INTRODUCTION

*Nezara viridula* is one of the insect pests feeding immature soybean pods, beside *Piezodorus hybneri* and *Riptortus linearis*. As a polyphagous insect, *N. viridula* includes feeds more than 30 plant families. Soybean is one of the legumes that is suitable food for this stink bug (Olson et al., 2018). Stink bugs have stylets, piercing-sucking mouthparts. The stylets pierce plants producing aesthetic damage (Lomate & Bonning, 2016). The imago and nymphs damage pods and seeds by sticking the stylet into the skin of the pod until it reaches the seed and then sucks the liquid of the seed (Depieri & Panizzi, 2011). The young pod attacked by *N. viridula* causes the seed to shrink and the pods to fall (Too1 & Turnipseed, 1974). Stink bug saliva and digestive enzymes are the important substrates for damaging the plants (Silva, da Silva, Depieri, & Panizzi, 2012). The higher stink bug population increases the damage of soybean pods and seeds (Venugopal, Coffey, Dively, & Lamp, 2014). The feeding of stink bug on the pod can have direct effect on the quantity and quality of the yield (Sosa-Gómez et al., 2019).

To overcome pest attacks on soybeans should use the concept of integrated pest control (IPM), by using resistant varieties, technical culture, the use of natural enemies, the use of synthetic biopesticides and chemical pesticides. From the various components, biological control is an important component because of its roles as a key ecosystem service of IPM (Naranjo, Ellsworth, & Frisvold, 2015). Usually, the use of chemical pesticides become the first control attempt because chemical pesticides result quickly impact on the pest population. However, this pest control has side effects, such as poisoning in humans and animals, decreasing natural enemies, the emergence of resistance and resurgence of the pests, and environmental pollution (Carvalho, 2017; Damalas & Koutroubas, 2016; Nicolopoulou-Stamati, Maipas, Kotampasi, Stamatis, & Hens, 2016). Beside, insecticide resistance appears if the control relies exclusively on the use of classical chemical insecticides (Snodgrass & Scott, 2003).
The use of resistant varieties can be an alternative way to control the pest because it has some advantages in culture technique, environmentally friendly, and economic benefit (Smith, 2005). The use of varieties can be done by identifying the varieties to the behavior of the insects. Insects attracted to plants are for oviposition (Endo, 2016), shelter, and food (Olson, Ruberson, & Andow, 2016). The vegetative parts of plants that are used as food by the stink bugs (Lomate & Bonning, 2016), such as leaves, stalks, flowers, fruit, roots, and the liquid of plant. Naturally, insect pests will be able to choose the preferred food source. Insects will have a certain tendency to access the food source. The difference in texture and structure, the type of variety and the chemical composition contained in an ingredient will have a major influence on the nature of the preference (Ederli et al., 2017; Gordon, Haseeb, Kanga, & Legaspi, 2017; Lomate & Bonning, 2016).

Considering the remarkable losses in productivity and quality of soybeans, and finding out biology control to the stink bug, this study was conducted to evaluate twenty soybean varieties to Nezara viridula infestation.

MATERIALS AND METHODS

Study Sites
Research was conducted in 2016 at Muneng Research Station, Probolinggo Regency, East Java Province, Indonesia. The coordinate of the site is 7° 48′ 7.2″S 113° 9′ 32.4″E, while the altitude is 10 m above sea level. The soil type of Muneng Research Station is Alfisols.

Plant Materials
Twenty soybean varieties were used as plant materials, consisted of Argomulyo (Arm), Argopuro (Arp), Burangrang (Bur), Cikuray (Cik), Dering 1 (Der1), Detam 1 (Det1), Detam 2 (Det2), Galunggung (Gal), Gepak Kuning (Gep), Grobogan (Gro), Gumitir (Gum), Kaba (Kab), Malabar (Mal), Merbabu (Mer), Panderman (Pan), Seulawah (Seu), Sibayak (Sib), Sinabung (Sin), Tamponas (Tam), and Tanggamus (Tan). The background of the varieties used was diverse from various release targets, such as agroecological adaptation, resistance to pests and diseases, plant morphology, and nutritional content of the seeds.

