

DENSITY-DEPENDENT REDUCTION AND INDUCTION OF MILKWEED CARDENOLIDES BY A SUCKING INSECT HERBIVORE

JOHN W. MARTEL¹ and STEPHEN B. MALCOLM^{1,*}

¹*Department of Biological Sciences, Western Michigan University
Kalamazoo, Michigan 49008, USA*

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Abstract—The effect of aphid population size on host-plant chemical defense expression and the effect of plant defense on aphid population dynamics were investigated in a milkweed-specialist herbivore system. Density effects of the aposematic oleander aphid, *Aphis nerii*, on cardenolide expression were measured in two milkweed species, *Asclepias curassavica* and *A. incarnata*. These plants vary in constitutive chemical investment with high mean cardenolide concentration in *A. curassavica* and low to zero in *A. incarnata*. The second objective was to determine whether cardenolide expression in these two host plants impacts mean *A. nerii* colony biomass (mg) and density. Cardenolide concentration ($\mu\text{g/g}$) of *A. curassavica* in both aphid-treated leaves and opposite, herbivore-free leaves decreased initially in comparison with aphid-free controls, and then increased significantly with *A. nerii* density. Thus, *A. curassavica* responds to aphid herbivory initially with density-dependent phytochemical reduction, followed by induction of cardenolides to concentrations above aphid-free controls. In addition, mean cardenolide concentration of aphid-treated leaves was significantly higher than that of opposite, herbivore-free leaves. Therefore, *A. curassavica* induction is strongest in herbivore-damaged tissue. Conversely, *A. incarnata* exhibited no such chemical response to aphid herbivory. Furthermore, neither host plant responded chemically to herbivore feeding duration time (days) or to the interaction between herbivore initial density and feeding duration time. There were also no significant differences in mean colony biomass or population density of *A. nerii* reared on high cardenolide (*A. curassavica*) and low cardenolide (*A. incarnata*) hosts.

Key Words—*Aphis nerii*, *Asclepias*, cardenolide, chemical defense, herbivory, induction, milkweed, reduction, sequestration, specialist, trophic interactions.

* To whom correspondence should be addressed. E-mail: steve.malcolm@wmich.edu

INTRODUCTION

Many plant families utilize rapidly mobilized, damage-induced chemical defense systems in response to insect herbivory. Phytochemical induction in response to mandibulate insect herbivory has been widely demonstrated where chewing insects cause conspicuous plant tissue damage and foliar biomass reduction (Karban and Baldwin, 1997). However, little is known about the defensive response of plants to haustellate insect herbivory, where photosynthate is typically sucked from phloem vasculature, causing little plant tissue damage or foliar biomass loss. Nevertheless, haustellate herbivory generates a sink for the products of primary metabolism that increases as individual herbivores grow and their local populations increase in size.

Malcolm and Zalucki (1996) found that common milkweed *Asclepias syriaca* (Apocynaceae, Asclepiadoideae) induces chemical defense expression in response to mechanical leaf damage like that caused by mandibulate chewing of herbivores such as larvae of the monarch butterfly, *Danaus plexippus*. The oleander aphid, *Aphis nerii* B. de F., is another widely distributed milkweed herbivore that, in contrast to monarch larvae, does not cause conspicuous host tissue damage because it feeds by inserting haustellate maxillary stylets directly into phloem tissue (Botha et al., 1977; Malcolm, 1981). Nothing is known about the effect that *A. nerii* feeding has on milkweed chemical defense expression, or the effect of variable defense expression on aphid fitness correlates.

A. nerii is a bright yellow, aposematic aphid with a worldwide distribution that is restricted to feeding on plants in the Apocynaceae, especially the subfamily Asclepiadoideae (Patch, 1938; Ismail and Swailem, 1971; Price and Willson, 1979; Malcolm, 1991). This common aphid is found wherever milkweeds and oleander occur and it is known to sequester cardenolides, phloem-mobile host allelochemicals, for use as a chemical defense against natural enemy attack (Rothschild et al., 1970; Pasteels, 1978; Duffey, 1980; Malcolm, 1981, 1986, 1989, 1990, 1992; Dixon, 1998). Cardenolides are potent, steroidal Na^+/K^+ -ATPase inhibitors that are toxic to most consumers and are characteristic defenses of plants in the Apocynaceae (Malcolm, 1991). *A. nerii* feed in the cardenolide-rich internal phloem of host bicollateral vascular bundles (Botha et al., 1977; Malcolm, 1981), and these chemicals are present in *A. nerii* honeydew and body tissue (Bailey, 1974; Malcolm, 1990).

We investigated the impact of *A. nerii* colony density and feeding duration on host cardenolide expression, and the effect of host cardenolide concentration on *A. nerii* colony size and biomass. Our first objective was to determine whether *A. nerii* feeding influences cardenolide expression in two milkweed species that vary in constitutive chemical defense investment. *Asclepias curassavica* L. has high constitutive leaf cardenolide content, while *A. incarnata* L., the swamp milkweed, has low constitutive leaf cardenolide content (Roeske et al., 1976; Malcolm, 1990,

1991). Our second objective was to determine whether cardenolide expression in these two host plants differentially impacts *A. nerii* colony size and biomass.

