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INVESTIGATION OF OVIPOSITION DETERRENT IN LARVAL FRASS OF *Spodoptera littoralis* (BOISD.)

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Abstract—Previous experiments demonstrated an oviposition-deterring effect of larval frass in the Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd.). In this study, females were shown to perceive the oviposition-deterring substance(s) with their antennae. During dark, airtight, and cold (−10°C) storage, the deterrent was persistent for at least 395 days. On the other hand, larval frass retained its activity for only two days when applied to cotton leaves. The deterrent activity of frass was independent of larval density. Frass of larvae reared at high densities deterred oviposition as well as frass of larvae feeding separately or in small groups. For significant oviposition deterrence, the minimum amount of frass was in the range of 5–10 mg frass per cotton leaf. An acetone extract of larval frass was highly deterrent, in contrast to extracts prepared with water, ethanol, chloroform, or pentane.

Key Words—*Spodoptera littoralis*, Lepidoptera, Noctuidae, Egyptian cotton leaf worm, oviposition behavior, oviposition deterrence, larval frass.

INTRODUCTION

Host plants visited for oviposition by a phytophagous insect should offer sufficient food for the progeny. Since laying eggs on infested host plants might result in a lack of space and food for the hatching larvae, females may regulate ovi-

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position on a host plant according to the infestation density to obtain optimal developmental conditions for the hatching larvae. It has been shown for a wide array of phytophagous insect species that chemical messengers of either insect or plant origin inform females about the infestation of a host plant and thus regulate oviposition (Prokopy, 1981; Prokopy et al., 1984).

In several species of Lepidoptera, feeding larvae and larval frass respectively indicate occupancy of the host plant and deter egg deposition (e.g., Dittrick et al., 1983; Mitchell and Heath, 1985; Renwick and Radke, 1980, 1981; Rothschild and Schoonhoven, 1977; Williams et al., 1986). Recently we found that oviposition is also deterred by larval frass in the Mediterranean noctuid moth *Spodoptera littoralis* (Boisd.) (Hilker, 1985).

To date, nothing was known about biological and chemical properties of this oviposition deterrent in *S. littoralis*. We hypothesized that only larvae at high densities excrete oviposition-deterring substances to which females respond by avoiding egg deposition. Several studies of *S. littoralis* indicate a change of metabolism when larval density increases (e.g., Hodjat, 1970; Rivnay and Meisner, 1965; Zaher and Moussa, 1961). Metabolic changes might cause a change of frass compounds. These changed substances in the frass of larvae feeding at high densities might signal to gravid females when a site is unsuitable for oviposition. We therefore investigated whether the activity of larval frass is dependent on larval density. We have also studied the perception of the oviposition deterrent, its stability during cold storage, the persistence of the deterring activity of larval frass on the host plant, and the solubility of the deterrent, and we have determined the minimum amount of frass that is necessary for significant oviposition deterrence. The results of these studies are reported here.

METHODS AND MATERIALS

For oviposition bioassays, moths of *S. littoralis* were obtained from a laboratory culture reared on semiartificial diet (Patana, 1969; Shorey and Hale, 1965). Only 3- to 4-day-old moths were used. Frass was collected daily from larvae of the fifth and sixth instar, feeding on cotton plant leaves. Only these late-instar larvae produced so much frass that sufficient material was available after a short time of collection. A determination of the fresh weight of frass production per day revealed that larvae feeding on cotton leaves excreted about 20–50 mg frass during the fifth instar and about 50–350 mg frass during the sixth instar, whereas third- and fourth-instar larvae produced only about 2–5 mg frass per day. Unless mentioned otherwise, the tested frass was 1–10 weeks old. After collection, frass was stored in dark, airtight conditions at $-10°C$. Cotton plants, *Gossypium barbadense* Mill., were grown in a greenhouse.

Bioassays were conducted in screened cages (50 × 50 × 70 cm) situated in a chamber with constant temperature (27 ± 1°C) and a 14 hr : 10 hr light–dark cycle according to conditions in Egyptian summer. Each bioassay began with the onset of the light period and lasted 24 hr. Three females and five males were placed in each cage. For oviposition, moths were offered two treated and two control cotton leaves. Each leaf was deposited in a 100 ml vial filled with water and situated in a corner of the cage.

