Susceptibility of six corn varieties (*Zea mays* L.) to *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae)

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Abstract

Agricultural produce is commonly stored in warehouses after the harvest period, before being utilized and fulfilling the crop stock. However, crop threats are not only present during cultivation and harvest, but crops can also be infected during storage, which leads to agricultural loss. This research aimed to observe the resistance level of corn varieties of Bisma, Bisi 18, Bisi 19, Pioneer 21, Pioneer 29, and Pertiwi 3 to the *Sitophilus zeamais* pest of stored product insects. This resistance was based on the mechanism of non-preference (antixenosis) resistance, measured with the Free Choice Test Method (FCTM), and antibiosis resistance mechanism, measured with the No Choice Test Method (NCTM). The results showed that based on the susceptibility index (Dobie, 1974), corn varieties of Pertiwi 3 were categorized as resistant to *S. zeamais* pests, while the varieties of Bisi 19, Bisma, Bisi 18, Pioneer 21 and Pioneer 29 were moderately resistant.

Introduction

The agricultural loss incurred due to pest of stored product insects have become a serious problem that threatens livestock.[1] Farmers tend to grow hybrid corn if both seed and input costs are affordable, and this also depends on the preferences of farmers.[2] Technological improvements in farming have meant that local farmers have been able to reduce their use of synthetic pesticides, which can have a negative effect on the environment and farmers' health.[3] Recently, several varieties of corn that have high yield potential have been developed, but their resistance to pest of stored product s is not yet known. During the storage period of corn production, damage can be caused by *Sitophilus zeamais*. *S. zeamais* pests commonly attack corn kernels from harvest time until the storage period.[4] The corn post-harvest losses due to *S. zeamais* have been recognized as an increasingly important problem in the world.[5] Losing weight of crops due to pest attack can reach up to 30%.[6] Utilization of resistant crop varieties is relatively easy, environmentally friendly, and is economically more profitable as an alternative way to reduce losses due to insect pests when in storage. This research aims to assess the resilience of the corn varieties Bisma, Bisi 18, Bisi 19, Pioneer 21, Pioneer 29, and Pertiwi 3 to *S. zeamais* pests based on susceptibility index values.

Materials and Methods

This research was conducted from May to September 2016 in the Plant Pest Laboratory, Department of Pest and Plant Disease, Faculty of Agriculture, University of Brawijaya (27±2ºC, 60±5% RH and 12:12 L:D radiation period). The research was conducted based on a mechanism of non-preference (antixenosis) resistance with the Free Choice Test Method (FCTM) and antibiosis resistance mechanisms with the No Choice Test Method (NCTM).

Hardness analysis of corn grains was conducted in the Agricultural Food Technology Laboratory, Faculty of Agricultural Technology, University of Gadjah Mada, Yogyakarta, using a Universal Testing Machine (BDO-FB0.5TS). The analysis of the phenol content was performed at the Technical Implementation Unit of Analytical Service and Measurement Department of Chemistry, Faculty of Mathematics and Natural Sciences, with spectrophotometric apparatus method.[7] The proximate content was analyzed using the Pendl et al. method.[8] Protein analysis were conducted using the Kjeldahl method in Quality Assay and Food Safety Laboratory, Department of Agricultural Product Technology, Faculty of Agricultural Technology, University of Brawijaya, Malang, Indonesia.

Six corn varieties were obtained from the Technical Implementation Unit of Palawija Seeds Development in Singosari, Malang, and the varieties Bisi 18, Bisi 19, Pioneer 21, Pioneer 29, and Pertiwi 3 were obtained from farmers in Jatikerto Kromengan, Malang. Before being used for the study, corn was sterilized following the method of Heinrichs *et al*.[9]

S. zeamais insects used in the research originated from reared in the Plant Pest Laboratory, Department of Pest and Plant Disease, Faculty of Agriculture, University of Brawijaya. The difference between the male and female imago phases is indicated by the shape of the muzzle and the tip of the abdomen.[9] The FCTM test was performed using a preference cage with an aerated cover. The inside of the preference cage was divided into six spaces (d=17.5 cm, h=7.5 cm). Each room was filled with corn (30 g) according to the treatment. Then, these were infested with 30 pairs of 1-2-week-old *S. zeamais* adult insects released in the middle of the preference cage. The top of the preference cage was then closed. The infestation of *S. zeamais* in a preference cage was performed over a period of 7 days since *S. zeamais* adult insects need time for oviposition.

