THE IMPORTANCE OF PLANT ONTOGENY FOR TRI-TROPHIC INTERACTIONS

By

CAROLINA QUINTERO

Lic. Universidad Nacional del Comahue, Argentina 2003

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This thesis for the Doctor of Philosophy degree by

Carolina Quintero

has been approved for the department of Ecology and Evolutionary Biology

by

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M. Deane Bowers

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Yan B. Linhart

_____

William D. Bowman

_____

Michael Breed

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Kasey E. Barton

_____

Dena M. Smith

The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.

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The importance of plant ontogeny for tri-trophic interactions

Thesis directed by Professor M. Deane Bowers

ABSTRACT

Plant-herbivore-natural enemy interactions can vary markedly in space and time. Yet, most studies assessing changes in plant defensive traits, and their consequences for higher trophic levels, have been conducted at single host plant developmental stages. The goal of this study was to assess the extent to which resource allocation constraints throughout plant development (i.e. ontogeny) influence the expression of plant constitutive and herbivore-induced defensive traits and their effects on plant-herbivore-natural enemy interactions. A series of experiments using multiple developmental stages of *Plantago lanceolata* (Plantaginaceae), and a specialist herbivore, the Buckeye butterfly, *Junonia coenia* (Nymphalidae), were performed. Results showed that investment in constitutive chemical (iridoid glycosides) and physical defenses significantly increased, while leaf water and nitrogen concentrations decreased with plant age. Moreover, plant age significantly influenced the ability of *P. lanceolata* to tolerate or induce defenses after damage. Ontogenetic changes in plant growth rate and allelochemical synthesis helped to explain why induction was mostly achieved earlier during plant development, whereas compensatory growth showed the opposite pattern. In contrast, extrinsic factors such as frequency and intensity of damage failed to modify age-dependent responses to leaf damage. Finally, ontogenetic patterns in *P. lanceolata* defensive traits significantly influenced tri-trophic interactions. Oviposition tests showed that Buckeye butterflies significantly preferred younger ontogenetic stages, laying 60% more eggs on juvenile than on reproductive plants. In turn, caterpillars feeding on juvenile plants showed faster growth and increased digestive efficiency, yet they acquired two to five times less sequestered defenses, suggesting that *J. coenia* predation risk should decrease with host plant age. However, higher sequestered iridoid

glycosides decreased larval immunocompetence by up to 30%, suggesting higher susceptibility to parasitoid attack as host plants age increases. Thus, I demonstrated for the first time that ontogenetic patterns in plant defensive traits, mediated by caterpillar performance and palatability, can alter the strength of the top-down control of herbivores. This study improves our understanding of the role of ontogenetic variation in plant quality and defenses for tri-trophic interactions, providing a framework for testing hypotheses related to the evolution of plant defenses and the context dependency of bottom-up and top-down controls shaping herbivore population dynamics.

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CHAPTER 1

INTRODUCTION

1.1. Research Rationale and Goals

Plant-animal interactions, ranging from mutualistic (e.g. pollination, seed dispersal) to antagonistic (e.g. herbivory, parasitism) relationships, have been shown to vary markedly in space and time (e.g. Herrera 1988, Horvitz and Schemske 1990, Brody 1997, Thies et al. 2003, Kolb et al. 2007). However, studies assessing changes in the direction and magnitude of these interactions have traditionally examined the role of spatial variation more than the role of temporal change (e.g. Travis 1996, Polis et al. 1997, Thies and Tscharntke 1999, Irwin and Maloof 2002, Fedriani et al. 2004, McGeoch and Price 2005, Thompson 2005, Andrew et al. 2007, Morales and Vazquez 2008; but see Irwin and Maloof 2002). Despite the fact that multiple traits known to influence species interactions change over the course of an organism's ontogeny (i.e. throughout their development), ontogeny has been little studied as an intrinsic factor able to influence plant-animal interactions. In particular, empirical studies that evaluate both the patterns and mechanisms explaining changes in plant-animal interactions throughout plant growth and development are still limited (Boege and Marquis 2005). Therefore, the overall aim of my dissertation research was to assess the extent to which variation in plant traits throughout plant ontogeny can modify tri-trophic interactions between herbivores, their host plants, and their natural enemies.

As plants develop from seedling to senescent stages, termed ontogeny, many morphological and physiological traits vary continuously, reflecting the underlying variation in resource acquisition, allocation and functional priorities (e.g. growth vs. reproduction). For instance, the transition between seedling and juvenile stages encompasses a shift in relative growth rate and often a transition of nutrient dependency from internal to external sources. In other words, once seedlings reach their maximum relative growth rate and, in many cases,

become functionally independent of their own stored nutrient supplies, it is believed that they have ended their seedling stage (Hanley et al. 2004). Alternatively, the transition between juvenile and mature life stages encompasses the allocation of resources into not only growth and maintenance, but also to the formation of reproductive tissues (Poethig 1990). Consequently, it is not surprising that the expression of numerous plant traits relevant to herbivores, such as nutritional content (Kozlowski 1971, Mattson 1980), physical defenses (Hanley et al. 2007), and chemical defenses (Boege and Marquis 2005) also vary throughout plant ontogeny. In some cases, empirical studies have reported that changes in plant defenses from one developmental stage to the next can sometimes rival the magnitude of differences observed among plant genotypes and/or species (e.g. Lawrence et al. 2003, Donaldson et al. 2006, Holeski et al. 2009). These shifts in phenotypic expression of plant defenses as plants age can be the result of dramatic changes in the levels of herbivory plants experience and/or internal resource allocation constraints. Nevertheless, while knowledge regarding ontogenetic patterns in plant constitutive and induced defenses has improved in the last three decades (reviewed in Boege and Marquis 2005, Barton and Koricheva 2010), less attention has been given to investigating the mechanisms that explain such patterns, such as variable costs and benefits of these defenses as plants develop (Ohnmeiss and Baldwin 2000, Van Dam et al. 2001, Boege et al. 2007, Orians et al. 2010).

These documented ontogenetic patterns in plant defenses should also be expected to play a significant role in mediating plant-herbivore-natural enemy interactions. Changes in plant defenses and nutritional value are known to influence tri-trophic interactions by directly altering herbivore host selection and performance, and/or indirectly altering herbivore predation risk (reviewed in Awmack and Leather 2002). In the last decade, a few studies have suggested an effect of plant ontogenetic stage on differential herbivore damage (Boege 2005a, Fonseca et al. 2006, Thomas et al. 2010), herbivore performance (Wallace and Eigenbrode 2002, Barrett and Agrawal 2004), and herbivore predation risk (Van Bael et al. 2003, Boege 2005a, Boege and

Marquis 2006). However, because most studies of plant-herbivore interactions have been traditionally focused on a single plant developmental stage (Boege and Marquis 2005), relatively little is known about how variation in plant defenses throughout the life-span of a plant may alter herbivore preference, performance and herbivore-predator interactions.

This dissertation explores how resource allocation constraints throughout plant ontogeny influence the magnitude of expression of plant constitutive and herbivore-induced defensive traits leading to significant changes in plant-herbivore and herbivore-natural enemy interactions (Figure 1.1). Specifically, using multiple plant developmental stages, the primary goals of my dissertation research were: (1) to provide a better understanding of the mechanisms that explain the often non-linear ontogenetic patterns in plant constitutive defenses, (2) to examine the influence of intrinsic (i.e. plant vigor) and extrinsic (i.e. variation in intensity and frequency of damage) factors mediating herbivore-induced responses following leaf damage, namely induced chemical defenses and compensatory growth, over plant ontogeny, and (3) to investigate the extent to which ontogenetic patterns in plant quality and defenses can influence the direction and magnitude of tri-trophic interactions: *directly* via herbivore host selection and performance, and/or *indirectly* via herbivore susceptibility to predation risk by predators and parasitoids.

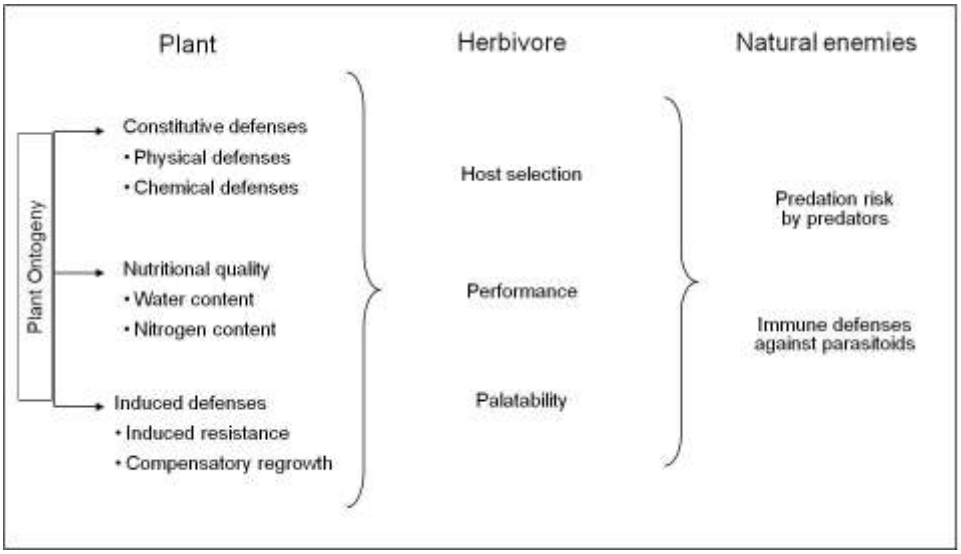

Figure 1.1. A conceptual model describing the variables I will explore to assess the influence of plant ontogeny on plant-herbivore-natural enemy interactions.

1.2. Plant Ontogeny and Defense Strategies

Plants have evolved a number of traits that reduce the negative impacts of herbivory on plant fitness. These defensive traits may decrease plant susceptibility to damage: (i) by repelling herbivores from ovipositing and/or feeding on plant tissues, and/or (ii) by decreasing consumption via reduction of herbivore survival, growth, or reproduction. Traits conferring repellent functions include chemical volatile cues and physical defenses such as leaf hairs, spines, trichomes, and toughness or hardness of leaf and stem surfaces (Fernandes 1994). Once herbivores bypass these initial barriers, production of toxic chemical compounds, called plant secondary metabolites or allelochemicals, may further limit damage by non-specialist herbivores (Hadacek 2002).

A wide array of plant allelochemicals, including but not limited to phenolics, terpenes, iridoids, alkaloids, tannins, furanocoumarins, glucosinolates, cardenolides and defensive proteins, have been described (Bennett and Wallsgrove 1994, Ayres et al. 1997, Dobler et al. 2011); usually lessening herbivore performance at very low concentrations or showing dosage-dependent effects. All plant species, even in the absence of damage, express baseline levels of certain suites of physical and chemical defenses, called constitutive defenses (Karban and Baldwin 1997). Since Fraenkel (1959) highlighted the role of plant secondary compounds in governing plant-herbivore interactions, the study of plant-herbivore-predator dynamics has become more tightly associated with plant chemical defenses (Berenbaum and Zangerl 2008, Hartmann 2008). Today, an overwhelming body of evidence has demonstrated extensive qualitative and quantitative variation in constitutive defenses within and among plant species and their importance in mediating plant-herbivore interactions (Hartmann 1996, Hadacek 2002). Therefore, identifying the environmental, genetic, and ecological factors responsible for explaining variation in plant defense strategies has become a central goal in ecological research.

Phenotypic variation in constitutively expressed defensive traits is typically due to three main factors: genotypic variation, resource availability and plant ontogeny. Interspecific genetic

variation accounts for a large portion of variation in plant qualitative and quantitative defenses (Massad et al. 2011). In addition, intraspecific variation can be substantial across clones or populations within a species. For example, estimated salicin concentrations in willow clones (*Salix oristera*, Salicaceae) vary as much as 100-fold (i.e. 0.05 - 5% dry weight) across the Sierra Nevada mountains of California (Smiley et al. 1985). In turn, several hypotheses have been proposed to explain how variation in resource availability can alter patterns of defense investment within and among plant species, such as the plant apparency hypothesis (Feeny 1976), the growth rate hypothesis (Coley et al. 1985), the carbon-nutrient balance hypothesis (Bryant et al. 1983), and the growth-differentiation balance hypothesis (Herms and Mattson 1992). In the last 35 years, empirical tests of these hypotheses, via altering environmental conditions such as soil nitrogen or phosphorus, water and light availability, CO_2 enrichment, and ozone exposure, have supported or refuted these hypotheses and their predictions (reviewed in Koricheva et al. 1998, Endara and Coley 2011, Massad et al. 2011). In contrast, while genotypic variation and resource availability have received considerable attention, our knowledge of how plant defenses change during ontogeny remains more limited (Boege and Marquis 2005, Hanley et al. 2007).

Ontogeny is the process that encompasses the origin and development of an individual organism from embryo to adulthood. In plants, this process is characterized by continuous or abrupt changes in plant anatomy and physiology, but due to the indeterminate growth of apical meristems (Jones 1999), it is often difficult to define distinctive developmental stages. However, in general, it is accepted that plants progress through at least four stages: seed, seedling, juvenile (non-reproductive plant) and adult (reproductive plant). The transition from one ontogenetic stage to the next is usually accompanied by shifts in several plant traits, including gradual to abrupt changes in leaf morphology and anatomy (Sylvester et al. 2001, Niinemets 2005, Zotz et al. 2011), architectural complexity (Barthelemy and Caraglio 2007), root:shoot ratios (Gedroc et al. 1996, McConnaughay and Coleman 1999), photosynthesis rates (Ishida et

al. 2005, Jaya et al. 2010), hormone production (Farnsworth 2004), and carbon and nitrogen metabolism (Wiedemuth et al. 2005). Furthermore, as plants' tissues form and differentiate, their access to environmental resources also varies. For instance, intrinsically regulated by the production of hormones such as auxins, roots grow through reiteration and elongation of organs, from primary to secondary roots, to tertiary, quaternary and/or adventitious roots (Malamy 2005), altering access to water and nutrients. Therefore, even if environmental conditions remain the same, changes in resource acquisition throughout ontogeny can shape variation in resources allocation to growth and differentiation.

As a function of the above mentioned shifts and constraints in resource acquisition, allocation, and functional priorities (i.e. growth vs. reproduction), several constitutively expressed physical and chemical defenses can also vary considerably over plant ontogeny. Indeed, specific predictions have been proposed regarding how plant ontogeny should influence plant defenses. The plant-age hypothesis (Bryant et al. 1992), which has been mainly supported by data from boreal woody plants (Swihart and Bryant 2001), argues that herbivore selective pressures will shape ontogenetic trajectories in plant defenses, leading to high levels of defenses in juvenile plants that decrease as they reach adult stages (i.e. as tissues become less accessible to mammalian browsers). In contrast, the growth-differentiation balance hypothesis (Herms and Mattson 1992) proposed that intrinsic forces, namely resource acquisition and allocation constraints, limit the expression of plant defenses, thereby predicting an increase in plant defenses as plants age. Expanding the idea of resource allocation constraints as a pivotal trait shaping plant defenses and using empirical data, Boege and Marquis (2005) proposed a detailed model encompassing all stages from cotyledon to senescent plants at the whole plant level. This model predicts that multiple resource allocation and architectural constraints throughout plant development will lead to a non-linear relationship between plant age and defenses. Specifically, the model predicts an early decline in plant defenses during the seedling stage, explained by the diminishing of stored resources in

cotyledons and seeds; followed by a significant increase from juvenile to mature stages as photosynthesis rates exceed growth rates and root:shoot ratios decrease. Finally, when plants reach reproductive maturity, allocation of resources to reproduction may again result in decreased levels of defenses or they may remain constant depending on the plant's reproductive strategy (Boege and Marquis 2005).

In support of this non-linear model, a recent meta-analysis conducted by Barton and Koricheva (2010), expanding previous reviews on this subject (Swihart and Bryant 2001, Boege and Marquis 2005), but increasing their scope by considering a wide array of studies (i.e. 116 studies and 153 plant species) across all plant life forms and defensive traits, concluded that plant defenses tend to show non-linear patterns across ontogeny. Yet, despite this generality, the shape of the relationship seems to significantly vary as a function of plant life form (i.e. woody plants, herbs or grasses) and defensive traits (i.e. constitutive or induced physical and chemical traits, and tolerance) (Barton and Koricheva 2010). Therefore, notwithstanding the increased interest in this subject, much remains to be studied. In particular, for any single plant species, we have a poor understanding of complete ontogenetic patterns (i.e. contrasting more than two developmental stages) in suites of constitutive defenses (i.e. as opposed to single traits) across multiple tissues (but see Williams and Ellis 1989, Bellostas et al. 2007, Mcarthur et al. 2010). Moreover, only a few empirical studies have assessed the potential mechanisms driving these previously observed shifts in the expression of defensive traits, such as resource allocation constraints (e.g. Bryant and Julkunentiitto 1995), architectural constraints (e.g. Brouat and McKey 2001), and age-dependent costs of investment in defenses (e.g. Briggs and Schultz 1990, Orians et al. 2010).

In addition to constitutive defenses, plants can also alter the quality and/or quantity of synthesized and released defenses following damage, called induced defenses. Plants deploy two basic types of induced defenses: (i) *resistance traits* that aim to reduce subsequent herbivore damage, by generally increasing baseline levels of physical and chemical defenses,

and (ii) *tolerance traits* that intend to minimize the negative effects of damage on plant fitness, by replacing the tissues lost to herbivores (i.e. compensatory growth) and/or allowing shifts in phenology (Karban and Baldwin 1997). These induced defensive traits can be achieved by enhancing photosynthesis rates, nutrient uptake or utilization of stored reserves (Tiffin 2000). Because the ability of a plant to up- or down-regulate these physiological processes will also depend on resource allocation constraints during plant development, strong age-dependent induced responses to herbivory should be expected.

Contrasting trends have being predicted for resistance and tolerance traits. Induced chemical defenses, usually considered a cost-saving strategy for plants, are associated with scenarios where resource allocation constraints limit constitutive defenses or are dependent on actively growing and differentiating tissues (Karban and Baldwin 1997, Cipollini et al. 2003). Because younger plant stages are often more susceptible to herbivory, have lower levels of constitutive defenses, and proportionally more undifferentiated and actively growing tissues than older plant stages, the ability of plants to induce defenses is predicted to be greater in younger developmental stages and lessen as plants mature (Karban and Baldwin 1997). In support of this prediction, Barton and Koricheva (2010) showed that, at least for herbaceous plants, induced resistance considerably decreased as plants progressed from seedling to senescent stages. Alternatively, compensatory growth that aims to restore the tissue lost to damage requires the reallocation of resources stored in undamaged tissues as well as enhancement of photosynthetic rates and nutrient uptake from the soil. Therefore, tolerance to herbivory is predicted to increase with plant age due to greater capacity to acquire resources and/or higher probability of having already stored reserves (Strauss and Agrawal 1999, Haukioja and Koricheva 2000). However, current evidence suggest that a plant's ability to tolerate damage for both woody and herbaceous plants did not differ throughout plant development (Barton and Koricheva 2010). Hence, concentrating on the relative importance of various intrinsic (i.e. growth rate and metabolic rate) and/or extrinsic (i.e. herbivore identity and frequency of damage)

factors driving these ontogenetic trends in resistance and tolerance to herbivory will help us improve theoretical predictions relating plant age and induced defenses.

Lastly, the extent of these increases or decreases in qualitative and quantitative changes as plants age can also be remarkable. For example, constitutive production of sesquiterpenes in leaf and root tissues of maize (*Zea mays* L., Poaceae) showed an overall dramatic decrease of 97%, whereas volatile blend complexity increased in leaves but decreased in roots as plants developed from seedling to mature plants (Kollner et al. 2004). Similarly, concentrations of condensed tannins and phenolic glycosides increased as much as 300% and 600%, respectively, in as little as six weeks during seedling development in two species of *Salix* (Salicaceae) (Fritz et al. 2001). In many cases, developmental variation in defense traits mirrors or even surpasses the variation described among populations (i.e. due to genetic or environmental factors) (Bowers and Stamp 1993, Lawrence et al. 2003, Donaldson et al. 2006, Barton 2007, Holeski et al. 2009). For instance, in clonal Aspen (*Populus tremuloides*, Salicaceae), while the expression of condensed tannins and phenolic glycosides varied from two to four times among mature clones, variation within a clone as ramets aged was found to increase (condensed tannins) or decrease (phenolic glycosides) 4 to 14 times between 1 and 25+ year old ramets (Donaldson et al. 2006). In other cases, plant age can interact with genotypic variation in plant defenses by modulating the expression of those defensive traits. For instance, Thyme (*Thymus vulgaris* L., Lamiaceae) genotypes carrying the most susceptible chemotype, linalool, against both specialist and generalist herbivores, do not express their linalool phenotype until after they reach three months of age. Prior to that age, they exhibit the least preferred phenol chemotype, probably increasing their chances of survival during the most vulnerable stage of development (Linhart and Thompson 1999). Given these ecologically relevant qualitative and quantitative changes in plant defenses as plants age, addressing the mechanisms driving these ontogenetic patterns, their interactions with other intrinsic and

extrinsic sources of variation, and the consequences for species interactions, especially at higher trophic levels, should be a priority of future research.

1.3. Plant Ontogeny and Insect Herbivore Preference and Performance

Empirical evidence suggests that changes in plant quality and defenses throughout plant ontogeny can significantly affect herbivore host selection. In general, invertebrate herbivores, in contrast to vertebrates, show a consistent preference for young rather than mature plant stages (reviewed in Fenner et al. 1999, Boege and Marquis 2005). Such preference is usually inferred by observations of greater insect herbivore damage (Price 1991, Spiegel and Price 1996, Albrectsen et al. 2004, Fonseca et al. 2006), or higher insect density and diversity (Waltz and Whitham 1997, Cuevas-Reyes et al. 2004, Boege 2005a, Thomas et al. 2010), on younger over older plant stages. While in several cases, higher herbivore damage in a certain developmental stage is associated with higher herbivore preference for those tissues (e.g. Pires and Price 2000, Del Val and Dirzo 2003, Lawrence et al. 2003, Cuevas-Reyes et al. 2004, Johnson and Zalucki 2005, Heckel et al. 2010), ontogenetic patterns in tissue damage may not necessarily reflect herbivore host selection. Rather, these patterns may arise as a consequence of (i) higher survival, due to superior herbivore performance and/or lower predation rates, or (ii) higher feeding rates, if herbivores need to ingest larger quantities of tissue to meet their nutritional needs, on younger versus mature plants. In most insect herbivores, although immature larvae can potentially disperse long distances as early as the neonate stage (Zalucki et al. 2002), it is common that larvae often feed upon the plant on which they hatched (Mayhew 1997). Thus, patterns of herbivory may often depend on female oviposition choice. Hence, more research is needed to directly evaluate the effect of plant ontogeny on patterns of herbivore host selection, especially at the levels of both female oviposition and larval feeding choice.

Studies assessing insect herbivore performance on different host-plant developmental stages, although scarce, also suggest that changes in plant quality and defenses as a function

of plant ontogeny can strongly influence herbivore performance (i.e. growth rate, developmental time, fitness, etc). Nonetheless, the pattern here is less clear. Herbivore performance has shown both patterns: to decrease as host plant age increases (e.g. Price et al. 1987, Diawara et al. 1994, Karban and Thaler 1999, Campos et al. 2003), or to increase as host plant age increases (e.g. Wallace and Eigenbrode 2002, Barrett and Agrawal 2004, Liu et al. 2010). Discrepancies in overall patterns of insect performance as a function of plant ontogeny should not be surprising given that several defensive strategies show non-linearity as plants age (see section 1.2). In fact, in most cases, this variation in herbivore performance throughout plant development tightly matches ontogenetic patterns in plant defenses (but see Barton and Koricheva 2010), with herbivores decreasing performance on those stages that are more highly defended. In addition, differential herbivore performance on different host plant developmental stages may be explained by differences in herbivore guild and/or degree of specialization. For instance, while the gall-forming aphid *Pemphigus betae* was 70 times as common on mature as on juvenile narrowleaf cottonwood trees (*Populus angustifolia*, Salicaceae), the leaf-feeding beetle *Chrysomela confluens* (Chrysomelidae) exhibited the opposite distribution, with densities 400 times as high on juvenile as on mature trees (e.g. Kearsey and Whitham 1989).

In summary, ontogenetic patterns in plant quality and defenses can lead to changing attractiveness and resistance to different suites of herbivores, generating non-overlapping herbivore distributions. Despite the increased interest in reporting trends of insect herbivore damage, richness and abundance across host plant development, there is still a lack of observational or manipulative experiments that aim to address the mechanisms underlying these patterns. Thus, expanding our knowledge of the behavioral (i.e. oviposition and/or feeding choice) and physiological (i.e. growth rate, digestibility and fitness) mechanisms that lead to differential patterns of herbivory across plant ontogeny may have key implications for understanding temporal shifts in herbivore population dynamics and community structure.

1.4. *Plant Ontogeny and* H*erbivore-Natural Enemy Interactions*

Variation in plant traits throughout plant ontogeny can influence the relationship between herbivores and their natural enemies in two ways: (i) *directly* by altering plant indirect defenses that promote the effectiveness of predators and parasitoids to locate prey on plant tissues, or (ii) *indirectly* by modifying predators' choice or performance among different quality prey.

In the first scenario, several studies have shown that plant indirect defenses, such as plant rewards (i.e. provision of extrafloral nectaries, food bodies and domatia), volatile organic compounds, and plant architectural traits (Price et al. 1980), can vary considerably as plants develop. For example, obligate ant-plant species (i.e. myrmecophytes) that provide both food and shelter to ants have consistently shown two main trends: (i) an initial lack of indirect defenses during the seedling and/or sapling stage until architectural constraints allow for the development of functional domatia (reviewed in Brouat and McKey 2001), followed by (ii) an ontogenetic increase in all plant rewards such as food body production (Heil et al. 1997, Itino et al. 2001, Webber et al. 2007), extrafloral nectaries (Young et al. 1997), and domatia abundance and size (Fonseca 1999, Brouat and McKey 2001, Fonseca and Benson 2003). Because, several plant species rely on these indirect defenses in order to decrease damage via facilitating the recruitment and/or residence of carnivorous natural enemies on plant tissues, ontogenetic changes in plant indirect defense traits can alter the predation risk of herbivores (Van Bael et al. 2003, Boege 2005a, Trager and Bruna 2006). Indeed, ontogenetic trends in plant rewards, for instance, have been shown to significantly alter the identity and abundance of mutualistic ants (e.g. Fonseca 1999, Feldhaar et al. 2003, Fonseca and Benson 2003, Djieto-Lordon et al. 2004, Dejean et al. 2008), which, in turn, mediates herbivore pressure (e.g. Djieto-Lordon et al. 2004, Izzo and Vasconcelos 2005, Trager and Bruna 2006) and, ultimately, plant fitness (e.g. Miller 2007, Palmer et al. 2010).

In the second scenario, changes in nutritional value and chemical defenses as plants age can also scale up to influence herbivore-natural enemy interactions by modifying the quality

of herbivores as prey. However, no empirical data to date have tested this assumption. Several possible scenarios can be predicted depending on the identity of both the herbivore and the natural enemy interacting throughout the development of their host plant. For example, in the case of specialist herbivores that employ plant allelochemicals as feeding stimulants and/or sequester these compounds as defenses against their own enemies (Nishida 2002, Opitz and Muller 2009), shifts in plant constitutive or inducible defenses as they develop can translate into temporal shifts in herbivore performance and palatability. Because higher levels of sequestered defenses often deter predators (Opitz and Muller 2009) but have shown to decrease herbivore immune defenses against parasitoids (Smilanich et al. 2009a), the selective pressure of natural enemies on specialist herbivores during plant ontogeny may differ between parasitoids and predators. In the case of generalist herbivores, similar plant-mediated effects on higher trophic levels can also be expected. For instance, increases in plant defenses as plants age can directly translate into lower growth rate and extended larval development time, potentially increasing mortality due to a prolonged exposure to natural enemies (e.g. Benrey and Denno 1997), or decreasing it if poor quality prey subsequently decreases the performance of their parasitoids and predators (e.g. Havill and Raffa 2000, Gols et al. 2008a, Gols et al. 2008b).

In summary, while it is likely that variation in plant traits during development can influence herbivore performance and palatability and thus, indirectly affect the behavior, prey selection, and fitness of natural enemies, the outcome of these tri-trophic interactions throughout plant ontogeny can be hard to predict. Yet, the complexity of outcomes mediated by ontogenetic changes in plant defenses offer a challenging opportunity to study the evolution of complex phenotypes, as well as their implications for understanding temporal variation in tri-trophic interactions. Moreover, uncovering patterns in ontogenetic trajectories in plant-herbivore-natural enemy interactions, and their underlying mechanisms, can shed light on why bottom-up and top-down controls of herbivore populations are often context dependent (temporally variable in direction and strength) or even hard to find.

1.5. Study System

Plantago lanceolata

Plantago lanceolata L., narrowleaf or ribwort plantain, (Plantaginaceae) is a common short-lived polycarpic herbaceous plant (annual or facultative perennial) that germinates in fall and spring and flowers in midsummer, retaining a green rosette year round (Roach et al. 2009). Originally native to Eurasia, it has become a species of cosmopolitan distribution, thriving in recently disturbed areas such as roadsides and mown fields (Cavers et al. 1980). In North America, it was introduced approximately 200 years ago (Cavers et al. 1980), being currently naturalized in all states (http://www.plants.usda.gov/java/profile?symbol=PLLA). In its vegetative form, it consists of a green rosette formed by alternate lanceolate-shaped leaves with three to five strong parallel veins and attached to the base by a narrowed short petiole. Based on the position that leaves occupy in the rosette and their physiological status, these leaves can be easily categorized into three leaf-age classes: new, intermediate and old leaves. Tough, fully expanded leaves located in the basal part of the rosette and showing some degree of discoloration are usually classified as old leaves. Tender and yet not fully expanded leaves found in the center of the rosette are new leaves, while fully expanded but still quite tender and not discolored leaves in between are typically classified as intermediate leaves (Bowers and Stamp 1992, 1993). As seen in other short-lived perennials, temporally variable environments can produce dramatic differences in optimal reproductive timing in *P. lanceolata*, showing a high degree of plasticity in reproductive output and number of potential reproductive seasons (i.e. one to six reproductive events, Shefferson and Roach 2010). However, in general, Shefferson and Roach (2010) reported that over 45% of the reproducing individuals only reproduced once and did so mostly in the first or second year of life. When plants reach maturity, they produce few to several leafless scapes (10–40 cm in length) ending in an oblong inflorescence that contains several small self-incompatible wind-pollinated or insect-pollinated flowers (4mm),

each with a pointed bract. Each flower can produce up to two seeds. Thus, reproductive output has been shown to fluctuate between 30 and 10,000 seeds per plant (Cavers et al. 1980).

Plantago lanceolata produces, in addition to iridoid glycosides (Ronsted et al. 2000), a number of bioactive phenolic compounds such as flavonoids and phenylethanoid glycosides (Chiang et al. 2003, Galvez et al. 2005). However, iridoid glycosides are the primary allelochemicals found in large concentrations across vegetative and reproductive tissues (i.e. up to 10-12% dry weight) and influencing feeding preferences of insect herbivores and pathogens (e.g. Bowers and Puttick 1989, Biere et al. 2004, De Deyn et al. 2004). Thus, in my research, I specifically focused on variation in constitutive and induced iridoid glycosides throughout plant ontogeny.

Iridoid glycosides (hereafter IGs) are a group of cyclopentanoid monoterpene-derived compounds that occur in over 50 families of plants (Bowers 1991, Jensen 1991). They are bitter compounds that consist of an 8-, 9- or 10-carbon skeleton with an attached monosaccharide at C-1, normally β-D-glucose (Boros and Stermitz 1991). Similar to several other plant secondary compounds, iridoids have deterrent effects and/or act as digestibility reducers on non-adapted insects (Bowers 1991, Dobler et al. 2011). For example, several of the over 1,700 IGs described to date (Elnaggar and Beal 1980, Boros and Stermitz 1990, 1991, Dinda et al. 2007b, a, 2009, 2011) have demonstrated detrimental post-ingestive effects that can increase larval mortality, or decrease relative growth rate and biomass gained (Dobler et al. 2011). These detrimental physiological effects may vary as a function of IG quality and quantity. For instance, differential effects were observed among various individual iridoid glycosides depending on their chemical structure and the presence or absence of functional groups, some of them showing dose-dependent effects (Puttick and Bowers 1988, Bowers and Puttick 1989). Although we lack specific knowledge regarding IG production sites (i.e. shoots vs. roots), these carbon-based defenses are synthesized through the isoprenoid biosynthetic pathway and are usually stored in cell vacuoles (Croteau 1987). They are also likely phloem mobile (Gowan et al. 1995, Beninger

et al. 2007) and concentrations across plant tissues have shown to be positively correlated (e.g. De Deyn et al. 2009).

Plantago lanceolata produces primarily two IGs: aucubin and catalpol (Figure 1.2), and amounts of these compounds may be as high as 10-12% dry weight (Bowers and Stamp 1992, 1993). As seen in several other plant species, levels of IGs vary among tissues, with the highest content being reported in reproductive tissues and the lowest in roots, with intermediate levels in leaf tissues (Darrow and Bowers 1999). In the case of leaves, there is also significant variation among leaf-age classes, with newer leaves containing higher nitrogen concentrations and two to three times more IGs and a higher proportion of catalpol than older leaves (Bowers and Stamp 1992, 1993). Because aucubin is the biosynthetic precursor of catalpol (Ronsted et al. 2000), high concentrations of IGs, especially of catalpol, indicate not only a greater biosynthetic investment by the plant, but also may result in a higher unpalatability and/or toxicity to herbivores and pathogens (Bowers and Stamp 1993). Finally, in addition to IGs, *P. lanceolata* also invests in physical defenses such as tough leaves (Schippers and Olff 2000) and glandular and non-glandular trichomes (de la Fuente 2002). Although we have considerably less information regarding within plant variation in physical defenses, a few studies have reported that as leaf age increases trichome density generally decreases (de la Fuente 2002), while leaf toughness increases (Schippers and Olff 2000).

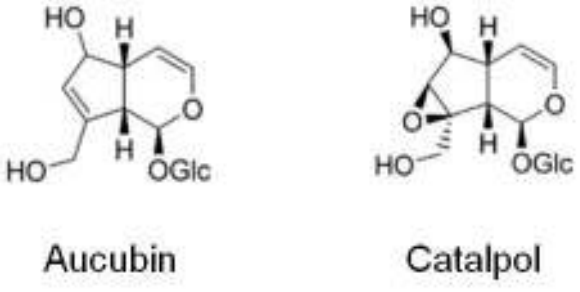

Figure 1.2. Structural conformation of two iridoid glycosides (IGs), aucubin and catalpol, commonly found in *P. lanceolata* tissues.

Multiple intrinsic and extrinsic factors may influence constitutive and induced levels of IGs in *P. lanceolata,* such as plant genotype (Marak et al. 2002, Barton 2007, Reudler Talsma et al. 2011), resource availability and temperature (Fajer et al. 1992, Darrow and Bowers 1999, Jarzomski et al. 2000, Tamura 2001), herbivore damage or identity (Bowers and Stamp 1993, Wurst and van der Putten 2007), and mutualistic associations with arbuscular mycorrhizal fungi (Bennett et al. 2009). In addition, plant age has also been identified as an important intrinsic factor affecting *P. lanceolata* expression of IGs (e.g. Bowers and Stamp 1993, Fuchs and Bowers 2004, Barton 2007); and these ontogenetic patterns in IG production significantly vary among both maternal families and populations (Bowers and Stamp 1993, Barton 2007). Specifically, earlier empirical studies looking at constitutive levels of IGs across two or more developmental stages demonstrated that, in general, IG content increases as plants age (e.g. Bowers and Stamp 1993, Fuchs and Bowers 2004, Barton 2007). However, these previous studies were limited in scope, only considering a few developmental stages at a time and/or limited their ontogenetic trajectories to only leaf tissues. Thus, a more comprehensive study is required to assess the overall patterns as well as the mechanisms explaining these ontogenetic trajectories.

Similarly, plant age has also been identified as an intrinsic factor that may alter a plant's ability to induce IGs and/or tolerate damage (e.g. Fuchs and Bowers 2004, Hanley and Fegan 2007, Barton 2008). Yet, no consistent trends illustrating how plant ontogeny alters defensive strategies following insect attack have emerged. For instance, previous evidence for induction of IGs after insect attack in *P. lanceolata* has ranged from no detectable induction (Adler et al. 1995, Fontana et al. 2009), to induced resistance (Bowers and Stamp 1993, Darrow and Bowers 1999, Marak et al. 2002) or induced susceptibility (Barton 2008). Furthermore, ontogenetic changes in other plant traits also known to mediate the interaction between *P. lanceolata* and specialists and generalist herbivores, such as nutritional content and physical defenses (see below) have been more rarely explored. Accordingly, how plant age influences

both constitutive and induced chemical and physical defenses, nutritional quality, and compensatory growth, throughout most of the life span of *P. lanceolata*, needs further investigation.

Finally, *P. lanceolata* has been incorporated into the diet of many native North American insect herbivores and thus, multiple specialist and generalist herbivores in both the introduced and native range can affect and be affected by IG content in this species. For example, high IG concentrations usually deter generalist herbivores and pathogenic fungi feeding on shoot or root tissues (e.g. Bowers and Puttick 1989, Biere et al. 2004, De Deyn et al. 2004, Harvey et al. 2005, Wurst and van der Putten 2007). Yet, several specialist herbivores use IGs as feeding and/or oviposition stimulants (e.g. Bowers 1983, 1984, Pereyra and Bowers 1988, Reudler Talsma et al. 2008b), and some have the ability to sequester these compounds from leaves and roots and use them for their own protection against natural enemies (e.g. Bowers and Puttick 1986, Baden and Dobler 2009, Opitz et al. 2010). Furthermore, although most herbivores associated with this species primary eat either leaves or roots, there is at least one example of a species of *Longitarsus* (Chrysomelidae) flea beetle that feeds on and sequesters IGs from both roots and leaves, as larvae and adults (Willinger and Dobler 2001, Baden and Dobler 2009). As a result, ontogenetic patterns of shoot and root IGs in this species can have profound consequences on the identity, abundance and performance of the herbivore community associated with it throughout its lifetime.

Junonia coenia

The buckeye butterfly, *Junonia coenia* Hubner (Nymphalidae) is a New World butterfly that can have one to three broods per year under temperate conditions, or more in the tropics where it can breed year-round (http://www.nearctica.com/butter/plate17/Jcoenia.htm). This species has been described as a specialist herbivore of plants containing IGs (Bowers 1984), *P. lanceolata* being one of its more frequent host plants (Graves and Shapiro 2003 and references therein).

Adult female butterflies use IGs as oviposition stimulants (Pereyra and Bowers 1988), choosing host plants or tissues within plants with higher IG concentrations, in particular catalpol (Pereyra and Bowers 1988, Klockars et al. 1993, Prudic et al. 2005). In addition, other host plant traits may also influence oviposition choice, although to a lesser extent; and thus, need to be taken into account. For instance, while adult Buckeye butterflies tend to choose plants higher in foliar nitrogen concentrations (Prudic et al. 2005), leaf trichome density does not seem to significantly influence Buckeye oviposition choice (de la Fuente 2002).

Buckeye larvae are oligophagous herbivores that have been documented to feed on members of four plant families: Cornaceae, Plantaginaceae, Scrophulariaceae and Verbenaceae (Bowers 1984). Buckeye caterpillars not only use IGs as feeding stimulants (Bowers 1984), but they are also able to sequester aucubin and catalpol in their hemolymph. Sequestration begins as soon as larvae start feeding, but when larvae reach their last instar, they begin to metabolize them before pupation, eventually eliminating them in the meconium just after adult eclosion (Bowers and Collinge 1992). Therefore, while immature larvae are highly unpalatable, sequestering IGs as a function of their concentration in host plant tissues (Camara 1997a), adult butterflies do not contain iridoids (Bowers and Collinge 1992) explaining their high palatability to birds (Bowers and Farley 1990). Levels of IGs in buckeye caterpillars, usually concentrating two to three times the levels of IGs found in their diet, vary normally from less than 5% to over 20% dry weight, and can reach up to 25% dry weight (Camara 1997a, Theodoratus and Bowers 1999).

The process of sequestration of ingested plant secondary compounds involves three main steps: (i) the resorption of plant allelochemicals through the gut wall, (ii) their transportation to the hemolymph, and (iii) their deposition in a particular site of the body (Nishida 2002); all of which can be energetically costly. In the case of *J. coenia*, while higher levels of IG concentrations on diets have shown to, in general, increase larval performance, some physiological costs associated with sequestration were also reported (Adler et al. 1995, Camara

1997a, Smilanich et al. 2009a). For instance, Camara (1997a) demonstrated that *J. coenia* reared on diets rich in IGs (~10% dry wt.) tend to experience a reduction in the efficiency of dry matter incorporation due to reduced digestibility and a decrease in sequestration efficiency due to decreased post-digestive efficiency. Thus, this study suggested that sequestration of high concentrations of IGs may come at a cost to growth. Furthermore, it has been proposed that insect herbivores that invest energy into plant allelochemical detoxification and/or sequestration may divert energy from other functions such as immune defenses (Moret and Schmid-Hempel 2000). Relevant to this, Smilanich et al. (2009a) recently demonstrated that high levels of IGs in *J.* coenia diets, leading to high IG content in their hemolymph, may enhance larval susceptibility to parasitoid attack. Nevertheless, caterpillars feeding on higher IG diets have decreased mortality by generalist herbivores such as ants, stink bugs, spiders, and predatory wasps (see below).

Natural Enemies

The importance of the third and higher trophic levels on interactions between herbivores and host plants has been acknowledged for several decades (Price et al. 1980), and few systems have been as intensely studied as those including IG-producing host plants, their herbivores, and natural enemies (i.e. predators and parasitoids) (reviewed in Bowers 1991, Dobler et al. 2011). Bottom-up interactions among IG-producing plants, herbivores and predators have been demonstrated repeatedly, as consumption of IG-producing plants by sequestering herbivores not only significantly decreases their predation risk but also confers a physiological cost to predators that consume sequestering prey (see below). Parasitoids may also be affected by changes in host quality, if the herbivores themselves experience lower performance or acquire higher levels of sequestered defenses. These effects on herbivores can then affect parasitoid oviposition choice, clutch size, development time and fitness (Ode 2006). Nevertheless, recent evidence demonstrated that the relationship between IGs and parasitoids may be opposite to

the one reported for generalist predators, with herbivores feeding on rich IGs diets showing only minimal effects on parasitoid fitness or higher parasitism rates (Smilanich et al. 2009a, Lampert and Bowers 2010b, Lampert et al. 2010, Reudler Talsma et al. 2011, Smilanich et al. 2011).

Sequestration of IGs by larvae and/or adult stages reduces the palatability of several herbivorous species to various vertebrate and invertebrate predators. For instance, a number of laboratory feeding experiments have shown that varying concentrations of IGs in adult butterflies and moths contributes to their unpalatability to birds and bats (Bowers 1980, 1981, Bowers and Farley 1990, Hristov and Conner 2005). On the other hand, species such as *Junonia coenia* (Nymphalidae) and *Ceratomia catalpae* (Sphingidae) that lack these defenses during their adult stages (Bowers 1991), were highly palatable as adults to Gray jays, *Perisoreus canadensis* (Corvidae) (Bowers and Farley 1990). Nevertheless, in these latter examples, larvae of these two species have warning coloration and sequester high concentrations of IGs, showing decreased larval mortality under field conditions (Camara 1997b). In general, sequestration of IGs by herbivore larvae serves as an effective defense against generalist invertebrate predators, including hymenopterans, hemipterans, coleopterans, and spiders (reviewed in Bowers 1991).

Choice- and non-choice tests between palatable and IG-sequestering lepidopteran larvae have consistently shown that sequestering species are consumed less than palatable species by such predators as jumping spiders, wolf spiders, and stinkbugs (Strohmeyer et al. 1998, Theodoratus and Bowers 1999). In addition, some invertebrate predators may also show dose-dependent responses to IG content in prey tissues. For example, while *J. coenia* larvae with high IG concentrations were more likely to be rejected and escape attacks by ants than those containing low levels of sequestered IGs (de la Fuente et al. 1995, Dyer and Bowers 1996), no concentration threshold differences were observed for *Phidippus audax* (Araneae: Salticidae) preying on *J. coenia* of varying IG content (Strohmeyer et al. 1998). Finally, social vespid wasps, considered major predators of lepidopteran larvae (Richter 1990, 2000), not only

have shown a reduced preference for IG sequestering prey, but also, colonies perform poorly when forced to consume them (Stamp 2001, Stamp and Meyerhoefer 2004). Specifically, two studies assessing the effect of *J. coenia* consumption by the social paper wasp *Polistes fuscatus* (Vespidae) demonstrated that IGs can have major negative fitness effects on the wasps by decreasing number of eggs produced, mean weight of female workers, and proportion and size of males (Stamp 2001, Stamp and Meyerhoefer 2004). Therefore, substantial evidence indicates that, for several specialist herbivores and in particular for Buckeye butterflies, consumption of plants containing IGs not only significantly decreases their predation risk but they may also confer physiological costs to predators that consume them.

