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RAPID COMMUNICATIONS

Host Plant Influences on Iridoid Glycoside Sequestration of Generalist and Specialist Caterpillars

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Abstract The effect of diet on sequestration of iridoid glycosides was examined in larvae of three lepidopteran species. Larvae were reared upon *Plantago major*, or *P. lanceolata*, or switched from one to the other in the penultimate instar. *Junonia coenia* is a specialist on iridoid glycoside-producing plants, whereas the arctiids, *Spilosoma congrua* and *Estigmene acrea*, are both polyphagous and eat iridoid-producing plants. All species sequestered iridoids. The specialist *J. coenia* sequestered from three to seven times the amounts sequestered by the two generalist species. *Junonia coenia* iridoid glycoside content depended on diet, and they sequestered from 5 to 15% dry weight iridoid glycosides. *Estigmene acrea* iridoid glycoside sequestration was relatively low, around 2% dry weight and did not vary with diet. *Spilosoma congrua* sequestration varied with diet and ranged from approximately 3 to 6% dry weight.

Key Words Arctiidae · Buckeye · *Estigmene acrea* · Iridoid glycosides · *Junonia coenia* · Nymphalidae · *Plantago* · *Spilosoma congrua* · Tiger moth

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Introduction

A variety of insect species in several different orders have the ability to sequester defensive compounds from the plants on which they feed (Nishida, 2002). Levels of these defenses may vary due to differences in the efficiency with which particular species or individuals store these compounds, as well as to variation in the amounts available in their host plants. Most often, sequestration is found in insects that specialize on plants containing a particular group of defense compounds; however, some generalist insects may sequester defense compounds as well. Insects that consume several plant species may be exposed to different classes of compounds as well as different levels of individual compounds in their diet (Bernays and Minkenberg, 1997).

Iridoid glycosides are produced by species in over 50 plant families. Several groups of insects have acquired the ability to sequester iridoid glycosides and use these compounds as defenses against predators (Bowers, 1991). Insects that sequester higher concentrations of iridoid glycosides are less palatable to both vertebrate and invertebrate predators (Bowers, 1991; Theodoratus and Bowers, 1999). However, because qualitative and quantitative variation in the levels of iridoid glycosides in plants can vary substantially among species, as well as among individuals within species (Bowers, 1991), the acquired chemical defenses of insects that feed on those plants may vary as well, with significant implications for selection on herbivore populations and tritrophic interactions.

Here, we examined how host plant species affects iridoid glycoside sequestration of larvae of three lepidopteran species that vary in their degree of specialization on plants containing iridoid glycosides. Larvae were fed 1. *Plantago lanceolata*, which contains two iridoid glycosides, aucubin and catalpol (Fig. 1), with combined amounts as high as

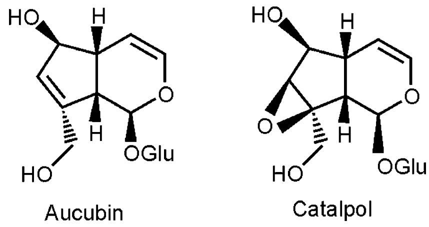

Fig. 1 Chemical structures of aucubin and catalpol

10–12% dry weight in leaves, although amounts can vary with leaf and plant age and among individuals (Bowers and Stamp, 1993); 2. *P. major*, which produces only aucubin in relatively low amounts of 0.5–2.0% in leaves (Barton and Bowers, 2006); or 3. both. *Junonia coenia* Hübner (Nymphalidae) is a specialist on iridoid glycoside producing plants, and larvae can sequester high amounts of iridoid glycosides, which render them unpalatable (Theodoratus and Bowers, 1999). *Estigmene acrea* (Drury) (Arctiidae) is a conspicuous grazing generalist (Bernays et al., 2004) that preliminary analyses suggested can sequester iridoids (Bowers, 2009), while *Spilosoma congrua* (Walker) (Arctiidae) is a cryptic, grazing generalist that can also sequester iridoids (Bowers and Stamp, 1997). All these species feed on both *Plantago* species.

