MECHANISMS PROMOTING SPATIAL AND TEMPORAL VARIABILITY IN PLANT DEFENSE

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
In partial fulfillment of the requirements for the degree of
Doctor of Philosophy
in
The Department of Biological Sciences

by
Alyssa Stocks Hakes
B.S., University of Illinois at Urbana-Champaign
August, 2010

ACKNOWLEDGMENTS

This dissertation project was completed due to the support of many people. I would like to first thank my advisor, Jim Cronin, for providing me with quality scientific training and for encouraging me to persevere through various disasters that complicated my field work. I am also very appreciative of the thorough nature in which Jim reviewed and improved my manuscript drafts. My graduate committee members Kyle Harms, Michael Stout, and Michael Hellberg, and Dean's representative, Carl Motsenbocker, also guided my professional development and to them I am grateful. I learned much from the graduate courses Kyle Harms and Michael Stout led, and Michael Stout and Michael Hellberg provided me with equipment for conducting chemical analyses and DNA extraction. I would also like to thank the administrative staff in the Department of Biological Sciences, especially Prissy Milligan and Jill Atwood, whose extensive knowledge and cheerful disposition helped make the necessary paperwork as easy as possible.

I would like to extend a special thanks to landowners Baton Rouge Recreation & Park Commission, Bluebonnet Land & Development, Entergy Gulf Coast States Utilities, and to the families of Roger Fritchie, Robert McVea, and Mostafa Khosravanipour for allowing me to conduct field research on their properties. Despite miserable conditions during the summer, Kelly Briggs, Farrar Brown, Katrina Florek, Charlotte Gates, Jon Hakes, Erick Rietscher, Maria Waight, and Amanuel Zeccarias provided me with welcomed field assistance. I cannot thank them enough for their hard work. Funding for my dissertation project was provided by NSF grants DEB 0211359 and DEB-0515764, Association of Women in Science, American Museum of Natural History, SDE Graduate Women in Science, LSU Biograds, and Sigma Xi.

My labmates were a huge source of support and friendship throughout my time at LSU. Kyle Haynes helped me to navigate Baton Rouge and adapt to life as a graduate student during my first year at LSU. Forrest Dillemuth was an indispensible member of the Cronin lab and I enjoyed his companionship on many research and conference trips. Heather Jackson was a wonderful friend and a prodigy of statistics and R programming. These qualities made her the ideal person to turn to whenever I needed help solving problems, including those involving data. Her passalid beetle condos gave the lab some character and her personal condo near my field site gave me refuge from hot summer days and afternoon storms. When Amanda Accamando joined the Cronin lab, we became very close in terms of friendship, as well as desk proximity. Amanda shared my interest in plant defense and always made me aware of important journal articles that I happened to miss. Ganesh Bhattarai was a welcome addition to the lab during my final year at LSU and was always willing to attend a social function with me. Kristen Baum and Elizabeth Jackson also enriched my life during their time with the lab. Many additional friends in the Department of Biological Sciences made my experience at LSU memorable and enjoyable. Lastly, the people at St. Paul Lutheran church and Girl Scouts Louisiana East helped me balance work with much needed recreation and community involvement. I will especially miss my Girl Scout troop whose members I have watched grow into wonderful young adults over these years.

My parents, John and Wendy Stocks, were instrumental in fostering my interest in the natural sciences by providing me with many outdoor experiences including numerous family vacations to State and National Parks and scouting trips to wilderness areas. They and my brothers, Jack and Kyle, along with the Hakes family deserve acknowledgment for their steadfast support and encouragement throughout my graduate education. Lastly, I want to offer my sincere gratitude to my amazing husband, Jon Hakes, for his constant love and support. He has sacrificed his time and energy in helping me realize my dream of becoming an ecologist and I wouldn’t have wanted to undertake this project without him by my side.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Mechanisms responsible for the diversity of plant defenses in natural populations are poorly understood. This dissertation examines how environmental heterogeneity and succession influence the distribution, expression, and adaptive value of resistance and tolerance of goldenrod (*Solidago altissima*). Within replicate early- and late-successional fields, I quantified the spatial structure of herbivore damage and goldenrod resistance, tolerance, and fitness traits, and assessed whether the spatial structure of defense was correlated with edaphic conditions and neighboring plant community. I then conducted a common garden experiment to empirically measure genetic variability of resistance and tolerance and quantify their associated fitness costs and selection gradients. Lastly, I conducted a field experiment to test the prediction that successional changes in the environment alter the adaptive value of resistance and tolerance. To this end, I transplanted goldenrod genets into early- and late-successional fields and measured defense levels, fitness costs, and selection gradients.

Field surveys revealed that defense traits were strongly spatially structured (i.e., autocorrelated), and late stages were more strongly structured than early stages. There were stage-specific relationships between defense and neighbor variables. Damage was correlated with vegetative cover in early stages, and tolerance traits were positively correlated with canopy cover in late stages. In the common garden, I found genetic variability in resistance and tolerance, but low heritability. Herbivores imposed strong selection for increased tolerance but not resistance. Results from the field experiment revealed an apparent succession-induced shift in the expression and adaptive value of resistance and tolerance. Resistance was 41% greater and tolerance was 97% lower for goldenrods planted in early than in late stages. Resistance was more adaptive in early stages and tolerance may be beneficial in mitigating the effects of both herbivory and low light availability in late stages.

This represents the first study to examine plant resistance and tolerance to herbivory in a spatiotemporal context. I conclude that environmental heterogeneity and succession may be important mechanisms promoting the spatial and temporal variability of plant defense expression in nature. Fluctuating adaptive landscapes may have important implications for trait evolution and should be incorporated into future studies of plant defense.

CHAPTER 1- INTRODUCTION

Resistance and tolerance are well recognized as alternative defense strategies plants can express to reduce the negative fitness effects of herbivore damage (e.g., Painter 1958, Fineblum and Rausher 1995). Resistant plants reduce damage suffered from herbivores, whereas tolerant plants reduce the negative fitness effects of damage without affecting the amount of damage plants incur. Plant resistance traits encompass morphological or chemical traits that reduce herbivore preference or performance, and tolerance traits include physiological mechanisms that increase compensatory growth or photosynthesis after damage (reviewed in Stamp 2003). Both resistance and tolerance strategies may yield similar benefits of higher fitness in the presence of herbivores relative to non-defended plants (e.g., Tiffin 2000). However, both defense strategies may incur fitness costs when herbivores are rare or absent if resources are allocated towards traits at the expense of plant growth or reproduction (reviewed in Bergelson and Purrington 1996). It is assumed that the type and level of defense traits plants express is influenced by the balance of the fitness costs and benefits associated with each trait (Stamp 2003).

Conventional defense theory has predicted through simple models that plant populations should eventually become fixed with the most adaptive defense trait or strategy (Herms and Mattson 1992, Mauricio et al. 1997). However, numerous studies have found that natural plant populations often express intermediate levels of both tolerance and resistance (e.g., Mauricio et al. 1997, Tiffin and Rausher 1999, Fornoni et al. 2004a). An emerging, yet untested theory predicts that mixed levels of resistance and tolerance may be selected and maintained within populations when there are conditions of variable costs and benefits (Fornoni et al. 2004a, Núñez-Farfan et al. 2007). Studies examining the defense phenotypes of plants in multiple community types and identifying the mechanisms responsible for variation in levels of defense within natural landscapes are needed (Agrawal et al. 2006).

It is known that environmental factors can influence the expression of plant resistance and tolerance in a variety of ways, though there is still no generality regarding the direction of the effect. For example, plants may not invest in resistance when resources are limited (e.g., Herms and Mattson 1992, Bergelson 1994). Alternatively, resistance may increase when resources are low if plants invest more in defense when growth is costly (e.g., Coley et al. 1985). The relationship between resource availability and plant tolerance is likewise inconsistent (reviewed in Hawkes and Sullivan 2001). The ability of a plant to tolerate damage may be greater when there are more resources available for growth (e.g., Maschinski and Whitham 1989). Alternatively, compensatory growth may be greater in low resource areas where plant relative growth rate remained below its potential prior to damage (e.g. Hilbert et al. 1981). Newer models predict that the relationship between resource levels and tolerance will vary depending on which resources are limiting and which parts of the plant are affected by damage (Wise and Abrahamson 2005, 2007). In addition to resource availability, competition with neighbors may alter resistance and tolerance levels. Competition may decrease defense expression if there is a tradeoff between defenses against herbivory and competition (Bazzaz et al. 1987, Bergelson and Purrington 1996, Cipollini and Bergelson 2001, 2002). Alternatively, competition may increase defense expression when defense traits serve a dual purpose mitigating against herbivory and competitive stress (e.g., Siemens et al. 2002, Siemens et al. 2003, Jones et al. 2006). Herbivore damage may also be indirectly influenced by neighbor interactions. For example, spatial proximity to more resistant/less palatable neighbors may reduce a plant's probability of being found and damaged by herbivores (Hay 1986, Baraza et al. 2006, Bergvall et al. 2006, Miller et al. 2007, Barbosa et al. 2009). Given that these variables (i.e., neighboring plant composition and resource availability) are heterogeneous in nature, we may expect geographic variation in selection landscapes (e.g., Thompson and Cunningham 2002, Rudgers and Strauss 2004) and complex spatial patterns in plant resistance and tolerance expression. Studies examining the spatial distributions of plant defense traits in natural populations are needed to assess potential causes for diverse levels of resistance and tolerance traits (e.g., Covelo and Gallardo 2004, Andrew et al. 2007).

In addition to being spatially variable, environmental conditions also change over time. For example, during old-field succession, the neighboring plant community transitions from an open canopy/dense understory dominated by forbs and grasses to a closed canopy/open understory forest dominated by trees and shrubs (Hartnett and Bazzaz 1985, Tilman 1987). There are many long-lived forbs and trees that appear early in succession and persist for long periods of time and thus are exposed to substantial community change during their lifetime. Until now, there has been no consideration of how successional changes in the community may influence the selection and maintenance of adaptive traits such as plant defense. I predict that successional changes in the environment will influence general changes in the costs, selection gradients, and consequently levels of resistance and tolerance.

In this dissertation, I incorporated a spatio-temporal approach to examining the mechanisms contributing to variable levels of plant resistance and tolerance traits in complex, natural landscapes. I used late goldenrod (*Solidago altissima*; Asteraceae) and its diverse herbivore assemblage as my study system. *S. altissima* is a widely distributed perennial in eastern North America and is a dominant component of mid-successional old-field habitats. Goldenrods are host to over 100 species of specialist and generalist insect herbivores, and goldenrod resistance to insect-herbivore attack (e.g., *Eurosta solidaginis*, *Philaenus spumarius*) has been well studied (Maddox and Root 1987, Pilson 1992, Root 1996, Abrahamson and Weis 1997, Meyer 1998b, a, Uriarte 2000, Cronin and Abrahamson 2001a, Cronin et al. 2001, Wise and Abrahamson 2008b).

Goldenrods reproduce sexually and asexually (via rhizomes); genets can persist in old-fields and young forests for up to 50-75 years (Hartnett and Bazzaz 1985, Maddox et al. 1989). Thus, a goldenrod genet that becomes established soon after field creation may persist throughout its successional transition to a young forest habitat (approximately 20 years). For these reasons, goldenrods are ideally suited to test whether spatial and temporal environmental heterogeneity is an important mechanism influencing variability in plant defense.

Uncovering the mechanisms influencing diverse types and levels of plant defense traits is an active area of research in the field of plant defense and this dissertation project makes several important contributions to this body of work. The field survey represents the first study to quantify the spatial distributions of both plant resistance and tolerance traits and examine correlations between the spatial structure of defense levels and environmental variables. The common garden experiment examines not only heritable genetic variability in plant defense, but also quantifies costs and selection gradients for resistance and tolerance traits for the purpose of evaluating their adaptive value. In the field experiment, I test the novel hypothesis that succession is an important mechanism promoting temporal variability in plant defense and examine whether the adaptive landscape (i.e., costs and selection gradients) for resistance and tolerance changes with respect to field successional-stage. Together, these studies highlight the context dependence of plant trait values and provide support for the recent hypothesis that spatio-temporal environmental heterogeneity may contribute to the diversity of plant defenses observed in nature (Núñez-Farfan et al. 2007). Lastly, this dissertation provides the first experimental measures of tolerance to herbivory in goldenrod, as well as the first evaluation of the adaptive values of goldenrod defense traits. Goldenrods are considered to be well-suited for studies pertaining to plant-insect interactions (Abrahamson and Weis 1997) and my findings add to the large body of work regarding its defense against insect-herbivore attack.

In chapter 2, I conducted spatially-explicit field surveys to examine how environmental heterogeneity influences the distribution and levels of putative resistance and tolerance traits of goldenrod. Here, I quantified the spatial patterns of herbivore damage and traits associated with resistance (leaf toughness, phenolics), tolerance (specific leaf area, relative growth rate, leaf addition rate, leaf senescence rate), and fitness (ramet height, diameter, inflorescence biomass) of goldenrods within replicate early- and late-successional fields. Also, I characterized the local neighborhood (stem density, canopy cover, vegetation cover) and edaphic conditions (soil moisture, pH, N) surrounding the target ramet, and determined relationships between these environmental variables and goldenrod trait levels. I hypothesized that if environmental heterogeneity is an important mechanism influencing defense traits, then trait levels will be non-randomly distributed within fields and correlated with environmental variables. Lastly, I compared spatial patterns and relationships among defense traits and environmental variables between early- and late-successional stages to examine how the spatial structure of plant defense traits and environmental variables changes over time.

In chapter 3, I conducted a common garden study to examine the genetic variability, costs, and selection for resistance, tolerance, and various fitness- and defense-related traits of 103 goldenrod genets. Genets originated from two field populations differing in successional stage (one early- and one late-successional field approximately 3 and 15 years old, respectively) that had been previously surveyed (see Chapter 2). Thus, I also compared trait levels of *S. altissima* genets in the common garden with those measured in their field of origin. These relationships, together with broad-sense heritability estimates for each trait, were used to evaluate whether there was a strong genetic basis to defense traits. I also quantified costs and selection coefficients for all traits to gauge the adaptive landscape for resistance and tolerance in the common garden environment.

In chapter 4, I conducted a field experiment to test whether costs, selection gradients, and levels of resistance and tolerance differ between early- and late-successional stages (approx. 5 and 15 years in age, respectively). I planted goldenrod genets exhibiting diverse levels of resistance and tolerance (selected from the common garden experiment; Chapter 3) into replicate early- and late-successional fields, and experimentally measured resistance, tolerance, costs, and selection coefficients. I predicted that owing to strong correlations between defense traits and successional stage-dependent environmental variables (see Chapter 2) and the possibility of strong plasticity in defense traits (see Chapter 3), successional changes in the environment would alter the adaptive landscape for defense strategies and promote temporal variability in plant resistance and tolerance.

In chapter 5, I summarize the main findings of my field survey, common garden study, and field experiment, and discuss their unique contributions to the field of plant defense. I conclude by discussing future directions in plant defense research.

CHAPTER 2. ENVIRONMENTAL HETEROGENEITY AND SPATIOTEMPORAL VARIABILITY IN PLANT DEFENSE

Introduction

Herbivores can exert strong selective pressures on plant traits (e.g., Ehrlich and Raven 1964, Mauricio and Rausher 1997, Agrawal 2005). In response to herbivore pressures, plants can express resistance traits to reduce herbivore damage or tolerance traits to reduce the negative fitness effects of herbivore damage. An active area of interest in plant defense theory focuses on understanding how variable defense traits co-occur and are maintained within natural populations (e.g., Fornoni et al. 2004a, Núñez-Farfan et al. 2007)

Environmental heterogeneity is thought to be a strong force selecting for and maintaining variation in the type and level of plant defense in natural plant populations (e.g., Fornoni et al. 2004a, Agrawal et al. 2006). For example, plants may invest less in resistance traits when resources are limited (e.g., Bergelson 1994). Alternatively, resistance trait levels may increase when resources are low if plants invest more in defense when growth is costly (e.g., Coley et al. 1985). The relationship between resource availability and plant tolerance is likewise equivocal (reviewed in Hawkes and Sullivan 2001) and may depend on the type of limiting resource and part of the plant that is damaged (e.g., Wise and Abrahamson 2005).

Neighboring plants may alter the defense levels of a focal plant, directly via competition or indirectly via associational resistance/susceptibility. Competition may decrease defense if there is a resource allocation tradeoff between defense traits and competitive ability (e.g., Cipollini and Bergelson 2002). Alternatively, competition stress may increase defense expression if defense traits serve a dual purpose, mitigating herbivory and competitive stress (e.g., Siemens et al. 2003, Jones et al. 2006). Even without competition, neighboring plants may reduce or enhance a plant's probability of being discovered and damaged by herbivores (reviewed in Barbosa et al. 2009).

Despite the diverse evidence that defense expression can be influenced by the environment, no clear generalizations have emerged. Most defense studies have been conducted in carefully controlled environments where few aspects of the environment are manipulated (e.g., Mauricio et al. 1997, Siemens et al. 2003, Fornoni et al. 2004a). Recent studies have shown that geographic variation in interactions among organisms may lead to complex patterns of trait selection (e.g., Thompson and Cunningham 2002, Rudgers and Strauss 2004). It is likely that environmental heterogeneity also influences selection for plant defense traits and is an important mechanism promoting defense-trait diversity. Studies examining the spatial distributions of plant defense traits in natural populations are needed to assess potential causes for diverse levels of resistance and tolerance traits.

Another area of plant defense research that has received very little attention is the distribution of plant defenses in response to temporal changes in the environment (but see van der Putten 2003). Soil conditions, herbivore assemblages, species composition, and competitive interactions among plants can change dramatically as a community ages or undergoes succession (e.g., Tilman 1987). For long-lived perennial plants that experience these environmental changes, the expression of plant defense traits as well as their spatial patterns may be temporally dynamic. For example, if the establishment of individuals occurs by lottery (e.g., Chesson and Warner 1981), then plants in both early- and late-successional fields may exhibit random distributions of defense trait levels. However, if local selection of resistant or tolerant genotypes is important, defense levels may become more non-random in their spatial distribution (i.e., autocorrelated) and exhibit stronger correlations with environmental variables over time. Given what we know about community succession, temporal effects on defense expression are likely.

We examine how spatial and temporal heterogeneity influence the diverse expression of plant defenses by quantifying the spatial distributions of herbivore damage, putative-resistance, tolerance, and fitness-related traits of *Solidago altissima* ramets (Late goldenrod) within replicate early- and late-successional, old-field habitats. We also characterize the relationships between defense traits and neighbor variables (neighbor stem density, vegetative ground cover, canopy cover) and edaphic conditions (soil moisture, pH, N) to identify potential mechanisms driving defense distributions. Lastly, we determine the strength of correlations among defense traits as well as between defense and fitness traits. Strong negative correlations would suggest tradeoffs or costs associated with those traits (Siemens et al. 2003). We hypothesize that if spatial heterogeneity is an important mechanism promoting and maintaining diverse defenses in nature, then defense trait levels will be non-randomly distributed (i.e., high spatial autocorrelation and/or correlations between traits and environmental variables). If local adaptation of defense expression is occurring over succession, we predict that late-successional fields will be more strongly spatially structured and have stronger spatial correlations among defense traits and environmental variables. To our knowledge, this is the first study to examine the potential causes of the spatial patterns of both resistance and tolerance traits within heterogeneous environments and investigate how successional changes in the environment influence spatial patterns of defense.

Methods

The Study System:

Late goldenrod (*Solidago altissima* L. subsp. *altissima*) is common throughout eastern North America (Semple and Cook 2006) and a dominant plant of mid-successional, old-field habitats. Goldenrods are insect pollinated and are typically self-incompatible but also reproduce vegetatively via rhizomes (Abrahamson and Weis 1997).

Goldenrods are model organisms for studying plant-insect interactions (Abrahamson and Weis 1997). More than 100 species of generalist and specialist herbivores feed on goldenrods (Maddox and Root 1990). Dominant herbivores include spittlebugs (*Philaenus spumarius*), gall-making flies (*Eurosta solidaginis*), and various grasshopper and beetle species. Previous studies have shown that these herbivores may variously affect goldenrod biomass, photosynthetic rates, leaf senescence, and reproduction (e.g., McCrea and Abrahamson 1987, Cronin and Abrahamson 1999). Even low herbivore densities can significantly affect goldenrod fitness (e.g., Root 1996).

Goldenrod Resistance and Tolerance to Herbivory:

A number of studies have revealed considerable genetic variability in goldenrod resistance to insect-herbivore attack (e.g., McCrea and Abrahamson 1987, Maddox and Root 1990, Cronin and Abrahamson 1999). Also, resistance of goldenrod genotypes to at least one herbivore, *E. solidaginis*, has been shown to be temporally dynamic (Cronin et al. 2001). We examined leaf toughness (LT) and total phenolics (TP) in the context of *S. altissima* resistance. These traits are assumed to be related to goldenrod resistance, although evidence for this is equivocal (Abrahamson et al. 1991, Abrahamson and Weis 1997, Siska et al. 2002). Tolerance traits have been studied less extensively but include physiological traits that aid in compensatory growth and photosynthesis. Leaf addition rate, increased relative growth rate, specific leaf area, and delayed senescence are mechanisms that have been experimentally shown to be associated with tolerance to herbivory in *S. altissima* (Meyer 1998a) and other plant species (e.g., Moriondo et al. 2003). In addition to tolerating herbivory, these traits may be associated with plant vigor and serve in tolerating other types of environmental stresses (Chapin 1991, Siemens et al. 2003, Jones et al. 2006).

Field Survey:

Field surveys of *S. altissima* were conducted from March to November, 2006, in three early- and three late-successional fields located in East Baton Rouge and East Feliciana Parishes, LA. Early fields had not been mown within the past two years and were dominated by forbs and grasses (e.g., *S. altissima*, *Ambrosia* spp., *Liatris* spp., *Dichanthelium* spp.; Figure 2.1a). Late fields were approximately 15 years post mowing and were dominated by trees and shrubs (e.g., *Triadica sebiferum*, *Cornus foemina*, *Acer negundo*, *Rubus* spp.; Figure 2.1b). Fields ranged from 1-3 ha in size and we focused our surveys on an approximately 1 ha area in each field. Colonization by goldenrods occurs through wind-dispersed achenes soon after field creation, and genets can persist for up to 75 years (Hartnett & Bazzaz 1985). Continued colonization and rapid clonal expansion occurs through the 5th year, at which time clonal density and diversity are maximal (Hartnett and Bazzaz 1985, Maddox et al. 1989). Over time, the number of clones declines and eventually, within about 20 years, woody plants begin to dominate the landscape (e.g., Maddox et al. 1989). Thus, the early-successional fields represent a clonal recruitment and expansion phase for *S. altissima* and the late-successional fields represent a clonal decline phase.

In each field, we mapped a grid with 80-121 nodes at 10 m intervals using a Trimble GeoXT GPS (Trimble Navigation Limited, Sunnyvale, CA) with sub-m precision. The closest *S. altissima* ramet to each node was selected and marked with a plastic tag. Every six weeks from Mar-Nov, 2006 we measured two fitness-related traits: ramet height and diameter at 10 cm. At the end of the growing season, newly-opened inflorescences were collected, dried in an oven at 65^{o} C for 4 days, and weighed as an estimate of potential short-term sexual fitness.

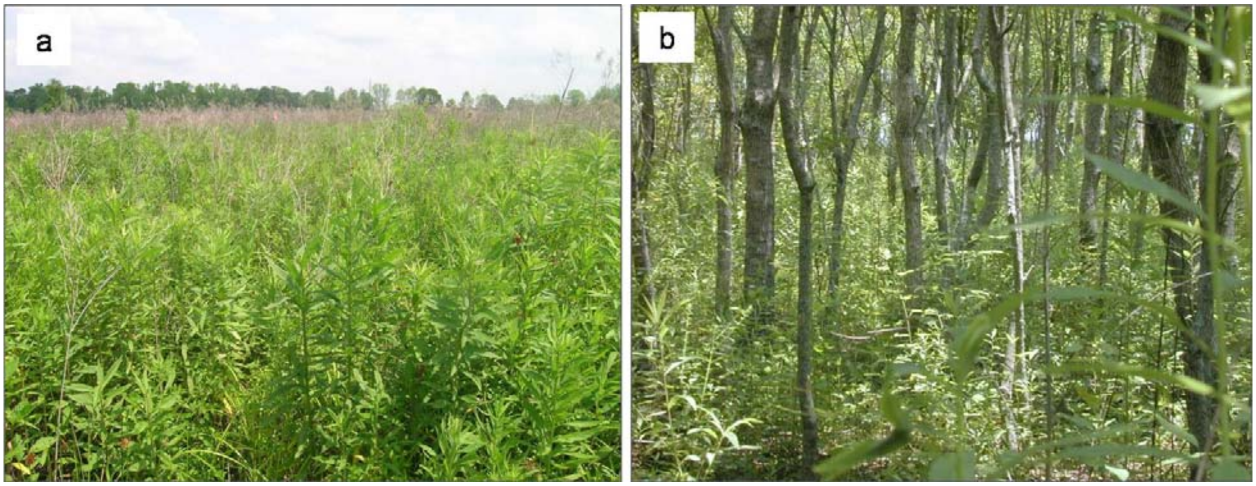


Figure 2.1. Early-successional field approximately 2 years since disturbance (a) and late-successional field approximately 15 years since disturbance (b). Fields were located in Baton Rouge, Louisiana, USA. Photo credits: A.S. Hakes.

