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ORIGINAL PAPER

First detection of resistance to deltamethrin in Spanish populations of the Mediterranean fruit fly, *Ceratitis capitata*

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Abstract

The control of the Mediterranean fruit fly (Medfly), *Ceratitis capitata*, in citrus orchards in Spain is mainly based in three insecticides (spinosad, lambda-cyhalothrin and deltamethrin) and the liberation of sterile males. However, Medfly control is compromised by the development of lambda-cyhalothrin resistance and the detection of spinosad-resistant alleles in field populations. We report here, for the first time, resistance to deltamethrin in populations collected in fields under different management strategies, including MagnetMed™ traps coated with this insecticide and/or spinosad and lambda-cyhalothrin used as bait sprays, and even in populations obtained from non-treated fields. Two deltamethrin-resistant strains (BP-delta and Rfg-delta) were generated from the descendants of some of the field populations that showed lower susceptibility to deltamethrin. Both strains showed low susceptibility to MagnetMed™ traps, moderate susceptibility to Ceratipack traps, and lacked cross-resistance to spinosad and lambda cyhalothrin. Our data suggest that deltamethrin resistance was mediated by P450 enzymes, since bioassays with synergists showed that PBO reverted resistance in a field population and the laboratory strains, whereas the effect of DEF and DEM was minor and no mutations were found in the VGSC gene. The inheritance of resistance for both strains was completely recessive, autosomic and did not fit the mortality expected for a recessive character under a monogenic or digenic model. We also found that deltamethrin resistance presented a fitness cost in terms of males' weight, males' and females' longevity and lifetime fecundity, with a more pronounced effect in the BP-strain than in the Rfg-delta strain. Our results highlight the need to implement insecticide resistance management strategies to prevent control failures.

Keywords Medfly · MagnetMed™ traps · Cross-resistance · Synergists · Inheritance · Fitness cost

Introduction

Resistance to insecticides is recognized as a major problem for the control of tephritid flies of economic importance (Vontas et al. 2011). This is the case of the Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Wiedemann), a pest of special relevance for citrus production in the Spanish Mediterranean region. Resistance to malathion was first reported in field populations collected from different geographical areas in 2004–2005 (Magaña et al. 2007). Since 2009, malathion was restricted in the European Union and other insecticides gained importance in Medfly control, such as spinosad and the pyrethroid lambda-cyhalothrin used as bait sprays. However, field resistance to lambda-cyhalothrin was shortly detected (Arouri et al. 2015) and resistance rates have remained stable during the last decade (Guillem-Amat et al. 2022). Field resistance to spinosad has not been reported yet (Ureña et al. 2019), but resistant alleles to this insecticide have been already detected at low frequency in field populations (Guillem-Amat et al. 2020a). Simulation experiments and modeling studies suggest that treatment strategies based on rotations (which involve temporal cycles) or mosaics (spatial patterns of applications) of lambda-cyhalothrin and spinosad are essential to maintain the efficacy of both insecticides for Medfly control (Guillem-Amat et al. 2020a, b, c, 2022). However, further proactive insecticide resistance

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management (IRM) strategies need to be implemented to preserve their utility (Sparks and Nauen 2015). This includes the harmonization of current insecticide treatments with other available insecticides and control methods.

The use of bait stations designed for mass-trapping or lure-and-kill applications represents an alternative strategy for Medfly control (Navarro-Llopis et al. 2013, 2015). These devices attract as many flies as possible by combining visual (as the color of the trap) and olfactory (as food and/or sexual) attractants, and are impregnated with insecticides that kill the flies once they come into contact with them. Bait stations impregnated with the pyrethroid deltamethrin were incorporated for Medfly control in small areas at the Generalitat Valenciana (Spain) in 2012. Since then, the use of different bait stations with this insecticide, especially MagnetMED™, increased to cover a surface of 10.600 ha in 2016 (Generalitat Valenciana 2016) and 12.500 ha in 2018 (communicated by Vicente Dalmau, Servicio de Sanidad Vegetal, Generalitat Valenciana). Since 2019, Ceratipack traps are also distributed to farmers by the "Conselleria de Agricultura" of Generalitat Valenciana, reaching a surface of 12.400 ha in 2021. This technique (recommended 50 traps/ha) provides a feasible management option when used on low-density pest populations and isolated orchards, but in many cases it is also necessary to reinforce the control measures using lambda-cyhalothrin and/or spinosad spray treatments. At present, etofenprox, phosmet and azadirachtin are also registered for Medfly control in citrus crops in Spain (MAPA 2022), although their use is very limited. Other strategies such as the sterile insect technique (SIT) are implemented in some areas, but Medfly outbreaks in the fall require the use of insecticides for a satisfactory management (Juan-Blasco et al. 2014).

The deployment of bait stations coated with deltamethrin is expected to increase in the future, providing an additional tool for IRM programs. Modeling studies indicates that the best option is to use it in combination with insecticides with different modes of action and no cross-resistance (Guillem-Amat et al. 2022). However, since lambda-cyhalothrin and deltamethrin are both pyrethroids, there is the possibility that cross-resistance occurs between them. Indeed, Arouri et al. (2015) reported that the lambda-cyhalothrin resistant strain W-1Kλ, derived from a malathion-resistant field population collected in Castelló (Spain), showed cross-resistance (more than 100-fold) to deltamethrin. However, it is not known: (i) to which extent field populations are susceptible to deltamethrin; (ii) if those field populations that showed resistance to lambda-cyhalothrin are also resistant to deltamethrin; and (iii) if there is cross-resistance between both insecticides, conferred by a common mechanism, or if different mechanisms are responsible for the resistance to each insecticide. Since current control practices include the use of lambda-cyhalothrin bait sprays in orchards where lure-and-kill traps with deltamethrin are also deployed, the answer to these questions is critical for the correct implementation of IRM strategies.

The goal of this work was to contribute to the sustainability of Medfly control programs by assessing the susceptibility of Spanish field populations to deltamethrin, analyzing some of the factors (cross-resistance, inheritance, mechanisms and fitness cost) underlying deltamethrin resistance in field-derived selected strains, and discussing their implications for resistance management.

Materials and methods

Field populations

Field populations were obtained by collecting infested fruits from fruit orchards (citrus, cherimoya and loquat), that had received different insecticide treatments, at different localities in Spain during the period 2017–2019 (Online Resource). The infested fruits were placed in plastic trays (15 × 21 × 28 cm) inside ventilated containers, both with several layers of filter paper, and kept in an environmentally controlled rearing room, at a photoperiod of 16 h light and 8 h dark, and a temperature of 26 ± 3 ºC, until pupation occurred (rearing room standard conditions). Every 2–3 days, pupae were harvested and maintained in ventilated boxes (12 cm in diameter and 5 cm in height) in an environmentally controlled climatic chamber (Sanyo MLR-350-H, Sanyo, Japan), at 25 ± 1 °C and 16 h light and 8 h dark photoperiod (climatic chamber standard conditions). Emerged adults from field-collected fruits (F0) were provided with water and adult rearing diet (4:1 sugar/yeast) and kept at climatic chamber standard conditions until used for susceptibility bioassays.

Laboratory strains

The laboratory susceptible strain (C) was established from wild *C. capitata* collected from non-treated experimental fields at the Instituto Valenciano de Investigaciones Agrarias (València, Spain) in 2001 and reared in the laboratory without any exposure to insecticides (Magaña et al. 2007). The malathion-resistant strain (W-4 km) derives from a field population collected in 2004 in Castelló (Spain) (Magaña et al. 2007) and was selected under laboratory conditions by exposing adults to increasing concentrations of malathion by ingestion (Couso-Ferrer et al. 2011). The lambda-cyhalothrin resistant strain (W-1Kλ) was generated from the W-4 km strain, by laboratory selection with lambda-cyhalothrin (Arouri et al. 2015). The spinosad-resistant strain JW-100 s was generated by laboratory selection from field individuals collected from Xàbia (Spain) in 2007 (Ureña et al. 2019).

