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ORIGINAL PAPER

Does secretory canal architecture determine the sabotage behaviors of insect folivores?

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Abstract

Insect folivores on plants protected by secretory canals commonly sever leaf veins or cut trenches before feeding beyond the cuts. Previous studies reported that vein cutting occurs when canals have an arborescent arrangement, whereas trenching is found when canals have a net-like arrangement. However, some danaine species, such as the monarch caterpillar, *Danaus plexippus*, show both behaviors on the same milkweed plant; early instars cut circular trenches and later instars chew furrows in the leaf midrib. This study tests the hypothesis that milkweed canals differ in arrangement at different scales, thus requiring different behaviors from early and late instars. I compared common milkweed, *Asclepias syriaca* (Apocynaceae) with prickly lettuce, *Lactuca serriola* (Asteraceae). Leaves were damaged with standard wounds and the response of the laticifers was compared by measuring latex exudate. With *L. serriola*, severing either the primary or secondary veins failed to reduce latex emission beyond the cuts. The veins and associated laticifers form an interconnected network; plusiine caterpillars on *L. serriola* disarm the network with a trench. With *A. syriaca,* transecting the midrib virtually eliminated distal exudation. However, severing a secondary vein caused only a partial reduction. To decrease exudation beyond secondary veins, milkweed insects need either to sever multiple adjacent veins (as shown by *Labidomera clivicollis* beetles) or to cut a trench (as in early instar danaine larvae). Thus, in both *A. syriaca* and *L. serriola*, herbivore behaviors match the laticifer systems as predicted by the hypothesis that canal architecture has a central role in determining behavior.

Keywords Secretory canal · Laticifer · Vein cutting · Trenching · *Danaus plexippus* · *Asclepias syriaca*

Introduction

Insects biting into a leaf frequently rupture secretory canals and encounter latex or resin exudates. Laticifers (living cells with latex) and resin ducts (intercellular canals) are broadly distributed; they occur in almost 20% of all plant families (Prado and Demarco 2018; Foisy et al. 2019). Fluids within these canals are often stored under pressure (Buttery and Boatman 1976; Pickard 2008). Thus, when a leaf is damaged, secretion exudes from the wound, confronting the prospective herbivore with a toxic, sticky barrier to feeding (Dussourd 1993; Agrawal and Konno 2009; Konno 2011). Insect folivores on these plants commonly use their mandibles to damage leaf veins before feeding distal to the cuts. This behavior termed canal cutting has been reported in approximately 100 species classified in 13 families in three orders (Lepidoptera, Coleoptera, Orthoptera) (Dussourd 2009 and references cited; Rodrigues et al. 2010; Kalaisekar and Sarma 2019; Lees and Zilli 2019). The insects exhibit canal cutting on plants with latex canals (9 families), ducts (3 families), or exuding phloem sap (one family) (Dussourd 2009). Well-known examples include monarch caterpillars (*Danaus plexippus*, Nymphalidae) and their danaine relatives on milkweeds (Apocynaceae) and plusiine caterpillars such as cabbage loopers (*Trichoplusia ni*, Noctuidae) on lettuce (Asteraceae: Lactuceae).

The behavior of canal-cutting insects matches the architecture of veins in the leaf and their associated secretory canals (Dussourd and Denno 1991; Dussourd 2009). On plants with canals that branch off a central midrib in an

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arborescent arrangement, the insects sever individual veins (vein cutting); the cuts reduce or eliminate exudation during feeding beyond the cuts. For example, diverse folivores on the milkweed, *Asclepias syriaca*, chew a furrow in the midrib or bite into individual veins; these species include larvae and adults of the chrysomelid beetle, *Labidomera clivicollis* (Fig. 1a, c), adults of cerambycid and curculionid beetles, and caterpillars of arctiine moths and the monarch butterfly (Fig. 1b) (Dussourd and Eisner 1987; Fordyce and Malcolm 2000; Dussourd and Denno 1991). In contrast, on plants with canals in a net-like arrangement, insects cut a trench, a continuous line of bites that isolate a portion of the leaf. The insects then feed within or beyond the trench. For example, on the wild lettuce, *Lactuca serriola*, five species of noctuid caterpillars cut trenches, including *Autographa precationis* (Fig. 1d) (Dussourd and Denno 1991). Canal arrangements have been classified as arborescent or net-like according to how the canals respond to a midrib cut (Dussourd and Denno 1991). With arborescent canals, severing the midrib isolates distal branches of the secretory canals, thereby preventing pressure-driven flow of canal contents to distal locations where the insect feeds. On plants with canals in a net-like arrangement, the midrib cut does not prevent distal exudation because secretion flows within canals that loop around the midrib cut.

This correspondence between canal architecture and canal-cutting behaviors has been documented in numerous insect–plant associations that represent multiple independent origins of both secretory canals and canal cutting (Dussourd 2009). However, exceptions do exist. For example, early instar larvae of the monarch and other danaines on milkweeds often cut a circular or semicircular trench before feeding within or beyond the trench (Fig. 1e) (DeVries 1987; Dussourd and Denno 1991; Zalucki and Brower 1992; Hirai and Ishii 2002; Ferreira and Rodrigues 2015). In some species, the caterpillars simply cut closely spaced individual veins giving the appearance of a trench (Clarke and Zalucki 2000), but in others they make a continuous line of cuts (Dussourd 1990; Agrawal 2017). When larger, the same larvae on the same plant chew furrows in the midrib (Fig. 1b). If different stages of the same species on the same plant show such strikingly different behaviors, how could the arrangement of secretory canals determine behavior? This apparent contradiction has led some to question if laticifer anatomy mediates insect sabotage behaviors (Ferreira and Rodrigues 2015).

