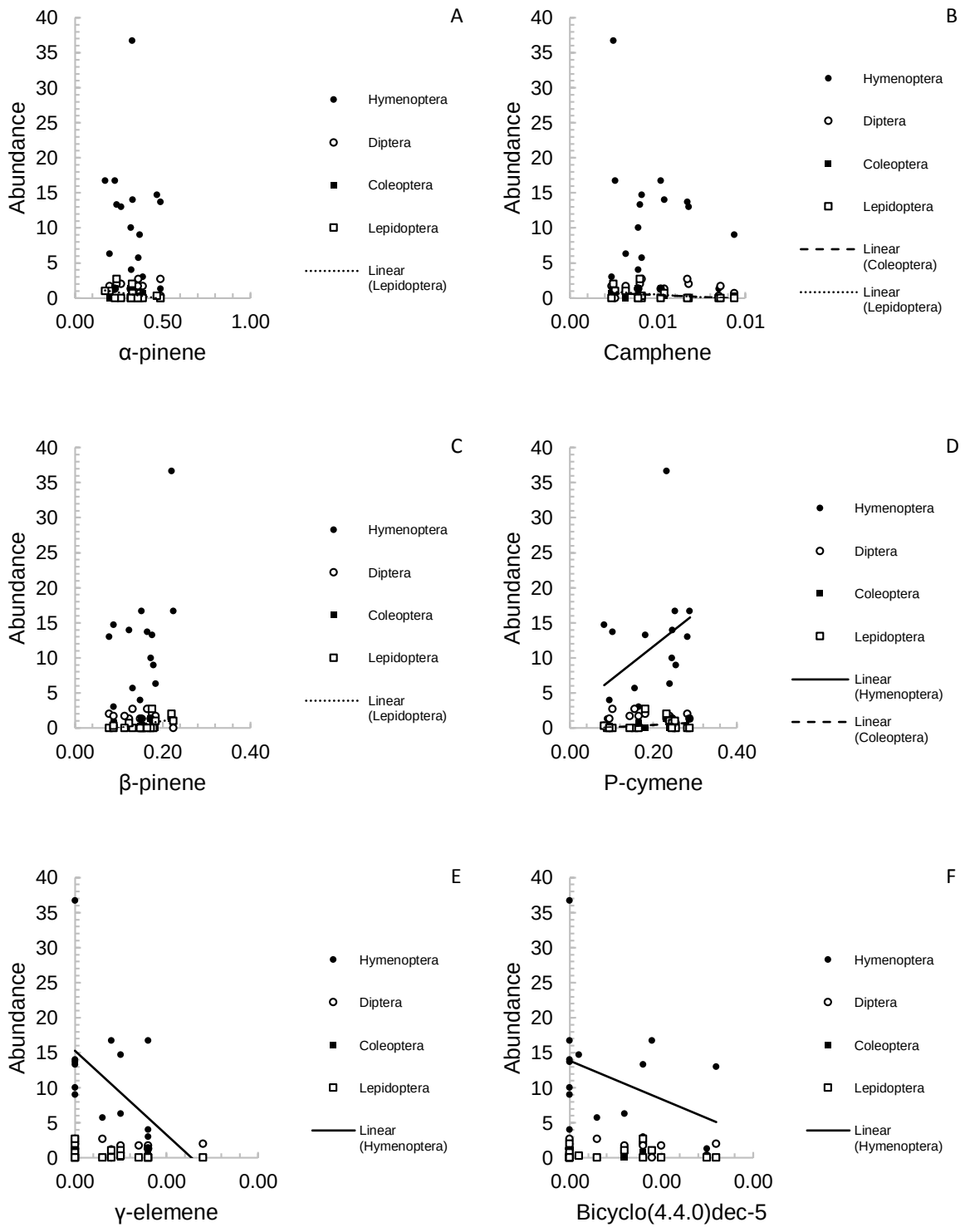

Fig. 26. Linear regressions of mean abundance for orders Hymenoptera (solid line), Diptera, Coleoptera (broken line) Lepidoptera (dotted line), and individual terpene proportions.

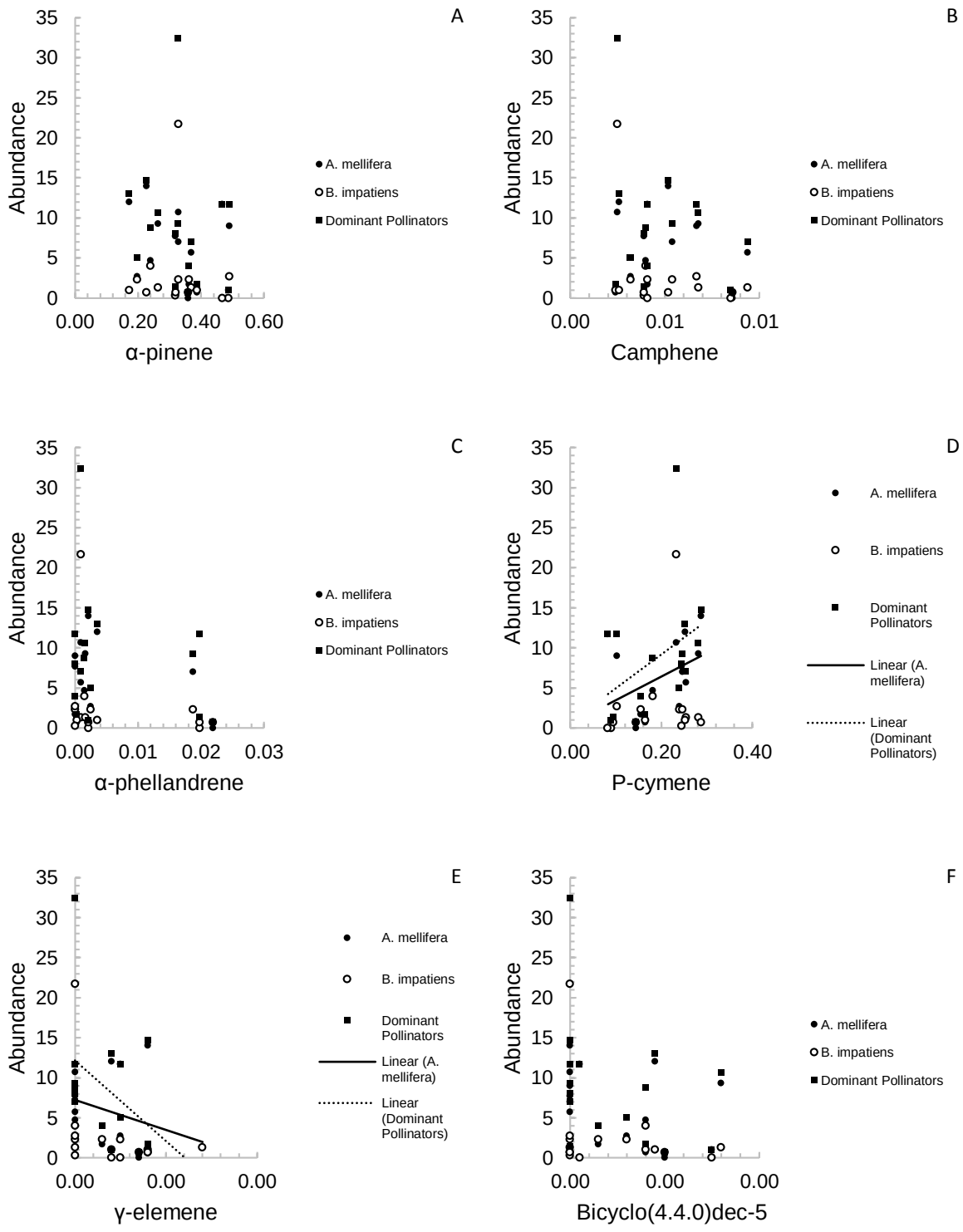

Fig. 27. Linear regressions of mean abundance for *A. mellifera*, *B. impatiens*, and dominant pollinators and individual terpene proportions.

