Chapter 1

Resistance to Insecticides in Populations of the Coffee Leafminer

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Abstract

Coffee leafminer *Leucoptera coffeella* is an important pest on coffee. The continued use of chemicals can result in loss of efficacy and selection of leafminer-resistant populations. We aimed to identify *L. coffeella* populations resistant to old and new neurotoxic insecticides in regions of Brazil. We collected seven populations of *L. coffeella* in Brazil. Low levels of resistance were observed for the insecticides chlorantraniliprole (1.02-3.23 times), abamectin (1.19-4.80 times), and deltamethrin (1.05-5.35 times). High resistance levels were observed for profenofos (65.3-522 times) and chlorpyrifos (4.53-18.63 times). We conclude that Brazilian *L. coffeella* populations showed greater resistance to organophosphate insecticides. Furthermore, resistance may be associated with the distance between the coffee-producing regions.

Keywords: Anthranilamide, *Coffea* spp, Lepidoptera, lethal time, organophosphate

1. Introduction

The coffee leafminer *Leucoptera coffeella* (Guérin-Méneville, 1842) (Lepidoptera: Lyonetiidae) is originally from Africa and has become a pest species of great significance in many countries producing coffee (*Coffea arabica* and *Coffea canephora*) [1,2]. The extremely variable life cycle of this species and their damage to coffee crops make them a pest with a high destruction capacity [3-5]. Insecticides provide the most efficient method of controlling this pest, with more than 30 different active pesticides registered for use against this *L. coffeella* in Brazil [6]. Despite the existence of several active ingredients, the overuse of pesticides by farmers has led to the insects becoming resistant [7].

The first documented case of resistance was in 1914, in San Jose Scale (*Quadraspidiotus perniciosus)* (Comstock, 1881) (Hemiptera: Diaspididae) exposed to repeated doses of sulfur powder [8]. Reports of insect resistance began to increase in the 1940s as insecticides and miticides emerged. There are over 7740 reported cases of resistance, involving 331 compounds and more than 540 species of insects and mite pests [9]. From 1914 to 2007, the vast majority of cases of resistance occurred in Lepidoptera, with 1799 confirmed cases.

Lepidopteran species such as *Alabama argillacea* (Lepidoptera: Noctuidae) [10], *Plutella xylostella* (Lepidoptera: Plutellidae) [11], and *Tuta absoluta* (Lepidoptera: Gelechiidae) [12] have shown resistance to several groups of insecticides. These authors studied insect populations from different locations, using different groups of insecticides with varying mechanisms of action. Studies with *L. coffeella*, however, have focused only on the organophosphate group with no studies on other chemical groups [13,14]. As such, studies of different populations and various insecticide groups are needed.

Among the insecticides used, most are neurotoxins, and it is this group that presents the most problems of insect resistance [9]. These neurotoxic insecticides (e.g., organophosphates and pyrethroids) cause rapid death of susceptible insects, and abamectin, neonicotinoids, and diamides are slower in causing death of insects [15].

It is therefore possible to detect resistance to a particular active ingredient by comparing the time of death of each population to different neurotoxic insecticides. Similar experiments have been done with other insects, such as the mosquito *Culex tarsalis* (Diptera: Culicidae) [16]. Slower deaths may indicate the population is beginning to become resistant. Delayed mortality could be compared to the effect of sublethal doses, which put the insects in a state of stress and reduce their metabolism before death [17]. One way to detect resistance using the lethal time of death (LT) is to collect geographically distant populations to obtain more precise information and compare populations across regions since the resistance is relative. Thus, based on the mechanism of action of each insecticide group, it is possible to compare resistance by measuring how quickly the insecticides act on a population.

There are two studies focusing on the detection of insecticide resistance among populations of *L. coffeella* and just with organophosphate insecticides. Our proposal is to study different groups and regions. This study aimed to recognize populations of *L. coffeella* in different regions of Brazil that were resistant to neurotoxic insecticides by comparing the lethal time.

