Research Article

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Economic injury levels and economic thresholds for *Leptoglossus zonatus* (Dallas) (Hemiptera: Coreidae) infesting seed maize

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Abstract

BACKGROUND: Although *Leptoglossus zonatus* is considered a secondary pest of maize grown for grain, when grown for seed, this pest can cause significant economic damage. There are no records of management recommendations for this pest. The objectives of this work were to quantify losses caused by *L. zonatus* to different genotypes used for seed maize production and to estimate the acute toxicity of selected insecticides to *L. zonatus* using different application technologies. The ultimate goal was to calculate economic injury levels (EILs) and develop economic thresholds (ETs) for *L. zonatus* infesting seed maize.

RESULTS: EILs and ETs varied, respectively, from 3 to 8 and from 2 to 6 adults per 1000 plants, depending on the genotype, insecticide and application technology. Effective control was obtained by using aerial or ground application of λ-cyhalothrin (10.6%) + thiametoxan (14.1%) or permethrin (38.4%) at the rates of 1.48 mL ha^{-1} and 27.10 mL ha^{-1}, respectively, or ground application of methomyl (21.5%) at the rate of 244.06 mL ha^{-1}.

CONCLUSION: Maize grown for seed is susceptible to *L. zonatus* damage during reproductive stages, and the pest densities requiring management vary with genotype, insecticide, and application technology. These results contribute significantly to integrated pest management (IPM) for seed maize.

Keywords: western leaffooted bug; *Zea mays*; chemical control; lethal concentrations; integrated pest management

1 INTRODUCTION

Maize grown for seed production demands a high monetary investment because the final product needs to meet specific quality standards related to seed viability (e.g. high vigor and germination rates).[1] Seed that does not meet the necessary standards is sold as grain or is returned to the beneficiation phase to reduce the amount of non-viable and damaged seed to a tolerable threshold (85% germination or 98% of seeds without insect injury).[2]

Insects are among the biotic factors that reduce seed quality, primarily bugs belonging to the genus *Leptoglossus* Guérin, which encompasses several species belonging to the Coreidae family.[3] Insects of this genus are distributed worldwide, occurring from southern Canada, throughout the USA, to Mexico, the Antilles, Central and South America, with some species also being detected in Africa, Southeast Asia, the Pacific Islands and Australia.[4,5] Recently, the species *Leptoglossus occidentalis* Heidemann (Heteroptera: Coreidae) was introduced into Europe, being first observed in northern Italy and rapidly expanding its occurrence to other countries.[6,7]

The species *Leptoglossus zonatus* (Dallas) occurs in the USA, Mexico and Central and South America.[4,5] This species is polyphagous with records of occurrence on many hosts.[8–18]

Adults and nymphs of *L. zonatus* feed on maize kernels by inserting their mouth stylets through the husks to suck the internal content of the kernels, causing them to shrink and rot, which can lead to yield losses reaching 15%.[19] Feeding begins at tasseling and can continue until physiological maturity of the kernels. Sometimes the injury and damage will not be noticed until harvest or seed processing.[20] Maize cultivated for seed production can also have its physiological condition and the number of filled seeds greatly altered, reducing the final quality or even causing seed to be unviable, as reported in conifer species when injured by some species of *Leptoglossus*.[21–23] *Leptoglossus* bugs can cause seed abortion,[24] reduce the stored amount of seed constituents (lipids and proteins), decrease seedling emergence,[25] and also act as a potential vector of some pathogens.[26,27] There is no information currently available on treatment thresholds or the impact of

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L. zonatus damage to maize ears, although this information already exists for other Pentatomidae bugs.[28,29]

The absence of such information does not mean that insecticide application targeting the pest[19] is avoided, application being based simply on the presence of the insect and varying in number from three to six applications, depending on maize genotype (Foresti J, 2016, personal communication). However, there are no records of insecticide efficacy with respect to *L. zonatus* infesting maize. The selection of insecticides and spray application rates are therefore based on recommendations available for other insect pests of maize.

Treatment thresholds are ideally based on economic injury levels (EILs), which can be calculated as proposed by Pedigo[30]: $EIL = C/V \times b \times k$, where C is the management cost per production unit (e.g. US\$ ha^{-1}); V is the market value per production unit (e.g. US\$ ha^{-1}); b is the unit loss caused per insect (e.g. the slope of the adjusted model set between insect densities and yields); and k is the proportional reduction in injury with management (e.g. 0.8 for an insecticide killing 80% of a population).[30,31] Because the market price of seed maize is far higher than that of maize grown for grain,[32] EILs and ETs will necessarily differ between the two production goals. In addition, EILs and ETs for seed maize will need to take into account the diversity of situations faced during its production, such as the genotype being cultivated (inbred strains and single hybrids) and the final control cost (e.g. US\$ ha^{-1}, including insecticide and application cost).

The objectives of this work were to quantify losses caused by the leaf-footed bug *L. zonatus* for different maize genotypes used in the production of maize seed (inbred lines and a single hybrid) and to estimate the acute toxicity of selected insecticides to the pest while using different application technologies. The ultimate goal was to estimate EILs and ETs for *L. zonatus* infesting seed maize fields.

