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Antixenosis and tolerance to *Diabrotica speciosa* (Coleoptera: Chrysomelidae) in common bean cultivars

Arlindo Leal Boiça Júnior[1], Eduardo Neves Costa[1,], Bruno Henrique Sardinha de Souza[1], Zulene Antônio Ribeiro[1], and Sergio Augusto Morais Carbonell[2]*

Abstract

Diabrotica speciosa (Germar, 1824) (Coleoptera: Chrysomelidae) is a polyphagous insect pest that attacks several crops of agricultural importance including common beans (*Phaseolus vulgaris* L.; Fabales: Fabaceae). *Diabrotica speciosa* larvae feed on the plant root system whereas the adults are leaf defoliators. *Diabrotica speciosa* control relies mainly on pesticide applications resulting in ecosystem contamination and other problems. This research aimed to identify common bean cultivars that express antixenosis to *D. speciosa* adult feeding and/or tolerance to larval injury, as well as to determine the resistance levels of the cultivars. To evaluate antixenosis, 10 common bean cultivars were tested for *D. speciosa* adult feeding preference in free-choice and no-choice tests. The cultivars were 'IAC-Una', 'Diamante Negro', 'IAC-Diplomata', 'FT-Nobre', 'Ônix', 'IAC-Uirapuru', 'IPR-Tiziu', 'BRS-Explendor', 'IAC-Maravilha' and 'BRS-Supremo'. Then, 6 cultivars were selected from the adult feeding assay and used in tests that assessed their tolerance to injury by *D. speciosa* larvae. In the free-choice test, the cultivars 'IAC-Una' and 'IAC-Uirapuru' were the least preferred by *D. speciosa* adults for feeding, whereas the cultivars 'IAC-Diplomata' and 'Ônix' were the least preferred in the no-choice test. The 'Ônix' and the 'IAC-Una' cultivars had the lowest reductions in plant height and dry weight of the aerial part after injury by *D. speciosa* larvae; the cultivars 'BRS-Explendor' and 'IAC-Diplomata' had intermediate reduction percentages; and the other cultivars were susceptible to injury by *D. speciosa* larvae. Field studies should evaluate the performance of the cultivar 'Ônix' infested with adults and larvae of *D. speciosa* in order to confirm the promising results obtained in the current study under laboratory and greenhouse conditions.

Key Words: *Phaseolus vulgaris* L.; pest management; host plant resistance; non-preference; leaf beetle; corn rootworm

Resumen

Diabrotica speciosa (Germar, 1824) (Coleoptera: Chrysomelidae) es un crisomelido polífago, que causa daño a varios cultivos, como el frijol común (*Phaseolus vulgaris* L.). Las larvas atacan el sistema radicular, mientras que los adultos se alimentan de las hojas de las plantas de frijol. Para controlar esta plaga, los plaguicidas se aplican cada año, contaminando las aguas subterráneas, ríos y muchos otros ambientes. Esta investigación tuvo como objetivo identificar los cultivares de frijol, que expresan resistencia de antixenosis contra la alimentación de los adultos y larvas de *D. speciosa*, y / o tolerancia al ataque de las larvas; y para determinar el nivel de resistencia o tolerancia involucradas. Para la prueba de antixenosis a los adultos, se utilizaron las siguientes 10 variedades de frijol con 10 repeticiones: 'IAC-Una', 'Diamante Negro', 'IAC-Diplomata', 'FT-Nobre', 'IAC-Uirapuru', 'IPR-Tiziu',' BRS-Explendor ',' IAC-Maravilha ', y' BRS-Supremo '. En los ensayos de antixenosis y la tolerancia de los cultivares de frijol al ataque de larvas de *D. speciosa*, se seleccionaron 6 cultivares, con 5 y 10 repeticiones, respectivamente. Un diseño completamente al azar fue adoptado para todas las pruebas, excepto en la prueba antixenosis de alimentación de las larvas. En una prueba antixenosis de libre elección de alimentos para los adultos, 'IAC-Una' y 'IAC-Uirapuru' fueron moderadamente resistentes. En una prueba antixenosis no-elección, los cultivares 'IAC-Diplomata' y 'Onix' manifiestan una resistencia moderada a la alimentación de los escarabajos. En el experimento de tolerancia, los cultivares 'Onix' y 'IAC-Una' sufrio una pequeña reducción en los porcentajes de la altura y peso seco de la parte aérea; 'BRS-Explendor' y 'IAC-Diplomata' sufrio una reducción intermedia en los porcentajes y los otros cultivares fueron susceptibles. Los estudios de campo deben evaluar el rendimiento del cultivar 'Onix' infestada de adultos y larvas de *D. speciosa* con el fin de confirmar los prometedores resultados obtenidos en el presente estudio en condiciones de laboratorio y de invernadero

Palabras Clave: *Phaseolus vulgaris* L.; manejo de plagas; resistencia genética a los insectos; no preferentes; escarabajo de hoja; gusano de la raíz del maíz

The genus *Diabrotica* has 15 species and subspecies identified as pests of 61 species of cultivated plants. Among those species, *Diabrotica speciosa* (Germar, 1824) (Coleoptera: Chrysomelidae) is the most known pest. It is broadly distributed in South America (Krysan 1986) and is established in several states of Brazil (Laumann et al. 2003). Due to its polyphagous feeding habit, *D. speciosa* causes injuries on several crops of agricultural importance including common beans (*Phaseolus vulgaris* L.; Fabales: Fabaceae), soybeans (*Glycine max* L.; Fabales: Fa-

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baceae), maize (*Zea mays* L.; Poales: Poaceae), peanuts (*Arachis hypogaea* L.; Fabales: Fabaceae), potatoes (*Solanum tuberosum* L.; Solanales: Solanaceae), and many others (Laumann et al. 2003).

Both larvae and adults of *D. speciosa* can damage common beans plants. The larvae have an aggregated distribution in the field and attack the plant's roots. Larvae-injured common bean plants are yellowed and their leaves progressively dry (Amante & Figueiredo Júnior 1971). The adults cause defoliation during the whole plant development process resulting in reduced photosynthetic efficiency. The most significant damage caused by the adults in common bean plants occurs at the seedling stage when they feed on the plant apical buds what can cause plant death in case of high population densities of *D. speciosa* (Quintela 2005). According to Silva et al. (2003), 2 adults per plant can cause defoliation of up to 16% within 24 h.

Management strategies for controlling *D. speciosa* mainly rely on applications of chemical insecticide. The adults are controlled by repeated foliar insecticide sprayings during crop development, whereas the larvae are controlled by soil-applied insecticides, which are expensive and can be harmful to the environment (Arruda-Gatti & Ventura 2003). Thus, alternative methods for controlling adults and larvae of *D. speciosa* in common beans are worth to be evaluated.