Two deltamethrin-resistant strains were obtained by laboratory selection of individuals from field populations (about 200–300 adults from each population), collected in: Benaguasil and Picassent in 2018 (BP-delta); and in Rafelguaraf-N1 in 2019 (Rfg-delta) (Online Resource 1). Males of BP-delta and Rfg-delta were crossed with females of the control C strain at F3 and F1, respectively, to obtain descendent females able to lay eggs through the net of rearing cages. Every generation, 5 groups of about 100 adults (about 50% of each sex) of 3–7 days old were selected with increasing concentrations of Decis Protech (1.5% deltamethrin p/v). The insecticide was diluted in absolute ethanol, applied with a pipette on the inside surfaces of the upper and bottom lids (2 ml in each surface) of Petri dishes (15 cm in diameter and 2 cm in height), and kept in a fume hood for 30 min to allow the solvent to evaporate completely. The concentration of insecticide was adjusted every generation to cause approximately 50% lethality, calculated previously through concentration-mortality bioassays (see below). Six small holes were drilled in the upper lid of the treated Petri dishes for ventilation. The flies were kept in the refrigerator (5 ± 0.5 ºC) for about 30 min and then placed inside the treated Petri dishes. The protocol was optimized from BP-delta F18 and Rfg-delta F14 by placing the insects in small plastic trays (3 cm in diameter and 0.2 cm in height), deployed inside the Petri dish, to allow the flies to recover from cold before they were exposed to the insecticide when started to walk on the treated surfaces. After 2 h, all flies were transferred to clean ventilated plastic dishes (8.9 cm in diameter, 2.3 cm in height), containing water and adult rearing diet and kept at standard conditions for 48 h. The surviving adults were then recovered and transferred to adult rearing cages ($20 \times 20 \times 20$ cm, with gauze on one side and containing water and adult rearing diet), and kept at rearing room standard conditions.

All strains were maintained in the laboratory at rearing room (adults) and climatic chamber (eggs, larvae and pupae) standard conditions, as previously described (Magaña et al. 2007), using larval (Albajes and Santiago-Álvarez 1980) and adult (see above) rearing diets.

Chemicals

The insecticides used were: Decis protech (deltamethrin 15 g liter^{-1}, EW, Bayer Cropscience S.A., Lyon, France); Karate Zeon (lambda-cyhalothrin 100 g liter^{-1} CS, SyngentaAgro S. A., Madrid, Spain); spinosad (880 g kg^{-1} technical, Dow AgroSciences LLC, Indianapolis, USA); MagnetMed™ traps (lure-and-kill device of 16×18 cm soaked with deltamethrin in their entire surface [10 mg per dispositive, 17.36 µg/cm^2] and baited with a BioLure® Unipack dispenser placed inside, Suterra Europe Biocontrol SL, Valencia, Spain); and Ceratipack traps (mass-trapping device with a diffuser inside that contains specific attractants and with the lid of the trap impregnated with deltamethrin [15 mg deltamethrin per dispositive, 92.5 µg/cm^2], SEDQ Healthy Crops S.L., Barcelona, Spain). The synergists tested were piperonyl butoxide (PBO; 90% technical, Aldrich, Milwaukee, WI), S,S,S-tributyl phosphorotrithioate (DEF; 97.2% technical, Chem Service, West Chester, PA) and diethyl maleate (DEM, 97% technical, Aldrich).

Susceptibility bioassays

At least otherwise stated: i) the assays were performed with young adult flies (3–5 days old); ii) the range of concentrations tested (4 to 7) was adjusted for each population/strain to obtain mortalities in the range 5–95%; iii) mortality was recorded after 48 h; and iv) flies were considered dead if they were ataxic (remained on their backs, unable to walk, with no further sign of movement).

MagnetMed™ and Ceratipack trap assays

The assays were performed in ventilated aluminum boxes ($50 \times 65 \times 60$ cm) installed in a greenhouse under controlled temperature 25 ± 2 ºC and the natural photoperiod of October–November (approximately, 11:13 h light:dark) in Madrid (Spain). Each cage contained a trap hanging from the upper side of the cage, and water and food were provided ad libitum. Three replicates and one control (without the trap), containing 20–30 flies each, were performed for each population. Mortality was recorded after 48 h of exposure for MagnetMed™ and 7 days of exposure for Ceratipack, based on the time needed in each trap to reach 90–100% mortality with adults of the control C strain.

Contact assay

The assay was performed with Decis Protech (1.5% deltamethrin p/v). The insecticide was diluted in absolute ethanol and applied to Petri dishes (15 cm in diameter and 2 cm in height) as described above for the selection of resistant strains. Three replicates per concentration were set up, each one consisting on 20 flies, that were kept 2 h inside the deltamethrin impregnated Petri dishes and then transferred to clean ventilated plastic dishes and kept at climatic chamber standard conditions. Flies exposed to Petri dishes treated with absolute ethanol were used as control.

Topical assay

The assay was performed with Decis Protech (1.5% deltamethrin p/v) by application of a 0.5 µl drop of insecticide solution in acetone to the dorsal thorax of adult flies. The flies were anesthetized with CO_2 and the treatment performed

with an automatic microapplicator 900X (Burkard Manufacturing Co., Hertfordshire, United Kingdom). Three replicates of 15–20 adults were performed per concentration, and acetone was used as a control. After the treatment, the flies were transferred to ventilated plastic dishes (8.9 cm in diameter, 2.3 cm in height), containing water and adult rearing diet, and kept at climatic chamber standard conditions.

Feeding assay

The assay was performed with Karate Zeon (10% lambda-cyhalothrin p/v) and technical spinosad (88% p/v). Adult flies were starved for 24 h before the exposure to insecticide. Twenty adults were then confined in ventilated plastic dishes (8.9 cm in diameter, 2.3 cm in height) and fed with water and adult rearing diet containing the appropriate concentration of insecticide (0.9 diet: 0.1 insecticide (w/w)) or the solvent alone (control). Dilutions were prepared with water in the case of lambda-cyhalothrin, and with a buffer composed of acetic acid/sodium acetate (1:3, pH 4.7) in the case of spinosad. Three replicates were performed for each concentration. Assays were conducted at climatic chamber standard conditions.

Assays with synergists

The synergists PBO, DEF and DEM were diluted in acetone and applied topically on the dorsal thorax to adult flies using an automatic microapplicator, as described above. The applied doses (0.5 µg PBO, 1 µg DEF, or 1 µg of DEM per insect) showed no mortality on adults of the tested populations and strains. Three replicates of 15–20 adults were performed, and acetone was used as a control. After 2 h, flies were treated with deltamethrin (contact bioassay) or lambda-cyhalothrin (feeding bioassay) as previously described.

