CROP PROTECTION

Nymphal Development, Lipid Content, Growth and Weight Gain of *Nezara viridula* (L.) (Heteroptera: Pentatomidae) Fed on Soybean Genotypes

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Desenvolvimento de Ninfas, Conteúdo de Lipídios, Crescimento e Ganho de Peso de *Nezara viridula* (L.) (Heteroptera: Pentatomidae) Alimentada com Genótipos de Soja

RESUMO - Os efeitos de cinco genótipos de soja [*Glycine max* (L.) Merrill] ('BR-16', 'IAC-100,' PI 227687, PI 229358, PI 274454) no desenvolvimento ninfal, peso de adulto e quantidade de lipídio em *Nezara viridula* (L.) foram avaliados em laboratório. Quando as ninfas se alimentaram de vagens de PI 227687 (resistente) e 'BR-16' (suscetível), sua mortalidade foi de 66,2% e 27,5%, respectivamente. Ninfas de segundo ínstar, mantidas desde a eclosão em placas de Petri contendo vagens de PI 274454 e 'BR-16', pesaram 1,1 mg e 1,0 mg, respectivamente; sendo mais pesadas que aquelas alimentadas com vagens da PI 227687 (0,7 mg) e 'IAC-100' (0,7 mg). O peso fresco de adulto foi 146,9 mg quando as ninfas se alimentaram em 'BR-16,' 127,0 mg em PI 227687 e 125,5 mg em 'IAC-100'. Os dois últimos genótipos também afetaram negativamente o ganho de peso do percevejo. Fêmeas acumularam menos lipídio quando se alimentaram em 'IAC-100' (4,8 mg) e PI 227687 (4,3 mg), em comparação com aquelas alimentadas em outros genótipos. Por seus efeitos deletérios, PI 227687 confirma-se como alimento inadequado para *N. viridula* e como o genótipo mais promissor para ser usado em programas de melhoramento como fonte de resistência a percevejos.

PALAVRAS-CHAVE: Resistência de plantas, antibiose, *Glycine max*

ABSTRACT - The effects of five soybean [*Glycine max* (L.) Merrill] genotypes ('BR-16', 'IAC-100', PI 227687, PI 229358, PI 274454) on nymphal development, adult weight and lipid content of the stink bug *Nezara viridula* (L.), were evaluated in the laboratory. When fed on the pods of PI 227687 (resistant) and of 'BR-16' (susceptible) nymph mortalities were respectively 66.2% and 27.5%. Second instar nymphs, maintained since hatching in petri dishes containing PI 274454 and 'BR-16' pods, weighed 1.1 mg and 1.0 mg, respectively; they were heavier than those fed with PI 227687 (0.7 mg) and 'IAC-100' (0.7 mg) pods. Adult fresh weights were 146.9 mg when fed on 'BR-16', 127.0 mg with PI 227687 and 125.5 mg with 'IAC-100'. The later two genotypes also negatively affected stink bug weight gain. Females accumulated less lipid when fed on 'IAC-100' (4.8 mg) and PI 227687 (4.3 mg) than those fed on the other genotypes. By their deleterious effects, PI 227687 is confirmed as an inadequate food to *N. viridula* and as the most promising genotype for use in soybean breeding programs as sources of resistance to stink bug.

KEY WORDS: Plant resistance, antibiosis, *Glycine max*

Nezara viridula L. is considered one of the most important species of the stink bug complex affecting soybean [*Glycine max* (L.) Merrill] in Brazil. This complex includes *Piezodorus guildini* (Westwood) and *Euschistus heros* (Fabricius) (Villas Bôas *et al.* 1990, Fernandes *et al.* 1991). Stink bugs decrease soybean seed yields and quality, increase seed-protein content and reduce oil content, as well as delay plant maturity (Villas Bôas *et al.* 1990, Lourenção *et al.* 1999).

