



Biological performance of *Leptoglossus zonatus* (Dallas) (Hemiptera: Coreidae) on corn genotypes

Vinícius Fernandes Canassa^{1,*} , Fabiana Campos Medeiros¹ , Luiz Felipe Souza Bastos¹ , Rodrigo Donizeti Faria¹ , Thais Lohaine Braga dos Santos¹ , Isabella Rubio Cabral¹ , André Luiz Lourenção² , Edson Luiz Lopes Baldin¹ 

1. Universidade Estadual Paulista  – Faculdade de Ciências Agrônômicas - Departamento de Proteção Vegetal - Laboratório de Resistência de Plantas a Insetos e Plantas Inseticidas - Botucatu, São Paulo, Brasil.
2. Universidade de São Paulo  – Escola Superior de Agricultura “Luiz de Queiroz” - Departamento de Entomologia e Acarologia - Piracicaba, São Paulo, Brazil.

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*Corresponding author: vf.canassa@unesp.br

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ABSTRACT: Corn, *Zea mays* (L.), is one of the most expressive crops in Brazil, due to its wide-ranging uses as animal feeding, biofuel production or used for human consumption. However, there is a huge gap between the actual yield and the natural yield potential of the crop, due to insects' attack. *Leptoglossus zonatus* (Dallas) (Hemiptera: Coreidae) is a key corn pest in seed producing regions, due to its attack on corn ears. Plant resistance is a valuable tool for Integrated Pest Management systems and may maintain insect populations at a level that is below an economic or damage threshold. This study aimed to evaluate the biological performance of *L. zonatus* in different corn genotypes, under laboratory conditions, searching for possible characterization of antibiosis/antixenosis. To achieve this goal, ten nymphs (1st instar) were transferred to polyvinylchloride (PVC) ring containing a corn ear (R6) of each genotype. Each ring corresponded to one replicate, for a total of five replicates per genotype in a completely randomized design. The parameters assessed included the duration of each instar, nymphal period, nymphal survival (%), percentage of mortality of each nymphal stage, adult longevity, and adult weight. The results demonstrate that XB 8018 Bt, 30F53, IAC Airan, XB 8030 and 30F53 YHR genotypes reduced the mean survival and caused high mortality of *L. zonatus* nymphs at the second instar (above 75.00%) indicating the occurrence of antibiosis/antixenosis. The higher mortality rates were observed at the second nymphal instar.

Key words: leaf-footed bug, antibiosis, antixenosis, *Zea mays*.

INTRODUCTION

Corn, *Zea mays* (L.), is one of the most consumed crops in the world and its main use is as a grain in animal feed. On the other hand, humans consume corn from its derivatives: oil, flour, starch, margarine, glucose syrup, among others (Artuzo et al. 2019). In addition, corn grains have been employed as raw material for ethanol production (Fasiku and Wakil 2022). Brazil is the third largest corn producer in the world, behind only the United States and China. Total planted area was estimated at 22,156.6 hectares in the 2022/2023 crop year, with production around of 127,767.0 tons and a mean productivity of 5,767 kg·ha⁻¹ (Conab 2023).

Although corn has a huge production capacity, the crop may be subject to the occurrence of pests and diseases, which can severely compromise crop yield. The leaf-footed bug, *Leptoglossus zonatus* (Dallas) (Heteroptera: Coreidae), a poliphagous pest widely found in the North and South American continents (Pires et al. 2011) attacks corn, sorghum, and some fruit plants (Schaefer and Panizzi 2000). The nymphs and adults are present throughout the crop entire life cycle. However, the damage related to yield losses is mainly related due to the suction of the nymphs (3rd, 4th, and 5th instar) and adults from the

grains of the corn ear. Therefore, the direct damage is related to its continuous suction resulting in physiological disorders that impairs grain weight and makes the product unviable for consumption and commercialization (Zucchi et al. 1993). In seed production fields, problems can be even more severe because quality parameters required for seed marketing are compromised (Foresti et al. 2017).

Currently, there are no products registered to control populations of *L. zonatus* in corn crops in Brazil, and its management is based on the use of agrochemicals available for the species *L. gonagra* (Fabr.) (Agrofit 2023). This situation stimulates the use of agrochemicals that are not efficient and encourages its application in higher doses than necessary to control the insect (Foresti et al. 2017). Moreover, the continuous and indiscriminate use of synthetic insecticides has caused the elimination of beneficial insects, selection of resistant populations, as well as concerns regarding human health and the environment (Almeida et al. 2017). Thereby, it is necessary searching for new control effective strategies and environmental safer. Among alternative methods to chemical control, plant resistance presents itself as a valuable option for the control of pest insects. Varietal resistance allows the maintenance of the pest insect population below the level of economic damage, reduces negative impacts on the environment and are highly compatible with other management tactics (Baldin et al. 2019; Smith and Clement 2012).

Painter (1951) defined 'resistant' as that plant which, due to the relative sum of its hereditary characteristics, influences the level of damage caused by insects. The host-plant resistance is divided into three categories: antixenosis (non-preference), antibiosis, and tolerance. Antixenosis is characterized by the presence of chemical or morphological factors in the plant, which negatively affects the insect's behavior during the colonization process. In antibiosis, the plant negatively affects the biology of the insect, interfering with its developmental time, reproduction, and survival. Tolerance, on the other hand, is the ability of the plant to resist or recover from insect injuries, maintaining productivity and/or quality, at the same level of infestation, without affecting the biology and behavior of the insect (Baldin et al. 2019; Painter 1951; Smith 2005). The present study focused on the category antibiosis and antixenosis resistance.

