American Journal of Botany 94(1): 56–66. 2007.

EARLY ONTOGENETIC PATTERNS IN CHEMICAL DEFENSE IN *PLANTAGO* (PLANTAGINACEAE): GENETIC VARIATION AND TRADE-OFFS[1]

KASEY E. BARTON[2]

Ecology and Evolutionary Biology, University of Colorado, Boulder, Colorado 80309 USA

Predictions based on the plant age and growth–differentiation balance hypotheses of defense were tested in two congeneric species, *Plantago lanceolata* and *P. major*, by quantifying iridoid glycosides, defensive chemicals, in seeds and leaves during the first 6 wk of growth. Concentrations decreased from the seed to 2-wk-old seedling stage in *P. lanceolata*, but increased during this period in *P. major*. In both species, levels were similar for 2- and 4-wk-old plants, then significantly increased from 4 to 6 wk. Genetic variation in the ontogeny of iridoid glycoside production was significant in both species at the maternal family level and at the population level. To examine whether allocation costs could explain the low production of iridoid glycosides in seedlings, relationships between growth and defense (iridoid glycosides) were characterized. Growth and defense had a positive or null relationship in all age groups, indicating that there was no trade-off in these plants at any age. This study provides some support for the growth–differentiation balance hypothesis, but offers no support for the plant age hypothesis. Measuring how herbivory affects plant fitness at different ontogenetic stages may shed light on these patterns in *Plantago* and on the evolution of the ontogeny of defense.

Key words: allocation constraints; growth defense trade-offs; iridoid glycosides; ontogenetic drift; plant age; Plantaginaceae; secondary chemistry; seedling.

Extensive experimental and observational evidence indicates that plant secondary chemistry is highly variable in most plant species and that this variation is a key aspect mediating interactions between plants and their natural enemies, herbivores and pathogens (Romeo et al., 1996). Factors contributing to this variation include, but are not limited to, genetics, resource availability, competition, and previous damage. Examinations of patterns in plant secondary chemistry have led to the development of numerous hypotheses of plant defense, notably the optimal defense, resource availability, carbon nutrient balance, growth rate, and growth-differentiation balance hypotheses (reviewed in Stamp, 2003). Despite this considerable interest in variation in secondary chemistry, relatively little is known about the roles of plant age and ontogeny, potentially important sources of variation.

Certain of these hypotheses predict that ontogeny should influence secondary chemistry and, specifically, that seedlings may have different levels of secondary compounds than older plants (juveniles and adults). An aspect of the optimal defense hypothesis that focuses specifically on ontogeny, the plant age hypothesis (Bryant et al., 1992; sensu Spiegel and Price, 1996), and the growth–differentiation balance hypothesis (Herms and Mattson, 1992) are especially interesting because both use adaptive explanations to predict patterns of variation in secondary chemistry for seedlings vs. adult plants, but the predictions are in opposite directions. The plant age hypothesis predicts a decrease in plant secondary chemistry with ontogeny, while the growth–differentiation balance hypothesis predicts an increase. Evidence exists to support both of these hypotheses. Some studies have documented ontogenetic decreases in plant defensive chemistry (e.g., Macedo and Langenheim, 1989; Erwin et al., 2001; Schaffner et al., 2003), while other studies have shown ontogenetic increases in defensive chemistry (e.g., Cipollini and Redman, 1999; Fritz et al., 2001; Russell and Southwell, 2003).

Because the plant age and growth–differentiation balance hypotheses consider the role of natural selection in structuring the ontogeny of defense, they both rely on the assumption that there is a genetic component to the ontogeny of defense production. Yet, despite extensive evidence that levels of defensive compounds vary genetically (reviewed in Kennedy and Barbour, 1992), few studies have explicitly documented genetic variation in ontogenetic patterns of defensive chemistry (but see Adler et al., 1995; Schappert and Shore, 2000). Therefore, the primary goals of this study were to compare the secondary chemistry of seedlings with that of older plants and to test for genetic variation in these ontogenetic patterns in two common species of *Plantago* (Plantaginaceae), *P. lanceolata* L. and *P. major* L. A secondary goal was to test the prediction of the growth–differentiation balance hypothesis that trade-offs between growth and defense drive ontogenetic patterns in defensive chemistry. Because the plant age hypothesis does not directly consider trade-offs, this study has the potential to expand our current view of the ontogeny of plant defense.

Observations from natural populations suggest that ontogenetic patterns in plant secondary chemistry are likely to have important consequences for plant fitness. Seedlings can experience high rates of damage and mortality (Clark and Clark, 1984; Klink, 1996; Clarke and Davison, 2004) due to a variety of biotic and abiotic factors, including competition, pathogens, herbivores, desiccation, and trampling (Harper,

[1] Manuscript received 14 February 2006; revision accepted 6 November 2006.

The author thanks M. D. Bowers, Y. Linhart, G. Carey, and D. Liptzin for valuable discussions on the experimental design and statistical analyses; K. Keefover-Ring and M. D. Bowers for advice with chemical analyses; J. Blake and E. Hughes for assistance with laboratory procedures; M. D. Bowers, Y. Linhart, B. Bowman, J. Maurer, C. Quintero, M. Jamieson, B. Breland, and two anonymous reviewers for insightful comments that improved the manuscript. Financial support for this research was provided by the Department of Ecology and Evolutionary Biology at the University of Colorado, Boulder.

[2] E-mail: bartonke@colorado.edu

1977). A recent review suggested that herbivory and disease are the most common mechanisms reducing seedling performance (Moles and Westoby, 2004) and often lead to seedling mortality (Augspurger, 1983; Maron, 1997; Shaw et al., 2002). Enemy-mediated seedling mortality patterns can affect plant population dynamics (Watkinson, 1997) and may influence higher organizational levels, such as communities (Hanley et al., 1995; Hulme, 1996) and ecosystems (Bishop, 2002; Sousa et al., 2003). Yet, despite the substantial effects disease and herbivory can have on plant fitness via seedling damage and mortality, there is relatively little known about seedling defense and how it may differ from adult plant defense (Boege and Marquis, 2005).

The plant age hypothesis extends predictions about optimal patterns in plant defense to ontogeny and predicts that if natural enemies reduce plant fitness more at the seedling stage than at the adult stage, defense allocation should be relatively high in young plants and decrease as plants mature and become less vulnerable to attacks (Bryant et al., 1992). However, seedlings may fail to produce defensive secondary chemicals or produce low amounts if growth demands for carbon and other nutrients constrain resource allocation to secondary chemistry, as predicted by the growth–differentiation balance hypothesis (Herms and Mattson, 1992). In many species, seedlings experience intense competition during recruitment, both with other seedlings (Gilbert et al., 2001) and with neighboring herbs and grasses (Van der Toorn and Pons, 1988; Maron, 1997). Allocation to defense may lower potential growth and thus compromise a seedling's competitive ability, potentially making it more adaptive for seedlings to allocate to growth at the expense of defense (Kurokawa et al., 2004; Kelly and Hanley, 2005). Moreover, seedlings are often carbon-limited due to relatively small photosynthetic surfaces, and so production of new aboveground tissues is likely to be a higher functional priority than the production of chemical defenses (Bryant et al., 1992; Boege and Marquis, 2005). Thus, although competition and resource allocation are undoubtedly also important for adult plants (Grace and Tilman, 1990), the growth–differentiation balance hypothesis explicitly predicts that allocation constraints for defense are greater in seedlings than in older plants (Herms and Mattson, 1992) and that this leads to an ontogenetic increase in defensive chemistry.