Mean pollinator richness was marginally related to camphene concentration (Table 11, Figure 28B), where pollinator richness declined with increasing concentration of this compound. Community evenness was marginally related to α-pinene concentration (Table 11, Figure 30A), with a trend for the the community to become more similar as the concentration of α-pinene increased. No other significant relationships were found between pollinator richness, adjusted richness, or evenness and individual terpene concentrations (Table 11).

Table 11. The *P* value*, r^2, and *df* (Linear Regression, JMP 12) for pollinator diversity measures and individual terpene concentrations.

	Richness		Adjusted Richness		Evenness	
	p	r^2	*p*	r^2	*p*	r^2
α-pinene	0.2151	0.108	0.6290	0.017	**0.0836**	0.213
camphene	**0.0692**	0.217	0.1467	0.144	0.8492	0.003
β-pinene	0.5538	0.026	0.4654	0.039	0.9996	0.000
α-phellandrene	0.6468	0.015	0.1514	0.152	0.1954	0.125
p-cymene	0.9164	0.001	0.2366	0.098	0.4111	0.049
β-elemene	0.9905	0.000	0.8752	0.002	0.7764	0.006
caryophyllene	0.7544	0.007	0.6076	0.019	0.638	0.016
germacrene-D	0.6876	0.012	0.9155	0.001	0.7736	0.006
azulene	0.8105	0.004	0.1637	0.144	0.1701	0.140
γ-elemene	0.4687	0.038	0.3599	0.060	0.6273	0.017
ledene oxide	0.5290	0.029	0.7831	0.006	0.6831	0.012
bicyclo(4.4.0)dec-5	0.2491	0.094	0.7115	0.010	0.5904	0.021

Note: $p \leq 0.10$ (marginally significant) presented in **bold** text.
df = 1,14 for individual terpene concentrations and pollinator richness, adjusted richness, and evenness.
df = 1,13 for α-pinene and evenness, α-phellandrene and adjusted richness, α-phellandrene and evenness, azulene and adjusted richness, and azulene and evenness.
*A Bonferonni correction would set significance of *P* at 0.0042.

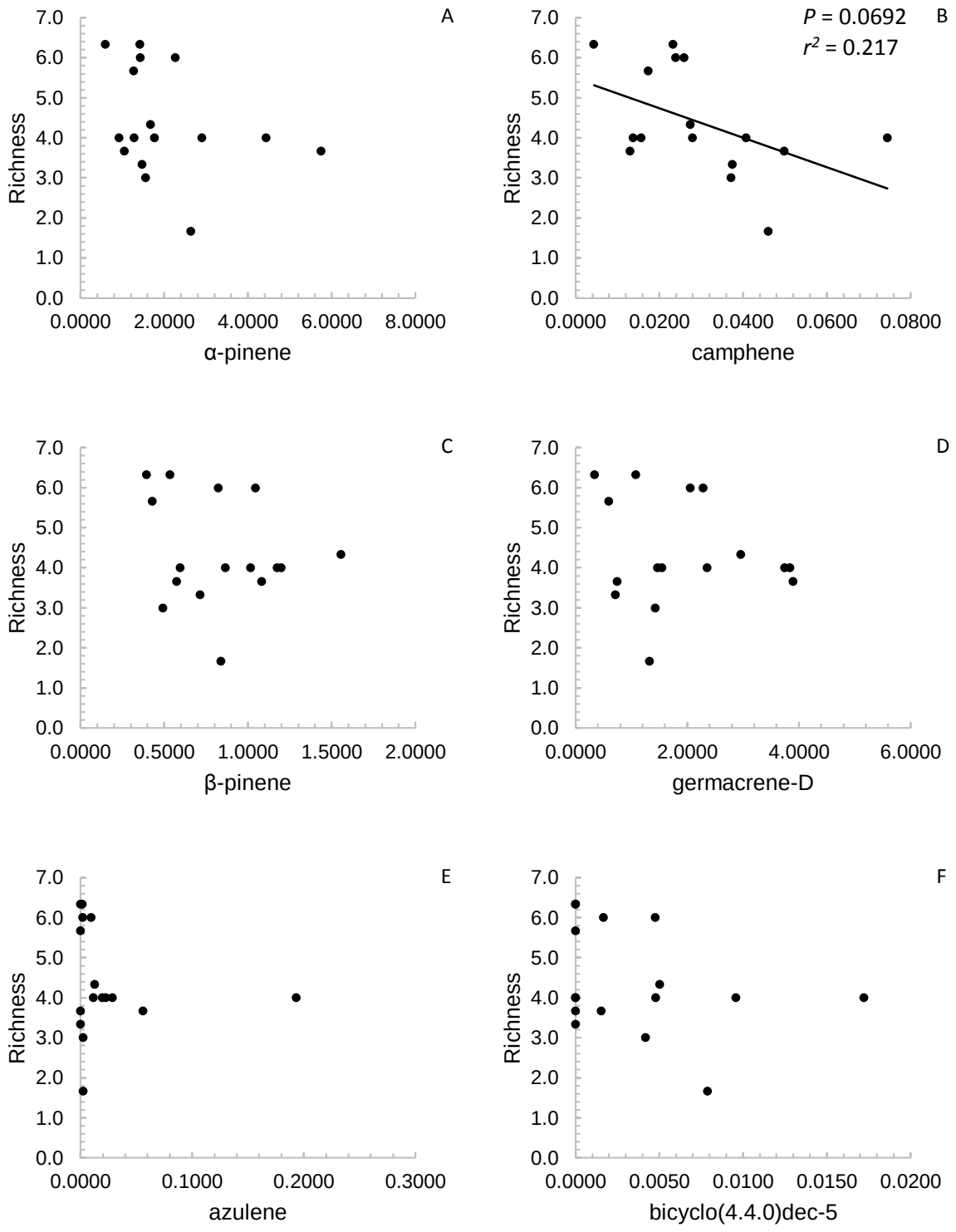

Fig. 28. Linear regressions of pollinator mean richness and α-pinene (A), camphene (B), β-pinene (C), germacrene-D (D), azulene (E), and bicyclo(4.4.0)dec-5 (F).

