

Resistance of Advanced Soybean Lines to Pod Borrer (*Etiella zinckenella*)

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INTRODUCTION

One of the limiting factors in increasing and stabilization of soybean product is pest attack (Tengkano et al., 2006). Pod borer, *Etiella zinckenella* Treitschke (Lepidoptera: Pyralidae) is the one of major pests in soybean (Baliadi et al., 2008a). Fluctuation and peak of eggs and larvae population vary according to the season, rainfall, variety, growing pattern, other host availability, natural enemy, and pest control activity using insectiside (Wagiman et al., 1987). The highest population of pod borer in soybean occurs in dry season, although there is *Crotalaria* sp. as the host along the year. Host plant can be served as source of pest population and as direct or indirect pest controller (Baliadi et al., 2008b).

Usually, pod borer controlling in Indonesia is conducted by using chemical insecticide causing negative effect to human health and ecosystem stability (Baliadi et al., 2008b). Beside, insecticide controlling is also inefficient because requires high cost (Tabata & Yasuda, 2011). Some of alternative environment friendly for controlling strategy to *E. zinckenella* are conducted by aplying resistant variety, trap crop, release of *Trichogramma* sp. paracitoid, sex-pheromon, and resistant gene transfer through biotechnology. The usage of resistant variety is able to decrease pesticide residue in environment and ecoomicaly benefit (Oki et al., 2012).

There are variability responses of soybean genotypes to pod borer *Etiella zinckenella* Treitschke (Amro et al., 2009; Taghizadeh et al., 2012). Variety of Grobogan is less preferred by imago pod borer as site for laying eggs with eggs population about 0.6–2 eggs per hill (Tengkano et al., 2010). Soybean genetic variability to pod borer are need to be identified to provide gene sources in development of soybean that resistant to pod borer. Soybean lines derived from crossing activity are also need to be identified to find out the combination of resistance trait and agronomical traits in a soybean line. Resistant cultivars is one of integrated control components (Inayati & Yusnawan, 2016).

The objective of the research was to study non preference resistance level of soybean genotypes to pod borer *E. zinckenella* Treitschke, and to find out the genotypes that resistance to this pod borer. The finding of the resistance level can be studied further to understand the resistance mechanism and the role of plant resistance gene. Besides, the resistant genotypes will benefit to be used in improving soybean resistance to pod borer or as promising lines that can be tested further for their yield potential, and then can be released as a new variety for pod borer resistance.

METHODS

The experiment was in laboratory and greenhouse of Indonesian Legume and Tuber Crops Research Institute (ILETRI), Malang. The design was a randomized complete block design (RCBD) with three replications. The materials consisted of 50 genotypes from crosses, five resistant genotypes (IAC 100, IAC 80, G 100 H, Detam 1, and No. 29) and two susceptible genotypes (Ichyou and Wilis).

Soybean variety "Wilis" was grown sequentially in ILETRI's greenhouse with one week interval for seven times planting date in the area of 7 m x 5 m every planting date. Plants were fertilized using Urea 0.4 g/hill and NPK 1.2 g/hill. Bean fly pests were controlled with insecticides of cypermethrin on 8 DAP, and leaf pests were controlled with insecticides of cyhalothrin at 14, 21, and 28 days after planting. After the plant was 28 DAP, controls were carried out manually.

Pod borer insects were obtain from collecting of instar 5 larva in Ngale Research Station, Ngawi Regency, East Java Province, Indonesia. The collected larva were taken to ILETRI's pest laboratory. Larva were reared in the plastic container, where in the container was filled with sawdust. Larvae were reared until transforming to be pupae and then the pupae moved to copulation cage. At the top of the inside cage, there were hanging cotton layer filled with 10% honey solution for feeding the imago that out from the pupa. At three days after the imago appeared, imago was identified for the sex, and the damages of foot and antene by using binocullar microscop. At four days age, the selected imagos were ready to be applied in treatment plants.

Planting was conducted in RCBD with three replications, where every replication consisted of 6 polybags/genotype with four seeds/ polybag. Planting was conducted based on flowering age of each genotype in order pod setting start simultaneously. Fertilizers were applied by using Urea 0.4 g and NPK 1.2 g per polybag at sowing date. Watering were carried out by monitoring the water availability in the soil and water will be added to maintain field capacity. Thinning was applied at 14 DAP with remaining 2 plants/ polybag. Similar to the feed preparation, bean fly pests were controlled with insecticides of sipermetrin on 8 DAP, and leaf pests were controlled with insecticides of sihalothrin at 14, 21, and 28 days after planting.

