

## RESISTANCE OF SOME GROUNDNUT CULTIVARS TO SOYBEAN POD BORER, *ETIELLA ZINCKENELLA* TREIT. (LEPIDOPTERA: PYRALIDAE)

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### ABSTRACT

**Resistance of some groundnut cultivars to soybean pod borer, *Etiella zinckenella* Treit. (Lepidoptera: Pyralidae).** Five groundnut cultivars: Badak, Panther, Sima, Gajah, and Simpai, were grown in field in June–August, 2006 to determine their resistance/susceptibility to *Etiella zinckenella* Treit. Two local cultivars (big and small seeds) were included as comparison (controls). All cultivars were grown in experimental plots arranged in a randomized complete block design (RCBD), replicated three times. The incidence of soybean pod borer and damaged pods were observed at 9, 11, 13 weeks after sowing (WAS) at 10 sample plants taken randomly from each plot. All cultivars were harvested at 13 WAS. Number of damaged pods was counted and percentages per plant were calculated. Larvae observed inside pod or in the soil were counted and collected. The seed yield per plant and weight of 100 seeds from 100 sample plants taken randomly at harvest were weighted to nearest gram at 10% water content. Pod toughness (hardness) was measured with penetrometer. Resistance level of each cultivar was determined based on cultivar's means and overall mean and standard deviation of the percentages of damaged pods. Data were analyzed with analysis of variance (ANOVA) and means were separated with DMRT. The result revealed that mean percentages of damaged pod differed significantly between cultivars. Seed yield of cultivar Panther, Sima and Badak were significantly higher than those of the other two and local cultivars. Cultivar Panther was categorized as resistant, cultivar Sima and Badak as moderately resistant, while the others as susceptible. The relative resistance of groundnut cultivar seems, at least in part, to correlate with the structural hardness of pod.

**Key words:** groundnut, resistance, *Etiella zinckenella*

### INTRODUCTION

Soybean pod borer, *Etiella zinckenella* Treit., has long been known as the most destructive soybean pest in Indonesia as well as in many other tropical countries. Its occurrence as groundnut pest, though, has not been considered as a threat and is mentioned only superficially, noting that groundnut is one of its host plants (e.g. Kalshoven, 1981; Marwoto *et al.*, 1999). The other species, the lucerne seed web moth (*Etiella behrii* Zeller) is known as pest of groundnut in Australia (Wightman and Rao, 1994; Anonymous, 2004), but there is no report of *Etiella* causing serious damage on groundnut in Indonesia. There are three *Etiella* species, two aforementioned above and *Etiella hopsoni* Butler (Naito *et al.*, 1983). All three occur in soybean in Indonesia with different but overlapping geographic distributions (Naito *et al.*, 1983; Hattori *et al.*, 2001) none reported their occurrence on groundnut.

In Bengkulu, *E. zinckenella* causes damage on groundnut pod in several areas in north and southern Bengkulu (Seluma) (0-300 asl.) (Apriyanto *et al.*, 2008)

and in Rejang Lebong (800-1200 asl.) (Apriyanto, personal communication with farmers). We speculate that it also occurs in other regions of Sumatera, especially in regions where groundnut are more widely grown. In North Bengkulu, this insect was reported to cause yield reduction of 31 – 48% and in Seluma it destroyed almost entirely two farmer's field surveyed (Apriyanto *et al.*, 2008). According to farmers, it often caused total lost and discouraging them to grow groundnut, recently.

Studies on management/control of *Etiella* spp. on soybean and other crops have been reported in many publications, including, the use of insecticides (Apriyanto & Toha, 1995; Marwoto *et al.*, 1999), parasitoid (Marwoto & Saleh, 2003), crop rotations and planting date (Naito, 2006), and sex pheromone (Hattori *et al.*, 2001). However, researchers consider *Etiella* as one of the most difficult soybean pests to control due to larval feeding behavior (i.e. spend most of the time during larval stage inside the pod).

There are several groundnut cultivars released in Indonesia (e.g. by Indonesian Research Institute for

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Biotechnology and Genetic Resources in Bogor and by Research Institute for Legume and Tuber Crops in Malang). None of this cultivar has been evaluated for resistance to pod borer. Resistant cultivar is often considered as core component of integrated pest management (IPM) concept (e.g. Panda & Kush, 1995) and adoption of such cultivars is the best option to overcome yield losses due to pest and diseases (Dwivedi *et al.*, 2002).