2 EXPERIMENTAL METHODS

2.1 Insects and plants

Mass rearing was initiated with 2000 hand-collected adults from maize fields. These insects were arranged in groups of 500 and maintained in the laboratory within voile insect-rearing cages constructed of polyvinyl chloride (PVC) (0.5 by 0.5 by 0.7 m). Environmental conditions were regulated to 25 ± 2 °C and 60 ± 10% relative humidity, with a photoperiod of 14:10 (light:dark), and the cages received maintenance every 3 days. Each cage received substrate for oviposition made of PVC pipes shaped in a rectangle measuring 0.3 by 0.4 m with white cotton thread (containing 85% of cotton fiber and 15% of other fibers) all around the largest side. The substrate was fitted to the top of the cage and egg masses were collected at 7-day intervals and transferred to a new cage of the same type as described above, where the emerged nymphs stayed until they reached the third instar. At this point, the nymphs were separated into two different cages where they stayed until they reached the adult stage. Insects were provided with fresh maize ears (stage R_2 to R_4) from hybrid 'A' and a portion of cotton soaked in distilled water until the onset of the experiment. Adults used for the infestations were sex distinguished via examination of male (pygophore with only one plate) and female (split pygophore) genitalia.[33–35]

Insects used in the LC_{50} (lethal concentration that kills 50% of the population or the amount of a given insecticide applied all at once which causes the death of 50% of a group of test animals (*L. zonatus*)) assays (both initial and final) were collected from commercial maize fields in order to include the variability existing in the field. Insects were maintained as described above until their use. The insects remained in the laboratory conditions for a maximum of 2 days before testing.

Plants used for insect rearing and experimentation were grown in seed maize fields belonging to DuPont of Brasil S.A. Pioneer Seeds Division (Itumbiara, Goiás, Brazil; 15° 43′ 32.33′′ S, 47° 36′ 36.43′ W). The genotypes inbred line 'A' (a homozygous parental which is used to generate the commercial maize seed) and hybrid 'A' (the female obtained from the cross between two inbred lines) were cultivated using normal production practices for the area, but without the use of insecticide.

Plants used in the experiments were cultivated at a row spacing of 0.4 m and within-row spacing of 0.2 m. Each experimental plot had four uniform plants (Figure 1a) and was enclosed by a structure made of wood (0.04 by 0.04 by 2.50 m) with voile screens (0.6 by 0.6 by 2.4 m) that were attached at the top to 6-mm nylon strings (Figure 1b). The screens also had a lateral zipper shaped as an inverted L (Figure 1c). Tassels were removed before infestation in accordance with the procedures adopted in a seed field. Also, at the beginning of maize silking, only the main ear of each plant was manually pollinated using tassels obtained from plants cultivated on the border of the field. Pollination was performed three times, at 2-day intervals.

2.2 Estimation of the qualitative yield losses caused by *Leptoglossus zonatus* in seed maize fields

Each plot (i.e. cage containing four plants) was infested with 10 pairs of 1 – 4-day-old male and female *L. zonatus*. This density was based on the observed gregarious behavior of this species and other species of Leptoglossus,[36,37] which is probably based on emission of the aggregation pheromone as observed in *L. occidentalis*,[36] as well as sex pheromones as observed in *Leptoglossus clypealis* Heidemann (Heteroptera: Coreidae).[37]

All plots were infested at the same time. The exposure time intervals among the plots were 0 days in control plots and 0 – 10, 0 – 20, 0 – 40 and 0 – 60 days in the treatments, which corresponded to the following maize phenological stages: V_T (tasseling) – R_1 (silking); V_T (tasseling) – R_2 (blister); V_T (tasseling) – R_4 (dough), and V_T (tasseling) – R_6 (physiological maturity). The different exposure times were arranged in a complete randomized block, with five replications.

The cages were visually inspected every 2 days and dead insects as well as egg masses and emerged nymphs were removed. Insects found dead within the infestation interval were replaced. These measures were taken to guarantee that the initial density was maintained for a given interval throughout the entire experiment.

For each tested interval, the insects were removed every 10 days, followed by a re-infestation with the same density as used in the initial infestation. This procedure was adopted for the plots submitted to exposure times > 10 days (0 – 20, 0 – 40 and 0 – 60 days).

The exposure intervals of 0, 0 – 10, 0 – 20, 0 – 40, and 0 – 60 corresponded to the following densities of *L. zonatus*: 0 (control), 10, 20, 40 and 60 adults per plot, respectively.

After 60 days the ears were manually and individually harvested, husked, and taken to a dryer where they remained until reaching a moisture content of 11.5%. The ears were then manually threshed and the numbers of shrunken (SS), embryo damaged (EbD), endosperm damaged (EdD) and non-damaged (ND) seeds were visually identified. The total number of damaged seeds (SS + EbD + EdD), as well as the number in each individual damage category, were regressed against the density of *L. zonatus* and

