

It's the first bites that count: Survival of first-instar monarchs on milkweeds

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Abstract Mortality of first instars is generally very high, but variable, and is caused by many factors, including physical and chemical plant characters, weather and natural enemies. Here, a summary of detailed field-based studies of the early-stage survival of a specialist lepidopteran herbivore is presented. 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, as well as other plant characters, such as leaf hairs, microclimate and concentration of secondary metabolites. Even for a so-called 'milkweed specialist', larval performance and survival appears to be related to the concentration of cardenolides produced by the plants (a potential chemical defence against herbivory). This case study of monarchs and milkweeds highlights the need for field-based experiments to assess the effect of plant characters on the usually poor survival of early instar phytophagous insects. Few similar studies concerning the performance and survival of first-instar, eucalypt-specific herbivores have been conducted, but this type of study is considered essential based on the findings obtained using *D. plexippus*.

Key words: *Asclepias*, assays, cardenolide, *Danaus plexippus*, growth rate, latex, laticifer, neonate larvae, plant defence, survival.

INTRODUCTION

Over the years, there has been much debate about the factors that influence the temporal variation in abundance of phytophagous insects. Analyses of long-term abundance data and sequential life-table studies (e.g. Dempster 1983; Strong *et al.* 1984; Stiling 1988) have identified various 'key factors' that account for most of the change in abundance over time. These include weather, failure to lay egg complement, starvation, natural enemies and plant resources; in many cases, early immature mortality is identified as a crucial stage.

Detailed life-table studies provide equivocal evidence for causes of mortality in early-instar plant chewing insects. Titmarsh (1992) reviewed published life-table studies that purportedly detail both the level and cause of mortality of various Lepidoptera. Most mortality occurs in the early stage and the most frequent cause of death is 'unknown factors' (see also Kyi *et al.* 1991). Life-tables, in the absence of controlled field experi-

ments, may not be able to distinguish between causes of death (Room *et al.* 1991). We propose that a high proportion of this 'unknown' mortality may be attributed to plant factors and interactions among factors.

Much of the work on the effect of plant characters on herbivorous insects, particularly plant toxins, is based on laboratory bioassays where material is exposed to large larvae, usually in artificial diets (e.g. Reese 1979; Ishaaya 1986). Such bioassays measure the doses needed to affect larvae, at least under the limited conditions of such experiments, but may be misleading in estimating the effects under field conditions, particularly as plants vary in many characters that may impinge on feeding insects. We suggest that field based studies are more 'ecologically relevant' to understanding insect–plant interactions (Chapman *et al.* 1983; Eigenbrode *et al.* 1991).

Host plant quality can affect the abundance of herbivores in two ways. First, plant quality can affect the number of colonizing herbivores by either influencing feeding larvae or adults, or by influencing the behaviour of ovipositing females. Second, the quality of the host plant can affect survival and larval performance. Larval survival influences the number of adults

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emerging in the same generation, whereas larval performance can affect the potential fecundity of females. Both the number surviving and their fecundity will influence the abundance of individuals in the subsequent generation.

Here, we present evidence for the importance of plant attributes to early-stage herbivore survival and growth rate in the field in a host plant specialist. We use monarch–milkweed interactions in the field as a model system to illustrate the approach. In the discussion, we present our thoughts on the relevance of the monarch–milkweed model system to insect–eucalypt interactions.

MONARCH–MILKWEED INTERACTIONS

Small toxic plant molecules may be effective defences against generalist herbivores but not against specialist herbivores (Feeny 1976; Rhoades & Cates 1976). Both the apparency theory (Feeny 1976) and the optimal defence theory (Rhoades & Cates 1976) argue that the products of secondary plant metabolism, such as cyanogenic glycosides, alkaloids and cardenolides, are effective defences against most generalist herbivores. The generalization implicit in these theories is that ‘plant toxins’ have little effect on specialists and this has been corroborated by evidence that ‘plant toxins’ can have little negative impact on the growth and survival of specialized herbivores (e.g. McCloud *et al.* 1995). However, it is becoming increasingly clear that small toxic molecules negatively affect many specialist herbivores (Baldwin 1991; Karban 1991; Zalucki *et al.* 2001).