Parasitoids, because they consume and utilize another animal as the sole source of nutrition and microhabitat throughout development, are also particularly sensitive to the quality of their host. Therefore, it is not surprising that plant allelochemicals in herbivore diets may translate into negative effects on the survival, development, size, and fitness of these antagonists (Ode 2006). Negative effects of IGs on endoparasitoids' prey choice and subsequent fitness would be expected in this system, as specialist herbivores that sequester IGs have been reported to contain high amounts of IGs in their hemolymph (i.e. up to 25 or 50% dry wt. Theodoratus and Bowers 1999, Bowers 2003), and generalist herbivores tend to show decreased performance in response to increasing IG content in their diets (Bowers and Puttick 1988, Puttick and Bowers 1988, Biere et al. 2004, Harvey et al. 2005, Beninger et al. 2008, Reudler Talsma et al. 2011). However, in contrast to predators, recent studies assessing the bottom-up effects of plant IGs on parasitoid survival and performance indicate neutral to only minimal or positive effects. Neutral to minimal effects have been reported for both generalist (Lampert and Bowers 2010b, Reudler Talsma et al. 2011, Smilanich et al. 2011) and specialist herbivores (Lampert et al. 2010, Reudler Talsma et al. 2011). In the case of the generalist *Trichoplusia ni* (Noctuidae), lack of negative effects on parasitoid fitness were explained in part due to metabolizing of IGs in the insect gut and a lack of IGs in the hemolymph; thus the

parasitoids did not directly contact IGs in hemolymph (Lampert and Bowers 2010b). In the other examples, although some detrimental effects of IGs on herbivore development time or pupal weight were observed, parasitism rate was mostly unaffected due to a slightly lower to no change in caterpillar immune responses (Reudler Talsma et al. 2011, Smilanich et al. 2011, respectivelly).

Decreased immunocompetence due to high levels of IGs in herbivore diets has shown more pronounced effects on specialist sequestering lepidopterans, leading to the previously mentioned positive effects of IGs on parasitoid fitness. In particular, Smilanich et al. (2009a), using caterpillars reared on diets varying in their IG concentrations and challenged by implanted glass beads, demonstrated that Buckeye larvae reared on diets rich in IGs are more likely to have their immune response compromised than those feeding on diets low in these compounds, suggesting a potential increase in parasitoid survival, performance and fitness as plant IGs increase. Nevertheless, natural variation in field parasitism rates as a function of varying levels of plant allelochemicals and sequestered IGs may be system specific. For example, while parasitism rates under field conditions did not correlate with levels of sequestered catalpol in *Ceratomia catalpa* (Sphingidae) (Lampert et al. 2010), Nieminen et al. (2003) demonstrated that parasitism rates decreased in *Melitaea cinxia* (Nymphalidae) caterpillars feeding on host plants with high levels of IGs. In summary, the patterns described above suggest that predators and parasitoids can exert different selective pressures on herbivore species feeding on IG-containing plant species, suggesting that optimal herbivore feeding choice may depend on the spatiotemporal variation of their natural enemy community.

1.6. Research Overview

This dissertation examined how variation in host plant defensive traits throughout plant ontogeny can modify the direction and magnitude of tri-trophic interactions, using the model system *Plantago lanceolata* (Plantaginaceae), the specialist sequestering herbivore *Junonia*

coenia (Nymphalidae), and the natural enemies of this herbivore (i.e. predators and parasitoids). In particular, my main objective was to assess the extent to which ontogenetic changes in host plant defenses and nutritional quality can alter plant-herbivore interactions via three main mechanisms: (i) varying plant ability to respond to herbivore damage (i.e. tolerance and resistance traits), (ii) varying herbivore host selection and performance, and (iii) varying herbivore predation risk from predators and parasitoids.

In Chapter 2 of my dissertation, I present the results of a greenhouse study describing ontogenetic patterns in several traits relevant to insect herbivores in *P. lanceolata*. Specifically, using seven plant age classes, I report variation in plant biomass, root:shoot ratios, nutritional quality (i.e. water and nitrogen concentrations) and constitutive levels of chemical defenses (i.e. iridoid glycosides) for both above- and below-ground tissues. In addition, I also present data evaluating whether resource allocation constraints, namely root:shoot ratios, would allow me to explain whole-plant investment in chemical defenses over time. In Chapter 3, I present the results of a related greenhouse experiment that examined how herbivore-induced plant responses to herbivory (i.e. tolerance and resistance traits) change as a function of plant developmental stage. This study, using four juvenile age classes, also provides evidence on the mechanisms that can explain the often complex responses observed, specifically assessing the role of plant vigor in tolerance and resistance traits as plants age. Furthermore, even though in most natural systems herbivore attack is seasonal, surprisingly little is known about how plant responses to herbivory change with successive damage events, and even less about how that might interact with age-dependent herbivore responses to herbivory. Thus, in Chapter 4, I studied the combined role of ontogenetic changes in plant growth and defenses with variation in the frequency and/or intensity of damage over a season. Finally, in order to assess whether these ontogenetic patterns in constitutive and induced defenses in *P. lanceolata* can significantly alter interactions with higher trophic levels, I explored in Chapter 5 the bottom-up effects of ontogenetic changes in plant traits on: *J. coenia* adult butterfly oviposition choice,

caterpillar performance, and caterpillar defensive chemistry to investigate susceptibility to generalist predators as well as to parasitoid attack. Lastly, in Chapter 6, I summarized the main conclusions of my dissertation research.

This work documents some of the complex ecological consequences of temporal variation in plant traits for plant-herbivore-natural enemy interactions. Despite the longstanding interest in understanding temporal variation in herbivore damage, the direct and indirect mechanisms that could explain these patterns have been rarely investigated. Here, results from my experiments support previous assessments that plant age can strongly shape plant constitutive and inducible defenses. Moreover, my results reveal that ontogenetic patterns in plant resistance and/or susceptibility to damage can have strong effects on adult oviposition choice, larval performance and larval susceptibility to predators and parasitoids. Furthermore, I illustrate that bottom-up effects on higher trophic levels may vary among functional groups with the direction and magnitude of the interaction varying between predators and parasitoids. Hence, this research provides a framework for testing hypotheses related to the evolution of plant defenses and the context dependency of bottom-up and top-down controls of herbivore populations.

CHAPTER 2

CHANGES IN PLANT CHEMICAL DEFENSES AND NUTRITIONAL QUALITY AS A FUNCTION OF ONTOGENY IN *PLANTAGO LANCEOLATA* (PLATAGINACEAE) [1]

ABSTRACT

Numerous empirical studies have examined ontogenetic trajectories in plant defenses but only few have explored the potential mechanisms underlying those patterns. Furthermore, most documented ontogenetic patterns in plant defenses have generally concentrated on aboveground tissues; thus, our knowledge regarding whole-plant trends in plant defenses throughout development or potential allocation constraints between growth and defenses is limited. Here, I document changes in plant biomass, nutritional quality and chemical defenses for below- and aboveground tissues across seven age classes of *Plantago lanceolata* (Plantaginaceae) to evaluate: (1) partial and whole-plant ontogenetic trajectories in constitutive chemical defenses and nutritional quality, and (2) the role of resource allocation constraints, namely root:shoot ratios, in explaining whole-plant investment in chemical defenses over time. Overall investment in iridoid glycosides (IGs) increased significantly, while water and nitrogen concentrations in shoot tissues decreased with plant age. Significant variation in IG concentrations between shoot and root tissues across development was observed: allocation of IGs into root tissues linearly increased from younger to older plants, while non-linear shifts in allocation of IGs during ontogeny were observed for shoot tissues. Finally, root:shoot ratios only weakly explained overall allocation of resources into defenses, with young stages showing a positive relationship, while older stages showed a negative relationship between R:S ratios and IG concentrations. Ontogenetic changes in plant quality and defenses within and among plant tissues can strongly influence insect herbivores' performance and/or predation risk; thus, they

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are likely to play a significant role in mediating species interactions.

INTRODUCTION

Plant susceptibility to herbivory, as well as the impact that herbivore damage has on plant fitness, vary markedly over a plant's lifetime. For example, variation in herbivore damage from the seedling stage to senescence can change by several orders of magnitude for the same plant-herbivore interaction (e.g. Pires and Price 2000, Fonseca et al. 2006, Loney et al. 2006), and may even involve considerable changes in the composition of the herbivore community inflicting damage throughout plant ontogeny (Waltz and Whitham 1997, Boege 2005a, Thomas et al. 2010). Similarly, the impact of herbivore damage on plant fitness also depends on the ontogenetic stage being attacked, ranging from complete compensation by the host plant to long-lasting reductions in flower production and fruit set (Warner and Cushman 2002, Boege et al. 2007, Hanley and Fegan 2007). As a result, the selective pressure that herbivores exert on plant traits that confer resistance should vary with plant ontogeny, leading to qualitative and/or quantitative changes in plant resistance traits during plant development. Despite the relevance that assessing developmental changes in plant defenses may have for the improvement of plant defense theories, as well as our understanding of ecological interactions, relatively little is known about how allocation of resources to plant defenses varies throughout plant development (Boege and Marquis 2005, Hanley et al. 2007).

Numerous models have been proposed to explain patterns of variation in plant constitutive chemical defenses as a function of plant development. With the exception of boreal woody plants, where the ontogenetic trajectories in plant defenses have been proposed to strongly reflect the selective pressure exerted by mammalian herbivores (Bryant et al. 1992), most arguments propose that intrinsic forces, namely resource acquisition and allocation constraints, are responsible for ontogenetic variability in plant defenses (Bryant et al. 1991, Herms and Mattson 1992, Boege and Marquis 2005). Among these, the theoretical model

proposed by Boege and Marquis (2005) is the more comprehensive and detailed model, encompassing all stages from cotyledon to senescent plants at the whole plant level. This model predicts that multiple resource allocation and architectural constraints throughout plant development will lead to a non-linear relationship between plant age and defenses. Specifically, the model predicts relatively higher levels of defenses in younger over older seedlings until resources stored in cotyledons and seeds are depleted, followed by a decrease in defenses in response to large root:shoot ratios and a disproportional allocation of resources to the production of photosynthetic area. Once root:shoot ratios start to decrease, growth rate might be more limited than photosynthetic rate allowing for a significant increase in defenses throughout juvenile stages. Finally, when plants reach reproductive maturity, constraints due to reproduction may again result in decreased levels of defenses or they may remain constant depending on the plant's reproductive strategy (Boege and Marquis 2005). A recent meta-analysis conducted by Barton and Koricheva (2010), including 116 studies and 153 plant species, concluded that ontogenetic trajectories in plant defenses tend to show non-linear patterns. However, while non-linearity appears to be the norm, the shape of the relationship seems to depend on plant life form (i.e. woody plants, herbs or grasses) and defensive traits (i.e. physical traits, chemical traits, tolerance) (Barton and Koricheva 2010).

The absence of a generalized pattern describing the ontogeny of plant defenses suggests that allocation constraints and costs of defenses through plant development are not consistent across all plant life forms and defensive traits. Nevertheless, despite the large number of studies documenting ontogenetic patterns in plant defenses, only a few have tested the potential mechanisms driving these patterns, such as those looking at age-specific allocation costs and trade-offs between growth and defenses (e.g. Goodger et al. 2006, Barton 2007, Boege et al. 2007, Orians et al. 2010). Hence, the potential of differences in allocation constraints among grasses, herbs and woody plants, as well as among different kinds of defensive traits, as the source of discrepancies in ontogenetic patterns of defenses remains

unclear. Furthermore, inconsistency among results from investigations of ontogenetic patterns of plant defense might reflect the lack of studies assessing ontogenetic trajectories at the whole plant level. Most studies to date concentrate on aboveground tissues, especially leaves, while ontogenetic patterns in root defenses have been largely ignored. However, the few studies that have explored ontogenetic changes in below- and aboveground tissues have consistently documented dissimilar ontogenetic patterns of defenses among tissues (Hartmann and Zimmer 1986, Williams and Ellis 1989, Bellostas et al. 2007, Beninger et al. 2009). Therefore, without a complete assessment of defensive traits at the whole plant level, meaningful variability within and among tissues during plant development may obscure overall patterns of plant investment in defenses over time, as well as potential trade-offs between growth and defenses.

In summary, in the last two decades, numerous attempts to predict theoretical patterns and drivers of ontogenetic variation in constitutive plant defenses have been made; yet, few studies to date have empirically tested the relationship between plant development and defenses across multiple developmental stages and plant tissues; and/or tested the mechanisms driving these patterns. In this study, I used seven pre-reproductive age classes of *Plantago lanceolata* L. (Plantaginaceae) to investigate ontogenetic variation in below- and aboveground constitutive plant chemical defenses (iridoid glycosides), and the role of resource allocation constraints as the potential mechanism driving these ontogenetic trajectories. In addition, I assessed ontogenetic variation in other plant traits relevant to herbivores, including leaf water and nitrogen concentration, as well as below- and aboveground biomass. Because my interest was to assess overall variation in constitutive trajectories at the whole plant level, I did not assess here within-tissue variation (e.g. young, intermediate and old leaf ages) which may add considerable variation in levels of defenses and nutritional quality for herbivores (e.g. Bowers and Stamp 1992, 1993). In particular, I addressed the following three questions: (1) How do plant traits relevant to herbivores, including constitutive chemical defenses and nutritional quality, change throughout plant development?; (2) Do plant chemical defenses in

below- and aboveground tissues follow similar ontogenetic trajectories?; and (3) Can root:shoot ratios help to predict the allocation of resources to constitutive chemical defenses at the whole plant level?

MATERIALS AND METHODS

Study system

Plantago lanceolata is a cosmopolitan, short-lived perennial herb with a rosette growth form introduced to North America from Eurasia more than 200 years ago (Cavers et al. 1980). This plant species produces, in addition to iridoid glycosides (Ronsted et al. 2000), a number of bioactive phenolic compounds such as flavonoids and phenylethanoid glycosides (Tamura 2001, Galvez et al. 2005). However, iridoid glycosides are the primary allelochemicals found in large concentrations in both below- and aboveground tissues and influencing feeding preferences of generalist and specialist rhizophagous and folivorous herbivores (e.g. Bowers and Puttick 1989, Biere et al. 2004, De Deyn et al. 2004); thus, here I specifically focused on the ontogenetic changes of these compounds.

Iridoid glycosides (IGs) are a group of cyclopentanoid monoterpene-derived compounds that occur in over 50 families of plants (Bowers 1991, Jensen 1991). *Plantago lanceolata* produces primarily two IGs: aucubin and catalpol, and amounts of these compounds may be as high as 10-12% dry weight (Bowers and Stamp 1992, 1993). High concentrations of IGs, especially of catalpol, indicate not only a greater biosynthetic investment by the plant, but also may result in a higher unpalatability and/or toxicity to generalist herbivores (Bowers and Stamp 1993). Although we lack specific knowledge regarding IG production sites (i.e. shoots vs. roots), these carbon-based defenses are synthesized through the isoprenoid biosynthetic pathway and are usually stored in cell vacuoles (Croteau 1987). They are also likely phloem mobile (Gowan et al. 1995, Beninger et al. 2007) and concentrations in leaves and roots in *P. lanceolata* were found to be positively correlated (Beninger et al. 2009). Previous studies have identified plant

age as an important intrinsic factor affecting *P. lanceolata* chemistry (e.g. Bowers and Stamp 1993, Fuchs and Bowers 2004, Barton 2007); and these ontogenetic patterns in IG production significantly vary among both maternal families and populations (Bowers and Stamp 1993, Barton 2007).

In addition, *P. lanceolata* has been incorporated into the diet of many North American insect herbivores and thus, multiple specialist and generalist herbivores can affect and be affected by IG content in this species. For example, high IG concentrations usually deter generalist herbivores feeding on shoot or root tissues (e.g. Bowers and Puttick 1989, Biere et al. 2004, De Deyn et al. 2004, Wurst and van der Putten 2007). Yet, several specialist herbivores use IGs as feeding and/or oviposition stimulants (e.g. Bowers 1984, Pereyra and Bowers 1988, Reudler Talsma et al. 2008b), and some have the ability to sequester these compounds from leaves and roots and use them for their own protection against natural enemies (e.g. Bowers and Puttick 1986, Baden and Dobler 2009, Opitz et al. 2010). Furthermore, although most herbivores associated with this species are either rhizophagous or folivorous, there is at least one example of a species of *Longitarsus* (Chrysomelidae) flea beetles that feeds on and sequesters IGs from both tissues, as larvae and adults (Willinger and Dobler 2001, Baden and Dobler 2009). As a result, ontogenetic patterns in constitutive variation of shoot and root IGs in this species can have profound consequences on the identity, abundance and performance of the herbivore community associated with it throughout its lifetime.

Ontogenetic variation in plant traits

In order to evaluate the effect of plant ontogeny on plant quality and chemical defenses, seven distinct age classes of *Plantago lanceolata* (3, 5, 8, 10, 12, 15, and 18 weeks old since germination) were grown at the University of Colorado greenhouse during the summer of 2008. The first two stages (3 and 5 wk-old) correspond to two distinctive seedling stages, the next three stages (8, 10 and 12 wk-old) correspond to juvenile stages, and the last two age classes

(15 and 18 wk-old) to pre-reproductive stages. All age classes were harvested simultaneously in order to control for confounding environmental factors that could influence plant performance such as photoperiod and temperature. Synchronization among ontogenetic stages occurred by germinating seeds at intervals of 15-30 days from March to July. In this way, although average environmental conditions from sowing to harvest varied among age classes, I ensured that all individuals were exposed to the same conditions at harvest time. Since IG concentrations in this as well as other IG containing plant species, can vary substantially over days and even hours (Hogedal and Molgaard 2000, Fuchs and Bowers 2004), or be influenced by temperature (Tamura 2001), simultaneous harvest across all age classes should minimize variables other than plant age that could influence IG content.

Seeds were collected from >20 maternal plants from a population in Boulder County, Colorado, mixed, and randomly assigned to treatments. Seeds were germinated in Fafard mix and transplanted to a growth medium of equal parts Metro Mix 350, sterilized sand, and turface after 15 days. Plants were grown in extra-deep 13 liter pots in order to minimize root-binding effects. I grew 20 plants in each age class, for a total of 140 plants. Replicates were randomly placed on a single 1.2 x 8m greenhouse bench, exposed to natural day light, and watered daily. Fertilizer was supplied to all plants every three to four days throughout the length of the experiment. The fertilizer solution was the standard solution used in the University of Colorado greenhouses, which was Scotts Peter's Excel (Scotts-Sierra Horticultural Products Company, Marysville, Ohio) mixed on a ratio of 15-5-15 N-P-K which also provided trace amounts of micronutrients. Temperatures fluctuated over the course of the season but greenhouse temperatures did not exceed 32°C or fall below 15°C at any time. Extra-deep pots were used in order to ensure natural growth rate of root as well as shoot tissues and to prevent pot-binding, which can affect plant secondary metabolite production (Baldwin 1988). However, I believe that the combination of abundant resources and nutrient availability together with moderate

greenhouse temperatures delayed the onset of flowering (usually recorded at 15wks after sowing under greenhouse conditions, pers. obs.) during this first growing season.

At harvest, plants were separated into roots and shoots. Shoot tissues represented mostly leaves but in few of the oldest plants (N = 14) these also contained immature inflorescences accounting for less than 1% of the total shoot biomass. Statistical analyses with and without these fourteen individual plants yielded similar results; thus, I present the results for the complete data set. All plant tissues were weighed fresh immediately following their harvest, oven-dried at 50°C for 48hrs to a constant mass and weighed again to the nearest 0.01g. The ratio between root and shoot dry biomass was calculated for each individual plant.

Analyses of plant water, nitrogen and iridoid glycoside concentrations

To assess variation in plant quality throughout development, I measured plant water and nitrogen concentrations. Percent water was calculated as [(wet weight –dry weight) / wet weight] x 100. Nutritional quality was measured as total leaf nitrogen, and quantified by Micro-Dumas combustion on a NA1500 C/H/N analyzer, using a sample size of 10 plants per age class. Due to the low biomass of plants at the 3wk stage, I was unable to measure leaf nitrogen concentrations for those plants.

To assess concentrations of IGs, roots and shoots were separately ground into a fine powder, and 2-50mg subsamples (entire above- and/or belowground tissue for some seedlings) were processed for IG extraction and analyzed by gas chromatography following previously described methods (Bowers and Stamp 1993, Barton 2007). Briefly, samples were extracted overnight in 95% methanol, the methanol extract was filtered to remove the plant material and the residue evaporated to dryness. Phenyl-β-D-glucose (PBG) was added as an internal standard and the samples were then partitioned between water and ether to remove hydrophobic compounds. An aliquot of the remaining solution was removed, evaporated, and derivatized with Tri-Sil-Z^{TM} (Pierce Chemical Company) and injected into a HP 7890A gas

chromatograph (Agilent Technology) using an Agilent DB-1 column (30m, 0.320mm, 0.25µm particle size). Amounts of aucubin and catalpol were quantified using ChemStation B-03-01 software and they are presented as percent dry weight for comparative purposes (e.g. Fuchs and Bowers 2004, Barton 2007, Wurst and van der Putten 2007).

Statistical analyses

Developmental variation in root and shoot biomass, Root:Shoot ratios (hereafter R:S), and percentage of water and nitrogen in aboveground tissues were analyzed using one-way ANOVAs, followed by Bonferroni post-hoc tests to distinguish mean differences among age classes if overall significant differences were found. Variation in production of IGs across plant ontogeny was first assessed simultaneously for both plant tissues, shoots and roots, and both iridoids, aucubin and catalpol. Because aucubin and catalpol are correlated and their concentrations are also potentially correlated between shoot and root tissues, I used multivariate analysis of variance (MANOVA) to examine variation in these four variables due to plant age. When significant effects were detected by MANOVA, I followed up with univariate ANOVAs for each of the four variables. Bonferroni post-hoc tests were used to assess significant differences among the seven age classes. Lastly, I did a repeated measures ANOVA in order to test overall variation in total IGs (aucubin + catalpol) among age classes, between plant tissues (shoots vs. roots; the repeated measure), and their interaction. Water, nitrogen, and IG concentrations as well as R:S ratios were arcsine square-root transformed to meet assumptions of normality. Biomass measures did not require transformation.

To assess whether investment in chemical defenses at the whole plant level as well as for below- and aboveground tissues showed a linear or non-linear increase with plant age, I regressed the concentration of total IGs (aucubin + catalpol) at the whole plant level (shoots + roots), as well as at the shoot and root tissue level separately, on plant age and tested whether the linear and quadratic slopes associated with age differ significantly from zero. Because age is

treated as a continuous variable, for these analyses I first needed to center this predictor variable (Judd et al. 2009). Then, to evaluate whether the shape of the curve of total IG concentrations as a function of plant age differ between shoot and root tissues, I first computed a new dependent variable that describes the difference in chemical defenses between tissues (i.e. within-subject difference variable IG_{diff} = IGs shoots – IGs roots) and regressed it on plant age. When a within-subject difference is regressed on a between-subject predictor, the resulting slopes will be testing for an interaction between the within-subject independent variable (tissue) and the between-subject one (age). Thus, the linear and quadratic term in this case will assess whether the linear or quadratic effect of age on plant defenses interacts with plant tissue (i.e. whether the linear and quadratic slopes for shoot and root tissues differ from each other) (Judd et al. 2009).

Finally, in order to assess whether R:S ratios can help to explain total IG production across developmental stages, I performed a linear regression to test the prediction of a negative relationship between R:S ratios and plant investment in defenses as proposed by Boege and Marquis (2005). In addition, in order to test whether the relationship between R:S ratios and IG concentrations shifts with plant age, I performed an analysis of covariance with total plant IG levels as a function of age using R:S ratios as a covariate and testing for an age by R:S ratio interaction. For the first regression I used all seven plant stages (N = 140), then after I confirmed a significant interaction between age and R:S ratios (see Results), I performed two separated linear regressions for younger life stages (age classes 3, 5, and 8wk-old, N = 60) and older life stages (age classes 10, 12, 15, and 18wk-old, N = 80).

RESULTS

There were pronounced differences in plant biomass, nutritional quality and chemical defenses among the seven *Plantago lanceolata* age classes. Plant age significantly influenced total plant biomass ($F_{6,132}$ = 103.4, P<0.0001) and R:S ratios ($F_{6,132}$ = 8.9, P<0.0001) (Figure 2.1a, b). As

expected, overall biomass of both shoot and root tissues increased with plant age; however, some exceptions can be observed such as lower biomass in shoots of the 15wk-old class and in roots of the 18wk-old class than previous age classes (Figure 2.1a). Given my experimental design, this unexpected variation is unlikely to be the result of potential genetic biases or limited resources altering growth rates. Furthermore, because I did not follow individual plants during a growing season but rather harvested all age classes simultaneously from cohorts germinated at different times from March to July, these changes in biomass cannot be interpreted as loss of biomass. Instead, they may have emerged as a result of differential average environmental conditions (i.e. day length and temperature) shaping developmental trajectories in biomass partitioning, as seen in other systems (McConnaughay and Coleman 1999, Moriuchi and Winn 2005). Nevertheless, after performing all statistical analyses with and without these age classes, because overall patterns remained the same (except where indicated), I present here the results of the complete data set.

Plant age significantly influenced shoot nutritional quality measured as percentage of water ($F_{6,133} = 85.5$, $P<0.0001$) (Figure 2.1c), and nitrogen ($F_{5,54} = 34.2$, $P <0.0001$) (Figure 2.1d), both of which declined as plants aged. Mean water (Figure 2.1c) and nitrogen (Figure 2.1d) concentrations were significantly higher for seedling and young juvenile stages (i.e. 3 to 8 wk-old) than older juvenile and pre-reproductive plants (i.e. 10 to 18wk-old). In contrast, constitutive production of total IGs (aucubin + catalpol) increased over time, ranging from as low as 0.003 to as high as 8.86% dry weight from seedling to pre-reproductive plants, respectively. Aucubin and catalpol in shoot and root tissues varied considerably throughout plant development as shown by the MANOVA (Wilks' $\lambda = 0.229$, $F_{4,126} = 9.67$, $P < 0.0001$) (Figure 2.2). Univariate ANOVAs also demonstrated a significant effect of plant age on shoot aucubin and catalpol concentrations ($F_{6,129} = 43.31$, $P < 0.0001$ and $F_{6,129} = 11.63$, $P < 0.0001$, respectively) as well as on root aucubin and catalpol concentrations ($F_{6,129} = 16.44$, $P < 0.0001$ and $F_{6,129} = 5.26$, $P < 0.0001$, respectively).

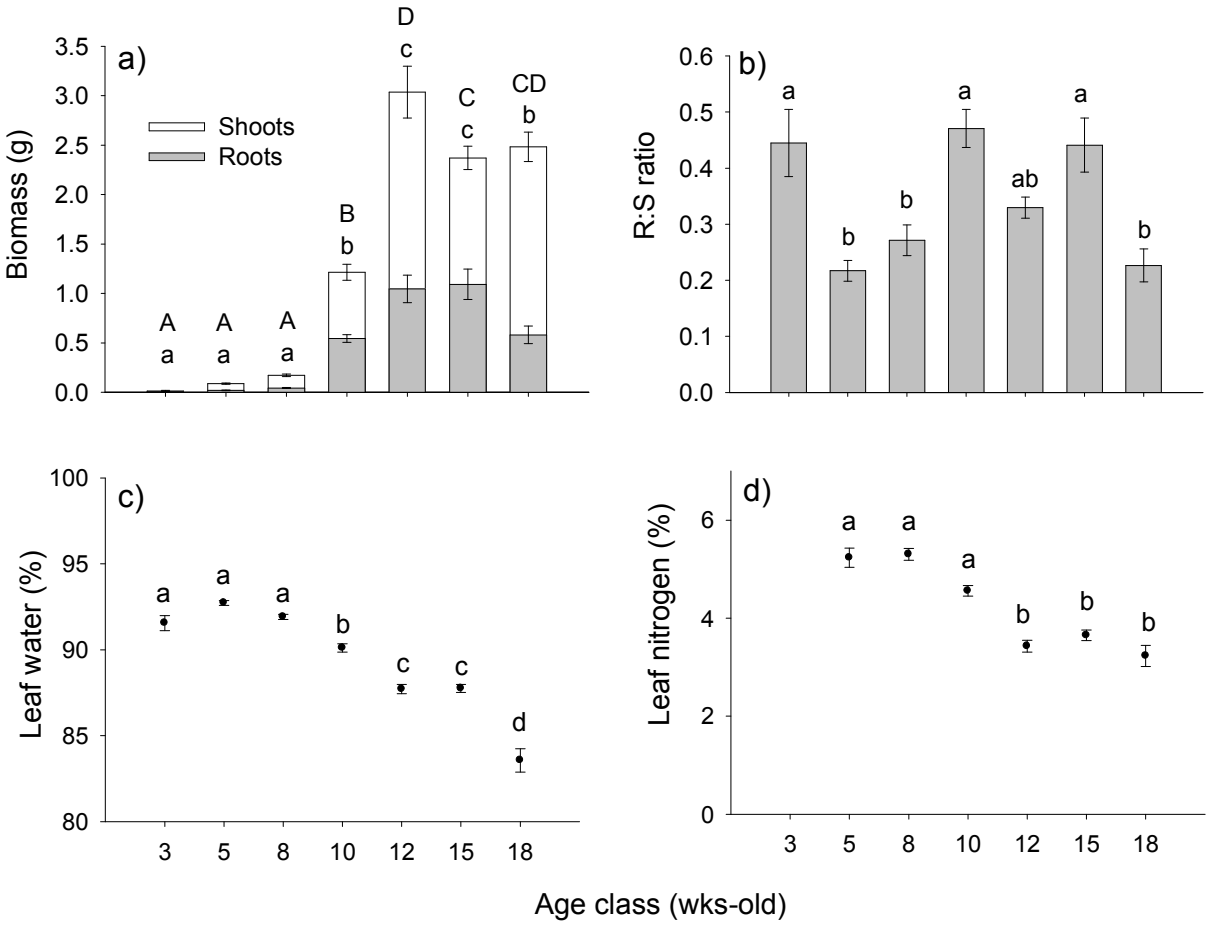


Figure 2.1. *Plantago lanceolata* ontogenetic variation (Mean ± 1SE) in **a)** shoot and root biomass, **b)** Root:Shoot ratios, and leaf nutritional quality measured as **c)** percentage water, and **d)** percentage nitrogen across seven age classes. Except for biomass, original data were arcsine-square root transformed for statistical analyses; actual values are shown for illustrative purposes only. Letters indicate mean group differences as tested by a Bonferroni post-hoc test ($p<0.05$). In panel a) capital letters were used to represent group mean differences for shoot biomass and lower-case letters were used to represent group mean differences for root biomass.

In turn, repeated measures ANOVA demonstrated that total IG concentration was significantly influenced by plant age ($F_{6,129}$ = 38.6, P < 0.0001) and plant tissue ($F_{1,129}$ = 104.3, P < 0.0001), with higher IG concentrations in shoot compared to root tissues and overall increase in IGs as plants aged, although in a non-linear fashion (Figure 2.2). Furthermore, there was a significant interaction between plant age and tissue ($F_{6,129}$ = 26.02, P < 0.0001), indicating that differences in IG concentrations between shoot and root tissues changed throughout plant development. Thus, three main trends emerged from these data. First, concentrations of IGs in shoot tissues were, on average, three times higher than were concentrations in root tissues. Second, mean group differences in aucubin and catalpol among all seven age classes followed similar trends for both shoot (Figure 2.2a) and root tissues (Figure 2.2b). Third, aucubin concentrations in shoot tissues drive most of the changes in total IGs throughout plant ontogeny.

Regression models demonstrated that constitutive variation in total IGs increased non-linearly with plant age for shoots and linearly for roots, indicating that ontogenetic patterns in defenses between tissues were dissimilar (Figure 2.3). At the whole plant level, ontogenetic patterns in constitutive IG production were also non-linear (Table 2.1, Figure 2.3a). This non-linear trend was mostly driven by shoots since partial investment in defenses for shoot tissues also showed a significant linear and quadratic term (Table 2.1, Figure 2.3b); but roots, with significantly less variation across age classes than shoots, showed a significant linear increase in IGs with plant age, but not a significant quadratic trend (Table 2.1, Figure 2.3c). Furthermore, the shape of the curves describing IG concentrations as plants age differed between tissues, demonstrated by a significant interaction between plant tissue and the linear or quadratic effect of age on plant defenses (for IG_{diff} linear t = 7.72, P <0.0001; IG_{diff} quadratic t = 6.37, P <0.0001). Specifically, I found that the linear and quadratic effect is significantly stronger in shoots than in roots, indicating that, as plants age, more abrupt differences in IG concentrations can be observed for shoots than for roots (see Figure 2.3).

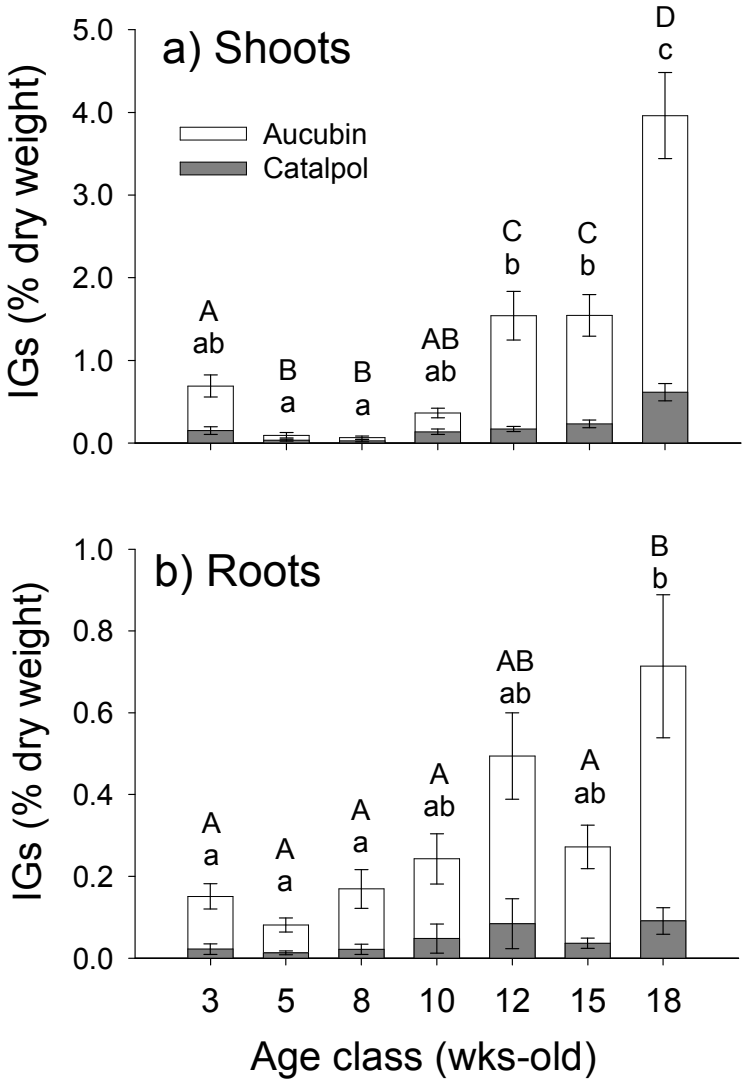


Figure 2.2. *Plantago lanceolata* ontogenetic variation (Mean ± 1SE) in constitutive defenses measured as proportion of aucubin and catalpol in **a)** shoot and **b)** root tissues. Original data were arcsine-square root transformed for statistical analyses, percentage data are shown for illustrative purposes only. Letters indicate mean group differences among age classes as tested by Bonferroni post-hoc tests ($p<0.05$). Capital letters were used to represent group mean differences for aucubin and lower-case letters were used to represent group mean differences for catalpol.

Table 2.1. Total IG concentrations at the whole plant level, as well as separately for shoots and roots, regressed as a function of plant age in *Plantago lanceolata*, from 3 to 18wks-old plants. Parameter estimates (β) for the constant and the linear and quadratic slopes are presented. Slopes significantly different from zero are highlighted in bold type.

	Whole plant			Shoots			Roots		
	β	t	p	β	t	p	β	t	p
Constant	0.060	12.4	**0.0001**	0.063	11.1	**0.0001**	0.048	14.1	**0.0001**
Age									
Linear	0.008	12.6	**0.0001**	0.010	12.1	**0.0001**	0.003	6.7	**0.0001**
Quadratic	0.001	7.8	**0.0001**	0.001	7.2	**0.0001**	0.000	0.9	0.33

Figure 2.3. Proportion of total IGs (arcsine sqrt transformed dry weight) for each individual plant measured (N=140, 20 per age class) at the **a)** whole plant level as well as separated for **b)** shoot and **c)** root tissues regressed across seven pre-reproductive age classes of *Plantago lanceolata*. Lines of best fit for linear (solid) and quadratic (dashed) models are graphed for illustrative purposes.

Finally, variation in R:S ratios across all age classes did not follow the negative association predicted by Boege and Marquis (2005) to explain plant allocation of resources to chemical defenses (slope = -0.09, R^2 = 0.001, P = 0.29). Similarly, the ANCOVA showed that total IG concentrations at the whole plant level were not explained by R:S ratios once age was included in the model (R:S ratio: $F_{1,120}$ 0.03, P = 0.85; plant age: $F_{6,120}$ 28.4, P <0.0001); but a significant age by R:S ratio interaction was observed ($F_{1,20}$ 5.61, P <0.0001), indicating that the relationship between R:S ratios and IGs varied among age classes. Because seedling R:S ratios were not higher than R:S ratios of older age classes (Figure 2.1d) and a significant age by R:S ratio interaction was detected, two separate linear regressions were run on the 3 to 8 wk-old individuals and the older 10 – 18 wk-old individuals. In this case, although much of the variation in IGs across plant age classes is still poorly explained by differences in R:S ratios, an interesting pattern arose. For seedling and young juvenile stages, contrary to predictions by the non-linear model, larger R:S ratios had a weak but significant positive relationship with proportion of total IGs (slope = 0.31, R^2 = 0.08, P = 0.02) (Figure 2.4a). In contrast, for older juvenile and pre-reproductive stages, the decrease in R:S ratios as plants grew showed a significant negative relationship with plant investment in defenses (slope = -0.47, R^2 = 0.22, P < 0.0001) as predicted by the model (Figure 2.4b). However, this negative relationship is mostly driven by the older pre-reproductive age class (18wk-old), as removal of this stage leads to a non-significant relationship between R:S ratios and IG concentrations in older stages (slope =0.04, R^2 = 0.002, P = 0.74).

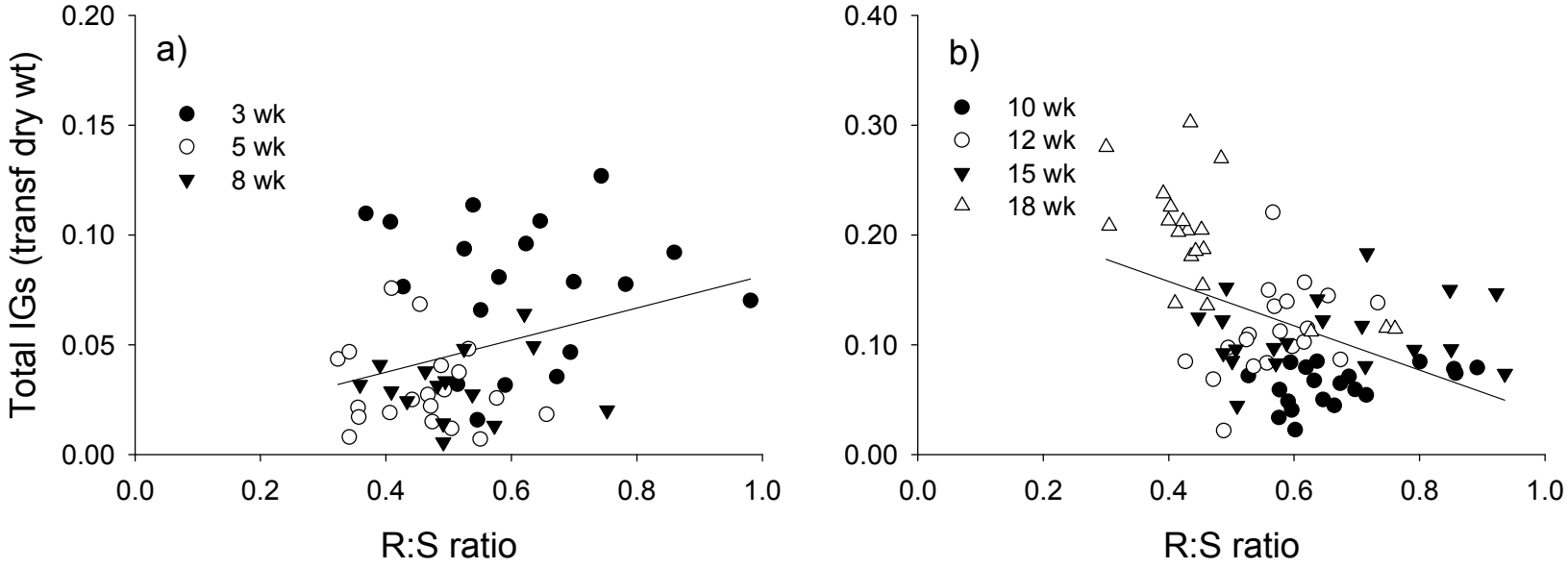


Figure 2.4. Linear regression between R:S ratios and proportion of total IGs production across **a)** young life stages (3, 5 and 8wk-old plants), and **b)** older life stages (10, 12, 15 and 18 wk-old plants). Proportion of total IGs at the whole plant level were arcsine-square root transformed.

DISCUSSION

Plant defense theories predict that defenses should be allocated to plant tissues as a function of the value of those tissues for plant fitness and their susceptibility to damage by natural enemies (Feeny 1976, McKey 1979). Although both the value and the susceptibility to damage of a tissue change as plants develop, few studies to date have explored changes in chemical defenses of different plant tissues across multiple developmental stages. Here, I showed that the defensive chemistry of shoot and root tissues significantly varies during plant development and that this variation is mostly non-linear, as predicted by Boege and Marquis (2005). Thus, my data add to the growing number of empirical studies supporting non-linear trends in ontogenetic trajectories of plant defenses (Barton and Koricheva 2010 and references therein). These ontogenetic trajectories in plant defenses indicate that population level demographics may contribute to the observed spatiotemporal variation in plant defenses, and may help to explain ecological interactions among plants and herbivores (Lawrence et al. 2003, Boege 2005a, Fonseca et al. 2006, Thomas et al. 2010).

In this study, the youngest seedling stage (3wk-old plants), consisting of the cotyledons and up to two true leaves, had twice as much total concentration of IGs than older seedling and young juvenile plants with six to 10 leaves (5 and 8wk-old). Similar to these results and supporting the proposed model by Boege and Marquis (2005), a few other empirical studies reported decreases in plant allelochemicals during early seedling development (Wallace and Eigenbrode 2002, Orians et al. 2010). However, this trend does not seem to apply across taxa (see Barton and Koricheva 2010), and is slightly different from a previous study showing positive ontogenetic trajectories in IGs in *P. lanceolata* seedlings (Barton 2007). Following this initial decrease, my study showed that allocation of resources to defenses during the juvenile stages (from 8 to 18wk-old) increased almost exponentially, from an average of less than 0.4% to over 4% dry weight (Figure 2.2). This result agrees with the well documented trend of

increases in plant defenses during immature plant stages (Barton and Koricheva 2010), also supporting the Boege and Marquis (2005) model.

Given that plant secondary compounds are differentially synthesized and stored among plant tissues, whole-plant assessment of defenses is needed for understanding plant partial and total investment of defenses over time. In this system, concentration of IGs were three times larger in shoot as compared to root tissues across all developmental stages, suggesting that resource allocation to defenses in aboveground tissues may prevail over belowground tissues during *P. lanceolata* development. Furthermore, although not as pronounced as in other systems, the pattern of variation in total IGs in leaves and roots differed, indicating that IG concentrations changed differently in shoot and root tissues throughout plant development. Specifically, allocation of IGs into root tissues constantly increased from younger to older plants while non-linear shifts in allocation of IGs during ontogeny were observed for shoot tissues (Table 2.1, Figure 2.3). Differential ontogenetic patterns between below- and aboveground tissues were also observed in other systems (Hartmann and Zimmer 1986, Williams and Ellis 1989, Bellostas et al. 2007, Beninger et al. 2009), but no consistent trends can be described. For example, in two IG containing plants, *Linaria vulgaris* and *Antirrhinum majus*, Beninger et al. (2009) reported a linear decrease as well as a lack of variation in root iridoids from seedling to reproductive plants, respectively, while defenses in aboveground tissues of both species seemed to vary in a non-linear fashion. In contrast, total alkaloid concentrations in *Papaver somniferun* reported by William and Ellis (1989) showed significant non-linear trends in both shoot and root tissues, although maximum and minimum peaks did not necessarily match (see also Hartmann and Zimmer 1986). This disparity in ontogenetic trajectories between shoot and root tissues highlights the need to consider all plant tissues available at any stage to accurately test for overall plant investment in defenses throughout development.

