

DEFENSIVE REGURGITATION BY  
A NOCTUID MOTH LARVA (*LITOPROSOPUS FUTILIS*)<sup>1</sup>

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ABSTRACT

Larvae of the noctuid moth *Litoprosopus futilis* regurgitate when disturbed. The oral effluent proved deterrent to ants on near-contact, and topically irritating in a scratch test with a cockroach. Larvae regurgitated when attacked by lycosid spiders and derived some protection from this behavior. Caterpillars were able to regurgitate even when emerging from the eggs; however, at this stage, they proved vulnerable to attack by chrysopterid larvae and ants.

INTRODUCTION

Regurgitation of gut contents is a common defensive strategy of insects, including orthopterans, isopterans, coleopterans, lepidopterans, dipterans, and hymenopterans (reviewed by Eisner 1970, 1980; Blum 1981; Brower 1984; Whitman *et al.* 1990; Bowers 1993). Repellent fluids are disgorged by both herbivorous (Eisner 1970, Eisner *et al.* 1974, Morrow *et al.* 1976, Peterson *et al.* 1987, Brower 1984, Smedley *et al.* 1990) and insectivorous larvae (Eisner *et al.* 1980). Foregut diverticula for storage of the expellable materials can be a morphological feature associated with this defense (Eisner *et al.* 1974, Morrow *et al.* 1976, Common & Bellas 1977), but such specialization is not requisite (Eisner *et al.* 1980, Brower 1984).

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We here show that larvae of the noctuid moth *Litoprosopus futilis* (Grote & Robinson), occurring in the southeastern United States, regurgitate when disturbed. We demonstrate by bioassays of the larval vomit and observations of encounters of the caterpillars with sympatric predators, that the emitted fluid has repellent and irritant properties. Further, we note that the larva is able to regurgitate even as it emerges from the egg, before onset of feeding on the host plant.

#### MATERIALS AND METHODS

##### *Field Observations*

Field observations were made at the Archbold Biological Station, Lake Placid, Highlands County, Florida, USA, where we found larvae of *L. futilis* feeding on saw palmetto (*Serenoa repens*). During 21–23 March 1989, we observed the behavior of larvae on two *S. repens* plants (Southern Ridge Sand Hill habitat on Red Hill). Larvae for experimental use were collected from other *S. repens* plants. Field-collected larvae and reared adults are deposited in the Cornell University Insect Collection, voucher lot # 1219.

##### *Larval Vomit and Ant Repellency Bioassay*

Larval regurgitant was presented in microcapillary tubes at close range to ants feeding at a sucrose solution bait, and its repellent effect was scored relative to that of controls (tap water).

These tests were done at natural foraging trails of the ant *Paratrechina longicornis* (Formicinae). A plastic plate with four conical feeding wells (positioned at the corners of an imaginary square, 1.3 cm/side) replete with sucrose solution (10% aqueous) served to lure the ants. After the ants had gathered to feed at the wells, two wells were randomly selected, and the number of ants at each of the two was recorded.

Regurgitant was collected by pinching individual, recently-fed, mid- to late-instar caterpillars gently between a finger and glass microscope slide. The effluent was taken up in a microcapillary tube. The fluid was then squeezed from the tube with a rubber bulb until it collected as a suspended drop at the opposite end of the tube. Presentation to ants involved positioning the capillary tube

vertically above (1–3 mm) the assigned well. The tap water control (similarly presented as a suspended droplet) was brought into position above its designated well at the same time as the regurgitant sample. After 5 sec of such paired presentation, the ants at the two wells were again counted.

It was noted that the regurgitant gradually changed in color from green to brown following discharge. A regurgitant sample was thus tested both fresh (within 10 sec after emission) and aged (after it remained in the tube for 3–4 min). Twenty-four presentations were performed, 12 with fresh and 12 with aged material.

A sample's repellency was scored as the difference in the number of ants before and after presentation. This scoring procedure was justified since the number of ants per well prior to sample presentation did not vary between the vomits and their controls: mean  $\pm 1$  S. E. ants/well for fresh regurgitant and its control =  $4.1 \pm 0.5$  and  $3.8 \pm 0.5$ , respectively, (paired  $t_{11} = 0.58$ ,  $p = 0.57$ ); aged regurgitant and its control =  $3.8 \pm 0.4$  and  $3.9 \pm 0.5$ , respectively, (paired  $t_{11} = -0.12$ ,  $p = 0.90$ ). The repellency of the fresh and aged regurgitant was compared to that of the respective controls by means of paired t-tests. To maintain an experiment-wide  $\alpha = 0.05$ , the resultant probabilities were adjusted using the sequential Bonferroni procedure (Rice 1989).