The increased planting areas that incorporate *Bacillus thuringiensis* (Bt) technology has provided a significant modification in sprayed insecticides and caused changes in the economic status of pests associated with crops. This is especially true for sucking insects that were not previously considered key pests (Cruz 2012). There are currently no studies that assess the behavior and biological performance of *L. zonatus* on corn germplasm, including Bt and non-Bt corn genotypes. For this reason, this study sought to evaluate the biological performance of *L. zonatus*, at different corn genotypes under laboratory conditions for possible characterization of antibiosis/antixenosis.

METHODS

Leptoglossus zonatus stock rearing

The adults of *L. zonatus* were collected from corn fields with the assistance of entomological nets and the insects were placed in metal cages (40 × 40 × 60 cm) covered with anti-aphid screens, in maximum of 50 adults per cage. The screens allowed air passage and prevented the escape of insects (Souza and Baldin 2009). The cages with the insects were kept in a greenhouse, under partially controlled conditions (T = 25 °C, with a maximum of 40.7 °C and a minimum of 19.0 °C; relative humidity (RH) = 60±10%; and natural light).

To supply the leaf-footed bug with water and maintain humidity inside the metal cages, portions of cotton ball moistened with distilled water was placed in Petri dishes (4 cm Ø). To feed the insects (nymphs and adults), squash, *Cucurbita pepo* (L. 1753) (cv 'Menina brasileira'), orange, *Citrus sinensis* (L. Osbeck 1759) (cv 'Pêra'), and corn ears, *Zea mays* (L. 1753) (genotypes different from the ones evaluated) (cv 'AL Avaré') were placed inside the metal cage simultaneously. The food and the moistened cotton portions were replaced weekly to avoid the development of microorganisms and contamination of the environment. Dry tree branches, *Caesalpinia peltophoroides* (Benth. 1844), were used as oviposition substrate for the leaf-footed bugs. Egg postures were collected daily and were placed in polyvinylchloride material (PVC) (35 cm high × 15 cm Ø) covered on the bottom with filter paper and closed with a 'voil' until the nymphs hatched, maintaining the nymphs and adults in separate rings.

Bioassay

The leaf-footed bug biological performance on each of the 10 corn genotypes was evaluated through a no-choice assay, under controlled laboratory conditions ($25\pm 2^{\circ}\text{C}$, $60\pm 10\%$ RH, photoperiod 14:10 h). Accordingly, one corn ear with husks from each genotype, in phenological phase R6, were individualized with rings made of PVC (35 cm high \times 15 cm \varnothing). Subsequently, 10 nymphs of *L. zonatus* (first instar) were released inside the ring and it was covered with filter paper and closed with a 'voil' fabric. A portion of cotton moistened with distilled water was placed inside the Petri dishes (4 cm \varnothing) to supply water to the insects. Each ring corresponded to one replicate, for a total of five replicates per genotype ($n = 50$ nymphs per genotype) in a completely randomized design (CRD).

The corn ears were obtained from plants grown in the experimental area without any spraying of the Plant Protection Department, School of Agriculture - FCA/UNESP, Botucatu Campus ($22^{\circ}85'09''\text{S}$ and $48^{\circ}43'16''\text{W}$). The genotypes used in the experiments and their features are described in Table 1. The corn planting was staggered in order to obtain the ears during the entire experimental period. The corn ears, the filter paper, and the cotton portions were replaced as necessary, never exceeding 10 days, to avoid the accumulation of excrement, the development of microorganisms, and the contamination of the rings.

Table 1. Name, characteristics, and origins of 10 corn genotypes evaluated for resistance to *Leptoglossus zonatus*.

Genotype	Features	Source
XB 6012	Simple hybrid (HS), Cry1Ab carrier that confers resistance to Lepidoptera.	Semeali®
XB 8018	Double hybrid (HD), Cry1Ab carrier that confers resistance to Lepidoptera.	Semeali®
XB 7116	Simple hybrid (HT), Cry1Ab carrier that confers resistance to Lepidoptera.	Semeali®
XB 8018	Double hybrid (HD), non-Bt.	Semeali®
XB 8030	Double hybrid (HD), non-Bt.	Semeali®
30F53	Tolerant to glyphosate herbicide	Corteva®
30F53 YHR	Resistant to insects of the order Lepidoptera and tolerant to the herbicide glufosinate ammonium.	Corteva®
IAC Airan	Non-Bt.	IAC
JM2M77	Non-Bt.	Jmen
DKB 290 VTPRO2	Carrier of Cry1A and Cry2Ab2 that confer resistance to Lepidoptera and tolerance to glyphosate herbicide	Bayer®

Source: Companies information.

The insects were evaluated daily and always at the same time each morning. Records for the insects at the immature stage included the duration of each nymphal instar, the nymphal period, the nymphal survival rate (%), and the mortality percentage during each nymphal instar. In the adult stage, the insects were maintained under the same conditions, evaluating the adult longevity of each insect. In addition, the weight of 24 h old adults were assessed, using analytical balance (Marte AY 220, accuracy of 0.0001 g). For test maintenance, exuvia and dead insects were removed daily at the time of the assessments, using a metal clamp with the tip wrapped in moistened cotton.

Statistical analysis

The data were submitted to an analysis of variance by F-test. The normality was verified by the Shapiro-Wilk test and homogeneity was verified by the Levene test. The significance of the treatment effects was determined using the Fishers Least Significant Difference (LSD) to compare the means. For the analysis, we used the statistical package PROC MIXED-SAS 9.2 (SAS Institute 2008).

Survival analysis were performed using Kaplan-Meier estimators using the Log-Rank test (Sigmaplot, 12.5 version). From this analysis, survival curves were obtained to access the time required to cause mortality in 50% of the individuals (ST50) and total survival time (ST) for each genotype. The curves were compared using the Holm-Sidak multiple comparison method at the significance level of 0.05 (Sigmaplot 12.5 version).

