Substrate Factors Involved in Oviposition Response of
the Limabean Pod Borer, *Etiella zinckenella*
TREITSHKE (Lepidoptera: Pyralidae)

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Factors involved in the oviposition response of the limabean pod borer, *Etiella zinckenella* TREITSHKE were examined, using soybean plants as the substrate under a net cage (30 × 30 × 40 cm) condition. Gravid females preferred to oviposit among hairs on the plant surface for a pubescent variety, while they oviposited preferentially under the sepals and stipels for a glabrous one. When the pod was taped with a wax paper band, most eggs were laid crowded together under it, particularly in the pod banded at the proximal part. In a pod taped with double papers, the number of eggs laid between the two papers was almost the same as those between the lower paper and the pod surface. Ovipositional preferences to the fresh, dried, and water-sucking (dried) pod were compared in both free-choice and no-choice situations. In both tests, the eggs deposited on the dried pod were much fewer than those on the other two pods. A few more eggs were found on the fresh pod than on the water-sucking one. These results suggest that the physical texture and moisture of the substrate act strongly on the oviposition response of *E. zinckenella*, and odor influences only weakly. Contact chemostimuli seem not to be contributive, at least, in the final step of oviposition behavior.

INTRODUCTION

The limabean pod borer, *Etiella zinckenella* TREITSHKE, is widely distributed in the world and is one of the most serious pests of soybean in the southwestern half of Japan. Although this species has been reported from a large variety of leguminous plants (ABUL-NASR and AWADALA, 1957; NAITO, 1961), HIRAI et al. (1980) observed that certain species of the leguminous genus *Vicia* were not visited or oviposited by the insect in the fields. In a preliminary experiment in the laboratory, a certain surface of the soybean host plant elicited much oviposition by gravid females, in contrast to artificial surfaces such as a paper towel, filter paper or cheesecloth on which little oviposition was observed. This suggests that various factors of the host plant influence the oviposition response of *E. zinckenella*. The substrate factors of soybean concerned with the ovipositional response of the insect were then explored. These studies should be useful in developing control measures and a device for egg collection in mass rearing which have been not yet established, as pointed out by HATTORI and SATO (1980).

MATERIALS AND METHODS

Insects. Larvae were obtained from soybean pods in the fields in August, 1980,

and reared to adult in the laboratory under 16L 8D (16 hr light, 8 hr dark) photoperiod at $25 \pm 2^\circ\text{C}$ and 50–60% RH.

General method for testing oviposition. Five to ten pairs of mated moths 4 to 7 days old were confined in net cages ($30 \times 30 \times 40$ cm) for oviposition tests. A cup of 5% sucrose solution was provided as food. Tests were always initiated after the first oviposition on fresh soybean pods. All pods used were at the middle boring stage. The substrate for oviposition was put in the cage just prior to light-off, and eggs laid on it were counted the next morning.

Comparison of distribution of eggs laid between pubescent and glabrous varieties. Soybean twigs of a pubescent variety (Tohoku 61) and a glabrous variety (Wasehadaka) were separately introduced into the net cage containing moths. The site of oviposition and the number of eggs laid on each variety were recorded the next morning.

Comparison of oviposition responses in relation to the size and position of artificial sepals. A wax paper band, 1 cm $\times$ 2 cm or 2 cm $\times$ 2 cm, was bound around the distal, middle or proximal position of soybean pods with adhesive tape. Each treated pod was hung by a wire clip from a wooden stick (25 cm long) which was placed upright in a 200 ml Erlenmeyer flask. The number of eggs laid under these bands in a free-choice experiment was compared.

Oviposition responses to artificial double sepal. Artificial double sepals were tested to determine whether or not the contact chemostimuli of the fresh pod were involved in the final step of oviposition. These sepals consisted of upper and lower paper bands (1 cm- and 2 cm-wide, respectively). The lower band was exposed about 1 cm beyond the upper one (Fig. 2). Thus, two slits were provided for oviposition sites, namely, one between the upper and lower paper bands, and the other between the lower band and the pod surface. Two pods taped singly with either 1 cm- or 2 cm- wide paper band were also used as controls.

