The First Int. Egyptian - Romanian Conf., Zagazig, Egypt, Dec., 6 - 8'\*, 2003

OVIPOSITION DETERRENT IN LARVAL FRASS OF *SPODOPTERA LITTORALIS* (BOISD.)

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ABSTRACT: The aim of the present work is to investigate the relationship between oviposition *of Spodoptera littoralis* (Boisd.) and larval frass extracts as well as to assess the electrophysiological effect on tarsus sensilla. The obtained data revealed that oviposition was significantly deterred by frass extract taken from larvae reared at high densities. However with low densities (small groups larvae) the inhibition effect of larval frass was not significant. On the other hand, the minimum concentration of frass extract (with which the leaves were sprayed) to cause significant oviposition deterrence was ranged from 5-10 % in case of *Neriunt oleander* leaf. A petroleum ether extract of larval frass was highly deterrent, as compared with water, ethanol or acetone extracts. Moreover, contact ; chemoreceptors on tarsus (sensilla chaeticum) plays an important . role to find out a suitable place of mated femles moths for egg laying.

Key words - *Spodoptera littoralis,* oviposition behaviour, oviposition

deterrence, larval frass , contact chemoreceptors, sensilla chaeticum.

INTRODUCTION (White and Chapman, 1990). Most

insect contact chemoreceptors and

The relationship between contact many olfactory sensilla contain

chemoreceptors and landing of more than one sensory neuron

female moths in the field to find (Zacharuk, 1980). On the ovipositor

out a suitable place for egg laying (Kalogianni, 1995 and 1996), contact

is very important to discover a chemoreceptors assist with identification

good place for the progeny to live, of suitable oviposition sites (Ma and

Chemoreception plays an important Schoonhoven, 1973). The antennae,

role in mediating a diverse range of which often point forward to

behaviours, including avoidance encounter sensory stimuli first and

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are endowed with many distance chemoreceptors, some contact chemoreceptors and many mechanoreceptors. The legs, particularly the tarsi that are in contact with the substrate (Gaaboub, 1990 and 2000), also carry many chemoreceptors (Gaaboub and Hustert, 1998). In butterflies *Pieris brassica* stimulation of the tarsi by sugar solutions evokes an automatic extension of the proboscis (Ma and Schoonhoven, 1973.). In several species of Lepidoptera, feeding larvae and larval frass indicate occupancy of the host plant and deter egg deposition (Dittrick *et a/.,* 1983; Mitchell and Heath, 1985; Renwick and Radke, 1980 and 1981, Rothschild and Schoonhoven, 1977 and Williams *el al.,* 1986). Oviposition is also deterred by larval frass in the Medierranean noctuid moth *Spodoptera littoidis* (Boisd.) (Hilker, 1985; Hilker and Klein, 1989). There is a lake of knowledge for biological and chemical properties of this oviposition deterrent in S. *littoralis.* One of the hypotheses is 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

(Hodjat, 1970; Rivnay and Meisner, 1965; Zaher and Moussa, 1961; Gaaboub, 1990 and Hilker and Klein, 1989). Metabolic changes might cause a change of frass compounds. These changes in frass of larvae, which were reared at high densities, might be a signal to gravid females indicates that the site is unsuitable for oviposition. The classification of deterrent substances is based on the elicited behaviour or lack of behaviour in each insect. A compound or a combination of compounds, which deter oviposition in one situation, may elicit a different type of behaviour in another situation. An example was found in oviposition experiments with *Ephestia kuehnella* and *Plodia inteipunctella.* Larvae of these species emit a secretion oviposition behaviour of females at the site (Corbet, 1973).