To investigate the predictions of the plant age and growth–differentiation balance hypotheses, a greenhouse study was conducted on two widespread and commonly co-occurring congeners, *P. lanceolata* and *P. major*. To examine genetic variation in the ontogeny of defense, secondary compounds were quantified at two levels of genetic structure, maternal families and populations, during the first 6 wk of growth. Within each species, seedlings from multiple maternal families ($N = 3$–4) within each of several populations were assayed. Relationships between plant growth rate and secondary chemistry production rate were characterized to test whether allocation constraints explained ontogenetic patterns of defense. Specifically, the following questions were addressed: (1) What are the concentrations of iridoid glycosides in the seeds and during the first 6 wk of growth in *P. lanceolata* and *P. major*? The hypotheses make opposite predictions for this question. The plant age hypothesis predicts that iridoid glycoside concentrations decrease during the first 6 wk. The growth–differentiation balance hypothesis predicts that iridoid glycoside concentrations increase during the first 6 wk. (2) Is there genetic variation in the ontogeny of iridoid glycoside production? Both hypotheses predict that there is genetic variation in the ontogeny of secondary chemistry. (3) Is there a trade-off between growth and defense? The plant age hypothesis does not address this question. The growth–differentiation balance hypothesis predicts that allocation constraints result in a growth–defense trade-off. (4) Do trade-offs between growth and defense change as plants age? The plant age hypothesis does not address this question. The growth–differentiation balance hypothesis predicts that trade-offs are stronger in young seedlings than in older plants.

MATERIALS AND METHODS

Study system—*Plantago lanceolata* and *P. major* are short-lived perennial herbs native to Eurasia, and both species now have naturalized weed populations all over the world (Van der Aart and Vulto, 1992). These species were selected for several reasons. First, their widespread distribution and the fact that they often co-occur in mixed populations (Haeck, 1992; K. Barton, personal observation) make them ecologically relevant and a meaningful species comparison. Second, *Plantago* is a model system for studying evolutionary ecology and has been a focal group for investigating the role secondary chemistry plays in mediating plant–herbivore interactions (e.g., Bowers and Stamp, 1997; Theodoratus and Bowers, 1999; Harvey et al., 2005), providing a well-developed context for the present study. Third, observations from field studies suggest that seedling interactions are common and important in *P. lanceolata* and *P. major*. Seedling mortality rates can be very high (98–100% in *P. major* and 36–99% in *P. lanceolata*; Blom, 1992), and herbivores have been observed to contribute to this mortality, at least in *P. lanceolata* (Hanley et al., 1996). Competition is also common for *P. lanceolata* and *P. major* seedlings and may influence establishment success (Van der Toorn and Pons, 1988). These interactions are likely to lead to contrasting selection pressures for high levels of secondary compounds in seedlings and make these ideal species for testing the plant age and growth–differentiation balance hypotheses.

Despite interest in the ecology of *Plantago* seedlings, no reports have been published on the early ontogeny of secondary chemistry. *Plantago lanceolata* and *P. major* produce iridoid glycosides, secondary compounds that impact both generalist and specialist herbivores (reviewed in Bowers, 1991), as well as fungal pathogens (Marak et al., 2002). The two most abundant iridoid glycosides in *P. lanceolata* are aucubin and catalpol, which occur in quantities as high as 10–12% dry mass (Bowers and Stamp, 1993). *Plantago major* does not produce catalpol (Rønsted et al., 2000) and produces aucubin in relatively low amounts (see Results, subsection *P. major iridoid glycosides*). In *P. lanceolata*, iridoid glycoside production is heritable (Marak et al., 2000), and significant genetic variation occurs among adult plants (Bowers et al., 1992; Bowers and Stamp, 1992, 1993; Adler et al., 1995). Concentrations of iridoid glycosides in *P. lanceolata* increase as plants develop from 6 wk through maturity (Bowers et al., 1992; Stamp and Bowers, 1994; Jarzomski et al., 2000; Fuchs and Bowers, 2004). Levels of iridoid glycosides in seedlings have not been previously investigated.

Due to differences in the breeding systems, siblings within maternal families likely differed in relatedness between the two species. *Plantago lanceolata* is self-incompatible (Van Damme, 1992), so seedlings within maternal families are almost certainly half-sibs, although receiving multiple pollen from a single source could lead to the presence of full-sibs. In contrast, *P. major* is self-compatible and in its native range has quite low documented rates of outcrossing (0–0.08) (Van Damme, 1992). Thus, in *P. major*, maternal families are likely to include mostly, if not exclusively, full-sibs. Considering these differences in breeding systems, I predicted that differences among maternal families would be easier to detect in *P. major* than in *P. lanceolata* because full-sib families are likely to have less within-family variation than are half-sib families (Hartl, 2000).

Sample summary—Seeds were collected from several maternal plants and stored separately. Genetic family thus refers to plants from a single maternal sibship. In *P. major*, four genetic families were used from each of two populations from Boulder County, Colorado, USA: "Creek" is a creekside population from a protected prairie at Chautauqua Park; "Lawn" is a population of lawn weeds on the University of Colorado campus. There were

approximately 10 replicate plants in each family × age group for a total sample size of 274 plants.

In *P. lanceolata*, seeds from 3–4 genetic families in each of three populations were used: "France" is a native population of weedy plants located within an experimental research preserve in Montepellier, France; "Road" is a roadside weed population near residential ranchland in Boulder County, Colorado, USA; "Prairie" is a population within the State Natural Tallgrass Prairie Area at South Boulder Creek in Boulder County, Colorado, USA. There were approximately 10 replicate plants in each family × age group for a total sample size of 298 plants.

Seed study—To test whether seedlings might be provisioned with iridoid glycosides from the seeds, I analyzed the secondary chemistry of seeds. To have samples large enough to detect trace amounts of iridoid glycosides, each sample was a composite of seeds from multiple maternal plants (mean number in *P. major*, 740 seeds; in *P. lanceolata*, 87 seeds). The difference in the mean number of seeds analyzed reflects the mean difference in seed size between these two species (see Results, subsection *Seeds*). Due to the high number of seeds required for each sample, there was not sufficient replication to test for genetic variation in seed secondary chemistry. Ten composite samples were analyzed for each species.

Ontogeny study—Greenhouse studies were conducted in spring 2003 (*P. major*) and spring 2004 (*P. lanceolata*). Seeds were sown in flats filled with Fafard (Conrad Fafard, Agawam, Massachusetts, USA) nursery mix and germinated in the greenhouse the first week of March. As seeds germinated, they were marked and transplanted 3 days later into 1-L pots filled with a medium composed of equal parts sterilized sand, Metro Mix 350 (50–60% vermiculite, 25–40% peat moss, 9–19% bark ash, Scotts-Sierra Horticultural Products, Marysville, Ohio, USA), and Turface MVP (Turface Athletics, Buffalo Grove, Illinois, USA). Seedlings were randomly assigned to one of three age groups, to be harvested at 2, 4, or 6 wk after transplantation. These age groups were selected because they include an obvious seedling stage (2 wk) as well as two stages during the juvenile to adult transition (4 and 6 wk). This range allowed me to look at how secondary chemistry changes during early ontogeny, and in particular, how seedling secondary chemistry differs from the secondary chemistry of older plants. Seeds germinated over a 2-wk period, which resulted in continuous harvest dates between 29 March–11 May in 2003 and 5 April–25 May in 2004. Replicates were randomly placed on one of three greenhouse benches within the same greenhouse room (2003) or on a single greenhouse bench (2004). Pots were routinely moved within a bench and the placement of replicates re-randomized. Plants were watered daily but were not fertilized at any time during the study.