METHODS AND MATERIALS

Plant Culture. Ninety North American *A. incarnata* were purchased from a grower in Kalamazoo, Michigan, USA (Van Bochove's Greenhouse Direct: plants grown from Michigan-collected seeds), and 90 neotropical *A. curassavica* were pot-reared from seed at the Western Michigan University greenhouse complex (seeds originated from greenhouse plants grown at Michigan State University). All plants were approximately 4 months old at the initiation of the experiment, were grown in MetroMix™ 410 soilless medium, and were maintained insect-free in a sealed greenhouse room until application of *A. nerii* in clip cages in the field (no pesticides were used on these plants). In addition to the marked difference in constitutive cardenolide content, the two species were chosen for this experiment because their leaf morphologies and growth forms are similar. Both species are erect perennials with smooth, entire, lanceolate leaves of equivalent leaf area (Woodson, 1954). Woodson (1954) describes *A. curassavica* as a neotropical annual; however, greenhouse-grown plants do perennate for several years.

Phytochemical Analysis. Cardenolides were extracted from harvested leaf samples by solid phase extraction and analyzed by high performance liquid chromatography (HPLC) using the methods of Wiegrebe and Wichtl (1993) as modified by Malcolm and Zalucki (1996). Mean foliar cardenolide concentrations for herbivore-free control plants harvested throughout the experiment were *A. curassavica* = 1421 $\mu\text{g/g}$ (SEM = 216 $\mu\text{g/g}$, $N = 15$) and *A. incarnata* = 0 $\mu\text{g/g}$ ($N = 15$).

Insect Culture. Aphid cultures were established from natural populations feeding on *A. syriaca* and *A. incarnata* in Kalamazoo County, Michigan, USA. Prior to experimentation, aphids to be used on *A. curassavica* were maintained on *A. curassavica*, while aphids to be used on *A. incarnata* were maintained on *A. incarnata* for at least five generations. To start the experiment, fifth instar, apterous *A. nerii* were placed in one of six logarithmically increasing starting densities (0, 3, 7, 20, 55, or 148), onto a single leaf of the third leaf pair from the plant apex. Each plant contained a single aphid colony confined to this location. The insects were enclosed in foam-backed PVC (polyvinylchloride) clip cages. The 4-cm diam clip cages were made from two 3-mm sections of PVC pipe held on opposite sides of a leaf by a spring hair clip. Nylon sheer mesh (0.1 mm^2) was glued on the outside of each PVC section to form an enclosure. In an effort to limit the potential for clip-cage-induced leaf tissue damage, only the foam made direct contact with the leaf surface. Clip cages were supported by twist-ties attached to 3-ft support stakes used to anchor each potted plant. All insects were allowed to reproduce and feed

freely within the confines of their respective enclosures until harvest. Aphids were confined to the lower, abaxial leaf surface, so excreted honeydew did not contact the surface of leaves analyzed for cardenolide content.

Experimental Procedure. The six insect starting density treatments (0 [control], 3, 7, 20, 55, 148) reflect a range of single leaf colony densities commonly found in nature (Ismail and Swailem, 1971; Malcolm, 1976; Hall and Ehler, 1980; Groeters, 1989; Groeters and Dingle, 1989; Bristow, 1991). The experimental design included 5 sampling days \times 6 initial herbivore treatment densities \times 2 milkweed species \times 3 replicates/plant species = 180 plants (90 of each species). The two groups of 90 plants were each divided into three, 30 plant replicate groups/plant species as illustrated (Table 1).

All greenhouse-grown plants were maintained outside under ambient conditions for 6 days (June 10–15) prior to the initiation of the experiment (June 16). The assay was conducted in a flat, 240 m² area of mowed lawn divided equally into six units (3 replicates \times 2 plant species) (Table 1). The day before the experiment began, all plants were arranged in conspecific replicate units 1 m from each nearest neighbor. Each replicate contained 25 treatment (+aphids) and 5 control plants (–aphids). Plants in a given replicate were arranged into rows, each containing six plants. Therefore, all rows were composed of plants representing the full complement of aphid starting densities (Table 1). The particular sequence of aphid starting density treatments in each row was predetermined to limit the potential for interactions between colony density and plant position.

To assess both the effects of aphid starting density and feeding duration time on cardenolide expression, one row was removed/replicate/plant species every 24 hr for 5 days. Two leaf samples were harvested from each plant (third leaf pair) to test whether phytochemical responses were systemic or localized to aphid-damaged tissue ($N = 360$). At the time of leaf harvest, all leaves were weighed wet. Samples were then lyophilized and reweighed, and cardenolides were extracted using the phytochemical analysis procedure described in Malcolm and Zalucki (1996). Aphids were counted according to three instar groups (first + second, third + fourth, and fifth alates + apterae) and then weighed collectively in grouped instars. These weights were then summed to give the total aphid biomass.

Statistical Analyses. Collected data were analyzed with both StatViewTM, SuperAnovaTM, and JMPTM v5 software (SAS Institute, Cary, NC) and tested for normality (Shapiro–Wilk W) and homogeneity of variances (O'Brien, Brown–Forsythe, Levene, or Bartlett tests) followed by ANOVA, ANCOVA, and regression analyses. Most of the 480 measures of aphid (aphid N , Ln aphid $N + 1$, aphid biomass) and plant response (leaf cardenolide content) were normally distributed among treatments and replicates, with the exception of 19 measures (4%): these included aphid measures at density 7 on *A. incarnata* on days 1 and 2, density 148 on *A. incarnata* on day 1, density 20 on *A. curassavica* and *A. incarnata* on day 3, and density 3 on *A. curassavica* on day 4. These data were excluded

TABLE 1. EXPERIMENTAL DESIGN AND ORIENTATION OF BOTH HOST PLANT AND 6 APHID DENSITY TREATMENTS (0 = CONTROL, 3, 7, 20, 55, 148) DISTRIBUTED AMONG 3 REPLICATES