2. Materials and methods

2.1. Insect populations

This study was conducted at the Laboratory of Integrated Pests Management at Universidade Federal de Viçosa, Rio Paranaíba Campus (UFV-CRP). We selected six municipalities with coffee cultivation of the species *C. arabica* and *C. canephora*, located in producing regions of the Brazilian states of Minas Gerais, Espírito Santo, São Paulo, and Pernambuco. These areas were selected because they are the largest coffee producing regions in Brazil. In these regions, we

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collected leaves from the middle third of plants randomly selected in commercial crops during the 2012-2013 crop season, with active mines (live caterpillars) of *L. coffeella*. These crops were georeferenced with the help of a portable Garmin E-trex Summit Hc GPS (Figure 1).

Regions City-State	Geographic coordinates	Average temperature (°C)	Precipitation (mm/year)	Altitude (m)
Rio Paranaiba-MG	-19.21,-46.14	21.33	1478	1071
Abaeté dos Mendes-MG	-19.17,-46.09	21.31	1475	849
Carmo do Paranaiba-MG	-19.04,-46.22	21.25	1472	1120
Guaraciaba-MG	-20.34,-43.00	21.11	1611	1120
Franca-SP	-20.58,-47.43	21.05	1644	578
Santa Teresa-ES	-19.93,-40.59	21.54	1162	174
Guaranhuns-PE	-08.89,-36.49	22.05	857	900

Figure 1. Location and characterization of *Leucoptera coffeella* collection in coffee-producing regions. Dark spots represent coffee-producing regions. White spherical symbols within the dark spots represent collection sites of leafminer populations.

The leaves collected in each region were transported to the laboratory in separate plastic bags for visual selection of mines that did not present any harm (e.g., open or with signs of parasitism/predation). Selected mined leaves were combined for insect rearing in a greenhouse (20 × 10 m). These leaves were placed in vials with water (25 mL) inside wooden cages covered with organza. The larvae were fed seedlings coffee of Catuaí cultivar grown in a greenhouse without insecticide application. Only larvae with at least one generation in the laboratory were used in bioassays to prevent the expression of insecticide tolerance due to differing environmental conditions at the different sampling sites (i.e., differences without any genetic basis).

2.2. Insecticides

Six neurotoxic insecticides were selected for bioassays of *L. coffeella* resistance to the concentrated active ingredients abamectin 18 g l^{-1} EC (emulsifiable concentrate) (Syngenta, São Paulo, Brazil), chlorantraniliprole 350 g l^{-1} WG (water-dispersible granules) (DuPont, Paulínia, Brazil), chlorpyrifos 480 g l^{-1} EC (Fersol, Mairinque, Brazil), deltamethrin 25 g l^{-1} EC (Bayer SA, São Paulo, Brazil), profenofos 550 g l^{-1} EC (Syngenta, São Paulo, Brazil), and thiamethoxam 250 g l^{-1} WG (Syngenta, São Paulo, Brazil) (Table 1).