The study was a complete randomized design and was repeated four times. Observations of the number of adult insects and the production of *S. zeamais* eggs was performed by calculating all the *S. zeamais* adult insects appearing in each treatment and calculating the oviposition pits on the corn grains infested by *S. zeamais* pests 7 days after infestation, using a microscope. The observation of the feed preference was carried out at the end of the study, by calculating the percentage of feed weight loss using the Adams and Schulten formula: weight decrease (%) = [(Wu × Nd) − (Wd ×

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Nu) / Wu × (Nd + Nu) × 100], where Wu is the weight of undamaged seeds, Nu is the number of undamaged seeds, Wd is the weight of damaged seeds, and Nd is the number of damaged seeds.[10] For the NCTM test, 30 g of each corn variety was weighed and inserted into glass tubes (d=6.5 cm, h=9 cm); these were then infested with 15 pairs of 1-2-week-old *S. zeamais* adult insects.[9] The infestation of *S. zeamais* into the glass tube was carried out for 7 days. The observed variable was *S. zeamais* adult insect mortality which was measured from the remaining infesting adult insects at 7 days after infestation.[11] Observation of the number of eggs was performed as in the FCTM experiment. The observation of the number of larvae was performed 25 days after infestation using light irradiation (above the lamp). At 31 days after the infestation, an observation of the number of pupae was conducted. An indication that corn seeds contain a pupae is that the corn germ looks increasingly transparent, lightweight, and fragile. Observations of the number of new adult insects were performed every day from the time the new imago phase was seen from outside the grains of corn kernels until 56 days or until all F1 ancestry were expected to appear.[12] All newly emerged adult insects were counted. Observation of the feed weight loss was performed at the end of the research. Decreased feed weight was calculated using the Adams and Schulten formula as in the FCTM experiment.[10]

For egg phase duration of *S. zeamais,* 10 grains of corn seeds were infested with *S. zeamais* eggs on the same day and placed in a vial (d=3.3 cm, h=4 cm); this was observed until the eggs hatched into larvae. The observation of the *S. zeamais* larval phase was carried out by sampling 10 grains of corn seeds in which there was a newly hatched larva from the egg. The grains of corn samples were placed into vials (d=3.3 cm, h=4 cm) and observed until the larvae developed into pupae. The observation of the pupal phase was performed by taking a sample of 10 grains of corn seeds that contained *S. zeamais* pupae formed on the same day and observing these until they become adult insects. The life cycle of *S. zeamais* was calculated from the total developmental time from eggs being oviposited until the F1 adult insects laid their first egg.

The data obtained from the experiments were analyzed using analysis of variance (ANOVA). If a significant difference was found, the data will be tested with the least significant difference (LSD) test at the 5% error level. The susceptibility index value was calculated using the Dobie and Kilminster formula :susceptibility index = [(log F) / D × 100], where F is the number of F1 from eggs laid by 15 pairs of *S. zeamais* adult insects infested for 7 days and D is the median development time estimated as time (days) starting from the middle of oviposition until the formation of 50% F1 adult insects.[13] The susceptibility index values ranged from 0 to 11, with the following categories: 0-3 = resistant, 4-7 = moderately resistant, 8-10 = susceptible and ≥11 = highly susceptible.[14]

Results and Discussion

The results of the experiment using FCTM showed that the number of present adult insects indicates a significant difference among the six tested varieties of corn. The mean value of the *S. zeamais* adult insects' presence in Pioneer 21 varieties was the highest (14.75 individuals). In contrast, the mean value of *S. zeamais* adult insects in corn varieties of Pertiwi 3 was the lowest (3.25 individuals) (Table 1). Chemical factors in the form of volatile compounds produced by each variety of corn are suspected to affect the presence of *S. zeamais* adult insects. *S. zeamais* insects have a positive response to volatile compounds in corn feed in the form of hexanoic acid.[15] The corn grains resistant to *S. zeamais* contain phenolic acids, inhibition of trypsin, and high fiber content, while susceptible varieties have high protein and starch content.[16] The lower phenol content (0.32%) in Pioneer 21 than the other corn varieties caused the *S. zeamais* adult insects to choose this variety as their host. The mean values of adult insects appearing, the number of eggs and the decrease in feed weight is presented in Table 1.