It is worthy to note that oviposition by females of *E. kuehniella* was strongly deterred by specific amount of secretion from conspecific larvae, while oviposition by females of *P. interpunctella* was stimulated by the same amount secretion. Thus, the females of the iwo species showed an oppsite behavioural response to the same stimulus. Deterrents affecting oviposition choice in moths have been found

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in damaged host plants (Rothschild and Schoonhoven 1977, Turlings *et al.* 1991). Derterring compounds produced by conspecific insects are called oviposition deterring pheromones (ODPs). Evidence for ODPs has been found in about 50 different insect species from four orders (Papaj, 1994). Three different sources of ODPs have been detected. They can either be associated with the eggs, or be deposited by the larvae or by female. In moths, ODPs associated with eggs and larvae have been identified (Prokopy 1981). Therefore investigation whether the deterrent activity of larval frass is dependent on larval density or not is very important. The persistence of the deterring activity of larval frass on the host plant, and its solubility, the rninimurn amount of frass that is necessary for significant oviposition deterrence and the relation between frass and contact chemoreceptor on tarsus were studied.

MATERIALS AND METHODS

Insects: for oviposition bioassays, moths of *S. littoralis* were obtained from the laboratory of department of Entomology, Faculty of Agriculture Moshtohor, kept in special cages for mating and

deposition. Larvae of the different instars were reared on castor bean leaves. Pupae were collected and kept in wooden box till moth emergence. Three to four day old moths were used in the present experiments. During the fifth and sixth larval instar, frass was collected daily. These larvae produced a sufficient amount of frass. The daily fresh weights of frass were found to be about 20-50 and 50-350 mg during the fifth and sixth larval instars respectively, whereas third and fourth instar larvae produced only 2-5 mg frass per day (Hilker and Klein, 1989).

Bioassay test: bioassays were conducted in the screened cages (50X50X50 cm) situated in a chamber with constant temperature (27 ± 1 °C) and 14: 10 hr lightdark cycle, relatively similar to the Egyptian summer. Each bioassay began with the onset of the light period and lasted for 24 hr. Three females and five males were placed in each cage. For oviposition, moths were offered two treated and two untreated *Nerium oleander* branches. Each branch ended with two leaves was fixed in 100-ml vial filled with water for continuous freshing of leaves and situated in the corner of the cage.

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Except the bioassay tests of the solubility of the deterrent, leaves were treated with a water suspension of frass in which the concentration of frass ranged from 5-10 %. This suspension was prepared in a Potter homogenizer and applied to the undersurface of *Nerhim oleander* with a brush because eggs are usually laid only on the undersurface of the leaf.

Frass extraction: Extraction of frass was carried out on 100 g of larvae frass, were successively extracted with petroleum ether 60:80, acetone, ethanol and water, for 48 hr. at room temperature. Each extract was evaporated separately under vacuum to complete dryness (Table 1).

Table (1) Extracted materials (%) from lOOg larval frass *{Spodoptera littoralis (Boisd.)}by* each solvent.

|  |  |
| --- | --- |
| Solvents used | Crude extraction/ lOOg Frass |
| Petroleum ether | 2.44 |
| Acetone | 6.139 |
| Ethanol | 1.827 |
| Water | 5.71 |

Electrophysiology experiments:

Responses from individual sensilla (sensilla chaeticum.) to chemical stimuli on the ventral side of the tarsus of leg were recorded using the tip recording technique (Hodgson *et al.* 1955). The potentials were amplified and filtered using AC amplifiers. A blunt glass microelectrode filled with different solutions was placed over the shaft of the sensillum. . Electrodes containing salt (0.1 M of NaCl mixed with the extracts at

the concentration of either 5 or 10 % extract petroleum ether, acetone, ethanol and water), were used to stimulate the chemosensory afferents. Controlled movements of this electrode were used to deflect the sensillum so as to elicit spikes in the mechanosensory afferents. The same electrode was therefore used simultaneously to evoke and record the spikes of the afferents. The displacement of a sensillum did ot deform its short and stout shaft.