With the exception of the bioassay testing the solubility of the deterrent, leaves were treated with a water suspension of frass. This suspension was prepared in a Potter homogenizer and applied to the undersurface of a leaf with a brush. Eggs are usually laid only on the undersurface of a cotton leaf. A test leaf was treated with 1 ml suspension and a control leaf with 1 ml water. Unless cited otherwise, the concentration of the suspension was 100 mg/ml H_2O.
Six different experiments were conducted.

Experiment I. In order to determine the stability of the oviposition deterrent during storage, we tested larval frass stored in 15 ml vials at −10°C for either 6 or 395 days in airtight, dark conditions.

Experiment II. This experiment was conducted to determine the persistence of frass activity on the host plant. We bioassayed test and control leaves for 24 hr either immediately after treatment (persistence day 1), or during the second day after treatment (persistence day 2), or during the third day (persistence day 3). Prior to testing persistence day 2 and persistence day 3, leaves were kept in vials filled with water at room conditions.

Experiment III. In order to investigate the perception of the oviposition deterrent, antennae of female moths were removed. One hour before the antennae were ablated, the test females were put into a cold (−2°C) room. One hour later, the antennae of the motionless females could easily be cut at the base with small scissors. About 15 min after the moths had been taken out of the cold room, they showed normal activity. For control, females with intact antennae were used. The control females were also chilled at −2°C for 1 hr prior to testing.

Experiment IV. In order to determine the amount of frass that is necessary for significant oviposition deterrence, we tested suspensions in the following concentrations: 5 mg frass/ml H_2O, 10 mg frass/ml H_2O, and 100 mg frass/ml H_2O.

Experiment V. In order to analyze a possible influence of larval density on the activity of frass, we tested frass produced by larvae of the fifth and sixth instar kept at different densities. The following densities were calculated from the number of larvae per square cm area, where they could move: 0.002 larvae/cm^2 (one single larva per 500 cm^2); 0.01 larvae/cm^2 (a small group of five larvae per 500 cm^2, moderate density); 0.07 larvae/cm^2 (35 larvae per 500 cm^2, overcrowded). All larvae were fed with cotton leaves in surplus.

Experiment VI. In order to get information on the solubility of the oviposition deterrent, we tried to extract it with different solvents. Extracts were prepared as follows: 1.1 g frass was added to 80 ml *n*-pentane, stirred for 12 hr at room temperature, and then filtered to obtain an *n*-pentane extract. The vacuum-dried residue was added to 80 ml chloroform, stirred for 12 hr, and newly filtered to obtain a chloroform extract. The extraction was continued in this way with acetone, ethanol, and water. The undersurface of each test leaf was treated with 1 ml of the various extracts using a soft brush. Each control leaf was treated with 1 ml of the respective solvent.

At the end of a bioassay, the number of egg masses on each leaf was counted. The number of replicates for the experiments was 23 with the exception of 20 in experiment VI. Statistical significance was tested by using the Wilcoxon test for paired differences.

RESULTS

The results of this study are compiled in Table 1.

Experiment I. Both larval frass stored for 6 days and for 395 days significantly deterred oviposition. Thus the oviposition deterrent was very stable when held in dark, airtight, and cold (−10°C) storage.

Experiment II. Larval frass distributed on cotton leaves deterred oviposition for two days. On the third day, cotton leaves treated with larval frass completely lost their oviposition-deterring activity.

Experiment III. The oviposition deterrent was perceived with the antennae. Females without antennae were unable to differentiate between control leaves and leaves treated with larval frass. This was in obvious contrast to females with antennae.

Experiment IV. Frass at 5 mg per leaf did not significantly reduce oviposition, but a small effect was seen with 10 mg frass per leaf. On leaves treated with 10 mg frass, significantly fewer egg masses were deposited than on control leaves. The strongest oviposition deterrence was caused by 100 mg frass per leaf. The threshold value for statistical significance in oviposition deterrence was in the range of 5–10 mg frass per leaf.

Experiment V. The oviposition-deterring effect of larval frass was independent of larval density. Frass of larvae feeding singly deterred oviposition as well as frass of larvae reared in moderate or high density.

