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FIELD EVALUATION OF SEVERAL CULTIVATED SOYBEAN VARIETIES AGAINST *EMPOASCA TERMINALIS* (HEMIPTERA: CICADELLIDAE)

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Abstract

Empoasca terminalis Distant (Hemiptera: Cicadellidae) is a new and increasingly important pest of soybean in South Sulawesi Province of Indonesia. Soybean producers rely heavily on insecticide use to control the insect; hence there is an urgency of finding alternative control measures that are effective and safer. The use of resistant cultivars well meet these criteria. Field studies were initiated to evaluate the susceptibility of several commercial soybean varieties against the leafhopper. Twelve soybean cultivars: 'Gepak Kuning', 'Kaba', 'Mahameru', 'Gema', 'Detam-2', 'Grobogan', 'Gepak Ijo', 'Willis', 'Tidar', 'Detam-1', 'Argomulyo', and 'Anjasmoro' were evaluated against *E. terminalis* in 2012 and 2013. The amounts of yield loss due to the leafhopper was also determined in 2012 and 2013. The lowest numbers of adults and nymphs were found on 'Gepak Kuning'; while the highest numbers of adults and nymphs were found on 'Mahameru'. Similarly, the lowest and highest scores of hopperburn were also found on these 2 cultivars, respectively. 'Tidar', 'Gepak Ijo', and 'Kaba' were also resistant to the leafhopper. Leafhopper infestation significantly reduced the yields on susceptible cultivars but not on the resistant ones. Our results also showed that trichome density was not associated with the level of resistance of the cultivars to the leafhopper. Our results suggested that 'Gepak Kuning', 'Gepak Ijo', 'Tidar', and 'Kaba' are resistant to *E. terminalis* and should be used by soybean producers to suppress the pest population.

Key Words: *Empoasca terminalis*, yield, hopperburn, cultivar, resistance

Resumen

Empoasca terminalis Distant (Hemiptera: Cicadellidae) es una nueva y cada vez más importante plaga de la soya en la Provincia Sulawesi del Sur, Indonesia. Se iniciarone studios del campo para evaluar la susceptibilidad de algunas variedades de soya comerciales contra la chicharrita (o saltahoja). El número más bajo de adultos y ninfas se encontrósobre 'Gepak Kuning', mientras que se encontró el mayor número de adultos y ninfas sobre 'Mahameru'. Del mismomodo, los resultados más bajos y más altos de que Madura hecha por la schicharitas se encontró en estos mismos cultivares, respectivamente. Las variedades 'Tidar', 'Gepak Ijo', y 'Kaba' tambien fueron resistentes a la chicharrita. Las infestaciones de las chicharitas redujieron significativamente los rendimientos de cultivares susceptibles, pero no de los resistentes. Nuestros resultados también muestran que la densidad de lastricomas no fue asociada con el nivel de resistencia de los cultivares a las chicharritas. Nuestros resultados sugierenque 'Gepak Kuning', 'Gepak Ijo', 'Tidar', y 'Kaba' son resistentes a *E. terminalis* y deben ser utilizados por los productores de soya para suprimir la población de la plaga. Además, estas variedades se pueden utilizer también como recursos genéticos para el mejoramiento de lasvariedades de soya.

Palabras Clave: *Empoasca terminalis*, rendimiento, quemadura de chicharita, resistencia de cultivares

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Empoasca terminalis Distant (Hemiptera: Cicadellidae) has been reported as a pest on soybean (Parsai & Tiwari 2002), a minor pest on sesame, groundnut (Biswas & Das 2011), and mungbean (Chhabra et al. 1981). The insect is now one of the major insect pests of soybean, and it has been inflicting substantial damage to crops, since it was reported for the first time in South Sulawesi, Indonesia in 2008. The pest can cause yield losses up to 70% and even 24% plant death on susceptible cultivars without insecticide applications during the dry season (Nasruddin 2010).

During the feeding process, both adults and nymphs of the leafhopper imbibe plant sap directly from the vascular system in the leaflet, petiole, and stem of the plant. The feeding process causes not only mechanical damage but also physiological injury due to toxic proteins present in the saliva. The crop physiological disruptions are expressed as hopperburn symptoms on the leaves (Kabrick & Bacus 1990; Eacle & Backus 1994). Reduced photosynthesis and transpiration rates can be substantial in injured plants, and these in turn reduce maturation and growth rates, seed number and weight, and nutrient level (Hutchins et al. 1990). *Empoasca terminalis* can also inflict indirect damage to plant by transmitting phytoplasma diseases (Munyaneza et al. 2008). Up to now, soybean yield losses due to this leafhopper in South Sulawesi are solely caused by direct damage. Leafhopper adult samples collected from the study sites and then sent to Dr. Joseph Munyaneza, Yakima Agricultural Research Laboratory (USDA-ARS), Washington, USA for molecular detection, turned out to be phytoplasma-free (unpublished data).