Insecticide	**Population**	**LT_{50} [a] ($CI_{95\%}$)[b]**	**n**	**RT_{50} [c]**	**χ^2 [d](df)[e]**	***P*[f]**
	Rio Paranaíba-MG	13.29 (11.29–15.34)	40	1.74	2.87 (3)	0.59
	Abaeté dos Mendes-MG	14.70 (12.59–16.53)	40	1.92	6.95 (3)	0.07
	Carmo do Paranaíba-MG	36.75 (33.63–40.97)	40	4.80	4.05 (3)	0.26
Abamectin	Santa Teresa-ES	9.11 (6.03–11.52)	40	1.19	2.89 (3)	0.59
	Guaranhuns-PE	17.85 (15.87–19.71)	40	2.33	4.97 (3)	0.17
	Franca-SP	12.41 (10.99–15.12)	40	1.62	3.42 (3)	1.12
	Guaraciaba-MG	7.65 (6.85–10.11)	40	1.00	5.63 (3)	3.11
	Rio Paranaíba-MG	8.16 (7.02–9.20)	40	8.08	7.35 (4)	0.12
	Abaeté dos Mendes-MG	17.18 (15.68–18.75)	40	17.01	9.07 (4)	0.06
	Carmo do Paranaíba-MG	16.39 (15.12–17.76)	40	16.23	1.66 (3)	0.65
Chlorpyrifos	Santa Teresa-ES	4.58 (3.62–5.54)	40	4.53	7.56 (5)	0.18
	Guaranhuns-PE	8.59 (6.70–10.21)	40	8.50	2.39 (3)	0.50
	Franca-SP	18.82 (17.54–20.15)	40	18.63	8.20 (4)	0.08
	Guaraciaba-MG	1.01 (0.35–2.07)	40	1.00	6.32 (7)	0.06
	Rio Paranaíba-MG	27.70 (24.70–31.56)	40	1.98	3.66 (3)	0.30
	Abaeté dos Mendes-MG	26.30 (22.15–34.79)	40	1.88	1.57 (2)	0.54
	Carmo do Paranaíba-MG Santa Teresa-ES	14.01 (11.87–16.47)	40	1.00	7.51 (5)	0.18
Chlorantraniliprole	Santa Teresa-ES	31.53 (28.44–35.74)	40	2.25	5.50 (3)	0.14
	Guaranhuns-PE	18.82 (17.54–20.15)	40	3.23	8.20 (4)	0.08
	Franca-SP	14.28 (11.00–18.23)	40	1.02	6.30 (5)	1.22
	Guaraciaba-MG	8.59 (6.70–10.21)	40	1.88	2.39 (3)	0.50
	Rio Paranaíba-MG	31.12 (27.59–36.20)	40	5.35	4.96 (4)	0.17
	Abaeté dos Mendes-MG	25.73 (23.34–28.56)	40	4.42	3.83 (3)	0.28
Deltamethrin	Carmo do Paranaíba-MG	28.18 (24.46–34.29)	40	4.84	2.22 (3)	0.53
	Santa Teresa-ES	5.82 (4.23–7.65)	40	1.00	5.99 (4)	0.07
	Guaranhuns-PE	20.38 (17.53–23.23)	40	3.50	6.04 (3)	0.11

Insecticide	Population	LT_{50} [a] ($CI_{95\%}$)[b]	n	RT_{50} [c]	χ^2 [d](df)[e]	*P*[f]
	Franca-SP	18.82 (17.54–20.15)	40	3.23	8.20 (4)	0.08
	Guaraciaba-MG	6.11 (5.03–7.84)	40	1.05	5.81 (4)	0.06
Profenofos	Rio Paranaíba-MG	15.66 (13.96–17.17)	40	522	0.85 (5)	0.66
	Abaeté dos Mendes-MG	12.25 (11.10–13.19)	40	408	1.35 (6)	0.51
	Carmo do Paranaíba-MG	6.96 (4.28–9.00)	40	232	3.85 (5)	0.28
	Santa Teresa-ES	1.96 (0.08–3.00)	40	65.3	3.71 (3)	2.32
	Guaranhuns-PE	10.96 (8.50–11.00)	40	365	1.36 (4)	0.44
	Franca-SP	12.96 (8.24–14.53)	40	432	4.12 (3)	0.21
	Guaraciaba-MG	0.03 (0.01–0.50)	40	1.00	1.58 (3)	0.23
Thiamethoxam	Rio Paranaíba-MG	37.29 (33.32–43.21)	40	4.41	2.54 (3)	0.53
	Abaeté dos Mendes-MG	23.10 (21.11–25.27)	40	2.73	0.43 (3)	0.93
	Carmo do Paranaíba-MG	89.93 (61.70–180.00)	40	10.61	6.54 (4)	0.16
	Santa Teresa-ES	10.49 (9.13–11.78)	40	1.24	8.65 (4)	0.07
	Guaranhuns-PE	13.57 (12.07–14.87)	40	1.61	7.69 (3)	0.06
	Franca-SP	8.45 (7.07–10.95)	40	1.00	5.66 (3)	1.05
	Guaraciaba-MG	9.36 (7.01–10.34)	40	1.11	6.71 (3)	0.06

[a]LT_{50} = time (h) lethal to kill 50% of the population.