#### *Cockroach Scratch Bioassay*

Topical application of chemical irritants induces scratch reflexes in the cockroach *Periplaneta americana* (Eisner *et al.* 1976). The technique has been used for assay of irritancy of defensive glandular products of insects. Decapitated roaches are used for this purpose, since these are non-ambulatory. To assess the irritancy of the regurgitant of *L. fulvipes*, twenty last instar *P. americana* nymphs were tested as per protocol in Eisner *et al.* (1976). Only fresh regurgitant was used, collected as for the preceding assay. Deionized water provided the control. Each cockroach was tested with both regurgitant and control. An interval of several minutes transpired between the two applications. The droplet volume ( $0.5 \mu\text{L}$ ) was the same for both samples, and was applied either to the left or right half of the fourth abdominal tergite. The application procedure controlled for treatment sequence (regurgitant or control first) and position (right or left side). The criterion for response was scratching with the hindleg directed at the site of

application within 30 sec of droplet delivery. Individuals not responding within 30 sec were scored as non-respondent.

The response of roaches to fresh vomit and to the control was compared using  $2 \times 2$  contingency test [log-likelihood ratio (G-statistic) with the Williams correction (Sokal & Rohlf 1981)].

#### *Encounters of Larvae with Lycosid Spiders*

To examine the interactions of *L. futilis* larvae with spiders, lycosids (*Lycosa* spp., including *L. ceratiola*) were collected at the Archbold Biological Station and placed in individual sand-lined containers (10 × 10 cm base; 6 cm height). The lycosids were maintained without food for one or two days before trials. A larva was placed with a spider and interactions were noted.

#### *Encounters of Emerging Larvae with Chrysopid Larvae and Ants*

Encounters between partially eclosed *L. futilis* caterpillars and predatory chrysopid larvae (*Ceraeochrysa cubana*) were examined. Field-collected egg masses were used. A portion of frond bearing an egg cluster with emerging larvae was placed in a 15 × 50 mm petri dish and observed under a stereomicroscope. A chrysopid larva was then introduced to this arena. Chrysopid predation attempts and *L. futilis* defensive behavior were noted. Two such trails were performed.

A frond bearing an egg mass with emerging larvae was taped to a microscope slide and placed in the path of ants [*P. longicornis* and *Solenopsis invicta* (Myrmicinae)] that had laid trails to bait (10% sucrose solution or chopped mealworm pieces). Maternal hairs covering the eggs (Fig. 1) were removed to facilitate observation. The reaction of the emerging larvae to ants was monitored with a stereomicroscope.

## RESULTS

#### *General Observations*

At night the two aggregations of *L. futilis* larvae (7 and 11 caterpillars/aggregation) were visibly feeding on the unopened flower buds of *S. repens*. With the onset of daylight, larvae sought dark refuges (frond petiole bases, sheaths of flower petioles, or patches of frass formed by a pyralid moth larva on petiole stalk). Often larvae would enter and leave several refuges before final