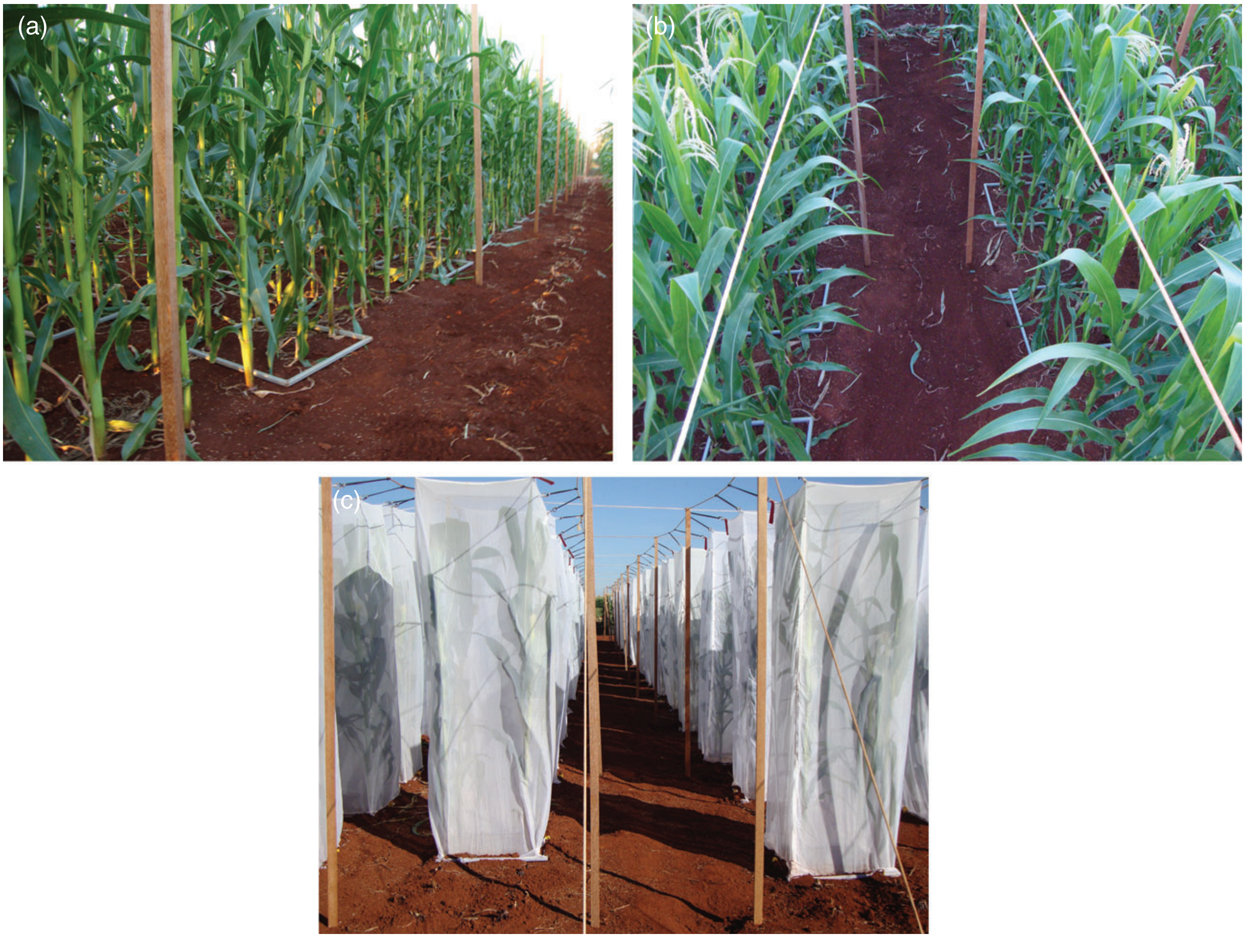

Figure 1. Experimental plots consisting of four uniform plants (a), a structure made of wooden sticks (0.04 by 0.04 by 2.5 m) and 6-mm nylon strings (b) bearing voile screens (0.6 by 0.6 by 2.4 m) having a lateral zipper shaped as an inverted L (c).

used in the estimation of qualitative yield losses caused by *L. zonatus* (the *b* component of the EIL formula).[30] The visually identified damage was confirmed through germination and vigor tests, but these data are not shown here.

2.3 Determination of the insecticide's lethal concentration against *Leptoglossus zonatus*

2.3.1 LC_{50} (lab assays)

In order to estimate the EILs and ETs, it is necessary to calculate the control cost and the proportional reduction in injury with management, which are both dependent on the insecticide effective dose to control the insect. This information was not available for *L. zonatus*.

The first step when selecting products for pest control is to define the discriminating dose or concentration (acute toxicity) which will effectively kill the pest. We chose to use the LC_{50}, which when doubled is expected to kill approximately 100% of the population, because it has a high degree of reproducibility and is more reliable when making assumptions for a population.[38]

The insecticides tested are presented in Table 1. The commercial products or formulations used were Lannate BR (methomyl) (DuPont do, Barueri, Brasil), Lorsban 480 BR (chlorpyrifos) (Dow Agrosciences, São Paulo, Brasil), Platinum Neo (λ-cyhalothrin + thiamethoxam) (Syngenta, São Paulo, Brasil), Piredan (permethrin) (FMC Química, Campinas, Brasil) and Avaunt 150 (indoxacarb) (DuPont do, Barueri, Brasil).

Initial bioassays were performed to define the concentration ranges that caused insect mortality from 0 to approximately 100%. These concentrations were further tested in the final bioassays, and adjusted for a water volume equivalent to 200 L ha^{-1}. In the final bioassays, five to six concentrations of each insecticide were tested (Table 1) plus the control (water). The treatments were arranged in a completely randomized design with five replications. In both bioassays (initial and final), three pairs of *L. zonatus insects* per replication were used. However, when taking into account the possibility of expanding the existing registration, the maximum tested dose was that already in use to control other pest species in maize (Brasil; http://www.planalto.gov.br/ccivil_03/decreto/2002/D4074compilado.htm).

Each plot consisted of transparent gerboxes measuring 11 by 11 by 3.5 cm (250 mL capacity), with a small opening in the lid, which was covered with an anti-aphid screen, and filter paper on the bottom, according to procedures adopted by the Insecticide Resistance Action Committee (IRAC) (http://www.irac-online.org/methods/euschistus-heros-adults/). Gerboxes were sterilized in a germicide chamber and the filter paper sheets were autoclaved before use.

The insecticides were diluted in distilled water until reaching the desired concentration. Distilled water at the same volume employed to prepare the solutions was also used as a control and as the solvent.

