

BIRD-REPELLENT PROPERTIES OF SECRETIONS FROM NYMPHS OF THE AZALEA LACE BUG¹

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Abstract. Many insect species possess chemical defenses against avian predators. Here, we present a series of behavioral investigations designed to assess the repellency of secretions produced by nymphs of the azalea lace bug (*Stephanitis pyriodes*). In Experiment 1, adult and nymph lace bugs were presented to Red-winged Blackbirds (*Agelaius phoeniceus*). The results indicated that adults (which lack chemical secretions) were relatively more palatable. In Experiment 2, we dipped nymphs in methylene chloride to remove secretion, and then presented dipped and undipped insects to birds. Consumption of the former nymphs was significantly higher than consumption of the latter, providing strong evidence that nymphs are avoided because of secretions. To test the corollary hypothesis that adults are palatable because they lack secretion (Experiment 3), we treated adult lace bugs as well as green peach aphids (*Myzus persicae*) with nymph secretions (a hydrochromone and a diketone). Treated insects of both species were avoided while untreated insects were not. Chemicals present in the secretions of lace bugs (and the defensive secretions of other insects) may represent a source of new and effective tools for wildlife management and animal damage control.

Key words: *Agelaius phoeniceus*; avoidance; feeding; lace bug; Red-winged Blackbird; repellent; *Stephanitis pyriodes*.

INTRODUCTION

Many insect species possess chemical defenses against vertebrate predators (Wickler 1968). Defensive substances include simple acids, aldehydes, quinones, terpenoids, alkaloids, steroids, and other diverse materials (e.g., hydrogen cyanide and pederine) (Mandava 1985). In some instances, these chemicals are obtained from plants. Monarch butterflies (*Danaus plexippus*), for example, sequester a variety of cardiac glycosides from milkweed plants (*Asclepias curassavica*) that are both unpalatable and emetic to potential avian predators (Parsons 1965, Brower et al. 1982).

Other insects synthesize their own defensive chemicals. The North American darkling beetle (*Eleodes* spp.) stands on its hind legs when disturbed and sprays a defensive mixture of hydroquinones from glands in

the abdomen (Eisner and Meinwald 1966). Likewise, the bombardier beetle (*Brachinus* sp.) ejects a mixture of hot water, benzoquinone, and toluquinone when disturbed (Schildknecht and Holoubek 1961). The beetle synthesizes and stores a mixture of hydrogen peroxide, hydroquinone, methyl hydroquinone, catalase, and peroxidase (Schildknecht et al. 1968): mixing of these ingredients gives quinones, water, and heat. Nymphs of the azalea lace bug (*Stephanitis pyriodes*) secrete a clear fluid from setae or hairs on the antennae and globulated spines on the dorsal and lateral aspects of the abdomen (Oliver et al. 1985). In spite of the gregarious feeding and social habits of lace bug nymphs (Drake and Ruhoff 1965), neither parasites nor predators of these nymphs have been reported. Because gregariousness is not uncommon among chemically protected (often distasteful) insects (Sillen-Tullberg and Leimar 1988), we decided to test whether the secretions of the azalea lace bug nymph might have bird-repellent properties.

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MATERIALS AND METHODS

Insects

Third through fifth instar azalea lace bug (*Stephanitis pyriodes*) nymphs (LBN), adult azalea lace bugs (LBA), and adult green peach aphids (*Myzus persicae*; GPA) were provided by the Florist and Nursery Crops Laboratory, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland, USA. At the Monell Center, these insects were kept in screened enclosures (60 × 60 × 75 cm) with free access to azalea (*Rhododendron nudiflorum* L., Krume hybrid, "Blaauw's Pink") or tobacco (*Nicotiana tabacum* L., variety MD-609) plants.

To collect LBN and GPA for the tests described in *Experiments 1-3*, we used a pair of forceps to gently grasp the insects and remove them from their respective host plants. Lace bug adults were first vacuumed into a small flask, and then gently removed from the flask with forceps. Presentation of all insects involved applying double-sided sticky tape to the bottom of metal food cups (7.5 cm diameter), and then pressing the ventral surface of the insects against the tape.