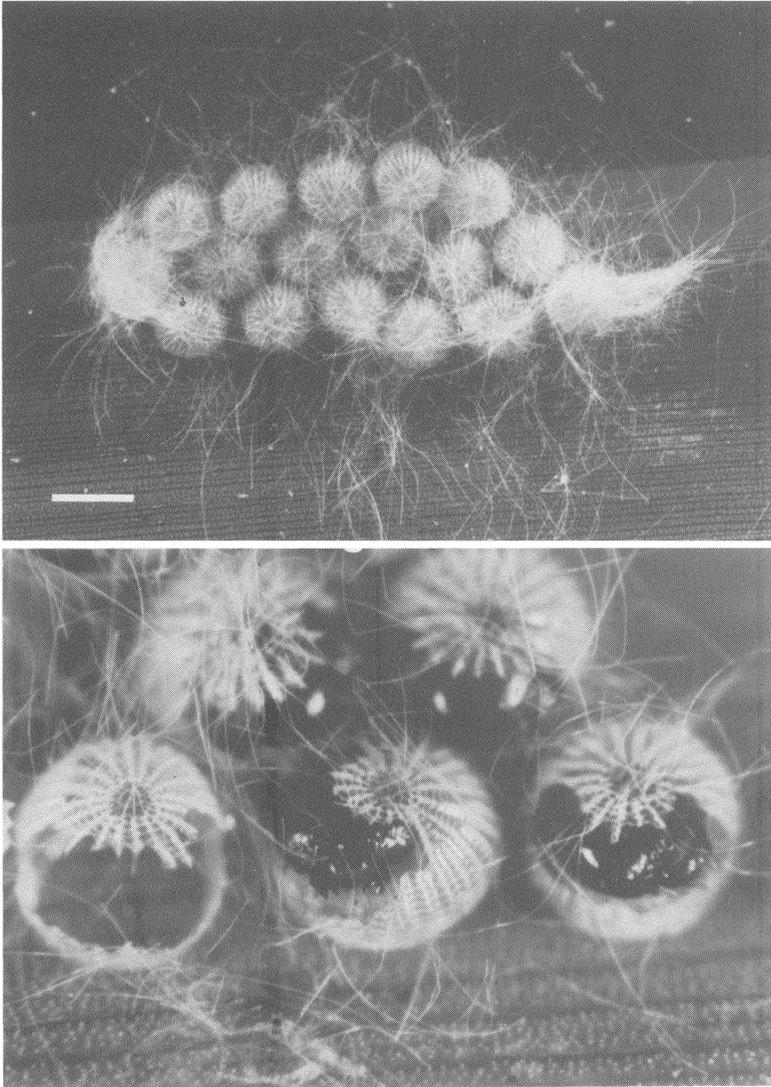


Figure 1. (Top) Natural egg cluster of *Litoprosopus futilis*. The hairs covering the eggs are presumably detached modified scales from the mother's body (Bar = 1 mm). (Bottom) Eggs in the process of hatching; note mandibles agape on larva in center foreground.

disappearance. During daylight hours, no larvae were seen on the developing inflorescences or other plant parts. At dusk caterpillars emerged from their shelters and began to feed.

Cannibalism was observed among larvae taken from the field and kept collectively in the laboratory.

#### *Larval Vomit and Ant Repellency Bioassay*

The vomit of *L. futilis* repelled *P. longicornis*. Fewer ants remained after presentation of fresh (paired  $t_{11} = 2.97$ ,  $p = 0.026$ ), but not aged vomit (paired  $t_{11} = 0.30$ ,  $p = 0.767$ ) (Fig. 2).

#### *Cockroach Scratch Reflex Bioassay*

Fresh vomit proved an irritant to *P. americana*. Vomit elicited a scratch reflex much more frequently than did the distilled water

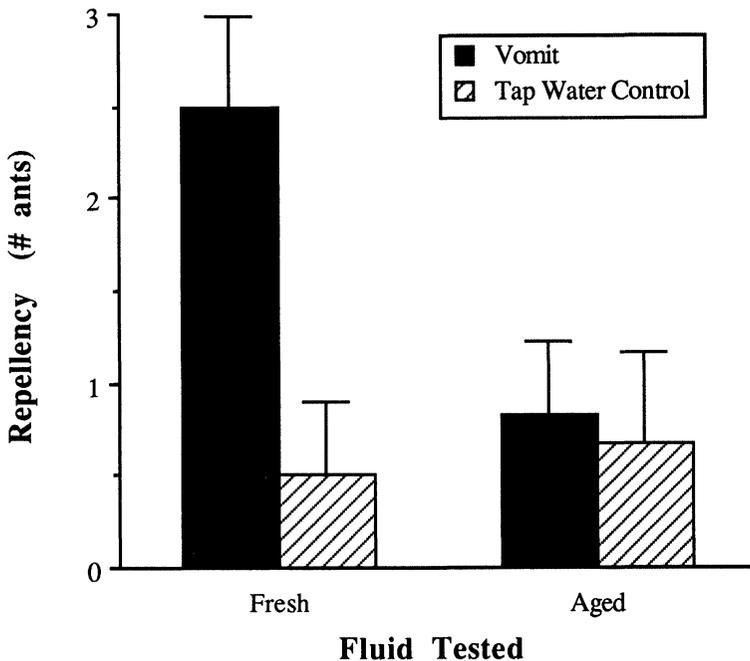


Figure 2. Repellency of fluid stimulant (*L. futilis* vomit or control) to ants (*P. longicornis*), as a function of fluid age. Mean repellency (# ants before - # ants after exposure to stimulant) + 1 S.E.;  $n = 12$  paired presentations of vomit and control for each of the two sets of data. Additional details in text.