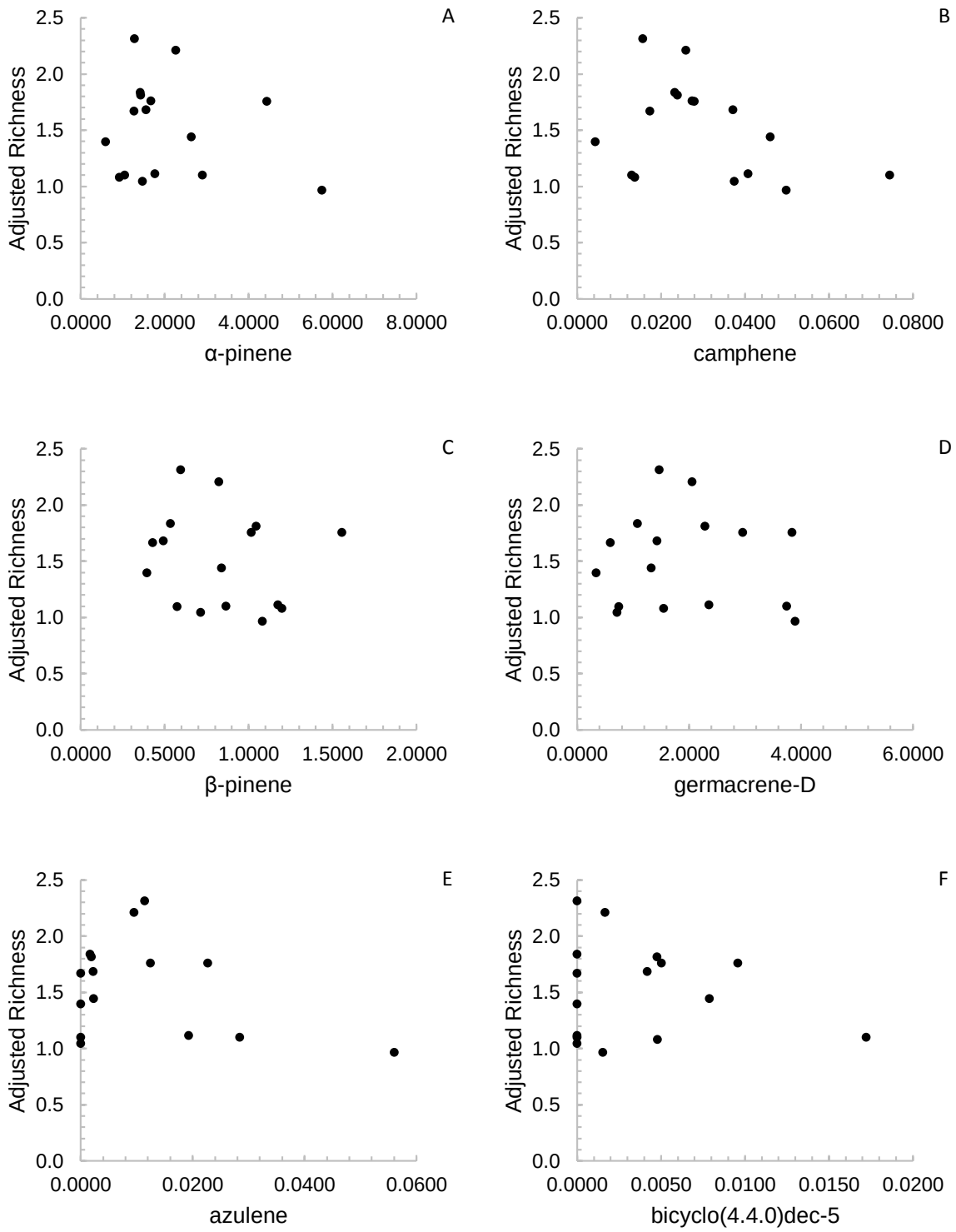

Fig. 29. Linear regressions of pollinator mean adjusted richness and α-pinene (A), camphene (B), β-pinene (C), germacrene-D (D), azulene (E), and bicyclo(4.4.0)dec-5 (F).

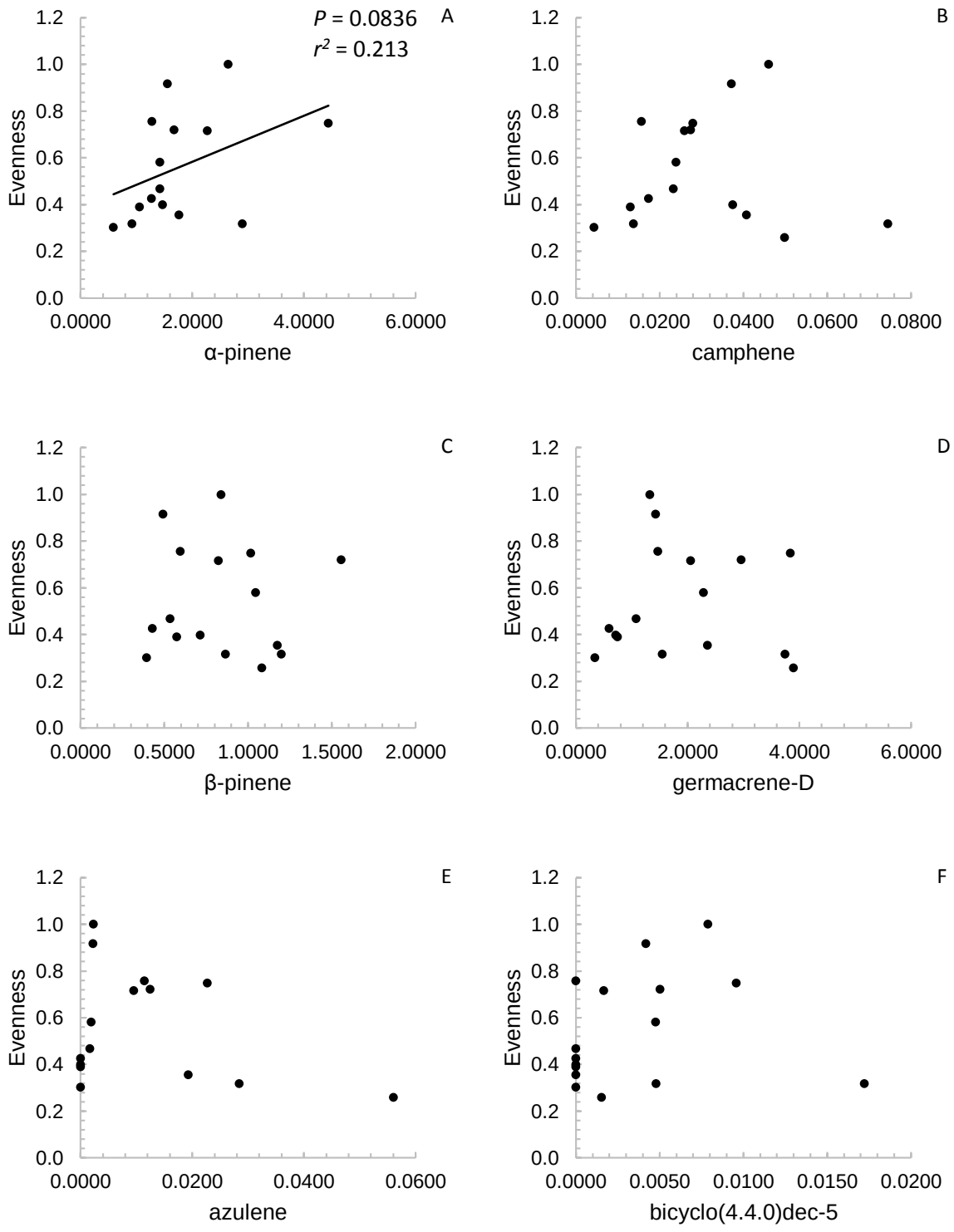

Fig. 30. Linear regressions of pollinator mean evenness and α-pinene (A), camphene (B), β-pinene (C), germacrene-D (D), azulene (E), and bicyclo(4.4.0)dec-5 (F).