The monarch butterfly, *Danaus plexippus*, feeds almost exclusively on milkweeds in the genus *Asclepias* (Malcolm & Brower 1986). Milkweed defences include cardenolides, a group of toxic steroids, with low molecular weights, that target the ubiquitous Na⁺, K⁺–ATPase receptor sites of all animals (Horisberger 1994). Cardenolides occur throughout the plant and in particular in the white milky latex that is characteristic of almost all *Asclepias* species (Malcolm 1995) and, similar to some other secondary compounds, they are inducible by larval damage to leaf tissue (Malcolm & Zalucki 1996).

Early-instar monarch larvae are known to have variable, but generally poor, survival (Zalucki & Kitching 1982), as is common in the Lepidoptera. 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*. Zalucki and Brower (1992) subsequently confirmed this observation experimentally and suggested that some of the high early larval instar losses might be related to the cardenolide concentration

in latex, or to the latex per se. This was confirmed by manipulative experiments with this milkweed species in the field (Zalucki *et al.* 2001).

We provide an overview of our studies on how milkweed defences influence first-instar monarch larval survival and growth. We summarize growth rate data for monarchs on nine species of milkweed, along with various measures of plant quality (category of latex production, mean plant cardenolide content, presence of leaf hairs) studied under field conditions. We also present survival and growth rate data for larvae at various densities with and without predation on one North American species, *Asclepias syriaca*. The data clearly indicate a major role for plant variables in early stage survival in this host specialist.

CASE STUDY METHODS: FIELD EXPERIMENTS WITH MONARCHS AND MILKWEEDS

Monarch larvae reduce latex flow by various feeding behaviours that trench or partially sever leaf petioles or veins, feeding on leaf tissue distal to the trenched or partially severed areas. In our field experiments we mimic this behaviour and reduce latex flow by partially severing the petioles of milkweed plants. By exposing first-instar larvae to intact and partially severed leaves, we assess the effectiveness of a plant’s defences against this ‘specialist’ herbivore. Experiments were conducted in Florida (Zalucki *et al.* 2001), Michigan (Zalucki & Malcolm 1999), California and Australia (M. P. Zalucki, unpubl. data) from 1993 to 1999 (Table 1).

Plant latex and cardenolide measurement

Non-articulated laticifers ramify throughout milkweed stem and leaf tissue. Latex production in different milkweeds was compared by regressing estimates of latex volume (μL) produced against the cross-sectional area (mm^2 , measured with callipers) of the source areas: leaf lateral veins, midrib veins, petiole and stem. Flowing latex was collected in volumetric capillary tubes (or small flasks) following rapid severance of the plant part at each cross-sectional point. The cumulative volume of latex 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. Here we simply categorize the volumes as high and low. Plant cardenolide levels were based on literature values or measures based on standard spectrophotometric assays as digitoxin equivalents ($\mu\text{g } 0.1\text{g}^{-1}$ dry weight of leaf tissue; Brower *et al.* 1975).

Larval experiments

We used monarch eggs (synchronized to hatch simultaneously) on leaf discs, which were glued to experimental plants (see Zalucki *et al.* 2001) or occasionally newly hatched larvae (see subsequent discussion). Eggs, laid by either wild-caught or laboratory-reared monarchs, confined within mesh bags that were tied around fresh stems of potted *Asclepias curassavica* plants, were removed by using a hole punch, then incubated to the 'black head' stage (just prior to larval emergence) for a day-degree accumulation of approximately 45 dd (Zalucki 1982). Discs (5 mm diameter) with eggs about to hatch were glued onto experimental plants by using a small drop of latex from an adjacent stem or with chicken egg albumin. To reduce both losses due to predation and larvae wandering off the plant, we confined larvae in some experiments to treatment leaves by using small clip cages (made from 5 mm deep by 23 mm diameter PVC piping, covered in fine gauze and held in place over leaves with clips). After 2–4 days, the surviving larvae were counted and taken to the laboratory, where they were weighed wet, usually dried in a freeze drier and then re-weighed.