Birds

Birds were mist-netted in the vicinities of Sandusky, Ohio or Bowling Green, Kentucky and then shipped to the Monell Center. Upon arrival, each was uniquely banded and individually caged (61 × 36 × 41 cm) in a room with a constant ambient temperature of 23°C, and a constant 14:10 light : dark cycle. Water was freely available and before experiments began, birds were permitted free access to Purina Flight Bird Conditioner (Purina Mills, Saint Louis, Missouri, USA), mealworms, and crushed shell grit.

Initially, adult male Red-winged Blackbirds (*Agelaius phoeniceus*), European Starlings (*Sturnus vulgaris*), and Brown-headed Cowbirds (*Molothrus ater*) were evaluated as potential predators. We eventually selected red-wings for use in our experiments because this species (unlike the other two) consistently ate insects presented to them in our laboratory.

Chemicals

The material topically applied to insects in *Experiments 2 and 3* was a synthetic mixture (≈ 1:1) of the two most abundant components secreted by LBN, namely the diketone 1-(2,6-dihydroxyphenyl)-dodecan-1,3-dione and the chromone 5-hydroxy-2-nonylchromone (Oliver et al. 1985). To estimate the amount of secretion actually applied to insects, LBN were dipped (≈ 10 s) in methylene chloride (to remove secretions), then air-dried on filter paper, dipped 2-3 s in the synthetic mixture of secretions, and again air-dried. Twenty such insects were introduced into a vial, 400 μL of ethyl alcohol was added, and the gas-liquid chromatographic responses of the diketone and chromone were compared to those of standard solutions. The 20 insects

carried 55 μg of the two substances (≈ 2.8 μg per insect). This amount of secretion was higher than the amount of secretion previously estimated for individual LBN (0.8 μg, Oliver et al. 1985).

General procedures

All testing occurred during the first 2 h of light. During the test periods, maintenance diet was removed from the cages, and test insects were the only available food. Following each test session, maintenance diet was returned to the cages, and the birds were left undisturbed until the following morning. Prior to the start of each experiment, naive birds were randomly selected, and their willingness to consume insects in our laboratory was assessed. On each of two consecutive days, maintenance diet was removed from the cages, and each bird was presented with a cup containing 16 mealworm segments. Only those birds that consumed ≥ 80% of the segments within 2 h on both days were used in the following experiments.

Experiment 1

We first assessed whether LBA were more palatable than LBN. Of the 20 birds randomly selected for the experiment, 15 consumed ≥ 80% of the mealworms presented and were given 2-cup tests between 10 LBA in one cup and 10 LBN in the other on each of four consecutive days.

Mean percent consumption by each bird of each insect type was calculated, and these means were assessed in a two-factor (insect type and days) repeated-measures analysis of variance (ANOVA). Tukey honestly significant difference (HSD) tests were used to isolate significant differences among means ($P < .05$).

Experiment 2

Here, we assessed whether secretion, per se, conferred unpalatability to LBN. To accomplish this evaluation, two samples of nymphs were collected. One sample was killed by refrigeration at 4.4° immediately following collection. The other sample was killed by dipping in methylene chloride (2 s each), which also removed secretion; they were then placed on paper towels for 30 min to allow the methylene chloride to evaporate, and finally refrigerated (4.4°). Both samples remained in the refrigerator until 30 min prior to testing. Twenty birds were randomly selected for use in the experiment. All of these birds passed the screening procedure and were randomly assigned to two groups (10 individuals/group). Group r-LBN was presented with rinsed LBN (1-cup tests, 10 nymphs/test) while the control group was presented with plain LBN (1-cup tests, 10 nymphs/test) on each of four consecutive days.

