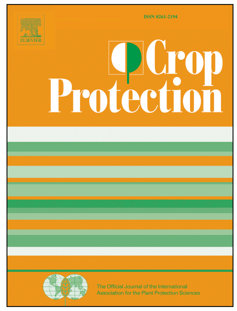

Deltamethrin resistance in *Ceratitis capitata* (Diptera: Tephritidae): Selections, monitoring and effect of synergist

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Deltamethrin resistance in *Ceratitis capitata* (Diptera: Tephritidae): selections, monitoring and effect of synergist

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Highlights

There is variability in deltamethrin susceptibility in Brazilian populations of *Ceratitis capitata*

A medfly population from Pelotas-RS was 26 times more resistant to deltamethrin than a population from Petrolina-PE

Esterases are involved in deltamethrin resistance in *C. capitata*

ABSTRACT

The Mediterranean fruit fly, *Ceratitis capitata* (Wied.) (Diptera: Tephritidae), is considered one of the pests with the greatest importance for fruticulture in the world, causing significant losses to fruit production and limiting the free transit of fruits for exportation. The objectives of this research were to evaluate the potential development of deltamethrin resistance in a Brazilian population of *C. capitata*; evaluate a possible metabolic resistance using a synergist, and compare the susceptibility to deltamethrin in populations of *C. capitata* from different regions of Brazil. The bioassays were performed with adult females via insecticide ingestion. Selection for resistance and susceptibility to deltamethrin with a population of *C. capitata* [from Campinas, State of São Paulo (SP)], under laboratory conditions, led to a resistance ratio (LC_{50} R / LC_{50} S) of 7.23. Deltamethrin susceptibility was evaluated in populations of *C. capitata* from: Campinas and Pedra Branca, State of São Paulo; Pelotas, State of Rio Grande do Sul; and Petrolina, State of Pernambuco. Differences in the susceptibility to the pyrethroid insecticide were observed among the populations. The population from Pelotas was 26 times more resistant to deltamethrin than the population from Petrolina. Studies with the synergist DEF (SSS-tributylphosphorotriothioate) indicated the involvement of esterases in the resistance of *C. capitata* to deltamethrin.

Keywords: Mediterranean fruit fly; pyrethroid insecticide; chemical control; esterases

1. Introduction

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is considered one of the world's most serious pests of fruit crops due to its diversity of host plants, the nature of the damage and its great adaptability to different climate conditions (Metcalf, 1995). In Brazil, where its presence has been recorded since the beginning of the twentieth century (Von Ihering, 1901), it is considered one of the most important quarantine pests, preferentially infesting introduced fruit species (Malavasi et al., 1980). Host fruits of 59 species, belonging to 21 botanical families, have been recorded as hosts for *C. capitata* in the country (Zucchi, 2000).

Control of fruit flies (*C. capitata* and *Anastrepha* spp.) has been carried out mainly with organophosphorus and pyrethroid insecticides, applied as cover spray or toxic baits for citrus and other crops, for more than 50 years (Raga and Sato, 2016). Chemical control with sequential use of insecticides, with the same mechanism of action, favors the selection of resistant populations of insects. In the case of Tephritid flies, their biological characteristics such as ease of dispersion, high population mobility and large numbers of alternative hosts are considered to be important factors in minimizing or delaying the evolution of insecticide resistance under field conditions (Georghiou and Taylor, 1976). However, recent reports indicated the development of insecticide resistance in fruit flies [*Bactrocera oleae* (Rossi), *Bactrocera dorsalis* (Hendel), *Bactrocera cucurbitae* (Coquillett), *C. capitata*] in different parts of the world, leading to a serious problem for the control of these pests in the field (Vontas et al., 2011).