Table 1. Description of active ingredients found in the insecticides tested and concentrations used for LC_{50} estimation for *Leptoglossus zonatus* (Dallas) (Hemiptera: Coreidae) under laboratory conditions (temperature: 25 ± 2 °C; relative humidity: 60 ± 10%; photophase: 14 h)

Active ingredient	Concentration (mg μL^{-1})[a]	Amount of commercial product ($\mu l\ L^{-1}$) (active ingredient (mg L^{-1}))[b]						
		[0]	[1]	[2]	[3]	[4]	[5]	[6]
λ-Cyhalothrin + thiamethoxam	0.106 and 0.141	Control	1.25 (0.13 + 0.18)	2.50 (0.27 + 0.35)	5.00 (0.53 + 0.71)	10.00 (1.06 + 1.41)	15.60 (1.65 + 2.2)	24.50 (2.60 + 3.45)
Chlorpyrifos	0.48	Control	2.50 (1.20)	12.50 (6.00)	30.00 (14.40)	40.00 (19.20)	55.00 (26.40)	-
Permethrin	0.384	Control	5.00 (1.92)	20.00 (7.68)	50.00 (19.20)	75.00 (28.80)	100.00 (38.40)	-
Indoxacarb	0.15	Control	79.00 (11.85)	125.00 (18.75)	215.75 (32.36)	373.65 (56.05)	646.00 (96.90)	854.00 (128.10)
Methomyl	0.215	Control	90.00 (19.35)	160.00 (34.40)	280.00 (60.20)	500.00 (107.50)	900.00 (193.50)	1,365.00 (293.48)

[0]... [1]... [2] etc. refers to a designated codification used for treatments.
[a] Concentration of the active ingredient in the commercial formulation of the insecticides.
[b] Tested concentrations of the commercial products and active ingredients.

Each filter paper received 2 mL of the test solution, using a 10-mL Eppendorf microsyringe (model H45172B) (Eppendorf, Hamburg, Germany). In addition, 1.5-cm-long R2 (blister kernels) to R4 (dough-milky kernels) ear sections (hybrid 'A') were immersed in the solutions for 5 s, drained on paper towels for 1 min, and fed to the insects.

Mortality was recorded 24, 48, 72, and 96 h after treatment. During the evaluations, dead insects were removed. The maximum accepted mortality in the control plots was 20%, otherwise the assay was discarded; and for the LC_{50} analysis only the mortality recorded 96 h after treatment was considered. Mortality data for each concentration were corrected based on mortality in the control[39] and submitted to Probit analysis using SAS.[40]

2.3.2 *Doubled LC_{50} (field conditions)*

Field-tested insecticides were those whose mortality rates fitted to Probit distributions and LC_{50s}, when doubled, were equal to or lower than the maximum dose already registered to control other maize insect pests (Table 2). The insecticides and concentrations tested are listed in Table 3. Distilled water was used in the same final volume of the solutions as a control. Insecticides were applied to plants and insects together. The treatments were arranged in complete randomized blocks with seven replicates, using six pairs of *L. zonatus* insects per plot.

Maize cultivation, arrangement and management practices were conducted as previously described (Figure 1a–c). Hybrid 'A' was used in this assay.

Both plants and insects were sprayed with the aid of a precision CO_2 sprayer at 30 kgf cm^{-2} pressure with a hollow cone JA-2® (Jacto, Pompéia, Brazil) nozzle tip and a final water volume of 200 L ha^{-1}. Initially, plants were sprayed for 3 s by moving the sprayer wand up and down until all plants were covered by the solution. Next, insects were treated after confinement within 0.4 x 0.5 m tulle sacs laid on kraft paper on the ground, where all the sample units for the same concentration were sprayed at the same time. The treated insects were then immediately assigned to the cages to which they belonged (corresponding to a given treatment and replication). Mortality was evaluated at 24, 48, 72 and 96 h after spraying. Mortality rates after 96 h were corrected for mortality of the control plots according to Püntener.[39] The different insecticides were compared by analysis of variance followed by the Tukey test at $P < 0.05$ using SAS proc GLM.[40]

2.4 Estimation of the insecticide control cost

The control cost (C) (US\$ ha^{-1}) used in the EIL formula includes the insecticide and application costs. The estimation of this cost was based on data obtained from mortality at 96 h after spraying. Because only λ-cyhalothrin + thiamethoxam, permethrin and methomyl caused mortality >75% (Table 3), the insecticide control cost was calculated for these insecticides. The efficacy of these products was used to estimate the k component of the EIL formula (proportional reduction in injury with management). The insecticide control cost also considered ground application and aerial application (Table 4), depending on the insecticide and the label recommendations.[41] These values were obtained by the companies selling this type of service within Brazil. The cost of the insecticides was local market value (Table 4).

2.5 Estimation of the crop market value

The crop market value (V) of the EIL formula is estimated using the market price (US\$ ha^{-1}) and is dependent upon the crop yield. Hence, the average yield was based on the values obtained for the control plots of the assay with varying densities of *L. zonatus*, and was 274 bags ha^{-1} of 60 000 kernels. The crop value was then calculated for three types of inbred line genotypes possessing: (a) high genetic potential (US\$106.32 bag^{-1}); (b) medium genetic potential (US\$81.01 bag^{-1}) and (c) low genetic potential (US\$55.70 bag^{-1}). The genetic potentials were based on the yield expressed by maize genotypes in commercial fields and their value on the market.

2.6 EIL calculation and ET estimation

The slope of the linear adjusted model (b) together with the control cost (C), estimated crop values (V), and experimental efficacies (k) were used to calculate the EIL as proposed by Pedigo[30] and presented above. The EIL was estimated for each combination of condition mentioned above, which included: three insecticides,

Table 2. Mortality 96 h after exposure of *Leptoglossus zonatus* (Dallas) (Hemiptera: Coreidae) to insecticides applied both over the food source and the filter paper (temperature: 25 ± 2 °C; relative humidity: 60±10%; photophase: 14 h)

Active ingredient	n^a	Slope (±SE)	LC_{50} (95% CI) (μl c.p. L^{-1})	df	χ^2	*P*-value
λ-Cyhalothrin + thiametoxam	210	2.28 (±0.32)	3.69 (2.64 – 4.82)	5	2.26	0.69
Chlorpyrifos	180	2.09 (±0.52)	13.00 (4.70 – 19.73)	3	3.31	0.35
Permethrin	180	5.97 (±1.86)	67.76 (49.59 – 78.59)	3	4.32	0.23
Indoxacarb	210	2.30 (±0.39)	215.71 (144.09 – 287.61)	5	1.35	0.85
Methomyl	210	3.06 (±0.93)	610.14 (270.39 – 795.02)	5	8.48	0.13

[a]Number of tested insects.
[b]Slope of the dose – mortality curve and its standard error (SE).
LC_{50}, lethal concentration that kills 50% of the tested insects in μL of commercial product (c.p.) per liter of water; df, degrees of freedom.