RESULTS

Regarding to the nymphal period, the duration of the first instar of *L. zonatus* on the genotype 30F53 (6.14 days) differed significantly from XB 6012 *Bt* (4.08 days) and XB 8030 (3.83 days) genotypes, whereas the others had intermediate values (Table 2). For the second instar duration, the genotype IAC Airan (35.81 days) stood out with the highest mean, differing significantly from 30F53YHR (7.62 days). At this instar, nymphs on the XB 8018 *Bt* died and were not able to complete the second instar. For the third nymphal instar, genotypes XB 8030 (31.00 days) and 30F53YHR (27.00 days) resulted in the longest period, differing significantly from the other genotypes. There was no significant difference among the genotypes in the fourth and fifth nymphal instar duration and nymphal period (Table 2). Regarding adult weight and longevity, there were no significant differences between genotypes (Table 3).

Table 2. Mean (\pm SE) of each instar and nymphal period (N1–N5) of *Leptoglossus zonatus* in 10 corn genotypes under laboratory conditions.

Genotype	Mean of each instar (days) ¹					
	1 st instar	2 nd instar	3 rd instar	4 th instar	5 th instar	Nymphal period
30F53	6.14 \pm 0.56 (n = 26) a	13.40 \pm ----- (n = 5) ab	7.50 \pm ----- (n = 2) b	8.00 \pm --- (n = 1)	13.00 \pm --- (n = 1)	46.00 \pm ----- (n = 1)
XB 8018 <i>Bt</i>	5.48 \pm 0.63 (n = 34) ab	-----	-----	-----	-----	-----
30F53YHR	5.18 \pm 0.33 (n = 31) ab	7.62 \pm ----- (n = 4) b	27.00 \pm ----- (n = 2) a	21.00 \pm --- (n = 1)	16.00 \pm --- (n = 1)	70.00 \pm ----- (n = 1)
XB 7116 <i>Bt</i>	5.05 \pm 0.46 (n = 49) ab	16.21 \pm 1.81 (n = 25) ab	11.70 \pm 1.45 (n = 14) b	13.65 \pm 2.42 (n = 12)	13.50 \pm 1.26 (n = 7)	61.50 \pm 8.39 (n = 7)
IAC Airan	4.88 \pm 0.03 (n = 50) ab	35.81 \pm 6.31 (n = 12) a	10.50 \pm 0.31 (n = 5) b	9.25 \pm 1.42 (n = 5)	15.25 \pm 1.10 (n = 5)	67.37 \pm 3.55 (n = 5)
JM2M77	4.88 \pm 0.32 (n = 44) ab	17.41 \pm 4.52 (n = 17) ab	8.70 \pm 1.28 (n = 13) b	11.21 \pm 1.41 (n = 12)	14.20 \pm 0.59 (n = 12)	57.52 \pm 5.94 (n = 12)
DKB 290 VTPRO2	4.80 \pm 0.45 (n = 40) ab	14.26 \pm 1.61 (n = 14) ab	7.91 \pm 0.81 (n = 6) b	9.33 \pm 0.25 (n = 5)	13.44 \pm 1.40 (n = 5)	50.33 \pm 4.52 (n = 5)
XB 8018	4.36 \pm 0.29 (n = 41) ab	20.75 \pm 4.84 (n = 21) ab	9.56 \pm 1.02 (n = 9) b	9.23 \pm 0.33 (n = 9)	12.63 \pm 0.83 (n = 8)	39.63 \pm 1.37 (n = 8)
XB 6012 <i>Bt</i>	4.08 \pm 0.16 (n = 49) b	20.70 \pm 3.75 (n = 30) ab	10.17 \pm 0.73 (n = 17) b	11.32 \pm 1.36 (n = 12)	18.75 \pm 2.59 (n = 9)	68.56 \pm 7.39 (n = 9)
XB 8030	3.83 \pm 0.24 (n = 38) b	14.50 \pm 3.47 (n = 5) ab	31.00 \pm ----- (n = 1) a	13.00 \pm --- (n = 1)	15.00 \pm --- (n = 1)	73.00 \pm ----- (n = 1)
<i>p</i>	0.0120	0.0396	<0.0001	0.1609	0.4404	0.2496

¹Mean followed by the same lower-case letter per column do not differ by LSD test ($p \leq 0.05$); Source: Elaborated by the authors.

Table 3. Mean (\pm SE) of adult weight (grams) and longevity (days) of *Leptoglossus zonatus* in 10 corn genotypes under laboratory conditions

Genotype	Adult weight (g) ¹	Longevity (d) ¹
XB 8018	0.1093 \pm 0.005 (n = 8)	109.13 \pm 27.37 (n = 8)
JM2M77	0.0885 \pm 0.017 (n = 12)	73.67 \pm 10.40 (n = 12)
IAC Airan	0.0948 \pm 0.005 (n = 5)	63.62 \pm 9.88 (n = 5)
30F53	0.2700 \pm ----- (n = 1)	62.00 \pm ----- (n = 1)
XB 7116 <i>Bt</i>	0.1771 \pm 0.034 (n = 7)	55.90 \pm 21.63 (n = 7)
DKB 290 VTPRO2	0.1533 \pm 0.041 (n = 5)	53.44 \pm 19.93 (n = 5)
30F53YHR	0.0903 \pm ----- (n = 1)	45.00 \pm ----- (n = 1)
XB 6012 <i>Bt</i>	0.1512 \pm 0.027 (n = 9)	37.87 \pm 11.25 (n = 9)
XB 8030	0.1100 \pm ----- (n = 1)	16.00 \pm ----- (n = 1)
XB 8018 <i>Bt</i>	-----	-----
<i>p</i>	0.2549	0.5198

¹Mean followed by the same lower-case letter per column do not differ by LSD test ($p \leq 0.05$). Source: Elaborated by the authors.

