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Insecticide resistance of corn weevil populations from semi-arid regions

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Abstract

Corn weevil (*Sitophilus zeamais*) is a key insect pest of stored corn in Brazil. Corn weevil infestations are traditionally controlled using insecticides. The intensive use of insecticidal compounds, however, may result in the development of insecticide-resistant populations and ultimately cause insecticide-based control measures to fail. The objective of the present work is to define the permethrin and pirimiphos–methyl resistance of *S. zeamais* populations collected from the semi-arid region of Pernambuco, Brazil. Insects were placed in glass bottles impregnated with dry insecticide residues at different concentrations. Deceased insects were counted 24 h after exposure. Concentration–response curves were generated by subjecting mortality results to Probit analysis. The Sete Lagoas population exhibited the lowest 50% mortality (LC_{50}) to permethrin, whereas the Lajedo population exhibited the lowest LC_{50} to pirimiphos–methyl. These populations were used the reference peremthrin- and pirimiphos–methyl-susceptible populations. The order of the LC_{50}-based susceptibility of the populations to the two insecticides differed from that of the LC_{95}-based susceptibility. Six and eight populations showed a significant resistance ratio (RR_{50}) against permethrin and pirimiphos–methyl, respectively. The Brejão population presented the maximum resistance to pirimiphos–methyl (RR_{50} = 26.7 and RR_{95} = 57.4). All populations exhibited low but greatly varied pyrethroid resistance levels. This result, however, does not imply that the problem of pyrethroid resistance is minimized when resistance is substantial in the Brejão population.

Keywords: Development of insecticide-resistant populations; Key insect pest; Probit analysis; *Sitophilus zeamais*; Stored corn in Brazil.
Abbreviations: CI 95%_Confidence interval at 95% probability; LC_Lethal concentration; RR_Resistance ratio; SE_Standard error of the estimate; ?²_Chi-square.

Introduction

Corn weevil (*Sitophilus zeamais* Motschulsky, Coleoptera: Curculionidae) is a main insect pest of stored grains and is one of the most destructive and widely distributed pest insects in the world (Faroni and Souza, 2006). Guedes (1991) reported that corn weevils are a key pest of corn in Brazil. Corn weevil infestations can result in losses of up to 20% of the total production of stored grains. Corn weevil infestations are intensified in tropical regions given the favorable environmental conditions for the corn weevil development and reproduction throughout the year (Lorini et al., 2015).

Corn infestation by *S. zeamais* generally begins in the field long before storage. This fact, allied with the broad distribution and destructive capability of this insect, results in massive losses during the post-harvest period. Corn weevils are classified as an internal primary pest given their ability to attack the intact grain; their intensified actions in corn cause considerable quantitative and qualitative damages to corn production (Rees, 1996). Damages result from a reduction in the dry mass of the grain, the physical and physiological qualities of seed corn, and the nutritional and commercial value of the final products (Lorini, 2002).

Ribeiro et al. (2007) stated that corn weevil infestations under storage conditions are traditionally controlled with chemicals given their quick action, low cost, and easy application. Nonetheless, the indiscriminate use of chemical pesticides has favored the selection of populations with resistance against different chemical compounds, such as pyrethroids and organophosphates, in many countries, including Brazil (Santos et al., 2009). In addition, pest populations can remain resistant to a certain insecticide even after long periods without insecticidal exposure (Corrêa et al., 2008). The stability and severity of the insecticidal resistance of corn weevil populations from different regions of Brazil have been documented (Fragoso et al., 2007; Freitas et al., 2009; Pereira et al., 2009; Braga et al., 2011; Silva et al., 2013). Guedes et al. (1995) concluded that these populations have varying levels of resistance to pyrethroids and organophosphates. The occurrence of pyrethroid and organophosphate resistance has led to the use of constantly increasing concentrations and frequent applications of these pesticides or their substitution with highly toxic and expensive chemicals (Guedes et al., 2006).

However, despite the well-known resistance of *S. zeamais* to pyrethroids and organophosphates, scientific work on the pyrethroid and organophosphate resistance of *S. zeamais* populations from the semi-arid region of Pernambuco, Brazil, remain lacking. Therefore, the present study aimed to determine the level of permethrin and pirimiphos–methyl resistance within regional populations of *S. zeamais*. The results of this study can be used to identify the most effective insecticide for controlling corn weevil infestations in this region.