In each experiment, we singly glued 5–10 leaf discs onto each control or treated plant stem. Discs bearing eggs were placed on the underside of leaves, with no more than 2–4 per leaf. The number of replicate plants varied depending on the availability of both plants and eggs (Table 1). Partial petiole severance to reduce the latex flow to leaves was achieved by notching across the underside of the petiole three times (notches were approximately 5 mm apart) with a pair of forceps. The effectiveness of this technique, as measured by latex volumes taken from a sample of intact and partially severed leaves, resulted in a reduction of latex flow by 52–94%.

Partially severed leaves remained turgid and green and were indistinguishable from intact control leaves over the short duration of each experiment. At the final assessment, larvae were counted and the instar recorded, then larvae were collected and placed on ice. The number of unhatched eggs and the number and fates of dead larvae were recorded.

When comparing the effects of experimental treatments within plant species, we used the wet weight 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 in the natural environment at different times and places. Daily maximum and minimum temperatures were recorded locally and/or within 5–10 km of the

plant locations. Measurements from these weather stations were representative of local conditions (unpubl. data).

Survival with and without predation

Eggs at the advanced black head stage were attached singly to 25 *A. syriaca* plants with intact leaves and enclosed in clip cages on 24 July 1995 at Arcadia, near Kalamazoo, Michigan. Eggs were also placed on plants with intact leaves (one per plant; $n = 25$) and enclosed in an organza white mesh bag, or left unbagged ($n = 50$ plants). Because of time constraints, we placed out a second series of eggs onto bagged and unbagged plants on 25 July at the following densities per plant: two ($n = 10$ plants per treatment), four ($n = 10$ plants per treatment), eight ($n = 5$), 16 ($n = 2$), and 25 ($n = 2$). Surviving larvae from eggs placed out on 24 July were recorded on 28 July, and those put out on 25 July were recorded on 29 July. We noted whether the eggs had hatched, the fates of larvae, and the presence of feeding damage.

RESULTS

Variation in plant characters

The volume of latex produced as a function of severed cross-sectional area was described by significant linear regressions for each milkweed species (M. P. Zalucki, unpubl. data). The latex volumes differed significantly among the nine *Asclepias* species, as did the regression slopes (M. P. Zalucki, unpubl. data). Thus, most latex was produced by *Asclepias erosa*, *Asclepias eriocarpa*, *Asclepias californica*, *A. humistrata* and *A. syriaca*, and the least by *Asclepias tuberosa* and *Asclepias incarnata* (Table 1).

Table 1. Details of milkweed (*Asclepias*) species used in field experiments to assess the effects of plant variables on first-instar monarch survival and growth

Species	No. experiments	No. plants (stems)	No. eggs
<i>A. erosa</i>	1	20 (20)	130
<i>A. eriocarpa</i>	2	20 (40)	376
<i>A. californica</i>	3	19 (38)	350
<i>A. humistrata</i>	1	29 (62)	744
<i>A. syriaca</i>	3	60 (60)	600
<i>A. tuberosa</i>	1	6 (20)	200
<i>A. incarnata</i>	1	8 (20)	200
<i>A. curassavica</i>	1	11 (22)	220
<i>A. fruticosa</i>	1	11 (22)	220

The five high latex species also have higher cardenolide contents than the two with the least latex (Table 2). However, *A. syriaca* (one of the high latex volume plants) has considerably lower cardenolide levels than the southern *A. humistrata*, or the Californian species (*A. erosa*, *A. eriocarpa* and *A. californica*). *Asclepias tuberosa* produces small amounts of a clear, watery latex and *A. incarnata* produces small amounts of a more viscous, creamy-yellow latex. These two species contrast with *A. erosa*, *A. eriocarpa*, *A. californica*, *A. humistrata* and *A. syriaca*, which produce copious white latex. The latex of *A. erosa* was also noticeably aromatic.

