

Oviposition Deterrent In Larval Frass of *Spodoptera littoralis* (Boisd.)

Ibrahim Gaaboub and Safaa Halawaa

Dept. of Plant Protection, Fac. of Agric. Moshtohor, Zagazig Univ.

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 *Nerium 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 females moths for egg laying.

Key words – *Spodoptera littoralis*, oviposition behaviour, oviposition deterrence, larval frass, contact chemoreceptors, sensilla chaeticum.

Introduction

The relationship between contact chemoreceptors and landing of female moths in the field to find out a suitable place for egg laying is very important to discover a good place for the progeny to live. Chemoreception plays an important role in mediating a diverse range of behaviours, including avoidance (White and Chapman, 1990). Most insect contact chemoreceptors and many olfactory sensilla contain more than one sensory neuron

(Zacharuk, 1980). On the ovipositor (Kalogianni, 1995 and 1996), contact chemoreceptors assist with identification of suitable oviposition sites (Ma and Schoonhoven, 1973). The antennae, which often point forward to encounter sensory stimuli first and 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 al.*, 1983; Mitchell and Heath, 1985; Renwick and Radke, 1980 and 1981, Rothschild and Schoonhoven, 1977 and Williams *et al.*, 1986). Oviposition is also deterred by larval frass in the Mediterranean noctuid moth *Spodoptera littoralis* (Boisd.) (Hilker, 1985; Hilker and Klein, 1989). There is a lack 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-detering 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 kuehniella* and *Plodia interpunctella*. 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 two species showed an opposite behavioural response to the same stimulus. Deterrents affecting oviposition choice in moths have been found in damaged host plants (Rothschild and Schoonhoven 1977, Turlings *et al.* 1991). Detering compounds produced by conspecific insects are called oviposition detering 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 minimum 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 light:dark cycle, relatively similar to the Egyption summr. 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. 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 *Nerium 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) Percentage of each extract of different solvent based on 100/g larval frass (*Spodoptera littoralis* (Boisd.)).

Solvents used	Crude extraction/ 100g 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

not deform its short and stout shaft.

Results and discussion

As shown in Tables (2,3) 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 al.*, 1983). Water extract of frass showed a -deterrent in *S. littoralis*. (Fig.1). 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-detering 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-hydroxypalmitoyl]taurine. The period during which the oviposition-detering pheromone of *Rhagoletis cerasi* L. retained its activity was at least 12 days (Katsoyannos, 1975). The oviposition-detering 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).

possibly set free by damaging the leaves. In larval frass, the oviposition-attracting substances might be digested so that undigested oviposition-deterrent 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-deterrent activity. During cold, dark, airtight storage the oviposition-deterrent was stable 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-deterrent substances are still present (Hilker, 1985).

Table (2) Mean squares of egg masses, egg numbers and egg hatching affected by material, concentration and their interactions.

S.O.V.	df.	Nr. of Mass	Nr. of Eggs	Nr. Of Hatching
Rep.	4	0.095	2.074	1.93
Material (M)	3	0.347*	2.18**	2.124**
Conc. (C)	2	0.475*	5.7**	0.601**
M X C	6	0.025*	2.07**	2.125**
Error	44	0.119	.725	0.362

Table (3) Mean of egg masses, egg numbers and egg hatching affected by material and concentration

	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

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

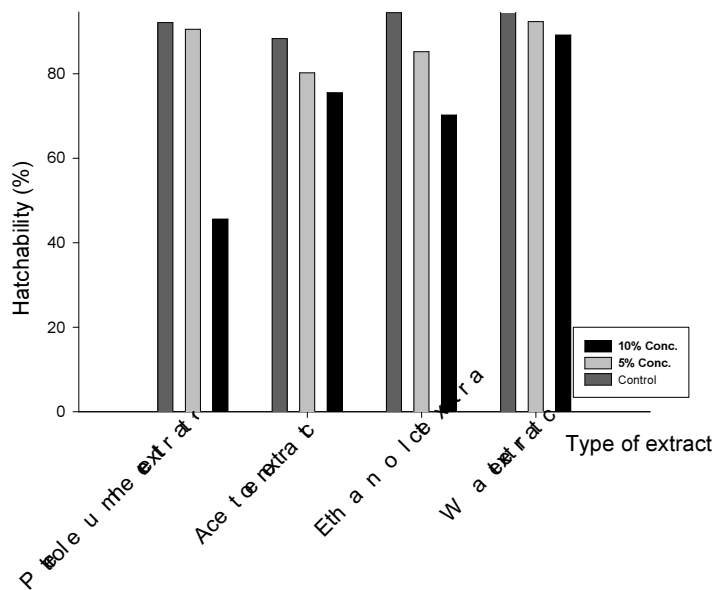
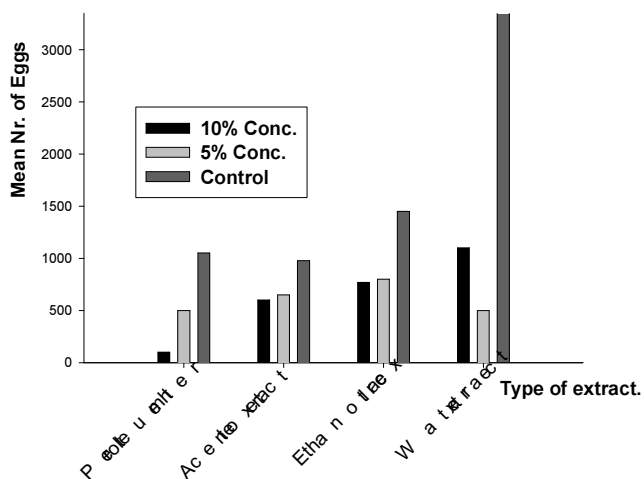