The cultivation of plants expressing some form of inherited resistance traits to pest arthropods has been practiced for many centuries (Smith 2005). Host plant resistance is divided into 3 categories: antixenosis, antibiosis, and tolerance. Antixenosis is defined as a non-preference reaction of arthropods towards a resistant plant. Antixenosis-resistance occurs when morphological or chemical factors in the plant adversely affect the arthropod behavior leading to delayed acceptance and possibly absolute rejection of the plant as a host for feeding, oviposition, or shelter. Antibiosis-resistance occurs when the plant adversely affects the life history of the arthropod attempting to use the plant as a host. Tolerance is a complex set of genetic traits that allow the plant to withstand or recover from pest damage. However, tolerance-resistance does not affect pest growth and survival (Smith & Clement 2012).

Recent information on common bean cultivars expressing resistance to *D. speciosa* is lacking in the literature, especially for common bean cultivars adapted for the Brazilian conditions. Therefore, the current study was performed with 2 main purposes: to identify common bean cultivars that express antixenosis against *D. speciosa* adult feeding and/or tolerance against larval injury and to determine the resistance levels of the cultivars assessed.

Materials and Methods

All assays were carried out using seeds of common bean cultivars provided by the Center of Analysis and Technological Research of the Agribusiness of Grains and Fibers (Centro de Análise e Pesquisa Tecnológica de Agronegócio de Grãos e Fibras), Agronomic Institute of Campinas (Instituto Agronômico de Campinas – IAC), Campinas, state of São Paulo, Brazil.

INSECT BREEDING

Diabrotica speciosa adults were collected from common bean plants cultivated in an experimental field of the College of Agricultural and Veterinary Sciences (Faculdade de Ciências Agrárias e Veterinárias – FCAV), University of São Paulo State (Universidade Estadual Paulista – UNESP), located in the municipality of Jaboticabal, state of São Paulo, Brazil. The breeding method followed the method described by Ávila et al. (2000). Briefly, the adults were confined in glass cages (30 cm width × 40 cm length × 30 cm height) under environmentally controlled conditions (25 ± 2 °C temperature, 70 ± 10% relative humidity, and 12:12 h L:D photoperiod). Petri dishes (14 cm diameter × 2 cm height) lined with moistened hydrophilic cotton topped with black gauze were placed into the cages and served as oviposition substrate for females. The eggs were withdrawn from the oviposition substrate by rinsing the gauze in running water into a 900 cm^2 synthetic polyester fabric (0.03 × 0.03 mm mesh size). To avoid contamination by microorganisms during egg incubation, the eggs were treated with 1% copper sulfate ($CuSO_4$) solution for 2 min and transferred to Petri dishes (9 cm diameter × 1 cm height) lined with moistened filter paper.

The initial infestation of plants with larvae was performed in cylindrical plastic containers (17 cm diameter × 9 cm height) filled with 40 g fine-grained vermiculite moistened with 50 g deionized water. Seventy corn seedlings ('CATIVERDE') were placed on the vermiculite and 70 larvae were carefully transferred to the corn roots with the aid of a fine paintbrush. Then, the same amounts of vermiculite and deionized water were placed on top of the corn roots so that all the larvae and the plant's root system were covered by the vermiculite. Ten days later, the larvae were transferred to larger plastic containers (16 cm width × 27 cm length × 9.5 cm height) and the amounts of deionized water, vermiculite, and corn seedlings were doubled to allow for sufficient plant material and space for development of larvae and pupae. Upon emergence, the adults were placed into glass cages (30 cm width × 40 cm length × 30 cm height) and fed with young common bean plants ('Pérola'). The corn seeds used for larval rearing were previously treated with carbendazim + thiram at the rate of 200 mL of the commercial product (Derosal Plus®) per 100 kg of seeds to prevent fungal infection.

ANTIXENOSIS TO *DIABROTICA SPECIOSA* ADULTS IN BEAN CULTIVARS

The assays were performed in the Laboratory of Host Plant Resistance to Insects of the UNESP, Jaboticabal Campus, state of São Paulo, Brazil, under environmentally controlled conditions (25 ± 2 °C temperature, 70 ± 10% relative humidity, and 12:2D h L:D photoperiod) . The following common bean cultivars were evaluated: IAC-Una, Diamante Negro, IAC-Diplomata, FT-Nobre, Ônix, IAC-Uirapuru, IPR-Tiziu, BRS-Explendor, IAC-Maravilha, and BRS-Supremo. Free-choice and no-choice tests were performed to detect antixenosis-resistance in these cultivars. Both assays were arranged in a completely randomized design and used leaf discs prepared from young leaflets collected from the apical trifoliate of 20-d-old plants. Ten replications were used for each assay.

In the free-choice test, 2.54-cm-diameter leaf discs of each cultivar were equidistantly arranged in glass containers (26.2 cm diameter × 5.0 cm height), which were lined with moistened filter paper. Then, 10 adults were released in each container. Each glass container represented 1 replicate. The attractiveness of the leaf disc of each cultivar to adults was evaluated at intervals of 1, 3, 5, 10, 15, and 30 min and 1, 2, 6, 12, and 19 h after release of adult. In the no-choice test, one 2.54-cm-diameter leaf disc was placed in one Petri dish (9.0 cm diameter × 1.2 cm height), which was lined with moistened filter paper. Then, 1 adult was released in each container. Each Petri dish containing 1 leaf disc represented 1 replicate. Assessments were made at intervals of 1, 3, 5, 10, 15, and 30 min and 1, 2, 6, 12, and 24 h after release of adults. In both assays, the attractiveness of the common bean cultivars was evaluated by counting the number of adults feeding on the leaf disc of each cultivar in the aforementioned time intervals. The average attractiveness was calculated considering all time intervals assessed. The leaf intake (cm^2) by adults was assessed by measuring the leaf discs of each cultivar immediately after the experiment ended with the aid of

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a leaf area meter (Model 3000A, LI-COR, Lincoln, Nebraska, USA). The feeding preference index (FPI) was calculated with the leaf intake data of the free-choice and no-choice tests using the formula: $C = 2A / (M + A)$, where C = feeding preference index, A = leaf intake of the tested cultivar, and M = leaf intake of the cultivar adopted as the susceptible standard (BRS-Explendor) (Kogan & Goeden 1970). The genotype BRS-Explendor was chosen as the susceptible standard according to our previous knowledge of its susceptibility to *D. speciosa* and other insects. Classification of the common bean cultivars was based on the C value obtained in pairwise comparison between the tested cultivar and the susceptible standard cultivar, as follows: $C > 1$, the tested cultivar was preferred (feeding stimulant) in comparison with the susceptible standard cultivar; $C = 1$, the tested cultivar was similar to the susceptible standard cultivar (neutral); $C < 1$, the tested cultivar was less preferred (feeding deterrent) than the standard susceptible cultivar.

Cultivar attractiveness and leaf intake data were analyzed for normality (Kolmogorov-Smirnov's test) and variance homogeneity (Bartlett's test), using the statistical software Assistat, version 7.7 Beta. Data with non-normal distribution were transformed to $(x + 0.5)^{1/2}$ to meet analysis of variance (ANOVA) assumptions. Next, data were subjected to 1-way ANOVA, and Tukey's HSD test was used at 5% probability for post-hoc mean comparisons.