The occurrence of insecticide resistant populations of fruit flies (Tephritidae) were recorded in Greece (*B. oleae*), United States (California: *B. oleae*, Hawaii: *B. dorsalis*), Taiwan (*B. dorsalis*, *B. cucurbitae*), Pakistan [*Bactrocera zonata* (Saunders)], China (*B. dorsalis*), and Spain (*C. capitata*), with varying levels of resistance to pyrethroid, organophosphate and spinosyn insecticides (Hsu and Feng, 2006; Magaña et al., 2007, 2008; Margaritopoulos et al., 2008; Ahmad et al., 2010; Vontas et al., 2011; Jin et al., 2011).

For pyrethroids, resistance may be associated with reduced affinity between the insecticides and their respective sodium gated sodium channel (VGSC) sites, caused by a single mutation or multiple amino acid substitutions (Soderlund and Knipple, 2003; Davies et al., 2008). Target site resistance was investigated in pyrethroid resistant populations of *B. oleae* from Greece; however, analysis of the VGSC in resistant insects failed to identify any resistant mutations (Margaritopoulos et al., 2008; Vontas et al., 2011). Insensitivity of the target site was associated with fenvalerate resistance in *B. dorsalis* (Vontas et al., 2011).

Some enzymes, such as esterases, carboxylesterases (COEs) (Oakeshott et al., 2005), glutathione S-transferases (GSTs) (Ranson and Hemingway, 2005) and cytochrome P450 dependent monooxygenases (Feyereisen, 2005), may also be involved in the development of insecticide resistance in fruit flies. A study on the mechanism of resistance in a laboratory selected lambda-cyhalothrin resistant strain (W-1Kλ) of *C. capitata* (205-fold resistance) showed that resistance was almost completely suppressed by the P450 inhibitor PBO; the evaluation of the expression of 53 of the 74 currently annotated P450 genes in the *C. capitata* genome indicated that CYP6A51 was overexpressed (13-18-fold) in this resistant strain. The W-1Kλ strain also presented high levels of cross-resistance to etofenprox (240-fold) and deltamethrin (150-fold) (Arouri et al., 2015).

Despite the importance of fruit flies and the frequent use of insecticides to control these pests in several Brazilian regions, there is no program for detection and monitoring of insecticide resistance in *C. capitata* or any other fruit fly species in Brazil. In this respect, several fruit growers have reported difficulties in controlling fruit flies with insecticides in recent years, but there is insufficient information on the susceptibility of these pests to the main insecticides registered in the country, in order to define adequate strategies for the management of these pests in the different Brazilian regions.

This study reports on selections for resistance and susceptibility to deltamethrin in *C. capitata* under laboratory conditions. In addition, the paper presents results on the effect of a synergist on the toxicity of the insecticide to the fruit fly. Furthermore, the paper presents information on deltamethrin susceptibility in *C. capitata* populations from different regions of Brazil, to provide basic information for the establishment of an effective management program for this pest in the country.

2. Materials and methods

2.1. Fruit fly population

The unselected population of *C. capitata* originated from medfly infested fruits [carambola (*Averrhoa carambola* L.), guava (*Psidium guajava* L.)] collected in the State of São Paulo (mainly in Campinas municipality) and maintained under laboratory conditions for more than four years; however, annual reintroductions were made with medfly adults originated from coffee berries collected from plantations without any insecticide treatments in Campinas municipality (22º54'S, 47º01'W). This population was named the Campinas (unselected) population.

After collection, the insects were reared continuously under laboratory conditions, before conducting the selection processes for resistance and susceptibility to deltamethrin. Medfly larvae were reared in artificial media (Raga et al., 1996) and the adults were kept in rearing cages made of polyethylene boxes of 20 x 20 x 20 cm (approximately 1,500 flies per cage) with voile fabric on both sides for oviposition. An artificial diet composed of sucrose, yeast extract and water, in a ratio of 4:1:0.1 were provided to the adults. The water was supplied through moistened polyurethane foam, placed in a Petri dish, inside the breeding cage.