Host plant	Replicate 1			Replicate 2			Replicate 3											
	3	7	20	55	148	C	3	7	20	55	148	C	3	7	20	55	148	C
<i>A. incarnata</i>	3	7	20	55	148	C	3	7	20	55	148	C	3	7	20	55	148	C
	C	148	3	7	20	55	C	148	3	7	20	55	C	148	3	7	20	55
	55	7	C	148	3	20	55	7	C	148	3	20	55	7	C	148	3	20
<i>A. curassavica</i>	148	3	20	7	C	55	148	3	20	7	C	55	148	3	20	7	C	55
	7	C	55	20	3	148	7	C	55	20	3	148	7	C	55	20	3	148
	3	7	20	55	148	C	3	7	20	55	148	C	3	7	20	55	148	C
N↑	C	148	3	7	20	55	C	148	3	7	20	55	C	148	3	7	20	55
	55	7	C	148	3	20	55	7	C	148	3	20	55	7	C	148	3	20
	148	3	20	7	C	55	148	3	20	7	C	55	148	3	20	7	C	55
	7	C	55	20	3	148	7	C	55	20	3	148	7	C	55	20	3	148

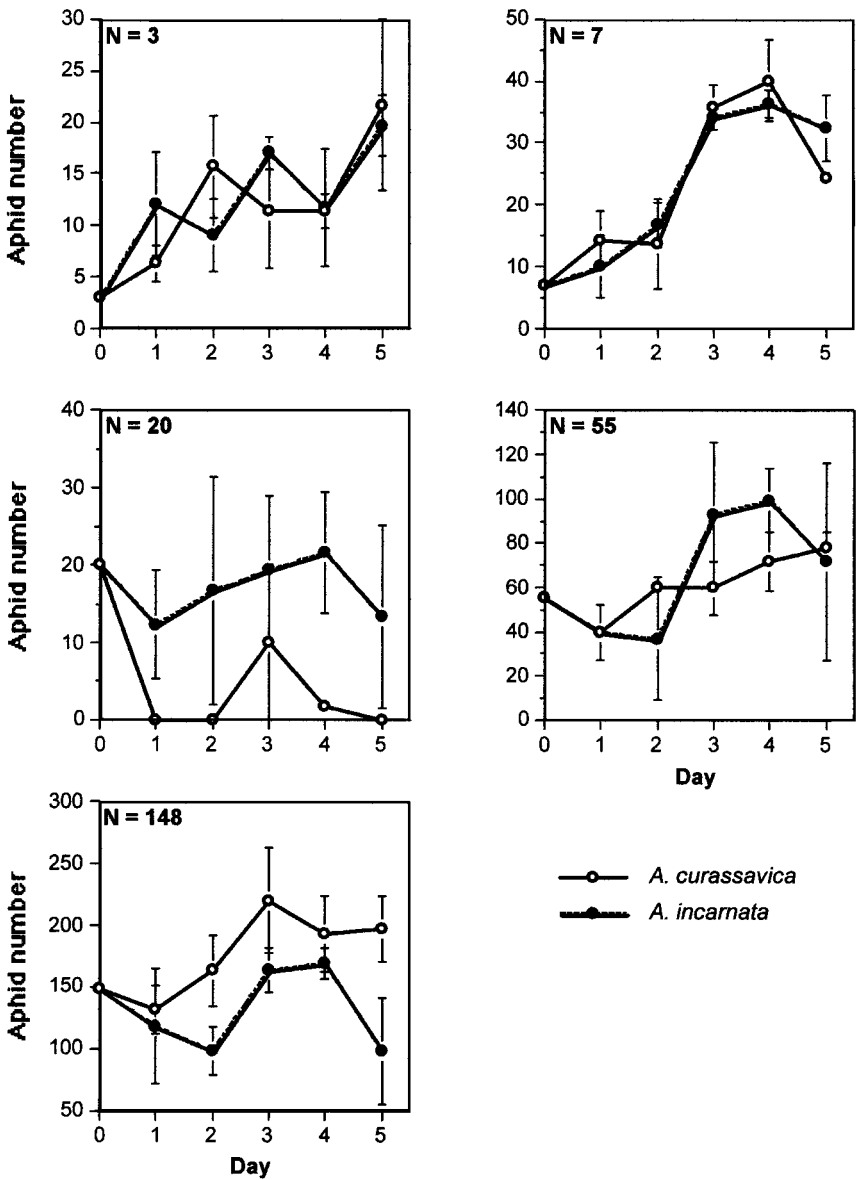


FIG. 1. Mean aphid numbers (\pm SEM) plotted against time for each initial density treatment on *A. curassavica* and *A. incarnata*.

from subsequent analyses because transformation made little difference to their distributions. In ANOVA comparisons of aphid performance between the two host plants, or plant cardenolide responses to aphid densities across days, all variances were homogeneous for one or more tests of homogeneity.

RESULTS

Aphid Population Dynamics. In each treatment, aphid populations increased significantly with time, except for the 20 density treatment on both host plants and the 55 and 148 density treatments on *A. incarnata* (Figure 1, Table 2). Density treatment 3 showed an overall increase, while density 7 growth on both host plants suggested sigmoidal increase to a possible asymptote after 4 days. This asymptote might result from interference competition for space within the clip cages. Such competition may also have limited the higher density treatments to less ($N = 20$) and more variable increase ($N = 55, 148$) in aphid density with time (Figure 1).

