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ARTICLE

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# Effective selection criteria for assessing the resistance of stink bugs complex in soybean

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**Abstract –** *Soybean plants with resistance to the stink bug complex are currently selected by extremely labor-intensive methods, which limit the evaluation of a large number of genotypes. Thus, this paper proposed the use of an alternative trait underlying the selection of resistant genotypes under field conditions with natural infestation: the weight of healthy seeds (WHS). To this end, 24 genotypes were evaluated under two management systems: with systematic chemical control of insects (management I), and without control (management II). Different indices were calculated using grain weight ($Y_P$) of management I and WHS of management II ($Y_S$). The high correlation between $Y_S$ and the indices mean productivity, stress tolerance and geometric mean productivity, plus the agreement in determining the groups of genotypes with resistance and high yield indicate that WHS is a useful character in simultaneous selection for these traits.*

**Key words:** *Glycine max*, *Nezara viridula*, *Piezodorus guildinii*, *Euschistus heros*.

## INTRODUCTION

Soybean is a legume of great worldwide importance, however its production can be affected by a number of both biotic and abiotic stresses. In this scenario, insect pests are influential, causing both direct (when attacking the marketable plant parts) and indirect damage to crops, and may also act on pathogen transmission (Gallo et al. 2002).

Phytophagous pentatomids (sucking bugs) are among the main pests of soybean (Godoi and Pinheiro 2009, Guedes et al. 2012). Known as stink bug complex, the species *Nezara viridula* (L.), *Piezodorus guildinii* (West.) and *Euschistus heros* (Fabr.) attack mainly during pod formation and maturation (Panizzi and Slansky Junior 1985, Godoi et al. 2002). The damage is caused basically by larger nymphs, from the 3rd to the 5th instars, and adults that feed directly on soybean seeds, piercing the pods and extracting nutrients from the seed with their piercing-sucking mouthpart (McPherson and McPherson 2000), resulting in losses in grain yield and quality. Plant maturation can also be delayed when the seeds are significantly injured (Leonard et al. 2011).

As a means to mitigate the effects of these insect pests on crops, insecticides have been intensively applied. However, this control method is harmful to the environment, leaving waste and promoting the selection of resistant populations (Maia et al. 2009). In 2000, decreased susceptibility of *Euschistus heros* (Fabr.) to the insecticide methamidophos (Sosa-Gómez et al 2001) was found in the State of São Paulo; and more recently a higher number of resistant genotypes was observed in the State of Paraná (Sosa-Gómez and Silva 2010). Moreover, there is a trend in the current Brazilian scenario to reduce the number of active ingredients available for stink bug control, prohibition of some organophosphate insecticides in addition to the lack of innovation and introduction of new insecticides (Guedes et al. 2012).

Thus, the development of soybean cultivars resistant to the stink bug complex is extremely meaningful for the maintenance and/or increase in yield levels of this crop. However, current strategies, such as the percentage index of pod damage (Rossetto et al. 1986, Nagai et al. 1987) and percentage of spotted seeds (Hoffmann-Campo et al. 1988) for selection of resistant genotypes are extremely labor-intensive, which limits the evaluation of a large number of genotypes without ensuring the selection of the highest-yielding. Thus, the objective of this study was to show, based on resistance indices, that the weight of healthy seeds can be used as an alternative trait for the selection of

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soybean genotypes resistant to the stink bug complex and with high yield potential.

## MATERIAL AND METHODS

The study was carried out in the 2011/12 growing season in Piracicaba, São Paulo, at the Experimental Station Anhumas. The reaction resistance of 24 genotypes (two of which are transgenic cultivars) to the stink bug complex was evaluated in two experiments, in a randomized block design with five replications under two management systems: with chemical insect control (management I), and without control measures (management II). Among the genotypes, cultivar IAC 100, developed by the Agronomic Institute of Campinas - IAC, is known to be moderately resistant to sucking (stink bug complex) and chewing insects (caterpillars and beetles) (Veiga et al. 1999). Other lines included in this research, denominated "LQ" (lines of soybean plant breeding program at the "Luiz de Queiroz" College of Agriculture), are also resistant to stink bug.