Design and Planting
Randomized complete block design (RCBD) with three replicates was applied in the experiment. Planting was carried out using planting space of 40 cm x 15 cm. There were two rows with 3 m length for each variety. The fertilizers were 50 kg/ha Urea, 75 kg/ha SP36, and 75 kg/ha KCl. Before planting, the seed was applied with 12.5 g carbosulfan per kg seed to prevent the seedling fly-pests. Weeding was applied at 3, 6 and 9 weeks after planting.

Pest Infestation
Forty days before the main experiment was conducted, the plants for rearing naturally of N. viridula were grown around the main experiment plots. The plants of the infestation plots were harvested at pod filling period to allow the N. viridula population migrated to the main experiment plots.

Resistance to pest was calculated as:

\[
\text{Resistance to pest} = \frac{\text{number of uninfilled pods}}{\text{number of total pods}} \times 100
\]

The resistance category was determined according to Chiang & Talekar (1980) methods using averages and standard deviations, and then grouped into (1) Highly resistant (HR):< X-2SD; (2) Resistant (R): X-2SD to X-SD; (3) Moderately resistant (MR): X-SD to X; (4) Susceptible (S): X to X+SD; and (5) Highly susceptible (HS): >X+2SD.

The correlation among characters was analyzed by using Pearson correlation.

RESULTS AND DISCUSSION

The analysis of variance shows that all agronomical characters differed significantly (Table 1). This shows that there are differences between the varieties which were expressed on the agronomics characters. This difference occurred because the genetic background of the varieties tested was different, including the target agroecological differences of the variety. The implications of agroecological differences lead to differences in agronomical character. Finally, agronomical performance impacts its response to stink bug (Nezara viridula) attacks. Some authors also reported differences in the response of soybean genotypes to stink bug (da Graça et al., 2016; Nomayanja, Tukamuhubwa, & Kyamanywa, 2000; Souza et al., 2016). The different response may be due to different content of soluble leaf phenolics (Zavala, Mazza, Dillon, Chludil, & Ballaré, 2015), the developmental stage of the plant (Kamminga, Koppel, Herbert Jr., & Kuhar, 2012) and developmental stage of the stink bug (Molina & Trumper, 2012).

The days to flowering of the varieties tested varied, where the earliest days to flowering was achieved by Grobogan with 25 days followed by Argomulyo and Argopuro with 29 days (Fig. 1).
The latest days to flowering was achieved by Seulawah with 43 days. There were no other varieties that had days to flowering more than 40 days, except Seulawah. Seulawah is a variety developed for acid dry land agroecology. Other varieties released for acid dry land are Tanggamus and Sibayak. Tanggamus had days to flowering of 38 days, while Sibayak had days to flowering of 37 days. The difference of days to flowering impacts on the development of the stink bug because it causes different initial reproductive stage which initiates in providing feed for stink bug. The period during bloom and early pod stages are the stages when stink bugs are highly attracted to soybean (McPherson, Todd, & Yeargan, 1994).

### Table 1. Analysis of variance of agronomical characters of soybean varieties

| Agronomical character                          | Rep. | Genotype | Error |
|-----------------------------------------------|------|----------|-------|
| Days to flowering                             | 8.52 | 45.46**  | 3.01  |
| Days to maturity                              | 29.82** | 45.22** | 5.57  |
| Duration of reproductive phase (days)         | 48.87** | 125.60** | 8.52  |
| Plant height (cm)                              | 141.96** | 756.63** | 27.19 |
| Number of branches per plant                   | 0.61  | 3.84**   | 0.23  |
| Number of reproductive nodes per plant         | 0.88  | 11.07**  | 0.83  |
| Number of filled pods per plant                | 702.82 | 2203.64** | 377.09 |
| Number of unfilled pods per plant              | 334.92* | 425.8** | 87.55 |
| Number of total pods per plant                 | 70.53 | 2627.11** | 397.7 |
| Resistance to pest                             | 0.06* | 0.11**   | 0.02  |
| Weight of 100 seeds (g)                        | 0.89  | 28.39**  | 3.33  |
| Seed yield (t/ha)                              | 0.58  | 1.63**   | 0.33  |

