

Full Length Research Paper

The influence of trichome characters of soybean (*Glycine max* Merrill) on oviposition preference of soybean pod borer *Etiella zinckenella* Treitschke (Lepidoptera: Pyralidae) in Indonesia

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Study of oviposition behavior of *Etiella zinckenella* Treitschke on several Indonesian local cultivars of soybean, *Glycine max* (L.) Merrill was conducted with multiple choice and no choice tests. This study confirmed that sequence of eggs oviposition of *E. zinckenella* on tropical region does not differ with other results on non-tropical region. Total number of egg deposited and oviposition frequency of *E. zinckenella* do not correlate with trichome density, trichome length and pod surface when they do not have a choice of host plants. On the contrary, on the condition of many host plants to choose, *E. zinckenella* prefers to deposit eggs on the cultivar with many short trichomes. The preference test based on soybean phenology showed that the *E. zinckenella* prefers R4 stage (fully filled pod) for oviposition.

Key words: Oviposition, *Etiella zinckenella*, *Glycine max*, phenology, trichome.

INTRODUCTION

The ability of female insect to correctly choose the perfect spot to oviposit its eggs is an important factor to ensure the survival of offspring. Host selection of insect consists of series of behavior which is essentially a response to external stimuli, resulting in the selection or rejection of a particular plant. The morphological characters of a plant could act as physical stimuli for insect feeding and its oviposition behavior (Hattori, 1988; Bailey and Smith 1991; Shanower et al., 1996). Variation in leaf size, shape, color, plant tissue rigidity, as well as the presence of hair (trichome) and papilla could determine preference of insect likely to a plant (Metcalf and Luckmann, 1982).

Soybean is one of the major agricultural commodities consumed in Indonesia. This plant species is mainly attacked by *Etiella zinckenella* and *Helicoverpa armigera*,

but the former is considered as high priority pest of soybean in Indonesia as its larvae significantly destroy the seeds (Talekar and Lin, 1994). Report on *E. zinckenella* in Indonesia showed that this insect may attack 9% of total pods with total seed loss up to 12% per plant (van den Berg et al., 1998). In order to control the population of *E. zinckenella*, most farmers in Indonesia applied insecticide. However, it caused more problems as spraying does not affect the density of moth larvae or damage to pods and seeds. Rather, it lowers the population of parasitoid (van den Berg et al., 2000).

Referring to this condition, the resistant plant tactic has become the alternative methods to reduce the impact of *E. zinckenella* attack on soybean. This tactic is actually based on the characteristics of trichomes present on the stem, leaf, flower, and pod and they vary among different cultivars (Kogan and Herzog, 1980; PPPT, 1993). Trichome characteristics have been reported to correlate with damage level caused by pod borer (Talekar and Lin,

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1994) and oviposition preference (Suharsono and Suntono, 2004; Suharsono, 2006).

In this study, we tested the effect of different trichome characteristics of various soybean cultivars on the oviposition behavior and preference of *E. zinckenella*. Unlike earlier research work carried out by Suharsono and Suntono (2004) and Suharsono (2006) where only no choice test was used, we applied both no choice and multiple test in the present study. The logical explanation for this is that local farmer use the combination of soybean cultivars in their fields. Further, we also observed the oviposition preference of *E. zinckenella* on different phenological stages of soybean in order to test the hypothesis whether *E. zinckenella* has preferred phenological stage.

MATERIALS AND METHODS

Etiella zinckenella rearing

Larvae of *E. zinckenella* were collected from Sanggar Penelitian Latihan dan Pengembangan Pertanian Universitas Padjajaran Unit Arjasari. The collected *E. zinckenella* larvae were kept in cylinder container (70 ml) and fed with natural food (soybean soaked in water for several hours) at Laboratory of Entomology, School of Life Science and Technology, ITB. The larvae conditions were checked daily, the food were added or renewed if necessary. Rearing containers were kept in a room with the temperature of 27°C, relative humidity of 70 to 80% and a photoperiod of 12:12.

When the larvae were about to complete the fifth-instar stage, they were transferred into separate container filled with tissue papers. When larvae pupated, the pupae were shifted into fresh container until imago emerged. The emerging imagoes were fed with 10% sucrose solution and placed in a cage (40 × 40 × 60 cm). In the following days, adults mated.