control ( $G_{\text{adj}} = 24.52$ , d.f. = 1,  $p < 0.001$ ; Table 1). For roaches responding to vomit, scratch initiation time was  $5.7 \pm 1.6$  sec. (mean  $\pm 1$  S.E.,  $n = 17$ ). Three nymphs responded neither to vomit nor control, but none responded to the control alone.

#### *Encounters of Larvae with Lycosid Spiders*

In many trials (18/35) spiders did not actively pursue the caterpillars. When attacks did take place, they occurred almost immediately after larval introduction. In 10 of the 17 instances where spiders seized caterpillars, the larva vomited upon the spider directly or on the sand in its vicinity (in the latter cases, we might have failed to notice fluid that contacted the spider). In most instances following larval regurgitation (7/10), the spider cleaned its mouthparts by wiping them in the sand, a typical response to irritants (Eisner *et al.* 1972). Caterpillars that did not regurgitate when attacked (7/17) did, however, raise their front end as they characteristically do prior to regurgitation.

The outcome of an attack was dependent, at least in part, on the size relationship of the contenders. Large spiders (2.0–2.3 cm body length) successfully killed mid- to late-instar larvae (3 killed/3 attacked), whereas medium-sized spiders (1.0–1.5 cm) were more successful in attacks on early (6 killed/8 attacked) than on late instar (0 killed/6 attacked) larvae. Five of the late instar larvae that escaped following attack appeared healthy 48 hr after the encounter.

#### *Encounters of Emerging Larvae with Chrysopid Larvae and Ants*

*L. futilis* larvae that were emerging from eggs (Fig. 1) displayed mandibular spreading and regurgitation when stimulated with forceps (Fig. 3) or when attacked by larval chrysopids (Fig. 4). This

Table 1.  $2 \times 2$  contingency table examining topical irritancy of fresh *L. futilis* vomit as assessed in the cockroach (*P. americana*) scratch bioassay.

Applied Fluid	Number of Cockroaches		Totals
	Respondent	Non-Respondent	
Vomit	17	3	20
Control	2	18	20
Totals	19	21	40