Much has been done on the study and breeding for groundnut resistance against diseases (Wynne & Beute, 1991; Dwivedi *et al.*, 2002; Sharma *et al.*, 2003). Such effort against insect pests is still lacking, especially to pod feeders. Some studies on resistant cultivars to insects have been done particularly for leaf feeders (Lych, 1990; Sharma *et al.*, 2003; Herselman *et al.*, 2004). The presence of resistant cultivar(s) for pod borer management among those that have been released to farmer is advantageous as component of IPM for groundnut pests, since there is no need to put more budget for breeding program for those cultivars. Host plant resistance is one of the most economically and environmentally friendly methods for keeping pest populations below economic injury levels (EILs) (Sharma *et al.*, 2003). Furthermore, the information about genetic resources for resistance is indispensable for farmers and researchers. Here we report our field trial to examine relative resistance of several groundnut cultivars to the soybean pod borer in Bengkulu.

## MATERIALS AND METHODS

Seeds of five cultivars: Badak, Panther, Sima, Gajah, and Simpai, were obtained from Research Institute for Biotechnology and Genetic Resources (BB-Biogen), Bogor. Two local cultivars, one with small seeds and the other with big seeds, obtained from local farmers were included as controls. The local cultivars have been grown by farmers for years and their origins were unknown. Both were heavily damaged by pod borer in field from previous observation (Apriyanto *et al.*, 2008). Each cultivar was grown in 3 m x 5 m plots in Ultisol soil (pH 4.6; 60 m asl.) in North Bengkulu (50 km northeastern of Bengkulu City) in July-September, 2006. Cultivars were arranged in plots in randomized complete block design (RCBD) replicated three times. The experiment field had been used for growing groundnut for many years, and at the same time ca. 50 m from experiment plot, a farmer was planting local cultivar of ca. four times as large as our plots. The experiment plots were surrounded by cassava and cowpea bean, and some fallow fields own by farmers.

Seeds were sown one per hill, at 30 cm between and 20 cm within rows, respectively. Plants were fertilized with 50 kg<sup>-1</sup> of Urea, 112.5 kg<sup>-1</sup> of SP-36 and 50 kg<sup>-1</sup> of KCl. Weeding was done as necessary. Half doses of Urea and all SP-36 and KCl were applied at planting date, while the other half was applied at 30 days after sowing.

The occurrence of damaged pods as well as number of larvae inside and outside pods was thoroughly observed at 9, 11, and 13 week after sowing (WAS), at 10 sample plants per plot. Sample plants were randomly selected. Numbers of damaged pods were counted and the percentage of those per plant for each plot was calculated based on total pod number per plant. At the day of harvesting, numbers of damaged pods were counted again from 30 sample plants taken randomly and the percentages were calculated as above. Seed yield taken from sample plants as well 100 seeds of each cultivar were weighed to nearest gram at 10% water content.

Data were analyzed with PROC GLM (SAS Institute, 1997) and means were separated with DMRT whenever significant difference was found. The mean percentage of damaged pods of each cultivar was used to place it in resistance category. Resistance categories were determined based on mean percentages and standard deviations of overall mean of all cultivars. Cultivar was classified as highly susceptible, susceptible, moderately resistance, resistance or highly resistance (Chiang & Talekar, 1980 cit. Nurdin *et al.*, 1994), based on data of harvested pods from 30 sample plants.

The categories of resistance are as follow: a) highly resistance ( $P < \bar{x} - 2SD$ ), b) resistance ( $P = -2SD$  to  $-1SD$ ), c) moderately resistance ( $P = -1SD$  to  $0$ ), d) susceptible ( $P = 0$  to  $+1SD$ ) and e) highly susceptible ( $P > +1SD$ ); where P was mean percentage of damaged pods of each cultivar; and SD were overall mean and standard deviation of percentage of damaged pods.

To evaluate possible explanation for resistance mechanism, pods of each cultivar were measured for their toughness (hardness) with a penetrometer (PRECISION Scientific, BF-6, Petroleum Instrument Co., Il.) set at 100 g cm<sup>-2</sup>, constant force. The sharpness of cone tip was 35°. The scale was read as millimeter, which measured the depth of penetration of penetrometer cone tip into pods. Therefore the deeper the cone tip penetrated the pods, the less the hardness of those pods was.