Host plant nurture

During this study, we used the common seeds of local soybean cultivar (Sindoro, Dieng, Wilis, Tidar, Galunggung, Leuser, dan Guntur cultivars). The seeds were obtained from Balai Penelitian Tanaman Kacang-Kacangan dan Umbi-Umbian (Balitkabi), Malang. The seeds were sown in a polybag, which were filled with soil that was enriched with NPK fertilizer (3 g/polybag), and kept at greenhouse of School of Life Science and Technology, Institut Teknologi Bandung. The soybean plants were watered after every 2 days and kept for 56 days. Before they were used in this study, the plants have started to produce flowers.

Oviposition behavior test

No-choice test

Seven soybean cultivars were placed in 7 different cages (each with size 40 × 40 × 60 cm). Then, 15 couples of experiment insects were released into the cage. During this study, 3 cages were used for each cultivar. Every day, numbers of oviposited eggs and oviposition behavior of the females were observed which included observing a series of behavior, starting from the host selection alighting of the insect on the plant and until the insect leaved the plant. The observations were taken 2 h prior to the dark period and throughout the dark period, with the aid of flashlight with low light

Table 1. Criteria of soybean stage stated by Indonesian center for food crops research and development (PPTP, 1993).

Stage	Explanation
V	Vegetative stage
R2	Fully flowered
R3	Start fruiting
R4	Full filled pod
R5	Start seeding

intensity and used indirectly to produce enough light to observe the behaviour.

Multiple choice test

Seven soybean cultivars were placed in a cage, each with the size of 85 × 75 × 100 cm. The cultivars were arranged in orderly manner, forming a hexagon with one cultivar in the middle. Then, 15 couples of experiment insects were released into the cage. This procedure were replicated four times. All oviposited eggs and frequency of oviposition were counted and recorded daily. In the same time we also switched each of the cultivar position randomly. The observation procedures for this test is same as procedures for no-choice test.

Effect of trichome characteristics to oviposition preference

The density of trichomes of each cultivar was measured under binocular microscope by placing small paper of 1.4 × 1.4 mm with grid under sample as guidance. Thus, the density of trichomes stated as number of trichomes per 1.96 mm². In addition, length of trichomes (mm) was also measured with the help of ocular guidance scale. Further, the surface area of the pods (mm²) was measured using small scale paper. These procedures were carried out for 10 to 20 plants (depend on the numbers of available plants) of each cultivar. All the data were presented as average values.

Effect of phenological stage of plant to oviposition preference

No-choice test

The soybean cultivar that was preferred most by *E. zinckenella* as host plant (based on oviposition behavior test) was chosen to observe the effect of host plant phenology to its oviposition preference. We used 5 plants for each phenological stage (V, R2, R3, R4 and R5) based on criteria set by Pusat Penelitian dan Pengembangan Tanaman Pangan (PPTP) (Indonesian Center for Food Crops Research and Development, ICFORD) (1993) (Table 1). Each plant was placed in different cage having the dimensions of 40 × 40 × 60 cm. Then, 10 pairs of *E. zinckenella* were released for each repetition and numbers of oviposited eggs were counted daily.

Multiple choice test

Plants of different phenological stages, of the same cultivar that was used in no-choice test were placed inside 85 × 75 × 100 cm-sized cage. These plants were arranged in orderly manner, forming a rectangle with 4 plants at each corner and 1 plant in the middle.

Table 2. Differences in some of characters of soybean cultivar used in this study.

Cultivar	No. of trichomes	Length of trichomes (mm)	Pod's surface area (mm ²)	Number of eggs oviposited	
				No-choice method	Multiple choice method
Sindoro	7.400 ± 0.21 ^a	1.60 ± 0.01 ^a	236.11 ± 7.72 ^a	63.50 ± 6.06 ^{ab}	107.00 ± 10.80 ^a
Dieng	7.373 ± 0.24 ^a	1.50 ± 0.01 ^b	232.84 ± 4.95 ^{ab}	78.75 ± 11.96 ^a	122.50 ± 8.91 ^a
Wilis	5.673 ± 0.11 ^b	2.00 ± 0.04 ^c	210.10 ± 8.30 ^b	72.75 ± 3.64 ^a	47.00 ± 1.87 ^{bcd}
Tidar	5.620 ± 0.20 ^{bc}	1.60 ± 0.01 ^a	221.82 ± 6.88 ^{ab}	45.25 ± 5.65 ^{bc}	66.25 ± 5.88 ^{cd}
Galunggung	3.147 ± 0.10 ^d	2.30 ± 0.04 ^f	239.67 ± 8.25 ^a	69.25 ± 4.64 ^a	42.50 ± 5.45 ^{bc}
Leuser	4.667 ± 0.17 ^e	1.95 ± 0.01 ^d	226.16 ± 10.04 ^{ab}	37.00 ± 4.88 ^{cd}	54.25 ± 3.17 ^{cdc}
Guntur	5.247 ± 0.15 ^b	1.90 ± 0.01 ^d	215.54 ± 10.10 ^{ab}	67.75 ± 10.23 ^a	72.50 ± 16.30 ^d

Average number of trichomes was measured for 1.96 mm² surface area. Average value followed by same letter are not significantly different ($P < 0.05$, $n = 16$). Average number of eggs was measured from 4 females at both no-choice method and multiple choice method.