Currently, growers rely heavily on insecticide use to suppress the leafhopper population. Although, most insecticides recommended for leafhopper control on other crop species were effective against *E. terminalis* (Nasruddin 2011); the use of those chemicals in wide areas from time to time could pose detrimental impacts to non-target organisms and the environment (Pedigo 1996). Thus, alternative control measures, which are effective and safer to the environment, need to be sought. Plant resistance against insect pests very well fits these criteria. Therefore, the purpose of the current study was to evaluate the susceptibility of different soybean cultivars against *E. terminalis* in the field.

MATERIALS AND METHODS

Study Site

Field trials were conducted in farmers' rice fields in Kecamatan Simbang, Kabupaten Maros (S 5° 01' 23" E 119° 39' 39"), South Sulawesi Province, Indonesia. The experiment sites were under the influence of the west coast climate of the province where the rainy and dry seasons are from Nov to Apr and May to Oct, respectively. In the area, most soybeans are planted in the rice paddy during the dry season. In this study, leafhopper infestation occurred naturally. The experiment sites were irrigated weekly in 2012 and for the last 3 weeks in 2013 by inundating the whole site up to 10 cm deep for 4 to 6 h.

Leafhopper Population and Hopperburn Rate

Twelve cultivars ('Gepak Kuning', 'Kaba', 'Mahameru', 'Gema', 'Detam-2', 'Grobogan', 'Gepak Ijo', 'Willis', 'Tidar', 'Detam-1', 'Argomulyo', and 'Anjasmoro') were evaluated for their susceptibility to infestation by *E. terminalis* in the field. Seeds were manually planted on 8 Aug 2012 (mid dry season) and 17 Apr 2013 (early dry season) using a pointed wooden pole. The cultivar treatments were arranged in a randomized complete block design with 4 replications. Each replication consisted of a plot of 8 rows wide and 5 m long. Planting space used was 0.4 m between row centers and 0.2 m within a row. The whole experimental area was fertilized, weeded, and irrigated, following the local cultural recommendations, except that no insecticides were applied during the whole season.

Leafhopper populations were assessed weekly for 5 weeks, starting 2 weeks after plant emergence. Leafhopper adults were sampled using a sweep net (38 cm diam) with 5 round-sweeps per plot (Harper et al. 1993). The leafhoppers were then brought back to the laboratory for identification and count. The number of nymphs was determined weekly by direct count on 16 middle leaves randomly selected from 2 middle rows in each plot 3 days before adult sampling.

Ten plants per plot were observed for hopperburn damage on 15 Sep 2012 and 20 May 2013 (the peak dates of leafhopper density). The following 0–5 scale was used to assess the rate of hopperburn injury: 0, no visible symptoms; 1, slight cupping of leaves; 2, slight cupping of leaves with yellowing of leaf margins; 3, many leaves cupped and yellowed; 4, plants stunted and showing leaf scorch; and 5, all leaves with severe hopperburn and plants severely stunted. This scale is based on the one used for scoring foliar damage caused by the potato leafhopper (*Empoasca fabae* Harris) on soybean (Schaafsma et al. 1998).

Because there were conflicting reports on the association of leaf pubescence with soybean resistance to leafhoppers (Schaafsma 1998; Pillemer & Tingey 1978), the trichome densities of the cultivars were determined using 5 uppermost fully developed leaflets randomly collected from each plot; hence 20 leaflets were sampled for each cultivar during mid-season (15 Sep 2012 and 20 May 2013). The number of hairs per 1 cm^2 of the lower leaf surface was assessed by placing a template with 1-cm^2 opening next to the midrib at the middle of each leaflet and then observed under a dissecting microscope (40–100X).

