

Chemical egg defense in a green lacewing (*Ceraeochrysa smithi*)*

(Neuroptera/Chrysopidae/parental investment/fatty acids/aldehydes)

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Contributed by Thomas Eisner, December 21, 1995

ABSTRACT The green lacewing *Ceraeochrysa smithi* (Neuroptera, Chrysopidae), like other members of its family, lays its eggs on stalks, but it is unusual in that it coats these stalks with droplets of an oily fluid. The liquid consists of a mixture of fatty acids, an ester, and a series of straight-chain aldehydes. Relative to the eggs of a congeneric chrysopid that lacks stalk fluid, the eggs of *C. smithi* proved well protected against ants. Components of the fluid, in an assay with a cockroach, proved potentially irritant. Following emergence from the egg, *C. smithi* larvae imbibe the stalk fluid, thereby possibly deriving nutritive benefit, defensive advantage, or both.

The neuropteroid insects of the family Chrysopidae, the so-called green lacewings, are unusual in that they lay their eggs at the tip of stalks (1). To produce an egg, a female first applies a droplet of clear gelatinous fluid from the tip of the abdomen to the substrate. It then flexes the abdomen sharply upward so as to pull the droplet into a thread, pauses momentarily, and squeezes out the egg. Thread hardening occurs quickly, before the egg is entirely extruded. Chrysopids commonly lay their eggs singly or in clusters, and on rare occasions grouped, with the stalks tightly bundled (1). The stalks have long been assumed to provide the eggs with protection, and it was indeed shown that destalked eggs are more vulnerable to coccinellid beetle predation than normal eggs attached to their stalks (2).

In 1967, near Lake Placid, FL, one of us (T.E.) discovered a cluster of chrysopid eggs whose stalks were conspicuously beset with droplets of fluid. We eventually found such eggs to be relatively common and to belong to a known species, *Nodita floridana*. Subsequently, again in Central Florida, two of us (E.M. and T.E.) independently found the egg stalks of a second known chrysopid, *Ceraeochrysa smithi*, also to be endowed with droplets. The nature of the droplets appeared to be different in the two species: they were viscous and sticky in *N. floridana* and oily in *C. smithi*. We suspected the fluid in both species to provide added protection to the eggs beyond what was provided by the stalks alone.

We have had occasion since to study the egg stalk fluid of *C. smithi* in some detail. We elucidated the chemical composition of the fluid, showed it to be defensive against ants, and proved some of its components to be strongly active in a topical irritancy test with a cockroach. Furthermore, we noted the fluid to be ingested by the newly hatched larvae, which thereby obtain their first meal. We present here these results.

MATERIALS AND METHODS

Field observations, collection of chrysopids, and tests with ants were done on the grounds of a wildlife preserve, the Archbold Biological Station, Lake Placid, Highlands County, FL.

C. smithi. We found this chrysopid to lay its eggs in clusters, in a characteristic spiral arrangement, with the egg stalks tilted slightly toward the spiral center (Fig. 1A and B). The number of fluid droplets per stalk was in the range of 3–6 (Fig. 1C and D), with some variation depending on droplet size. We have, over the years, located a number of such clusters in the field on various substrates, including fronds of palmetto (*Serenoa repens*, *Sabal palmetto*) and leaves of an introduced creeping fig tree (*Ficus pumila*). In clusters where the eggs had hatched, the stalks were conspicuously free of droplets (Fig. 1B).

Eggs were obtained in the laboratory from gravid females collected outdoors near light sources to which they had flown at night. Such females readily oviposited on paper when confined in vials lined with paper.

Ceraeochrysa cubana. This congener of *C. smithi* lays unclustered eggs devoid of stalk fluid. Such eggs provided a convenient control in the predation tests with ants. These eggs were also obtained, as with *C. smithi*, from field-collected females that oviposited on paper in vials.

Ant Predation Tests. These tests were carried out on the foraging territory of a natural nest of the common ant, *Monomorium destructor*. The ants were baited with dilute honey solution, presented on small circular glass discs, to which the ants quickly laid trails. For a test, a group of 10 chrysopid eggs, 5 of *C. smithi* and 5 of *C. cubana*, were placed in regular alternation in a circle (3- to 4-cm diameter) around one of the discs. The eggs had all been isolated from laboratory-laid clusters by cutting away a small square of the paper to which they were attached. Each egg was thereby provided with a basal platform by which it could be stood upright. Tests were of 12 hr duration and consisted of checking frequently on the fate of the eggs. Five replicates were done.