At harvest, plants were separated into leaves and roots. Plants were oven-dried at 50°C to a constant mass and weighed to the nearest 0.01 g. Leaves were analyzed for iridoid glycosides. All leaves for each plant were ground into a fine powder, and 5–50 mg subsamples (entire aboveground tissue for many 2-wk-old seedlings) were processed for chemical analysis by gas chromatography using previously described methods (Gardner and Stermitz, 1988; Bowers and Stamp, 1993) although extraction methods were adjusted to detect low amounts (see Results). Briefly, samples were extracted in methanol and then partitioned between water and ether to remove hydrophobic compounds and chlorophyll. An internal standard (phenyl-β-D-glucose [PBG]) was added, and an aliquot was derivatized with Tri-Sil-Z (Pierce Chemical, Rockford, Illinois, USA). Derivatized samples were injected into a Hewlett-Packard 5890A GC (Agilent Technologies, Santa Clara, California, USA), and chromatograms were integrated with HP 3365 Series II ChemStation (v. A.03.34 Hewlett-Packard).

Statistical analyses—Data were analyzed using SAS for Windows V9 mixed (proc mixed), regression (proc reg), and means (proc means) procedures (SAS, 2003). In all analyses, genetic family was nested within population. Aucubin and catalpol data were analyzed as proportions of dry mass (concentration) and were arcsine transformed to meet assumptions of normality. Seed mass was analyzed with a mixed-model three-way factorial ANOVA with species (fixed), population (fixed), and genetic family (random) as the main effects. Seed iridoid glycoside concentrations were compared between the two species with a *t* test. For all other traits, data for *P. major* and *P. lanceolata* were analyzed separately. Total plant biomass (shoot + root), aucubin and catalpol concentrations in leaves were each analyzed with a mixed-model three-way factorial ANCOVA. The full model included a block effect (2003 only), germination time (days to germination) as a covariate, and three main effects: age (fixed), population (fixed), and genetic family (random) plus the two-way interactions population × age and family × age. No three-way interactions were significant during preliminary analyses, and they were pooled into the error term. The significance of the random effects (and interactions with random effects) were tested by running models with and without the random effect of interest and calculating the likelihood-ratio statistics, which can be compared to a chi-square distribution (Littell et al., 1996). Thus, results from the ANCOVAs are reported as *F* values for fixed factors and chi-square values for random factors.

To test for trade-offs between growth and defense, the relationship between growth rate and iridoid glycoside (IG) production rate was investigated in each species. Rates were calculated for each plant for the 2 wk preceding harvest. For 2-wk-old plants, growth rate was calculated by dividing the 2-wk total biomass (roots and shoots) by 14 d, and IG rate was calculated by dividing iridoid glycoside content by 14 d. For 4- and 6-wk-old plants, I used the family-level means from the previous harvest to estimate initial plant size for the 2-wk period used to calculate rates. For example, to calculate growth rate for a 4-wk-old plant from the genetic family L2, the difference between the 4-wk-old plant's total biomass and the L2 family mean for total biomass at 2-wk was divided by 14. IG production rates were calculated by dividing the difference between the 4-wk iridoid glycoside content and the 2-wk family mean for iridoid glycoside content by 14 d. A similar set of calculations produced growth and IG production rates for 6-wk-old plants, but in these, 4-wk family means were used. Although this technique uses means as estimates of "initial" biomass and iridoid glycoside content, because calculations were made only within genetic families, it is reasonable to assume that the actual initial plant size and iridoid glycoside content were closely approximated. Furthermore, the strength of this technique is that it allows allocation investments to growth and defense to be related at each age class.

The relationship between growth rate and IG production rate was analyzed in several ways. First, a regression analysis on all data for each species was conducted to test for an overall relationship between growth and IG production rates; a negative relationship would indicate a trade-off (Koricheva, 1999). Second, to determine whether the relationship between growth rate and IG production rate varied among age groups, genetic families, or populations, I used mixed-model ANCOVA models with IG production rate as the dependent variable, and the main effects of age, genetic family, and population. Growth rate was used as a covariate, and significant interactions between growth rate and the main effects were interpreted as variation among groups (age, families, populations). For example, if the interaction between growth rate and age was significant, I would conclude that the relationship between growth and IG production rates varied among age groups. If the interaction between growth rate and genetic family was significant, I would conclude that the relationship between growth and IG production rates varied among genetic families. Finally, in the case that the growth–defense relationship differed significantly among age groups, separate regression analyses were run for each age group in order to understand the nature of these differences.

RESULTS

Seeds—Seed mass was an order of magnitude higher in *P. lanceolata* (mean ± 1 SE, 1.625 ± 0.033 mg) than in *P. major* (mean ± 1 SE, 0.175 ± 0.004 mg; $F_{1,10} = 436.24$, $P < 0.0001$). Within species, seed mass varied significantly among genetic families ($\chi^2 = 46.9$, $P < 0.0001$) but not among populations ($F_{1,10} = 0.46$, $P = 0.5121$). Iridoid glycosides were detected in the seeds of both species, but concentrations were significantly higher in *P. lanceolata* than in *P. major* (Fig. 1B, D; $t_{18} = 10.95$, $P < 0.0001$). In *P. lanceolata*, aucubin concentrations were nearly twice as high as catalpol concentrations (Fig. 1E).

Plantago major—*Iridoid glycosides*—In general, concentrations of aucubin increased significantly with age, although this pattern varied among genetic families (Table 1, Fig. 1A). Aucubin concentrations were higher in plants from the Creek population than in plants from the Lawn population, although this difference was only marginally statistically significant ($P = 0.0578$) and was consistent among age groups (Table 1, Fig.

1B). Concentrations of aucubin were significantly influenced by greenhouse bench ($F_{2,262} = 8.11$, $P = 0.0004$).

To determine if differences in ontogenetic patterns of iridoid glycosides among genetic families were due to differences in plant size, an additional analysis was conducted that included total biomass as a covariate. Inclusion of biomass as a covariate did not reduce the significance of the family × age effect on aucubin (with biomass: $\chi^2 = 6.1$, $P = 0.0068$; without biomass: $\chi^2 = 3.1$, $P = 0.03915$), suggesting that genetic variation in the ontogenetic patterns of iridoid glycoside production was not driven merely by differences among genetic families in plant size.

Biomass—Plant biomass increased with age, although the ontogeny of plant size varied among genetic families (Table 1, Fig. 2A). Mean biomass did not differ between plants from the two populations (Table 1, Fig. 2B). Germination time contributed significantly to variation in total plant biomass (Table 1), such that plants that germinated later were larger than plants that germinated early. Greenhouse bench contributed to variation in total biomass ($F_{2,255} = 3.25$, $P = 0.0402$).

Growth–defense trade-offs—Overall, there was a highly significant positive relationship between growth rate and IG production rate ($R^2 = 0.809$, $P < 0.0001$), indicating that there was not a trade-off between growth and defense. The growth–defense relationship varied genetically at both the family ($\chi^2 = 59.8$, $P < 0.0001$) and population ($F_{1,6.19} = 6.86$, $P = 0.0385$) levels, although in all cases, the relationship was nonetheless positive. Furthermore, although the growth–defense relationship significantly varied among age groups ($F_{2,258} = 3.30$, $P = 0.0383$), the patterns were again consistently positive (Fig. 3).