TOLERANCE TO *DIABROTICA SPECIOSA* LARVAE IN BEAN CULTIVARS

The tolerance assay was conducted in a greenhouse under ambient conditions, in the municipality of Jaboticabal, state of São Paulo, Brazil. Sieved sand previously sterilized in an oven (Model AS200S, QUIMIS, Diadema, São Paulo, Brazil) at 110 °C for 24 h was used as substrate for plant development. The 6 common bean cultivars used in this assay were selected based on the results of the antixenosis assay. The cultivars selected for the tolerance assay were IAC-Una, Diamante Negro, IAC-Diplomata, Ônix, IAC-Uirapuru, and BRS-Explendor.

Plants of the 6 cultivars were grown in 770 mL plastic cups (one plant per container), which were arranged in the greenhouse in a complete randomized design comprising 10 replicates for each cultivar. The 15-d-old plants were infested with two 8-d-old larvae by using a fine paintbrush. The larvae were collected from the laboratory colony following the methods previously described. Fifteen days after infestation with larvae, the plants were carefully removed from the cups and their roots were washed in running water. Thereafter, the plant height and number of leaflets of larvae-infested and non-infested plants of each cultivar were recorded.

In the laboratory, the plants were dried in an oven (Model AS200S, QUIMIS) at 60 °C for 48 h. Further, plant materials were weighed on a precision analytical scale (Model AS200S, Florham Park, New Jersey, USA), and the dry weight of the aerial part and dry weight of the root system were separately recorded. The reduction percentage of the plant growth parameters (plant height, number of leaflets, dry weight of aerial part, and dry weight of root system) of the cultivars were calculated as follows: $X = (MA - MD) / MA \times 100$, where X = reduction percentage (%); MA = plant growth parameter of non-infested plants; MD = plant growth parameter of larvae-infested plants (Reese et al. 1994).

Plant growth parameters of larvae-infested and non-infested plants were analyzed for normality (Kolmogorov-Smirnov's test) and variance homogeneity (Bartlett's test) using the statistical software Assistat, version 7.7 Beta. Afterwards, data were transformed into arcsine to meet ANOVA assumptions. Data were then subjected to 1-way ANOVA, and means were separated by Tukey's HSD test at 5% probability whenever ANOVA was significant. The Hierarchical Clustering Analysis (HCA) was performed adopting the Ward's method and the Euclidian distance as the dissimilarity measure using the plant growth parameters of each cultivar. In addition, the Principal Component Analysis (PCA) was performed using the same parameters aiming to group the common bean cultivars that exhibited the highest similarity. The HCA and PCA were performed using the software Statistica 7.0 (Statsoft Incorporation 2004). Lastly, to test for a possible correlation between the reduction percentage of dry weight of the root system and dry weight of the aerial part, the Pearson's simple linear correlation was performed for each cultivar using the software Assistat, version 7.7 Beta.

Results

ANTIXENOSIS TO *DIABROTICA SPECIOSA* ADULTS IN BEAN CULTIVARS

In the free-choice test, differences in leaf disc attractiveness to *D. speciosa* adults were observed at 5 min and 12 h after the release of adults (Table 1). After 5 min, few adults were recorded feeding on the cultivar Diamante Negro (0.10 insects), whereas higher number of adults were found on the cultivar IPR-Tiziu (1.10 insects). At 12 h, the cultivar IAC- Uirapuru was the least attractive (0.10 insects), whereas the cultivar BRS-Supremo had the highest number of adults (1.10 insects). The cultivars IAC-Una and IAC-Uirapuru had leaf intakes of 0.11 cm^2 and 0.13 cm^2, respectively, being the least consumed by adults. On the other hand, the cultivars FT-Nobre and BRS-Supremo were the most preferred by adults with leaf intakes of 1.17 cm^2 and 1.16 cm^2, respectively. In the free-choice test (Fig. 1), the cultivars IAC-Una, IAC-Diplomata, Ônix, IAC-Uirapuru, IPR-Tiziu, and Diamante Negro were classified as feeding deterrent to adults; the cultivar IAC-Maravilha was classified as neutral; and the other cultivars were classified as stimulants for adult feeding after comparison with the results from the standard susceptible cultivar (BRS-Explendor).

In the no-choice test, significant differences of leaf disc attractiveness to adults were observed at 1, 5, and 15 min and 1 h after adult release (Table 2). Moreover, considering the average of all time intervals assessed, the leaf disc attractiveness differed significantly between the common bean cultivars. One minute after the release of adults, none was observed feeding on the cultivar Ônix, whereas the cultivars IAC-Maravilha and BRS-Supremo were the most attractive to adults (0.90 insects both). At 5 min, few adults were recorded on the cultivar IPR-Tiziu (0.30 insects), whereas the highest number of adults was recorded on the cultivar IAC-Maravilha (1.00 insects). Fifteen minutes after adult release, the cultivars IPR-Tiziu and FT-Nobre attracted fewer adults (both 0.30 insects) than the cultivar IAC-Maravilha (1.00 insect). In the average of all the time intervals assessed, the least attractive cultivar was IPR-Tiziu (0.44 insects) and the most attractive cultivars were IAC-Maravilha and BRS-Supremo (0.86 insects both). In the no-choice test, the cultivar IAC-Diplomata was significantly less consumed (0.86 cm^2) by adults than the cultivars Diamante Negro (2.17 cm^2) and BRS-Explendor (2.26 cm^2). In the no-choice test (Fig. 2), the cultivars IAC-Una, FT-Nobre, Ônix, IAC-Uirapuru, IPR-Tiziu, IAC-Diplomata, BRS-Supremo, FT-Nobre, and IAC-Maravilha were classified as deterrent for adult feeding, and the cultivar Diamante Negro was classified as neutral.

TOLERANCE TO *DIABROTICA SPECIOSA* LARVAE IN BEAN CULTIVARS

Significant differences were found in the reduction percentage of plant height and dry weight of the aerial part between cultivars (Table 3). The plant height of the cultivar Ônix was not reduced and was not significantly different from that of the cultivars IAC-Una, BRS-Explendor, and IAC-Diplomata, which had 0.18%, 0.88%, and 2.97% reduction, respectively. On the other hand, the cultivar Diamante Negro had the highest reduction

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Table 1. Number (mean ± SE) of *Diabrotica speciosa* adults attracted to leaf discs and leaf intake (LI) (mean ± SE) values of common bean cultivars in the free-choice test.

