

# Alkaloid Uptake Increases Fitness in a Hemiparasitic Plant via Reduced Herbivory and Increased Pollination

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**ABSTRACT:** It has been historically difficult to manipulate secondary compounds in living plants to assess how these compounds influence plant-herbivore and plant-pollinator interactions. Using a hemiparasitic plant that takes up secondary compounds from host plants, I experimentally manipulated secondary compounds *in planta* and assessed their effects on herbivores and pollinators in the field. Here, I show that the uptake of alkaloids in the annual hemiparasite *Castilleja indivisa* resulted in decreased herbivory, increased visitation by pollinators, and increased lifetime seed production. These results indicate that resistance traits such as alkaloids can increase plant fitness directly by reducing herbivore attack and indirectly by increasing pollinator visitation to defended plants. Thus, selection for production of secondary compounds may be underestimated by considering only the direct effect of herbivores on plant fitness.

**Keywords:** herbivores, indirect effects, parasitic plants, plant-animal interactions, pollinators, secondary compounds.

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Herbivores and pollinators can each exert selective pressure on plant resistance traits, such as secondary compounds (Simms and Bucher 1996; Armbruster et al. 1997; Strauss 1997). Our current understanding of how secondary compounds confer resistance to herbivory has emerged from a combination of field studies that correlate secondary chemistry with resistance and laboratory studies that manipulate these compounds via artificial diet or extracts applied to leaf tissue (Berenbaum et al. 1986; Duffey and Stout 1996). Although this approach has led to many insights about the role of secondary compounds in plant-herbivore interactions, there are limits to what can be inferred from these methods. Field studies that correlate

secondary compounds with resistance are difficult to interpret because secondary compounds are also affected by plant environment and stress, which can have independent effects on herbivore resistance (Tuomi et al. 1984; Larsson et al. 1986; Edwards 1992). Manipulations of secondary compounds in artificial diet provide clean contrasts, but removing compounds from their natural milieu can give misleading results (Duffey and Stout 1996). Experimental manipulation of secondary compounds in living plants would provide definitive evidence that secondary compounds are causal agents of plant resistance.

Although resistance to herbivores may influence plant-pollinator as well as plant-herbivore interactions (Strauss and Armbruster 1997), the difficulty of manipulating secondary compounds *in planta* has made detection of these multispecies interactions impracticable. Secondary compounds could affect pollinators by making floral rewards less attractive to pollinators; correlative evidence for this has been found in populations of *Brassica rapa* selected for high or low resistance to beetle herbivores. Pollinators spent less time foraging on high-resistance plants, although populations did not differ in the amount of floral rewards (Strauss et al. 1999). Floral tissue contains glucosinolates (Rosa 1997), suggesting that changes in pollinator foraging behavior could be governed by differences in reward palatability. Alternatively, plant secondary compounds could increase attractiveness to pollinators if decreased herbivory results in improved displays or rewards; evidence of pollinator preference for less damaged plants has been found in several systems (Karban and Strauss 1993; Cunningham 1995; Lohman and Berenbaum 1996; Juenger and Bergelson 1997; Lehtil and Strauss 1997; Krupnick et al. 1999; Strauss et al. 1999; Mothershead and Marquis 2000). Thus, plant secondary compounds have the potential to affect directly or indirectly pollinators as well as herbivores, but their role in plant-pollinator interactions is largely unknown.

Our limited ability to manipulate secondary compounds in living plants can be addressed with the use of hemiparasitic plants, which provide a unique opportunity to study chemically mediated interactions between plants,

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herbivores, and pollinators. The term “hemiparasite” describes plants that contain chlorophyll and are photosynthetic, but obtain water and nutrients from host plants via haustorial connections (Kuijt 1969). Many hemiparasites take up secondary compounds from their host plants (Schneider and Stermitz 1990; Boros et al. 1991; Stermitz and Pomeroy 1992). Thus, the presence of certain secondary compounds varies within and among populations of parasitic plants, depending on the host association of individual parasites (Stermitz and Harris 1987). The ability to take up host compounds provides a novel tool for manipulating secondary compounds in living plants and assessing consequences of these compounds in the field.