[b]CI = confidence interval of 95%.

[c]RT_{50} = ratio of lethal time to kill 50% of the population.

[d]χ^2 = chi-square.

[e]*df* = degrees of freedom.

[f]*P* = probability.

Table 1. Time and mortality curves (LT_{50}) of Brazilian populations of *Leucoptera coffeella* under the effect of seven insecticides at the recommended doses.

The registered label rates of the respective active ingredients in Brazil were 0.18 mg mL^{-1} (0.026 mg a.i. mL^{-1}) for abamectin, 0.072 mg mL^{-1} and 0.078 mg a.i. mL^{-1} for chlorantraniliprole, 0.05 mg mL^{-1} (4.800 mg a.i. mL^{-1}) for chlorpyrifos, 0.032 mg mL^{-1} (0.013 mg a.i. mL^{-1}) for deltamethrin, 0.4 mg mL^{-1} (1.100 mg a.i. mL^{-1}) for profenofos, and 0.024 mg mL^{-1} (2.000 mg a.i. mL^{-1}) for thiamethoxam.

2.3. Time-mortality bioassay

For time-mortality analysis, circular discs (diameter 90 mm) of filter paper were dipped into the insecticide solutions diluted in distilled water, using the recommended doses to control *L. coffeella*. The control used embedded disks with distilled water. The discs containing the insecticides and the water were fixed on a clothesline to dry in the shade and then placed separately into Petri dishes (9.0 × 1.5 cm). Ten larvae of *L. coffeella* reared in the lab were transferred to each Petri dish using a fine-tipped brush. The Petri dishes with the larvae were kept in the BOD incubator (model SP-500) at 25°C ± 1°C until the time of evaluation. The experiments were conducted in a completely randomized design with four replications.

Preliminary tests using only discs soaked in water were carried out to observe caterpillar mortality over a 48-h period. This was necessary to estimate the maximum evaluation time after bioassay assembly that causes 20% lower mortality in the control [18]. Thus, to have a mortality range from 0% to 100%, evaluations were made at 2, 6, 12, 16, 24, 32 and 48 h (treatments) after bioassay assembly. The time intervals were assessed in independent experimental units, to avoid pseudoreplicates. We considered insects dead when they did not move after being touched with the fine-tipped brush.

2.4. Spatial dependence of insecticide resistance

To determine the spatial dependence of *L. coffeella* insecticide resistance, the semivariance statistical model of LT_{50} values to *L. coffeella* populations for each insecticide and the distance between sampling locations of each population were used. The distance between the sampling sites of each insect population was determined using geographic coordinates with a global positioning system (GPS 12, Garmin International, Olathe, KS). The semivariograms were estimated from the semivariance data of the LTs_{50} of each population for each insecticide and used as dependent variables in regression analysis, with the distance between the sampling sites as an independent variable. The first inflection point of the semivariogram curve represents the maximum distance of interference between the populations of *L. coffeella* in relation to susceptibility to a given insecticide.

3. Results and discussion

Resistance to neurotoxic insecticides varied generally among the different populations of *L. coffeella* in Brazil. RT_{50} varied from 1.02 to 522. Low resistance levels were observed for chlorantraniliprole insecticides (1.02-3.23 times), abamectin (1.19-4.80 times), and deltamethrin (1.05-5.35 times).