There are many possible explanations for why early and late instar danaines differ in behavior. Perhaps the mandibles of young larvae are too small or weak to sever veins. Or perhaps vulnerable early instars use exudate oozing from trenches as a defensive moat to protect against predators such as ants (DeVries 1991). Here I test the hypothesis suggested by Dussourd (2017) that the secretory canals differ in arrangement at different scales. Perhaps entire milkweed leaves have an overall arborescent arrangement, but portions of a leaf on a scale relevant to an early instar caterpillar have a net-like arrangement. This hypothesis is suggested by the architecture of leaf veins. Leaves of the milkweed *Asclepias syriaca* (Apocynaceae) have a single primary vein, the midrib, with prominent parallel secondary veins branching off the midrib (Fig. 2a). This arborescent organization differs strikingly from the orientation of the tertiary veins that connect the secondary veins with a ramifying network (Fig. 2c). The parallel secondary veins are also often connected by one or two veins that run adjacent to and

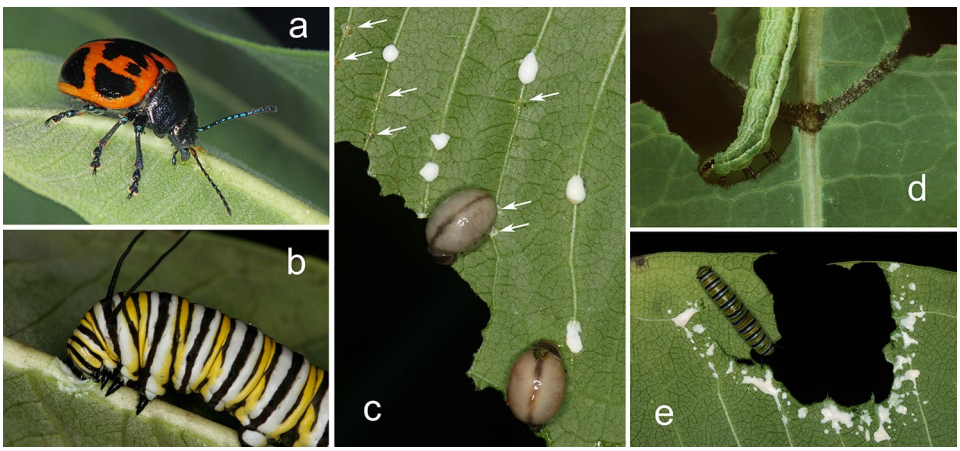

Fig. 1 **a** Adult of *Labidomera clivicollis* (Coleoptera: Chrysomelidae) biting into the midrib of an *Asclepias syriaca* leaf before feeding on the leaf tip. **b** Final instar of the monarch, *Danaus plexippus* (Lepidoptera: Nymphalidae), chewing a furrow into an *A. syriaca* midrib before feeding distal to the cut. **c** Larvae of *L. clivicollis* feeding from the edge of an *A. syriaca* leaf after cutting secondary veins repeatedly. Arrows indicate vein cuts that elicited little or no latex exudation. **d** *Autographa precationis* (Lepidoptera: Noctuidae: Plusiinae) feeding beyond a trench in a *Lactuca serriola* leaf. **e** Early instar monarch larva feeding on *A. syriaca* within a semicircular trench

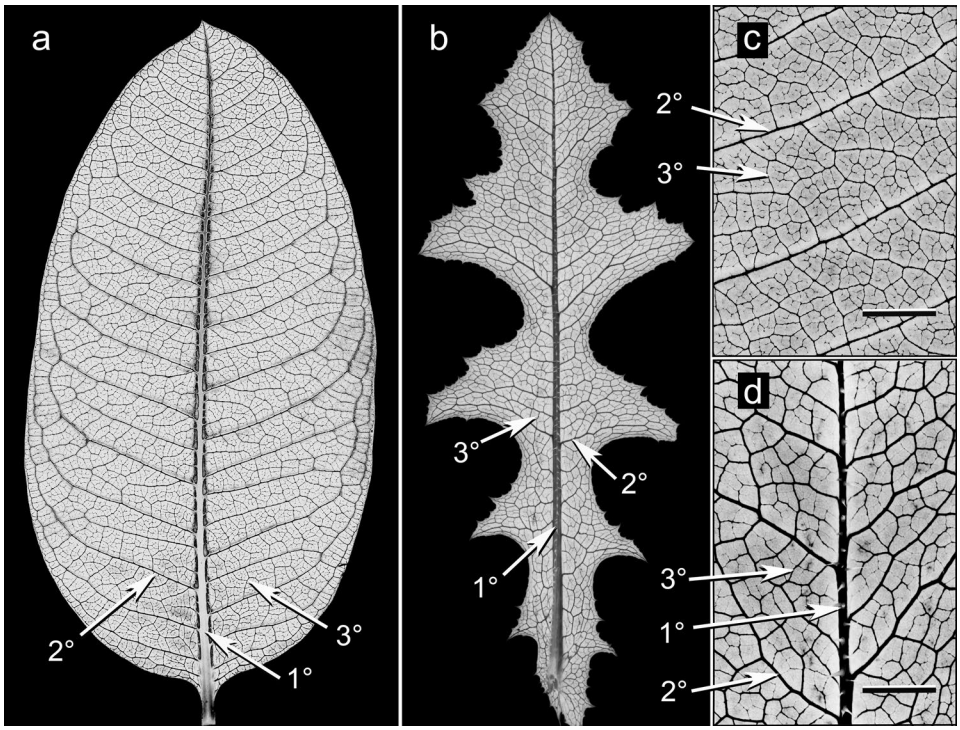

Fig. 2 Mature leaf of *Asclepias syriaca* 18.5 cm long (**a**) and of *Lactuca serriola* 17.3 cm long (**b**). Close-up of secondary and tertiary veins in the same *A. syriaca* (**c**) and *L. serriola* leaves (**d**). Leaves were photographed with backlighting. The resulting images were converted to black and white, then inverted (black and white switched). Scale bars equal 0.5 cm

parallel to the leaf edge. The organization of the smaller leaf veins resembles the arrangement of the secondary and tertiary veins of plants with trenching herbivores such as prickly lettuce, *Lactuca serriola* (Asteraceae) (Fig. 2b, d). Laticifers in leaves tend to follow the vascular bundles (Fahn 1979; Metcalfe and Chalk 1983). If milkweed laticifers are arranged like the milkweed leaf veins, then vein cutting would suffice for insects able to sever primary or secondary veins, but trenching might be required for smaller insects feeding among the ramifying tertiary veins.

