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SOLVENT DEACTIVATION OF MIMOSA WEBWORM LARVAL WEBBING (LEPIDOPTERA: PLUTELLIDAE)

Robert C. North[1], Elwood R. Hart[2] and Lu MingXian[3]

ABSTRACT

Untreated larval webbing of the mimosa webworm, *Homadaula anisocentra* stimulated oviposition. Six-week-old webbing was as active as two-day-old webbing. Stimulatory activity of webbing was lost after rinsing with highly polar solvents, but not after rinsing with nonpolar solvents. Addition of the polar solvent rinses did not induce activity in other substrates nor restore activity to rinsed webbing. No differences in structure were found in a scanning electron microscope examination of unrinsed webbing and webbing rinsed with solvents of varying polarity.

Numerous studies of conspecific oviposition-deterring mechanisms, especially pheromones, have been conducted (e.g., Prokopy 1981, Prokopy et al. 1984, Averill and Prokopy 1987). Such communication among adults is commonly encountered, but intraspecific communication that stimulates oviposition, especially between immatures and adults of the same species, is less well documented.

A mandibular gland secretion of final instar Mediterranean flour moth, *Anagasta kuehniella* Zeller, influenced oviposition behavior of adults of the species (Corbet 1973). Larval-adult communication in the navel orangeworm, *Amyelois transitella* (Walker) was noted and it was hypothesized that a pheromone produced by the larvae may have been responsible for the increased attraction and oviposition by females on infested mummy nuts (Andrews and Barnes 1982). In these and similar studies, it is difficult to separate plant effects from larval effects. A clear demonstration of larva-produced factors is not easily accomplished.

Larval trail marking functions of silk are known to occur in a number of lepidopterous species (e.g., Fitzgerald and Edgerly 1979, Peterson 1988). The use of larval silk as an intraspecific oviposition marker is less documented. In our previous studies of the mimosa webworm, *Homadaula anisocentra* Meyrick (Lepidoptera: Plutellidae), we reported that oviposition was stimulated by the presence of larval webbing, even in the absence of plant material (North and Hart 1983). We also noted that old larval webbing (no active larval feeding evident) in infested trees did not induce oviposition.

In a preliminary examination of this phenomenon, we observed that webbing that had been wetted during tree watering seemed to have a reduced

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ability to induce oviposition. A parallel phenomena has been noted in the reduction by water of the trail marking function of lepidopterous silk (Roessingh 1990). In this paper we report a series of experiments that examine this deactivation in the mimosa webworm. The major objective of these experiments was to define the effects of water on the activity of webbing. We also examined the effects of solvents of differing polarity on the activity of the webbing, as well as the role of physical factors in the activity of the webbing.

MATERIALS AND METHODS

Two series of multiple-choice experiments were designed to determine the effects and interactions of different solvents and substrates on webbing activity. The first series, consisting of four sets, was designed to determine if activity could be modified by solvents of differing polarity. Different substrate combinations were rinsed with selected solvents and then bioassayed for oviposition-inducing activity. The second series, consisting of seven sets, was designed to determine whether or not that activity, once removed, could be recovered by adding those solvent rinses to different substrates. Different combinations of substrate were rinsed with the solvent and the wash solution was pipetted back onto the original substrate or onto other selected substrates. Those substrates were then bioassayed for activity.

To determine if physical changes in webbing were involved in the ability of webbing to stimulate oviposition, we conducted a bioassay of silken substrates as well as a scanning electron microscope study of treated webbing.

With only minor modifications, the oviposition cages, rearing methods, light regimes, and treatment preparations as described in North and Hart (1983) were used. Larger, rectangular, nylon-screen, wooden frame cages (64 x 64 x 40 cm) were used to accomodate an increase in the number of treatments for some of the multiple choice experiments.