**RESULTS AND DISCUSSION**

Pod damage by soybean pod borer was typical: small hole when larva still inside, small and larger holes with partly or completely damage seeds (empty pods) when larva had already left pods. Whenever seeds were not completely consumed by the larvae, they were covered by fungus, especially *Aspergillus* and

contaminated with aflatoxin, rendering them unedible for further feeding by pod borer. The presence of aflatoxin in groundnut seed (kernels) in field due to insect feeding has been mentioned in many publications (e. g. Hill *et al.*, 1983; Diener *et al.*, 1987). Holes made by larvae of pod borers provide way of entrance for fungus producing aflatoxin. Therefore, any damage to pod

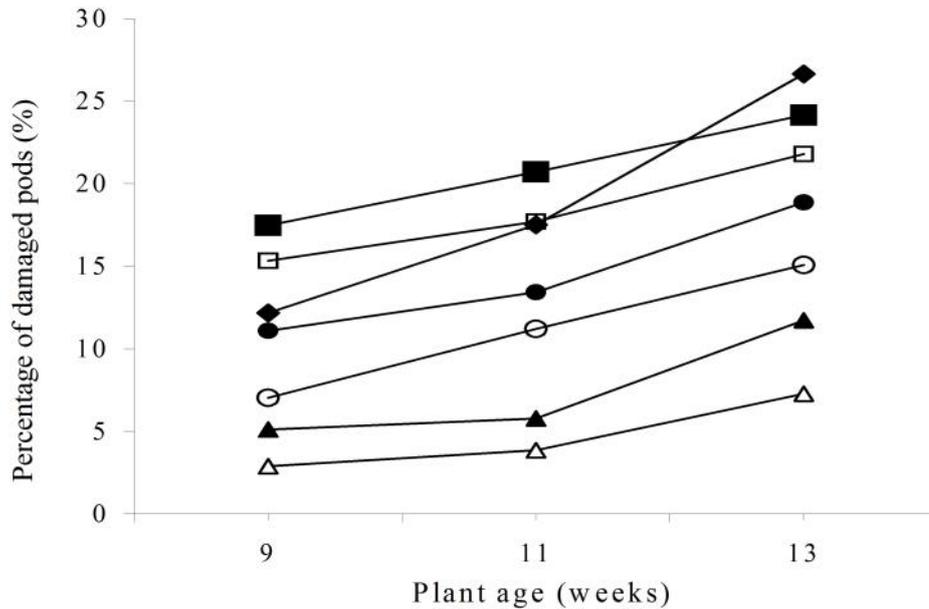


Figure 1. Percentages of damaged pods by *Etiella zinckenella* at plant age of 9-13; ■: Local cultivar (small seed), □: Local cultivar (big seed), ◆: cv. Gajah, ●: cv. Simpai, ○: cv. Badak, ▲: cv. Sima, △: cv. Panther.

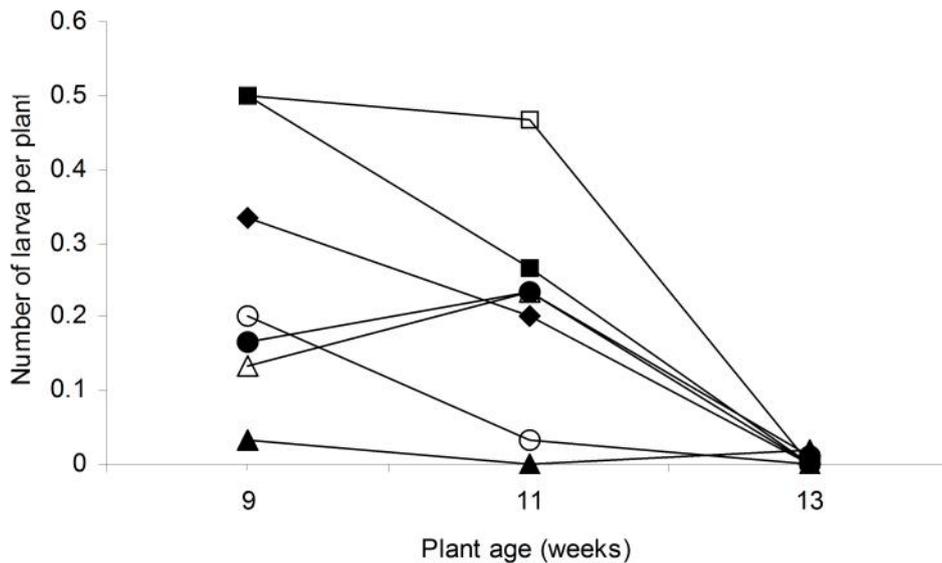


Figure 2. *Etiella zinckenella* larval densities per plant at plant age of 9-13; ■: Local cultivar (smaller seed), □: Local cultivar (bigger seed), ◆: cv. Gajah, ●: cv. Simpai, ○: cv. Badak, ▲: cv. Sima, △: cv. Panther.