On the other hand, intermediate resistance was observed for thiamethoxam (1.11-10.61 times) and chlorpyrifos (4.53-18.63 times), while resistance was high for profenofos (65.3-522 times) (Table 1). Higher levels of organophosphate resistance were observed in Minas Gerais (Abaeté dos Mendes, Rio Paranaíba and Carmo do Paranaíba), Pernambuco (Guaranhuns), and São Paulo (Franca).

The RT_{50} values are supported by the LT_{50} values, which were variable among populations and insecticides. The population from Carmo do Paranaíba-MG was noteworthy as it took 89.93 h for 50% of the population to die after contact with the insecticide thiamethoxam. The organophosphate and pyrethroid insecticides had lower lethal times. Chlorantraniliprole showed lower LT_{50} of 8.59 h.

Two canonical axes were significant among the five canonical axes identified, showing linear associations between LT_{50} of the insecticides with the geographical regions of the population origins of *L. coffeella*, which showed that the four canonical axes were significant, with the first three axes explaining 90% of the total variance data (Table 1 and Figure 2). The highest absolute values of the canonical coefficients show which insecticides most contributed to the standard deviation of resistance among the different localities. For the first canonical axis of greater importance in the analysis, the insecticides chlorpyrifos, profenofos, and deltamethrin showed positive correlations and higher values of coefficients and thus higher contributions to the differences between the resistant populations (Table 2). Profenofos and deltamethrin, with a positive relationship, contributed to the pattern of divergence on the second axis.

The opposite relationship was observed for assistance with the chlorpyrifos insecticide on the third and fourth axes. On the fifth and sixth axes, a positive relationship was observed between the profenofos insecticide and the standard deviation. It is important to highlight that the new insecticide chlorantraniliprole did not contribute to the resistance of populations (Table 2). Graphs of this analysis done with the first two axes explained 92% of the total variance of the data to show the grouping between locations (Table 2 and Figure 2).

The weight of organophosphate (profenofos and chlorpyrifos) and pyrethroid (deltamethrin) insecticides on the first two axes enhanced the resistance process since they are among the main groups with examples of insect resistance (quotation). Two grouping patterns were observed, with one group for the populations of *L. coffeella* Rio Parnaíba-MG, Carmo do Paranaíba-MG, and Abaeté dos Mendes-MG and a second group for the populations of Santa Teresa-ES and Guaraciaba-MG, but these patterns did not occur in the other populations (Figure 2).

Variables/mortality	**Canonical axes**					
1	2	3	4	5	6	
Abamectin	–0.21	–0.10	–0.21	–0.46	–0.38	–0.05
Chlorpyrifos	0.77	–0.65	–0.33	–0.45	–0.10	–0.35
Chlorantraniliprole	–0.10	0.00	–0.21	0.00	0.04	0.00
Deltamethrin	0.51	0.68	0.17	0.05	0.39	0.55
Profenofos	0.64	–0.59	0.38	0.55	0.64	0.64
Thiamethoxam	0.40	0.42	0.11	0.19	0.40	0.38
F	31.02	25.51	20.36	15.02	13.55	9.11
df_x^a	68; 181	54; 181	46; 181	32; 181	20; 181	16; 181
R^2x^b	0.90	0.89	0.78	0.66	0.61	0.53

[a]df_x = degrees of freedom (numerator/denominator).

[b]R^2x^b = canonical correlation square.

Table 2. Canonical axes and coefficients (grouped in the canonical structure) of mortalities of *Leucoptera coffeella* caused by six neurotoxic insecticides.