Table 3. Mortality (%) of *Leptoglussus zonatus* (Dallas, 1852) (Hemiptera: Coreidae) corrected by the control mortality, 96 h after insecticide application to maize plants and insects

Active ingredient	Tested concentration ($2 \times LC_{50}$)[a]	Mortality (%) (mean ± SEM)[b]
λ-Cyhalothrin + thiametoxam	7.40	79.45 ± 18.51 A
Chlorpyrifos	26.00	36.03 ± 22.71 B
Permethrin	135.50	77.60 ± 17.53 A
Indoxacarb	431.40	23.40 ± 21.36 B
Methomyl	1,220.30	75.04 ± 21.72 A

[a] Doubled LC_{50} is 2 × the lethal concentration that kills 50% of the tested insects in μl of commercial product (c.p.) per liter of water.
[b] Means followed by the same letter do not differ by Tukey's test at $P < 0.05$.
SEM, standard error of the mean.

two application methods and inbred genotypes possessing three different genetic potentials. The ETs were set 20% below the EIL values as suggested by Mujica and Kroschel.[42]

2.7 ET validation

The ET validation was performed by evaluating the number of *L. zonatus* in 100 maize ears per hectare, sampled in five cultivated maize fields of the hybrid 30F53. The size of each field was: field 1, 15 ha; field 2, 35 ha; field 3, 45 ha; field 4, 38 ha, and field 5, 27 ha. At harvest, 100 maize ears per hectare per field were assessed, manually threshed and visually rated with respect to *L. zonatus* damage, and a subsample of 100 seeds was used to estimate the dry weight in grams. The number of damaged seeds and the weight of non-damaged seeds were correlated (Pearson correlation at $P < 0.05$) using the SAS system.[40]

3 RESULTS

3.1 Qualitative yield loss caused by *L. zonatus* in seed maize fields

There was a significant linear increase in the number of lost seeds per ear ($r^2 = 0.93$) with the number of *L. zonatus* adults per ear (Figure 2a), despite the absence of significance for the regression model set between the number of each category of damaged seed (embryo and pericarp damaged or withered) and the number of *L. zonatus* per ear (Figure 2b; $P > 0.05$). The *b* component, or the loss caused per unit insect (slope of the linear model adjusted for the relationship between the number of adults of *L. zonatus* and the seed losses per ear), of the EIL formula was 0.07180 (Figure 2a).

3.2 Insecticide lethal concentration for *L. zonatus*

3.2.1 LC_{50} (lab assays)

The LC_{50} values for λ-cyhalothrin + thiametoxam, chlorpyrifos, permethrin, indoxacarb and methomyl are presented in Table 2. The components of each model, the χ^2 and *P* values, the number of tested insects, and the slope and degrees of freedom for each insecticide tested are also presented in Table 2. All five insecticides tested fitted a probit distribution ($P > 0.05$; Table 2), and the estimated LC_{50}, when doubled, was lower than the maximum field rate already registered to control a different insect pest in maize (Brasil; http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons).

3.2.2 Doubled LC_{50} (field conditions)

There were no significant differences in *L. zonatus* mortality among the insecticides λ-cyhalothrin + thiamethoxam, permethrin and methomyl (Table 3). These insecticides caused the highest mortality, killing >75% of the tested insects (Table 3). Chlorpyrifos and indoxacarb caused low and similar (not significantly different) *L. zonatus* mortality (< 75%), which was significantly different from that caused by the other three insecticides. Therefore, only λ-cyhalothrin + thiametoxam, permethrin and methomyl were selected for the EIL calculations.

3.3 Economic variables

The chemical control costs (*C* of the EIL formula) for ground and aerial application of the insecticides are presented in Table 4. Ground application ranged from US$5.10 to 6.10, while the costs for aerial application ranged from US$6.62 to 6.73.

The market value of cultivated seed maize with genotypes of different genetic potential ranged from US$15 261.80 to 29 131.68 for low and high genetic potential genotypes, respectively (Table 5).

3.4 EIL calculation and ET estimation

The EIL and ET values for *L. zonatus* infesting cultivated seed maize with inbred genotypes possessing low, medium and high genetic potential using ground and aerial application of λ-cyhalothrin + thiamethoxam, permethrin and methomyl are presented in Table 6. The EIL varied from 3 to 8 adults per 1000 plants (0.003 to 0.008 adults per ear) and the ET varied from 2 to 6 adults per 1000 plants (0.002 to 0.006 adults per ear) (Table 6). Economic thresholds for ground application were generally lower than for aerial application. However, EILs and ETs for ground application of

Table 4. Estimated control cost (application + insecticide) of one spraying of each insecticide to control *Leptoglossus zonatus* (Dallas, 1852) (Hemiptera: Coreidae) in seed maize fields, using different application technologies

Active ingredient	Insecticide cost (US$ L^{-1})	Concentration (mL ha^{-1})	Cost of one insecticide spray (US$ ha^{-1})	Application technology	Application cost (US$ ha^{-1})	Total cost (spray + insecticide) (US$ ha^{-1})
λ-Cyhalothrin + thiametoxam	24.00	1.48	0.04	Ground application	5.06	5.10
				Aerial application	6.58	6.62
Permethrin	5.62	27.10	0.15	Ground application	5.06	5.21
				Aerial application	6.58	6.73
Methomyl	4.25	244.06	1.04	Ground application	5.06	6.10

