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SPECIALIST WEEVIL, *Rhyssomatus lineaticollis*, DOES NOT SPATIALLY AVOID CARDENOLIDE DEFENSES OF COMMON MILKWEED BY OVIPOSITING INTO PITH TISSUE

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Abstract—*Rhyssomatus lineaticollis* is a milkweed specialist whose larvae feed upon pith parenchyma in ramet stems of the common milkweed, *Asclepias syriaca*. Compared with other specialist insect herbivores on milkweeds, this curculionid beetle is unusual in that it is cryptically colored and does not sequester cardenolides characteristic of milkweed chemical defense. Based upon optimal defense theory, we predicted that pith tissue would be low in defensive compounds and that oviposition into the pith would spatially avoid cardenolides. We rejected this hypothesis because we found that pith tissue has a relatively high cardenolide concentration compared to cortex, epidermis, and leaf tissues. Moreover, we found total plant cardenolide concentration was lower in plants that contained the beetle eggs. Cardenolide concentrations were different among tissues in intact stems without the pith herbivore compared to stems where it was present. Furthermore, the overall polarity of the cardenolides present varied among plant tissues and between plants with and without *R. lineaticollis* eggs. Although we found lower concentrations of cardenolide in piths where the eggs were present, the cardenolides present in the pith contained more nonpolar forms, indicating that the plant may be responding to herbivory by increasing toxic efficacy of cardenolide defenses while lowering the total concentration. We suggest that preoviposition behavior by female beetles, which includes feeding on new leaves of the plant, is a mechanism by which females manipulate plant chemistry and assess quantitative and qualitative changes in cardenolide chemistry in response to herbivory prior to oviposition.

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Key Words—Milkweed, *Asclepias syriaca*, Asclepiadaceae, *Rhyssomatus lineaticollis*, Curculionidae, herbivory, induced chemical defense, specialist herbivore, cardenolide, defense theory, oviposition, allelochemical tissue distribution, feeding guild.

INTRODUCTION

Herbivores in different feeding guilds present plants with a variety of challenges because they attack different tissues. Defenses against leaf chewers are likely to be different from those against leaf miners or against stem borers, either because the tissues or organs attacked have different values to the plant (Zangerl and Bazzaz, 1992) or because internal plant morphology, physiology and metabolism constrains the deployment of chemical defenses (Jones et al., 1993). Nevertheless, little is known about how plants invest in different chemical defenses or delivery systems against different herbivore feeding guilds. It is likely that herbivores specializing on groups of plants with characteristic chemical defense mechanisms will have behavioral strategies (Tallamy, 1986; Compton, 1987; Dussourd and Eisner, 1987; Stamp and Casey, 1993; Dussourd, 1997) or physiological means (Lindroth, 1991; Berenbaum and Zangerl, 1992) to overcome these defenses. Furthermore, plant defenses can influence herbivore phenology (Feeny, 1976; Chew and Courtney, 1992; Jordano and Gomariz, 1994) and where on the plant herbivory occurs (Malcolm, 1990, 1991; Zangerl and Rutledge, 1996).

Optimal defense theory predicts that allocation of chemical defenses should depend upon the relative value of tissues or structures to the plant (Feeny, 1976; Rhoades and Cates, 1976; McKey, 1979; Rhoades, 1979; Nitao and Zangerl, 1987; Zangerl and Bazzaz, 1992; Zangerl and Nitao, 1998). For example, Berenbaum et al. (1986) showed that higher chemical defense investment in reproductive tissues conferred higher fitness to the plant. Furthermore, tissues that are low in nutritive content for potential herbivores are predicted to have low concentrations of defensive compounds. For example, stem tissues, which are high in nutritionally deficient materials such as cellulose and lignin, may be low in defense (Zangerl and Bazzaz, 1992). However, if herbivores specialize on regions of the plant assumed to be of lower value to the plant, selection may then favor defense for these regions since any herbivore damage has the potential to reduce plant fitness (Strong et al., 1984; Karban and Baldwin, 1997).

In this study we tested the hypothesis that pith feeding by the larvae of a specialist herbivore may be an effective life history strategy to circumvent the cardenolide defenses of the common milkweed, *Asclepias syriaca* (Malcolm et al., 1989). Because the pith is primarily composed of parenchyma and lacks most of the laticifer system characteristic of other milkweed tissues, we predicted that pith herbivory may be an effective means of spatially avoiding plant defenses. We also investigated whether allocation of cardenolide defenses in this

milkweed varied in quantity and quality among tissues and if this allocation differed in plants with the herbivore present compared to plants in which it was absent.