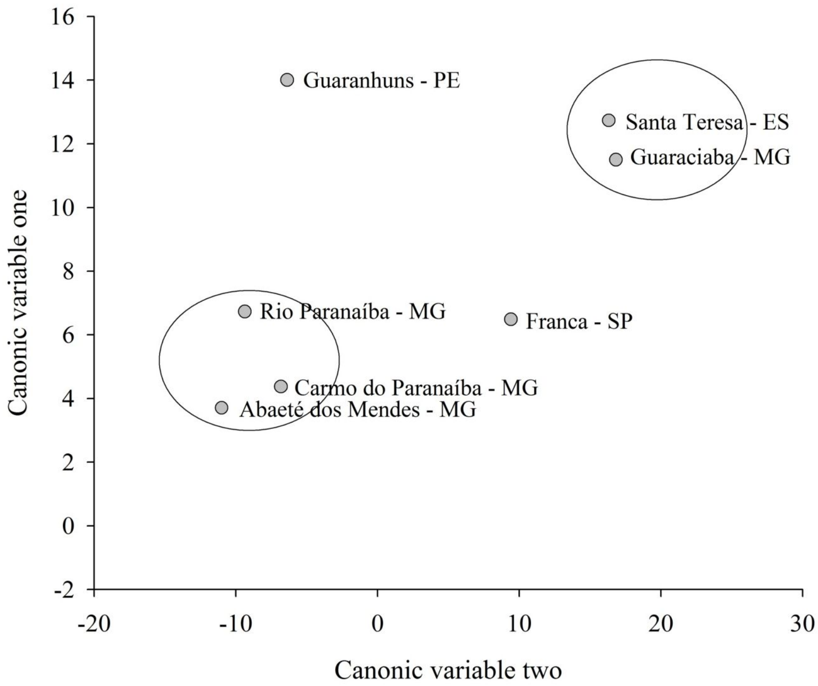

Figure 2. Ordination diagram showing discrimination of insecticide resistance between Brazilian populations of *Leucoptera coffeella*. Spherical gray symbols are centroids of treatments and represent the average of canonical variable classes. Large circles indicates treatment groups with no significant difference between them (approximate *F* test, *P* < 0. 05), based on the Mahalanobis distance (D_2) between averages.

The semivariogram models related to the LT_{50} values of *L. coffeella* with the distance between the sampling sites obtained for only two insecticides, the organophosphates chlorpyrifos and the pirimiphos. The first inflection points for the models were lengths of 169 and 1,956 km for the insecticides chlorpyrifos and pirimiphos (Figure 3). Therefore, these were the maximum distances between the interference resistance levels of the *L. coffeella* sampling sites.

Our study reported high variations in the resistance ratio (RT_{50}) of the organophosphates profenofos (522 times) and chlorpyrifos (19 times) compared to the susceptible population of *L. coffeella*. This large variation represented by RT_{50} indicates that populations show differences in susceptibility and greater or lesser sensitivity to the enzyme acetylcholinesterase since variations were observed between populations that died the fastest and those that died more slowly.

This shows that this group of insecticides is extremely important in managing resistance because of its intense use, with this group being highly toxic and presenting higher neurotoxic action [19]. Many studies on resistance to the organophosphate insecticide group showed high variation in the mortality of the resistant population compared to other lepidopteran populations [20,21]. Extensive insecticide use in coffee crops and high death speed are among the main factors of resistance [22]. Fragoso et al. [13] observed up to 22 applications of organophosphate insecticides, detecting high levels of resistance when larvae were kept exposed to the discriminating concentration. These concentrations were higher than those tested for profenofos and chlorpyrifos in our study.