2.2. Toxicity tests

Fifteen adult females (9-10 day-old) of *C. capitata* were placed inside a rearing cage (18 x 11 x 9 cm) made of polyethylene boxes, prepared as described above. The adult flies were fed on the same artificial diet (sucrose, yeast extract and water) used for rearing and maintained at 25 ± 2ºC, relative humidity of 70 ± 10% and photoperiod of 14 hours. The insecticide deltamethrin (Decis® 2.5% EC, Bayer) was diluted in distilled water at different concentrations (100.0; 50.0; 25.0; 12.5; 6.25; 3.12; 1.56; 0.781 mg a.i./L), placed in polyurethane foam (0.5 cm thick) in a Petri dish and offered to adult females of *C. capitata* inside the rearing cage. Mortality assessments were conducted at 24, 48 and 72 hours after the beginning of the exposure to the insecticide. The flies that fell on the bottom of the containers (irreversible knockdown), without any movement in the appendages, were considered dead (Raga and Sato, 2011). Tests in which control mortality was equal to or higher than 10% were not considered in this study. Each experiment was replicated at least four times. Mortality data were subjected to Probit analysis (Finney, 1971), using Polo Plus program (LeOra Software, 2003), after adjusting for control mortality using Abbott's formula (Abbott, 1925).

The bioassay method was based on the procedure described by Ferrer (2012), with the exception of offering the insecticide in the drinking water instead of mixing it with the adult diet. This procedure was adopted to reduce the influence of the diet on the mortality of adult flies, considering the difficulty to standardize the adult diet (e.g., sources of yeast extract), resulting in a non-uniform attraction of the fruit flies to the toxic bait in each bioassay. The toxicity tests for the Campinas unselected population of *C. capitata* were carried out in November 2016.

2.2. Selection for resistance

For the selection bioassay, the insecticide was diluted in water and offered in polyurethane foam in a Petri dish for adult females of *C. capitata*. The concentration of 12.5 mg of active ingredient (a.i.) of deltamethrin per liter was used for selection, which led to mortalities between 50 and 70% of adults. This concentration of the pyrethroid corresponds to the recommended concentration used for the control of *C. capitata* in several crops in Brazil (AGROFIT, 2018). The surviving individuals were maintained in the cages for oviposition. The eggs, used for the formation of the new generation, were collected only after the death of the susceptible adults, 72 hours after the beginning of adult exposure to the insecticide. For each selection, at least 2,000 adults of *C. capitata* were used.

2.3. Selection for susceptibility

Selection for susceptibility was performed to eliminate the resistant insects from the population. Lots of five mated females of *C. capitata* were isolated in rearing cages of 18 x 11 x 9 cm for oviposition. Females were kept in these cages for 48 hours after initial oviposition. All eggs were collected in plastic trays with water. After this period, females of *C. capitata* were transferred to new rearing cages of 18 x 11 x 9 cm, containing water and diet for adults. The insecticide was offered in mixture with water.

Selection for susceptibility was made using the concentration of 12.5 mg a.i./L. The eggs, corresponding to the cages in which the females of *C. capitata* died within 72 hours after the beginning of the treatment, were used to form the new generation. After selection for susceptibility and resistance to deltamethrin, the susceptible [S1 (once selected) and S2 (twice selected)] and resistant strains [R1 (once selected) and R2 (twice selected)] of *C. capitata* were obtained.

Mortality data for *C. capitata* strains, selected for deltamethrin resistance and susceptibility, were submitted to Probit analysis (LeOra Software, 2003). The percentages of survival of selected strains for resistance and susceptibility to deltamethrin at the recommended concentration (12 mg a.i./L) of the pyrethroid were compared using the chi-squared (X^2) test at 5% significance.

2.5. Tests with synergist

The effect of the synergist DEF (SSS-tributylphosphorotriothioate), which is an esterase inhibitor (Oakeshott et al., 2005), was evaluated on the deltamethrin resistant and susceptible strains of *C. capitata*. The experiment was carried out using the method proposed by Ferrer (2012). Fruit flies were placed in a freezer at -3ºC for five minutes, to reduce the movement of adult females and facilitate the application of the synergist using an automatic microapplicator (Burkard Manufacturing Co., UK). A volume of 0.5 µl of the synergist (1.0 µg of DEF diluted in acetone) was applied on the dorsal surface of the thorax of each fly (Ferrer, 2012).