Crosses for inheritance study

Pupae from BP-delta, Rfg-delta and C strains were collected, individualized and their sex determined immediately after adult emergence. To assure virginity, males and females from each strain were placed separately into ventilated plastic dishes and maintained in an environmentally controlled chamber at standard conditions for 3–5 days. Reciprocal crosses (50 ♂BP-delta × 50 ♀C, 50 ♀BP-delta × 50 ♂C, 50 ♂Rfg-delta × 50 ♀C and 50 ♀Rfg-delta × 50 ♂C) were performed to obtain the F1 generation (F1A-BP, F1B-BP, F1A-Rfg and F1B-Rfg, respectively). The F1s were pooled and kept in the absence of selection pressure to produce the F2 generations ([50 ♂ F1A-BP + 50 ♂ F1B-BP] x [50 ♀F1A-BP + ♀ 50 F1B-BP] to obtain F2-BP; and [50 ♂ F1A-Rfg + 50 ♂ F1B-Rfg] x [50 ♀F1A-Rfg + ♀ 50 F1B-Rfg] to obtain F2-Rfg). F1s were also crossed to parent strains to obtain the backcrosses ([50 ♂ F1A-BP + 50 ♂ F1B-BP] × 50 ♀C to obtain BcA-BP-C; [50 ♀F1A-BP + 50 ♀F1B-BP] × 50 ♂ BP-delta to obtain BcB-BP-BP; [50 ♂ F1A-Rfg + 50 ♂ F1B-Rfg] × 50 ♀C to obtain BcA-Rfg-C; and [50 ♀F1A-Rfg + 50 ♀F1B-Rfg] × 50 ♂ Rfg-delta to obtain BcB-Rfg-Rfg). The dominance value (D_{LC}) of resistance was calculated using Bourguet's modification (Bourguet et al. 2000) of Stone's formula (Stone 1968): $D_{LC} = \{[(2\ \log LC_{50}\ F1 - \log LC_{50}\ P1 - \log LC_{50}\ P2)/(\log LC_{50}\ P1 - \log LC_{50}\ P2)] + 1\}/2$ where P1 and P2 corresponded to parental resistant (BP-delta or Rfg-delta) and susceptible (control C) strains, respectively. Values ranged between 0 for completely recessive and 1 for completely dominant.

Assessment of life history traits

Adult longevity was assessed by placing 30 females or 30 males (2–3 days old adult flies) of each strain in ventilated plastic dishes (5 × 11 cm diameter), feeding them with water and adult rearing diet, and keeping them at climatic chamber standard conditions to measure daily survival. Thirty females and 30 males were kept in the same way for 3 days and weighed with a precision balance (AM100, Mettler-Toledo, Zurich, Switzerland).

Lifetime fecundity was analyzed placing 7–10-day-old adult flies (30 males and 30 females) of each strain in ventilated plastic boxes (20 × 20 × 20 cm) with water and adult rearing diet and keeping them at rearing room standard conditions. The boxes were kept as described before until the flies died. Eggs were collected weekly and measured volumetrically.

Embryo to pupal viability and developmental time to pupation was determined by collecting a volume of 50 µl of eggs (containing at least 500 eggs, estimated visually) laid within 24 h, which were spread on larval rearing medium (Albajes and Santiango-Álvarez 1980) (160 g approximately) in containers (130 × 90 × 25 mm) covered with an aluminum foil to avoid desiccation. Containers were placed in 2 L ventilated plastic boxes and kept at climatic chamber standard conditions. Third instar larvae that jumped from the food container and pupated were daily recorded and removed from the box.

Two experiments with at least three replicates of each strain per experiment were performed for all parameters analyzed.

Detection of mutations in the voltage-gated sodium channel (VGSC) gene

The domains II and III of the voltage-gated sodium channel (VGSC) gene (XM_020861574) were partially sequenced to cover most of the codons associated to knockdown resistance (kdr) mutations in insect species, as previously described

(Guillem-Amat et al. 2022). We analyzed 20 flies from: (i) the field populations collected in Betxi in 2017 and in Benaguasil and Picassent in 2018 that survived to the bioassays with MagnetMed™; and (ii) the deltamethrin-resistant strains BP-delta and Rfg-delta, that survived to the exposure to Decis Protech in contact bioassays. The oligonucleotides used were NaCh899_F (5'-TCGAGTTTTTAAACTTGCCAAA) and NaCh932_R(5'-TTTCCGAACAGTTGCATTCC) for region 899–932, Kdr_F (5'-TCGTTTTTCGTGTGCTATGC) and Kdr_R (5'-CCAGGCTTTAAAACGCGATA) for region 977–1058, and NaCh1528_F (5'-AAGCAACCAATCCGTGAAAC) and NacCh1575_R (5'-TCGGTCTAGGAATGGCTTTT) for region 1528–1575 (Guillem-Amat et al. 2022). The amplicons were visualized on 1% agarose (Agarosa D2, Conda Pronadisa, Madrid, Spain) gels (Tris 40 mM, EDTA 1 mM, pH 8.0), purified using the QIAquick PCR Purification Kit (QIAGEN, Germany), and sequenced by Sanger at Secugen S.L. (Madrid, Spain) facilities. The sequences were analyzed with Geneious 11.0.5 (https://www.geneious.com), and EditSeq and MegAlign (DNASTAR LASERGENE SUITEv.15.3, Madison, WI).

Statistics

Data were statistically analyzed with Levene and Shapiro–Wilk tests to check homogeneity and normality, respectively. The susceptibility of field populations and laboratory strains to MagnetMed™ and Ceratipack traps was analyzed by ANOVA followed by Dunnett post hoc test (percentage data were arcsin-sqrt transformed in both cases). Susceptibility to insecticides in contact, topical and feeding bioassays was analyzed using mortality data to estimate the concentration/dose needed to cause 50% mortality (LC_{50} or $LD_{50,}$ respectively) by Probit analysis [program POLO-PC, LeOra Software14, LeOra, Berkeley, CA, USA, which corrects samples' mortality by control mortality using Abbott's transformation (Abbott, 1925)]. Resistance ($RR = LC_{50}$ (tested population/strain)/LC_{50} (C strain)) and synergistic ($SR = LC_{50}$ (without synergist)/LC_{50} (with synergist)) ratios were considered significant if their 95% fiducial limits (FL) did not include 1 (Robertson and Preisler 1992). In the inheritance study, $\chi 2$ tests were performed to check the fit of the mortality data to different inheritance models. Life history traits were analyzed by one-way ANOVA, followed by Tukey's post hoc test. The Kaplan–Meier method was used to analyze adult survival, and their distributions were compared by the Mantel-Cox log-rank test.

Results

Susceptibility of field populations to deltamethrin

All field populations tested, except Algarrobo Costa which was obtained from non-treated experimental fields, showed significantly lower susceptibility to MagnetMed™ traps than the susceptible control C strain (Table 1). The populations from Alcalà de Xivert, Vila-real and Sagunt showed moderate susceptibility, with mortalities ranging between 58 and 66%. The rest of the populations showed low levels of susceptibility, with mortalities below 40%. This last group included populations obtained from fields where MagnetMed™ traps have been deployed, in combination with the application of insecticide (lambda-cyhalothrin or spinosad) bait formulations, for the control of *C. capitata* (Betxí, Vinaròs, Benaguasil and Picassent), but also populations from fields where only spinosad (Puçol) or lambda-cyhalothrin (Logroño) were used, and even populations from non-treated fields (Rafelguaraf) or whose regime of treatments is unknown (Antella) (Online Resource 1). When available, some of these field populations (Antella, Rafelguaraf-N1 and -N2, Sagunt and Genovés) were also tested with Decis Protech by contact application (Table 1). In all cases, field populations were more resistant than the susceptible C strain with resistance ratios ranging between 3.7- and 6.2-fold.

