

PLANT LATEX AND FIRST-INSTAR MONARCH
LARVAL GROWTH AND SURVIVAL ON THREE
NORTH AMERICAN MILKWEED SPECIES

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Abstract—First-instar larvae of the monarch butterfly, *Danaus plexippus*, a milkweed specialist, generally grew faster and survived better on leaves when latex flow was reduced by partial severance of the leaf petiole. The outcome depended on milkweed species and was related to the amount of latex produced. The outcome also may be related to the amount of cardenolide produced by the plants as a potential chemical defense against herbivory. Growth was more rapid, but survival was similar on partially severed compared with intact leaves of the high-latex/low-cardenolide milkweed, *Asclepias syriaca*, whereas both growth and survival were unaffected on the low-latex/low-cardenolide milkweed *A. incarnata*. On the low-latex/low-cardenolide milkweed *A. tuberosa*, both growth and survival of larvae were only marginally affected. These results contrast sharply to previous results with the milkweed, *A. humistrata*, in Florida, which has both high latex and high cardenolide. Larval growth and survival on *A. humistrata* were both increased by partially severing leaf petioles. Larval growth rates among all four milkweed species on leaves with partially severed petioles were identical, suggesting that latex and possibly the included cardenolides are important in first-instar monarch larval growth, development, and survivorship.

Key Words—*Asclepias*, cardenolide, *Danaus plexippus*, growth rate, latex, laticifer, milkweed, neonate larvae, plant defense, survival.

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INTRODUCTION

Small, toxic molecules in plants have been argued to be effective defenses against generalist herbivores but not against specialist herbivores (Feeny, 1976; Rhoades and Cates, 1976). Both apparency theory and optimal defense theory argue that toxic products of secondary plant metabolism, such as cyanogenic glycosides, alkaloids, and cardenolides, are effective defenses against most generalist herbivores. The generalization implicit in these theories is that "plant toxins" have little effect on specialists, and this has since been corroborated by evidence that "plant toxins" can have little negative impact on the growth and survival of specialized herbivores (e.g., Berenbaum, 1981; Scriber, 1984; Tallamy and McCloud, 1991; McCloud et al., 1995). However, it is now becoming clear that many specialist herbivores are negatively affected by small, toxic molecules (Gould, 1988; Baldwin, 1989, 1991; Karban, 1991) and that these defenses can be extraordinarily variable in time and many are strongly and rapidly inducible by specialist herbivore feeding (Baldwin and Ohnmeiss, 1994; Baldwin et al., 1994a,b; Karban and Niiho, 1995; Karban and Adler, 1996).

Here we report the results of field experiments to investigate the effects of highly mobile, toxic plant defenses on the growth and survivorship of a specialist insect herbivore. The monarch butterfly, *Danaus plexippus* L., feeds almost exclusively on milkweeds in the genus *Asclepias* (Ackery and Vane-Wright, 1984; Malcolm and Brower, 1986; Malcolm, 1991, 1995). Both monarch larvae and adults are thought to benefit from host-plant-derived chemical defenses, which they sequester for use in their own defense against natural enemies (Malcolm, 1991, 1995), rather than suffer growth or survival costs associated with these plant chemicals. These defenses include cardenolides, a group of toxic steroids with low molecular weights, that target the ubiquitous Na^+ , K^+ -ATPase receptor sites of all animal consumers (Horisberger, 1994). Cardenolides occur constitutively throughout the plant and in the white, milky latex characteristic of almost all *Asclepias* species (Malcolm, 1995), and like some other secondary compounds, they are inducible by damage to leaf tissue (Malcolm and Zalucki, 1996).

Early-instar monarchs are known to have variable but generally poor survival (Zalucki and Kitching, 1982a), as is common in the Lepidoptera (Dempster, 1983; Kyi et al., 1991). Based on field observations of naturally laid eggs, Zalucki et al. (1990) showed that survival in the first instar was weakly negatively correlated with plant cardenolide concentration in *Asclepias humistrata* in Florida. Zalucki and Brower (1992) subsequently confirmed this observation with a field experiment. They suggested that some of the high, early-instar losses might be related to the cardenolide concentration in latex or to latex per se. However, observations of larval behaviors by others and ourselves suggest that the larvae are adept at avoiding latex and circumventing the mechanical stickiness and possible toxicity of latex (Brewer, 1977; Rothschild, 1977; Dixon et al.,

1978; Dussourd and Eisner, 1987; Dussourd, 1990, 1993; Zalucki and Brower, 1992). Larvae reduce latex flow by various feeding behaviors to trench or partially sever leaf petioles or veins and then they feed on leaf tissue distal to the trenched or partially severed areas. These behaviors suggested that we could mimic this behavior, and pilot experiments showed that we could reduce latex flow in a similar manner by partially severing the petioles of milkweed plants growing in the field.