Methods and Materials

Freshly hatched larvae were reared in groups of 10 until the third instar, when they were placed individually into 100 mm Petri dishes for the remainder of the trial. Larvae were reared upon one of four leaf diets: freshly excised leaves of *P. major*, *P. lanceolata*, *P. major* until the antepenultimate instar then changed to *P. lanceolata*, or *P. lanceolata* until the antepenultimate instar then changed to *P. major*. Leaves of a mixture of ages were collected daily from many individual plants in stands around Boulder, CO, USA. Leaves were of various ages, and no attempt was made to collect leaves from particular plants or of a certain age. Iridoid glycoside concentrations of leaves were not measured. Larvae were frozen at −80°C immediately upon reaching the final instar. Sample sizes ranged from 8–10 individuals/diet, except for *E. acrea* reared on *P. major* followed by *P. lanceolata*, where mortality was high and only 2 larvae were available. Larvae were prepared for analysis and iridoid glycosides [derivatized using Tri-sil Z (Pierce Chemical Company)] quantified by gas chromatography (methods in Bowers and Stamp, 1997). Fresh to dry weight conversions were obtained from separate sets of caterpillars, and all results are reported on a dry weight basis. Bonferroni-adjusted 1-way analyses of variance (ANOVA) were performed for each species, comparing the percent dry weight of total iridoid glycosides (analyzed as the arc-sine transformed proportion) and the proportion of catalpol (arc-sine transformed) sequestered among diets. Tukey's HSD post-hoc tests were applied where appropriate.

Results

Junonia coenia larvae sequestered the greatest concentration of iridoids, while *E. acrea* sequestered the lowest concentration, and *S. congrua* was intermediate (Fig. 2). All three species sequestered both aucubin and catalpol when both were available, although the relative proportion of the two compounds varied among both diets and species. Catalpol has not been reported in *P. major* (Barton and Bowers, 2006 and references therein), and thus, none was found in the larvae reared solely on this plant, so these larvae were not included in analyses of catalpol sequestration.

Estigmene acrea was a poor sequesterer and was particularly inefficient at sequestering catalpol, which could not be compared statistically (Fig. 2). Diet did not influence

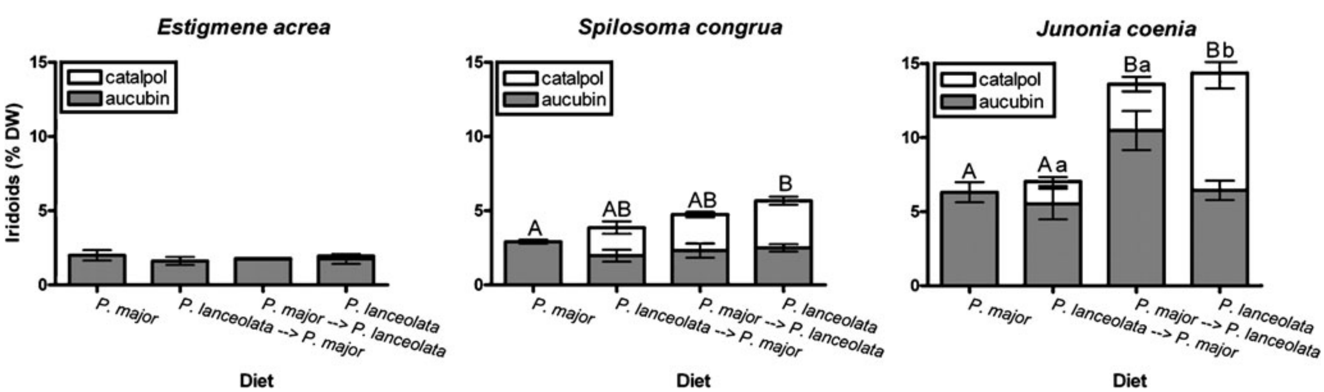

Fig. 2 Average (±1 SEM) iridoid glycoside sequestration of three caterpillar species varying in degree of specialization. *Uppercase letters* indicate significantly ($P<0.05$) different total iridoid glycoside means according to Tukey's Honestly Significant Difference post-hoc tests, while *lowercase letters* indicate significant differences in the proportion of catalpol