Changes in resource allocation among plant tissues during ontogeny reflect variation in growth rate, physiological and anatomical constraints, as well as changing priorities (e.g. growth vs. reproduction) of an organism during the course of its development. Thus, researchers have used these allocation patterns, such as R:S ratios, to predict resource allocation to plant defenses (e.g. Boege and Marquis 2005). Here, I demonstrate that R:S ratios can partially help to predict ontogenetic changes in plant defenses. While juvenile to pre-reproductive plants follow the predicted trend of a negative relationship between R:S ratios and constitutive defenses (Figure 2.4b), allocation of defenses in early ontogenetic stages showed the opposite trend (Figure 2.4a). The early decrease in IGs as well as R:S ratios from young seedling to young juvenile stages may reflect a trade-off between investment in photosynthetic area versus defenses or simply a dilution effect due to higher growth rate compared to biosynthetic synthesis of chemical defenses. Following this initial positive relationship, the decrease in R:S ratios was associated with an increase in IGs, which is consistent with an increase in synthesis of defenses as growth rate becomes more limiting than photosynthetic rate (Herms and Mattson 1992). Furthermore, this shift in the relationship between R:S ratios and IG concentrations may suggest potentially transient costs of defenses, as recently demonstrated by Orians et al. (2010). Nevertheless, these trends need to be interpreted with caution since the amount of variation in constitutive levels of IGs explained by R:S ratios was very low and some trends may be driven by a single age class, indicating that production of IGs depends on several other factors besides the allocation of biomass to different tissues. Alternatively, lack of strong correlations between R:S ratios and IG concentrations may be due to partial assessment of *P. lanceolata* overall investment in defenses. Because *P. lanceolata* also invest in phenolic compounds (Galvez et al. 2005) as well as in physical defenses such as leaf toughness and trichomes (Schippers and Olff 2000, de la Fuente 2002) I cannot rule out the potential predicted value of R:S ratios in overall defenses.

Whatever the mechanism, the ontogenetic variation in IGs described here, encompassing four to five-fold increases in shoot and root tissues, can have important consequences for plant-herbivore interactions. Laboratory and field experiments have shown that specialist butterflies prefer to oviposit on *P. lanceolata* tissues with higher IG concentrations (Prudic et al. 2005, Reudler Talsma et al. 2008b), and that larvae of these herbivores grew better on diets with higher amounts of IGs (e.g. Bowers and Puttick 1989, Harvey et al. 2005, Saastamoinen et al. 2007). In contrast, generalist herbivores and pathogenic fungi damaging below- or aboveground tissues are usually deterred by high IG concentrations (Strohmeyer et al. 1998, Biere et al. 2004, De Deyn et al. 2004, Harvey et al. 2005, Wurst and van der Putten 2007). As a consequence, the relative palatability of *P. lanceolata* to below- and aboveground herbivores and pathogens will significantly vary through the season, and may determine the impact of these antagonists on plant fitness. Lastly, for sequestering specialist herbivores, ontogenetic changes in plant defenses can, in turn, modify herbivore unpalatability and thus, can indirectly mediate tri-trophic interactions (e.g. Strohmeyer et al. 1998, Harvey et al. 2005, Baden and Dobler 2009, Smilanich et al. 2009a). Thus, mainly for specialist herbivores that consume and sequester IGs from single or multiple tissues, these ontogenetic patterns in plant constitutive defenses may impact herbivore population dynamics and community structure through seasonal changes in host selection, herbivore performance, and levels of resistance to natural enemies.

Ontogenetic patterns in leaf nutritional quality were also evident, but the trend was opposite to the one described for chemical defenses, showing a decrease in leaf water and nitrogen concentration as plants aged. Similar decreases in nutritional quality have been observed in numerous plant taxa (e.g. Bowers and Stamp 1993, Gaudet et al. 2001, Goodger et al. 2006, Merilo et al. 2009), directly influenced by plant age or indirectly mediated through their correlation with other ontogenetic shifts in leaf anatomy and physiology (Apple et al. 2002, Ishida et al. 2005). In this study, ontogenetic variation in nutritional quality encompassed an

average decrease of 10% in percentage water and 30% in nitrogen concentration from 3wk to 18wk-old plants (Figure 1a, b). This variation can also have important implications for plant-insect interactions since diets with low water and nitrogen concentrations typically have negative impacts on herbivore host selection, growth rates, and fitness (Mattson 1980, Awmack and Leather 2002). Specifically for this system, similar variation in leaf nitrogen concentrations (~30%) among individuals of *P. lanceolata* resulted in a significant effect on oviposition preferences of the specialist Buckeye butterfly (*Junonia coenia*, Nymphalidae), with females showing preference for high-nitrogen plants (Prudic et al. 2005). Thus, the ontogenetic patterns in nutritional quality showed here may also help to explain temporal variation in species interactions as seen in other systems (e.g. Gaudet et al. 2001, Barrett and Agrawal 2004).

In summary, developmental variation in chemical defenses across *Plantago lanceolata* ontogeny appears to be similar, and sometimes greater, in magnitude than genetic or environmental variation in a single age class (Bowers et al. 1992, Bowers and Stamp 1993, Barton 2007). Recent empirical studies have shown similar patterns in other systems (e.g. Lawrence et al. 2003, Donaldson et al. 2006, Holeski et al. 2009). Thus, these studies highlight the importance of separating the proportion of variation in plant defenses that has a developmental basis from strict genetic or environmentally dependent variation, in order to improve our understanding of the evolution of plant defenses. Furthermore, these ontogenetic patterns in plant defenses and nutritional quality are likely to influence below- and aboveground herbivores alike, providing a potential mechanism to explain temporal variation in herbivore community composition and species interactions over time.

CHAPTER 3

PLANT ONTOGENY AND HERBIVORY: INFLUENCES ON TOLERANCE AND RESISTANCE TRAITS

ABSTRACT

Plants can minimize the negative impact of herbivory via two well-recognized mechanisms: tolerance and resistance. Such herbivore-induced responses can be influenced by numerous extrinsic and intrinsic factors such as resource availability, herbivore identity, plant genotype, and plant developmental constraints. Among these, plant ontogeny has been the less well explored, and the mechanisms underlying the potential age-dependent induced responses are still poorly understood. Here, I assessed how plant ontogeny can alter tolerance and resistance traits in *Plantago lanceolata* (Plantaginaceae), and associated this variation with intrinsic changes in plant relative growth rate (RGR) and allelochemical synthesis rate throughout plant development. Four juvenile age classes of *P. lanceolata* were simultaneously damaged by the specialist herbivore, *Junonia coenia* (Nymphalidae), until ~30% of the available leaf tissue was consumed. Short- and long-term responses in compensatory growth (i.e. tolerance) and induction of iridoid glycosides (= IG) (i.e. resistance), were assessed one, three and five weeks after damage. Plant age significantly influenced the ability of *P. lanceolata* to tolerate or induce defenses after damage. In terms of compensatory growth, intermediate juvenile stages showed a higher compensatory growth capability as compared to younger or older juvenile stages; and this variation seems to be explained, at least in part, by ontogenetic changes in plant RGR. In terms of induced chemical defenses, results showed a complex pattern. While young juvenile plants showed no significant differences in IG concentrations between damaged and control plants, older juvenile stages showed responses ranging from no induction to induced susceptibility or induced resistance. However, the use of graphical vector analyses (GVA) demonstrated that IG synthesis was reduced more, compared with growth rate, in older juvenile

stages, while the opposite was observed for younger juvenile stages. Thus, compensatory growth and induced synthesis of IGs showed constantly fluctuating patterns as plants aged, with some stages being able to employ both strategies, while others employed only one or neither of them. These results demonstrate that shifts in defensive strategies as a function of plant ontogeny can be very complex, but that intrinsic RGR and metabolic rates (i.e. IG synthesis rate) can help predict under which conditions different responses to herbivory should be favored. Furthermore, these results suggest that the outcome of plant-herbivore interactions may significantly vary depending on the timing of herbivory.

INTRODUCTION

Plants can minimize the negative impact of herbivory via two well-recognized mechanisms: *resistance traits* that aim at reducing subsequent herbivore damage and *tolerance traits* that minimize the negative effects of damage on plant fitness (Karban and Baldwin 1997). These responses are typically achieved by enhancing photosynthesis rates, increasing nutrient uptake, up-regulation of defense compound synthesis, more efficient utilization of stored reserves, and activation of dormant meristems, among others (Tiffin 2000). As plants age, several morphological and physiological traits often change from one developmental stage to the next. For instance, while architectural complexity, resource acquisition (i.e. access to water and nutrients) and storage capacity typically increase (Poethig 1990, Malamy 2005, Barthelemy and Caraglio 2007, Niinemets 2010), photosynthesis and growth rate, root:shoot ratios, and hormone production and metabolic activity usually decrease as plants develop (Gedroc et al. 1996, West et al. 2001, Farnsworth 2004, Ishida et al. 2005, Wiedemuth et al. 2005). Hence, it is not surprising that plants' ability to induce chemical defenses and/or tolerate damage vary as plants age (Boege and Marquis 2005, Barton and Koricheva 2010). However, despite the increasing interest in describing ontogenetic trends in plant induced defenses, the mechanisms

that explain the variation observed in tolerance and resistance traits as plants age are still poorly understood.

Tolerance has been defined as the reaction norm of the fitness response of a given genotype to a gradient of herbivore damage (Stowe et al. 2000). However, when genotypes are unknown, the replacement of damaged tissues, termed compensatory growth, represents the most commonly studied plastic response to damage (Mcnaughton 1983, Strauss and Agrawal 1999, Stowe et al. 2000). In terms of plant ontogeny, the capacity to replace the tissue lost to herbivores is predicted to increase as plants age (Strauss and Agrawal 1999, Haukioja and Koricheva 2000), due to a greater number of available meristems, smaller root to shoot ratios, greater capacity to acquire resources, and higher probability of mobilization of stored reserves at older developmental stages. Support for this prediction comes from both woody (e.g. Warner and Cushman 2002, Hodar et al. 2008) and herbaceous plants (Stout et al. 2002, Boege et al. 2007, Tucker and Avila-Sakar 2010, Gruntman and Novoplansky 2011).

Alternatively, there are also numerous cases where higher tolerance or compensatory growth was achieved at younger, compared to older, life stages (e.g. Maschinski and Whitham 1989, Gedge and Maun 1992, Weltzin et al. 1998, Boege 2005b). Decreases in tolerance traits throughout ontogeny may be linked to the decline in metabolic rate and growth rate as plants age. During growth, the resources acquired to fuel metabolism can be allocated to the production of new tissue or to the maintenance of existing ones (West et al. 2001). Thus, as plants age and individual size increases, more carbon needs to be allocated to the maintenance of photosynthetic and non-photosynthetic structural tissues (Enquist et al. 1999, West et al. 2001), resulting in a decrease in relative growth rate (hereafter RGR); and thus, tolerance capabilities. Additionally, higher compensatory growth early during plant development may be due to the lack of induction of resource sequestration. Induced resource sequestration refers to an increase in export of existing or newly acquired resources from attacked tissues into storage organs, in such a way that these resources become unavailable for growth or defense (reviewed

in Orians et al. 2011). Because there are costs associated with resource sequestration, Orians and coworkers (2011) predicted that resource sequestration should be less beneficial at younger developmental stages, where susceptibility to herbivores and fitness costs are higher, while it should be favored in mature stages, where export of resources to short-term storage pools may later benefit reproduction or overwintering strategies.

Given these conflicting ontogenetic changes in plant physiology, architecture and functional priorities (i.e. growth vs. defense vs. reproduction), it is unlikely that a single overall increase or decrease in tolerance capabilities will be observed as plants age. Indeed, when more than two developmental stages were tested, trajectories often varied in a non-linear fashion (Hare 1980, Lennartsson et al. 1998, Ohnmeiss and Baldwin 2000, Del-Val and Crawley 2005). Moreover, a recent meta-analysis summarizing ontogenetic patterns across multiple plant life histories and developmental stages illustrated that, on average, the ability of both woody and herbaceous plants to tolerate damage did not differ as plants aged (Barton and Koricheva 2010). Therefore, the simultaneous assessment of both patterns and mechanisms will help to better understand and predict under which conditions a positive or negative change in tolerance capabilities should be expected as plants age.

Induced defenses, defined as the increase in chemical and morphological defense traits which may deter current and/or subsequent herbivores, also vary throughout plant ontogeny. Induced chemical defenses, usually considered a cost-saving strategy, are associated with scenarios where resource allocation constraints limit constitutive defenses or are dependent on actively growing and differentiating tissues (Karban and Baldwin 1997, Cipollini et al. 2003). Because younger plant stages are often more susceptible to herbivory, have lower levels of constitutive defenses, and have proportionally more undifferentiated and actively growing tissues than older plant stages; the ability of plants to induce defenses is predicted to be greater in younger developmental stages and lessen as plants mature (Karban and Baldwin 1997). In support of this prediction, Barton and Koricheva (2010) showed that, at least for herbaceous

plants, induced defenses considerably decreased as plants progress from seedling to senescent stages. However, there are also examples where higher induction in defensive traits was attained at later developmental stages (Mattiacci et al. 2001, Zhu and Park 2005, Du et al. 2008, Santos and Fernandes 2010). Higher induction in defensive traits at older life stages may be common in species where metabolic costs of defense production are high, such as those that depend on the previous development of biosynthetic machinery and/or complex storage structures (i.e. resin ducts and glandular trichomes; Gershenzon 1994). Furthermore, although induced defenses can vary with plant ontogeny, the result can be a decrease in levels of defenses as compared with undamaged plants, termed 'induced susceptibility' (e.g. Barton 2008). Hence, while age-related changes in concentration of plant allelochemicals beyond constitutive levels can be useful to predict subsequent herbivory events, this approach may not necessarily reflect changes in plant resource allocation to defenses.

Changes in allelochemical concentrations can reflect variation in synthesis rates and/or biomass accumulation (Koricheva 1999). Adaptive defense strategies, such as the increased synthesis of plant allelochemicals following damage, involve the production of new molecules (quantities) rather than concentrations (= content/biomass). Consequently, to better understand how plant ontogeny directly mediates allocation of resources to the production of plant allelochemicals, we need to be able to separate whether changes in concentration are primarily due to concentration-dilution effects (changes in biomass) or synthesis effects (changes in content). A technique that allows separating these confounding effects is graphical vector analyses (Haase and Rose 1995, Koricheva 1999). This technique is particularly important in studies assessing induced defenses throughout plant development, as significant changes in both growth and production of defenses are expected.

Here, using four juvenile stages of the herbaceous weed, *Plantago lanceolata* L. (Plantaginaceae), I examine ontogenetic variation in the expression of tolerance and resistance traits following leaf damage by the specialist caterpillar, the Buckeye (*Junonia coenia*,

Nymphalidae). Tolerance traits were assessed as compensatory growth ability (Mcnaughton 1983), and resistance traits were assessed as induction of iridoid glycosides, the main allelochemicals synthesized by *P. lanceolata* (Ronsted et al. 2000). To explain ontogenetic variation in compensatory growth, I assessed the role of plant RGR, under the assumption that higher rates of growth should increase the ability of a plant to recover the tissue lost to herbivores (Hilbert et al. 1981, Anten et al. 2003). In addition, I used graphical vector analyses (GVA) to assess whether changes in concentrations of plant allelochemicals reflect changes in synthesis rates and/or indirect changes in biomass accumulation as plants develop (Haase and Rose 1995, Koricheva 1999).

MATERIALS AND METHODS

Study system

Plantago lanceolata L., narrowleaf or ribwort plantain, (Plantaginaceae) is a common, short-lived, herbaceous plant (annual or facultative perennial) introduced to North America around 200 years ago (Cavers et al. 1980). This plant species produces, in addition to iridoid glycosides (Ronsted et al. 2000), a number of bioactive phenolic compounds such as flavonoids and phenylethanoid glycosides (Tamura 2001, Galvez et al. 2005). However, iridoid glycosides (hereafter IGs) are the primary allelochemicals found in large concentrations across tissues and influencing feeding preferences of insect herbivores and pathogens (e.g. Bowers and Puttick 1989, Biere et al. 2004, De Deyn et al. 2004). Thus, here I focused on variation in constitutive and induced IGs throughout plant ontogeny. Specifically, *P. lanceolata* produces primarily two IGs: aucubin and catalpol, and amounts of these compounds can reach up to 10-12% dry weight in leaf tissues (Bowers and Stamp 1992, 1993). Because aucubin is the biosynthetic precursor of catalpol (Ronsted et al. 2000), high concentrations of IGs, especially of catalpol, indicate a greater biosynthetic investment by the plant. In addition, high concentrations of IGs deter several generalist herbivores and pathogenic fungi (Bowers and Puttick 1989, Strohmeyer

et al. 1998, Biere et al. 2004, De Deyn et al. 2004, Harvey et al. 2005, Wurst and van der Putten 2007). Therefore, enhanced concentrations of IGs, especially of catalpol, may result in higher unpalatability and/or toxicity to most generalist antagonists (Bowers and Stamp 1993). In contrast, high IG concentrations may potentially increase damage by specialist herbivores that use IGs as oviposition and feeding stimulants (Bowers 1983, 1984, Reudler Talsma et al. 2008a), although some negative physiological and ecological consequences were reported for specialist herbivores as well (Adler et al. 1995, Camara 1997a, Smilanich et al. 2009a).

Earlier studies have identified plant age as an important intrinsic factor affecting *P. lanceolata* constitutive defenses, showing that, in general, IG content increases as plants age (Bowers and Stamp 1993, Fuchs and Bowers 2004, Barton 2007, Quintero and Bowers 2011a). Similarly, plant age also has been identified as an intrinsic factor altering the ability of *P. lanceolata* to induce defenses and/or tolerate damage. For example, Hanley and Fegan (2007) showed that following cotyledon damage, seedlings incur in both short- and long-term reduction in biomass and/or number of inflorescences when damaged earlier (1 and 2wk-old plants) but not later (3wk-old plants) in their development. In contrast, Barton (2008) found that 2 and 4wk-old seedlings suffering 50% leaf damage did not statistically differ in terms of compensatory growth capabilities. Nevertheless, older stages (4wk-old plants) showed lower compensatory growth, or, if they achieved compensation, it was at the expense of root biomass, while younger seedlings showed higher or complete compensation during the same period (Barton 2008). Similarly, in terms of induction, while lack of induced defenses was reported for young seedlings (2wk-old plants, Barton 2008), juveniles (5, 6 or 7wk-old plants, Fuchs and Bowers 2004) and mature stages (14wk-old plants, Quintero and Bowers 2011b), some juvenile stages demonstrated induced susceptibility (4wk-old plants, Barton 2008) or resistance (9wk-old plants, Quintero and Bowers 2011b) following 50% damage. These previous studies demonstrated that the ontogenetic stage at which damage occurred plays a critical role in *P. lanceolata* induced defenses. Nevertheless, it is still unclear whether contrasting patterns among studies may be

due to variation in environmental conditions, type (mechanical vs. herbivore) and amount of damage, time elapsed following damage, or to intrinsic variation in plant vigor. Furthermore, previous studies only reported changes in overall biomass and/or concentrations of IGs; and therefore, were unable to evaluate whether those changes reflected variation in plant IG synthesis or concomitant changes in biomass. By assessing short- and long-term responses to herbivory across four age classes and controlling for multiple factors that may alter plant responses to herbivory, this study attempts to relate age-dependent induced responses to herbivory with intrinsic variation in plant growth rate and IG synthesis rate as plants age.

The buckeye butterfly, *Junonia coenia* Hubner, (Nymphalidae) is a New World butterfly that can have one to multiple broods per year under natural conditions (Brock and Kaufman 2003). This species is a specialist herbivore of plants containing IGs (Bowers 1984). Adult female butterflies use IGs as oviposition stimulants, choosing host plants or tissues within plants with higher IG concentrations (Pereyra and Bowers 1988, Klockars et al. 1993). Buckeye larvae also use IGs as feeding stimulants and are documented to feed on members of four plant families: Cornaceae, Plantaginaceae, Scrophulariaceae and Verbenaceae (Bowers 1984). Furthermore, buckeye caterpillars are able to sequester these compounds as a function of their concentration in host plant tissues (Camara 1997a). As a result, caterpillar performance and predation risk can strongly depend on levels of host plant defenses. For instance, caterpillars feeding on higher IG diets are typically avoided by invertebrate predators (Dyer and Bowers 1996, Strohmeyer et al. 1998, Theodoratus and Bowers 1999, Stamp 2001), but show enhanced susceptibility to parasitoid attack (Smilanich et al. 2009a; Chapter 5). In North America, Buckeye butterflies have incorporated *P. lanceolata* into their diet and use this species as a preferred host (Graves and Shapiro 2003 and references therein). Because *P. lanceolata* forms natural populations with diverse age structures (Shefferson and Roach 2010) and Buckeyes can have multiple generations within a growing season, it is possible that plants can

be damaged in nature at any developmental stage making this greenhouse experimental design (see below) realistic under natural conditions.

Compensatory growth and induced defenses following leaf damage

The study was performed at the University of Colorado greenhouse during spring-summer 2008. Buckeye larvae used in this study were from a laboratory colony reared at the University of Colorado at Boulder. Prior to the experiment, larvae were fed on *P. lanceolata* leaves and kept in growth chambers with a photoperiod of 14hr day: 10 night, and day-night temperatures of 27°C /22°C.

Plant ability to induce defenses after herbivory, measured as the ability of a plant to increase chemical defenses (resistance), and/or increase growth rate to compensate for the lost tissue (compensatory growth) were evaluated at four juvenile age classes: 3, 6, 10, and 14 week-old plants from germination (see Table 3.1 for descriptions). To control for potential environmental factors that could influence plant responses to herbivory other than plant age (i.e. photoperiod and temperature), plants of different age classes were analyzed simultaneously. Synchronization among age classes occurred by germinating seeds at intervals of 20-30 days from April to July. In this way, although average environmental conditions from sowing to harvest varied among the four juvenile age classes, I ensured that all individuals were exposed to the same conditions at the time of exposure to herbivore damage, as well as at harvest time. Since IG concentrations in this as well as other IG containing plant species, can vary substantially over days and even hours (Hogedal and Molgaard 2000, Fuchs and Bowers 2004), or be influenced by temperature (Tamura 2001), simultaneous damage and harvest across all age classes should minimize variables other than plant age that could influence constitutive and induced IG concentrations.

Seeds were collected from >20 maternal plants of a population in Boulder County, Colorado, and mixed before the experiment started. Sub-groups of seedlings were germinated

in Fafard mix and then transplanted after 15 days to a growth medium of equal parts Metro Mix 350, sterilized sand, and turface. Each time, all seedlings used for this experiment were selected to be equivalent in size and number of leaves at the time of transplanting. Plants were grown in extra-deep 13 liter pots in order to minimize root-binding effects, which can affect both compensatory growth and plant secondary metabolite production (Karban and Baldwin 1997). Replicates were randomly placed on four $5m^2$ greenhouse benches, exposed to natural daylight and watered daily. Fertilizer was supplied to all plants every three to four days throughout the length of the experiment, using Scotts Peter's Excel solution (Scotts-Sierra Horticultural Products Company, Marysville, Ohio) mixed at a ratio of 15-5-15 N-P-K, which also provides trace amounts of micronutrients. Temperatures fluctuated over the course of the season but greenhouse temperatures did not exceed 32°C or fall below 15°C at any time.

On July 20th, when all age classes were present, 90 plants per ontogenetic stage were randomly assigned to one of two herbivore treatments: C = control (no herbivore) and H = herbivory by leaf-chewing specialist buckeye caterpillars, *Junonia coenia*. The herbivory treatment consisted of one to six 4^{th} instar larvae added to the plants for a period of a few hours to three days, the time in which the caterpillars consumed approximately 30% of the plant tissues. To confine caterpillars on the plants during the treatment, control and treatment plants were caged using mesh bags constructed of Remay™. As treatment plants reached 30% damage, caterpillars and mesh bags from control and treatment plants were removed. Because induction of IGs in *P. lanceolata* appears to reach its highest point six days after damage (Fuchs and Bowers 2004), to test for short-term responses to herbivory (i.e. induction of IGs and compensatory growth), a subset of 15 plants per age class/treatment were harvested a week after herbivore removal. Furthermore, because the ability of *P. lanceolata* to induce chemical defenses or compensate for lost tissue may vary considerably with time after initial damage (Fuchs and Bowers 2004, Hanley and Fegan 2007, Barton 2008), to fully assess the role of plant age on induced plant responses I added two additional harvest times: 3 and 5 weeks after

Table 3.1. Description of the four juvenile age classes before herbivory treatment started. Values calculated by harvesting a subsample of 15 plants per age class the same day that caterpillars where placed on treatment plants (Mean ± 1SE). Constitutive defenses (total IGs in percent dry weight) and nutritional quality (percent water and nitrogen concentrations) are reported only for aboveground tissues.

Age class (weeks-old)	Number of leaves	Shoot biomass (g)	Root biomass (g)	R:S ratio	Constitutive total IGs (% dry wt.)	Water (%)	Nitrogen (%)
3	4.01 ± 0.11	0.014 ± 0.001	0.0057 ± 0.0006	0.44 ± 0.06	0.84 ± 0.14	91.54 ± 0.45	5.64 ± 0.13
6	7.6 ± 0.23	0.088 ± 0.005	0.0198 ± 0.0025	0.22 ± 0.02	0.13 ± 0.04	92.72 ± 0.14	5.23 ± 0.19
10	23.0 ± 2.5	1.21 ± 0.08	0.54 ± 0.04	0.46 ± 0.03	0.50 ± 0.08	89.88 ± 0.26	4.55 ± 0.11
14	33.75 ± 3.84	3.03 ± 0.26	1.05 ± 0.14	0.33 ± 0.02	1.71 ± 0.03	87.71 ± 0.27	3.43 ± 0.12

herbivore removal, again using 15 plants per age class/treatment combination. The 3 and 5 week harvest times, termed here as long-term responses, were selected as a trade-off between providing sufficient time for plants to recover from their 30% tissue loss and avoiding the considerable changes in photoperiod and temperature at the end of the summer, which may further alter plant growth rate and allelochemical synthesis rate as plants prepare to overwinter. Total sample size was 360 plants, with 15 replicates per age class, treatment and harvest time combination.

At harvest, all aboveground tissues from both control and herbivory groups were weighed fresh, oven-dried at 50°C for 48hrs to a constant mass and weighed again to the nearest 0.01g. To assess variation in concentrations of IGs, all leaves were ground into a fine powder, and 10-30mg subsamples (entire available tissue for some seedlings) were processed for IG extraction and analyzed by gas chromatography following previously described methods (Bowers and Stamp 1993, Barton 2007, Quintero and Bowers 2011a).

Statistical analyses

Differences in compensatory growth and induced concentrations of IGs following herbivory, as a function of plant age (i.e. 3, 6, 10, and 14 weeks-old plants) and harvest time (i.e. 1, 3, and 5 weeks after damage), was assessed as changes between control and treatment plants (i.e. C vs. H) in aboveground biomass, total levels of IGs, and proportion of catalpol/total IGs. Biomass data were square root transformed and IG concentrations were arcsine square root transformed to meet assumptions of normality. The data were first fitted to an initial full factorial model with plant age, treatment (C vs H), harvest time, and their interactions as the main effects. Then, for each of the three dependent variables, non significant variables were progressively removed from the model until a minimal appropriate model was obtained (i.e., a simplified model in which all terms are significant). Single factors or variables incorporated into significant interactions were maintained in the minimum adequate model (Crawley 1993). I compared all alternative

models for each comparison using the Akaike Information Criteria (AIC) of the R software environment (the R freeware statistical package, R Development Core Team, 2008).

Changes in compensatory growth capabilities across plant age class and harvest time following damage, measured as differences in aboveground biomass between C and H plants, can be the result of differential growth rates among plant developmental stages throughout the season (Hilbert et al. 1981, Anten et al. 2003). Thus, to test whether changes in growth rate vary over plant ontogeny and following damage, I tested for differences in relative growth rates (RGR) between control and damaged plants across plant age classes and harvest times. Since changes in biomass as a function of plant age and treatment were calculated using destructive sampling, I was unable to use individual plant biomass before and after treatment. Hence, I calculated RGR as the increment in total aboveground biomass for each plant age class and treatment combination (ωat) between successive harvests (h) divided by the total mean biomass at the previous harvest (ω̃ath-1) and the number of weeks between harvests (t).

(1) RGR at$i_{1\text{-}15}$ = (ωath$i$ - ω̃ath-1) ω̃ath-1^{-1} t^{-1}

The only exception was the assessment of RGR of treatment plants at the first harvest (i.e. 3wks-old plants one week following damage), where mean for the treatment group (i.e. ω̃ath-1 of damaged plants) was the mean biomass of control plants before the experiment started minus 30% to account for the original lost tissue by caterpillar feeding. RGR values were log transform to meet assumption of normality. Finally, a three-way ANOVA was used to assess whether changes in RGR depended on plant age, treatment, harvest time and the interactions. As before, non significant variables were progressively removed from the model until a minimal appropriate model was obtained, and the difference between the full and minimal appropriate model was assessed using the Akaike Information Criteria (AIC).

To assess whether changes in concentrations of IGs following herbivory, as a function of plant age and time elapsed following initial attack (i.e. harvest time), reflect allocation of

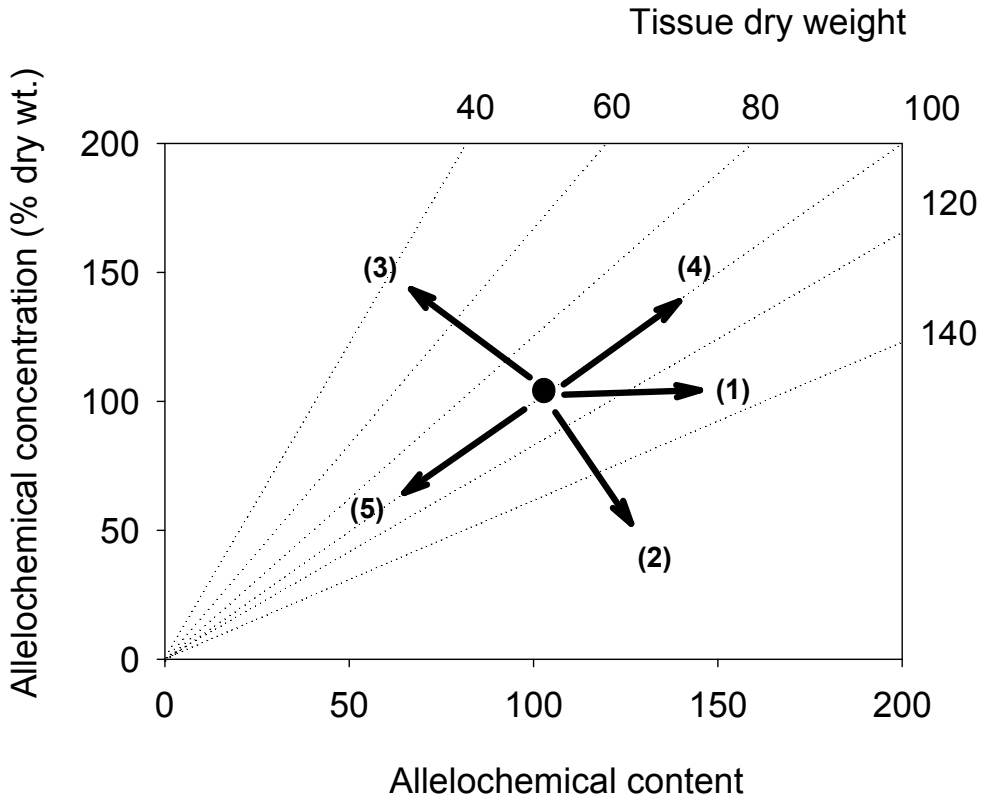
resources to plant allelochemicals, I used GVA (Koricheva 1999). Since plant responses to herbivory usually encompass a combination of changes in plant growth and allelochemical synthesis rate, this method helped to discern whether observed shifts in concentrations of plant IGs (mg g^{-1} dry wt) were due to active changes in IG synthesis rate or to indirect changes in biomass accumulation (Haase and Rose 1995). The construction of the vector diagram and the application of GVA in plant nutrient and chemical analyses have been previously described in detail (see Haase and Rose 1995, Koricheva 1999). Briefly, GVA allows for the simultaneous comparison of relative changes in plant biomass (Z), chemical content (X) and concentration (Y) of plant chemical defences. Relative changes are calculated based on a reference treatment, which in this case was the mean of the control group (undamaged plants). As a result, changes between control (C) and treatment group (H) are interpreted based on examination of both the direction and length of vectors extending beyond this reference point (relative difference between the C and H groups) (see Figure 3.1).

RESULTS

Plant age and compensatory growth

Mean aboveground biomass of treatment plants was always lower than that of control plants, but statistically significant differences were only found for certain plant age classes and harvest times, indicating that some *P. lanceolata* stages were better than others at compensating for the 30% lost tissue. Thus, difference in plant biomass significantly varied with plant age class, herbivory treatment, and time following damage (Figure 3.2, Table 3.2). Time following damage did not interact with the treatment factor and therefore was excluded from the model, as was the three-way interaction (Table 3.2). The significant age by treatment interaction indicated that plant ability to tolerate damage depended on plant age at the time of herbivory (Table 3.2). In general, intermediate juvenile stages (6 and 10wk-old) showed a higher compensatory growth

Figure 3.1. Graphical vector analysis (GVA) allows for the simultaneous comparison between relative changes in plant biomass (Z), chemical content (X), and concentration of plant allelochemicals (Y), where concentration=content/biomass. Relative changes are calculated based on a reference treatment, which in most cases is the mean of the control group. Then, these changes are interpreted based on examination of both the direction and length of vectors extending beyond this reference point (relative difference between the control and treatment groups). In this way, this technique enables one to distinguish between cases where shifts in concentrations are due to changes in compound uptake or synthesis and cases where these shifts are simply due to changes in biomass accumulation. Five possible scenarios can be expected: **(1) Steady state** represents a situation where concentrations do not change but both content and biomass increase, indicating that allelochemical synthesis matches changes in biomass accumulation. **(2)** A **Dilution effect** indicates a decrease in concentrations accompanied by an increase in content and biomass, which signifies periods of active plant growth when allelochemical synthesis cannot keep pace with the high rate of biomass accumulation. **(3)** A **Concentration effect**, which represents the inverse of the dilution effect, occurs when plant growth is reduced more than allelochemical synthesis, and is characterized by increased concentrations but a reduced content and biomass. **(4) Excess synthesis**, in turn, represents an increase in concentrations accompanied by corresponding increases in content and biomass, indicating that the allelochemical synthesis rate is higher than the biomass accumulation rate. **(5)** Finally, a decrease in concentrations observed together with a decrease in content and a decrease or no change in biomass indicates **reduced synthesis**, where allelochemical synthesis is reduced more than biomass accumulation. Figure and legend modified from Koricheva (1999).



capability as compared to younger (3wk-old) or older (14wk-old) juvenile stages (Figure 3.2). This variation is hard to visualize in Figure 3.2 given the scale of the y-axis; however, the magnitude of biomass reduction (i.e. Δ biomass H - C / C) across the three harvest times was greater for 3 and 14wk-old age classes (damaged plants were, on average, 37% and 25% smaller than control plants, respectively) than for 6 and 10wk-old age classes (damaged plants were, on average, 20% and 17% smaller than control plants, respectively) ($F_{3,12}$ = 4.81, P = 0.034). Furthermore, the significant age by harvest interaction (Table 3.2), indicated that, independently of responses due to damage, changes in plant biomass as plants progressed over the growing season also varied among plant age classes. In particular, while younger age classes (3, 6 and 10wk-old) showed significant increases in biomass through time, older juvenile stages (14wk-old) did not notably increase their aboveground biomass over time (Figure 3.2).

Changes in RGR among plant age classes may have partially accounted for the variation seen in compensatory growth ability. RGR significantly varied with plant age class and harvest time but did not change with herbivory treatment (Table 3.2). This indicates that herbivory treatment did not affect the predetermined growth rate of plants at any developmental stage. In contrast, in all cases, RGR showed a consistent decrease over time, with age and across harvest time (Figure 3.3). Because the RGR did not change with the treatment factor, this result suggests that when both control and treatment groups have greater RGR, damaged plants have a more difficult time “catching-up” with the undamaged plants. This can explain why compensatory growth capabilities increased from 3 to 10 wk-old age classes, but the subsequent decrease at the 14 wk-old stage may be a response to different mechanisms or constraints.

Table 3.2. Summary of significant factors of minimal appropriate models assessing changes in aboveground biomass, total levels of iridoid glycosides, proportion of catalpol/total IGs, and plant relative growth rate (RGR) as a function of plant age (Age), herbivory (Treatment), and time following damage (Harvest), as well as their interactions, in *Plantago lanceolata*. ΔAIC indicates the difference between the full factorial model and the minimal appropriate model using Akaike Information Criteria (AIC).

	Biomass			**Total IGs**			**Prop Catalpol**			**RGR**		
	df	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
Model	15	630.89	**0.0001**	23	458.18	**0.0001**	12	47.50	**0.0001**	12	384.29	**0.0001**
Age	3	553.53	**0.0001**	3	339.28	**0.0001**	3	12.91	**0.005**	3	324.20	**0.0001**
Harvest	2	208.09	**0.0001**	2	147.29	**0.0001**	2	12.15	**0.002**	2	107.15	**0.0001**
Treatment	1	35.91	**0.0001**	1	4.94	**0.026**	1	10.52	**0.001**	1	0.006	0.940
Age*Harvest	6	65.49	**0.0001**	6	126.91	**0.0001**	6	14.32	**0.026**	6	34.43	**0.0001**
Age*Treatment	3	10.17	**0.017**	3	2.98	0.395						
Harvest*Treatment				2	0.50	0.777						
Age*Harvest*Treatment				6	18.45	**0.005**						
Error	344			336			347			347		
ΔAIC			12.03			0.11			11.75			17.58

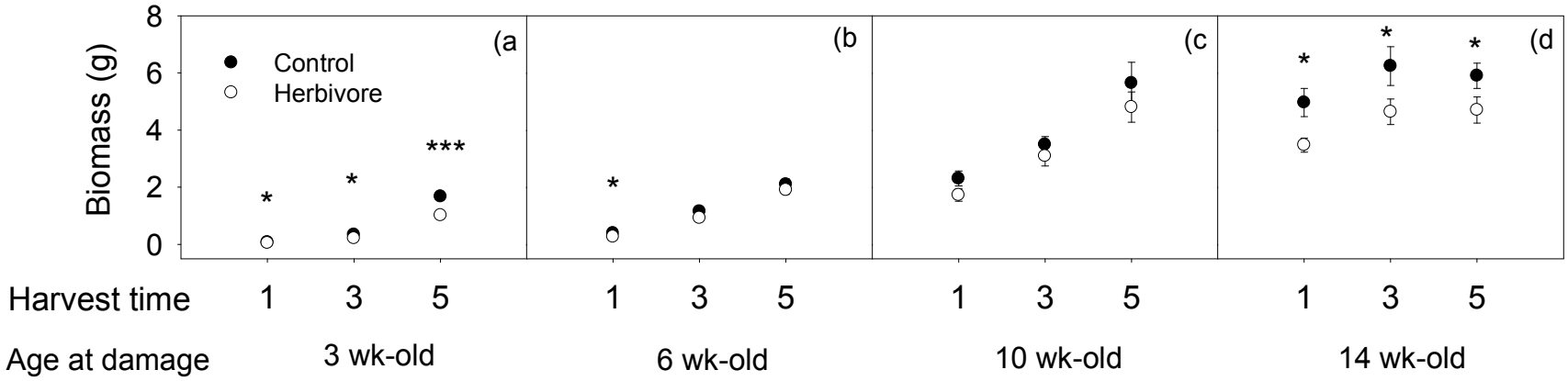


Figure 3.2. *Plantago lanceolata* compensatory growth ability measured as the difference in mean total aboveground dry biomass of plants subject to herbivory versus undamaged control plants (mean ± 1SE, N=15 per treatment) at 1, 3 or 5 weeks following initial damage (i.e. harvest time) across four juvenile age classes: (**a**) 3 week-old plants, (**b**) 6 week-old plants, (**c**) 10 week-old plants, and (d) 14 week-old plants. Standard errors are calculated and graphed for all treatments, but some are too small to be visible given the size of the symbols. Original data were square root transformed for statistical analyses; untransformed data are shown for illustrative purposes only. Asterisks represent mean group differences among herbivore and control treatments as tested by single degree of freedom contrasts. Significance is displayed as $P < 0.0001$ (***), $P < 0.01$ (**), $P < 0.05$ (*).

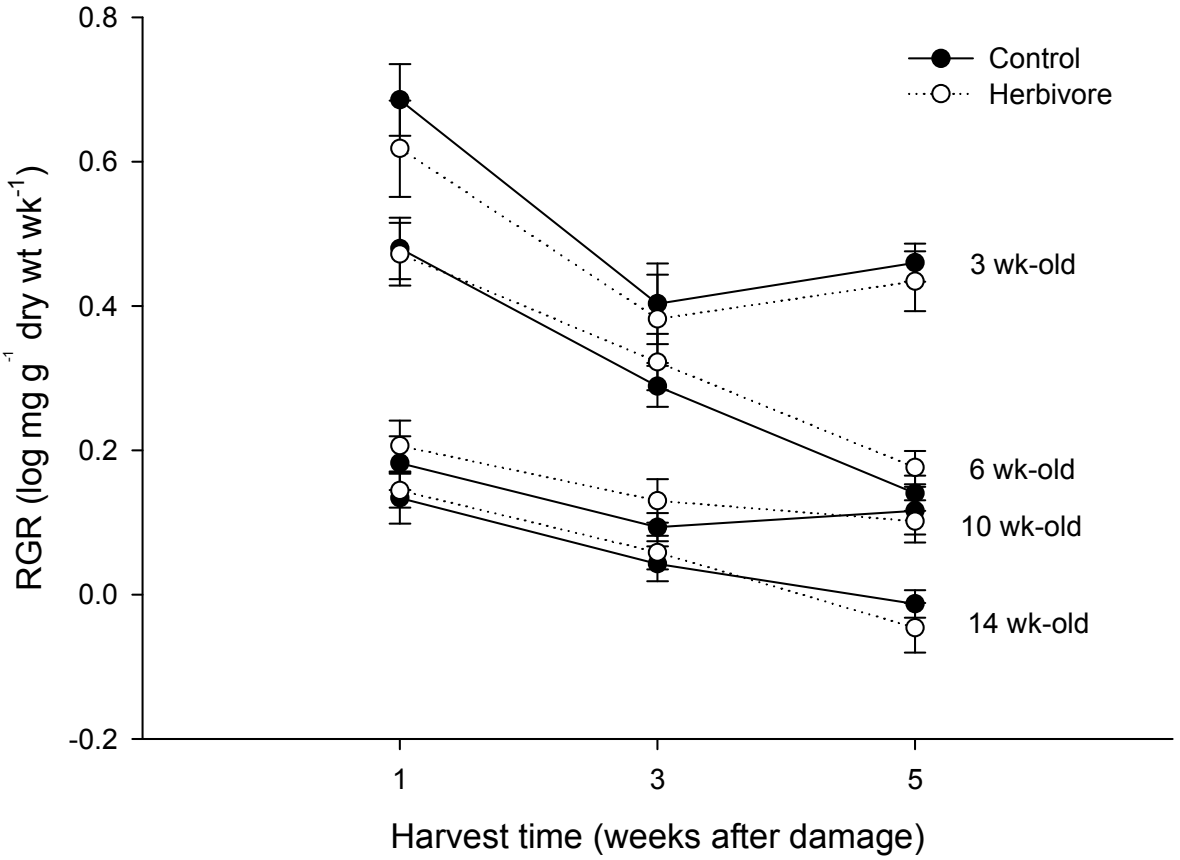


Figure 3.3. Changes in relative growth rate (RGR) of control and treatment plants from the initial damage event to one, three, or five weeks following damage for all four juvenile *P. lanceolata* age classes.

Plant age and induced defenses

Induced defenses after damage, assessed as changes in total levels of IGs, or proportion of catalpol/total IGs, significantly varied as a function of plant age at the time of damage, herbivory treatment and time following damage (Table 3.2, Figure 3.4). In addition, in both cases there was a significant age by harvest interaction (Table 3.2), indicating that, independently of the effect of treatment, changes in IG concentrations during this five week window varies among plant age classes. For example, in the case of total IG concentrations, while younger age classes (i.e. 3, 6 and 10 wk-old) showed a dramatic increase in defenses from early to late harvests, older juvenile plants (i.e. 14 wk-old) showed significantly less variation in total IG concentration during the same period (Figure 3.4). Finally, for total levels of IGs, a significant three-way interaction was observed (Table 3.2), indicating that, not only the ability of a plant to induce defenses changed as a function of plant age at the time of herbivore attack, but also that induced defense strategies varied with time following damage (Figure 3.4). For example, in the case of 6 and 10wk-old plants, whereas total levels of IGs in damaged plants completely matched those of control plants one week following damage (Figure 3.4), total IGs decreased when assessed at three weeks (for 6wk-old plants) or five weeks (for 10wk-old plants) after damage (Figure 3.4). Thus, plant ability to alter total IG concentrations varied with time following damage, but the pattern was not consistent across age classes. Finally, another interesting pattern is that while concentration of total IGs in damaged plants sometimes decreased compared to control plants, the proportion of catalpol increased (see 6wk-old plants at 3wk harvest time and 14wk-old plants and 1 wk harvest time). As aucubin is the precursor of catalpol in the IG biosynthetic pathway (Ronsted et al. 2000), shifts between proportion of catalpol and total IGs should represent changes in synthesis rate.