Table 1 shows that *S. zeamais* adult insects laid more eggs in Pioneer 21 varieties (111.50 eggs) than other varieties (Table 1). This is possibly influenced by nutritional factors and the hardness level of corn seeds that support the survival of its offspring. The protein content in Pioneer 21 varieties (8.86%) was higher than in other varieties. Adult insects, especially adult female insects, need more protein in their diets than male insects for egg production and during egg development.[17] There was a positive correlation (r=0.609) between the number of female adult insects appearing in each treatment and the number of eggs laid.

The determination of *S. zeamais* adutl insects eating preference on six corn varieties based on the variable feed weight-loss percentage. The resulting analysis of the weight loss percentage showed a significant effect on the six corn varieties that were tested. The feed weight-loss percentage was higher in Pioneer 21 corn varieties (4.04%) compared to other varieties (Table 1).

The results of the research using NCTM showed that *S. zeamais* adult insects' mortality of Pertiwi 3 varieties was higher (15%) than the others (Table 2). This is presumably because the secondary metabolite compounds of phenol present in the corn seeds of the Pertiwi 3 variety are quite high (0.37%). Secondary metabolites that have a nutritional balance content in the feed are expected to have affected the high percentage of feed weight loss on the corn variety Pioneer 21. The corn variety Pioneer 21 has a protein content (8.86%) higher than other the varieties tested by using mild hardness of seed (277.37 N); this facilitates *S. zeamais* and thus results in higher damage. Antika *et al.*[18] suggested that the protein

Table 1. Mean value of *S. zeamais* adult insects presence, eggs, and weight loss of feed (%) due to an *S. zeamais* infestation using FCTM on the six varieties of tested corn.

Varieties	Imago presence (individual), $\overline{X}$ ± SE	Number of eggs, $\overline{X}$ ± SE	Weight loss of feed (%), $\overline{X}$ ± SE
Bisma	11.75±2.93 bc	53.50±5.87 ab	1.10±0.48 a
Bisi 18	13.25±3.04 c	50.50±12.18 ab	0.48±0.16 a
Bisi 19	6.25±1.25 ab	26.75±2.84 a	0.29±0.11 a
Pioneer 21	14.75±2.93 c	111.50±17.89 c	4.00±1.38 b
Pioneer 29	10.75±0.48 bc	75.50±17.29 b	1.71±0.61 a
Pertiwi 3	3.25±0.75 a	19.75±8.26 a	0.09±0.07 a
LSD (5%)	2.17	35.94	1.93

The mean values accompanied by the same letters in the same column show no significant difference (α<0.05).

content in the feed affects the number of new emerging adult insects and the rate of feed weight loss. Zakka *et al.*[19] stated that seeds with a mild texture will support the growth and development of insects since insects require feed and proper nutrition for growth and development. The nutrition contained in corn varieties has a chronic effect on the reproductive physiology of *S. zeamais* adult insects.[20] In general, corn contains chemical toxic to insects in the form of alkaloids, terpenoids, phenol compounds or benzoate compounds.[21] In addition, the ash content (1.37%) in Pertiwi 3 varieties is high enough to reduce the extent of damage caused by an *S. zeamais* attack. Khan *et al.*[22] stated that ash content affects the low level of pest infestations so it reduces the level of pest attack.

Six corn varieties showed significant differences in the number of eggs, larvae, pupae, and new *S. zeamais* adult insects for NCTM. The production of *S. zeamais* eggs in the Pioneer 29 variety was the highest among the varieties studied (137.25 eggs) (Table 3). The number of larvae, pupae, and newly emerging adult insects was also highest in this variety.