Plant lines bred for high and low levels of secondary compounds have been found to differ in resistance to herbivory (Wink 1988; Giamoustaris and Mithen 1995), providing the most convincing evidence to date that secondary compounds confer resistance. Because secondary compounds can have pleiotropic effects on many other aspects of plant function, it is difficult to determine whether observed ecological effects are due to the manipulated trait or to closely linked alleles. The use of a hemiparasite-host system to manipulate defensive compounds brings several advantages to address this issue. By assigning hemiparasites from different maternal lines to each host treatment, the effects of secondary compounds can be evaluated across varied genetic backgrounds and provide a level of removal from pleiotropic or linked effects in the host plant. In addition, because the hemiparasite does not manufacture compounds taken up from a host, costs of producing compounds (“allocation costs”) can be separated from ecological (Simms 1992) and autotoxic (McKey 1974; Chew and Rodman 1979; Fowden and Lea 1979) costs of possessing secondary compounds. Finally, because hemiparasites naturally take up secondary compounds from their hosts, manipulations do not represent the introduction of novel compounds to naive herbivores and pollinators. Rather, the herbivores and pollinators of hemiparasites may encounter host-obtained secondary compounds frequently (L. S. Adler, personal observation).

To assess the effect of alkaloid uptake on herbivory, pollination, and plant fitness, I grew individuals of the annual hemiparasitic plant *Castilleja indivisa* (or Indian paintbrush) with one of two hosts: sweet (low-alkaloid) or bitter (high-alkaloid) near-isogenic lines of the lupine *Lupinus albus*. These lines are similar morphologically and in resource availability but differ in their alkaloid content (L. S. Adler and C. Huyghe, unpublished data). Two populations of Indian paintbrush were chosen that differed in their historic levels of herbivory and pollination. In order to determine the impact of herbivory and pollination on seed production, Indian paintbrush from each population and with each host were assigned to herbivory (natural or

reduced) and pollination (natural or supplemental) treatments in a fully factorial design. These plants were used in a field experiment to ask whether alkaloid uptake influences levels of herbivory and/or pollination, and ultimately lifetime seed production, in a hemiparasitic plant.

### Material and Methods

*Castilleja indivisa* (Scrophulariaceae), or Indian paintbrush, is an annual hemiparasite endemic to Texas (Loughmiller and Loughmiller 1984). Indian paintbrush is self-incompatible (L. S. Adler and C. Huyghe, unpublished data) with inconspicuous flowers and brightly colored bracts. Indian paintbrush parasitize hosts by establishing connections to the host vascular system via root haustoria (Kuijt 1969). Indian paintbrush do not produce alkaloids but take up the alkaloid lupanine when parasitizing a prevalent host, *Lupinus texensis* (Stermitz and Pomeroy 1992). These alkaloids are produced in the chloroplasts of lupines but are transported via the phloem to all plant parts, including the roots (Wink 1989).

“Sweet” lines of many annual lupines have been developed that produce trace quantities of alkaloids, although alkaloid composition is unchanged relative to bitter (wild-type) lines (Saito et al. 1993; Wink 1993). Sweet lines of *L. texensis* are not available; however, near-isogenic bitter and sweet lines are available for the annual *Lupinus albus*, which has an alkaloid profile similar to that of *L. texensis* (Wink et al. 1995). Near-isogenic lines were created by crossing sweet and bitter autumn-sown indeterminate genotypes of *L. albus*. Sweet parents were homozygous recessive for the *pauper* allele (Cowling et al. 1998). From the F2 progeny of each cross, the F3 and F4 generations were obtained by single-seed descent. Only plants that were heterozygous for alkaloid production were retained. The near-isogenic lines were created by the selection of one sweet and several bitter plants in the progeny of single heterozygous F4 plants. In the progeny, only true-breeding bitter plants were retained. Seeds from pairs were produced under isolation conditions to avoid cross-pollination (C. Huyghe, personal communication).