Selection of deltamethrin-resistant strains

Two deltamethrin-resistant strains were generated from the descendants of some of the field populations that showed lower susceptibility to deltamethrin (Benaguasil and Picassent to obtain BP-delta and Rafelguaraf-N1 to obtain Rfg-delta) (Table 2). The resistance ratio of both strains rapidly recovered the levels observed in field populations (3–six-fold), after crossing with the C strain to obtain descendent females that lay eggs through the net of rearing cages, and reached 8–12-fold after several generations of selection. BP-delta and Rfg-delta showed low susceptibility to MagnetMed™ traps, with mortalities below 40%, whereas their susceptibility to Ceratipack was moderate (reached 60–70%) (Table 3) and their resistance ratios to Decis Protech by topical application were only 2–3-fold (Table 4).

Cross-resistance to approved insecticides for Medfly control in citrus crops

Both deltamethrin-resistant strains showed resistance against lambda-cyhalothrin by ingestion, but their levels decreased or were lost during the selection process (Table 4). For BP-delta, a resistance ratio of 3.6 was obtained at generation F6, but the susceptibility at

Table 1 Susceptibility to deltamethrin (MagnetMed™ traps and Decis Protech) of field populations of *Ceratitis capitata*

Population [1]	MagnetMed™ traps % mortality ± SE (*n*) [2]	Decis Protech by contact application [3]					
		n	Slope ± S.E	LC_{50} (95% FL)	χ^2	d*f*	RR (95%FL)
Alcalà de Xivert	66 ± 9 (90) *						
Vila-real	58 ± 4 (90) *						
Betxí	36 ± 5 (78) ***						
Algarrobo Costa	71 ± 6 (90)						
Vinaròs	25 ± 4 (85) ***						
Benaguasil	18 ± 3 (60) ***						
Picassent	27 ± 14 (40) ***						
Antella	15 ± 3 (60) ***	210	3.72 ± 0.48	4.88 (4.07–5.72)	4.2*	5	3.9 (3.2–4.8) #
Rafelguaraf-N1	20 ± 9 (90) ***	480	2.80 ± 0.27	7.71 (5.69–10.56)	54.9	16	6.2 (5.1–7.5) #
Rafelguaraf-N2	38 ± 4 (90) ***	240	3.53 ± 0.54	4.81 (3.92–6.30)	9.4*	7	3.9 (3.2–4.8) #
Sagunt	63 ± 8 (90) **	380	1.59 ± 0.18	5.28 (3.38–9.75)	29.4	12	4.3 (3.1–5.9) #
Logroño	21 ± 1 (84) ***						
Puçol	38 ± 1 (90) ***						
Genovés [4]	-	214	5.75 ± 0.90	4.53 (3.64–5.49)	16.3	8	3.7 (3.1–4.3) #
Control (C)	90 ± 6 (90)	592	3.14 ± 0.23	1.24 (1.05–1.50)	68.5	26	-

[1] Sampling site, year and insecticides used against *C. capitata* are indicated in Online Resource 1

[2] Mortality after 48 h of exposure to MagnetMed™ traps (10 mg deltamethrin per dispositive, 17.36 µg/cm^2, Suterra) in a greenhouse (25 ± 2 ºC and natural photoperiod). The total number of flies tested is indicated between brackets (3 replicates of 20–30 flies each). The mortality of a non-treated replica (20–30 flies) of each population, maintained under identical conditions, was always below 5%. The asterisks indicate statistically significant differences with respect to the susceptible Control (C) strain (ANOVA followed by Dunnett post hoc test, $*P \leq 0.05$; $**P \leq 0.01$: $***P \leq 0.001$; percentage data were arcsin-sqrt transformed)

[3] Bioassays were performed by contact with Decis Protech (1.5% deltamethrin p/v). *n* = number of flies considered in the Probit analysis (including non-treated). Lethal concentration (LC_{50}) in µg of insecticide/cm^2. * Good fit of the data to the probit model ($P > 0.05$). Resistance ratio (RR) = LC_{50} (field population) / LC_{50} (C strain). The fiducial limits for RR were calculated according to Robertson and Preisler (1992). # RR is significant ($P < 0.05$) if the 95% FL does not include 1

[4] The bioassays were performed with the F1 generation from flies collected at Genovés

generation F26 was similar to that of the control C strain. In the case of Rfg-delta, the resistance ratio decreased from 9.9 at generation F11 to 4.5 at generation F21. Indeed, the resistance levels of Rafelguaraf-N1, the field population from which Rfg-delta was derived, were even higher (LC_{50} = 863 (524–1758), RR = 50 (7–350), Guillem-Amat et al. (2022)]. These results indicate that although the parental field populations were resistant to both pyrethroids, the mechanisms that mediate resistance may be different, since resistance to lambda-cyhalothrin is lost in the absence of selection pressure with this insecticide. None of the deltamethrin-resistant strains showed resistance to spinosad by ingestion (Table 4).

Remarkably, the lambda-cyhalothrin resistant strain W-1Kλ showed resistance to Decis Protech by both topical (4.8 fold) and contact (3.8 fold) application (Table 4), and MagnetMed™ traps were totally inefficient (3% mortality) against this strain (Table 3), suggesting the existence of cross-resistance, since W-1Kλ has never been exposed to deltamethrin. A significant but moderate level of resistance to MagnetMed™ traps (70% mortality) was also observed for the malathion-resistant W-4 km strain (Table 3), which was susceptible to Decis Protech by topical and contact application (Table 4). The spinosad-resistant strain JW-100 s was susceptible to both MagnetMed™ traps and Decis Protech (Tables 3 and 4).

Effect of synergists on the toxicity of pyrethroids

Topical treatment with PBO (inhibitor of cytochrome P450s) on both deltamethrin-resistant BP-delta and Rfg-delta strains and on the field population Rafelguaraf-N1 completely reverted deltamethrin resistance, with synergistic ratios (SR) of 23, 33 and 211, respectively (Table 5). Lambda-cyhalothrin resistance in the BP-delta strain was also partially (SR = 5.9) suppressed by PBO. A slight but significant reduction on LD_{50}, with synergistic ratios ranging between 1.5 and 1.8, was obtained with DEF

Table 2 Selection of resistance to deltamethrin by contact application of the insecticide to obtain the BP-delta and Rfg-delta strains

Strain Generation	SC [1]	n[2]	Slope ± SE	LC_{50}[3] (95%FL)	χ^2	d.f	RR (95%FL) [4]
BP-delta [5]							
F4	–	600	2.32 ± 0.23	3.76 (3.08–4.72)	36.6	22	3.0 (2.5–3.7) #
F5	3.25	420	1.94 ± 0.18	5.54 (4.35–7.10)	23.3*	16	4.5 (3.6–5.6) #
F6	6.5	360	3.87 ± 0.36	5.09 (4.31–5.97)	17.9*	13	4.1 (3.5–4.8) #
F15	6.5–9	549	2.52 ± 0.20	3.36 (2.38–4.40)	93.0	22	2.7 (2.3–3.2) #
F16	6.5–9	371	2.93 ± 0.32	10.5 (8.3–15.5)	60.7	18	8.5 (7.0–10.3) #
F24	30	300	6.16 ± 0.75	17.8 (16.2–19.6)	4.0*	10	8.9 (7.7–10.3) #
F26	10	270	5.34 ± 0.76	19.3 (16.9–22.1)	11.8*	10	9.6 (8.3–11.2) #
Rfg-delta [6]							
F2	3.25	360	3.08 ± 0.30	4.25 (2.90–6.42)	62.2	12	3.4 (2.8–4.2) #
F3	3.25	360	2.65 ± 0.25	3.82 (3.03–4.84)	22.1	13	3.1 (2.5–3.7) #
F6	6.5–13	1000	2.35 ± 0.14	5.49 (4.39–6.66)	56.8	15	4.4 (3.8–5.1) #
F11	6.5–9	753	3.72 ± 0.31	7.55 (6.48–8.81)	99.3	28	6.1 (5.3–6.9) #
F12	6.5–9	360	4.56 ± 0.46	5.94 (4.38–7.62)	64.2	13	4.8 (4.1–5.6) #
F13	9	420	3.57 ± 0.33	6.15 (4.84–7.49)	46.3	16	4.9 (4.2–5.8) #
F16	20	420	4.47 ± 0.44	21.0 (18.6–23.7)	23.0*	16	10.5 (9.1–12.1) #
F18	25	300	3.28 ± 0.46	24.0 (19.5–32.4)	20.3*	10	12.0 (9.9–14.6) #
F21	10	270	5.01 ± 0.61	13.9 (12.5–15.5)	9.3*	13	7.0 (6.0–8.1) #