The mean percent consumption by the birds in both groups was calculated, and these means were assessed in a two-factor ANOVA with repeated measures on the second factor (days). Tukey HSD tests were used

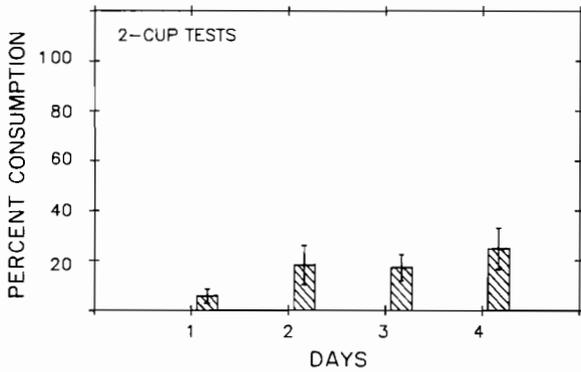


FIG. 1. Experiment 1. Percent consumption of adult (hatched bars) and nymph lace bugs in 2-cup tests. No nymphs were consumed in any test. Data are means \pm 1 SE.

to isolate significant differences among means ($P < .05$).

Experiment 3

Of 24 birds randomly selected for use, 20 were randomly assigned to two groups (10 individuals/group). One group received 20 LBA, and the other, 20 GPA in 1-cup 2-h tests on each of 12 d. During pretreatment (4 d), birds were presented with LBA and GPA that had been dipped in methylene chloride (as described in *Experiment 2* above). During treatment (4 d), insects that had been dipped in a 2.0% LBN secretion/methylene chloride solution were presented. During the final 4 (posttreatment) d, both groups were again given 1-cup tests with LBA and GPA that had been dipped in methylene chloride only.

The mean percent consumption of secretion-dipped and methylene chloride-dipped insects by each bird was calculated, and the data were assessed in a three-factor ANOVA with repeated measures on the second (period) and third (cups) factors. Tukey HSD tests were used to isolate significant differences among means ($P < .05$).

RESULTS AND DISCUSSION

Experiment 1

Significantly more ($P < .01$) LBA (16.4 ± 6.0 individuals) were consumed than LBN (0.0 ± 0.0 individuals) (Fig. 1). There were no other significant effects. These results are consistent with (but do not directly test) the hypothesis that secretions protect LBN from avian predators.

Experiment 2

Significantly more ($P < .01$) rinsed (60.6 ± 14.9 individuals) than plain (24.1 ± 8.7 individuals) LBN were consumed (Fig. 2). There were no other significant differences. These data lend further support to the hypothesis that LBN avoidance by red-wings is mediated by microdroplets of secretion present on the body surface of nymphs but absent on adults.

Experiment 3

There were no significant differences between groups of birds presented with the different insects. Pretreatment consumption of both GPA and LBA was high (Fig. 3). During treatment, when secretion-dipped insects were presented, consumption of both LBA and GPA significantly decreased ($P < .01$). During the post-treatment period, when insects dipped in methylene chloride only were again presented, consumption returned to pretreatment levels.

The results of Experiment 3 are consistent with the findings of Experiments 1 and 2. Although the concentration of secretion on dipped insects in the present experiment was higher ($2.8 \mu\text{g}/\text{insect}$) than that which occurs naturally ($0.8 \mu\text{g}/\text{nymph}$), the data suggest that LBN secretions can afford protection to otherwise palatable prey.

GENERAL DISCUSSION

We suspect that the repellency of LBN secretion is mediated by some aversive chemosensory characteristic, although our data do not specifically address this point. While further experimentation is necessary to clarify the issue, there is good evidence that passerines have olfactory sensitivities comparable to those of rats (Clark and Mason 1989). In addition, at least some birds (e.g., starlings) exhibit excellent taste and trigeminal (Mason et al. 1989) acuity, although stimulants for avian trigeminal system appear quite different from those for mammalian trigeminal system.

From an ecological viewpoint, it is interesting that LBN are cryptic and not aposematic. Many chemically defended invertebrates advertise their unpalatability to avian predators with bright (usually red or orange) coloration and simple, bold striping (Wickler 1968). Also, aposematic insects often make no attempt to conceal themselves from avian predators. Lace bug nymphs prefer to forage on abaxial leaf surfaces.