The percentage of nymph survival was null in nymphs fed on XB 8018 *Bt*, while the genotype JM2M77 led to the highest survival rate (24.00%) (Fig.1). The survival of *L. zonatus* nymphs exposed to genotypes was significantly reduced over time (Log-rank test: 161.66; $P < 0.001$). *Leptoglossus zonatus* nymphs showed rapid reduction in survival when exposed to XB 8030 ($LT_{50} = 7.00$ days; $CI_{95} = 5.11-8.89$), XB 8018 *Bt* ($LT_{50} = 8.00$ days; $CI_{95} = 6.95-9.04$), 30F53 YHR ($LT_{50} = 8.00$ days; $CI_{95} = 6.46-9.53$), and 30F53 YHR ($LT_{50} = 11.00$ days; $CI_{95} = 9.21-12.79$) (Fig. 2). On the other hand, XB 6012 *Bt* ($LT_{50} = 26.00$ days; $CI_{95} = 19.07-32.92$), XB 7116 *Bt* ($LT_{50} = 22.00$ days; $CI_{95} = 14.08-29.91$), and IAC Airan ($LT_{50} = 22.00$ days; $CI_{95} = 19.69-24,30$), prolonged the survival time (LT_{50}) (Fig. 2 and Table 4) .

Table 4. Mean (\pm SE) of survival time (days) of *Leptoglossus zonatus* in 10 corn genotypes under laboratory conditions.

Genotype	ST ₅₀ ¹ (d)	ST ² (d)
JM2M77	13.00 \pm 1.75	41.82 \pm 7.77
XB 8018	18.00 \pm 2.94	41.06 \pm 8.33
XB 6012 <i>Bt</i>	26.00 \pm 3.53	39,02 \pm 4.89
XB 7116 <i>Bt</i>	22.00 \pm 4.03	38.16 \pm 5.40
IAC Airan	22.00 \pm 1.17	36.12 \pm 5.58
DKB 290 VTPRO2	12.00 \pm 3.53	23.90 \pm 4.61
30F53YHR	8.00 \pm 0.78	12.00 \pm 2.51
30F53	11.00 \pm 0.91	11.62 \pm 2.24
XB 8030	7.00 \pm 0.96	9.84 \pm 1.80
XB 8018 <i>Bt</i>	8.00 \pm 0.53	9.40 \pm 0.85
<i>p</i>		<0.0001
Log-Rank Test		161.66

¹ ST₅₀ - Time required to cause mortality in 50% of the individuals. ² ST - Total survival time. Source: Elaborated by the authors.

The highest means of nymphal mortality percentage was observed in the second instar for almost all corn genotypes. The genotypes XB 8018 *Bt* (100%), 30F53 YHR (91.1%), XB 8030 (88.5%), 30F53 (84.3%), IAC Airan (76.0%) stood out among the others. Moreover, DKB290 VTPRO2 (65.8%), JM2M77 (60.6%) and XB 8018 (56.0%) genotypes showed intermediate nymphal mortality percentage (Fig. 3).

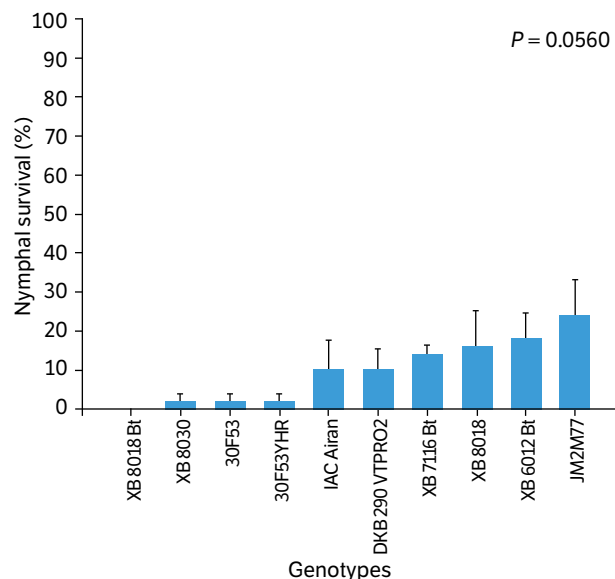


Figure 1. Mean (\pm SE) of percentage of nymphal survival of *Leptoglossus zonatus* in 10 corn genotypes under laboratory conditions.

Source: Elaborated by the authors.

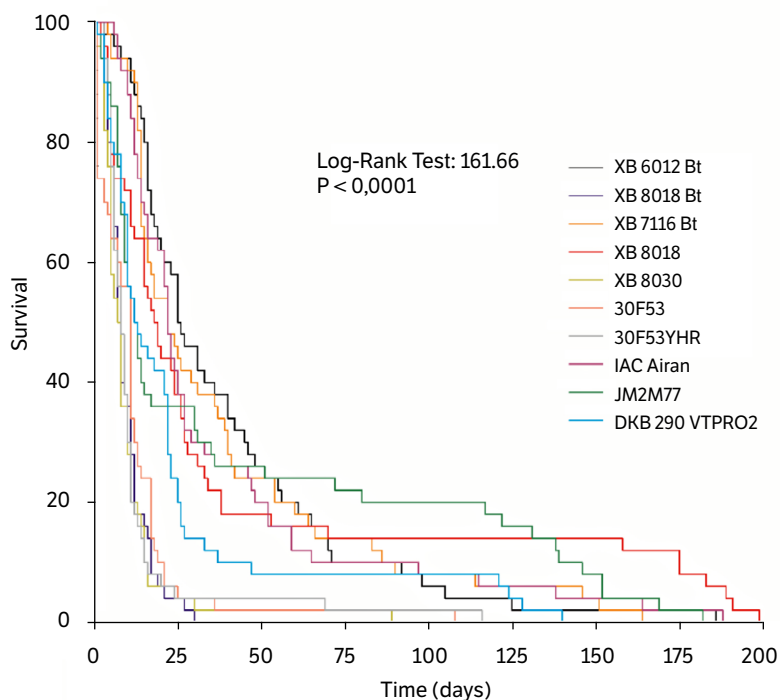


Figure 2. Survival curves of *Leptoglossus zonatus* in different corn genotypes under laboratory conditions.