The synergist was applied on the adult females of S1 and R1 strains of *C. capitata*, two hours before the treatment with deltamethrin, to allow the synergist to act in the flies before the exposition of the insects to the pyrethroid. Five to six concentrations (25.0; 12.5; 6.25; 3.12; 1.56; 0.781 mg a.i./L) of deltamethrin (offered to the adult females in the drinking water) were used to estimate the LC_{50} of the insecticide. The observed mortalities, for the S1 and R1 strains of medfly, previously exposed and not exposed to DEF, were corrected according to the formula proposed by Abbott (1925) and subjected to Probit analysis (LeOra Software, 2003).

2.6. Monitoring of deltamethrin susceptibility

The deltamethrin susceptibility was evaluated in seven populations/strains of *C. capitata* from different Brazilian regions:

1) Campinas (unselected) population (described above);

2) Campinas susceptible strain (S2) (described above);

3) Campinas population II: originated from pupae (more than 300) of Campinas unselected population, collected in February 2018, after a period of 16 months (without the exposition of the insects to any pesticide) from the first toxicity tests with deltamethrin, carried out for Campinas population in November 2016. During this time interval, a new introduction of adult medflies from coffee berries was done in July 2017. These coffee fruits, infested with *C. capitata*, were collected from a plantation free of any insecticide treatment, in Campinas municipality.

4) Pedra Branca population: originated from medfly infested carambola (*Averrhoa carambola* L.) fruits, collected from a commercial orchard in the rural district of Pedra Branca (22º59'S, 47º04'W), located in the southern region of Campinas municipality, State of São Paulo, in January 2018. During the year before fruit collection, this carambola orchard was sprayed with phosmet (four times), dimethoate (once) and malathion (once) for fruit fly control. No pyrethroid insecticide was applied in this period (12 months); however, insecticides of this chemical group (e.g., lambda-cyhalothrin, deltamethrin) were previously used for control of insect pests in this orchard.

5) Pedra Branca population II: originated from carambola fruits collected from the same orchard in Pedra Branca District (described above) in May 2018. The orchard was sprayed with the insecticide phosmet on February 03, 2018. No other insecticide was applied in the orchard from January to May 2018.

6) Pelotas population: originated from medfly infested fruits [peach (*Prunus persica* (L.) Batsch), Japanese persimmon (*Diospyros kaki* L.) and cattley guava (*Psidium cattleianum* Sabine)] collected in Pelotas municipality (31°46'S, 52°21'W), State of Rio Grande do Sul, in 2009. After collection, the population was maintained in laboratory conditions, with periodical (annual) introductions of fruit flies from the field (mainly from peach orchards). These orchards

received frequent treatments (average of five treatments per year, in the last 10 years) with insecticides (deltamethrin, phosmet and malathion).

7) Petrolina population: originated from medfly infested mango fruits, collected from a commercial orchard with few insecticide treatments in Petrolina municipality (9°6'S, 40°17'W), State of Pernambuco, in 2016. No pyrethroid insecticide was applied in this orchard during the last two years.

After collection, insects of all populations were maintained under laboratory conditions, free of any insecticide treatment. The toxicity tests were carried out in 2016 and 2018, following the same method described above. In the case of field populations, the toxicity tests were carried out using adult females of the second generation, after the establishment of the population under laboratory conditions. Five to six deltamethrin concentrations were used to determine the concentration-mortality curves and estimate LC_{50} values for each population/strain. The data were analyzed by Probit analysis (Polo Plus, LeOra Software, 2003), after correcting the mortality data using the formula of Abbott (1925).