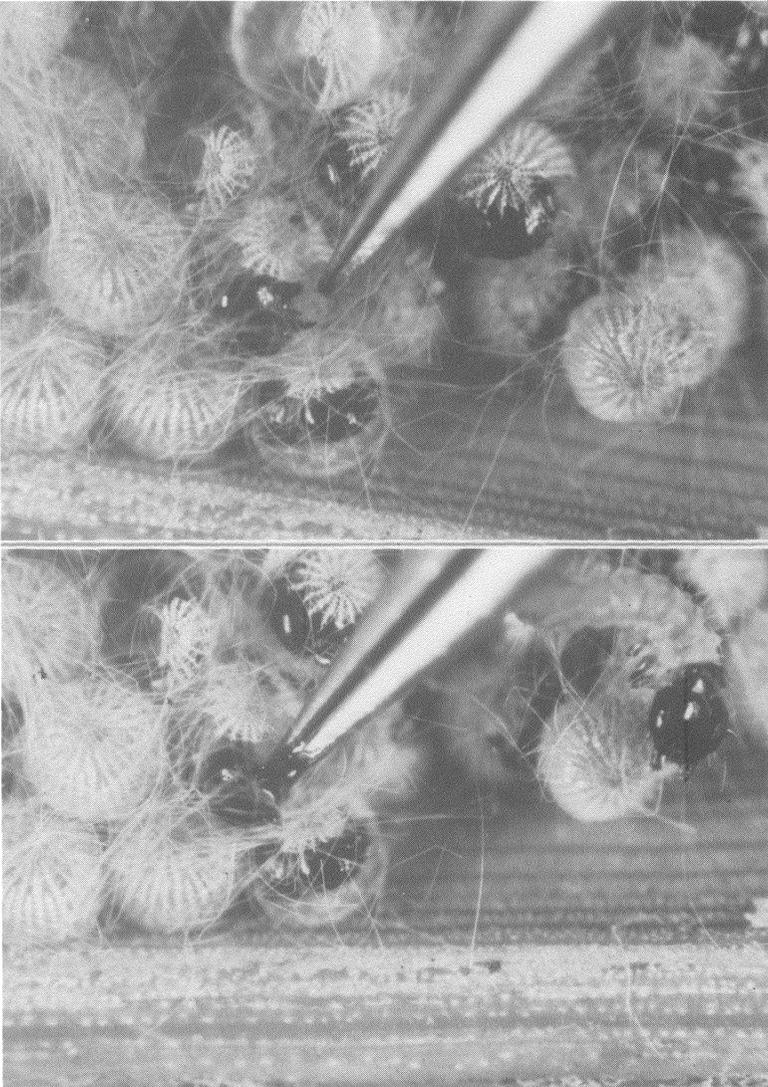


Figure 3. (Top) Emergent larva responding to prodding with forceps by biting with its mandibles. (Bottom) Same, showing larva regurgitating directly upon the contacting forceps.

defensive behavior, however, did not prevent the chrysopids from killing and eating such larvae. The chrysopids also consumed unhatched eggs.

Two of three larvae that had recently emerged from eggs regurgitated when probed by the chrysopid's mandibles. Nevertheless, all three of these encounters proved fatal to *L. futilis*.

When attacked by ants, larvae that were hatching from eggs responded by spreading their mandibles. Ants were observed carrying off eggs containing larvae. The reaction of recently emerged caterpillars to the attacks included head swaying, mandibular spreading, and vomiting. These responses seemed to have no deterrent effect on the ants.

#### DISCUSSION

Regurgitation in larval *L. futilis* appears to be a defensive behavior. The oral effluent of mid- to late-instar larvae is repellent to ants and irritant to cockroaches, properties that could well be indicative of general anti-arthropodan detergency. The finding that the effluent induced cheliceral cleaning in lycosids, and that larvae had a good chance of surviving after their rejection by the spiders, provides direct evidence for the defensive potential of the vomiting behavior.

In the scratch test with *P. americana*, the larval regurgitant proved as effective as two well-known chemical defensive agents, one a common component of arthropodan secretions (2-methyl-1,4-benzoquinone), the other a defensive metabolite of plants (pulegone). The time-delay to scratching with these two compounds had previously been determined (Eisner *et al.* 1990) and is comparable to what we found it to be with the larval regurgitant.

The fact that the effluent proved repellent on near-contact in the tests with the ant *P. longicornis* indicates that one or more components of the fluid can effect their detergency as vapors. No definitive explanation can be given for the decline in repellency following aerial exposure. Such decline could be due to evaporative loss of active principles, or to any number of reactive transformations (oxidations?) triggered in the fluid upon emission.

A question of obvious interest concerns the origin of the active principles in the oral effluent. Whether the chemicals are produced by the larva itself, are derived from the diet, or are of dual

endogenous and exogenous origin, remains unknown. We cannot even rule out the possibility that activity is attributable to a single compound rather than a mixture.

Vomiting on the part of eclosing larvae proved ineffective *vis à vis* the predators that we selected for testing (the chrysoiid *C. cubana* and the ants *P. longicornis* and *S. invicta*). We are reluctant to conclude from this that eclosing larvae are generally vulnerable, and that they would have fared similarly in tests with other predators. Mandibular snapping and oral emission could well prove effective in predation contexts that remain to be examined. Conversely, it is possible that the effluent does not become active until the caterpillar has commenced feeding on its host plant.

We found *L. futilis* to have the habit, shared with many other lepidopteran larvae, of ingesting their egg shells after hatching (Fig. 4). Whether by doing so they acquire not only nutrients, but also chemicals that contribute to the detergency of the regurgitant, remains unknown. The question may be pertinent to other species as well. Does shell ingestion provide newly eclosed insects with the option of reusing defensive chemicals bestowed upon the eggs by the mother?