Overall, total aphid biomass on each host plant increased significantly and linearly with aphid numbers [*A. curassavica* aphid biomass (mg) = 0.46 aphid N , $r^2 = 0.80$, $F_{(1, 72)} = 289.4$, $P < 0.001$; *A. incarnata* aphid biomass (mg) = 0.50 aphid N , $r^2 = 0.73$, $F_{1,84} = 228.5$, $P < 0.001$], and there was no significant difference between the slopes of these linear regressions constrained through the origin [ANCOVA, $F_{1,155} = 0.19$, ns]. Thus, aphid numbers were used in the analysis of aphid dynamics rather than aphid biomass. In the case of the *A. curassavica* 20 treatment, we collected insufficient data because the aphid populations crashed in all three replicates for unknown reasons. Consequently, we are unable to compare these data with those from *A. incarnata*.

TABLE 2. LINEAR REGRESSIONS OF APHID NUMBERS AGAINST TIME IN DAYS FOR EACH INITIAL APHID DENSITY ON *A. curassavica* AND *A. incarnata* AND ANALYSES OF COVARIANCE OF REGRESSION SLOPES BETWEEN THE TWO HOST PLANTS

Host	Density	Linear regression				ANCOVA	
		Regression	r^2	$F_{(1, 17)}$	P	Host $F_{(1, 31)}$	P
<i>A. curassavica</i>	3	$N = 4.11 + 2.98 \text{ d}$	0.32	7.68	0.014	0.05	ns
<i>A. incarnata</i>	3	$N = 5.05 + 2.71 \text{ d}$	0.41	10.20	0.006		
<i>A. curassavica</i>	7	$N = 9.21 + 5.31 \text{ d}$	0.43	12.28	0.003	0.01	ns
<i>A. incarnata</i>	7	$N = 8.62 + 5.96 \text{ d}$	0.73	40.08	<0.001		
<i>A. curassavica</i>	20	—	—	—	—	—	—
<i>A. incarnata</i>	20	$N = 21.13 + 0.42 \text{ d}$	0.004	0.04	ns		
<i>A. curassavica</i>	55	$N = 45.57 + 6.03 \text{ d}$	0.32	7.49	0.015	0.02	ns
<i>A. incarnata</i>	55	$N = 43.19 + 9.10 \text{ d}$	0.13	2.30	ns		
<i>A. curassavica</i>	148	$N = 140.71 + 13.93 \text{ d}$	0.23	4.70	0.046	0.04	ns
<i>A. incarnata</i>	148	$N = 134.64 - 0.77 \text{ d}$	0.001	0.01	ns		

TABLE 3. TWO-WAY ANOVA OF THE EFFECTS OF *A. nerii* FEEDING DURATION TIME (DAYS), INITIAL *A. nerii* DENSITY, AND INTERACTION, ON THE CARDENOLIDE CONCENTRATIONS ($\mu\text{g/g}$ LEAF DRY WEIGHT) OF *A. curassavica* AND *A. incarnata*

<i>A. curassavica</i> ($\mu\text{g/g}$) ^a						<i>A. incarnata</i> ($\mu\text{g/g}$) ^a					
Density	Mean	SE	Day	Mean	SE	Density	Mean	SE	Day	Mean	SE
0	1419	216	1	1401	1102	0	0	0	1	0	0
3	661	110	2	1120	911	3	7.6	7.6	2	7.6	7.6
7	685	95	3	959	675	7	23.6	17.3	3	4.6	4.6
20	563	113	4	1328	880	20	46.0	25.9	4	40.7	22.3
55	1332	129	5	1248	1358	55	14.4	10.6	5	23.5	14.7
148	2608	336				148	0	0			

Source	df	<i>A. curassavica</i>		<i>A. incarnata</i>	
		F	P	F	P
Time (d)	4	1.04	ns	1.84	ns
<i>A. nerii</i> treatment density	5	16.90	<0.001	1.68	ns
Time \times Density interaction	20	0.92	ns	1.01	ns
Residual	60				

^aCardenolide concentrations by day ($N = 18$ each day) and density ($N = 15$ for each density).

When slopes of linear regressions of aphid numbers against time for each aphid density treatment were compared between *A. curassavica* and *A. incarnata* using analysis of covariance (ANCOVA), we found no significant differences (Table 2). Thus, host-plant species had no effect on the rates of increase of aphid populations for all starting densities.

Leaf Cardenolide Content. There was a significant effect of aphid density on the cardenolide content of *A. curassavica* leaves, but not *A. incarnata* leaves (Table 3). However, there was no time effect of aphid feeding duration on the cardenolide content of leaves for either host plant (Table 3). In addition, there were no significant Feeding Duration \times Aphid Density interactions (Table 3). Thus, the cardenolide responses of leaves that supported each aphid density, and leaves opposite these aphid colonies, showed little change through time (Figures 2 and 3). Both clip cage leaves with the highest initial aphid density of 148, and leaves opposite these colonies on *A. curassavica* showed a decrease in cardenolide content on days 2 and 3. This was followed by an increase back to the original cardenolide concentrations. Control plants without aphids all showed cardenolide concentrations that were higher than all aphid density treatments, except the highest initial density of 148 aphids (Figure 2).