3. Results

3.1. Selection for resistance and susceptibility to deltamethrin and tests with synergist

The selections for resistance and susceptibility to deltamethrin in Campinas population of *C. capitata*, using the concentration of 12.5 mg a.i./L of the insecticide, were effective in altering the susceptibility of medfly to the pyrethroid. In the case of the selection for resistance to deltamethrin, a variation of 3.8-fold in the LC_{50} was observed, increasing from 6.23 to 23.4 mg a.i./L, after two cycles of selection under laboratory conditions. In the selection for susceptibility to the pyrethroid, a significant reduction in the LC_{50} was also observed, decreasing from 6.23 to 3.24 mg a.i./L. Regarding the selection for resistance and susceptibility to deltamethrin in *C.*

capitata, the insecticide resistance ratio reached 7.23 (Table 1). Relatively low chi-square values ($X^2 \leq 3.39$; d.f. ≥ 2; $P \geq 0.18$) indicated that all equations (linear regression) presented in Table 1 fit the Probit model.

Table 1. Selection for resistance and susceptibility to deltamethrin in a population of *Ceratitis capitata* from Campinas municipality, State of São Paulo, and effect of the synergist DEF on the selected populations: estimation of LC_{50} (mg a.i./L), slope, resistance ratios and synergism ratio.

Strain/ population	Concentration for selection (mg a.i./L)	n^a	LC_{50} (95% CI)	Slope ± SE	X^2	P	df	RR[b]	SR[c]
Campinas R2	12.5	182	23.42 (16.43 – 30.63)	1.90 ± 0.35	0.009	0.99	2	7.23 (3.89 – 11.97)	-
Campinas R1	12.5	245	17.09 (12.04 – 25.09)	1.47 ± 0.25	1.11	0.57	2	5.27 (2.98 – 8.73)	8.06 (3.10 – 70.25)
Campinas R1 + DEF	-	329	2.12 (0.26 – 4.66)	1.45 ± 0.39	1.50	0.68	3	0.65 (0.08 – 1.82)	1
Campinas (unselected population)	-	396	6.23 (5.87 – 7.92)	2.00 ± 0.20	2.67	0.44	3	1.92 (1.22 – 3.24)	-
Campinas S1	12.5	189	3.80 (2.69 – 4.95)	2.49 ± 0.82	3.39	0.18	2	1.17 (0.86 – 1.72)	-
Campinas S1 + DEF	-	179	2.52 (0.71 – 4.12)	1.39 ± 0.47	0.38	0.82	2	0.78 (0.20 – 1.63)	-
Campinas S2	12.5	157	3.24 (2.01 – 4.59)	1.78 ± 0.43	0.16	0.92	2	-	-

[a] Total number of insects used

[b] Resistance ratio (LC_{50} of resistant strain divided by LC_{50} of susceptible strain) and 95 % CL (Robertson et al.,

2007)

[c] Synergism ratio (LC_{50} without synergist divided by LC_{50} with synergist) and 95 % CL (Robertson et al. 2007)

The Campinas unselected population of *C. capitata* was already slightly resistant to deltamethrin, before starting the selection process. The initial LC_{50} (6.23 mg a.i./L) was 1.92 times (95% CI: 1.29 – 2.75) higher than the LC_{50} (3.24 mg a.i./L) observed for the susceptible strain, after the second selection for susceptibility.

With regard to the survival of medfly to deltamethrin at its recommended concentration (12.5 mg a.i./L), an increase was observed (X^2 = 20.189, P< 0.0001) in the percentage of survival from 46.6% (before the selection) to more than 76% after selection for resistance to the pyrethroid insecticide. In the case of the selection for susceptibility, the survival of the flies to the recommended concentration was reduced (X^2 = 58.294, P< 0.0001) from 46.6% to zero (Fig. 1).