Graphical vector analyses (GVA) were also useful for elucidating differences in plant ability to induce defenses across age classes (Figure 3.5). When responses to the herbivory treatment were examined only in terms of total IG concentrations (Figure 3.4), my results

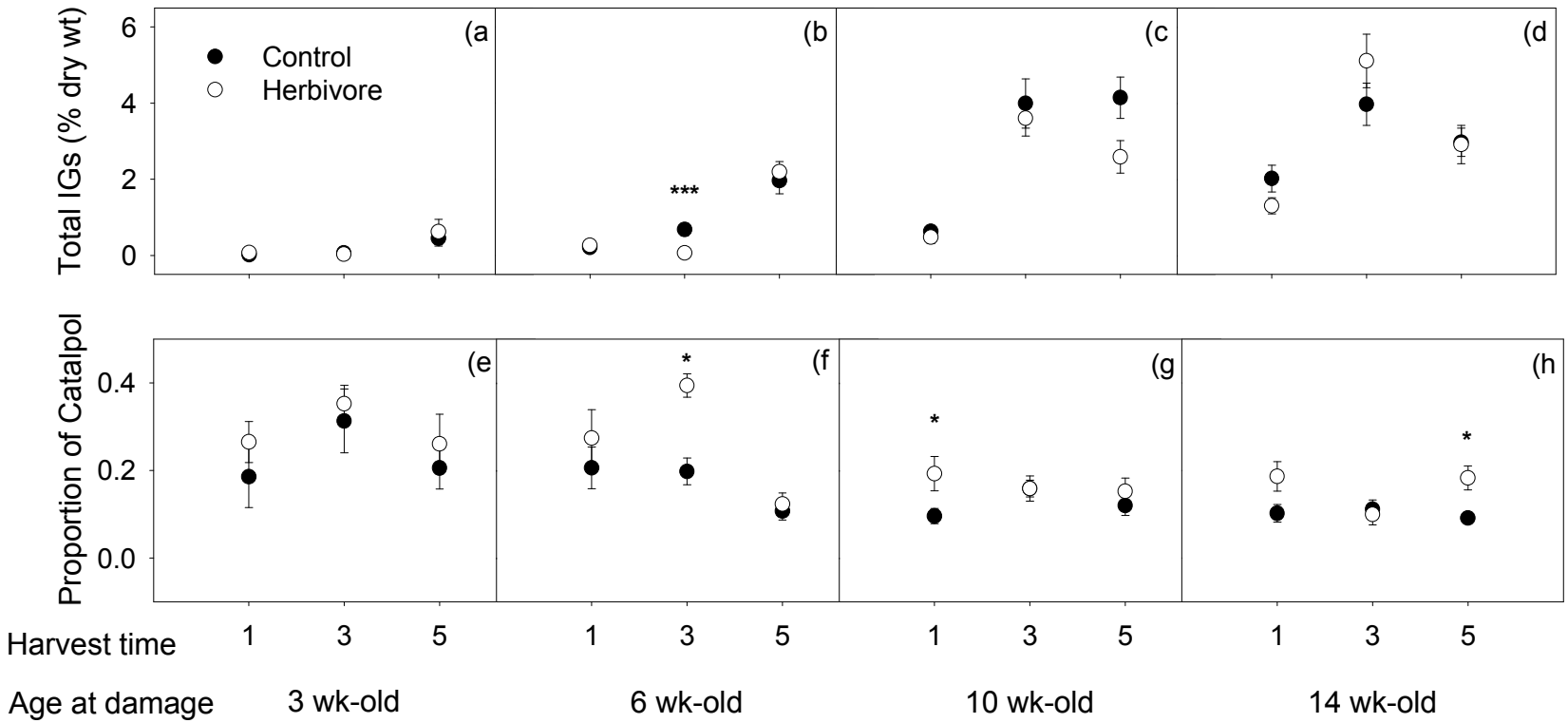


Figure 3.4. *Plantago lanceolata* induced defenses measured as the difference in mean total concentration of IGs (**a**, **b**, **c, d**) and proportion of catalpol/total IGs (**e, f, g, h**) between plants subject to herbivory versus undamaged control plants (mean ± 1SE, N = 15 per treatment) at 1, 3 or 5 weeks following initial damage (i.e. harvest time) across four juvenile age classes: (**a, e**) 3 week-old plants, (**b, f**) 6 week-old plants, (**c, g**) 10 week-old plants, and (**d, h**) 14 week-old plants. Standard errors are calculated and graphed for all treatments, but some are too small to be visible given the size of the symbols. Original data were arcsine-square root transformed for statistical analyses; percentage data are shown for illustrative purposes only. Asterisks represent mean group differences among herbivore and control treatments as tested by single degree of freedom contrasts. Significance is displayed as $P < 0.0001$ (***), $P < 0.01$ (**), $P < 0.05$ (*).

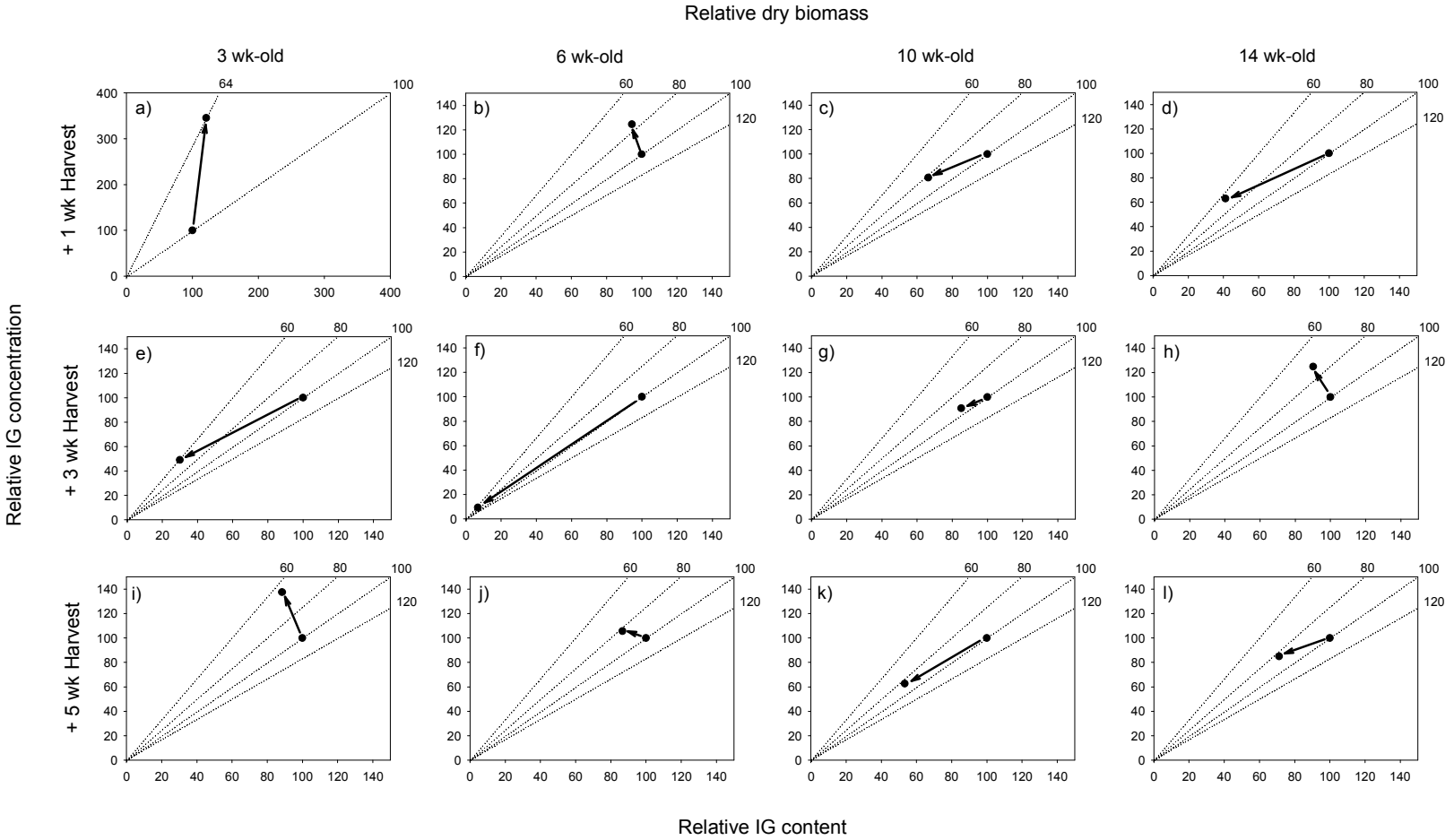


Figure 3.5. Graphical vector analyses showing changes in total concentrations and total amounts of leaf iridoid glycosides (IG) and dry weight following 30% tissue removal by specialist caterpillars in *P. lanceolata* across four developmental stages (3, 6, 10 and 14 wk-old plants) and three harvest times following initial damage (1, 3 and 5 wks after damage). In each panel, mean IG concentration (y axis) for control and damage treatments is plotted against corresponding mean total IG amount per plant (x axis). Diagonal lines correspond to the mean biomass of dried leaves. Concentrations, amounts and biomass are expressed in relative values to allow easy comparison of plant responses to herbivory across plant developmental stages and short versus long term responses. Direction of vector from control to damaged treatment plants indicates the effect of tissue removal on plant ability to alter concentrations and/or amounts of IGs as a function of plant age and time following damage (see Figure 3.1 for interpretation of results). Panel a indicates excess synthesis, panels b, h, I and j show concentration effects, and panels c, d, e, f, g, k and l show reduced synthesis.

showed a complicated picture. For instance, total IG concentrations varied only slightly between control and damaged plants in the case of 3 wk-old plants, which can be interpreted as a lack of induction; while older ontogenetic stages (i.e. 6 to 14 wk-old) showed a larger variation in IG induction, suggesting all possible outcomes: no-induction, induced susceptibility and induced resistance (Figure 3.4). Based on these results, I conclude that, disregarding variation in direction (i.e. induced resistance or susceptibility), older juvenile plants showed a more plastic response to damage than younger juvenile stages. However, GVA allowed clearer insight into the actual changes in IG synthesis rate and growth rate that may underlie ontogenetic variation in plant induction of allelochemicals in response to herbivory.

As shown in Figure 3.5, IG content increased only in 3 wk-old plants one week following damage, indicating that excess IG synthesis (i.e. higher IG synthesis rate than biomass accumulation rate) was only a short-term response strategy restricted to very young juvenile stages of *P. lanceolata*. Although that was the only clear case where I detected excess synthesis of IGs in response to damage, short- and long-term responses to herbivory by younger plant stages (3 and 6wk-old plants) more often than not showed that plant growth was reduced to a larger extent than IG synthesis, indicating a concentration effect (Figure 3.5). In contrast, older plant stages (10 and 14wk-old plants) showed an opposite trend, demonstrated by the decrease in IG concentration as well as IG content and biomass. Thus, older juvenile stages almost exclusively showed a proportionally larger reduction in IG synthesis than biomass accumulation, indicating a reduction in IG synthesis (Figure 3.5). Hence, these results indicate that variation in IG concentrations following damage, as a function of plant age, was mostly driven by changes in biomass between treatments, rather than induced IG synthesis.

DISCUSSION

Although an increasing number of empirical studies have reported age-dependent induced responses to herbivory (reviewed in Barton and Koricheva 2010), few have compared more

than two developmental stages (e.g. Hare 1980, Lennartsson et al. 1998, Del-Val and Crawley 2005), and even fewer have attempted to describe potential mechanisms that may explain the patterns observed (e.g. Ohnmeiss and Baldwin 2000, Tucker and Avila-Sakar 2010). Here, I found that plant RGR may partially explain the observed changes in compensatory growth capabilities across four distinct juvenile stages of *Plantago lanceolata*, suggesting that developmental stages that have intrinsically higher RGRs (i.e. in this study, younger stages) were proportionally less able to compensate for their lost tissue. In turn, the combined assessment of the relative change between plant growth rate and allelochemical synthesis rate using GVA facilitated the interpretation of complex variability in concentrations of IGs between treatment and control plants over time, showing that young juvenile plants were the only stage where excess synthesis of IGs was observed following damage.

Compensatory growth through plant development

Compensatory growth has been predicted to increase (Strauss and Agrawal 1999, Haukioja and Koricheva 2000) or to decrease (Orians et al. 2011) throughout plant ontogeny. While there is support for both predictions, it is becoming evident that the ability of a plant to tolerate damage may often fluctuate during ontogeny (Hare 1980, Lennartsson et al. 1998, Ohnmeiss and Baldwin 2000, Del-Val and Crawley 2005). In this study, I observed that higher compensatory growth in *P. lanceolata* was achieved at intermediate juvenile stages (6 and 10wk-old plants), while younger and older stages (3 and 14wk-old plants, respectively) were unable to fully compensate for the 30% tissue removal even after five weeks following damage.

Lack of compensation early during plant development has been associated with proportionally fewer available meristems, greater root to shoot ratios, and decreased capacity to acquire resources or have stored reserves (Strauss and Agrawal 1999, Haukioja and Koricheva 2000). While decreased compensatory growth or tolerance in younger as compared with older juvenile stages has been reported for other systems (Stout et al. 2002, Boege et al. 2007,

Tucker and Avila-Sakar 2010, Gruntman and Novoplansky 2011), my results only partially agree with previous studies assessing compensatory growth in early life stages of *P. lanceolata*. For instance, Hanley and Fegan (2007) showed that young *P. lanceolata* seedlings (1 and 2wk-old plants) failed to compensate, even after 15 weeks following damage; whereas 3wk-old seedlings achieved the same shoot and reproductive biomass as undamaged plants in the same period of time. In contrast, Barton (2008) found that, although 2 and 4wk-old plants did not statistically differ in terms of compensatory growth capabilities, older stages (4wk-old plants) showed lower compensatory growth. Or, if they achieved compensation, it was at the expense of root biomass; while younger seedlings showed proportionally higher or complete compensation in the same time period. A possible explanation for these differences could be the variation in the period of recovery allowed following damage (i.e. a maximum of 5 weeks in the present study versus a maximum of 6-8 to 15 weeks in former studies). Differences can also be due to variation in type and amount of tissue damage. For example, while Hanley and Fegan's (2007) study used a fixed amount of artificial damage to only cotyledon tissues, Barton (2008) and the present study used a specialist herbivore to inflict a proportionally similar amount of damage across stages (50% and 30%, respectively). Thus, the higher tolerance response in older seedling stages in the Hanley and Fegan (2007) study may be due to the proportionally lower damage received in older, as compared with younger, seedling stages. Finally, because I only evaluated aboveground biomass, I cannot neglect the possibility that in young stages resources were allocated to root biomass following damage. Allocation of resources to root tissues in order to enhance nutrient uptake needed for the production of new photosynthetic tissue has been previously reported (reviewed in Orians et al. 2011), potentially explaining the lack of aboveground compensatory growth observed here for young juvenile stages.

The observed increase in compensatory growth from 3 to 10wk-old juvenile stages can be explained by the intrinsic ontogenetic patterns in plant RGR. Contrary to what I found, in general, previous research predicts that plants with higher RGR should achieve greater

compensation (Hilbert et al. 1981, Anten et al. 2003). However, this should be true if the RGR of damaged plants was greater than that of undamaged plants (i.e. due to induced-RGR following damage). In the present study, because damage treatment did not interact with the plants' aboveground RGR at any point in time, I believe that intrinsically higher RGR would impose a greater challenge, as damaged plants will be unlikely to "catch-up" with their undamaged counterparts. Had the experiment continued for longer, when RGR would begin to decrease, it is likely that all these stages may have achieved full compensation as previously seen in this species (Hanley and Fegan 2007, Barton 2008). Alternatively, as mentioned above, lack of belowground biomass data does not allow determination of whether changes in resource partitioning between below- and aboveground biomass may further explain the observed ontogenetic patterns in aboveground RGR and compensation.

Finally, lack of compensatory growth in the older juvenile age class assessed here (i.e. 14wk-old plants) may contradict previous predictions suggesting that older plants should be better at tolerating damage than younger life stages (Strauss and Agrawal 1999, Haukioja and Koricheva 2000). However, decreased compensatory growth as plants age may not be surprising, since older plants are probably prioritizing resources to be put into flowers and/or roots for overwintering. Indeed, sequestration of resources from already damaged or vulnerable photosynthetic tissues into short-term storage pools has recently been suggested to be common in older as compared with younger ontogenetic stages (Orians et al. 2011). This strategy was predicted to be more common in (i) perennial species where resources are mostly stored in leaves as compared with roots and stems, and in (ii) mature stages that have passed their leaf expansion period but are just before the onset of reproduction. Older juvenile stages of the short-lived, facultative perennial, *P. lanceolata,* satisfy all these requirements. Thus, although this study does not provide the root biomass data to assess this potential outcome (but see Ilmarinen et al. 2005), such a strategy may explain the lower intrinsic RGR and the decrease in compensatory ability observed at this later juvenile stage.

Induced defenses through plant development

In those studies where age-dependent induced chemical defenses have been assessed using more than two developmental stages, complex non-linear patterns were observed (e.g. Santos and Fernandes 2010). Here, changes in IG concentrations between control and damaged plants varied from no significant differences in young juvenile stages (3wk-old plants) to high variation in older juvenile stages, ranging from no induction to induced resistance or susceptibility. Furthermore, herbivore-induced chemical defenses significantly changed with time following damage. One week following caterpillar damage, all stages showed comparable responses by maintaining similar levels of total IGs but increasing the proportion of catalpol/total IGs in shoot tissues (although it was significantly higher only for 10wk-old plants, Figure 3.4). In contrast, three and five weeks after damage only a few developmental stages at a time showed some degree of induced susceptibility or resistance as compared with control plants.

These results agree with previous empirical studies that demonstrated a complex pattern of age-dependent induced IGs in *P. lanceolata*. In particular, lack of induced defenses was reported across multiple developmental stages of *P. lanceolata* such as young seedlings (2wk-old plants, Barton 2008), juveniles (5, 6 or 7wk-old plants, Fuchs and Bowers 2004) and mature stages (14wk-old plants, Quintero and Bowers 2011b). Nevertheless, variable induced defenses as a function of plant age was reported for this species. In particular, one week following 50% damage by the specialist caterpillar *J. coenia*, Barton (2008) showed that while 2wk-old seedlings did not vary in IG concentrations, 4wk-old plants showed induced susceptibility; and Quintero and Bowers (2011b) showed that induced resistance was restricted to juvenile (9wk-old plants) and was not evident in mature stages (14wk-old plants). Although previous studies in this species failed to detect trade-offs between constitutive IG and plant growth (Darrow and Bowers 1997, Barton 2007), this complex pattern in herbivore-induced defenses may reflect

shifts in age-dependent costs of defense, as seen in other systems (e.g. Briggs and Schultz 1990, Boege et al. 2007, Orians et al. 2010).

Lack of changes in IG concentrations may not necessarily imply that metabolic patterns were unaffected. Sutter and Muller (2011) recently revealed that although concentrations of IGs and verbascoside in *P. lanceolata* changed minimally following mechanical damage, herbivory or treatment with phytohormones, metabolic fingerprinting revealed pronounced chemical changes beyond these targets. Thus, only focusing on variation in concentration of plant defenses may not be appropriate for assessing age-dependent resource allocation constraints to defenses.

Here, by using graphical vector analyses (GVA), I was able to illustrate that IG synthesis was reduced more than growth rate in older juvenile stages, while the opposite was observed for younger juvenile life stages. This trend is consistent with predictions that induced defenses should be greater in younger developmental stages, given their high susceptibility to herbivory, lower constitutive defenses, and proportionally higher undifferentiated and actively growing tissues (Karban and Baldwin 1997). However, a significant increase in synthesis rate was only observed in 3wk-old plants a week following damage, indicating that excess IG synthesis was only a short-term response strategy restricted to very young juvenile stages of *P. lanceolata*. In addition, the two youngest juvenile stages (i.e. 3 and 6wk-old plants) showed strong temporal variation in IG synthesis following damage, as represented by shifts between increased synthesis of IGs one week after damage, followed by reduced synthesis at three weeks, and a subsequent increase in IG synthesis at five weeks post damage (Figure 3.5). In contrast, older juvenile stages (i.e. 10 and 14wk-old plants) showed a consistent decrease in IG synthesis rate throughout the whole period. These results are also consistent with the patterns reported for plant RGR. Synthesis of terpenoids, in particular IGs, has been associated with actively growing tissues (Bowers and Stamp 1992, 1993, Gershenzon 1994, Fuchs and Bowers 2004). Hence, it is not surprising that younger life stages, with higher RGR, showed the highest allocation of

resources to synthesis of defenses. However, it is important to note that this pattern was unapparent when I compared overall concentrations between damaged and undamaged plants, due to concomitant changes in plant biomass between treatments.

Regardless of the combination of factors explaining these complex patterns, shifts in the expression of defense traits can greatly influence higher trophic levels. Changes in concentrations of total IGs, or in the relative abundance of aucubin and catalpol, can render plants more or less susceptible to a wide change of herbivores and pathogens (reviewed in Dobler et al. 2011). As a result, the differences in IG concentrations reported here, as a function of *P. lanceolata* age and time following damage, can strongly influence plant fitness and the arthropod community associated with this plant species over its lifetime. Yet, as previously reported for this (Fuchs and Bowers 2004, Barton 2008, Quintero and Bowers 2011b) and other systems (Ohnmeiss and Baldwin 2000, Van Dam et al. 2001), constitutive variation in plant biomass and defenses across plant stages was as much as three orders of magnitude larger than differences between control and damaged plants at any single stage. This trend may be particularly common in annual and short-lived perennial plant species, where the transition through key developmental stages may occur relatively quickly, overriding herbivore-induced responses. Thus, these results imply that constitutive ontogenetic trajectories in plant growth and defenses may play a more significant role in mediating multitrophic interactions than changes following previous damage events.

Conclusions

In summary, both strategies, compensatory growth and induced synthesis of allelochemicals, showed complex patterns as plants aged, with some stages being able to employ both strategies (some 6wk-old stages), while other employed only one (3wk and 10wk-old stages) or neither of them (14wk-old stages). To uncover the complexity of ontogenetic patterns in *P. lanceolata*'s induced defenses, further research should explore the role of several factors such

as age-dependent costs of tolerance and resistance traits, biomass partitioning between shoots and roots, variation between local and systemic induction of chemical defenses, and the consequences for overall plant fitness. Nonetheless, here I illustrated how the assessment of plant RGR and IG synthesis rate (appraised through GVA), was central for detection and comprehension of herbivore-induced response patterns as plants age. Future studies, explicitly testing the mechanisms underlying ontogenetic patterns in plant defensive traits, especially those that incorporate more than two developmental stages, will better predict the potential constraints on the expression of resistance and tolerance traits throughout ontogeny.

CHAPTER 4

PLANT INDUCED DEFENSES DEPEND MORE ON PLANT AGE THAN PREVIOUS HISTORY OF DAMAGE: IMPLICATIONS FOR PLANT-HERBIVORE INTERACTIONS [2]

ABSTRACT

Herbivore-induced plant responses can significantly change as a function of plant developmental stage and previous history of damage. Yet, empirical tests assessing the combined role of multiple damage events and age-dependent constraints on the ability of plants to induce defenses within and among tissues are scarce. This question is of particular interest for annual and/or short-lived perennial plant species, whose responses to single or multiple damage events over a growing season are likely to interact with ontogenetic constraints in affecting a plant's ability to respond to herbivory. Using *Plantago lanceolata* and one of its specialist herbivores, *Junonia coenia*, I examined the effect of plant ontogeny (juvenile vs. mature developmental stages) and history of damage (single and multiple damage events early and/or late in the season) on plant responses to leaf damage. Plant responses to herbivory were assessed as induced chemical defenses (iridoid glycosides) and compensatory growth, in both above- and belowground tissues. I found that constitutive concentration of iridoid glycosides markedly increased as plants matured, but plant ability to induce chemical defenses was limited to juvenile, but not mature, plant stages. In addition, induced defenses observed seven days following herbivory in juvenile plants disappeared five weeks after the first herbivory event, and mature plants that varied considerably in the frequency and intensity of damage received over five weeks, did not significantly differ in their levels of chemical defenses. Also, only small changes in compensatory growth were detected. Finally, I did not observe changes in belowground tissues' defenses or biomass a week following 50% removal of leaf tissues at

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either age class or history of damage. Together, these results suggest that in *P. lanceolata* and perhaps other systems, ontogenetic trajectories in plant growth and defenses leading to strong age-dependent induced responses may prevail over herbivore-induced indirect interactions.

INTRODUCTION

Herbivore-induced plant responses that lead to changes in localized or systemic nutritional quality, physical and chemical defenses, growth, physiology and phenology are common and widespread in plants (Karban and Baldwin 1997). As a result, conspecific and/or heterospecific herbivores that share the same host may compete with or facilitate each other by altering plant traits for future herbivores (Ohgushi 2005, Kaplan and Denno 2007) or by altering the ability of a plant to respond to subsequent damage (Thaler et al. 2002, Viswanathan et al. 2007). Even though in most natural systems herbivore attack is seasonal, surprisingly little is known about how plant responses to herbivory change with successive damage events. Furthermore, short- and long-term plant responses to herbivory throughout a season occur concurrently with a plant's natural development. Thus, ontogenetic trajectories in plant constitutive defenses can interact with herbivore-induced defenses leading to changes in the occurrence and strength of induction in unpredictable ways. This question is of particular interest for annual and/or short-lived perennial plant species, whose responses to single or multiple damage events over a growing season are likely to interact with ontogenetic constraints in affecting a plant's ability to respond to herbivory. Yet, empirical tests assessing the combined role of multiple damage events and age-dependent constraints on the ability of plants to induce defenses are scarce.

Constitutive defenses have been shown to vary as a function of plant development, with defensive traits increasing or decreasing over time as resource allocation constraints and plant functional priorities change (Boege and Marquis 2005). Furthermore, dissimilar ontogenetic patterns in constitutive chemical defenses have been reported among plant tissues, such as between above- and belowground tissues (Williams and Ellis 1989, Bellostas et al. 2007).

These ontogenetic trajectories can encompass small to large qualitative and quantitative changes in defensive traits, which may in turn influence the identity of herbivores associated with different plant developmental stages (Waltz and Whitham 1997, Thomas et al. 2010). Despite the increasing interest in documenting developmental variation in constitutive defenses, the role of plant age in explaining herbivore induced defenses within and among tissues continues to be largely unexplored. Yet, in those systems where age-dependent induction of defenses was assessed, the ability of plants to resist or tolerate herbivory has been shown to strongly depend on plant developmental stage (Van Dam et al. 2001, Warner and Cushman 2002, Boege et al. 2007, Muola et al. 2010).

Physiological and morphological constraints during plant ontogeny can lead to strong age-dependent induced responses to herbivory, with opposite trends being predicted for resistance and tolerance. Induced chemical defenses, usually considered a cost-saving strategy for plants, are associated with scenarios where resource allocation constraints limit constitutive defenses or are dependent on actively growing and differentiating tissues (Karban and Baldwin 1997, Cipollini et al. 2003). Because younger plant stages are often more susceptible to herbivory, have lower levels of constitutive defenses, and proportionally more undifferentiated and actively growing tissues than older plant stages, the ability of plants to induce defenses is predicted to be greater in younger developmental stages and lessen as plants mature (Karban and Baldwin 1997). Alternatively, secondary growth that aims to compensate for lost tissue requires the reallocation of resources stored in undamaged tissues as well as enhancement of photosynthetic rates and nutrient uptake from the soil. Therefore, tolerance to herbivory is predicted to increase with plant age due to greater capacity to acquire resources and/or higher probability to have already stored reserves (Strauss and Agrawal 1999, Haukioja and Koricheva 2000). While there is some support for such patterns (Boege and Marquis 2005, Barton and Koricheva 2010), this is mostly based on comparisons of single herbivore damage events during

plant development, and as such, do not reflect potential plant responses due to natural variation in multiple and successive damage events over the course of development.

Sequential damage over a growing season can alter age-dependent induced responses to herbivory in two ways. First, repeated damage events by an herbivore species can induce an enhanced response as compared to a single damage event (e.g. Ruuhola et al. 2007, Roitto et al. 2009), as a result of what has been called 'immunological memory' (Baldwin and Schmelz 1996). Alternatively, plant responses to a previous damage event may reduce the ability of a plant to defend itself against a later attacker (e.g. Viswanathan et al. 2007, Poelman et al. 2008a). This pattern, usually termed 'priority effect', can arise as a consequence of physiological limitations such as negative cross-talk between pathways elicited by different herbivores (Verhage et al. 2010) or due to resource limitations if resources used during an initial response are unavailable for use in later responses. Thus, history of damage over a season or throughout plant development can strongly enhance or diminish expected age-dependent induced responses, potentially altering key plant-herbivore interactions. Furthermore, because allocation patterns and synthesis of chemical defenses may vary within and among tissues over time as a response to plant development and history of damage (Williams and Ellis 1989, Van Dam et al. 2001, Bellostas et al. 2007, Kaplan et al. 2008), it is essential that we incorporate both above- and belowground plant responses to current and past damage to fully understand the complexity of factors shaping plant defenses and, as a consequence, plant-mediated herbivore interactions.

Here, using *Plantago lanceolata* L. (Plantaginaceae) and one of its specialist chewing herbivores, *Junonia coenia* Hübner (Lepidoptera: Nymphalidae), I examine the effect of plant ontogeny (two developmental stages) and history of damage (single and multiple damage events early or late in the season) on plant responses to herbivory in both above- and belowground tissues. I specifically ask the following questions: 1) Do levels of constitutive defenses, as well as induced defenses and compensatory growth following leaf damage by a

specialist chewing herbivore, vary with plant age?, 2) For mature plants, does variation in frequency and intensity of herbivory early during a growing season influence plant induced chemical defenses and compensatory growth later in the season?, and 3) To what extent do changes in aboveground plant responses to leaf chewing damage, due to plant age or previous history of damage, translate into changes in belowground biomass and defenses?

METHODS AND MATERIALS

Study System

Plantago lanceolata L., narrowleaf or ribwort plantain, (Plantaginaceae) is a common short-lived weed (annual or facultative perennial) introduced to North America from Eurasia ca. 200 years ago (Cavers et al. 1980). This plant species forms natural populations with diverse age structures (Shefferson and Roach 2010) and thus, herbivore communities are naturally exposed to a wide diversity of host age classes in wild populations. *Plantago lanceolata* produces iridoid glycosides (IGs) (Ronsted et al. 2000) as its primary allelochemicals influencing feeding preferences of generalist and specialist rhizophagous and folivorous herbivores. For example, several laboratory and field experiments have shown that specialist lepidopterans use IGs as oviposition and feeding stimulants (Bowers 1983, 1984, Reudler Talsma et al. 2008b), and that larvae of these herbivores grew better on diets with higher concentrations of IGs (e.g. Bowers and Puttick 1989, Harvey et al. 2005, Saastamoinen et al. 2007). In contrast, generalist herbivores and pathogenic fungi damaging below- or aboveground tissues are usually deterred by high concentrations of IGs (Bowers and Puttick 1989, Strohmeyer et al. 1998, Biere et al. 2004, De Deyn et al. 2004, Harvey et al. 2005, Wurst and van der Putten 2007). *Plantago lanceolata* produces primarily two IGs: aucubin and catalpol, and amounts of these compounds may be as high as 10-12% dry weight (Bowers and Stamp 1993). Because aucubin is the biosynthetic precursor of the more toxic catalpol (Ronsted et al. 2000), high relative concentrations of catalpol indicate not only a greater biosynthetic investment of the plant but

also may be important in determining how generalist and specialist herbivores respond to the plant. Finally, constitutive concentrations of IGs have been shown to increase non-linearly during plant ontogeny (Fuchs and Bowers 2004, Quintero and Bowers 2011a) leading to significant seasonal variation. The variation in constitutive defenses in both above- and belowground tissues can be as much as an order of magnitude, even during relatively short periods of time (Bowers and Stamp 1993, Fuchs and Bowers 2004, Barton 2007, Quintero and Bowers 2011a), which can lead to significant variation in a plant's ability to induce chemical defenses within and among tissues.

The common buckeye butterfly (*Junonia coenia* Hübner, Nymphalidae) is a New World butterfly that can have one to three broods per year under temperate conditions, or more in the tropics where it can breed year-round (http://www.nearctica.com/butter/plate17/Jcoenia.htm). The larvae have been documented to feed on members of four plant families: Cornaceae, Plantaginaceae, Scrophulariaceae and Verbenaceae, and this species is considered a specialist on plants containing IGs (Bowers 1984). In *P. lanceolata* the two major IGs, aucubin and catalpol, serve as feeding and oviposition stimulants for buckeyes (Bowers 1984, Pereyra and Bowers 1988). In addition, buckeye larvae not only sequester IGs from their hosts, but also larval IG content is positively correlated with plant IG content (Camara 1997a); and thus, caterpillar performance and predation risk can strongly depend on levels of host plant defenses (e.g. Dyer and Bowers 1996, Theodoratus and Bowers 1999). Nevertheless, high concentrations of IGs may also have some detrimental effects by decreasing larval performance (Adler et al. 1995, Camara 1997a) and/or enhancing larval susceptibility to parasitoid attack (Smilanich et al. 2009a).

Plant and Caterpillar Colony Maintenance

The study was performed at the University of Colorado during spring-summer 2006. Seeds were collected from >20 maternal *P. lanceolata* plants from several populations in Boulder County,

Colorado, and mixed before the experiment started. Seedlings were germinated in Fafard mix on April 26th and transplanted after 15 days to a 1.5 L plastic pots containing growth medium of equal parts Metro Mix 350TM, sterilized sand, and turface. These extra-deep pots were used in order to ensure natural growth of root as well as shoot tissues and to prevent pot-binding, which can affect both compensatory growth and plant secondary metabolite production (Karban and Baldwin 1997). All seedlings used for this experiment were selected to be equivalent in size and number of leaves at the time of transplanting. Buckeye larvae used in this study were from a laboratory colony reared at the University of Colorado at Boulder. Larvae were fed on a mix of *P. lanceolata* leaves and kept in growth chambers with a photoperiod of 14hr day: 10 night, and day-night temperatures of 27°C /22°C.

Plant Age and Responses to Herbivory

To assess the effect of plant age on plant induced responses to herbivory, juvenile and mature *P. lanceolata* plants were damaged by *J. coenia* caterpillars when plants reached 9 and 14wk-old, respectively. I chose five weeks as a meaningful period between age classes because this is a good approximation of the average generation time of *J. coenia* (personal observation). In addition, 9 and 14-wk-old age classes were *a priori* selected to represent two distinct but still comparable developmental stages. These stages are comparable in the sense that both are already independent from reserves stored in the seed, contain several new and intermediate leaves but lack mature leaves, and from previous research they have shown to do not considerably vary in terms of nutritional quality and physical defenses (i.e. < 1% difference in nitrogen concentrations, <4% difference in percent water, and <10% difference in leaf toughness; unpublished data). However, while 9 wk-old plants, designated juvenile plants, still have a high relative growth rate and lower constitutive levels of IGs, 14 wk-old plants, designated mature plants, are usually reaching the onset of flowering, have started to decrease

their vegetative growth rate and constitutive defenses are often double those of juvenile stages (see results).

At both age classes, 40 previously undamaged plants were randomly assigned to one of two treatments: no herbivore (C: control) or damage by *J. coenia* caterpillars (H: herbivore). In order to account for potential differences between treatments before the experiment started, initial plant size of all plants at the beginning of the herbivory treatments was measured as the product of the length of the longest leaf and the total number of leaves, which provides a good index of plant size (Jarzomski et al. 2000). To confine caterpillars on the plants, control and treatment plants were caged using mesh bags constructed of Remay™. For juvenile plants, two newly molted 4th instar larvae per plant were placed in the center of the plants in the herbivory treatment. For mature plants, I applied from four to seven 4th instar caterpillars per plant in order to reach approximately 50% damage during an equivalent period of time. In both cases, two days after larvae were placed on plants or when larvae had eaten approximately 50% leaf tissue, all caterpillars and mesh bags were removed. Since induction of IGs was estimated to reach its highest point six days after damage (Fuchs and Bowers 2004), to test for short-term plant induced defenses as a function of plant age, all plants per treatment per age class were harvested seven days after herbivore removal.

Harvested plants were separated into above- and belowground tissues. Aboveground tissues consisted of new and intermediate leaf age classes (see Bowers and Stamp 1993) for both juvenile and mature stages. Thus, although I did not asses variation in plant defenses within different leaf age classes, I kept equal representation of leaf ages classes across plant developmental stages. Root tissues were washed until roots were totally clean from remaining soil material. Due to time constraints, root tissues from only half of the plants were processed (N = 10 per treatment). Harvested tissues were weighed fresh, oven-dried at 50°C for 48hrs to a constant mass and weighed again to the nearest hundredth of a gram. By the time plants reached their mature stage (14wk-old plants), few plants per treatment had started flowering.

However, because inflorescence biomass was usually <10% of the total aboveground biomass and because the proportion of plants flowering did not vary among treatments ($P > 0.05$), I added reproductive tissue data to leaf data in order to report overall aboveground (i.e. shoot) values for biomass and chemical defenses. Furthermore, I ran all analyses for the complete data set as well as separately, using just flowering or non-flowering individuals; but because trends were not significantly different (data not shown) I present here the results for the complete data set.

To assess variation in concentrations of IGs, dried shoots and roots were ground into a fine powder, and subsamples of 25 to 50mg were processed for IG extraction and analyzed by gas chromatography following previously described methods (Bowers and Stamp 1993, Jarzomski et al. 2000). Briefly, samples were extracted overnight in 95% methanol, and then partitioned between water and ether to remove hydrophobic compounds, using Phenyl-β-D-glucose (PBG) as the internal standard. An aliquot of the solution was derivatized with Tri-Sil-Z™ (Pierce Chemical Company) and injected into a HP 7890A gas chromatograph (Agilent Technology) using an Agilent DB-1 column (30m, 0.320mm, 0.25μm particle size). Amounts of aucubin and catalpol were quantified using ChemStation B-03-01 software and they are presented as percent dry weight for comparative purposes (e.g. Fuchs and Bowers 2004, Barton 2007, Wurst and van der Putten 2007).

Independent sample *t-tests* were used to test for possible differences in initial plant size between treatments of 9 or 14wk-old plants before the herbivory treatments were applied. Plant responses to herbivory (C vs. H) at juvenile or mature plant stages were assessed as changes in shoot and root biomass (dry mass), total concentration of IGs (% dry weight), and proportion of catalpol/total IGs, using a series of two-way analyses of variance (ANOVA) with age and treatment as the main effects and an interaction term included. Iridoid glycoside concentration data were arcsine-square root transformed for statistical analyses, but biomass did not required transformation. Separate two-way ANOVAs were performed for shoot and root tissues since

root tissues were available for only half of the plants (N = 10 for roots and N = 20 for shoots for each herbivory: age treatment combination). When the herbivory treatment effect was significant, I followed with single degree of freedom contrasts to assess differences between treatments (C vs. H) at the two different age classes.

History of Damage and Plant Responses to Herbivory

To determine how variation in the frequency and intensity of damage might affect plant performance and IG concentration, plants received a combination of damage and non-damage events early and later in the season. First, 80 juvenile plants (i.e. 9wk-old) were divided in two treatments: control and herbivory (N = 40 for each) as described above. Five weeks following the first damage event to juvenile plants (50% damage), 14 week-old plants (40 undamaged and 40 previously damaged plants) were exposed to a second generation of Buckeye caterpillars (50% damage). Thus, for this experiment there were four treatments: 1) plants that were never damaged (control-control: CC), 2) plants damaged by the first generation of Buckeyes but not by the second generation (herbivory-control: HC), 3) plants not damaged by the first generation of Buckeyes but damaged by the second generation (control-herbivory: CH), and 4) plants damaged by both generations of buckeyes (herbivory-herbivory: HH), with 20 replicates per treatment. As before, when caterpillars achieved 50% damage during both first and second damage events, all caterpillars and exclusion cages from control and treatment plants were removed. Seven days after the last damage event all plants per treatment were harvested and separated into shoot and root tissues to assess differences in plant biomass and induced defenses as explained above.

To assess the effect of previous herbivory on subsequent plant responses to damage, one week after the second generation of damage, differences in biomass, total concentration of IGs, and proportion of catalpol/total IGs in shoot and root tissues were evaluated using one-way ANOVAs. Because sample sizes were different between root and shoot tissues (see above)

these data were analyzed separately. In all cases, when significant effects were detected by ANOVAs, I followed up with a priori contrasts comparing (1) control vs. herbivory treatments (CC vs. HC + CH + HH), (2) plant responses to damage received early vs. late in the season (HC vs. CH), and (3) mature plant responses to a single herbivory event vs. multiple events (CH vs. HH).

RESULTS

Plant Age and Responses to Herbivory

At the beginning of the experiment, aboveground size of plants in the two herbivory treatments (C and H) were not significantly different from each other for either juvenile plants ($t_{1,38}$ -0.07; P = 0.51), or for mature plants ($t_{1,38}$ 5.6; P = 0.13). Based on the subset of plants harvested the same day that treatments started, juvenile plants at the beginning of the experiment had, on average, 12 leaves, their average aboveground biomass (dry weight) was 0.5 ± 0.04g, and their belowground biomass was 0.13 ± 0.01g. Mature plants had, on average, 56 leaves, their aboveground biomass was 6.05 ± 0.29g, and their belowground biomass was 4.14 ± 0.24g (dry weight).

One week following the first generation of damage, aboveground plant responses to herbivory were strongly influenced by plant age (Table 4.1, Figure 4.1). Aboveground biomass was significantly higher in control plants and older plants, but no interaction was observed (Table 4.1). Thus, one week following 50% tissue removal, damaged plants in both age classes were unable to compensate for the lost tissue (Figure 4.1a), with damaged plants being on average 46% and 24% smaller than control plants for juvenile and mature plants, respectively (juvenile: $F_{1,76}$ 6.5, P = 0.013; mature: $F_{1,76}$ 24.5, P<0.001). Therefore, although there was no significant interaction between age and herbivory, mature plants were better than juvenile plants at compensating for the lost tissue one week following damage. In turn, induced defenses after herbivory in aboveground tissues were observed only for proportion of catalpol but not for total

TABLE 4.1. Summary of two-way ANOVA's comparing plant responses in shoot and root tissues one week after herbivory treatments for juvenile and mature plants. Plant responses to first damage event (50% tissue removal) was assessed as changes in biomass (dry mass), total concentration of iridoid glycosides (% dry weight IGs), and proportion of catalpol/total IGs.

	Shoot tissues							**Root tissues**						
		Biomass		**Total IGs**		**Prop Catalpol**			**Biomass**		**Total IGs**		**Prop Catalpol**	
	df	*F*	*P*	*F*	*P*	*F*	*P*	df	*F*	*P*	*F*	*P*	*F*	*P*
Plant age	1	452.1	**<0.001**	541.7	**<0.001**	43.3	**<0.001**	1	124.1	**<0.001**	20.2	**<0.001**	12.5	**<0.001**
Herbivory	1	28.2	**<0.001**	0.2	0.67	17.0	**<0.001**	1	1.9	0.17	0.01	0.95	1.1	0.30
Age*Herbivory	1	2.9	0.095	0.06	0.80	10.4	**0.002**	1	0.2	0.63	0.38	0.54	3.4	0.07
Error	76							36						

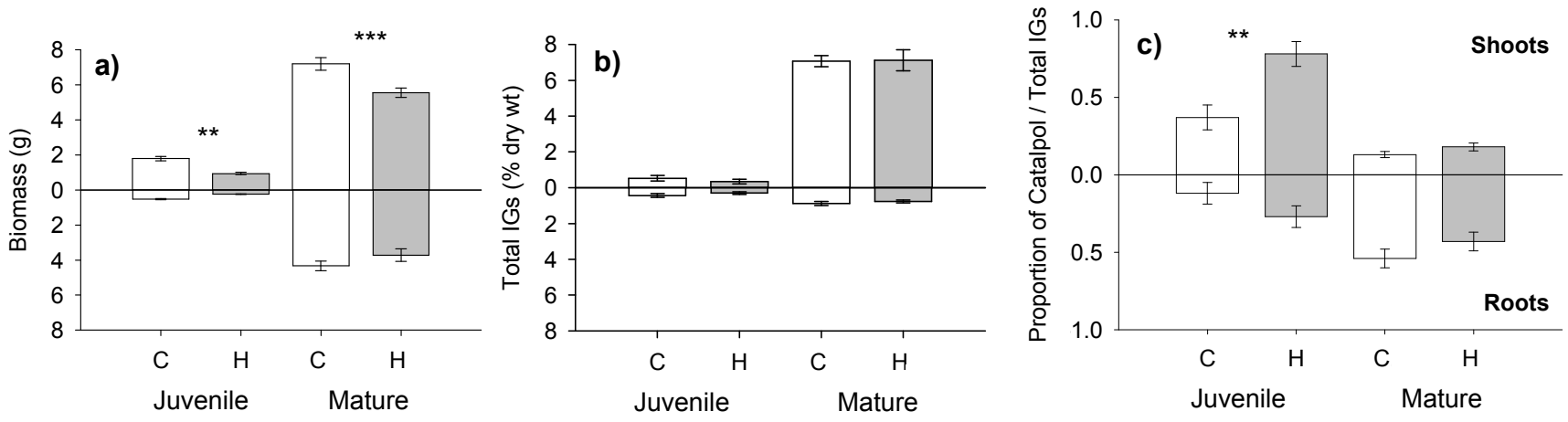

Figure 4.1. Plant responses to herbivory in *P. lanceolata*'s above- and belowground **a)** biomass, **b)** total concentration of iridoid glycosides (IGs), and **c)** proportion of catalpol/total IGs for control (C; white bars) and herbivore damaged plants (H; gray bars) during juvenile or mature plant stages. Original data were arcsine-square root transformed for statistical analyses, percentage data are shown for illustrative purposes only. Asterisks represent mean group differences among treatments as tested by single degree of freedom contrasts. Significance is displayed as $P < 0.0001$ (***), $P < 0.01$ (**), $P < 0.05$ (*).

levels of IGs (Table 4.1). Total levels of IGs were 16 times greater in mature plants when compared to juvenile plants; but they did not significantly vary between control and herbivory treatments in either age class (Figure 4.1b). In contrast, the proportion of catalpol not only varied with plant age, showing an average decrease of 47% from juvenile to mature plants, but also the ability of a plant to induce aboveground chemical defenses depended on host plant age (Table 4.1). Single degree of freedom contrasts showed that one week following 50% tissue removal, damaged juvenile plants showed a significant 45% increase in their levels of catalpol/total IGs ($F_{1,76}$ 9.7, P = 0.003), while mature plants did not significantly differ from control plants in the proportion of catalpol (Figure 4.1c) ($F_{1,76}$ 0.3, P = 0.58). This difference is illustrated by a significant interaction between plant age and treatment in aboveground proportion of catalpol (Table 4.1).