[1] Selection concentration (SC) in µg of deltamethrin/cm^2. The absence of treatment is indicated as "-"

[2] Number of flies considered in the Probit analysis (including non-treated)

[3] Lethal concentration (LC_{50}) and fiducial limits (95% FL) in µg of deltamethrin/cm^2

[4] Resistance ratio (RR) = LC_{50} (selected strain) / LC_{50} C strain [1.24 (see Table 1) for BP-delta generations F4-16 and Rfg-delta generations F2-13; and 2.00 (see Tables 4 and 6) for BP-delta generations F24-26 and Rfg-delta generations F16-21]. The fiducial limits for RR were calculated according to Robertson and Preisler (1992). # RR is significant ($P < 0.05$) if the 95% FL does not include 1

[5] BP-delta was obtained by selecting the field populations collected in Benaguasil and Picassent in 2018 (survivors of MagnetMed™ bioassays, see Table 1) with increasing concentrations of deltamethrin. Males of BP-delta were crossed with females of the control C strain at F3

[6] Rfg-delta was obtained by selecting the field population collected in Rafelguaraf-N1 in 2019 (see Table 1) with increasing concentrations of deltamethrin. Males of Rfg-delta were crossed with females of the control C strain at F1

* Good fit of the data to the Probit model ($P > 0.05$)

(esterase inhibitor) and DEM (inhibitor of glutathione S-transferases) for deltamethrin resistance in both resistant strains (Table 5). These results suggest a major contribution for P450s, and a minor contribution of esterases and glutathione S-transferases, on the mechanisms of resistance to deltamethrin and lambda-cyhalothrin of the field and laboratory strains tested.

Mutations in the voltage-gated sodium channel (VGSC) gene

The three regions of the VGSC gene that concentrate most of the kdr mutations associated to pyrethroids resistance in insect species (Dong et al. 2014) were sequenced in flies from three field populations (Betxí, Benaguasil and Picassent) and two laboratory strains (BP-delta F-15 and Rfg-delta F11), that survived to the bioassays with MagnetMed™ (Table 1) and Decis Protech in contact bioassays (Table 2), respectively. We did not find mutations in any of the 20 individuals analyzed from each population/strain, suggesting that target-site resistance was not associated with deltamethrin resistance in the populations and strains analyzed.

Inheritance of deltamethrin resistance

Reciprocal crosses between the susceptible C strain and the deltamethrin-resistant BP-delta and Rfg-delta strains to obtain the corresponding F1s resulted in complete reversion of resistance (RR ranging between 0.5 and 1.2), indicating

Table 3 Susceptibility to MagnetMed™ and Ceratipack traps of laboratory strains of *Ceratitis capitata*

Strain	Generation	Mortality % ± SE (*n*) [(1)]	
		MagnetMed™	Ceratipack
BP-delta	F4	33 ± 3 (60) ***	
	F20		64 ± 1 (90) ***
	F27		69 ± 4 (90) ***
Rfg-delta	F6	35 ± 7 (84) ***	
	F15		88 ± 1 (90) **
	F23		69 ± 3 (90) ***
W-1Kλ		3 ± 3 (90) ***	
JW-100 s		90 ± 0 (90)	
W-4 km		70 ± 3 (90) *	
Control (C)		89 ± 4 (90)	98 ± 2 (180)

[(1)] Mortality after 48 h of exposure to MagnetMed™ traps (10 mg deltamethrin per dispositive [17.36 µg/cm^2], Suterra) or 7 days of exposure to Ceratipack traps (15 mg deltamethrin per dispositive [92.5 µg/cm^2], SEDQ Healthy Crops S.L.) in a greenhouse (25 ± 2 ºC and natural photoperiod). The total number of flies tested is indicated between brackets (3–6 replicates of 20–30 flies each). The mortality of a non-treated replica (20–30 flies) of each population, maintained under identical conditions, was always below 5% for assays with MagnetMed™ and below 17% for assays with Ceratipack. The asterisks indicate statistically significant differences with respect to the susceptible Control (C) strain (ANOVA followed by Dunnett post hoc test, *$P \leq 0.05$; **$P \leq 0.01$: ***$P \leq 0.001$; percentage data were arcsin-sqrt transformed)

that deltamethrin resistance was inherited as a completely recessive autosomal trait [$D_{LC} = 0.09$ for F1 (♂BP-delta x ♀C); $D_{LC} = 0.06$ for F1 (♀BP-delta x ♂C), $D_{LC} = -0.33$ for F1 (♂Rfg-delta x ♀C); $D_{LC} = -0.19$ for F1 (♀RfgP-delta x ♂C)] (Table 6).

Backcrosses of the F1s with the susceptible parent resulted in low levels of resistance (RR = 1.8- and 1.9-fold for BP-delta and Rfg-delta, respectively), whereas backcross with their corresponding resistant parental maintained intermediate levels of resistance (RR = 4.9- and 4.4-fold for BP-delta and Rfg-delta, respectively), and F2 crosses also resulted in low levels of resistance (RR = 2.5 and 1.9 for BP-delta and Rfg-delta, respectively) (Table 6), which is consistent with recessive resistance.

The observed mortality at F1, F2 and backcrosses when using the discriminating concentration of 6.5 µg of deltamethrin/cm^2 (90% mortality for susceptible parental C and 0% and 4% mortality for resistant parental BP-delta and Rfg-delta strains) did not fit the expected mortality for a recessive character under a monogenic or digenic inheritance model (Online resource 2). These results are inconsistent with only one or two genes under Mendelian genetics, suggesting polygenic inheritance of deltamethrin resistance in both selected strains.

Fitness cost associated to deltamethrin resistance

Different biological parameters were evaluated to determine whether deltamethrin resistance presented a fitness cost (Table 7). Our results indicate that individuals from the BP-delta strain showed a reduced fitness, compared to susceptible individuals from the C strain, in terms of males' weight (4% reduction), males' and females' longevity (1.6 and 3.5 days shorter, respectively) and lifetime fecundity (42% reduction). The individuals for the Rfg-delta strain also showed reduced lifetime fecundity (34% reduction), but the rest of the parameters tested were not significantly different from the C strain. Kaplan–Meier survival curves showed significant differences for both BP-delta and Rfg-delta males and females with respect to their corresponding controls C males and females (Fig. 1). Taken together, a more pronounced reduction in the fitness appears to be associated with deltamethrin resistance in the BP-strain than in the Rfg-delta strain.