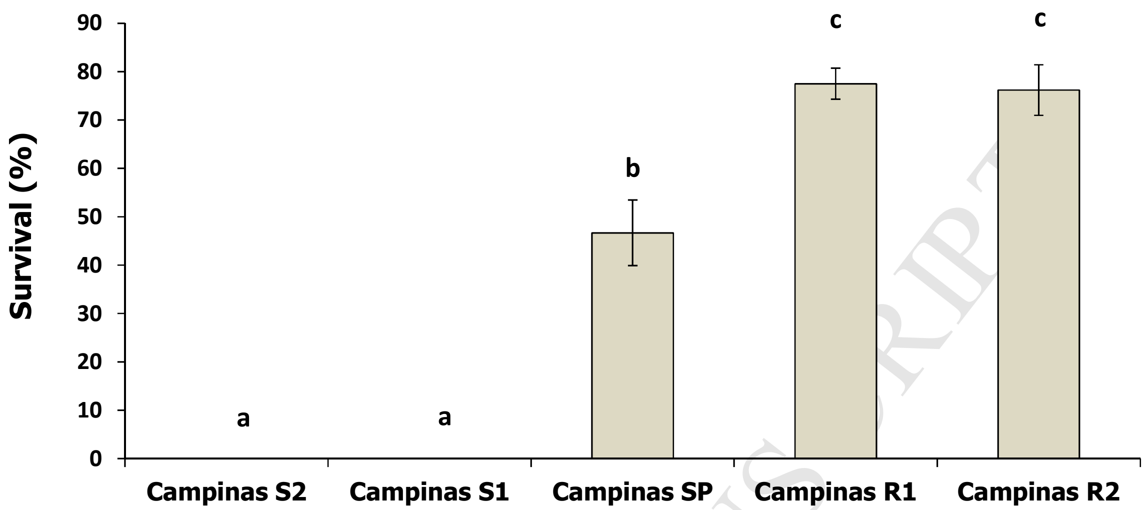

Fig. 1. Survival (mean ± SE) of adult females of *Ceratitis capitata* after treatment with deltamethrin at its recommended concentration (12.5 mg a.i./L), in an unselected population (Campinas) and in selected strains for resistance (Campinas R1, Campinas R2) and susceptibility (Campinas S1, Campinas S2) to the insecticide. Columns with the same letter do not differ from each other using chi-square (X^2) tests at 5% significance.

In the tests with the synergist DEF, which is an esterase inhibitor, an eight-fold increase in the toxicity of deltamethrin was observed with the resistant strain (Campinas R1) of *C. capitata*; however, no significant synergistic effect was observed when tested with the susceptible strain (Campinas S1) of the Mediterranean fruit fly (Table 1). The LC_{50} of deltamethrin for the Campinas R1 strain was similar to that observed for the Campinas S1 strain when the flies of both strains were previously treated with the synergist DEF. The results indicate the involvement of esterases in the resistance of *C. capitata* to deltamethrin.

Considering the toxicity tests with deltamethrin in different populations of *C. capitata*, significant contrasts in the susceptibility to the pyrethroid were observed among populations.

The least susceptible population was from Pelotas, State of Rio Grande do Sul, which was 17.7 times more resistant than the Campinas S2 strain of *C. capitata*. The greatest contrast among populations was observed for the populations from Pelotas and Petrolina, with a 26.8-fold difference in LC_{50} values (Table 2).

The most susceptible population was from Petrolina, State of Pernambuco, with an LC_{50}value (2.14 mg a.i./L) similar to that estimated for the Campinas S2 strain selected for the susceptibility to deltamethrin. The LC_{50} values estimated for Campinas population II and Pedra Branca population II were also similar to that of Campinas S2 strain, but slightly higher than that of Petrolina population (based on the criterion of non-overlapping 95% confidence intervals of LC_{50}values). Relatively low chi-square values ($X^2 \leq 4.53$; d.f. ≥ 2; $P \geq 0.21$) were estimated for all concentration-mortality curves for the populations of *C. capitata* collected in different Brazilian regions, indicating that all the equations presented in Table 2 fit the Probit model.

Table 2. Toxicity tests on adult females of *Ceratitis capitata* populations/strains from different Brazilian regions: estimation of LC_{50} (mg a.i./L; 95% confidence interval), slope and resistance ratios.