Then, 10 pairs of experimental insect were released into the cage. This test was replicated four times. The numbers of oviposited eggs were counted each day and the position of each plant was switched daily.

Data analysis

The data were analyzed by analysis of variance (ANOVA), continued by least significant difference test. We also carried out general linear model test to find the correlation of trichome density, trichome length, and pod surface area with number of eggs deposited. All the data were analyzed using Statistica 6.0 for Windows.

RESULTS AND DISCUSSION

Oviposition behavior

No-choice test method

Female insects began moving their antennae for about 15 min, just before the dark period began. After that, they began flying around the host plants randomly for about 30 to 60 min. Some female insects moved closer to host plant, flew around the plant, and mostly landed on the lower part of the stem, possibly aiming for the young pods. After alighting on pods, they touched for a short time the surface of the pods with their antennae, and they protruded their ovipositor to the lower surface of the pod, forming an angle of 45° relative to their body surface. Female insects moved forward dragging their ovipositor against plant surface while tapping it with their antennae.

The female stopped walking, lifted their antennae, and by raising the abdomen they laid an egg. They continued sensing the surface of the young pod with the end of their ovipositor and antennae sometimes only with the end of their ovipositor. Sensing was paused when they stopped walking, and by raising their abdomen, another egg was laid on new area. This series of behavior was performed repetitively, and an egg was laid in each oviposition attempt which lasted in 4 to 4.5 min. During the

oviposition process, some female took a brief rest or normal walk on the host plant or sometimes moved away. After completion of oviposition behavior, females often take a rest. The frequency of oviposition was higher at 2 h after dark and generally the female insects stayed till the end of the dark period.

Multiple choice test method

Female insects displayed the oviposition behavior same as shown in no choice test accept the number of eggs laid on each cultivar that was different (Table 2). They began by moving their antennae at the beginning of the dark period, and continued their flying around the host plant. This activity was shown for 30 to 60 min to find the perfect host to land and oviposit. Some females moved closer to the host plant, flew around them, and landed on it. During oviposition process, the females laid eggs individually on different pods in each visit.

The results obtained are in accordance with the previous work done on oviposition behaviour of *E. zinckenella* in Japan (Hattori, 1986). The finding indicates that oviposition behavior of *E. zinckenella* might be the result of evolutionary process and it is possibly not affected by differences in climate even though further researches are needed to confirm this. However, Hattori did not report the oviposition behaviour on different hosts. Our study showed that *E. zinckenella* has rigid oviposition behavior as it carries out the same behavioral pattern and spent relatively the same time on each available plant in the cage. It was also observed that even after more tapping on plants, the female insects did not always oviposit egg on each visit.

Effect of trichome characteristics to oviposition preference

Table 2 shows that Dieng and Sindoro cultivars had significantly higher number of trichomes (7.400 trichomes and 7.373 per 1.96 mm², respectively) than other