Chemistry. Stalk fluid was collected from a single, unhatched, field-collected *C. smithi* egg cluster. Stalk droplets were taken up from four egg stalks with glass microcapillaries, which were sealed without solvent in a glass capsule (1.5-mm diameter, 20-mm length). This sample was analyzed directly by gas chromatography using a convenient direct solid-sampling technique (3, 4). GC/MS analysis was performed using a DB-5-coated capillary column (0.22 mm × 30 m) in a Hewlett Packard (HP) 5890 gas chromatograph linked to a HP mass selective detector (35°C for 4 min, increased to 270°C at 15°C/min). For derivatization, another sample (fluid from 17 stalks, also collected with microcapillaries) was extracted with hexane (5 μl) and mixed with 1 μl of *N,N*-dimethylhydrazine (DMH) (5). After 10 min at room temperature, the reaction mixture was concentrated and analyzed by GC/MS as described above.

Cockroach Irritancy Test. When a droplet of irritant chemical is placed on one side or the other of the fifth abdominal tergite of a decapitated nymph of the cockroach *Periplaneta*

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Abbreviation: DMH, *N,N*-dimethylhydrazine.

*This paper is no. 135 in the series *Defense Mechanisms of Arthropods*; paper no. 134 is ref. 19.

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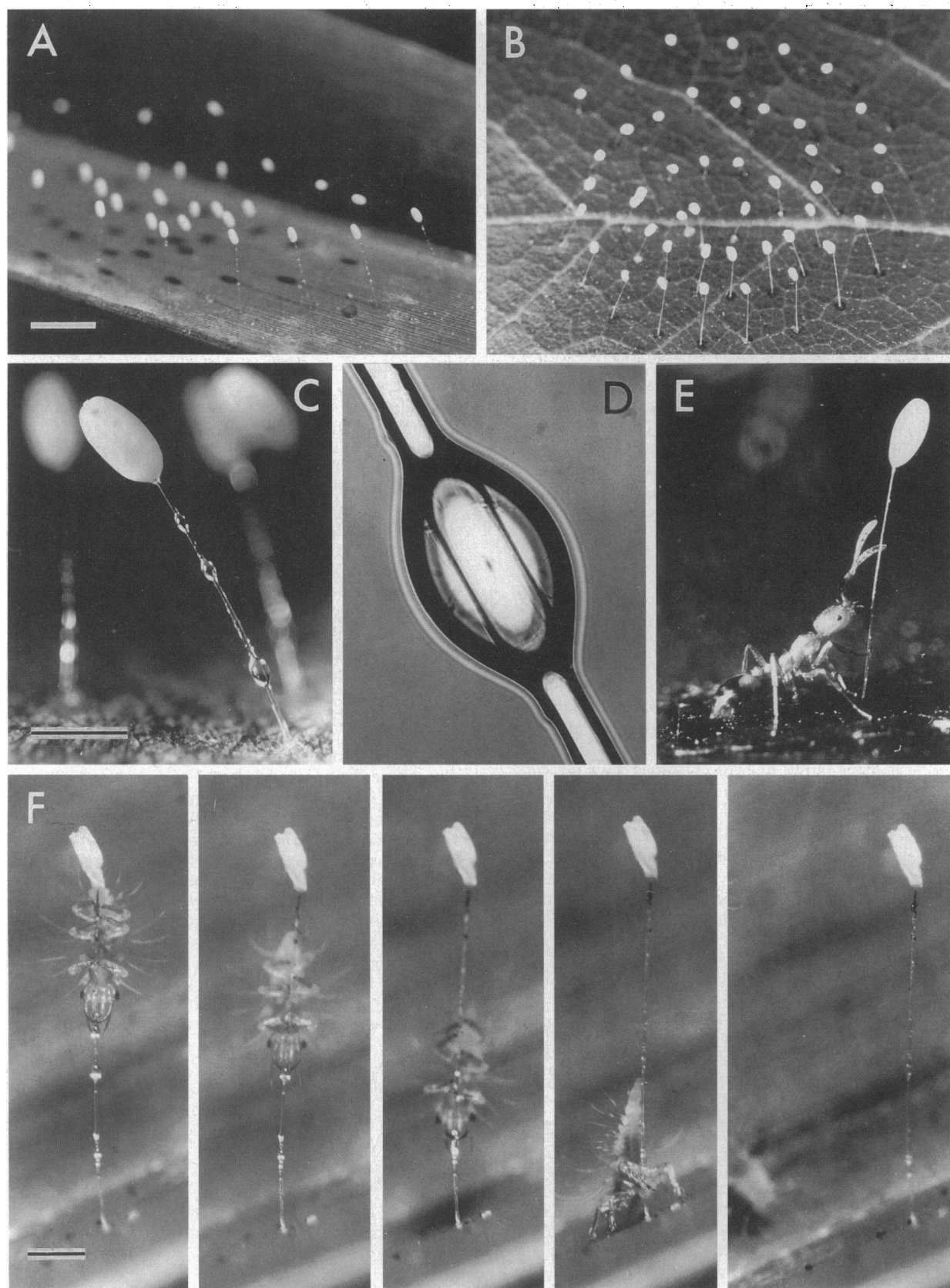