No regressions of cardenolide content against time were significant (Table 4) for clip cage and opposite leaves from *A. curassavica*. This reaffirmed the lack of influence of feeding duration in the two-way ANOVA (Table 3). There was so

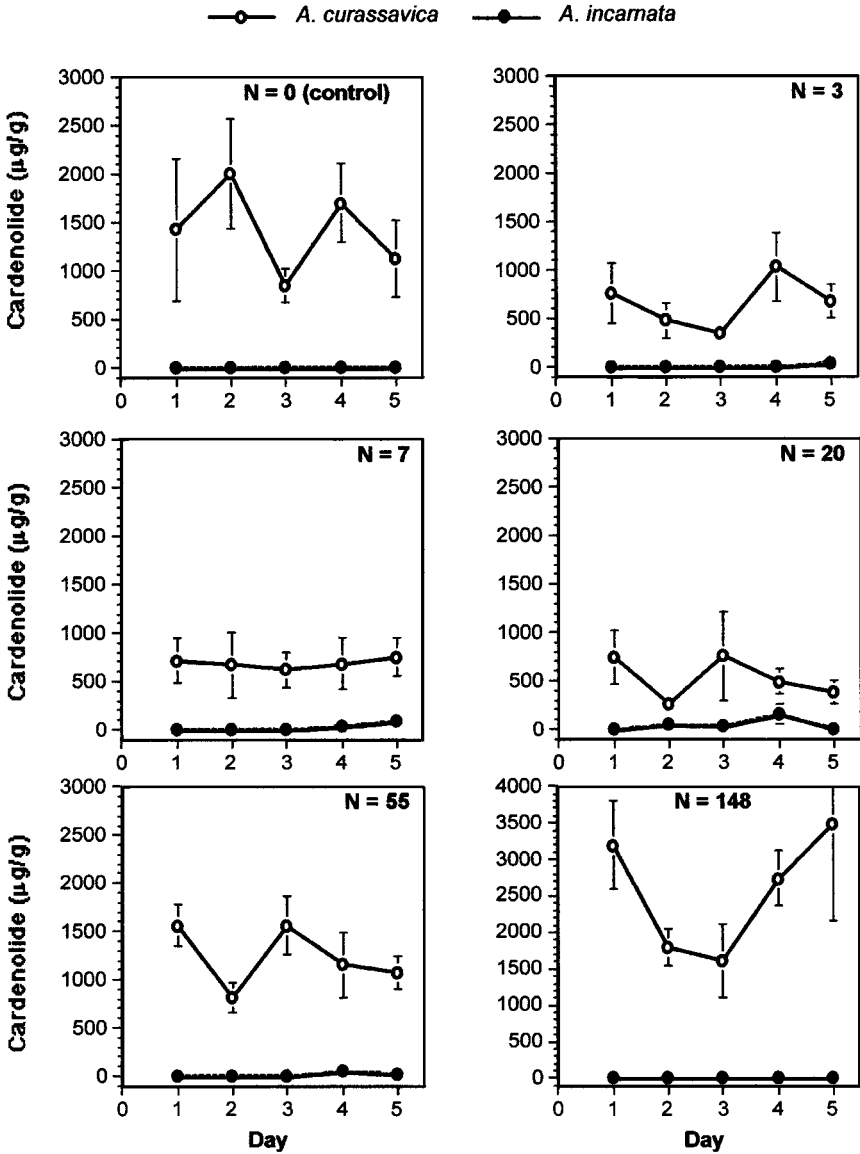


FIG. 2. Change in mean cardenolide concentrations in aphid leaves of *A. curassavica* and *A. incarnata* with time.

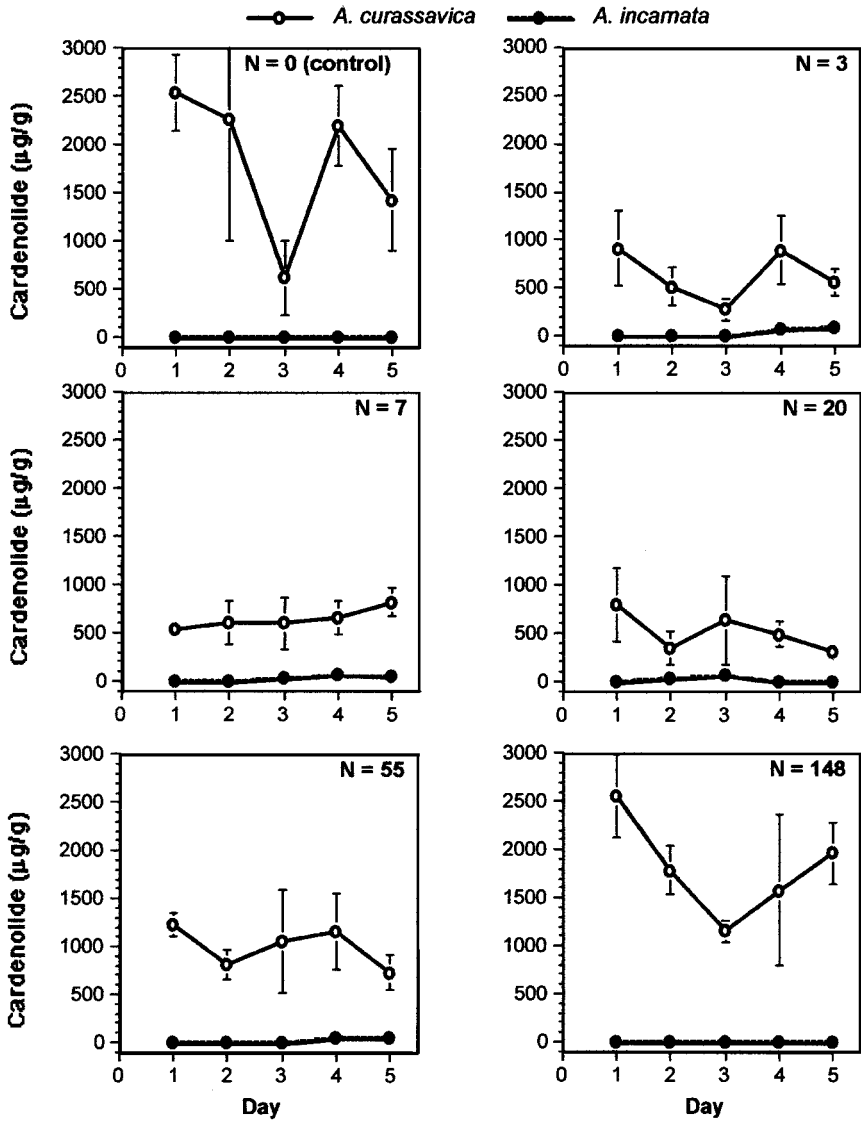


FIG. 3. Change in mean cardenolide concentrations in opposite leaves of *A. curassavica* and *A. incarnata* with time.