The plants were caged by using screen textile at 35 DAP. When the plant age was 21 days after flowering (DAF), pod borer infestation in treatment plants was carried out at 14.00 Western Indonesian Time with two couples of imago for every genotype in one replication. Hence, there were 144 couples or 228 imago in one replication. Number of eggs was observed 2 days after infestation (DAI) at the pods by using binocular microscope. Each observation of larva population and percentage of pod borer attacking were recorded at two polybags consisted of four plants at 14 DAI, before larva got down to the soil for transforming to be pupa. Data were analyzed by using analysis of variance and continued with least significant difference at 5% significance level.

lows:

Pod damage =

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$$
\frac{Number of damage pools}{Number of total pools} \times 100\%
$$
\nSeed damage =

\n
$$
\frac{Number of damage seeds}{Number of total seeds} \times 100\%
$$

Attacking percentage was calculated as fol-

Determination of resistance criteria based on the formula bellow (Chiang & Talekar, 1980):

Where:

RESULTS AND DISCUSSION

Imago population

Analysis of variance showed that the differences of soybean genotype affected significantly on population of imago, eggs, and larva, and number of attacked pod and seed damage. Imago population of *E. zinckenella* on 50 evaluated genotypes, six check resistant genotypes, and one check susceptible genotypes revealed that imago population ranged 2.33–8.33 imago/ genotype (Fig. 1). The highest imago population were on genotype of Tgm/Anj-599 and Tgm/ Anj-764 (8.33 imago/genotype), while the lowest imago population were on genotype of Tgm/ Anj-833 (2.33 imago/genotype). Imago population on check resistant genotype ranged between 5-5.67 imago/genotype, while imago population on check susceptible genotype were 5.67 imago/genotype. Based on imago population, there were two genotype indicated highly resistant of non-preference manner or were not chosen as an alighting site, i.e. Tgm/Anj-833 and Anjasmoro. Beside, there were seven genotype indicated as

resistant genotypes, i.e. Tgm/Anj-530, Tgm/Anj-847, Tgm/Anj-889, Tgm/Anj-910, Tgm/Anj-912, Tgm/Anj-918, and Tgm/Anj-959. Probably, these genotypes had short trichome, because the genotypes with many short trichomes are prefered by *E. zinckenella* in depositing eggs (Permana et al., 2012).

Population of eggs, and larvae of *E. zinckenella*

E. zinckenella eggs population on 57 tested genotypes were between 3.67-43.67 eggs/hill. The highest eggs population was on genotypes Tgm/Anj-571 (43.67 eggs/hill), while the lowest population was on genotype IAC 100 (3.67 eggs/ hill) less lower than genotype G100H (4.67 eggs/ hill). Those two genotypes were the check of resistant genotypes. Based on eggs population, there were three genotypes indicated as resistant genotypes in non-preference manner or were not chosen by imago as the site to put the eggs, i.e. Tgm/Anj-778, Tgm/Anj-847, and Tgm/Anj-909 with eggs population 5.00, 9.33 and 8.67 eggs/ hill respectively.

In this study, varieties of Tanggamus, Anjasmoro, and Detam 1 showed criteria of moderately resistant or less chosen by *E. zinckenella* imago as site for putting the eggs with eggs population 12, 10 and 15.67 eggs/hill. This study was supported by Tengkano et al. (2011) that varieties of Tanggamus and Anjasmoro were not chosen by pod borer imago as site for putting the eggs with eggs population 0 egg/hill, while Detam 1 was less chosen by imago for putting the eggs. Taghizadeh et al. (2012) also reported different mean number of eggs laid among the cultivars.

Genotypes of Ichyou and Wilis, as the check susceptible genotypes, also showed low eggs population namely 4.67 and 11 eggs/hill respectively. Hence, Ichyou and Wilis were indicated as resistant and moderately resistant respectively. This result is different to Santi et al. (2014) that stated Ichyou was chosen by *E. zinckenella* imago as site for putting the eggs. Presumably, this difference caused by the number of genotypes, where in this study we used 57 genotypes that was more than Santi et al. (2014) leading lower possibility to be chosen as eggs place. Ichyou is a genotype with hairless pod. Genotype with hairless pods is not preferred by the imago to lay eggs because they do not have hair that can protect the eggs (Susanto & Adie, 2008). However, based on the observations of Ichiyou pods in laboratory, pod borer eggs were laid under the petals at the base of the pod (Santi et al.*,* 2014). Perhaps, Ichyou has secondary chemical compounds that can attract imago pod borer to lay their eggs on the

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Figure 1. Population of imago of *E. zinckenella*

pods by hiding them under the petals pods. On hairy pods, eggs were laid by pod borer imago on the pod skin among the hairs. This may be due to the pod borer imago want the eggs laid among the hair can not be taken by predators such as imago of *Paederus* sp. Observation showed that when the pod borer eggs removed from the pod, it would soon be devoured by imago *Paederus* sp.