FIG. 1. (A) Egg cluster of *C. smithi*, unhatched. (B) Comparable cluster but hatched (note absence of stalk droplets). (C) *C. smithi* egg. (D) *C. smithi* egg-stalk droplet. (E) Ant inspecting an egg (*C. cubana*) devoid of stalk fluid. (F) Freshly emerged larva of *C. smithi* pausing to ingest one stalk droplet after another as it descends along the stalk. (Bars: A, 3 mm; C, 1 mm; F, 0.5 mm.)

americana, the animal scratches the site with the hindleg of the side stimulated. The time interval between application of sample and scratching provides a measure of the irritant

potency of the chemical. Details of the assay, which we have used previously for assessment of irritancy of insect and plant products, are given elsewhere (6-8).

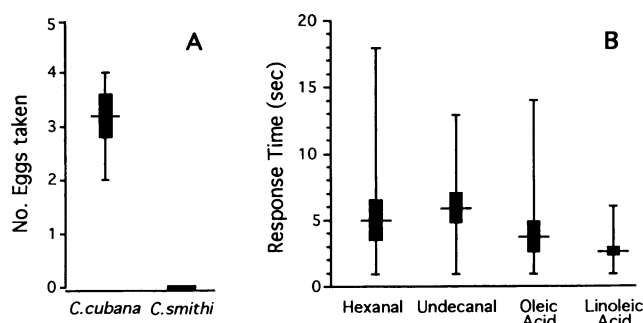


FIG. 2. (A) Relative acceptability of the two *Ceraeochrysa* eggs in predation tests ($n = 5$) with ants (*M. destructor*). Data are given as means \pm SE and range. (B) Sensitivity of cockroach (*P. americana*) to topical application of components of *C. smithi* egg stalk fluid. Sensitivity is expressed as delay to onset of the scratch reflex induced. Data are given as means \pm SE and range.

We used the assay to test for the potency of four representative components of the *C. smithi* egg stalk fluid: hexanal, undecanal, oleic acid, and linoleic acid. Droplets were applied in fixed volumes ($0.1 \mu\text{l}$), and only last-instar nymphs were used. Fifteen cockroaches were tested per sample. Responses were timed to 1 s accuracy with a foot-operated stopwatch.

RESULTS

Ant Predation Tests. In not a single test were any of the *C. smithi* eggs taken by the ants (Fig. 2A). In contrast, the *C. cubana* eggs (Fig. 1E) proved vulnerable: from two to four per test were carried off, each by an individual ant. Typically an ant ascended a stalk, straddled the egg, and cut the egg from the stalk with its mandibles. It then fell to the ground with the egg, grasped the egg in the mandibles, and scurried off along the trail. Discrimination against the *C. smithi* eggs appeared to be effected on contact rather than near contact with the stalk. Upon touching a stalk, ants abruptly backed off and walked away.