Four significant relationships were found between soil nutrient content and the pollinator community measures. Pollinator richness is significantly related to soil carbon (mg/g) (Table 12, Figure 31B). Pollinator adjusted richness is significantly related to all 3 measures: soil nitrogen (mg/g), carbon (mg/g), and carbon to nitrogen ratio (Table 12, Figure 31A, C, D). The concentration of one terpene, ledene oxide, was marginally related to soil carbon to nitrogen ratio (Table 13).

Table 12. The *P* value*, r^2, and *df* (Linear Regression, JMP 12) for pollinator community measures and soil nutrient measures.

	Nitrogen g/mg soil		Carbon g/mg soil		C:N	
	p	r^2	*p*	r^2	*p*	r^2
Abundance	0.883	0.002	0.943	0.000	0.658	0.014
Richness	0.100	0.181	**0.092**	0.189	0.428	0.046
Adjusted Richness	**0.008**	0.407	**0.011**	0.384	**0.081**	0.201
Evenness	0.123	0.161	0.184	0.122	0.334	0.067

Note: $p \leq 0.05$ (significant) and $p \leq 0.10$ (marginally significant) presented in **bold** text.
df = 1,14.
*A Bonferroni correction would set significance of *P* at 0.012.

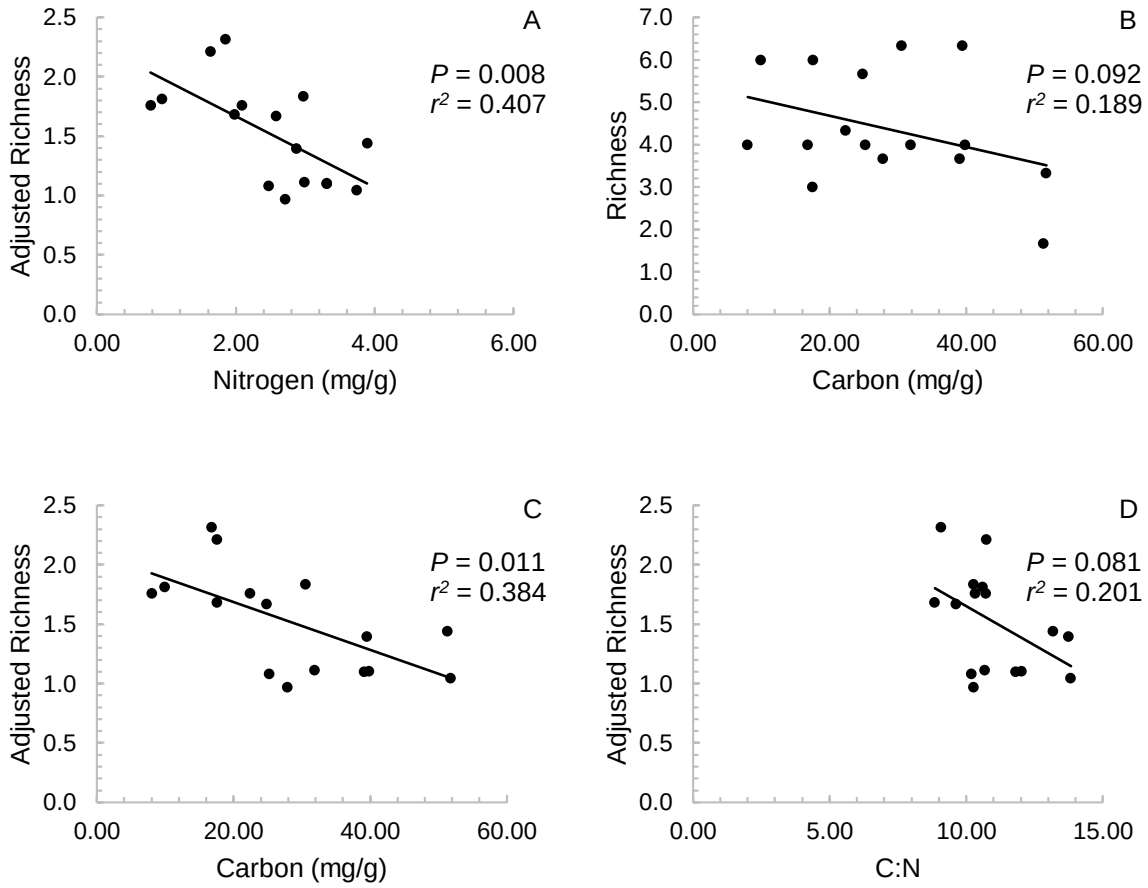

Fig. 31. Linear regressions of pollinator community measures and soil nutrient measures for adjusted richness and nitrogen (mg/g) (A), richness and carbon (mg/g) (B), adjusted richness and carbon (mg/g) (C), and adjusted richness and C:N (D).

Table 13. The *P* value*, r^2, and *df* (Linear Regression, JMP 12) between soil nitrogen and carbon to nitrogen ratio for individual terpene concentrations.

	Nitrogen g/mg soil		C:N	
	p	r^2	*p*	r^2
α-pinene (mg/g)	0.598	0.020	0.681	0.012
camphene	0.249	0.094	0.630	0.017
β-pinene	0.389	0.053	0.751	0.007
α-phellandrene	0.989	0.000	0.120	0.164
p-cymene	0.787	0.005	0.921	0.001
β-elemene	0.510	0.032	0.429	0.045
caryophyllene	0.426	0.046	0.134	0.153
germacrene-D	0.111	0.171	0.224	0.104
azulene	0.885	0.002	0.458	0.040
γ-elemene	0.819	0.004	0.743	0.008
ledene oxide	0.174	0.128	**0.078**	0.206
bicyclo (4.4.0) dec-5	0.773	0.006	0.711	0.010

Note: $p \leq 0.05$ (significant) and $p \leq 0.10$ (marginally significant) presented in **bold** text.

df = 1,14.

*A Bonferroni correction would set significance of *P* at 0.0042.

Partial Least Squares Regression

I used the multivariate technique Partial Least Squares Regression (PLSR) to model pollinator abundance in relation to terpenes. In order to build the most appropriate and robust model, only pollinator measures with substantial abundance within and between plots were used. These included: total pollinator abundance, mean pollinator abundance, *A. mellifera* abundance, *B. impatiens* abundance, and dominant pollinators (*A. mellifera* and *B. impatiens*) abundance. For each measure a significant relationship between abundance and terpenes was observed. (Table 14, Figure 32 A-E). Terpenes explained 45% of the variation in both total and mean pollinator abundance (all pollinators observed) (Table 14) and over half of the variation in abundance for *A. mellifera* and dominant pollinators. Because genotypes varied in terpene concentrations and proportions, these results suggest that terpenes seem to contribute to the observed responses of the pollinators in my study.