Dissection of *L. futilis* larvae revealed no special morphological refinements of the foregut, such as diverticula. We noted no diverticula, such as are known for storage of regurgitable oils by euca-lyptus-feeding larvae of the lepidopteran genus *Myrascia* (Oecophoridae) (Common & Bellas 1977).

A number of larvae that we collected in the field and that subsequently pupated in the laboratory succumbed to a tachinid fly parasitoid (adults are deposited in the Cornell Insect Collection, voucher lot no. 1219). *L. futilis* that we raised indoors from field-collected eggs remained unparasitized. In another noctuid moth, *Agrotis ipsilon*, larval regurgitant functions as a kairomone that elicits larviposition by the tachinid *Bonnetia comta* (Clement *et al.* 1986). One wonders whether in *L. futilis* the oral effluent has a similar signal function.

Finally, our observations of larval cannibalism corroborate the earlier field and laboratory studies of Semlitsch and West (1988), who noted larval cannibalism during an outbreak of *L. futilis* in a maritime forest in South Carolina. Unlike our observation of strictly nocturnal activity, they found larvae active during both day and night.

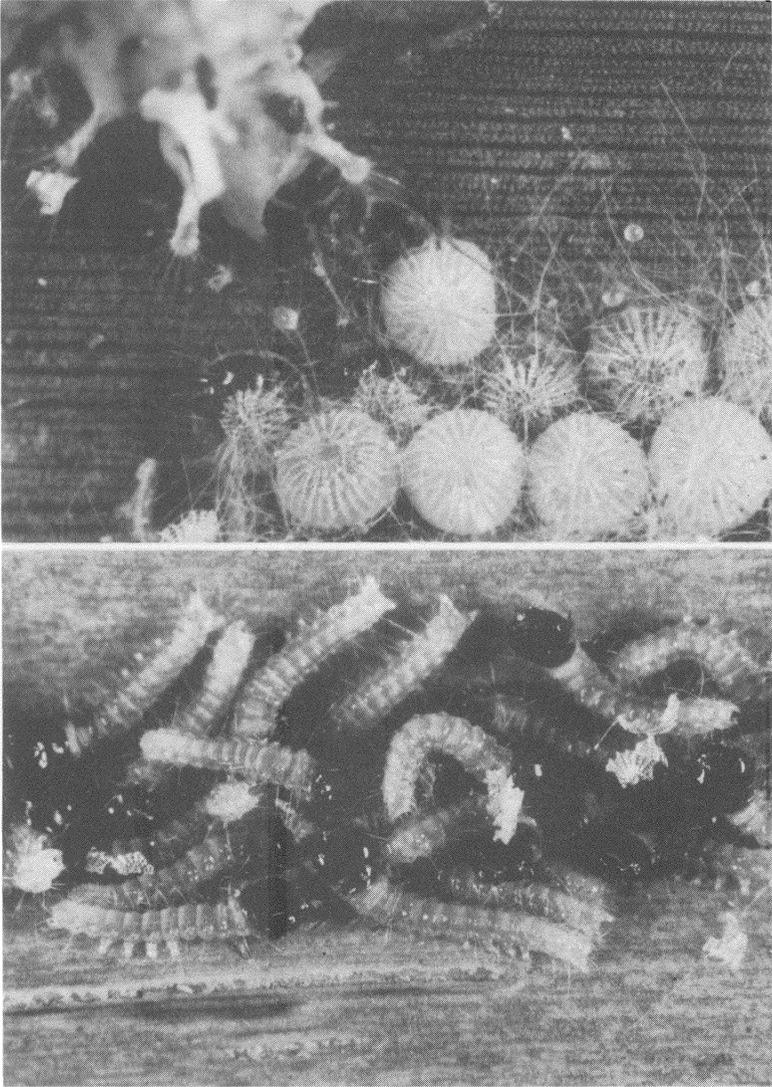


Figure 4. (Top) Chrysopid larva (*C. cubana*) feeding on *L. futilis* egg. (Bottom) Newly emerged larvae immediately after consuming egg shells.