In contrast, although belowground tissues followed similar patterns to the ones seen for aboveground tissues, ANOVA results showed only a significant effect of plant age on root biomass and defenses; but leaf damage by Buckeye caterpillars did not translate into changes in root biomass or chemical defenses between control and herbivory treatments one week following damage (Table 4.1, Figure 4.1).

History of Damage and Plant Responses to Herbivory

One week following the second generation of damage by Buckeye caterpillars, ANOVA results showed that herbivory treatment had a significant negative effect on shoot biomass ($F_{3,76}$ = 21.46, P< 0.001) and a negative nearly significant effect on root biomass ($F_{3,36}$ 2.77, P = 0.056) (Figure 4.2a). Therefore, compensatory growth was not achieved a week or even five weeks following herbivore damage. Single degree of freedom contrasts showed that a significant decrease in shoot biomass was observed between undamaged control plants and damaged plants (CC vs. HC + CH + HH): $F_{1,76}$ 44.9, P<0.001), as well as between mature plants damaged once versus those damaged twice (CH vs. HH: $F_{1,76}$ 18.7, P<0.001) (Figure 4.2a). However, no

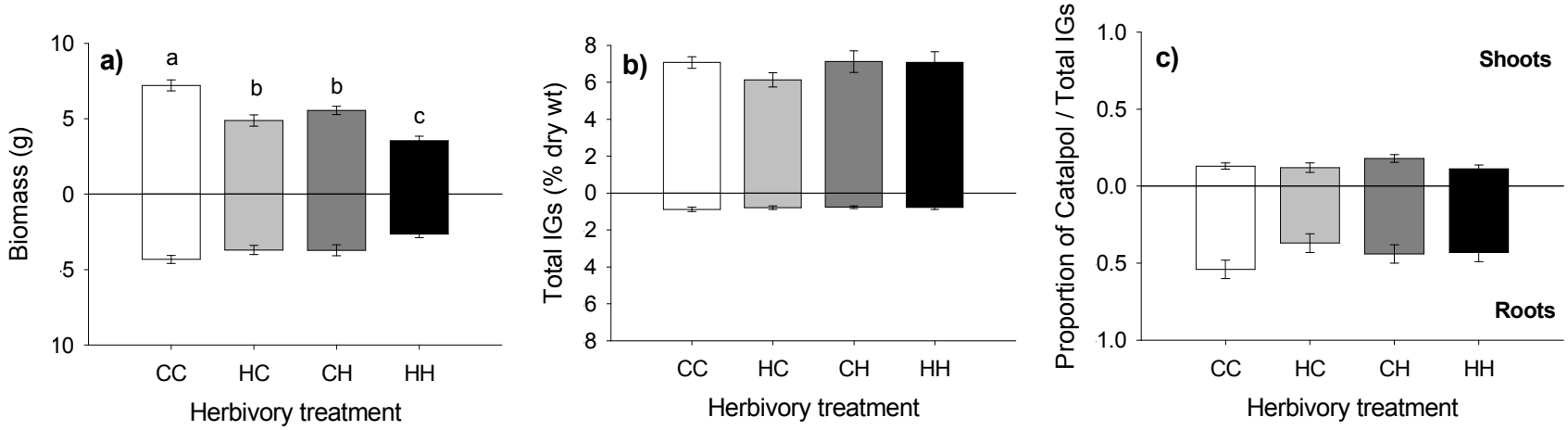

Figure 4.2. Effects of herbivory on *P. lanceolata*'s above- and belowground **a)** biomass, **b)** total concentration of iridoid glycosides (IGs), and **c)** proportion of catalpol/total IGs one week after second generation of damage. Bars represent the combination of first and second damage events, leading to four treatments: 1) plants that were never damaged (CC), 2) plants damaged by the first generation of Buckeyes but not by the second generation (HC), 3) plants not damaged by the first generation of Buckeyes but damaged by the second generation (CH), and 4) plants damaged by both generations of Buckeyes (HH), with 20 replicates per treatment. Original data were arcsine-square root transformed for statistical analyses, percentage data are shown for illustrative purposes only. Lowercase letters represent mean group differences among treatments as tested by Bonferroni post-hoc tests ($p<0.05$).

significant difference in shoot biomass was observed between plants with one single damage event early versus late in the season (HC vs. CH: F1,76 2.1, P = 0.15) (Figure 4.2a).

In contrast, I did not observe any significant changes in plant IGs as a response to different histories of damage: production of total IGs and proportion of catalpol/total IGs did not vary with history of damage either for aboveground tissues ($F_{3,76}$ = 0.99, P = 0.40 and $F_{3,76}$ = 2.39, P = 0.08, respectively) or belowground tissues ($F_{3,36}$ = 0.13, P = 0.94 and $F_{3,36}$ = 0.59, P = 0.63, respectively). Thus, mature plants varying in intensity and frequency of herbivory, had very similar levels of defenses across all four history of damage treatments, and induction of defenses was not observed (Figure 4.2b and c).

DISCUSSION

This study revealed novel patterns regarding the extent to which plant age and history of damage over a growing season can modify *Plantago lanceolata*'s ability to respond to herbivory. Most notably, I showed that while constitutive concentration of IGs markedly increased as plants matured, plant ability to induce chemical defenses was limited to juvenile, but not mature, plant stages. In addition, induced defenses observed seven days following herbivory in juvenile plants disappeared five weeks after the first herbivory event, and mature *P. lanceolata* plants that varied considerably in the absolute amount and frequency of damage received over five weeks, did not significantly differ in their levels of chemical defenses. Finally, contrary to what I expected, I did not observe changes in belowground tissues' defenses or biomass a week following 50% removal of aboveground tissues at either age class or history of damage. Together, these results suggest that in *Plantago lanceolata* and perhaps other systems, ontogenetic patterns in plant growth and defenses can play a stronger role in mediating plant-herbivore interactions than history of damage.

Plant ontogeny strongly influenced plant defenses in both above- and belowground tissues. Synthesis and accumulation of constitutive IGs increased 13 times in aboveground

tissues and doubled in belowground tissues from juvenile to mature stages. In addition, plant allocation patterns also varied considerably from almost equal investment in total IG concentrations between shoot and root tissues during the juvenile stage to a large increase in plant total IGs in shoots as compared with roots during the mature stage (i.e. ratio of total levels of IGs between shoot and root tissues varied from roughly 1:1 in juveniles to 8:1 ratio in mature plants) (Figure 4.1). Comparable trends in constitutive defenses within and among tissues as plants age have been reported previously for *P. lanceolata* (Darrow and Bowers 1999, Fuchs and Bowers 2004, Quintero and Bowers 2011a) as well as for other IG-containing plants (Hogedal and Molgaard 2000, Beninger et al. 2007, Jamieson and Bowers 2010). These shifts suggest that, even in the absence of damage, plant palatability can dramatically change in a short period of time with strong implications for plant-herbivore interactions and plant fitness. For instance, even smaller differences in concentration of IGs in *P. lanceolata* as the ones reported here have shown to significantly decrease leaf consumption and growth rate of generalist herbivores and pathogens as IG increases (Biere et al. 2004). As a result, herbivore pressure may be higher on younger developmental stages, decreasing their chances of survival or future reproductive output (e.g. Shefferson and Roach 2010).

While the role of ontogenetic trajectories in constitutive defenses has received considerable attention, our knowledge regarding the role of plant age in explaining herbivore induced defenses is still limited. A recent meta-analysis summarizing ontogenetic patterns in plant defense traits illustrated that, in the case of herbaceous plants, ability to induce defenses after damage usually decreased with plant age while no significant differences in tolerance were observed across plant development (Barton and Koricheva 2010). In accordance with this trend, I show that a week following damage, juvenile *P. lanceolata* plants were significantly more capable of induction (i.e. 45% increase in the proportion of catalpol/total IGs) than mature plants, but no significant differences were observed for compensation to herbivory. Nevertheless, lack of differences in compensatory growth between age classes should be interpreted carefully

since I only assessed changes in biomass between treatment and control plants a week following 50% damage and thus, these short-term responses may not reflect long-term compensatory growth strategies nor be indicative of potential fitness consequences. In terms of induction, because the defensive properties of IGs have been well documented (reviewed by Bowers 1991), my data suggest that increased ability to induce defenses at the juvenile stage is likely to increase plant resistance to generalist herbivores. Increased levels of IGs, especially catalpol, usually deter generalist herbivores and/or decrease consumption rate (e.g. Bowers and Puttick 1989, Biere et al. 2004, De Deyn et al. 2004, Wurst and van der Putten 2007), potentially allowing induced juvenile plants to decrease subsequent damage by generalist herbivores. However, this increase in defenses may also enhance *P. lanceolata* susceptibility to specialist herbivores such as Buckeye butterflies (e.g. Bowers 1984, Pereyra and Bowers 1988, Prudic et al. 2005, Reudler Talsma et al. 2008b). Therefore, whether a high induction capability at younger life stages in *P. lanceolata* is adaptive remains to be tested, but may strongly depend on the community structure of antagonist species present in the habitat. Alternatively, mature plant stages with already 16 times more constitutive defenses than juvenile stages may not benefit by investing in induced defenses. Thus, as seen in other plant species, age-dependent induced defenses in *P. lanceolata* may respond to variable cost and benefits of these defensive strategies as plants develop (Ohnmeiss and Baldwin 2000, Van Dam et al. 2001, Boege et al. 2007, Orians et al. 2010).

Plant responses to sequential damage over a growing season may depend on and/or interact with age-dependent induced responses to herbivory. Two alternative scenarios can be expected, one where age-dependent induced responses can prevail over plant responses to sequential damage or, the opposite, where herbivore-induced indirect interactions alter plant defenses beyond the expected age-dependent induced response. To my knowledge, we currently lack empirical data testing these opposite scenarios. On one hand, recent empirical studies have shown strong age-dependent induced responses to herbivory (Van Dam et al.

2001, Boege et al. 2007, Barton 2008, Tucker and Avila-Sakar 2010), but these studies usually lack a consideration of the role of multiple and successive damage events over the course of plant development. Alternatively, studies assessing the effects of previous history of damage on plant responses to herbivory have shown both that previous damage events can increase plant induced responses after subsequent damage (Ruuhola et al. 2007, Roitto et al. 2009), or decrease plant ability to mount a localized or systemic induced response following subsequent damage (Van Zandt and Agrawal 2004, Viswanathan et al. 2007, Poelman et al. 2008a, Erb et al. 2011). Although these latter studies assess the role of history of damage over extended periods of time, where plant ontogeny may interact with herbivore-induced indirect interactions, conclusions on the independent or combined effects of these factors on plant induced responses are hard to draw. Most of these studies vary widely in terms of plant life form (i.e. annual or perennial), herbivore-mediated indirect interactions (i.e. conspecific or heterospecific interactions), and time elapsed between initial and subsequent damage (i.e. from 48hrs to >5 years); making it difficult to assess whether changes in plant defense phenotypes and/or subsequent herbivore communities are the product of immunological memory and/or priority effects, age-dependent induced responses, or a combination of both. My results show, that a week following the second damage event, mature plants with diverse frequency and intensity of damage (i.e.CC, CH, HC, HH) had all similar levels of defenses. Thus, for this plant-herbivore system and considering potential conspecific interactions occurring over successive generations (i.e. five weeks), plant age seems to influence plant allocation of resources to defense more than past history of damage.

Evaluating changes in plant induced responses within and between above- and belowground tissues as a function of plant age or history of damage is essential to understand temporal variation in plant-herbivore interactions. Herbivore-induced defenses not only may change the palatability and nutritional quality of a tissue for future herbivores, but also may lead to altered phytochemical expression across multiple plant tissues (Ohgushi 2005, Kaplan et al.

2008). However, in this study, *P. lanceolata*'s short-term responses to 50% aboveground tissue removal by a specialist caterpillar did not translate into significant changes in root biomass or defenses (as also found in Darrow and Bowers 1999). In accordance with these data, a recent meta-analysis of plant induced defenses within and across tissues following leaf and root herbivory demonstrated that herbivore damage to aboveground tissues usually results in stronger induced responses in leaves than in roots (Kaplan et al. 2008). Thus, if these observed short-term responses hold true throughout the growing season, then we should expect stronger temporal changes in aboveground herbivore population and/or community structure following leaf damage (especially in the case of juvenile plants) than in belowground communities. Nevertheless, it is important to emphasize that the patterns described here may be system-specific. Here, I only assessed *P. lanceolata* responses to variable timing and severity of damage by a single specialist leaf-chewing herbivore, larvae of the Buckeye butterfly. It is the case for *P. lanceolata* that induced defenses within and across tissues can vary considerably as a function of herbivore identity. For instance, Bowers and Stamp (1993) showed that *P. lanceolata* damaged by the specialist *J. coenia* induced higher levels of IGs than plants damaged by the generalist *Spilosoma congrua* (Arctiidae). Similarly, Wurst and van der Putten (2007) demonstrated not only that induction of IGs depends on herbivore identity but also that herbivore-induced indirect interactions can vary as a function of initial and subsequent above- and belowground herbivore species. Therefore, the patterns reported here may differ for different herbivore species and/or a combination of multiple antagonists damaging above- and belowground tissues over time. Hence, future studies assessing the ecological consequences of history of damage and plant age-dependent induction within and across tissues for a wide diversity of above- and belowground antagonists as well as more plant developmental stages will shed light on the ubiquity of the patterns observed here.

In summary, these results suggest that plant ontogeny not only dictates important shifts in plant investment in constitutive defenses, but it can also influence the ability of a plant to

respond to herbivory, making ontogenetic trajectories a key feature in mediating plant-herbivore interactions. In contrast, variable frequency and intensity of damage in mature plant stages did not affect plant investment in defenses within as well as between tissues; suggesting that plant-mediated herbivore interactions throughout a season may strongly depend on plant age during initial and subsequent damage. For example, age-dependent induced resistance may shift the relative abundance or performance of generalist and specialist herbivores following initial damage to juvenile stages but not mature plant stages. Thus, in *P. lanceolata* and perhaps other systems, it is possible that ontogenetic patterns in plant growth and defenses leading to strong age-dependent induced responses may prevail over herbivore-induced indirect interactions. Yet, because herbivore-induced indirect interactions also vary as a function of herbivore identity and the lag of the localized and/or systemic induced response over time (Van Zandt and Agrawal 2004, Muola et al. 2010), more studies addressing the effect of sequential damage by multiple shoot and root herbivores on plant defense phenotype and subsequent herbivore community structure throughout plant development are needed.

CHAPTER 5

PLANT ONTOGENY MEDIATES TRITROPHIC INTERACTIONS BETWEEN PLANTS, HERBIVORES, AND NATURAL ENEMIES

ABSTRACT

Plant-herbivore-natural enemy interactions may vary markedly in space and time. However, studies assessing changes in the direction and magnitude of these interactions have traditionally examined the role of spatial variation more than the role of temporal change. Physiological and anatomical constraints during plant ontogeny affect the expression of numerous plant traits relevant to higher trophic levels, such as nutritional content and physical and chemical defenses. Yet, we know little about how these sources of temporal variation can directly and/or indirectly mediate tri-trophic interactions. Using four distinct ontogenetic stages of *Plantago lanceolata* (Plantaginaceae) and the specialist herbivore *Junonia coenia* (Nymphalidae), I evaluated: butterfly oviposition choice, caterpillar performance (survival rate, relative growth rate and nutritional indices), caterpillar sequestration, and caterpillar immune defenses. Plant defensive traits changed significantly as *P. lanceolata* developed, with leaf tissues increasing in toughness and concentration of plant allelochemicals (iridoid glycosides), while decreasing in nutritional quality (water and nitrogen concentrations) as plants aged. These ontogenetic changes significantly influenced plant-herbivore-natural enemy interactions both directly and indirectly. Buckeye butterflies significantly preferred younger developmental stages of *P. lanceolata* over older stages, laying on average 60% more eggs on juvenile plants than on reproductive stages. In accordance with the preference-performance hypothesis, caterpillars feeding on juvenile *P. lanceolata* plants showed faster relative growth rate and increased digestive efficiency compared with those feeding on plants in the reproductive stage. In contrast,

caterpillars feeding on younger developmental stages acquired lower levels of sequestered chemical defenses, which may increase their susceptibility to predation. Finally, host plant age also altered the ability of a caterpillar to mount an immune response against simulated parasitoid eggs, with caterpillars reared on older life stages, and thus having higher levels of sequestered iridoid glycosides, showing a compromised immune response compared to those feeding on younger plant age classes. These results demonstrate that ontogenetic variation in plant defenses and nutritional quality can alter tri-trophic interactions both directly and indirectly, emphasizing the importance of plant ontogeny in regulating temporal shifts in herbivore population dynamics and community structure.

INTRODUCTION

Plants can minimize the damage caused by herbivores via the expression of traits that diminish the ability of herbivores to locate and consume plant tissues (i.e. bottom-up forces) or via traits that enhance herbivore predation risk by predators and parasitoids (i.e. top-down forces) (Price et al. 1980). As plants develop, all major traits that influence their quality as food for herbivores such as secondary metabolites (Boege and Marquis 2005), physical defenses (Hanley et al. 2007), and water and nutrient content (Kozlowski 1971, Mattson 1980), change from seedling to mature stages. In addition, plant traits that influence the strength of top-down forces on herbivore population dynamics, such as volatile organic compounds, architectural traits and shelter or food rewards, have also demonstrated considerable variation as plants develop (Quintero et al. *in prep.*). Thus, it is not surprising that several studies have reported considerable variation in herbivore diversity, abundance and damage as host plants develop (Kearsley and Whitham 1989, Hanley et al. 1995, Fenner et al. 1999, Pires and Price 2000, Swihart and Bryant 2001, Fonseca et al. 2006, Thomas et al. 2010). However, only a few studies have explored whether these patterns are shaped primarily by bottom-up forces, such as plant traits that alter herbivore host selection and performance (e.g. Lawrence et al. 2003,

Albrectsen et al. 2004, Johnson and Zalucki 2005, Heckel et al. 2010), or by top-down forces such as prey vulnerability to natural enemies (e.g. Van Bael et al. 2003, Riihimaki et al. 2006, Bohm et al. 2011). Furthermore, with few exceptions (Stein and Price 1995, Pires and Price 2000, Del Val and Dirzo 2003, Boege 2005a, Fonseca et al. 2006), most studies have only assessed one of these forces at a time, failing to consider potential synergisms or trade-offs. The goal of this study was to evaluate how ontogenetic variation in plant defenses and nutritional quality might affect tri-trophic interactions: (i) directly, through changes in butterfly oviposition choice and caterpillar performance, or (ii) indirectly, through changes in caterpillar vulnerability to natural enemies.

One way in which changes in insect herbivore diversity, abundance and damage over the course of plant development can arise is if herbivores select different developmental stages of their host due to species-specific feeding needs. This selection can occur trough either female oviposition choice or larval/adult foraging choice. Empirical evidence shows that, in general, invertebrate herbivores cause greater damage (Price 1991, Spiegel and Price 1996, Albrectsen et al. 2004, Fonseca et al. 2006), or reach higher density and diversity (Waltz and Whitham 1997, Cuevas-Reyes et al. 2004, Boege 2005a, Thomas et al. 2010) on younger compared to older plant stages. While this pattern can indeed reflect herbivore preferences, it may also result from higher herbivore performance or lower predation rates. Thus, to tease these possible mechanisms apart, more studies explicitly assessing host selection across host plant ontogeny are needed. Empirical studies so far have revealed highly species-specific responses. For instance, there are cases in which females or free living larvae prefer to feed on younger (e.g. Pires and Price 2000, Del Val and Dirzo 2003, Cuevas-Reyes et al. 2004) or mature host plant stages (e.g. Lawrence et al. 2003, Johnson and Zalucki 2005, Fernandes et al. 2010, Heckel et al. 2010). These examples emphasize that herbivore host selection cannot be the sole mechanism driving the general pattern described for insect herbivore diversity and damage across host plant ontogeny.

The ontogenetic stage of the host plant may also affect herbivore growth rate, development time and feeding efficiency, leading to differences in the rate or absolute amount of tissue lost to herbivores. For example, upon consumption of plant tissues, plant allelochemicals can inhibit digestive proteases in the insect gut, affect membrane permeability to primary nutrients, and inhibit DNA synthesis (Schmeller et al. 1997, Howe et al. 2005, Constabel and Barbehenn 2011), all of which can critically reduce growth. Alternatively, herbivorous insects can compensate for suboptimal nutritional quality or higher levels of physical defenses by increasing food consumption or extending development time (Hagele and Rowell-Rahier 1999, Clissold et al. 2009). Because plant nutritional and defensive traits are known to vary as plants age (Kozlowski 1971, Mattson 1980, Boege and Marquis 2005, Hanley et al. 2007), their overall effect on herbivore performance will depend on whether the effects are synergistic, antagonistic or neutral. In several plant species, when more than one trait was assessed, contrasting ontogenetic patterns were described within and among both plant allelochemicals and nutritional quality (Donaldson et al. 2006, Rehill et al. 2006, Mcarthur et al. 2010, Quintero and Bowers 2011a). As a result, these complex phenotypic changes can render plants more or less susceptible to a diverse suite of generalist and specialist herbivores (e.g. Kearsley and Whitham 1989),

The strength of the top-down control of herbivores is also likely to change throughout plant ontogeny, thereby altering herbivore diversity and/or damage. At higher trophic levels, ontogenetic changes in plants traits can alter predator-prey interactions in two ways: (i) directly by altering plant cues used by natural enemies to find their prey or (ii) indirectly by modifying prey quality and thus, predators' choice and performance among different quality prey. Evidence for the former comes from studies demonstrating that the top-down control of herbivores can be mediated by ontogenetic changes in plant size and architectural complexity (Van Bael et al. 2003, Boege 2005a, Riihimaki et al. 2006), release of volatile organic compounds in response to damage (Zhu and Park 2005, Rostas and Eggert 2008, Hare 2010),

and changes in the availability of food rewards (Del Val and Dirzo 2003, Miller 2007, Palmer et al. 2010). In contrast, although ontogenetic patterns in quality and quantity of secondary compounds can also indirectly alter the magnitude of the top-down control through reduced prey quality (Hunter 2003), data confirming this assumption are limited.

Changes in host plant defenses, as well as nutritional quality, may have different effects on herbivores and their associated natural enemies (reviewed in Ode 2006, Poelman et al. 2008b). First, plant quality can influence herbivores and their enemies in the same direction, such that highly defended or less nutritious plants decrease the performance of both the insect herbivores and their natural enemies (e.g. Reitz and Trumble 1997, Havill and Raffa 2000, Teder and Tammaru 2002, Gols et al. 2008b). Alternatively, natural enemies can respond differently than herbivores to variation in host plant quality, if, for example, highly defended plants increase the performance of specialist sequestering herbivores, but natural enemies show decreased performance or fitness due to increased prey toxicity or unpalatability (e.g. Fordyce 2001, Ode et al. 2004, Singer et al. 2009, Chaplin-Kramer et al. 2011, Kos et al. 2011). Furthermore, natural enemies such as predators and parasitoids may also vary in their response to prey quality. For example, while increased levels of plant allelochemicals in insect tissues usually decrease predation, they may enhance susceptibility against parasitoids (Gentry and Dyer 2002, Lampert et al. 2010, Le Guigo et al. 2011).

One way in which endoparasitoids may benefit from increased plant defenses is via the direct effects of plant defenses on their host's immune system (Turlings and Benrey 1998). Because the ability of insect herbivores to successfully encapsulate and kill their endoparasitoids depends on their energetic and physiological resources (Blumberg 1997, Cotter et al. 2011), herbivores exposed to high levels of allelochemicals and/or poor nutritional quality may experience decreased immunocompetence (e.g. Haviola et al. 2007, Bukovinszky et al. 2009, Smilanich et al. 2009a, Shikano et al. 2010). Hence, as host plants progress from seedling to senescent stages, the changes in nutritional quality and defenses that translate into

variation in herbivore performance and quality as prey or host can indirectly mediate the selective pressure imposed by predators and parasitoids on herbivore population dynamics.

Here, I present a series of experiments that assess the extent to which ontogenetic variation in plant defenses and nutritional quality might affect (i) butterfly oviposition choice, (ii) caterpillar performance (survival rate, growth rate, and digestive efficiency), and (iii) caterpillar vulnerability to natural enemies, as mediated by prey quality and palatability. Greenhouse and laboratory experiments assessing changes in plant-herbivore-natural enemy interactions as plants develop were conducted across four distinctive ontogenetic stages using a model system composed of Ribwort or Narrow-leafed plantain, *Plantago lanceolata* L. (Plantaginaceae), and one of its specialist herbivores, the Buckeye butterfly (*Junonia coenia* Hübner, Lepidoptera: Nymphalidae). Significant ontogenetic patterns in *P. lanceolata*'s traits relevant to herbivores have been reported, with previous studies showing an overall increase in plant allelochemicals (i.e. iridoid glycosides), but significant decreases in nutritional quality (i.e. water and nitrogen concentrations) as plants age (Bowers and Stamp 1993, Fuchs and Bowers 2004, Barton 2007, Quintero and Bowers 2011a). Results from this study show that consideration of the combined role of direct and indirect effects of plant ontogeny on tri-trophic interactions may have key implications for understanding temporal shifts in herbivore population and community structure.

MATERIALS AND METHODS

Study system

Plantago lanceolata (Plantaginaceae), Ribwort or Narrow-leafed plantain, is a common short-lived weed (annual or facultative perennial) introduced to North America from Eurasia ca. 200 years ago (Cavers et al. 1980). This plant species forms natural populations with diverse age structures (Shefferson and Roach 2010) and, thus, herbivore communities are naturally exposed to a wide diversity of host age classes in wild populations. *Plantago lanceolata*

produces iridoid glycosides (IGs) (Ronsted et al. 2000) as its primary allelochemicals influencing generalist and specialist herbivores. In general, high levels of IGs deter or decrease damage inflicted by generalist herbivores (Bowers and Puttick 1989, Strohmeyer et al. 1998, Biere et al. 2004, De Deyn et al. 2004, Harvey et al. 2005, Wurst and van der Putten 2007), although several insect herbivore species have evolved to overcome, and in some cases sequester, those defenses in both their native and introduced range (Bowers 1983, 1984, Reudler Talsma et al. 2008a, Baden and Dobler 2009, Opitz et al. 2010). Primarily two IGs are produced by this species, aucubin and catalpol, and amounts of these compounds can reach up to 10-12% dry weight (Bowers and Stamp 1992, 1993). Besides IGs, *P. lanceolata* also invests in physical defenses such as leaf toughness (Schippers and Olff 2000) and glandular and non-glandular trichomes (de la Fuente 2002). Strong ontogenetic patterns in nutritional quality and constitutive concentrations of IGs have been previously reported (e.g. Fuchs and Bowers 2004, Quintero and Bowers 2011a), suggesting that herbivores are often exposed to significant seasonal variation in host plant quality. The variation in constitutive defenses can be as much as an order of magnitude, changing over relatively short periods of time (Bowers and Stamp 1993, Fuchs and Bowers 2004, Barton 2007, Quintero and Bowers 2011a), and sometimes exceeding the variation reported as a function of plant genotype, nutrient availability or herbivore damage (Bowers et al. 1992, Bowers and Stamp 1993, Barton 2007, Quintero and Bowers 2011b).

Junonia coenia Hubner (Nymphalidae), the common buckeye butterfly, is a New World butterfly that can have one to three broods per year in temperate regions (Brock and Kaufman 2003). This species is a specialist on plants containing IGs (Bowers 1984), *P. lanceolata* being a common host plant (Graves and Shapiro 2003 and references therein). Adult female butterflies use IGs as oviposition stimulants (Pereyra and Bowers 1988), choosing host plants or tissues within plants with higher IG content, in particular catalpol (Klockars et al. 1993, Prudic et al. 2005). In addition, *J. coenia* butterflies lay a single egg at a time (http://www.butterfliesandmoths.org/species/Junonia-coenia), and thus, in the oviposition tests

(see below), each egg represents an individual choice. Furthermore, buckeye caterpillars not only use IGs as feeding stimulants (Bowers 1984), but they are also able to sequester aucubin and catalpol in their hemolymph (Bowers and Puttick 1986). Levels of IGs in buckeye caterpillars, which are positively correlated with levels of IGs in their diet, vary normally from less than 5% to over 20% dry weight (Camara 1997a). Caterpillars reaching higher levels of IGs in their tissues benefit from decreased mortality by predators such as ants, stink bugs, spiders, and predatory wasps (de la Fuente et al. 1995, Dyer and Bowers 1996, Strohmeyer et al. 1998, Theodoratus and Bowers 1999, Stamp 2001, Stamp and Meyerhoefer 2004). Nevertheless, some physiological and ecological costs associated with sequestration have also been reported (Adler et al. 1995, Camara 1997a, Smilanich et al. 2009a). Most recently, Smilanich et al. (2009a) showed that high IG diets, leading to high IG content in caterpillar hemolymph, may enhance larval susceptibility to parasitoid attack by weakening cellular immune responses.

Experimental design

Plants used in these experiments were grown at the University of Colorado greenhouse during the summer of 2009 and 2010. In both years, I wanted to expose butterflies (2009) and caterpillars (2010) simultaneously to all host plant developmental stages. Therefore, I synchronized host plant developmental stages by germinating seeds at intervals of 30 days from March to June. In this way, although average environmental conditions from sowing to harvest varied among age classes, I ensured that all individual plants were exposed to the same environmental conditions prior to exposure to herbivores or harvest. In this way, I reduced potential problems associated with serial sowing by reducing the effects of other confounding environmental factors such as photoperiod and temperature on plant quality and defenses (Hogedal and Molgaard 2000, Tamura 2001, Fuchs and Bowers 2004). Seeds were collected from >20 maternal plants from a population in Boulder County, Colorado, and mixed before sowing them in seed flats at intervals over the spring-summer season. Seeds were germinated

in Fafard mix and transplanted, after 15 days, to a growth medium of Metro Mix 350 and turface in 4.5 liter pots. Buckeye larvae used in this study were from a laboratory colony reared at the University of Colorado at Boulder. Larvae were fed on a mix of field collected *P. lanceolata* leaves and kept in growth chambers with a photoperiod of 14hr day: 10 night, and day-night temperatures of 27°C /22°C.

In both years, I wanted to compare distinctive developmental stages to assess the effect of host plant ontogeny on higher trophic levels. Four age classes were used: J1- which represents young juvenile plants soon after they ended their seedling stage (i.e. containing from 5 to 15 new leaves, and averaging 0.40g dry mass), J2- which represents juvenile plants that have reached a complete rosette with new, intermediate and old leaves but have not yet developed any reproductive structures (~1.60 to 4.30g dry mass), FL- representing mature rosette plants that have started flowering and contain from few to many scapes with buds or open flowers (~6 to 11.5g dry mass), and FR- representing mature rosette plants with their inflorescences ranging from fruit development to seed release (~14.20g dry mass). I simultaneously grew 20 to 180 plants in each of these four age classes per year, to ensure sufficient independent replicates for butterfly choice tests (2009) as well as for caterpillar rearing (2010). Replicates were randomly placed on four to six 2.5 x 4m greenhouse benches, exposed to natural daylight, and watered daily. Scotts Peter's Excel (Scotts-Sierra Horticultural Products Company, Marysville, Ohio) mixed in a ratio of 15-5-15 N-P-K with trace micronutrients fertilizer was supplied to all plants every three to four days throughout the duration of the experiment.

Female oviposition choice

Plant quality and defenses- Immediately following inspection, counting, and egg removal from all plant tissues used in butterfly oviposition tests (see below), I measured variation in plant nutritional quality (leaf water and nitrogen concentration) and defenses (leaf toughness and

concentration of IGs) as a function of plant ontogeny. All aboveground tissues (new, medium and old leaves and inflorescences) available per plant were weighed fresh immediately following harvest, oven-dried at 50°C for 48hrs to a constant mass, and weighed again to the nearest 0.01g. Leaf water content was calculated as [(wet weight –dry weight) / wet weight] x 100. Total leaf nitrogen concentrations was quantified by Micro-Dumas combustion on a NA1500 C/H/N analyzer, using approximately 3mg of finely ground leaf tissue per sample.

To assess variation in concentrations of IGs, all tissues were ground into a fine powder, and 10-30mg subsamples (entire available tissue for some J1 plants) were processed for IG extraction and analyzed by gas chromatography following previously described methods (Bowers and Stamp 1993, Barton 2007, Quintero and Bowers 2011a). Briefly, samples were extracted overnight in 95% methanol, the methanol extract was filtered to remove the plant material and the residue evaporated to dryness. Phenyl-β-D-glucose (PBG) was added as an internal standard and the samples were then partitioned between water and ether to remove hydrophobic compounds. An aliquot of the remaining solution was removed, evaporated, and derivatized with Tri-Sil-Z^{TM} (Pierce Chemical Company) and injected into a HP 7890A gas chromatograph (Agilent Technology) using an Agilent DB-1 column (30m, 0.320mm, 0.25µm particle size). Amounts of aucubin and catalpol were quantified using ChemStation B-03-01 software and they are presented as percent dry weight for comparative purposes (e.g. Fuchs and Bowers 2004, Barton 2007, Wurst and van der Putten 2007).

To determine leaf toughness, a Wagner Fruit Tester series penetrometer (Model #U0801, with a 0.1-mm tip) was used to measure the grams of force (GF) required to fracture the leaf lamina. Leaf toughness measurements were taken before leaf tissues were oven dried. Specifically, the amount of force needed to fracture foliage (defined here as leaf toughness, Sanson et al. 2001) was assessed by randomly selecting 3 to 6 leaves, per tissue category and age class, and taking four measures per leaf in between major leaf veins. Mean grams of force needed to punch each leaf with a 0.1mm steel rod was used as the dependent variable.

Although I recorded all plant traits (i.e. biomass, leaf water and nitrogen concentrations, leaf toughness and % dry weight aucubin and catalpol) for each of the four available tissues, here I present differences in plant quality and defenses only for new leaves for two reasons. First, new leaves are the only tissue category present across all four plant developmental stages, allowing comparison across treatments. Second, as previously observed by Klockars et al. (1993), Buckeye butterflies preferentially oviposit on new leaves regardless of their availability in the plant (see results). Thus, assessing variation in quality traits of new leaves across all age treatments should highlight ontogenetic changes relevant for ovipositing butterflies.

Developmental variation in new leaf biomass, leaf toughness, and water and nitrogen concentrations was analyzed using one-way ANOVAs, followed by Bonferroni post-hoc tests to distinguish mean differences among age classes if overall significant differences were found. Multivariate analysis of variance (MANOVA) was used to examine concurrent variation in aucubin and catalpol concentrations as a function of plant age, due to correlation between these two variables within tissues. When significant effects were detected by MANOVA, I followed up with univariate ANOVAs for each IG, and mean group differences among plant age classes were assessed using Bonferroni post-hoc tests. Biomass data were square-root transformed and percent water, nitrogen, and IG concentrations were arcsine square-root transformed to improve normality and homogeneity of variance.

Oviposition tests - To evaluate butterfly oviposition preference among multiple developmental stages of *P. lanceolata*, two sets of 3-way choice tests were performed from June 16th to 30th, 2009: (i) J1 - J2 - FL and (ii) J2 - FL - FR. For each test, plants in each of the three developmental stages were placed inside a circular wired cage (1.2 m diameter x 1m height), covered with a fine mesh and secured with clothes pins on top. Plants were equally spaced from each other, 30cm apart, and a source of sugar water was placed in the center of the cage,

equidistant from all plants. One to 3-day-old naïve butterflies, four *J. coenia* males and one female, were placed inside each cage for 72hr, allowing sufficient time for the female butterfly to mate and lay eggs. All experiments were performed in the field under natural light and temperature conditions; and thus, plants used in this experiment were acclimated to the field conditions for a week before the experiment started. The cages were checked every 12 to 24hr to refill the food supply and to water plants as needed. After 72hrs, all plants were removed from the cages, harvested, and aboveground tissues were separated into four categories: new, medium and old leaves, and inflorescences (see Bowers and Stamp 1992, Klockars et al. 1993). Number of leaves and inflorescences per tissue per plant were counted as well as the total number of eggs laid on each tissue category per plant. Plant tissues were then prepared for subsequent assessments of nutritional quality and defenses (see above). Although rarely found, eggs deposited on the cage or pot were not included in the analyses.

Twenty replicates per choice test were completed, but I excluded replicates where females laid less than 20 eggs total. Friedman two-way analysis of variance by ranks was used to assess female choice across the two sets of 3-way choice tests. When an overall significant effect of plant age was found, I followed with Wilcoxon signed-rank tests, with their respective Bonferroni adjustment, to test for mean group differences among the three age classes. Total number of eggs laid differed widely among individual females (i.e. 60 to 370 eggs); therefore, I used the arcsine-square root transformed proportion of eggs laid by each female on each plant age class as the dependent variable. In addition, because plants of different developmental stages show significant variation in leaf biomass available (see results), I performed the same analyses as before with number of eggs over number of available leaves as a more meaningful dependent variable. To assess whether butterflies significantly prefer new leaves over other plant tissues, the proportion of eggs laid on new leaves versus the proportion of eggs laid in medium, old and inflorescences together was assessed, when available, by paired t-tests.

Larval performance

Plant quality and defenses – All larval performance experiments were conducted during spring-summer 2010, rearing caterpillars on plants on one of four plant age classes: J1, J2, FL, and FR. Experiments assessing caterpillar performance, digestibility, IG sequestration, and susceptibility to simulated parasitoid attack were performed under controlled growth chamber conditions with a photoperiod of 14hr day: 10 night, and day-night temperatures of 27°C /22°C. Individual plants reared at the greenhouse were harvested every two to three days in order to supply caterpillars with a constant source of fresh leaves, previously undamaged. At harvest, only new and intermediate leaves were saved, and when present, old leaves and inflorescences were discarded. New and intermediate leaves from multiple individual plants per age-class per week were separated into two categories: (1) fresh material used to rear the larvae assigned to each plant age treatment, and (2) material that was weighed fresh, oven-dried at 50°C for 48hrs to a constant mass and weighed again to the nearest 0.01g. Variation in nutritional quality (water and nitrogen concentrations) and chemical defenses (% dry weight IGs) was assessed as before. The only difference was the measurement of leaf toughness, which in this case was assessed as specific leaf area (SLA). Specific leaf area was calculated as *A/M*, where *A* is the area of a disk ~2cm in diameter (cut with a cork borer) and *M* is the leaf disk dry mass (Milla et al. 2008). Twenty leaf disks collected separately from at least 10 leaves per age class were used, measuring their dry weight after drying them at 50°C for 48hr in the oven. SLA has shown to inversely correlate with fiber concentration, such that lower SLA indicates higher concentrations of fiber and thus, higher leaf toughness (e.g. Choong 1996, Gras et al. 2005).

Developmental variation in new leaf percent water, percent nitrogen, and SLA was analyzed using one-way ANOVAs, followed by Bonferroni post-hoc tests to distinguish mean differences among age classes. As before, MANOVA was first used to examine concurrent variation in aucubin and catalpol concentrations as a function of plant age. When significant effects were detected by MANOVA, I followed up with univariate ANOVAs for each IG, and

mean group differences among plant age classes were assessed using Bonferroni post-hoc tests. Percent water, nitrogen, and IG concentrations were arcsine square-root transformed to improve normality and homogeneity of variance.

Caterpillar performance and fitness - Caterpillar performance from hatching to pupation was assessed as a function of host plant ontogeny using the four developmental stages above described: J1, J2, FL, and FR. Under growth chamber conditions, neonate larvae in groups of ten individuals per petri dish were assigned randomly to feed on a mix of new and intermediate leaves from each of these four developmental stages. Twelve replicates were performed for each of these non-choice tests for a total of 480 larvae. Larvae were monitored every day and a constant supply of fresh leaves was provided. At the beginning of the experiments, as well as every three days, all live larvae per petri dish were counted and weighed as a group to the nearest 0.01mg. Three measures of caterpillar performance were calculated: mortality rate, relative growth rate, and time to pupation. Mortality rate was calculated as the proportion of individuals that died over the initial number of larvae per replicate (N = 10). Relative growth rate (RGR), dry mass increase per unit dry mass per day, was calculated as $[(W_f - W_i)/W_i]/t$ where W_f is final biomass, W_i is initial biomass and t is the total number of days from neonate to newly molted 5th instar. To estimate fitness, once caterpillars pupated, pupae were weighed fresh to the nearest 0.01mg. Variation in larval mortality rate, RGR, time to pupation and pupal weight were tested by one-way ANOVA followed by Bonferroni post-hoc tests to assess differences among the four diet treatments. Time to pupation and pupal weight data were square root transformed and proportion data (mortality rate and RGR) were arcsine-square root transformed to improve normality and homogeneity of variance.

Caterpillar feeding efficiency – In a separate experiment, newly molted 3rd, 4th and 5th instar caterpillars, previously reared on J1, J2, FL and FR plants from neonates as described above,

were separated for detailed assessments of feeding efficiency. Feeding efficiency can be used to estimate the metabolic costs of feeding on diets varying in multiple traits such as nutritional quality, physical and chemical defenses. Four nutritional indices were calculated according to the standard gravimetric method (Waldbauer 1968): consumption index (CI), approximate digestibility (AD), efficiency of conversion of ingested food (ECI), and efficiency of conversion of digested food (ECD) (see Table 5.1). Briefly, CI provides a measure of the total amount of leaf consumed relative to the body mass gained, and AD, or assimilation efficiency, reflects the proportion of ingested food that is actually digested. Trade-offs among these indices may also arise. For instance, many insects increase food consumption rates in response to low concentrations of critical nutrients such as protein. However, increased consumption will accelerate passage of food through the gut and thereby reduce AD. In turn, ECI (growth efficiency) and ECD (metabolic efficiency) provide a measure of the overall efficiency with which the food ingested or digested, respectively, is converted into caterpillar biomass. All measurements were gathered over a 24hr interval starting with newly molted 3^{rd}, 4^{th} and 5^{th} instar larvae. To obtain these indices, 20 individual larvae per instar (e.g. 3^{rd}, 4^{th} and 5^{th}) per treatment diet (e.g. J1, J2, FL and FR) were placed in small sealed containers (160 mm^2) and provided with sufficient leaf material. Five measurements were collected per replicate: initial and final food mass (fresh weight), initial and final larval mass (fresh weight), and final fecal mass (dry weight). Prior to this 24hr period, caterpillars were starved for four to eight hours to ensure an empty gut before the trials begin. Similarly, after removal of the remaining leaf material following the 24hrs, caterpillars were starved for four additional hours before larval fresh weight and fecal dry weight were measured. A separate subset of larvae and leaves from each instar and treatment diet combination was dried and weighed at the beginning and the end of the experiment to obtain dry weight conversion factors. To avoid problems with the statistical analysis of ratios, all nutritional indices were analyzed using two-way ANCOVAs (Raubenheimer and Simpson 1992). In all cases, except for CI, the numerator of the formula used to calculate

TABLE 5.1. Formulas used to calculate larval nutritional indices according to the standard gravimetric method (Waldbauer 1968). CI = consumption index, AD = approximate digestibility, ECI = efficiency of ingested food, and ECD = efficiency of conversion of digested food.

Index	Formula
CI	Dry weight of food ingested / (Larval mass gain x Number of days)
ECI	Larval mass gain / Dry weight of food ingested
AD	(Dry weight of food ingested - Dry weight of feces) / Dry weight of food ingested
ECD	Larval mass gain / (Dry weight of food ingested - Dry weight of feces)

analysis of ratios, all nutritional indices were analyzed using two-way ANCOVAs (Raubenheimer and Simpson 1992). In all cases, except for CI, the numerator of the formula used to calculate each nutritional index was the dependent variable, while the denominator was used as a covariate (see Table 5.1). In the case of CI, the covariate was initial larval mass. Host plant age and larval instar were included as fixed factors. Dependent and covariate variables were square root transformed to improve normality and homogeneity of variance.

Caterpillar sequestration and immune defenses

Caterpillar IG sequestration – Following the nutritional indices experiment, all 3rd, 4th and 5th instar larvae per treatment diet were freeze-killed and later processed for extraction of sequestered IGs. Sample size per instar per diet treatment varied between 15 and 20 individuals. To measure sequestration of IGs, whole caterpillars were ground inside 15ml test tubes using sterilized sand and a glass rod, and the mixture was extracted overnight in 95% methanol. The methanol extract was subsequently filtered to remove caterpillar tissues and sand, and the residue evaporated to dryness. Sample purification and quantification of IG extraction using gas chromatography followed previously described methods (see also Bowers and Stamp 1993, Barton 2007, Quintero and Bowers 2011a). Variation in sequestration was assessed using a two-way MANOVA with percent dry weight aucubin and catalpol as dependent variables, and host plant age and larval instar as fixed factors. When a significant effect was detected, I followed with univariate ANOVAs for each compound separately. Proportion data were arsine square root transformed to improve normality and homogeneity of variance.