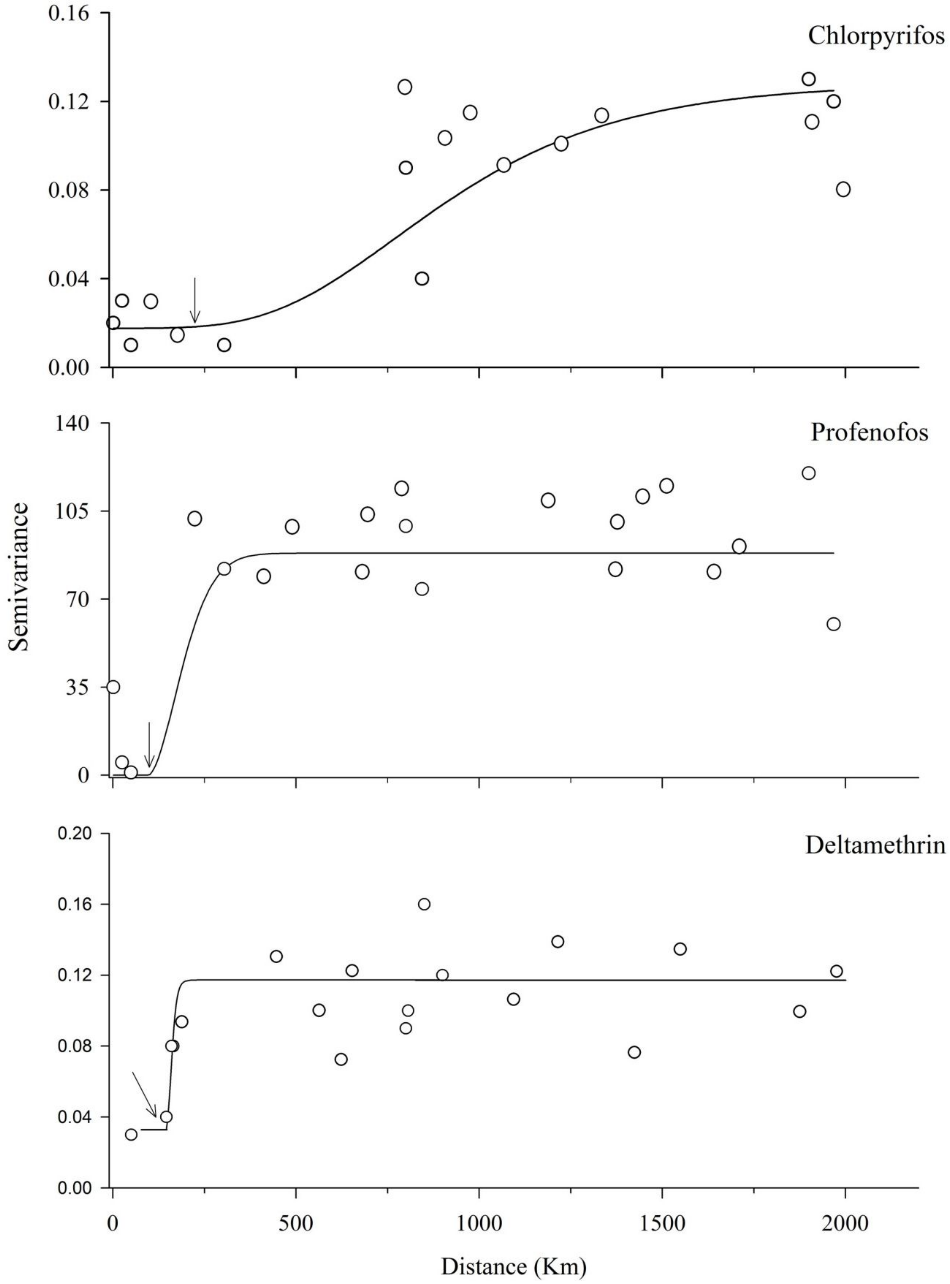

Figure 3. Semivariogram of the LT_{50} of chlorpyrifos, profenofos, and deltamethrin according to the distance between sampled points from populations of *Leucoptera coffeella*. The first inflection point of the semivariogram curve, represented by a down ward-pointing arrow, represents the maximum distance of interference of the resistance to the insecticides.

On the other hand, chlorantraniliprole, abamectin, and deltamethrin insecticides showed low levels of RT_{50} variation. The result with the chlorantraniliprole insecticide was as expected since this insecticide has only recently been commercialized [23-25] and has a highly efficient molecule since low doses of this insecticide (31.5 g a.i. ha^{-1}) cause high mortality to *L. coffeella*; moreover, it is selective for wasps [26].