Source: Elaborated by the authors.

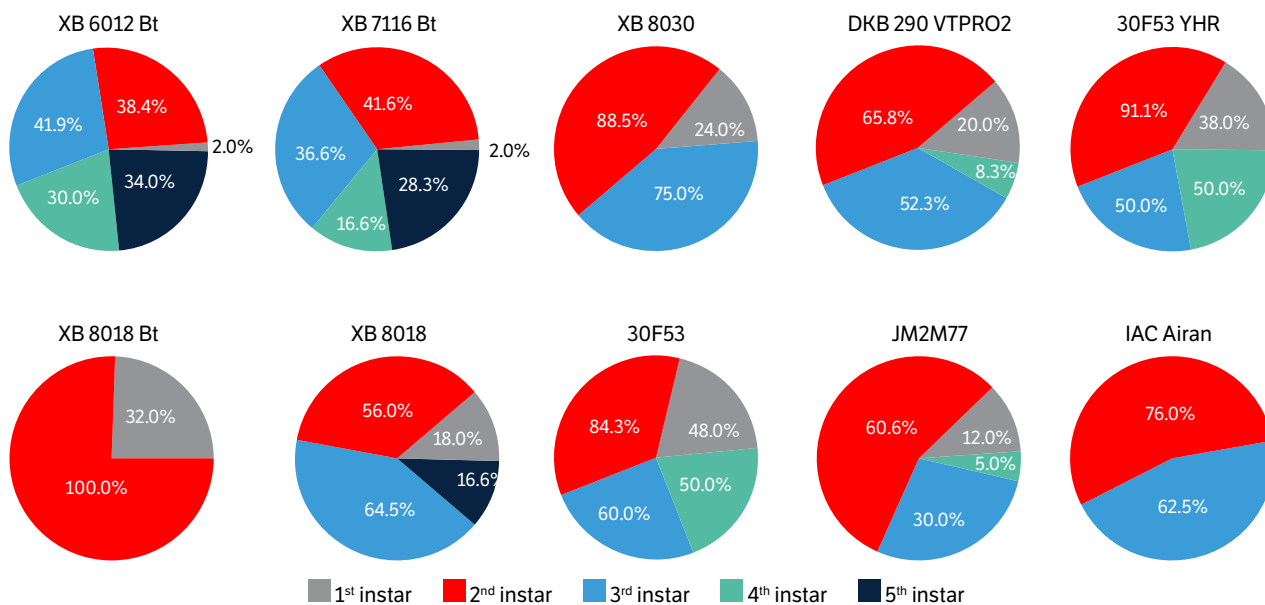


Figure 3. Mean percentages of nymphal mortality per instar of *Leptoglossus zonatus* in 10 corn genotypes under laboratory conditions.

Source: Elaborated by the authors, adapted from Canassa et al., 2017.

The different corn genotypes significantly affected the biological parameters of the leaf-footed bug, indicating the occurrence of resistance (Baldin et al. 2019; Painter 1951). Antibiosis-resistant genotypes and those with antixenotic factors can have deleterious effects on the biology of insects, especially at the early instars of development (Lara 1991; Smith 2005).

Based on the results, the performance of *L. zonatus* varied in the different genotypes. The genotypes 30F53, IAC Airan, XB 8030 and 30F53 YHR resulted in prolonged developmental nymphal periods and showed high nymphal mortality in the second and third instar, in addition to XB 8018 *Bt* with mortality of all insects in this period, which suggests the occurrence of antibiosis and/or antixenosis (Baldin et al. 2019; Smith and Clement 2012). The other genotypes showed intermediate resistance or were susceptible.

The husk leaves covering the ears and the corn grains in a real field condition, may vary in quantity, thickness, and hardness for each genotype, making it difficult for the insect to reach the milky grains at the insertion time of its mouth apparatus on the ears. In studies under field conditions with other corn genotypes, nymphs and adults migrate to different regions of the plant (leaves and tassels) when they find an obstacle to feed on the ears, selecting the structure of each genotype that is most ideal for feeding (Matrangolo and Waquil 1994; Souza and Baldin 2009). In the present study, only green corn ears with husks were provided for the feeding nymphs and adults of the leaf-footed bug, simulating a real field condition.

Our research results showed variability among genotypes during the evaluated periods of the biological assays, and this may be associated with the presence of anti-nutritional and/or antibiotic/antixenotic compounds in the genotypes and to decrease insect consumption of host cultivars, plants have the ability to use toxic compounds that confers resistance by delaying the development of immature stages of pests (Panda and Kush 1995). Chemical compounds of corn plant defenses can include saponine, glucoside, ethylene and jasmonate (Geyter et al. 2007; Louis et al. 2015; Meihls et al. 2013; Yan et al. 2012).