My approach to testing if laticifer arrangement differs at different scales was to damage leaves of *A. syriaca* and *L. serriola* with standardized wounds and then measure the secretory response of the laticifer systems. Specifically, I tested if leaves respond differently to cuts in primary and secondary veins. The goal was to determine if *A. syriaca* leaves resemble *L. serriola* in their response to severed secondary veins due to the interconnecting tertiary veins, in which case trenching would be expected of small caterpillars. In most plant species, defenses such as toxins and deterrents and insect counter-adaptations such as digestive enzymes are invisible and thus sophisticated analytical methods are required to quantify defensive responses. But with *Asclepias* and *Lactuca* laticifers, the plant defense (latex exudation) and insect behavioral counterploys are both visible and easily quantified, and therefore, relatively simple tests can be employed.

Methods

Experiment 1. Response of laticifers to vein cuts and pinpricks

To deduce how laticifers respond to vein cuts, I severed a single primary or secondary vein 2 cm from the leaf edge with a hole punch (hole diameter 3.175 mm). Punching a hole through the vein insured that it was completely severed. When latex outflow into the hole ceased, the leaf was then punctured completely through the blade 12 times with a #4 insect pin. The punctures provided an indication of how profusely laticifers ramify in the leaf and if the laticifers beyond a vein cut remained pressurized. Punctures were evenly spaced between the vein cut and leaf edge and were made within ½ cm of the severed vein (Fig. 3). Similar punctures were made in control leaves next to undamaged veins. I counted the number of punctures that elicited a visible release of white latex. Four treatments were tested in random order with a total of 40 *A. syriaca* ramets and 40 *L. serriola* plants: midrib intact, midrib severed, secondary vein intact, and secondary vein severed (10 leaves/treatment/species). Both *A. syriaca* and *L. serriola* were grown in garden plots in Conway, Arkansas. *Asclepias syriaca* plants have underground rootstocks bearing adventitious buds capable of producing multiple stems (Bhowmik and Bandeen 1976). I used a single mature *A. syriaca* leaf per

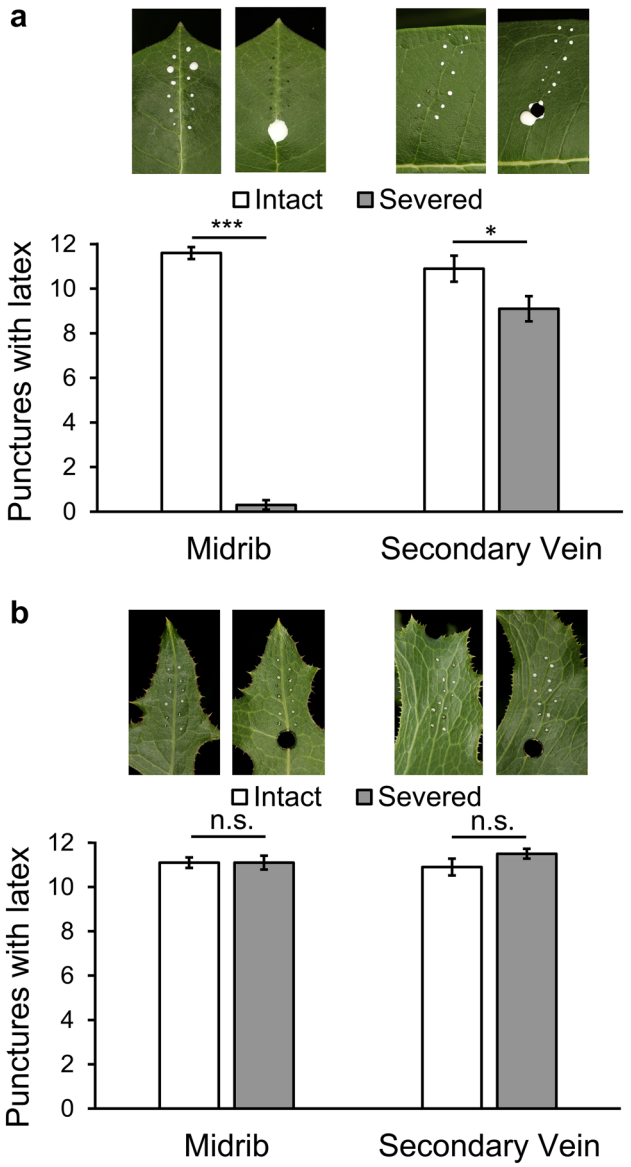

Fig. 3 Number of punctures eliciting a visible drop of latex exudate from leaves of *Asclepias syriaca* (a) and *Lactuca serriola* (b). Either the midrib (left) or a secondary vein (right) was intact or severed before the leaf was punctured 12 times with a pin ($n = 10$ leaves/treatment/species). Punctures in *A. syriaca* leaves released a greater volume of latex than *L. serriola* punctures, and thus, latex is more visible in the *A. syriaca* photographs. Bars represent means ± 1 SE. Treatments were compared with Wilcoxon rank sum tests (*$P < 0.05$, ***$P < 0.0005$, n.s. not significant)

ramet and one mature *L. serriola* leaf per plant. Many of the *A. syriaca* stems had flowers or fruits. The *L. serriola* plants were bolted, but mostly lacked reproductive structures. Bolted *L. serriola* plants release more latex than unbolted plants (Dussourd and Denno 1991).