Table 14. The *P* value, *F* ratio, and r^2 (Partial Least Squares Regression, JMP Pro 10) for total abundance, mean abundance, *Apis mellifera*, *Bombus impatiens*, and dominant pollinators.

	p	*F*	r^2
Total Abundance	**0.0064**	10.53	0.447
Mean Abundance	**0.0064**	10.51	0.447
Apis mellifera	**0.0014**	16.28	0.560
Bombus impatiens	**0.0192**	6.70	0.333
Dominant Pollinators	**0.0016**	15.78	0.548

Note: $p \leq 0.05$ (significant) presented in **bold** text.
df=1,13 for total abundance, mean abundance, *Apis mellifera*, and dominant pollinators; *df*=1,14 for *Bombus impatiens*.

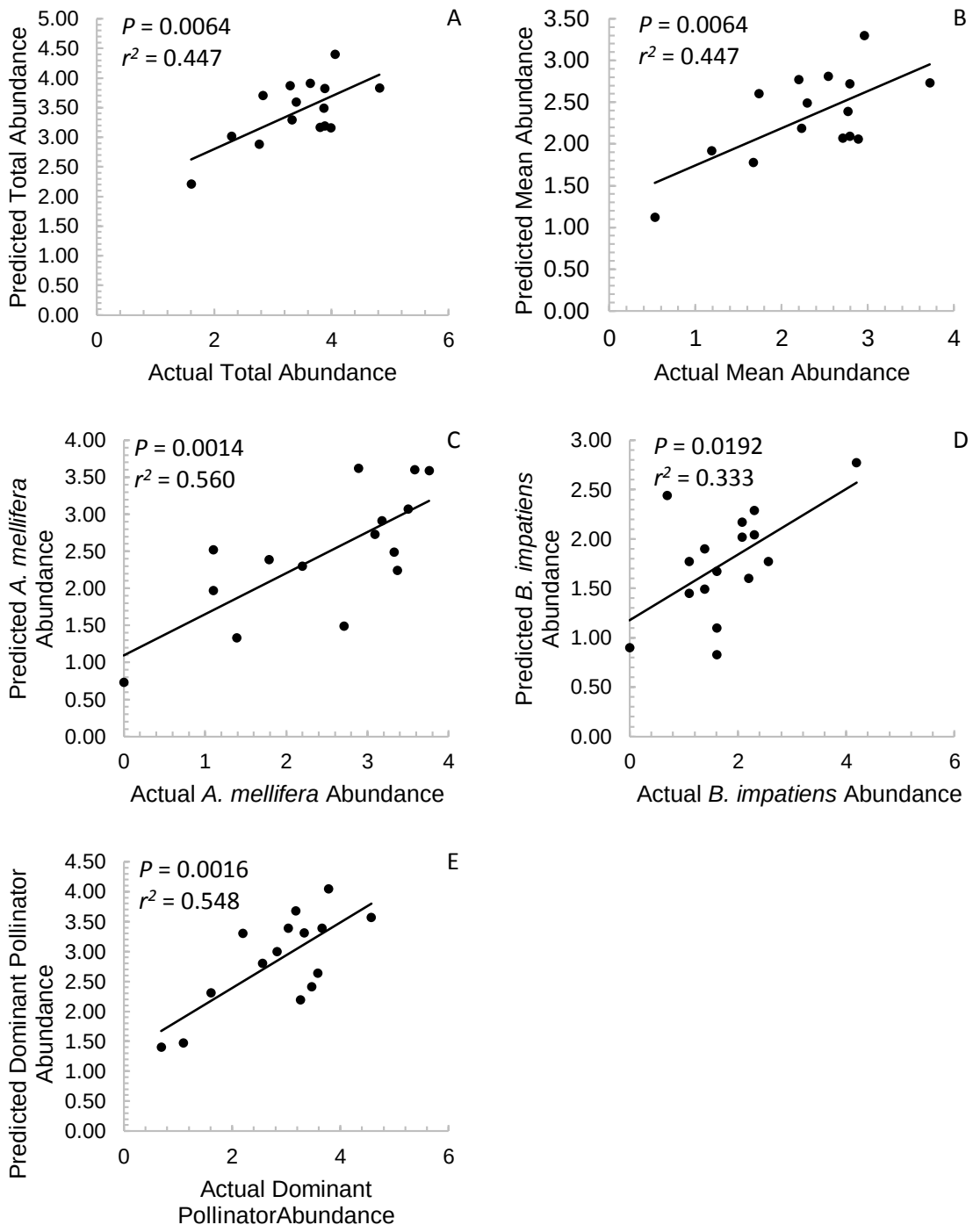

Fig. 32. Partial Least Squares Regression (PLSR) of total abundance (A) mean abundance, (B) *Apis mellifera*, (C) *Bombus impatiens* (D) and dominant pollinators (E).

Our PLSR model demonstrates statistically significant relationships between the pollinator community and terpenes, such that terpene variation explained 36-46% of the variation in pollinator richness, adjusted richness, and evenness (Table 15). The actual values pulled from our community data are strongly related to the predicted values from our PLSR model, indicating that terpenes are predictors of the associated insect pollinator community of *S. altissima* (Figure 33A-C).

Table 15. The *P* value, *F* ratio, and r^2 (Partial Least Squares Regression, JMP Pro 10) for pollinator diversity measures.

	p	*F*	r^2
Richness	**0.0135**	7.97	0.363
Adjusted Richness	**0.0036**	12.14	0.464
Evenness	**0.0061**	10.42	0.426

Note: $p \leq 0.05$ (significant) presented in **bold** text.
df=1,15 for richness and adjusted richness; *df*=1,14 for evenness.