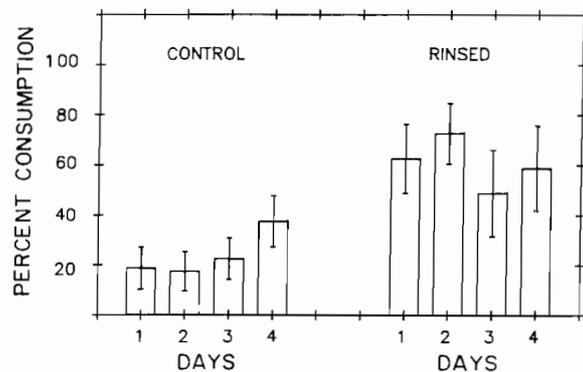


FIG. 2. Experiment 2. Percent consumption of lace bug nymphs rinsed in methylene chloride vs. consumption of control (unrinsed) nymphs in 1-cup tests. Data are means \pm 1 SE.

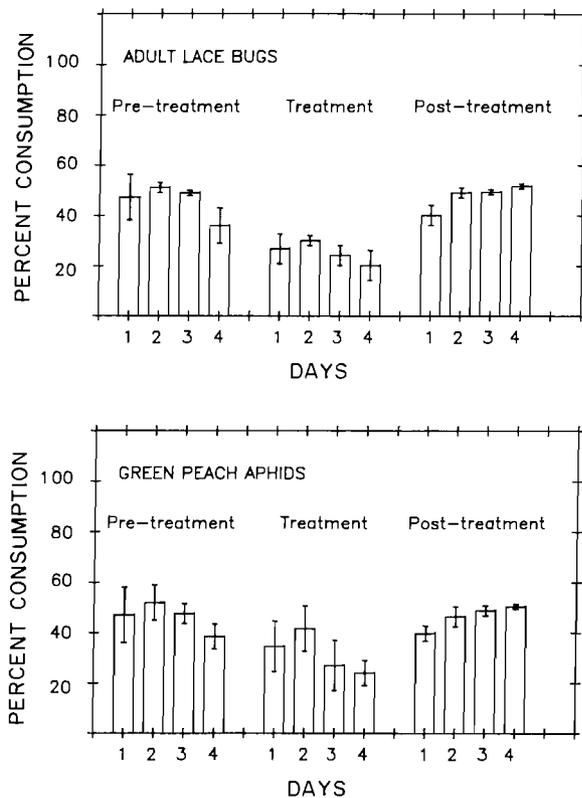


FIG. 3. Experiment 3. Percent consumption of adult lace bugs (Upper panel) and green peach aphids (Lower panel). During pretreatment, plain insects were presented. During treatment, insects dipped in LBN secretion were presented. During post-treatment, plain insects were again presented. Data are means \pm 1 SE.

MANAGEMENT IMPLICATIONS

Despite increasing need, few chemical repellents are available for the control of avian depredation and nuisance problems. One new source of repellents may be compounds present in the defensive secretions of insects. This approach seems especially promising because little predictive information is available at present concerning the nature of avian repellents. Although the morphological organization of the peripheral trigeminal system (i.e., the fifth cranial nerve, which mediates irritant/repellent perception in mammals) in birds is not very different from that in mammals, there appear to be broad functional (e.g., behavioral) discrepancies. Of those avian species tested to date, not one has shown avoidance of prototypical mammalian repellents such as capsaicin (Mason and Maruniak 1983, Szolcsanyi et al. 1986, Mason and Otis 1989). Consequently, we believe that it is prudent to study avian predator/insect prey interactions with the aim of uncovering new candidate repellents.

To commercialize particularly effective substances, it will be necessary to identify materials that are both durable and cost efficient. Regarding the former point,

behavioral studies that examine the habituation of avoidance responses are required. Quite possibly, higher concentrations of repellent than those used here will be necessary to abolish the consumption of preferred diets, especially when alternative foods are unavailable (Rogers 1978). Regarding cost efficiency, it probably will be necessary to identify and bioassay inexpensive and already commercially available materials that are structurally similar to repellents identified in insect secretions. Relatively little capital exists for the synthetic development of new bird repellents, the overall size of the market is small, and relatively high concentrations of chemical are required for consistent effects (e.g., Mason et al. 1989).

We now plan to examine the secretions of other lace bug species to determine whether these insects also secrete defensive compounds against avian predators. Already, the chemical nature of several species' secretions have been characterized (Lusby et al. 1987, Oliver et al. 1987). Detection of repellency in other compounds would promote further studies, including comparisons of repellent effectiveness vs. the species and genus of origin as well as structure-activity relationships of synthetic compounds.

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