E. acrea sequestration ($F_{3,26}$=0.26, P=0.85). *Spilosoma congrua* was particularly effective at sequestering catalpol. Catalpol made up the majority of the sequestered iridoids and proportion of catalpol was not influenced by diet ($F_{2,21}$=0.71, P=0.50); however, diet did influence total iridoid sequestration ($F_{3,30}$=8.18, P<0.001) (Fig. 2). Diet influenced both the proportion of catalpol ($F_{2,23}$= 82.68, P<0.001) and total iridoids ($F_{3,31}$=5.80, P=0.003) sequestered by *J. coenia* (Fig. 1).

Discussion

The three species differed substantially in their sequestration ability. *Junonia coenia*, a specialist on iridoid glycoside containing plants, sequestered the highest amounts. The amount of total iridoid glycosides was almost three times higher when these larvae were fed solely *P. lanceolata* than when they were fed *P. major*. Feeding experiments with wolf spiders showed that such a difference renders *P. lanceolata*-reared *J. coenia* less palatable than those reared on *P. major* (Theodoratus and Bowers, 1999). In addition, however, catalpol appears to be a more toxic iridoid than aucubin (Puttick and Bowers, 1988), which may also contribute to this difference in palatability. *Spilosoma congrua* sequestered about one third the levels of iridoids sequestered by *J. coenia*. Of three *Spilosoma* species tested, *S. congrua* was the only species with the ability to sequester iridoid glycosides; its congeners, *S. virginica* and *S. latipennis* were unable to sequester iridoids (Bowers and Stamp, 1997). Interestingly, although *E. acrea* was able to sequester aucubin in about the same amounts as *S. congrua*, it was poor at sequestering catalpol. *Estigmene acrea* thus was particularly effective at breaking down or eliminating catalpol.

In both *S. congrua* and *J. coenia*, diet had a strong effect on the amount of iridoids sequestered. Since most food is eaten and most iridoids are ingested in the later instars, the host plant fed on during the later instars most affected the chemical content of the caterpillars. This was most apparent in *J. coenia*, in which feeding on *P. lanceolata* in the later instars doubled the amount of sequestered iridoids compared to larvae fed only *P. major* or *P. lanceolata* followed by *P. major*. Indeed, larvae fed *P. lanceolata* after *P. major* had levels of total iridoid glycosides almost as high as larvae reared exclusively on *P. lanceolata* (Fig. 2). However, feeding exclusively on *P. lanceolata* resulted in much higher levels of catalpol being sequestered compared to larvae fed on *P. major* followed by *P. lanceolata*. Although less dramatic, similar results were found for *S. congrua*. These results emphasize the importance of host plant choice in later instars, as that affects sequestration and consequent protection against natural enemies.

Both arctiid species in this study graze among several plant species, including species that vary widely in secondary chemistry (Bernays et al., 2004). Depending on the plant species consumed, these caterpillars can sequester quite different profiles of secondary compounds, and potentially vary dramatically in their palatability to natural enemies. Furthermore, *E. acrea* can sequester a quite different group of compounds, pyrrolizidine alkaloids, and retain these compounds through to the adult stage in amounts as high as approximately 4% dry weight (Hartmann et al., 2005). Thus, larvae of *E. acrea* are better at sequestering pyrrolizidine alkaloids than iridoids. However, given the polyphagous feeding behavior of this species, it could encounter both pyrrolizidine alkaloid and iridoid glycoside containing host-plants and could sequester both kinds of compounds.

In summary, this study provides the first quantification of sequestration of iridoid glycosides by *E. acrea*, and showed that only low levels are sequestered and that catalpol is inefficiently sequestered, if at all. Second, we showed that host plant species affects sequestration of iridoids in *S. congrua* and *J. coenia*, but not *E. acrea*. Third, the variation in sequestration among diets was greatest for *J. coenia*, the species sequestering the highest amounts and concentrations of iridoid glycosides. Overall, our results show that although plant defense compounds may be sequestered by both generalist and specialist insects, the amounts that different species contain and the efficiency with which those compounds are sequestered may vary considerably.

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