Here we investigate further the hypothesis that milkweed defenses influence monarch larval growth and survival. First, we measured latex flow in three common northern species of *Asclepias* found in Michigan, and then we performed field experiments that measured the effect of manipulated latex flow on the growth and survival of first-instar monarchs. The three milkweed species were: *A. syriaca* (common milkweed), which is the major host of the monarch butterfly in the summer part of its range (Malcolm et al., 1989, 1993; Malcolm, 1995), *A. incarnata* (swamp milkweed), and *A. tuberosa* (butterflyweed). These species offer an interesting contrast to the southern *A. humistrata* in that their cardenolide levels are much lower, but latex levels are either comparable (as in *A. syriaca*) or much lower (as in *A. incarnata* and *A. tuberosa*).

METHODS AND MATERIALS

Plant Latex Measurement. Latex production in different milkweeds was compared by regressing latex volume samples against the cross-sectional area of their sources. We measured with calipers the cross-sectional diameter of leaf lateral veins, midrib veins, petiole, and stem at various points on plants so that we could calculate the cross-sectional latex source area. Nonarticulated laticifers ramify throughout stem and leaf tissues (Wilson and Mahlberg, 1980; Dussourd, 1993), and so the cross-sectional area was used as the independent variable against which we regressed latex volume. Latex volume was measured by rapid severance at each cross-sectional point and collection of the flowing latex in volumetric capillary tubes. The cumulative volume produced until flow ceased was measured. Each section was cut from a different plant module (stems or ramets from one or more genets) so that prior damage did not influence latex flow.

Larval Experiments. We used monarch eggs, synchronized to hatch simultaneously, glued on leaf disks of experimental plants as illustrated and described in Zalucki and Brower (1992). Eggs laid by wild-caught monarchs bagged around fresh stems of potted *A. curassavica* plants were removed by using a hole punch and were incubated to the "black-head" stage (just prior to larval emergence) for a day-degree (dd) accumulation of 45 dd (Zalucki, 1982). Disks (7 mm diameter) with eggs about to hatch were then glued onto experimental plants (free of wild monarch eggs) by using a small drop of latex from an adja-

cent stem (not included in the experiment). After three to four days (depending on field temperature for the duration of the first instar), surviving larvae were counted and taken to the laboratory where they were weighed wet, dried in a freeze drier, and then reweighed on a Mettler AT261 DeltaRange balance.

In each experiment, we placed 10 leaf disks on each of 10 control plants and 10 on each of 10 treated plants. The partial petiole severance treatment consisted of one of us (for consistency this was M.P.Z. in each case) reducing the latex flow to all leaves on a plant by notching the underside of the petiole three times across approximately 5 mm with a pair of forceps. We recorded the effectiveness of this technique to reduce latex flow by measuring latex volumes taken from a sample of 10 intact and 10 partially severed leaves on different stems of *A. syriaca* 10 min after partial petiole severance. Latex volumes were recorded by weight on preweighed filter paper and were collected from each intact and partially severed leaf from a point cut across the midrib, 4–5 cm from the leaf apex. Partial petiole severance caused a significant reduction in latex volume for both wet weights ($F_{1,18} = 21.9$, $P < 0.001$) and dry weights ($F_{1,18} = 22.1$, $P = 0.002$). Wet weights of latex were reduced by a mean of 52% (22–60%), and dry weights were reduced by a mean of 71% (49–89%).

All partially severed leaves remained turgid and green and were indistinguishable from intact, control leaves. At initiation of each experiment, we recorded plant height, number of leaf pairs, plant flowering status, and the presence and likely cause of preexisting herbivore damage. Disks bearing eggs were placed on the underside of leaves, with no more than three per leaf. At the final assessment, larvae were counted, collected into labeled vials, and placed on ice. The number of unhatched eggs and the number of dead larvae were recorded.