The presence of eggs, larvae, pupae, and new adult insects in Pertiwi 3 was the lowest (Table 3). This is probably because the corn seeds of Pertiwi 3 variety are smaller than the other varieties; therefore, egg production is very low. Research from Groote *et al.*[23] discovered that the stored product insects (*S. zeamais* and *Prostephanus truncatus*) preferred smaller seed grains (d≤8 mm) to larger ones (d≥11 mm). The selection of corn seeds by *S. zeamais* adult insects for laying their eggs is also affected by the ability of corn seeds to accommodate more than one egg. Research by Mathias *et al.*[24] demonstrated that the female *S. zeamais* adult insects can find the eggs in the corn seeds previously filled with eggs and choose the corn seeds that can hold more than one *S. zeamais* egg.

The presence of eggs, larvae, pupae, and new adult insects was the highest for Pioneer 29 (84.75). This was probably due to the nutrient content in the corn seeds that affected the reproduction of *S. zeamais* on these varieties. The fat content in the corn seeds of Pioneer 29 (4.99%) was higher than the other varieties. According to Parra,[15] fat is needed in the feed by insects as an energy reserve. Insects need fat for growth, development, and reproduction. The ash content of Pioneer 29 is not toxic to *S. zeamais* offspring, so the growth and development occurs normally.

The six varieties of corn showed significant effects of the percentage of feed weight loss. The percentage of feed weight loss of Pioneer 29 was the highest (6.50%), whereas Pertiwi 3 was the lowest (0.23%). Based on the results of the study, the number of larvae was smaller in corn varieties of Pertiwi 3 than the other varieties, and this caused the percentage of feed weight loss in this variety to be lower. On the other hand, the number of larvae found was greater in the corn seeds of Pioneer 29 than the other varieties, so that the percentage of feed weight loss was higher (Table 4).

Larvae presence affected feed weight loss due to feeding activity. The larval phase is a stage that requires more feed than adult insects; therefore, it is called the eating stage.[25] There was a positive correlation (r=0.858) between the number of larvae at each treatment with the percentage of feed weight loss, which means any additional

Table 2. Mean value of *S. zeamais* adult insects mortality (%) on six corn varieties using NCTM.

Varieties	Mean value of S. zeamais adult insects mortality (%), $\overline{X}$ ± SE
Bisma	3.33±2.36 a
Bisi 18	2.50±2.50 a
Bisi 19	6.67±1.36 a
Pioneer 21	1.67±0.96 a
Pioneer 29	1.67±0.96 a
Pertiwi 3	15.00±2.89 b
LSD (5%)	5.92

The mean values accompanied by the same letters in the same column show no significant difference (α<0.05).

Table 3. Mean value of eggs, larvae, pupaee, and new emerging *S. zeamais* Imago in six corn varieties.

Varieties	Eggs production (eggs), $\overline{X}$ ± SE	Larvae presence (individual), $\overline{X}$ ± SE	Pupaee presence (individual), $\overline{X}$ ± SE	New adult insects presence (individual), $\overline{X}$ ± SE
Bisma	82.50±7.64 c	57.25±5.41 b	42.25±2.95 b	25.50±0.96 b
Bisi 18	79.25±6.49 bc	58.25±3.07 b	38.75±2.84 b	26.25±1.38 b
Bisi 19	64.00±3.94 b	50.50±3.30 b	37.50±2.06 b	19.75±5.92 b
Pioneer 21	100.50±6.34 d	77.00±2.74 c	65.25±5.51 c	29.00±5.05 b
Pioneer 29	137.25±5.51 e	115.50±3.95 d	98.50±4.79 d	84.75±5.86 c
Pertiwi 3	44.75±2.10 a	28.00±1.47 a	15.25±1.93 a	4.50±1.32 a
LSD (5%)	16.67	10.49	10.72	12.10

The mean values accompanied by the same letters in the same column show no significant difference (α<0.05).

Table 4. Mean value of feed weight loss percentage (%) in six corn varieties caused by *S. zeamais* attack, susceptibility index and category on tests using NCTM.