Effect of Leafhopper Infestation

Effects of *E. terminalis* infestation on several plant variables of 5 soybean cultivars (('Gepak Kuning', 'Mahameru', 'Gepak Ijo', and 'Anjasmoro') and 8 soybean cultivars ('Gepak Kuning', 'Kaba','Burangrang', 'Mahameru', 'Willis', 'Gepak Ijo', 'Argomulyo', and 'Anjasmoro') were evaluated in the field in 2012 and 2013, respectively. Seeds were manually planted on 8 Aug 2012 and 17 Apr 2013 in Kecamatan Simbang, Kabupaten Maros. The experiments were arranged in a split-plot design with cultivar as the main plot and leafhopper population level as the sub-plot. Two levels of leafhopper populations were used in this experiment: leafhopper absent and leafhopper present, which were achieved by weekly insecticide applications and without insecticide applications for the whole season, respectively. Each treatment combination had 4 replications, and each replication consisted of a plot of 8 rows wide and 4 m long. Planting spaces used were 0.4 m between row centers and 0.20 m within a row. The whole experimental area was fertilized, weeded, and watered, following the local cultural recommendations.

Before harvest, 20 plants per plot were randomly selected to determine plant height. The plants were harvested by cutting their stems close to the ground and then placed in separate plastic bags. The plants were used to determine several variables: pod number per plant and dry seed weight per plant (2012) and wet biomass weight per plant, pod number per plant, dry seed weight per plant, and weight per 100 dry seeds (2013). Beans were dried under the sun until their water content was about 18% before seeds were weighed to determine the yield weight (g) per plant. The dry yield weight per plant was used to calculate the equivalent yield per ha. The yield loss due to leafhopper was calculated using the following formula: Yield loss = Yield (leafhopper absent) – Yield (leafhopper present).

Data Analysis

Data of leafhopper population were transformed by log (n + 1) before being subjected to a two-way analysis of variance (ANOVA) to determine the effects of year and soybean cultivar. Since year effect was not significant on trichome density, the data were pooled before ANOVA was performed. Average number of adults, nymphs, and hopperburn rates were separated using Tukey's HSD test at 5% level. Pearson's correlation coefficients were calculated to determine the relationship between the trichome density and the leafhopper populations. For each cultivar, the differences between plant with leafhopper presence and plant with leafhopper absent in each plant variable were compared using a paired-T test at 5% level (BioStat 2009).

Results

Leafhopper Population and Hopperburn Rate

Year effect on the number of adults and nymphs was significant in this experiment ($P < 0.001$ for both adult and nymph). Average numbers of adults and nymphs were significantly greater in 2012 than 2013. The average number of adults was highly variable among soybean cultivars in both years ($P < 0.001$). Similarly, the average number of nymphs was also highly variable among soybean cultivars in each year ($P < 0.001$ (2012) and ($P < 0.01$ (2013)] (Table 1). In both years, 'Gepak Kuning' consistently had the fewest numbers of adults. The numbers of nymphs found on 'Gepak Kuning', 'Tidar', 'Willis', and 'Kaba' were not significantly different from each other but lower than those of the other cultivars. 'Mahameru' had the most adults and nymphs among cultivars evaluated. Similarly, ANOVA detected a significant difference among cultivar entries in

Table 1. Mean numbers of leafhopper adult and nymph, and hopperburn scores on 12 soybean cultivars exposed to natural infestation of *E. terminalis* in 2012 and 2013 in South Sulawesi Province, Indonesia.

	Adult /5 sweeps[1]		Nymphs/16 trifoliates[1]		Hopperburn Score[2]	
Cultivar	2012	2013	2012	2013	2012	2013
'Argomulyo'	47.7 ef	23.0 b	27.6 b	14.7 ab	3.3 cde	3.2 de
'Grobogan'	34.7 cd	31.0 c	27.9 b	21.3 cd	3.0 cd	2.2 cd
'Kaba'	57.7 gh	22.0 b	15.3 ab	10.7 ab	1.5 b	1.0 ab
'Detam-1'	38.7 de	28.3 bc	24.5 ab	17.3 bc	2.5 c	2.4 cde
'Mahameru'	65.3 gh	51.3 d	52.4 c	34.7 f	4.6 f	4.4 f
'Gema'	68.3 h	50.3 d	18.3 ab	13.3 ab	4.0 ef	3.4 ef
'Detam-2'	55.3 fg	47.0 d	36.8 b	29.3 ef	3.9 def	3.2 de
'Anjasmoro'	51.0 ef	26.3 bc	23.8 ab	22.7 cd	2.8 c	1.4 abc
'Tidar'	33.3 cd	22.0 b	20.5 ab	13.4 ab	0.7 ab	0.6 a
'Willis'	25.7 bc	11.7 a	15.8 ab	8.0 a	2.5 c	1.8 bc
'Gepak Kuning'	12.3 a	10.7 a	13.1 a	7.3 a	0.5 a	0.4 a
'Gepak Ijo'	17.3 ab	19.3 b	19.8 ab	26.7 de	0.7 ab	0.4 a

Means within the same column with different letters differ significantly ($P < 0.05$) by a Tukey's test.