During each census, herbivores were surveyed by visual counts and identified to family, and proportion of leaves damaged and leaf area damaged were calculated. Leaf area lost to herbivores was assessed through digital photographs of three haphazardly chosen damaged leaves using the program UTHSCSA *ImageTool* (University of Texas Health Science Center at San Antonio, TX). At the end of each census, a copper wire was tied under each apical bud. In the subsequent census, it was then possible to identify and record chewing damage for leaves that were produced since the last census. This procedure allowed for a cumulative measure of damage without repeatedly measuring the same leaves. Recording damage exclusively on new leaves was appropriate since leaves from previous censuses tended to accumulate little additional damage (A.S. Hakes unpublished data). The percent of total plant tissue damaged by leaf-chewing herbivores was estimated from this procedure.

Relative resistance is often measured as 1 - the proportion of damage incurred by the plant from leaf-chewing herbivores (e.g., Fineblum and Rausher 1995, Fornoni et al. 2004a) and as herbivore density for mining, sucking, or galling herbivores (e.g., Maddox and Root 1990). This operational measure of resistance assumes that plants are equally accessible to uniformly distributed herbivore populations, which is almost certainly not the case in natural environments. For this reason, we avoid using our direct measure of herbivore damage to infer ramet resistance. Instead, we quantified leaf toughness (LT) and concentration of total phenolics (TP), which are traits that are putatively associated with resistance (see "Goldenrod Resistance and Tolerance to Herbivory"). Tolerance is ideally measured as a fitness norm of reaction among clonal replicates subjected to a gradient of herbivore damage or density (e.g., Mauricio et al. 1997, Fornoni et al. 2004a). Because our study focused on naturally occurring ramets of unknown genetic background, we could not measure tolerance directly. Instead, we measured traits shown to be associated with compensatory growth and photosynthesis in response to herbivory (i.e., tolerance) in *S. altissima* (leaf addition rate (LAR), leaf senescence rate (LSR), relative growth rate (RGR), and specific leaf area (SLA) (see Meyer 1998a)). LAR and LSR were measured as the number of leaves added or senesced per day and RGR was calculated as the difference in *ln*-transformed height divided by the number of days between measurements (Meyer 1998a).

In the summer censuses, we collected leaf tissue samples for assessment of LT, TP, and SLA. Three leaves were collected haphazardly from the upper two-thirds of the stem. LT, measured as the average force needed to push a metal rod through leaf tissue, was assessed using a penetrometer (Itin Scale Co., Inc., Brooklyn, NY; Siska et al. 2002). The collected leaves were transported to the laboratory on dry ice, lyophilized (72 hr), and weighed. Leaf area was calculated from digital photographs of each leaf using ImageTool. SLA, or leaf area per unit dry leaf mass, was calculated. SLA is positively correlated with mass-based photosynthetic rate and higher SLA in re-growth leaves allows damaged plants to gain more leaf area for a given biomass investment (Meyer 1998a). Leaves were ground with a mortar and pestle and we determined the concentration of TP (micromoles of ferulic acid equivalent per gram dry weight) in leaf samples using the Folin-Ciocalteau spectrophotometric method (Haynes et al. 2007).

Measurements of edaphic conditions were obtained for each ramet during mid-summer. A 25-cm deep soil core was extracted from two random locations 0.5 m from the target ramet and the top 5 cm of the cores were discarded. We combined the core samples, obtained their wet and dry weights, and computed the percent soil moisture. Soil pH was determined with a bench-top pH meter after mixing 10 g of dried soil sample with 100 ml deionized water for an hour, and then allowing the mixture to sit an additional hour. Percent total nitrogen content in soil was determined using a dry combustion procedure (Leco CN Analyzer). Due to logistic constraints, soil pH and N analyses were conducted on every soil sample from only one early- and one late-successional field and on 20 randomly chosen soil samples from the remaining fields.

Plants are affected most by interactions with their nearest neighbors (Molofsky et al. 2002), thus, we assessed the neighboring plant community by randomly placing two 0.25 m x 0.25 m sampling frames within a 0.5 m radius of each ramet during mid-summer. This distance largely encompasses the rhizosphere of a goldenrod ramet (e.g., Meyer and Schmid 1999). The stem densities of all grasses, forbs, and woody plants inside the frames were totaled and percent ground vegetation cover was estimated. Digital photographs of the canopy were taken with a Nikon Coolpix 5400 camera and 0.42x fisheye lens and percent canopy cover (an indicator of light availability), was measured using Gap Light Analyzer v.2 (Simon Fraser University, British Columbia, and Institute of Ecosystem Studies, New York).

Data Analysis:

We first tested whether goldenrod ramet defense levels and local environmental conditions varied significantly within individual fields and between early- and late-successional fields using separate nested ANOVAs for each variable. Fields were nested within successional stage and tests were performed using SYSTAT 11 (SYSTAT 2004). All variables (see Table 2.1) required transformations to normalize distributions with the exception of ramet height, LT, and TP. Percent vegetative cover, canopy cover, and soil moisture were arcsine square-root transformed and percent leaf damage was logit transformed. All remaining variables were *ln*-transformed and sequential Bonferroni corrections to α were made for multiple tests.

To test the prediction that plant traits and environmental variables in late-successional fields are more strongly spatially structured than in early-successional fields, we calculated Moran's *I* coefficients of autocorrelation separately for each variable at 10 distance classes using the spatial analysis program PASSAGE v.2 (Rosenberg 2008). Moran's *I* is similar to Pearson's *r* in that $0 > I \geq -1$ indicates a negative autocorrelation (nearest neighbors are most dissimilar), $0 < I \leq 1$ indicates a positive autocorrelation (nearest neighbors are most similar), and the strength of autocorrelation increases with the absolute value of *I*. Our objective was to examine patterns of autocorrelation for suites of similar traits in early- and late-successional fields. Therefore, variables were grouped into like categories of putative defense traits (SLA, RGR, LAR, LSR, LT, TP), fitness-related traits (ramet height, diameter, inflorescence weight), edaphic variables (soil moisture, pH, N), neighbor variables (canopy cover, ground vegetative cover, stem density), and herbivore damage. For each site and trait category, we computed the mean Moran's *I* at each distance class from the individual variables that make up that category. Differences in autocorrelation between early (n = 3) and late (n = 3) successional fields for a particular category were assessed by comparing the mean Moran's *I* ± 95% CI (mean of the site means) across distances. We conducted *t*-tests using SYSTAT 11 (SYSTAT 2004) to assess whether the strength of autocorrelation differed from zero, and differed between early and late fields at each distance class. Sequential Bonferroni corrections were made to α to account for multiple tests.

We tested the predictions that defense traits were correlated with environmental variables, tolerance traits were negatively correlated with resistance traits, and defense traits were positively correlated with fitness traits. An important caveat associated with examining correlations among variables in nature is that the presence of significant autocorrelation implies that data points are not independent (Legendre 1993). To account for potential non-independence, we used partial Mantel's tests to assess the partial correlation between the distance values of two variables (e.g., damage and canopy cover) while controlling for the effect of spatial distance. The partial Mantel coefficient, r_M ranges from -1 to 1, but is generally lower and not directly comparable to Pearson's *r* coefficient (Fortin and Dale 2005). Instead, the magnitude of r_M is to be used in a comparative way with other r_M values (Fortin and Dale 2005). For each field, we performed partial Mantel's tests between each defense trait (or damage) and each fitness-related trait and environmental variable. We also performed partial Mantel's tests among defense traits for each field. Whenever r_M was found to be significant, we performed a follow-up test to determine the nature of the association between the distance values of two variables. We performed a correlation analysis on the raw data that would give an indication of whether the association was due to a positive or negative correlation between the two variables. Partial Mantel tests were performed in PASSAGE v.2 (Rosenberg 2008) and correlation analyses were conducted using SYSTAT 11 (SYSTAT 2004).

Meta-analyses were used to summarize stage-specific relationships between all possible pairs of defense traits and environmental variables, defense traits and fitness traits, and among defense traits. Partial Mantel's (r_M) coefficients from each field were grouped by stage (early *n*=3 and late *n*=3) to yield a mean effect size r_M ± 95% CI. Using this approach, we tested the prediction that the magnitude of relationships (i.e., r_M) between defense traits and fitness-related traits, defense traits and environmental variables, and among defense traits differed from zero and differed between stages. Meta-analyses were performed using Comprehensive Meta Analysis v.2 (Borenstein et al. 2005) and random effects models for summary analyses. Sequential Bonferroni corrections to α were made for multiple tests.

Results

Among the 17 variables examined in this study, early- and late-successional fields differed by an average of 70 ± 16% in trait values (Table 2.1). However, in only a few cases were the mean differences between successional stages statistically significant (following sequential Bonferroni corrections of α). Goldenrod ramets in late-successional fields grew 34% shorter and had a stem diameter 46% smaller than ramets in early successional fields ($P < 0.05$, Table 2.1). Relative to ramets in early-successional fields, 56% fewer ramets flowered in late-successional fields and those that flowered had 24% lower inflorescence biomass. This difference was marginally significant following a sequential Bonferroni correction (uncorrected $P = 0.028$). We also note here that four of our six defense traits (LT, RGR, LAR, LSR) had uncorrected *P*-values less than 0.05. All putative defense traits had greater mean values (i.e. RGR = 10%, LAR = 141%, LSR = 44%, LT = 63%, and TP = 40% greater) in early- than in late-successional fields with the exception of SLA which was 25% greater in the latter field type.

With regard to neighboring plant variables, late-successional fields had a 52% lower density of neighboring stems, 41% lower ground vegetation cover, and 260% greater canopy cover than early-successional fields.

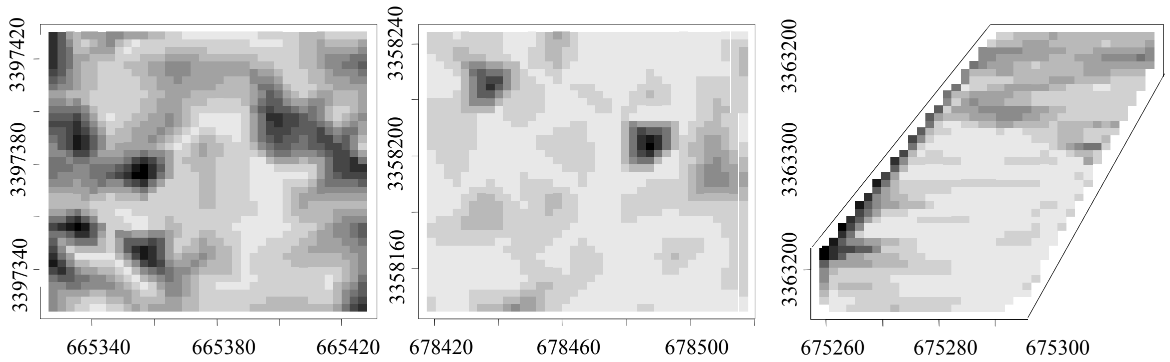
Table 2.1. Effect of field successional-stage (early vs. late) on goldenrod fitness traits, herbivore damage, putative defense traits, neighbor variables, and edaphic variables (mean ± SE). F statistic and *P* value determined from separate nested ANOVAs (field (n=3) nested within successional stage (n=2); see Methods). Proportion of ramets flowering was calculated using *t*-test with df = 4.

TRAIT	EARLY	LATE	$F_{1,4}$ φ	*P*
FITNESS				
Ramet Height (cm)	152.59 ± 7.33	100.21 ± 6.36	29.27	0.006*
Ramet Diameter (mm)	9.33 ± 2.97	5.00 ± 0.39	5.81	0.07
Proportion of Ramets Flowering	0.75 ± 0.06	0.33 ± 0.10	3.38 γ	0.028
Inflorescence Biomass (g)	12.84 ± 1.19	9.77 ± 0.27	2.74	0.17
DAMAGE				
% Leaf Tissue Damaged	8.01 ± 3.75	10.51 ± 2.03	0.33	0.59
DEFENSE TRAITS				
LT (g)	102.20 ± 5.66	61.67 ± 11.14	10.85	0.03
TP (mmoles/g)	369.46 ± 23.37	258.94 ± 66.04	2.76	0.17
LAR (# per day)	1.99 ± 0.47	0.72 ± 0.14	11.90	0.02
LSR (# per day)	1.29 ± 0.18	0.83 ± 0.05	16.06	0.01
RGR (cm^{-1} cm^{-1}/day)	$7.2e^{-3} \pm 1.3e^{-3}$	$5.4e^{-3} \pm 3.3e^{-3}$	12.22	0.02
SLA (cm^2/mg)	0.15 ± 0.00	0.18 ± 0.02	4.59	0.09
NEIGHBOR VARIABLES				
Total Stem Density (0.0625 m^2)	19.17 ± 5.23	9.28 ± 1.30	4.87	0.09
% Ground Vegetative Cover	69.37 ± 5.22	41.06 ± 11.73	5.25	0.08
% Canopy Cover	8.80 ± 0.80	30.76 ± 12.62	2.70	0.17
EDAPHIC VARIABLES				
Soil % Moisture	14.75 ± 2.42	14.10 ± 1.45	0.03	0.88
Soil pH	6.38 ± 0.18	6.77 ± 0.44	0.47	0.53
Soil Total % Nitrogen	0.13 ± 0.02	0.11 ± 0.01	0.74	0.44

φ = Analyses were performed using transformed data (see Methods)
γ = *t*-statistic
* *P* values < the critical level of α of 0.05 following sequential Bonferroni corrections.
SLA = specific leaf area, RGR = relative growth rate, LAR = leaf addition rate, LSR = leaf senescence rate, LT = leaf toughness, TP = total phenolics.

Despite large effect sizes with regard to neighbor variables, there were no significant differences between successional stages. Edaphic conditions (soil moisture, pH and nitrogen levels) were quite similar between early and late successional fields (mean difference: 9 ± 4%). Finally, percentage of leaf area damaged by chewing herbivores (various grasshoppers) averaged 8.0 ± 3.7% (range: 0 -51%) and 10.5 ± 2% (range: 0 – 62%) in early and late fields, respectively, but the difference was not significant. Other types of herbivores and plant damage were scarce. Therefore, we focus the remainder of our paper on damage from chewing herbivores.

Even though we found few significant differences between early and late successional fields for the 17 variables in Table 2.1, there were strong within-field spatial patterns in trait levels, and the relationships among variables differed in important ways between successional stages. The distributions of trait levels for all of the variables considered in Table 2.1 (with the exception of proportion of ramets flowering which was measured per field and not per ramet), were strongly spatially structured (statistically significant spatial autocorrelation at one or more distance classes in 69 of 96 cases). Goldenrods that were close neighbors expressed more similar trait values, and nearby locations tended to be more similar in environmental conditions, than goldenrods and locations that were far apart. For illustration, Figure 2.2 shows the spatial variation in one variable, herbivore damage, for each of the six fields. On average, defense trait levels in late-successional fields exhibited strong, positive autocorrelations ($I > 0$) at 10-30 m, and strong, negative autocorrelations ($I < 0$) at 60-100 m (uncorrected P-values < 0.05 for 8 of 10 distance classes; Figure 2.3a). Defense traits in early-successional fields followed a similar pattern of positive autocorrelation at distances < 40 m and negative autocorrelation at distances > 40 m, but only exhibited significant negative autocorrelation at 90 m (Figure 2.3a). As predicted when local adaptation and/or other non-random processes occur over successional time (see



LATE-SUCCESSIONAL FIELDS

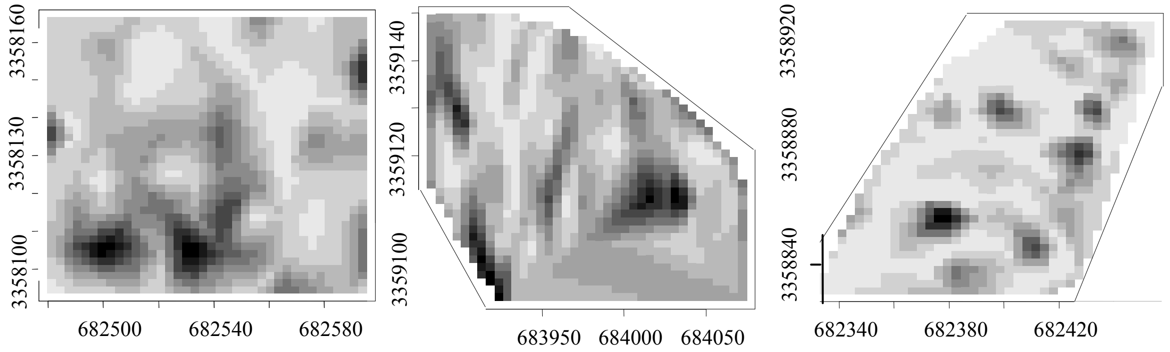


Figure 2.2. Distribution of damage intensity by leaf-chewing herbivores in three early and three late-successional fields (field size averaged approximately 1 ha). Damage ranged from 0% of leaf tissue lost to herbivory (white) to 60 % of leaf tissue lost to herbivory (black). Coordinates are given in meters in UTM zone 15N.

Introduction), defense traits were an average of 7.2 ± 2.9 times more strongly autocorrelated (based on absolute values of Moran's *I*) in late-successional than in early-successional fields (uncorrected *P*-values < 0.05 for 5 of 10 distance classes), and this difference was significant at 20 m and 70 m (Figure 2.3a). Neighbor and edaphic variables generally followed a similar trend of positive autocorrelation up to 30 m and negative autocorrelation beyond 60 m. However, the differences in strength of autocorrelation between early and late fields were not significant (Figure 2.3b,c), and the only distance class where Moran's *I* differed from zero was for edaphic variables at 10 m (Figure 2.3c). Fitness traits and herbivore damage were not significantly autocorrelated and did not differ in strength between successional stages (Figure 2.3d,e).

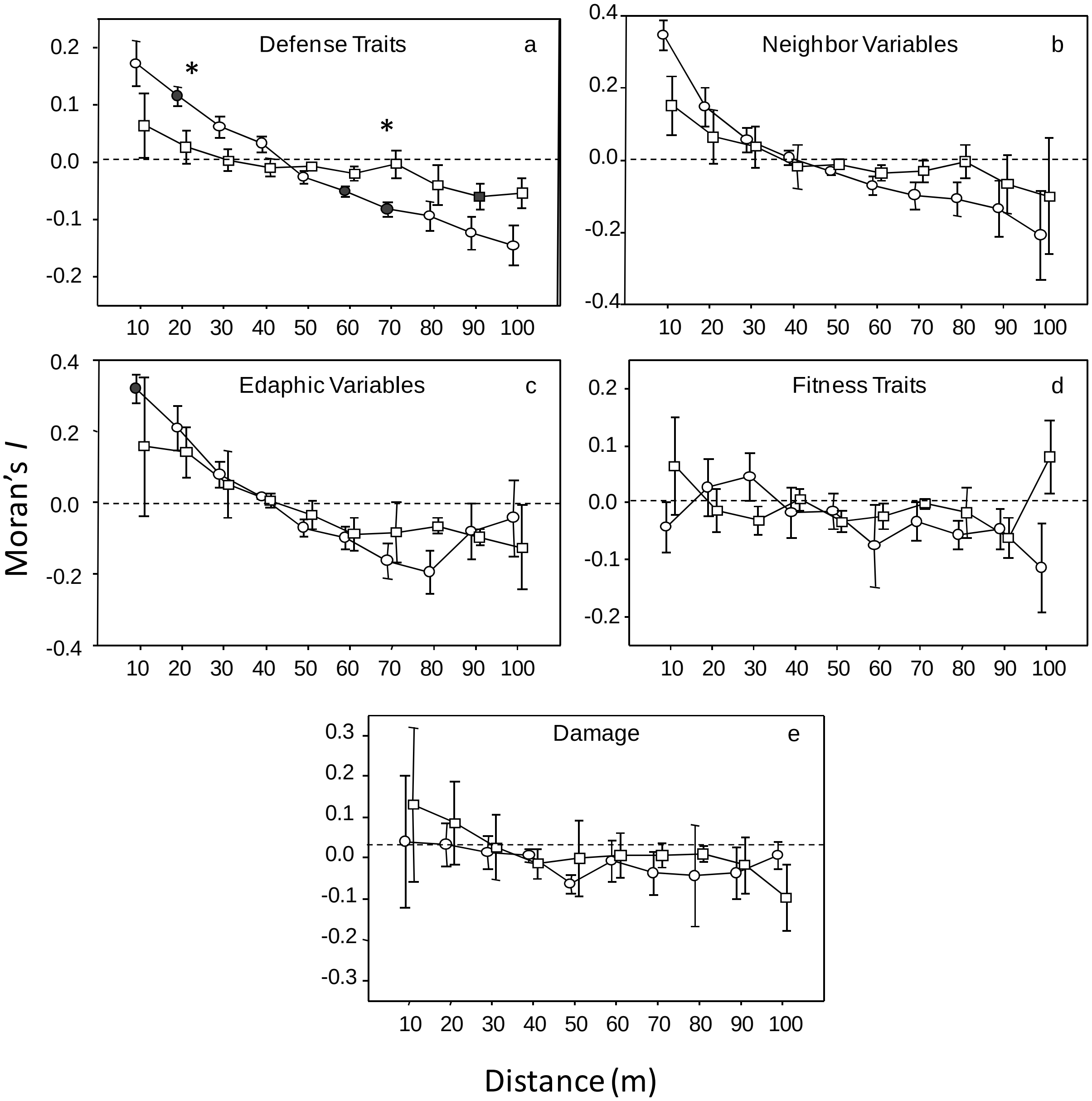


Figure 2.3. Correlograms of spatial autocorrelation for (a) defense traits (*n*=6); (b) neighbor variables (*n*=3); (c) edaphic variables, n=3; (d) fitness-related traits (*n*=3); and (e) herbivore damage across 10 m distance classes. Moran's *I* coefficient of spatial autocorrelation (mean ± 95% CI) for three early (*squares*) and three late (*circles*) successional fields. Filled shapes represent coefficients that are significantly different from zero, and * denotes coefficients that are significantly different between early- and late-successional fields. Significance was determined using sequential Bonferroni corrections to α to account for multiple tests.

After correcting for spatial autocorrelation (using a partial Mantel's test), we found several important significant correlations among traits and environmental variables, and the strength of these relationships (indicated by r_M values) varied with successional stage. Of the various putative resistance (LT, TP) and tolerance traits (SLA, RGR, LAR, LSR), only the tolerance traits were strongly associated with goldenrod ramet fitness traits (based on the mean ± 95% CI partial Mantel's r_M for sites within a successional stage). RGR and LAR were significantly correlated with ramet height in late-successional stages, but only LAR was correlated with height in early-successional stages (Figure 2.4a). No defense traits were significantly correlated with ramet diameter in either successional stage (Figure 2.4b). LAR and LSR were significantly correlated with inflorescence biomass in late-successional fields and LAR was also significantly correlated in early-successional fields (Figure 2.4c). Correlations between tolerance and fitness traits in early- and late-successional fields did not differ statistically between successional stages, however, correlations between inflorescence biomass and LAR and LSR trended stronger (based on r_M values) in early- than in late- successional fields (Figure 2.4c). For the partial Mantel r_M relationships that were significant, correlation analyses of the raw data indicated that tolerance traits were positively correlated with fitness-related traits (mean $r = 0.54 \pm 0.02$, n = 17).

The neighboring plant community was significantly associated with tolerance traits and damage levels only for a particular successional stage. The tolerance trait SLA was significantly associated with canopy cover and neighbor stem density in late-successional stages and these relationships were also stronger in late- than in early-successional fields (Figure 2.5a, c). The relationship between damage and vegetation cover was significantly stronger in early- than in

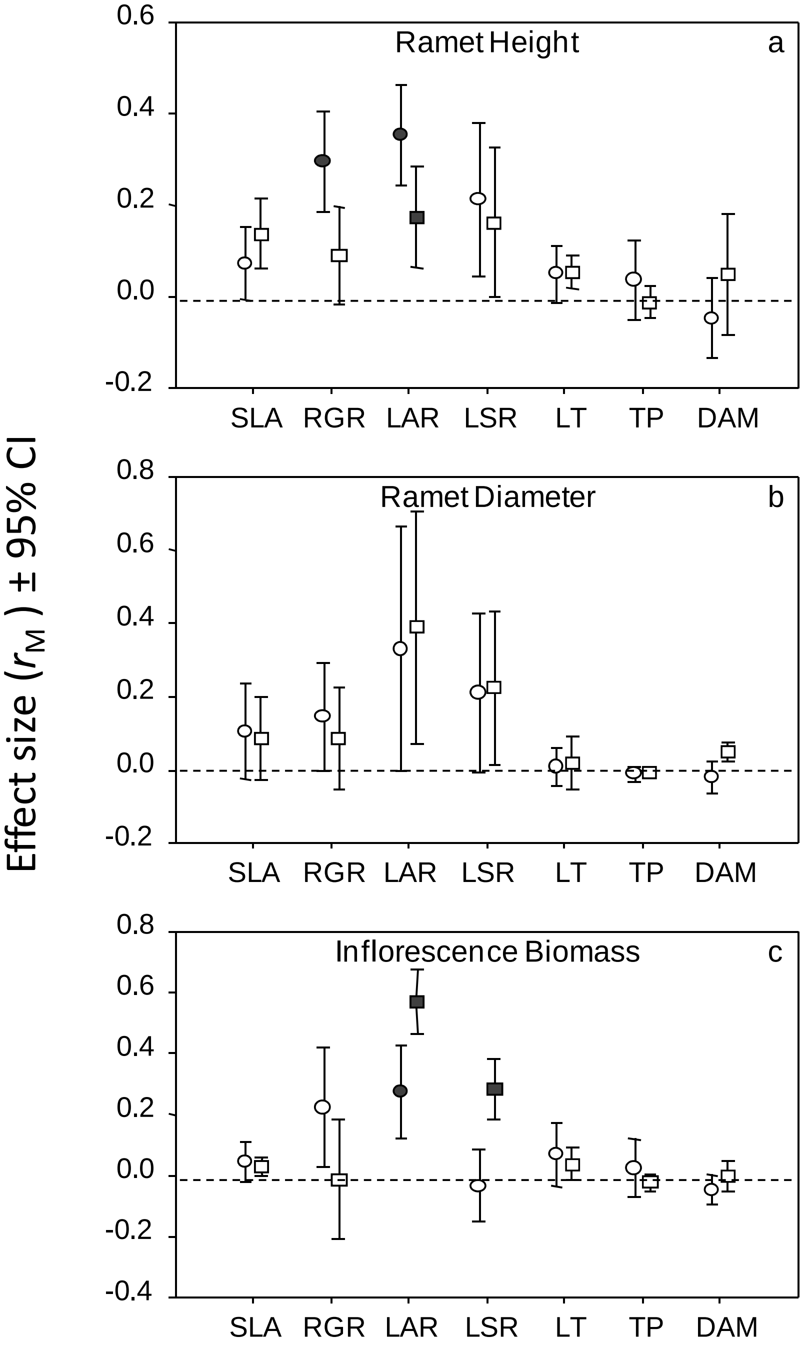


Figure 2.4. Relative strengths of relationships between each defense trait (or damage) and fitness-related traits (a) ramet height, (b) ramet diameter, and (c) inflorescence biomass after controlling for spatial distance between individuals. Effect sizes are represented as mean partial Mantel's r_M ± 95% CI for three early (*squares*) and three late (*circles*) successional fields. Filled shapes represent correlations that are significantly different from zero, and * denotes correlations that are significantly different between early- and late-successional fields. Significance was determined using sequential Bonferroni corrections to α to account for multiple comparisons.