The plants also differ in the presence of surface hairs (Table 2). *Asclepias humistrata*, *A. curassavica* and *A. fruticosa* are glabrous species; *A. erosa* leaves are hairy to start with, but become less so as they age. The other species were all hirsute (Table 2).

We did not compare latex viscosity or flow rate following laticifer puncturing. Our observations suggest that these properties differ among the species. For

Table 2. Physical and chemical characteristics of nine *Asclepias* species

Species	Mean (range) cardenolide* ($\mu\text{g } 0.1 \text{ g}^{-1} \text{ DW}$)	Latex volume	Leaf hairs present
<i>A. erosa</i>	562 (79–2102)	High	Yes/No
<i>A. eriocarpa</i>	421 (102–919)	High	Yes
<i>A. californica</i>	66 (9–199)	High	Yes
<i>A. humistrata</i>	389 (170–645)	High	No
<i>A. syriaca</i>	50 (4–229)	High	Yes
<i>A. tuberosa</i>	3 (0–6)	Low	Yes
<i>A. incarnata</i>	111 (42–199)	Low	No
<i>A. curassavica</i>	415 (227–638)	Low	No
<i>A. fruticosa</i>	345 (119–719)	Low	No

*taken from Seiber *et al.* (1982), Malcolm & Brower (1989), Zalucki & Brower (1992), Nelson (1993), Zalucki *et al.* (1990, 2001).

Table 3. Comparative survival and growth rates of first-instar monarch larvae reared on plants or stems with either intact leaves or leaves with partially severed petioles on nine species of *Asclepias*

<i>Asclepias</i> species	Per cent survival (%)		Growth rate (10^{-5} g wet weight per day degree)	
	Intact	Severed	Intact	Severed
<i>A. erosa</i>	0	10	2.5	9.2
<i>A. eriocarpa</i>	57	67	4.2	6.6
<i>A. californica</i>	7	56	3.5	6.5
<i>A. humistrata</i>	28	59	3.5	7.8
<i>A. syriaca</i>	61	67	4.4	6.9
<i>A. tuberosa</i>	39	55	6.5	7.6
<i>A. incarnata</i>	57	63	7.7	7.6
<i>A. curassavica</i>	57	41	4.8	5.0
<i>A. fruticosa</i>	45	45	5.4	4.7

example, the latex of *A. humistrata* and *A. erosa* appears to have low viscosity, flowing rapidly to form many small globules depending on the size of the puncture. In *A. syriaca*, *A. californica* and *A. eriocarpa*, the latex is more viscous and oozes out, and, at least on the pubescent surfaces of leaves, the copious latex can spread by surface forces from hair to hair. In *A. incarnata*, *A. curassavica*, *A. fruticosa* and *A. tuberosa*, the latex tends not to flow beyond the immediate area of the puncture.

We also have not compared leaf hardness and toughness (see Sanson *et al.* 2001) but these characters differ among individual plants and species and no doubt influence larval performance, growth rate and survival.

Larval experiments

Growth was more rapid and survival much higher on partially severed leaves of *A. californica*, *A. eriocarpa*, *A. erosa* and *A. humistrata* compared with the intact treatments (Table 3). All these are species with a high latex volume, and except for *A. californica*, they are high-cardenolide milkweeds (Table 2). The latter is covered with a dense mat of plant hairs (M. P. Zalucki, unpubl. data).

Survival and growth rate were similar on partially severed and intact leaves of the low latex volume and high-cardenolide milkweeds *A. curassavica* and *A. fruticosa*. Both have soft glabrous leaves and larvae do not appear to have any mechanical problems in handling the relatively low latex flow from these plants. This is similar to results for *A. incarnata*, another smooth-leaved plant with relatively low latex content (Table 2; see Zalucki & Malcolm 1999).

Generally, growth rates on the intact leaves of most milkweed species were low compared with the usual elevated growth rates on treated leaves, suggesting a negative effect of plant constituents, possibly the high cardenolide content in the latex. Larval growth rates on *A. californica*, *A. eriocarpa*, *A. erosa*, *A. humistrata*, *A. incarnata*, *A. syriaca* and *A. tuberosa* on leaves with partially severed petioles were similar to each other but differed on intact leaves (Table 3), suggesting that latex and possibly cardenolides are important in first-instar monarch larval growth, development and survivorship.