	Time						
Cultivar	1 min	5 min	15 min	1 h	12 h	Average	LI (cm^2)[a]
IAC-Una	0.40 ± 0.22 a	0.70 ± 0.21 abc	0.60 ± 0.16 a	0.60 ± 0.22 a	0.20 ± 0.13 ab	0.49 ± 0.10 a	0.11 ± 0.08 a
Diamante Negro	0.20 ± 0.13 a	0.10 ± 0.10 c	0.20 ± 0.13 a	0.70 ± 0.33 a	0.20 ± 0.13 ab	0.32 ± 0.10 a	0.24 ± 0.12 ab
IAC-Diplomata	0.20 ± 0.13 a	0.20 ± 0.13 bc	0.40 ± 0.22 a	0.30 ± 0.15 a	0.30 ± 0.15 ab	0.35 ± 0.12 a	0.27 ± 0.14 ab
FT-Nobre	0.40 ± 0.16 a	0.70 ± 0.21 abc	0.70 ± 0.30 a	0.90 ± 0.31 a	0.80 ± 0.25 ab	0.70 ± 0.18 a	1.17 ± 0.41 b
Ônix	0.10 ± 0.10 a	0.30 ± 0.15 bc	0.80 ± 0.20 a	0.40 ± 0.16 a	0.30 ± 0.15 ab	0.44 ± 0.09 a	0.47 ± 0.11 ab
IAC-Uirapuru	0.20 ± 0.13 a	0.20 ± 0.13 bc	0.20 ± 0.13 a	0.30 ± 0.15 a	0.10 ± 0.10 a	0.22 ± 0.07 a	0.13 ± 0.07 a
IPR-Tiziu	0.50 ± 0.22 a	1.10 ± 0.41 a	0.90 ± 0.38 a	0.70 ± 0.26 a	0.60 ± 0.22 ab	0.73 ± 0.19 a	0.55 ± 0.24 ab
BRS-Explendor	0.70 ± 0.21 a	0.80 ± 0.25 ab	1.00 ± 0.33 a	0.80 ± 0.29 a	1.00 ± 0.21 ab	0.85 ± 0.17 a	0.98 ± 0.17 ab
IAC-Maravilha	0.30 ± 0.15 a	0.80 ± 0.21 ab	0.80 ± 0.29 a	0.60 ± 0.31 a	0.80 ± 0.25 ab	0.57 ± 0.16 a	1.03 ± 0.28 ab
BRS-Supremo	0.30 ± 0.15 a	0.80 ± 0.15 ab	0.60 ± 0.22 a	0.50 ± 0.22 a	1.10 ± 0.31 b	0.49 ± 0.11 a	1.16 ± 0.23 b
F	1.05^{NS}	2.21*	1.18^{NS}	0.54^{NS}	3.06**	1.30^{NS}	4.37***
df	9, 81	9, 81	9, 81	9, 81	9, 81	9, 81	9, 81
P	0.4055	0.0295	0.3181	0.8404	0.0033	0.2503	< 0.0001

Means followed by the same letter in a column did not differ significantly by the Tukey's test at 5% probability.
^{NS} = non-significant; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.
[a]After 19 h of *D. speciosa* adult feeding.

percentage of plant height (18.29%). The cultivar Ônix had the lowest reduction of the dry weight of the plant aerial part (0.25%), whereas the cultivars Diamante Negro (19.40%) and IAC-Uirapuru (19.69%) had the highest reduction percentage of this plant growth parameter.

According to the HCA results, the common bean cultivars were divided into 4 groups when a Euclidian distance at 1.10 for group separation was adopted (Fig. 3). The groups were formed according to the similarity degree of the cultivars considering the plant growth parameters assessed as follows: the cultivar IAC-Uirapuru was individually separated in the 1st group; Diamante Negro was separated in the 2nd group; BRS-Explendor and IAC-Diplomata formed the 3rd group; and Ônix and IAC-Una composed the 4th group (Fig. 3). Regarding the PCA, the 1st principal component (PC1) concentrated 88.99% of the variability contained in the original variables, and the parameters that most influenced the group separation were plant height (−0.93), number of leaflets (−0.93), and dry weight of the aerial part (−0.96) (Fig. 4). The 2nd principal component (PC2) held only 7.40% of the variability contained in the original variables indicating lack of relevance for this analysis.

The PCA results were similar to those obtained with the HCA, and the common bean cultivars were distinctly divided into the same 4 groups (Fig. 4). The cultivars Ônix and IAC-Una were positioned in the 1st quadrant and had the lowest reduction percentages in plant height and number of leaflets (Fig. 4). The cultivar Diamante Negro was isolated in the 2nd quadrant and exhibited highest reduction percentages of plant height, dry weight of the aerial part, and number of leaflets. The cultivar IAC-Uirapuru was isolated in the 3rd quadrant and showed the highest reduction percentages of number of leaflets and dry weight of the aerial part, in addition to intermediate reduction percentage of plant height (Fig. 4). Lastly, the cultivars IAC-Diplomata and BRS-Explendor were positioned together in the 4th quadrant mainly due to the low reduction percentage of plant height and intermediate reduction percentage of dry weight of the aerial part.

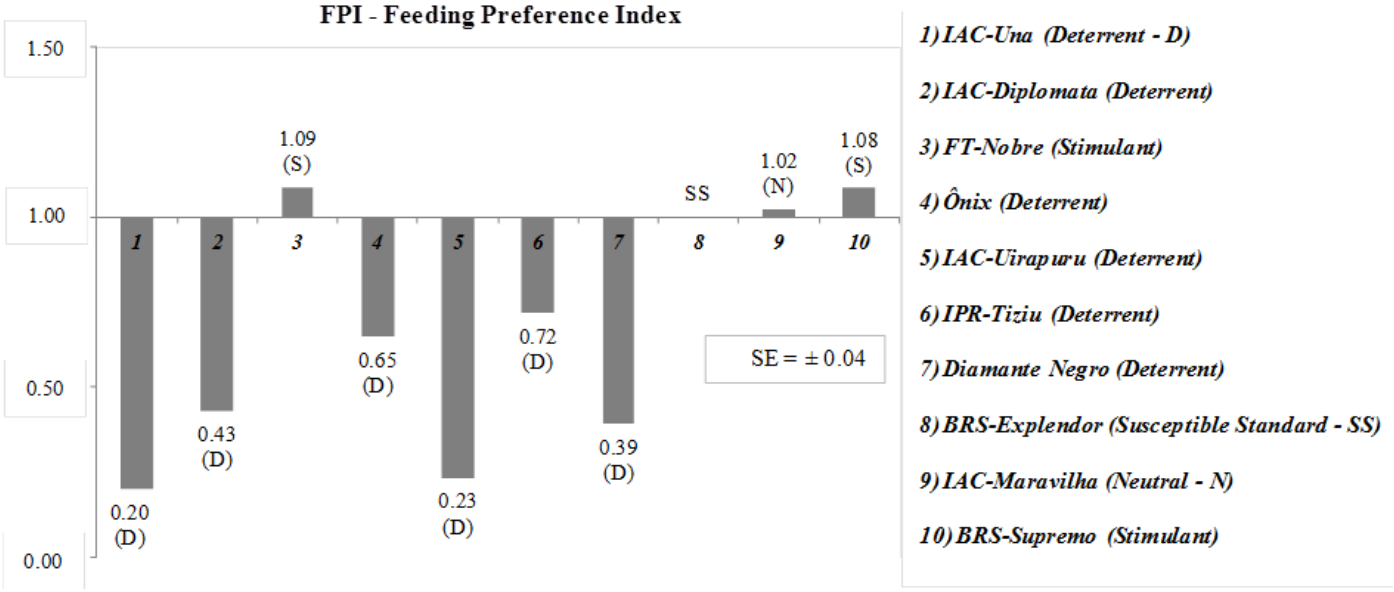


Fig. 1. Feeding preference index of *Diabrotica speciosa* adults to common bean cultivars in the free-choice test.