Remarks: The numbers which are followed by the same letter are not similar based on the HSD 5%; HR = highly resistant, R = resistant, MR = moderately resistant, S = Susceptible, HS = highly susceptible
Resistance to a pest is an important character because it can describe the response of varieties to stink bug infestation. Of the 20 varieties tested, Argomulyo was the most preferred by stink bug, followed by Grobogan and Malabar. The resistance to the pest in these three varieties were 0.83, 0.70 and 0.68, respectively. This condition showed that Argomulyo was susceptible to stink bug infestation until 83% of the pods did not fill properly. Varieties with the least resistance to pest were shown by Gepak Kuning, Seulawah and Sinabung, with a ratio of 0.17, 0.17 and 0.19 (Table 2). These three varieties were not favored by the stink bug. This non preference was not due to differences in availability of pods, but it was caused by other plant factors. This is because Gepak Kuning is early maturing, while Sinabung is medium maturing and Seulawah is late maturing (Balitkabi, 2016). However, in this study the three varieties had moderate maturing (Fig. 2), which allegedly changes in age due to the stink bug infestation. This study similar to Da Fonseca Santos, Möller, Clough, & Pinheiro (2018) stating that resistant genotypes are indicated by lower ratio of pod damage.

From the resistance to a pest, the criteria of soybean resistance to stink bug were obtained. Three varieties were resistant to stink bug, namely Gepak Kuning, Seulawah and Sinabung, Argomulyo was the only variety that had a category as highly susceptible. Other varieties were moderate resistant (Argopuro, Cikuray, Dering 1, Gumitir, Kaba, Merbabu, Sibayak, Tamponas, and Tanggamus), moderately susceptible (Burangrang, Detam 1, Detam 2, Galunggung, and Panderman), and susceptible (Grobogan and Merbabu). However, Grobogan and Malabar need to get more attention because even though they were included as susceptible, the resistance to the pest of these two varieties was high (Table 2). This study is different from that reported by Nomayanja, Tukamuhubwa, & Kyamanywa (2000) that varieties with long maturity period are preferred by the insects. This study is similar to Daugherty et al. (1964) who demonstrated that varieties with shorter duration of reproductive are less damaged by a stink bug. Argopuro, Grobogan, and Argomulyo were varieties with long reproductive duration, i.e. 71.34, 65.33, and 62.66 days respectively (Fig. 3). Argomulyo and Grobogan were included as highly susceptible and susceptible, respectively. Although the duration of the reproductive phase of Argopuro was long, the response to stink bug was moderate resistant. Seulawah, Gepak Kuning and Tanggamus had a reproductive duration of 42.33, 47.00, and 48.67 days. Seulawah and Gepak Kuning was resistant, while Tanggamus was moderate resistant.

| Variety     | Resistance to pest | Resistance criteria |
|-------------|--------------------|---------------------|
| Argomulyo   | 0.83*              | HS                  |
| Argopuro    | 0.34*              | MR                  |
| Burangrang  | 0.43*              | MS                  |
| Cikuray     | 0.26*              | MR                  |
| Dering 1    | 0.29*              | MR                  |
| Detam 1     | 0.43*              | MS                  |
| Detam 2     | 0.42*              | MS                  |
| Galunggung  | 0.57*              | MS                  |
| Gepak Kuning| 0.17*              | R                   |
| Grobogan    | 0.70*              | S                   |
| Gumitir     | 0.22*              | MR                  |
| Kaba        | 0.21*              | MR                  |
| Malabar     | 0.68*              | S                   |
| Merbabu     | 0.31*              | MR                  |
| Panderman   | 0.54*              | MS                  |
| Seulawah    | 0.17*              | R                   |
| Sibayak     | 0.30*              | MR                  |
| Sinabung    | 0.19*              | R                   |
| Tamponas    | 0.22*              | MR                  |
| Tanggamus   | 0.36*              | MR                  |
| HSD (5%)    | 0.4                |                     |