Effect of plant cardenolide concentration and latex overall

As in Zalucki *et al.* (2001), we used the difference in growth rate of first-instars between partially severed and intact treatments as an indicator of host plant cardenolides and latex effects. The greater the difference, the greater the latex and cardenolide effect is likely to be (Fig. 1). As was found within a milkweed species among

plants (Fig. 1; for *A. humistrata* the best fit regression line is shown, Zalucki *et al.* 2001), the concentration of cardenolides affected first-instar growth rates in a similar manner across species, at least for high latex volume and generally hairy species. The differences were not as marked for glabrous and low latex volume plants (Table 3, Fig. 1).

Using the differences in growth rate as a measure

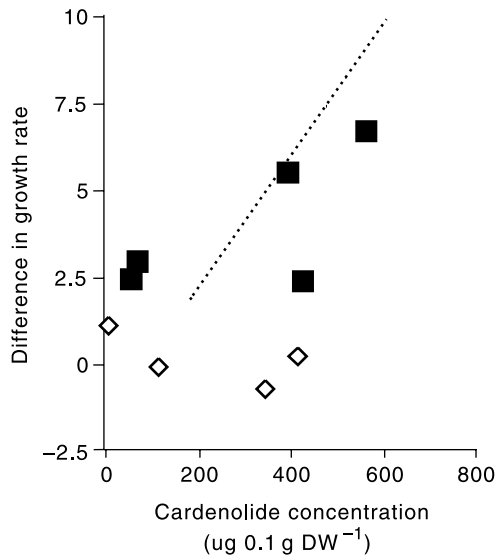


Fig. 1. Mean difference in mean growth rate between partially severed and intact leaf treatments plotted against leaf cardenolide concentration for *Asclepias* species with (■) high and (◇) low latex flow. Also shown is the regression line only for the same type of data for individual plants of *Asclepias humistrata* (taken from Zalucki *et al.* 2001).

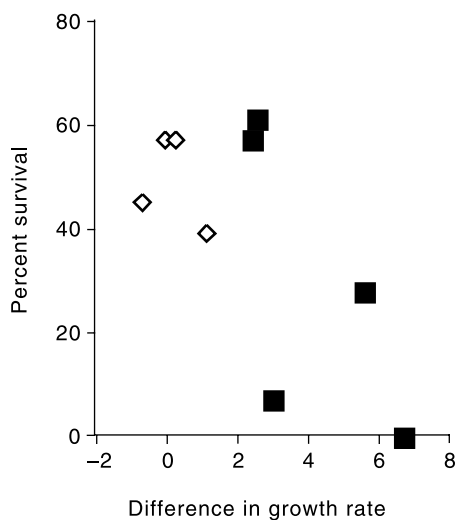


Fig. 2. Percentage survival in the first instar recorded on intact treatments of nine *Asclepias* species plotted against the difference in larval growth rate between severed and intact treatments. (■), High latex; (◇), low latex.

of host species quality, we found that survival was generally higher for the low latex and low cardenolide species (Fig. 2), decreasing in high latex, high cardenolide species (Fig. 2; analysis on arcsin square-root-transformed proportion surviving; survival = $0.851 - 0.10 \times$ 'host quality', $F_{1,7} = 9.94$, $P = 0.0161$, $r^2 = 0.53$; slope significantly different from zero, $t = -3.15$, $P < 0.05$).

Density and predation effects

On *A. syriaca*, single larvae confined by clip cages had a higher survival rate (92%) than larvae allowed to wander freely on bagged (72%) and unbagged plants (24%). In the density experiment, some 64% of larvae survived on the bagged plants to the end of the first instar, a significantly higher proportion than survived on unbagged plants (25%, $P < 0.05$). There was no effect of density (expressed as initial number of eggs per leaf) on these results (Fig. 3). The absence of a density-dependant effect suggests that either predation is consistent across all plants, or that on bagged plants, larvae that become dislodged are more likely to relocate the test plant and appear to have a higher survival rate (or both). We have not investigated how or why larvae become dislodged from or leave plants.