Study System. The curculionid beetle, *Rhyssomatus lineaticollis* (Say), is a specialist herbivore of milkweeds (Asclepiadaceae). Unlike most other specialist insect herbivores of milkweeds that are characteristically aposematically colored and sequester cardenolides from the plant (Scudder and Duffey, 1972; Isman et al., 1977; Cohen and Brower, 1983; Seiber et al., 1984; Malcolm, 1991, 1995), *R. lineaticollis* is a nonsequestering, cryptic specialist (Nishio et al., 1983). It is the earliest and most common herbivore attacking the common milkweek (*Asclepias syriaca*) in Michigan as soon as the ramets of this highly modular milkweed appear in late May. On average, we observed that 37% of *A. syriaca* stems (ramets) per patch (genet) are attacked by the beetle (min. 11%, max. 56%: $N = 12$ patches), making it an extremely important herbivore of *A. syriaca*.

Little information exists concerning the life history of this beetle. We observed that adult beetles feed primarily on newly flushing leaves and cause extensive damage to the apical meristem of *A. syriaca* ramets as soon as they appear in May and June. Conflicting reports state that the larvae only feed as stem borers (Wilbur, 1976) or on seeds (Blatchley and Leng, 1916; Price and Willson, 1979; Nishio et al., 1983). In Michigan, the species may be univoltine or bivoltine. In 1993, during an uncharacteristically cool summer, we found only one generation of adults that laid eggs exclusively in the stems of milkweeds where the larvae fed on pith tissues within stem internodes. In 1994, 1995, and 1996, however, we observed a small second brood in September that laid eggs in the pericarp of the follicular fruit and we observed larvae feeding on the pericarp tissue of the seed pod. All observations herein describe the behavior of the much larger early summer generation that produced only stem-boring larvae.

In late May through mid-July, ovipositing females were observed chewing a small hole in the lower stem of *A. syriaca* ramets and then turning around to lay a single egg in the hole directly at the interface between the vascular tissue and the pith. This procedure was repeated numerous times, always directly above the most recently deposited egg and within the same internode to form a longitudinal, vertical scar 2–4 cm long on the stem that became covered with solidified latex and eventually turned black. Despite the large investment of time and effort during oviposition behavior, abortive behavior was common in which there were ovipositor insertions but no egg depositions. In fact, only 30% of ovipositor insertions into the stem resulted in egg deposition ($N = 143$ stems). When the larvae emerged they fed exclusively on pith tissue within a single internode, and they are apparently unable to pass between nodes. Larvae most likely pupate and overwinter in the soil, as has been observed in a congener (Strathie and Hoffmann, 1993), although this has not been confirmed for *R. lineaticollis*.

METHODS AND MATERIALS

Collection of Plant Material. Plant samples ($N = 370$ plants) were collected between June 25 and July 15, 1993, in Kalamazoo County, Michigan, USA. Each sampling consisted of *A. syriaca* ramets with *R. lineaticollis* oviposition scars and intact nearest neighbors. The scarred region of the stem and the superior leaf adjacent to the internode were packaged in Whirl Paks and frozen at −80°C. Control stems from intact neighbors were prepared in the same way using an internode from the same region of the plant. From these samples, a subsample of 12 scarred stems with *R. lineaticollis* eggs and 12 intact stems were randomly selected for analysis of cardenolides to determine if pith tissue contained cardenolide defenses and whether distribution of cardenolides agreed with our hypothesis that oviposition into the pith tissue spatially avoids these plant toxins. Of the scarred stems, only those stems containing unhatched eggs were considered for this subsample.

Extraction Procedure. Frozen stems were freeze dried and then dissected to separate epidermal, cortex, vascular, and pith tissues. Together with leaf samples, this generated a total of 12 replicates × 5 tissues × 2 treatments for a total of 120 samples for cardenolide analyses. Leaf tissue and each separated stem tissue were grown in a mortar and then weighed and placed in a centrifuge tube with 4 ml of 70% methanol. Each sample was heated to 55°C and sonicated for 20 min. Pigments were precipitated using 1 ml of 15% lead acetate, followed by 1 ml 4% sodium phosphate after mixing and cooling on ice (Wiegrebe and Wichtl, 1993). Samples were diluted to 10 ml with 4 ml water and centrifuged at high speed for 10 min.