[1]Average of 5 weekly counts made from Aug 29 to Sep 26, 2012 and from May 8 to Jun 5, 2013.

[2]Hopperburn damage rated at mid season (Sep 19, 2012 and May 29, 2013).

Hopperburn scale: 0, no visible symptom; 1, slight cupping of leaf; 2, slight cupping of leaf with yellowing of leaf margin; 3, many leaves cupped and yellowed; 4, plants stunted and showing leaf scorch; and 5, all leaves with severe hopperburn and plants severely stunted (Schaafsma et al. 1998).

hopperburn rates (Table 1). In both years, 'Gepak Kuning', 'Gepak Ijo', 'Tidar', and 'Kaba' sustained the least hopperburn damage; while Mahameru suffered the most hopperburn damage. 'Gepak Ijo' hosted significantly more adults and nymphs than did 'Gepak Kuning' in 2013 but hopperburn rate on the cultivar was not significantly different from the rate on 'Gepak Kuning' (Table 1). On the other hand, 'Willis' had low number of nymphs but sustained higher hopperburn damage.

Trichome densities (number of hairs per cm^2) among the soybean cultivars differed significantly (Fig. 1). However, trichome density was not correlated with the number of adults or nymphs found on each cultivar [adult: $r = 0.2$, $P > 0.05$ (2012), $r = 0.22$, $P > 0.05$ (2013)]; [nymph: $r = 0.55$, $P > 0.05$ (2012), $r = 0.45$, $P > 0.05$ (2013)]. 'Mahameru' had the highest number of hairs per cm^2 but also had the highest numbers of adults and nymphs. On the other hand, 'Gepak Kuning' had the lowest trichome density but also had the lowest numbers of adults and nymphs.

Effect of Leafhopper Infestation

Leafhopper presence differently affected the plant variables observed (plant height, wet biomass, pod number, dry seed weight, and weight of 100 dry seeds) among the cultivars (Table 2). For 'Gepak Kuning', leafhopper presence did not significantly affect those variables; while for 'Mahameru', 'Burangrang', and 'Argomulyo', leafhopper presence significantly affected all plant variables. For other cultivars, effects of leafhopper presence varied. All plant variables except plant height were significantly affected by the leafhopper infestation of 'Kaba'. For 'Anjasmoro' and 'Gepak Ijo', only wet biomass and pod number per plant were significantly affected by the leafhopper infestation; while for 'Willis' only wet biomass and dry seed weights were significantly affected by the leafhopper attack.

Leafhopper inflicted yield losses on all cultivars with varying degrees from 3.5% ('Gepak Kuning') to 39.3% ('Argomulyo') in 2012; and 2.5% ('Gepak Kuning') to 25.1% ('Mahameru') in 2013. However, the leafhopper presence did not significantly reduce yield in 'Gepak Kuning', 'Anjasmoro', 'Kaba', and 'Gepak Ijo' (Table 3).

Discussion

Leafhopper density in 2012 was higher than 2013. This is probably due to the higher rainfall during 2013 season than in the 2012 season. Higher leafhopper population on grapevines was associated with lower rainfall (Marais 1988). Besides that, the lower leafhopper population in 2013 can also be attributable to the early planting time (14 Apr 2013), which was just right after the second rice planting was harvested; therefore, the initial infestation of leafhopper was low.

Substantial variation among soybean cultivars in their susceptibility against *E. terminalis* was documented in this study. The adult leafhopper abundance on the susceptible cultivar ('Mahameru') was more than 5-fold greater than on the resistant cultivar ('Gepak Kuning') in both years. This finding contradicts a previous report that the number of adult leafhoppers was not affected by the level of cultivar resistance against the insect (Kornegay et al. 1989). 'Mahameru' is probably