I chose two populations of Indian paintbrush for this experiment that differed in their historic levels of herbivory and pollination. The Stengl population (seed collected from the Stengl House Reserve in Bastrop, Tex., operated by the University of Texas at Austin) came from a high-herbivory, high-pollination environment, whereas the Wildflower Center population (seed collected from the former site of the Lady Bird Johnson Wildflower Center; 2600 FM 973 North, approximately 3 miles north of Highway 71) experienced lower natural levels of both herbivory and pollination (L. S. Adler, personal observation). Individuals of Indian paintbrush from these two populations

were grown from seed and randomly assigned to one of two hosts: sweet (low-alkaloid) or bitter (high-alkaloid) near-isogenic lines of *L. albus*. Two lupine hosts were planted with multiple Indian paintbrush per 10-cm pot in a greenhouse in January 1998. Plants grew in a 1 : 1 ratio of University of California at Davis soil mix : vermiculite (Baker 1972; Evans 1998) under a day : night regime of 16L : 8D created with a 1,000 W metal halide light. In March, plants were thinned to one parasite and two host individuals per pot and transplanted to a fenced natural area in the Lady Bird Johnson Wildflower Center, Austin, Texas. Each parasite-host pair was surrounded by a buried cylinder of nonwoven polypropylene fabric (Root Control, Oklahoma City, Okla.), 22 cm in depth with a 25-cm diameter, to allow water penetration but to prevent parasitism on other wild plants.

I determined the effects of alkaloid uptake on herbivory and pollination by observing pollination for 70 h during the flowering season and measuring herbivory during and at the end of the season. The most common herbivores included larvae of the moths *Endothenia hebesana* (Tortricidae), *Junonia coenia* (Noctuidae), and *Plusia biloba* (Noctuidae). Inflorescences were pollinated primarily by the black-chinned hummingbird *Archilochus alexandri*. At the end of the season, the fate of every flower (filled or unfilled fruit, and damaged or undamaged) was recorded, and seeds were counted for every filled fruit.

Within this experiment, Indian paintbrush from each population and with each host were assigned to herbivory (natural or reduced) and pollination (natural or supplemental) treatments in a fully factorial design to determine the impact of herbivory and pollination on seed production. In the herbivory treatment, half of the Indian paintbrush were sprayed with *Bacillus thuringiensis*, a biodegradable nonsystemic pesticide specific to Lepidoptera (Thuricide Concentrate, Bonham, Tex.), to assess the direct effect of herbivores on plant fitness. Control plants were sprayed with water. Host plants were not sprayed, and there were no detectable effects of pesticides on host plant biomass ( $F = 0.09$ ,  $df = 1, 109$ ,  $P > .75$ ). *Bacillus thuringiensis* application is unlikely to affect vertebrates such as hummingbirds, which made up the vast majority of pollinating visitors in 1998. *Bacillus thuringiensis* in artificial diet did not affect larval survival or pupal dry weight in a study of the honey bee *Apis mellifera* (Arpaia 1996), and bees were the second most common category of pollinators in this study (L. S. Adler, personal observation). Thus, it is unlikely that pesticide application had direct effects on pollinators.

In the pollination treatment, all Indian paintbrush were exposed to natural pollination, and half the plants received supplemental hand-pollination twice weekly to determine whether pollinators were limiting seed set.

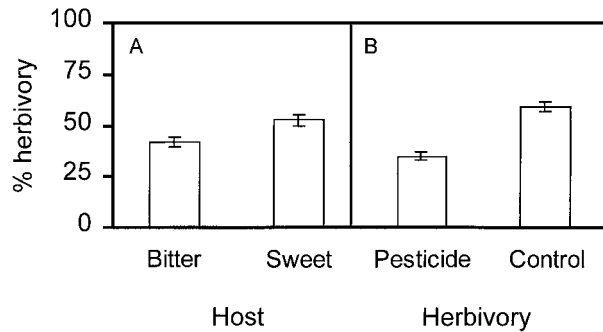
Plants were randomized within 15 blocks in a split-plot design, with host treatment as the subplot and herbivory treatment, pollination treatment, and paintbrush population as the main plots. Each block contained one replicate of each population-host-herbivory-pollination combination, for a total of 240 plants.