The number of experiments and variations on this procedure are detailed for each plant species below. When comparing effects of experimental treatments within plant species, we used the wet and dry weights of larvae and the proportion that survived. For comparisons among plant species, we converted weights to weight per day degree (dd, above a development threshold of 11.5°C; see Zalucki, 1982), as growth rate will depend (in part) on temperature, and this necessarily varied among experiments run at different times. Daily maximum and minimum temperatures were recorded locally within 10 km of the plant locations.

Host Plant Species. *Asclepias syriaca* (common milkweed) is the most abundant milkweed in North America and is common throughout the Great Lakes region of the United States and Canada in old fields, agricultural fields, and by roadsides (Woodson, 1954; Malcolm et al., 1989). Plants grow to approximately 1.25 m and have large, pubescent leaves. The plant grows in large patches as clonal genets within which the separate, unbranched stems are ramets of the same genet connected by underground, rhizomelike roots (Woodson, 1954; Bhowmik and Bandeen, 1976; Polowick and Raju, 1982). We conducted three

experiments with this species: July 8–11 at a small patch (ca. 700 plants) near the university campus in Kalamazoo; July 17–20 and August 5–8, both at adjacent large patches (ca. 120,000 ramets/patch) on the northeast side of Kalamazoo. The first and last dates represent the set-up and harvest dates, respectively. In all experiments we deliberately chose pairs of similar stems that were close together (1.5–62 cm apart; mean = 23 cm). One plant was designated the control and the other was treated by partial petiole severance (see above). In the first two experiments we removed the top of each plant to prevent flower development. In the second experiment we included five extra control plants from which the tops had not been removed. The results from this treatment suggested an effect of removing plant tops; thus, in the third experiment we discontinued this practice.

Asclepias tuberosa (butterflyweed) is the second most common milkweed in southern Michigan and grows mostly in sandy, well-drained soils. It is a small, much less modular milkweed with a rosette of mostly unbranched stems growing from a single vertical, fleshy root. The leaves are small, narrow, and pubescent. We conducted a single experiment from July 18 to 21 at a patch of approximately 100 plants. This milkweed has multiple stems per plant, and hence treatments were allocated to stems within plants. We treated 10 plants with each stem treatment receiving 10 egg disks.

Asclepias incarnata (swamp milkweed) grows in low-lying wet areas. It is a relatively tall milkweed (approximately 1.5 m) with narrow, smooth leaves. Plants may have multiple or single branched stems. We conducted a single experiment on this species from July 22 to 25 at a small patch (ca. 15 plants) near the university campus. Treatments were either allocated to adjacent plants (five pairs) or to stems within plants (one plant with three treatment pairs and another with two).

RESULTS

Plant Latex Measurement. The volume of latex produced as a function of severed cross-sectional area was described by significant linear regressions for each of the three species measured in Michigan (Table 1). For comparison, data for *A. humistrata* (from Malcolm, 1995) are given, and the four species are ranked by level of latex production by using regression slopes. Analysis of covariance among the regressions showed that there was a difference among the four species ($F_{3,66} = 2.9$, $P = 0.04$). There was also a difference among the slopes of latex volume regressed against latex source cross-sectional area for the four species ($F_{3,66} = 15.7$, $P < 0.001$). However, source area did not predict latex volume from untransformed data ($F_{1,66} = 1.2$, $P = 0.27$), and so the data were log-transformed and the ANCOVA was repeated. By using log-transformed data,

TABLE 1. REGRESSION DATA FOR LATEX VOLUME AGAINST CROSS-SECTIONAL AREA SEVERED IN FOUR MILKWEED SPECIES^a

	Regression data				Area (mm ²) range (x)	Volume (μl) range (y)	Mean cardenolide ^a
	Slope	Intercept	r	N			
<i>A. humistrata</i> ^b	9.7 ^f	18.5	0.90	13	0.01–26	0.3–380	389
<i>A. syriaca</i> ^c	6.6 ^f	62.3	0.87	30	0.07–79	3.8–598	50
<i>A. tuberosa</i> ^d	1.2 ^g	0.4	0.94	6	0.02–3.7	0.1–4.2	3
<i>A. incarnata</i> ^e	1.1 ^g	10.5	0.62	25	0.20–50	0.1–119	14

^aData from Malcolm (1991), cardenolide concentrations were measured in whole leaves. The sample sizes, ranges for *x* and *y* variates, and mean plant cardenolide levels (μg cardenolide/0.1 g dry leaf tissue) are also shown. Regression slopes (untransformed data) followed by the same letter do not differ significantly (*t*-test comparisons of least squares means for log-transformed data, $P > 0.05$); other differences are significant at $P = 0.0001$.