Discussion

We have detected for the first time resistance to deltamethrin in Spanish Medfly field populations. Resistant populations included those collected in orchards where MagnetMed™ traps were deployed, but also populations from fields where other insecticides (spinosad or lambda-cyhalothrin) were applied, and even populations obtained from non-treated fields. This novel case of resistance adds to other reported cases in Spanish field populations of *C. capitata*, such as to malathion in 2004 (Magaña et al. 2007) and to lambda-cyhalothrin in 2009–2010 (Arouri et al. 2015), highlighting the potential of Medfly populations to develop resistance to different classes of insecticides. Resistance to deltamethrin has also been recently reported for Medfly Brazilian populations (Demant et al. 2019), whereas no significant levels of resistance to this insecticide has been found in field populations from Greece (Voudouris et al., 2018). Another remarkably result of this work is that resistance to deltamethrin was already widespread when first detected, as occurred with malathion (Magaña et al. 2007) and lambda-cyhalothrin (Arouri, et al. 2015), probably related to the high rates of gene flow among Spanish Medfly populations (Beroiz et al. 2012). Currently, the repertoire of effective insecticides against this pest in Spain is becoming very limited, farmers being constrained to use only one or a few effective insecticides. Thus, we are in a situation in which Medfly control may be seriously compromised if IRM strategies are not reinforced.

An important component of IRM is the use of alternative insecticides to delay the onset of resistance to a particular insecticide and avoid the combined use of insecticides

Table 4 Cross-resistance to approved insecticides for *Ceratitis capitata* control in Spanish citrus crops in laboratory strains resistant to deltamethrin (BP-delta and Rfg-delta), lambda-cyhalothrin (W-1Kλ), malathion (W-4 km) and spinosad (JW-100 s)

Insecticide [(1)]	Bioassay	Strain	Generation	$n^{(2)}$	Slope ± S.E	LC_{50} [(3)] (95%FL)	χ^2	d.f	RR (95%FL) [(4)]
Deltamethrin	Topical	Rfg-delta	F13	487	2.34 ± 0.21	2.96 (2.54–3.41)	16.6*	18	3.3 (2.8–4.0) #
		BP-delta	F18	520	2.68 ± 0.24	1.91 (1.61–2.22)	22.3*	18	2.2 (1.8–2.6) #
		W-1Kλ		300	3.37 ± 0.38	4.31 (2.06–7.63)	114.1	14	4.8 (3.8–6.2) #
		W-4 km		598	1.92 ± 0.15	1.11 (0.85–1.53)	52.9	22	1.3 (1.0–1.6)
		JW-100 s		600	1.65 ± 0.14	1.13 (0.88–1.50)	34.2	22	1.3 (0.9–1.6)
		Control C		1509	2.13 ± 0.16	0.89 (0.77–1.01)	95.1	66	–
	Contact	W-1Kλ		361	1.69 ± 0.23	7.60 (5.15–14.4)	18.7*	11	3.8 (2.6- 5.5) #
		W-4 km		240	4.33 ± 0.57	2.36 (2.07–2.75)	2.9*	7	1.2 (0.8–1.7)
		JW-100 s		298	1.44 ± 0.23	2.81 (1.69–4.04)	13.5*	10	1.4 (0.9–2.0)
		Control C		765	3.03 ± 0.20	2.00 (1.67–2.37)	106	40	–
Lambda-cyhalothrin	Feeding	BP-delta	F6	440	0.52 ± 0.09	54 (22–103)	6.2*	16	3.6 (2.6–4.9) #
			F26	225	2.59 ± 0.31	16 (12–22)	14.4*	10	1.1 (0.7–1.7)
		Rfg-delta	F11	471	0.77 ± 0.07	146 (99–219)	17.3*	19	9.9 (5.7–17.4) #
			F21	270	1.46 ± 0.17	66 (47–98)	13.1*	13	4.5 (2.7–7.5) #
		Control C		360	3.15 ± 0.74	15 (8–19)	14.8*	13	–
Spinosad	Feeding	BP-delta	F26	375	3.48 ± 0.36	0.22 (0.18–0.26)	33.7	20	1.0 (0.8–1.2)
		Rfg-delta	F21	270	3.80 ± 0.46	0.23 (0.20–0.27)	11.8*	13	1.1 (0.9–1.3)
		Control C		265	4.28 ± 0.49	0.22 (0.18–0.26)	19.8*	13	–

[(1)] Bioassays were performed by contact and topical application with Decis Protech (1.5% deltamethrin p/v), and by ingestion with Karate Zeon (10% lambda-cyhalothrin p/v) and technical spinosad (88% p/v)

[(2)] Number of flies considered in the Probit analysis (including non-treated)

[(3)] Lethal concentration (LC_{50}) in µg of deltamethrin/cm^2 for the contact bioassays and in ppm of insecticide in the adult rearing diet for the feeding bioassays. Lethal dose (LD_{50}) in µg insecticide/g of insect (fresh weight assuming an average weight of 10 mg) for the topical bioassays. * Good fit of the data to the Probit model ($P > 0.05$)

[(4)] Resistance ratio (RR) = LC_{50} (tested population/strain) / LC_{50} (C strain). The fiducial limits for RR were calculated according to Robertson and Preisler (1992). # RR is significant ($P < 0.05$) if the 95% FL does not include 1

with cross-resistance. We have found deltamethrin resistance in geographic areas where lambda-cyhalothrin resistance already existed (Arouri et al. 2015; Guillem-Amat et al. 2022). Thus, it is possible that resistance to lambda-cyhalothrin is conferring cross-resistance to deltamethrin, though they may also have evolved independently by different resistance mechanisms. Interestingly, one of the field populations (Rafelguaraf-N1) with higher resistance levels to deltamethrin was also highly resistant to lambda-cyhalothrin by ingestion (Guillem-Amat et al. 2022). However, whereas resistance to deltamethrin was maintained in the resistant strain Rfg-delta derived from this field population by selection with deltamethrin, resistance to lambda-cyhalothrin decreased during the selection process. Likewise, other resistant strain (BP-delta), derived from field populations resistant to deltamethrin, also showed resistance against lambda-cyhalothrin at first, but this last resistance was lost after several generations of selection with deltamethrin. Thus, though the parental field populations were resistant to both pyrethroids, lambda-cyhalothrin resistance is lost in the absence of selection pressure with this insecticide, indicating that, in this particular case, the mechanisms that mediate resistance to each insecticide may be different. However, other scenarios may also be able to evolve in the field. Indeed, we have shown that the lambda-cyhalothrin-resistant strain W-1Kλ was also resistant to deltamethrin by both topical and contact application, and MagnetMED™ traps were totally inefficient against it, in agreement with Arouri et al. (2015) who showed that this strain was highly resistant to deltamethrin when tested by ingestion. Since this strain derives from a field population collected in Castelló (Spain) in 2004 (Arouri et al. 2015), before bait stations coated with deltamethrin were deployed in the field, and has never been selected with deltamethrin, we can conclude that in this case resistance to lambda-cyhalothrin confers cross-resistance to deltamethrin. The malathion-resistant W-4 km strain, derived from the same field population collected in Castelló in 2004 and never exposed to deltamethrin (Couso-Ferrer et al. 2011), also showed moderate level of resistance to MagnetMed™ traps, reinforcing the possibility that cross-resistance between lambda-cyhalothrin and deltamethrin may develop in the field. Thus, care should be taken when combining deltamethrin traps and lambda-cyhalothrin sprays for Medfly control in the area of study, since different

Table 5 Effects of synergists on the resistance to deltamethrin and lambda-cyhalothrin of *Ceratitis capitata* laboratory strains (BP-delta and Rfg-delta) and a field population (Rafelguaraf-N1)