Results were analyzed with ANOVA (GLM procedure of SAS). All main effects and interactions were tested except interactions with block. Block was treated as a random factor, and population was treated as a fixed effect because populations of paintbrush were not chosen at random. Host treatment and interactions including host were tested over the mean squared error term (MSE) because host treatment was the subplot factor (Littell et al. 1991). All other terms were tested over the block  $\times$  herbivory  $\times$  pollination  $\times$  population interaction term, and the RANDOM/TEST option of SAS was used to construct appropriate error terms for testing a mixed-model ANOVA (Littell et al. 1991). All proportional data were arcsine(square root(x)) transformed, and all other data were log transformed to meet assumptions of normality. Pollinator visitation was scored as the number of plants that were visited during the entire observation period and was tested with a  $\chi^2$  statistic using the TABLES/CHISQ option of the FREQ procedure in SAS.

Alkaloid content of Indian paintbrush inflorescences was determined using gas chromatography. Inflorescences were collected at the end of the field season, dried at 50°C for 1 wk, and ground to pass through a 40-mesh screen using a Wiley Mill (Thomas Scientific, Swedesboro, N.J.). Alkaloids were extracted in 0.5 M HCl with cinchonidine HCl added as an internal standard to 0.1% dry weight. The resulting extract was then made basic with addition of  $\text{NH}_4\text{OH}$ , and methylene chloride was added to extract alkaloids as free bases (Johnson et al. 1989). This methylene chloride extract was injected into a HP 5890A gas chromatograph (Hewlett Packard, Wilmington, Del.) with a DB-1 megapore capillary column 30 m long with 0.25-mm internal diameter and 0.25- $\mu\text{m}$  film thickness (J&W Scientific, Folsom, Calif.). Alkaloids were eluted by temperature programming from 200° to 300°C over a 10-min period followed by 5 min at 300°C. This temperature program provided a sufficient amount of time to detect peaks from representative *Lupinus* alkaloids (B. Bentley, personal communication). Alkaloids were identified by comparison to known standards (Johnson et al. 1989).

## Results

Analysis of Indian paintbrush samples using gas chromatography demonstrated that these hemiparasites obtained primarily the alkaloid lupanine from their hosts. Other alkaloids, when detectable, were present only in



**Figure 1:** A, Herbivore damage to Indian paintbrush parasitizing bitter (high alkaloid) and sweet (low alkaloid) lupine hosts. B, Herbivore damage to Indian paintbrush sprayed with pesticide compared to natural herbivory controls sprayed with water (*Control*). Herbivory was measured as the percentage of initiated flowers that were damaged at any stage for each plant. Data shown are averaged over all other treatments, as there were no significant two-, three-, or four-way interactions between any main effects. Bars represent standard errors. Sample sizes ranged from 107 to 113 Indian paintbrush per treatment.

trace amounts, with the exception of two plants that contained an unidentified alkaloid also found in the host (L. S. Adler and C. Huyghe, unpublished data). Because lupanine was the principal alkaloid detected, the term “alkaloid” will be used to refer to lupanine content in the remainder of the article. Indian paintbrush parasitizing bitter hosts contained between 0% and 0.15% alkaloids (dry weight) in their inflorescences; Indian paintbrush parasitizing sweet hosts did not contain detectable alkaloids.

In the field, Indian paintbrush parasitizing bitter lupines received less damage from herbivores than Indian paintbrush parasitizing sweet lupine hosts (fig. 1A;  $F = 13.6$ ,  $df = 1, 95$ ,  $P = .0004$ ). Most herbivory was on buds, flowers, and fruits (a mean of 47% of initiated flowers were damaged, whereas <5% of leaves were damaged, the majority with <10% area removed). Therefore, the measure of damage used here is the proportion of total flowers initiated that received damage at any stage. There was a significant negative regression between damage and alkaloid content of inflorescences ( $F = 6.97$ ,  $df = 1, 217$ ,  $P = .0089$ ).