To identify soybean genotypes resistant to *N. viridula*, Gilmar *et al.* (1982) evaluated 894 plant introductions and twenty-six cultivars at field condition for five years; among the insect-resistant plant introductions (PI 171451, PI 227687 and PI 229358), only one genotype (PI 227687) was moderately resistant to *N. viridula* damage. The cultivar IAC-100, which in addition to other sources of resistance has PI 274454 in its genealogy, was released by the breeding program of the Instituto Agronômico (Campinas, São Paulo State, Brazil). 'IAC-100' presents resistance characteristics to stink bugs and to leaf feeders, and possess various other desirable agronomic traits (Rossetto *et al.* 1995, Veiga *et al.* 1999).

The objective of the present study was to evaluate the effect of soybean genotypes on biological and nutritional aspects of *N. viridula* and to identify genotypes to be used in breeding programs as sources of resistance to stink bug.

Material and Methods

Egg masses of *N. viridula* were obtained from a laboratory mass rearing at Embrapa Soja (Londrina, Paraná State), where the insects are maintained in cages containing dried soybean, peanut (*Arachis hypogaea* L.) and privet (*Ligustrum lucidum* Ait.) seeds and soybean plants cv. Paraná. Egg masses were divided into smaller pieces and randomly placed in five plastic petri dishes (8.5 x 1.5 cm), lined with moist filter paper and cotton ball. They also contained a pod of each of the following five soybean genotypes: 'BR-16' (susceptible control), PI 229358, PI 274454, PI 227687 and 'IAC-100' (insect-resistant genotypes), at the R5/R6 development stage (Fehr & Caviness 1977). On the first day of the second instar, the nymphs were weighed and kept individually until the end of the experiment. Pods were replaced every day, and the dishes replaced as needed. The dishes were maintained in controlled environmental chambers at 26 ± 1ºC, 60 ± 10% RH with 14L:10D of photoperiod. The insects were observed daily to evaluate the mean duration of each nymphal stage, until they reached adult stage; the numbers of dead insects were recorded to calculate percent of mortality.

Growth and Weight Gain. The analysis of covariance (ANCOVA), proposed by Raubenheimer & Simpson (1992), followed by bicoordenate plots (Raubenheimer & Simpson 1994), was used to estimate the growth and weight gain of *N. viridula* individuals. The adult fresh weights were adjusted for the covariates development time and initial weight to obtain insect growth and weight gain, respectively. After performing ANCOVA, when the interaction between covariate and treatments was significant, the treatments were adjusted for the covariate. Otherwise, when the interaction between covariate and treatment was not significant, the parallel line model was used, and only the effect of treatment was considered; means were then compared by the ANCOVA means or least square means.

Lipid and Water Contents. The insects were weighed on the first day of adult stage, placed in numbered tubes, and freeze-dried for lipid extraction and determination of water and lipid contents. The insects were oven-dried for two days and the dry weight was recorded; the water content was determined as the difference between insect fresh and dry weights. After 4h in a Soxhlet extractor, in hexane reflux, the insects were placed in the oven for another 24h to eliminate hexane residues, and re-weighed. The lipid content was calculated by the difference between the dry weight before and after the extraction.

Statistical Analyses. Two bioassays were carried out in a period of six months, in a completely randomized design, with 40 replicates each (80 in total). The mean square analysis allowed the two data sets to be combined. The homogeneity of mortality for the different treatments was examined by using the chi-squared (χ^2) test. All data were analyzed by SAS statistical package (SAS Institute 1996). The effects of the treatment (genotype) on the initial weight (first day of the second instar), fresh adult weight (first day of adult stage), development time (total and for each instar) and lipid and water contents (male and female) were analyzed by analyses of variance (ANOVA). If the ANOVA, performed by the general linear models (GLM) procedure, indicated a significant effect of the treatments at 5% probability ($P < 0.05$), the means were compared by Tukey test.