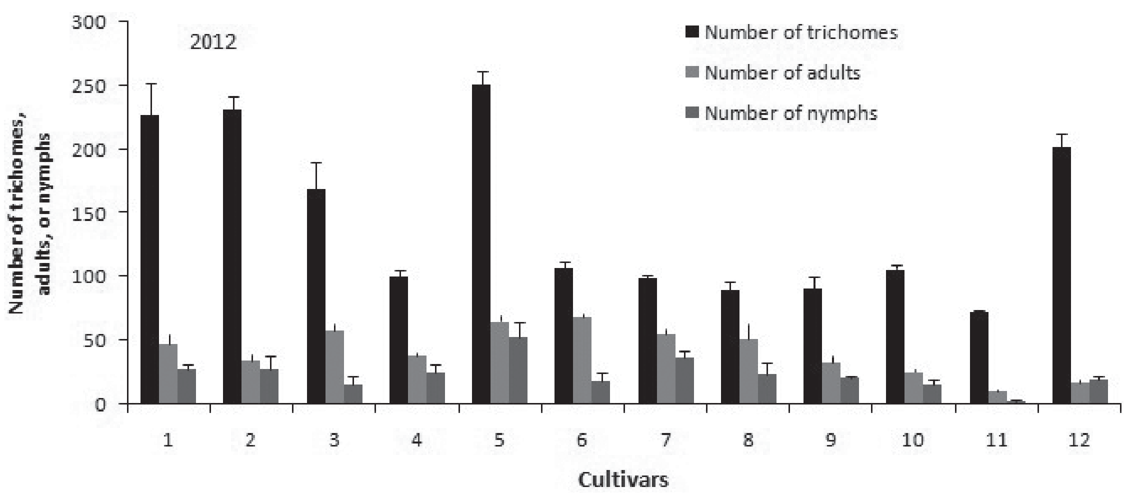

Fig. 1. Average numbers of trichomes per cm^2, leafhopper adults per 5 sweeps, and leafhopper nymphs per 16 trifoliates on twelve cultivated soybean varieties in 2012 and 2013. Cultivated soybean varieties: 1, 'Argomulyo'; 2, 'Grobogan'; 3, 'Kaba'; 4, 'Detam-1'; 5, 'Mahameru'; 6, 'Gema'; 7, 'Detam-2'; 8, 'Anjasmor'o; 9, 'Tidar'; 10, 'Willis'; 11, 'Gepak Kuning'; and 12, 'Gepak Ijo'. Means + SE.

TABLE 2. PLANT HEIGHT, WET BIOMASS WEIGHT, POD NUMBER, DRY SEED WEIGHT, AND WEIGHT OF 100 DRY SEEDS OF THE PLANTS WITH OR WITHOUT LEAFHOPPER INFESTATION IN 2012 AND 2013.

	Plant height		Wet biomass (g/plant)		Pod number per plant		Dry seed weight (g/plant)		Weight of 100 dry seeds (g)	
Cultivar	LH absent	LH present	LH absent	LH present	LH absent	LH present	LH absent	LH present	LH absent	LH present
2012										
‘Gepak Kuning’	51.2 a	49.5 a	—	—	54.0 a	46.6 a	10.2 a	8.4 a	—	—
‘Anjasmoro’	53.3 a	48.0 a	—	—	29.8 a	22.5 b	7.8 a	6.4 b	—	—
‘Gepak Ijo’	52.4 a	48.0 a	—	—	35.1 a	26.8 b	7.3 a	6.7 a	—	—
‘Argomulyo’	46.8 a	34.7 b	—	—	21.8 a	14.6 b	8.1 a	5.4 b	—	—
‘Mahameru’	59.3 a	37.7 b	—	—	42.1 a	24.3 b	7.9 a	5.9 b	—	—
2013										
‘Gepak Kuning’	48.6 a	44.9 a	49.2 a	48.6 a	49.7 a	46.2 a	8.2 a	7.6 a	9.2 a	9.2 a
‘Kaba’	45.9 a	39.8 a	65.8 a	41.5 b	37.1 a	26.0 b	9.4 a	5.6 b	10.9 a	10.4 b
‘Burangrang’	53.1 a	44.5 b	41.2 a	19.2 b	23.4 a	12.4 b	5.0 a	4.1 b	16.3 a	14.6 b
‘Anjasmoro’	49.4 a	43.0 a	51.0 a	36.6 b	30.9 a	24.2 b	7.4 a	6.8 a	10.9 a	10.3 a
‘Gepak Ijo’	53.7 a	47.5 a	44.4 a	34.8 b	39.1 a	24.7 b	7.0 a	6.8 a	11.2 a	11.0 a
‘Willis’	48.2 a	43.0 a	48.2 a	32.7 b	35.7 a	32.7 a	7.2 a	5.5 b	10.9 a	10.3 a
‘Argomulyo’	47.5 a	33.8 b	41.7 a	27.7 b	24.8 a	12.6 b	7.6 a	4.6 b	15.8 a	14.2 b
‘Mahameru’	55.2 a	34.5 b	71.2 a	52,3 b	41.7 a	22.0 b	7.4 a	5.2 b	15.4 a	11.3 b

For each plant variable, means within the same row with different letters differ significantly ($P < 0.05$) by a paired-T test.