^b $F_{1,12} = 45.3$, $P < 0.001$ (data from Malcolm, 1995).

^c $F_{1,29} = 90.0$, $P < 0.001$.

^d $F_{1,5} = 28.3$, $P = 0.006$.

^e $F_{1,24} = 14.2$, $P = 0.001$.

source area predicted latex volume ($F_{1,66} = 218.9$, $P < 0.001$), and the latex volumes differed among the four *Asclepias* species ($F_{3,66} = 47.7$, $P < 0.001$), as did the regression slopes ($F_{3,66} = 2.98$, $P = 0.038$, Table 1). Thus, most latex is produced by *A. humistrata* and *A. syriaca*, and least latex is produced by *A. tuberosa* and *A. incarnata*.

The cross-sectional areas of the four plant species varied widely (Table 1), which reflects the overall size differences of the plant stems, petioles, and leaf veins, and so the range of latex volumes varied widely among the four species. These sizes show that *A. syriaca* is by far the largest of the milkweeds with the largest stems and vascular tissue, *A. humistrata* is next, followed by *A. incarnata* and *A. tuberosa*.

The two species with the most latex, *A. humistrata* and *A. syriaca*, also have higher cardenolide contents than the species with the least, *A. incarnata* and *A. tuberosa* (Table 1). However, *A. syriaca* has considerably lower cardenolide levels than the southern *A. humistrata*. During our field trials, we found that *A. tuberosa* produces small amounts of a clear, watery latex and *A. incarnata* produces small amounts of a more viscous, creamy yellow latex, in contrast to the copious white latex produced by *A. humistrata* and *A. syriaca*.

Thus, *A. syriaca* appears to be exceptional in that it has large latex-carrying cross-sectional areas (petioles, side veins, etc.) and produces high latex volumes, but it has characteristically low cardenolide levels. *A. syriaca* is also by far the most modular of the four milkweed species with single individuals commonly comprised of several thousand ramets. We have not compared latex viscosity or flow rate once a laticifer has been punctured. Our observations suggest, how-

TABLE 2. INITIAL CHARACTERISTICS, HEIGHT, AND LEAF NUMBER OF TREATMENT AND CONTROL PLANTS (OR STEMS) WITH SAMPLE REPLICATES (*N*) IN 5 EXPERIMENTS WITH THREE MILKWEED SPECIES TO ASSESS GROWTH RATE AND SURVIVAL OF FIRST INSTAR MONARCHS

<i>Asclepias</i> species	Exp.	<i>N</i>	Plant height (m, mean \pm SD)		Initial leaf number (mean \pm SD)	
			Treatment	Control	Treatment	Control
<i>A. syriaca</i>	1	10	1.05 \pm 0.12	1.02 \pm 0.14	16.6 \pm 2.3	16.7 \pm 2.2
	2	10	1.00 \pm 0.09	1.02 \pm 0.09	21.1 \pm 3.7	20.0 \pm 2.2
	3	10	0.71 \pm 0.15	0.76 \pm 0.13	16.5 \pm 3.4	18.4 \pm 3.1
<i>A. tuberosa</i>	1	10	0.58 \pm 0.02	0.59 \pm 0.03	36.5 \pm 9.9	40.9 \pm 9.3
<i>A. incarnata</i>	1	10	1.04 \pm 0.14	1.01 \pm 0.14	34.6 \pm 20.0	33.5 \pm 9.8

ever, that these properties differ among the species. For example, the latex of *A. humistrata* appears to be nonviscous and flows rapidly to form many small globules depending on the size of the puncture. In *A. syriaca*, the latex is more viscous and oozes out and, at least on the pubescent undersides of leaves, the copious material can spread by attraction from hair to hair. In *A. incarnata* and *A. tuberosa*, the latex fluid is restricted to the immediate area of the puncture.