Results

Concentration–response curves (Tables 1 and 2) were generated by fitting the Probit model to the mortality data obtained through definitive tests (χ^2 significant, $p \leq 0,05$). The Sete Lagoas population showed the lowest 50% mortality (LC_{50}) to permethrin (Table 1), whereas the Lajedo population showed the lowest LC_{50} to pirimiphos–methyl (Table 2).

The LC_{50} values of permethrin ranged from 7.6 (Sete Lagoas-MG) to 51.4 (Jacarezinho-PR) $\mu g \cdot cm^{-2}$. Thus, the LC_{50}-based resistance ratios (RR) of the studied populations were higher by 1.10- (Jupi-PE) to 6.76-fold (Jacarezinho-PR) than those of the reference susceptible population. Although the populations had different responses, their resistance levels were not substantially higher than those of populations collected from other localities. Excluding the value of 1.0 from the confidence intervals of the ratios (Robertson and Preisler, 1992) revealed that six populations had significant permethrin RRs. LC_{95} values varied from 37.4 (Caruaru-PE) to 565.1 (Jacarezinho-PR) $\mu g \cdot cm^{-2}$. The angular coefficients of the curves varied from 1.33 (Garanhuns Natto-PE) to 3.39 (São João-PE) for permethrin. Thus, the order of susceptibility of the populations based on LC_{50} differed from that based on LC_{95} (Table 1).

The LC_{50} values of pirimiphos–methyl ranged from 0.6 (Lajedo-PE) to 32.0 (Jacarezinho-PR) $\mu g \cdot cm^{-2}$. Excluding the value of 1.0 from the confidence interval of the ratios (Robertson and Preisler, 1992) revealed that eight *S. zeamais* populations had significant pirimiphos–methyl ratios. The LC_{50}-based ratios of these populations were 1.17- (Jupi-PE) to 53.3-fold (Jacarezinho-PR) higher than those of the reference pirimiphos–methyl-susceptible population. The RR_{50} = 26.7 and RR_{95} = 57.4 of the Brejão population was significantly higher than those of the Pernambuco populations. The LC_{95} of pirimiphos–methyl varied from 1.0 (Lajedo-PE) to 261.0 (Jacarezinho-PR) $\mu g \cdot cm^{-2}$. The angular coefficients of the curves for pirimiphos–methyl varied from 1.80 (Jacarezinho-PR) to 8.52 (Bom Conselho-PE). Thus, similar to that for permethrin, the order of LC_{50}-based susceptibility of the populations to pirimiphos–methyl differed from that of LC_{95}-based susceptibility of the populations to pirimiphos–methyl (Table 2). High angular coefficients are indicative of genetic variation among the individuals of a population. Thus, susceptibility tends to vary widely in response to the intensity of the stimulus (pesticide dose, concentration, and exposure duration). The range of responses justifies the use and discussion of LC_{95} in this work.

Discussion

The insecticide resistance of the Jacarezinho population has been intensively studied (Guedes et al., 2009). The insensitivity mechanism may be responsible for the insecticide resistance of the population, which has been maintained under laboratory conditions since 1987.

Guedes et al. (1995) posited that the recurrent use of the organochloride dichlorodiphenyltrichloroethane (DDT) during the 1980s may have provided an intense selection pressure that promoted the evolution of pyrethroid cross-resistance in insect populations in Brazil (Côrrea et al., 2008; Braga et al., 2011; Silva et al., 2013). New insecticidal compounds, such as pyrethroids, organophosphates, or mixtures of the two, are now commonly used for controlling insect infestations after the agricultural use of DDT was prohibited. Nevertheless, the intensive use of these compounds have resulted in the development of insecticide-resistant populations and ultimately caused insecticide-based control measures to fail, particularly when used to control infestations in warehouses (Guedes et al., 1995). Corrêa et al. (2011) reported that the majority of 27 *S. zeamais* populations in Brazil are resistant to pyrethroids and pyrethroid + organophosphate mixtures.

The lack of susceptibility baseline values indicated that *S. zeamais* populations have evolved organophosphate resistance. In addition, mortality data indicated the emergence of a possible cross-resistance or multidrug resistance of these populations (Freitas et al., 2009).

The Brejão population was more resistant to pirimiphos–methyl than the Lajedo population. This difference was significant considering that both populations are derived from Pernambuco and suggested that control failures are already a potential problem in this region. The pesticide resistance of these populations is a likely consequence of the pest management and discontinuous nature of the corn storage process. The Brejão county has an intense historical practice of coffee cropping, which requires the use of phosphates and carbamates. Thus, the populations likely developed cross-resistance in response to these conditions.