For both the midrib and secondary veins of each plant species, Wilcoxon rank sum tests were used to compare intact versus vein-cut leaves in the number of punctures with visible latex. Additional Wilcoxon rank sum tests examined the effects of severing the midrib versus a secondary vein. Nonparametric rank sum tests were selected because the data did not have a normal distribution. JMP v. 11 (SAS Institute Inc., Cary, North Carolina, USA) was used for all statistical analyses.

Experiment 2. Effect of vein cuts on the amount of exudate released

Pinpricks illuminate the distribution of laticifers, but do not indicate how much latex is released. To quantify the amount of exudate beyond vein cuts, I used the hole punch to sever either a primary or secondary vein of *A. syriaca* 2 cm from the leaf edge. Latex oozing from the hole was collected onto pre-weighed filter paper until exudation ceased. After re-weighing the filter paper to obtain wet weight of exudate, a second hole was punched in the same vein midway between the first hole and the leaf edge and exudate oozing from the second hole was similarly weighed. If the second hole released less latex, the reduction could be due either to the first hole depressurizing distal laticifers or to fewer laticifers being present closer to the leaf edge. To distinguish these two possibilities, holes also were produced in the reverse order, where the first hole was punched 1 cm from the leaf edge and the second at 2 cm. A total of four treatments were tested in random order: midrib with basal hole first, distal second; midrib with distal hole first, basal second; secondary vein with basal hole first, distal second; and secondary vein with distal hole first, basal second. The same procedure was followed with *L. serriola* except that latex amounts were quantified by collecting latex with 2 µl capillaries (Drummond Microcaps, Broomall, Pennsylvania, USA). The *L. serriola* plants were growing in the wild in Conway, Arkansas. Latex volumes were estimated by measuring the length of white fluid in the capillaries. A total of 40 *A. syriaca* ramets were tested (1 leaf/stem, 10 stems/treatment using the same stems as in the first experiment), together with 60 bolted *L. serriola* plants (1 leaf/plant, 15 plants/treatment). Wilcoxon signed rank tests were used to compare the amount of latex emitted by paired basal and distal holes, whereas unpaired Wilcoxon rank sum tests compared the impact of severing the midrib versus a secondary vein.

Experiment 3. Response of laticifers to cuts in one versus three secondary veins

Larger insect herbivores on milkweed typically either transect the midrib (Fig. 1a, b) or sever several adjacent secondary veins (Fig. 1c). To test if cutting multiple secondary veins reduces distal latex outflow more effectively than transecting a single vein, I compared the response of *A. syriaca* laticifers to three treatments: no veins cut, one secondary

vein severed 2 cm from the leaf edge with the 3.175 mm hole punch, and three adjacent secondary veins each severed 2 cm from the leaf edge. In all three treatments, secondary veins near the center of the leaf were selected. Latex exudation was quantified by puncturing the leaf 12 times with a #4 insect pin. The punctures were evenly spaced along a single secondary vein within 0.5 cm of the vein; the middle severed vein was chosen in the third treatment (3 veins cut). The same *A. syriaca* ramets were used as in the previous two experiments with eight stems per treatment, one leaf per stem, and treatments tested in random order. Since the data were normally distributed and variances were unequal, a Welch's ANOVA followed by Games-Howell post hoc tests was used to compare the three treatments.

Results

Experiment 1. Response of laticifers to vein cuts and pinpricks

Nearly all punctures in intact *A. syriaca* leaves elicited a visible release of white latex whether the punctures were made along the midrib or next to a secondary vein (Fig. 3a). The laticifer system clearly extends throughout the leaf, not just in the major veins. Severing the midrib virtually eliminated latex release from distal punctures. Comparing leaves with intact versus severed veins, the number of punctures with latex differed when the midrib was cut ($P < 0.0001$ Wilcoxon rank sum test) and also when the secondary vein was cut ($P = 0.0437$). However, cutting a secondary vein only reduced the number of punctures with latex by 16.5%. The leaves with severed secondary veins had significantly more punctures with latex than did leaves with severed midribs ($P = 0.0001$, Wilcoxon rank sum test). In contrast, with *L serriola*, nearly all punctures caused a visible release of latex in both intact leaves and leaves with severed veins (Fig. 3b). Comparing leaves with intact or severed veins, there was no difference in the number of punctures with latex whether the midrib ($P = 0.84$, Wilcoxon rank sum test) or a secondary vein ($P = 0.27$) was severed. Thus, in *L.serriola*, individual vein cuts were completely ineffective at preventing exudation from distal punctures. In contrast, cutting the midrib of *A. syriaca* eliminated distal outflow, but severing a secondary vein did not. The response of laticifers in *A. syriaca* to secondary vein cuts resembled the response of *L. serriola* laticifers.