Results and Discussion

Mortality of nymphs varied from 27.5% to 66.2% when insects were reared on 'BR-16' pods (control) and PI 227687 pods, respectively (Table 1) and the χ^2 test (24.7, 4 df, $P < 0.05$) showed that these percentages of mortality were dependent on the treatment. Jones & Sullivan (1979) evaluating genotypes for stink bug resistance in laboratory feeding tests also observed higher *N. viridula* mortality when the nymphs were fed on PI 227687, compared to those fed on the susceptible cultivar 'Bragg' (8%).

Table 1. Mortality by nymphal stage and total of *N. viridula* fed on different soybeans genotypes (Temp.: 26 ± 1ºC; RH: 60 ± 10%; 14L:10D).

Genotypes	Instar / mortality (%)				Total (%)
	2nd	3rd	4th	5th	
'BR-16'	8.7	7.5	3.7	7.5	27.5
PI 229358	28.7	8.7	5.0	8.5	50.0
PI 274454	31.2	11.2	6.2	3.7	52.5
PI 227687	37.5	16.2	0.0	12.5	66.2
'IAC-100'	26.2	12.5	5.0	5.0	48.8
χ^2	18.9*	3.6 ^{ns}	2.0 ^{ns}	5.3 ^{ns}	24.7*

*significant at 5% probability level. ^{ns}- not significant

The highest mortality was observed during the second instar (Table 1); 37.5% of nymphs fed on PI 227687 died during this period, while only 8.7% of nymphs died after feeding on 'BR-16' pods. Chemical defenses of soybean and/or physical characteristics of pods, such as the distance between the seed and pod walls, usually prevent young nymph feeding (Panizzi 1991) and, depending on the intensity of food deterrence or inaccessibility, these characteristics can lead to insect mortality. Compared to other genotypes, PI 227687 caused the highest mortality and also possesses the highest concentration of the isoflavones genistin and daidzin (Carrão-Panizzi & Kitamura 1995, Piubelli 2000). Bioactivity of isoflavones in insects has been reported by Sutherland *et al.* (1980) and Rao *et al.* (1990) and it is likely that genistin and daidzein have a role in stink bug resistance. Trichomes were also mentioned as cause of nymph death by Panizzi (1991). However, when PI 227687 pod trichomes were mechanically removed from the pods, the numbers of stylet sheaths left by second instar nymphs, after eating, was similar to that observed for PI 227687 pods, with trichomes (Piubelli 2000). In general, mortality of the fourth instar nymphs was low (below 7%), but tended to increase again in the fifth instar, which is also a critical period for stink bug development.

The development time (days) of the insects was also affected by genotype, especially in the second and fifth instars (Table 2). The second instar was longer when nymphs were fed on PI 274454, and shorter on cv. BR-16. In contrast, nymphs fed on PI 229358 had a shorter fifth instar, reaching the adult age before those reared on the other genotypes. In general, the fifth instar tended to be longer than the others. This is common in Heteroptera, since intense changes occur when the insects are nearing maturity (Panizzi 1991) and a larger amount of nutrients is required for molting and to produce adults with a good reproductive potential.

The initial fresh weight of nymphs (first day of the second instar) and adult (first day of adult stage) varied with genotype in which the insects were fed on (Table 3). Initial weight of nymphs maintained in petri dishes containing PI 274454 and 'BR-16' pods, since the egg hatching, were heavier than those maintained on PI 227687 and 'IAC-100'. First instar nymphs are not able to feed. Pod morphology can constrain young nymphs from feeding, and as the fragility and size of the stink bug stylet can render the seeds inaccessible to them (Panizzi 1991). Thus, it is likely that these genotypes influenced the first instar nymph aggregation behavior. In this instar, nymphs remain grouped, mainly on the pods. Lockwood & Story (1986) suggest that this gregarious behavior by first-instar nymphs of *N. viridula*, caused by olfactory stimuli, helps to maintain humidity, protecting them against desiccation. Another reason could be volatile substances found in some soybean genotypes. Volatiles from PI 227687 leaves were repulsive to *Trichoplusia ni* larvae and *Epilachna varivestis* adults (Liu *et al.* 1989) and, although information on soybean pod volatiles are scarce, it is likely that they may have an effect by inducing disruption in *N. viridula* young nymphs, causing variation in their initial weight.