Following Wiegrebe and Wichtl (1993), cardenolides were extracted from the supernatant using a C_{18}-modified polymeric solid phase extraction column (Polysorb MP-1, 100 mg column from Interaction Chromatography from Transgenomic Inc., Omaha, Nebraska, USA). The 10-ml supernatant was passed through the column followed by 2 ml of water. The pooled "load" fraction was saved for HPLC analysis because, unlike the results of Wiegrebe and Wichtl (1993), very polar cardenolides were not retained on the column. However, those cardenolides retained on the column were eluted with 2 × 2 ml of 100% methanol as the methanol fraction. Each fraction was dried using a Savant Speed Vac Plus vacuum centrifuge. Samples were resuspended in 1 ml 100% methanol and passed through 0.45-μm Nylon Acrodisc syringe filters into 1-ml autosampler vials before high performance liquid chromatography (HPLC). Because of errors, one stem with *R. lineaticollis* absent and two infected stems were discarded, leaving 11 uninfected stems and 10 infected stems for analysis.

HPLC Procedure. Sample analyses were performed using the method of Wiegrebe and Wichtl (1993) on a Waters gradient HPLC system with WISP autosampler, 600E pump, 996 diode array detector, and Millennium 2010 chro-

matography software. The reverse-phase elution gradient was acetonitrile–water at 1.2 ml/min at 40°C, with 20% acetonitrile at the start, to 32% after 35 min, 40% after 45 min, 50% after 55 min, then back to 20% at 61 min, and 20% at 65 min, on a 250-4 LiChroCART RP-18 column packed with 5 μm LiChrospher 100 (E. Merck), with a 10-mm guard column packed with the same material. Sample injections were 20 μl. Load fractions were chromatographed for 35 min (single gradient from 20% to 32% acetonitrile), and methanol fractions were separated for 65 min. Each sample was run after a 15-min column equilibration at 20% acetonitrile.

Cardenolides were detected at 218.5 nm and identified by their symmetrical spectra between 205 and 235 nm and a λ_{max} of between 214 and 225 nm. Only cardenolide peaks reported by Millennium 2010 software as consistently pure were considered for analysis. Data for load and methanol fractions for each sample were pooled to give the total cardenolide concentration per sample.

Data Analysis. We used multivariate analysis of variance (MANOVA) to determine if there were significant overall effects of the presence or absence of *R. lineaticollis* on the amount of cardenolide (micrograms per 0.1 g dry wt) found in each of the tissues analyzed. Subsequent univariate ANOVAs were used to determine which of the tissues contributed most to the overall effect. Intraplant differences in cardenolides among tissues for ramets with or without *R. lineaticollis* oviposition was determined using a blocked ANOVA, with each ramet treated as a block.

Qualitative analyses of cardenolides present were carried out in two distinctly different ways: one to determine overall polarity of the mixture of cardenolides present, and the other to detect differences in the amounts of each particular cardenolide. We used a MANOVA to determine if the overall lipophilicity (i.e., the retention time and amount of each cardenolide detected) differed among intact ramets without *R. lineaticollis* compared to scarred ramets where it was present. This was done because we were interested in the potential toxic efficacy of the cardenolides present, here interpreted as lipophilicity because lipophilic cardenolides are generally regarded as bitter tasting and can easily pass through membranes and disrupt cellular functions (Malcolm, 1991). Similarly, we used a block ANOVA to characterize how tissues differ in the polar character of cardenolides present. Both of these analyses were carried out using rank transformed data (Conover, 1999). Rank transformation was used because of heteroscedasticity of the data and because ranks are in essence categorical (distribution free), as is each cardenolide.

Differences in the concentration of each cardenolide present among the tissues were analyzed using a Friedman test (Conover, 1999). Comparisons between the concentration of each cardenolide in ramets with and without *R. lineaticollis* eggs were carried out using Mann-Whitney *U* tests (Sokal and Rohlf, 1995). Nonparametric tests were used for these analyses because the data did

not meet the assumptions of parametric techniques. Additionally, we compard the relative allocation of each cardenolide (i.e., percentage of total cardenolide concentration in each tissue that each cardenolide contributes) between ramets, with and without the pith herbivore. We did this analysis because it is another way to assess qualitative change and we were interested whether the relative amount of each cardenolide change as the total amount of cardenolide changed (quality vs. quantity). These analyses were also carried out using the Mann-Whitney *U* test on the percent contribution of each cardenolide between ramets with and without the herbivore (Sokal and Rohlf, 1995).

RESULTS

Quantitative Analysis. Scarred ramets, where *R. lineaticollis* was present, had a 33% lower cardenolide concentration than ramets without the herbivore (Table 1; Figure 1). Results of subsequent univariate tests indicated that vascular and cortex tissue were most responsible for the lower concentration of cardenolide in stems where *R. lineaticollis* was present compared to stems where it was absent. Leaf, epidermis, and pith tissue showed no detectable difference in the mean cardenolide concentration between ramets where *R. lineaticollis* was present or absent (Table 1).