TABLE 4. LINEAR REGRESSIONS OF CARDENOLIDE CONCENTRATIONS ($\mu\text{g/g}$ LEAF DRY WEIGHT) IN APHID LEAVES (FIGURE 2) AND OPPOSITE LEAVES (FIGURE 3) AGAINST TIME (d)

Host	Density	Leaf	Regression	r^2	$F_{(1, 14)}$	P
<i>A. curassavica</i>	0	aphid	$\mu\text{g} = 1697.2 - 92.6 \text{ d}$	0.03	0.35	ns
<i>A. curassavica</i>	0	opposite	$\mu\text{g} = 2504.4 - 232.0 \text{ d}$	0.08	1.06	ns
<i>A. curassavica</i>	3	aphid	$\mu\text{g} = 547.5 + 37.9 \text{ d}$	0.02	0.22	ns
<i>A. curassavica</i>	3	opposite	$\mu\text{g} = 722.1 - 30.6 \text{ d}$	0.009	0.12	ns
<i>A. curassavica</i>	7	aphid	$\mu\text{g} = 663.2 + 7.1 \text{ d}$	0.001	0.92	ns
<i>A. curassavica</i>	7	opposite	$\mu\text{g} = 462.8 + 60.6 \text{ d}$	0.09	1.33	ns
<i>A. curassavica</i>	20	aphid	$\mu\text{g} = 647.5 - 28.3 \text{ d}$	0.009	0.12	ns
<i>A. curassavica</i>	20	opposite	$\mu\text{g} = 760.9 - 81.5 \text{ d}$	0.07	0.98	ns
<i>A. curassavica</i>	55	aphid	$\mu\text{g} = 1676.3 - 114.8 \text{ d}$	0.11	1.65	ns
<i>A. curassavica</i>	55	opposite	$\mu\text{g} = 1197.1 - 65.9 \text{ d}$	0.04	0.48	ns
<i>A. curassavica</i>	148	aphid	$\mu\text{g} = 2213.0 + 131.6 \text{ d}$	0.02	0.29	ns
<i>A. curassavica</i>	148	opposite	$\mu\text{g} = 2228.3 - 140.2 \text{ d}$	0.07	0.90	ns

little cardenolide in *A. incarnata* that there were insufficient data for regression analyses (Figures 2 and 3).

Regression analysis of leaf cardenolide content in *A. curassavica* against two measures of aphid biomass (natural logarithms of numbers and weight) all showed significant influences of increases in aphid biomass on leaf cardenolide (Figure 4). Cardenolide increased significantly with initial aphid density in both clip cage leaves and opposite leaves, and with final aphid density and biomass on clip cage leaves (Figure 4). There was also a significant regression relationship between cardenolide contents of opposite and clip cage leaves (Figure 5) with a slope of 0.58. This indicates that opposite leaves induced cardenolide production in concert with infested clip cage leaves but at a lower rate (slope < 1). Despite the induction of both leaves, leaves with aphids in a clip cage had higher cardenolide contents (mean = 1310 $\mu\text{g/g}$) than the opposite leaves (mean = 1009 $\mu\text{g/g}$) ($t_{62} = 3.27$, $P = 0.002$).

DISCUSSION

We found that although aphid populations increased with time from each starting density (Table 2, Figure 1), there were no significant differences between aphid numbers or biomass on either host plant (Table 2). Thus, although the two host plants differed markedly in their constitutive cardenolide content, this difference had no impact on aphid numbers or biomass. Thus cardenolides in *A. curassavica* and *A. incarnata* had no measurable effect on aphid population dynamics. Interference competition for space within the clip cages may have limited the higher density treatments to a lower and more variable increase in aphid density with