Insecticide [1]	Strain/population	Synergist [2]	n[3]	Slope ± S.E	LC_{50} [4] (95%FL)	χ^2	d.f	SR (95%FL) [5]
Deltamethrin	BP-delta (F24)	–	300	6.16 ± 0.75	17.8 (16.2–19.6)	4.0*	10	–
		PBO	270	1.25 ± 0.18	0.76 (0.35–1.31)	24.8	13	23 (15–38) #
		DEF	315	1.89 ± 0.21	9.83 (6.27–15.45)	47.0	16	1.8 (1.4–2.4) #
		DEM	315	2.78 ± 0.34	9.94 (6.74–13.54)	44.1	16	1.8 (1.4–2.3) #
	Rfg-delta (F18)	–	300	3.28 ± 0.46	24.0 (19.5–32.4)	20.3*	10	–
		PBO	240	5.57 ± 0.65	0.72 (0.62–0.84)	9.4*	7	33 (27–41) #
		DEF	225	5.23 ± 0.72	15.5 (13.1–18.4)	13.1*	10	1.6 (1.3–1.9) #
		DEM	225	5.07 ± 0.68	15.7 (12.0–21.9)	31.9	10	1.5 (1.2–1.9) #
	Rafelguaraf-N1[6]	–	480	2.80 ± 0.27	7.71 (5.69–10.56)	54.9	16	–
		PBO	238	1.29 ± 0.44	0.04 (0.00–0.09)	5.6*	6	211 (13–3407) #
Lambda-cyhalothrin	BP-delta (F6)	–	440	0.52 ± 0.08	52.2 (21.7–103.3)	6.2*	16	–
		PBO	240	0.64 ± 0.11	8.90 (4.23–18.98)	4.3*	10	5.9 (1.9–18.2) #

[1] Bioassays were performed by contact with Decis Protech (1.5% deltamethrin p/v) and by ingestion with Karate Zeon (10% lambda-cyhalothrin p/v)

[2] The synergists PBO (0.5 µg/insect), DEF (1 µg/insect) and DEM (1 µg/insect) were diluted in 0.5 µl acetone and topically applied (acetone was used as control) on the dorsal thorax to adult flies (3–5 days old) using an automatic microapplicator. After 2 h, the flies were treated with the insecticide

[3] Number of flies considered in the Probit analysis (including non-treated)

[4] Lethal concentration (LC_{50}) in µg of deltamethrin/cm^2 or ppm of lambda-cyhalothrin in the adult rearing diet

[5] Synergistic ratio (SR) = LC_{50} (without synergist) / LC_{50} (with synergist). The fiducial limits for SR were calculated according to Robertson and Preisler (1992). # SR is significant ($P < 0.05$) if the 95% FL does not include 1

[6] Data from Table 1

(*) Good fit of the data to the Probit model ($P > 0.05$)

scenarios of cross-resistance between these two pyrethroids may occur. On the contrary, deltamethrin-resistant strains were susceptible to spinosad by ingestion and the spinosad-resistant strain JW-100 s was susceptible to deltamethrin, indicating that spinosad is a good candidate for spray, when needed, in fields where bait stations with deltamethrin are deployed. Interestingly, negative cross-resistance between pyrethroid resistance mediated by P450 and organophosphates has been reported in mosquitoes (Wipf et al. 2022). However, the only organophosphate approved for Medfly control in citrus crops in Spain is phosmet, whose use is very limited. Nevertheless, further investigation is required to test the potential of this and other organophosphates for the control of deltamethrin- and/or lambda-cyhalothrin-resistant populations.

Knowledge of the factors and mechanisms by which resistance is acquired and evolves are also essential for devising effective IRM strategies. We have assessed some of these factors in two field-derived deltamethrin selected strains (Rfg-delta and BP-delta). Both strains showed resistance to MagnetMed™ traps (mortality below 40%) and reached 8–12-fold resistance to deltamethrin by contact after 16 generations of selection. These levels of resistance are similar to those reported for Brazilian field populations (up to 18 fold) and selected strains (4–seven fold) when tested by ingestion (Demant et al. 2019). Interestingly, the resistant strains Rfg-delta and BP-delta also showed reduced susceptibility to Ceratipack traps, use of which has increased since 2019, though the levels of resistance in this case were lower (mortality 60–70%). The differences in susceptibility to both types of traps may be related to the differences in the amount of deltamethrin deployed in the surface of each trap: MagnetMed™ (17.36 µg/cm^2), and Ceratipack (92.5 µg/cm^2). Thus, it is expected that the susceptibility of field populations to Ceratipack will also be higher than to MagnetMed™, though monitoring and testing will be required to confirm this hypothesis in the next years.

Resistance to pyrethroids is mainly caused by two mechanisms: target-site insensitivity (knockdown resistance or kdr) (Dong et al. 2014; Scott 2019) and metabolic detoxification mediated by P450 enzymes, esterases and/or GSTs (Li et al. 2007; Davies et al. 2008). We analyzed whether individuals from three field populations (Betxí, Benaguasil and Picassent) resistant to deltamethrin and the two laboratory strains (BP-delta and Rfg-delta) presented alterations at the VGSC gene, the physiological target of pyrethroids. Mutations were not found in those regions of the VGSC gene that concentrate most of the point mutations previously associated with kdr and super kdr resistance in other species (Dong et al. 2014). On the contrary, the bioassays with synergists showed

Table 6 Inheritance of resistance to deltamethrin in *Ceratitis capitata* BP-delta and Rfg-delta strains

Strain/Cross	n [(1)]	Slope ± SE	LC_{50} [(2)] (95% FL)	χ^2	df	RR (95%FL) [(3)]	D_{LC} [(4)]
Parents							
Control (C)	765	3.03 ± 0.20	2.00 (1.67–2.37)	106	40	-	
BP-delta (F26)	270	5.34 ± 0.76	19.3 (16.9–22.1)	11.8*	10	9.6 (8.3- 11.2) #	
Rfg-delta (F21)	270	5.01 ± 0.61	13.9 (12.5–15.5)	9.3*	13	7.0 (6.0- 8.1) #	
F1 crosses							
F1A-BP (♂ BP-delta x ♀ C)	315	3.36 ± 0.31	2.47 (2.07–2.99)	20.1*	16	1.2 (1.0- 1.5)	0.09
F1B-BP (♀ BP-delta x ♂ C)	315	3.47 ± 0.33	2.31 (1.94–2.78)	20.3*	16	1.2 (0.9- 1.4)	0.06
F1A-Rfg (♂ Rfg-delta x ♀ C)	360	3.02 ± 0.310	1.05 (0.89–1.23)	22.31*	19	0.5 (0.4- 0.6) #	-0.33
F1B-Rfg (♀ Rfg-delta x ♂ C)	225	8.84 ± 2.02	1.36 (1.25–1.59)	4.18*	10	0.7 (0.6- 0.8) #	-0.19
F2 crosses							
F2-BP (F1A-BP and F1B-BP interbred)	360	2.24 ± 0.25	4.98 (3.98–5.99)	23.0*	19	2.5 (2.0- 3.1) #	
F2-Rfg (F1A-Rfg and F1B-Rfg interbred)	480	2.23 ± 0.21	3.96 (3.21–4.72)	40.1*	27	1.9 (1.6- 2.4) #	
Backcrosses							
BcA-BP-C ([♂ F1A-BP + ♂ F1B-BP] x ♀ C)	313	2.55 ± 0.28	3.59 (2.51–4.62)	24.3*	16	1.8 (1.4- 2.3) #	
BcB-BP-BP ([♀ F1A-BP + ♀ F1B-BP] x ♂ BP-delta)	315	5.64 ± 0.66	9.73 (8.60–10.97)	30.5	16	4.9 (1.4- 16.5) #	
BcA-Rfg-C ([♂ F1A-Rfg + ♂ F1B-Rfg] x ♀ C)	405	2.39 ± 0.20	3.97 (3.212–4.88)	34.7	22	1.9 (1.6- 2.4) #	
BcB-Rfg-Rfg ([♀ F1A-Rfg + ♀ F1B-Rfg] x ♂ BP-delta)	315	7.52 ± 0.94	8.70 (8.11–9.26)	13.1*	16	4.4 (3.8- 4.9) #	