Selectivity is an important factor in managing resistance in pest insects [27]. Many studies with basic lines of susceptibility have been done with chlorantraniliprole insecticide and Lepidoptera, and the observations are that populations show susceptibility with low variation in mortality [28,29]. The insecticide abamectin is not considered old and has been effective in controlling this pest insect, with no flaws detected in its control of *L. coffeella* as of yet. Despite the abamectin insecticide not being among those at risk of resistance in *L. coffeella*, this insecticide has not been studied. However, many arthropod pests have been classified as being at risk for resistance to this group. Among them are *Leptinotarsa decemlineata* (Say) [30], *Musca domestica* [31], *P. xylostella* [32], *Frankliniella occidentalis* [33], and *Tetranychus urticae* [34]. Abamectin resistance has been observed in populations of *F. occidentalis* [35] and *Liriomyza trifolii* [36]. Deltamethrin had surprising results, with low discrepancy between the resistant and the susceptible populations (5 times) compared to their insecticides such as thiamethoxam (10 times) that are less used in coffee plantation. In recent years, however, the number of pyrethroid applications in coffee production has been greatly reduced. Despite the low resistance to pyrethroids, however, the variation has been observed in Brazil for the moth *P. xylostella* [37] as well as with other pyrethroids (cypermethrin, β-cypermethrin, deltamethrin, and esfenvalerate) in Pakistan, India, China, and Korea [38,39]. Although deltamethrin has affected fewer Brazilian populations of *L. coffeella*, a difference of 5 times is cause for concern since it should have been more effective.

Insects usually have a resistance mechanism that confers nerve insensitivity, known as knockdown resistance (Kdr), as first reported in *M. domestica* (L.) (Diptera: Muscidae) [40]. This type of resistance is found in other agricultural pests based on patterns of cross-resistance and the absence of compound synergism that inhibits the activity of cytochrome P_{450} and esterase enzymes [41].

The insecticide thiamethoxam has been frequently used and can be applied as a spray or via the soil [42]. There are no studies of lepidopteran resistance to this insecticide. Control failures were observed depending on the time of application, however, for example [43] observed effectiveness of 4.1%, 50.6%, 62.1%, and 69.0%.

The grouping of populations from Rio Paranaíba, Carmo do Paranaíba, and Abaeté (Group I) and Santa Teresa with Guaraciaba (Group II), coupled with the significant response of the effect of distance on the LT_{50} of the chlorpyrifos, profenofos, and deltamethrin insecticides, showed that resistance was affected by the collection distance of these populations since more closely connected populations had similar resistance responses.

Studies have shown a strong relationship between collection distance and resistance patterns [44,10,45,12]. All of these studies showed significant association of resistance with distance, and nearby populations tended to show more similar responses, as is the case for *P. xylostella* (L.) (Lepidoptera: Plutellidae). Chen et al. [46] studied the resistance of pyrethroids to *Culex pipiens* (Diptera: Culicidae) and found different frequencies of resistance at different locations, ranging from 21.4% to 79.8%. Moreover, this type of response may be associated with the large dispersal capacity of adult *L. coffeella* and the sampling characteristics.

Adults of *L. coffeella* disperse easily between coffee crops and have different densities in different environments [47-49]. Moreover, there is a geographic corridor between the largest-producing Brazilian states (Figure 1). Isaaks and Srivastava [50] also found that in order to detect differences among geostatistical studies of spatial distribution, it was necessary to collect both near and distant samples.

We conclude that Brazilian populations of *L. coffeella* showed greater resistance to organophosphates. Furthermore, resistance may be associated with the distance between the producing regions, and local selection favored by dispersal seem important for insecticide resistance evolution among Brazilian populations of *L. coffeella* and should be considered in designing pest management programs. The insecticides that do not show mortality to *L. coffeella* should be sprayed in such conditions, and a higher variety of insecticides (out of the cross-resistance and multiple-resistance spectra) should be used in rotation to reduce the danger of evolution of resistance.

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