Experiment VI. The oviposition deterrent was extractable from larval frass with acetone. The number of egg masses on cotton leaves treated with an acetone extract of frass was significantly lower than the number of egg masses on

TABLE 1. RESPONSE OF GRAVID *Spodoptera littoralis* FEMALES TO LARVAL FRASS AT DIFFERENT TEST CONDITIONS

Experiment	Test parameter	Total egg masses (%)		Significance[a]	Total No. egg masses (*N*)
		Test leaves	Control leaves		
I	Storage period				
	6 days[b]	25.0	75.0	*	40
	395 days	22.2	77.8	**	36
II	Persistence				
	1 day[b]	25.0	75.0	*	40
	2 days	25.7	74.3	*	35
	3 days	48.6	51.4	NS	37
III	Perception				
	♀ ♀ with antennae	29.7	70.3	*	37
	♀ ♀ without antennae	49.1	50.9	NS	55
IV	Amount of Frass				
	5 mg/leaf	34.3	65.7	NS	35
	10 mg/leaf	30.4	69.6	**	46
	100 mg/leaf[b]	25.0	75.0	*	40
V	Larval Density				
	0.002 larvae/cm^2 (a single larva)	29.4	70.6	*	34
	0.01 larvae/cm^2 (a small group of larvae)[b]	25.0	75.0	*	40
	0.07 larvae/cm^2 (overcrowded)	31.1	68.9	*	61
VI	Solubility				
	Pentane extract	35.7	64.3	NS	28
	Chloroform extract	55.2	44.8	NS	29
	Acetone extract	14.3	85.7	***	28
	Ethanol extract	53.3	46.7	NS	30
	Water extract	46.7	53.3	NS	30

[a] Statistically significant difference between number of egg masses on test and control leaves: * ($0.05 \geq P \geq 0.01$), ** ($0.01 \geq P \geq 0.001$), *** ($P \leq 0.001$). NS, no significant difference.

[b] One single test listed several times for comparison. In this test we used larval frass stored under dark, airtight, and cold conditions for six days. Frass was obtained from larvae provided with cotton leaves and reared in small groups (0.01 larvae/cm^2). Frass, 100 mg, was applied to each test leaf and bioassayed during the first day after application.

control leaves. All other tested extracts did not cause a significantly provable oviposition deterrence.

DISCUSSION

The results of this study provided information on the oviposition deterrent in *S. littoralis* and the first hints of its chemical nature. Moreover, useful clues resulted for further studies of the chemistry of the deterrent.

The investigation of the stability of the deterrent during cold storage demonstrated that there is no necessity of using fresh frass in order to show its oviposition-deterring activity. During cold, dark, airtight storage the oviposition-deterrent was stabile for longer than one year. Therefore, in chemical studies of old frass, stored at the above described conditions, it can be certain that active oviposition-deterring substances are still present.

In contrast to the high stability of the oviposition deterrent during cold storage, its persistence was rather short when frass was applied to cotton leaves. Activity was lost after two days. Oviposition deterrents in other phytophagous insects showed a longer period of biological activity. The oviposition deterrents in larval frass of the corn borer, *Ostrinia nubilalis* Hb. (Dittrick et al., 1983), and the cabbage looper, *Trichoplusia ni* (Hb.) (Renwick and Radke, 1980), remained active for at least three days. The period during which the oviposition-deterring pheromone of *Rhagoletis cerasi* L. retained its activity was at least 12 days (Katsoyannos, 1975). The oviposition-deterring pheromone produced by females of *Pieris brassicae* L. was still active after it had been dried for seven weeks at room conditions in a desiccator (Schoonhoven et al., 1981).

In *S. littoralis* the development of all individuals of a population is not completely simultaneous. Therefore, it is possible that emergence of females coincides with the occurrence of feeding larvae. There should be no necessity for a long-lived oviposition deterrent, because feeding larvae continously excrete fresh frass and, thereby, further oviposition-deterring substances. The loss of activity of larval frass may be due to either evaporation or damage by oxygen or light.

Perception of the oviposition deterrent by the antennae does not provide evidence for olfactory perception. Gravid females often could be observed touching the leaves with their antennae. Therefore, perception by chemotactile sensilla should be considered. Helal and Abdel Gawaad (1984) investigated the antennae of *S. littoralis* males and females by means of scanning electron microscopy and found seven different types of sensilla. Electrophysiological

experiments are necessary in order to determine the sensilla responding to the oviposition deterrent in *S. littoralis*.