The days to maturity of the varieties tested was also varied. The earliest days to maturity was achieved by Gepak Kuning although the days to flowering was longer than Grobogan. Argopuro was the variety that had the longest days to maturity (100 days) (Fig. 2). It seems that the days to maturity in this study is influenced by pod sucking pests because all varieties had longer days to maturity than those listed in the description of varieties (Balitkabi, 2016). Early maturing soybeans such as Malabar and Grobogan which had the days to maturity of 70 and 76 (Balitkabi, 2016), in this study the days to maturity of Malabar and Grobogan reached 87 and 90 days, respectively. Boethel et al. (2000) also reported delays in soybean maturity. This maturity delay is called “green stem syndrome” which is resulted from Nezara viridula infestation.
Fig. 2. Days to maturity of twenty soybean varieties

Fig. 3. Duration of reproductive phase of twenty soybean varieties
The delay varies from several days to weeks, without leaves senescing and pods ripening. This syndrome has a relationship with the density of the stink bug, where a density of 8 adults per 0.3 m increases the number of green leaves (Vyavhare, Way, & Medina, 2015). However, Hobbs et al. (2006) demonstrated that stink bug feeding did not affect the incidence of “green stem syndrome”.

The duration of reproductive phase is the phase between the days to flowering and days to maturity. The duration of the longest reproductive phase was achieved by Argomulyo, Argopuro, and Burangrang, with 62.66, 71.34, and 56.33 days respectively, while the shortest was achieved by Tanggamus, Tampomas and Sinabung, with 48.67, 49.33, and 52 days respectively (Fig. 3). In this reproductive phase, the stage from flowering to pod formation is important because the maximum stink bug infestation occurs in this stage (Biswas, 2013). The population of *N. viridula* at soybean reproductive stage is abundant (Herbert & Toews, 2012). Molina & Trumper (2012) reported that fifth instar nymphs to have a higher attraction than the adult females on the R7 and R8 of soybean pods stage. Oliveira & Panizzi (2003) demonstrated that the performance of *N. viridula* increases when feeding soybean pods of the R7 phenological stage.

The plant heights of the tested varieties were high, except for early maturity varieties such as Malabar, Grobogan, and Argomulyo that had medium plant height. Pandeman which is a medium-aged variety (Balitkabi, 2016) also had a medium plant height in this study. The highest plant height achieved by Seulawah followed by Sibayak with 106 and 103 cm, respectively (Fig. 4). In general, the plant heights in this study were higher than those listed in the description of varieties (Balitkabi, 2016). It is suspected that this condition occurs because of the “green stem syndrome” which causes the plant stem always to be green which indicates the stem of the plant in the vegetative period due to the high nutrients accumulation in the stems. Egli & Bruening (2007) reported a linear relationship between soluble sugar accumulation in the stem and “green stem syndrome”.

The highest number of branches was achieved by Sibayak (6 branches), while the least was achieved by Pandeman (1.57 branches) (Fig. 5). Similar to plant height, early maturing varieties had fewer branches than the late maturing varieties.

![Plant height of twenty soybean varieties](image)
Branch is one of the plant organs that is favored by Stink bugs to perch. Zavala, Mazza, Dillon, Chludil, & Ballaré (2015) stated that stink bugs prefer branches with attenuated UV-B treatment which correlates with isoflavonoid content. It indicates that stink bug preference to the plant branches depends on the branches traits, including antibiosis. The chemical concentration and composition is not necessarily the same between plant organs in the same plant (Schoonhoven, van Loon, & Dicke, 2005). The chemical composition of the plants affects the stink bug’s food selection because the food resources quality affects the performance of stink bugs (Scheirs, Jordaens, & De Bruyn, 2005).

The number of reproductive nodes is similar to plant height and number of branches per plant, where early maturing varieties had fewer reproductive nodes than late maturing varieties. Grobogan was the least number of reproductive nodes, followed by Malabar and Argomulyo. Panderman, the medium-aged variety, also had a small number of reproductive nodes (Fig. 6). Reproductive nodes are important for stink bugs because reproductive nodes are the location of pods as their main food.