Differences soybean genotypes also significantly affected populations of *E. zinckenella* larvae (Table 1). The population of pod borer larvae on these genotypes ranged between 37.67- 255 larvae/hill. The highest larval population was found on the Tgm/Anj-784, while the low-

est population was found on Anjasmoro variety. Based on the larval population and the analysis of variance it can be argued that Anjasmoro was indicated as a highly resistant variety, where the resistance was non-preferences or not selected by the larvae as their food. Beside Anjasmoro, there were three genotypes that indicated as resistant genotypes based on larval population i.e. Tgm/ Anj-744 (92.33 larvae/hill), Tgm/Anj-833 (93.67 larvae/hill), and Tgm/Anj-897 (86 larvae/hill). In addition, there were 20 genotypes that indicated as medium resistant with larval population between 113.33-143.67 larvae/hill.

In this study, genotype IAC 100 and

Table 1. Population of egg and larva of *E. zinckenella*

	Egg		Larva	
Genotype	popula- tion per	Cri- teria	popula- tion per	Cri- teria
	hill		hill	
Tgm/Anj-502	34.00 a-g	HS	158.33 b-i	S
Tgm/Anj-522	42.33 a-b	HS	140.33 b-i	MR
Tgm/Anj-530	17.33 a-j	ΜR	161.00 b-i	S
Tgm/Anj-537	32.33 a-f	HS	156.33 b-i	S
$Tgm/Anj-558$	$14.00 b-i$	ΜR	156.67 b-i	S
$Tgm/Anj-561$	29.00 a-h	S	127.00 b-i	MR
Tgm/Anj-565	18.33 c-j	MR	197.67 a-e	HS
Tgm/Anj-571	43.67 a-b	HS	188.67 a-e	HS
Tgm/Anj-597	14.67 c-i	MR	156.33 b-i	S
Tgm/Anj-599	16.67 a-j	ΜR	142.33 b-i	MR
$Tgm/Anj-743$	11.00 e-j	MR	121.67 d-j	MR
Tgm/Anj-744	20.00 a-j	S	92.33 f-j	R
Tgm/Anj-764	24.00 a-h	S	127.00 b-i	MR
Tgm/Anj-773	$15.67 a-j$	ΜR	207.67 a-d	HS
$Tgm/Anj-777$	21.33 a-h	S	122.67 c-i	MR
$Tgm/Anj-778$	5.00 i-j	R	140.67 b-i	ΜR
Tgm/Anj-780	16.33 a-j	MR	120.00 d-j	MR
Tgm/Anj-784	$10.00 f-j$	MR	255.00 a	HS
Tgm/Anj-789	13.00 c-i	MR	125.33 b-i	MR
Tgm/Anj-790	19.33 a-i	ΜR	135.33 b-i	ΜR
Tgm/Anj-795	15.33 с-ј	MR	143.67 b-i	ΜR
Tgm/Anj-796	26.00 a-h	S	156.00 b-i	S
Tgm/Anj-799	16.00 a-j	MR	157.67 b-i	S
Tgm/Anj-803	12.00 e-i	MR	169.00 a-g	S
Tgm/Anj-824	13.00 c-i	MR	133.00 b-i	MR
Tgm/Anj-833	15.00 c-j	ΜR	93.67 f-j	R
Tgm/Anj-834	11.67 d-j	MR	177.33 a-f	S
Tgm/Anj-844	27.00 a-h	S	210.67 a-c	HS
Tgm/Anj-846	12.67 f-j	MR	194.67 a-e	HS
Tgm/Anj-847	9.33 g-j	R	190.00 a-e	HS
Tgm/Anj-856	34.33 a-f	HS	113.33 e-j	ΜR
Tgm/Anj-871	17.67 c-j	MR	121.00 d-j	MR
Tgm/Anj-889	23.67 a-j	S	165.33 b-h	S
Tgm/Anj-890	32.33 а-е	HS	169.67 a-g	S
Tgm/Anj-894	38.33 a-e	HS	152.67 b-i	S
Tgm/Anj-897	17.33 a-j	MR	86.00 g-j	R
Tgm/Anj-898	19.33 a-i	MR	132.67 b-i	MR
Tgm/Anj-908	18.00 a-j	ΜR	138.67 b-i	MR
Tgm/Anj-909	8.67 g-j	R	153.33 b-i	S
Tgm/Anj-910	18.67 a-j	ΜR	197.33 а-е	HS

The number followed by the same letter is not different based on least significant different at 5% level (LSD 5%), $HR =$ highly resistant, $R =$ resis $tant, MR = moderately resistant, S = susceptible,$ $HS =$ highly susceptible

G100H as check resistant genotypes were consistanly resistant with larval population 72.67 and 80.33 larva/hill respectively. However, genotype IAC 80 as resistant check genotype showed as susceptible criteria with larval population 165.33 larvae/hill, while Wilis as susceptible check variety showed medium resistant cirteria with larvae population of 140 larva/hill. There may be other factors that caused genotype IAC 80 selected by larvae as their food. The resistance of the tested genotypes were non preference, it meant that the genotype is not chosen or not favored by pod borer larvae as their food. The genotypes were not chosen by the larvae as food materials alleged to contain chemical compounds that can be detected and disliked by pod borer larvae.