Larval Experiments. For *Asclepias syriaca*, control and experimental plant stems were similar in height and initial number of leaves (Table 2). Stems were generally close together with mean between-stem distances (\pm SD) of 8 ± 9 cm, 33 ± 19 cm, and 28 ± 13 cm, for experiments 1, 2, and 3, respectively. Each of the three experiments was performed within an area of approximately 25 m² within a patch of *A. syriaca*. Within each of these areas, it is likely that stems or ramets belonged to the same genet. Our growth and survivorship results reflect variation among ramets from the three separate genets. For the purposes of analysis of weights, we excluded larvae in experiment 1 that had gone through to the second instar. The period of the experiment (July 8–11) was particularly warm (more than 45 dd accumulated above the development threshold of 11.5°C), and the larvae developed much more quickly than expected. Furthermore, a storm on the day prior to harvest stripped leaves with damaged petioles from the treated plants, greatly reducing the number of larvae recovered. We therefore compared only the weights of first instars that we recovered. During the second and third experiments all larvae were first instars, having accumulated only 29 dd and 14.3 dd, respectively.

Larvae were larger (both wet and dry weights) on leaves with partially severed petioles in all three experiments (Table 3). During experiment 2 we included five stems whose tops had not been removed. Leaves on these plants were left intact. Larvae were smaller ($P < 0.05$), with a mean wet weight of 132×10^{-5} g

TABLE 3. WET AND DRY WEIGHTS AND GROWTH RATES OF FIRST-INSTAR MONARCHS FED INTACT LEAVES OF *Asclepias syriaca* OR LEAVES WITH PARTIALLY SEVERED PETIOLES

Experiment	Treatment	Larval weight		<i>t</i> test	
		Mean \pm SE	<i>N</i>	<i>t</i>	<i>P</i>
Wet weight ($\times 10^{-5}$ g)					
1	Intact	252 \pm 10	29	2.8	0.009
	Severed	324 \pm 26	5		
2	Intact	197 \pm 11	53	1.8	0.067
	Severed	228 \pm 13	47		
3	Intact	62.1 \pm 1.9	75	5.8	<0.001
	Severed	85.2 \pm 3.7	66		
Dry weight ($\times 10^{-5}$ g)					
1	Intact	39.8 \pm 1.4	29	2.4	0.022
	Severed	48.8 \pm 3.6	5		
2	Intact	34.7 \pm 1.7	53	2.0	0.05
	Severed	39.7 \pm 1.9	47		
3	Intact	12.1 \pm 0.5	75	5.4	<0.001
	Severed	16.3 \pm 0.7	66		
Growth rate (10^{-5} g wet weight/dd)					
1	Intact	5.60 \pm 0.22	29	2.8	0.009
	Severed	7.20 \pm 0.58	5		
2	Intact	6.81 \pm 0.36	53	1.8	0.067
	Severed	7.85 \pm 0.43	47		
3	Intact	4.35 \pm 0.13	75	5.8	<0.0001
	Severed	5.96 \pm 0.26	66		

(SE = 15.3, *N* = 14) on these stems, compared to larvae from stems with either intact or partially severed petioles but with tops removed in experiment 2 (Table 3). During the third experiment we left the tops of all stems intact.

In order to compare larval growth rates among the three experiments, we have expressed larval growth as a rate per day degree. We concentrate here on wet weights only, as the results are essentially the same for dry weights (Table 3). Damaging leaves and topping plants had effects on larval growth rates ($F_{1,285} = 34.4$ and $F_{1,285} = 56.7$, $P < 0.001$, respectively, Table 4). There was no interaction between leaf damage and topping ($F_{1,285} = 0.118$, NS). Larvae grew fastest on plants with both severed leaves and tops removed and slowest on intact, untopped plants (Table 4). Growth rates did not differ between larvae from topped plants with leaf petioles intact and larvae from untopped plants with severed petioles (Table 4).