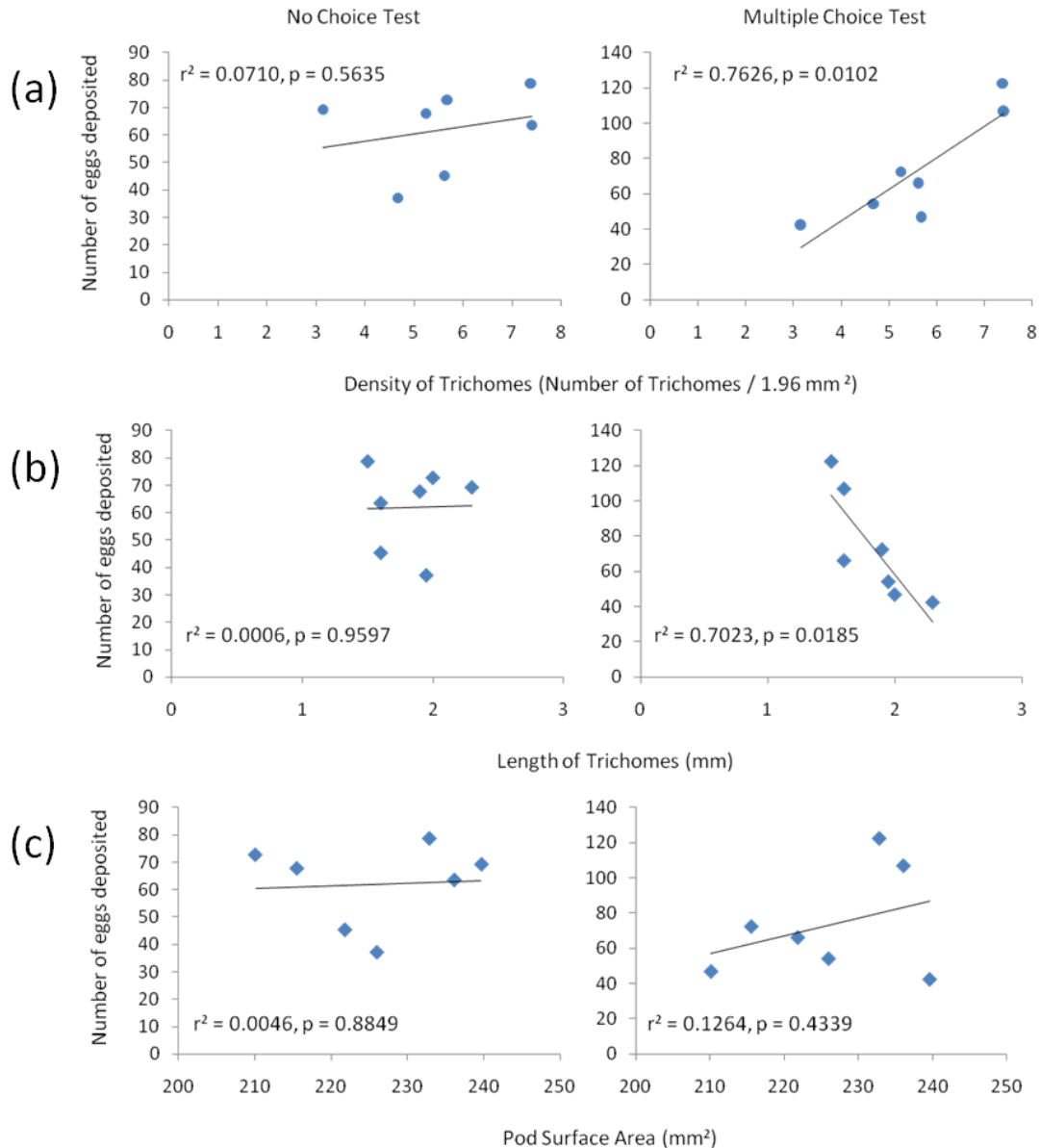


Figure 1. Correlation between trichomes characteristics and number of eggs deposited during no-choice and multiple choice tests. (a) Density of trichomes; (b) length of trichomes; (c) pod surface area.

cultivars. High variations were found in the average length of trichomes of each cultivar - Galunggung had the longest (2.3 mm) and Dieng had the shortest (1.5 mm). Further, Sindoro had significantly bigger pod (236.11 mm²) and Wilis had significantly smaller pod (210.1 mm²) ($p < 0.05$) compare with other cultivars.

We did not find any significant difference in number of eggs deposited during no-choice test for all the soybean cultivars. On the other hand, significant preference of *E. zinckenella* was found to oviposit in Sindoro and Dieng cultivars (Table 2). This result indicated that local *E. zinckenella* population has preferred these cultivars over others. When these preferred soybean cultivars are

absent, the insect species is also capable to use other cultivars for oviposition.

We found significant positive correlation between trichome density and number of eggs deposited ($R^2 = 0.76, p < 0.05$) and negative correlation between the length of trichome and number of eggs deposited ($R^2 = 0.70, p < 0.05$) only on multiple choice test. On the other hand, we did not find strong correlation between pod surface area and number of egg deposited (Figure 1a, b and c).