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Table 2. Number (mean ± SE) of *Diabrotica speciosa* adults attracted to leaf discs and leaf intake (LI) (mean ± SE) values of common bean cultivars in the no-choice test.

	Time						
Cultivar	1 min	5 min	15 min	1 h	12 h	Average	LI (cm^2)[a]
IAC-Una	0.30 ± 0.15 abc	0.50 ± 0.17 ab	0.50 ± 0.17 ab	0.50 ± 0.17 ab	0.70 ± 0.15 a	0.56 ± 0.10 abc	1.38 ± 0.28 abc
Diamante Negro	0.60 ± 0.16 abc	0.60 ± 0.16 ab	0.60 ± 0.16 ab	0.90 ± 0.10 b	0.60 ± 0.16 a	0.63 ± 0.07 abc	2.17 ± 0.33 c
IAC-Diplomata	0.50 ± 0.17 abc	0.80 ± 0.13 ab	0.90 ± 0.10 ab	0.70 ± 0.15 ab	0.70 ± 0.15 a	0.61 ± 0.08 abc	0.86 ± 0.18 a
FT-Nobre	0.20 ± 0.13 ab	0.50 ± 0.17 ab	0.30 ± 0.15 a	0.60 ± 0.16 ab	0.80 ± 0.13 a	0.50 ± 0.07 ab	1.14 ± 0.22 abc
Ônix	0.00 ± 0.00 a	0.40 ± 0.16 ab	0.60 ± 0.16 ab	0.70 ± 0.15 ab	0.60 ± 0.16 a	0.51 ± 0.09 ab	0.87 ± 0.15 ab
IAC-Uirapuru	0.60 ± 0.16 abc	0.70 ± 0.15 ab	0.70 ± 0.15 ab	0.80 ± 0.13 b	0.80 ± 0.13 a	0.72 ± 0.09 abc	2.03 ± 0.26 bc
IPR-Tiziu	0.40 ± 0.16 abc	0.30 ± 0.15 a	0.30 ± 0.15 a	0.20 ± 0.13 a	0.50 ± 0.17 a	0.44 ± 0.08 a	1.93 ± 0.28 abc
BRS-Explendor	0.80 ± 0.13 bc	0.90 ± 0.10 ab	0.90 ± 0.10 ab	1.00 ± 0.00 b	0.80 ± 0.13 a	0.83 ± 0.06 bc	2.26 ± 0.30 c
IAC-Maravilha	0.90 ± 0.10 c	1.00 ± 0.00 b	1.00 ± 0.10 b	1.00 ± 0.00 b	0.70 ± 0.15 a	0.86 ± 0.03 c	1.92 ± 0.27 abc
BRS-Supremo	0.90 ± 0.10 c	0.90 ± 0.10 ab	0.90 ± 0.10 ab	1.00 ± 0.00 b	0.70 ± 0.15 a	0.86 ± 0.06 c	1.57 ± 0.26 abc
F	4.86***	2.90**	3.56**	4.58***	0.43[NS]	4.29***	3.68**
df	9, 90	9, 90	9, 90	9, 90	9, 90	9, 90	9, 90
P	< 0.0001	0.0047	0.0008	< 0.0001	0.9133	< 0.0001	0.0006

Means followed by the same letter in a column did not differ significantly by the Tukey's HSD test at 5% probability.
[NS] = non-significant; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.
[a]After 24 h of *D. speciosa* adult feeding.

Significant positive correlation between reduction of plant root system and reduction of plant aerial part was found for 4 out of the 6 cultivars evaluated in the tolerance assay: Diamante Negro (Fig. 5B), IAC-Diplomata (Fig. 5C), IAC Uirapuru (Fig. 5E), and BRS-Explendor (Fig. 5F). On the other hand, there was no significant correlation between reduction of plant root system and reduction of plant aerial part for the cultivars IAC-Una (Fig. 5A) and Ônix (Fig. 5D). In other words, the aerial part of the cultivars IAC-Una and Ônix was not affected by larval feeding injury.

Discussion

ANTIXENOSIS TO *DIABROTICA SPECIOSA* ADULTS IN BEAN CULTIVARS

This experiment found promising results for antixenosis- and tolerance-resistance against adults and larvae of *D. speciosa* in common bean cultivars under laboratory and greenhouse conditions. Whereas some cultivars displayed less adult leaf intake in the free-choice and the no-choice feeding preference assays, others did not have the plant growth significantly affected following larval feeding injury in the roots. Furthermore, resistance levels were assigned for each cultivar based on the parameters herein evaluated.

The cultivars IAC-Una and IAC-Uirapuru were the least preferred by adults for feeding in the free-choice test, although differences were not found for cultivar attractiveness when all time intervals assessed were considered. However, the cultivars IAC-Una and IAC-Uirapuru did not have the same performance in the no-choice test (Table 2), in which they were moderately preferred by the adults. Although the cultivar IAC-Diplomata was the least consumed and the cultivars Diamante Negro and BRS-Explendor were the most consumed by adults under no-choice conditions, they were equally attractive to the adults when the average of all time intervals assessed was considered.