Fig (3) Effect of larval frass extract type and concentration on the mean number of eggs.



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 water:frass 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 100mg 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 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.

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

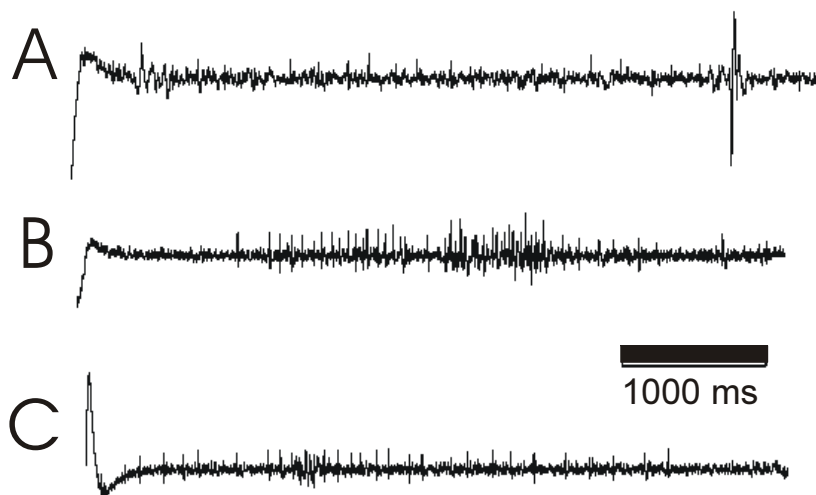
the larval density is correlated with numerous changes, e.g., larval color changes, activity of larvae increases, and fat and water content of the resulting pupae are different (e.g., Zaher and Moussa, 1961; Rivnay and Meisner, 1965; Hodjat 1970). The results indicate metabolic changes at higher larval 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 oviposition sites. Examination of this hypothesis revealed that the oviposition-detering 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-deterrent pheromone deposited by females of *Rhagoletis 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-deterrent pheromone of the eggs (Behan and Schoonhoven, 1978; Klijnsma 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 chaetica). 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.

Fig. (4) Recording from a tarsus sensilla (sensilla chaetica) 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.



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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التأثير المانع للبيض في براز يرقات دودة ورق القطن المصرية

إبراهيم عبد الله جعوب و صفاء محمود حلاوة

قسم وقاية النبات، كلية الزراعة، بمشتهر، جامعة الزقازيق.

الملخص العربي

يهدف هذا البحث إلى دراسة تأثير معاملة أوراق التفلة بمستخلص براز يرقات دودة ورق القطن ووضع فراشات هذه الآفة للبيض حيث تم قياس التأثير الألفروفسولوجي على شعيرات الرسغ. أثبتت النتائج أن المعاملة بالمستخلص الناتج من براز تجمعات كبيرة من اليرقات سبب تأثيراً معنوياً في الانخفاض الذي حدث في وضع البيض بينما المعاملة بالمستخلص الذي أخذ من تجمعات محددة الكثافة فكان غير معنوياً. على الجانب الآخر فإن أقل تركيز من مستخلص البراز الذي عوملت به أوراق التفلة سبب تأثيراً فعالاً في منع وضع البيض تراوح بين 5-10%. وقد كان مستخلص الأثير البترولي هو الأعلى فاعلية عند مقارنته بمستخلصات الماء، الإيثانول والأسيتون. بالإضافة إلى ذلك ثبت أن المستقبلات الكيماوية على

الرسغ تلعب دورا هاما في قدرة إناث فراشات دودة ورق القطن على تحديد المكان المناسب
لوضع البيض.