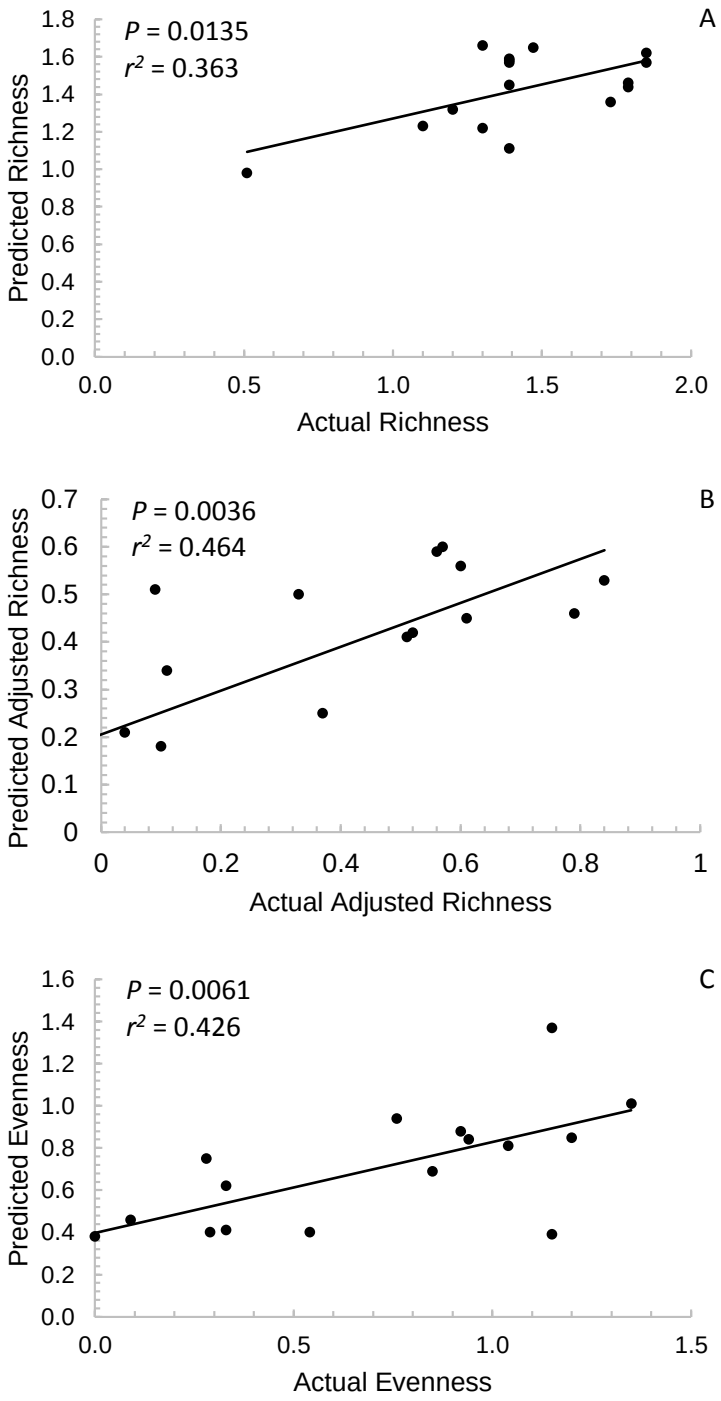

Fig. 33. Partial Least Squares Regression (PLSR) between the pollinator community and terpenes: richness (A), adjusted richness (B), and evenness (C).

DISCUSSION

Solidago altissima exhibits considerable intraspecific genetic variation, including in traits such as flowering phenology and floral production (Gross and Werner 1983; Genung et al. 2012b; Burkle et al. 2013) and ecosystem processes such as above- and belowground productivity (Crutsinger et al. 2006; Breza et al. 2012). Several studies have reported the effects that this variation has on the arthropod community of *S. altissima* and other foundation plant species (Crutsinger et al 2006; Genung et al. 2012b), but the importance of intraspecific genetic variation in this species with respect to floral phytochemistry and its impact on associated pollinators remains largely unexplored. The goal of my observation field study was to determine if relationships existed between flower phytochemistry (terpenes) and the insect pollinator community among genotypes of *S. altissima* at different spatial scales. I investigated presumed plant intraspecific trait variation within and between fields so that the role of genetic variation within patches (genetic identity effect) could be compared to effects of spatial scale (environment effect). Understanding the role of genotypic variation in flower phytochemistry within this plant-pollinator system addresses important questions about how terpenes might influence pollinator selection of *S. altissima* genotypes. In addition, my study investigated the possibility that spatial separation of genotypes could be a significant contributor to observed pollinator community and phytochemistry measures. Though some site effects on chemistry and pollinator abundances were observed, by and large my study found that variation at the level of patches (i.e., genotypes) was much more important than where they were located. Due to the nature of my

experimental design I had to make assumptions about the role of genetic variation, though I am very confident in my interpretation of the results. My analysis relating terpenes to pollinator measures supports the conclusion that terpenes play a role in genotype choice, while site has little influence.

Though other studies in the field of community genetics have examined the importance of the role that plant intraspecific genetic variation might play for insect associations (Johnson and Agrawal, 2005; Crutsinger et al. 2006), including for floral visitors (Genung et al. 2010; Burkle et al. 2013), specific reasons for genotype choice remain poorly understood. Using *S. altissima* clones, Burkle et al. (2013) found that the effects of host-plant genetic variation were more influential in structuring the pollinator community than were environmental effects or genotype by environment interactions. I asked if genotype and site affect the pollinator community and found that pollinator abundance and diversity were more influenced by differences among patches of *S. altissima* (representing genotypes) than by sites where patches were located. This result addresses previous concerns in community genetics studies that reported effects of genotypic variation on arthropod communities do not sufficiently consider the potential for spatial variation when drawing conclusions on the importance of genetic variation (Tack and Roslin 2011; Tack et al. 2012). Studies such as these suggest an overall inflation of the importance of host-plant genetic variation in structuring communities without considering spatial scale (environment effects). Incorporating spatial scale into community genetics studies allows for comparing the relative effects of genetic variation and the environment (Stratton and Bennington 1998; Johnson and Agrawal 2005). The scale-dependent hypothesis proposes that biotic factors, such as genetic variation, are more important in structuring communities at local levels and that abiotic

factors become more important at larger spatial scales (Menge and Olson 1990, Jackson et al. 2001). To address these issues and concerns that have arisen, I used naturally occurring populations of *S. altissima* that ranged from 6km to 16km apart from each other. When examining four fields separated by over 6km, I found almost no effect of spatial scale on the pollinator community (Table 1, 3). My data support the conclusion that genetic variation (i.e., differences between patches as determined by my statistical model) is more important in this widespread old-field plant species for pollinators than is the spatial separation of discrete patches. I make this conclusion with some caution as I was unable to replicate genotypes in my experiment, which would have provided a more robust way to investigate differences. While other factors could have contributed to the error term in my ANOVA model, which allowed me to make conclusions about genotype, I am confident my data fully support an important contribution of trait variation among my patches. My analysis that terpenes varied by genotype and not site demonstrated a role for these chemicals in pollinator choice of genotypes.

My study found that, in addition to total and mean abundance measures of pollinating species, the species richness of the pollinator community was influenced much more by differences among patches than among sites (Table 3). This result was different when adjusted richness was calculated, but the marginally significant effect of site in this measure still leads to conclusions about the importance of genetic variation in my study. Using flowering time and abundance as mechanisms through which pollinators choose host plants, Genung et al. (2010) investigated the importance of plant intraspecific genetic variation and diversity for the pollinator community. Their results indicated that underlying genetic traits in *S. altissima*, and subsequent genotypic variation in flowering phenology, impacted the

abundance and richness of floral communities, but that any impact on pollinator richness was indirectly caused by plant traits influencing insect abundance. In a related study comparing the effects of genetic identity, genotypic diversity, and nutrient enrichment of *S. altissima* clones, Burkle et al. (2013) found that it was genetic variation that had the strongest effect on the floral visitor community. Their findings were largely due to intraspecific variation in the time of flowering. My experiment tried to control for genotypic variation in phenology (see Materials and Methods), so it seems unlikely my observed differences among patches in the pollinator community was due to differing flowering times. Though my data largely show variation among *S. altissima* genotypes rather than the spatial separation of fields affected pollinator abundance there were exceptions. For example, insects in the Order Coleoptera, which can contain common pollinators, and the eastern carpenter bee, *Xylocopa virginica*, were more influenced by site. It is perhaps not surprising that due to the diversity of the pollinator community that spatial effects would be found. But when the most common and abundant pollinators are considered (*Apis mellifera* and *Bombus impatiens*) patch variation is most evident. These are without doubt wide ranging pollinators in *Solidago* fields and likely make choices based on a variety of factors. Bees are known to use floral features including color, scent, and texture, when locating host-plants.