Experiment 2. Effect of vein cuts on the amount of exudate released

The midribs of *A. syriaca* leaves released significantly more latex from a basal hole than from a second hole

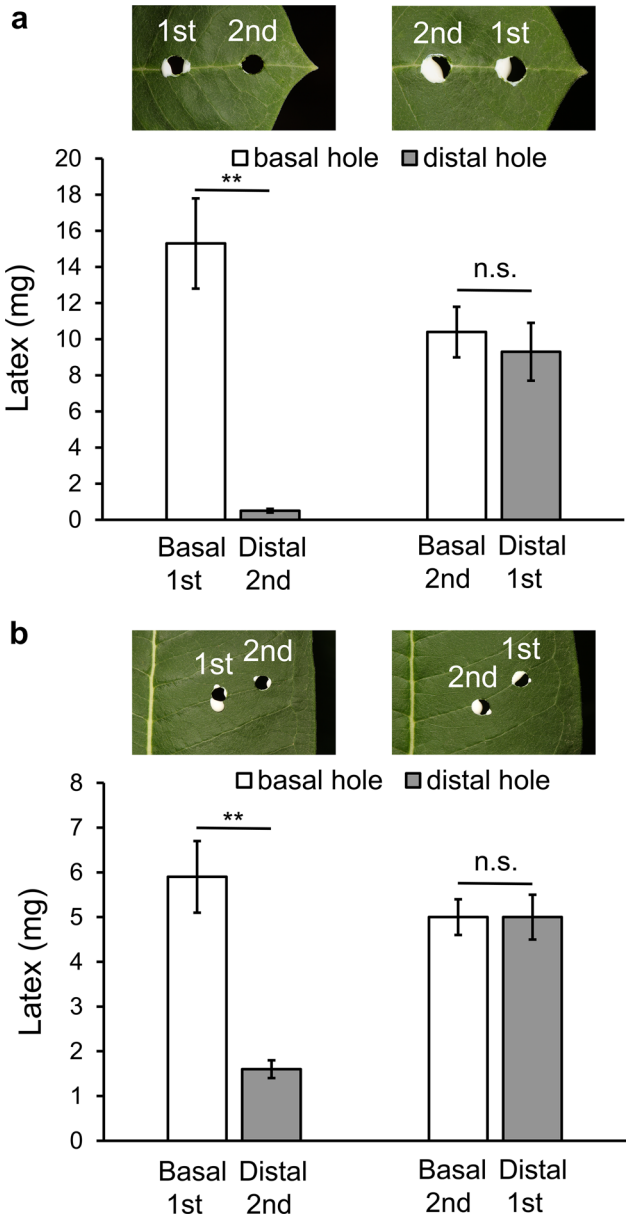

Fig. 4 Weight of latex emitted by *A. syriaca* leaves after the midrib (**a**) or a secondary vein (**b**) was severed twice with a 3.175 mm hole punch. Either the basal hole was punched first and the distal second (left side) or the distal hole was made first and the basal second (right side). Bars represent means ± 1 SE; $n = 10$ leaves/treatment. Paired treatments were compared with Wilcoxon signed rank tests (**$P < 0.005$, n.s. not significant)

produced distal to the first ($P = 0.002$ Wilcoxon signed rank test) (Fig. 4a). In most cases, no white latex exudate was visible at the second hole. The reduced outflow from the distal second hole cannot be attributed just to fewer laticifers or lower latex production at this location. When the distal hole in the midrib was made first, substantial exudation occurred (Fig. 4a). The distal hole made first released 18.6 times more latex than a distal hole made

second, a significant difference ($P = 0.0002$, Wilcoxon rank sum test).

Likewise, when a secondary vein was severed, outflow from the distal second hole was significantly lower than from the first basal hole ($P = 0.002$ Wilcoxon signed rank test) (Fig. 4b). However, the reduction in outflow from the second hole was not as substantial as with the midrib cut. The distal second hole in the secondary vein exuded significantly more latex than the distal second hole in the midrib ($P = 0.0011$ Wilcoxon rank sum test) even though the midrib normally emits more latex. For example, a basal first hole in the midrib emitted significantly more latex (2.6 times more) than a basal first hole in the secondary vein ($P = 0.0011$, Wilcoxon rank sum test). The impact of severing the midrib versus secondary veins can be visualized by dividing average exudation from the second-cut distal hole by average exudation from the first-cut distal hole. The reduction caused by a midrib cut was greater than the reduction produced by a secondary vein cut (Fig. 5). Thus, with *A. syriaca*, both the pinprick experiment and this experiment document that cutting a secondary vein does not eliminate distal latex outflow as effectively as severing the midrib.

With *L. serriola*, a basal cut in the midrib released significantly more latex than a second distal hole ($P = 0.001$, Wilcoxon signed rank test) (Fig. 6a). However, this 50% reduction can be attributed to lower latex amounts closer to the leaf tip. When the distal hole was punched first, it still released less latex than the basal hole produced second ($P = 0.0175$ Wilcoxon signed rank test)(Fig. 6a). Remarkably, distal holes made first (before the basal hole) or second (after the basal hole) released similar amounts of latex ($P = 0.7863$ Wilcoxon rank sum test).

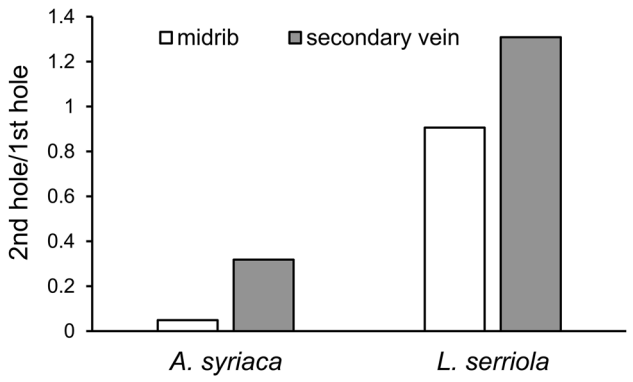

Fig. 5 Average amount of latex emitted by a second hole punched in a vein (distal to a previous hole) divided by the amount produced by a comparable first hole in a vein. With *A. syriaca*, cutting the midrib substantially reduced outflow from the second distal hole resulting in a ratio close to zero. Severing a secondary vein caused a less substantial reduction in distal outflow than cutting the midrib. With *L. serriola*, a prior cut caused little or no reduction in latex exudation beyond the cut (ratios are closer to one)