TABLE 3. AVERAGE YIELDS (X ± SE) OF SEVERAL SOYBEAN VARIETIES INFESTED AND NON-INFESTED BY *E. TERMINALIS* AND YIELD LOSSES DUE TO THE LEAFHOPPER IN 2012 AND 2013.

Cultivar	Average Yield (kg/ha)[1]	Yield (kg/ha) Leafhopper absent[2]	Yield (kg/ha) Leafhopper present[3]	Yield loss (kg/ha) due to leafhopper (%)[4]
2012				
'Gepak Kuning'	2,860	2,780 ± 231 a	2,602 ± 213 a	98 (3.5)
'Anjasmoro'	2,250	2,010 ± 296 a	1,890 ± 115 a	120 (6.0)
'Gepak Ijo'	2,680	2,250 ± 181 a	2.110 ± 245 a	140 (6.2)
'Argomulyo'	2,000	1,857 ± 96 a	1,425 ± 125 b	750 (39.3)
'Mahameru'	2.160	1,814 ± 74 a	1.345 ± 102 b	675 (36.2)
2013				
'Gepak Kuning'	2,860	2,657 ± 251 a	2,591 ± 185 a	66 (2.5)
'Kaba'	2,130	2,350 ± 272 a	2,254 ± 89 a	96 (4.1)
'Burangrang'	2,500	2,225 ± 242 a	1,825 ± 64 b	400 (18)
'Anjasmoro'	2,250	1,850 ± 334 a	1,700 ± 295 a	150 (8.1)
'Gepak Ijo'	2,680	1,750 ± 281 a	1,700 ± 550 a	50 (2.9)
'Willis'	1,600	1,800 ± 78 a	1,682 ± 85 b	118 (6.5)
'Argomulyo'	2,000	1,907 ± 104 a	1,552 ± 225 b	355 (18.6)
'Mahameru'	2.160	1,890 ± 74 a	1.415 ± 102 b	475 (25.1)

For each variety, yield means within the same row with different letters differ significantly ($P < 0.05$) by a paired-T test.
[1]Average potential yields published in "Deskripsi Varietas Unggul Kedelai"
[2]Plants weekly sprayed with insecticide
[3]Plants unsprayed for the whole season
[4]Numbers in parentheses indicating percentages of yield loss due to the leafhopper

more preferred by adult leafhoppers due to its physical appearance with its taller stem and larger and lighter-colored leaves than 'Gepak Kuning'. Bullas-Appleton et al. (2004) pointed out that the potato leafhopper (*E. fabae*) preference was significantly influenced by the leaf color of edible beans.

Despite some reports suggesting an association between the density of foliar trichomes and the numbers of leafhopper adults and nymphs (Pillemer & Tingey 1978), our results showed that trichome densities of the varieties was not associated with their resistance levels against *E. terminalis*. This result is in agreement with Broersma et al. (1972), Kornegay et al. (1989), and Schaafsma et al. (1998), who reported that trichome density was a poor indicator for plant resistance to leafhoppers. The smallest number of leafhoppers was found on 'Gepak Kuning', though the variety had the least dense trichome; and vice versa, the greatest number of leafhoppers was present on 'Mahameru' whose trichomes were the densest among cultivar entries. Therefore, antibiosis may play a role in the resistance mechanism of the cultivars in addition possibly to the non-preference mechanism for the adults as described before. 'Gepak Ijo' could host larger numbers of leafhoppers while sustaining lower hopperburn damage and yield loss, suggesting that the cultivar may react as tolerant to the leafhopper infestation. Lines with high nymphal populations but lower hopperburn scores predominantly express a tolerance defense mechanism (Schaafsma et al. 1998).

This study had successfully identified 4 soybean cultivated varieties resistant to *E. terminalis*, a new yet damaging pest of soybean. 'Gepak Kuning', 'Gepak Ijo', 'Tidar', and 'Kaba' had smaller leafhopper populations and sustained less hopperburn damage than the other cultivars studied. Leafhopper infestations on these resistant cultivars did not cause significant yield reduction. These varieties should be adopted by soybean producers to control the pest and to reduce the amount of insecticide used in their operations. However, further studies are necessary to elucidate the exact resistance mechanism and identify genetic materials that can be incorporated into breeding programs for the improvement of tropical soybean cultivars.

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