The percentage of pods and seeds that attacked by *E. zinckenella*

Analysis of variance showed that the differences in soybean genotypes significantly affected the percentage of pod damage and seeds by pod borer (Table 2). The percentage of infected pods on 57 tested genotypes ranged from 37.29% to 81.58%. The highest percentage of pod damage by *E. zinckenella* larvae was found on No. 29 variety with 37.29%, while the lowest percentage was found on Tgm/Anj-790 with 81.58%. Based on the percentage of infected pods, there are 8 resistant genotypes i.e. Tgm/Anj-744, Tgm/Anj-824, Tgm/Anj-796, Tgm/Anj-856, Tgm/Anj-871, Tgm/Anj-897, Tgm/Anj-908, Tanggamus, and Anjasmoro. Beside, there were 12 genotypes that indicated as moderately resistant (Table 1).

Figure 2. Outside view of soybean pods attacked by *Etiella zinckenella*

Figure 3. Inside view of soybean pods attacked by *Etiella zinckenella*

Table 2 also shows that the differences in soybean genotype significantly affected percentage of attacked seed by *E. zinckenella*. The percentage of seed damage of the 57 tested genotypes ranged from 30.17% to 70.74% per hill. The highest percentage of seed damage by *E. zinckenella* larvae was found on genotype Tgm/Anj-790, while the lowest percentage of pod damage found on genotype Tgm/Anj-871. Based on the percentage of seed damage, there were five resistant genotype i.e. Tgm/Anj-846, Tgm/Anj-871, Tgm/Anj-897, Tgm/Anj-908, and Anjasmoro. Beside, there were 17 genotypes that indicated as moderately resistant (Table 2).

The number followed by the same letter is not different based on least significant different at 5% level (LSD 5%), $HR =$ highly resistant, $R =$ resis $tant, MR = moderately resistant, S = susceptible,$ $HS =$ highly susceptible

Based on Table 1 and 2, it can be seen that the resistance of soybean genotypes against *E. zinckenella* determined based on eggs population, larval population, the percentage of pod damage, and the percentage of seed damage. Imago population was not used as a resistance determinant because imago was mobile, especially when they were observed. Based on the four determinants, there were nine soybean lines that consistently resistant to *E. zinckenella*, i.e. Tgm/Anj-599, Tgm/ Anj-743, Tgm/Anj-778, Tgm/Anj-789, Tgm/ Anj-795, Tgm/Anj-824, Tgm/Anj-871, Tgm/ Anj-897, and Tgm/Anj-908. Of the seven resistant check genotypes, there were three varieties (Anjasmoro, Detam 1, and No. 29) and two accessions (IAC 100 and G100H) that were indicated as resistant based on eggs population, larval population, percentage of pod damage and percentage of seed damage; whereas genotypes of IAC 80, Tanggamus and Wilis were susceptible. IAC 80 was suscaptible on three determinant variables, while Tanggamus and Willis were susceptible on one determinant variable. Probably, the location of IAC 80 based on randomization in the greenhouse was positioned close to the sun at noon and light at night, affecting the imago in choosing a place to lay their eggs and larvae to eat IAC 80 than other genotypes.

The nine soybean line can be assessed further for the potential yield if they be released as the new high yielding varieties that resistant to *E. zinckenella*. The three varieties and two accessions identified as *E. zinckenella* resistant can be studied further to ensure the gene resistance role and its mechanism in physiological aspect. These varieties are able to be used as gene sources for development a new resistant superior variety. Also, they can be cultivated in farmer field to reduce the impact of *E. zinckenella* incident. By using these varieties, the yield loss due to the *E. zinckenella* infestation can be decreased.

CONCLUSION

There were 10 genotypes that consistently resistant to *E. zinckenella*, i.e. Tgm/Anj-599, Tgm/Anj-743, Tgm/Anj-778, Tgm/Anj-789, Tgm/Anj-795, Tgm/Anj-824, Tgm/Anj-871, Tgm/Anj-897, Tgm/Anj-908, dan Anjasmoro. The resistance of the resistant genotypes were antixenosis resistance (non-preference resistance) or the rejection of plants because of the morphological characters on the plant causing the insects did not like the plants as food and roost and shelter.

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