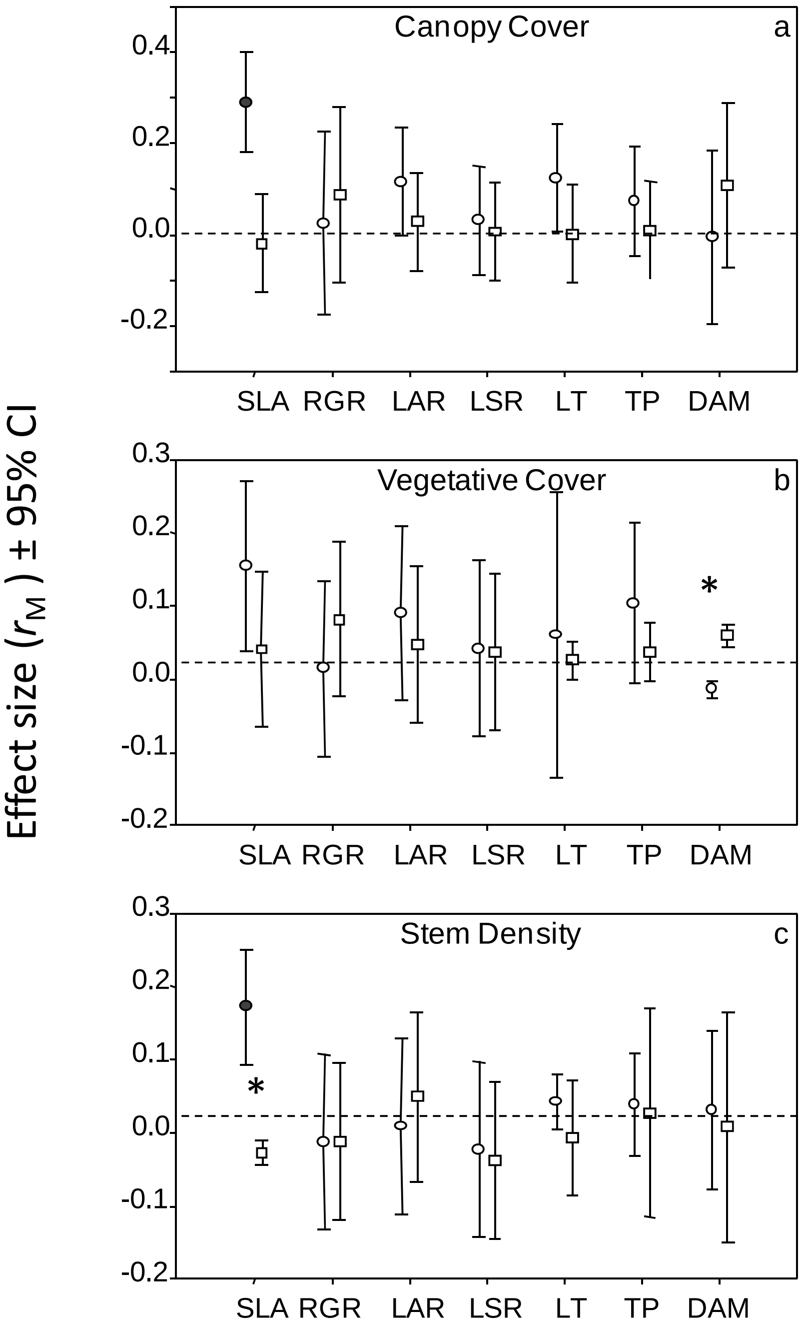


Figure 2.5. Relative strengths of relationships between each defense trait (or damage) and neighbor variables (a) canopy cover, (b) ground vegetative cover, and (c) neighbor stem density after controlling for spatial distance between individuals. Effect sizes are represented as mean partial Mantel's r_M ± 95% CI for three early (*squares*) and three late (*circles*) successional fields. Filled shapes represent correlations that were significantly different from zero, and * denotes correlations that are significantly different between early- and late-successional fields. Sequential Bonferroni corrections to α were made to account for multiple comparisons.

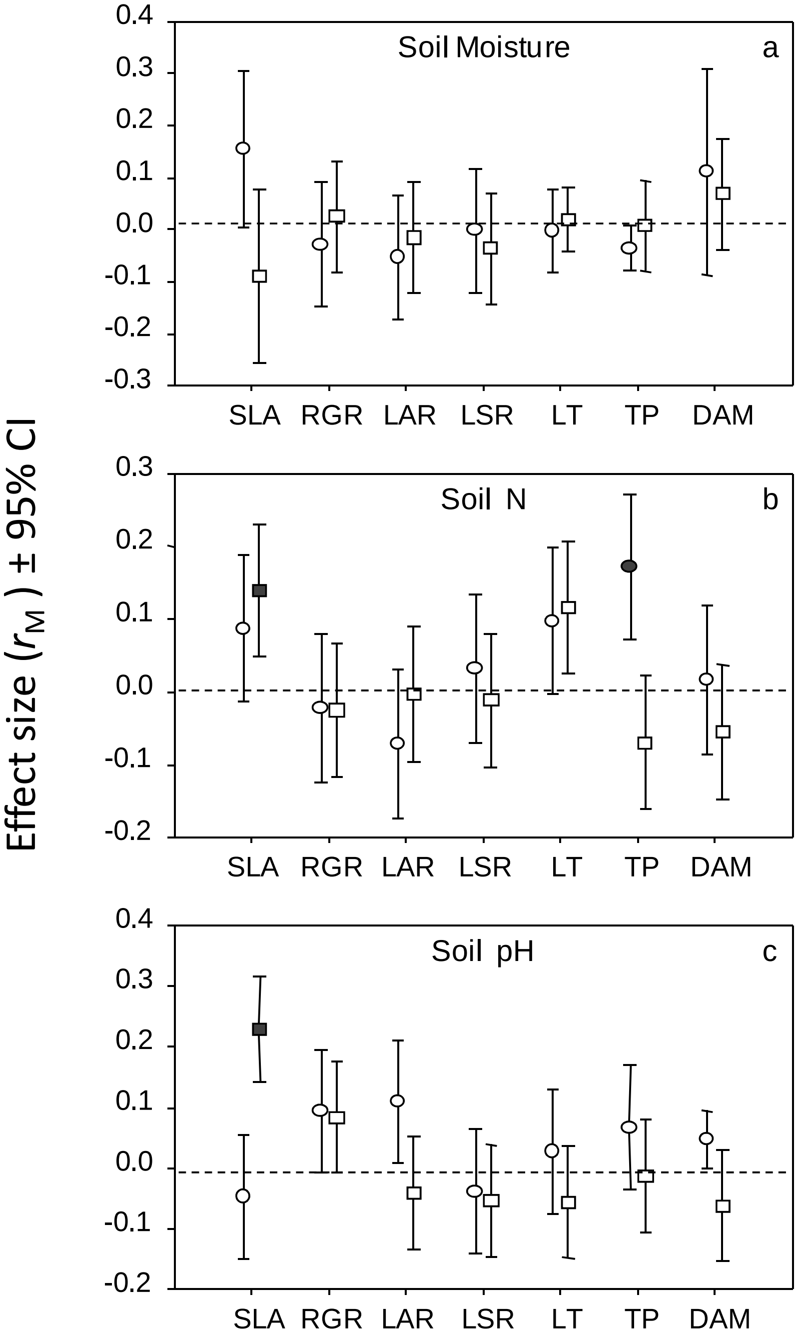


Figure 2.6. Relative strengths of relationships between each defense trait (or damage) and edaphic variables (a) soil moisture, (b) soil nitrogen, and (c) soil pH after controlling for spatial distance between individuals. Effect sizes are represented as mean partial Mantel's r_M ± 95% CI for three early (*squares*) and three late (*circles*) successional fields for soil moisture levels (a). Partial Mantel's r_M ± 95% CI for Soil N (b) and soil pH (c) were determined from only one early- and one late-successional field. Filled shapes represent correlations that were significantly different from zero based on meta-analysis (a) or permutation test (b & c). Sequential Bonferroni corrections to α were made to account for multiple, non-independent comparisons.

late-successional fields (Figure 2.5b). The nature of these associations (as indicated from the partial Mantel's tests) was revealed through correlation analyses using the raw data. In each late-successional field, SLA was positively correlated with canopy cover (mean $r = 0.50 \pm 0.09$) and negatively correlated with neighbor stem density (mean $r = -0.43 \pm 0.07$). Damage was positively correlated with neighbor stem density in 2/3 of early-successional fields ($r = 0.22$, $r = 0.07$) and negatively correlated to neighbor stem density in the remaining field ($r = -0.25$).

Edaphic conditions were correlated with defense traits within individual fields, but no overall patterns emerged between successional stages. We found no significant relationships between soil moisture and defense-traits or damage level for either successional stage (Figure 2.6a). Because soil pH and percent total nitrogen were measured completely for only one early- and one late-successional field (see Methods), we could not assess whether correlations involving these variables were characteristic of the successional stage, or were unique to the particular field. In the late-successional field, the only significant relationship was between soil N and TP (Figure 2.6b) and the direction of this relationship was positive ($r = 0.216$; based on correlation analyses using raw data). In the early-successional field, SLA had a significant negative association with soil N ($r = 0.079$; Figure 2.6b) and a significant positive association with soil pH ($r = 0.04$; Figure 2.6b,c).

Finally, contrary to predictions about tradeoffs between defense traits, relationships between putative resistance and tolerance traits were weak after removing spatial autocorrelation ($r_M < 0.10$, Table 2.2). The only exception was the significant positive correlation between LAR and LSR, in early ($r_M = 0.455$), and late ($r_M = 0.612$) stages (Table 2.2). Lastly, damage levels were not significantly correlated with defense traits.

Table 2.2. Relative strengths of relationships (r_M) between all possible pairs of putative defense traits and damage after controlling for spatial distance between individuals. Partial Mantel's r_M are averaged for early (n=3) and late (n=3) successional fields.

		DEFENSE TRAITS					
		SLA	RGR	LAR	LSR	LT	TP
DAM	Early	0.050	0.026	-0.042	-0.006	0.063	-0.041
	Late	0.061	-0.005	-0.047	-0.066	0.006	-0.040
SLA	Early	.	0.075	0.007	-0.006	0.146	0.053
	Late	.	0.003	0.045	0.071	0.063	0.044
RGR	Early		.	0.046	0.031	-0.023	0.050
	Late		.	0.122	0.010	0.044	-0.032
LAR	Early			.	0.455*	0.005	0.003
	Late			.	0.612*	0.006	0.074
LSR	Early				.	-0.002	-0.031
	Late				.	0.016	0.024
LT	Early					.	0.082
	Late					.	0.002
TP	Early						.
	Late						.

*Notes significance after sequential Bonferroni corrections to α.
P values (not shown) were calculated using Comprehensive Meta Analysis v.2.
SLA = specific leaf area, RGR = relative growth rate, LAR = leaf addition rate, LSR = leaf senescence rate, LT = leaf toughness, TP = total phenolics, DAM = herbivore damage.

Discussion

To our knowledge, our study is the first to find evidence that environmental heterogeneity and its changes over the course of old-field succession may influence the expression of diverse types and levels of plant defense strategies in natural plant populations. Defense traits were found to be more similar among nearest neighbors in late- than in early-successional fields. Neighbor variables were found to be more important than soil moisture in influencing the spatial distribution of plant defenses, and the relationships between neighbor variables and defense traits differed with respect to field successional-stage. Specifically, SLA was more strongly correlated with canopy cover and neighbor stem density in late-successional fields, and damage was more strongly correlated with vegetation ground cover in early-successional fields. Our data also suggest the possibility of successional shifts in defense expression, with increased levels of traits conferring tolerance later in succession (see below).

Many plant species, particularly clonal species, exhibit morphologies, defense levels, etc, that are more similar among nearest neighbors (i.e., positive spatial autocorrelation). Our finding that plants within 20 m of each other had similar defense trait values was similar to the findings by Andrew et al. (2007) and Covelo & Gallardo (2004) that neighboring *Eucalyptus* trees within 20 m and *Quercus* trees within 6-10 m, respectively, had similar levels of defense chemicals (positive spatial autocorrelation).

The greater similarity of defense trait levels among goldenrods at short distances in late- than in early-successional fields may be due to the underlying spatial genetic structure of *S. altissima*. Studies examining spatial distributions of clonal plant and animal species have attributed their high positive autocorrelation to the expansion of genets over time (e.g., Hammerli and Reusch 2003). Goldenrod achenes are wind dispersed and not likely to be limited to the 1 ha scale of our investigation, thus, it is unlikely that dispersal limitation is influencing spatial patterns of *S. altissima* defense traits. We are currently examining the clonal structure of *S. altissima* in our fields using microsatellite markers (A. S. Hakes, D.A. Moeller, & J.T. Cronin, unpublished data).

Another explanation for the greater similarity in trait levels among nearest neighbors in late- versus early-successional fields is local adaptation to environmental variables which are themselves spatially autocorrelated (Legendre 1993). Of the three studies that have considered the spatial distributions of resistance traits (Covelo and Gallardo 2004, Brenes-Arguedas and Coley 2005, Andrew et al. 2007), two have suggested that these patterns may have been influenced by the spatial structure of environmental variables such as light availability (Covelo and Gallardo 2004, Brenes-Arguedas and Coley 2005). However, no explicit tests were performed in these studies to evaluate the relationships between environmental variables and defense levels.

Our study not only supports the growing body of evidence that neighboring plants can influence damage and defense expression, but also provides evidence that neighbor effects can vary during the course of succession. Correlations between putative defense traits and environmental variables were statistically significant only for a particular successional stage. For example, the strong correlation between the tolerance trait SLA and canopy cover was only evident in late-successional fields where canopy cover was 2.6 times greater. Canopy cover could indirectly affect tolerance trait levels if, for example, herbivore foraging increases in shaded habitats (e.g., Henriksson et al. 2003). The relationship between herbivore damage and canopy cover was extremely weak in late-successional stages ($r_M = -0.005$), providing little support to this explanation. More likely, the strong, positive correlation between SLA and canopy cover in late-successional fields suggests that SLA functions in tolerating low light conditions (e.g., Janse-ten Klooster et al. 2007) in addition to tolerating defoliation (e.g., Meyer 1998a). It is known that physiological mechanisms that allow plants to tolerate herbivory may also allow plants to deal with a broader range of environmental stress (Chapin 1991, Siemens et al. 2003, Jones et al. 2006). Therefore, plant traits that promote tolerance to both herbivory and environmental stress may be adaptive and expressed at greater levels when both types of stress are present (Siemens et al. 2003, Jones et al. 2006). Interestingly, the relationship between SLA and neighboring stem density was significantly stronger in late- than early successional fields, even though stem density averaged 106% greater in early-successional fields. The negative correlations (from Pearson's *r*) between SLA and neighboring stem density suggest a possible tradeoff between SLA and competitive ability (e.g., Herms and Mattson 1992) that is only manifested in late-successional fields.

Another important neighbor effect involved the relationship between vegetative cover and damage that was significantly stronger (based on r_M values) in early than in late fields. We found that damage was positively correlated with vegetative cover in 2/3 of the early fields, which suggests that neighboring plant cover may attract and retain herbivores (reviewed in Barbosa et al. 2009), although there was variability in this relationship among fields. Experimental tests are needed to uncover the specific mechanisms responsible for the relationships between defense traits, damage, and neighbor variables in our system.

A novel insight from this study is the possibility that changes in the environment over the course of succession may shift the adaptive value of defense expression from a resistance strategy to a tolerance strategy in our system. This suggestion is based on our findings that 1) the positive correlation between damage (commonly used to infer resistance; see Methods) and vegetation cover was significantly stronger in early-successional fields (Fig. 4b), and 2) the significant positive correlations between the tolerance trait SLA and canopy cover and neighbor stem density were evident only in late-successional fields (Fig. 4a). In old-field and grassland habitats, succession results in a high turnover in community composition, including a decrease in density of forbs and grasses and an increase in woody plants and canopy cover (e.g., Hartnett and Bazzaz 1985). When suites of environmental stresses change predictably during succession (i.e., canopy cover increases and neighbor stem density and vegetation cover decreases) and plants are long-lived, then the optimal defense strategy may involve either plastic or ontogenetic changes to increase tolerance over time. Plastic (or induced) defenses to herbivory have been the focus of numerous studies (reviewed in Callaway et al. 2003). Recently, ontogenetic shifts from a resistance to a tolerance strategy have been documented in some plants (e.g., Böege et al. 2007).

Alternatively, the absence of significant relationships between SLA and canopy cover in early fields and between damage and vegetation cover in late fields could be a statistical artifact. A priori, one might expect little variation in canopy cover and vegetation cover in early- and late-successional fields, respectively. This lack of variation in one of the variables could make it statistically difficult to detect a significant relationship. However, coefficients of variation for canopy cover were similar in both successional stages (mean CV ± SE early = 0.86 ± 0.05, late = 0.73 ± 0.31), and contrary to the above prediction, coefficients of variation for vegetation cover were higher in late rather than early fields (early = 0.25 ± 0.03, late = 0.58 ± 0.12). These findings suggest that the weak correlations between SLA and canopy cover in early fields and between damage and vegetation cover in late fields were not an artifact of low statistical power.

The intriguing hypothesis suggested by our results, that a successional shift in selection for resistance and tolerance expression may be taking place, warrants additional research. In a subsequent field experiment in which replicate goldenrod clones were transplanted into early- and late-successional fields, genotypes exhibited higher resistance and tolerance to herbivory in early- and late-successional fields, respectively (see chapter 4). Together these results suggest strong phenotypic plasticity in expression of defense trait levels and that trait expression is subject to stage-specific environmental pressures. Successional changes in the environment have until now been neglected in plant defense theory as an important mechanism influencing temporal changes in defense expression for long-lived plants.

This study adds support to the recent hypothesis that mixed patterns of plant resistance and tolerance may be maintained in natural populations when spatial and temporal environmental heterogeneity alters the expression of tolerance and resistance traits (e.g., Agrawal et al. 2006, Núñez-Farfan et al. 2007). This finding is contrary to conventional defense theory which has predicted through simple models that plant populations should eventually become fixed with the most adaptive defense trait or strategy (Herms and Mattson 1992, Mauricio et al. 1997). Instead, spatial patterns of defense traits are likely to be dependent on a plant's environmental context and may change in quantitative and qualitative ways during succession. For long-lived plants in successional habitats, the most adaptive defense strategy in response to this spatial and temporal variability in the environment may be to exhibit ontogenetic changes or phenotypic plasticity in defense strategies. We suggest that future plant defense research should consider incorporating a spatiotemporal approach to better understand the evolution of these traits in complex environments.

CHAPTER 3. SOLIDAGO ALTISSIMA RESISTANCE AND TOLERANCE TO HERBIVORY: EXAMINING COSTS, SELECTION, AND GENETIC VARIABILITY OF MULTIPLE TRAITS

Introduction

In response to herbivore pressure, plants have evolved a variety of defense traits to reduce herbivore damage or the negative fitness effects of herbivore damage (e.g., Painter 1958, Ehrlich and Raven 1964, Pilson 2000, Weinig et al. 2003). Within a population, plants often express various levels of both resistance and tolerance traits (Mauricio et al. 1997, Fornoni et al. 2004b, Stevens et al. 2007). This observation runs counter to theoretical expectations that resistance and tolerance represent mutually exclusive defense strategies owing to separate costs and similar benefits (e.g., van der Meijden et al. 1988, Herms and Mattson 1992, Mauricio et al. 1997). An active area of research in plant defense theory focuses on understanding the evolution of diverse defense traits and uncovering the mechanisms maintaining their variability within natural populations (e.g., Tiffin 2000, Fornoni et al. 2004a, Núñez-Farfan et al. 2007). To this end, it is necessary to determine the extent to which these traits are genetically based, characterize their adaptive value (i.e., fitness benefits relative to costs) across a gradient of herbivory, and examine correlations among defense-trait levels.

There is much that we do not know about the adaptive value and heritability of resistance and tolerance traits, or associations among defensive traits. Few studies have examined both resistance and tolerance in the same plant population (e.g., Simms and Triplett 1994, Mauricio et al. 1997, Shen and Bach 1997, Fornoni et al. 2003, 2004b, Ivey et al. 2009), and fewer studies have quantified costs and benefits of both resistance and tolerance levels (e.g., Simms and Triplett 1994, Mauricio et al. 1997, Pilson 2000, Siemens et al. 2003, Fornoni et al. 2004b, Ivey et al. 2009) or explored how defense levels covary with each other and other plant traits (e.g., Carr et al. 2006, Leimu and Koricheva 2006, Travers-Martin and Muller 2008).

Late goldenrod (*Solidago altissima*: Asteraceae) is a cosmopolitan species whose interactions with its diverse herbivore assemblage have been well studied (Maddox and Root 1987, Meyer and Whitlow 1992, Pilson 1992, Root 1996, Abrahamson and Weis 1997, Meyer 1998b, a, Uriarte 2000, Cronin and Abrahamson 2001a, Cronin et al. 2001, Wise and Abrahamson 2008b). Many of these studies have demonstrated that there is considerable genetic variability in goldenrod resistance to insect-herbivore attack (Maddox and Root 1987, McCrea and Abrahamson 1987, Maddox and Root 1990, Cronin and Abrahamson 2001b, Wise 2009), but few have examined costs and/or selection for resistance (Uriarte et al. 2002, Wise et al. 2008). These few studies suggest that there is generally strong selection for increased resistance but the trait has low broad-sense heritability. To date, no study has experimentally measured tolerance to herbivory in goldenrod. Clearly, more studies that explicitly examine costs, selection gradients, and genetic variation (and heritability) of resistance, tolerance, and other traits are needed to develop a framework for understanding the evolution of defenses in this, and other ecologically important plant species.

We conducted a common garden experiment to quantify genetic variability, costs, and selection gradients for, and correlations among, *S. altissima* resistance, tolerance, and various defense- and fitness-related traits (e.g., relative growth rate, leaf addition and senescence rate, specific leaf area, leaf toughness, ramet height, and inflorescence biomass). In this study, we used 103 goldenrod genets propagated from ramets previously surveyed in one early- and one late-successional field (chapter 2). The common garden environment controlled for variability in neighboring plants, light and water availability that may otherwise affect the phenotypic expression of plant defensive and fitness-related traits (e.g., Horner and Abrahamson 1992, Dudt and Shure 1994, Cipollini 2005, Wise and Abrahamson 2007). For the various goldenrod traits that were measured in the field of origin and again in the common garden, we examined whether they were correlated. If goldenrod defense traits are mainly genetically-based, then we expect to find significant variation among genets for defense traits, high broad-sense heritability, and strong correlations between goldenrod trait levels from the field survey and common garden experiment. We expect strong selection gradients favoring increased defense and expect that defense traits will exhibit costs when herbivores are absent.

Methods

The Goldenrod System:

Late goldenrod (*Solidago altissima* L. subsp. *altissima*) is common throughout eastern North America (Semple and Cook 2006) and is a dominant plant of mid-successional, old-field habitats. Goldenrods are host to over 100 species of herbivores (Maddox and Root 1987, 1990) and dominant herbivores include spittlebugs (*Philaenus spumarius*), gall-making flies (*Eurosta solidaginis*), and various grasshopper and beetle species (e.g., *Acrididae* spp. *Trirhabda* spp. Herbivore damage can decrease goldenrod biomass, increase photosynthetic rates, and delay leaf senescence and flowering time (e.g., Cain et al. 1991, Meyer and Whitlow 1992, Meyer 1993, Meyer and Root 1993, Meyer 1998b, Carson and Root 1999, Cronin and Abrahamson 1999, 2001b, Wise 2009).