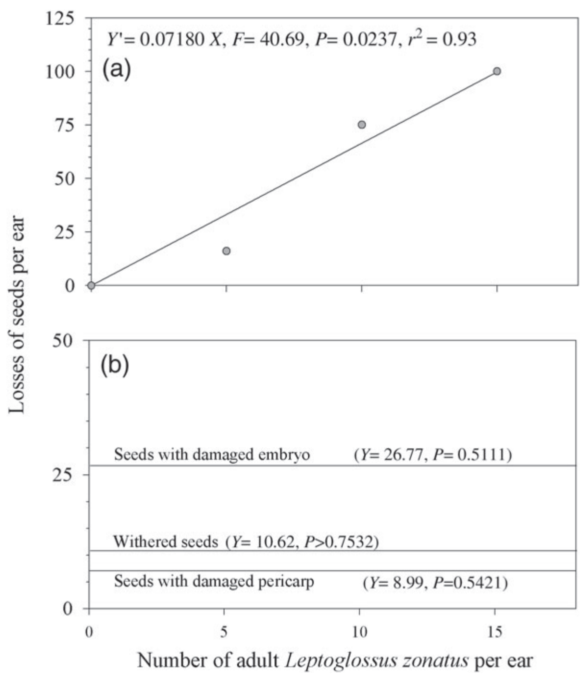

Figure 2. Number of lost seeds per maize ear (a), and seeds with damaged embryo, withered seeds, and seeds with damaged pericarp (b) as a function of the number of *Leptoglossus zonatus* (Dallas) (Hemiptera: Coreidae) adults per ear.

methomyl are equal to those for aerial application of λ-cyhalothrin + thiametoxam and permethrin (Table 6). Inbred genotypes possessing low genetic potential had higher ETs than those of medium and high genetic potential.

3.5 ET validation

The number of *L. zonatus* adults varied from 0.1 to 5.1 per 100 ears sampled in 1 ha among the fields (Figure 3), reaching the ET in four out of the five sampled fields using the lowest ET calculated in this work (Table 6). In field 2 (Fig. 3a), the decision to initiate control would only occur at the lowest ET (0.002 *L. zonatus* per ear; ground application of λ-cyhalothrin + thiametoxam; high genetic potential genotype), while in the remaining cases (fields 3, 4 and 5) (Figure 3a), the decision to control would be taken in any case, independent of the insecticide or application technology used (Table 6).

There was a significant and negative correlation between the number of *L. zonatus* per 100 ears and the weight of 100 non-damaged seeds. The lowest weight of 100 non-damaged seeds occurred where there was the highest number of *L. zonatus* (fields 3, 4 and 5). The highest weight of 100 maize seeds occurred where the lowest number of *L. zonatus* was observed (Figure 2b).

Table 5. Yield, market price and market value of cultivated seed maize with genotypes possessing varying genetic potential

Genetic potential of the genotypes[a]	Yield (bags ha^{-1})[b]	Market price (US$ bag^{-1})	Market value (US$ ha^{-1})
High	274.00	106.32	29 131.68
Medium	274.00	81.01	22 196.74
Low	274.00	55.70	15 261.80

[a] Genetic potential based on yield: higher than average (high), average (medium) and lower than average (low).
[b] Bag of 60 MK (60 000 kernel unities) with size 16.5/64 to 26/64 inches according to seed industry classification. For the genotype yield estimates, neither area occupied by the males nor harvest and processing losses were considered.

4 DISCUSSION

Although *L. zonatus* is described as a polyphagous species,[8–11,14,16] this pest is especially important for maize. This is partly a consequence of the nutritional value of maize, as, according to a study by Panizzi[43] on the effect of different food sources on the biology of *L. zonatus*, maize is the most nutritionally complete source of food. Matrangolo and Waquil[44] noticed an increase in the survival of immature individuals when they were fed maize compared with sorghum (*Sorghum bicolor*). This could account for the linear increase in maize seeds lost as the number of adult *L. zonatus* rises. This scenario, together with the introduction of *Bacillus thuringiensis* (*Bt*) maize and the subsequent reduction in insecticide application,[45] has increased outbreaks of pests that are not susceptible to the expressed toxins,[46] as has been reported for hemipteran pests in maize. This is problematic because there is little information on how and when to treat maize infested with these and other hemipteran pests, such as *L. zonatus*, in the USA.[29] This is also true for Brazil, although information concerning the treatment threshold and management of stink bugs is well defined for cotton (*Gossypium hirsutum*)[47] and soybean (*Glycine max*).[48] For instance, *L. zonatus* is not even listed among the pests for which there is a registered insecticide for their control on the website of the Brazilian Ministry of Agriculture and Poultry (Brasil; http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons).

In maize, the only true bugs with insecticides registered for their control are *Dichelops furcatus* (Fabr.) and *Dichelops melacanthus*

Table 6. Economic injury level (EIL) and economic threshold (ET) for *Leptoglossus zonatus* (Dallas, 1852) (Hemiptera: Coreidae) infesting cultivated seed maize with genotypes possessing varying genetic potential and employing different application technologies; the production cost is shown at the bottom of the table

		EIL and ET (insects per ear) as a function of the genetic potential of the maize genotypes					
		Low		Medium		High	
Insecticide	Application technology	EIL	ET	EIL	ET	EIL	ET
λ-Cyhalothrin + thiametoxam	Ground application	0.006	0.005	0.004	0.003	0.003	0.002
	Aerial application	0.008	0.006	0.006	0.004	0.004	0.003
Permethrin	Ground application	0.006	0.005	0.004	0.003	0.003	0.002
	Aerial application	0.008	0.006	0.006	0.004	0.004	0.003
Methomyl	Ground application	0.008	0.006	0.005	0.004	0.004	0.003