Plantago lanceolata—*Iridoid glycosides*—Concentration of total iridoid glycosides increased as plants aged, and genetic families differed in their ontogenetic patterns of increase (Table 1, Fig. 1C). Populations differed significantly in iridoid glycoside concentrations, although differences depended on age (Table 1). Concentrations of iridoid glycosides were similar for 2- and 4-wk-old seedlings from all three populations, but by 6 wk of age, iridoid glycoside concentrations were significantly higher in plants from the Prairie population than in plants from either the Road or France population (Fig. 1D). Separate analyses on aucubin and catalpol yielded some differences, although both were influenced mostly by age (Table 1, Fig. 1D). Ontogenetic changes in aucubin concentration were similar among genetic families, but differed significantly among populations (Table 1). Aucubin concentrations were similar in 2- and 4-wk-old plants from all three populations, but by 6 wk, levels were lowest in plants from France and highest in Road plants (Fig. 1D). In contrast, ontogenetic patterns in catalpol concentration did not differ among populations, but did vary significantly among genetic families (Table 1). The proportion of catalpol making up the total iridoid glycosides changed with age, and this pattern varied both among populations and among genetic families within those populations (Table 1, Fig. 1E). Additionally, days to germination influenced concentrations of aucubin, but not catalpol (Table 1). Plants that germinated later had more aucubin than plants that germinated early.

To test whether biomass influenced the result that genetic families varied in ontogenetic patterns of chemistry, additional analyses were conducted that included total biomass as a covariate. Inclusion of biomass as a covariate did not reduce the significance of the family × age effects for concentrations of total iridoid glycosides (with biomass: $\chi^2 = 10.0$, $P = 0.0008$; without biomass: $\chi^2 = 5.3$, $P = 0.0107$), catalpol (with biomass: $\chi^2 = 34.5$, $P < 0.0001$; without biomass: $\chi^2 = 31.7$, $P < 0.0001$), or proportion catalpol (with biomass: $\chi^2 = 17.7$, $P < 0.0001$; without biomass: $\chi^2 = 18.1$, $P < 0.0001$). Aucubin was not tested because families did not differ in their ontogenetic patterns of increase.

Biomass—Plants increased in total biomass with age, and the pattern of increase was similar among genetic families (Table 1, Fig. 2C). Differences among populations in mean plant biomass did not become apparent until the age of 6 wk, at which time, plants from France were the smallest, and plants from the Prairie were the largest (Table 1, significant pop × age interaction; Fig. 2D). Plants that germinated later were significantly larger than plants that germinated early (Table 1).

Growth–defense trade-offs—As with *P. major*, there was no evidence for a trade-off between growth and defense in *P. lanceolata*, as demonstrated by the highly significant positive relationship between growth rate and iridoid glycoside production rate ($R^2 = 0.804$, $P < 0.0001$). The relationship was consistently positive, but did vary in slope among genetic families ($\chi^2 = 20.4$, $P < 0.0001$) and among populations ($F_{2,9.13} = 4.27$, $P = 0.0490$). Furthermore, the growth–defense relationship differed significantly among age groups ($F_{2,282} = 29.50$, $P < 0.0001$); positive relationships were detected for both 4- and 6-wk-old plants, but there was no growth–defense relationship for 2-wk-old plants (Fig. 3).

DISCUSSION

In this study, clear ontogenetic patterns were found in plant defensive chemistry that could not be explained by growth–defense trade-offs. Furthermore, in contrast to my prediction based on differences in the breeding systems, genetic variation in the traits studied was detected in both *P. major* and *P. lanceolata*. Most importantly, this study reveals genetic variation in the ontogeny of defense production, supporting a key assumption of hypotheses about the evolution of ontogenetic patterns of defense. Revisiting my initial questions, I found that (1) levels of iridoid glycosides increased during the first 6 wk of growth, although these patterns differed between the two species. This finding supports the predictions of the growth–differentiation balance hypothesis. (2) As predicted by both the plant age and growth–differentiation balance hypotheses, genetic variation in the ontogeny of iridoid glycoside production was detected in both species. (3) Refuting the growth–differentiation balance hypothesis, there was no evidence for a trade-off between growth and defense. (4) The relationship between growth and defense varied among age groups, but when present, it was always positive, further refuting the prediction of the growth–differentiation balance hypothesis that growth constrains defense more at the young seedling stage than at older stages.

Concentrations of iridoid glycosides increased with age in both species of *Plantago*, a pattern inconsistent with the prediction of the plant age hypothesis (Bryant et al., 1992). This increase was especially marked between 4 and 6 wk of age. These results would not have been predicted from a

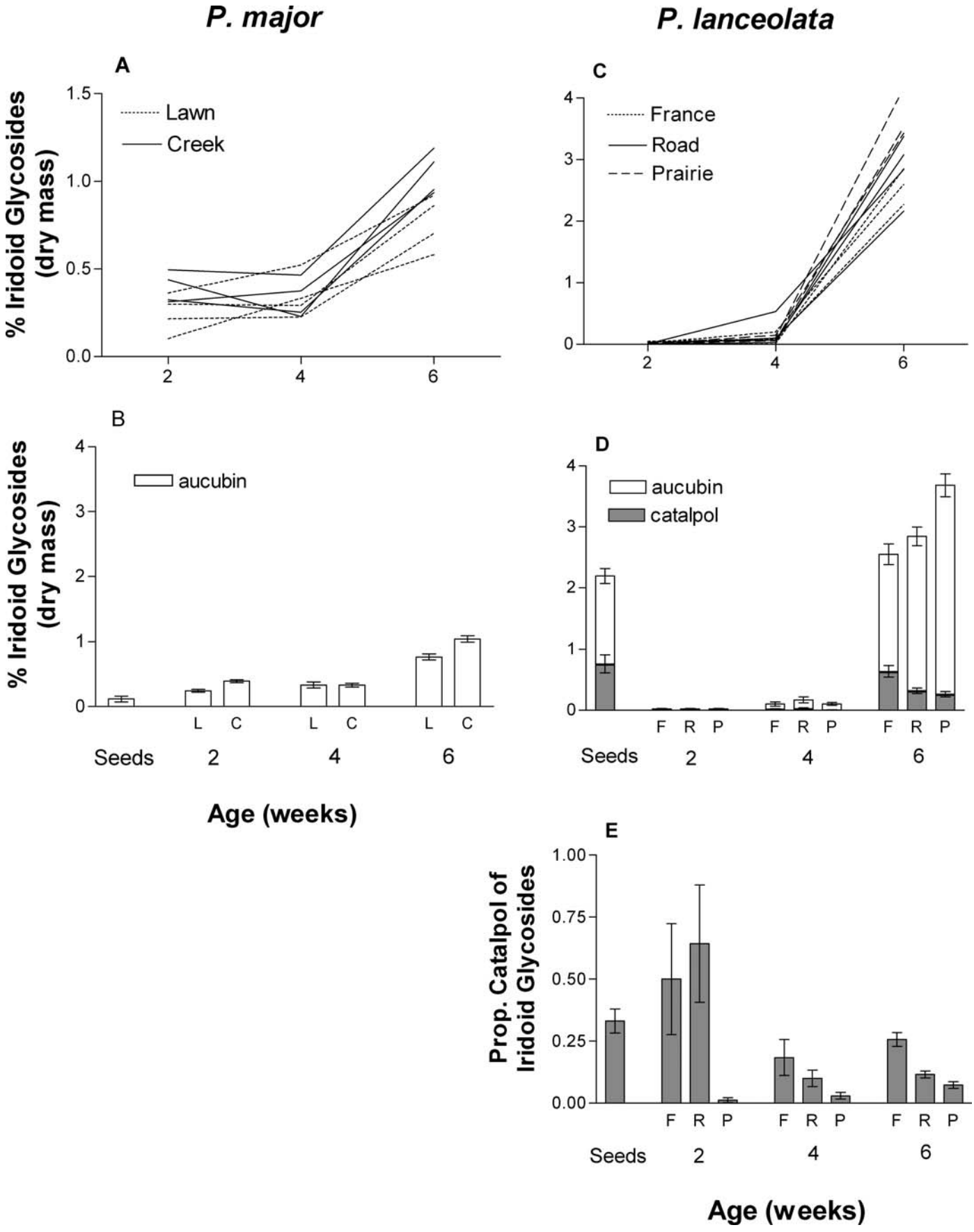

P. major
P. lanceolata
A
Lawn
Creek
% Iridoid Glycosides (dry mass)
C
France
Road
Prairie
B
aucubin
D
aucubin
catalpol
L C
F R P
Seeds
2
4
6
Age (weeks)
E
Prop. Catalpol of Iridoid Glycosides

TABLE 1. Summary of ANCOVAs for each of the following *Plantago major* and *P. lanceolata* plant traits. *F* values are reported for germination time, age, population, and the population by age (pop × age) interaction. Chi-square values are reported for the genetic family by age (fam × age) interaction because it is a random effect. Significance is displayed as $P < 0.0001$ (***), $P < 0.01$ (**), $P < 0.05$ (*).