Goldenrod resistance and tolerance traits:

In goldenrods, a variety of traits have been linked to resistance and tolerance Morphological resistance traits may include leaf toughness, although evidence for this is equivocal (e.g., Choong 1996, Siska et al. 2002; see also chapter 2). It has recently been identified that goldenrods with a "nodding" apex morphology experience increased resistance to galling herbivores (Wise, 2009). Goldenrods also may employ secondary defense chemicals such as diterpenes and phenolics for defensive purposes (Gershenzon 1994, Abrahamson and Weis 1997, Hull-Sanders et al. 2007). Putative tolerance mechanisms have been explored in some detail. Tolerance mechanisms in goldenrods may include physiological traits that aid in compensatory growth and photosynthesis. Specifically, increased leaf addition rate, relative growth rate, specific leaf area, and delayed leaf senescence are mechanisms that have been experimentally shown to be associated with compensatory growth following damage in *S. altissima* (Meyer 1998a) and other species (e.g., Oesterheld 1992, Moriondo et al. 2003). In addition to tolerating herbivory, these traits may be associated with plant vigor and serve in tolerating other types of environmental stresses (e.g., Siemens et al. 2003, Jones et al. 2006). None of these studies have explored the fitness costs and benefits of any defensive traits.

Source of goldenrod genets:

Genets originated from one early- and one late-successional field in Baton Rouge, Louisiana. The early field had been mown within the past three years and was dominated by goldenrods and other forbs and grasses (e.g., *S. altissima*, *Ambrosia* spp., *Liatris* spp., *Dichanthelium* spp.). The late field had remained fallow for approximately 15 years and was dominated by trees and shrubs (e.g., *Triadica sebiferum*, *Cornus foemina*, *Acer negundo*, *Rubus* spp.). Both fields were approximately 2 ha in size. In 2006, we surveyed levels of various defense- and fitness-related traits from goldenrod ramets spaced 20 m apart in a rectangular grid within each field (chapter 2). This spacing was selected to maximize the number of ramets sampled in each field as well as the likelihood that each ramet represented a unique genet (Meyer and Schmid 1999). A thorough description of these field surveys of is provided in chapter 2 of this dissertation. In February 2007, we excavated the root bundles of these ramets. Clonal replicates were obtained from rhizome cuttings (5 cm sections) from one individual per genet. Rhizome material was propagated in flats of vermiculite for 4 weeks in a greenhouse at Louisiana State University.

Common garden:

We created the common garden plot inside a 1 ha, mid-successional field (5-years in age) that had been cleared two months prior to the start of our experiment. The field was previously dominated by goldenrods. We planted 6 replicates of each genet into a 30 m by 46 m grid with a spacing among ramets of 1.5 m. Assignment of goldenrods to locations in the garden was determined by a random draw. To promote vigorous growth and minimize micro-site differences, ramets were fertilized at the start of the experiment and watered in times of drought. Also, all plants within a 0.25 m radius of each ramet were routinely clipped at ground level to discourage neighbor competition. We administered an insecticide treatment to encourage a gradient of damage among clonal replicates for the purpose of estimating tolerance (see section "Resistance and tolerance measures"). One half of the replicates per genet were randomly assigned to an herbivory-reduced treatment and sprayed biweekly with Sevin® carbaryl insecticide (Bayer CropScience). The remaining one half were assigned as controls and exposed to natural levels of herbivory. Because the common garden was created within a field dominated by goldenrods, the herbivore community in our garden was similar those of other nearby goldenrod fields (A.S. Hakes personal observation).

Every eight weeks from Mar-Nov, 2006 we measured ramet height and stem diameter at 10 cm**,** and the number of new leaves produced since the last census. Proportion of leaves damaged by leaf-chewing insects and leaf area removed were calculated for new leaves at each census. Leaf area lost to herbivores was assessed through digital photographs of three haphazardly chosen damaged leaves using the program UTHSCSA *ImageTool* (University of Texas Health Science Center at San Antonio, TX). The proportion of total plant tissue damaged by leaf-chewing herbivores was estimated from this procedure. Herbivores on ramets were surveyed by visual counts and identified to family. Other types of herbivores (i.e., galling and sucking herbivores) were scarce in our fields and are ignored in this study.

We measured plant traits known to be associated with compensatory growth in response to herbivory in *S. altissima* (the tolerance traits leaf addition rate [LAR], leaf senescence rate [LSR], relative growth rate [RGR], and specific leaf area (SLA; see Meyer 1998a). LAR and LSR were measured as the number of leaves added or senesced per day, respectively, and RGR was calculated as the difference in *ln*-transformed height divided by the number of days between measurements (Meyer 1998a). In the early summer census, we collected leaf tissue samples for assessment of leaf toughness and SLA. Three leaves were collected haphazardly from the upper two-thirds of the stem. Leaf toughness was measured as the average force (in grams) needed to push a metal rod through leaf tissue using a penetrometer (Itin Scale Co., Inc., Brooklyn, NY; Siska et al. 2002). The collected leaves were transported to the laboratory on dry ice, lyophilized (72 hr), and weighed. Leaf area was calculated from digital photographs of each leaf using ImageTool and used to compute SLA (leaf area per unit dry leaf mass). SLA is positively correlated with mass-based photosynthetic rate and higher SLA in re-growth leaves allows damaged plants to gain more leaf area for a given biomass investment (e.g., Meyer 1998a, Gunn et al. 1999). At the end of the growing season, newly-opened inflorescences were collected, dried in an oven at 65^{o} C for 4 days, and the biomass determined as an estimate of short-term sexual reproduction. We used height as our estimate of short-term fitness. Studies have shown that perennials that are larger in size have higher survival and life-time fitness in terms of sexual and clonal reproduction (e.g., Gardner and Mangel 1999, e.g., Haukioja and Koricheva 2000).

Resistance and tolerance measures:

Resistance per ramet was measured as 1- the proportion of tissue damaged by chewing herbivores (Rausher and Simms 1989, Simms and Triplett 1994, Stinchcombe and Rausher 2001, Fornoni et al. 2004a). Resistance at the genet level was determined as the average resistance among the control ramets (i.e., the three ramets exposed to herbivory). Tolerance per genet was measured as the slope of a linear regression of *ln*-height (our proxy for fitness) against the level of damage sustained by each individual ramet (Simms and Triplett 1994, Mauricio et al. 1997, Strauss and Agrawal 1999, Tiffin and Rausher 1999). Tolerance levels increase as the slope decreases. A positive slope indicates overcompensation to damage (e.g., Belsky et al. 1993, Tiffin and Rausher 1999, Agrawal 2000)

Data analysis:

Differences among genets for resistance and each of the defense and fitness-related trait levels (i.e., RGR, LAR, LSR, SLA, leaf toughness, ramet height, and inflorescence biomass) were assessed with separate nested ANOVAs. Source population (early field or late field) and insecticide treatment (control or pesticide) were fixed factors and genotype nested within source population (48 early genets, 55 late genets) was a random effect in the model. Without replicate fields for each successional stage, a source-population effect is open to interpretation. We include this source of variation in the model only to partition the effects of site of origin from our estimates of a genet effect on plant-trait variation. However, in all of our analyses, we did not find a significant source-population effect on the expression of any trait (average *P*-value = 0.17 ± 0.1). To simplify the presentation of our data, we subsequently omitted this term from the model, reducing it to a 2-way ANOVA with genet and insecticide treatment as the main effects. A significant genotype effect would indicate genetic variability for a particular trait, a significant insecticide treatment effect would indicate that fitness and/or defense levels varies with the level of herbivory, and a significant interaction would indicate that the effect of herbivory on trait levels varied among genets.

To determine whether there was variability among genets in tolerance levels, we conducted an ANCOVA using *ln*-height (fitness estimate) as the dependent variable, herbivore damage as the covariate, and genotype as a random effect. The degree of tolerance is indicated by the slope of the relationship between damage and fitness (specifically, -1/slope; under the expectation that the relationship is negative). A significant damage by genotype interaction would indicate that tolerance differs among genets (i.e., the slopes differ among genets).

All plant defense and fitness variables required transformations to normalize distributions with the exception of leaf toughness. Resistance was logit-transformed and the remaining variables were *ln*-transformed. Tests were conducted using R (R Development Core Team 2008) and sequential Bonferroni corrections to α were made to account for multiple, non-independent tests (Rice 1989).

Broad-sense heritabilities for resistance and defense traits were computed using the methods of Falconer (1989) and Mitchell and Shaw (1993). Broad-sense heritability (i.e., clonal repeatability) was estimated as V_g/V_p, where V_p = total variance and $V_g = (MS_{genotype} - MS_{error})$/(number of ramets per genet) from the ANOVA models described above (Falconer 1989, Mitchell and Shaw 1993). Because tolerance is measured at the level of genet rather than individual ramets, we were unable to obtain a broad-sense heritability estimate for tolerance.

We also gauge whether defense levels have a strong genetic basis by examining correlations between trait levels of genets measured in the common garden and trait levels of the same genets measured in their field of origin. Correlation analyses between field and garden trait values were performed separately for each trait. If goldenrod fitness and defense traits are under strong genetic control, then we would expect that the trait values for a particular genet would be similar whether they are growing naturally in the field or in a common garden (i.e., a positive correlation should exist among genets). Alternatively, the absence of a correlation would suggest that the traits have low heritability or strong phenotypic plasticity. We also examined correlations among putative defense traits and fitness, resistance, and tolerance levels.

Costs of defense traits are best determined from the relationship between defense level and fitness in the absence of herbivores (Simms and Rausher 1989, Mauricio et al. 1997). In situations where herbivores are not completely excluded, fitness in the absence of herbivory can be estimated using the following procedure. For each genet, we determined the *y*-intercept of the regression of fitness (*ln*-height) over the gradient of damage among ramets (e.g., Mauricio et al. 1997, Tiffin and Rausher 1999). This quantity (W_A) provides an estimate of fitness in the absence of herbivory for a particular genet. Fitness costs of resistance and tolerance, and other defense traits (RGR, LAR, LSR, SLA, leaf toughness) were estimated as the slope of the regression of genet W_A against its defense trait value. A significant negative relationship between W_A and defense trait level would suggest a cost for that particular trait (Mauricio et al. 1997, Tiffin and Rausher 1999, Fornoni et al. 2004a). A quadratic term was added to the model to examine nonlinear relationships between W_A and trait levels. Because using W_A as an estimate of genet fitness produces a bias in the estimation of the cost of tolerance owing to non-independence between the y-intercept (fitness in absence of herbivory) and slope (tolerance; see

Mauricio et al. 1997, Tiffin and Rausher 1999, Fornoni et al. 2004b), we used a second method to test for a cost of tolerance by estimating the true covariance (corrected for the bias on sample covariance; see appendix B in Mauricio et al. 1997). Standard errors of the estimated corrected covariance between tolerance and fitness were obtained using a jackknife procedure (Tiffin and Rausher 1999). An estimate of the true covariance with a negative value and an interval that does not overlap zero indicates a significant cost of tolerance (Mauricio et al. 1997, Tiffin and Rausher 1999, Fornoni et al. 2004b).

The fitness benefits of defense traits are estimated in the presence of herbivores (i.e., the control plants). Selection gradients for tolerance and resistance, and other defense traits (RGR, LAR, LSR, SLA, and leaf toughness) were estimated as the slope of the regression of mean *ln*-height of control ramets (i.e., genet fitness in the presence of herbivores; W_P) against its value of defense. Selection gradients favoring increased or decreased levels of defense traits would be indicated by a significant positive or negative linear relationship, respectively, between W_P and defense trait level (Mauricio et al. 1997, Tiffin and Rausher 1999). Stabilizing or disruptive selection would be evident if there were significant positive or negative nonlinear relationships, respectively (Mauricio et al. 1997, Tiffin and Rausher 1999).

Results

Damage by leaf-chewing herbivores ranged from 0-50% of the total plant tissue removed. On average, ramets in the insecticide treatment experienced 67% lower damage than ramets in the control treatment (mean ± SE control = 7.87 ± 0.48%, sprayed = 2.63 ± 0.31% damage). Even though the insecticide treatment did not eliminate all herbivores, it did serve its intended purpose to increase the range of variability in damage to ramets of the same genet and allow for meaningful estimates of tolerance, costs, and selection coefficients (see Methods).

We found significant genetic variability for many of the goldenrod traits examined. Resistance varied significantly among goldenrod genets, ranging from 0.72 to 1.00 (mean ± SE = 0.92 ± 0.05; $F_{102,102} = 1.58$, $P = 0.01$; Table 3.1). There were also significant genotype effects on LSR ($F_{102,102} = 1.90$, $P < 0.001$), leaf toughness ($F_{102,102} = 1.70$, $P < 0.001$), ramet height ($F = 1.32$, $P = 0.08$), and inflorescence biomass ($F_{102,102} = 1.68$, $P = 0.005$; Table 3.1). Only 70% of the ramets planted into the common garden flowered, and of those ramets that flowered, inflorescence biomass ranged from 0.45 g to 92.0 g (mean ± SE = 14.7 ± 0.6 g). Inflorescence biomass was significantly positively correlated with ramet height (r = 0.65, $P < 0.001$). However, we found no evidence for differences among genets in height, RGR, LAR, and SLA (Table 3.1).

Table 3.1. Results from separate two-way ANOVAs for the effects of genotype (n=103), insecticide treatment (control vs. sprayed), and their interaction on goldenrod resistance and various goldenrod traits associated with fitness and defense.

Variables	Genotype		Insecticide		Genotype x Insecticide	
	$F_{102,102}$	*P*-value	$F_{102,412}$	*P*-value	$F_{102,142}$	*P*-value
Resistance	1.58	0.010*	166.98	<0.001*	0.82	0.887
Ramet Height	1.32	0.083	20.99	<0.001*	1.22	0.091
Inflorescence biomass	1.68	0.005*	2.48	0.116	0.95	0.615
RGR	1.25	0.131	0.66	0.417	1.09	0.278
LAR	1.23	0.152	2.42	0.121	1.19	0.122
LSR	1.90	<0.001*	5.55	0.019	1.26	0.057
Toughness	1.70	<0.001*	15.67	<0.001*	1.62	0.001*
SLA	1.13	0.271	19.27	<0.001*	3.22	0.001*

* *P* values < the critical level of 0.05 following sequential Bonferroni corrections to α. SLA= specific leaf area, RGR=relative growth rate, LAR=leaf addition rate, LSR=leaf senescence rate.

On average, genets appeared to be quite tolerant of herbivore damage. Ramet height (proxy for fitness) was unaffected by damage level ($F_{1,412} = 0.43$, $P = 0.51$; Table 3.2). However, we did find a marginally significant interaction between genet and damage on ramet height ($F_{102,412} = 1.27$, $P = 0.055$; Table 3.2), suggesting that tolerance levels (i.e. slopes of the regression of ramet height against damage differed among genets) varied among genets. Tolerance levels ranged from -0.21 to 0.29 among genets (mean ± SE = -0.01 ± 0.07), with a nearly equal number of positive (indicating overcompensation in growth following damage) and negative slopes.

Table 3.2. Results from an ANCOVA for the effects of genotype (n=103), herbivore damage (covariate), and their interaction on ramet height (proxy for fitness).

Source of Variation	df	Mean Sq	F	*P*-value
Genotype	102	0.56	1.56	0.001
Damage	1	0.16	0.43	0.511
Genotype*Damage	102	0.46	1.27	0.055
Residuals	412	0.36		

There was no evidence for tradeoffs between resistance and tolerance, nor among any of the goldenrod traits (i.e., there were no significant negative correlations between traits; Table 3.3). There were, however, many positive correlations among traits (30 out of 36). Tolerance was significantly positively correlated with putative tolerance traits RGR (r = 0.25), LAR (r = 0.40), LSR (0.20), and fitness-related traits ramet height (r = 0.40) and inflorescence biomass (r = 0.28). There were also significant, positive correlations between LAR and traits RGR and LSR (r = 0.48 and r = 0.62, respectively), and these traits also exhibited strong, positive correlations with ramet height and inflorescence biomass (with the exception of LSR and inflorescence biomass; Table 3.3). Resistance, leaf toughness, and SLA were not significantly correlated with any traits measured in the common garden experiment (Table 3.3).

Table 3.3. Pearson's product moment correlations (r) among all possible pairs of defense-related traits and fitness-related traits of goldenrod measured in the common garden experiment.

	Tolerance	RGR	LAR	LSR	SLA	Toughness	Ramet Height	Inflorescence Biomass
Resistance	0.10	0.03	0.02	0.03	0.08	0.16	0.06	0.01
Tolerance	.	0.25*	0.40*	0.20*	0.08	0.02	0.40*	0.28*
RGR	.	.	0.48*	0.06	0.10	0.07	0.71*	0.48*
LAR	.	.	.	0.62*	-0.07	0.01	0.81*	0.66*
LSR	.	.	.	.	-0.12	-0.14	0.38*	-0.02
SLA	.	.	.	.	.	0.01	-0.06	-0.09
Toughness	.	.	.	.	.	.	0.02	0.13
Ramet height	.	.	.	.	.	.	.	0.65*

* *P* values < the critical level of 0.05 following sequential Bonferroni corrections to α. SLA= specific leaf area, RGR=relative growth rate, LAR=leaf addition rate, LSR=leaf senescence rate.

Broad-sense heritability estimates for defense and fitness-related traits were low (Table 3.4). LSR had the highest heritability ($H^2 = 0.17 \pm 0.07$) and the remainder of the traits had an $H^2 \leq 0.09$. In addition, fitness- and defense-related trait levels measured from genets in the common garden were not significantly positively correlated with trait levels measured from the source genets in their field of origin (Table 3.5). In fact, most of the correlations were negative (7 of 8), 4 of which had P values < 0.05 (leaf toughness, RGR, LAR, and ramet height). However, after employing a sequential Bonferroni correction to control for the possibility of an inflated type I error rate associated with multiple tests on the same dataset, these correlations were deemed non significant (Table 3.5).

There was no evidence for a fitness cost of resistance in the common garden (r = 0.17, P = 0.085, Figure 3.1a), but the significant, negative relationship between tolerance levels and W_A indicates a significant cost of tolerance (r = -0.47, P <0.001; Figure 3.1b). We also examined the cost of tolerance by testing whether the corrected covariance was less than zero (see Methods). Application of the correction for the bias in the relationship yielded an estimate of the true covariance of -3.14 ± 8.13e^{-5}, confirming a cost of tolerance. We did not find fitness costs for any other goldenrod trait. On the contrary, there were significant, positive correlations between W_A and the putative tolerance traits SLA (r = 0.23, P = 0.02), RGR (r = 0.26, P = 0.008), and LAR (r = 0.23, P = 0.018) (Figure 3.1c-g). Finally, we found no evidence for nonlinear relationships between W_A and the various goldenrod traits (i.e., no significant quadratic regressions).

Table 3.4. Broad-sense heritability estimates (H^2 ± se) for goldenrod resistance and various fitness- and defense-related traits.

Traits	H^2 ± SE
Resistance	0.05 ± 0.05
Ramet height	0.08 ± 0.06
Inflorescence biomass	0.09 ± 0.07
Relative growth rate (RGR)	0.08 ± 0.06
Leaf addition rate (LAR)	0.06 ± 0.06
Leaf senescence rate (LSR)	0.17 ± 0.07
Leaf Toughness	0.02 ± 0.05
Specific leaf area (SLA)	0.07 ± 0.06

Table 3.5. Correlations between goldenrod trait levels measured from source ramets in their field of origin and trait levels measured from the same genets in the garden environment (genet-level average). Pearson's product moment correlations (r) and *P*-values are reported. A significant positive correlation is indicative that genets express similar trait levels in field and garden environments. Tolerance could not be estimated from source genets in the field.

Traits	r	*P*-value
Resistance	-0.08	0.41
Ramet height	-0.23	0.03
Inflorescence biomass	-0.14	0.31
Relative growth rate (RGR)	-0.22	0.02
Leaf addition rate (LAR)	-0.21	0.04
Leaf senescence rate (LSR)	0.13	0.19
Leaf toughness	-0.20	0.04
Specific leaf area (SLA)	-0.08	0.40

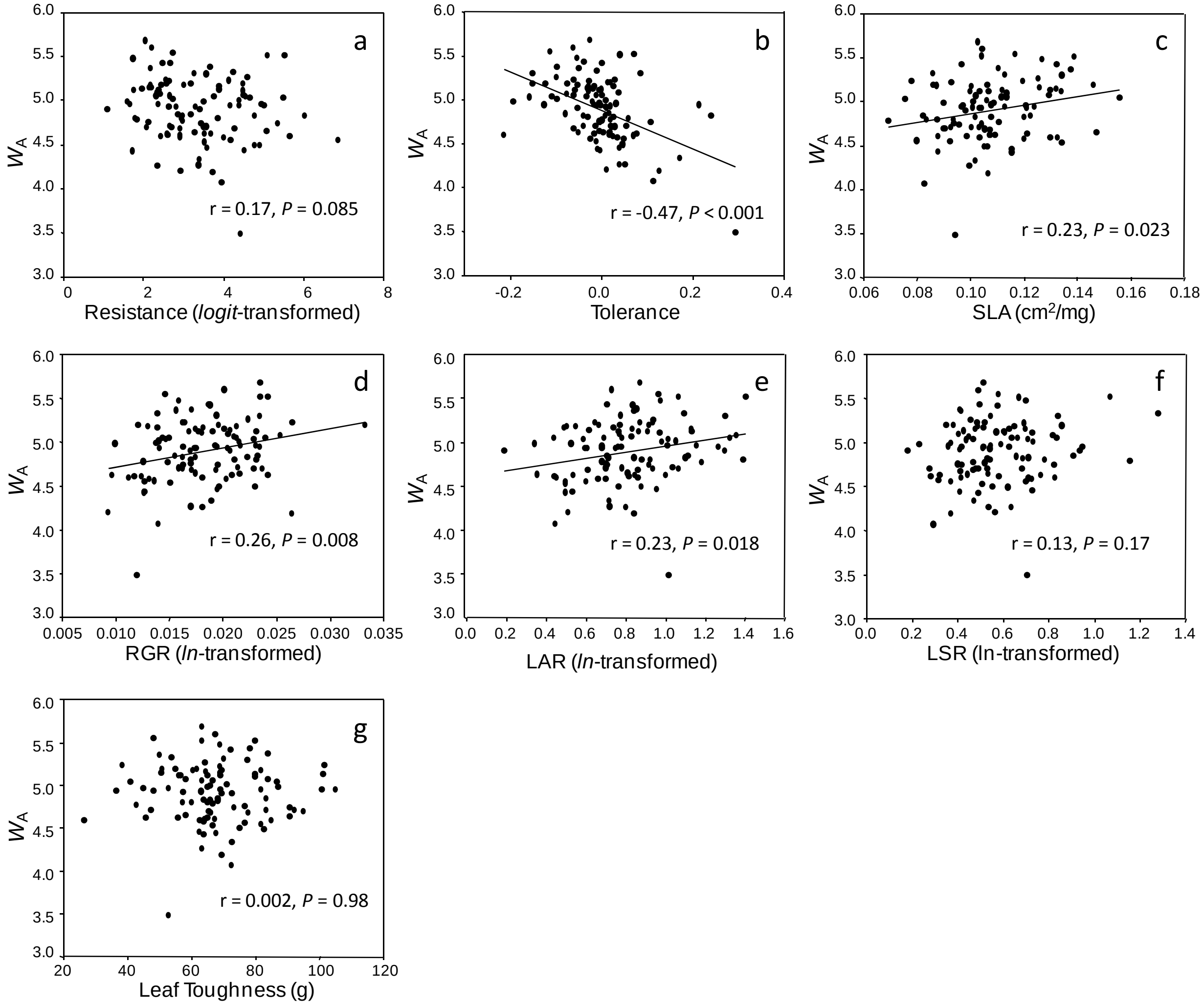


Figure 3.1. The relationships between various goldenrod defense traits (i.e., resistance (a), tolerance (b), SLA (c), RGR (d), LAR (e), LSR (f), and LT (g)) and ramet height in the absence of herbivory (W_A). A significant negative relationship is indicative of a cost associated with the associated trait. Each data point represents the genet average.

We found no evidence that fitness in the presence of herbivory (W_P) was correlated with resistance level in the common garden (r = 0.06, $P = 0.52$, Figure 3.2a). In contrast, there was a significant, positive relationship between W_P and tolerance, indicating strong selection for increased tolerance to herbivory (r = 0.38, $P < 0.001$; Figure 3.2b). We also found significant, positive selection gradients for RGR (r = 0.72, P <0.001), LAR (r = 0.81, P < 0.001), and LSR (r = 0.38, P < 0.001), but no evidence of selection gradients for SLA or leaf toughness (Figure 3.2c-g). There was no evidence for stabilizing or disruptive selection for resistance, tolerance, or for any of the traits (i.e., quadratic regressions between W_P and trait levels were not significant).