As shown in Figure 2, strong positive correlations of density of trichomes ($R^2 = -0.72, p < 0.05$) and number of eggs deposited ($R^2 = -0.9459, p < 0.05$) was observed with

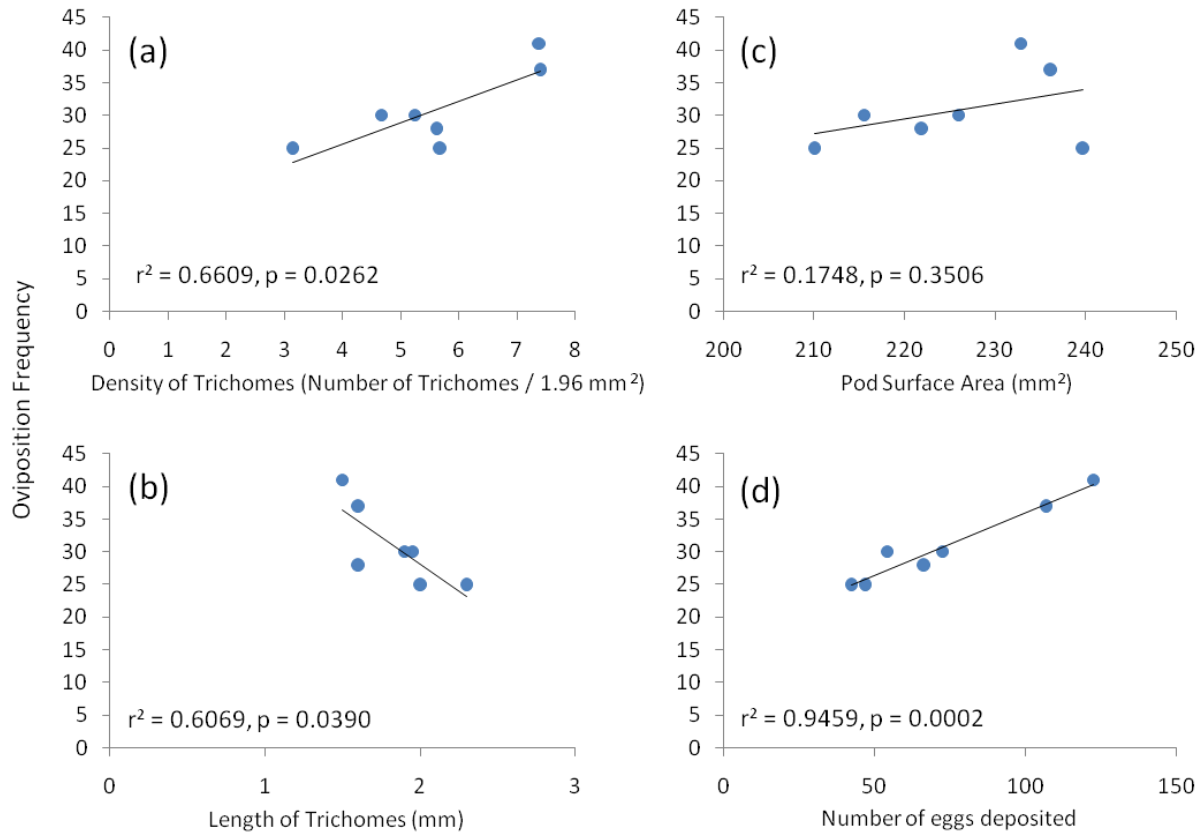


Figure 2. Correlation between (a), Density of trichomes; (b), length of trichomes; (c), pod surface area; (d), number of eggs deposited with oviposition frequency (data only gathered by multiple choice test).

oviposition frequency. On the other hand, length of trichomes showed negative correlation ($R^2 = -0.6069, p < 0.05$) with oviposition frequency as pod surface area did not affect it ($R^2 = -0.1748, p > 0.05$).

The present study showed that *E. zinckenella* prefers to oviposit on soybean pods having denser short trichomes which support the results of Talekar and Lin (1994) and Susanto and Adie (2008) who conducted their study, respectively, outside tropical region and in Indonesia. However, our finding does not support the result of another similar research work in Indonesia carried out by Suharsono and Suntono (2004). The difference in the method might be the reason for the deviation in results. Unlike us, Suharsono and Suntono (2004) performed only no-choice test method that could affect the oviposition behavior of female insects. Ohgushi (1992) suggested that the placement of only one host plant per cage could not give female insect the opportunity to choose the perfect place to oviposit. In this circumstance, insect could not fully utilize the host plant which it did not really prefer for its offspring's survival. Notably, Metcalf and Luckmann (1982) emphasized that in certain situations (also in the field) when no better choice was available, the insect species could eat and oviposit on a host plant which they did not really prefer.