Varieties	Mean value of feed weight loss (%), $\overline{X}$ ± SE	Susceptibility index	Susceptibility category
Bisma	1.55±0.68 ab	3.89	Moderately resistant
Bisi 18	1.05±0.26 ab	4.01	Moderately resistant
Bisi 19	0.81±0.20 ab	3.37	Moderately resistant
Pioneer 21	2.11±0.22 b	4.26	Moderately resistant
Pioneer 29	6.50±1.20 c	5.96	Moderately resistant
Pertiwi 3	0.23±0.09 a	1.47	Resistant
LSD (5%)	1.74		

The mean value accompanied by the same letter in the same column show no significant difference (α<0.05).

Table 5. Mean value of larval phase, and life cycle of *S. zeamais* in six varieties of corn using NTCM.

Varieties	Larval phase duration (days), $\bar{X}$ ± SE	Life cycle (days), $\bar{X}$ ± SE
Bisma	27.07±0.30 c	43.70±0.26 c
Bisi 18	27.27±0.12 cd	44.27±0.44 cd
Bisi 19	27.90±0.06 cd	45.87±0.38 d
Pioneer 21	23.30±0.80 a	39.47±0.69 a
Pioneer 29	24.80±0.44 b	41.33±0.09 b
Pertiwi 3	28.40±0.23 d	47.6±0.66 e
LSD (5%)	1.25	1.45

The mean value accompanied by the same letter in the same column show no significant difference (α<0.05).

larvae will cause an increase of feed weight loss. Pertiwi 3 is categorized as the resistant variety, and the five other varieties are categorized as a moderately resistant category. Askanovi stated that the susceptibility index value is a parameter used to measure the effectiveness of feed on the growth and development of insects.[26] The higher the susceptibility index value, the more susceptible the crop warehouses are to a pest attack. The results showed that Pertiwi 3 was included in the resistant category. The resilience of corn varieties of Pertiwi 3 to *S. zeamais* attack is suspected to be due to the incompatibility of its seeds to the growth and development of *S. zeamais*, so that the number of larvae and the number of F1 offspring present on this variety is lower than for the other varieties. The lower the number of larvae and emerging F1, the lower the susceptibility index. The lower the susceptibility index of a variety,[14] the more resistant the variety is to *S. zeamais* attack. If the susceptibility index value is less than 4, then the varieties are included in the resistant category. Abebe *et al.*[11] stated that if there is a greater number of emerging F1 in different corn varieties, the susceptibility index will be higher because the number of F1 offspring is a factor that affects the susceptibility index value. Besides the number of larvae and F1, the development time median also affects the susceptibility index value. The longer the development time median is, the lower the susceptibility index value. Astuti *et al.*[27] stated that the susceptibility index value is influenced by the number of newly emerging adult insects and the median development time.

No corn variety showed differences in the duration of egg phase or pupae of *S. zeamais*. On the contrary, the duration of the larval phase and the life cycle showed a significant difference. The larval phase was faster in Pioneer 21 (23.30 days) compared to other varieties, and the larval phase of Pertiwi 3 (28.40 days) was longer than the other varieties (Table 5). This was likely due to the phenol content of Pioneer 21 being low (0.32%). The results by Astuti *et al.*[27] showed that rice varieties with high phenolic content and higher granular hardness were more resistant to *Rhyzopertha dominica* pest attack. Furthermore, there was a tendency for a shorter life cycle in that the life cycle of *S. zeamais* in Pioneer 21 varieties (39.47 days) than in other corn varieties (Table 5). This is influenced by the nutrient content in each variety. It appears that protein content was higher in Pioneer 21 corn seeds. In addition to nutritional factors, the level of grain hardness also affected the development of insects. Research from Astuti *et al.*[27] discovered that the level of grain hardness affects the development of *R. dominica*. Moreover, the *S. zeamais* pest feeds on the corn germ because it is the soft part of the corn seed. The softer this part is, the shorter the insect development duration will be because they have access to sufficient nutrition.

Conclusions

Corn varieties of Pertiwi 3 are categorized as resistant to the *S. zeamais* pest, whereas the other varieties Bisi 19, Bisma, Bisi 18, Pioneer 21, and Pioneer 29 are categorized as moderately resistant based on the susceptibility index value.

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