For experiments 2 and 3 that were not affected by thunderstorms, survival was generally high, ca. 70%, and unaffected by plant treatment. For experiments

TABLE 4. MEAN GROWTH RATES (MEAN \pm SE, *N*) OF FIRST INSTAR MONARCHS (10^{-5} g WET WEIGHT/DAY DEGREE) FED INTACT LEAVES OF *A. syriaca* OR LEAVES WITH PARTIALLY SEVERED PETIOLES FROM PLANTS WITH TOPS REMOVED OR INTACT^a

Plant	Leaves	
	Intact	Severed
Intact	4.38 \pm 0.14 ^b (89)	5.96 \pm 0.26 ^a (66)
Top removed	6.38 \pm 0.26 ^a (82)	7.79 \pm 0.40 ^c (52)

^aLarval growth rates differed significantly between intact and severed leaves (ANOVA $F_{1,285} = 34.4$, $P < 0.001$) and between topped and intact plants (ANOVA $F_{1,285} = 56.7$, $P < 0.001$). Means followed by the same letter are not significantly different at $P < 0.05$.

2 and 3 survival was 62 and 72% on petiole-damaged plants and 67 and 73% on plants with intact petioles, respectively. Experiment 1 was conducted in a small, unrelated patch, and survival on the intact plants that were not adversely affected by the storm was 44%. Experiments 2 and 3 were harvested before the end of the first instar. Extrapolation of mean survival rates to the end of the first instar yielded survival levels of 47 and 30%, respectively.

As with *A. syriaca*, control and treatment plants of both *A. tuberosa* and *A. incarnata* had similar characteristics (Table 2). Unlike the results for *A. syriaca*, there was no effect of treatment on wet or dry weights of larvae from *A. incarnata* (ANOVA $F_{1,110} = 0.065$, Table 5). Partial petiole severance did affect the size of larvae on *A. tuberosa* (ANOVA $F_{1,75} = 4.786$, $P = 0.032$). Again, as for *A. syriaca*, larvae fed *A. tuberosa* leaves with partially severed petioles were heavier than those with intact leaves (Table 5). Not surprisingly, mean growth rates (10^{-5} g/dd) were similar on severed (7.6) and intact (7.7) leaves of *A. incarnata* (Table 6), whereas they were different ($P = 0.032$, *t* test) for *A. tuberosa*, at 7.6 and 6.5 for severed and intact treatments, respectively.

Survival did not differ between severed (63%) and intact (57%) treatments on *A. incarnata*, but was higher on severed leaf plants (55%) than on intact leaf plants (39%) of *A. tuberosa* (one tailed *t*-test on arcsin square root transformed proportions, $P = 0.038$).

DISCUSSION

Monarch butterflies in North America have been recorded ovipositing, or feeding as larvae, on 27 of the 108 local *Asclepias* species (Malcolm and Brower, 1986), and most evidence suggests that monarchs are *Asclepias* specialists (Ackery and Vane-Wright, 1984). The basis of this specialization is not clear. Milkweeds are noted for containing a group of steroids known as cardenolides, and

TABLE 5. WET AND DRY WEIGHTS AND GROWTH RATES OF FIRST-INSTAR MONARCHS FED INTACT LEAVES OR LEAVES WITH PARTIALLY SEVERED PETIOLES OF *Asclepias incarnata* AND *A. tuberosa*

Experiment and treatment	Larval weight		<i>t</i> test	
	Mean \pm SE	<i>N</i>	<i>t</i>	<i>P</i>
<i>A. incarnata</i>				
Wet weight ($\times 10^{-5}$ g)				
Intact	247 \pm 8.9	55	0.26	0.80
Severed	244 \pm 10.4	57		
Dry weight ($\times 10^{-5}$ g)				
Intact	39.7 \pm 1.65	54	0.40	0.69
Severed	40.6 \pm 1.29	57		
Growth rate (10^{-5} g wet weight/dd)				
Intact	7.66 \pm 0.27	55	0.26	0.80
Severed	7.55 \pm 0.32	57		
<i>A. tuberosa</i>				
Wet weight ($\times 10^{-5}$ g)				
Intact	166 \pm 9.1	33	2.19	0.03
Severed	195 \pm 9.0	44		
Dry weight ($\times 10^{-5}$ g)				
Intact	31.9 \pm 1.65	33	0.89	0.38
Severed	34.0 \pm 1.65	44		
Growth rate (10^{-5} g wet weight/dd)				
Intact	6.51 \pm 0.36	33	2.19	0.03
Severed	7.63 \pm 0.36	44		