The adults were heavier when nymphs fed on 'BR-16' pods (Table 3), followed by nymphs reared on PI 229358 and PI 274454. The lowest weights were observed for nymphs fed on 'IAC-100' and PI 227687.

Growth of *N. viridula*. The interaction between the development time (covariate) and the treatment (genotype) was significant (Table 4a). When *N. viridula* was fed on 'BR-16', PI 274454, PI 227687 and 'IAC-100', a negative relationship between development time and fresh adult weight was observed (Fig. 1); insects fed for longer periods of time without showing proportional increase in insect weight. In contrast, when they were fed on PI 229358 they reached the adult stage before the other insects, showing a positive effect. The insects were heavier as the development time increased, suggesting that they could use the increase in time to reach the ideal weight and molt to adult stage.

Weight Gain of *N. viridula*. The ANCOVA for weight gain showed no interaction between nymph fresh weight and treatment (Table 4b), consequently this relationship can be supported by the parallel line model. The main effect of genotype was significant, showing that, even after removing the effect of initial weight, the weight gain remained affected by genotype (Fig. 2). ANCOVA means or least square means indicated that when the nymphs fed on 'BR-16', PI 229358,

Table 2. Total mean development times (± SE), and by nymphal stage (days) of *N. viridula* fed on different soybeans genotypes (Temp.: 26 ± 1°C; RH: 60 ± 10%; 14L:10D).

Genotypes	Instar (days)				Total (days)
	2nd	3rd	4th	5th	
'BR-16'	4.4 ± 0.10 c	4.2 ± 0.23	5.4 ± 0.18	8.7 ± 0.26 a	22.7 ± 0.51 ab
PI 229358	5.0 ± 0.12 ab	4.5 ± 0.28	4.8 ± 0.22	7.0 ± 0.31 b	21.4 ± 0.61 b
PI 274454	5.3 ± 0.12 a	4.8 ± 0.29	5.3 ± 0.22	8.4 ± 0.36 a	23.9 ± 0.66 a
PI 227687	5.0 ± 0.14 b	4.9 ± 0.35	5.4 ± 0.26	8.3 ± 0.38 a	23.3 ± 0.75 ab
'IAC-100'	4.8 ± 0.12 bc	5.0 ± 0.28	5.4 ± 0.21	8.2 ± 0.31 ab	23.5 ± 0.61 ab
F values	9.90*	1.49 ns	1.58 ns	4.90*	2.49*

Means followed by the same letter are not significantly different by Tukey test at 5% probability level.
*significant at 5% probability level. ns- not significant

Table 3. Means (± SE) of initial (first day of second instar) and adult fresh weight (mg) of *N. viridula* fed on different soybeans genotypes (Temp.: 26 ± 1°C; RH: 60 ± 10%; 14L:10D).

Genotypes	Initial weight (mg)	Adult fresh weight (mg)
'BR-16'	1.0 ± 0.03 a	146.9 ± 3.68 a
PI 229358	0.8 ± 0.04 b	141.0 ± 4.13 ab
PI 274454	1.1 ± 0.04 a	139.1 ± 4.24 ab
PI 227687	0.7 ± 0.05 b	127.0 ± 5.16 b
'IAC-100'	0.7 ± 0.04 b	125.5 ± 4.08 b
F values	20.53*	5.32*

Means followed by the same letter are not significantly different by Tukey test at 5% probability level.
*significant at 5% probability level

Table 4. ANCOVA testing for the effect of resistant genotypes on the weight of adults adjusted for development time (a), and initial weight as covariates (b).