The number of total pods is the highest potential of the number of filled pods if all pods can grow and develop properly. The total number of pods is most widely achieved by Sibayak and Merbabu, which were 144.9 and 137.3 pods, respectively. Another variety with more than 100 pods per plant was Tanggamus with 106.63 pods. Grobogan was the variety with the least number of total pods, namely 28.3 pods. Grobogan followed by Merbabu, while the least number of filled pods was achieved by Grobogan and Argomulyo (Fig. 8). The number of filled pods in these two early maturity varieties of Grobogan (7.90 pods) and Argomulyo (8.53 pods) were very small compared to other varieties. Argomulyo, which had the least number of filled pods, turned out to have a number of unfilled pods (42.17 pods) equivalent to Malabar (52.4 pods), Merbabu (43.6 pods), and Sibayak (44.1 pods) as the varieties with the most numerous unfilled pods. It is different to Grobogan, where the number of unfilled pods was 20.4 pods, less than Argomulyo (Fig. 9).

Fig. 5. Number of branches of twenty soybean varieties
Fig. 6. Number of reproductive nodes of twenty soybean varieties

Fig. 7. Number of total pods of twenty soybean varieties
Fig. 8. Number of filled pods of twenty soybean varieties

Fig. 9. Number unfilled pods of twenty soybean varieties
The weight of 100 seeds from the 20 varieties varied from 8.80-19.81 g. The smallest seed size was achieved by Seulawah and Gepak Kuning, namely 8.80 and 8.90 g/100 seeds, while the largest seeds were achieved by Argomulyo and Grobogan with 19.81 and 19.19 g/100 seeds (Fig. 10). The size of the seed is in accordance with the description of the variety (Balitkabi, 2016), so that the stink bug attack did not decrease the size of the seeds. Varieties with large seed size, namely Argomulyo (19.81 g/100 seeds) and Grobogan (19.19 g/100 seeds), were highly susceptible to stink bug infestation. Da Fonseca Santos, Möller, Clough, & Pinheiro (2018) also reported the soybean with small seed to be resistant to stink bug infestation. However, the large seed size varieties of Argopuro (16.43 g/100 seeds) were moderately resistant to stink bug attack (Table 2). This variety may have antixenosis or antibiosis to defense from stink bug attack. Antixenosis mechanisms have been reported, such as pod hardness (Silva, Baldin, Canassa, Souza, & Lourenço, 2014), the rigid epidermis of the pod (de Santana Souza, Lopes Baldin, da Silva, & Lourenço, 2013), cell walls hardness of seed (Giacometti, Ilina, Eduardo, & Zavala, 2018), and trichome densities (Nomayanja, Tukamuhubwa, & Kyamanywa, 2000). Antibiosis mechanisms also have been reported, such as daidzin and genistin content in pods (Zavala, Mazza, Dillon, Chludil, & Ballaré, 2015) and daidzin and genistin content seed (Piubelli, Hoffmann-Campo, De Arruda, Franchini, & Lara, 2003), and flavonoids content in immature seeds (da Graça et al., 2016; Piubelli, Hoffmann-Campo, De Arruda, Franchini, & Lara, 2003).

The seed yield is the result of the response of various yield components to the stink bug infestation. Seed yields from the 20 varieties varied (Fig. 11). Varieties with the highest seed yield were Sinabung (2.95 t/ha) followed by Merbabu (2.68 t/ha) and Sibayak (2.62 t/ha). Seed yield is one of the criteria for plant resistance to pests besides the small seed and lower ratio of pod damage (Da Fonseca Santos, Möller, Clough, & Pinheiro, 2018). Based on the resistance to a pest, two of the three high yielding varieties, namely Sinabung and Sibayak, were classified as resistant and moderately resistant to stink bug, while Merbabu was moderately resistant to stink bug. Gepak Kuning as resistant variety only had seed yield of 1.82 t/ha. The low seed yield of Gepak Kuning due to the size of the seeds was small (8.90 g/100 seeds), although the number of filled pods per plant was high (83 pods).