Table 1. Means  $\pm$  SE percentages of damaged pods by soybean pod borer, pod hardness and resistance categories of six evaluated cultivars<sup>1)</sup>

Cultivars	% damaged pods	Pod hardness at harvest <sup>2)</sup>	Resistance categories <sup>3)</sup>
Local (small seed)	26.64 a	2.03 a	Highly susceptible
Local (big seed)	24.16 ab	1.00 bc	Susceptible
Gajah	21.79 abc	1.23 b	Susceptible
Simpai	18.87 bcd	1.23 b	Susceptible
Badak	15.08 cd	1.83 a	Moderately resistance
Sima	11.73 de	0.87 c	Moderately resistance
Panther	7.28 e	0.77 c	Resistance

1) Means followed by same letter within same column do not differ significantly (DMRT;  $\alpha = 0.05$ ).

2) Depth of penetration of penetrometer cone tip into evaluated pods (in mm).

3) Criteria based on Chiang and Talekar (1980) *cit* Nurdin *et al.* (1994).

would predispose pod to infection by fungus (Anonymous, 2004).

Percentages of damage pods increase significantly from plant age of 9 to 13 weeks (ANOVA; repeated measure, plant age as sub-plot;  $df = 2$ ,  $F = 44.49$ ,  $P < 0.0001$ ) as showed in Figure 1. The larva densities per plant, on the other hand, decreased significantly between plant ages ( $F = 20.74$ ,  $P < 0.0001$ ) (see Figure 2). In general, there seem to show correlation between larval densities and percentages of damaged pods in each cultivar (i.e. cultivars with higher percentages of damaged pods are in accordance with those with higher larval densities).

Figure 1 also tells us that three cultivars, Panther, Badak, and Sima had distinctly lower pod damage than the other four in all observations dates, suggesting that they might be less attractive or more resistant to pod borer. If there were the case, those cultivars, particularly Panther, could be the best choice to be incorporated into IPM system for combating the pod borer attacking groundnut. The decrease of larval density through time and the disappearance of larvae in the last observation (13 WAS) might indicate that most of them had gone to pupation. Unfortunately, we did not sample soil for pupal density.

Percentages of damaged pods at harvest ranged 7.28–26.64%. The differences between means of evaluated cultivars were highly significant (ANOVA;  $df = 6$ ,  $F = 9.39$ ,  $P = 0.0006$ ). Cultivar Panther suffered the least pod damage and significantly differed from others, except cultivar Sima. Among five others, the percentage of damaged pod of cultivar Badak was the lowest, although it did not differ significantly, except with

that of local cultivar (small seed). Percentage of damage pods on cultivar Gajah did not differ significantly to those of local cultivars, which suffered the highest damage (Table 1).

Cultivar Panther was the only cultivar categorized as resistant. Whereas, cultivars Badak and Sima fall within moderately resistant, cultivars Simpai and Gajah and local cultivar (big seed) were included as susceptible, and the other local cultivar (small seed) as highly susceptible to soybean pod borer. Data in Table 1 also suggest that there is correlation between mean percentages of damaged pods and the depth of penetrometer cone-tip penetration, indicating that pod structure might have contributed, at least in part, to the resistance of evaluated cultivars. Even though the correlation does not differ significantly from zero when all cultivars were included ( $r = 0.5452$ ,  $t = 1.735$ ,  $P > 0.10$ ), the correlation was significant ( $r = 0.75134$ ,  $t = 3.4506$ ,  $P < 0.05$ ), when cultivar Badak was excluded. The performance of cultivar Badak was odd; it fell within moderately resistance category but showed lower value of pod hardness (i.e. has deeper of cone-tip penetration than that of cultivars Gajah or Sima). Structural toughness has been considered as one of important resistant mechanism to insects on other crops; for example, resistance to second generation of European corn borer (*Ostrinea nubiulalis*) was contributed more by the high content of silica and lignin in corn leaf sheath and collard tissue, rather than by the presence of DIMBOA (review; Panda and Khush, 1995). Scriber and Slansky (1981) argued that plants with high cell wall components more likely to deter herbivores. Despite narrow genetic base, low genetic diversity and lack of

Table 2. Dry seed yield and some characters of evaluated groundnut cultivars related to seed size and nutrition contain<sup>1)</sup>