Source of variation	DF	F values for adult fresh weight
(a) Time (covariate)	1	4.46*
Genotype	4	4.28*
Time x genotype	4	3.49*
Residual	194	-
(b) Initial weight (covariate)	1	0.16 ^{ns}
Genotype	4	1.12 ^{ns}
Initial weight x genotype	4	0.47 ^{ns}
Residual	194	-
Genotype	4	3.94 *
Initial weight	1	0.19 ^{ns}
Residual	198	-

*P < 0.05; ^{ns}- not significant

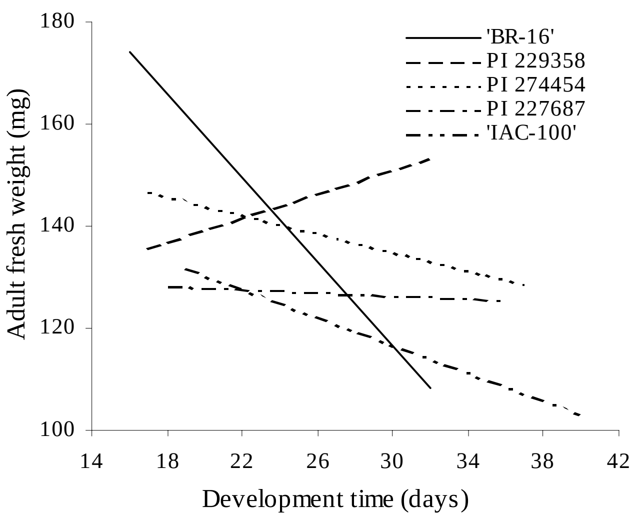

Figure 1. Growth of *N. viridula*, fed on different genotypes, estimated by the relationship between development time and adult weight (Temp.: 26 ± 1ºC; RH: 60 ± 10%; 14L:10D).

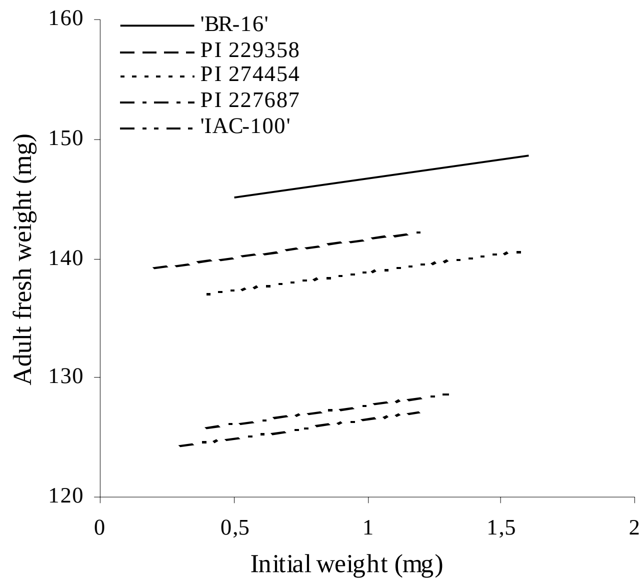

Figure 2. Weight gain of *N. viridula*, fed on different genotypes, estimated by the relationship between initial and adult weight (Temp.: 26 ± 1ºC; RH: 60 ± 10%; 14L:10D).

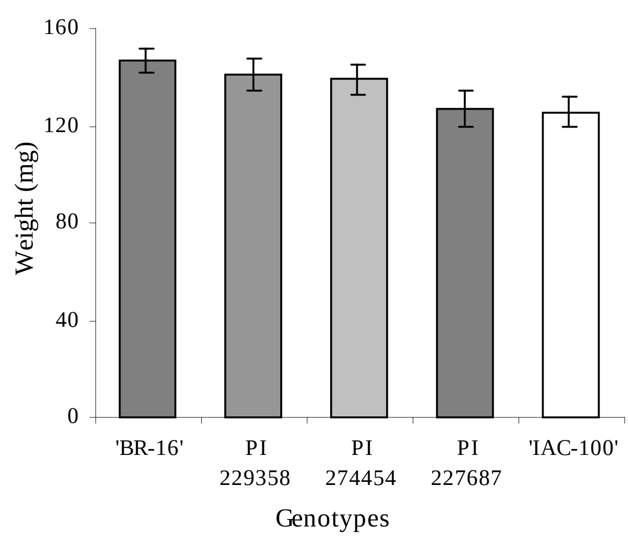

Figure 3. ANCOVA means of adult fresh weight of *N. viridula* fed on different soybean genotypes.

or PI 274454, they gained more weight than those fed on PI 227687 and 'IAC-100' (Figs. 2 and 3).