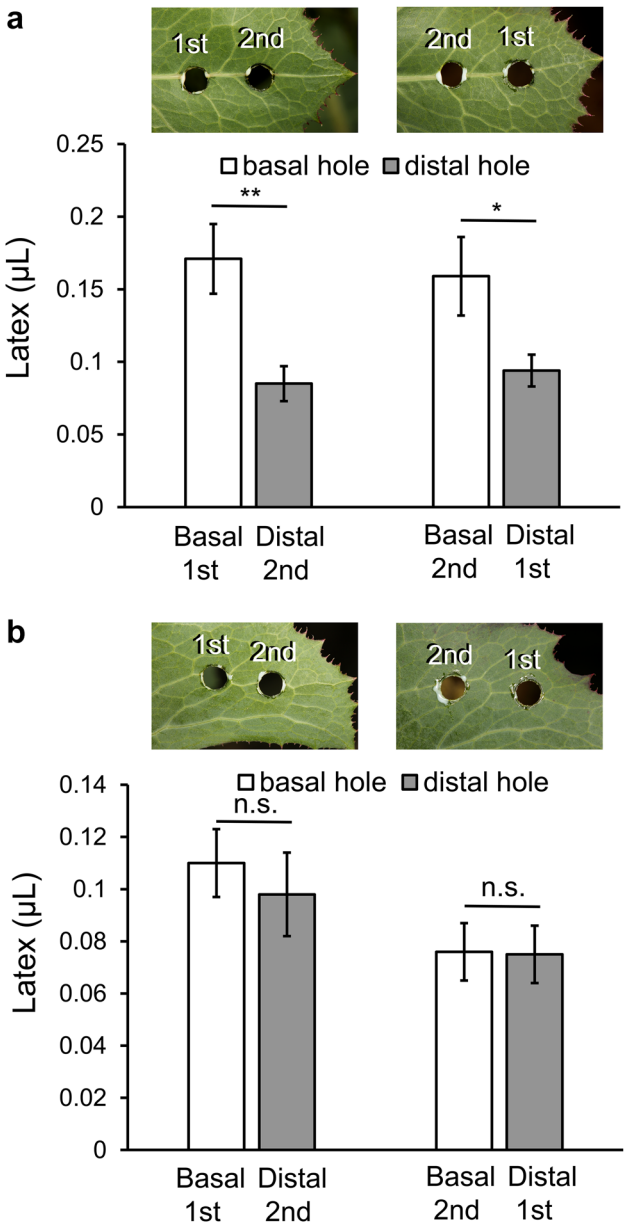

Fig. 6 Volume of latex emitted by *L. serriola* leaves after the midrib (**a**) or a secondary vein (**b**) was severed twice with a 3.175 mm hole punch. Either the basal hole was made first and the distal second (left side) or the distal first and the basal second (right side). Bars represent means ± 1 SE; $n = 15$ leaves/treatment. Paired treatments were compared with Wilcoxon signed rank tests (*$P < 0.05$, **$P < 0.005$, n.s. not significant)

With the secondary veins of *L. serriola*, first and second holes released similar amounts of latex, whether the basal hole was made first ($P = 0.326$) or the distal hole first ($P = 0.986$, Wilcoxon signed rank tests) (Fig. 6b). Thus, cutting either the midrib or a single secondary vein in *L. serriola* did not reduce distal exudation. Dividing exudation from the second-cut distal hole by exudation from the first-cut distal hole documents that *L. serriola* responds differently to

a vein cut than *A. syriaca* (Fig. 5). Unlike in *L. serriola*, *A. syriaca* distal holes made second released much less latex. However, the reduction was not as profound with *A. syriaca* secondary veins. The response of *A. syriaca* secondary veins to a cut was intermediate between the response from *A. syriaca* midribs and *L. serriola* midribs (Fig. 5).

The laticifers in *A. syriaca* emitted much more latex than *L. serriola* laticifers. As a result, the amount of latex emitted beyond a severed secondary vein was actually greater in *A. syriaca* than in *L. serriola* even though the *A. syriaca* vein cut caused a greater reduction in outflow. When the distal cut was made second, secondary veins of *A. syriaca* emitted 1.6 ± 0.2 mg latex ($= 1.55 \pm 0.23$ µl, Dussourd 1999) (Fig. 4b), whereas the secondary veins of *L. serriola* emitted only 0.10 ± 0.02 µl (Fig. 6b), a significantly lower amount ($P < 0.0001$, Wilcoxon rank sum test). Even if early instar danaines could sever a secondary milkweed vein, they would still encounter substantial latex during feeding beyond the cut.

Experiment 3. Response of laticifers to cuts in one versus three secondary veins

The number of pinpricks eliciting latex release from *A. syriaca* leaves with 0, 1, or 3 secondary veins cut differed significantly ($P < 0.0005$, Welch's ANOVA $F_{2,\ 9.9} = 18.8$). Severing three veins more effectively eliminated distal latex release from punctures than cutting just one vein, and one vein cut reduced latex more than the control (0 veins cut) (Fig. 7).