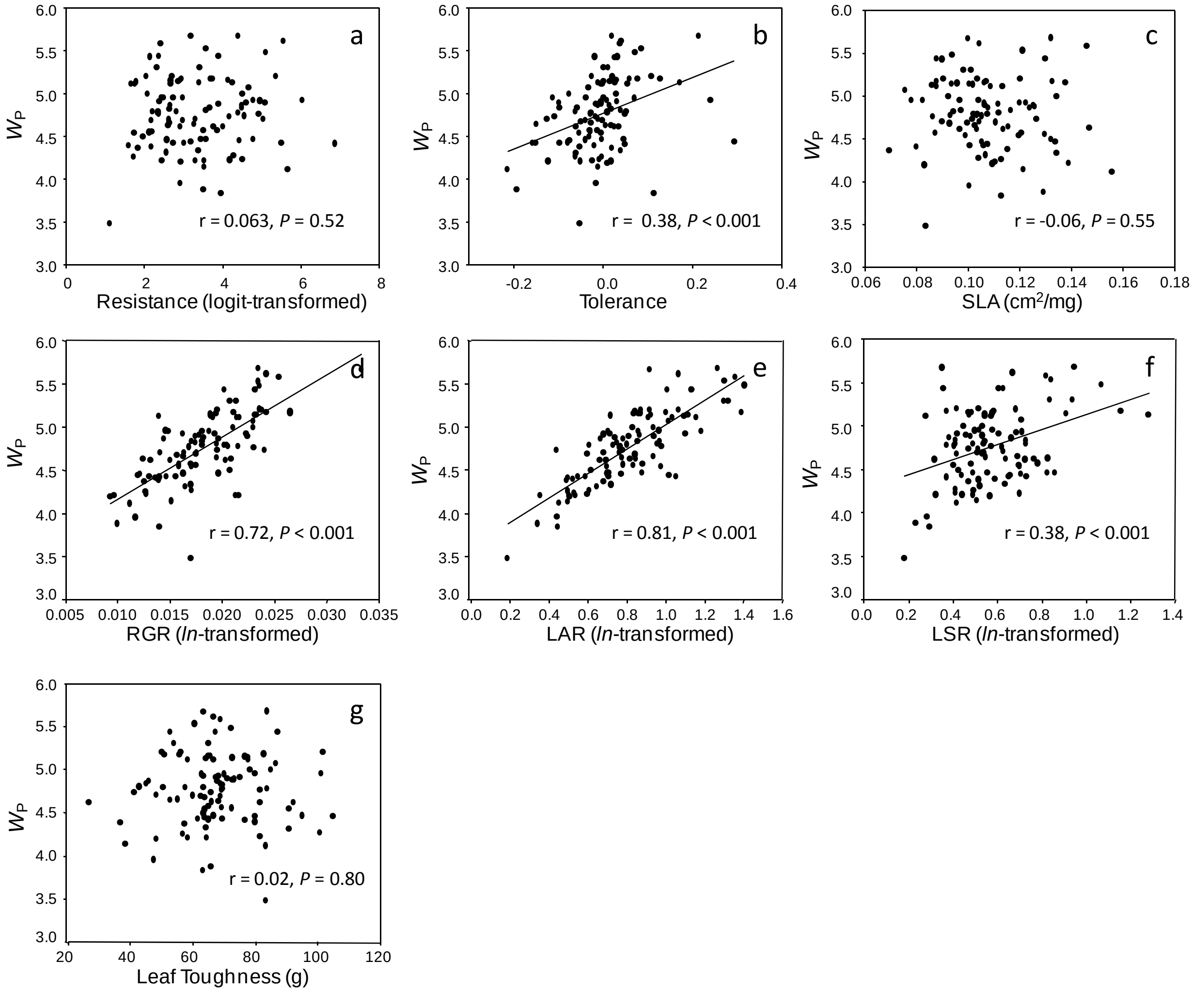


Figure 3.2. The relationships between various goldenrod traits (i.e., resistance (a) and tolerance (b) SLA (c), RGR (d), LAR (e), LSR (f), and LT (g)) and ramet height in the presence of herbivory (W_P). A significant positive or negative relationship is indicative of selection for high or low trait levels, respectively.

Discussion

We report evidence for genetic variability in goldenrod resistance and, for the first time, tolerance to herbivory and many defense- and fitness-related traits of goldenrod (i.e., LSR, leaf toughness, inflorescence biomass). Goldenrods had previously only been examined for genetic variation in resistance to herbivores (Maddox and Root 1987, McCrea and Abrahamson 1987, Maddox and Root 1990, Cronin and Abrahamson 2001b, Wise 2009), although significant genetic variability in both resistance and tolerance has been documented for other plant species (e.g., Simms and Triplett 1994, Mauricio et al. 1997, Shen and Bach 1997, Fornoni et al. 2003, 2004b, Carr et al. 2006, Ivey et al. 2009).

Despite significant genetic variability in goldenrod traits, we found very low broad-sense heritability estimates for resistance ($H^2 = 0.05 \pm 0.05$) and all of the traits examined (ranging from 0.02 to 0.17). This finding is contrary to a number of studies that have found high heritability of resistance in other plant species (e.g., Carr et al. 2006, Agrawal and Konno 2009, Johnson et al. 2009). Similar to our findings, Wise (2007) found significant genetic variation in *Solanum carolinense* resistance, but relatively low broad-sense heritability estimates in resistance towards herbivores (ranging from 0.04 to 0.30). In an earlier study of goldenrods, Maddox and Root (1987) also found low broad-sense heritability of resistance in 18 *S. altissima* genets (ranging from 0.05 to 0.2). In the same study, however, Maddox and Root (1987) found that the narrow-sense heritability estimates (0.05-0.92) were generally much higher than broad-sense heritability estimates, which suggests that broad-sense heritability should not necessarily be interpreted as an upper-limit estimate of heritability (Wise 2007). Nevertheless, low broad-sense heritability estimates, such as those reported here, have the potential to constrain trait evolution (e.g., Falconer 1989).

The lack of positive correlations between defense trait levels measured from the same genets in their field of origin and common garden may suggest that goldenrod defense traits have strong phenotypic plasticity. Plastic defense expression is common in many plants (reviewed in Callaway et al. 2003, see also Barton 2008, Holeski et al. 2010), and studies that have examined goldenrod resistance in multiple environments and over several years report that resistance rankings among genets can differ between environments and can change over time (Maddox and Cappuccino 1986, e.g., Horner and Abrahamson 1992, Meyer and Root 1993, Cronin et al. 2001, but see Wise 2009). If goldenrod traits are plastically expressed, then the lack of positive correlations we found between goldenrod traits measured in their original fields and common garden may be a result of different environmental conditions influencing defense levels. By maintaining an environment with reduced vegetative ground cover and canopy cover (see Methods), the common garden environment differed from that of the early- and late-successional field that our genets originated from. These characteristics of the neighboring plant community are potential mechanisms influencing plant defenses (e.g., Horner and Abrahamson 1992, Cipollini and Bergelson 2001, 2002, Siemens et al. 2003, Jones et al. 2006). We found that goldenrod resistance and tolerance plastically respond to different successional environments, and found strong correlations between resistance/tolerance traits and vegetative cover/canopy cover within early and late-successional fields, respectively (see chapters 2 and 4).

Alternatively, the lack of a positive correlation between field and garden estimates of goldenrod resistance could be explained by an uneven distribution of herbivores in the source populations as compared to the common garden. The operational measure of resistance equating 1- (the proportion of damage) (see Fineblum and Rausher 1995, Tiffin and Rausher 1999, Pilson 2000, Fornoni et al. 2004a) assumes that plants are equally accessible to uniformly distributed herbivore populations. This assumption is more likely to be met in our common garden where genets were planted in close proximity (1.5 m spacing between ramets), but is almost certainly not the case in the natural field where genets were spaced 20 m apart (see chapter 2). It is possible that a non-resistant plant (as determined from the common garden) may have received little damage in its field of origin because of low encounter rates with herbivores, but it would be scored as highly resistant.

The only variable exhibiting a significant fitness cost was tolerance to herbivory. Resistance and the remaining goldenrod traits examined either showed non-significant, or positive relationships with ramet height in the absence of herbivory, indicating fitness benefits of these traits, irrespective of herbivore pressures. There was no evidence that goldenrod resistance was under selection by herbivores, but there was strong selection for increased tolerance and tolerance traits RGR, LAR, and LSR. Overcompensation to damage was highly adaptive in the common garden (i.e., the fitness benefits of tolerance outweighed the fitness costs for goldenrod genets exhibiting positive tolerance levels; Figure 3.3). We conclude that herbivores are imposing selection for high tolerance to herbivory in the common garden, but the evolutionary response to this pressure may be limited by the low heritability of tolerance traits. There was no evidence of selection for increased goldenrod resistance in our study and the lack of fitness costs associated with this trait suggests that resistance is selectively neutral and may be subject to genetic drift. Other studies examining costs and benefits of resistance and tolerance within plant populations have found not found mixed evidence for defense costs and selection gradients (e.g., Simms and Triplett 1994, Mauricio et al. 1997, Fornoni et al. 2004b). There is a growing consensus that the adaptive landscape for plant defenses may be highly dependent on a plant's environmental context (e.g., Pilson 2000, Siemens et al. 2003, Fornoni et al. 2004b). In a subsequent field experiment where we out-planted goldenrods into early- and late-successional fields, we found strong selection for increased resistance in early-successional fields and no selection gradients associated with tolerance (see chapter 4). Together, these findings lend support to the existence of environmentally variable adaptive landscapes for resistance and tolerance within plant populations.

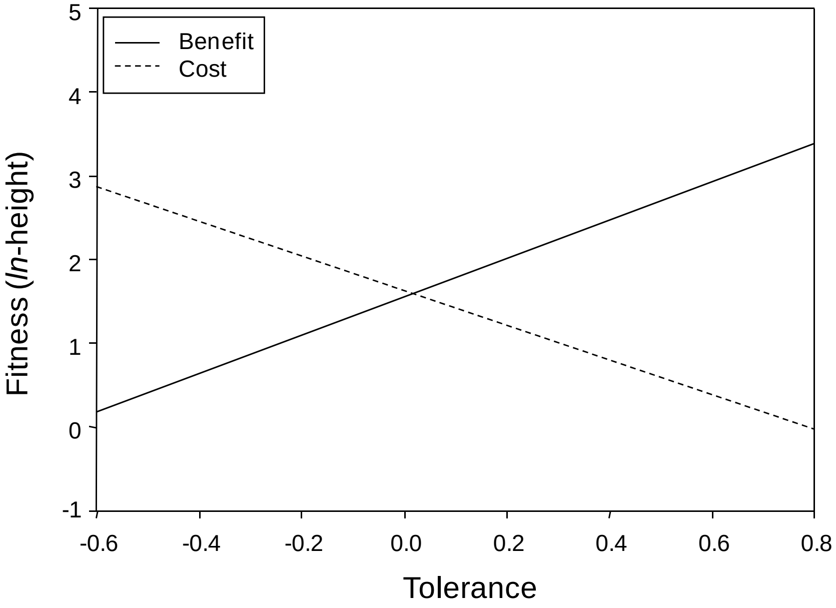


Figure 3.3: The slope of the regression of goldenrod fitness in the absence of herbivores against tolerance levels (i.e. cost of tolerance), and the slope of regression fitness in the presence of herbivores against tolerance levels (i.e., benefit of tolerance). The point at which these slopes intersect indicates the level at which increased tolerance is adaptive (i.e., fitness benefits outweigh costs).

High tolerance was adaptive in the common garden environment. Because the competitive interactions among neighboring plants in our garden were low relative to un-manipulated goldenrod fields, our results may follow the expectation of the compensatory continuum hypothesis that predicts that high tolerance should be favored in plants located in environments with minimal competition stress from neighboring plants (e.g., Maschinski and Whitham 1989, Pilson 2000). For this hypothesis to be supported, an experiment testing for whether the adaptive value of tolerance decreases (i.e., costs outweigh benefits) under conditions of greater competition is required (e.g., Tiffin 2002, but see Wise and Abrahamson 2005). Alternatively, the benefit-cost ratio of tolerance may increase under competition stress if tolerance traits serve a dual function in tolerating both competition stress and herbivory (Siemens et al. 2003, Jones et al. 2006). The tolerance trait SLA (which was not significantly correlated with tolerance levels in our common garden environment) may be more important in environments with neighbor shading where it can mitigate stress from both low light availability (e.g., Janse-ten Klooster et al. 2007, see also chapter 2 of this dissertation) and herbivory (e.g., Oesterheld 1992, Meyer 1998a).

In goldenrod, a widespread and ecologically important plant, we found low genetic basis to resistance, tolerance, and various defense and fitness-related traits. We found strong selection favoring increased tolerance under a gradient of herbivory. However, we expect the evolution of tolerance to be constrained by low heritability in tolerance traits and a strong fitness cost. Our results suggest that goldenrod defenses appear to have great phenotypic plasticity. Given the potential for the environment to influence goldenrod defense expression, the adaptive nature of resistance and tolerance may co-vary with spatial and temporal environmental variability.

CHAPTER 4. SUCCESSIONAL CHANGES IN PLANT RESISTANCE AND TOLERANCE TO HERBIVORY

Introduction

Resistance and tolerance are two alternative defense strategies that plants can express to reduce herbivore damage or the negative fitness effects of herbivore damage, respectively (e.g., Fineblum and Rausher 1995, Stamp 2003). Both strategies may yield benefits of higher fitness in the presence of herbivores relative to non-defended plants. However, both defense strategies may incur fitness costs when herbivores are rare or absent if resources are allocated towards maintaining resistance or tolerance traits at the expense of plant growth or reproduction (reviewed in Stamp 2003). The type and level of defense strategy expressed is assumed to be influenced by their associated fitness costs and benefits (Stamp 2003).

The adaptive value of resistance and tolerance will fluctuate if their associated costs and benefits vary spatially and/or temporally. A fluctuating adaptive landscape may favor the evolution of plasticity in defense expression (Via and Lande 1985) and promote spatio-temporal variability in defense levels for long-lived plant species (e.g., Laine and Tellier 2008). Recent studies have shown that plant defense traits can vary spatially owing to heterogeneity in environmental variables such as light, nutrient, and water availability (Covelo and Gallardo 2004, Brenes-Arguedas and Coley 2005, see also chapter 2). Fewer studies have documented temporal variability in plant defenses (Cronin et al. 2001, Tiffin 2002, Takahashi and Yamauchi 2010). Mechanisms promoting temporal variability in plant defense may include induction of defenses following exposure to herbivores (reviewed in Karban and Baldwin 1997) and ontogenetic changes in the plant (Böege et al. 2007, reviewed in Barton and Koricheva 2010). To date, there has been no consideration of how temporally fluctuating environmental conditions, such as those occurring during succession, influence the level or adaptive value (i.e., in terms of selection gradients and costs) of plant resistance and tolerance.

During succession, environmental conditions and community composition change in predictable ways (e.g., Huston and Smith 1987, Tilman 1987) that could potentially affect the costs, benefits, and expression of plant defenses. For example in old-field habitats, the neighboring plant community transitions from an open canopy/dense understory dominated by forbs and grasses to a closed canopy/open understory forest dominated by trees and shrubs . A growing list of studies have demonstrated that the density and/or identity of neighboring plants can alter resistance and tolerance levels of a focal plant, directly via competition or indirectly via associational resistance or susceptibility (Agrawal et al. 2006). For example, a high density of neighboring plants may increase costs and decrease plant defense if there is a resource-allocation tradeoff between defense traits and competitive ability (e.g., Bazzaz et al. 1987, Cipollini and Bergelson 2002). Alternatively, the presence of neighbors may decrease costs and increase defense expression if defense traits serve a dual purpose, mitigating herbivory and competitive stress (e.g., Strauss and Agrawal 1999, Siemens et al. 2003, Jones et al. 2006). As succession progresses and woody plants begin to dominate the landscape, neighboring plant density in the understory decreases and canopy cover increases (Hartnett and Bazzaz 1985, see also chapter 2). The existing literature provides equivocal support for whether shading should cause resistance levels to increase (e.g., Cipollini 2005) or decrease (e.g., Izaguirre et al. 2006). In contrast, the well supported limiting-resource model of Wise and Abrahamson (2007, 2008) predicts that tolerance to herbivory should decrease when the acquisition of a limiting resource is further exacerbated by herbivore damage. In this case, tolerance to leaf-chewing herbivores is expected to be lower in shaded than in open canopy environment. Overall, the literature strongly suggests that changes in the plant community composition during succession are likely to alter plant defense levels.

We conducted a field experiment to test whether late goldenrod (*Solidago altissima*; Asteraceae) resistance and tolerance levels, fitness costs, and selection gradients differ with respect to field successional stage and genotype. We out-planted clones of 14 genets varying in resistance and tolerance into replicate early- and late-successional fields. We predicted that the transition from an open-canopy environment dominated by an herbaceous understory (i.e., early fields) to a closed-canopy environment dominated by trees and shrubs (i.e., late fields) will elicit strong phenotypic changes in resistance and tolerance levels, as well as changes in their associated costs and selection gradients. For example, based on the predictions from the limiting-resource model (Wise and Abrahamson 2005, 2007, 2008), we expected to find reduced costs, strong selection for, and greater levels of tolerance in goldenrods planted into early than in late fields. We also tested whether a genet by stage interaction was evident, which would suggest a fundamental change in defenses among genets during succession. Lastly, we examined whether the adaptive landscape for resistance and tolerance was temporally dynamic by testing for differences in costs, selection gradients, and defense tradeoffs between successional stages.

Methods

The Goldenrod System:

Solidago altissima L. subsp. *altissima* is common throughout eastern North America (Semple and Cook 2006) and is a dominant plant of mid-successional, old-field habitats. Goldenrods reproduce sexually and asexually (i.e., via rhizomes). More than 100 species of generalist and specialist herbivores feed on goldenrod, including spittlebugs (*Philaenus spumarius*), gall-making flies (e.g., *Eurosta solidaginis*), and various grasshoppers and beetles (e.g., Acrididae spp., Trirhabda spp.; Maddox and Root 1987, Abrahamson and Weis 1997). These herbivores have been found to significantly decrease goldenrod biomass, sexual and asexual reproduction, increase photosynthetic rates, and delay leaf senescence and flowering (Cain et al. 1991, Meyer 1993, Abrahamson and Weis 1997, Meyer 1998b, Cronin and Abrahamson 1999, 2001b).

Goldenrods can persist in old-field habitats for up to 50-75 years (Hartnett and Bazzaz 1985, Maddox et al. 1989). In early-successional fields, continued colonization by seed and rapid clonal expansion occurs through approximately the 5th year after field abandonment, at which time genet density and diversity are maximal (Hartnett and Bazzaz 1985, Maddox et al. 1989). After the 5th year, recruitment of new genets generally ceases (Hartnett and Bazzaz 1985). As old-field succession progresses, the number of goldenrod genets begins to decline and herbaceous plants become displaced by woody plants (Maddox et al. 1989).

Experimental Design:

The goldenrod genets used for our field experiment were selected from a common-garden plot that had been maintained during the previous year. A total of 103 genets, excavated from one early-successional field (n = 55) and one late-successional field (n = 48) in February 2007, were propagated from rhizome material and re-planted into a common garden. Throughout the subsequent season, the resistance and tolerance levels of all 103 genets were evaluated. A description of the methods used to create the garden and compute resistance and tolerance are provided in Chapter 3 of this dissertation. Genets varied significantly in resistance and tolerance levels. Resistance ranged from 0.80 to 1.00 (1-proportion of plant damaged) and tolerance ranged from -0.40 to 0.80 (slope of the regression of fitness over damage levels; see section "Resistance and tolerance measures"). For this experiment, we selected 14 genets representing a wide range in resistance and tolerance levels (Figure 4.1). By propagating genets in the common garden for a year, we reduced any maternal effects on fitness and defense levels that might derive from their habitat of origin.

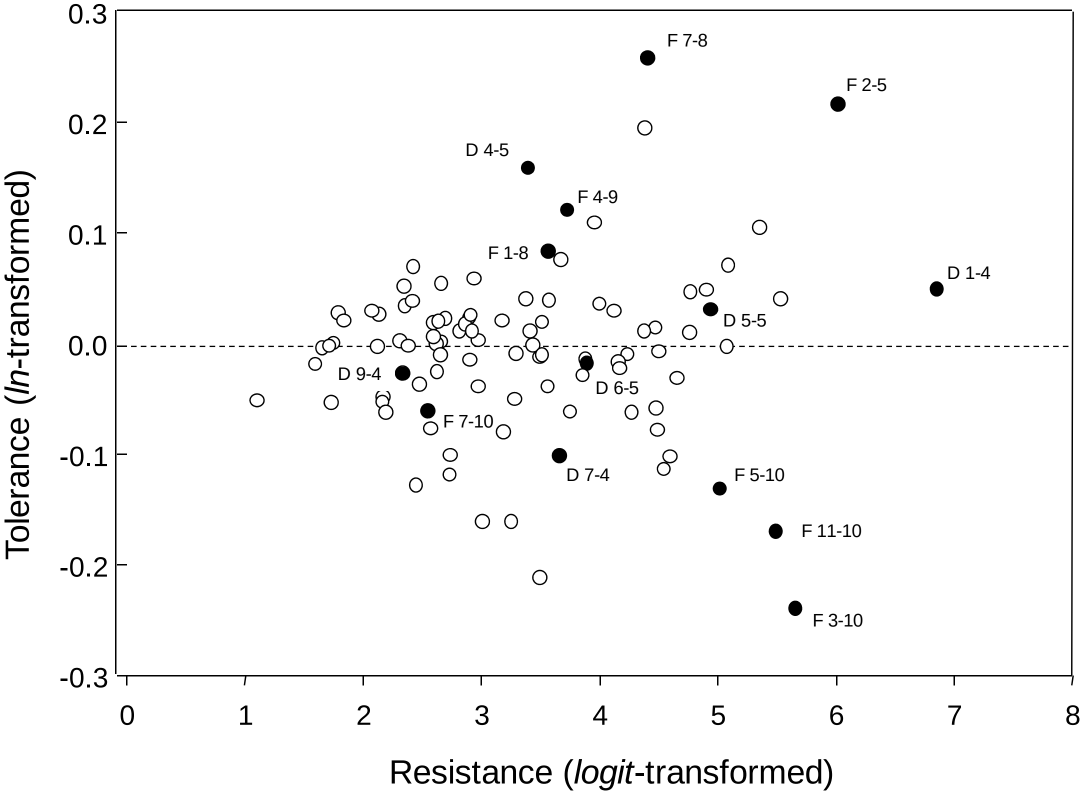


Figure 4.1. Mean resistance and tolerance of 103 genotypes propagated in a common garden for one year. The genotypes used in the experiment (solid circles; n = 14) were specifically selected to represent a broad range of resistance and tolerance levels.

In early February 2008, we excavated the root bundles of the 14 selected genets from the common garden, cut the rhizome material into 5-cm sections, and propagated the cuttings in flats of vermiculite in a greenhouse at Louisiana State University. After four weeks, 48 replicates of each genet were out-planted into three early- and three late-successional field sites in East Baton Rouge Parish, LA. Early fields had been mown within the past three years and were dominated by forbs and grasses (e.g., *S. altissima*, *Ambrosia* spp., *Liatris* spp., *Dichanthelium* spp.). Late fields were approximately 15 years post mowing and were dominated by trees and shrubs (e.g., *Triadica sebiferum*, *Cornus foemina*, *Acer negundo*, *Rubus* spp.). Fields ranged in size from 1-3 ha, and our experiment was conducted within a 240 m^2 area in each of the fields. Within each field, 8 replicates of each of the 14 genets were assigned at random to a position within an 8 x 14 grid (vertices 1.5 m apart). We did not attempt to alter the composition of the neighboring plants or environment surrounding each out-planted ramet and avoided trampling vegetation within 0.5 m of each ramet. An insecticide treatment was applied to encourage a gradient of damage among replicates for the purpose of estimating tolerance levels for each genotype (see section *Resistance and tolerance measures*). One-half of the replicates per genet were randomly assigned to the insecticide treatment and sprayed biweekly with Sevin® carbaryl insecticide (Bayer CropScience, Research Triangle Park, NC). The remaining ramets were assigned as controls and exposed to natural levels of herbivory.

Every eight weeks from Mar-Nov, 2008, we measured ramet height and the number of new leaves produced since the previous census. The proportion of leaves damaged by leaf-chewing herbivores was determined and leaf area removed per damaged leaf was assessed from digital photographs of three randomly chosen leaves using the program UTHSCSA *ImageTool* (University of Texas Health Science Center at San Antonio, TX). In addition to damage from insect herbivores (mainly grasshoppers in the families *Acrididae* and *Tettigoniidae*, and the genus *Romalea*), goldenrods experienced damage by white-tailed deer (*Odocoileus virginianus*) and eastern cottontail rabbits (*Sylvilagus floridanus*). Damage by mammals typically resulted in the apical meristem being completely grazed. We estimated damage from mammal as the proportional change in ramet height since the previous census. We recorded other types of plant damage (i.e., by galling and sucking herbivores) but they were scarce and thus are ignored in this study. During each census, all herbivores on ramets were identified to family and their numbers recorded. In late spring, the insect community within the grid was surveyed using a sweep net (four 10 m transects consisting of 20 back-and-fourth sweeps per field). The relative abundance (number per sweep net) of herbivores known to feed on goldenrod was estimated per field.

At the end of the growing season, newly-opened inflorescences, stems, and root bundles were collected and dried in an oven at 65^{o} C for 4 days. Only 24% of ramets planted in early fields and none planted in late fields flowered. Thus, we used total biomass (inflorescence, stem, and rhizome) as our estimate of short-term fitness. Goldenrod biomass is positively correlated with survival and reproduction (e.g., Goldberg 1988), and studies have shown that perennials with a large end-of-season biomass have higher survival and life-time fitness in terms of future sexual and clonal reproduction (e.g., Gardner and Mangel 1999).