The lowest seed yield was achieved by Grobogan (0.42 t/ha), Galunggung (0.48 t/ha), and Argomulyo (0.68 t/ha). Grobogan and Argomulyo were susceptible and highly susceptible respectively, while Galunggung was moderate susceptible. Vyavhare, Way, & Medina (2015) reported that decreasing seed yield in susceptible varieties is caused by decreasing seed weight and increasing number of unfilled pods. In this study, the number of unfilled pods increased, but the seed weight did not decrease. Varieties with low seed yields were Detam 2 and Malabar. Both varieties differed in resistance to stink bugs, where Detam 2 was moderate susceptible, and Malabar was susceptible. Therefore, seed yield was not only determined by the resistance of a variety to stink bugs, but also by the yield potential of a variety.

Correlation between characters shows that resistance to pest was significantly correlated with all characters observed, except the number of branches per plant. In general, the correlations were negative, except the correlation between resistance to pest with the days to maturity and the number of unfilled pods. This means that resistance to pest increase with increasing days to maturity and unfilled pods (Table 3). The increase in days to maturity was caused by the presence of green stem syndrome due to the increase of damaged pods (Boethel et al., 2000). Nomayanja, Tukamuhubwa, & Kyamanywa (2000) reported that days to maturity has a positive correlation with stink bug populations which indicates the longer plant stays in the field causes longer plant exposed to stink bug infestation.

The interesting in this study is the longer days to flowering and the high plant height decreased resistance to pest which had implications on the increasing resistance. This occurred because the days to flowering is the end of the vegetative period. The longer days to flowering means the vegetative period is also longer. Stink bug attack the plant during the generative period, so that with the longer vegetative period, pod attacked by stink bug can be delayed. Likewise, with higher plant height, the mobility of the stink bug to different pods is also more inhibited. This is different from Nomayanja, Tukamuhubwa, & Kyamanywa (2000) which states that the high soybean plant is important for the attraction of stink bugs. It can also be seen from the number of reproductive nodes that was negatively correlated with resistance to the pest. A reproductive node is a place where pods grow, so that the higher plants will have more reproductive nodes than short plants.
Fig. 10. Weight of 100 seeds of twenty soybean varieties

Fig. 11. Seed yield of twenty soybean varieties
Seed yield per plant correlated with all the characters observed, except the days to maturity, plant height, and a number of unfilled pods. In general, the correlations were positive, except the correlation between seed yield with resistance to pests and seed size (Table 3). This indicates that resistance to a pest, as a representation of plant susceptibility, negatively affected seed yield. Previous studies also reported that seed size had a negative correlation with seed yield (Kuswantoro, 2017; Kuswantoro, Hapsari, Sulistyo, & Supeno, 2017). Seed size in this study also had a negative effect on the seed yield. It may be caused by the stink bug preference on the varieties with large seed rather than varieties with small seed (Da Fonseca Santos, Möller, Clough, & Pinheiro, 2018). Allegedly, the larger seed size the easier the pods to be sucked, so the plants are more susceptible.

CONCLUSION

Responses of twenty soybean varieties to stink bug were significantly different. Based on the resistance to a pest, the varieties of Gepak Kuning, Seulawah, and Sinabung were resistant to stink bug. There was only one variety that highly susceptible to stink bug, namely Argomulyo, and two susceptible varieties, i.e. Grobogan and Malabar. These three varieties also had the lowest seed yield. The resistance soybean varieties to stink bug had a negative correlation to days to maturity, duration of the reproductive phase, the number of unfilled pods, and weight of 100 seeds. The negative correlation was also found between seed yield with duration of the reproducive phase, resistance to a pest, and weight of 100 seeds. *Nezara viridula* may prefer variety with long duration of reproductive phase and large seed size rather than the variety with short duration of reproductive phase and small seed size. Therefore, the soybean resistance to *Nezara viridula* can be determined through the duration of reproductive phase and seed size.

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