Removal of Activity. In sets 1, 2, 3, and 4, mature honeylocust leaves (more than 6 weeks old), either: (a) alone, (b) in combination with old larval webbing (more than 6 weeks old) or, (c) in combination with new larval webbing (less than 2 d old) were removed from infested 'Shademaster' honeylocust trees. After removal, the petiole of each leaf was placed immediately into a florist's Aquapic filled with tap water. The exposed portion of the leaf was rinsed individually by swirling it in 50 ml of a fresh select solvent for 30 sec. The Aquapic with its rinsed leaf was placed into the drainage hole in the bottom of an inverted clay flower pot (d = 17 cm, h = 16.5 cm) and allowed to dry. The inverted pot was then placed into a large screen cage, with the top of the leaf approximately 15 cm from the top of the cage, well within the searching pattern of mated mimosa webworm.

For each replication in each set, nine treatments (Table 1) were assigned randomly to positions within a cage. Three cages were used during a replication. The cages were rotated randomly in position within the greenhouse each day, and were washed and rotated randomly between replications. Sets 1, 2, and 3 were replicated three times and set 4 was replicated twice. For each replication, five mated females were released in each cage in sets 1, 2, and 3, and ten mated females were released in each cage in set 4. Eggs were counted for 3 d in each replication for sets 1, 2, and 3, and for 2 d in each replication for set 4.

In set 1, mature leaves, mature leaves with old webbing, and mature leaves with new webbing were used. Two polar solvents (distilled water and 95% ethanol) and a nonpolar solvent (hexane) were used as a rinse for selected

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Table 1. Mimosa webworm eggs oviposited on rinsed substrates.

Oviposition substrate	Rinse solvent	n	No. eggs ($\bar{x}$ ± SD)[a]
	Set 1		
leaves + old webbing	none	9	12.0 ± 4.8
leaves + new webbing	none	9	11.4 ± 4.5
leaves + old webbing	hexane	9	8.2 ± 5.0
leaves + old webbing	ethanol (95%)	9	1.6 ± 0.9
leaves	water	9	0.2 ± 0.3
leaves	none	9	0.0
leaves + old webbing	water	9	0.0
leaves	hexane	9	0.0
leaves	ethanol (95%)	9	0.0
	Set 2		
leaves+ new webbing	none	9	26.9 ± 11.4
leaves + old webbing	none	9	24.1 ± 10.2
leaves + old webbing	hexane	9	14.2 ± 10.1
leaves + new webbing	hexane	9	13.2 ± 8.5
leaves + larval silk wrap	none	9	10.1 ± 6.3
leaves + larval silk wrap	hexane	9	7.0 ± 6.0
leaves + new webbing	water	9[b]	4.0 ± 6.2
leaves + old webbing	water	9	0.3 ± 0.5
leaves + larval silk wrap	water	9	0.0
	Set 3		
leaves + new webbing	hexane	9	9.3 ± 5.3
leaves + new webbing	none	9	9.0 ± 4.4
leaves + new webbing	acetone	9	2.8 ± 1.5
leaves + new webbing	ethanol (95%)	9	1.0 ± 1.3
leaves	acetone	9	0.7 ± 1.1
leaves + new webbing	water	9	0.1 ± 0.3
leaves	hexane	9	0.0
leaves	ethanol (95%)	9	00
leaves	water	9	0.0
	Set 4		
leaves + new webbing	none	6	20.8 ± 11.1
leaves + new webbing	hexane	6	16.5 ± 7.3
leaves + new webbing	methanol	6	2.8 ± 1.8
leaves	none	6	0.3 ± 0.6
leaves	methanol	6	0.2 ± 0.3
leaves (simulated feeding)	none	6	0.0
leaves	water	6	0.0
leaves	hexane	6	0.0
leaves + new webbing	water	6	0.0

[a] $\bar{x}$ = number of eggs oviposited by single female over 3 days.
[b] One experimental unit contaminated by larva during replication three, cage one.

combinations of leaves and webbing. Following rinsing, the substrates were tested for activity, as measured by oviposition.

In set 2, we used a polar solvent (distilled water) and a nonpolar solvent (hexane). The rinsed substrates included leaves with old webbing and leaves with new webbing. A third combination, leaves with a freshly spun larval silk wrap (North and Hart 1983), was used to isolate the silk from other possible larval-produced substances (e.g., frass) that may have influenced ovipositional activity.