TABLE 6. COMPARATIVE GROWTH RATES OF FIRST-INSTAR MONARCHS REARED ON INTACT LEAVES OR LEAVES WITH PARTIALLY SEVERED PETIOLES OF FOUR SPECIES OF *Asclepias*

<i>Asclepias</i> species	Growth rate (10^{-5} g wet wt/dd)	
	Intact	Severed
<i>A. humistrata</i> ^a	3.5	7.8
<i>A. syriaca</i>	4.4	6.0-7.8
<i>A. tuberosa</i>	6.5	7.6
<i>A. incarnata</i>	7.7	7.6

^aData from M. Zalucki, L. P. Brower, A. Alonso-M., and T. Van Hook (unpublished data).

some authors have suggested a role for cardenolides in host-plant selection (Brower, 1961; Cohen and Brower, 1982; Zalucki and Kitching, 1982b; Malcolm and Brower, 1986), while others have disputed these findings (Dixon et al., 1978; Zalucki et al., 1989; see Oyeyele and Zalucki, 1990 for a review).

Like many plant secondary compounds these steroids may act as toxins to potential herbivores (Malcolm, 1991, 1995). As the name milkweed implies, plants in this family are noted for their milky latex that is usually rich in cardenolides (Nelson et al., 1981; Seiber et al., 1982; Van Emon and Seiber, 1985). The latex is contained under pressure in a reticulated, sealed system of vessels called laticifers (Lucansky and Cloug, 1986). When a leaf is punctured or its petiole cut, latex flows out and coagulates on contact with air. This system of latex-bearing canals, pressurized with a high concentration of toxic cardenolides in a quick-setting glue, has been interpreted as a plant defense, particularly against generalist herbivores (Dussourd and Eisner, 1987; Dussourd, 1990, 1993).

Monarchs are considered to have evolved the ability to circumvent these physical defenses of milkweeds and subsequently to exploit the chemical defenses of the plant by sequestering them for use against third trophic level enemies (Malcolm, 1991, 1995). Monarch larvae of all stages show various behaviors that may effectively disable the canal-based defenses of milkweeds. Early instars use various forms of vein snipping and trenching (Dussourd, 1990, 1993; Zalucki and Brower, 1992), and late instars can partially sever the petiole before consuming the leaf (Brewer, 1977; Dussourd and Eisner, 1987; Zalucki and Brower, 1992). Monarch larvae feeding on milkweeds concentrate cardenolides above the concentration in the plant (Malcolm and Brower, 1989; Nelson, 1993; Malcolm, 1995) and conserve a mixture of cardenolides through to the adult stage (Brower et al., 1988), possibly as a form of storage excretion in the cuticle. This aspect of monarch biology has attracted considerable attention, particularly with respect to adult cardenolide content and its effect on predation by birds (Brower, 1984; Brower et al., 1988).

More recently, we have investigated the relationship between plant cardenolides, the latex system of milkweeds, and early-instar growth and survival. Most eggs are found on plants with intermediate cardenolide levels (Zalucki et al., 1989; Oyeyele and Zalucki, 1990; van Hook and Zalucki, 1991), and females display postlighting discrimination against plants with low and high cardenolide concentrations (Zalucki et al., 1990). Survival of the resulting first instars to the second instar on various milkweed species is generally poor, ranging from 3.4 to 40% (see Zalucki and Brower, 1992 for a review), and is generally skewed. In field experiments, Zalucki and Brower (1992) found only 3.4–11.5% of newly hatched larvae survived the first instar on *A. humistrata*. Early-stage survival was negatively correlated with plant cardenolide level (see also Cohen and Brower, 1982; Zalucki et al., 1990) and was not affected by ground-dwelling predators. About 30% of larvae were found mired in the leaf latex and glued to the leaf sur-

face. This was despite the elaborate latex-“sabotaging” behavior of first instars. We observed that larvae frequently encountered latex while sabotaging veins and feeding on leaf tissue. During such encounters, latex often adhered to the mouthparts and head, which generally resulted in vigorous cleaning behaviors. The larvae also apparently inadvertently imbibed latex, which generally resulted in catalepsis (as noted in Zalucki and Brower, 1992). As the concentration of cardenolide is much higher in the latex than in leaf tissue (Roeske et al., 1976; Seiber et al., 1982; Zalucki and Brower, 1992), we suspected that this may have been responsible for the catalepsis. However, it was not clear whether cardenolides per se or something else in the latex was the cause of the high mortality that we observed.