	Sources of variation				
	Covariate				
	Days to germination	Age	Population	Fam × Age	Pop × Age
P. major					
Aucubin	0.78	122.24 ***	5.58	3.1 *	2.49
Total biomass	22.98 ***	284.23 ***	1.74	20.3 ***	2.47
P. lanceolata					
Total iridoid glycosides	22.49 ***	597.69 ***	8.51 **	5.3 *	4.80 **
Aucubin	26.20 ***	759.71 ***	19.63 ***	2.4	11.29 ***
Catalpol	2.51	40.01 ***	1.94	31.7 ***	2.33
Proportion catalpol	0.20	39.19 ***	3.51 *	18.1 ***	2.93 *
Total biomass	46.81 ***	1338.86 ***	19.21 ***	0.0	3.90 **

previous study that found that a generalist slug preferred adult plants (6- to 10-wk-old) over seedlings (1- to 3-wk-old) in both *P. lanceolata* and *P. major* (Fenner et al., 1999). However, because Fenner et al. (1999) used ground plant tissue incorporated into artificial diet, differences in preference could reflect other differences in plant quality. Nonetheless, the ontogenetic increases in iridoid glycosides found in this study would be expected to result in a decrease in palatability with plant age, especially for a generalist herbivore.

Genetic variation in the ontogeny of iridoid glycoside production was detected in both species. In *P. major*, genetic families varied significantly in the ontogeny of aucubin production; in some families, mean levels of aucubin were actually higher in 2-wk-old seedlings than in 4-wk-old seedlings. *Plantago major* populations did not differ in their patterns of chemical defense with age, although overall levels of aucubin were marginally higher in plants from the Creek population than in plants from the Lawn population. In *P. lanceolata*, genetic families varied in the ontogenetic patterns of total iridoid glycosides, catalpol, and proportion catalpol of total iridoid glycosides, but not aucubin. In *P. lanceolata* genetic variation in the ontogeny of defense was also detected at the population level for total iridoid glycosides, aucubin, and proportion catalpol of total iridoid glycosides, but not for catalpol. At 6 wk of age, total iridoid glycoside concentrations were higher in plants from the Prairie population than they were in plants from either the France or Road population. Differences in secondary chemistry among *P. lanceolata* populations could reflect differential herbivore selection pressure in these populations. Additional studies quantifying levels of herbivory and demonstrating how herbivory affects plant fitness in the field would shed light on this pattern.

In *P. lanceolata*, the chemical profile changed with age. Despite the increased production of both aucubin and catalpol, the proportion of catalpol making up total iridoid glycosides decreased with age. Considering that catalpol is the more toxic of the two iridoid glycosides (Bowers and Puttick, 1988; Puttick and Bowers, 1988), having relatively higher amounts of catalpol could reflect an adaptive strategy by seedlings to maximize the toxicity of chemical defense when overall levels are low. Changes in chemical profile with age have been documented in several other plant species, including monoterpenes in Australian tea tree (*Melaleuca alternifolia*, Myrtaceae; Russell and Southwell, 2003), terpenes in the tropical rainforest tree *Nectandra ambigens* (Lauraceae; Sánchez-Hidalgo et al., 1999), and alkaloids in opium poppy (*Papaver somniferum*, Papaveraceae; Williams and Ellis, 1989). Although originally referring to tree species, Langenheim and Stubblebine (1983) suggested that ontogenetic changes in secondary chemical profile could be an adaptive strategy allowing seedlings to escape from herbivores and pathogens adapted to the chemical profiles of nearby conspecific adults. Additionally, it is possible that changes in iridoid glycoside profile during the first 6 wk of growth contribute to a moving target mechanism of defense (sensu Adler and Karban, 1994).

Although *P. lanceolata* and *P. major* were studied in separate years and cannot be quantitatively compared, because studies were done in the same greenhouse room with the same protocol and at exactly the same time of year, some reliable qualitative comparisons can be made. Ontogenetic patterns of secondary chemistry within species are markedly different for *P. lanceolata* and *P. major*. Iridoid glycoside concentrations increased from the seed to 2-wk seedling stage in *P. major* (mean 0.12–0.32%), but decreased precipitously in *P. lanceolata* (mean 2.19–0.014%). These early changes in iridoid glycoside concentrations did not merely reflect dilution as biomass increased. Total iridoid glycoside content also decreased from the seed (mean 36.0 μg) to 2-wk seedling

←

Fig. 1. Patterns of variation in secondary chemistry for both *Plantago major* and *P. lanceolata*. (A) Reaction norms for family chemistry data in *P. major*. There are four families from each population, and the number of replicates per family ranges from 7 to 16. (B) Mean iridoid glycoside concentrations (% ± 1 SE) for *P. major*; means for the two populations are referred to as L for Lawn and C for Creek. (C) Reaction norms for family chemistry data in *P. lanceolata*. There are three families from each of the France and Prairie populations and four families from the Road population; the number of replicates per family ranges from 8 to 13. (D) Mean iridoid glycoside concentrations (% ± 1 SE) for *P. lanceolata*. (E) Proportion catalpol of total iridoid glycosides for *P. lanceolata*. Means for the three populations are referred to as F for France, R for Road, and P for Prairie.