In sets 3 and 4 we used two additional solvents of intermediate polarity. These were added after evidence indicated that the highly polar solvent, dis-

tilled water (also present in the 95% ethanol), was eliminating oviposition activity in the webbing and silk wraps. In set 3, we used distilled water, 95% ethanol, acetone, and hexane as solvent rinses. Leaves with and leaves without new webbing were used as substrates. In set 4, we used distilled water, absolute methanol, and hexane as solvent rinses. Leaves with and without new webbing again were used as substrates. An additional treatment, simulated damage in the absence of larvae or webbing, was used to test for possible oviposition-stimulating effects of damaged or senescent foliage. The simulated damage was prepared by removing the dorsal surface of leaflets with a razor blade.

Restoration of Activity. To determine whether any activity could be transferred to other material by the rinse solvent after rinsing leaves, silk, or webbing, a second series of tests was conducted. The objective of these tests was to determine if activity could be restored to a deactivated webbing substrate or conferred to other selected substrates by addition of the rinse material.

This second series of tests involved rinsing fresh webbing, larval silk, or macerated honeylocust leaves with solvents of differing polarity. The rinse material was pipetted onto various substrates and the ovipositional activity of webworm adults on the rinse plus substrate was recorded.

Nine treatments were used in each of sets 5, 6, 7, and 8. Position assignment and cage manipulation were carried out as described. Eggs were counted daily for 2 d. Ten mated females were used in each cage. Each experiment was replicated twice. The treatments were prepared by rinsing 10 mature honeylocust leaves and their associated new larval webbing in 50 ml of solvent for 1 min. The solvents evaluated were distilled water, 95% ethanol, acetone, and hexane. The solvent was allowed to evaporate until a volume of 10 ml remained. The assumption was made that any dissolved material was relatively nonvolatile. One ml of this concentrate was then pipetted onto filter paper (set 5), rubber septa (set 6), cotton dental wick (set 7), or a glass slide (set 8), and allowed to dry before being bioassayed.

In sets 9, 1 0, and 1 1, four treatments were assigned randomly, each to one of four positions within one of 14 cages on a plywood sheet (North and Hart 1983). The cages were washed with water and exchanged randomly between replications. In set 9, three mated females were placed into each cage during each of three replications. In sets 10 and 11, two mated females were placed into each cage during each of two replications. In set 9, mature honeylocust leaves with new larval webbing were rinsed with 50 ml of distilled water for 30 sec and the rinse material discarded. The leaves were allowed to dry and then placed into cages with gravid adult females. Rinse material from fresh larval silk that had been immersed in 4 ml solvent for 1 min was placed on the leaves. Solvents included distilled water, 95% ethanol, acetone, and hexane. If no oviposition took place after 2 d, 1 ml of the silk rinse was pipetted onto the seemingly inactive webbing and leaves. Filter paper (set 10) or cotton dental wick (set 11) was used as a substrate for rinse material obtained from macerated honeylocust leaves in a further attempt to determine any possible role that leaf material might play in the chemical aspect of oviposition. A mature leaf was macerated in 10 ml of solvent (distilled water, acetone, or hexane) and 1 ml of the supernatant was pipetted onto the substrate prior to bioassay.

Physical Influences on Oviposition. Two investigations of possible physical influences of the silk also were performed. In the first of these, four treatments were tested in the smaller cages: mature honeylocust leaves alone, leaves with commercial silk fibers of the same approximate diameter as mimosa webworm silk, leaves with fresh larval silk, and leaves from which the larval silk had been removed mechanically. A single mated female was placed in each of the 14 cages for each treatment; there were three replications.

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To examine if the larval silk might be modified physically by solvents of differing polarity, a test was designed to detect possible surface changes of the silk when wetted. Four replications of new silk were collected from a greenhouse colony and each divided into four fractions. Each fraction was placed onto Whatman #1 filter paper in a glass petri dish. Fraction 1 was untreated, and fractions 2, 3, and 4 were rinsed with triple-distilled water, reagent grade n-butyl alcohol, and hexane, respectively. The dishes were covered and placed into a drying oven at 37°C for 24 hr. Each fraction was placed into an individual gelatin capsule before being coated with gold-palladium for scanning electron microscope examination.

Data were submitted to the Statistical Analysis System (SAS Institute 1979) for ANOVA among treatments.