The prolonged development period may be also associated with the presence of morphological traits (e.g., leaf thickness/toughness, very tight husk cover, kernel hardness) that can minimize the damage by insects (Prasanna et al. 2022; Smith 2005). Faria et al. (2021) found a significant correlation among morphological factors, such as leaf hardness and vein stiffness, and the resistance of different corn genotypes. However, the overlap of chemical and morphological resistance factors with inhibitory effects on insect performance, make it difficult to distinguish the causes related to antibiosis and/or antixenosis (Stout 2013). For this reason, to differentiate both categories of resistance, further studies should be carried out to specifically examine the insect consumption (Baldin et al. 2014; Canassa et al. 2020; Morando et al. 2017).

Some of the genotypes evaluated in our study prolonged developmental nymphal periods of *L. zonatus*. This result has already been reported in other studies involving other polyphagous insects (Bentivenha et al. 2018; Canassa et al. 2017; Silva et al. 2014). This indicates that nymphal stage is the period most susceptible of the insects to antinutritional and/or antibiotics compounds that may be present in resistant genotypes (Smith 2005). In another previous study of nymphal survival of *L. zonatus* under laboratory conditions, the authors used nymphs of second and third instar that were fed green beans and peanuts (Daane et al. 2019), and the observed mortality rate was also significant. The prolonged nymphal period development in 30F53, IAC Airan, XB 8030 and 30F53 YHR genotypes and the high nymphal mortality in XB 8018 *Bt* in the second instar suggests few stimuli for the initiation of feeding are present and/or the presence of unpalatable compounds, which can affect *L. zonatus* (Baldin et al. 2019).

The adoption of transgenic *Bt* corn and soybeans by Brazilian growers may have further contributed to the attack of sucking insects on corn plants. *Bt* corn has led to less insecticide use for controlling the fall armyworm, *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) (Burtet et al. 2017). This may have resulted in the rise of secondary pests, such as stink bugs and leaf-footed bugs, which were also controlled by those insecticide applications (Cruz 2012; Cruz et al. 2016; Netto et al. 2015).

In other studies, three different *Bt* genes were used (Cry1Ab, Cry1A and Cry1A2) and in studies conducted with other sucking insects, *Diceraeus melacanthus* Dallas (Hemiptera: Pentatomidae) and *Dalbulus maidis* (DeLong & Wolcott) (Hemiptera: Cicadellidae), the authors found no evidence that these same *Bt*-toxins acted on the mortality of the insects, as we did in our study *L. zonatus* (Bueno et al. 2021; Faria et al. 2021). In general, the impacts of *Bt* corn like other *Bt* crops on sucking pests may be caused indirectly by unintended alteration of host plants induced by inserted transgenes (Akhtar et al. 2010) but not directly by *Bt* insecticidal proteins (Alcantara et al. 2004).

Knowing fundamental aspects of the biological cycle of *L. zonatus*, such as the duration of each development instar or detecting the instar where the insect is most susceptible to death, is important when making a decision that allows the producer to take preventive and control measures under field conditions. Although some studies have evaluated the influence

of different maize genotypes on *L. zonatus* (Foresti et al. 2017; Souza and Baldin 2009), studies on the characterization of resistance of maize genotypes on this species are scarce. Thus, the present study demonstrates, in an unprecedented way, the resistance of the studied genotypes to *L. zonatus*.

CONCLUSION

Based on the results obtained in our study, the genotypes 30F53, IAC Airan, XB 8030 and 30F53 YHR induced prolonged periods of nymphal development and high nymphal mortality in the second and third instar of *L. zonatus*. In addition, we verified that the genotype XB 8018 *Bt* caused high mortality of second instar nymphs, indicating expression antibiosis and/or antixenosis. The genotypes assessed in these experiments have never been tested for expression of resistance to *L. zonatus*. Thus, further studies should be conducted aiming to isolate the causes of resistance. The results of this study are novel and can serve as a basis for choosing genotypes to be cultivated in the field (XB 8018 *Bt*, 30F53, IAC Airan, XB 8030 and 30F53 YHR), as well as for directing programs for the genetic improvement of corn, focusing on resistance to sucking insects.

AUTHORS' CONTRIBUTION

Conceptualization: Canassa, V.F., Medeiros, F.C., Bastos, L.F.S., Faria, R.D., Santos, T.L.B., Cabral, I.R., Lourenção, A.L. and Baldin, E.L.L.; **Methodology:** Santos, T.L.B; Investigation: Medeiros, F.C., Bastos, L.F.S., Faria, R.D; Cabral, I.R.; **Writing – Original Draft:** Canassa, V.F., Cabral, I.R.; **Writing – Review and Editing:** Canassa, V.F., Cabral, I.R.; **Funding Acquisition:** Baldin, E.L.L; Supervision: Lourenção, A.L.; Baldin, E.L.L.

DATA AVAILABILITY STATEMENT

All dataset were generated and analyzed in the current study.