A separate test demonstrated that the eggs of both species, when deprived of stalks, are intrinsically acceptable to the ants. Five eggs of each species, carefully cut from stalks, were laid out as part of a circular arrangement of food items around one of the baiting dishes with honey solution. The ants grasped the

eggs of both types unhesitatingly, and carried all away in quick order.

Chemistry. GC/MS analysis of the first fluid sample showed the presence of trace quantities of many volatile constituents. The mass spectra corresponding to most of these components suggested that the signals represented a homologous series of saturated aldehydes. This supposition was confirmed by GC/MS analysis of the sample derivatized by treatment with DMH. The mass spectra of aldehyde *N,N*-dimethylhydrazones are particularly useful analytically because they show not only significant molecular ions but also a diagnostic McLafferty rearrangement peak at m/z 86 (9). Fig. 3 shows a selected ion retrieval chromatogram (m/z 86) obtained from the derivatized mixture. The mass spectrum corresponding to each of 15 identified gas chromatographic peaks showed the expected dimethylhydrazone molecular ions at the appropriate retention time (Table 1). These identifications were confirmed by direct comparison with GC/MS data obtained from authentic samples.

While these aldehydes occur in the stalk fluid in only trace amounts (0.2–1.2 ng per stalk), several other compounds were found in much larger quantities. Comparison of the GC/MS data obtained from these additional components with library spectra led to identification of myristic, palmitic, linoleic, oleic, stearic, and a docosaenoic acid in approximately equal amounts. The quantity of oleic acid was estimated to be ≈ 30 ng per egg stalk by use of a solution of oleic acid of known concentration as an external standard. Finally, GC/MS analysis showed the presence of one fatty acid ester, isopropyl myristate, in amounts comparable to those of the free fatty acids.

Cockroach Irritancy Test. All four compounds proved topically active (Fig. 2B). The cockroaches scratched promptly, on average within 3–6 s after application of the droplet. With each sample there were individuals that responded virtually instantaneously (within 1 s).

Behavior of Emerged Larvae. As is typical for chrysopids (1), the newly emerged larvae of *C. smithi* remained astride their chorions for some hours before descending along the stalks. When they eventually walked down, they did so head-first, pausing along the way to consume the stalk fluid. They halted by each droplet for as long as it took to imbibe the liquid with their hollow, sickle-shaped jaws (Fig. 1F). Upon arrival at the base of the stalk, they briskly walked away. We observed this behavior with a number of clusters and found it to be

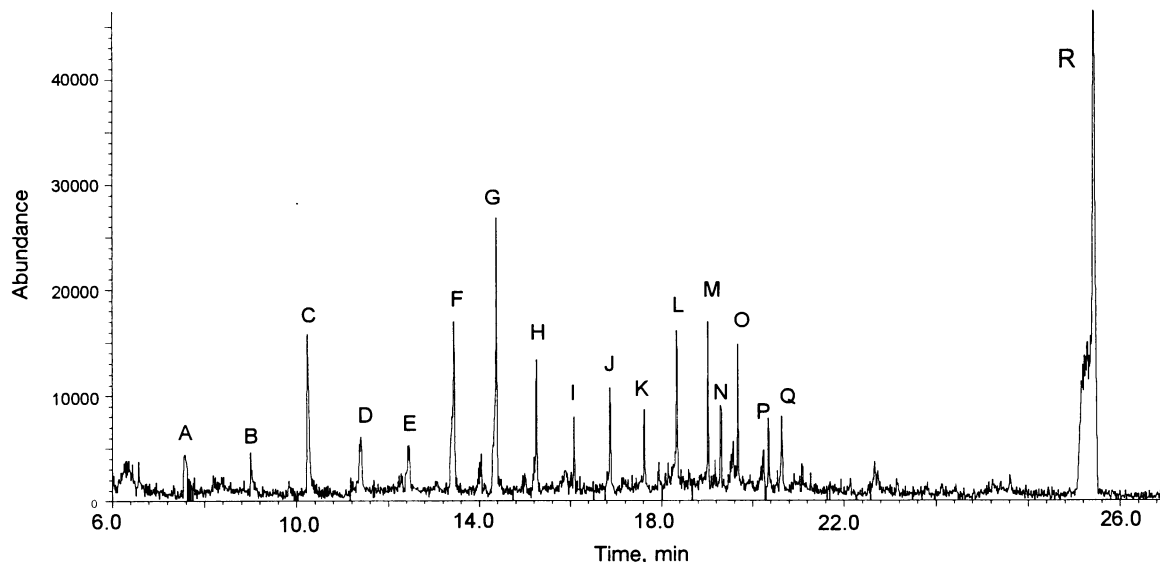


FIG. 3. A selected-ion retrieval chromatogram from m/z 86 obtained by GC/MS analysis of the volatiles extracted from *C. smithi* egg stalk fluid after derivatization with DMH.