In management I, systematic and preventive spraying was applied five times while no insect control measures were used in management II. The experimental plot consisted of four 5-m long rows, spaced 0.5 m apart. The insects were sampled in the experimental area by a drop cloth (two meters of a row), with 20 droppings per experiment and daily assessment. The characters grain yield (GY) and weight of healthy seeds (WHS) were evaluated, both in kg plot$^{-1}$. The WHS was determined by discarding empty, green and malformed grains, with the use of a spiral, in which the seeds are separated by the action of gravity and centrifugal forces.

To evaluate the resistance of the different genotypes to the stink bug complex, indices were used, based on the GY of management I representing the potential yield of the genotype ($Y_P$), i.e., yield in the absence of stress (no stink bug damage), and WHS of management II, representing the yield of genotypes under stress ($Y_S$).

The resistance indices of genotypes to the stink bug complex were estimated by the equations proposed by Fernandez (1992):

Stress susceptibility index (SSI): $SSI_i = \dfrac{1-\left(Y_{Si}/Y_{Pi}\right)}{1-\left(\bar{Y}_S/\bar{Y}_P\right)}$

Tolerance (TOL): $TOL_i = Y_{Pi} - Y_{Si}$

Mean productivity (MP): $MP_i = \dfrac{Y_{Si}+Y_{Pi}}{2}$

Stress tolerance index (STI): $STI_i = \dfrac{Y_{Si} \times Y_{Pi}}{\bar{Y}_P^{\,2}}$

Geometric mean productivity (GMP): $GMP_i = \sqrt[2]{Y_{Pi} \times Y_{Si}}$

where $Y_{Si}$ represents the yield of the i$^{th}$ genotype under stress (WHS management II), $Y_{Pi}$ the yield potential of the i$^{th}$ genotype in the absence of stress (GY under management I), $\bar{Y}_S$ and $\bar{Y}_P$ the genotype means in both environments, with and without stress, respectively.

Analyses of individual variance, F-test and Pearson correlation for the traits $Y_S$, $Y_P$ and for the indices estimated from these were performed. When the F test detected significant differences, tests of treatment means (Scott-Knott probability level of 0.05) were performed.

## RESULTS AND DISCUSSION

As expected in the experiment without chemical insect control, there was a significant increase in the population of stink bugs at the end of the crop cycle (Figure 1). This can be explained by the presence of pods on the plants, which is directly related to the presence of stink bugs in the crop, and insect migration from already harvested neighboring areas (Panizzi et al. 2000). The average number of bugs ranged from 0 to 1 in the experiment with chemical insect control (Management I), and reached 13 in management II. According to Corrêa-Ferreira and Panizzi (1999), the control should be performed when the population reaches four bugs (nymphs of the third instar or adults) per drop cloth (in two meters of a row). Thus, stink bug infestation in management II was high enough to evaluate the reaction of genotypes, allowing a discrimination among them.

The analysis of variance for grain yield potential ($Y_{Pi}$), weight of healthy seeds ($Y_{Si}$) and the indices estimated from these (Table 1) demonstrates the variability among genotypes, allowing an identification of those with ability

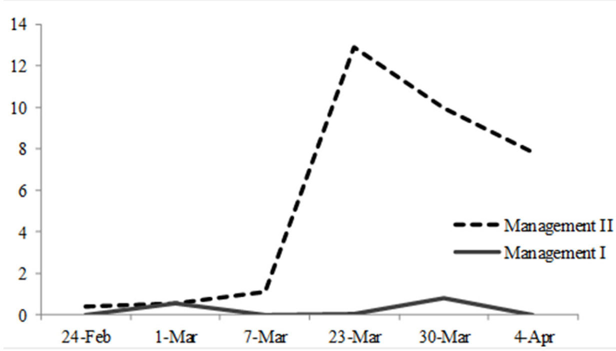


**Figure 1.** Average number of bugs per sample in two experiments: management I (with systematic chemical insect control) and management II (no insect control) in the period between the reproductive stages R3 and R8 of the soybean genotypes under study.