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RESULTS AND DISCUSSION

As shown in Tables (2 and 3) the

number of egg masses on the

*Nerium oleander* leaves treated

with petroleum ether extract of *S.*

*littoralis* larval frass was significantly

lower than the number of egg

masses on control leaves. The

same solvent alone did not give the

same result. Other tested extracts

did not cause a significant provable oviposition deterrence (Figures 1 and 2). The ability of petroleum ether to solubilize the oviposition deterrent substances in the frass of *S. littoralis* larvae indicates a moderate polar character of the deterring substances The oviposition deterrent in larval frass on the European corn borer, *Ostrinia nubilalis* Hb., showed a similar solubility, acetone and ethanol extracts of frass proved effective in reducing oviposition by 90% (Dittrick *et ai,* 1983). Water extract of frass showed a -deterrent in *S. littoralis.* (Fig.l). In

contrast, the oviposition deterrent

compounds in larval frass of *Spodoptera exigua* L. and *Spodoptera*

*eridania* (Cramer) could be extracted 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 obtained reveal that the oviposition-deterring substances of *S. littoralis* were chemically different from deterrents of other *Spodoptera* species.

According to Hurter *et al.* (1987)  
the identified oviposition deterrent  
materials which released by  
females of *Rhagoletis cerasi* L.  
were characterized as a pheromone,  
having the following chemical  
formulation:N[5(B-glucopyranosyl)  
oxy-8-hydroxvpalmitoyl]taurine. 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  
*Pieres brassicae* L. was still active  
after it had been dried for seven  
weeks at room conditions in a  
desiccator (Schoonhoven *et al.,*1981). ,

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Table (2) Analysis of variance for egg masses, egg numbers and egg hatching as affected by extraction materials and their concentrations.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| s.o.v. | df. | No. of Mass | No. of  Eggs .; | ; No, of Hatching |
| Rep. | 4 | 0.095 | 2.074 | 1.93 |
| Material (M) | 3 | 0.347\* | 2.18\*\* | 2.124\*\* |
| Cone. (C) | 2 | 0.475\* | 5.7\*\* | 0.601\*\* |
| MX C | 6 | 0.025\* | 2.07\*\* | 2.125\*\* |
| Error | 44 | 0.119 | .725 | 0.362 |

\* and \*\* denote significance at 0.05 and 0.01 probability levels,  
respectively. , -

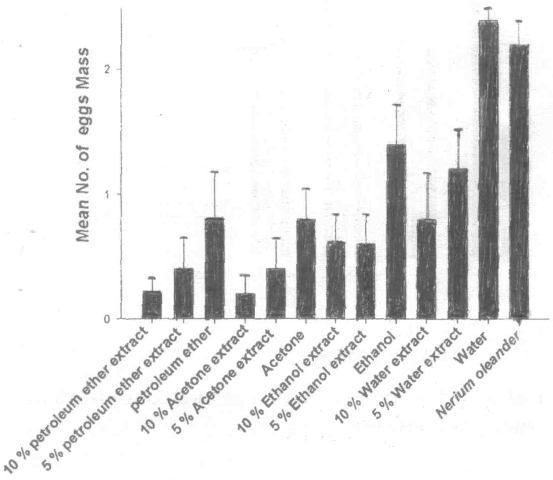
Table (3) Means of egg masses, egg numbers and egg hatching as affected by extraction materials and their concentrations.

|  |  |  |  |
| --- | --- | --- | --- |
|  | No. of Egg Masses | Egg Number | Egg Hatching |
| Material | |  | |
| Petroleum ether | 0.466 | 110 | 97.57  • \*•<\*-" . , ' " » |
| Acetone | 0.48 | 148.6 | 122.33 |
| Ethanol | 0.86 | 201.33 | 172.33 |
| Water | 1.46 | 330 | 306.93 |
| Concentration | | | |
| 10% | 0.45 | 128.5 | 100.93 |
| 5% | 0.65 | 122.5 | 105.7 |
| 0 | 1.35 | 341.5 | 318.9 |

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Fig. (1) Effect of larval frass extracts type and concentration on the mean number of the eggs mass.



*%* cone.