Resistance and tolerance measures:

We estimated resistance and tolerance levels for each genet in each field. Resistance was measured as 1 – (the proportion of tissue lost to chewing herbivores) (e.g., Fineblum and Rausher 1995, Tiffin and Rausher 1999, Fornoni et al. 2004b). Genet resistance was computed for each field as the average resistance of control ramets (i.e., ramets that were not treated with the insecticide and were thus exposed to natural levels of herbivory). Tolerance per genet was measured as the slope of a linear regression of *ln*-biomass (our proxy for fitness) against the level of damage per ramet (Mauricio et al. 1997, Tiffin and Rausher 1999, Fornoni et al. 2004b).

Statistical analyses:

We first tested whether goldenrod resistance to chewing herbivores (insects, mammals, both combined) and fitness (*ln*-biomass) was significantly affected by successional stage (early or late), field (n=3), genotype (n=14), and herbivory treatment (control or pesticide) using separate nested ANOVAs. Successional stage, genotype, and herbivory treatment were considered fixed effects, and field within successional stage was the nested effect. Resistance (1-(proportion damaged)) was logit-transformed prior to analysis to normalize the distribution (i.e., x' = *ln*(x)-*ln*(1-x), where x = resistance). A simpler two-way ANOVA was used to assess treatment effects on tolerance. Because tolerance was measured at the level of genet rather than individuals, field became our unit of replication (n =3). Tradeoffs between resistance and tolerance in early-and late-successional stages were examined using ANCOVA. Tolerance was the dependent variable, field successional stage (early vs. late) was the fixed factor, and resistance was the covariate. A negative relationship between resistance and tolerance levels would indicate a defense tradeoff, and a significant interaction between stage and the covariate would indicate the relationship between resistance and tolerance varies with successional stage.

Costs of defense are best measured when herbivores are rare or absent (Simms and Rausher 1989, Mauricio et al. 1997). For each genet, we determined the *y*-intercept of the regression of fitness (*ln*-biomass) against the level of herbivore damage among ramets (Mauricio et al. 1997, Tiffin and Rausher 1999, Fornoni et al. 2004b). This quantity (W_A) provides an estimate of fitness in the absence of herbivores for a particular genet. To determine fitness costs of tolerance and resistance in response to different successional stages, separate ANCOVAs were performed. W_A was the dependent variable, field successional stage (early vs. late) was the fixed factor, and defense level (resistance or tolerance) was the covariate. Both linear and quadratic terms for the covariate were included. A significant negative relationship between W_A and defense level would indicate a cost for defense (Mauricio et al. 1997, Tiffin and Rausher 1999, Fornoni et al. 2004b). A significant interaction between stage and resistance/tolerance would indicate succession-dependent differences in costs of defense between successional stages (i.e., slopes differ between stages). Because using W_A as an estimate of genet fitness produces a bias in the estimation of the cost of tolerance owing to non-independence between the y-intercept (fitness in absence of herbivory) and slope (tolerance; see Mauricio et al. 1997, Tiffin and Rausher 1999, Fornoni et al. 2004b), we used a second method to test for a cost of tolerance by estimating the true covariance (corrected for the bias on sample covariance; see appendix B in Mauricio et al. 1997). Standard errors of the corrected covariance between tolerance and fitness were obtained using a jackknife procedure (Tiffin and Rausher 1999). An estimated covariance with a negative value and an interval that does not overlap zero would indicate a significant cost of tolerance (Mauricio et al. 1997, Tiffin and Rausher 1999, Fornoni et al. 2004b).

In contrast to the calculation of costs, the fitness benefits of defense traits are estimated in the presence of herbivores (i.e., the control plants). To determine selection gradients for tolerance and resistance in early and late fields, we used the same ANCOVA model as for costs with the exception of the dependent variable which was the mean fitness of control ramets (i.e., fitness in the presence of herbivores; W_P). Selection gradients favoring increased or decreased levels of defense would be indicated by a significant positive or negative linear relationship, respectively, between W_P and defense level (Mauricio et al. 1997, Tiffin and Rausher 1999). Stabilizing or disruptive selection would be evident if the quadratic term of the covariate was significantly positive or negative, respectively (Mauricio et al. 1997, Tiffin and Rausher 1999**).** A significant interaction between stage and defense level would indicate succession-dependent differences in selection gradients between stages. All statistical tests were conducted using SYSTAT 12 (SYSTAT 2007) or R (R Development Core Team 2008).

Results

Goldenrods ranged in damage by chewing insects and mammals from 0 to 100% of the total plant tissue removed. In early fields, 87 ± 3% of plants received insect damage and 10 ± 4% of plants received mammal damage compared to 92 ± 3% and 54± 18% of plants, respectively, in late fields. Damage by chewing insects and mammals were significantly lower in early- than in late-successional fields (63% and 89% lower, respectively, Figure 4.2), resulting in a combined total of 85% lower damage by herbivores in early than in late fields (mean ± SE early = 0.05 ± 0.01, late = 0.31 ± 0.03; $F_{1,4} = 16.01$, $P = 0.012$; Figure 4.2). Contrary to the distribution of goldenrod damage between successional stages, we found that the relative abundance of chewing insect known to feed on goldenrod was 57% higher in early than in late stages, but this difference was not significant (mean ± SE early = 5.67 ± 2.38, late = 2.42 ± 1.12, $F_{1,4} = 1.52$, $P = 0.285$; Figure 4.3).

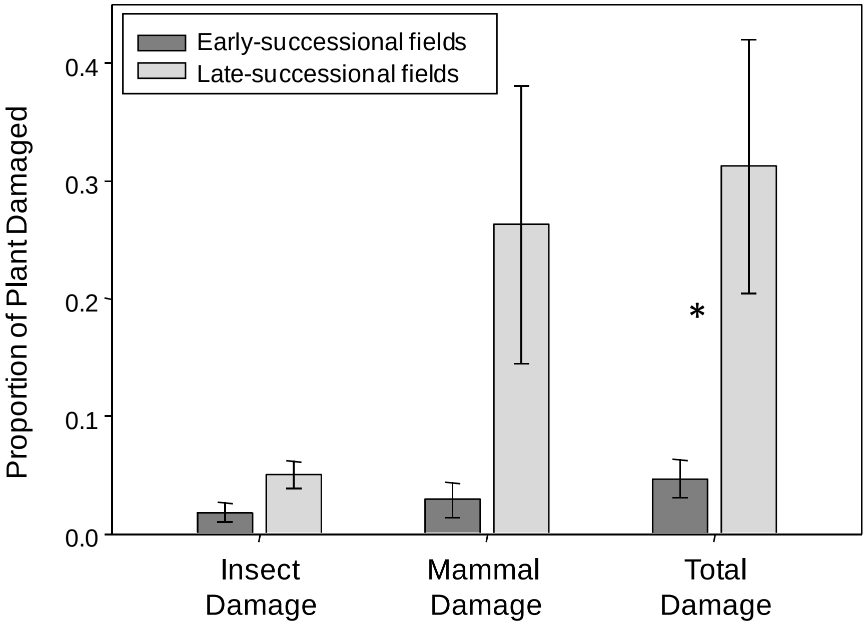


Figure 4.2. Mean proportion of plant damaged (± SE) by insect herbivores, mammalian herbivores, and combined insect and mammalian herbivores between early- and late-successional fields. * denotes $P < 0.05$ after sequential Bonferroni corrections to α.

Ramets treated with an insecticide experienced statistically less herbivore damage (insects and mammals combined) than control ramets, but the difference was small (mean ± se damage control = 20% ± 0.01, sprayed = 15% ± 0.01; $F_{1,445} = 27.32$, $P < 0.001$; Appendix B, Table A1). Although the insecticide treatment reduced insect damage by 25% ($F_{1,445} = 58.06$, $P < 0.001$), mammals were undeterred by the insecticide ($F_{1,445} = 0.001$, $P = 0.97$; Appendix A, Table A1). We note that even though the insecticide treatment did not eliminate herbivores, it served its intended purpose - to increase the range of damage to ramets within a genet to allow for meaningful estimates of tolerance, costs, and selection (see Methods).

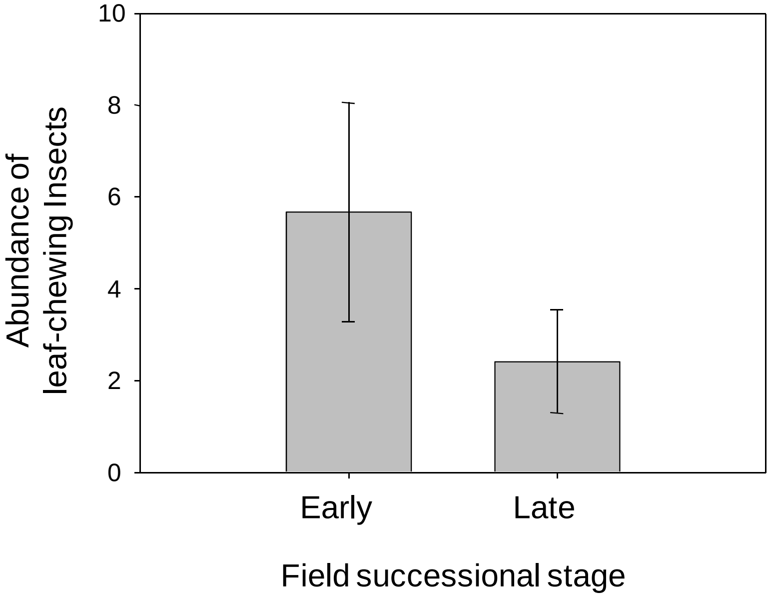


Figure 4.3. Mean abundance of leaf-chewing insects (± SE) per sweep sample between early- and late-successional fields (n=3). Only insects observed to feed on goldenrod are included in abundance data. * denotes $P < 0.05$.

Resistance, tolerance, and fitness:

Successional stage had a significant effect on resistance and tolerance levels. Specifically, plants in early-successional fields had 41% greater resistance to insect and mammalian herbivores combined than those in late fields ($F_{1,4} = 16.01$, $P = 0.012$; Appendix A,

Table A1; Figure 4.4a) and plants in late-successional fields were 120 times more tolerant of damage than those in early-successional fields ($F_{1,54} = 7.76$, $P < 0.01$, Appendix A, Table A2; Figure 4.4b). Interestingly, there was no effect of genotype or its interaction with stage on resistance or tolerance levels (Appendix A). We note that there was also a significant effect of field within successional-stage on resistance ($F_{4,52} = 15.35$, $P < 0.001$; Appendix A, Table A1).

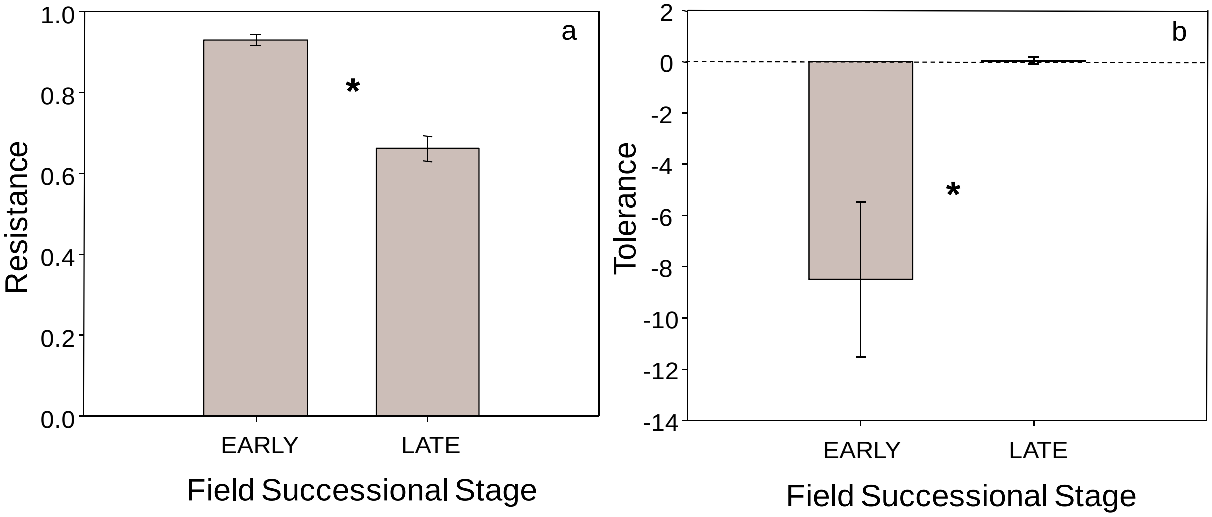


Figure 4.4. Resistance (a) and tolerance (b) of the goldenrods planted into of three early- and three late-successional fields. For each site, the average among genets was determined and the means ± SE of the site means was reported (n = 3). Resistance was measured as the 1-(proportion of plant damaged). Tolerance was measured as the slope of the regression of fitness (*ln*-biomass) over herbivore damage.* notes significance $P < 0.05$; see Appendix B.

Ramet biomass (our proxy for fitness) was not significantly affected by successional-stage ($F_{1,4} = 1.93$, $P = 0.237$; Appendix A, Table A1). Although successional-stage accounted for 20% of the variation in biomass, and biomass averaged six times greater in early- than in late-successional stages (mean ± se early = 5.31 ± 3.6 g, late = 0.87 ± 0.23 g), twice the amount of variation in biomass was explained by differences among fields within successional-stage (the nested factor; $F_{4,52} = 104.93$, $P < 0.001$; Appendix A, Table A1). Biomass did not differ among genets, nor was there a significant stage by genotype interaction (Appendix A, Table A1).

We found a significant relationship between goldenrod resistance and tolerance levels, but this relationship differed between successional stages. In the ANCOVA model, the covariate (resistance) had a significant effect on tolerance, as did the interaction between resistance and stage ($F_{1,77} = 8.15$, $P = 0.006$ and $F_{1,77} = 18.47$, $P < 0.001$, respectively; Appendix B, Table B1). Interestingly, resistance and tolerance exhibited a tradeoff in early fields ($r = -0.51$, $P < 0.001$, $n = 40$) and were positively correlated in late fields ($r = 0.34$, $P = 0.03$, $n = 42$; Figure 4.5).

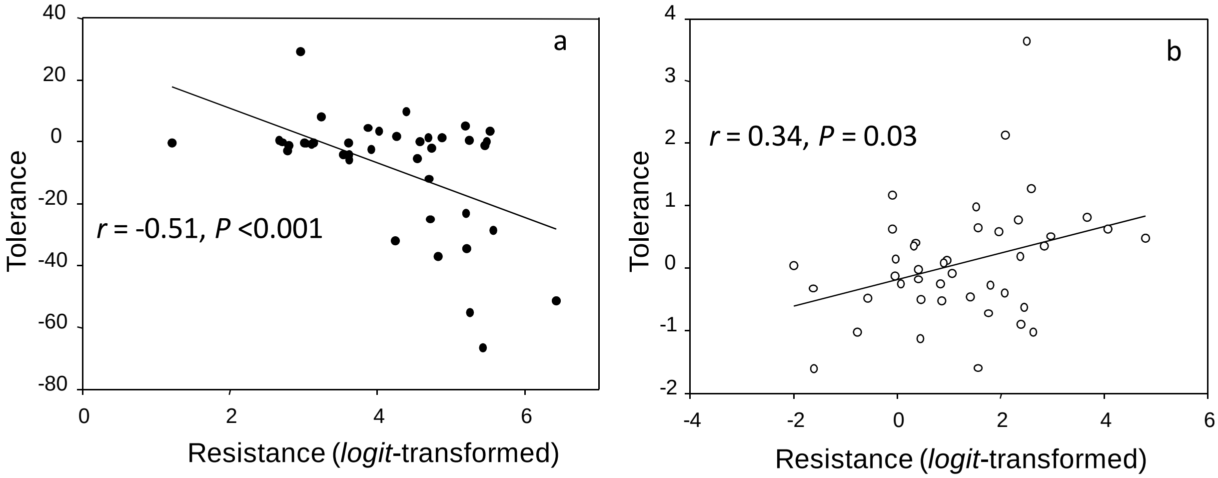


Figure 4.5. Relationships between tolerance and resistance in early-successional (a) and late-successional (b) fields. A negative relationship indicates a tradeoff between defenses. Each data point represents the genet average within separate fields. Line is fit by least-squares regression and the Pearson's product moment correlation (r) and p-value are reported.

Costs and selection gradients associated with defense:

In examining the factors that affect goldenrod fitness in the absence of herbivory (W_A), we found that W_A was nonlinearly related to resistance level (as indicated by a significant quadratic covariate term; $F_{1,80} = 8.94$, $P = 0.004$; Appendix B, Table B2) and was significantly affected by the stage-resistance interaction ($F_{1,80} = 4.35$, $P = 0.04$; Appendix B, Table B2). Opposing relationships between resistance and W_A in early versus late fields is likely the explanation for these significant terms in the ANCOVA model. In early fields, there was no evidence of a fitness cost of resistance. On the contrary, a strong positive relationship between resistance and W_A indicates a fitness benefit to increased resistance in early-field goldenrods in the absence of herbivory (r = 0.35, p = 0.02, Figure 4.6a). Late fields exhibited a strong negative relationship, signifying a cost of resistance (r = -0.50, $P < 0.001$; Figure 4.6a).

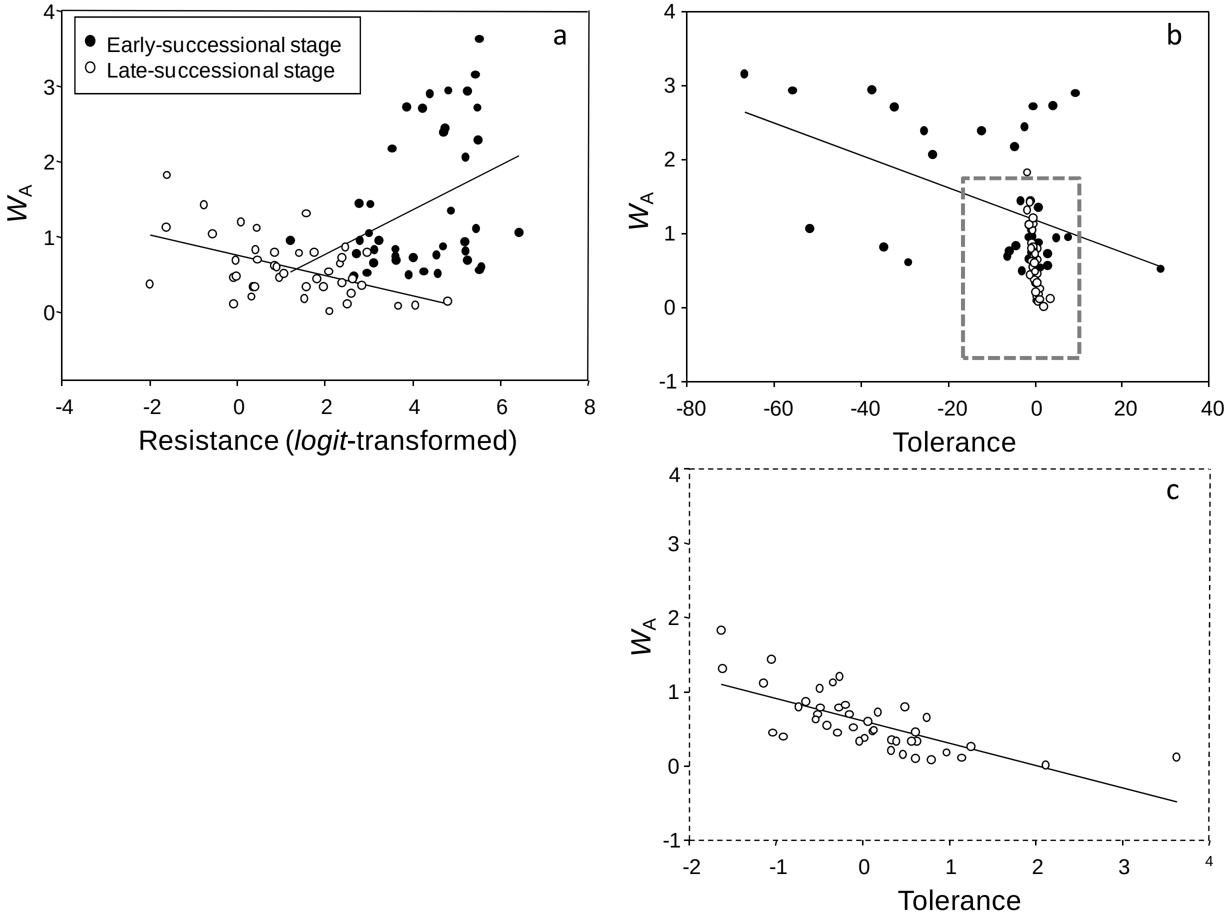


Figure 4.6. The relationship between goldenrod resistance (a) tolerance (b) and estimated biomass in the absence of herbivory (W_A) in early- and late-successional fields. A significant negative relationship is indicative of a cost associated with that defense. The relationship between tolerance and (W_A) in late fields is redrawn at a smaller scale to reveal the relationship more clearly (c). Each data point represents the genet average within separate fields; filled circles for genets in early fields and open circles for genets in late fields.

In contrast to resistance, tolerance was costly irrespective of successional stage (i.e., a significant negative relationship between W_A and tolerance; $F_{1,78} = 21.36$, $P < 0.001$; Figure 4.6). However, costs differed in strength between stages (i.e., significant interaction term, $F_{1,78} = 8.77$, $P = 0.004$; Appendix B, Table B2; early $r = -0.47$, $p < 0.002$; late $r = -0.75$, $p < 0.001$; Figure 4.6 b,c). Based on slope comparisons, tolerance was 14 times more costly in late than early fields (early: -0.02 ± 0.01, late: -0.30 ± 0.05). There was no evidence for a nonlinear cost of tolerance (i.e., non-significant quadratic term, Appendix B, Table B2). Application of a correction for the bias in the relationship between tolerance and W_A yielded a true covariance estimate of -6.06 ± 0.13 in early fields and -1.70 ± 0.01 in late fields, confirming a cost of tolerance in both stages.

Goldenrod fitness in the presence of herbivores (W_P) was independent of resistance level (Appendix B, Table B3). However, the relationship between resistance and W_P varied with successional stage (significant interaction term; $F_{1,80} = 5.82$, $P = 0.018$; Appendix B, Table B3; Figure 4.7a). In early fields, there was strong directional selection favoring increased resistance ($r = 0.38$, $P = 0.012$, $n = 42$); whereas in late fields, we did not find evidence for selection ($r = -0.08$, $P = 0.613$, $n = 42$). The significant quadratic covariate term (Table B3) is likely explained by these opposing patterns with regard to resistance and W_P.

In comparison, I found a significant negative, linear relationship between tolerance and W_P ($F_{1,78} = 10.7$, $P = 0.002$; Appendix B, Table B3). This negative relationship was similar between successional fields (as indicated by the non-significant interaction term; Table B3), but when stages were treated separately, we detected strong selection against tolerance in early fields ($r = -0.40$, $P = 0.01$, $n = 40$; Figure 4.7b) and no evidence for selection for tolerance in late fields ($r = 0.05$, $p = 0.75$, $n = 40$; Figure 4.7c). Finally, there was no evidence of stabilizing or disruptive selection acting on tolerance (i.e., non-significant quadratic term; $F_{1,78}=1.25$, $P=0.27$).

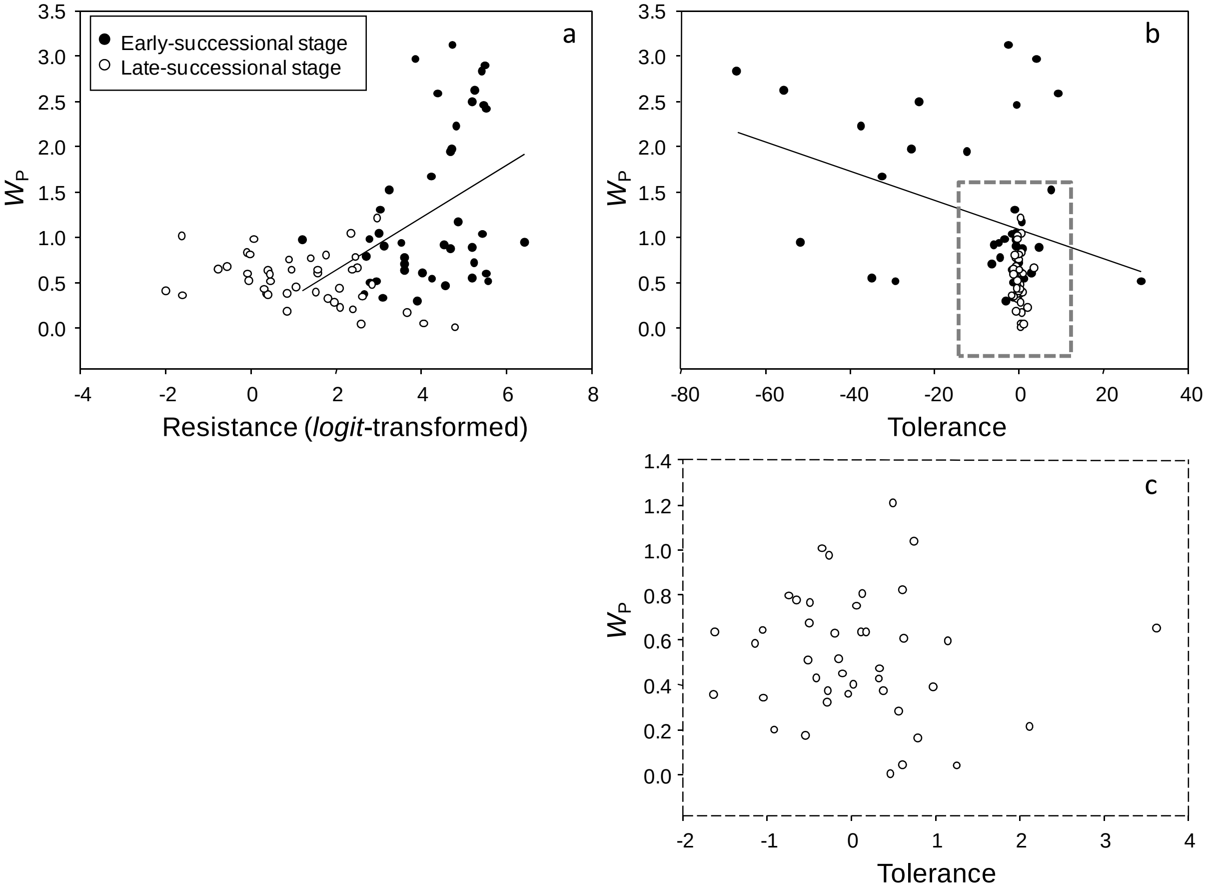


Figure 4.7. The relationship between goldenrod resistance (a) tolerance (b) and biomass in the presence of herbivory (W_{P}) in early- and late-successional fields. A significant negative/positive relationship indicates selection favoring low/high levels of defense, respectively. In late fields, the relationship is more clearly revealed at a smaller scale (c). Each data point represents the genet average within each field; filled circles for in early fields and open circles for late fields.