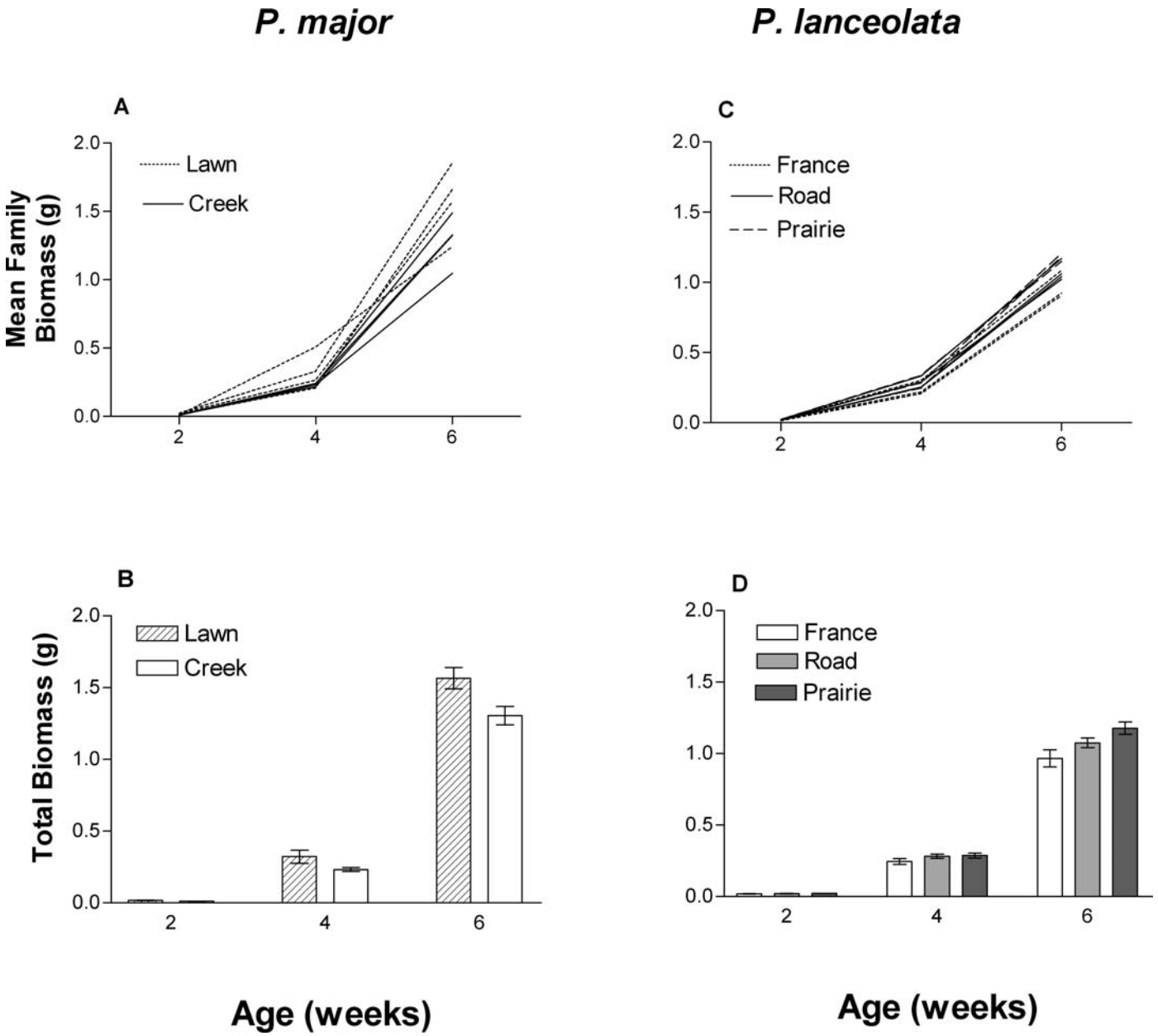
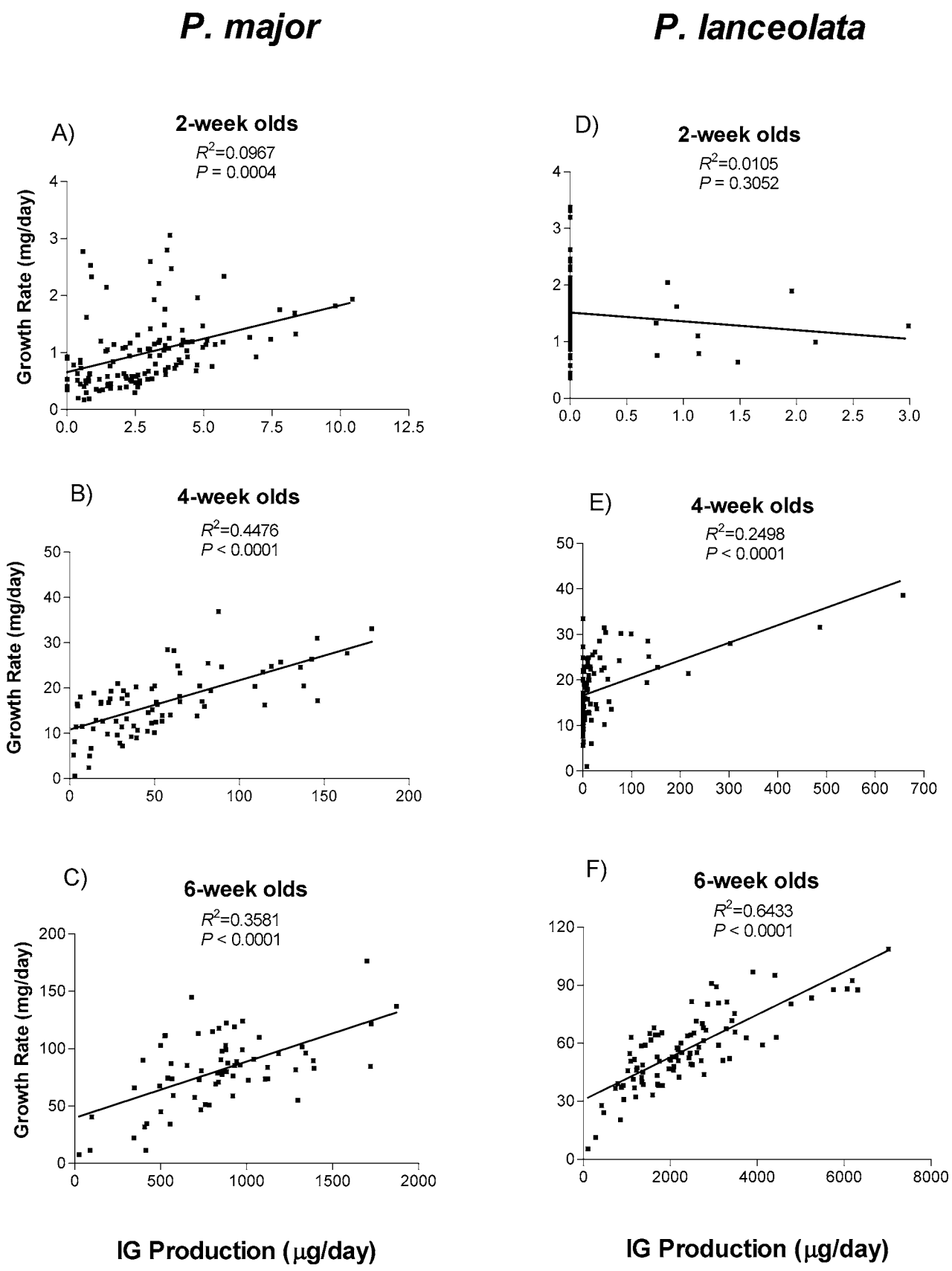


Fig. 2. Patterns of variation in biomass for both *Plantago major* and *P. lanceolata*. (A) Reaction norms for family biomass data in *P. major*. There are four families from each population, and the number of replicates per family ranges from 7 to 16. (B) Mean total biomass (±1 SE) for *P. major*. (C) Reaction norms for family biomass data in *P. lanceolata*. There are three families from each of the France and Prairie populations and four families from the Road population; the number of replicates per family ranges from 8 to 13. (D) Mean total biomass (±1 SE) for *P. lanceolata*.

(mean 2.8 μg) stage in *P. lanceolata*, suggesting that iridoid glycosides present in the seed were either lost in the seed coat during germination or were metabolized during early seedling growth. This pattern also indicates that iridoid glycoside production was very low or absent during the first 2 wk of growth in *P. lanceolata*. In contrast, iridoid glycoside production was higher in young *P. major* seedlings, as evidenced by an increase in iridoid glycoside content from the seed (mean 0.2 μg) to 2-wk seedling (mean 45.4 μg) stage. In both species, iridoid glycoside concentrations were similar for 2- and 4-wk-old plants, followed by a significant increase between 4 and 6 wk of age. It thus appears that the production

→

Fig. 3. Regression analyses to investigate trade-offs between growth rate and iridoid glycoside production rate in *Plantago major* and *P. lanceolata*. For each plant, rates were calculated over the 2-wk period preceding the harvest date. Separate analyses were conducted for each age class (2, 4, and 6 wk) in each species. The coefficient of determination (R^2) and P value are reported for each analysis.