Pernambuco populations are still predominantly sensitive to organophosphates. Thus, the development of organo-phosphate resistance should be evaluated and monitored. Insecticides are frequently applied at inadequate and/or incorrect dosages in Pernambuco counties. In addition, the systemic use of different chemical insecticides regardless of the pest insect's presence is a common practice among farmers. Ribeiro et al. (2007) reported that that the rapid evolution of insecticide resistance in natural populations has become a main obstacle in the management of pest insects.

Insects have a short life cycle and high fertility. These characteristics favor the development of populations with different genetic characters. The spread of resistance is related with the frequency of insecticide use. Moreover, insecticide resistance is a result of the selective pressure exerted by these toxic compounds and the inheritance of characteristics from resistant individuals. Individuals with mutations that confer insecticide resistance are more likely to survive insecticide treatments and contribute more offspring to the general population than susceptible individuals, thus increasing the frequency of insecticide resistance genes in coming generations (Li et al., 2007). The

Table 1. Susceptibility of *S. zeamais* populations against peremthrin.

S. zeamais populations	Angular coefficient ± SE	LC_{50} (CI 95%) μg.cm^{-2}	LC_{95} (CI 95%) μg.cm^{-2}	RR (CI 95 %)	χ^2
Sete Lagoas-MG	1.93 ± 0.23	7.6 (5.5 – 10.4)	53.9 (33.8 – 108.6)		71.57
Jupi-PE	2.42 ± 0.42	8.3 (5.7 – 12.4)	39.9 (23.5 – 107.9)	1.10 (0.70 – 1.75)	33.32
Caruaru-PE	2.82 ± 0.49	9.7 (7.2 – 13.6)	37.4 (23.7 – 89.5)	1.28 (0.86 – 1.94)	33.05
Lagoa do Ouro-PE	2.47 ± 0.87	11.7 (4.5 – 43.2)	54.5 (21.3 – 5266.3)	1.54 (0.81 – 2.98)	8.01
Lajedo-PE	1.95 ± 0.29	13.2 (8.8 – 20.1)	91.6 (51.0 – 245.8)	1.74 (1.05– 2.90)*	45.90
São João-PE	3.39 ± 0.40	14.3 (11.9 – 17.7)	43.9 (32.5 – 69.0)	1.88 (1.37 – 2.64)*	71.32
Brejão-PE	1.74 ± 0.16	27.9 (21.5 – 36.1)	246.0 (165.9 – 417.5)	3.67 (2.39 – 5.71)*	123.18
Garanhuns Natto-PE	1.33 ± 0.11	29.3 (21.6 – 39.4)	503.2 (310.2 – 962.0)	3.85 (2.25 – 6.69)*	130.84
Bom Conselho-PE	1.98 ± 0.23	35.3 (25.6 – 48.5)	238.7 (152.0 – 465.5)	4.64 (3.05 – 7.19)*	74.27
Jacarezinho-PR	1.58 ± 0.17	51.4 (35.8 – 73.5)	565.1 (329.7 – 1245.0)	6.76 (4.19 – 11.1)*	81.86

SE = Standard error of the estimate; LC = Lethal concentration; RR = Resistance ratio; CI 95% = Confidence interval at 95% probability; χ^2 = Chi-square.

Table 2. Susceptibility of *S. zeamais* populations against pirimiphos–methyl.