TABLE 1. MANOVA AND UNIVARIATE ANOVAS ON CARDENOLIDE CONCENTRATION (μg/0.1 g dw) OF PITH, VASCULAR, CORTEX, EPIDERMIS, AND LEAF TISSUES IN RAMETS WITH AND WITHOUT *R. lineaticollis*.

Source	*df*	Wilks' lambda	*F*	*P*
MANOVA				
R. lineaticollis presence	5, 13	0.353	4.742	0.011
ANOVA				
Pith	1		0.568	0.461
Error	17			
Vascular	1		5.131	0.037
Error	17			
Cortex	1		5.687	0.029
Error	17			
Epidermis	1		1.466	0.243
Error	17			
Leaf	1		1.898	0.186
Error	17			

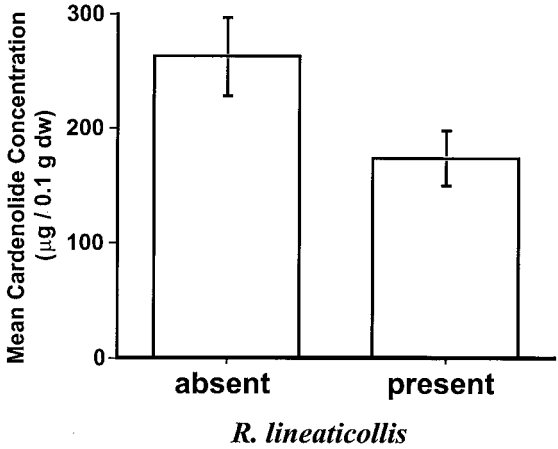

FIG. 1. Mean cardenolide concentration in milkweed ramets with and without *R. lineaticollis* (mean ± SE).

Comparisons among leaf, epidermis, cortex, vascular, and pith tissues show that allocation of cardenolides among tissues was not equal (Table 2) in both intact ramets and scarred ramets (Figure 2). Vascular tissue samples, which include much of the laticifer system of the stem, contained 56% of the total cardenolide concentration in intact ramets, significantly higher than all other tissues. Similarly, in scarred ramets, vascular tissue contained a higher concentration than other tissues analyzed. Pith tissue had the second highest concentration of cardenolide in groups with *R. lineaticollis* present and *R. lineaticollis* absent. Interestingly, leaf tissue, the tissue most often analyzed for plant cardenolide

TABLE 2. ANOVA TABLES FOR CARDENOLIDE CONCENTRATION (μg/0.1 g dw) OF PITH, VASCULAR, CORTEX, EPIDERMIS, AND LEAF TISSUES.

Source	*df*	MS	*F*	*P*
Rhyssomatus lineaticollis absent				
Ramet (Block)	10	3165.078	1.626	0.136
Tissue	4	25895.704	13.306	<0.001
Error	38	1946.206		
Rhyssomatus lineaticollis present				
Ramet (Block)	9	1135.930	1.584	0.157
Tissue	4	8008.644	11.171	<0.001
Error	36	716.921		