Cultivars	Seed yield (g) <sup>2)</sup>	Weight of 100 seeds (g)	Nutrition contain (%)		
			Protein <sup>3)</sup>	Fat <sup>3)</sup>	N total <sup>4)</sup>
Local (smaller seed)	762.9 a	34.5 bc	-	-	2.94
Local (bigger seed)	865.1 a	46.2 ab	-	-	2.80
Gajah	972.2 a	52.4 a	29.00	48.00	2.80
Simpai	1039.1 a	50.3 a	32.06	50.61	2.10
Badak	1148.5 a	35.9 bc	24.00	47.00	2.10
Sima	1107.2 a	37.6 bc	29.90	50.00	2.76
Panther	1222.8 a	34.8 c	21.50	43.00	2.66

- 1) Seed yield and weight of 100 seeds were measured at 10% water contain. Means followed by same letter within same column do not differ significantly (DMRT;  $\alpha = 0.05$ )
- 2) Per hill based.
- 3) Values adopted from seed description of groundnut (Indonesian Research Institute for Biology and Genetic Resources, Bogor).
- 4) Data from laboratory analysis done at Soil Science Laboratory, Faculty of Agriculture, University of Bengkulu.

sources for resistance to pests and diseases in cultivated *A. hypogaea* (Bertioli *et al.*, 2003), groundnut resistant to arthropods has been reported; for example, against sap-feeders such as *Aphis craccivora* Koch, *Empoasca kerri* Pruthi and *Empoasca fabae* (Harris); leaf feeders such as *Helicoverpa zea* (Boddie), *Spodoptera frugiperda* (J.E. Smith) and *Spodoptera litura* (F.), and root feeder such as *Diabrotica undecimpunctata howardi* Barber, pod feeders such *Odontotermes* sp. and *Eulepida mashona* Arrow and other insect species (Lynch, 1990; Sharma *et al.*, 2003).

Pabbage (1990) argued that differences in soybean pod damage by *E. zinckenella* were due to seed size (i.e. the less the seed size the more resistance the soybean cultivars were to pod borer). However, seed size, though differed significantly between resistant and susceptible cultivars, except for local one (small seed), as indicated by the weight of 100 seed (see Table 2), unlikely affected percentages of damaged pods and therefore could not have been possibly determined resistance status of groundnut cultivars in our study.

The lower protein and fat contain in cultivar Panther than those in others might also indicate chemical basis for resistance, but further study is needed because it was not so for cultivar Sima which fell within moderately resistant (Table 2). Primary metabolite such as protein and fat contains per see has not usually

considered as important mechanism contributing to resistance in plants. However, nutrition unbalance or in suboptimal concentration has been mentioned as one of possible plant defense mechanisms in plants against herbivores (Kyto *et al.*, 1996; Moran & Hamilton, 1980). Except for cultivar Sima, there is trend that resistance has connection with low protein (but not N total) and fat contains (see Table 2).

Extensive review on the role of secondary metabolite as defense mechanism in plant against herbivores has been done (e.g. Berembaum & Zangerl, 1992; Fenny, 1992; Schowalter, 2006). Resistance to insect in other species of Genus *Arachis* has been studied and some were suspected as based on antibiosis mechanism (Sharma *et al.*, 2003). Thus far, there has not been any study that looks insight the chemical basis for resistance to insects done in groundnut (species *hypogaea*).

Seed yield of each cultivar (Table 2), though did not differ statistically, provides some clues of correlation between levels of resistance/susceptibility of cultivars (i.e. percentages of damaged pods as in Table 1) with seed yields, ( $r = -0.95073$ ,  $t = -22.1177$ ,  $P < 0.001$ ), which indicates that in regions where soybean pod borer would likely to occur, resistance cultivar will give better yield. The higher seed yield of cultivar Panther than those of two others, is no doubt, due to the lower pod damage and therefore also to the level of resistance, at

least in part. However, it is necessary that this cultivar be studied further under no-choice tests before it is recommended, in combination with other techniques (i.e. in IPM), to overcome yield losses due to pod borer infestation.

## CONCLUSIONS

From this study we conclude that cultivar Panther is resistant to soybean pod borer. Whereas cultivars Sima and Badak are moderately resistant and the others are susceptible and highly susceptible to soybean pod borer. Structural toughness of pod might contribute to the mechanism of resistance to soybean pod borer in groundnut. It is necessary though that those cultivars be studied further in no-choice test before recommended to farmers for soybean pod borer management.

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