Comparison of the oviposition responses among fresh, dried and water-sucking soybean pods. Fresh pods were taken from the plant just prior to the start of the test. Dried pods were prepared by immersing the fresh pods in absolute ethanol for two weeks and drying them for a month. Water-sucking pods were prepared by soaking the dried pods in water for more than 7 hr and removing excessive water with a piece of filter paper before the tests. The three kinds of pods tested were compared both in free-choice and no-choice experiments. In the no-choice situation, two kinds of pods were alternately changed every day.

RESULTS

Difference in the distribution of eggs laid between pubescent and glabrous varieties

In the pubescent variety, eggs were laid on the hairy surface of the pod, petiol, leaf and stem as well as under the sepal and stipel. In the glabrous variety, on the other hand, about 85% of the eggs were found under the sepal and stipel, and no egg was on the pod or petiol surface (Fig. 1).

*Effects of the size and position of the wax paper band bound around the soybean pod on the oviposition of *E. zinckenella**

The number of eggs laid under 1 cm- and 2 cm-wide paper bands bound around the pod at the distal, middle or proximal position is summarized in Table 1. Significantly

Fig. 1. Distribution of eggs of *E. zinckenella* deposited on soybean twigs of pubescent and glabrous varieties. Each variety was tested separately. : Pubescent variety (Tohoku 61), : Glabrous variety (Wasehadaka).

Table 1. Effects of size and position of wax paper band bound around soybean pod on oviposition of *E. zinckenella*

Soybean pod taped with wax paper band			Mean number of eggs laid under band ^a	
Position banded	Size of band	Position within band		
Proximal part	1 cm	—	—	20.0±5.3 a
	2 cm	—	—	13.3±2.6 ab
Middle part	1 cm	Proximal side	4.2±0.9 a	8.7±1.4 bc
		Distal side	4.5±0.6 a	
	2 cm	Proximal side	3.6±1.0 a	9.6±1.9 bc
		Distal side	5.9±1.3 a	
Distal part	1 cm	—	—	11.2±3.7 abc
	2 cm	—	—	1.9±0.6 d

^a Replicated 14 times. Values (mean±SE) followed by the same letter in a column are not significantly different at 5% level by the Duncan's multiple range test.

Table 2. Oviposition of *E. zinckenella* on the two types of wax paper sepals

Type of artificial sepal		Oviposition site	Mean number of eggs laid between or under papers ^a
Single	1 cm-wide	under	16.3±4.0
Single	2 cm-wide	under	23.5±6.5
Double	1 cm-wide (upper)	between	12.5±2.9
	2 cm-wide (lower)	under	14.0±3.6

^a Relicated 6 times. Values are mean±SE. There are no significant differences between 1 cm- and 2 cm-sepals at the 5% level in either single or double layers.

more eggs were laid under the 1 cm-wide paper band at the proximal part than under the others. Both sides of the paper band at the middle part were equally oviposited.

Oviposition of E. zinckenella under artificial double sepals

The number of eggs laid under the single or double artificial sepals made of 1 cm- or 2 cm-wide paper bands is given in Table 2. In the single sepal type, there was no significant difference in the number between the two band widths. In the double sepal types likewise, females deposited an almost equal number of eggs in the slits between the 1 cm- and 2 cm-paper bands and between the 2 cm-paper band and the pod surface.

Difference in oviposition responses among fresh, dried and water-sucking pods

The results are shown in Table 3. The number of eggs laid were significantly greater on the fresh and water-sucking pods than on the dried ones in both free- and no-choice experiments. Although eggs were deposited much more on the fresh pod than on the water-sucking one in the free-choice situation, no significant difference was found in the no-choice experiment.