Discussion

Our main finding was an apparent non-genetic, succession-induced shift in goldenrod defense expression from high resistance and low tolerance in early stages to low resistance and high tolerance in late stages. Moreover, the adaptive value of these traits (in terms of their costs relative to benefits) also appears to change significantly during succession. These results suggest that temporal environmental variability, such as the predictable changes associated with successional communities, can dramatically alter the adaptive landscape for different defense strategies and favor strong plasticity in defense expression. Because all communities undergo succession, and succession is concomitant with anthropogenic activity, the general linkages we describe between goldenrod defenses and succession may be common to many long-lived plants.

The change in goldenrod defense expression from being highly resistant in early-successional fields to highly tolerant in late-successional fields suggests a successional tradeoff in defense strategies that has not previously been reported. Studies have found ontogenetic changes in plant defense expression (reviewed in Barton and Koricheva 2010), including a recent study with *Raphanus sativus* in which high levels of resistance during the juvenile stage were followed by high levels of tolerance in the mature stage (Böege et al. 2007). Although our goldenrods exhibited a similar temporal transition from high resistance to high tolerance, our experimental design controlled for ontogenetic effects by using similar-sized clonal fragments (i.e., a 5 cm rhizome cutting). Thus, the strong differences in goldenrod resistance and tolerance levels between early- and late-successional fields were driven by stage-specific environmental factors such as changes in the neighboring plant community and/or pressure from herbivores (see below). Moreover, these results suggest that there is tremendous plasticity in goldenrod defense. Phenotypic plasticity allows traits of plants located in spatially and temporally variable environments to respond to changing fitness costs and selection gradients (Lloyd 1984). Plasticity in defense expression is common (reviewed in Callaway et al. 2003) and has been reported previously for goldenrods (e.g., Horner and Abrahamson 1992, Cronin and Abrahamson 1999, 2001b), however, it has never been reported in the context of successional changes in the community. We expect plasticity to be beneficial to long-lived plants that are subjected to temporal community changes that alter the adaptive landscape for defense traits.

In addition to there being a general temporal tradeoff between resistance and tolerance, we found that the relationship between tolerance and resistance differed between successional stages. In the early stage, there is evidence of a classic defense tradeoff (see also Fineblum and Rausher 1995, Tiffin and Rausher 1999). However, in the late stage, there was a significant positive correlation between tolerance and resistance (see Fig. 3b). We are unaware of any study that has shown such a qualitative change in the relationship between defensive traits in response to a change in the environment. One possibility for the change in the resistance-tolerance relationship between successional stages is that in early stages where herbivore pressure is greatest, plants allocate resources to one or the other defense as early theoretical models have suggested (i.e., Fineblum and Rausher 1995, Mauricio et al. 1997, Tiffin and Rausher 1999). In late stages, goldenrods are under greater stress from competition for light, as evidenced by their reduction in height, biomass, and flower production relative to early fields (see also chapter 2). Because physiological traits conferring tolerance to herbivory may also allow plants to tolerate competition among neighboring plants (Siemens et al. 2003, Jones et al. 2006), and low light availability (e.g., Janse-ten Klooster et al. 2007), tolerance may be expected to increase in late succession, particularly in areas with heavy canopy cover. In our previous survey of goldenrod defense traits within early- and late-successional fields, we found strong positive correlations between a goldenrod tolerance trait, specific leaf area (SLA), and increased canopy cover within late-successional fields (see chapter 2). In sum, we suggest that herbivore pressure in early stages and the added stress from competition in late successional stages may explain the patterns observed in our study.

Despite our attempt to select genets that differed markedly in tolerance and resistance (Figure 4.1), we were surprised to find no evidence for genetic differences in resistance and tolerance. Numerous studies with *S. altissima* have revealed considerable genotypic variability in goldenrod resistance to insect-herbivore attack (e.g., Maddox and Root 1987, McCrea and Abrahamson 1987, Cronin and Abrahamson 2001b). Moreover, our common-garden study revealed significant genetic variability in resistance and tolerance among 103 goldenrod genets (see chapter 3), of which the 14 most different were used in this study (Figure 4.1). Thus, we suggest that at least some fraction of our genets represented different clones.

The adaptive landscape for goldenrod resistance and tolerance varied significantly with successional stage. In early fields, we found no cost of high resistance (in terms of fitness in the absence of herbivory), but strong selection against low resistance (in the face of herbivore damage). On the balance, there was a net benefit to being resistant (i.e., the fitness benefits outweigh the costs). In contrast, resistance was costly in late stages, and there was no evidence of selection for or against high resistance. Here, the costs should tip the balance against the occurrence of highly resistant ramets. For the highly plastic genets that were used in our experiment, these expectations were met – resistance levels were more than 40% higher in early- than in late-successional fields. Similar results were found in a survey of goldenrod defense traits within a different set of early- and late-successional fields (resistance, leaf toughness and total phenolics were 31%, 65% and 42% higher, respectively, in early versus late fields; see chapter 2). Higher resistance in early fields may be more adaptive given that herbivores tend to be more abundant in the early successional stages.

In addition to strong costs of tolerance within both successional stages, there was a strong selection gradient against tolerance in the early-successional stage. Our data suggest that in early fields, goldenrods should allocate few resources to tolerance traits. Indeed, we found that goldenrod genets had relatively low levels of tolerance in these fields. Interestingly, in late-successional fields, goldenrods had high tolerance levels despite the high costs. Although this might seem paradoxical, tolerance traits can serve a dual purpose in mitigating the effects of herbivory and competition (Siemens et al. 2003, Jones et al. 2006, Janse-ten Klooster et al. 2007). If as we suggested earlier, goldenrods in late-successional fields are under considerable stress from competition for light, and tolerance traits may be beneficial. To evaluate this hypothesis, we would need to determine whether tolerance to herbivory and tolerance to competitive stress are correlated and if tolerance to competitive stress is costly and beneficial in the absence and presence of competitors, respectively. We do not have this data at present.

Although numerous models have been proposed to explain how variation in resource availability affects tolerance levels, the limiting-resource model (LRM) by Wise and Abrahamson (2007, 2008a) has proven the most reliable. In this model, tolerance of herbivory depends on the type of resource and damage, and 95% of the studies examined by Wise and Abrahamson (2008) supported the predictions of this model. However, our study system is an exception. The LRM predicts that tolerance to leaf-chewing herbivores should be lower in light-limited environments (i.e., late-successional fields) because leaf removal hinders the plant's ability to acquire limiting light resources. Yet, we found that goldenrod genets expressed 120 times greater tolerance levels in closed canopy fields than in more open canopy fields. We suggest that the reason the LRM predictions did not match our data is because the model does not take into account the possibility that tolerance traits may serve dual purposes (mitigating herbivory damage and environmental stress).

It is possible that differences in herbivore pressure between early and late fields may have driven the differences in resistance and tolerance levels between successional stages. Because resistance is typically measured as 1- (proportion tissue damaged) (Fineblum and Rausher 1995, Tiffin and Rausher 1999, Fornoni et al. 2004b), low resistance may be a consequence of there being few herbivores available. Thus, the conclusion that early-successional fields have high resistance relative to late fields could be explained by low herbivore abundance in the former fields. However, our sweep-sample data suggest this is not the case for insect herbivores. The abundance of chewing insects was 57% greater in early versus late fields, although the difference was not statistically significant. This finding is consistent with other studies of old-field succession involving goldenrods that concluded the abundance of insect herbivores remained relatively constant among years (e.g., Root and Cappuccino 1992, Siemann et al. 1999, Fornoni et al. 2004b). Therefore, early-field goldenrods suffered less insect herbivore damage (and consequently, higher resistance) in spite of equivalent, if not higher, insect herbivore abundances (see Figure 4.3). In contrast, we cannot rule out the possibility that differences in resistance to mammalian herbivores in early and late fields (resistance was 89% higher in early fields) is an artifact of changing abundances during succession. We did not attempt to directly quantify rabbit and deer abundance between successional stages, but we did observe more fecal pellets from these herbivores in the late-successional fields (A.S. Hakes, personal observation).

Our study with goldenrods, a cosmopolitan group of successional species, strongly argues that defense strategies may be highly plastic and that successional changes in the environment are an important, and perhaps main, driver of temporal variability in plant defense. Few studies have demonstrated that spatially or temporally fluctuating environmental variables can alter the adaptive value of plant traits (Lankau and Strauss 2008, Punzalan et al. 2010, but see Tiffin 2002). A temporally dynamic landscape may have important implications for trait evolution by selecting for high trait plasticity (Lankau and Strauss 2008). Our results highlight the importance of incorporating spatio-temporal environmental complexity into future studies of plant defense. In light of increasing anthropogenic activity and land-use changes, an understanding of how plant traits respond to environmental change and the consequences of those changes for plant-plant and plant-insect interactions is becoming increasingly important.

CHAPTER 5. SUMMARY

In this dissertation, I examined how spatial and temporal environmental heterogeneity may promote diverse types and levels of plant defense strategies (i.e., resistance and tolerance) in natural landscapes. These experiments were conducted within successional old-field habitats using late goldenrod (*Solidago altissima*) and its various chewing herbivores as my study system. This research was divided into three separate studies. In the field survey described in chapter 2, I quantified the spatial patterns of putative defense traits and environmental variables. Defense traits were found to exhibit non-random distributions in space and were more strongly autocorrelated (i.e., more similar among nearest neighbors) in late- than in early-successional fields. I also found that neighbor variables were more important than soil moisture in influencing the spatial distribution of plant defenses, and that relationships between neighbor variables and defense traits differed with respect to field successional stage. For example, the tolerance trait specific leaf area was more strongly correlated with canopy cover and neighbor stem density in late-successional fields, and damage was more strongly correlated with ground vegetation cover in early-successional fields. I concluded that neighboring plants may influence the spatial distributions of herbivore damage and goldenrod defense trait levels within successional fields. A novel insight from this study was the suggestion that changes in predominant neighboring plant interactions over the course of succession (i.e., ground vegetation cover in early succession and canopy cover in late succession) may shift the adaptive value of defense from a resistance strategy to a tolerance strategy in our system, with increased levels of traits conferring tolerance later in succession. I investigated this hypothesis in a field experiment described in chapter 4.

In chapter 3, I conducted a common garden experiment to examine genetic variability, fitness costs, and selection gradients for goldenrod resistance, tolerance, and various fitness- and defense-related traits. Although there were significant differences among goldenrod genets for resistance, tolerance, and several other traits (i.e., leaf toughness, leaf senescence rate, inflorescence biomass), broad-sense heritability estimates for all traits were very low. Non-significant correlations between trait levels measured from source genets in the field and the same genets in the common garden suggests that there may be strong plasticity in goldenrod defense traits. Herbivores imposed strong selection for increased tolerance and tolerance traits RGR, LAR, and LSR in the common garden, but evolution in tolerance may be constrained by low broad-sense heritability estimates of tolerance traits and a significant fitness cost of tolerance when herbivores are absent. I concluded that genetic variability in tolerance may be maintained within the common garden if herbivore pressures vary spatially or temporally but that, owing to non-existent costs and selection pressures, genetic variability in resistance may persist within the garden environment.

In chapter 4, I described a field experiment testing the novel hypothesis suggested by my field survey (chapter 2) that the adaptive value of resistance and tolerance changes over the course of old-field succession. For this experiment, I measured resistance, tolerance, and fitness of genets selected from the common garden (chapter 3) and out-planted genets into replicate early- and late-successional fields. I found a significant effect of successional-stage on defense levels but no effect of genet or its interaction with stage. Resistance levels were 41% greater in genets planted in early- than in late-successional fields, whereas tolerance levels were 120 times greater in genets planted in late fields relative to early fields. In early-successional fields, I found no costs of, and strong selection for, increased resistance as well as a significant tradeoff between resistance and tolerance. In late fields, there were costs of resistance and tolerance, strong selection against resistance, and no evidence of a selection gradient for tolerance. I concluded that successional changes in the environment can alter the selection landscape for plant defense strategies and promote plasticity and temporal variability in resistance and tolerance. My results suggested that resistance may be more adaptive in early-successional stages and, despite a cost of tolerance in late stages, tolerance may be beneficial in mitigating the effects of both herbivory and environmental stresses (i.e., low light availability) that limit fitness in these fields. The consequence of this is that populations of goldenrod may plastically switch defense strategies from resistance to tolerance as old-field succession progresses. These patterns may be common in other types of long-lived plants in successional habitats.

The novel results from these studies offer several important contributions to the field of plant defense. First, my finding that defense traits were non-randomly distributed within fields and co-varied with characteristics of the neighboring plant community, suggests that environmental heterogeneity may be an important mechanism influencing resistance and tolerance expression. This, together with my finding of different adaptive landscapes within early- and late-successional fields adds empirical support to the emerging hypothesis that mixed patterns of plant resistance and tolerance may be maintained in natural populations when spatial and temporal environmental heterogeneity alters the expression or adaptive value of tolerance and resistance traits (e.g., Agrawal et al. 2006, Núñez-Farfan et al. 2007). Contrary to conventional defense theory which has predicted through simple models that plant populations should eventually become fixed with the most adaptive defense trait or strategy (Herms and Mattson 1992, Mauricio et al. 1997, Weinig et al. 2003), defense strategies are likely to be dependent on a plant's environmental context and may change in quantitative and qualitative ways as the environment changes.

My finding of a succession-induced shift in the expression of defense strategies has never before been reported. Until now, plant defense theory has considered mainly ontogenetic mechanisms to explain temporal variability in plant defense. Because succession affects all environments, succession may represent an important and common mechanism promoting phenotypic plasticity and temporal variability in plant defense expression for long-lived plants. In light of increasing anthropogenic effects on land-use change and global climate change, it is becoming increasingly important for future studies to investigate how plant traits respond to these often rapid changes in environmental conditions. Environmental changes may indirectly affect the evolution of plant traits by altering the intensity of species interactions or resource availability, which can influence the selective landscape for resistance and tolerance. A recent study by Lau and Tiffin (2009) documented an increased costs of tolerance under elevated CO^2, but no effects on costs of plant resistance, nor on the amount of damage incurred (see also Lau et al. 2008). Another study suggests that the production of phenolic compounds (a defense trait) may be enhanced by elevated CO^2 and UV light for many species (Agrell et al. 2000, Bidart-Bouzat and Imeh-Nathaniel 2008). It is not clear from these studies whether the elevated CO^2 influenced plant traits directly or indirectly by altering plant-plant or plant-herbivore interactions which in turn influenced defense traits.

The results from my dissertation have important implications both for continued work in the goldenrod system, as well as broader implications for plant defense theory. Future research examining plant defense in successional systems should empirically test whether specific neighbor effects are influencing resistance and tolerance expression. I highlight two potentially important variables, ground vegetation cover and canopy cover, that may be driving the patterns of greater resistance in early stages and greater tolerance in late stages, respectively, and discuss how taking a closer look at the mechanisms behind these relationships may help advance our general knowledge of defense evolution.

Although spatial associations and competition among neighboring plants are recognized as important factors influencing plant defense or damage levels (Hay 1986, Bazzaz et al. 1987, Bergelson and Purrington 1996, Cipollini and Bergelson 2001, 2002, Baraza et al. 2006, Bergvall et al. 2006, Miller et al. 2007, Barbosa et al. 2009), the circumstances under which plant competition may lead to associational effects on plant resistance (or vice versa) has not been addressed in the literature (Barbosa et al. 2009). In our early-successional fields, herbivore damage was strongly correlated with neighboring vegetative cover, but the direction of this relationship differed among early fields. The fact that a specific environmental variable can have opposite effects on herbivore damage in the same study suggests that the relationship between damage and vegetation cover may be complex. For example, neighboring plants might be influencing herbivore damage levels on a focal plant through competitive effects at particular density or at a specific spatial scale, and through associational effects at another density or spatial scale (Barbosa et al. 2009). The specific identity of neighbors may also influence the competitive/associational effect (e.g., Barton and Bowers 2006).

Whether variation for tolerance is subject to selection imposed by factors other than herbivory remains untested (Núñez-Farfan et al. 2007). Because plant traits associated with competitive ability also tend to enhance tolerance to herbivory (e.g., Chapin 1991, Strauss and Agrawal 1999) any selective agent acting on growth could potentially affect tolerance (Núñez-Farfan et al. 2007). The presence of multiple selective pressures on the same trait has the potential to constrain trait evolution when selection coefficients differ in their direction or magnitude (reviewed in Núñez-Farfan et al. 2007), or alternatively, may increase the adaptive value of the trait by decreasing its associated fitness costs:benefit ratio when both selective agents are present (Siemens et al. 2003). Studies that tests the dual function of tolerance and/or resistance by manipulating putative selection agents other than herbivory that may influence the adaptive value of defense traits are needed (e.g., Uriarte et al. 2002, Siemens et al. 2003, Jones et al. 2006). The goldenrod system may be ideal for testing this hypothesis. In late-successional fields, there were high tolerance levels despite a strong cost of tolerance and no evidence that herbivores imposed positive selection pressures for tolerance. Furthermore, tolerance trait SLA was found to exhibit strong, positive correlations with canopy cover, suggesting that SLA may function in tolerating low light conditions (e.g., Gunn et al. 1999, Awada et al. 2003, Janse-ten Klooster et al. 2007) in addition to tolerating defoliation (Bassman and Dickmann 1982, Hoogesteger and Karlsson 1992, Meyer 1998a). In this case, we might expect to find low costs and high fitness benefits of tolerance (i.e., high adaptive value) in low light environments, and manipulations of canopy cover and herbivory levels may suggest that canopy cover is the primary selective agent for goldenrod tolerance in late-successsional fields.

Overall, this dissertation highlights the importance of incorporating spatio-temporal environmental heterogeneity into future studies of plant defense. Only when we move from examining plant traits in simple, controlled environments to examining traits in the context of their complex, natural landscapes, will we be able to accurately estimate their adaptive value and evolutionary constraints or potential.

LITERATURE CITED

Abrahamson, W. G., K. D. Mccrea, A. J. Whitwell, and L. A. Vernieri. 1991. The role of phenolics in goldenrod ball gall resistance and formation. Biochemical Systematics and Ecology **19**:615-622.

Abrahamson, W. G. and A. E. Weis. 1997. Evolutionary Ecology Across Three Trophic Levels: Goldenrods, Gallmakers, and Natural Enemies. Princeton University Press, Princeton NJ.

Agrawal, A. A. 2000. Overcompensation of plants in response to herbivory and the by-product benefits of mutualism. Trends in Plant Science **5**:309-313.

Agrawal, A. A. 2005. Natural selection on common milkweed (*Asclepias syriaca*) by a community of specialized insect herbivores. Evolutionary Ecology Research **7**:651-667.

Agrawal, A. A. and K. Konno. 2009. Latex: a model for understanding mechanisms, ecology, and evolution of plant defense against herbivory. Annual Review of Ecology Evolution and Systematics **40**:311-331.

Agrawal, A. A., J. A. Lau, and P. A. Hamback. 2006. Community heterogeneity and the evolution of interactions between plants and insect herbivores. Quarterly Review of Biology **81**:349-376.

Agrell, J., E. P. McDonald, and R. L. Lindroth. 2000. Effects of CO2 and light on tree phytochemistry and insect performance. Oikos **88**:259-272.

Andrew, R. L., R. Peakall, I. R. Wallis, and W. J. Foley. 2007. Spatial distribution of defense chemicals and markers and the maintenance of chemical variation. Ecology **88**:716-728.

Awada, T., M. E. L. Perry, and W. H. Schacht. 2003. Photosynthetic and growth responses of the C-3 *Bromus inermis* and the C-4 *Andropogon gerardii* to tree canopy cover. Canadian Journal of Plant Science **83**:533-540.

Baraza, E., R. Zamora, and J. A. Hodar. 2006. Conditional outcomes in plant-herbivore interactions: neighbours matter. Oikos **113**:148-156.

Barbosa, P., J. Hines, I. Kaplan, H. Martinson, A. Szczepaniec, and Z. Szendrei. 2009. Associational resistance and associational susceptibility: Having right or wrong neighbors. Annual Review of Ecology Evolution and Systematics **40**:1-20.

Barton, K. E. 2008. Phenotypic plasticity in seedling defense strategies: compensatory growth and chemical induction. Oikos **117**:917-925.

Barton, K. E. and M. D. Bowers. 2006. Neighbor species differentially alter resistance phenotypes in Plantago. Oecologia **150**:442-452.

Barton, K. E. and J. Koricheva. 2010. The ontogeny of plant defense and herbivory: Characterizing general patterns using meta-analysis. American Naturalist **175**:481-493.

Bassman, J. H. and D. I. Dickmann. 1982. Effects of defoliation in the developing leaf zone on young populus x euramericana plants .1. Photosynthetic physiology, growth, and dry-weight partitioning. Forest Science **28**:599-612.

Bazzaz, F. A., N. R. Chiariello, P. D. Coley, and L. F. Pitelka. 1987. Allocating resources to reproduction and defense. Bioscience **37**:58-67.

Belsky, A. J., W. P. Carson, C. L. Jensen, and G. A. Fox. 1993. Overcompensation by plants - Herbivore optimization or red herring. Evolutionary Ecology **7**:109-121.

Bergelson, J. 1994. The effects of genotype and the environment on costs of resistance in lettuce. American Naturalist **143**:349-359.

Bergelson, J. and C. B. Purrington. 1996. Surveying patterns in the cost of resistance in plants. American Naturalist **148**:536-558.

Bergvall, U. A., P. Rautio, K. Kesti, J. Tuomi, and O. Leimar. 2006. Associational effects of plant defences in relation to within- and between-patch food choice by a mammalian herbivore: neighbour contrast susceptibility and defence. Oecologia **147**:253-260.

Bidart-Bouzat, M. G. and A. Imeh-Nathaniel. 2008. Global Change Effects on Plant Chemical Defenses against Insect Herbivores. Journal of Integrative Plant Biology **50**:1339-1354.

Böege, K., R. Dirzo, D. Siemens, and P. Brown. 2007. Ontogenetic switches from plant resistance to tolerance: minimizing costs with age? Ecology Letters **10**:177-187.

Borenstein, M., L. Hedges, J. Higgins, and H. Rothstein. 2005. Comprehensive Meta Analysis. Biostat, Englewood, NJ.

Brenes-Arguedas, T. and P. D. Coley. 2005. Phenotypic variation and spatial structure of secondary chemistry in a natural population of a tropical tree species. Oikos **108**:410-420.

Cain, M. L., W. P. Carson, and R. B. Root. 1991. Long-term suppression of insect herbivores increases the production and growth of *Solidago altissima* rhizomes. Oecologia **88**:251-257.

Callaway, R. M., S. C. Pennings, and C. L. Richards. 2003. Phenotypic plasticity and interactions among plants. Ecology **84**:1115-1128.

Carr, D. E., J. F. Murphy, and M. D. Eubanks. 2006. Genetic variation and covariation for resistance and tolerance to Cucumber mosaic virus in Mimulus guttatus (Phrymaceae): a test for costs and constraints. Heredity **96**:29-38.

Carson, W. P. and R. B. Root. 1999. Top-down effects of insect herbivores during early succession: influence on biomass and plant dominance. Oecologia **121**:260-272.

Chapin, F. S. 1991. Integrated responses of plants to stress. Bioscience **41**:29-36.

Chesson, P. L. and R. R. Warner. 1981. Environmental variability promotes coexistence in lottery competitive-systems. American Naturalist **117**:923-943.

Choong, M. F. 1996. What makes a leaf tough and how this affects the pattern of *Castanopsis fissa* leaf consumption by caterpillars. Functional Ecology **10**:668-674.

Cipollini, D. 2005. Interactive effects of lateral shading and jasmonic acid on morphology, phenology, seed production, and defense traits in *Arabidopsis thaliana*. International Journal of Plant Sciences **166**:955-959.

Cipollini, D. F. and J. Bergelson. 2001. Plant density and nutrient availability constrain constitutive and wound-induced expression of trypsin inhibitors in *Brassica napus*. Journal of Chemical Ecology **27**:593-610.