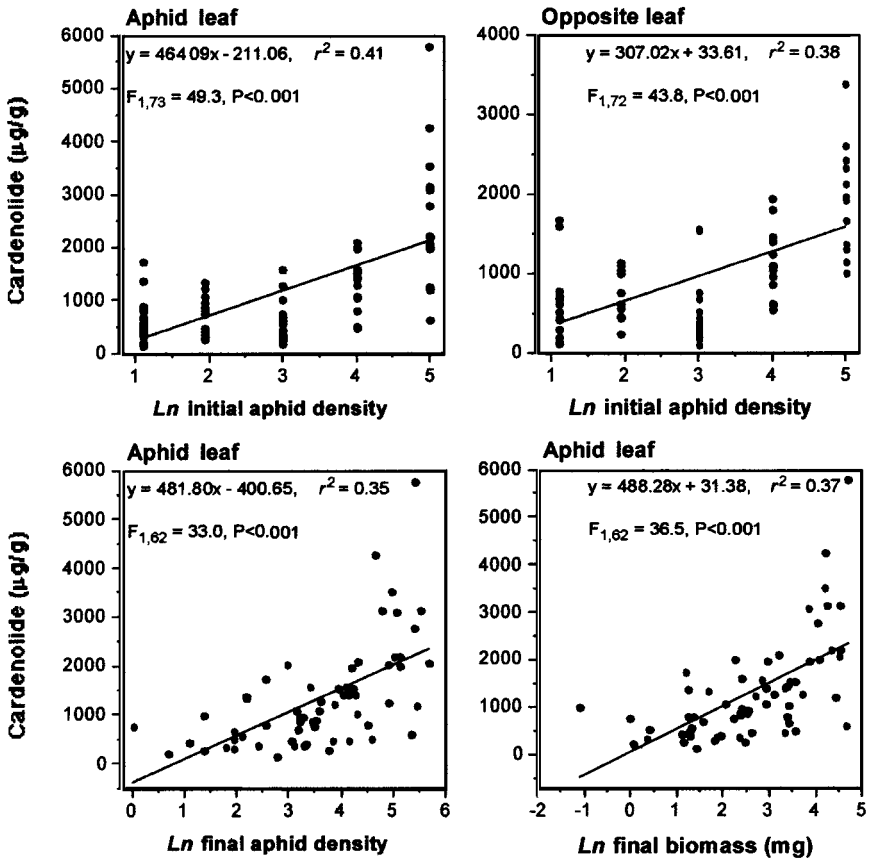


FIG. 4. Change in mean cardenolide concentrations in either aphid leaves or opposite leaves of *A. curassavica* with initial aphid density, final aphid density, or biomass.

time. Because *A. nerii* sequesters induced toxins for defense against natural enemies (Rothschild et al., 1970; Pasteels, 1978; Malcolm, 1981, 1986, 1989, 1990, 1992), intraspecific competition for these cardenolides may limit aphid population growth to a greater extent than competition for primary plant metabolites in phloem. The negative impact of such competition may be partially ameliorated from an evolutionary perspective by kin selection among apomictic relatives (Malcolm, 1986).

Similarly, we also found no significant effects of time on cardenolide expression in either *A. curassavica* or *A. incarnata*, measured in either leaves with aphids or adjacent, opposite leaves (Tables 3 and 4, Figures 2 and 3). We were surprised by this result because previous work with milkweeds (Malcolm and Zalucki, 1996)

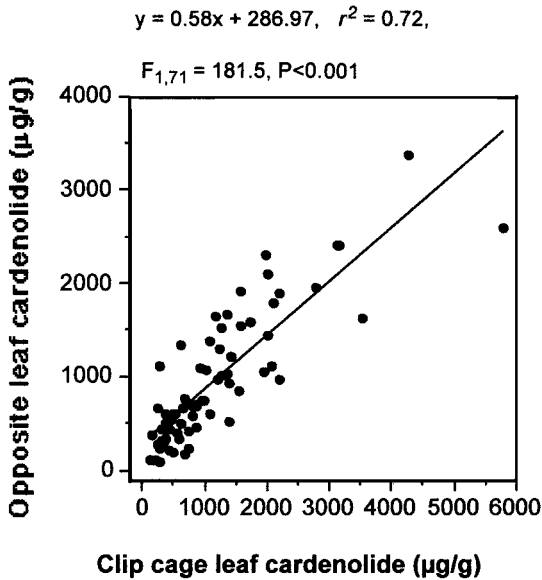


FIG. 5. Linear regression of cardenolide concentrations in opposite leaves against those in aphid leaves.

has shown that leaf damage to *A. syriaca* does induce cardenolide expression after 24 hr and that this expression decays back to approximately constitutive levels after 148 hr.

In contrast, we did find a significant effect of aphid density on cardenolide expression in *A. curassavica*, but not in *A. incarnata*. *A. incarnata* showed no measurable cardenolide response to aphids, but cardenolides in *A. curassavica* did respond to aphid density (Figure 4). The effect of aphid density on cardenolide expression by *A. curassavica* leaves surprised us because our *a priori* expectation was that the host with the lowest constitutive defense, *A. incarnata*, would induce cardenolide production more strongly in response to herbivory than the well-defended *A. curassavica* with high constitutive cardenolide. The rationale for this expectation was that low defense expression when herbivores are absent minimizes constitutive costs of defense, but that this low cost needs to be balanced by rapidly inducible defenses when a plant is attacked by herbivores. However, we found almost no detectable cardenolide in either control or aphid-infested *A. incarnata*.

Cardenolide contents increased significantly with initial aphid density in both clip cage leaves and opposite leaves, and with final aphid density and biomass on clip cage leaves from *A. curassavica* (Figure 4). Our data show that *A. curassavica* does have high constitutive levels of cardenolide in the absence of herbivores, but that cardenolide content actually *reduces* at low aphid densities, and then as aphid

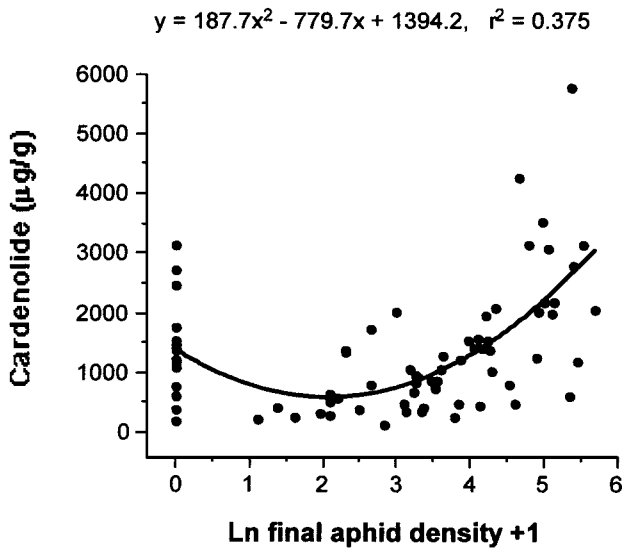


FIG. 6. Polynomial regression of leaf cardenolide content against aphid-free controls and final aphid densities.

density increases cardenolide content is induced, until at high aphid densities they are above the constitutive controls (Figure 6). Our data suggest a significantly curvilinear relationship that reflects both *reduction* and *induction* of cardenolides in response to aphid density as well as constitutive cardenolide in the absence of aphids.