**Table 1.** Summary of analysis of variance and estimated resistance indices of soybean to the stink bug complex, for 24 genotypes evaluated under two management systems: systematic chemical control of insects (Management I), and absence of control measures (management II)

| Variable | Genotype | Error | CV (%) | Mean |
|---|---|---|---|---|
| | Degrees of freedom | | | |
| | 23 | 92 | | |
| | Mean squares | | | |
| $Y_P$ | 0.0485* | 0.0239 | 21.31 | 0.7254 |
| $Y_S$ | 0.0808* | 0.0125 | 44.01 | 0.2544 |
| SSI | 0.4590* | 0.1096 | 33.93 | 0.9756 |
| TOL | 0.1095* | 0.0349 | 39.68 | 0.4709 |
| MP | 0.0373* | 0.0094 | 19.88 | 0.4899 |
| STI | 0.1935* | 0.0332 | 51.27 | 0.3553 |
| GMP | 0.0620* | 0.0110 | 25.81 | 0.4077 |

$Y_S$: genotype yield (kg plot$^{-1}$) under stress (management II – absence of control measures); $Y_P$: genotype yield (kg plot$^{-1}$) in absence of stress (management I – with chemical control of insects); SSI: stress susceptibility index; TOL: tolerance; MP: mean productivity; STI: stress tolerance index; GMP: mean geometric productivity (kg plot$^{-1}$).
*$P < 0.01$.

to support stink bug attack. The coefficients of variation (CV) ranged from 19.88 to 51.27%. High CV values can be explained because some genotypes are more affected than others by the stink bug attack.

Among all evaluated genotypes, BRS 133, BRS Invernada, LQ1043, LQ1194, LQ1413, LQ1421, LQ1505, JAB 00-05-6/763D, and JAB 00-02-2/2J3D had the highest yields in the environment without stress (management I) (Table 2), whereas in the environment with stress (management II) the genotypes BRS 133, LQ1050, LQ1421 and LQ1505 had the highest $Y_S$ values. These results show that selection for GY cannot ensure the identification of those with higher resistance. This is the case of BRS Invernada, LQ1043, LQ1194, LQ1413, JAB 00-05-6/763D, and JAB 00-02-2/2J3D, which despite the high yield potential, were extremely stress-sensitive, with reduction in $Y_S$ values (Table 2).

Considering the SSI index, three groups were formed. For this index, LQ1050 was classified as the most resistant genotype to the stink bug complex (Table 2). By the TOL index however, apart from LQ1050, genotypes BRS 133, LQ1421, LQ1505, LQ1402, LQ1504, IAC 100, LQ1519, L1-1-55, IAC 23, LQ1078, IAC 17, and BMX Potência RR had higher resistance to stink bugs than the others. Soybean cultivar IAC 100 was characterized as resistant to the stink bug complex, based on at least five mechanisms: shorter grain filling period, more seeds, abscission of damaged pods and replacement by regrowth, normal senescence with leaf fall at maturity and resistance to yeast *Eremothecium coryli* (Peglion) (Rossetto et al. 1995). The evaluated LQ lines were derived from IAC 100, and therefore expected to be grouped together.

However, both indexes, SSI and TOL, may favor the selection of genotypes with high values under stress conditions ($Y_S$) but with low yield potential in the absence of stress (Fernandes 1992). This was the case with LQ1050, which was classified as resistant by these two indices, but is not in the group of highest $Y_P$ values. These indices consider a proportion (SSI) or a difference (TOL) between the $Y_S$ and $Y_P$ values. Therefore, the smaller the amplitude of these values, the stronger the genotype resistance. Thus, if a genotype is low-yielding in the absence of stress and maintains these levels under stress, the difference between yields will be reduced and the genotype is considered resistant.

By index MP, the genotypes BRS 133, LQ1421, LQ1050, LQ1413, LQ1421, and LQ1505 performed best. By the indices STI and GMP, the genotypes BRS 133, LQ1421, and LQ1505 performed extremely well. Selection based on these indices favors genotypes with high yield under both management conditions (Abarshahr et al. 2011). However, GMP is less sensitive to extreme values (widely discrepant $Y_S$ and $Y_P$), making this index more suitable for the distinction of genotype groups (Fernandes 1992). According to Talebi et al. (2009), the correlation between GMP and STI is approximately one, as similarly found in this study (0.98). This explains why the groupings by both indices were the same.