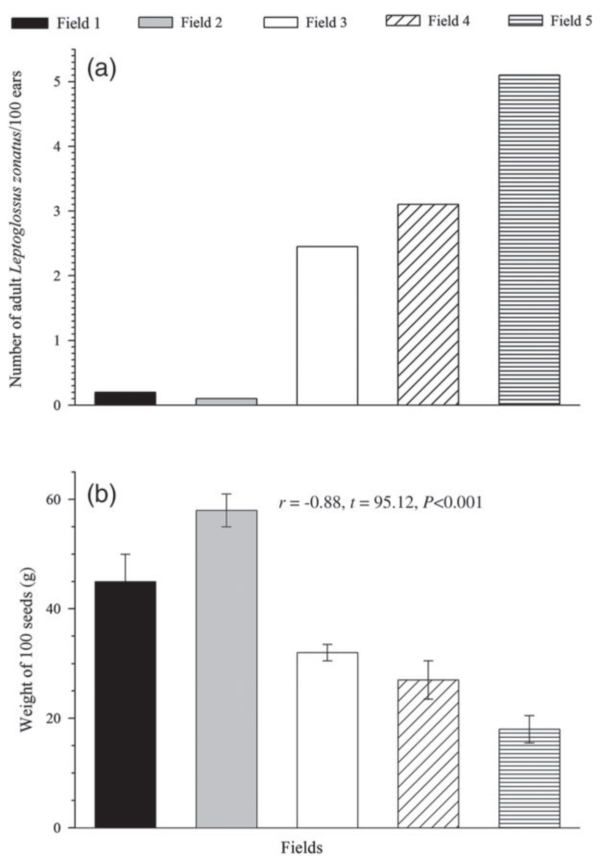

Figure 3. Densities of *Leptoglossus zonatus* (Dallas) (Hemiptera: Coreidae) in maize fields (a) and weight of 100 non-damaged seeds (b) obtained from ears collected in the sampled fields.

(Dallas) (Hemiptera: Pentatomidae), and those formulations are neonicotinoids or mixtures of pyrethroids and neonicotinoids (Brasil; http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons). The situation is even worse for seed maize, where a low threshold is admissible for seeds damaged by insects (2%) and germination must be >85%[2]. Where such gaps exist, it is common for growers to use products and adopt concentrations that they know work well to manage other maize insect pests, as well as to spray preventively. In both cases, this leads to problems such as failure to provide effective control, selection for resistance,[49] increased hazards of environmental contamination, and mortality of non-target organisms.[50–52] Although the starting point is to determine the pest density that allows decision-making on when to control the pest, in order to achieve economic control it is necessary to determine the control cost of the EIL, which is in turn dependent on the insecticide and the application technology to be used.

In the absence of insecticides registered to control *L. zonatus* infesting maize, LC_{50} tests were performed under lab conditions and the doubled LC_{50} values were tested under field conditions. The estimated LC_{50} values and the doubled LC_{50} values of the three insecticides that had acceptable efficiency in field tests were much lower than the concentration already in use to manage some other maize pests in Brazil (Brasil; http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons), and some other hemipteran pests elsewhere.[53–56] For example, the rates of λ-cyhalothrin + thiamethoxam registered to control *D. melacanthus* in maize vary from 200 to 250 ml of the commercial product ha^{-1} (Brasil; http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons), which is 135 – 168-fold higher than the values determined herein for *L. zonatus* (1.48 mL ha^{-1}). However, similar results to ours have been obtained in other studies. Pansa *et al.*[56] estimated the LC_{50} of λ-cyhalothrin (95 g a.i. L^{-1} of commercial product) for *Nezara viridula* L. (Hemiptera: Pentatomidae) and *Eurygaster maura* L. (Hemiptera: Scutelleridae) by application of the treatment solution (1 mL) to Petri dishes and to leaves and spikes of wheat at the same rate; both methodologies were similar to those used in this work. The authors obtained LC_{50} values of 0.428 and 4.464 $\mu l\ L^{-1}$ for *N. viridula* and *E. maura*, respectively, when treating Petri dishes, contrasting with the values of 2.10 and 1.17 $\mu l\ L^{-1}$ when treating leaves and spikes. Some of these values are close to the value of 3.69 $\mu l\ L^{-1}$ found in the present study, despite using a combined formulation consisting of λ-cyhalothrin (106 g a.i. L^{-1} of commercial product) and thiamethoxam (141 g a.i. L^{-1} of commercial product). Also, both Snodgrass *et al.*,[57] working with *Acrosternum hilare* (Say) (Hemiptera: Pentatomidae) and *N. viridula*, and López *et al.*,[58] working with *N. viridula*, found low LC_{50} values for λ-cyhalothrin (varying from 0.0039 to 0.47 μg per vial) using technical grade insecticide impregnated in a 20-mL scintillation vial. Even when evaluating a commercial formulation of λ-cyhalothrin against *Oebalus pugnax* (F.) (Hemiptera: Pentatomidae), Blackman *et al.*[55] found an LC_{50} of 0.2973 $\mu g\ mL^{-1}$ for the susceptible population and comparable control efficacy for formulations containing only half of the λ-cyhalothrin found in a mixture of λ-cyhalothrin + thiamethoxan and a similar concentration of thiametoxan. Therefore, these active ingredients used alone or in mixture should allow for effective control of true bugs. Also, if the concentrations of λ-cyhalothrin + thiamethoxam recommended for the control of *D. melacanthus* in maize were to be used for the control of *L. zonatus*, they would be more than 100-fold too high justifying the need for accurate estimations made in the present study.