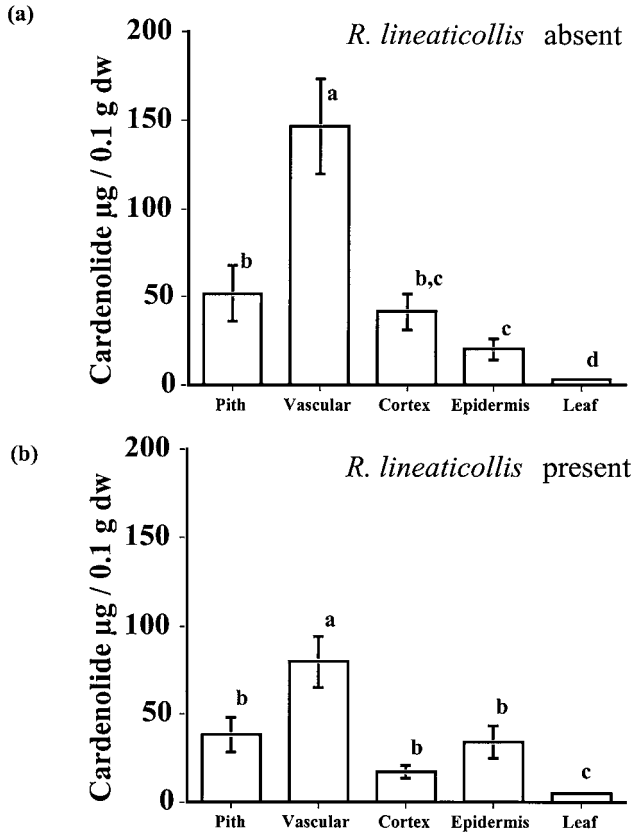

FIG. 2. Mean cardenolide concentration of tissues from ramets with andwithout *R. lineaticollis* (mean ± SE). Means followed by the same letter in each figure are not significantly different as $P < 0.05$ (Student-Newman-Keuls).

content (Malcolm, 1991, and references therein), had the lowest concentration of cardenolide.

Qualitative Analysis. A MANOVA on the rank transformed data of cardenolide retention times indicated that scarred ramets had more lipophilic cardenolides than intact ramets (Table 3). Subsequent univariate test indicated that pith and vascular tissues account for most of this difference in lipophilicity (Table 3; Figure 3). Leaf tissue contrasted with pith and vascular tissues by having significantly more polar cardenolides in scarred ramets than in intact ramets. Comparisons among the five tissues analyzed in this study revealed that pith and vascular tissue of intact ramets had mostly polar cardenolides compared to cortex, epi-

TABLE 3. MANOVA AND UNIVARIATE ANOVAS ON LIPOPHILICITY (RETENTION TIME) OF PITH, VASCULAR, CORTEX, EPIDERMIS, AND LEAF TISSUE CARDENOLIDES IN RAMETS WITH AND WITHOUT *Rhyssomatus lineaticollis*

Source	*df*	Wilks' lambda	*F*	*P*
MANOVA				
R. lineaticollis presence	5, 11	0.301	5.108	0.012
ANOVA				
Pith	1		4.803	0.045
Error	15			
Vascular	1		5.118	0.039
Error	15			
Cortex	1		0.511	0.486
Error	15			
Epidermis	1		0.646	0.434
Error	15			
Leaf	1		6.486	0.022
Error	15			

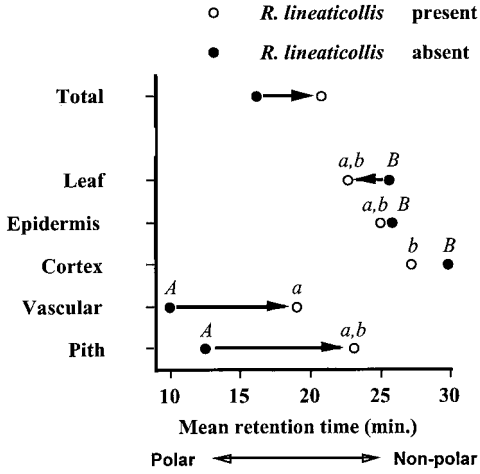

FIG. 3. Mean retention time for all cardenolides present in ramets with and without *R. lineaticollis*. Longer retention times correspond to higher lipophilicity. Comparisons of retention times among tissues are indicated with letters. Same letters of the same case are not significantly different at $P < 0.05$ (Student-Newman-Keuls) Capital letters indicate comparisons among tissues from ramets where *R. lineaticollis* was absent, lowercase from ramets where *R. lineaticollis* was present. Arrows indicate significant difference ($P < 0.05$) in lipophilicity between ramets with and without *R. lineaticollis*.

TABLE 4. ANOVA TABLES OF MEAN OVERALL LIPOPHILICITY OF PITH, VASCULAR, CORTEX, EPIDERMIS, AND LEAF CARDENOLIDES

Source	*df*	MS	*F*	*P*
Rhyssomatus lineaticollis absent				
Ramet (block)	10	3.855	2.812	0.011
Tissue	4	30.963	22.702	<0.001
Error	37	1.364		
Rhyssomatus lineaticollis present				
Ramet (block)	9	3.035	2.766	0.015
Tissue	4	3.415	3.113	0.027
Error	35	1.097		

dermis, and leaf tissues (Table 4; Figure 3). However, in scarred ramets, this distinction was less clear because pith, vascular, epidermal, and leaf tissues all had a similar range of cardenolide polarities due primarily to a decrease in the polarity of pith and vascular tissue cardenolides (Figure 3). Degrees of freedom in these analyses differ from the quantitative analysis because one intact stem had no detectable epidermal cardenolides, and thus no mean retention time.