Oviposition by £ *exigna, S. eridania,* 5. *frugiperda* was also deterred by extracts from the damaged host-plant material (Mtchell and Heath, 1985; williams *et aL,* 1986). Furthermore, deposition of eggs by the noctirid moth *Tnchoplusia 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 were produced from the host plants In *S. liftoralis,* a suspension of macerated *Nerii/m oleander* in water (100 mg/ml) did not deter oviposition. In a suspension of macerated *Neriiim oleander,* oviposition-attracting substances might compete with oviposition-

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Fig (2) Effect of larval frass extracts type and concentration on the % of hatching *ofSpodoptera littoralis.*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| 80 | :' |  |  |  |  |
| i s  i | |  |  |  |  |
| 60 | \* |  |  |  |  |
|  | ! |  |  |  |  |
| 40 | : |  |  |  |  |
|  | i |  |  |  |  |
|  | j |  |  |  | i^ 10% Cone. i^i^j 5% Cone. |
| 20 - | 1 |  |  |  | ••• Control |
| n |  |  |  |  |  |

Fig (3) Effect of larval frass extracts type and concentration on the mean number of eggs.

10% Cone. 5% Cone. Control

U 2000

•5

1500

liil

Type of extract

*J* \*

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deterring compounds that are possibly set free by damaging the leaves. In larval frass, the oviposition-attracting substances might be digested so that undigested oviposition-deterring plant substances would display their activity.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 substance. The stability of the deterrent during cold storage showed 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, stores at the above-described conditions, it can be certain that active oviposition-deterring substances are still present (Hilker, 1985). Spraying frass suspension at concentration of 5 % did not significantly reduce oviposition, but the strongest oviposition deterrence was caused by 10 % frass suspension. The minimum

percentage of frass in waterfrass  
suspension for statistical significance  
in oviposition deterrence was in the  
range of 5-10 % frass. Two days  
after moulting sixth-instar larva  
fed on castor leaves produced  
about lOOmg frass per day. About  
10% of this daily frass production  
of a late-instar larva was found to  
be sufficient to repellent the gravid  
females to another site more suitable  
for oviposition. This laboratory  
results need more cofirmation as in  
the field oviposition deterrence. Field  
observation by (Campion *et* a/.,  
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.  
As previously hypothesized that  
frass activity is dependent on  
larval density. This hypothesis is  
based on several studies, which are  
demonstrated that an increase in  
the 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., Zaher and Moussa,  
1961; Rivnay and Meisner, 1965;

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Hodjat 1970). The 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 opposition sites. Examination of this hypothesis revealed that the oviposition-deterring activity of frass was dependent on larval density.

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 and Gaaboub, 1990)  
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. The  
oviposition-deterring pheromone

deposited by females *ofRhagoletis pomonella* (Walsh) is principally perceived by sensilla located on the tarsi (Prokopy and Spatcher, 1977; Crnjar *et al.,* 1978). 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). Electrophysiological recordings were carried out to study the afferent responses to different concentrations of frass (5 and 10%) extracted with petroleum ether, acetone, ethanol and water extracts on the electrical activity of tarsus sensilla (sensilla chetica). The investigation showed that the sensilla were sensitive to The investigation showed that the sensilla were sensitive to all mentioned extracts (stimuli). The results indicated that both the frequency and the amplitude of afferents from sensilla differed according to the type of chemical and its concentration (Fig. 4). High concentrations of the stimulation were more effective than low concentrations.

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Fig. (4) Recording from a tarsus sensilla (sensilla chaeticum) to (A) 0.1M NaCl, (B) NaCl 0.1 M mixed with 10 % of petroleum ether extract and (C) NaCl 0.1 M mixed with 10 % of acetone extract were used to stimulate the chemosensory afferents.

A

|Nt\|^4^



B

*j\*\*t\*J>\*\*\*,mVfi<#Jfy»H*

1000ms

Two different response types occurred, in most cases the chemical sensitive neurone began to fire immediately upon stimulation, followed by a period of decreasing frequency as adaptation occurred. Some neurones, however, showed an initial latency of around 100ms,

followed by a period of increasing frequency. Both types were due to the activity of a single neurone in each sensillum, and in both cases, after a suitable recovery time (10 min.), it was possible to record another responses (White and Chapman, 1990, Gaaboub, 2000)

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ACKNOWLEDGMENTS

As this study was supported by the Dept. of Plant Protection, Faculty of Agriculture Moshtohor, we express appreciation to all members at our Dept. for kind help.

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