High and significant correlations were found, considering $Y_S$ and the indices (Table 3). Correlations of de $Y_S$ with SSI and TOL were negative, since higher $Y_S$ indicate genotypes with greater ability to withstand the insect attack, while for

TOL and SSI higher values indicate greater susceptibility of a genotype.

Conversely, correlations of $Y_S$ with MP, STI and GMP were positive, since higher values of $Y_S$ and these indices indicate increased resistance of the genotype (Table 3). The high correlations of $Y_S$ with these indices show that $Y_S$ can be used as an effective parameter in the selection of genotypes with resistance to the stink bug complex and high-yielding in the presence or absence of stress. The selection of genotypes with this performance is admittedly a challenge for plant breeders, while the yield in favorable environments was successfully increased (Richards et al. 2002).

The selected genotypes based on $Y_S$ or MP, SSI and GMP indices agree, with some exceptions. $Y_S$ is easy to estimate under stress, thus the selection based on this parameter facilitates the assessment for a high number of genotypes,

**Table 2.** Mean soybean yield of genotypes in the absence ($Y_P$) (management I: systematic chemical insect control) and presence of stress ($Y_S$) (management II: no insect control), and different resistance indices to the stink bug complex[1]

| Genotype | $Y_S$ | | $Y_P$ | | SSI | | TOL | | MP | | STI | | GMP | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BRS 133 | 0.52 | a | 0.82 | a | 0.54 | b | 0.30 | b | 0.67 | a | 0.81 | a | 0.64 | a |
| IAC 100 | 0.37 | b | 0.63 | b | 0.59 | b | 0.26 | b | 0.50 | b | 0.43 | c | 0.47 | b |
| IAC 17 | 0.20 | c | 0.67 | b | 1.05 | a | 0.46 | b | 0.44 | b | 0.26 | d | 0.37 | c |
| IAC 23 | 0.21 | c | 0.51 | b | 0.90 | b | 0.29 | b | 0.36 | b | 0.21 | d | 0.33 | c |
| IAC 24 | 0.10 | c | 0.65 | b | 1.31 | a | 0.55 | a | 0.37 | b | 0.12 | d | 0.24 | c |
| BRS Invernada | 0.18 | c | 0.84 | a | 1.20 | a | 0.66 | a | 0.51 | b | 0.29 | d | 0.38 | c |
| L1-1-55 | 0.27 | c | 0.67 | b | 0.88 | b | 0.40 | b | 0.47 | b | 0.35 | c | 0.41 | c |
| LQ1043 | 0.21 | c | 0.86 | a | 1.14 | a | 0.64 | a | 0.53 | b | 0.33 | c | 0.41 | c |
| LQ1050 | 0.47 | a | 0.65 | b | 0.13 | c | 0.18 | b | 0.56 | a | 0.58 | b | 0.54 | b |
| LQ1078 | 0.21 | c | 0.64 | b | 0.94 | b | 0.42 | b | 0.43 | b | 0.24 | d | 0.34 | c |
| LQ1119 | 0.14 | c | 0.74 | b | 1.26 | a | 0.60 | a | 0.44 | b | 0.20 | d | 0.31 | c |
| LQ1124 | 0.12 | c | 0.73 | b | 1.30 | a | 0.61 | a | 0.43 | b | 0.17 | d | 0.29 | c |
| LQ1188 | 0.15 | c | 0.69 | b | 1.21 | a | 0.54 | a | 0.42 | b | 0.19 | d | 0.32 | c |
| LQ1194 | 0.20 | c | 0.84 | a | 1.18 | a | 0.64 | a | 0.52 | b | 0.33 | c | 0.41 | c |
| LQ1402 | 0.31 | b | 0.69 | b | 0.84 | b | 0.38 | b | 0.50 | b | 0.41 | c | 0.45 | b |
| LQ1413 | 0.31 | b | 0.85 | a | 0.96 | b | 0.53 | a | 0.58 | a | 0.50 | b | 0.51 | b |
| LQ1421 | 0.47 | a | 0.86 | a | 0.66 | b | 0.38 | b | 0.67 | a | 0.76 | a | 0.60 | a |
| LQ1504 | 0.34 | b | 0.65 | b | 0.76 | b | 0.30 | b | 0.50 | b | 0.45 | c | 0.46 | b |
| LQ1505 | 0.44 | a | 0.87 | a | 0.77 | b | 0.43 | b | 0.65 | a | 0.72 | a | 0.61 | a |
| LQ1519 | 0.31 | b | 0.67 | b | 0.81 | b | 0.35 | b | 0.49 | b | 0.40 | c | 0.46 | b |
| BMX Potência RR | 0.20 | c | 0.64 | b | 1.06 | a | 0.44 | b | 0.42 | b | 0.26 | d | 0.35 | c |
| JAB 00-05-6/763D | 0.12 | c | 0.76 | a | 1.31 | a | 0.64 | a | 0.44 | b | 0.17 | d | 0.29 | c |
| JAB 00-02-2/2J3D | 0.12 | c | 0.84 | a | 1.33 | a | 0.73 | a | 0.48 | b | 0.18 | d | 0.30 | c |
| V-MAX RR | 0.13 | c | 0.67 | b | 1.26 | a | 0.55 | a | 0.40 | b | 0.17 | d | 0.29 | c |