Caterpillar immune defenses - To measure the immune response of *J. coenia* larvae, caterpillars previously reared on J1, J2, FL and FR plants from neonates as described above, were injected with silica beads (Lavine and Beckage 1996, Lovallo et al. 2002, Rantala and Roff

2007, Smilanich et al. 2009a). Previous studies have shown that exposing caterpillars with these positively charged glass beads produces a strong encapsulation response, which correlates with both immune response against real parasites and pathogens (Rantala and Roff 2007) and field parasitism rates (Smilanich et al. 2009b). Thus, larval immune response, measured as percent melanization, was assessed following previously described methods (Smilanich et al. 2009a).

Newly molted 5th instar larvae, anesthetized by exposure to CO_2 for approx. 30 seconds, were injected with silica beads. Beads used for injections were DEAE Sephadex-A25 silica chromatography beads (40–120 μm diameter), obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Prior to injections, beads were dyed with a 0.1% solution Congo Red Dye and allowed to dry completely, before storing them in Ringer's solution. Beads were injected into caterpillar hemolymph, at the base of the third proleg, using hand-made fine glass needles, and the wound was sealed with New Skin Liquid Bandage (Medtech Products, Jackson, Wyoming, USA). Injections were performed under a Leica S6D dissection microscope with 30x magnification, and each injection consisted of approx. 5 µL Ringer's solution, containing 15 ± 10 beads. Following injection, larvae were placed back in their individual rearing container with the same treatment diet as before (i.e. either J1, J2, FL or FR new leaves) for 24 h. After this period, larvae were freeze-killed and later dissected to retrieve beads. Caterpillar dissections were conducted under a Leica S6D dissection microscope with 60x magnification to facilitate bead identification and removal. Caterpillars were dissected immersed in 2ml 95% methanol to prevent IG breakdown. Once the dissection was complete and all beads retrieved, all caterpillar tissues were transferred to a 15ml test tube and later processed for extraction of sequestered IGs as described above. Beads were stored in Ringer's saline until photographed.

A Zeiss Axiocam Vision LE camera mounted onto a Stemi SVII dissection microscope with 80x magnification was used to photograph beads. Control (uninjected) beads and recovered beads from caterpillars were photographed under the same exposure and white

balance, and the encapsulation and melanization strength were quantified by measuring the red value (*r*-value) of each bead. The *r*-value is a numerical measure of the red saturation of an image on a scale ranging from 0 to 255, where 0 = pure grey, and 255 = pure red. *R*-values were obtained for each bead within a caterpillar, using ADOBE PHOTOSHOP version CS2 (Adobe Systems Inc., San Jose, California, USA), and these values were averaged to provide a single *r*-value score for each individual caterpillar. The mean *r*-value was transformed into percent melanization using the following formula: [1 - (*r*-value⁄control *r*-value)] for ease of interpretation. A total of 20 larvae per treatment were injected, but final sample size varied between 10 and 16 replicates per treatment. Analyses of Covariance (ANCOVA), with number of beads retrieved per caterpillar as a covariate, were used to compare percent melanization as a function of host plant developmental stage. Pearson's correlation coefficients were used to examine associations between percent melanization and sequestered IGs (aucubin, catalpol and total IGs).

RESULTS

Female oviposition choice

Plant quality and defenses- New leaves significantly differed in all plant traits measured across plant ontogeny (Figure 5.1). In the case of the J1-J2-FL choice test, a significant increase in biomass ($F_{2,48}$ = 103.6, P < 0.0001), physical ($F_{2,48}$ = 24.39, P < 0.0001) and chemical defenses (MANOVA: Wilks' λ = 0.504, $F_{4,88}$ = 9.0, P< 0.0001, ANOVAs aucubin: $F_{2,48}$ = 21.02, P < 0.0001, catalpol: $F_{2,48}$ = 14.74, P < 0.0001) was observed as plants aged (Figure 5.1a-c). In contrast, nutritional quality of new leaves decreased as plants aged, as observed for both water ($F_{2,48}$ = 68.6, P < 0.0001) and nitrogen concentrations ($F_{2,48}$ = 91.35, P < 0.0001) (Figure 5.1d,e). In addition, it is interesting to note that in the case of both plant defenses I did not observe significant differences between J1 and J2 new leaves (Figure 5.1b,c). Thus, leaf toughness and percent dry weight aucubin and catalpol in new leaves, although they showed an

increase between J1 and J2 stages, did not significantly differ between the two juvenile stages. Similar trends were observed for J2-FL-FR choice tests. While biomass ($F_{2,54}$ = 18.8, P < 0.0001), physical ($F_{2,54}$ = 83.81, P < 0.0001) and chemical defenses (MANOVA: Wilks' λ = 0.326, $F_{4,100}$ = 18.81, P< 0.0001, ANOVAs aucubin: $F_{2,54}$ = 48.36, P < 0.0001; catalpol: $F_{2,54}$ = 11.09, P < 0.0001) increased in new leaves as plants developed (but note the decrease in aucubin and catalpol between FL and FR stages), nutritional quality decreased (% water: $F_{2,54}$ = 44.98, P < 0.0001; % N: $F_{2,54}$ = 147.31, P < 0.0001) (Figure 5.1f-j).

Oviposition tests - Individual J. coenia females laid, on average, 202.5 ± 15.5 SE eggs, across all host plant stages. Of the 40 individual females tested, only six laid less than 20 eggs and thus, were excluded from the analyses. Overall, in both choice tests, butterflies preferred younger plant developmental stages over mature stages, but that choice was clearer when correcting for leaf tissue availability (Figure 5.2). Friedman tests, assessing variation in proportion of eggs laid among each set of choice tests, demonstrated that butterflies significantly prefer J2 and FL plants over J1 in the first choice test ($\chi^2_{2, 16}$ = 8.59, P = 0.014), and J2 plants over FL and FR in the second choice test ($\chi^2_{2, 18}$ = 19.44, P < 0.0001). *Post-hoc* analysis with Wilcoxon signed-rank tests were conducted with a Bonferroni correction applied, resulting in a significance level set at P < 0.017. Mean group treatment differences demonstrated a significant preference for J2 over J1 (Z = -3.19, P = 0.001), but no differences were observed between FL and J1 or J2 stages (Z = -2.15, P = 0.031 and Z = -0.5, P = 0.62, respectively) (Figure 5.2a). In the case of the choice test among older age classes, J2 stages showed a significantly higher proportion of eggs over both FL (Z = -3.16, P = 0.002) and FR (Z = -3.68, P = 0.0001), and no difference was observed between reproductive stages (FL and FR) (Z = -1.24, P = 0.21) (Figure 5.2b). Furthermore, butterflies not only showed a preference for

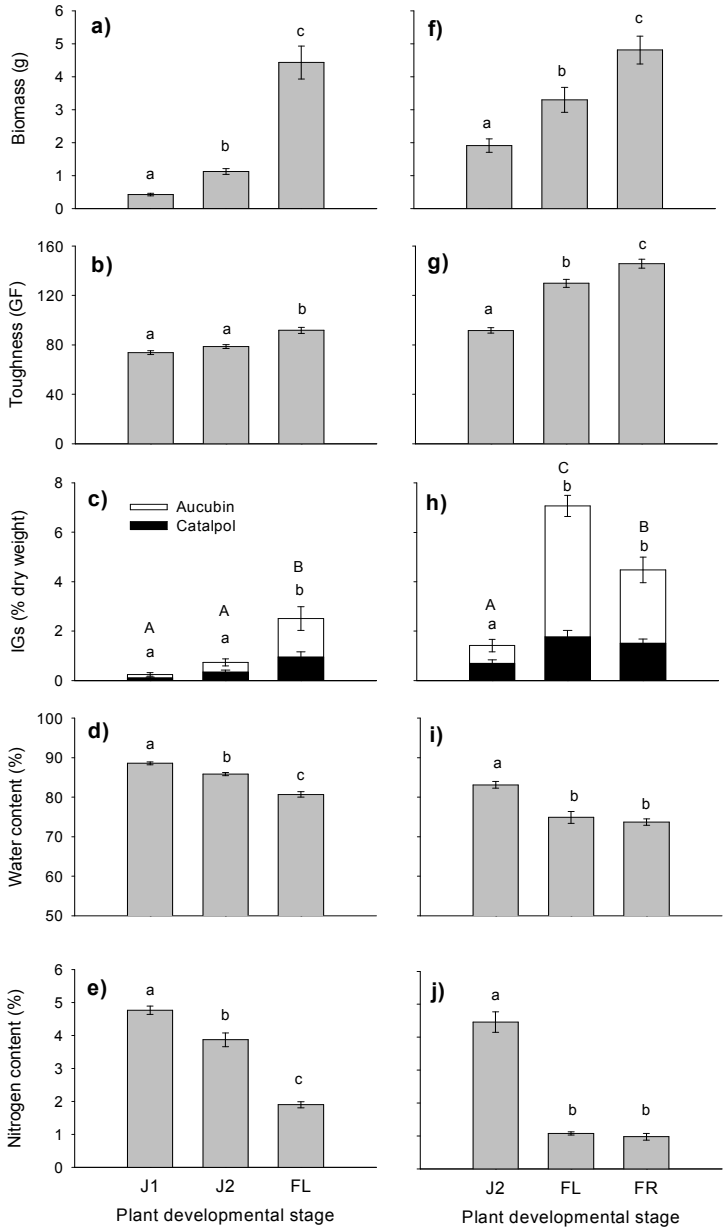

Figure 5.1. Ontogenetic variation in new leaves of *Plantago lanceolata* used in oviposition tests (Mean ± 1SE). Left panels are for J1, J2, FL tests (a to e) and right panels are for J2, FL and FR tests (f to j). Five traits were measured: biomass, physical defenses measured as leaf toughness (i.e. grams of force, GF), chemical defenses measured as percent dry weight IGs: aucubin and catalpol, and leaf nutritional quality measured as percentage water and nitrogen. Original data were square root or arcsine-square root transformed for statistical analyses, actual values are shown for illustrative purposes only. Letters indicate mean group differences as tested by a Bonferroni post-hoc test ($p<0.05$). In panel c) and h) capital letters were used to represent group mean differences for aucubin and lower-case letters were used to represent group mean differences for catalpol concentrations.

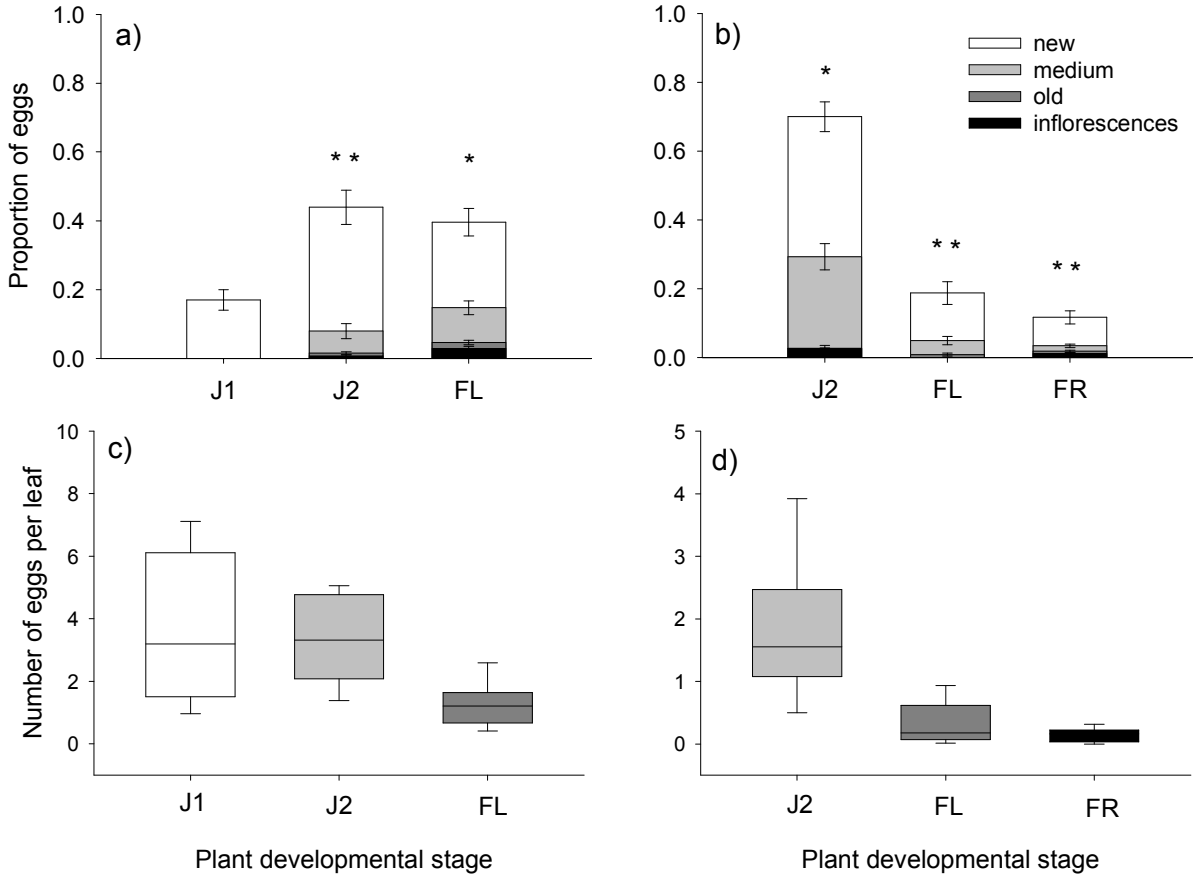

Figure 5.2. *Junonia coenia* oviposition choice across two sets of 3-way choice tests, represented as **a)** proportion of eggs laid across tissues and developmental stages for J1-J2-FL (N = 16) and **b)** J2-FL-FR (N = 18) *P. lanceolata* stages, or as number of eggs per total available leaf tissues for **c)** J1-J2-FL (N = 16) and **d)** J2-FL-FR (N = 18) *P. lanceolata* stages. In top panels, asterisks represent significant differences between new leaves and all other tissues using paired *t-tests*. Significance is displayed as $P < 0.0001$ (***), $P < 0.01$ (**), $P < 0.05$ (*). In bottom panels, the boxes represent the median and the 25th and 75th percentiles, and bars extend to the 5 and 95% values.

younger developmental stages (preferentially J2 stages), but also for younger leaf tissues. On average, butterflies laid 70-100% of their eggs on new leaves as compared with medium, old leaves and inflorescences, and that difference was statistically significant in all cases as demonstrated by paired *t-tests* (see Figure 5.2a,b).

Correcting for differences in available biomass between plant developmental stages revealed an even stronger preference of female Buckeye butterflies for juvenile plants (Figure 5.2c, d). In the case of the choice test J1-J2-FL, butterflies laid more than twice as many eggs per available leaf on juvenile stages than on reproductive stages ($\chi^2_{2, 16}$ = 13.76, P < 0.001). *Post-hoc* analysis with Wilcoxon signed-rank tests demonstrated that there were no significant differences between the J1 and J2 stages (Z = -0.26, P = 0.79), although a small reduction in egg number per available leaf was observed from J1 to J2 stages. In turn, there was a statistically significant reduction in butterfly preference between J1 and FL (Z = -2.67, P = 0.007) and between J2 and FL plants (Z = -3.34, P = 0.001) (Figure 5.2c). In the case of the choice test including older plant stages, J2-FL-FR, the differences were also more pronounced. Although, on average, females in the J2-FL-FR tests laid considerably fewer total eggs than those exposed to J1-J2-FL stages (note the scale difference in Figure 5.1), butterflies laid 5 to 14 times more eggs per leaf on juvenile plants than on reproductive stages ($\chi^2_{2, 18}$ = 24.33, P < 0.0001). *Post-hoc* analysis demonstrated a significant preference for J2 over FL (Z = -3.46, P = 0.001) and FR stages (Z = -3.72, P = 0.0001), but no differences were observed between FL and FR stages (Z = -1.63, P = 0.102) (Figure 5.2d).

Larval performance

Plant quality and defenses – Similar to the patterns described above for the plants harvested following butterfly oviposition in 2009, combined new and intermediate leaves used to feed *J. coenia* caterpillars in 2010 also varied in all measured traits as a function of host plant ontogenetic stage (Table 5.2). Specifically, while overall nutritional quality decreased, physical

and chemical defenses increased as plants developed. However, it is interesting to note the lack of variation between the two young juvenile stages (J1 and J2), which show similar levels across all variables measured except SLA (Table 5.2), suggesting that an increase in physical defenses might be the major difference between new and intermediate leaves in these stages. Furthermore, small changes between the 2009 and 2010 harvest can be observed (see Figure 5.1 and Table 5.2), which may correspond to annual variation in uncontrolled biotic and abiotic factors and/or to slight variation in the actual age of plants at the moment of harvest. In this regard, it is interesting to note that while in 2009 all plants were harvested within a one-week period; in 2010 plants were harvested over four weeks in order to account for variation in plant traits during the complete period of caterpillar development (which may also explain the lack of variation among the two juvenile stages).

Caterpillar performance and fitness – Changes in plant quality and defenses as host plant developmental stage significantly impacted caterpillar performance but not larval mortality rate or pupal weight (a correlate of fitness). While mortality rate of immature larvae did not vary as a function of host plant age ($F_{3,44} = 2.21$, $P = 0.1$), RGR significantly decreased two to three times for those larvae fed new and intermediate leaves of reproductive stages, as compared with leaves of the same age but from juvenile stages ($F_{3,44} = 13.35$, $P < 0.0001$) (Figure 5.3a,b). Time to pupation significantly varied as a function of diet ($F_{3,44} = 69.4$, $P < 0.0001$), with larvae reared on FR plants taking ~30% longer (i.e. 7-10 days) to reach the pupal stage as compared with larvae reared on J1, J2 and FL plants (Figure 5.3c). However, only marginally significant differences were detected for pupal weight across host plant treatments ($F_{3,126} = 2.6$, $P = 0.055$), suggesting that the extended development time compensated for low quality diets (Figure 5.3d).

TABLE 5.2. Nutritional quality, physical and chemical defenses of combined new and intermediate *P. lanceolata* leaves used to feed *J. coenia* caterpillars during summer 2011. Mean ± 1SE values for all plant traits and plant developmental stages are presented as well as the ANOVA results. Letters in parenthesis indicate mean group differences among plant age treatments as tested by Bonferroni post-hoc tests ($P < 0.05$).

Plant traits	J1	J2	FL	FR	*df*	*F*	*P*
Water (%)	88.06 ± 0.41 (a)	88.15 ± 0.26 (a)	84.39 ± 0.52 (b)	80.17 ± 0.50 (c)	3,141	79.91	**0.0001**
Nitrogen (%)	4.26 ± 0.20 (a)	4.21 ± 0.16 (a)	1.95 ± 0.10 (b)	1.25 ± 0.05 (c)	3,139	79.91	**0.0001**
SLA (cm^2 mg^{-1})	0.47 ± 0.05 (a)	0.24 ± 0.02 (b)	0.21 ± 0.02 (c)	0.17 ± 0.02 (d)	3, 76	417.43	**0.0001**
Total IGs (%)	0.99 ± 0.16 (a)	0.87 ± 0.09 (a)	2.78 ± 0.46 (b)	3.86 ± 0.47 (c)	3,141	79.91	**0.0001**
Aucubin (%)	0.73 ± 0.13 (a)	0.72 ± 0.08 (a)	1.98 ± 0.31 (b)	2.83 ± 0.36 (c)	3,141	79.91	**0.0001**
Catalpol (%)	0.26 ± 0.05 (a)	0.15 ± 0.04 (a)	0.80 ± 0.16 (b)	1.03 ± 0.14 (b)	3,141	79.91	**0.0001**

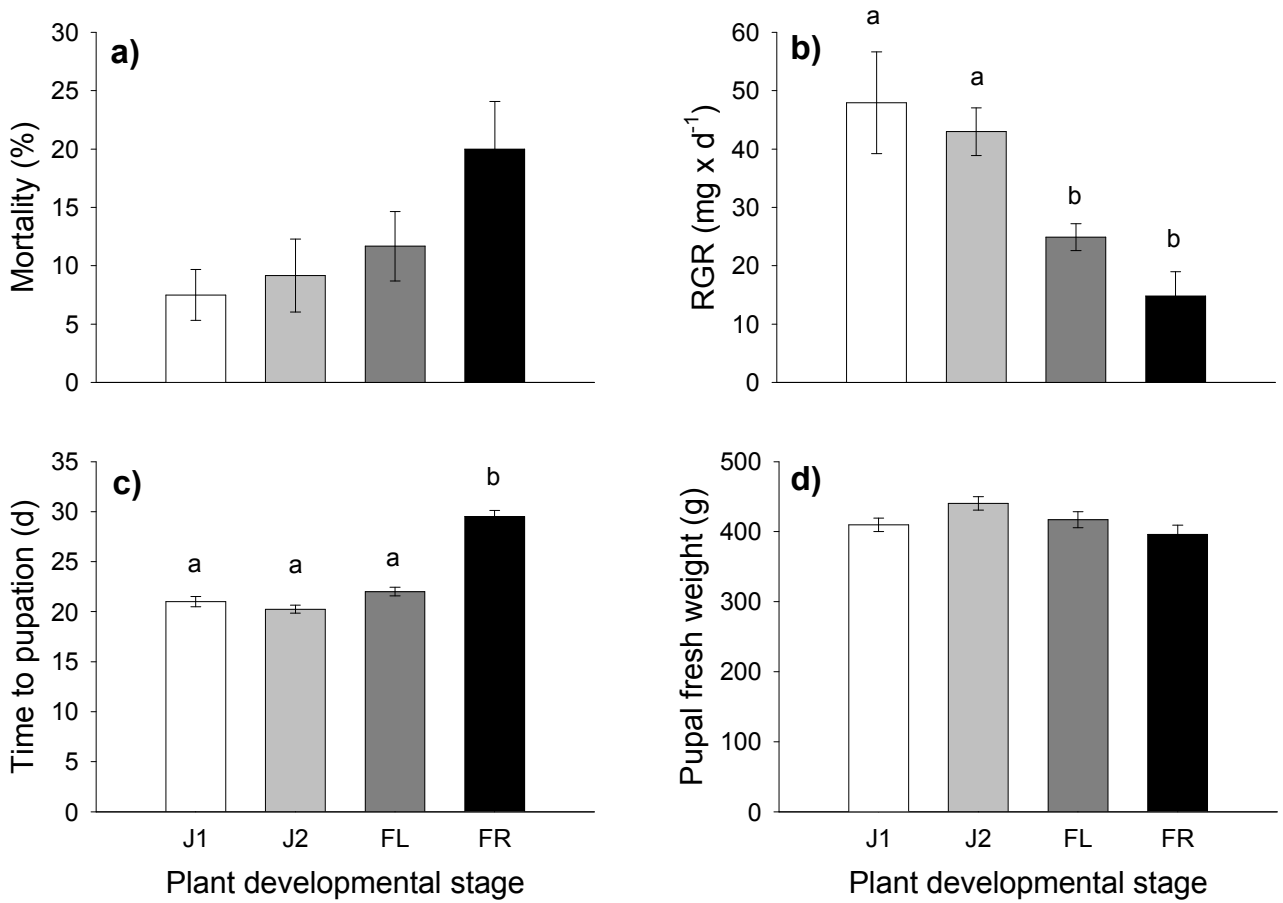

Figure 5.3. *Junonia coenia* caterpillar performance and fitness, reared from neonate to pupation, across four host plant developmental stages (J1, J2, FL, and FR), measured as **a)** mortality rate, **b)** relative growth rate, **c)** time to pupation, and **d)** pupal weight. Original data were square root or arcsine-square root transformed for statistical analyses, actual values are shown for illustrative purposes only. Letters indicate mean group differences as tested by a Bonferroni post-hoc test ($P<0.05$), when overall ANOVA was significant.

Caterpillar feeding efficiency - Feeding efficiency data showed that, in general, the ability of a larva to digest leaf tissue varying in nutritional quality and defenses as a function of host plant age significantly varied throughout larval development (see significant age*instar interactions in Table 5.3), with 3rd instar larvae being the most susceptible to ontogenetic changes in host plant leaf quality (Figure 5.4). The consumption index (CI) showed a consistent trend across larval stages, with a significant increase of two to four times more leaf material consumed as host plant age increased (Figure 5.4a). Approximate digestibility (AD), did not significantly vary across larval instars (Table 5.3) and showed little variation across host plant stages (Figure 5.4c). In contrast, the efficiency of food conversion indices (ECD and ECI) were in general significantly higher for larvae feeding on juvenile plants as compared to those feeding on mature plants (Figure 5.4b, d).

Caterpillar sequestration and immune defenses

Caterpillar IG sequestration - Host plant variation in IGs had a significant effect on caterpillar sequestration ability, showing an overall increase in caterpillar IG concentration as host plants aged, and thus host plant IG content increased (Figure 5.5). MANOVA and ANOVA results both showed a significant effect of host plant age, caterpillar instar and, in most cases, a significant plant age by instar interaction (Table 5.4). Therefore, variation in sequestered IGs throughout larval development changed as a function of host plant developmental stage. For instance, caterpillars feeding on older FR stages always sequestered high levels of IGs without substantial variation from 3rd to 5th instars (i.e. total IGs, aucubin + catalpol, from 3rd to 5th instar varied from 11 to 11.6% dry wt. respectively), while caterpillars reared on younger host plant developmental stages, J1 to FL, more than doubled their levels of sequestered IGs during the same period (Figure 5.5).

TABLE 5.3. Summary of two-way ANCOVAs comparing larval nutritional indices as a function of host plant developmental stage (caterpillar diet treatment: J1, J2, FL and FR) and larval instar (3rd, 4th and 5th instar). See text and Table 5.1 for description of covariate in each case and nutritional indices abbreviation. Significant effects are indicated in bold.

	CI			ECI			AD			ECD		
Source of variation	*df*	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Covariate	1	70.63	**0.0001**	1	9.99	**0.002**	1	280.12	**0.0001**	1	6.64	**0.011**
Plant age	3	63.83	**0.0001**	3	1.30	**0.033**	3	2.99	**0.032**	3	2.33	0.075
Instar	2	14.03	0.066	2	24.86	**0.0001**	2	0.99	0.373	2	129.88	**0.0001**
Age*Instar	6	16.14	**0.0001**	6	8.12	**0.0001**	6	4.9	**0.0001**	6	10.92	**0.0001**
Error	209			209			209			209		

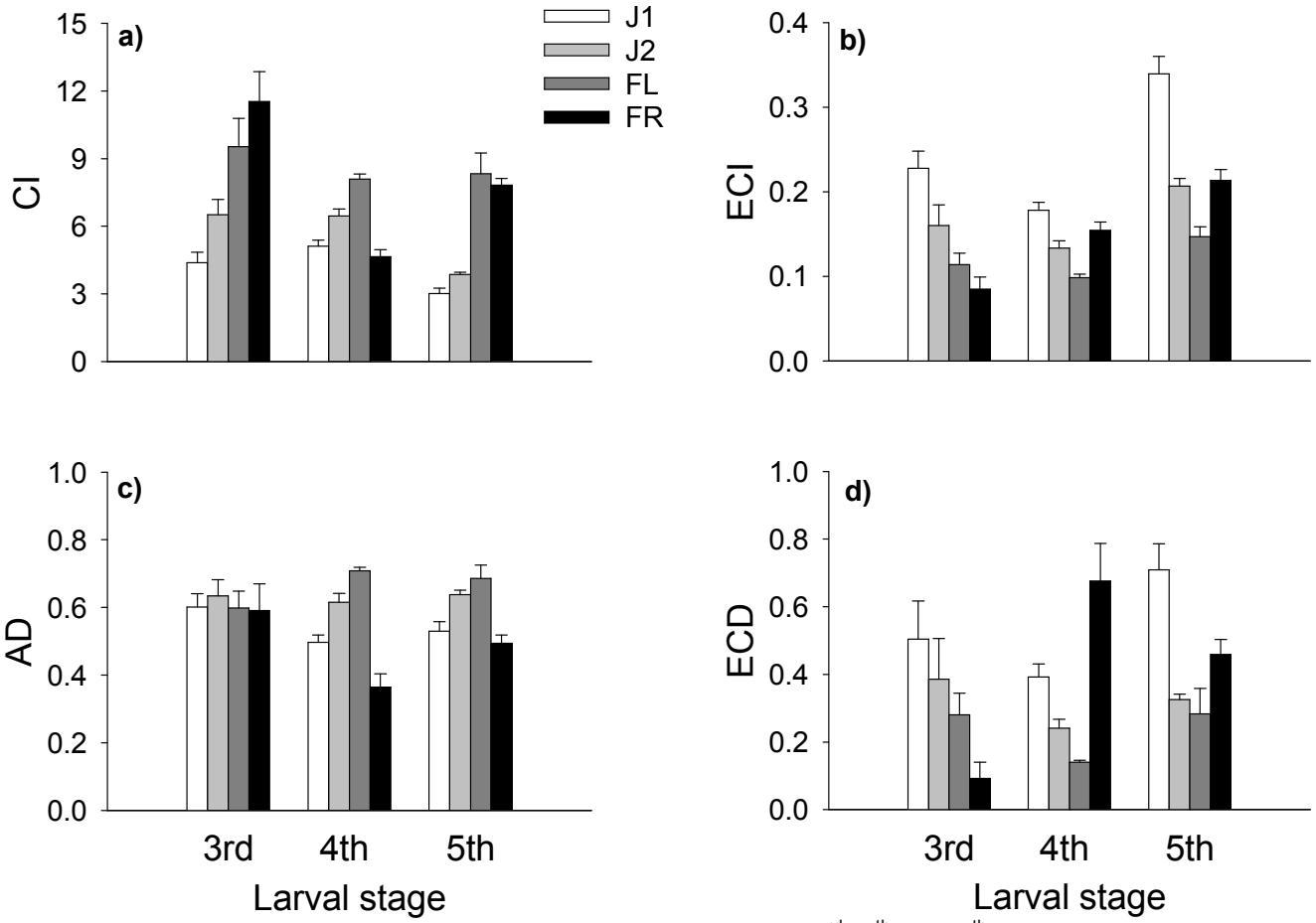

Figure 5.4. *Junonia coenia* larval nutritional indices (Mean ± 1 S.E.) for 3rd, 4th and 5th instar feeding for 24 hr on new leaves of four host plant developmental stages (J1, J2, FL and FR): **a)** consumption index (CI), **b)** efficiency of conversion of ingested food (ECI), **c)** approximate digestibility (AD), and d) efficiency of conversion of digested food (ECD).

TABLE 5.4. Summary of two-way MANOVA and ANOVAs comparing larval sequestration rate as a function of host plant developmental stage (caterpillar diet treatment: J1, J2, FL and FR), larval instar (3^{rd}, 4^{th} and 5^{th} instar) and their interaction.

	Source of variation	λ	*df*	*F*	*P*
Aucubin and Catalpol	Plant age	0.44	6,418	34.7	0.0001
	Instar	0.74	4, 418	17.3	0.0001
	Age*Instar	0.85	12,418	2.9	0.001
Aucubin	Plant age		3,210	60.86	0.0001
	Instar		2, 210	35.95	0.0001
	Age*Instar		6,210	3.56	0.002
Catalpol	Plant age		3,210	28.1	0.0001
	Instar		2, 210	15.37	0.0001
	Age*Instar		6,210	2.0	0.066

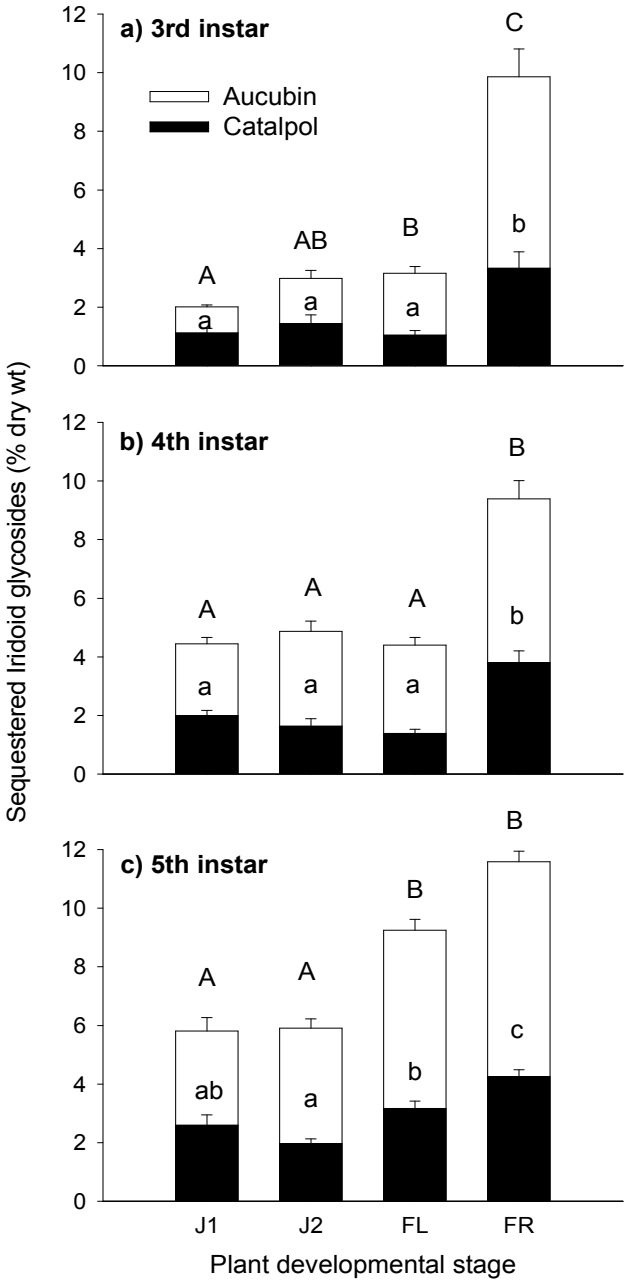

Figure 5.5. *Junonia coenia* IG sequestration (Mean ± 1 S.E.) for 3rd, 4th and 5th instar caterpillars, feeding from neonate on new leaves of four host plant developmental stages (J1, J2, FL and FR). Percent dry weight aucubin and catalpol was assessed when caterpillars just molted to the **a)** 3rd instar, **b)** 4th instar and **c)** 5th instar. Original data were arcsine-square root transformed for statistical analyses; actual values are shown for illustrative purposes only. Letters indicate mean group differences as tested by Bonferroni post-hoc tests (P <0.05). Capital letters were used to represent mean group differences for aucubin and lower-case letters were used to represent group mean differences for catalpol concentrations.

Caterpillar immune defenses - The ability of 5th instar caterpillars to encapsulate and melanize a foreign object significantly varied across host plant age classes ($F_{3,49}$ = 2.97, P = 0.04), showing a decrease in melanization rate as host plant age increases. Specifically, larvae feeding on FR plants showed a significant decrease in melanization rate (up to 30% difference) as compared to younger host plant stages. These results indicate that *J. coenia* larvae feeding on older host plant stages, and thus on high IG containing plants, might be less efficient at defending themselves again parasitoids (Figure 5.6). The number of beads recovered per individual caterpillar, used as a covariate, did not affect the melanization response ($F_{1,49}$ = 1.6, P = 0.21), indicating that the ability to mount an immune defense can be independent of level of attack. Finally, Pearson correlation coefficients demonstrated a negative correlation between caterpillar sequestration rate and melanization rate (total IGs: R = -0.32, P = 0.021, N = 52; aucubin: R = -0.29, P = 0.035, N = 52; catalpol: R = -0.31, P = 0.023, N = 52), indicating that diets rich in IGs, leading to higher IG larval sequestration, diminish caterpillar immune response to parasitoids.

DISCUSSION

Both direct and indirect species interactions have key roles in structuring communities. For example, plants can alter the identity, abundance and performance of invertebrate carnivores through their direct impact on the performance and foraging behavior of insect herbivores (Ode 2006, Inbar and Gerling 2008, Dobler et al. 2011, Unsicker et al. 2011). Similarly, herbivore damage that results in a cascade of induced changes in morphological and chemical traits in their host plants can result in often asymmetrical competition among herbivore species (Denno et al. 1995, Poelman et al. 2008b), and even scale up to alter the outcome of higher trophic level interactions (Bukovinszky et al. 2009, Poelman et al. 2011). Despite increasing knowledge that multi-trophic interactions vary in both space and time, relatively little is known regarding

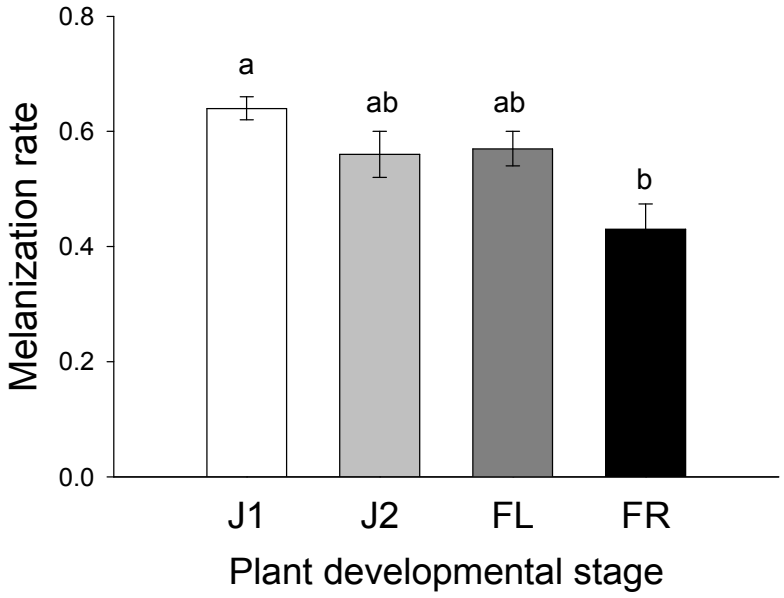

Figure 5.6. *Junonia coenia* larval immune response to simulated parasitoid eggs measured as percent melanization of injected silica beads, in response to host plant developmental stage (J1, J2, FL and FR). Letters indicate mean group differences as tested by a Bonferroni post-hoc test (P <0.05).

how the strength and direction of plant-herbivore-natural enemy interactions vary across host plant development. Here, I illustrated that the selective pressure of a herbivore may vary over its host plant's ontogeny, as plants become less preferred and more resistant to this specialist herbivore. However, overall levels of herbivore abundance and damage as plants age will depend on the community composition of the higher trophic level, as caterpillars feeding on younger life stages become more vulnerable to predators, but better defended against parasitoids. These results suggest that physiological and morphological constraints during plant ontogeny can alter the strength of the bottom-up versus top-down controls on herbivore population dynamics, potentially driving the observed changes in insect communities associated with a plant species over its lifetime.

Ontogenetic patterns in plant defensive traits

Changes in plant defensive traits as plants develop, altering the quality of food for herbivores, have been repeatedly demonstrated across all plant life forms (reviewed in Barton and Koricheva 2010). While it is expected that this temporal variation will impact tri-trophic interactions, the direction and magnitude of these changes can be hard to predict. One reason underlying this complexity is that, often, ontogenetic patterns encompass shifts in several defensive traits at a time, which can show parallel or opposing trajectories (e.g. Donaldson et al. 2006, Rehill et al. 2006, Mcarthur et al. 2010). In this study, for instance, while *Plantago lanceolata* leaf toughness doubled and levels of IGs in new leaves increased up to 16 times between juvenile and mature stages (i.e. 0.36 to 6% dry wt.), percent water decreased from 90 to 75% and nitrogen concentrations decreased from 4.5 to 1.25%, during the same period. This pattern is consistent with previous reports describing *P. lanceolata* increases in IG content and physical defenses as plants age (Bowers and Stamp 1993, Fuchs and Bowers 2004, Barton 2007, Quintero and Bowers 2011a), with concomitant decreases in SLA (Schippers and Olff 2000) and nutritional quality throughout ontogeny (Bowers and Stamp 1993, Quintero and

Bowers 2011a). Nevertheless, it is important to note that the patterns reported here only represent constitutive ontogenetic changes in mostly new leaves and excluded any effect of plant age on among tissue variation (i.e. new vs. old leaves) (Bowers and Stamp 1992) or induced defenses following damage (Fuchs and Bowers 2004, Barton 2008, Quintero and Bowers 2011b). Had the experiments been run under field conditions, where herbivores might be exposed to multiple sources of variation as plants age, the effect size of ontogenetic patterns in host plant constitutive and induced defenses on plant-herbivore-natural enemy interactions may be larger than those estimated here.

Plant age and female oviposition choice

Variation in insect herbivore richness and density on certain developmental stages over others may be due to selective female oviposition choice and/or immature herbivore foraging behavior. In this study, adult *Junonia coenia* selectively preferred younger developmental stages of the host plant, *P. lanceolata*, by laying 2 to 14 times more eggs per leaf on juvenile plants than on reproductive stages. Because the butterfly choice tests used whole plants, a combination of stimulants and deterrents most probably was responsible for the observed female preferences, as reported for other lepidopteran species (Honda 1995). Previous studies have demonstrated that Buckeye butterflies use IGs as oviposition cues (Pereyra and Bowers 1988), and that often higher IG concentrations in leaves, in particular catalpol, increases likelihood of oviposition (Pereyra and Bowers 1988, Klockars et al. 1993). In addition, Prudic et al. (2005) suggested that female oviposition behavior is also affected by host plant traits other than IG concentration, as females laid more eggs on high-nitrogen, low IG plants than on low-nitrogen, high IG plants. Lastly, because Buckeyes often select younger leaves over older leaves (Klockars et al. 1993), female preference may be driven by the combination of: (i) larger proportion of new leaves, (ii) higher proportion of catalpol despite lower total IG concentrations, and (iii) high nutritional quality and lower leaf toughness in younger juvenile stages (see Figure 5.1). Thus, although the

independent role of each trait on female oviposition choice in this system warrants further investigation, this study highlights that adult females can show strong preferences for certain host plant developmental stages over others.

Whether butterfly oviposition choice leads to optimal or suboptimal conditions for the offspring may depend on the relative importance of bottom-up versus top-down forces. The preference-performance hypothesis, first proposed by Jaenike (1978), states that female insects will preferentially oviposit on plants that maximize the survival and performance of their larvae. Over the past 30 years, this hypothesis has received substantial support (Gripenberg et al. 2010); however, many insects have also been found to make poor oviposition decisions. Possible explanations for 'bad motherhood' decisions include: optimal foraging, where adult insects choose oviposition sites that enhance their own long-term fitness at the expense of their individual offspring (Mayhew 2001), and enemy-free space, where females choose oviposition sites that decrease larval predation risk over enhanced larval performance (Denno et al. 1990, Bjorkman and Larsson 1991, Ballabeni et al. 2001, Sadek et al. 2010). Here, in accordance with the preference-performance hypothesis, caterpillars feeding on juvenile *P. lanceolata* plants, which were preferred by ovipositing females, showed faster relative growth rate, shorter development time, and increased digestive efficiency compared to those feeding on older, reproductive hosts. Nonetheless, these results suggest that this decision provides larvae with the best nutrition, but the least defensive compounds, which can render immature larvae more susceptible to predators but more resistant to parasitoids. Thus, whether this strategy may enhance larval performance in the presence of natural enemies may depend on the identity, abundance and behavior of predators and parasitoids in the field. Yet, because larval mortality is usually higher earlier during larval development (reviewed in Zalucki et al. 2002), when levels of sequestered defenses are quite low, the choice reported for *J. coenia* in this study suggests that increased larval growth rates in early susceptible instars may be more important than enemy-free space for enhancing overall herbivore fitness.

Plant age and caterpillar performance

The nutritional quality of forbs is distinguished by its high water (75-95% fresh wt.) and nitrogen concentrations (1.5-9.7% dry wt.) as compared with foliage of grasses and trees (Slansky and Scriber 1985). This high nutrient availability promotes rapid growth in chewing herbivores associated with forbs (Tabashnik and Slansky 1987), but those herbivores may be less adapted to high spatiotemporal variation in nitrogen and water content in their diets. Performance indices of many herbivorous insects are strongly correlated with both nitrogen and water concentrations of the food; but their relative importance is complicated by the fact that both traits tend to covary, especially when the proportion of structural carbohydrates increases over tissue development (Slansky and Scriber 1985). In general, insects respond to lower nitrogen content by increasing food consumption or the efficiency of nitrogen use (Tabashnik and Slansky 1987). Thus, the observed ten percent decrease in foliar water content and three fold decrease in nitrogen concentrations as *P. lanceolata* ages from J1 to FL stages (Table 5.2), may explain the higher consumption rate (CI) and lower larval relative growth rate (RGR) observed while feeding on those tissues. Leaf toughness (higher fiber and cellulose content) may also act in concert with lower nutritional quality as plants age, further decreasing larval performance. In particular, leaf toughness may decrease insect performance due to its direct effect on limiting the size of meals being eaten, slowing gut passage rates, and reducing nutrient supply and efficiency of assimilation of nutrients (Hochuli 1996, Clissold et al. 2009). Therefore, the low efficiency of conversion of ingested and digested food (ECI, ECD) and the extended development time of *J. coenia* reared on older host plant stages may be due, at least in part, to ontogenetic patterns in leaf physical defenses.

Alternatively, the amount and proportion of energy (e.g. protein, lipids, carbohydrates, and vitamins) and allelochemicals can vary greatly within and among forb species (Tabashnik and Slansky 1987), leading to varying insect performance and digestive efficiency. In the case of IGs, their toxic effects are induced when the compounds are activated by enzymes such as

the hydrolytic β-glucosidases (Dobler et al. 2011). The resulting iridoid aglycone can denature amino acids, proteins and nucleic acids, act as enzyme inhibitors, and inhibit the formation of prostaglandins and leucotrienes, all of which may either affect herbivores directly or may reduce the quality of ingested food by rendering proteins undigestible (reviewed in Dobler et al. 2011). To date, it is unknown whether these β-glucosidases stem from the food plant or are present in the gut as standard digestive enzymes (Dobler et al. 2011). Nevertheless, in either case, if an increase in food IG content augments β-glucosidase content in the insect gut, that may help to explain the decreased performance previously observed for this specialist herbivore (Adler et al. 1995, Camara 1997a) and in the current study.