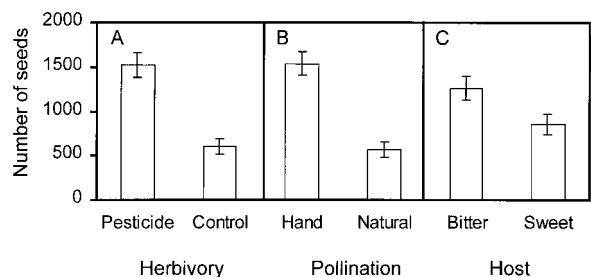
Natural levels of herbivory and pollination both strongly influenced lifetime seed production. Pesticide application reduced herbivory by over 40% relative to control plants (fig. 1B;  $F = 53.54$ ,  $df = 1, 107$ ,  $P < .0001$ ), and plants that were sprayed produced over twice as many seeds as control plants (fig. 2A;  $F = 30.26$ ,  $df = 1, 103$ ,  $P < .0001$ ). Thus, herbivory had a strong effect on seed production in Indian paintbrush. Hand-pollinated plants set over twice as many fruit and produced approximately three times as many

seeds as naturally pollinated control plants (fig. 2B;  $F = 51.18$ ,  $df = 1, 102$ ,  $P < .0001$ ), demonstrating that Indian paintbrush were strongly pollen limited under natural conditions.

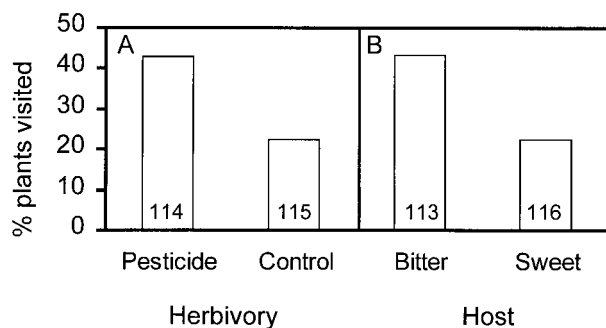
Pollinators discriminated between plants on the basis of damage by herbivores; hummingbirds visited Indian paintbrush that had been sprayed with pesticide almost twice as frequently as they visited control plants (fig. 3A;  $\chi^2 = 10.79$ ,  $df = 228$ ,  $P < .001$ ). Pollinators did not appear to discriminate on the basis of prior hand-pollination ( $\chi^2 = 0.009$ ,  $df = 228$ ,  $P > .9$ ).

In addition, pollinators visited Indian paintbrush parasitizing bitter hosts twice as often as Indian paintbrush parasitizing sweet hosts (fig. 3B;  $\chi^2 = 11.41$ ,  $df = 228$ ,  $P < .001$ ). The number of flowers pollinated per visit and time spent per flower were not significantly different between paintbrush parasitizing bitter and sweet hosts (number of flowers:  $F = 0.001$ ,  $df = 1, 49$ ; time per flower:  $F = 0.001$ ,  $df = 1, 47$ ;  $P > .9$  for both). Indian paintbrush parasitizing bitter and sweet lupines did not differ in measured components of floral display (plant height, number and length of inflorescences, number of open flowers per day, total bud production, and calyx length; MANOVA,  $F = 1.2184$ ,  $df = 6, 183$ ,  $P > .29$ ), suggesting that pollinators could discern actual damage to inflorescences.

Indian paintbrush parasitizing bitter lupine ultimately produced almost 50% more seed than Indian paintbrush parasitizing sweet lupine (fig. 2C;  $F = 10.55$ ,  $df = 1, 95$ ,  $P = .0016$ ). This effect could be due to greater resource availability from bitter hosts, direct effects of reduced herbivory causing increased seed production, and/or indirect



**Figure 2:** A, Mean lifetime seed production for Indian paintbrush sprayed with pesticide compared to natural herbivory controls (*Control*). B, Lifetime seed production for Indian paintbrush hand-pollinated twice a week (*Hand*) compared to natural pollination controls (*Natural*). C, Lifetime seed production for Indian paintbrush parasitizing bitter (*Bitter*) and sweet (*Sweet*) lupine hosts. Total number of seeds was natural log-transformed for analysis. Data shown are averaged over all other treatments, as there were no significant two-, three-, or four-way interactions between any main effects. Bars represent standard errors. Sample sizes ranged from 107 to 113 Indian paintbrush per treatment.