Population/ Strain	n^a	LC_{50} (95% CI)	Slope ± SE	X^2	P	d.f.	RR[b]
Pelotas	431	57.41 (33.50 – 98.75)	1.15 ± 0.30	3.71	0.29	3	17.72 (8.76 – 39.30)
Petrolina	382	2.14 (1.58 – 2.74)	1.36 ± 0.18	0.98	0.81	3	0.66 (0.41 – 1.26)
Campinas	396	6.23 (5.87 – 7.92)	2.00 ± 0.20	2.67	0.44	3	1.92 (1.22 – 3.24)
Campinas II	360	4.62 (3.89 – 5.01)	1.84 ± 0.21	4.53	0.21	3	1.42 (0.95 – 1.98)
Pedra Branca	360	7.96 (6.59– 9.34)	2.20 ± 0.25	0.99	0.80	3	2.45 (1.72 – 3.71)
Pedra Branca II	360	4.98 (3.57 – 6.36)	1.67 ± 0.22	0.34	0.95	3	1.54 (0.89 – 2.76)
Campinas S2	157	3.24 (2.01 – 4.59)	1.78 ± 0.43	0.16	0.92	2	-

[a] Total number of insects used

[b] Resistance ratio (LC_{50} of resistant strain divided by LC_{50} of susceptible strain) and 95 % CL (Robertson et al., 2007)

Discussion

The lowest susceptibility to deltamethrin was observed in a *C. capitata* population from the municipality of Pelotas, an important peach-growing area, with intensive use of insecticides (e.g., pyrethroids, organophosphates, neonicotinoids) for controlling fruit flies and other insect pests. The LC_{50} value of deltamethrin (57.4 mg a.i./L), estimated for the population from Pelotas, was 4.59 times higher than the recommended concentration (12.5 mg a.i./L) of the insecticide for the control of *C. capitata* in several crops in Brazil (AGROFIT, 2018). According to May-De Mio et al. (2014), up to 18 insecticide applications are made per year in conventional peach orchards in the southern region of Brazil. Another factor that may have contributed to the evolution of deltamethrin resistance is the long history of fruit fly control using insecticides in the Pelotas municipality, where the peach has been cultivated at a large scale since 1880 (Barbosa and Pio, 2013).

In Petrolina, which is located in the northeastern region of Brazil, fruit production (including mango) has increased only in recent years, particularly since 1992 (Correia et al., 2001). The shorter period of fruit production and, consequently, lower exposure of fruit flies to chemical treatments used for insect pest control, may have contributed to the susceptibility of *C. capitata* populations to the pyrethroid insecticide. Another factor is the integrated production system, adopted by many mango growers in this region, which favors fruit production with low usage of insecticides (especially pyrethroids) (Lacerda and Lacerda, 2004). These factors may explain the high contrast in deltamethrin susceptibility between the medfly populations from Pelotas-RS and Petrolina-PE.

The scientific literature describes a few cases of resistance to pyrethroid insecticides in fruit flies of the family Tephritidae (Ferrer, 2012). For *B. dorsalis*, two cases of lambda-cyhalothrin resistance have been reported in populations (selected for insecticide resistance under laboratory conditions) from Taiwan (Hsu and Feng, 2002; Hsu et al., 2004b). In the case of field populations, different levels (resistance ratios from 2.3 to 54.7) of resistance to the pyrethroid alpha-cypermethrin were detected in populations of *B. oleae* in Greece (Margaritopoulos et al., 2008). The insecticide resistance ratios observed for the populations of *B. oleae* in Greece were similar to, or a little higher than, those observed for the Brazilian populations of *C. capitata*. In both species, the pyrethroid resistance was associated with increased metabolic activities (P450 monoxigenases, esterases) in the resistant populations.