Lipid and Water Contents. The stink bug capacity for storing nutritional reserves as lipids can be affected by nutritional quality of the genotype (Panizzi 1991). In general, when the insects store more lipid, they accumulate less water in their bodies. The lowest water content was observed in males and females reared in 'BR-16' and PI 229358, respectively, compared with 'IAC-100' fed-nymphs (Table 5). The female tended to accumulate larger amounts of lipid than the male, in all genotypes but those fed on PI 227687 pods accumulated less lipid than those fed on PI 229358 and 'BR-16'. According to Panizzi (1991) they require more lipid to accomplish reproduction. Thus, the reproductive capacity of females fed on PI 227687 may be negatively affected.

Despite their high mortality, stink bugs reared on PI 229358, generally, showed adult fresh weights similar to those reared on 'BR-16' (Table 3). These insects also developed faster, especially in the fifth instar, suggesting that surviving nymphs recovered and produced adults of adequate weight. This can help to explain why PI 229358 was the only genotype that showed a positive slope in the growth assessment. Nymphs reared on PI 274454 had high mortality in the second instar (31.2%), but mortality decreased in the following instars; they tended to produce adults with weights similar to those fed on the susceptible genotype ('BR-16'). Normally they consume larger amount of food as a form of nutritional compensation when foods contain low nutrient content (Simpson & Simpson

Table 5. Means (± SE) of body water (%) and lipid contents (mg) of *N. viridula* fed on different soybeans genotypes (Temp.: 26 ± 1°C; RH: 60 ± 10%; 14L:10D).

Genotypes	Males		Females	
	Water (%)	Lipid (mg)	Water (%)	Lipid (mg)
'BR-16'	78.3 ± 1.72 b	4.7 ± 1.42 ab	78.9 ± 1.92 ab	7.0 ± 2.51 ab
PI 229358	78.9 ± 1.32 ab	6.1 ± 1.70 a	78.3 ± 2.12 b	7.7 ± 2.31 a
PI 274454	79.3 ± 1.97 ab	3.7 ± 0.74 b	78.8 ± 1.57 ab	6.7 ± 1.33 abc
PI 227687	78.7 ± 2.05 ab	3.3 ± 0.90 b	79.5 ± 1.46 ab	4.3 ± 1.40 c
'IAC-100'	81.3 ± 2.55 a	3.0 ± 1.50 b	81.0 ± 1.74 a	4.8 ± 2.40 bc
F values	3.76*	7.41*	2.68*	3.36*

Means followed by the same letter are not significantly different by Tukey test at 5% probability level.
*significant at 5% probability level

1990, Slansky & Wheeler 1992). In the field, such behavior can be a disadvantage, since the damage may reach economic limiting levels, requiring application of insecticides.

The mortality of nymphs fed on PI 227687, PI 274454 and PI 228358 which were considered potential sources of resistance were nearly two times higher than to those fed on 'BR-16', the susceptible control. This suggests that the major resistance mechanism may be antibiosis. However, the possibility of the presence of a non-preference or antixenosis mechanism cannot be excluded, as their effects are difficult to separate in controlled laboratory conditions; in field conditions, deterrence is widely correlated with toxicity (Berenbaum 1986).

PI 227687, one of the most studied genotypes in breeding programs for resistance to insects, negatively affected the life cycle of *N. viridula* by causing higher mortality, lower initial and final weight, as well as by decreasing lipid storage by females. Thus, the results suggest that PI 227687 is an inadequate food source for *N. viridula*, and may be confirmed as a promising genotype to be used in soybean breeding programs for resistance to stink bug attacks.

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