S. zeamais populations	Angular coefficient ± SE	LC_{50} (CI 95%) μg.cm^{-2}	LC_{95} (CI 95%) μg.cm^{-2}	RR (CI 95 %)	χ^2
Lajedo-PE	8.47 ± 1.17	0.6 (0.5 – 0.7)	1.0 (0.9 – 1.2)		52.36
Jupi-PE	3.52 ± 0.48	0.7 (0.6 – 0.8)	2.1 (1.7 – 3.2)	1.17 (0.94 – 1.33)	52.38
Garanhuns Natto-PE	6.79 ± 0.94	0.8 (0.7 – 0.9)	1.4 (1.2 – 1.7)	1.33 (1.11 – 1.27)*	51.94
Bom Conselho-PE	8.52 ± 1.21	0.8 (0.7 – 0.9)	1.2 (1.1 – 1.5)	1.33 (1.16 – 1.26)*	49.59
São João-PE	6.22 ± 0.98	1.0 (0.9 – 1.2)	1.9 (1.6 – 2.7)	1.67 (1.48 – 1.68)*	40.48
Sete Lagoas-MG	6.11 ± 0.98	1.1 (1.0 – 1.3)	2.1 (1.7 –3.0)	1.83 (1.60 – 1.83)*	38.84
Caruaru-PE	5.73 ± 1.81	1.4 (1.0 – 2.1)	2.7 (1.9 – 10.5)	2.33 (1.87 – 2.45)*	10.01
Lagoa do Ouro-PE	3.42 ± 0.36	1.6 (1.4 – 1.8)	4.8 (3.8 – 6.6)	2.67 (2.14 – 2.72)*	92.33
Brejão-PE	2.96 ± 0.51	16.0 (11.2 – 24.3)	57.4 (34.9 – 144.7)	26.7 (20.4 – 29.9)*	33.82
Jacarezinho-PR	1.80 ± 0.22	32.0 (23.2 – 46.3)	261.0 (146.5 – 654.6)	53.3 (37.3 – 63.9)*	64.80

SE = Standard error of the estimate; LC = Lethal concentration; RR = Resistance ratio; CI 95% = Confidence interval at 95% probability; χ^2 = Chi-square.

Table 3. Origin from populations of *S. zeamais* used to evaluate resistance against the insecticides peremthrin- and pirimiphos–methyl.

S. zeamais populations	City	Sampling location	Product	Sampling date
Susceptible	Sete Lagoas[1]	Laboratory		
Resistant	Jacarezinho[2]	Laboratory		
1	Jupi	Grain Warehouse	Dry corn	April/2013
2	Brejão	Storeroom	Wet corn	April /2013
3	Caruaru	Metallic silo	Dry corn	June/2013
4	São João	Storeroom	Dry corn	August/2013
5	Lagoa do Ouro	Metallic silo	Dry corn	August/2013
6	Bom Conselho	Metallic silo	Dry corn	November/2013
7	Lajedo	Metallic silo	Dry corn	November/2013
8	Garanhuns Natto	Metallic silo	Dry corn	January/2014

[1] = Standard population of susceptibility, preserved in laboratory since 1985; [2] = Standard population of resistance against pyrethroids since 1987; Populations were numbered by the sampling order.

application period of permethrin should be extended to decrease selection pressure and the frequency of resistant genotypes.

Pyrethroid resistance is characterized by stability. Thus, to manage pyrethroid resistance efficiently in agricultural areas, pyrethroids should be alternately or simultaneously applied with newly registered insecticides that have different modes of action and with negative cross-resistance. This application strategy will decrease the frequency of pyrethroid resistance genes in the population (Santos et al., 2009). Chemical rotation hinges on the possible disadvantage of adaptive resistant individuals.

As in previous studies, RR was evaluated on the basis of LC_{50}. LC_{95}, however, is also an effective control parameter for field populations. Braga et al. (2011) stated that LC_{95} has excellent representativeness and allows for the adjustment of the Probit model (less amplitude of the mean standard errors), thus providing further credibility to the evaluation of lethal concentration and RR. The determination of LC_{50} and RR allows the monitoring of temporal changes in the susceptibility of populations (Silva et al., 2013). The pirimiphos–methyl RR of the Jacarezinho and Brejão populations can be classified as severe. These populations may thus potentially harm stored grain.

The angular coefficient of the curve is inversely proportional to the mean standard error of the phenotypic distribution of insecticide tolerance in a population. According to Ribeiro et al. (2003), the variability between the individuals of the same population can be inferred from the slope of the curve. Fragoso et al. (2007) suggested that gradually sloping curves indicate greater genetic variability and suggest the presence of more than one genotype in the population. This pattern also indicates that insecticide response is highly heterogeneous and that the population has been exposed to different selective pressures. In addition, intra- and inter-population genetic diversity may explain the variability observed within these results. These scores will vary from year to year given the interference of various external factors. The data obtained by this study justify its implementation and corroborates its practical significance. The results of this study may support the development of management strategies against insecticide resistance. The successful implementation of these strategies may decrease losses incurred by the failure of agricultural pest control, especially in stored grains.