DISCUSSION

The monarch butterfly is a specialized larval herbivore on plants of the family Asclepiadaceae, and virtually to the genus *Asclepias*. Other authors have shown that

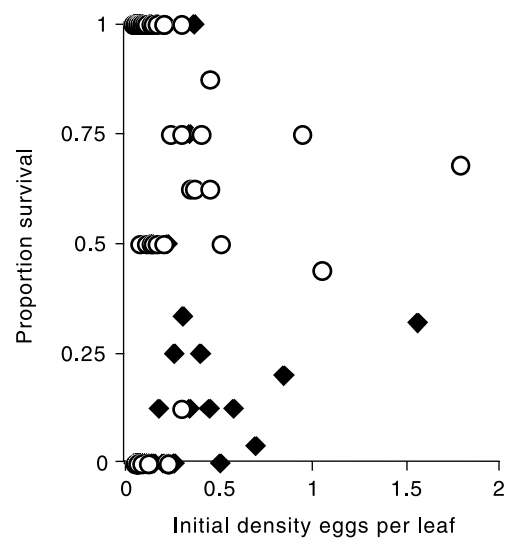


Fig. 3. Proportion of larvae surviving the first instar against initial density of eggs about to hatch (equals neonates) at various densities (per leaf available) per plant for *Asclepias syriaca* covered in (○) mesh bags or (◆) left unbagged.

these plants contain differing arrays of cardenolides that vary in amount and type within and between species, within various plant parts, and through time (Nelson *et al.* 1981; Brower *et al.* 1982; Malcolm 1991, 1995). *Asclepias* species are noted for a milky latex that is contained under pressure in a non-articulated sealed system of vessels known as laticifers (Lucansky & Clough 1986). When any part of the plant is punctured, latex flows rapidly out and coagulates on contact with air. Milkweed latex may contain high concentrations of cardenolides (Seiber *et al.* 1982), amyrin (a precursor of rubber) and other noxious chemicals (Farrell *et al.* 1991). The latex system has been interpreted as a plant defence, particularly against generalist herbivores that lack latex canal sabotaging behaviour (Dussourd & Eisner 1987; Dussourd 1993; Dussourd & Denno 1994).

Monarch caterpillars have evolved the ability both to circumvent the latex defence of milkweeds (Dussourd & Eisner 1987) and to appropriate the cardenolide chemical defences of the plant (Brower 1984). All larval instars show sabotaging behaviour and may effectively disable the latex flow in milkweeds. Early instars trench and cut small moats through the leaves (Dussourd 1990; Dussourd & Denno 1991; Zalucki & Brower 1992), whereas later instars sever the petioles or midribs of the leaves before consuming them (Zalucki & Brower 1992). These behavioural traits appear common in the Danaines (Clarke & Zalucki 2000). Monarch larvae generally concentrate cardenolides above the level found in the plant leaves (Malcolm & Brower 1989; Nelson 1993) and conserve the compounds through to the adult stage, possibly as a form of storage excretion (Brower *et al.* 1988).

Survival of first-instars on various milkweeds in the field is poor, ranging from 3 to 40%, with most studies finding low survivorship (Zalucki & Kitching 1982; Zalucki & Brower 1992; Zalucki & Malcolm 1999; Zalucki *et al.* 2001; M. P. Zalucki, unpubl. data). Using field experiments, Zalucki and Brower (1992) found that only 3–11% of newly hatched larvae survived through the first instar on *A. humistrata*. Larval survival was correlated negatively with the concentration of cardenolides in the leaves (see also Zalucki *et al.* 1990), but was not affected measurably by ground-dwelling predators. Approximately 30% of larvae that died were glued by the latex to the leaf surface. This occurred even though all the neonate larvae engaged in trenching behaviour to sabotage the latex outflow. The first bites into the leaves of this milkweed, even by specialist neonate larvae, are dangerous. Exactly how larvae become mired is not clear (see Zalucki *et al.* 1990).