P. major
P. lanceolata
A)
2-week olds
R^2=0.0967
P = 0.0004
D)
2-week olds
R^2=0.0105
P = 0.3052
B)
4-week olds
R^2=0.4476
P < 0.0001
E)
4-week olds
R^2=0.2498
P < 0.0001
C)
6-week olds
R^2=0.3581
P < 0.0001
F)
6-week olds
R^2=0.6433
P < 0.0001
Growth Rate (mg/day)
IG Production (μg/day)

of iridoid glycosides starts early but increases only gradually in *P. major*, while in *P. lanceolata*, production starts slowly and then undergoes a dramatic increase between 4 and 6 wk of age.

Despite sharing many life history characteristics, these congeners differ dramatically in the early ontogeny of chemical defense. Observations from previous studies may shed light on the significance of some of these key differences. *Plantago lanceolata* seeds generally remain viable in the seedbank longer than *P. major* seeds (Blom, 1992), which may be due, at least in part, to the higher levels of iridoid glycosides in *P. lanceolata* seeds. *Plantago lanceolata* seedlings also typically have greater establishment success than do *P. major* seedlings. In competitive grassland communities, *P. lanceolata* demonstrated significantly higher rates of seedling survival than *P. major*, reportedly because of faster growth rates (Van der Toorn and Pons, 1988). Despite the failure of this study to detect growth–defense trade-offs, it appears that the strategy of *P. lanceolata* seedlings is to grow quickly during the first 2 wk, while producing few or no iridoid glycosides, to achieve establishment. *Plantago major* seedlings grow more slowly, but those with higher growth rates also tend to be the ones that are better defended chemically. Understanding how the low levels of chemical defense in *Plantago* seedlings influence *Plantago* population dynamics requires additional research.

The growth–differentiation balance hypothesis predicts that low levels of secondary chemicals in seedlings result from strong allocation constraints imposed by growth. However, in this study, low levels of iridoid glycosides in *P. major* and *P. lanceolata* seedlings do not appear to result from trade-offs with growth, as revealed by the highly significant positive relationship between growth rate and iridoid glycoside production rate in all age classes except for 2-wk-old *P. lanceolata* seedlings, which showed no growth–defense relationship. In contrast, plants with fast growth rates also tended to be the ones with high iridoid glycoside production. Failure to detect allocation costs of defense is relatively common in adult plants (Bergelson and Purrington, 1996), but this is one of the first studies to demonstrate that growth–defense trade-offs may not occur in seedlings either. However, given the documented high costs of iridoid glycoside production (Gershenzon, 1994), it may be that allocation costs are merely difficult to detect. Levels of iridoid glycosides were very low in 2-wk-old plants (undetectable even in most *P. lanceolata* seedlings), perhaps reducing the statistical power necessary for detecting trade-offs. Alternatively, considering that the growth–defense relationship varied genetically in both species, perhaps natural selection has acted to minimize allocation costs, resulting in the positive relationship between growth and defense.

In conclusion, this study clearly refutes the plant age hypothesis and offers some support for the growth–differentiation balance hypothesis. *Plantago* seedlings produced lower levels of iridoid glycosides than older plants, but this pattern was not explained by trade-offs between growth and defense. Additional research is needed to investigate why *Plantago* seedlings have such low levels of iridoid glycosides. In particular, the following three scenarios must be explored. First, because constitutive secondary chemistry is not the only plant defense strategy, additional defense strategies, including chemical induction and compensation, must be tested. Seedlings may rely on these alternative defense strategies more than constitutive secondary chemistry. Second, despite previous evidence that seedling mortality rates are high (Blom, 1992) and that seedling herbivory is common (Hanley et al., 1996), there may not be consistently strong selection for high levels of seedling defense. Finally, low chemical defense in seedlings may simply reflect non-adaptive ontogenetic drift, such as that demonstrated for some vegetative traits (Gedroc et al., 1996). Future work measuring herbivory levels and enemy-mediated mortality rates of *Plantago* under natural conditions would allow selection estimates to be made, providing additional insight into these possible scenarios and the evolution of the ontogeny of defense.

LITERATURE CITED

ADLER, F. R., AND R. KARBAN. 1994. Defended fortresses or moving targets? Another model of inducible defenses inspired by military metaphors. *American Naturalist* 144: 813–832.

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.

AUGSPURGER, C. K. 1983. Seed dispersal of the tropical tree, *Platypodium elegans*, and the escape of its seedlings from fungal pathogens. *Journal of Ecology* 71: 759–771.

BERGELSON, J., AND C. B. PURRINGTON. 1996. Surveying patterns in the cost of resistance in plants. *American Naturalist* 148: 536–558.

BISHOP, J. G. 2002. Early primary succession on Mount St. Helens: impact of insect herbivores on colonizing lupines. *Ecology* 83: 191–202.

BLOM, C. W. P. M. 1992. Germination and establishment. *In* P. J. C. Kuiper and M. Bos [eds.], Plantago: a multidisciplinary study, 88–97. Springer-Verlag, Berlin, Germany.

BOEGE, K., AND R. J. MARQUIS. 2005. Facing herbivory as you grow up: the ontogeny of resistance in plants. *Trends in Ecology and Evolution* 20: 441–448.

BOWERS, M. D. 1991. Iridoid glycosides. *In* G. A. Rosenthal and M. R. Berenbaum [eds.], Herbivores: their interactions with secondary plant metabolites, vol. 1, 2nd ed., 297–326. Academic Press, San Diego, California, USA.

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 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 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. 1997. Fate of host-plant iridoid glycosides in Lepidopteran larvae of Nymphalidae and Arctiidae. *Journal of Chemical Ecology* 23: 2955–2965.

BRYANT, J. P., P. B. REICHARDT, T. P. CLAUSEN, F. D. PROVENZE, AND P. J. KUROPAT. 1992. Woody plant–mammal interactions. *In* G. A. Rosenthal and M. R. Berenbaum [eds.], Herbivores: their interactions with plant secondary metabolites, vol. 2, 2nd ed., 344–371. Academic Press, San Diego, California, USA.

CIPOLLINI, D. F., AND A. M. REDMAN. 1999. Age-dependent effects of jasmonic acid treatment and wind exposure on foliar oxidase activity and insect resistance in tomato. *Journal of Chemical Ecology* 25: 271–281.

CLARK, D. A., AND D. B. CLARK. 1984. Spacing dynamics of a tropical rain forest tree: evaluation of the Janzen–Connell model. *American Naturalist* 124: 769–788.

CLARKE, P. J., AND E. A. DAVISON. 2004. Emergence and survival of herbaceous seedlings in temperate grassy woodlands: recruitment limitations and regeneration niche. *Austral Ecology* 29: 320–331.

ERWIN, E. A., M. G. TURNER, R. L. LINDROTH, AND W. H. ROMME. 2001. Secondary plant compounds in seedling and mature aspen (*Populus tremuloides*) in Yellowstone National Park, Wyoming. *American Midland Naturalist* 145: 299–308.

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.

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.

GARDNER, D. R., AND F. R. STERMITZ. 1988. Host plant utilization and iridoid glycoside sequestration by *Euphydryas anicia* individuals and populations. *Journal of Chemical Ecology* 14: 2147–2168.

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.

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

GILBERT, G. S., K. E. HARMS, D. N. HAMILL, AND S. P. HUBBELL. 2001. Effects of seedling size, El Niño drought, seedling density, and distance to nearest conspecific adult on 6-year survival of *Ocotea whitei* seedlings in Panamá. *Oecologia* 127: 509–516.

GRACE, J. B., AND D. TILMAN. 1990. Perspectives on plant competition. Blackburn Press, Caldwell, New Jersey, USA.