**Figure 3:** Percentage of Indian paintbrush visited by pollinators in field array. *A*, Pollinator visits to Indian paintbrush with bitter (high alkaloid) and sweet (low alkaloid) lupine hosts. *B*, Pollinator visits to Indian paintbrush sprayed with pesticide compared to natural herbivory controls (*Control*). Numbers within each bar are sample sizes. Data for each graph are averaged over all other treatments.

effects of reduced herbivory making plants more attractive to pollinators and thereby increasing seed production. In greenhouse studies where herbivores were excluded, Indian paintbrush did not differ in biomass when grown on bitter compared to sweet hosts (L. S. Adler and C. Huyghe, unpublished data), suggesting that herbivory and/or pollination are responsible for the greater seed production of Indian paintbrush with alkaloids. A path analysis of the field data showed that alkaloids reduced herbivory but did not directly affect pollinator visitation; increased pollination in alkaloid-containing plants was related to reduced herbivory (L. S. Adler, R. Karban, and S. Y. Strauss, unpublished data). Herbivory and pollination both influenced total seed production in Indian paintbrush, indicating that reduced herbivory due to alkaloid uptake benefits plants directly and benefits them indirectly by influencing pollinator choice.

Populations of paintbrush differed in the levels of herbivory they experienced in a common environment ( $F = 10.22$ ,  $df = 1, 107$ ,  $P = .0018$ ). Paintbrush from the Stengl population (high natural herbivory) suffered less herbivory than paintbrush from the Wildflower Center population (low natural herbivory; proportion of buds damaged: Stengl,  $0.43 \pm 0.027$  SE; Wildflower,  $0.52 \pm 0.026$  SE). However, pollinators did not discriminate between paintbrush from different populations ( $\chi^2 = 0.499$ ,  $df = 225$ ,  $P > .48$ ), and paintbrush from the two populations did not differ in lifetime seed production ( $F = 0.51$ ,  $df = 1, 103$ ,  $P > .47$ ).

Although herbivory treatment, pollination treatment, host treatment, and population all significantly affected herbivory, and all factors except population significantly affected lifetime seed production, there were no significant two-, three-, or four-way interactions between these factors

(herbivory: all  $F < 1.85$ ,  $P > .17$ ; seed production:  $F < 2.48$ ,  $P > .11$ ).

### Discussion

In this study, Indian paintbrush taking up alkaloids from bitter hosts experienced decreased herbivory compared to Indian paintbrush parasitizing sweet hosts. Although alkaloids were not detected in the fruit, corolla, or nectar of Indian paintbrush, they were found in calices and floral bracts, which are the most conspicuous parts of the inflorescence (L. S. Adler and M. Wink, unpublished data). Adults of the most common herbivores oviposited on the outside of floral bracts and calices, and these tissues were chewed through by newly hatched larvae before inner tissues were damaged (L. S. Adler, personal observation). Therefore, alkaloids might decrease herbivory by affecting early larval establishment.

The results that pollinators preferred plants with less herbivore damage, that plants with alkaloids received less herbivore damage, and that pollinators preferred plants with alkaloids all suggest that decreased herbivory due to alkaloid uptake made plants more attractive to pollinators. The cue used by pollinators to choose less damaged plants is unknown. Pollinators may perceive actual damage to flowers, or plants with less damage may produce more attractive displays (Strauss et al. 1996). Indian paintbrush parasitizing bitter and sweet lupines did not differ in measured components of floral display, suggesting that actual damage may be the cue used by pollinators to distinguish between paintbrush with and without alkaloids. Although nectar production was not measured in this study, it is possible that damage to inflorescences reduced the amount or accessibility of nectar. Herbivores often severed corollas at the base, making it difficult for nectar to accumulate in the tubular flower (L. S. Adler, personal observation). Hummingbirds, the principal pollinator, often return to or defend good nectar sources (Feinsinger and Colwell 1978), and one might expect that the results here are a consequence of hummingbirds returning to plants that were rewarding on the first visit or avoiding those that were not. Of the 222 plants that flowered, during my observations <10% were visited more than once (132 were not visited, 70 were visited once, 14 twice, 5 three times, and 1 four times). This suggests that, while hummingbirds may avoid plants that were not rewarding on the first visit, there was little evidence for repeated visits to particularly rewarding or attractive plants. Also, host plant did not affect the number of flowers probed or time per flower for plants that were visited, suggesting that plants were selected using long-range rather than short-range cues.