The higher number of oviposited eggs by *E. zinckenella* on plant with denser short trichomes might correlate with the structure and function of soybean trichomes. The trichomes of soybean are glandular (Shanower et al., 1996) and secrete viscous liquid that harden as soon as it comes in the contacts of air (Metcalf and Luckmann, 1982) and acts like a sticky glue. Herbivores use this liquid to prevent their eggs from being attacked by natural enemies (Shanower et al., 1996). In addition, denser trichomes can negatively affect the walking speed and behavior of natural enemies (Kashyap et al., 1991).

Effect of plant phenology on oviposition preference of *E. zinckenella*

In this study, we found that *E. zinckenella* preferred to deposit eggs on the pod at developmental stage R4 (mean numbers of eggs for no-choice test and multiple choice test are 72.75 and 105, respectively). The number of eggs deposited increased constantly from stage V (having no eggs) to peak number in stage R4 followed by sharp decline in stage R5 (Table 3).

The present study confirmed other previous researches that suggest correlation between development stage of

Table 3. The average number of *E. zinckenella* eggs on several stage of soybean development by no-choice and multiple choice tests.

S/N	Stage	Average number of the eggs	
		No-choice test	Multiple choice test
1	V	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
2	R2	23.75 ± 2.69 ^b	27.50 ± 4.17 ^{bc}
3	R3	41.75 ± 3.84 ^b	69.00 ± 6.82 ^c
4	R4	72.75 ± 12.89 ^c	105.00 ± 10.66 ^d
5	R5	38.00 ± 4.78 ^b	39.25 ± 7.49 ^e

Average value followed by same letter are not significantly different ($P < 0.05$, $n = 16$). Average number of eggs was measured from 4 females at both no-choice method and multiple choice method.

the pod and total number of eggs deposited (Hattori and Sato, 1983; Kobayashi and Oku, 1980; Kamandalu et al., 1997). High preference of *E. zinckenella* females to oviposit in the pods at stage R4 may correlate with the emergence time of larvae as female choose the best possible food for their offspring. Pods at stage R4 provide best physical texture and moisture also which act strongly on the oviposition response of *E. zinckenella* (Hattori and Sato, 1983). We found that the numbers of eggs deposited by *E. zinckenella* in the pods at stages R2, R3, and R5 during no-choice test and multiple choice test were more or less same. This finding indicates that *E. zinckenella* has similar oviposition behavior for less favored phenological stages of their host plant.

The difference in density and length of trichomes in seven soybean cultivars affect the oviposition frequency but not the oviposition behavior of *E. zinckenella*. Agarwala (1996) suggested that the way an insect species gets and uses its host is a historical product of evolutionary adaptation that is established by natural selection in the dimension of space and time. Insect behavior responses in selecting host plants depend on particular genotype pool and environmental condition. Our study showed that *E. zinckenella* can tolerate changes in wide range of environmental conditions, which is a key factor for its successful life as major pest in soybean plantation in Indonesia.

Most insect species choose plants based on the patterns of sensorial information complex combined with several modalities such as taste, smell, sight, and touch that will generate complex neural patterns in central nervous system. The selection of plants by insect is mainly affected by sensorial capture that, in turn, affects behavior of the insect (Rani, 1996). Hirai et al. (1980) reported that several chemical and mechanical stimuli by young pods of soybean could act as the factors attracting *E. zinckenella* for oviposition even though not contribute, at least, in the final step of oviposition behavior (Hattori and Sato, 1983). This fact could be explained by the observation, as mentioned earlier, that *E. zinckenella* protrude its ovipositor on the pod surface, and walked

with its ovipositor still contacting the pod surface before oviposition.

Our results are in agreement with Ramaswamy (1988) who proposed that generalist moths do not depend on volatiles for host finding. These results also support other research studies on *Helicoverpa virescens* (F.), *Epyphyas postvittana* (Walker) and *Spodoptera frugiperda* (Smith) (Ramaswamy et al., 1987; Foster and Howard, 1998; Rojas et al., 2003)

Conclusion

This study extends our understanding on the host finding behavior of *E. zinckenella*. First, it suggests that *E. zinckenella* females use the combination of trichome density, trichome length, and pod surface area condition to select particular soybean cultivar as host within the spatial limit of our study. Secondly, we found that host finding and eggs deposition process by *E. zinckenella* in tropical region is similar to that in non-tropical region. This finding raised other questions on the origin of host finding mechanism and egg deposition behavior of this insect species. Finally, *E. zinckenella* may have fixed behavioral pattern for oviposition during the period when the number of preferred hosts are scarce.

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