Materials and methods

S. zeamais populations

Ten different *S. zeamais* populations were used in this experiment. One population was obtained from the EMBRAPA–Corn and Sorghum National Research Center, Sete Lagoas-MG, Brazil. This population was maintained under laboratory conditions without insecticide exposure and was used as the reference pyrethroid-susceptible population. Another population originated from populations collected from warehouses in Jacarezinho-PR, Brazil. Previous studies have identified this population as resistant against pyrethroids. Thus, it was used as the reference pyrethroid-resistant population in the present study. Another *S. zeamais* population, designated as the Natto population, was collected from silos at the Rations Factory, Notaro Alimentos Ltda (Natto). The Natto population was treated with insecticides every four months on a rotating basis (Table 3). Other populations were collected from grain-producing counties in the semi-arid region of Pernambuco, Brazil, where control failures of insecticide application have been frequently reported (Table 3). Each population was cultivated on clean and dry corn grains. The grains were previously frozen for seven days at −20 °C to eliminate any possible insect infestations. The populations were maintained at room temperature in glass containers (1.2 L) covered with thin, stiff, perforated cotton lids and were established from at least 500 individuals. The populations were individually preserved for several generations without exposure to insecticides at the Applied Entomology Laboratory of the Federal Rural University of Pernambuco of the Academic Unit of Garanhuns until the beginning of the experiments. Dry corn grains were periodically replaced to preserve populations.

Insecticides and solvent

The insecticides used in the bioassays (Technical Grade) were the pyrethroid permethrin (99.5%) and the organophosphate pirimiphos–methyl (99.3%). Both insecticides were procured from Sigma-Aldrich (Brazil) and were applied independently. Different concentrations of these chemicals were prepared with analytical grade acetone as a solvent (99.5%, Modern Chemical Industry and Trade Ltda.).

Concentration–response tests

In vivo bioassays were performed following a completely randomized design in biochemical oxygen demand climatic chambers under 25 ± 2 °C, relative humidity of 70% ± 5%, and photoperiod of 12 hours. All tests were performed in experimental units comprising transparent cylindrical glass flasks with a volume of 5 mL and internal area of 18.8 cm^2. The methodology was optimized to determine the optimal values for the following parameters: i- duration of insecticide exposure; ii- insecticide volume; and iii- number of insects per flask. The optimal parameters implemented in the model were as follows: 24 h of insecticide exposure, application volume of 0.1 mL, and 10 individuals per flask.

After optimization, preliminary concentration–response tests were performed using six sequential dilutions (1000, 100, 10, 1, 0.1, and 0.01 $\mu g \cdot cm^{-2}$) of the active compounds from permethrin and pirimiphos–methyl. Tests were performed separately with each insecticide. A total of 0.1 mL of pesticide solutions was added to each flask. The flasks were manually agitated to promote acetone evaporation and the uniform distribution of the insecticide in the solution. Subsequently, 10 unsexed adults of *S. zeamais* were placed into each flask. The number of dead individuals under each concentration was evaluated after 24 h of exposure. To standardize evaluation, insects with evidence of paralysis, which was defined as the inability to move after being touched with the bristles of a round-tip paintbrush for 60 seconds, were considered dead. After the evaluation, the lowest concentration of insecticide under which the maximum number of deaths occurred and the highest concentration of insecticide under which no death occurred were defined as the higher and lower extremes of insecticide concentration, respectively. Subsequently, new intermediary concentrations between these extremes were established. Additional tests were conducted with six different insecticide concentrations and one control (solvent only) with five replicates each and 350 individuals from each population. Definitive concentration tests were then performed with five replicates for each intermediary concentration in addition to the extremes. These tests constituted several treatments, which were varied on the basis of the extreme responses of each population, and a control (solvent only). A concentration–response curve for each population that had been exposed to a particular insecticide was constructed. A total of 350 or 400 individuals from each population were exposed. The mortality rates obtained through the definitive tests were adjusted in accordance with Abbott (1925). To obtain the final concentration–response curves, the adjusted results were then analyzed through Probit analysis with the PROC PROBIT procedure packaged in the statistical software SAS 9.0. RRs were calculated by dividing the LC of a population under study by its respective LC from the most susceptible population. These ratios were considered significant when the confidence intervals at 95% of probability did not include the value of 1.0 (Robertson and Preisler, 1992).

Conclusion

There are strong evidences of cross resistance against pyrethroids and/or multiple resistance to pyrethroids and organophosphates. Considering semi-arid region of Pernambuco state, Brazil, there are problems of control failure of *S. zeamais* with the use of phosphorous. Mortality ratios in Jacarezinho evidenced other mechanisms of resistance, in addition to insensibility of the target site.

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