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## LITERATURE CITED

- BLUM, M. S.  
1981. Chemical Defenses of Arthropods, Academic Press, New York. 562 pp.
- BOWERS, M. D.  
1993. Aposematic caterpillars: Life-styles of the warningly colored and unpalatable, pp. 331-371. In N. E. Stamp and T. M. Casey [eds.], Caterpillars: Ecological and Evolutionary Constraints on Foraging, Chapman and Hall, New York. 587 pp.
- BROWER, L. P.  
1984. Chemical defense in butterflies, pp. 109-133. In R. I. Vane-Wright and P. R. Ackery [eds.], The Biology of Butterflies, Symposium of the Royal Entomological Society of London, Number 11, Academic Press, London. 429 pp.
- CLEMENT, S. L., W. L. RUBINK, AND D. A. MCCARTNEY  
1986. Larviposition response of *Bonnetia comta* [Dipt.: Tachinidae] to a kairomone of *Agrotis ipsilon* [Lep.: Noctuidae]. Entomophaga 31: 277-284.
- COMMON, I. F. B. AND T. E. BELLAS  
1977. Regurgitation of host-plant oil from a foregut diverticulum in the larvae of *Myrascia megalocentra* and *M. bracteateella* (Lepidoptera: Oecophoridae). J. Aust. ent. Soc. 16: 144-147.
- EISNER, T.  
1970. Chemical defense against predation in arthropods, pp. 157-217. In E. Sondheimer and J. B. Simeone [eds.], Chemical Ecology, Academic Press, New York. 336 pp.  
1980. Chemistry, defense, and survival: case studies and selected topics, pp. 847-878. In M. Locke and D. S. Smith [eds.], Insect Biology in the Future, "VBW 80," Academic Press, New York. 977 pp.
- EISNER, T., J. S. JOHNESSEE, J. CARREL, L. B. HENDRY, AND J. MEINWALD  
1974. Defensive use by an insect of a plant resin. Science 184: 996-999.
- EISNER, T., A. F. KLUGE, J. C. CARREL, AND J. MEINWALD  
1972. Defense mechanisms of arthropods. XXXIV. Formic acid and acyclic ketones in the spray of a caterpillar. Ann. Entomol. Soc. Amer. 65: 765-766.
- EISNER, T., I. KRISTON, AND D. J. ANESHANSLEY  
1976. Defensive behavior of a termite (*Nasutitermes exitiosus*). Behav. Ecol. Sociobiol. 1: 83-125.

- EISNER, T., K. D. MCCORMICK, M. SAKAINO, M. EISNER, S. R. SMEDLEY, D. J. ANESHANSLEY, M. DEYRUP, R. L. MYERS, AND J. MEINWALD  
1990. Chemical defense of a rare mint plant. *Chemoecology* **1**: 30–37.
- EISNER, T., S. NOWICKI, M. GOETZ, AND J. MEINWALD  
1980. Red cochineal dye (carminic acid): its role in nature. *Science* **208**: 1039–1042.
- MORROW, P. A., T. E. BELLAS, AND T. EISNER  
1976. *Eucalyptus* oils in the defensive oral discharge of Australian sawfly larvae (Hymenoptera: Pergidae). *Oecologia* **24**: 193–206.
- PETERSON, S. C., N. D. JOHNSON, AND J. L. LEGUYADER  
1987. Defensive regurgitation of allelochemicals derived from host cyanogenesis by eastern tent caterpillars. *Ecology* **68**: 1268–1272.
- RICE, W. R.  
1989. Analyzing tables of statistical tests. *Evolution* **43**: 223–225.
- SEMLITSCH, R. D. AND C. A. WEST  
1988. Size-dependent cannibalism in noctuid caterpillars. *Oecologia* **77**: 286–288.
- SOKAL, R. R. AND F. J. ROHLF  
1981. *Biometry*. 2nd ed. W. H. Freeman and Co., San Francisco. 859 pp.
- SMEDLEY, S. R., K. D. MCCORMICK, AND T. EISNER  
1990. Interaction of *Pyrausta panopealis* (Pyrilidae) with a newly-reported host, the endangered mint *Dicerandra frutescens* (Labiatae). *J. Lepid. Soc.* **44**: 156–162.
- WHITMAN, D. W., M. S. BLUM, AND D. W. ALSOP  
1990. Allomones: Chemicals for defense, pp. 289–351. In D. L. Evans and J. O. Schmidt [eds.], *Insect Defenses: Adaptive Mechanisms and Strategies of Prey and Predators*. State University of New York Press, Albany, NY. 482 pp.



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