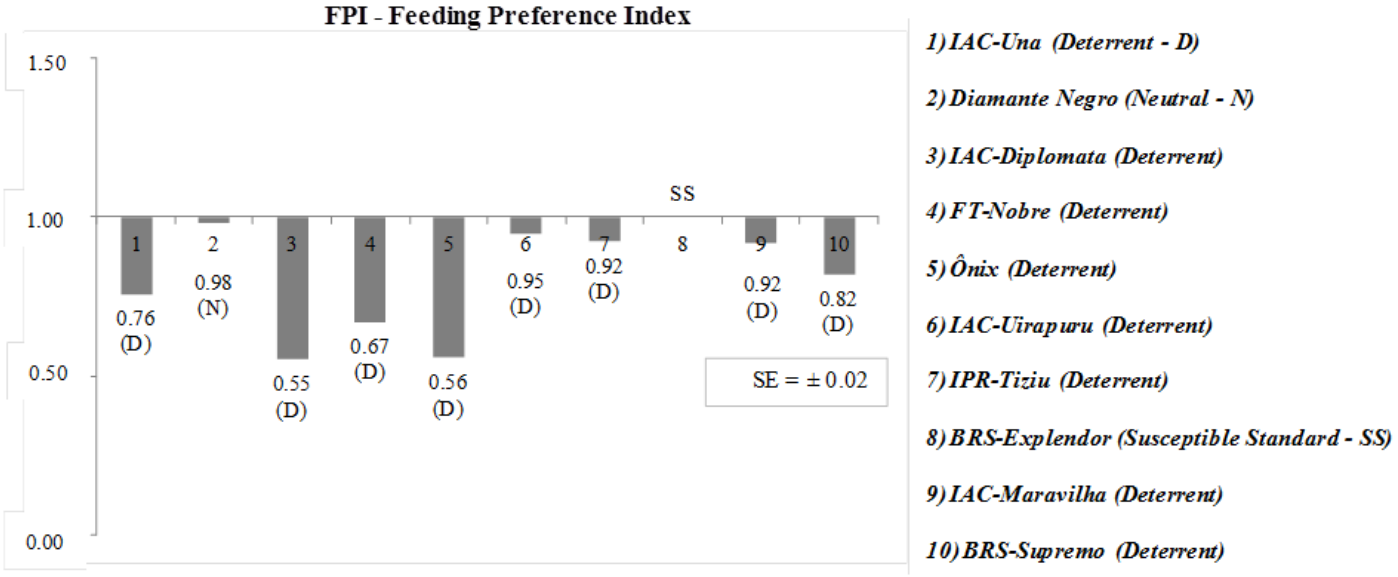


Fig. 2. Feeding preference index of *Diabrotica speciosa* adults to common bean cultivars in the no-choice test.

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Table 3. Reduction percentage (mean ± SE) in plant height, number of leaflets, dry weight of the aerial part, and dry weight of the root system of plants of common bean cultivars infested by *Diabrotica speciosa* larvae.

	Reduction percentage			
Cultivar	Height (cm)	Number of leaflets	Dry weight of the aerial part (mg)	Dry weight of the root system (mg)
IAC-Una	0.18 ± 0.18 a	0.74 ± 0.20 a	7.13 ± 4.05 ab	20.17 ± 5.14 a
Diamante Negro	18.29 ± 7.97 b	5.00 ± 3.82 a	19.40 ± 7.32 b	19.58 ± 6.74 a
IAC-Diplomata	2.97 ± 2.97 a	2.31 ± 2.31 a	1.05 ± 0.96 ab	13.26 ± 5.30 a
Ônix	0.00 ± 0.00 a	0.00 ± 0.00 a	0.25 ± 0.25 a	6.06 ± 2.89 a
IAC-Uirapuru	10.46 ± 5.94 ab	6.15 ± 6.15 a	19.69 ± 11.11 b	22.27 ± 8.61 a
BRS-Explendor	0.88 ± 0.88 a	3.51 ± 3.51 a	6.59 ± 4.0 7 ab	11.46 ± 3.65 a
F	2.92*	0.48^{NS}	2.93*	1.19^{NS}
df	5, 54	5, 54	5, 54	5, 54
P	0.0209	0.7883	0.0205	0.3266

Means followed by the same letter in a column did not differ significantly by Tukey's HSD test at 5% probability.
^{NS} = non-significant; * = $P < 0.05$.

Herbivorous insects are able to respond to several stimuli from host plants when selecting them for feeding and oviposition purposes (Panda & Khush 1995). The absence of favorable (attractive) stimuli and/or presence of unfavorable (repellent) stimuli in the plant are related to expression of antixenosis. Probably, the volatile blends emitted by the leaf discs were not too distinct between cultivars; therefore, they did not guide the adults towards the most suitable common bean cultivars or repelled the insects in the case of unsuitable cultivars. Based on these findings, we suggest that the volatiles from the common bean cultivars evaluated in the current study are not very efficient in repelling or attracting *D. speciosa* adults. However, it seems that some of the cultivars possess deterrent compounds allowing the adults to distinguish unsuitable cultivars only after they have started feeding on the foliage.

In this study, some cultivars were classified as feeding stimulants in the free-choice test (Fig. 1) but classified as feeding deterrents in the no-choice test (Fig. 2). Schlick-Souza et al. (2011) pointed out that in plant resistance studies, discrepancies in results between free-choice and no-choice tests are common. According to these authors, it is possible that a host plant initially considered as susceptible under free-choice condition can be unfavorable under no-choice condition. The same situation was observed in our study for some cultivars and demonstrates that a no-choice test is always recommended for evaluation of antixenosis-resistance among plant cultivars. Likewise, there are situations in which one cultivar is classified as resistant when the insects are allowed to choose between many cultivars, but it does not maintain this characteristic under no-choice conditions (Boiça Junior et al. 2013), what was also observed in our study. Furthermore, no-choice tests are more similar