HAECK, J. 1992. Phytosociology of *Plantago* habitats in the Netherlands and the relation with habitat characteristics. *In* P. J. C. Kuiper and M. Bos [eds.], *Plantago*: a multidisciplinary study, 20–28. Springer-Verlag, Berlin, Germany.

HANLEY, M. E., M. FENNER, AND P. J. EDWARDS. 1995. An experimental field study of the effects of mollusk grazing on seedling recruitment and survival in grassland. *Journal of Ecology* 83: 621–627.

HANLEY, M. E., M. FENNER, AND P. J. EDWARDS. 1996. Mollusc grazing and seedling survivorship of four common grassland plant species: the role of gap size, species and season. *Acta Oecologica* 17: 331–341.

HARPER, J. L. 1977. Population biology of plants. Academic Press, New York, New York, USA.

HARTL, D. L. 2000. A primer of population genetics, 3rd ed. Sinauer, Sunderland, Massachusetts, USA.

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.

HERMS, D. A., AND W. J. MATTSON. 1992. The dilemma of plants: to grow or defend. *Quarterly Review of Biology* 67: 283–335.

HULME, P. E. 1996. Herbivory, plant regeneration, and species coexistence. *Journal of Ecology* 84: 609–615.

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.

KELLY, C. K., AND M. E. HANLEY. 2005. Juvenile growth and palatability in co-occurring, congeneric British herbs. *American Journal of Botany* 92: 1586–1589.

KENNEDY, G. G., AND J. D. BARBOUR. 1992. Resistance variation in natural and managed systems. *In* R. S. Fritz and E. L. Simms [eds.], Plant resistance to herbivores and pathogens, 13–41. University of Chicago Press, Chicago, Illinois, USA.

KLINK, C. A. 1996. Germination and seedling establishment of two native and one invading African grass species in the Brazilian cerrado. *Journal of Tropical Ecology* 12: 139–147.

KORICHEVA, J. 1999. Interpreting phenotypic variation in plant allelochemistry: problems with the use of concentrations. *Oecologia* 119: 467–473.

KUROKAWA, H., Y. KITAHASHI, T. KOIKE, J. LAI, AND T. NAKASHIZUKA. 2004. Allocation to defense or growth in dipterocarp forest seedlings in Borneo. *Oecologia* 140: 261–270.

LANGENHAIM, J. H., AND W. H. STUBBLEBINE. 1983. Variation in leaf resin composition between parent tree and progeny in *Hymenaea*: implications for herbivory in the humid tropics. *Biochemical Systematics and Ecology* 11: 97–106.

LITTELL, R. C., G. A. MILLIKEN, W. W. STROUP, AND R. D. WOLFINGER. 1996. SAS system for mixed models. SAS Institute, Cary, North Carolina, USA.

MACEDO, C. A., AND J. H. LANGENHEIM. 1989. Microlepidopteran herbivory in relation to leaf sesquiterpenes in *Copaifera langsdorfii* adult trees and their seedling progeny in a Brazilian woodland. *Biochemical Systematics and Ecology* 17: 217–224.

MARAK, H. B., A. BIERE, AND J. M. M. VAN DAMME. 2000. Direct and correlated responses to selection on iridoid glycosides in *Plantago lanceolata* L. *Journal of Evolutionary Biology* 13: 985–996.

MARAK, H. B., A. BIERE, AND J. M. M. VAN DAMME. 2002. Two herbivore-deterrent iridoid glycosides reduce the in-vitro growth of a specialist but not of a generalist pathogenic fungus of *Plantago lanceolata* L. *Chemoecology* 12: 185–192.

MARON, J. L. 1997. Interspecific competition and insect herbivory reduce bush lupine (*Lupinus arboreus*) seedling survival. *Oecologia* 110: 284–290.

MCKEY, D. 1974. Adaptive patterns in alkaloid physiology. *American Naturalist* 108: 305–320.

MOLES, A. T., AND M. WESTOBY. 2004. What do seedlings die from and what are the implications for evolution of seed size? *Oikos* 106: 193–199.

PUTTICK, G. M., AND M. D. BOWERS. 1988. Effects of qualitative and quantitative variation in allelochemicals on a generalist insect—iridoid glycosides and the southern armyworm. *Journal of Chemical Ecology* 14: 335–351.

ROMEO, J. T., J. A. SAUNDERS, AND P. BARBOSA [EDS.]. 1996. Phytochemical diversity and redundancy in ecological interactions. *Recent Advances in Phytochemistry*, vol. 30, Plenum Press, New York, New York, USA.

RØNSTED, N., E. GÖBEL, H. FRANZYK, S. R. JENSEN, AND C. E. OLSEN. 2000. Chemotaxonomy of *Plantago*. Iridoid glucosides and caffeoyl phenylethanoid glycosides. *Phytochemistry* 55: 337–348.

RUSSELL, M. F., AND I. A. SOUTHWELL. 2003. Monoterpenoid accumulation in 1,8-cineole, terpinolene and terpinen-4-ol chemotypes of *Melaleuca alternifolia* seedlings. *Phytochemistry* 62: 683–689.

SÁNCHEZ-HIDALGO, M. E., M. MARTÍNEZ-RAMOS, AND F. J. ESPINOSA-GARCÍA. 1999. Chemical differentiation between leaves of seedlings and spatially close adult trees from the tropical rainforest species *Nectandra ambigens* (Lauraceae): an alternative test of the Janzen–Connell model. *Functional Ecology* 13: 725–732.

SAS. 2003. SAS/STAT software: changes and enhancements through release 9.1.3. SAS Institute, Cary, North Carolina, USA.

SCHAFFNER, U., K. VRIELING, AND E. VAN DER MEIJDEN. 2003. Pyrrolizidine alkaloid content in *Senecio*: ontogeny and developmental constraints. *Chemoecology* 13: 39–46.

SHAPPERT, P. J., AND J. S. SHORE. 2000. Cyanogenesis in *Turnera ulmifolia* L. (Turneraceae). II. Developmental expression, heritability and cost of cyanogenesis. *Evolutionary Ecology Research* 2: 337–352.

SHAW, M. T., F. KEESING, AND R. S. OSTFELD. 2002. Herbivory on *Acacia* seedlings in an East African savanna. *Oikos* 98: 385–392.

SOUSE, W. P., P. G. KENNEDY, AND B. J. MITCHELL. 2003. Propagule size and predispersal damage by insects affect establishment and early growth of mangrove seedlings. *Oecologia* 135: 564–575.

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. 2003. Out of the quagmire of plant defense hypotheses. *Quarterly Review of Biology* 78: 23–55.

STAMP, N. E., AND M. D. BOWERS. 1994. Effects of cages, plant age and mechanical clipping on plantain chemistry. *Oecologia* 99: 66–71.

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.

VAN DAMME, J. M. M. 1992. Breeding systems in *Plantago*. *In* P. J. C.

Kuiper and M. Bos [eds.], *Plantago*: a multidisciplinary study, 18–19. Springer-Verlag, Berlin, Germany.

VAN DER AART, P. J. M., AND J. C. VULTO. 1992. Biogeography and human effects. *In* P. J. C. Kuiper and M. Bos [eds.], *Plantago*: a multidisciplinary study, 5–6. Springer-Verlag, Berlin, Germany.

VAN DER TOORN, J., AND T. L. PONS. 1988. Establishment of *Plantago lanceolata* L. and *Plantago major* L. among grass. *Oecologia* 76: 341–347.

WATKINSON, A. R. 1997. Plant population dynamics. *In* M. J. Crawly [ed.], Plant ecology, 2nd ed., 359–400. Blackwell Science, Oxford, UK.

WILLIAMS, R. D., AND B. E. ELLIS. 1989. Age and tissue distribution of alkaloids in *Papaver somniferum*. *Phytochemistry* 28: 2085–2088.