If the leaf is hirsute, larvae must first graze down the hairs (see Malcolm 1995) before chewing through the leaf surface. The larva breaks the latex vessels, encounters latex outflow, and if avoidance behaviour is not

sufficiently fast, latex adheres to the mouthparts and head. The larva then attempts to clean itself vigorously but frequently imbibes the latex and become cataleptic (Zalucki & Brower 1992). The 34–47-fold higher concentration of cardenolides in the latex compared with the leaves may be responsible for the catalepsis, although it is not clear whether cardenolides per se or some other chemicals in the latex cause catalepsis and contribute to the subsequent high mortality (Zalucki *et al.* 2001).

Previous research on the latex defences of milkweeds has focused largely on the ability of specialist herbivores, including *D. plexippus* and *Danaus gilippus berenice* (Cramer), to defeat the system (Dussourd & Eisner 1987; Dussourd & Denno 1991, 1994; Dussourd 1993). Clearly, the latex system of milkweeds and their included cardenolides have a direct effect on the survivorship and growth rates of a dietary specialist. Our novel experimental method disrupts the flow of latex into the leaves by mimicking fifth-instar larval behaviour and allows three effects of the plant's defences on first-instar larval survival and growth to be partitioned out: (i) the lethal effects of miring and gluing by the latex; (ii) the toxic effects of cardenolides in the latex; and (iii) the toxic effects of cardenolides in the leaves.

Latex reduces first-instar larval growth rates

The latex had a detrimental effect on the growth rate of first-instar monarch larvae. Larvae feeding on leaves of which the petioles had been partially severed generally grew faster than larvae on intact control leaves. The outcome depended on the species of *Asclepias*. Larval growth rates on leaves of *A. californica*, *A. eriocarpa*, *A. erosa*, *A. humistrata*, *A. incarnata*, *A. syriaca* and *A. tuberosa* with partially severed petioles were similar to each other but differed from growth rates on intact leaves, suggesting that latex and possibly cardenolides are important in first-instar monarch larval growth. Growth rates on severed leaves of *A. fruticosa* and *A. curassavica* were low overall compared with the usual elevated growth rates on treated leaves, suggesting an effect of plant constituents in the leaf, possibly the amount and type of cardenolide.

Latex increases first-instar larval mortality

Only 28% of the first-instar larvae survived for 72 h when placed as eggs on intact leaves of *A. humistrata* compared with 59% survival when placed on partially severed leaves (Zalucki *et al.* 2001). Moreover, 27% of the mortality on the intact plants was due to the larvae

becoming mired in the latex, compared with only 2% miring on the partially severed stems. Thus, feeding on the intact leaves was nearly 15 times more likely to kill the larvae than when they fed on leaves to which the flow of latex had been cut off. Similarly, low survival was recorded on other high-cardenolide, high-latex plant species, but survival was better on low-cardenolide, low-latex plant species in California and Michigan (Table 3).

Growth reduction factors include the time larvae spend on latex avoidance behaviour and coping with plant characters, for example, moving to other plant parts if copious latex is encountered, removing hairs to get to the leaf surface and becoming inactive (cataleptic) after ingesting latex. Because the mortality of early instars because of Formicidae, Neuroptera and other entomophages must be time dependent, any factors that slow growth rates and increase movement will probably also reduce survival by exposing larvae to various predators for longer. The absence of any density-dependent effects on larval survival suggests that the constant high loss rate may well be related to larvae losing contact with their host plant.

Detrimental effects of various plant constituents on larvae

The growth rates of Lepidoptera on their host plants are influenced by many factors, but primarily nutritional composition and secondary plant compounds (Scriber & Slansky 1981; Herms & Mattson 1992; Slansky 1992). The latter will vary among plants, plant parts and developmental stages (Nelson *et al.* 1981; Brower *et al.* 1982). The experimental design enabled us to control for nutritional composition. Our technique suggests that there were other effects on larval growth rates, as indicated by the scatter of points and by the low variance explained by cardenolides measures alone. These may include other latex constituents (e.g. rubber compounds) and/or leaf chemistry, moisture, physical attributes and nutrient levels.