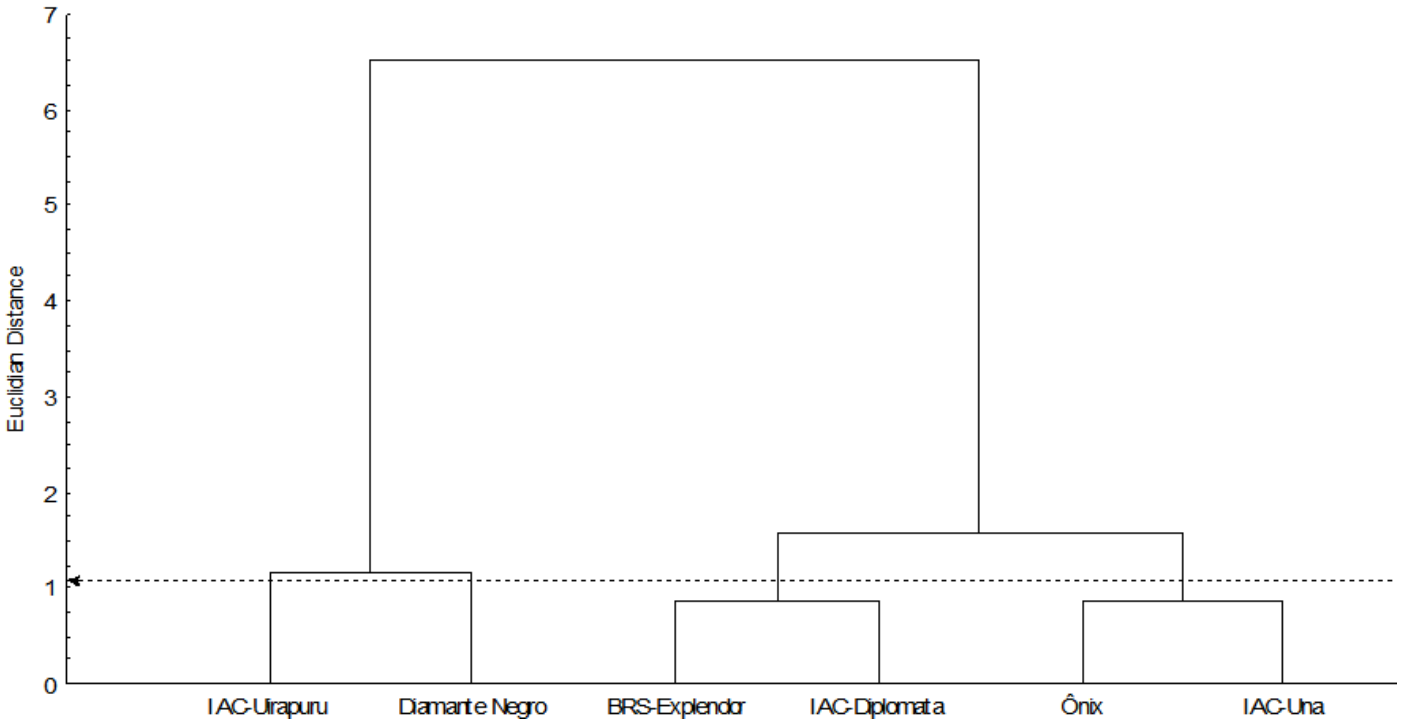


Fig. 3. Dendrogram based on plant growth parameters recorded in common bean cultivars infested by *Diabrotica speciosa* larvae. The arrow indicates the Euclidian distance used for group separation.

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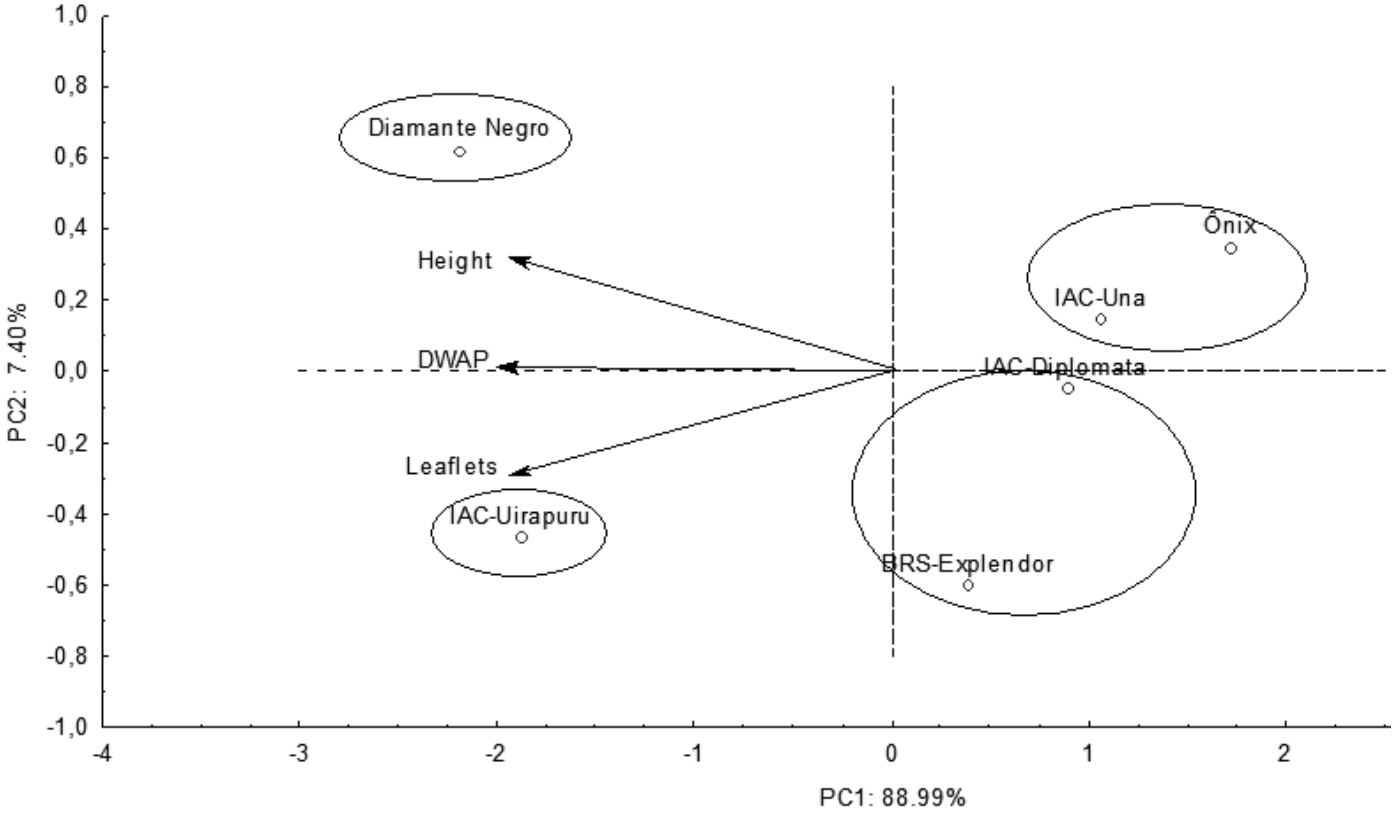


Fig. 4. Distribution of plant growth parameters and common bean cultivars following principal component analysis obtained from plants infested by *Diabrotica speciosa* larvae.

to field conditions where the crops usually are grown in monocultures. Thus, considering the leaf intake of *D. speciosa* adults recorded in the no-choice test, the common bean cultivars evaluated in the current study were classified as follows: IAC-Diplomata and Ônix were the least preferred; IAC-Una, FT-Nobre, IAC-Uirapuru, IPR-Tiziu, IAC-Maravilha, and BRS-Supremo were moderately preferred; and Diamante Negro and BRS-Explendor were the most preferred by *D. speciosa* adults.

Although laboratory studies are very useful to understand the mechanisms mediating plant–insect interactions (Bernays & Chapman 1994), results from field studies may be different from studies conducted in environmentally controlled conditions due to the complexity of the field's ecological factors. Thus, the natural order of performing plant resistance studies should be first in the laboratory, next in the greenhouse, and lastly in the field. However, only 1 study comparing the results of laboratory and field assays using bean cultivars infested with *D. speciosa* was found in the literature (Paron & Lara 2001).

In that study, Paron & Lara (2001) evaluated the feeding preference of *D. speciosa* to several bean genotypes from different domestication centers under laboratory and field conditions. They concluded that the Middle American genotypes (Emgopa 201 Ouro and IAPAR 57) were the most preferred by *D. speciosa* in feeding assays, whereas the Andean genotypes (Goiano Precoce, Jalo Precoce, PR 95105142, and PR 95105146) were the least preferred. Additionally, the relationship between laboratory and field results indicated that laboratory data could be very useful for predicting results of field studies. Despite this being true, we encourage field evaluations using the cultivars screened in the current study during different growing seasons to confirm whether any of the cultivars expresses antixenosis to *D. speciosa* adults under field conditions and whether resistance can be expressed in varying levels according to the plant growing conditions.