Such a response, where dynamic defenses both *reduce* and *induce* in response to variable herbivore load, is important to plants that are attacked by specialist herbivores that can co-opt plant defenses by sequestration for use in their own defenses against natural enemies in the third trophic level. Reduction of plant defense can make sequestering herbivores more susceptible to natural enemies, so that more natural enemies can become “included” and increase herbivore mortality as a function of defense expression. Evidence for this scenario is described by Malcolm (1992) for this same system of *A. nerii*, *A. curassavica*, and a suite of different species of predators and parasitoids recorded as natural enemies of *A. nerii* around the world.

The response of aphid-infested leaves was reinforced by the response of uninfested leaves adjacent to the site of feeding (Figure 5). However, this response was lower than that of the infested leaves because leaves with aphids in a clip cage had significantly higher cardenolide contents (mean = 1310 µg/g) than opposite leaves (mean = 1009 µg/g). This suggests that defense reduction and induction occur systemically throughout a ramet, but that the dynamic response is strongest closest to the site of herbivory.

Hunter and Price (1992) suggested the possibility that particular herbivores may adversely affect both the first and third trophic levels. If herbivore-sequestered phytochemicals can reduce natural enemy efficacy, herbivore population density might increase and exert a direct negative impact on host-plant fitness, especially in cases where herbivores are not adversely affected by plant chemistry. Malcolm (1995) and Malcolm and Zalucki (1996) suggested the existence of a *lethal plant defense paradox* operating such that chemical defense mechanisms impose a negative impact on herbivores that may not necessarily impact host plants in a positive manner. If cardenolide expression in milkweeds is a function of *A. nerii* density, cardenolide induction could be both metabolically expensive and ineffective against herbivory. *A. nerii* might sequester the induced toxin for defense against its own natural enemies (Rothschild et al., 1970; Pasteels, 1978; Malcolm, 1981, 1986, 1989, 1990, 1992) or excrete what it is unable to assimilate in honeydew.

Karban and Baldwin (1997) suggested that phytochemical induction might rely, in part, on host-plant exposure to herbivore salivary β -glucosidase. Although *A. nerii* does not reduce *A. curassavica* foliar biomass, its piercing/sucking foraging strategy does expose plant tissue to salivary β -glucosidase and causes host damage at the cellular level (Dixon, 1998). Botha et al. (1977) photographed *A. nerii* stylets penetrating phloem sieve tubes and companion cells of the internal phloem in four milkweed species. A preference for the internal phloem of the bicollateral vascular bundles is evidence for the aphid's specialized feeding habit and the sink it represents for milkweed resources. Such specialized foraging activity is apparently sufficient to elicit a density-dependent inductive response in the cardenolides of *A. curassavica* but not in *A. incarnata*. This does not mean that *A. incarnata* is not defended by allelochemicals, and it is possible that *A. incarnata* has shifted to reliance on different, but biochemically related chemical defenses, such as the steroidal pregnane glycosides suggested by Roeske et al. (1976).

The impact of haustellate aphid herbivory on plant defense expression is poorly understood. Although aphid herbivory typically does not reduce host-plant foliar biomass, it is a resource sink and may reduce simple sugar and amino acid concentrations in phloem sap. To fuel extraordinarily high population growth rates, phloem-feeding aphids must process particularly large quantities of food due to low nitrogen concentrations in plants (Dixon, 1970, 1998; McNeill and Southwood, 1978). Therefore, the foraging activities of aphids in dense colonies should lower host-plant fitness. This in turn suggests the likely operation of selection for defenses to protect plant fitness. Such a response was described by Muller (1959), who found that the roots of grape, *Vitis vinifera*, induce the production of "corky" necrotic zones in response to feeding by the vine louse, *Phylloxera vasatrix*. These zones function to isolate the herbivore from remaining healthy root tissue. More recently, Gianoli and Niemeyer (1996, 1997) found that wheat, *Triticum aestivum*, induces hydroxamic acid in response to feeding by a minimum of 25 bird-cherry

aphids, *Rhopalosiphum padi*, following 48 hr of infestation. Thus, it is possible that milkweeds may also engage in damage-induced cardenolide expression in response to piercing/sucking herbivory by *A. nerii*.

Despite the fact that *A. curassavica* induces cardenolides as a function of *A. nerii* density, the insect population data suggest that milkweed secondary chemistry exerts little adverse effect on herbivore colony biomass or population density, over the short-time period of our measurements. *A. curassavica* and *A. incarnata*-reared *A. nerii* colony biomass and population densities were virtually identical. This again suggests that *A. nerii* is an adapted specialist that largely avoids the deleterious effects of milkweed-induced resistance, regardless of cardenolide concentration. Because cardenolide sequestration reduces predator efficacy (Malcolm, 1981, 1986, 1989, 1990, 1992), cardenolide induction in some milkweed species may enhance the effectiveness of *A. nerii*-sequestered chemical defense against natural enemies in the third trophic level.

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