$Y_S$: genotype yield (kg plot$^{-1}$) under stress (management II – no insect control); $Y_P$: genotype yield (kg plot$^{-1}$) in the absence of stress (management I – with : systematic chemical insect control); SSI: stress susceptibility index; TOL: tolerance; MP: mean productivity; STI: stress tolerance index; GMP: geometric mean productivity.

[1] Means followed by the same letter in a column belong to the same group by the Scott-Knott test (P <0.05).

**Table 3.** Pearson correlation coefficient (r) between the yield of genotypes under stress ($Y_S$) (management II: no insect control) and the stress resistance indices (stink bug damage)

| | SSI | TOL | MP | STI | GMP |
|---|---|---|---|---|---|
| $Y_S$ | -0.8281 | -0.6574 | 0.7244 | 0.9294 | 0.9234 |
| Prob | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |

SSI: stress susceptibility index; TOL: tolerance; MP: mean productivity; STI: stress tolerance index; GMP: geometric mean productivity.

allowing an increase in the number of repetitions and experimental locations, with no need for testing different management types (with and without insect control). The results also indicate that WHS is a useful character in simultaneous selection for high yield and resistance to the stink bug complex. This fact is highly relevant, since farmers will only accept a new resistant cultivar if it is also high-yielding in the presence or absence of stink bug attack. Moreover, plant resistance is a very promising strategy because it generates no adoption cost while being compatible with the other forms of insect control.

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## Critério efetivo de seleção para avaliar a resistência ao complexo de percevejos em soja

**Resumo –** *A seleção de plantas em soja com resistência ao complexo de percevejos é feita atualmente com base em métodos laboriosos, o que limita a avaliação de um grande número de genótipos. Assim, este trabalho propôs uma nova alternativa para a seleção de genótipos resistentes, em condição de campo com infestação natural: a massa de sementes boas (MSB). Para isto, 24 genótipos de soja foram avaliados sob dois sistemas de manejo: com controle químico sistemático de insetos (manejo I), e sem nenhum controle (manejo II). Diferentes índices foram estimados utilizando a massa de grãos ($Y_P$) do manejo I e o MSB do manejo II ($Y_S$). A alta correlação entre $Y_S$ e os índices de produtividade média, tolerância ao estresse e média geométrica, aliada à concordância na determinação dos grupos de genótipos com resistência e alto rendimento indicam que a MSB é um caráter útil na seleção simultânea para estas características.*

**Palavras-chave:** *Glycine max*, *Nezara viridula*, *Piezodorus guildinii*, *Euschistus heros*.

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