Comparisons of the concentration of each cardenolide among the tissues are presented in Table 5. These peaks have yet to be identified and are currently only distinguished by their retention times. Seven of the 10 significant differences in overall polarity among the tissues occurred in scarred ramets where *R. lineaticollis* was present, possibly due to a reallocation of defenses among tissues after herbivore attack. Total concentration of each cardenolide compared between tissues in scarred and intact ramets showed that most changes occurred in pith and leaf tissues (Figure 4). Percent contribution of each cardenolide to the total cardenolide concentration of each tissue differ among tissues in intact ramets compared to scarred ramets (Figure 5). Most notably, the highly polar cardenolides of the pith are at lower concentrations in scarred ramet stems. Similarly, the percentage that each of these compounds contributes to the total cardenolide concentration of the pith suggests a lower allocation of highly polar compounds and a higher allocation of nonpolar compounds in ramets where the herbivore is present.

DISCUSSION

Our results lead us to reject our initial hypothesis that placement of eggs into pith tissue by *R. lineaticollis* is an effective strategy for spatially avoiding cardenolide defenses of *A. syriaca* because pith tissue contained a high concentration of cardenolides, significantly higher than leaf tissue. Pith feeding may, however,

TABLE 5. COMPARISONS OF TOTAL CONCENTRATION OF EACH CARDENOLIDE[a] AMONG PITH, VASCULAR, CORTEX, EPIDERMIS, AND LEAF TISSUES IN RAMETS WITH AND WITHOUT *R. lineaticollis* LARVAE

Cardenolide retention time (min)	*R. lineaticollis*	χ^2	*P*	Multiple comparisons[b] high ⟶ low concentration
(A) 3.7	*absent*	5.591	0.232	
	present	11.019	0.019	c_1, e_2, v_2, p_2, l_2
(B) 5.5	*absent*	28.736	<0.001	v_1, p_2, e_3, c_3, l_3
	present	19.660	<0.001	v_1, p_2, e_2, c_2, l_2
(C) 11.2	*absent*	3.648	0.456	
	present	13.721	0.008	p_1, $e_{1,2}$, $v_{1,2}$, $l_{1,2}$, c_2
(D) 15.6	*absent*	6.510	0.164	
	present	3.553	0.470	
(E) 21.3	*absent*	5.000	0.280	
	present	11.273	0.024	p_1, e_1, v_1, c_2, l_2
(F) 27.4	*absent*	3.634	0.458	
	present	7.844	0.097	
(G) 29.3	*absent*	2.385	0.665	
	present	6.240	0.182	
(H) 34.5	*absent*	8.706	0.069	
	present	17.667	0.001	v_1, p_2, c_2, e_2, l_2
(I) 39.5	*absent*	9.524	0.049	l_1, c_1, e_1, p_2, v_2
	present	8.000	0.092	
(J) 43.1	*absent*	20.750	<0.001	c_1, v_1, p_2, $e_{2,3}$, l_3
	present	23.455	<0.001	v_1, c_2, p_2, e_2, l_2
(K) 49.5	*absent*	4.000	0.406	
	present	2.077	0.722	
(L) 53.5	*absent*	3.500	0.478	
	present	10.593	0.032	e_1, $p_{1,2}$ $c_{1,2}$, $v_{1,2}$, l_2

[a] Friedman's test (Conover 1999).

[b] Multiple comparisons for Friedman's test as described by Conover (1999). Like subscript numbers indicate no significant difference at $P < 0.05$. Tissue abbreviations are p = pith, v = vascular, c = cortex, e = epidermis, l = leaf.

permit larvae to circumvent latex because the pith does not have the extensive laticifer system associated with other plant regions, most notably in the vascular and leaf tissues (Wilson and Mahlberg, 1978, 1980). Latex can be an effective means of deterring herbivory and many specialists have developed behaviors to disrupt latex flow (Compton, 1987, 1989; Dussourd and Eisner, 1987; Zalucki and Brower, 1992). We had predicted that there would be low investment in defensive compounds in pith tissue based upon an optimal defense theory argument (McKey, 1979), because pith tissue is loosely packed with large parenchymal cells and is not closely linked to plant function or reproduction. This pre-