Theoretical models have attempted to explain why plant defenses are variable both within and between species

(Rhoades and Cates 1976; Coley et al. 1985; Herms and Mattson 1992). Many of these models assume there is a cost of defense; traits that result in increased fitness in the presence of herbivores are predicted to have fitness costs in the absence of herbivores. Physiological costs of resistance are not always detected and are influenced by the type of plant, control of genetic background of the resistance allele, and genetic context of the allele (Bergelson and Purrington 1996). Ecological costs have been proposed as another mechanism by which resistance can be costly; for example, resistance to one herbivore might confer susceptibility to another (Simms 1992). One type of ecological cost could be decreased pollination in resistant plants; this could occur if resistance traits such as thorns or secondary compounds deterred pollinators (Detzel and Wink 1993; Strauss et al. 1999) and plants were pollen limited. The results from the current study suggest that if such a trade-off does exist, it is greatly outweighed by the increased attractiveness of resistant plants to pollinators that presumably results from decreased herbivory.

The possibility of additional differences between bitter and sweet lines due to linked alleles cannot be completely discounted. Bitter and sweet lines used in this study were near-isogenic rather than isogenic; that is, it is likely that these lines differ at other loci. Although it is not feasible to screen bitter and sweet plants for all possible metabolites that could differ, several lines of reasoning suggest that alkaloids, rather than linked traits, are responsible for the effects found in this study. In a greenhouse study, bitter and sweet lupines did not differ in nonalkaloid nitrogen content of roots, indicating that availability of this resource should not be different for hemiparasites on bitter compared to sweet hosts (L. S. Adler and C. Huyghe, unpublished data). Indian paintbrush parasitizing bitter and sweet *Lupinus albus* in the greenhouse did not differ in aboveground biomass, suggesting that the advantages of bitter hosts in the field were not found under greenhouse conditions. In fact, Indian paintbrush produced fewer seeds per fruit when parasitizing bitter lupine in the greenhouse (L. S. Adler and C. Huyghe, unpublished data). This result is in contrast with the field study, where Indian paintbrush parasitizing bitter hosts had increased seed set, along with reduced herbivory and increased pollination, compared to Indian paintbrush with sweet hosts. Reducing herbivory with pesticide application in the field also increased pollination and seed set, independent of alkaloid uptake. Thus, the simplest explanation for the increased seed production of alkaloid-containing Indian paintbrush in the field but not the greenhouse would be the benefits of reduced herbivory.

It could be argued that the reduced herbivory in Indian paintbrush parasitizing bitter hosts is due to uptake of other metabolites that differ between bitter and sweet

hosts. Although this possibility cannot be excluded, the link between alkaloid production and herbivore resistance has been established in many systems and provides the most parsimonious explanation for the differences found in this study. About 20%–30% of higher plants contain alkaloids, which represent one of the largest groups of natural products, with over 10,000 known compounds. In artificial diet, many taxa of insects tend to select a diet with no or low concentrations of alkaloids, and the evidence that alkaloids have antiherbivore, pathogenic, and allelopathic activity is extensive. The mechanism of action can be disruption of DNA or RNA replication, protein biosynthesis, electron chains, membrane transport, and/or cytoskeleton assembly (see review by Wink 1993). Given that alkaloids are related to herbivore resistance in a number of systems and that the near-isogenic lines in this study were bred specifically to differ in alkaloid content, the most plausible explanation for reduced herbivory in Indian paintbrush parasitizing bitter hosts is alkaloid uptake.

This study is unique in that uptake of secondary compounds was experimentally manipulated in living plants, and uptake of these compounds influenced interactions with pollinators as well as herbivores. Herbivory and pollination each had substantial effects on seed production, and pollinators preferred to visit less damaged plants regardless of alkaloid content. Both plant-herbivore and plant-pollinator interactions strongly affected plant fitness. These results indicate that resistance traits such as alkaloids can increase plant fitness directly by reducing herbivore attack and indirectly by increasing pollinator visitation to defended plants. Thus, selection for production of secondary compounds may be underestimated by considering only the direct effect of herbivores on plant fitness.

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