Cipollini, D. F. and J. Bergelson. 2002. Interspecific competition affects growth and herbivore damage of *Brassica napus* in the field. Plant Ecology **162**:227-231.

Coley, P. D., J. P. Bryant, and F. S. Chapin. 1985. Resource availability and plant antiherbivore defense. Science **230**:895-899.

Covelo, F. and A. Gallardo. 2004. Green and senescent leaf phenolics showed spatial autocorrelation in a *Quercus robur* population in northwestern Spain. Plant and Soil **259**:267-276.

Cronin, J. T. and A. G. Abrahamson. 2001a. Do parasitoids diversify in response to host-plant shifts by herbivorous insects? Ecological Entomology **26**:347-355.

Cronin, J. T. and W. G. Abrahamson. 1999. Host-plant genotype and other herbivores influence goldenrod stem galler preference and performance. Oecologia **121**:392-404.

Cronin, J. T. and W. G. Abrahamson. 2001b. Goldenrod stem galler preference and performance: effects of multiple herbivores and plant genotypes. Oecologia **127**:87-96.

Cronin, J. T., W. G. Abrahamson, and T. P. Craig. 2001. Temporal variation in herbivore host-plant preference and performance: constraints on host-plant adaptation. Oikos **93**:312-320.

Dudt, J. F. and D. J. Shure. 1994. The influence of light and nutrients on foliar phenolics and insect herbivory. Ecology **75**:86-98.

Ehrlich, P. R. and P. H. Raven. 1964. Butterflies and plants - a study in coevolution. Evolution **18**:586-608.

Falconer, D. S. 1989. Introduction to Quantitative Genetics. 3rd edition, New York, NY.

Fineblum, W. L. and M. D. Rausher. 1995. Tradeoff between resistance and tolerance to herbivore damage in a morning glory. Nature **377**:517-520.

Fornoni, J., J. Nunez-Farfan, P. L. Valverde, and M. D. Rausher. 2004a. Evolution of mixed strategies of plant defense allocation against natural enemies. Evolution **58**:1685-1695.

Fornoni, J., P. L. Valverde, and J. Nunez-Farfan. 2003. Quantitative genetics of plant tolerance and resistance against natural enemies of two natural populations of *Datura stramonium*. Evolutionary Ecology Research **5**:1049-1065.

Fornoni, J., P. L. Valverde, and J. Nunez-Farfan. 2004b. Population variation in the cost and benefit of tolerance and resistance against herbivory in *Datura stramonium*. Evolution **58**:1696-1704.

Fortin, M.-J. and M. R. T. Dale. 2005. Spatial Analysis: A Guide for Ecologists. Cambridge University Press, Cambridge, UK.

Gardner, S. N. and M. Mangel. 1999. Modeling investments in seeds, clonal offspring, and translocation in a clonal plant. Ecology **80**:1202-1220.

Gershenzon, J. 1994. Metabolic costs of terpenoid accumulation in higher plants. Journal of Chemical Ecology **20**:1281-1328.

Goldberg, D. E. 1988. Response of *Solidago canadensis* clones to competition. Oecologia **77**:357-364.

Gunn, S., J. F. Farrar, B. E. Collis, and M. Nason. 1999. Specific leaf area in barley: individual leaves versus whole plants. New Phytologist **143**:45-51.

Hammerli, A. and T. B. H. Reusch. 2003. Genetic neighbourhood of clone structures in eelgrass meadows quantified by spatial autocorrelation of microsatellite markers. Heredity **91**:448-455.

Hartnett, D. C. and F. A. Bazzaz. 1985. The genet and ramet population-dynamics of *Solidago canadensis* in an abandoned field. Journal of Ecology **73**:407-413.

Haukioja, E. and J. Koricheva. 2000. Tolerance to herbivory in woody vs. herbaceous plants. Evolutionary Ecology **14**:551-562.

Hawkes, C. V. and J. J. Sullivan. 2001. The impact of herbivory on plants in different resource conditions: A meta-analysis. Ecology **82**:2045-2058.

Hay, M. E. 1986. Associational plant defenses and the maintenance of species-diversity - Turning competitors into accomplices. American Naturalist **128**:617-641.

Haynes, K. J., F. P. Dillemuth, B. J. Anderson, A. S. Hakes, H. B. Jackson, S. E. Jackson, and J. T. Cronin. 2007. Landscape context outweighs local habitat quality in its effects on herbivore dispersal and distribution. Oecologia **151**:431-441.

Henriksson, J., E. Haukioja, V. Ossipov, S. Ossipova, S. Sillanpaa, L. Kapari, and K. Pihlaja. 2003. Effects of host shading on consumption and growth of the geometrid *Epirrita* *autumnata*: interactive roles of water, primary and secondary compounds. Oikos **103**:3-16.

Herms, D. A. and W. J. Mattson. 1992. The dilemma of plants - to grow or defend (Q Rev Biol, Vol 67, Pg 283, 1992). Quarterly Review of Biology **67**:478-478.

Holeski, L. M., R. Chase-Alone, and J. K. Kelly. 2010. The genetics of phenotypic plasticity in plant defense: Trichome production in *Mimulus guttatus*. American Naturalist **175**:391-400.

Hoogesteger, J. and P. S. Karlsson. 1992. Effects of defoliation on radial stem growth and photosynthesis in the mountain birch (*Betula Pubescens* Ssp Tortuosa). Functional Ecology **6**:317-323.

Horner, J. D. and W. G. Abrahamson. 1992. Influence of plant genotype and environment on oviposition preference and offspring survival in a gallmaking herbivore. Oecologia **90**:323-332.

Hull-Sanders, H. M., R. Clare, R. H. Johnson, and G. A. Meyer. 2007. Evaluation of the evolution of increased competitive ability (EICA) hypothesis: Loss of defense against generalist but not specialist herbivores. Journal of Chemical Ecology **33**:781-799.

Huston, M. and T. Smith. 1987. Plant succession - life-history and competition. American Naturalist **130**:168-198.

Ivey, C. T., D. E. Carr, and M. D. Eubanks. 2009. Genetic variation and constraints on the evolution of defense against spittlebug (*Philaenus spumarius*) herbivory in *Mimulus guttatus*. Heredity **102**:303-311.

Izaguirre, M. M., C. A. Mazza, M. Biondini, I. T. Baldwin, and C. L. Ballare. 2006. Remote sensing of future competitors: Impacts on plant defenses. Proceedings of the National Academy of Sciences of the United States of America **103**:7170-7174.

Janse-ten Klooster, S. H., E. J. P. Thomas, and F. J. Sterck. 2007. Explaining interspecific differences in sapling growth and shade tolerance in temperate forests. Journal of Ecology **95**:1250-1260.

Johnson, M. T. J., A. A. Agrawal, J. L. Maron, and J. P. Salminen. 2009. Heritability, covariation and natural selection on 24 traits of common evening primrose (*Oenothera biennis*) from a field experiment. Journal of Evolutionary Biology **22**:1295-1307.

Jones, T., S. Kulseth, K. Mechtenberg, C. Jorgenson, M. Zehfus, P. Brown, and D. H. Siemens. 2006. Simultaneous evolution of competitiveness and defense: induced switching in *Arabis drummondii*. Plant Ecology **184**:245-257.

Karban, R. and I. T. Baldwin. 1997. Induced Responses to Herbivory. Chicago University Press, Chicago, IL USA.

Laine, A. L. and A. Tellier. 2008. Heterogeneous selection promotes maintenance of polymorphism in host-parasite interactions. Oikos **117**:1281-1288.

Lankau, R. A. and S. Y. Strauss. 2008. Community complexity drives patterns of natural selection on a chemical defense of *Brassica nigra*. American Naturalist **171**:150-161.

Lau, J. A., J. Strengbom, L. R. Stone, P. B. Reich, and P. Tiffin. 2008. Direct and indirect effects of CO2, nitrogen, and community diversity on plant-enemy interactions. Ecology **89**:226-236.

Lau, J. A. and P. Tiffin. 2009. Elevated carbon dioxide concentrations indirectly affect plant fitness by altering plant tolerance to herbivory. Oecologia **161**:401-410.

Legendre, P. 1993. Spatial autocorrelation - Trouble or new paradigm. Ecology **74**:1659-1673.

Leimu, R. and J. Koricheva. 2006. A meta-analysis of tradeoffs between plant tolerance and resistance to herbivores: combining the evidence from ecological and agricultural studies. Oikos **112**:1-9.

Lloyd, D. G. 1984. Variation strategies of plants in heterogeneous environments. Biological Journal of the Linnean Society **21**:357-385.

Maddox, G. D. and N. Cappuccino. 1986. Genetic determination of plant-susceptibility to an herbivorous insect depends on environmental context. Evolution **40**:863-866.

Maddox, G. D., R. E. Cook, P. H. Wimberger, and S. Gardescu. 1989. Clone structure in 4 *Solidago altissima* (Asteraceae) populations - rhizome connections within genotypes. American Journal of Botany **76**:318-326.

Maddox, G. D. and R. B. Root. 1987. Resistance to 16 diverse species of herbivorous insects within a population of goldenrod, *Solidago-altissima* - genetic-variation and heritability. Oecologia **72**:8-14.

Maddox, G. D. and R. B. Root. 1990. Structure of the encounter between goldenrod (*Solidago altissima*) and its diverse insect fauna. Ecology **71**:2115-2124.

Maschinski, J. and T. G. Whitham. 1989. The continuum of plant-responses to herbivory - the influence of plant-association, nutrient availability, and timing. American Naturalist **134**:1-19.

Mauricio, R. and M. D. Rausher. 1997. Experimental manipulation of putative selective agents provides evidence for the role of natural enemies in the evolution of plant defense. Evolution **51**:1435-1444.

Mauricio, R., M. D. Rausher, and D. S. Burdick. 1997. Variation in the defense strategies of plants: Are resistance and tolerance mutually exclusive? Ecology **78**:1301-1311.

McCrea, K. D. and W. G. Abrahamson. 1987. Variation in herbivore infestation - Historical vs genetic-factors. Ecology **68**:822-827.

Meyer, A. H. and B. Schmid. 1999. Experimental demography of the old-field perennial *Solidago altissima*: the dynamics of the shoot population. Journal of Ecology **87**:17-27.

Meyer, G. A. 1993. A comparison of the impacts of leaf-feeding and sap-feeding insects on growth and allocation of goldenrod. Ecology **74**:1101-1116.

Meyer, G. A. 1998a. Mechanisms promoting recovery from defoliation in goldenrod (*Solidago altissima*). Canadian Journal of Botany-Revue Canadienne De Botanique **76**:450-459.

Meyer, G. A. 1998b. Pattern of defoliation and its effect on photosynthesis and growth of goldenrod. Functional Ecology **12**:270-279.

Meyer, G. A. and R. B. Root. 1993. Effects of herbivorous Insects and soil fertility on reproduction of goldenrod. Ecology **74**:1117-1128.

Meyer, G. A. and T. H. Whitlow. 1992. Effects of leaf and sap feeding insects on photosynthetic rates of goldenrod. Oecologia **92**:480-489.

Miller, A. M., C. McArthur, and P. J. Smethurst. 2007. Effects of within-patch characteristics on the vulnerability of a plant to herbivory. Oikos **116**:41-52.

Mitchell, R. J. and R. G. Shaw. 1993. Heritability of floral traits for the perennial wild flower *Penstemon-centranthifolius* (Scrophulariaceae)-Clones and crosses. Heredity **71**:185-192.

Molofsky, J., J. D. Bever, J. Antonovics, and T. J. Newman. 2002. Negative frequency dependence and the importance of spatial scale. Ecology **83**:21-27.

Moriondo, M., S. Orlandini, and F. J. Villalobos. 2003. Modelling compensatory effects of defoliation on leaf area growth and biomass of sunflower (*Helianthus annuus* L.). European Journal of Agronomy **19**:161-171.

Núñez-Farfan, J., J. Fornoni, and P. L. Valverde. 2007. The evolution of resistance and tolerance to herbivores. Annual Review of Ecology Evolution and Systematics **38**:541-566.

Oesterheld, M. 1992. Effect of defoliation intensity on aboveground and belowground relative growth-rates. Oecologia **92**:313-316.

Painter, R. H. 1958. Resistance of plants to insects. Annual Review of Entomology **3**:267-290.

Pilson, D. 1992. Aphid distribution and the evolution of goldenrod resistance. Evolution **46**:1358-1372.

Pilson, D. 2000. The evolution of plant response to herbivory: simultaneously considering resistance and tolerance in Brassica rapa. Evolutionary Ecology **14**:457-489.

Punzalan, D., F. H. Rodd, and L. Rowe. 2010. Temporally variable multivariate sexual selection on sexually dimorphic traits in a wild insect population. American Naturalist **175**:401-414.

Rausher, M. D. and E. L. Simms. 1989. The evolution of resistance to herbivory in *Ipomoea purpurea* .1. Attempts to detect selection. Evolution **43**:563-572.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution **43**:223-225.

Root, R. B. 1996. Herbivore pressure on goldenrods (*Solidago altissima*): Its variation and cumulative effects. Ecology **77**:1074-1087.

Root, R. B. and N. Cappuccino. 1992. Patterns in population-change and the organization of the insect community associated with goldenrod. Ecological Monographs **62**:393-420.

Rosenberg, M. S. 2008. PASSaGE. Pattern Analysis, Spatial Statistics, and Geographic Exegesis.

Rudgers, J. A. and S. Y. Strauss. 2004. A selection mosaic in the facultative mutualism between ants and wild cotton. Proceedings of the Royal Society of London Series B-Biological Sciences **271**:2481-2488.

Semple, J. C. and R. E. Cook. 2006. *Solidago*. Oxford University Press, Oxford, UK.

Shen, C. S. and C. E. Bach. 1997. Genetic variation in resistance and tolerance to insect herbivory in *Salix cordata*. Ecological Entomology **22**:335-342.

Siemann, E., J. Haarstad, and D. Tilman. 1999. Dynamics of plant and arthropod diversity during old field succession. Ecography **22**:406-414.

Siemens, D. H., S. H. Garner, T. Mitchell-Olds, and R. M. Callaway. 2002. Cost of defense in the context of plant competition: *Brassica rapa* may grow and defend. Ecology **83**:505-517.

Siemens, D. H., H. Lischke, N. Maggiulli, S. Schurch, and B. A. Roy. 2003. Cost of resistance and tolerance under competition: the defense-stress benefit hypothesis. Evolutionary Ecology **17**:247-263.

Simms, E. L. and M. D. Rausher. 1989. The evolution of resistance to herbivory in *Ipomoea purpurea* .2. Natural selection by insects and costs of resistance. Evolution **43**:573-585.

Simms, E. L. and J. Triplett. 1994. Costs and benefits of plant responses to disease - resistance and tolerance. Evolution **48**:1973-1985.

Siska, E. L., S. C. Pennings, T. L. Buck, and M. D. Hanisak. 2002. Latitudinal variation in palatability of salt-marsh plants: Which traits are responsible? Ecology **83**:3369-3381.

Stamp, N. 2003. Out of the quagmire of plant defense hypotheses. Quarterly Review of Biology **78**:23-55.

Stevens, M. T., D. M. Waller, and R. L. Lindroth. 2007. Resistance and tolerance in *Populus tremuloides*: genetic variation, costs, and environmental dependency. Evolutionary Ecology **21**:829-847.

Stinchcombe, J. R. and M. D. Rausher. 2001. Diffuse selection on resistance to deer herbivory in the ivyleaf morning glory, Ipomoea hederacea. American Naturalist **158**:376-388.

Strauss, S. Y. and A. A. Agrawal. 1999. The ecology and evolution of plant tolerance to herbivory. Trends in Ecology & Evolution **14**:179-185.

SYSTAT. 2004. SYSTAT for Windows. SYSTAT software, Richmond, CA.

SYSTAT. 2007. SYSTAT for Windows. SYSTAT software, Richmond, CA.

Thompson, J. N. and B. M. Cunningham. 2002. Geographic structure and dynamics of coevolutionary selection. Nature **417**:735-738.

Tiffin, P. 2000. Are tolerance, avoidance, and antibiosis evolutionarily and ecologically equivalent responses of plants to herbivores? American Naturalist **155**:128-138.

Tiffin, P. 2002. Competition and time of damage affect the pattern of selection acting on plant defense against herbivores. Ecology **83**:1981-1990.

Tiffin, P. and M. D. Rausher. 1999. Genetic constraints and selection acting on tolerance to herbivory in the common morning glory *Ipomoea purpurea*. American Naturalist **154**:700-716.

Tilman, D. 1987. Secondary succession and the pattern of plant dominance along experimental nitrogen gradients. Ecological Monographs **57**:189-214.

Travers-Martin, N. and C. Muller. 2008. Matching plant defence syndromes with performance and preference of a specialist herbivore. Functional Ecology **22**:1033-1043.

Uriarte, M. 2000. Interactions between goldenrod (*Solidago altissima* L.) and its insect herbivore (*Trirhabda virgata*) over the course of succession. Oecologia **122**:521-528.

Uriarte, M., C. D. Canham, and R. B. Root. 2002. A model of simultaneous evolution of competitive ability and herbivore resistance in a perennial plant. Ecology **83**:2649-2663.

van der Meijden, E., M. Wijn, and H. J. Verkaar. 1988. Defense and regrowth, alternative plant strategies in the struggle against herbivores. Oikos **51**:355-363.

van der Putten, W. H. 2003. Plant defense belowground and spatiotemporal processes in natural vegetation. Ecology **84**:2269-2280.

Via, S. and R. Lande. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. Evolution **39**:505-522.

Weinig, C., J. R. Stinchcombe, and J. Schmitt. 2003. Evolutionary genetics of resistance and tolerance to natural herbivory in *Arabidopsis thaliana*. Evolution **57**:1270-1280.

Wise, M. J. 2007. Evolutionary ecology of resistance to herbivory: an investigation of potential genetic constraints in the multiple-herbivore community of *Solanum carolinense*. New Phytologist **175**:773-784.

Wise, M. J. 2009. To duck or not to duck: resistance advantages and disadvantages of the candy-cane stem phenotype in tall goldenrod, *Solidago altissima*. New Phytologist **183**:900-907.

Wise, M. J. and W. G. Abrahamson. 2005. Beyond the compensatory continuum: environmental resource levels and plant tolerance of herbivory. Oikos **109**:417-428.

Wise, M. J. and W. G. Abrahamson. 2007. Effects of resource availability on tolerance of herbivory: A review and assessment of three opposing models. American Naturalist **169**:443-454.

Wise, M. J. and W. G. Abrahamson. 2008a. Applying the limiting resource model to plant tolerance of apical meristem damage. American Naturalist **172**:635-647.

Wise, M. J. and W. G. Abrahamson. 2008b. Ducking as a means of resistance to herbivory in tall goldenrod, *Solidago altissima*. Ecology **89**:3275-3281.

Wise, M. J., J. M. Partelow, K. J. Everson, M. K. Anselmo, and W. G. Abrahamson. 2008. Good mothers, bad mothers, and the nature of resistance to herbivory in *Solidago altissima*. Oecologia **155**:257-266.

APPENDIX A

Table A.1. Results from separate nested ANOVAs to test the effects of successional-stage (S), field within stage (F(S); nested effect), genotype (G), insecticide treatment (I) and their interactions on resistance to insects, resistance to mammals, total resistance (1-proportion damaged by both types of herbivores), and biomass (our proxy for fitness).

Source of		Resistance to insects[§]		Resistance to Mammals[§]		Total Resistance			
Biomass									
Variation	df	F	*P*-value	F	*P*-value	F	*P*-value	F	*P*-value
Stage	1,4	8.70	0.042	5.08	0.082	16.01	0.012*	1.93	0.237
Field(Stage)	4,52	6.66	<0.001*	34.89	<0.001*	15.35	<0.001*	104.01	< 0.001*
Genotype	13,445	0.84	0.620	0.79	0.672	0.85	0.607	0.10	0.237
Insecticide	1,445	58.06	<0.001*	0.01	0.970	27.32	<0.001*	1.42	0.234
G*I	13,445	0.82	0.644	0.39	0.973	0.50	0.925	1.42	0.145
T*S	1,445	5.06	0.025	1.69	0.195	0.01	0.932	0.69	0.408
S*G	13,445	1.09	0.362	0.51	0.917	0.38	0.976	0.90	0.550
S*I*G	13,445	1.42	0.147	0.72	0.747	1.29	0.218	1.96	0.022
G*F(S)	52,445	0.90	0.664	0.99	0.498	0.97	0.541	1.12	0.268
I*F(S)	4,445	2.18	0.070	0.15	0.963	1.84	0.120	0.97	0.421
G*I*F(S)	52,445	1.05	0.382	1.19	0.178	1.14	0.244	1.58	0.008*

[§] The above ANOVA results for resistance are the same as for damage.

* *P* values < the critical level of α of 0.05 after sequential Bonferroni corrections

Table A.2. Results from a two-way ANOVA for the effects of successional stage, genotype and their interaction on tolerance[δ] levels. Estimates of tolerance are based on a single measure per genotype per field.

Source of Variation	*df*	MS	F	*P*
Stage	1	1502.90	7.76	0.007
Genotype	13	140.60	0.72	0.730
S*G	13	158.40	0.82	0.640
Residuals	54	193.70		

[δ] In one early-successional field, all replicates of two genets (D9-4 and F1-8) received no damage. Consequently, we were unable to calculate tolerance estimates for those two cases.

APPENDIX B

Table B.1. Results from an ANCOVA for the effects of successional-stage (early vs. late), resistance (covariate), and their interaction on goldenrod tolerance. A significant covariate effect indicates an association between resistance and tolerance** and a significant interaction between stage and the covariate indicates that the relationship differs between successional stages.

	df	MS	F	*P*
Stage	1	1456.8	10.87	0.001
Resistance	1	1092.7	8.15	0.006
S*R	1	2475.4	18.47	< 0.001
Residuals	77	134.0		

** Note, switching resistance and tolerance (i.e., making resistance the dependent variable and tolerance the covariate) did not change the results.

Table B.2. Separate ANCOVAs to test for the effects of successional-stage (early vs. late), defense level (covariate), and their interaction on fitness of goldenrods in the absence of herbivory (W_A). Quadratic terms of the covariate are included to evaluate whether there is a nonlinear relationship between the defense and fitness. A significant, negative covariate would indicate cost of resistance or tolerance.

	df	MS	F	*P*
Cost of Resistance:				
Stage	1	15.33	33.31	< 0.001*
Resistance	1	0.05	0.11	0.746
Resistance^2	1	4.11	8.94	0.004*
S*R	1	2.00	4.35	0.040
Residuals	80	0.46		
Cost of Tolerance:				
Stage	1	12.43	36.41	< 0.001*
Tolerance	1	7.29	21.36	< 0.001*
Tolerance^2	1	0.07	0.21	0.652
S*T	1	3.01	8.77	0.004*
Residuals	78	0.34		

* *P* values < the critical level of 0.05 after sequential Bonferroni corrections to α.

Table B.3. Separate ANCOVAs to test for the effects of successional-stage (early vs. late), defense level (covariate), and their interaction on fitness of goldenrods subjected to herbivory (W_P). Quadratic terms of the covariate are included. A significant covariate would indicate selection for or against (depending on whether the slope was positive or negative) resistance or tolerance.

	df	MS	F	*P*
Selection for Resistance:				
Stage	1	12.41	33.61	< 0.001*
Resistance	1	0.59	1.60	0.211
Resistance^2	1	2.05	5.56	0.021*
S*R	1	2.15	5.82	0.018*
Residuals	80	0.37		
Selection for Tolerance:				
Stage	1	10.06	29.88	< 0.001*
Tolerance	1	3.62	10.70	0.002*
Tolerance^2	1	0.42	1.25	0.267
S*T	1	0.01	0.03	0.867
Residuals	78	0.34		

* *P* values < the critical level of 0.05 after sequential Bonferroni corrections to α

VITA

Alyssa Stocks Hakes was born in 1980 in northwest Indiana, to John and Wendy Stocks. Her interest in the natural sciences developed over the course of her childhood, stimulated by numerous outdoor hiking and camping trips with her family. For her Girl Scout Gold Award service project, Alyssa planned and constructed a self-guided wildflower trail at Sunset Hill Farm County Park in Porter County, Indiana. In the summer of 1999, Alyssa worked as a naturalist for Sunset Hill Farm County Park before attending the University of Illinois at Urbana Champaign to pursue a bachelor's degree in biology and minor in chemistry. As an undergraduate, Alyssa traveled to Barro Colorado Island in Panama to assist Dr. James Dalling in conducting ecological research. Alyssa subsequently completed a senior thesis examining the germination requirements of a tropical pioneer plant, *Clidemia hirta*, under the direction of Dr. Dalling. Alyssa was awarded the Helen E. Hess Award for undergraduate research and graduated from the University of Illinois with high distinction in May 2003. That summer, she and her husband, Jonathan Hakes, moved to Baton Rouge, Louisiana. Alyssa joined the laboratory of Dr. James Cronin as a doctoral student in the Department of Biological Sciences at Louisiana State University. Her dissertation research examined plant resistance and tolerance in a spatial and temporal context, using goldenrods in mid-successional fields as her study system. In addition to her research and graduate course work, Alyssa was an active volunteer for Girl Scouts Louisiana East and member of St. Paul Lutheran Church. Alyssa begins a postdoctoral research position in the Department of Biological Science at Florida State University in Tallahassee, Florida, in the summer of 2010.