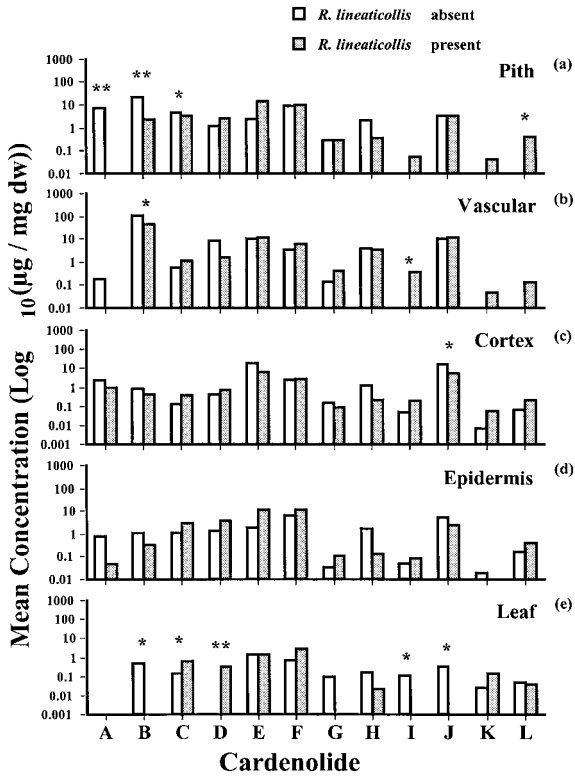

FIG. 4. Mann-Whitney *U* comparisons of mean concentration of each cardenolide per tissue between ramets with and without *R. lineaticollis*; $^{*}P < 0.05$, $^{**}P < 0.01$. Cardenolides are arranged alphabetically by increasing retention times. Retention time of each cardenolide is given in Table 5.

diction of optimal defense theory is supported by the work of Rosenthal and Welter (1995), who found that stem boring has minimal impact on plant fitness as measured by seed number and biomass. It is not known whether pith herbivory on *A. syriaca* directly affects plant fitness through either loss of biomass or increased susceptibility to other natural enemies including pathogens. However, optimal defense theory also predicts that allocation of defense is based in part on the probability of herbivore attack (Feeny, 1976; Rhoades and Cates, 1976; Rhoades, 1979; McKey, 1979). In the case of *R. lineaticollis* the probability of pith attack is high and so the plant may respond with an increased investment in cardenolides possibly triggered by chewing of oviposition scars by females. Although variation in cardenolide investment on a temporal scale

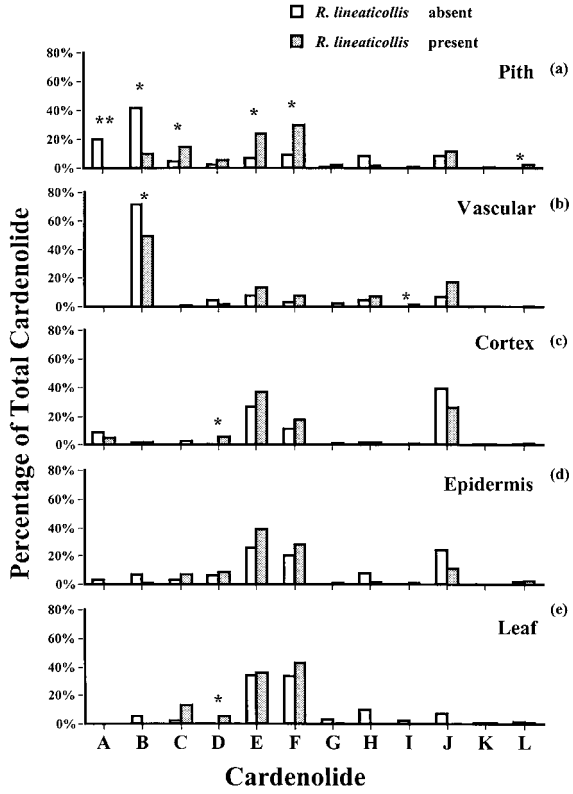

FIG. 5. Mann-Whitney *U* comparisons of the percent contribution of each cardenolide towards the total cardenolide concentration of each tissue between ramets without and with *R. lineaticollis*, $^{*}P < 0.05$, $^{**}P < 0.01$]. Cardenolides are arranged alphabetically by increasing retention times. Retention times of each cardenolide are given in Table 5.

longer than 148 hr (Malcolm and Zalucki, 1996) has not been determined for *A. syriaca*, Nelson et al. (1981) observed decreasing cardenolide levels in *A. eriocarpa* stems in California over the course of the growing season. The relatively low cardenolide levels found in our samples of *A. syriaca* leaf tissue may be explained by the absence of many of the important leaf herbivores that occur later in the growing season (Morse, 1985), after *R. lineaticollis* larvae have completed their pith-feeding stages.