Here we investigated the effects of latex on early-instar survival and growth by cutting off latex supply to leaves by partially severing leaf petioles of *A. humistrata* (M. P. Zalucki, L. P. Brower, A. Alonso-Mejia, and T. Van Hook, unpublished data), and *A. syriaca*, *A. tuberosa*, and *A. incarnata* (this study). The damage we inflicted to petioles is like that caused by late-instar monarchs, and two other insect specialists of milkweeds, the cerambycid beetle, *Tetraopes tetrophthalmus*, and the curculionid beetle, *Rhyssomatus lineaticollis*. Our experimental protocol significantly reduced latex flow to leaves (data in Methods and Materials) and had little effect on leaf moisture content. As Zalucki et al. (unpublished data) found with *A. humistrata*, damaging the leaf petiole can increase the growth rate and survival of first instars, but the result differs among milkweed species, possibly reflecting the amount of latex produced and its qualities. Larval growth rate increased on *A. syriaca* with both high latex and low cardenolide when leaf petioles had been damaged or plants had been topped. In either case, we interpret this to reflect reduced latex volume or pressure and greater time available for feeding on such plants because less time is wasted avoiding latex. Unlike *A. humistrata*, survival was unaffected by leaf severing, and this might reflect the generally low cardenolide content of *A. syriaca*. Survival was higher on *A. syriaca* (30–77%) than on any of the other milkweeds reviewed by Zalucki and Brower (1992), and during our experiments we made no attempt to restrict access by predators to experimental plants.

For *A. incarnata*, there was no influence of reduced latex flow on growth rates and survival of first-instar monarchs. This is both a low latex and low cardenolide plant species (Table 1), with smooth, soft, lanceolate leaves. Survival was high (57–63%), even on plants to which predators had easy access. This lack of a response to latex in a low-latex/low-cardenolide milkweed lends weight to the suggested role of both latex and cardenolides as integrated defenses against early-stage monarch larvae.

If this conclusion were generally true among milkweed species, we should have seen the same result with *A. tuberosa*, which is also a low-latex/low-cardenolide milkweed. However, restricting latex in *A. tuberosa* improved both survival

(both 39 to 55%), and growth rates (significantly from 6.5 in intact plants to 7.6 in severed plants; see Table 5). Interestingly, survival was also higher on *A. tuberosa* than on milkweed species with both high latex and high cardenolide (Zalucki and Brower, 1992). Nevertheless, the effect of our plant treatment may reflect other properties of the latex system and its contents in *A. tuberosa*. Only two cardenolide glycosides and their common genin have been isolated from *A. tuberosa* (Petricic, 1966), and it is curious that these particular cardenolides have only been isolated from African *Asclepias* (= *Gomphocarpus*) species and not from other North American *Asclepias* species (Roeske et al., 1976). Possibly, *A. tuberosa* is sufficiently unapparent to ovipositing monarch females that it is not exploited with sufficient frequency to select for defenses against adapted specialists.

There can be no doubt as to the suitability of milkweed leaf tissue for early stage monarch growth. Growth rates of first instars on four species are comparable, once the latex system is circumvented (Table 6). However, both the amount of latex a species produces once a laticifer is ruptured and its cardenolide level adversely affect the growth rate and survival of newly hatched larvae. Once the vulnerable first instar is passed, larvae of this specialized herbivore can probably increase or induce their ability to handle plant chemical and mechanical defenses as they grow larger mouthparts that can more readily trench and overcome the latex system.

Another problem for monarchs on milkweeds was recently raised by Malcolm and Zalucki (1996). Levels of cardenolides are induced above constitutive levels in damaged leaves, and this induction effect occurs rapidly with a peak after 24 hr and then declines over six days. Thus, not only do monarch larvae have to contend with constitutive levels of cardenolide and latex in plants, but they also have to handle cardenolides that are probably rapidly induced in response to feeding. If latex slows larval growth and reduces survivorship, induced cardenolides may also impact growth and survivorship either directly or indirectly by forcing larvae to move to a new leaf. The extensive movements characteristic of early instars among leaves and ramets of milkweeds may be a reflection of this multicomponent defense and a good indication that these "adapted specialists" do indeed incur a cost to feeding on milkweeds.

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