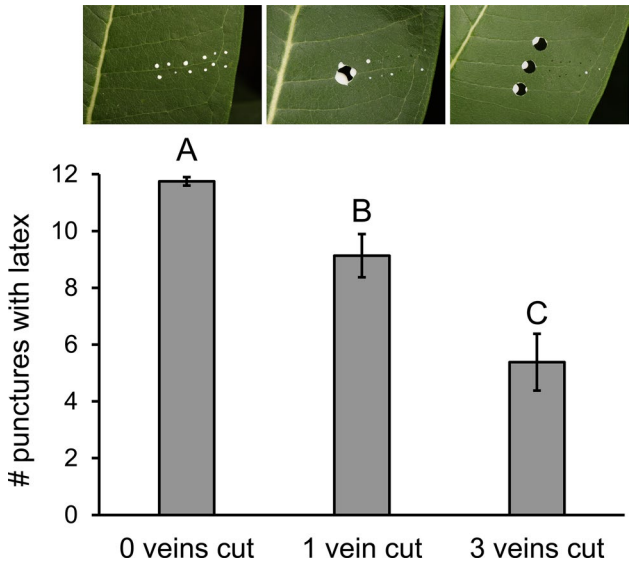

Fig. 7 Number of pinpricks eliciting a visible outflow of white latex from *A. syriaca* leaves with 0, 1, or 3 secondary veins severed with a hole punch ($n = 8$ stems/treatment, 1 leaf/stem). Data are presented as means ± 1 SE; bars with different letters differ significantly at $P < 0.05$ using Games-Howell post hoc tests

Discussion

Insect folivores on plants protected by secretory canals exhibit diverse behaviors; some sever individual veins, some cut trenches partway or completely through the leaf blade, some feed from the base or center of the leaf towards the periphery, some skeletonize leaves, etc. (Dussourd and Denno 1991; Dussourd 1993 and unpub. obs., Lewinsohn and Vasconcellos-Neto 2000). Why do different species and even different stages within a species differ? Insect species within a lineage tend to exhibit similar behaviors suggesting that phylogeny influences behavior. For example, multiple species of *Amblycorypha* katydids (Tettigoniidae) use their powerful mandibles to sever midribs of Anacardiacae, Apocynaceae, and Euphorbiaceae, whereas several species of plusiine caterpillars cut trenches in Asteraceae (Lactuceae), Apiaceae, Cucurbitaceae, and Moraceae (Compton 1989; Dussourd and Denno 1991; Dussourd 2009). Insect sabotage behaviors could also be affected by plant traits, such as inducible defenses, vein architecture, trichome distribution, leaf toughness, etc. For example, vein cutting by soybean loopers (*Chrysodeixis includes*) on geranium is triggered by exudate from glandular trichomes (Hurley and Dussourd 2015). However, for folivores on plants with secretory canals, only attributes of the canals, especially canal architecture, have been associated with variation between insect species in canal-cutting behaviors (Dussourd and Denno 1991; Dussourd 2009, 2017). This canal architecture-behavior hypothesis proposes that the arrangement of canals in leaves, including their distribution and interconnections, determines the sabotage behaviors of insect folivores. Architecture is distinct from development—the origin and elaboration of the canal system during plant growth and leaf expansion. Canal systems with markedly different origins could potentially develop identical architectures in the mature leaf. Likewise, canal systems with similar origins could produce different arrangements, for example if species differ in their propensity to form interconnections between canals.

The presence of trenching and vein-cutting insects on the same plant appears to directly contradict the canal architecture-behavior hypothesis. However, as documented here for the milkweed *A. syriaca*, the efficacy of a vein cut varies depending on location within a leaf. A midrib cut effectively eliminates distal exudation, but a comparable cut in a secondary vein only partially reduces distal latex outflow (Figs. 3, 4). Cutting a secondary vein may be less effective because laticifers branching from adjacent secondary veins overlap spatially. Due to the copious quantities of latex emitted by *A. syriaca* laticifers, exudation beyond a severed

secondary vein is substantial and actually greater than in *L. serriola* (Figs. 4b, 6b). Thus, the presence of trenching early instar monarch larvae and vein-cutting late instars does not contradict the architecture-behavior hypothesis. Quite the contrary, the change in behavior provides strong support. On the scale of an entire leaf, the veins and associated laticifers of *A. syriaca* branch off the midrib in an arborescent arrangement that is vulnerable to vein cutting. In contrast, secondary veins are connected by ramifying tertiary veins that resemble the net-like veins of *L. serriola*. Early instar larvae can most effectively disable laticifers within the tertiary veins with a trench.

The behaviors of *Labidomera clivicollis* on *A. syriaca* are also consistent with the canal architecture-behavior hypothesis. When the larvae and adults feed on the leaf tip, they only transect the midrib (Fig. 1a), which suffices to eliminate distal exudation (Fig. 4a). But when they feed on the side of the leaf, they invariably cut several adjacent secondary veins (Fig. 1c), which more effectively diminishes distal latex outflow than cuts in a single vein (Fig. 7). However, even cutting multiple adjacent veins does not eliminate exudation beyond the cuts (Fig. 7). The solution for *L. clivicollis* is to cut veins not just once, but repeatedly (Fig. 1c). The initial cuts cause copious latex emission, but subsequent cuts often elicit little or no exudation. Thus, milkweed folivores on *A. syriaca* show three main strategies: they sever the midrib, cut multiple secondary veins repeatedly, or produce trenches amongst the tertiary veins. Each behavior effectively reduces latex exudation where the insect feeds.

With *L. serriola* and other Lactuceae, the laticifers form an interconnected ramifying network (Vertrees and Mahlberg 1978; Gutiérrez and Luna 2013; Teixeira et al. 2020). Vein cuts in either the midrib or secondary veins of *L. serriola* do not reduce distal latex outflow (Figs. 3, 6). Insect herbivores on this plant cannot reduce their exposure to latex by severing a single vein. To isolate a portion of the laticifer system and to drain latex from it, they have to sever all strands of the network with a trench. Four species of plusiine noctuids and one species of amphipyrine noctuid all cut trenches in *L. serriola* leaves (Dussourd and Denno 1991).