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REFERENCES

- Agrofit (2023). Sistema de agrotóxicos fitossanitários. Ministério da Agricultura, Pecuária e Abastecimento. [Accessed: Aug. 2, 2023]. Available at: https://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons.
- Akhtar, Z. R., Tian, J. C., Chen, Y., Fang, Q., Hu, C. and Chen, M. (2010) Impacts of six *Bt* rice lines on nontarget rice feeding thrips under laboratory and field conditions. *Environmental Entomology*, 39, 715–726. <https://doi.org/10.1603/EN09095>
- Alcantara, E. P., Aguda, R. M., Curtiss, A., Dean, D. H. and Cohen, M. B. (2004). *Bacillus thuringiensis* delta-endotoxin binding to brush border membrane vesicles of rice stem borers. *Archives of Insect Biochemistry and Physiology*, 55, 169–177. <https://doi.org/10.1002/arch.10128>
- Almeida, W. A. D., Silva, I. H. L. D., Santos, A. C. V. D., Barros Júnior, A. P. and Sousa, A. H. D. (2017). Potencialização do óleo-resina de Copaíba com inseticidas sintéticos para o controle da Lagarta-do-cartucho. *Revista Caatinga*, 30, 1059-1066. <https://doi.org/10.1590/1983-21252017v30n427rc>
- Artuzo, F. D., Foguesatto, C. R., Mahado, J. A. D., Oliveira, L. and Souza, Â. R. L. O. (2019). Potencial produtivo brasileiro: uma análise histórica da produção de milho. *Revista em Agronegócio e Meio Ambiente*, 12, 515-540. <https://doi.org/10.17765/2176-9168.2019v12n2p515-540>
- Baldin, E. L. L., Schlick-Souza, E. C., Lourenção, A. L. and Camargo, R. S. (2014). Resistance of collard greens to *Ascia monuste* orseis (Lepidoptera: Pieridae). *Arthropod Plant Interact*, 9, 67-74. <https://doi.org/10.1007/s11829-014-9344-x>
- Baldin, E. L. L., Vendramim, J. D. and Lourenção, A. L. (2019). Resistência de plantas a insetos: fundamentos e aplicações. Piracicaba: Fealq.
- Bentivenha, J. P. F., Canassa, V. F., Baldin, E. L. L., Borguini, M. G., Lima, G. P. P. and Lourenção, A. L. (2018). Role of the rutin and genistein flavonoids in soybean resistance to *Piezodorus guildinii* (Hemiptera: Pentatomidae). *Arthropod-Plant Interactions*, 12, 311–320. <https://doi.org/10.1007/s11829-017-9578-5>
- Bueno, N. M., Baldin, E. L. L., Canassa, V. F., Ribeiro, L. D. P., Silva, I. F. D., Lourenção, A. L. and Koch, R. L. (2021). Characterization of antixenosis and antibiosis of corn genotypes to *Dichelops melacanthus* Dallas (Hemiptera: Pentatomidae). *Gesunde Pflanzen*, 73, 67-76. <https://doi.org/10.1007/s10343-020-00529-z>
- Burtet, L. M., Bernardi, O., Melo, A. A., Pes, M. P., Strahl, T. T. and Guedes, J. V. C. (2017). Managing fall armyworm, (Lepidoptera: Noctuidae), with *Bt* maize and insecticides in southern Brazil. *Pest of Management Science*, 73, 2569–2577. <https://doi.org/10.1002/ps.4660>
- Canassa, V. F., Baldin, E. L. L., Bentivenha, J. P. F., Pannuti, L. E. R. and Lourenção, A. L. (2017). Resistance to *Dichelops melacanthus* (Hemiptera: Pentatomidae) in soybean genotypes of different maturity groups. *Bragantia*, 76, 257-265. <https://doi.org/10.1590/1678-4499.068>
- Canassa, V. F., Baldin, E. L. L., Lourenção, A. L., Barros, D. R. P., Lopes, N. P. and Sartori, M. M. P. (2020). Feeding behavior of *Brevicoryne brassicae* in resistant and susceptible collard greens genotypes: interactions among morphological and chemical factors. *Entomologia Experimentalis et Applicata*, 168, 228-239. <https://doi.org/10.1111/eea.12897>
- [CONAB] Companhia Nacional de Abastecimento. (2023). Acompanhamento da Safra Brasileira de Grãos, Brasília, DF, v. 10, safra 2022/23, n. 10, décimo levantamento, julho 2023. [Accessed: Aug. 2, 2023]. Available at: <https://www.conab.gov.br/info-agro/safras/graos/boletim-da-safra-de-graos>
- Cruz, I. (2012). Manejo de pragas não alvo de milho *Bt* incidentes na parte aérea da planta. In M. Paterniani, A. P. Duarte, and A. Tsunehiro (Eds.), *Diversidade e inovações na cadeia produtiva de milho e sorgo na era dos transgênicos* (p. 321-340). Campinas: ABMS/IAC.
- Cruz, I., Bianco, R. and Maciel, C. (2016). Potential risks of losses in maize caused by *Dichelops melacanthus* (Dallas) (Hemiptera: Pentatomidae) in Brazil. *Revista Brasileira de Milho e Sorgo*, 15, 386–397. <https://doi.org/10.18512/1980-6477/rbms.v15n3p386-397>
- Daane, K. M., Yokota, G. Y. and Wilson, H. (2019). Seasonal dynamics of the leaf-footed bug *Leptoglossus zonatus* and its implications for control in almonds and pistachios. *Insects*, 10, 255. <https://doi.org/10.3390/insects10080255>