TOLERANCE TO *DIABROTICA SPECIOSA* LARVAE IN BEAN CULTIVARS

Differences were found for the reduction percentages of the plant growth parameters assessed after *D. speciosa* larval infestation between the common bean cultivars using univariate (Table 3) and multivariate statistical analyses (Figs. 3 and 4). The cultivars Ônix and IAC-Una had the lowest reduction of plant height and dry weight of the aerial part. The cultivars BRS-Explendor and IAC-Diplomata showed intermediate reduction of all plant growth parameters, and the other cultivars had the highest reduction of plant growth following *D. speciosa* larval feeding injury.

In our study, only the cultivars IAC-Una and Ônix did not have reduced dry weight of the plant aerial part following larval feeding injury on the roots (Fig. 5). According to Bernays & Chapman (1994), the roots tend to have low levels of nutrients or high levels of secondary metabolites. In this context, cultivars expressing tolerance probably possess significant amounts of substances that stiffen the root tissues, such as lignin, cellulose, and silicon. Thus, based on the results for the cultivars IAC-Una and Ônix, further studies should investigate the amount of lignin, cellulose, and silicon in their roots in order to correlate tolerance to *D. speciosa*–caused injury with the amount of these substances.

Studies evaluating the attack by *D. speciosa* larvae on common bean plants are scarce in the literature. In 1 of these studies, Ávila & Parra (2002) evaluated the development of *D. speciosa* on different hosts and concluded that in field conditions, larvae and adults can use bean plants for feeding and reproduction, respectively, as an alternative host in the absence of the preferential host. Cardona et al. (1982), while assessing the damages caused by larvae of *Diabrotica balteata* Le Conte on bean plants, concluded that the losses could reach up to 100% in the plant stand. In addition, *D. balteata* larval feeding injury

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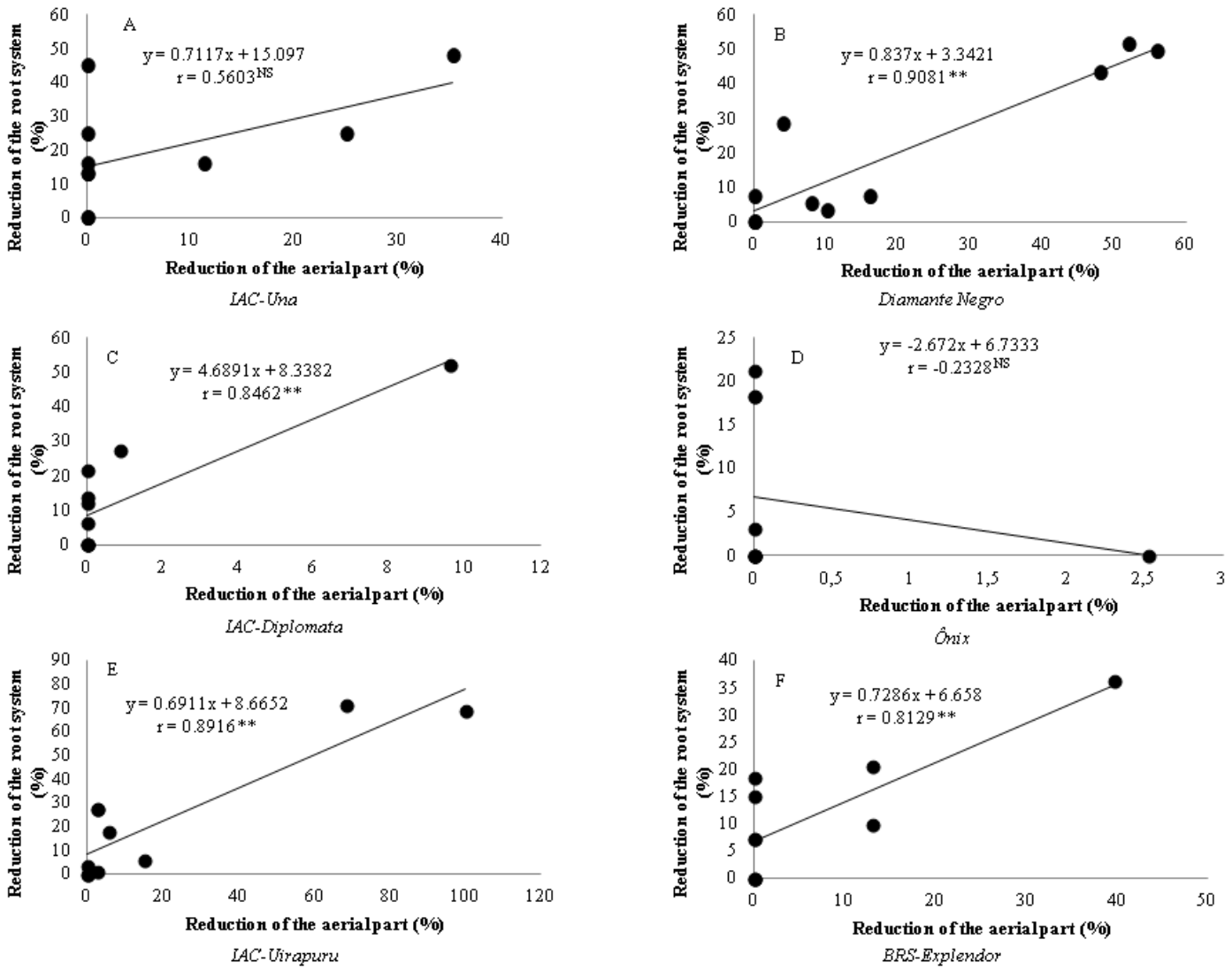


Fig. 5. Analysis of Pearson's simple linear correlation between reduction percentage of the dry weight of the plant root system and dry weight of the plant aerial part in common bean cultivars infested by *Diabrotica speciosa* larvae.

could significantly reduce the leaf area of plants that survived larval infestation at 1, 4, and 7 d after sowing.

In summary, the current study showed differences between the common bean cultivars regarding their tolerance levels to *D. speciosa* larval feeding injury and assessed the effects of *D. speciosa* infestation on the development of the plant aerial part. Thus, the next step should be the evaluation of the cultivars under field conditions to obtain more conclusive data, especially regarding to plant yield under *D. speciosa* larval infestation.

FUTURE PERSPECTIVES

Smith (2005) claimed that plants resistant to insects are found in low percentages among several plant genotypes assessed. The cultivar Ônix was one of the least preferred by *D. speciosa* adults for feeding and had practically no reduced growth of the plant aerial parts following larval feeding injury in the roots. Thus, the potential resistance of the cultivar Ônix to *D. speciosa* should be further investigated, because in the current study this cultivar had a good performance in antixenosis and tolerance assays with adults and larvae of *D. speciosa*, respectively. In addition, the underlying mechanisms of resistance of the Ônix cultivar merit exploration in order to transmit the resistance-related traits to high-yielding cultivars.

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