Table 1. Aldehydes identified from egg stalk fluid of *C. smithi*

Peak label*	Aldehyde	M ⁺ of DMH derivative
A	Butanal	114
B	Pentanal	128
C	Hexanal	142
D	Heptanal	156
E	Octanal	170
F	Nonanal	184
G	Decanal	198
H	Undecanal	212
I	Dodecanal	226
J	Tridecanal	240
K	Tetradecanal	254
L	Pentadecanal	268
M	Unidentified component	
N	Unidentified component	
O	Heptadecanal	296
P	Octadecanal	310
Q	Unidentified component	
R	Tetracosanal	394

*Peak labels are those designated in Fig. 3.

consistent. It provided an explanation for why the stalks of hatched *C. smithi* eggs in the field had been noted to be fluid-free.

DISCUSSION

It seems established that the egg stalk fluid of *C. smithi* serves for defense. The liquid is deterrent to ants and, judging from the effect of some of its components on *P. americana*, may derive its anti-insectan action from its irritancy. While ants may be the principal enemies of *C. smithi* eggs, other potential predators, including coccinellid beetles and mites, might also be deterred by the fluid.

Some of the constituents of the *C. smithi* stalk fluid are known from other insectan sources. A number of the aldehydes, for instance, have been isolated from the defensive secretions of Hemiptera, beetles, and cockroaches (10). Components comparable to the fatty acids and ester that figure as chief constituents of the stalk fluid are not usually present in arthropodan defensive products. A notable exception is an ester of palmitic acid, methyl palmitate, present in a secretion used by certain wasps to impregnate the stalk of their nest. This secretion also functions to guard against ants (10–14).

Of special interest is the finding that the newly emerged larvae, upon descending from the egg, imbibe the stalk fluid. By so doing they evidently avoid becoming contaminated with the liquid, which could be topically irritating to them as well. It is conceivable, however, that the larvae are themselves insensitive to the fluid, and that they reuse the acquired chemicals for their own defense, perhaps by excreting them (or some of them) through the integument. A further possibility is that they metabolize the chemicals and put them to nutritional use.

We are uncertain about the anatomical source of the stalk fluid. We suspect the liquid to stem from the colleterial gland of the adult female, an organ known to secrete the stalk itself (15) and which in *C. smithi* we found to possess a sizable extra lobe not present in the colleterial gland of other chrysopids.

Data that we have on the one other chrysopid known to produce stalk fluid, *N. floridana*, suggest that this species evolved its fluid-secreting capacity independently from *C. smithi*. *N. floridana* larvae do not ingest their stalk fluid upon emergence, and the fluid itself, so far as we can judge from preliminary analytical data, is chemically distinct from that of *C. smithi*.

A number of insects have been shown to endow their eggs with defensive chemicals (16–18). But in none of these cases are the chemicals ingested as such by the offspring. As an insectan “mother’s milk” that seemingly combines the attributes of “guns and butter,” the egg stalk fluid of *C. smithi* is evidently unusual.

Note Added in Proof. An excellent paper has recently appeared (20) on the chemistry of the secretion used by wasps to impregnate their nest stalks (pedicels). This paper presents evidence that unsaturated fatty acids, including oleic acid and linoleic acid, as are present in these secretions, are deterrent to ants.

We thank Dr. Catherine A. Tauber and Dr. P. A. Adams for confirming the chrysopid identifications, Dr. Mark Deyrup for helpful suggestions, and the staff of the Archbold Biological Station for personal kindnesses. This study was supported by Grants AI 02908 and AI 12020 from the National Institutes of Health and by Hatch Grant NYC 191-424.

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