Because larvae confined to low-quality diets commonly compensate by consuming more tissues (Tabashnik and Slansky 1987); such a compensatory strategy would increase *J. coenia* exposure to defensive compounds, which must subsequently be detoxified or sequestered, diverting energy that otherwise could be allocated to adding body mass (Smilanich et al. 2009a). As a result, a combination of higher levels of physical and chemical defenses and lower nutritional quality in leaves of older host plant stages slows the growth of this specialist insect by making digestive processes less efficient. Nevertheless, despite considerable variation in larval performance, neither larval mortality nor pupal weight varied across plant ontogeny, suggesting that overall herbivore fitness may not be strongly impacted beyond the potential effects that delayed development time may already confer (e.g. Benrey and Denno 1997).

Plant age and the third-trophic level

The effects of ontogenetic variation in plant traits on herbivores may also cascade up the food chain altering the foraging behavior and performance of higher trophic levels. Consequently, the strength of the top-down control of herbivores may change as host plants develop. Most research looking at this question has focused on the direct effects of plant traits, such as architectural complexity (Van Bael et al. 2003, Boege 2005a, Riihimaki et al. 2006, Obermaier et

al. 2008) or the provision of shelter and food rewards (Djieto-Lordon et al. 2004, Izzo and Vasconcelos 2005, Trager and Bruna 2006, Miller 2007) on natural enemies' foraging behavior. In contrast, indirect effects of ontogenetic trends in plant traits on natural enemy performance have received less consideration. My results suggest that temporal variation in plant traits may lead to opposing effects on predators versus parasitoids, as mediated by caterpillar ability to sequester host plant chemical defenses.

In terms of predator-prey interactions, my results suggest that mortality rate should be higher when *J. coenia* caterpillars feed on younger as compared with older host plant stages. Because larval sequestration correlates with IG content in the diet (as previously reported e.g. Camara 1997), the observed increase in *P. lanceolata* IG concentrations, from ~1 to 4% dry weight between juvenile and mature stages (Table 5.2), may considerably decrease larval predation risk against predators as host plant age increases. Earlier studies showed that sequestration of IGs by *J. coenia* larvae serves as an effective defense against invertebrate predators, including predatory wasps (Stamp 2001, Stamp and Meyerhoefer 2004), ants (de la Fuente et al. 1995, Dyer and Bowers 1996), stink bugs (Strohmeyer et al. 1998), and spiders (Theodoratus and Bowers 1999). In addition to decreasing predation risk, invertebrate predators may also show dose-dependent responses to IG content in prey tissues (Dyer and Bowers 1996), with concomitant decreases in performance and fitness if forced to consume highly unpalatable larvae (Strohmeyer et al. 1998, Stamp 2001, Stamp and Meyerhoefer 2004). In this study, caterpillar IG concentrations varied considerably with larval instar and host plant age, increasing as larvae progress from 3rd to 5th instar and reaching higher IG concentrations when larvae are fed on older plants. Moreover, diet-mediated differences were striking, with caterpillars reaching two to five times higher IG concentrations when feeding on mature FR plants (~10% dry wt. IGs) than on young juvenile stages (2-6% dry wt. IGs). As a result, although I did not directly assess the effect of *P. lanceolata* ontogeny on subsequent larval mortality, substantial evidence indicates that the variation I observed in 3rd to 5th instar IG

concentrations should scale up to not only decrease larval predation risk, but also confer physiological costs to predators that consume them, potentially weakening the top-down control of predators as host plants develop.

In contrast, in terms of parasitoid-prey interactions, this increase in larval sequestration ability while feeding in older host plant stages resulted in diminished larval immunocompetence, suggesting higher susceptibility of *J. coenia* against parasitoids and pathogens as their host plant develops. In particular, less defended *J. coenia* larvae reared on juvenile plants achieved 30% higher levels of melanization compared to larvae reared in older mature stages (Figure 5.6). This result agrees with previous evidence that higher levels of IGs in *J. coenia* diets decreased larval ability to mount an encapsulation and melanization response (Smilanich et al. 2009a). Because parasitoids are considered one of the most important sources of mortality for many caterpillars (Hawkins et al. 1997) and immune response is clearly one of the most effective defenses that caterpillars have against parasitism (Smilanich et al. 2009b), a 30% decrease in mortality due to enhanced immunocompetence might be ecologically relevant.

Decreased immunocompetence has been reported as a consequence of increased larval ingestion or sequestration of plant allelochemicals (Haviola et al. 2007, Smilanich et al. 2009a) or due to poor diet quality (Ojala et al. 2005, Lee et al. 2006, Klemola et al. 2007). In this study, I was unable to separate the relative contribution of sequestration and diet quality, and thus, both may have played a role in decreasing larval immunocompetence. Nevertheless, it is likely that the increase in IGs as host plants aged, from ~1 to 4% dry wt. total IGs, is the primary driver of the changes seen in melanization, as previous studies with *J. coenia* reared on artificial diets or using IG supplementation (i.e. same nutritional quality but different IG levels) demonstrated that high IG content, principally catalpol, significantly reduced larval immune response (Smilanich et al. 2009a). Thus, my results add to the increasing evidence that defenses that are effective against predators may render larvae more susceptible to parasitoids (Gentry and Dyer 2002, Barbosa and Caldas 2007, Smilanich et al. 2009b).

Conclusions

This research has revealed the degree to which temporal variation in plant defensive traits can scale up to higher trophic levels, significantly altering the direction and magnitude of interactions between plants, herbivores and their natural enemies. In particular, the observed ontogenetic patterns in plant defenses and nutritional quality were shown to: (1) decrease by two to 14 times the number of eggs per leaf laid by female butterflies, (2) decrease by three fold larval relative growth rate, and (3) increase two to five times sequestered defenses in caterpillar tissues, thereby (i) increasing their chances of escaping predation, but (ii) decreasing immunocompetence, and thus, the ability to defend against endoparasitoids. What is more remarkable is that these differences in *J. coenia* host selection, performance, sequestration, and estimated predation risk on different developmental stages of *P. lanceolata* can mirror or even surpass the variation seen in previous studies due to environmental or genetically mediated phenotypic variation in *P. lanceolata* quality and defenses (Fajer et al. 1991, Adler et al. 1995, Bowers and Stamp 1997b, Prudic et al. 2005) or even due to plant species identity (Theodoratus and Bowers 1999, Smilanich et al. 2009a, Lampert and Bowers 2010a).

While these controlled experiments can help us predict natural variation in *J. coenia* abundance and leaf tissue damage throughout *P. lanceolata* ontogeny, the outcome of these interactions under natural conditions may be harder to forecast (e.g. Hunter and Elkinton 2000). For example, the actual strength of the top-down control of *J. coenia* larvae across host plant development may depend on the combination of larval performance and relative abundance of natural enemies in the field. For instance, while lower levels of sequestered defenses should enhance larval susceptibility to invertebrate predators, faster development times achieved while feeding on *P. lanceolata* juvenile stages (i.e. approx. 10 days faster) may decrease larval chances of being found by enemies (Clancy and Price 1987, Benrey and Denno 1997), resulting in potentially similar levels of predation risk across host plant ontogenetic stages. Likewise, whether caterpillar immune response may predict temporal variation in field parasitism rate is

also questionable. Parasitism rates can respond to density-dependent factors such as prey abundance (i.e. expected to be higher in younger plant stages due to higher female preference) or to trait-mediated factors such as prey defenses and performance (e.g. Bukovinszky et al. 2008) evening out or enhancing the differences predicted due to larval immunocompetence. Furthermore, the role of IGs present in the caterpillar's hemolymph, beyond their indirect effect on encapsulation and/or melanization, may further alter parasitoid success, although current evidence only indicates minor negative direct effects to actually positive effects of IGs on parasitoid performance (Lampert et al. 2010, Reudler Talsma et al. 2011).

In summary, this research demonstrated that ontogenetic patterns in host plant traits can strongly modify the relative importance of bottom-up and top-down forces on herbivore population dynamics. From a more applied perspective, studies focused on economically important species should also incorporate field surveys in order to better understand how plant-herbivore, herbivore-predator and herbivore-parasitoid interactions change as host plants age, and how those changes may, in turn, shape overall levels of tissue damage, plant fitness and the resulting arthropod community.

CHAPTER 6

SUMMARY AND CONCLUSIONS

Early empirical studies have improved our knowledge of the direct effects that physiological, morphological, and resource allocation constraints during plant development have on shaping ontogenetic changes in several plant defensive traits. However, less attention has been given to elucidating potential indirect effects of this variation on higher trophic levels. The main focus of this dissertation was to explore the bottom-up effects of ontogenetic variation in plant allelochemistry and nutritional quality on the strength of tri-trophic interactions between herbivores, their host plants, and their natural enemies. Through a series of controlled greenhouse and laboratory experiments, this study documented some of the potential ecological consequences of temporal variation in plant traits on plant-herbivore-natural enemy dynamics.

6.1. Defense strategies throughout plant ontogeny

Many studies have reported ontogenetic patterns in constitutive defensive traits (reviewed in Barton and Koricheva 2010), but only a few have simultaneously assessed multiple defensive traits across several plant tissues and/or developmental stages. In general, most studies to date have focused on describing changes in one defensive trait at a time, typically focusing on leaf tissues and considering only two contrasting developmental stages. While these studies emphasized the need to incorporate ontogeny as a critical factor shaping plant defensive traits, they were limited in their ability to test: (i) partial and whole-plant investment in defenses during plant ontogeny, (ii) the overall relationship between plant age and investment in defensive traits, and (iii) the potential role of resource allocation constraints, namely root:shoot ratios, in explaining whole-plant investment in chemical defenses over time.

Here, in chapter 2, I showed that concentration of iridoid glycosides (IGs) were three times larger in shoot as compared to root tissues across all developmental stages, suggesting that resource allocation to defenses in aboveground tissues may prevail over belowground tissues during *P. lanceolata* development, potentially reflecting their differential susceptibility to herbivore damage. Furthermore, although not as pronounced as in other systems (e.g. Hartmann and Zimmer 1986, Williams and Ellis 1989, Bellostas et al. 2007, Beninger et al. 2009), the pattern of variation in total IGs in leaves and roots differed, indicating that IG concentrations changed differently in shoot and root tissues throughout plant development. Specifically, allocation of IGs into root tissues constantly increased from younger to older plants while non-linear shifts in allocation of IGs during ontogeny were observed for shoot tissues. Nevertheless, root:shoot ratios only weakly explained overall allocation of resources into defenses, indicating that biomass partitioning may not solely explain whole plant investment in defenses. In contrast, it may be possible that ontogenetic patterns in defensive traits may be driven by other selective forces such as herbivore and pathogen damage, mychorrhizae associations, competition, or photoprotection, also varying over time. Finally, in accordance with previous studies that have measured more than one trait throughout plant development (e.g. Donaldson et al. 2006, Rehill et al. 2006, Mcarthur et al. 2010), contrasting ontogenetic patterns were described within and among both plant defenses and nutritional quality. Specifically, results from Chapter 2 and 5 demonstrated that, while overall investment in chemical defenses (IGs) and physical defenses (leaf toughness) significantly increased, water and nitrogen concentrations in shoot tissues decreased as plants aged. These ontogenetic trajectories in plant defenses indicate that population level demographics may contribute to the observed spatiotemporal variation in plant defenses, and may help to explain ecological interactions among plants and herbivores.

Nonetheless, it is important to note that in my experiments, with the exception of Chapter 5, nutritional quality and defensive traits in aboveground tissues were assessed across leaf

classes (combined new, intermediate, and old leaf stages, see Bowers and Stamp 1992, 1993). Therefore, the ontogenetic patterns described at the whole plant level may be confounded with ontogenetic changes in leaf age (e.g. younger developmental stages have a higher proportion of new leaves). In consequence, future studies that assess ontogenetic patterns in plant defensive traits within as well as across leaf tissues as plants age may shed light on the relative contribution of these confounding factors explaining plant allocation of resources to defenses as plants develop.

In addition to constitutive defenses, plants can also alter the quality and/or quantity of synthesized and released defenses (resistance traits) and/or the allocation of resources to biomass accumulation to compensate for the lost tissue following damage (tolerance traits). These induced defenses often vary during plant ontogeny, as predicted shifts in metabolic rates, growth rate, and access to nutrients or stored reserves take place. In the past three decades, opposing trends have been predicted for both strategies (Karban and Baldwin 1997, Strauss and Agrawal 1999, Cipollini et al. 2003, Orians et al. 2011), and partial support for these predictions has been demonstrated (reviewed in Barton and Koricheva 2010). However, empirical tests of the underlying mechanisms responsible for modulating age-dependent induced defenses are limited (e.g. Boege et al. 2007, Gruntman and Novoplansky 2011). In this study, I emphasize that our understanding of the patterns and mechanisms leading to age-dependent induced responses to herbivory can be critical in several contexts.

In terms of plant defense theory, assessing age-dependent induced defenses can shed light on the conditionality of trade-offs between constitutive and induced defenses or between tolerance and resistance traits. Using four juvenile developmental stages, constantly fluctuating patterns in compensatory growth and induced synthesis of IGs as plants aged were found, with some stages being able to employ both strategies (some 6wk-old stages), while other employed only one (3wk and 10wk-old stages) or neither of them (14wk-old stages) (Chapter 3). Although my experimental design in this case did not allow me to test for potential trade-offs between

defensive strategies, these results suggest that possible negative correlations between tolerance and resistance traits may be present in some developmental stages (i.e. 3wk and 10wk-old stages) but not in others (i.e. 6wk and 14wk-old stages). Similarly, because constitutive defenses increased constantly as plants age but induced synthesis of IGs was only observed early during plant development (Chapter 3 and 4), these results agree with the predicted negative correlation between plant investment in constitutive versus induced defenses (Morris et al. 2006, Kempel et al. 2011).

The highly complex patterns in age-dependent induced defenses in *P. lanceolata* was only uncovered due to the simultaneous assessment of multiple developmental stages (Chapter 3) and various intrinsic (i.e. growth rate and metabolic rate, Chapter 3) and/or extrinsic (i.e. herbivore identity and frequency of damage, Chapter 4) factors driving these ontogenetic trends in resistance and tolerance to herbivory. In particular, here I exemplify that the assessment of plant relative growth rate (RGR) and IG synthesis rate (appraised through graphical vector analyses, GVA), were central to detect and comprehend patterns of resource allocation to herbivore-induced responses as plants age (Chapter 3). The traditional approach, measuring changes in plant biomass and concentration of IGs between damaged and undamaged plants, illustrated a rather complex picture. Compensatory growth was achieved at intermediate stages while resistance showed, in general, very low variation in IGs with few intermediate and older juvenile stages being able to increase or decrease concentration of IGs as compared with undamaged plants (Chapter 3). However, thanks to the assessment of plant RGR and allocation to growth (biomass) versus synthesis of allelochemicals (IG content and concentration), using GVA, I was able to highlight key changes in plant allocation to resistance and tolerance traits as they develop. Specifically, Chapter 3 demonstrated that, despite maintaining equivalent RGRs as undamaged plants, plants invested relatively more in synthesis of IGs early during juvenile development than on biomass accumulation, while the opposite was observed for older juvenile stages. Furthermore, extrinsic factors such as frequency and

intensity of damage failed to modify age-dependent responses to leaf damage (Chapter 4), suggesting that intrinsic physiological and anatomical constraints throughout plant ontogeny may be more important than extrinsic stressors shaping phenotypic plasticity in herbivore-induced defensive traits.

In terms of plant-herbivore interactions, induced defenses have been shown to play a critical role in indirect competition among herbivores with considerable impacts on the structure of herbivore-natural enemy communities (reviewed in Kessler and Halitschke 2007, Anderson et al. 2009). Therefore, assessing age-dependent induced defenses relative to ontogenetic changes in constitutive defensive traits can help us better predict temporal shifts in arthropod population dynamics and the selective pressure that they might impose. Research on *P. lanceolata* (Fuchs and Bowers 2004, Barton 2008, and Chapters 3 and 4) has consistently shown that constitutive ontogenetic patterns in plant biomass and defenses are considerably larger in magnitude than age-dependent induced defenses. This trend may be particularly common in annual and short perennial plant species, where the transition through key developmental stages may take place relatively quickly, overriding herbivore-induced responses. While further research testing this prediction across several herbaceous and woody plants is required; at least for *P. lanceolata*, these results imply that constitutive ontogenetic trajectories in plant growth and defenses may play a more significant role in mediating multitrophic interactions than changes following previous damage events.

6.2. Plant ontogeny and higher trophic levels

Despite the longstanding interest in understanding temporal variation in herbivore damage, the direct and indirect mechanisms that could explain these patterns have been rarely investigated. Here, data from Chapter 5 reveal that ontogenetic patterns in plant nutritional quality and physical and chemical defenses have strong effects on adult oviposition choice, larval preference and presumed larval susceptibility to natural enemies. Furthermore, these results

suggested that bottom-up effects on higher trophic levels may vary between functional groups with the direction and magnitude of the interaction differing between predators and parasitoids.

Host selection by adult female phytophagous insects depends on multiple traits such as volatile cues, leaf shape, color and surface texture, and qualitative and quantitative concentrations of key attractant and deterrent allelochemicals, among others (Honda 1995). Given the large and often contrasting ontogenetic trajectories in plant traits, it is becoming clear that throughout plant ontogeny host selection can be quite species-specific, partially explaining temporal variation in the arthropod community composition associated with a plant species over its lifetime. In this study, Buckeye butterflies choose younger over older (especially reproductive) plant stages, suggesting that a combination of higher proportion of new leaves, with higher nutritional quality but lower leaf toughness and concentrations of total IGs (although a considerably higher proportion of catalpol) are important traits during host selection. Yet, acknowledging that female choice may be mostly driven by one or a few of these measured traits, I am planning to use structural equation models, with a more complete data set than the one presented here, to estimate the relative importance of each trait and their autocorrelation in explaining female oviposition choice.

Whether butterfly oviposition choice leads to optimal or suboptimal conditions for the offspring has been largely discussed since the preference-performance hypothesis was first proposed (Jaenike 1978). This hypothesis, which states that female insects will preferentially oviposit on plants that maximize the survival and performance of their larvae, has received substantial support (Gripenberg et al. 2010); however, exceptions are not rare (e.g. Denno et al. 1990, Bjorkman and Larsson 1991, Ballabeni et al. 2001, Mayhew 2001). Here, in accordance with the preference-performance hypothesis, caterpillars feeding on juvenile *P. lanceolata* plants showed faster relative growth rate, shorter development time, and increased digestive efficiency compared to those feeding on older, reproductive hosts. Nonetheless, this decision provides larvae with the best nutrition, but the least amount of defensive compounds, which can render

them more susceptible to predators but more resistant to parasitoids. Altogether, these results highlight the fact that the likelihood of supporting or rejecting the preference-performance hypothesis may depend on the identity, abundance and behavior of predator and parasitoids in the field. In addition, these results suggest that potential mismatches between female oviposition choice and offspring performance may depend on the developmental stages available for both adults and larvae in natural populations or experimental assays. These considerations can be central in conservation biology if decreased availability of preferred developmental stages reduces the success of restoration projects or biocontrol strategies to manage invasive species. Likewise, knowledge regarding potential mismatches between female oviposition choice and offspring performance can help us improve pest management strategies for economically important crops.

As mentioned before, most studies assessing variation in the strength of the top-down control of herbivores during host plant ontogeny have been centered on changes in the foraging behavior of natural enemies (Van Bael et al. 2003, Djieto-Lordon et al. 2004, Boege 2005a, Izzo and Vasconcelos 2005, Riihimaki et al. 2006, Trager and Bruna 2006, Miller 2007, Obermaier et al. 2008). Alternatively, predation risk can be mediated through herbivore quality as prey and/or host, but evidence of this potential indirect effect of ontogenetic trajectories in plant defenses on higher trophic levels is still lacking. Thus, to my knowledge, I demonstrated here for the first time that ontogenetic patterns in plant defenses, as mediated by caterpillar performance and palatability, can indirectly alter the strength of the top-down control of herbivores (Chapter 5). Although the ability to uptake and store plant allelochemicals is certainly rare, more than 250 insect species have been shown to sequester plant metabolites from at least 40 plant families (Opitz and Muller 2009). Hence, it is certainly possible that temporal variation in the strength of top-down control of herbivores, as mediated by herbivore sequestration rate, may be more common than previously anticipated. Consequently, the study of indirect interactions among

trophic levels altered by ontogenetic patterns in plant defensive traits promises to be a fertile area of research.

Controlled experiments, like the ones used here to estimate larval vulnerability to natural enemies, are critical to elucidate the potential mechanisms driving temporal variation in herbivore population dynamics. However, given that numerous factors may alter the outcome of predator-prey interactions throughout host plant ontogeny, complementary field studies are needed. At least four possible features of herbivore and natural enemy foraging behavior may alter the outcome of tri-trophic interactions as predicted by laboratory assays. First, natural enemies may rely on a set of cues to locate prey, such as plant biomass, architecture, food rewards and volatile profiles, which can also vary as plants develop (Quintero et al. *in prep.*), but may not necessarily match the expected temporal variation in herbivore density, performance and palatability. Second, the top-down control of herbivores may be mostly driven by prey availability (density-dependent) or by prey quality and behavior (trait-mediated) (Preisser et al. 2005); in which case, trends predicted given larval unpalatability or performance may over- or underestimate actual risks of predation under natural conditions. Third, vulnerability to natural enemies can also vary throughout insect development. For example, some predators may be more limited by prey size than prey toxicity/quality (Dyer and Floyd 1993, Remmel et al. 2011) and/or the ability to encapsulate and melanize an invader may change considerably as larvae develop (e.g. Rantala and Roff 2005, Bukovinszky et al. 2009). Finally, the foraging behavior of insect prey may change when the perception of predation risk increases (Ives and Dobson 1987, Lima 1998, Werner and Peacor 2003). In the case of *P. lanceolata-J.coenia* interactions, previous studies have shown that the presence of two invertebrate predators, *Podisus maculiventris* (stink bugs) and *Polistes fuscatus* (social paper wasps), often led to a decline in larval growth rate due to forcing larvae to forage on lower quality food, cooler microhabitats, or by increasing dispersal and time spent hiding (e.g. Stamp and Bowers 1991, 1992, 1993). Moreover, although the presence of predators can reduce larval growth rate, it can also

simultaneously enhance leaf consumption (Stamp and Bowers 1993) and sequestration rate (Bowers and Stamp 1997a). Thus, the selective pressure of natural enemies on herbivore populations as plants age may diverge from the patterns predicted based just on larval performance and palatability.

6.3. Closing remarks and future directions

Considering plant ontogeny as a source of variation in plant-herbivore interactions can offer new perspectives for understanding the selective impact of herbivores on plant traits, and the conditionality of costs and benefits of diverse defense strategies at each life stage contributing to overall fitness. From the point of view of the plant, the optimal defense strategy is to reduce damage without impairing fitness. This may depend on the relative benefits of investing in constitutive and herbivore-induced allelochemical synthesis and tolerance strategies against the metabolic cost of their expression. Because the cost and benefit of investment in plant defenses change as plants develop, significant trajectories in defense strategies are expected. In this study, I demonstrated that ontogeny has a dramatic impact on *P. lanceolata*'s expression of chemical defenses, both constitutive and induced, as well as on several other traits relevant for higher trophic levels. However, future research should assess how these ontogenetic trends in defense strategies contribute to optimize overall plant fitness. In particular, I advocate that, if possible, future studies will benefit by incorporating multiple developmental stages at a time, several resistance and tolerance traits across both above- and belowground tissues, and an estimation of the potential intrinsic and extrinsic mechanisms underlying the observed changes in defensive traits as plants develop.

Ontogeny is a ubiquitous process in all plant life forms, certainly shaping plant traits and, as a result, temporal variation in the community structure of higher trophic levels. Understanding the forces that shape ontogenetic trajectories in herbivore diversity and damage can have key implications for theoretical and applied ecology. From a theoretical perspective, the assessment

of how herbivore preference, performance and predation risk vary for a suite of associated herbivores on a single plant species over its lifetime may improve our knowledge of the relative importance of bottom-up or top-down forces in driving herbivore population dynamics and arthropod community structure over time. From an applied perspective, assessing the direct and indirect mechanisms driving herbivorous pest abundance and damage in agroecosystems may improve pest management strategies. For example, if predators and parasitoids are differentially effective throughout host plant ontogeny, such as my data suggest for this system, it may be important to vary biocontrol agents over time. Future studies that focus on the relative importance of direct and indirect interactions mediating herbivore host selection, performance and predation risk will provide key new insights into the role of plant ontogeny at regulating temporal shifts in herbivore population dynamics and community structure.

REFERENCES

Adler, L. S., J. Schmitt, and M. D. Bowers. 1995. Genetic variation in defensive chemistry in *Plantago lanceolata* (Plantaginaceae) and its effect on the specialist herbivore *Junonia coenia* (Nymphalidae). Oecologia 101:75-85.

Albrectsen, B. R., H. Gardfjell, C. M. Orians, B. Murray, and R. S. Fritz. 2004. Slugs, willow seedlings and nutrient fertilization: intrinsic vigor inversely affects palatability. Oikos 105:268-278.

Anderson, K. E., B. D. Inouye, and N. Underwood. 2009. Modeling herbivore competition mediated by inducible changes in plant quality. Oikos 118:1633-1646.

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.

Anten, N. P. R., M. Martinez-Ramos, and D. D. Ackerly. 2003. Defoliation and growth in an understory palm: quantifying the contributions of compensatory responses. Ecology 84:2905-2918.

Apple, M., K. Tiekotter, M. Snow, J. Young, A. Soeldner, D. Phillips, D. Tingey, and B. J. Bond. 2002. Needle anatomy changes with increasing tree age in Douglas-fir. Tree Physiology 22:129-136.

Awmack, C. S. and S. R. Leather. 2002. Host plant quality and fecundity in herbivorous insects. Annual Review Of Entomology 47:817-844.

Ayres, M. P., T. P. Clausen, S. F. MacLean, A. M. Redman, and P. B. Reichardt. 1997. Diversity of structure and antiherbivore activity in condensed tannins. Ecology 78:1696-1712.

Baden, C. U. and S. Dobler. 2009. Potential benefits of iridoid glycoside sequestration in *Longitarsus melanocephalus* (Coleoptera, Chrysomelidae). Basic and Applied Ecology 10:27-33.

Baldwin, I. T. 1988. Damage-induced alkaloids in Tobacco - pot-bound plants are not inducible. Journal Of Chemical Ecology 14:1113-1120.

Baldwin, I. T. and E. A. Schmelz. 1996. Immunological "memory" in the induced accumulation of nicotine in wild tobacco. Ecology 77:236-246.

Ballabeni, P., M. Wlodarczyk, and M. Rahier. 2001. Does enemy-free space for eggs contribute to a leaf beetle's oviposition preference for a nutritionally inferior host plant? Functional Ecology 15:318-324.

Barbosa, P. and A. Caldas. 2007. Do larvae of species in macrolepidopteran assemblages share traits that influence susceptibility to parasitism? Environmental Entomology 36:329-336.

Barrett, R. D. H. and A. A. Agrawal. 2004. Interactive effects of genotype, environment, and ontogeny on resistance of cucumber (*Cucumis sativus*) to the generalist herbivore, *Spodoptera exigua*. Journal Of Chemical Ecology 30:37-51.

Barthelemy, D. and Y. Caraglio. 2007. Plant architecture: a dynamic, multilevel and comprehensive approach to plant form, structure and ontogeny. Annals of Botany 99:375-407.

Barton, K. E. 2007. Early ontogenetic patterns in chemical defense in *Plantago* (Plantaginaceae): genetic variation and trade-offs. American Journal Of Botany 94:56-66.

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

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.

Bellostas, N., J. C. Sorensen, and H. Sorensen. 2007. Profiling glucosinolates in vegetative and reproductive tissues of four Brassica species of the U-triangle for their biofumigation potential. Journal of the Science of Food and Agriculture 87:1586-1594.

Beninger, C. W., R. R. Cloutier, and B. Grodzinski. 2008. The iridoid glucoside, antirrhinoside, from *Antirrhinum majus* L. has differential effects on two generalist insect herbivores. Journal Of Chemical Ecology 34:591-600.
Beninger, C. W., R. R. Cloutier, and B. Grodzinski. 2009. A Comparison of antirrhinoside distribution in the organs of two related Plantaginaceae species with different reproductive strategies. Journal Of Chemical Ecology 35:1363-1372.
Beninger, C. W., R. R. Cloutier, M. A. Monteiro, and B. Grodzinski. 2007. The distribution of two major iridoids in different organs of *Antirrhinum majus* L. at selected stages of development. Journal Of Chemical Ecology 33:731-747.
Bennett, A. E., J. D. Bever, and M. D. Bowers. 2009. Arbuscular mycorrhizal fungal species suppress inducible plant responses and alter defensive strategies following herbivory. Oecologia 160:771-779.
Bennett, R. N. and R. M. Wallsgrove. 1994. Secondary metabolites in plant defense-mechanisms. New Phytologist 127:617-633.
Benrey, B. and R. F. Denno. 1997. The slow-growth-high-mortality hypothesis: a test using the cabbage butterfly. Ecology 78:987-999.
Berenbaum, M. R. and A. R. Zangerl. 2008. Facing the future of plant-insect interaction research: Le Retour a la "Raison d'Etre". Plant Physiology 146:804-811.
Biere, A., H. B. Marak, and J. M. M. van Damme. 2004. Plant chemical defense against herbivores and pathogens: generalized defense or trade-offs? Oecologia 140:430-441.
Bjorkman, C. and S. Larsson. 1991. Pine sawfly sefense and variation in host plant resin acids - a trade-off with growth. Ecological Entomology 16:283-289.
Blumberg, D. 1997. Parasitoid encapsulation as a defense mechanism in the Coccoidea (Homoptera) and its importance in biological control. Biological Control 8:225-236.
Boege, K. 2005a. Herbivore attack in *Casearia nitida* influenced by plant ontogenetic variation in foliage quality and plant architecture. Oecologia 143:117-125.
Boege, K. 2005b. Influence of plant ontogeny on compensation to leaf damage. American Journal Of Botany 92:1632-1640.
Boege, 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.
Boege, K. and R. J. Marquis. 2005. Facing herbivory as you grow up: the ontogeny of resistance in plants. Trends In Ecology & Evolution 20:441-448.
Boege, K. and R. J. Marquis. 2006. Plant quality and predation risk mediated by plant ontogeny: consequences for herbivores and plants. Oikos 115:559-572.
Bohm, S. M., K. Wells, and E. K. V. Kalko. 2011. Top-down control of herbivory by birds and bats in the canopy of temperate broad-leaved Oaks (*Quercus robur*). Plos One 6.
Boros, C. A. and F. R. Stermitz. 1990. Iridoids - an updated review .1. Journal of Natural Products 53:1055-1147.
Boros, C. A. and F. R. Stermitz. 1991. Iridoids - an updated review .2. Journal of Natural Products 54:1173-1246.
Bowers, M. D. 1980. Unpalatability as a defense strategy of *Euphydryas phaeton* (Lepidoptera, Nymphalidae). Evolution 34:586-600.
Bowers, M. D. 1981. Unpalatability as a defense strategy of Western Checkerspot butterflies (*Euphydryas scudder*, Nymphalidae). Evolution 35:367-375.
Bowers, M. D. 1983. The role of iridoid glycosides in host-plant specificity of Checkerspot butterflies. Journal Of Chemical Ecology 9:475-493.
Bowers, M. D. 1984. Iridoid glycosides and host-plant specificity in larvae of the Buckeye butterfly, *Junonia coenia* (Nymphalidae). Journal Of Chemical Ecology 10:1567-1577.
Bowers, M. D. 1991. Iridoid glycosides. Pages 297-326 *in* G. A. Rosenthal and M. R. Berenbaum, editors. Herbivores: Their interactions with secondary plant metabolites. Academic Press, Inc. , San Diego, California, USA.

Bowers, M. D. 2003. Hostplant suitability and defensive chemistry of the Catalpa sphinx, *Ceratomia catalpae*. Journal Of Chemical Ecology 29:2359-2367.
Bowers, M. D. and S. K. Collinge. 1992. Fate of iridoid glycosides in different life stages of the Buckeye, *Junonia coenia* (Lepidoptera, Nymphalidae). Journal Of Chemical Ecology 18:817-831.
Bowers, M. D., S. K. Collinge, S. E. Gamble, and J. Schmitt. 1992. Effects of genotype, habitat, and seasonal variation on iridoid glycoside content of *Plantago lanceolata* (Plantaginaceae) and the implications for insect herbivores. Oecologia 91:201-207.
Bowers, M. D. and S. Farley. 1990. The behavior of Gray Jays, *Perisoreus canadensis*, towards palatable and unpalatable Lepidoptera. Animal Behaviour 39:699-705.
Bowers, M. D. and G. M. Puttick. 1986. Fate of ingested iridoid glycosides in Lepidopteran herbivores. Journal Of Chemical Ecology 12:169-178.
Bowers, M. D. and G. M. Puttick. 1988. Response of generalist and specialist insects to qualitative allelochemical variation. Journal Of Chemical Ecology 14:319-334.
Bowers, M. D. and G. M. Puttick. 1989. Iridoid glycosides and insect feeding preferences - Gypsy moths (*Lymantria dispar*, Lymantriidae) and buckeyes (*Junonia coenia*, Nymphalidae). Ecological Entomology 14:247-256.
Bowers, M. D. and N. E. Stamp. 1992. Chemical variation within and between individuals of *Plantago lanceolata* (Plantaginaceae). Journal Of Chemical Ecology 18:985-995.
Bowers, M. D. and N. E. Stamp. 1993. Effects of plant-age, genotype, and herbivory on *Plantago* performance and chemistry. Ecology 74:1778-1791.
Bowers, M. D. and N. E. Stamp. 1997a. Effect of hostplant genotype and predators on iridoid glycoside content of pupae of a specialist insect herbivore, *Junonia coenia* (Nymphalidae). Biochemical Systematics And Ecology 25:571-580.
Bowers, M. D. and N. E. Stamp. 1997b. Fate of host-plant iridoid glycosides in lepidopteran larvae of Nymphalidae and Arctiidae. Journal Of Chemical Ecology 23:2955-2965.
Briggs, M. A. and J. C. Schultz. 1990. Chemical defense production in *Lotus corniculatus* L .2. Trade-offs among growth, reproduction and defense. Oecologia 83:32-37.
Brock, J. P. and K. Kaufman. 2003. Butterflies of North America. Houghton Miffin Company, Massachusetts.
Brody, A. K. 1997. Effects of pollinators, herbivores, and seed predators on flowering phenology. Ecology 78:1624-1631.
Brouat, C. and D. McKey. 2001. Leaf-stem allometry, hollow stems, and the evolution of caulinary domatia in myrmecophytes. New Phytologist 151:391-406.
Bryant, J. P., F. S. Chapin, and D. R. Klein. 1983. Carbon nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos 40:357-368.
Bryant, J. P. and R. Julkunentiitto. 1995. Ontogenic development of chemical defense by seedling resin Birch: energy-cost of defense production. Journal Of Chemical Ecology 21:883-896.
Bryant, J. P., P. J. Kuropat, P. B. Reichardt, and T. P. Clausen. 1991. Controls over the allocation of resources by woody plants to chemical anti-herbivore defense. Pages 83-103 *in* R. T. Palo and C. T. Robbins, editors. Plant defenses against mammalian herbivory. CRC Press, Boca Raton.
Bryant, J. P., P. B. Reichardt, T. P. Clausen, F. D. Provenze, and P. J. Kuropat. 1992. Woody plant-mammal interactions. Pages 344-371 *in* G. A. Rosenthal, and M. R. Berenbaum editor. Herbivores: their interactions with plant secondary metabolites. Academic Press, San Diego, California, USA.
Bukovinszky, T., E. H. Poelman, R. Gols, G. Prekatsakis, L. E. M. Vet, J. A. Harvey, and M. Dicke. 2009. Consequences of constitutive and induced variation in plant nutritional quality for immune defence of a herbivore against parasitism. Oecologia 160:299-308.

Bukovinszky, T., F. J. F. van Veen, Y. Jongema, and M. Dicke. 2008. Direct and indirect effects of resource quality on food web structure. Science 319:804-807.
Camara, M. D. 1997a. Physiological mechanisms underlying the costs of chemical defence in *Junonia coenia* Hubner (Nymphalidae): a gravimetric and quantitative genetic analysis. Evolutionary Ecology 11:451-469.
Camara, M. D. 1997b. Predator responses to sequestered plant toxins in buckeye caterpillars: are tritrophic interactions locally variable? Journal Of Chemical Ecology 23:2093-2106.
Campos, W. G., J. H. Schoereder, and M. C. Picanco. 2003. Performance of an oligophagous insect in relation to the age of the host plant. Neotropical Entomology 32:671-676.
Cavers, P. B., I. J. Bassett, and C. W. Crompton. 1980. The biology of Canadian weeds .47. *Plantago lanceolata* L. Canadian Journal Of Plant Science 60:1269-1282.
Chaplin-Kramer, R., D. J. Kliebenstein, A. Chiem, E. Morrill, N. J. Mills, and C. Kremen. 2011. Chemically mediated tritrophic interactions: opposing effects of glucosinolates on a specialist herbivore and its predators. Journal Of Applied Ecology 48:880-887.
Chiang, L. C., L. T. Ng, W. Chiang, M. Y. Chang, and C. C. Lin. 2003. Immunomodulatory activities of flavonoids, monoterpenoids, triterpenoids, iridoid glycosides and phenolic compounds of *Plantago* species. Planta Medica 69:600-604.
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., C. B. Purrington, and J. Bergelson. 2003. Costs of induced responses in plants. Basic and Applied Ecology 4:79-89.
Clancy, K. M. and P. W. Price. 1987. Rapid herbivore growth enhances enemy attack - sublethal plant defenses remain a paradox. Ecology 68:733-737.
Clissold, F. J., G. D. Sanson, J. Read, and S. J. Simpson. 2009. Gross vs. net income: how plant toughness affects performance of an insect herbivore. Ecology 90:3393-3405.
Coley, P. D., J. P. Bryant, and F. S. Chapin. 1985. Resource availability and plant antiherbivore defense. Science 230:895-899.
Constabel, C. P. and R. V. Barbehenn. 2011. Tannins in plant-herbivore interactions. Phytochemistry 72:1551-1565.
Cotter, S. C., S. J. Simpson, D. Raubenheimer, and K. Wilson. 2011. Macronutrient balance mediates trade-offs between immune function and life history traits. Functional Ecology 25:186-198.
Crawley, M. J. 1993. Glim for Ecologists Blackwell Scientific Publications, Oxford.
Croteau, R. 1987. Biosynthesis and catabolism of monoterpenoids. Chemical Reviews 87:929-954.
Cuevas-Reyes, P., M. Quesada, P. Hanson, R. Dirzo, and K. Oyama. 2004. Diversity of gall-inducing insects in a Mexican tropical dry forest: the importance of plant species richness, life-forms, host plant age and plant density. Journal Of Ecology 92:707-716.
Darrow, K. and M. D. Bowers. 1997. Phenological and population variation in iridoid glycosides of *Plantago lanceolata* (Plantaginaceae). Biochemical Systematics And Ecology 25:1-11.
Darrow, K. and M. D. Bowers. 1999. Effects of herbivore damage and nutrient level on induction of iridoid glycosides in *Plantago lanceolata*. Journal Of Chemical Ecology 25:1427-1440.
De Deyn, G. B., A. Biere, W. H. van der Putten, R. Wagenaar, and J. N. Klironomos. 2009. Chemical defense, mycorrhizal colonization and growth responses in *Plantago lanceolata* L. Oecologia 160:433-442.
De Deyn, G. B., C. E. Raaijmakers, J. van Ruijven, F. Berendse, and W. H. van der Putten. 2004. Plant species identity and diversity effects on different trophic levels of nematodes in the soil food web. Oikos 106:576-586.
de la Fuente, M. A. 2002. Variation in plant antiherbivore defenses: causes and consequences. University of Colorado, Boulder.

de la Fuente, M. A., L. A. Dyer, and M. D. Bowers. 1995. The iridoid glycoside, catalpol, as a deterrent to the predator *Camponotus floridanus* (Formicidae). Chemoecology 5:13-18.
Dejean, A., C. Djieto-Lordon, R. Cereghino, and M. Leponce. 2008. Ontogenetic succession and the ant mosaic: An empirical approach using pioneer trees. Basic and Applied Ecology 9:316-323.
Del-Val, E. K. and M. J. Crawley. 2005. Are grazing increaser species better tolerators than decreasers? An experimental assessment of defoliation tolerance in eight British grassland species. Journal Of Ecology 93:1005-1016.
Del Val, E. and R. Dirzo. 2003. Does ontogeny cause changes in the defensive strategies of the myrmecophyte *Cecropia peltata*? Plant Ecology 169:35-41.
Denno, R. F., S. Larsson, and K. L. Olmstead. 1990. Role of enemy-free space and plant-quality in host-plant selection by willow beetles. Ecology 71:124-137.
Denno, R. F., M. S. Mcclure, and J. R. Ott. 1995. Interspecific interactions in phytophagous insects - competition reexamined and resurrected. Annual Review Of Entomology 40:297-331.
Diawara, M. M., J. T. Trumble, C. F. Quiros, K. K. White, and C. Adams. 1994. Plant-age and seasonal-variation in genotypic resistance of Celery to Beat Armyworm (Lepidoptera, Noctuidae). Journal of Economic Entomology 87:514-522.
Dinda, B., D. R. Chowdhury, and B. C. Mohanta. 2009. Naturally occurring iridoids, secoiridoids and their bioactivity. An updated review, part 3. Chemical & Pharmaceutical Bulletin 57:765-796.
Dinda, B., S. Debnath, R. Banik, N. Sato, and Y. Harigaya. 2011. Iridoid glucosides from *Wendlandia tinctoria* roots. Natural Product Communications 6:747-748.
Dinda, B., S. Debnath, and Y. Harigaya. 2007a. Naturally occurring iridoids. A review, part 1. Chemical & Pharmaceutical Bulletin 55:159-222.
Dinda, B., S. Debnath, and Y. Harigaya. 2007b. Naturally occurring secoiridoids and bioactivity of naturally occurring iridoids and secoiridoids. A review, part 2. Chemical & Pharmaceutical Bulletin 55:689-728.
Djieto-Lordon, C., A. Dejean, M. Gibernau, M. Hossaert-McKey, and D. McKey. 2004. Symbiotic mutualism with a community of opportunistic ants: protection, competition, and ant occupancy of the myrmecophyte Barteria nigritana (Passifloraceae). Acta Oecologica-International Journal of Ecology 26:109-116.
Dobler, S., G. Petschenka, and H. Pankoke. 2011. Coping with toxic plant compounds - The insect's perspective on iridoid glycosides and cardenolides. Phytochemistry 72:1593-1604.
Donaldson, J. R., M. T. Stevens, H. R. Barnhill, and R. L. Lindroth. 2006. Age-related shifts in leaf chemistry of clonal aspen (*Populus tremuloides*). Journal Of Chemical Ecology 32:1415-1429.
Du, D. L., J. A. Winsor, M. Smith, A. Denicco, and A. G. Stephenson. 2008. Resistance and tolerance to herbivory changes with inbreeding and ontogeny in a wild gourd (Cucurbitaceae). American Journal Of Botany 95:84-92.
Dyer, L. A. and M. D. Bowers. 1996. The importance of sequestered iridoid glycosides as a defense against an ant predator. Journal Of Chemical Ecology 22:1527-1539.
Dyer, L. A. and T. Floyd. 1993. Determinants of predation on phytophagous insects - the importance of diet breadth. Oecologia 96:575-582.
Elnaggar, L. J. and J. L. Beal. 1980. Iridoids - a review. Journal of Natural Products 43:649-707.
Endara, M. J. and P. D. Coley. 2011. The resource availability hypothesis revisited: a meta-analysis. Functional Ecology 25:389-398.
Enquist, B. J., G. B. West, E. L. Charnov, and J. H. Brown. 1999. Allometric scaling of production and life-history variation in vascular plants. Nature 401:907-911.