RESULTS AND DISCUSSION

The results demonstrated that the oviposition-inducing activity of webbing was increasingly reduced as solvent polarity increased. All attempts to restore activity to deactivated webbing or to transfer activity to nonactive substrates were unsuccessful. Evidence also indicated that physical changes were not associated with changes in webbing activity.

Removal of Activity. Rinsing with a polar or weakly polar solvent removed or reduced oviposition on larval webbing (Table 1). There was no discernible difference in the number of eggs oviposited on old or on new webbing, old webbing continuing to stimulate oviposition following air exposure for 6 weeks in a greenhouse.

In set 1 there was a significant difference among treatments ($F = 19.6$, 12 d.f., $P < 0.01$). Female webworms oviposited on untreated leaves and leaves with webbing rinsed with hexane, whereas oviposition was reduced significantly or eliminated by using 95% ethanol or distilled water as a rinse. Leaves without webbing, rinsed or not rinsed, elicited little oviposition.

Significance among treatments also occurred in set 2 ($F = 32.3$, 12 d.f., $P < 0.01$). The webbing rinsed with hexane stimulated oviposition, whereas the webbing rinsed with distilled water did not. Oviposition activity was less on silk wraps than on webbing.

There was a significant difference among treatments in set 3 ($F = 15.1$, 12 d.f., $P < 0.01$). Webbing rinsed with acetone elicited reduced oviposition when compared with unrinsed webbing and with webbing rinsed with hexane; 95% ethanol reduced but did not eliminate oviposition. Distilled water again reduced or eliminated any oviposition. Only a few eggs were deposited on leaves rinsed with a polar solvent.

There also was a significant difference among treatments in set 4 ($F = 26.3$, 11 d.f., $P < 0.01$). Methanol reduced but did not eliminate oviposition on webbing. Distilled water again eliminated oviposition on webbing. Unaltered leaves and leaves with simulated feeding injury were seldom used as sites of oviposition.

Similar results were observed in the trail marking properties of larval silk of *Yponomeuta cagnegellus* (Hübner) (Lepidoptera: Yponomeutidae) (Roessingh 1990). Wetting with water removed most of the communication properties of the silk, hexane had little effect, and dichloromethane had but a slight influence.

Restoration of Activity. In the replacement sets, 5 through 11, the stimulatory properties of webbing were not restored to inactivated webbing nor conferred to other substrates upon the addition of any solvent rinse material. Only 10 eggs were oviposited on the treatments during the entire series.

Table 2. Mimosa webworm eggs oviposited on unrinsed substrates.

Oviposition substrate	n	No. eggs ($\bar{x} \pm SD$)[a]
leaves + larval silk wrap	42	3.7 ± 3.3
leaves with silk wrap removed	42	1.2 ± 2.0
leaves	42	0.4 ± 0.7
leaves + commercial silk wrap	42	0.0

[a] $\bar{x}$ = number of eggs oviposited by single female over 3 days.

Physical Influences on Oviposition. There was a significant difference among treatments in this bioassay ($F = 63.3$, 17 d.f., $P < 0.01$) (Table 2). No eggs were deposited on the commercial silk strands. Leaves from which fresh webbing had been completely removed maintained an ability, although significantly reduced, to induce oviposition. This suggests that chemical rather than physical factors may be responsible for the oviposition-inducing function of the webbing.

Larval silk examined at 20,000x and 40,000x presented no noticeable differences in surface structure of the silk among the treatments. This also supports a hypothesis that the stimulus probably involves chemical cues that may be modified by polar solvents. The exact nature and physiological origin of any chemicals that may be involved in this system are not known.

Ecologically, the major function of deactivation would seem to be, through periodic moisture-caused declines of stimulatory cues, the deterrence of overcolonization of food resources and the prevention of oviposition on exhausted materials. The adaptive value of this phenomenon in preventing oviposition mistakes, and possibly in reducing the time required to select the appropriate host, needs further study. Also, relative to the frequency of deactivating events such as rainfall or heavy dew, possible larval density effects and competition by females for oviposition sites need to be studied for this insect relative to the concept of optimal density range of individuals per unit of resource (Peters and Barbosa 1977, Prokopy 1981).

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