One of the main goals of my study was to investigate the importance of flower terpenes as an explanation for pollinator choice of patches. Variation among *S. altissima*, presumed to be largely explained by genotypes, explained much of the variation in pollinator abundance and diversity and flower terpene concentration, suggesting that genetically based traits such as phytochemistry could influence the insect pollinator community. Previous investigations have shown secondary chemicals exhibit variation among genotypes, including

tannins (Schweitzer et al. 2004, 2008) and terpenes (Dungey et al 2000; Williams and Avakian 2015), and that intraspecific genetic variation in phytochemistry can affect associated arthropod communities (Raguso et al. 2007; Johnson et al. 2009; Smith 2015). However, no studies relating flower terpenes and pollinators in *S. altissima* had been previously conducted.

In my study both concentrations and proportions of individual floral terpenes were primarily affected by differences among patches rather than site (Table 4-5, Figure 11-14). This result shows that differences in where patches are located was less important than differences between patches, supporting the conclusion that a genetic identity effect, and not an environment effect, occurred. As with abundance and community measures, differences among genotypes were greater than among sites for almost all the compounds I quantified. A study by Williams and Avakian (2015) that used *S. altissima* was one of the first to demonstrate an effect of host-plant genotype identity and subsequent terpene variation on an associated insect. Plant volatile organic compounds are emitted from foliage to deter herbivory and from flowers to attract pollinators (Caissard et al. 2004), and floral terpene emissions are known to vary among plant species partly due to the diversity in the species involved in plant-pollinator interactions (Byers et al. 2014a). Because insects in part rely on olfaction when locating host-plants (Chittka and Raine 2006; Milet-Pinheiro et al. 2015), it seems plausible that genotype-unique compositions of terpenes and other volatile compounds influenced genotype choice in my experiment. For my plants the effects of terpenes could have been due to either/or thei presence in nectar or emissions from flowers. My study did not allow me to separate the two. In a study by Morse et al. (2012), *B. impatiens* preferred to pollinate tomato flowers that produced less of the terpenes β-phellandrene and careen, thus

illustrating the importance of specific compounds. Whatever the perception method, my data support the use of some terpenes by pollinators.

In addition to changes among patches in the amount of terpenes produced, linear regression results demonstrated several marginally significant ($p \leq 0.10$) and statistically significant relationships ($p \leq 0.05$) between the abundance of various pollinator or pollinator groups and the concentrations and proportions of individual terpenes. Though some of my relationships were relatively weak (low r^2), these data lend evidence that terpenes could play a role in the choice of patches, and therefor genotypes, in my study. In many cases, there were similarities in the significance in relationships with certain terpenes among Order Hymenoptera, which included *A. mellifera*, *B. impatiens*, and dominant pollinators. These similarities are likely due to the greater abundance of both *A. mellifera* and *B. impatiens* compared to other pollinators. For many of the pollinator groups in my study, there were particular terpenes that seem to play important roles in attracting or deterring them from host-plants. The concentrations of α-pinene, camphene, α-phellandrene, caryophyllene, azulene, and bicyclo(4.4.0)dec-5 apparently act as deterrents for pollinators, as pollinator abundance declined at higher levels of concentrations (Table 7). Camphene was also negatively correlated to pollinator species richness (Figure 28B), yet as α-pinene increased, the pollinator community became more evenly represented (evenness closer to 1; Figure 30A). Additionally, higher γ-elemene proportions were associated with decreased abundances of bee pollinators, while higher p-cymene proportions seemed to attract bee pollinators to *S. altissima* genotypes. A study that investigated floral scent of six species in family Apiaceae found large quantities of species-specific fragrances – some including the terpenes α-pinene and β-pinene – which likely act as attractants for insect pollinators (Borg-Karlson et al.

1994). In my study there was not clear indication of the role these terpenes played in *S. altissima*, where with some pollinators α-pinene showing a negative relationship and β-pinene few if any. This is largely supported as well by the exclusion of this compound from the VIP factors in my PLSR analysis. It is interesting that separate taxonomic groups varied from being significantly related to both terpene concentrations and proportions to either terpene concentration or proportion. This variation might suggest differences in how certain pollinators perceive compounds, such that an individual terpene may be important to one taxonomic group, while a particular suite of terpenes is important for another taxonomic group.

PLSR analyses indicated that suites of compounds extracted from flowers related to pollinator abundance and diversity as groups of terpenes accounted for much of the variation observed in pollinator abundance and community measures. Because genotypes varied in terpene concentrations and proportions, these results suggest that terpenes contribute to the observed responses of the pollinators among patches in my study. Similar to previous studies (Avakian 2014; Howells 2014; Bonville 2016; Williams and Avakin 2015), that have demonstrated the importance of leaf terpenes in explaining the abundance of a specialist aphid species, these compounds appear to play a role in pollinator choice of genotypes.

Though I found few site effects on flower phytochemistry and pollinator abundance and diversity measures, there were significant differences in soil nitrogen, carbon, and C:N ratio among sites. Previous work with *S. altissima* found that soil nutrient availability affected the associated pollinator community, though the insect responses were much weaker than those due to genotype (Burkle et al. 2013). In my study adjusted richness was significantly related to each of the three soil measures, and richness was significantly related

to soil carbon. But with one exception there were no significant relationships found between soil nitrogen or C:N and individual terpene concentrations (Table 13). Although soil nutrient content was one of the few measures that significantly varied among sites in my study, the lack of significant relationships between soil nutrients and terpenes suggests that site effects in nutrients was not reflective of pollinator community responses with respect to terpene production and my identified relationships between soil nutrients and community measures stems from other factors not accounted for in my study.

In conclusion, by focusing on terpenes as a possible explanation for pollinator choice of site or genotypes in *S. altissima*, my study addresses previous concerns in community genetics research that plant-pollinator interactions have been largely unexplored, including for phytochemical explanations with respect to intraspecific genetic variation in a foundation plant species. I included a spatial component to tease out the relative importance of genetic variation compared to spatial scale and found that what I perceived as genetic variation was indeed a stronger influence on the associated insect pollinator community. My results somewhat counter previous suggestions that genetic variation effects are inflated at larger scales due to the effect of differing environments. Though my data support the role of terpenes in the choice of genotypes by pollinators, they could not specifically provide a mechanism and direct comparison among genotypes for observed differences among patches in pollinator abundance, richness, and community evenness. Even so, my study is strongly suggestive that further studies, including experiments designed to examine pollinator species preferences for terpenes, are warranted.