Permethrin and methomyl also proved effective, and were approximately 9- and 80-fold less toxic to *L. zonatus* adults than the mixture of λ-cyhalothrin + thiametoxam. Similar results were obtained by Snodgrass *et al.*[56] when comparing the LC_{50} values of permethrin and λ-cyhalothrin for *A. hilare* and *N. viridula*, which were 4- to 13-fold lower for λ-cyhalothrin. However, some authors have reported recovery after initial exposure of some invasive pentatomids to λ-cyhalothrin. This does not occur after exposure to permethrin and thiametoxan.[53] In this context, the use of formulations containing a mixture of λ-cyhalothrin + thiametoxan and permethrin alone should avoid a similar occurrence in *L. zonatus*. In the same study of Leskley *et al.*,[53] both methomyl and permethrin, applied at rates of 1112 and 1120 g ha^{-1}, respectively, showed high initial efficacy against *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), which was maintained throughout the entire evaluation period, giving them high lethality index values.[53] In another study performed with the same pest infesting apple and peach orchards, mortality caused by methomyl (90% a.i.) applied at a rate of 1.1 kg ha^{-1} early in the season reached 100%.[54] In our study, acceptable efficacy (>75%) for *L. zonatus* was obtained at much lower field rates of methomyl (52.47 g ha^{-1}) and permethrin (10.41 g ha^{-1}). The field concentrations estimated for the formulations containing methomyl and permethrin (e.g. 0.244 L ha^{-1} and 27.1 mL ha^{-1}, respectively), were also much lower than those used for control of the fall armyworm *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae), which are 0.4-0.6 L ha^{-1} and 100-130 mL ha^{-1}, respectively (Brasil; http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons). Based on the estimations made herein, expansion of the registration to include *L. zonatus* as a pest to be controlled by λ-cyhalothrin + thiamethoxam, methomyl and permethrin in maize fields would be appropriate (Brasil; http://www.planalto.gov.br/ccivil_03/decreto/2002/D4074compilado.htm). Based on this information, the EILs and ETs for these insecticides were calculated for all the application technologies prescribed on their labels, and for different inbred genotypes cultivated during seed maize production.

The ET will vary with the EIL, which assumes variable values depending on, among other things, control cost (*C* variable of the EIL formula) and crop or market value (*V* variable of the EIL formula).[30,59] The nature of the relationship established between *C* and the EIL and ET is direct,[30] while there is an inverse relationship between *V* and the EIL and ET.[30,59]

Management cost incorporates the cost of insecticide acquisition and application, assuming variable values depending on the cost and amount of formulation needed to spray a given area, as well as with the application method.[30] The lowest *C* value was for ground application of λ-cyhalothrin + thiamethoxan, because of both the low amount of formulation needed (lower LC_{50}) and the application technology used. Methomyl, which cannot be used in aerial application, had an intermediary *C* value, primarily because of the high amount of formulation needed (higher LC_{50}) compared with the other two insecticides. The maximum *C* value occurred for aerial application of permethrin, caused by both the high LC_{50} and the application technology used.

Crop or market value (*V*) varies with the market price of the crop and is dependent on, among other things, the crop yield.[30] More productive crops have higher *V* values than low production crops. That is why the *V* value was maximized by the more productive genotypes.

The *k* component (amount of damage avoided based on efficacy of the insecticide) of the EIL formula can have a significant effect on the EIls and ETs.[59,60] However, in our study, *k* varied little (75% to 79%), so did not account for the variations observed in the EILs and ETs, which were more dependent on greater variations of the *C* and *V* components of the EIL formula.

The ETs calculated herein ranged from 0.002 to 0.006 insects per ear (2 to 6 adults of *L. zonatus* per 1000 ears). Ni *et al.*[29] found that the kernel weight was not significantly reduced by 1 – 2 adult *Euschistus servus* (Say) per ear at stage V_T or R_1 for 9 days, and proposed an EIL of 0.5 bugs per ear for 9 d at stage V_T and less for stage R_1, numbers much higher than calculated here. However, damage to the seed will happen before a loss in weight is observed, and it is for this reason that ETs for seed maize fields are much lower than for grain production, although it should be noted that *L. zonatus* is also able to cause weight loss of seeds, as demonstrated by the significant and negative correlation found between the pest density and the weight of 100 kernels. For example, Depieri and Panizzi[61] studied the duration of stink bug feeding in relation to superficial and in-depth damage to soybean seeds and found that even a feeding time as short as 70 min for some species was sufficient to cause seed damage as a result of cell disruption and protein body dissolution. Sayers *et al.*[32] reported that EILs for European corn borer (*Ostrinia nubilalis*) were considerably lower for seed maize than for maize grown for grain, primarily because of the high monetary value of the seeds.

During the validation of the *L. zonatus* ETs, sampling indicated that in 60% of the cases the ET would be reached and management action should be taken, showing that densities requiring management can be relatively common for seed maize. Therefore, this work provides considerable and innovative contributions to IPM for seed maize, especially because there are no records of similar efforts involving this system either in use or in published literature.

5 CONCLUSIONS

Maize grown for seed production is susceptible to *L. zonatus* attack during the reproductive phase, and the pest densities requiring management vary with the genetic potential of the genotype cultivated, insecticide selected, and application technology used. Depending on those factors, EILs and ETs for *L. zonatus* infesting seed maize varied from 3 to 8 and from 2 to 6 adults per 1000 plants, respectively. In cases where the densities of *L. zonatus* reach the ET, control can be effective by using aerial or ground application of λ-cyhalothrin (106 g a.i. L^{-1}) + thiametoxan (141 g a.i. L^{-1}) at the rate of 1.48 mL ha^{-1}, aerial or ground application of permethrin (384 at g a.i. L^{-1}) at the rate of 27.10 mL ha^{-1}, or ground application of methomyl (215 g a.i. L^{-1}) at the rate of 244.06 mL ha^{-1}.

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