Erb, M., C. A. M. Robert, B. E. Hibbard, and T. C. J. Turlings. 2011. Sequence of arrival determines plant-mediated interactions between herbivores. Journal Of Ecology 99:7-15.
Fajer, E. D., M. D. Bowers, and F. A. Bazzaz. 1991. The effects of enriched CO_2 atmospheres on the Buckeye butterfly, *Junonia coenia*. Ecology 72:751-754.
Fajer, E. D., M. D. Bowers, and F. A. Bazzaz. 1992. The effect of nutrients and enriched CO_2 environments on production of carbon-based allelochemicals in *Plantago* - a test of the carbon nutrient balance hypothesis. American Naturalist 140:707-723.
Farnsworth, E. 2004. Hormones and shiftlng ecology throughout plant development. Ecology 85:5-15.
Fedriani, J. M., P. J. Rey, J. L. Garrido, J. Guitian, C. M. Herrera, M. Medrano, A. M. Sanchez-Lafuente, and X. Cerda. 2004. Geographical variation in the potential of mice to constrain an ant-seed dispersal mutualism. Oikos 105:181-191.
Feeny, P. 1976. Plant apparency and chemical defense. Pages 3-19 *in* J. W. Wallace and M. R. L., editors. Recent Advances in Phytochemistry. Plenum Press New York.
Feldhaar, H., B. Fiala, R. B. Hashim, and U. Maschwitz. 2003. Patterns of the Crematogaster-Macaranga association: The ant partner makes the difference. Insectes Sociaux 50:9-19.
Fenner, M., M. E. Hanley, and R. Lawrence. 1999. Comparison of seedling and adult palatability in annual and perennial plants. Functional Ecology 13:546-551.
Fernandes, G. W. 1994. Plant mechanical defenses against insect herbivory. Revista Brasileira de Entomologia 38:421-433.
Fernandes, G. W., E. D. Almada, and M. A. A. Carneiro. 2010. Gall-inducing insect species richness as indicators of forest age and health. Environmental Entomology 39:1134-1140.
Fonseca, C. R. 1999. Amazonian ant-plant interactions and the nesting space limitation hypothesis. Journal of Tropical Ecology 15:807-825.
Fonseca, C. R. and W. W. Benson. 2003. Ontogenetic succession in Amazonian ant trees. Oikos 102:407-412.
Fonseca, C. R., T. Fleck, and G. W. Fernandes. 2006. Processes driving ontogenetic succession of galls in a canopy. Biotropica 38:514-521.
Fontana, A., M. Reichelt, S. Hempel, J. Gershenzon, and S. B. Unsicker. 2009. The effects of arbuscular mycorrhizal fungi on direct and indirect defense metabolites of *Plantago lanceolata* L. Journal Of Chemical Ecology 35:833-843.
Fordyce, J. A. 2001. The lethal plant defense paradox remains: inducible host-plant aristolochic acids and the growth and defense of the pipevine swallowtail. Entomologia Experimentalis Et Applicata 100:339-346.
Fraenkel, G. 1959. The raison d'etre of secondary plant substances. Science 129:1466-1470.
Fritz, R. S., C. G. Hochwender, D. A. Lewkiewicz, S. Bothwell, and C. M. Orians. 2001. Seedling herbivory by slugs in a willow hybrid system: developmental changes in damage, chemical defense, and plant performance. Oecologia 129:87-97.
Fuchs, A. and M. D. Bowers. 2004. Patterns of iridoid glycoside production and induction in *Plantago lanceolata* and the importance of plant age. Journal Of Chemical Ecology 30:1723-1741.
Galvez, M., C. Martin-Cordero, P. J. Houghton, and M. J. Ayuso. 2005. Antioxidant activity of methanol extracts obtained from *Plantago* species. Journal of Agricultural and Food Chemistry 53:1927-1933.
Gaudet, D. A., A. Laroche, and B. Puchalski. 2001. Effect of plant age on water content in crowns of fall rye and winter wheat cultivars differing in snow mold resistance. Canadian Journal Of Plant Science 81:541-550.
Gedge, K. E. and M. A. Maun. 1992. Effects of simulated herbivory on growth and reproduction of 2 beach annuals, *Cakile edentula* and *Corispermum hyssopifolium*. Canadian Journal of Botany 70:2467-2475.

Gedroc, J. J., K. D. M. McConnaughay, and J. S. Coleman. 1996. Plasticity in root shoot partitioning: Optimal, ontogenetic, or both? Functional Ecology 10:44-50.
Gentry, G. L. and L. A. Dyer. 2002. On the conditional, nature of neotropical caterpillar defenses against their natural enemies. Ecology 83:3108-3119.
Gershenzon, J. 1994. Metabolic costs of terpenoid accumulation in higher-plants. Journal Of Chemical Ecology 20:1281-1328.
Gols, R., T. Bukovinszky, N. M. van Dam, M. Dicke, J. M. Bullock, and J. A. Harvey. 2008a. Performance of generalist and specialist herbivores and their endoparasitoids differs on cultivated and wild Brassica populations. Journal Of Chemical Ecology 34:132-143.
Gols, R., R. Wagenaar, T. Bukovinszky, N. M. van Dam, M. Dicke, J. M. Bullock, and J. A. Harvey. 2008b. Genetic variation in defense chemistry in wild cabbages affects herbivores and their endoparasitoids. Ecology 89:1616-1626.
Goodger, J. Q. D., R. M. Gleadow, and I. E. Woodrow. 2006. Growth cost and ontogenetic expression patterns of defence in cyanogenic *Eucalyptus* spp. Trees-Structure And Function 20:757-765.
Gowan, E., B. A. Lewis, and R. Turgeon. 1995. Phloem transport of antirrhinoside, an iridoid glycoside, in *Asarina scandens* (Scrophulariaceae). Journal Of Chemical Ecology 21:1781-1788.
Gras, E. K., J. Read, C. T. Mach, G. D. Sanson, and F. J. Clissold. 2005. Herbivore damage, resource richness and putative defences in juvenile versus adult *Eucalyptus* leaves. Australian Journal of Botany 53:33-44.
Graves, S. D. and A. M. Shapiro. 2003. Exotics as host plants of the California butterfly fauna. Biological Conservation 110:413-433.
Gripenberg, S., P. J. Mayhew, M. Parnell, and T. Roslin. 2010. A meta-analysis of preference-performance relationships in phytophagous insects. Ecology Letters 13:383-393.
Gruntman, M. and A. Novoplansky. 2011. Ontogenetic contingency of tolerance mechanisms in response to apical damage. Annals of Botany 108:965-973.
Haase, D. L. and R. Rose. 1995. Vector analysis and its use for interpreting plant nutrient shifts in response to silvicultural treatments. Forest Science 41:54-66.
Hadacek, F. 2002. Secondary metabolites as plant traits: current assessment and future perspectives. Critical Reviews in Plant Sciences 21:273-322.
Hagele, B. F. and M. Rowell-Rahier. 1999. Dietary mixing in three generalist herbivores: nutrient complementation or toxin dilution? Oecologia 119:521-533.
Hanley, M. E. and E. L. Fegan. 2007. Timing of cotyledon damage affects growth and flowering in mature plants. Plant Cell and Environment 30:812-819.
Hanley, M. E., M. Fenner, and P. J. Edwards. 1995. The effect of seedling age on the likelihood of herbivory by the slug *Deroceras reticulatum*. Functional Ecology 9:754-759.
Hanley, M. E., M. Fenner, H. Whibley, and B. Darvill. 2004. Early plant growth: identifying the end point of the seedling phase. New Phytologist 163:61-66.
Hanley, M. E., B. B. Lamont, M. M. Fairbanks, and C. M. Rafferty. 2007. Plant structural traits and their role in anti-herbivore defence. Perspectives In Plant Ecology Evolution And Systematics 8:157-178.
Hare, J. D. 1980. Impact of defoliation by the Colorado Potato Beetle (Coleoptera, Chrysomelidae) on potato yields. Journal of Economic Entomology 73:369-373.
Hare, J. D. 2010. Ontogeny and Season Constrain the Production of Herbivore-Inducible Plant Volatiles in the Field. Journal of Chemical Ecology 36:1363-1374.
Hartmann, T. 1996. Diversity and variability of plant secondary metabolism: a mechanistic view. Entomologia Experimentalis Et Applicata 80:177-188.
Hartmann, T. 2008. The lost origin of chemical ecology in the late 19th century. Proceedings Of The National Academy Of Sciences Of The United States Of America 105:4541-4546.

Hartmann, T. and M. Zimmer. 1986. Organ-specific distribution and accumulation of pyrrolizidine alkaloids during the life-history of 2 annual *Senecio* species. Journal of Plant Physiology 122:67-80.
Harvey, J. A., S. Van Nouhuys, and A. Biere. 2005. Effects of quantitative variation in allelochemicals in *Plantago lanceolata* on development of a generalist and a specialist herbivore and their endoparasitoids. Journal Of Chemical Ecology 31:287-302.
Haukioja, E. and J. Koricheva. 2000. Tolerance to herbivory in woody vs. herbaceous plants. Evolutionary Ecology 14:551-562.
Havill, N. P. and K. F. Raffa. 2000. Compound effects of induced plant responses on insect herbivores and parasitoids: implications for tritrophic interactions. Ecological Entomology 25:171-179.
Haviola, S., L. Kapari, V. Ossipov, M. J. Rantala, T. Ruuhola, and E. Haukioja. 2007. Foliar phenolics are differently associated with *Epirrita autumnata* growth and immunocompetence. Journal Of Chemical Ecology 33:1013-1023.
Hawkins, B. A., H. V. Cornell, and M. E. Hochberg. 1997. Predators, parasitoids, and pathogens as mortality agents in phytophagous insect populations. Ecology 78:2145-2152.
Heckel, D. G., Z. D. Liu, and J. Scheirs. 2010. Host plant flowering increases both adult oviposition preference and larval performance of a generalist herbivore. Environmental Entomology 39:552-560.
Heil, M., B. Fiala, K. E. Linsenmair, G. Zotz, P. Menke, and U. Maschwitz. 1997. Food body production in Macaranga triloba (Euphorbiaceae): a plant investment in anti-herbivore defence via symbiotic ant partners. Journal of Ecology 85:847-861.
Herms, D. A. and W. J. Mattson. 1992. The dilemma of plants - To grow or defend. Quarterly Review Of Biology 67:283-335.
Herrera, C. M. 1988. Variation in mutualisms - The spatio-temporal mosaic of a pollinator assemblage. Biological Journal Of The Linnean Society 35:95-125.
Hilbert, D. W., D. M. Swift, J. K. Detling, and M. I. Dyer. 1981. Relative growth-rates and the grazing optimization hypothesis. Oecologia 51:14-18.
Hochuli, D. F. 1996. The ecology of plant/insect interactions: Implications of digestive strategy for feeding by phytophagous insects. Oikos 75:133-141.
Hodar, J. A., R. Zamora, J. Castro, J. M. Gomez, and D. Garcia. 2008. Biomass allocation and growth responses of Scots pine saplings to simulated herbivory depend on plant age and light availability. Plant Ecology 197:229-238.
Hogedal, B. D. and P. Molgaard. 2000. HPLC analysis of the seasonal and diurnal variation of iridoids in cultivars of *Antirrhinum majus*. Biochemical Systematics And Ecology 28:949-962.
Holeski, L. M., M. J. C. Kearsley, and T. G. Whitham. 2009. Separating ontogenetic and environmental determination of resistance to herbivory in cottonwood. Ecology 90:2969-2973.
Honda, K. 1995. Chemical basis of differential oviposition by Lepidopterous insects. Archives of Insect Biochemistry and Physiology 30:1-23.
Horvitz, C. C. and D. W. Schemske. 1990. Spatiotemporal variation in insect mutualists of a neotropical herb. Ecology 71:1085-1097.
Howe, G. A., H. Chen, C. G. Wilkerson, J. A. Kuchar, and B. S. Phinney. 2005. Jasmonate-inducible plant enzymes degrade essential amino acids in the herbivore midgut. Proceedings Of The National Academy Of Sciences Of The United States Of America 102:19237-19242.
Hristov, N. and W. E. Conner. 2005. Effectiveness of tiger moth (Lepidoptera, Arctiidae) chemical defenses against an insectivorous bat (*Eptesicus fuscus*). Chemoecology 15:105-113.

Hunter, A. F. and J. S. Elkinton. 2000. Effects of synchrony with host plant on populations of a spring-feeding Lepidopteran. Ecology 81:1248-1261.
Hunter, M. D. 2003. Effects of plant quality on the population ecology of parasitoids. Agricultural and Forest Entomology 5:1-8.
Ilmarinen, K., J. Mikola, M. Nieminen, and M. Vestburg. 2005. Does plant growth phase determine the response of plants and soil organisms to defoliation? Soil Biology & Biochemistry 37:433-443.
Inbar, M. and D. Gerling. 2008. Plant-mediated interactions between whiteflies, herbivores, and natural enemies. Annual Review Of Entomology 53:431-448.
Irwin, R. E. and J. E. Maloof. 2002. Variation in nectar robbing over time, space, and species. Oecologia 133:525-533.
Ishida, A., K. Yazaki, and A. L. Hoe. 2005. Ontogenetic transition of leaf physiology and anatomy from seedlings to mature trees of a rain forest pioneer tree, *Macaranga gigantea*. Tree Physiology 25:513-522.
Itino, T., T. Itioka, A. Hatada, and A. A. Hamid. 2001. Effects of food rewards offered by ant-plant Macaranga on the colony size of ants. Ecological Research 16:775-786.
Ives, A. R. and A. P. Dobson. 1987. Antipredator behavior and the population-dynamics of simple predator-prey systems. American Naturalist 130:431-447.
Izzo, T. J. and H. L. Vasconcelos. 2005. Ants and plant size shape the structure of the arthropod community of Hirtella myrmecophila, an Amazonian ant-plant. Ecological Entomology 30:650-656.
Jaenike, J. 1978. Optimal oviposition behavior in phytophagous insects. Theoretical Population Biology 14:350-356.
Jamieson, M. A. and M. D. Bowers. 2010. Iridoid glycoside variation in the invasive plant Dalmatian Toadflax, *Linaria dalmatica* (Plantaginaceae), and sequestration by the biological control agent, *Calophasia lunula*. Journal Of Chemical Ecology 36:70-79.
Jarzomski, C. M., N. E. Stamp, and M. D. Bowers. 2000. Effects of plant phenology, nutrients and herbivory on growth and defensive chemistry of plantain, *Plantago lanceolata*. Oikos 88:371-379.
Jaya, E., D. S. Kubien, P. E. Jameson, and J. Clemens. 2010. Vegetative phase change and photosynthesis in *Eucalyptus occidentalis*: architectural simplification prolongs juvenile traits. Tree Physiology 30:393-403.
Jensen, S. R. 1991. Plant iridoids, their biosynthesis and distribution in angiosperms. Pages 133-158 *in* J. B. Harborne and F. A. Tomas-Barberan, editors. Annual proceedings of the phytochemical society of Europe: ecological chemistry and biochemistry of plant terpenoids. Oxford University Press, Oxford.
Johnson, M. L. and M. P. Zalucki. 2005. Foraging behaviour of *Helicoverpa armigera* first instar larvae on crop plants of different developmental stages. Journal of Applied Entomology 129:239-245.
Jones, C. S. 1999. An essay on juvenility, phase change, and heteroblasty in seed plants. International Journal of Plant Sciences 160:S105-S111.
Judd, C. M., G. H. McClelland, and C. S. Ryan. 2009. Data analysis: a model comparison approach. Routledge Taylor and Francis, New York.
Kaplan, I. and R. F. Denno. 2007. Interspecific interactions in phytophagous insects revisited: a quantitative assessment of competition theory. Ecology Letters 10:977-994.
Kaplan, I., R. Halitschke, A. Kessler, S. Sardanelli, and R. F. Denno. 2008. Constitutive and induced defenses to herbivory in above- and belowground plant tissues. Ecology 89:392-406.
Karban, R. and I. T. Baldwin. 1997. Induced Responses to Herbivory. Chicago, University of Chicago Press.

Karban, R. and J. S. Thaler. 1999. Plant phase change and resistance to herbivory. Ecology 80:510-517.
Kearsey, M. and T. J. Whitham. 1989. Developmental changes in resistance to herbivory: implications for individuals and populations. Ecology 70: 422-434.
Kearsley, M. J. C. and T. G. Whitham. 1989. Developmental-Changes in Resistance to Herbivory - Implications for Individuals and Populations. Ecology 70:422-434.
Kempel, A., M. Schadler, T. Chrobock, M. Fischer, and M. van Kleunen. 2011. Tradeoffs associated with constitutive and induced plant resistance against herbivory. Proceedings Of The National Academy Of Sciences Of The United States Of America 108:5685-5689.
Kessler, A. and R. Halitschke. 2007. Specificity and complexity: the impact of herbivore-induced plant responses on arthropod community structure. Current Opinion in Plant Biology 10:409-414.
Klemola, N., T. Klemola, M. J. Rantala, and T. Ruuhola. 2007. Natural host-plant quality affects immune defence of an insect herbivore. Entomologia Experimentalis Et Applicata 123:167-176.
Klockars, G. K., M. D. Bowers, and B. Cooney. 1993. Leaf variation in iridoid glycoside content of *Plantago lanceolata* (Plantaginaceae) and oviposition of the buckeye, *Junonia coenia* (Nymphalidae). Chemoecology 4:72-78.
Kolb, A., J. Ehrlen, and O. Eriksson. 2007. Ecological and evolutionary consequences of spatial and temporal variation in pre-dispersal seed predation. Perspectives In Plant Ecology Evolution And Systematics 9:79-100.
Kollner, T. G., C. Schnee, J. Gershenzon, and J. Degenhardt. 2004. The sesquiterpene hydrocarbons of maize (Zea mays) form five groups with distinct developmental and organ-specific distribution. Phytochemistry 65:1895-1902.
Koricheva, J. 1999. Interpreting phenotypic variation in plant allelochemistry: problems with the use of concentrations. Oecologia 119:467-473.
Koricheva, J., S. Larsson, E. Haukioja, and M. Keinanen. 1998. Regulation of woody plant secondary metabolism by resource availability: hypothesis testing by means of meta-analysis. Oikos 83:212-226.
Kos, M., P. Kabouw, R. Noordam, K. Hendriks, L. E. M. Vet, J. J. A. van Loon, and M. Dicke. 2011. Prey-mediated effects of glucosinolates on aphid predators. Ecological Entomology 36:377-388.
Kozlowski, T. T. 1971. Growth and development of trees. Academic Press, New York. .
Lampert, E. C. and M. D. Bowers. 2010a. Host plant influences on iridoid glycoside sequestration of generalist and specialist caterpillars. Journal Of Chemical Ecology 36:1101-1104.
Lampert, E. C. and M. D. Bowers. 2010b. Host plant species affects the quality of the generalist *Trichoplusia ni* as a host for the polyembryonic parasitoid *Copidosoma floridanum*. Entomologia Experimentalis Et Applicata 134:287-295.
Lampert, E. C., L. A. Dyer, and M. D. Bowers. 2010. Caterpillar chemical defense and parasitoid success: *Cotesia congregata* parasitism of *Ceratomia catalpae*. Journal Of Chemical Ecology 36:992-998.
Lavine, M. D. and N. E. Beckage. 1996. Temporal pattern of parasitism-induced immunosuppression in *Manduca sexta* larvae parasitized by *Cotesia congregata*. Journal of Insect Physiology 42:41-51.
Lawrence, R., B. M. Potts, and T. G. Whitham. 2003. Relative importance of plant ontogeny, host genetic variation, and leaf age for a common herbivore. Ecology 84:1171-1178.
Le Guigo, P., Y. Qu, and J. Le Corff. 2011. Plant-mediated effects on a toxin-sequestering aphid and its endoparasitoid. Basic and Applied Ecology 12:72-79.

Lee, K. P., J. S. Cory, K. Wilson, D. Raubenheimer, and S. J. Simpson. 2006. Flexible diet choice offsets protein costs of pathogen resistance in a caterpillar. Proceedings Of The Royal Society B-Biological Sciences 273:823-829.
Lennartsson, T., P. Nilsson, and J. Tuomi. 1998. Induction of overcompensation in the field gentian, *Gentianella campestris*. Ecology 79:1061-1072.
Lima, S. L. 1998. Nonlethal effects in the ecology of predator-prey interactions - What are the ecological effects of anti-predator decision-making? Bioscience 48:25-34.
Linhart, Y. B. and J. D. Thompson. 1999. Thyme is of the essence: biochemical polymorphism and multi-species deterrence. Evolutionary Ecology Research 1:151-171.
Liu, Z. D., J. Scheirs, and D. G. Heckel. 2010. Host plant flowering increases both adult oviposition preference and larval performance of a generalist herbivore. Environmental Entomology 39:552-560.
Loney, P. E., C. McArthur, B. M. Potts, and G. J. Jordan. 2006. How does ontogeny in a *Eucalyptus* species affect patterns of herbivory by Brushtail Possums? Functional Ecology 20:982-988.
Lovallo, N., B. A. McPheron, and D. L. Cox-Foster. 2002. Effects of the polydnavirus of *Cotesia congregata* on the immune system and development of non-habitual hosts of the parasitoid. Journal of Insect Physiology 48:517-526.
Malamy, J. E. 2005. Intrinsic and environmental response pathways that regulate root system architecture. Plant Cell and Environment 28:67-77.
Marak, H. B., A. Biere, and J. M. M. Van Damme. 2002. Systemic, genotype-specific induction of two herbivore-deterrent iridoid glycosides in *Plantago lanceolata* L. in response to fungal infection by *Diaporthe adunca* (Rob.) niessel. Journal Of Chemical Ecology 28:2429-2448.
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.
Massad, T. J., R. M. Fincher, A. M. Smilanich, and L. Dyer. 2011. A quantitative evaluation of major plant defense hypotheses, nature versus nurture, and chemistry versus ants. Arthropod-Plant Interactions 5:125-139.
Mattiacci, L., S. Rudelli, B. A. Rocca, S. Genini, and S. Dorn. 2001. Systemically-induced response of cabbage plants against a specialist herbivore, *Prieris brasicae*. Chemoecology 11:167-173.
Mattson, W. J. 1980. Herbivory in relation to plant nitrogen-content. Annual Review Of Ecology And Systematics 11:119-161.
Mayhew, P. J. 1997. Adaptive patterns of host-plant selection by phytophagous insects. Oikos 79:417-428.
Mayhew, P. J. 2001. Herbivore host choice and optimal bad motherhood. Trends In Ecology & Evolution 16:165-167.
Mcarthur, C., P. E. Loney, N. W. Davies, and G. J. Jordan. 2010. Early ontogenetic trajectories vary among defence chemicals in seedlings of a fast-growing eucalypt. Austral Ecology 35:157-166.
McConnaughay, K. D. M. and J. S. Coleman. 1999. Biomass allocation in plants: ontogeny or optimality? A test along three resource gradients. Ecology 80:2581-2593.
McGeoch, M. A. and P. W. Price. 2005. Scale-dependent mechanisms in the population dynamics of an insect herbivore. Oecologia 144:278-288.
McKey, D. 1979. The distribution of secondary compounds within plants. Pages 55-133 *in* G. A. Rosenthal and D. H. Janzen, editors. Herbivores: their interaction with secondary plant metabolites. Academic, London.
Mcnaughton, S. J. 1983. Compensatory plant-growth as a response to herbivory. Oikos 40:329-336.

Merilo, E., I. Tulva, O. Raim, A. Kukit, A. Sellin, and O. Kull. 2009. Changes in needle nitrogen partitioning and photosynthesis during 80 years of tree ontogeny in *Picea abies*. Trees Structure and Function 23:951-958.
Milla, R., P. B. Reich, U. Niinemets, and P. Castro-Diez. 2008. Environmental and developmental controls on specific leaf area are little modified by leaf allometry. Functional Ecology 22:565-576.
Miller, T. E. X. 2007. Does having multiple partners weaken the benefits of facultative mutualism? A test with cacti and cactus-tending ants. Oikos 116:500-512.
Morales, J. M. and D. P. Vazquez. 2008. The effect of space in plant-animal mutualistic networks: insights from a simulation study. Oikos 117:1362-1370.
Moret, Y. and P. Schmid-Hempel. 2000. Survival for immunity: The price of immune system activation for bumblebee workers. Science 290:1166-1168.
Moriuchi, K. S. and A. A. Winn. 2005. Relationships among growth, development and plastic response to environment quality in a perennial plant. New Phytologist 166:149-158.
Morris, W. F., M. B. Traw, and J. Bergelson. 2006. On testing for a tradeoff between constitutive and induced resistance. Oikos 112:102-110.
Muola, A., P. Mutikainen, L. Laukkanen, M. Lilley, and R. Leimu. 2010. Genetic variation in herbivore resistance and tolerance: the role of plant life-history stage and type of damage. Journal of Evolutionary Biology 23:2185-2196.
Nieminen, M., J. Suomi, S. Van Nouhuys, P. Sauri, and M. L. Riekkola. 2003. Effect of iridoid glycoside content on oviposition host plant choice and parasitism in a specialist herbivore. Journal Of Chemical Ecology 29:823-844.
Niinemets, U. 2005. Key plant structural and allocation traits depend on relative age in the perennial herb *Pimpinella saxifraga*. Annals of Botany 96:323-330.
Niinemets, U. 2010. Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: past stress history, stress interactions, tolerance and acclimation. Forest Ecology And Management 260:1623-1639.
Nishida, R. 2002. Sequestration of defensive substances from plants by Lepidoptera. Annual Review Of Entomology 47:57-92.
Obermaier, E., A. Heisswolf, H. J. Poethke, B. Randlkofer, and T. Meiners. 2008. Plant architecture and vegetation structure: Two ways for insect herbivores to escape parasitism. European Journal of Entomology 105:233-240.
Ode, P. J. 2006. Plant chemistry and natural enemy fitness: effects on herbivore and natural enemy interactions. Annual Review Of Entomology 51:163-185.
Ode, P. J., M. R. Berenbaum, A. R. Zangerl, and I. C. W. Hardy. 2004. Host plant, host plant chemistry and the polyembryonic parasitoid *Copidosoma sosares*: indirect effects in a tritrophic interaction. Oikos 104:388-400.
Ohgushi, T. 2005. Indirect interaction webs: herbivore-induced effects through trait change in plants. Annual Review Of Ecology Evolution And Systematics 36:81-105.
Ohnmeiss, T. E. and I. T. Baldwin. 2000. Optimal defense theory predicts the ontogeny of an induced nicotine defense. Ecology 81:1765-1783.
Ojala, K., R. Julkunen-Tiito, L. Lindstrom, and J. Mappes. 2005. Diet affects the immune defence and life-history traits of an Arctiid moth *Parasemia plantaginis*. Evolutionary Ecology Research 7:1153-1170.
Opitz, S. E. W., S. R. Jensen, and C. Muller. 2010. Sequestration of glucosinolates and iridoid glucosides in sawfly species of the genus *Athalia* and their role in defense against ants. Journal Of Chemical Ecology 36:148-157.
Opitz, S. E. W. and C. Muller. 2009. Plant chemistry and insect sequestration. Chemoecology 19:117-154.
Orians, C. M., C. G. Hochwender, R. S. Fritz, and T. Snall. 2010. Growth and chemical defense in willow seedlings: trade-offs are transient. Oecologia 163:283-290.

Orians, C. M., A. Thorn, and S. Gomez. 2011. Herbivore-induced resource sequestration in plants: why bother? Oecologia 167:1-9.
Palmer, T. M., D. F. Doak, M. L. Stanton, J. L. Bronstein, E. T. Kiers, T. P. Young, J. R. Goheen, and R. M. Pringle. 2010. Synergy of multiple partners, including freeloaders, increases host fitness in a multispecies mutualism. Proceedings of the National Academy of Sciences of the United States of America 107:17234-17239.
Pereyra, P. C. and M. D. Bowers. 1988. Iridoid glycosides as oviposition stimulants for the Buckeye butterfly, *Junonia coenia* (Nymphalidae). Journal Of Chemical Ecology 14:917-928.
Pires, C. S. S. and P. W. Price. 2000. Patterns of host plant growth and attack and establishment of gall-inducing wasp (Hymenoptera : Cynipidae). Environmental Entomology 29:49-54.
Poelman, E. H., C. Broekgaarden, J. J. A. Van Loon, and M. Dicke. 2008a. Early season herbivore differentially affects plant defence responses to subsequently colonizing herbivores and their abundance in the field. Molecular Ecology 17:3352-3365.
Poelman, E. H., R. Gols, T. A. L. Snoeren, D. Muru, H. M. Smid, and M. Dicke. 2011. Indirect plant-mediated interactions among parasitoid larvae. Ecology Letters 14:670-676.
Poelman, E. H., J. J. A. van Loon, and M. Dicke. 2008b. Consequences of variation in plant defense for biodiversity at higher trophic levels. Trends In Plant Science 13:534-541.
Poethig, R. S. 1990. Phase-change and the regulation of shoot morphogenesis in plants. Science 250:923-930.
Polis, G. A., W. B. Anderson, and R. D. Holt. 1997. Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Annual Review Of Ecology And Systematics 28:289-316.
Preisser, E. L., D. I. Bolnick, and M. F. Benard. 2005. Scared to death? The effects of intimidation and consumption in predator-prey interactions. Ecology 86:501-509.
Price, P. W. 1991. The plant vigor hypothesis and herbivore attack. Oikos 62:244-251.
Price, P. W., C. E. Bouton, P. Gross, B. A. McPheron, J. N. Thompson, and A. E. Weis. 1980. Interactions among 3 trophic levels - Influence of plants on interactions between insect herbivores and natural enemies. Annual Review Of Ecology And Systematics 11:41-65.
Price, P. W., H. Roininen, and J. Tahvanainen. 1987. Plant-age and attack by the bud galler, *Euura mucronata*. Oecologia 73:334-337.
Prudic, K. L., J. C. Oliver, and M. D. Bowers. 2005. Soil nutrient effects on oviposition preference, larval performance, and chemical defense of a specialist insect herbivore. Oecologia 143:578-587.
Puttick, G. M. and M. D. Bowers. 1988. Effect of qualitative and quantitative variation in allelochemicals on a generalist insect - Iridoid glycosides and the Southern Armyworm. Journal Of Chemical Ecology 14:335-351.
Quintero, C., K. E. Barton, and K. Boege. *in prep.* The ontogeny of plant indirect defense. To be sumbitted to Perspectives in Plant Ecology, Evolution and Systematics.
Quintero, C. and M. D. Bowers. 2011a. Changes in plant chemical defenses and nutritional quality as a function of ontogeny in *Plantago lanceolata* (Plantaginaceae). Oecologia DOI 10.1007/s00442-011-2114-x.
Quintero, C. and M. D. Bowers. 2011b. Plant induced defenses depend more on plant age than previous history of damage: implications for plant-herbivore interactions. Journal Of Chemical Ecology 37:992–1001.
Rantala, M. J. and D. A. Roff. 2005. An analysis of trade-offs in immune function, body size and development time in the Mediterranean field cricket, *Gryllus bimaculatus*. Functional Ecology 19:323-330.
Rantala, M. J. and D. A. Roff. 2007. Inbreeding and extreme outbreeding cause sex differences in immune defence and life history traits in *Epirrita autumnata*. Heredity 98:329-336.

Raubenheimer, D. and S. J. Simpson. 1992. Analysis of covariance - an alternative to nutritional indexes. Entomologia Experimentalis Et Applicata 62:221-231.
Rehill, B. J., T. G. Whitham, G. D. Martinsen, J. A. Schweitzer, J. K. Bailey, and R. L. Lindroth. 2006. Developmental trajectories in cottonwood phytochemistry. Journal Of Chemical Ecology 32:2269-2285.
Reitz, S. R. and J. T. Trumble. 1997. Effects of linear furanocoumarins on the herbivore *Spodoptera exigua* and the parasitoid *Archytas marmoratus*: host quality and parasitoid success. Entomologia Experimentalis Et Applicata 84:9-16.
Remmel, T., J. Davison, and T. Tammaru. 2011. Quantifying predation on folivorous insect larvae: the perspective of life-history evolution. Biological Journal Of The Linnean Society 104:1-18.
Reudler Talsma, J. H., A. Biere, J. A. Harvey, and S. van Nouhuys. 2008a. Oviposition cues for a specialist butterfly-plant chemistry and size. Journal Of Chemical Ecology 34:1202-1212.
Reudler Talsma, J. H., A. Biere, J. A. Harvey, and S. van Nouhuys. 2011. Differential performance of a specialist and two generalist herbivores and their parasitoids on *Plantago lanceolata*. Journal Of Chemical Ecology 37:765-778.
Reudler Talsma, J. H., K. Torri, and S. van Nouhuys. 2008b. Host plant use by the Heath fritillary butterfly, *Melitaea athalia*: plant habitat, species and chemistry. Arthropod-Plant Interactions 2:63-75.
Richter, M. R. 1990. Hunting social wasp interactions - Influence of prey size, arrival order, and wasp species. Ecology 71:1018-1030.
Richter, M. R. 2000. Social wasp (Hymenoptera : Vespidae) foraging behavior. Annual Review Of Entomology 45:121-150.
Riihimaki, J., H. Vehvilainen, P. Kaitaniemi, and J. Koricheva. 2006. Host tree architecture mediates the effect of predators on herbivore survival. Ecological Entomology 31:227-235.
Roach, D. A., C. E. Ridley, and J. L. Dudycha. 2009. Longitudinal analysis of *Plantago*: age-by-environment interactions reveal aging. Ecology 90:1427-1433.
Roitto, M., P. Rautio, A. Markkola, R. Julkunen-Tiitto, M. Varama, K. Saravesi, and J. Tuomi. 2009. Induced accumulation of phenolics and sawfly performance in Scots pine in response to previous defoliation. Tree Physiology 29:207-216.
Ronsted, N., E. Gobel, H. Franzyk, S. R. Jensen, and C. E. Olsen. 2000. Chemotaxonomy of *Plantago*. Iridoid glucosides and caffeoyl phenylethanoid glycosides. Phytochemistry 55:337-348.
Rostas, M. and K. Eggert. 2008. Ontogenetic and spatio-temporal patterns of induced volatiles in Glycine max in the light of the optimal defence hypothesis. Chemoecology 18:29-38.
Ruuhola, T., J. P. Salminen, S. Haviola, S. Y. Yang, and M. J. Rantala. 2007. Immunological memory of mountain birches: effects of phenolics on performance of the autumnal moth depend on herbivory history of trees. Journal Of Chemical Ecology 33:1160-1176.
Saastamoinen, M., S. van Nouhuys, M. Nieminen, B. O'Hara, and J. Suomi. 2007. Development and survival of a specialist herbivore, *Melitaea cinxia,* on host plants producing high and low concentrations of iridoid glycosides. Annales Zoologici Fennici 44:70-80.
Sadek, M. M., B. S. Hansson, and P. Anderson. 2010. Does risk of egg parasitism affect choice of oviposition sites by a moth? A field and laboratory study. Basic and Applied Ecology 11:135-143.
Sanson, G., J. Read, N. Aranwela, F. Clissold, and P. Peeters. 2001. Measurement of leaf biomechanical properties in studies of herbivory: opportunities, problems and procedures. Austral Ecology 26:535-546.

Santos, J. C. and G. W. Fernandes. 2010. Mediation of herbivore attack and induced resistance by plant vigor and ontogeny. Acta Oecologica-International Journal of Ecology 36:617-625.
Schippers, P. and H. Olff. 2000. Biomass partitioning, architecture and turnover of six herbaceous species from habitats with different nutrient supply. Plant Ecology 149:219-231.
Schmeller, T., B. LatzBruning, and M. Wink. 1997. Biochemical activities of berberine, palmatine and sanguinarine mediating chemical defence against microorganisms and herbivores. Phytochemistry 44:257-266.
Shefferson, R. P. and D. A. Roach. 2010. Longitudinal analysis of *Plantago*: adaptive benefits of iteroparity in a short-lived, herbaceous perennial. Ecology 91:441-447.
Shikano, I., J. D. Ericsson, J. S. Cory, and J. H. Myers. 2010. Indirect plant-mediated effects on insect immunity and disease resistance in a tritrophic system. Basic and Applied Ecology 11:15-22.
Singer, M. S., K. C. Mace, and E. A. Bernays. 2009. Self-medication as adaptive plasticity: increased ingestion of plant toxins by parasitized caterpillars. Plos One 4.
Slansky, F., Jr. and J. M. Scriber. 1985. Food consumption and utilization.*in* G. A. Kerkut and L. I. Gilbert, editors. Comprehensive Insect Physiology, Biochemestry and Pharmacology, Pergamon, Oxford.
Smilanich, A. M., L. A. Dyer, J. Q. Chambers, and M. D. Bowers. 2009a. Immunological cost of chemical defence and the evolution of herbivore diet breadth. Ecology Letters 12:612-621.
Smilanich, A. M., L. A. Dyer, and G. L. Gentry. 2009b. The insect immune response and other putative defenses as effective predictors of parasitism. Ecology 90:1434-1440.
Smilanich, A. M., J. Vargas, L. A. Dyer, and M. D. Bowers. 2011. Effects of ingested secondary metabolites on the immune response of a polyphagous caterpillar *Grammia incorrupta*. Journal Of Chemical Ecology 37:239-245.
Smiley, J. T., J. M. Horn, and N. E. Rank. 1985. Ecological effects of salicin at 3 trophic levels - New problems from old adaptations. Science 229:649-651.
Spiegel, L. H. and P. W. Price. 1996. Plant aging and the distribution of *Rhyacionia neomexicana* (Lepidoptera: Tortricidae). Environmental Entomology 25:359-365.
Stamp, N. E. 2001. Effects of prey quantity and quality on predatory wasps. Ecological Entomology 26:292-301.
Stamp, N. E. and M. D. Bowers. 1991. Indirect effect on survivorship of caterpillars due to presence of invertebrate predators. Oecologia 88:325-330.
Stamp, N. E. and M. D. Bowers. 1992. Foraging Behavior of Specialist and Generalist Caterpillars on Plantain (Plantago-Lanceolata) Altered by Predatory Stinkbugs. Oecologia 92:596-602.
Stamp, N. E. and M. D. Bowers. 1993. Presence of predatory wasps and stinkbugs alters foraging behavior of cryptic and non-cryptic caterpillars on Plantain (*Plantago lanceolata*). Oecologia 95:376-384.
Stamp, N. E. and B. Meyerhoefer. 2004. Effects of prey quality on social wasps when given a choice of prey. Entomologia Experimentalis Et Applicata 110:45-51.
Stein, S. J. and P. W. Price. 1995. Relative effects of plant-resistance and natural enemies by plant developmental age on sawfly (Hymenoptera, Tenthredinidae) preference and performance. Environmental Entomology 24:909-916.
Stout, M. J., W. C. Rice, and D. R. Ring. 2002. The influence of plant age on tolerance of rice to injury by the rice water weevil, *Lissorhoptrus oryzophilus* (Coleoptera : Curculionidae). Bulletin of Entomological Research 92:177-184.

Stowe, K. A., R. J. Marquis, C. G. Hochwender, and E. L. Simms. 2000. The evolutionary ecology of tolerance to consumer damage. Annual Review Of Ecology And Systematics 31:565-595.
Strauss, S. Y. and A. A. Agrawal. 1999. The ecology and evolution of plant tolerance to herbivory. Trends In Ecology & Evolution 14:179-185.
Strohmeyer, H. H., N. E. Stamp, C. M. Jarzomski, and M. D. Bowers. 1998. Prey species and prey diet affect growth of invertebrate predators. Ecological Entomology 23:68-79.
Sutter, R. and C. Muller. 2011. Mining for treatment-specific and general changes in target compounds and metabolic fingerprints in response to herbivory and phytohormones in *Plantago lanceolata*. New Phytologist 191:1069-1082.
Swihart, R. K. and J. P. Bryant. 2001. Importance of biogeography and ontogeny of woody plants in winter herbivory by mammals. Journal Of Mammalogy 82:1-21.
Sylvester, A. W., V. Parker-Clark, and G. A. Murray. 2001. Leaf shape and anatomy as indicators of phase change in the grasses: comparison of maize, rice, and bluegrass. American Journal Of Botany 88:2157-2167.
Tabashnik, B. E. and F. Slansky, Jr. 1987. Nutritional ecology of forb foliage-chewing insects. Page 1016 *in* F. Slansky, Jr. and J. G. Rodriguez, editors. Nutritional ecology of insects, mites, spiders, and related invertebrates. Wiley-Interscience.
Tamura, Y. 2001. Effects of temperature, shade, and nitrogen application on the growth and accumulation of bioactive compounds in cultivars of *Plantago lanceolata* L. Japanese Journal of Crop Science 70:548-553.
Team, R. D. C. 2008. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
Teder, T. and T. Tammaru. 2002. Cascading effects of variation in plant vigour on the relative performance of insect herbivores and their parasitoids. Ecological Entomology 27:94-104.
Thaler, J. S., A. L. Fidantsef, and R. M. Bostock. 2002. Antagonism between jasmonate- and salicylate-mediated induced plant resistance: Effects of concentration and timing of elicitation on defense-related proteins, herbivore, and pathogen performance in tomato. Journal Of Chemical Ecology 28:1131-1159.
Theodoratus, D. H. and M. D. Bowers. 1999. Effects of sequestered iridoid glycosides on prey choice of the prairie wolf spider, *Lycosa carolinensis*. Journal Of Chemical Ecology 25:283-295.
Thies, C., I. Steffan-Dewenter, and T. Tscharntke. 2003. Effects of landscape context on herbivory and parasitism at different spatial scales. Oikos 101:18-25.
Thies, C. and T. Tscharntke. 1999. Landscape structure and biological control in agroecosystems. Science 285:893-895.
Thomas, S. C., A. J. Sztaba, and S. M. Smith. 2010. Herbivory patterns in mature sugar maple: variation with vertical canopy strata and tree ontogeny. Ecological Entomology 35:1-8.
Thompson, J. N. 2005. The geographic mosaic of coevolution. The University of Chicago Press. Ltd., London.
Tiffin, P. 2000. Mechanisms of tolerance to herbivore damage: what do we know? Evolutionary Ecology 14:523-536.
Trager, M. D. and E. M. Bruna. 2006. Effects of plant age, experimental nutrient addition and ant occupancy on herbivory in a neotropical myrmecophyte. Journal Of Ecology 94:1156-1163.
Travis, J. 1996. The significance of geographical variation in species interactions. American Naturalist 148:S1-S8.
Tucker, C. and G. Avila-Sakar. 2010. Ontogenetic changes in tolerance to herbivory in *Arabidopsis*. Oecologia 164:1005-1015.

Turlings, T. C. J. and B. Benrey. 1998. Effects of plant metabolites on the behavior and development of parasitic wasps. Ecoscience 5:321-333.
Unsicker, S. B., G. A. Boeckler, and J. Gershenzon. 2011. Phenolic glycosides of the Salicaceae and their role as anti-herbivore defenses. Phytochemistry 72:1497-1509.
Van Bael, S. A., J. D. Brawn, and S. K. Robinson. 2003. Birds defend trees from herbivores in a Neotropical forest canopy. Proceedings Of The National Academy Of Sciences Of The United States Of America 100:8304-8307.
Van Dam, N. M., M. Horn, M. Mares, and I. T. Baldwin. 2001. Ontogeny constrains systemic protease inhibitor response in *Nicotiana attenuata*. Journal Of Chemical Ecology 27:547-568.
Van Zandt, P. A. and A. A. Agrawal. 2004. Community-wide impacts of herbivore-induced plant responses in milkweed (*Asclepias syriaca*). Ecology 85:2616-2629.
Verhage, A., S. C. M. van Wees, and C. M. J. Pieterse. 2010. Plant immunity: It's the hormones talking, but what do they say? Plant Physiology 154:536-540.
Viswanathan, D. V., O. A. Lifchits, and J. S. Thaler. 2007. Consequences of sequential attack for resistance to herbivores when plants have specific induced responses. Oikos 116:1389-1399.
Waldbauer, G. P. 1968. The consumption and utilization of food by insects. Advances in Insect Physiology 5:229-289.
Wallace, S. K. and S. D. Eigenbrode. 2002. Changes in the glucosinolate-myrosinase defense system in *Brassica juncea* cotyledons during seedling development. Journal Of Chemical Ecology 28:243-256.
Waltz, A. M. and T. G. Whitham. 1997. Plant development affects arthropod communities: opposing impacts of species removal. Ecology 78:2133-2144.
Warner, P. J. and J. H. Cushman. 2002. Influence of herbivores on a perennial plant: variation with life history stage and herbivore species. Oecologia 132:77-85.
Webber, B. L., B. A. Abaloz, and I. E. Woodrow. 2007. Myrmecophilic food body production in the understorey tree, Ryparosa kurrangii (Achariaceae), a rare Australian rainforest taxon. New Phytologist 173:250-263.
Weltzin, J. E., S. R. Archer, and R. K. Heitschmidt. 1998. Defoliation and woody plant (*Prosopis glandulosa*) seedling regeneration: potential vs realized herbivory tolerance. Plant Ecology 138:127-135.
Werner, E. E. and S. D. Peacor. 2003. A review of trait-mediated indirect interactions in ecological communities. Ecology 84:1083-1100.
West, G. B., J. H. Brown, and B. J. Enquist. 2001. A general model for ontogenetic growth. Nature 413:628-631.
Wiedemuth, K., J. Muller, A. Kahlau, S. Amme, H. P. Mock, A. Grzam, R. Hell, K. Egle, H. Beschow, and K. Humbeck. 2005. Successive maturation and senescence of individual leaves during barley whole plant ontogeny reveals temporal and spatial regulation of photosynthetic function in conjunction with C and N metabolism. Journal of Plant Physiology 162:1226-1236.
Williams, R. D. and B. E. Ellis. 1989. Age and tissue distribution of alkaloids in *Papaver somniferum*. Phytochemistry 28:2085-2088.
Willinger, G. and S. Dobler. 2001. Selective sequestration of iridoid glycosides from their host plants in *Longitarsus* flea beetles. Biochemical Systematics And Ecology 29:335-346.
Wurst, S. and W. H. van der Putten. 2007. Root herbivore identity matters in plant-mediated interactions between root and shoot herbivores. Basic and Applied Ecology 8:491-499.
Young, T. P., C. H. Stubblefield, and L. A. Isbell. 1997. Ants on swollen thorn acacias: Species coexistence in a simple system. Oecologia 109:98-107.
Zalucki, M. P., A. R. Clarke, and S. B. Malcolm. 2002. Ecology and behavior of first instar larval Lepidoptera. Annual Review Of Entomology 47:361-393.

Zhu, J. W. and K. C. Park. 2005. Methyl salicylate, a soybean aphid-induced plant volatile attractive to the predator Coccinella septempunctata. Journal of Chemical Ecology 31:1733-1746.
Zotz, G., K. Wilhelm, and A. Becker. 2011. Heteroblasty-a review. Botanical Review 77:109-151.