- Faria, R. D., Baldin, E. L. L., Takaku, V. S. and Canassa, V. F. (2021). Variable levels of antibiosis and/or antixenosis of *Bt* and non-*Bt* maize genotypes on *Dalbulus maidis* (Hemiptera: Cicadellidae). *Arthropod-Plant Interactions*, 15, 457-465. <https://doi.org/10.1007/s11829-021-09832-6>
- Fasiku, S. A. and Wakil, S. M. (2022). Screening of factors responsible for conversion of maize straw into bioethanol: Bioethanol. *Journal of Microbiology, Biotechnology and Food Sciences*, 12, e5901-e5901. <https://doi.org/10.55251/jmbfs.5901>
- Foresti, J., Bastos, C. S., Fernandes, F. L. and Silva, P. R. D. (2017). Economic injury levels and economic thresholds for *Leptoglossus zonatus* (Dallas) (Hemiptera: Coreidae) infesting seed maize. *Pest Management Science*, 74, 149-158. <https://doi.org/10.1002/ps.4671>
- Geyter, E. D., Lambert, D., Geelen, G. and Smaghe, G. (2007). Novel advances with plant saponins as natural insecticides to control pest insects. *Pest Technology*, 1, 96-105. [Accessed: Sep. 9, 2023]. Available at: http://future-cannabis.s3.amazonaws.com/downloads/Clackamas_Coot_PDF_Library/Saponins_natural_plant_insecticide_IPM.pdf.
- Lara, F. M. (1991). Princípios de resistência de plantas a insetos. São Paulo: Ícone.
- Louis, J., Felton, G. W., Luthe, D. S., Jiang, V., Williams, W. P., CastanoDuque, L., Basu, S. and Varsani, S. (2015). Ethylene contributes to maize insect resistance-mediated maize defense against the phloem sap-sucking corn leaf aphid. *Plant Physiology*, 169, 313-324. <https://doi.org/10.1104/pp.15.00958>
- Matrangolo, W. J. R. and Waquil, J. M. (1994). Biologia de *Leptoglossus zonatus* (Dallas) (Hemiptera: Coreidae) alimentados com milho e sorgo. *Anais da Sociedade Entomológica do Brasil*, 23, 419-423. [Accessed: Sep. 9, 2023]. Available at: <https://ainfo.cnptia.embrapa.br/digital/bitstream/item/41732/1/Biologia-leptoglossus.pdf>
- Meihls, L. N., Handrick, V., Glauser, G., Barbier, H., Kaur, H., Haribal, M. M., Lipka, A. E., Gershenson, J., Buckler, E. S., Erb, M. and Kollner, T. G. (2013). Natural variation in maize aphid resistance is associated with 2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one glucoside methyltransferase activity. *Plant Cell*, 25, 2341-2355. <https://doi.org/10.1105/tpc.113.112409>
- Morando, R., Baldin, E. L. L., Cruz, P. L. and Lourenção, A. L. (2017). Assessing common bean cultivars for resistance to the soybean looper *Chrysodeixis includens* (Lepidoptera: Noctuidae). *Neotropical Entomology*, 46, 561-570. <https://doi.org/10.1007/s13744-017-0486-2>
- Netto, J. C., Michelotto, M. D., Fernando, J., Grigolli, J., Galli, J. A., Pirotta, M. Z. and Busoli, A. C. (2015). Damages caused by *Dichelops melacanthus* (Heteroptera: Pentatomidae) in conventional and transgenic corn hybrids. *Bioscience Journal*, 31, 1092-1101. <https://doi.org/10.14393/BJv31n4a2015-26323>
- Painter, R. H. (1951). Insect resistance in crop plants. New York: McMillan.
- Panda, N. and Khush, G. S. (1995). Host plant resistance to insects. Wallingford: CAB International Rice Research Institute.
- Pires, E. M., Bonaldo, S. M., Ferreira, J. A. M., Soares, M. A. and Candan, S. (2011). New record of *Leptoglossus zonatus* (Dallas) (Heteroptera: Coreidae) attacking starfruit (*Averrhoa carambola* L.) in Sinop, Mato Grosso, Brazil. *Entomo Brasiliis*, 4, 33-35. <https://doi.org/10.12741/ebrasilis.v4i1.114>
- Prasanna, B. M., Bruce, A., Beyene, Y., Makumbi, D., Gowda, M., Asim, M., Martinelli, S., Head, G. P. and Parimi, S. (2022). Host plant resistance for fall armyworm management in maize: relevance, status and prospects in Africa and Asia. *Theoretical and Applied Genetics*, 135, 3897-3916. <https://doi.org/10.1007/s00122-022-04073-4>
- SAS Institute. (2008). PROC Users Guide. Cary: SAS Institute.
- Schaefer, C. W. and Panizzi, A. R. (2000). Heteroptera of economic importance. Boca Raton: CRC press. <https://doi.org/10.1201/9781420041859>
- Silva, J. P. G. F., Baldin, E. L. L., Canassa, V. F., Souza, E. S. and Lourenção, A. L. (2014). Assessing antixenosis of soybean entries against *Piezodorus guildinii* (Hemiptera: Pentatomidae). *Arthropod Plant Interaction*, 8, 349-359. <https://doi.org/10.1007/s11829-014-9316-1>.
- Smith, C. M. (2005). Plant resistance to arthropods. Netherlands Dordrecht: Springer Science & Business.

- Smith, C. M. and Clement, S. L. (2012). Molecular bases of plant resistance to arthropods. *Annual Review of Entomology*, 57, 309-328. <https://doi.org/10.1146/annurev-ento-120710-100642>
- Souza, E. S. and Baldin, E. L. L. (2009). Preferência alimentar e aspectos biológicos de *Leptoglossus zonatus* Dallas, 1852 (Hemiptera: Coreidae) em diferentes genótipos de milho. *Boletín de Sanidad Vegetal - Plagas*, 35, 175- 185. [Accessed: Sep. 9, 2023]. <https://dialnet.unirioja.es/servlet/articulo?codigo=3105707>
- Stout, M. J. (2013). Reevaluating the conceptual framework for applied research on host plant resistance. *Insect Science*, 20, 263-272. <https://doi.org/10.1111/1744-7917.12011>
- Yan, Y, Christensen, S., Isakeit, T., Engelberth, J., Meeley, R., Hayward, A., Emery, R. J. N. and Kolomiets. M. V. (2012). Disruption of OPR7 and OPR8 reveals the versatile functions of jasmonic acid in maize development and defense. *Plant Cell*, 24, 1420-1436. <https://doi.org/10.1105/tpc.111.094151>
- Zucchi, R. A., Silveira Neto, S. and Nakano, O. (1993). Guia de identificação de pragas agrícolas. Piracicaba: Fealq.