To our knowledge there is no other study that addresses sensory perception of oviposition deterrents when the deterrent is in larval frass. The oviposition-deterring pheromone deposited by females of *Rhagoletis pomonella* Walsh is principally perceived by sensilla located on the tarsi (Crnjar et al., 1978; Prokopy and Spatcher, 1977). In addition to tarsal and probably abdominal contact chemoreceptors, in females of *Pieris brassicae* L. also olfactory sensilla located on the antennae show electrophysiological responses to the inherent oviposition-deterring pheromone of the eggs (Behan and Schoonhoven, 1978; Klijnstra and Roessingh, 1986).

Females of *S. littoralis* need to perceive a minimum amount of larval frass before decreasing egg deposition. The threshold value for the quantity of frass causing a significant deterrence of egg laying was in the range of 5–10 mg frass per cotton leaf. Two days after moulting, a sixth-instar larva feeding on cotton leaves produces about 100 mg frass per day. About 10% of this daily frass production of a late-instar larva on a cotton leaf obviously indicated such a high feeding activity that a gravid female "decides" her offspring will not be sufficiently provided for at this site and therefore seeks another, more suitable, oviposition site. The result of this laboratory bioassay needs to be checked in the field in order to get information on ecological consequences of oviposition deterrence in *S. littoralis*. Field observations by Campion et al. (1977) revealed that *S. littoralis* emigrated from areas with high population densities. Possibly *S. littoralis* females respond to oviposition deterrents by emigration, in order to look for a place where the offspring will find suitable developmental conditions.

We previously hypothesized that frass activity is dependent on larval density. This hypothesis is based on the following background. Several studies demonstrated that an increase of larval density is correlated with numerous changes, e.g., larval color changes, activity of larvae increases, and fat and water content of the resulting pupae are different (e.g., Hodjat 1970; Rivnay and Meisner, 1965; Zaher and Moussa, 1961). These results indicate metabolic changes at higher larval density. Such metabolic changes may be correlated with changes of frass compounds. Possibly gravid females only avoid egg deposition in response to such changed frass compounds. These changed frass compounds would then indicate high larval density and, thus, unsuitable oviposition sites. Examination of this hypothesis revealed that the oviposition-deterring activity of frass was independent of larval density. The oviposition-deterring substances were obviously not subjected to metabolic changes when larval density increased.

The acetone solubility of the oviposition deterrent in frass of *S. littoralis* larvae indicates a moderate polar character of the deterring substances. The oviposition deterrent in larval frass of the European corn borer, *Ostrinia nubilalis* Hb., showed a similar solubility: methanol and acetone extracts of frass proved effective in reducing oviposition by 90% (Dittrick et al., 1983). A water extract of frass showed no activity in *S. littoralis*.

In contrast, the oviposition deterrent in larval frass of *Spodoptera exigua* L. and *Spodoptera eridania* (Cramer) could be extracted both with water and organic solvents like ethanol and dichloromethane (Mitchell and Heath, 1985). Oviposition in *Spodoptera frugiperda* (J.E. Smith) was also deterred by aqueous extracts of larval frass (Williams et al., 1986). The results of our study suggest that the oviposition-deterring substances of *S. littoralis* will be chemically different from deterrents of other *Spodoptera* species. To date, the only identified oviposition deterrent is that released by females of *Rhagoletis cerasi* L.: Hurter et al. (1987) characterized this pheromone as *N*[15(β-glucopyranosyl)oxy-8-hydroxypalmitoyl]taurine.

Oviposition by *S. exigua*, *S. eridania*, and *S. frugiperda* was also deterred by extracts of damaged host-plant material (Mitchell and Heath, 1985; Williams et al., 1986). Furthermore, deposition of eggs by the noctuid moth *Trichoplusia ni* (Hb.) was reduced not only by larval frass, but also by damaged leaves of the host plants (Renwick and Radke, 1981). These results indicate that oviposition-deterring substances in the larval frass of these species are undigested, allelochemical substances of the host plants. In *S. littoralis*, a suspension of macerated cotton leaves in water (100 mg/ml) did not deter oviposition (Hilker, 1985). This result does not prove that the oviposition deterrent in *S. littoralis* is a pheromone produced by the larvae themselves. In a suspension of macerated cotton leaves, oviposition-attracting substances might compete with oviposition-deterring compounds that are possibly set free by damaging the leaves. In larval frass, the oviposition-attracting substances might be digested so far that undigested oviposition-deterring plant substances would display their activity.

In our current work we hope to determine whether the oviposition deterrent in the larval frass of *S. littoralis* is a pheromone or an allelochemical plant substance.

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