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APPENDICES

Appendix 1

GPS Coordinates of Patches

State Farm	Coordinates (N)	Coordinates (W)	Elevation (m)
Patch 1	36.20???°	081.64???°	946
Patch 2	36.20913°	081.64898°	944
Patch 3	36.20890°	081.64855°	947
Patch 4	36.20876°	081.64801°	942

Tom Jackson	Coordinates (N)	Coordinates (W)	Elevation (m)
Patch 1	36.26632°	081.61517°	1001
Patch 2	36.26665°	081.61482°	1009
Patch 3	36.26671°	081.61525°	1011
Patch 4	36.26667°	081.61567°	1007

US 421	Coordinates (N)	Coordinates (W)	Elevation (m)
Patch 1	36.23735°	081.74656°	887
Patch 2	36.23756°	081.74718°	887
Patch 3	36.23822°	081.74590°	893
Patch 4	36.23774°	081.74622°	891

Appendix 2

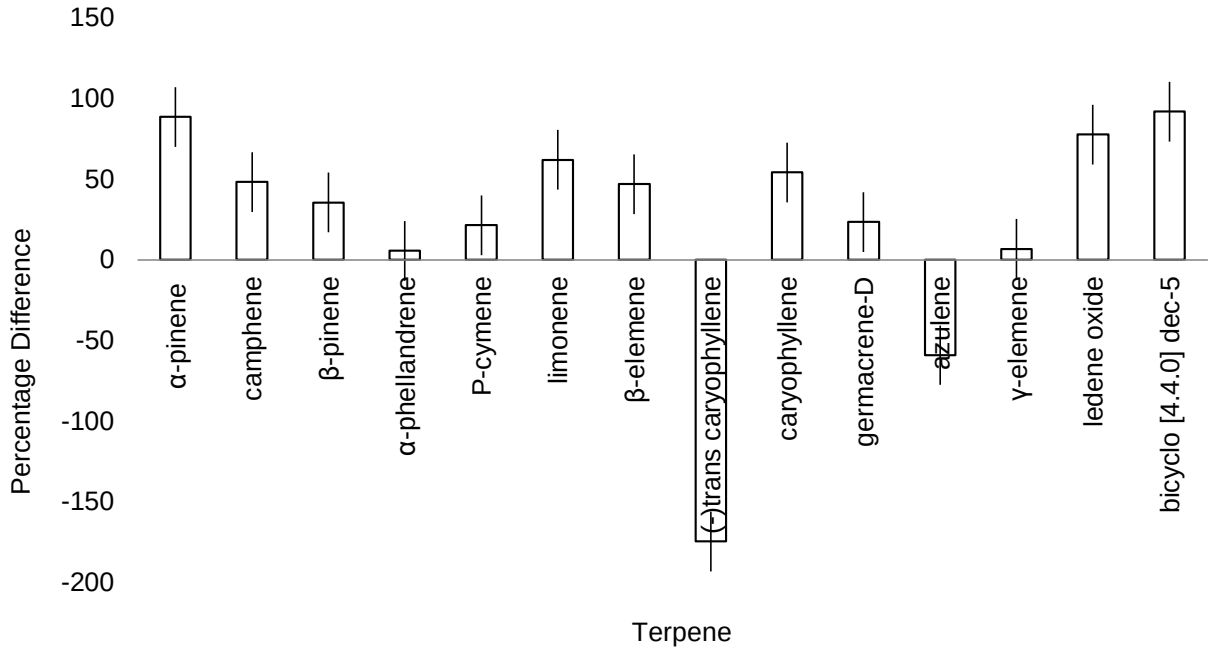
Mass of flowers used for terpene extraction

State Farm	Mass (g)	Tom Jackson	Mass (g)
Sample 1-1	2.0405	Sample 1-1	2.0174
Sample 1-2	2.0083	Sample 1-2	2.0450
Sample 1-3	2.0511	Sample 1-3	2.0263
Sample 1-4	2.0204	Sample 1-4	2.0402
Sample 1-5	2.0245	Sample 1-5	2.0344
Sample 2-1	2.0199	Sample 2-1	2.0369
Sample 2-2	2.0185	Sample 2-2	2.0336
Sample 2-3	2.0150	Sample 2-3	2.0596
Sample 2-4	2.0161	Sample 2-4	2.0139
Sample 2-5	2.0136	Sample 2-5	2.0064
Sample 3-1	2.0348	Sample 3-1	2.0359
Sample 3-2	2.0449	Sample 3-2	2.0278
Sample 3-3	2.0146	Sample 3-3	2.1193
Sample 3-4	2.0128	Sample 3-4	2.0635
Sample 3-5	2.0548	Sample 3-5	2.0938
Sample 4-1	2.0627	Sample 4-1	2.0424
Sample 4-2	2.0368	Sample 4-2	1.9840
Sample 4-3	2.0336	Sample 4-3	2.0146
Sample 4-4	2.0301	Sample 4-4	2.0281
Sample 4-5	2.0399	Sample 4-5	2.0451
Parkway	Mass (g)	US 421	Mass (g)
Sample 1-1	2.0301	Sample 1-1	2.3321
Sample 1-2	2.0212	Sample 1-2	2.0805

Sample 1-3	1.9372	Sample 1-3	2.1877
Sample 1-4	2.0212	Sample 1-4	2.0331
Sample 1-5	2.1321	Sample 1-5	2.0558
Sample 2-1	2.0534	Sample 2-1	2.2365
Sample 2-2	2.0325	Sample 2-2	2.2880
Sample 2-3	2.0036	Sample 2-3	2.2997
Sample 2-4	2.1830	Sample 2-4	2.2806
Sample 2-5	2.0773	Sample 2-5	2.1343
Sample 3-1	2.0730	Sample 3-1	2.2349
Sample 3-2	2.1051	Sample 3-2	2.1163
Sample 3-3	2.0294	Sample 3-3	1.9558
Sample 3-4	2.2350	Sample 3-4	2.2685
Sample 3-5	2.2585	Sample 3-5	2.1158
Sample 4-1	2.0405	Sample 4-1	2.0615
Sample 4-2	2.0744	Sample 4-2	2.1444
Sample 4-3	1.9189	Sample 4-3	2.0811
Sample 4-4	2.1037	Sample 4-4	2.2239
Sample 4-5	2.0350	Sample 4-5	2.0286

Appendix 3

Percentage difference in individual terpene concentration between *S. altissima* flowers and calyces.



Vita

Julie Anna Ragsdale was born in 1990 in the sunny city of Tallahassee, Florida to her mother Gaye Anne Groot and father Burr Augustus Ragsdale III. She knew at a young age that she was interested in plants and insects. Her first science fair experiment entailed surveying the bugs found under rocks in her back yard, awarding her the nickname "Julie Bug". She maintained this interest in biology, and upon graduating high school she left the sunshine state behind her and ventured to North Carolina for college. Julie attended Appalachian State University, where she earned her Bachelor of Science in Biology. She discovered her passion for plant sciences early on in her academic career. Her love of the school and town of Boone led her to stay for a graduate program in biology, during which she worked with her advisor Ray S. Williams. During this time, she worked as a teaching assistant instructing biology labs at the university. After three years of rigorous study, she received the Master of Science degree in Ecology and Evolutionary Biology. Julie is the first in her immediate family to earn a bachelor's degree and the first to continue her education for a master's degree.