Our studies leave two questions unanswered. Specifically, are larvae on intact leaves better protected from predators because of this higher cardenolide level? The possible trade-off between lower predation rate or higher mortality caused by the latex and cardenolides requires further research. Also, does latex ingestion cause catalepsy and disorientation in first-instars and cause them to fall off the plants and/or increase the probability that predators or adverse conditions will kill them? Classic life-table studies would simply record such larvae as dead because of either 'unknown' causes or possibly erroneously attribute the loss to predation. Our experiments show that a substantive proportion of neonate larval mortality is related to plant factors.

Insect-eucalypt interactions post-script

Most eucalypt-feeding insects appear to be host specialists and very few studies have demonstrated any adverse effects of the terpenoid constituents of leaves on growth and survival (Ohmart & Edwards 1991). This may not be surprising given that some insect herbivores of eucalypts are known to consume foliage of tree species not related to the Myrtaceae but of similar terpenoid composition (Steinbauer & Wanjura 2000). Furthermore, field-based studies of insect-eucalypt interactions have often been correlative and simply related the degree of herbivory (plant damage) to various measures of plant quality (usually various terpenoids, nitrogen; e.g. Fox & Macauley 1977; Macauley & Fox 1980; Morrow & Fox 1980; Edwards *et al.* 1993). This sort of study does not enable one to distinguish between the effect of plant characters, if any, on insect feeding, colonization, survival and development. In contrast, manipulative field-based studies of insect-plant interactions are suggested to be more 'ecologically relevant' because they allow for interactions between organisms that are difficult, if not impossible, to mimic by means of laboratory-based studies (see Chapman *et al.* 1983; Eigenbrode *et al.* 1991).

To date, most of the detailed insect-eucalypt studies have found strong effects of leaf toughness (Steinbauer *et al.* 1998; Howlett 2000), nitrogen (Ohmart *et al.* 1985) and eucalypt species-specific reactions to eggs and larvae of some insects (Mazanec 1985). Clearly, the monarch-milkweed model system differs somewhat from the insect-eucalypt system. Most notably, eucalypts do not exude latex when damaged. The only known reaction to resemble this in eucalypts is the release of the saccharine exudate known as 'manna' following damage to eucalypts by insect herbivores (see Steinbauer 1996, 1997). At present, this appears to be the only 'rapidly induced response' known in eucalypts. Recently, a group of naturally occurring chemicals known as diformyl-phloroglucinol compounds (DFPCs) have been found to deter feeding by eucalypt-specific marsupials (Lawler *et al.* 1998a, 2000). Like cardenolides, DFPCs appear to induce nausea, thereby creating an aversion to eucalypts in which these compounds are found (Lawler *et al.* 1998b). The presence of DFPCs in certain eucalypts preingestion appears to be detected by animals via specific volatile terpenoids, for example, cineole. Cineole can condition the aversive response because its concentration varies linearly with the concentration of a common DFPC, namely sideroxylonal (Lawler *et al.* 1999). The role of DFPCs in insect herbivory is currently being investigated (see also Cooper 2001). If DFPCs affect eucalypt-specific insects similarly to the way in which cardenolides affect monarchs, then their influence on survival and performance clearly warrants investigation.

To determine whether there are any adverse effects of eucalypt secondary chemicals, not to mention adverse effects of other leaf characters, detailed field studies will need to be conducted (e.g. Maddox 1995; Patterson *et al.* 1996), in which measurements are taken in an ecologically relevant way. We suggest that manipulative experiments that are based on an understanding of the life history of the study insect, as well as a clear identification of the eucalypt trait (e.g. leaf toughness, water or other primary component content, manna, terpenoids and possibly also DFPCs) of particular interest, are the most likely means of advancing our understanding of insect–eucalypt interactions.

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