The significance of qualitative variation in cardenolides present, both between ramets with and without *R. lineaticollis* and among tissues, is difficult to assess. Here we used a mean retention time for all the cardenolides present as a measure of lipophilicity. Nothing is known about how *R. lineaticollis* manages

cardenolides, but other milkweed specialists, like the monarch butterfly (*Danaus plexippus*) metabolize cardenolides of lower polarity to more polar forms (Malcolm and Brower, 1989; Malcolm, 1991; Nelson, 1993), perhaps because polar cardenolides are easier to store in the sequestered defense characteristics of the monarch (Malcolm, 1995). Because of the lipophilic nature of nonpolar cardenolides, they can easily pass across the gut membranes and thus may have a greater-toxic effect than polar forms, although once across the gut membrane polar cardenolides are likely to be more toxic (Malcolm, 1991). This may explain why those tissues that an herbivore encounters first, such as the leaf and stem epidermis, had the most lipophilic cardenolides. Additionally, these less polar cardenolides may be more easily detected by herbivores than more polar compounds and may act as a deterrent or repellent signal to herbivores (Malcolm, 1991). Thus, qualitative changes in plant chemistry may play a role in plant defense that is as important as that of more general quantitative changes in response to herbivory. We found that pith tissue in scarred stems where *R. lineaticollis* was present had a lower overall cardenolide concentration compared to pith tissue in intact stems. However, this lower concentration of cardenolides contained more lipophilic forms and may increase the efficacy of plant-derived cardenolides against developing larvae of *R. lineaticollis*.

The difference in both the quantity and quality of cardenolides present in plants with and without *R. lineaticollis* can be interpreted in two ways. First, if we assume that females choose when and where they oviposit based upon plant chemical signals, they may sample a range of ramets or even internodes within a plant and choose to lay eggs in stems that are likely to be most suitable for their offspring. Second, because females feed and mate on the flushing leaves at the top of *A. syriaca* ramets before they lay eggs in the stem, the females may manipulate plant defense to be favorable to their offspring by causing it to respond to herbivore cues before the larvae are present.

Induced responses to herbivory resulting in changes in secondary compounds that are believed to function as defenses have been well documented (Karban and Baldwin, 1997). Malcolm and Zalucki (1996) found that cardenolides in *A. syriaca* are rapidly induced by damage and then quickly return to, or below, constitutive levels. They suggested that this was a plant response to the specialist herbivore *Danaus plexippus* and may resolve the paradox of a plant investing in chemical defenses that benefit sequestering specialists because rapid induction compromised larval survivorship and growth. Unlike *R. lineaticollis*, *D. plexippus* adults can only assess plant quality at the time of oviposition (Zalucki et al., 1990) and are incapable of determining the future induced chemical response. Induced plant responses to herbivory can be an effective strategy for plants to reduce herbivore pressure (Karban and Baldwin, 1997; Agrawal, 1998). *R. lineaticollis* offers a unique problem to milkweeds in that induction of plant chemicals in the region of larval feeding may be caused by the adult beetle

and not the larvae. Although we did not explicitly test whether female *R. lineaticollis* feeding prior to oviposition causes a similar induction of cardenolides, adult female *R. lineaticollis* exhibit feeding behaviors similar to *D. plexippus* larvae. Specifically, they also sever or puncture the leaf midrib to disrupt latex and feed on the distal portion of the leaf blade (Compton, 1987; Dussourd and Eisner, 1987). If the induced response of the plant is similar for these two herbivores, it may explain why ramets in which *R. lineaticollis* larvae were found contained a lower concentration of cardenolides compared to ramets where the weevil was absent, because initially induced cardenolides would have had sufficient time to reduce back to, or below, constitutive levels (Malcolm and Zalucki, 1996).

The variation in both the quality and the quantity of the cardenolides found among the tissues we analyzed demonstrates that each feeding guild on *A. syriaca* is confronted with unique defenses. Each of these guilds may not be independent of one another, however, even if they are separated spatially and temporally because feeding on one tissue may influence the defensive chemistry of other tissues. Herbivore-induced changes in plant chemistry make herbivore communities on a single plant dynamic systems where the plant is a medium for inter- and intraspecific interactions because plant responses to one herbivore can impact performance of subsequent herbivores (Hartley and Lawton, 1987; Agrawal, 1999). Preoviposition feeding behaviors of *R. lineaticollis* adults may be a means for larvae to temporally avoid the early induced response of plant cardenolides, rather than spatially avoiding them, as we had initially hypothesized. Female *R. lineaticollis* may be manipulating the plant chemistry to which larvae will be exposed and subsequently oviposit on plants that they assess having the least risk to larval fitness.

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