In the original paper describing the association between canal architecture and behavior, Dussourd and Denno (1991) noted that the behaviors of insects on latex plants corresponded not only to canal arrangements (as deduced through simple tests that simulated insect sabotage behaviors), but also to categories of laticifers described by plant anatomists (Esau 1965; Fahn 1979). Vein-cutting insects occur on plants reported to have nonarticulated laticifers. These laticifers originate as a small number of initials in the embryo that elongate through intrusive growth. The multinucleate latex tubes often branch, but do not interconnect to form networks (Evert 2006). Examples include the Apocynaceae (*Asclepias* with 16 initials in the embryo, Wilson 1986; *Nerium* usually 28 initials, Mahlberg 1961), Euphorbiaceae (*Euphorbia* 4, 8, or 12, Evert 2006; *Jatropha* 5 to 7, Cass 1985), and Moraceae (*Morus* 8, van Veenendaal and den Outer 1990). In contrast, trenching herbivores are found typically on plants with anastomosing articulated laticifers, which originate as chains of cells. The end walls between adjacent cells break down resulting in tubes that interconnect to form networks (Evert 2006). Finally, some plants in the Convolvulaceae have nonanastomosing articulated laticifers restricted to the major veins in leaves (Condon and Fineran 1989; Kennedy and Crafts 1931). Small insects such as tortoise beetles on these plants eat holes between the veins, thus avoiding the laticifers (Dussourd and Denno 1991). This correspondence between laticifer type and insect behavior documented across diverse insect–plant associations suggested that different canal types produce different canal architectures that determine insect behavior (Dussourd and Denno 1991).

The laticifer classification described above was developed over a century of anatomical research (Fahn 1979; Mahlberg 1993; Evert 2006; Hagel et al. 2008). The presence of nonarticulated laticifers in Apocynaceae, for example, was reported by multiple investigators (Chauveaud 1891; Blaser 1945; Mahlberg 1961). However, recent anatomical studies challenge these earlier results. Most or all of the plant groups with nonarticulated laticifers have been re-interpreted as having articulated laticifers (Prado and Demarco 2018; Teixeira et al. 2020), including Apocynaceae (Demarco et al. 2006; Demarco and Castro 2008; Gama et al. 2017; Naidoo et al. 2020). According to Demarco and Castro (2008), *Asclepias curassavica* has articulated anastomosing laticifers, the same category as *Lactuca* and other Lactuceae (Olson et al. 1969; Vertrees and Mahlberg 1978), with no intrusive growth and no predetermined number of initial cells. Other studies of various plant families continue to report the presence of nonarticulated laticifers (Araújo et al. 2014; Kajii et al. 2014; Dghim et al. 2015; Castelblanque et al. 2016) or of articulated laticifers capable of intrusive growth (Canaveze and Machado 2016; Canaveze et al. 2019) or of two laticifer systems in the same plant (Demarco et al. 2013), which may include both articulated and nonarticulated laticifers (Dehgan and Craig 1978). With so much variation and conflicting interpretations, a clear-cut association between insect behavior and laticifer classification is no longer apparent. If *A. syriaca* has anastomosing articulated laticifers as reported in *A. curassavica* (Demarco and Castro 2008), then why do laticifers in *A. syriaca* and *L. serriola* respond differently to midrib cuts? Perhaps *A. syriaca* and *L. serriola* laticifers differ in their ability to form connections between adjacent tubes or their leaves simply have different architectures of veins resulting in different arrangements of laticifer. Of course, from the perspective of an insect herbivore confronting toxic, adhesive exudate,

how laticifers originate in the embryo and develop is probably irrelevant; how the laticifers respond to vein cuts and trenches is still of critical importance.

In this study, laticifer architecture was deduced from the responses of laticifers to wounding. Clearly, anatomical studies of laticifer arrangement, branching, and interconnections would be helpful in further clarifying the relationship between architecture and secretory response. Additional research is also needed to determine if canal cuts serve just to sever secretory canals or if they also function to disrupt the xylem and phloem. By severing vascular tissues, insects could potentially block the movement of signaling molecules and/or defensive compounds induced by feeding damage. The occurrence of both vein cutting and trenching herbivores on plants lacking secretory canals documents that these behaviors can have functions unrelated to exudates (Dussourd 2017). In *A. syriaca*, feeding by monarchs increases levels of cardenolides (Agrawal et al. 2012) and induces the release of volatiles attractive to natural enemies (Wason and Hunter 2014). Whether canal cutting by milkweed herbivores affects these responses has apparently not yet been investigated. In contrast, multiple lines of evidence link canal cutting with secretory canals (Dussourd 2009). The exudates of milkweeds and other plants are clearly detrimental to herbivores due to their toxic and adhesive properties (Agrawal and Konno 2009; Konno 2011). Even specialists can be severely affected (Zalucki and Brower 1992). Canal cuts decrease insect ingestion of exudate by reducing outflow beyond the cuts, thereby increasing the acceptability of this distal section (references in Dussourd 2009; Oppel et al. 2009). Finally, the exudates themselves trigger vein cutting and trenching, including midrib cutting by final instar monarchs (Dussourd 1997; Helmus and Dussourd 2005). Individual compounds, such as the sesquiterpene lactone lactucin in *Lactuca* latex, have been identified that trigger trenching (Dussourd 2003). These results support the conclusion that canal cutting on plants with secretory canals serves specifically, if not necessarily exclusively, to reduce insect exposure to exudate.

In summary, this study documents that laticifer response to damage varies not only between plant species, but also within an individual leaf of *A. syriaca*. Vein cuts in the midrib virtually eliminated distal outflow, whereas cuts in secondary veins were less effective. Smaller insects unable to transect multiple secondary veins cut a trench amid the tertiary veins. Larger insects with more powerful mandibles, such as late instar danaines and chrysomelid beetles, sever the midrib or repeatedly cut multiple adjacent secondary veins, thereby isolating and draining laticifers over a larger portion of the leaf. Although canal-cutting insects on plants with secretory canals could potentially achieve many benefits by severing veins and associated canals, to date, only one has been documented: reduction in exposure to exudate during feeding.

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Availability of data Data are included in the manuscript as Online Resource 1.

Compliance with ethical standards

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