DISCUSSION

In many lepidopterous insects, the surface texture of the host plants influences their oviposition response, e.g. a pubescent surface is preferred by adults of the soybean pod borer, *Grapholitha glycinivorella* (UCHIDA and OKADA, 1937), the green cloverworm, *Plathypena scabra* (PEDIGO, 1971) and the tobacco hornworm, *Manduca sexta* (SPARKS,

Fig. 2. *E. zinckenella* ovipositing in between double paper sepals without contact of her ovipositor, antennae or legs with pod surface.

Table 3. Difference in oviposition responses of *E. zinckenella* among types of pods in free- and no-choice experiments

Oviposition substrate	Mean number of eggs ^a	
	Free-choice test	No-choice test
Fresh pod	28.2±0.4	39.7±8.6
Dried pod	3.9±2.2**	8.9±0.3**
Water-sucking pod	28.7±4.2	53.1±10.0
Dried pod	6.3±2.1**	15.8±5.0*
Fresh pod	28.4±2.5	36.6±5.4
Water-sucking pod	15.6±2.7*	25.5±5.3

^a Replicated 10 times except 30 times of comparison between fresh and water-sucking pods in free-choice test. Values are mean±SE.

* and ** indicate significant difference at 5% and 1% levels, respectively.

1973), and a dimpled surface is preferred by the diamondback moth, *Plutella maculipennis* (GUPTA and THORSTEINSON, 1960) and the potato tuber moth, *Phthorimaea operculella* (FENEMORE, 1978) for their oviposition. On the other hand, a smooth surface is preferred by the southern corn borer, *Diatraea brandiosella* (POSTON et al., 1979) and the codling moth, *Cydia pomonella* (HAGLEY et al., 1980). The difference in the number of eggs of the limabean pod borer between pubescent and glabrous varieties of soybean (Fig. 1) confirmed the result by TSUTSUI (1950) who also examined oviposition of this insect between these two varieties. Most eggs (about 85%) laid under the sepals and stipels suggest that the physical texture of the substrate is important in inducing ovipositional response of this species. This assumption seems to be supported by the results of experiments with artificial paper sepals in which almost all eggs were laid under the paper sepals. Some physical stimuli may play an important role in the determination of the oviposition site on the substrate. However, a certain positional preference of the moth also seems to contribute to some extent to this determination, since the pod banded with a single paper sepal at the proximal part was significantly more laid than the other treated pods (Table 1).

The results of the experiment on double artificial sepals (Table 2) indicate that the female can oviposit normally without touching the pod surface with her ovipositor tip at the final step of oviposition behavior. It is obvious that neither antennae nor legs contact the pod surface because the proximal part of the pod is covered with a paper band (Fig. 2). *E. zinckenella* seems, therefore, not to perceive contact chemostimuli of the plant just prior to oviposition, differing from the behavior of *Pieris brassicae* (DAVID and GARDINER, 1962), *Manduca sexta* (YAMAMOTO et al., 1969) and *Papilio protenor demetrius* (ICHINOSE and HONDA, 1978). However, that fact does not deny the possibility that these organs might perceive some chemostimuli from the substrate surface before its oviposition.

Odor of the host plant has been known to influence the oviposition response of *Manduca sexta* (YAMAMOTO et al., 1969; SPARKS, 1973), *Plutella maculipennis* (GUPTA and THORSTEINSON, 1969), the cabbage looper, *Trichoplusia ni* (SHOREY, 1964) and *Laspeyresia pomonella* (WEARING et al., 1973). That *E. zinckenella* deposited significantly more eggs on the fresh pod than on the water-sucking pod in the free-choice experiment might suggest some relations of odor from the fresh pods.

The results in which a greater number of eggs were laid on the water-sucking pod than on the dried one may suggest the important role of moisture of the substrate for oviposition, as already proved in many insects, e.g. *Trichoplusia ni* (SHOREY, 1964), *Manduca sexta* (SPARKS, 1973), *Papilio protenor demetrius* (ICHINOSE and HONDA, 1978) etc.

Further studies will be necessary to analyze the behavioral process in ovipositional sequence of this insect and to define the precise role of the various factors involved.

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