The LC_{50} of deltamethrin, estimated for the Campinas S2 strain, did not differ significantly from the LC_{50} observed for the Petrolina population; however, the LC_{50} for the Campinas R2 strain (23.4 mg a.i./L) was lower than that observed for the Pelotas population (57.4 mg a.i./L). In this aspect, the relatively low (7.23-fold) resistance ratio of *C. capitata* to deltamethrin after the selection process may be associated with the small number of selections for resistance and susceptibility to the pyrethroid. Couso-Ferrer et al. (2011) obtained a strain (1Klamda) of *C. capitata* 35 times more resistant to lambda-cyhalothrin than a susceptible strain of reference. The pyrethroid-resistant strain was obtained by selection for resistance to the insecticide, under laboratory conditions, from a malathion-resistant strain (W-4Km) of the fruit fly (Couso-Ferrer et al., 2011).

Studies with the synergist DEF suggested the involvement of esterases in the resistance of *C. capitata* to lambda-cyhalothrin in a pyrethroid-resistant strain selected under laboratory conditions in Spain (Arouri et al., 2010; Vontas et al., 2011), corroborating results of the present study that indicated the possible involvement of esterases in deltamethrin resistance in the Campinas R1 strain of the Mediterranean fruit fly. In the case of *B. dorsalis*, Hsu et al. (2004a) reported the involvement of monooxygenases in the resistance to pyrethroids in some populations of the fruit fly in Taiwan. Increased monooxygenase activity was also associated with alpha-cypermethrin resistance in *B. oleae* field populations in Greece (Margaritopoulos et al., 2008).

The relatively low susceptibility of the Campinas unselected population of *C. capitata* to deltamethrin, despite the annual introduction of field insects from coffee plantations free of insecticide treatment, may be associated with a migration of resistant medflies (in low proportion) from different crops [e.g., citrus (*Citrus sinensis* [L.] Osbeck), guava] (Bateman, 1972; Raga et al., 2004) cultivated around the coffee plantation and eventually sprayed with pyrethroids.

A trend toward increased susceptibility to deltamethrin was observed in the Campinas population II (LC_{50} = 4.62 mg a.i./L) in comparison with the Campinas unselected population (LC_{50}= 6.23 mg a.i./L) after a period of 16 months without fruit flies being exposed to insecticides. The same trend was observed for the Pedra Branca population II (LC_{50} = 4.98 mg a.i./L), which was collected five months after the collection of the Pedra Branca population (LC_{50} = 7.96 mg a.i./L). During this period, no pyrethroid insecticide was applied in the carambola orchard. A possible explanation for this trend for the reversal of deltamethrin resistance is the fitness cost associated with the metabolic resistance (involvement of esterases), as noted for the Campinas R1 strain of *C. capitata* in the present study. In this aspect, an increased production of detoxifying enzymes (e.g., esterases) may affect some aspects of the fitness (e.g., fecundity, fertility) of the pest insect (Raymond et al., 2001; Queiroz and Sato, 2016), leading to a decrease in the percentage of resistant insects in the population (Roush and McKenzie, 1987).

The magnitude of deltamethrin resistance was relatively low (RR = 2.45), with a tendency of increased susceptibility to the insecticide, in the Pedra Branca population of *C. capitata* in spite of the frequent applications of organophosphate insecticides (phosmet, dimethoate, malathion) in the carambola orchard. In this case, the mechanisms of deltamethrin resistance (Vontas et al., 2011) in this population of *C. capitata* seem to be different from those associated with organophosphate resistance in this fruit fly (Magaña et al., 2008; Couso-Ferrer et al., 2011). Metabolic resistance mediated by esterases and P450 has been associated with cross-resistance between organophosphates (e.g., malathion) and pyrethroids in different insect species (Chen and Sun, 1994, Heidari et al., 2005, Arouri et al., 2015); however, the importance of insecticide cross-resistance for the development of deltamethrin resistance is still unknown for Brazilian populations of *C. capitata*. Further studies with a higher number of field populations of *C. capitata* are necessary to understand the actual importance of deltamethrin resistance in the Mediterranean fruit fly in Brazil.

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