(1) Number of flies considered in the Probit analysis (including non-treated)

(2) Lethal concentration (LC_{50}) in µg of deltamethrin/cm^2. Contact bioassays were performed with Decis Protech (1.5% deltamethrin p/v)

(3) Resistance ratio (RR) = LC_{50} (strain or cross) / LC_{50} (Parent C strain). The fiducial limits for RR were calculated according to Robertson and Preisler (1992). # RR is significant ($P < 0.05$) if the 95% FL does not include 1

(4) Dominance value (D_{LC}) calculated following the formula $D_{LC} = \{[(2\ logLC_{50}F1 - logLC_{50}P1 - logLC_{50}P2)/(logLC_{50}P1 - logLC_{50}P2)] + 1\}/2$; where P1 and P2 corresponded to parental resistant (BP-delta or Rfg-delta) and susceptible (control C) strains, respectively. $D_{LC} = 0$ for completely recessive and 1 for completely dominant

Table 7 Biological parameters of flies from deltamethrin-resistant (Rfg-delta and BP-delta) and susceptible (Control C) strains of *Ceratitis capitata*

	Control C	Rfg-delta	BP-delta
Embryo to pupal viability (number of pupae)	756 ± 79 (ab)	566 ± 44 (a)	863 ± 141 (b)
Developmental time (days from egg to pupae)	8.4 ± 0.2 (a)	8.2 ± 0.2 (a)	8.6 ± 0.2 (a)
Females' weight (mg)	9.35 ± 0.1 (a)	9.52 ± 0.08 (a)	9.31 ± 0.1 (a)
Males' weight (mg)	8.44 ± 0.07 (a)	8.49 ± 0.08 (a)	8.13 ± 0.08 (b)
Females' longevity (days)	24.3 ± 0.7 (a)	22.6 ± 0.6 (ab)	20.8 ± 0.7 (b)
Males' longevity (days)	23.7 ± 0.7 (a)	22.1 ± 0.6 (ab)	21.2 ± 0.7 (b)
Lifetime Fecundity (cm^3 eggs/30 females)	1.20 ± 0.07 (a)	0.80 ± 0.09 (b)	0.70 ± 0.05 (b)

Data are mean ± standard error. Different letters within each row indicate significant differences (ANOVA, Tukey post hoc test, $P \leq 0.05$)

that PBO reverted deltamethrin resistance in both field (Rafelguaraf-N1) populations and laboratory (BP-delta and Rfg-delta) strains, whereas the effect of DEF and DEM was minor, suggesting that deltamethrin resistance was mediated by P450 enzymes. Likewise, Guillem-Amat et al. (2022) reported the absence of mutations at the VGSC gene in other three field populations (Blanca, Vinaròs and Rafelguaraf-N1) resistant to lambda-cyhalothrin collected from the same region, and the reversion of resistance to lambda-cyhalothrin by PBO in Rafelguaraf-N1. The implication of Medfly P450s (Papanicolaou et al. 2016) in lambda-cyhalothrin resistance in laboratory strains has already been associated with the overexpression of the P450 gene *CcCYP6A51* (Arouri et al. 2015; Tsakireli et al. 2019). However, further studies are needed to determine which P450s are involved in field resistance, and whether they are specific or not for lambda-cyhalothrin and deltamethrin, which will determine cross-resistance.

Two of the factors that may condition the development and spread of resistant populations in the field are the inheritance (Devine and Denholm 2009) and fitness cost (Kliot and Ghanim 2012) of resistance; factors that in some cases it has been possible to correlate with their molecular mechanism (Bourguet and Raymond 1998). Thus, the inheritance

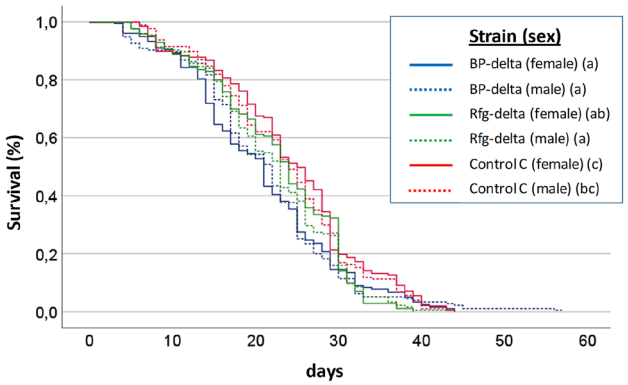

Fig. 1 Kaplan–Meier survival curves of males and females from deltamethrin-resistant (Rfg-delta and BP-delta) and susceptible (Control C) strains of *Ceratitis capitata.* Survival curve legends followed by different lowercase letters (between brackets) accounts for statistically significant differences (Log-Rank Mantel–Cox test, $P \leq 0.05$)

of pyrethroids associated with VGSC mutations is in most cases recessive (Scott 2019), whereas resistance to pyrethroids mediated by P450s varies from dominant (Abbas et al. 2014; Khan et al. 2015) to recessive (Li and Liu 2010). We found that deltamethrin resistance was inherited as a completely recessive autosomal trait in both Medfly (BP-delta and Rfg-delta)-resistant strains. This result contrasts with that of the lambda-cyhalothrin W-1Kλ resistant strain, where resistance is inherited as a dominant trait (Guillem-Amat et al. 2020b), supporting our previous conclusion that different resistance mechanisms may be involved. Besides, in both cases resistance appears to be polygenic, as already reported for other cases of pyrethroid resistance mediated by P450s (Abbas et al. 2014; Khan et al., 2015). Polygenic traits associated with pyrethroids resistance can include multiple P450 genes, but also other detoxification genes and genes associated with pesticide metabolism (Scott 2017). Regarding the fitness cost of pyrethroids resistance mediated by P450s, it has been reported that the overexpression of these enzymes can be energetically costly and can negatively affect the life traits of resistant strains (Ffrench-Constant and Bass 2017), as observed for permethrin resistance in *Culex pipiens* (Hardstone et al. 2010) and deltamethrin resistance in *Aedes aegypti* (Alvarez-Gonzalez et al. 2017). We have found that deltamethrin resistance presented a fitness cost in terms of males' weight, males' and females' longevity and lifetime fecundity, with a more pronounced effect in the BP-strain than in the Rfg-delta strain. Similar results were reported for the lambda-cyhalothrin W-1Kλ-resistant strain (Guillem-Amat et al. 2020b), highlighting that trade-offs occur between metabolic resistance to pyrethroids and life history traits in this species, though unspecific pleiotropic effects cannot be discarded (Lenormand et al. 2018).

In conclusion, the detection of deltamethrin resistance in Spanish field populations hinders further Medfly control in the area of study. Hence, the implementation of IRM strategies is required to prevent possible control failures. Our results indicate that it is advisable to use spinosad in those orchards where lure-and-kill traps of deltamethrin are deployed. This strategy, as well as the rotation of spinosad and lambda-cyhalothrin bait sprays (Guillem-Amat et al. 2020a), combine two insecticides with no cross-resistance, avoiding the repeated use of a single insecticide in the same field, which is expected to delay resistance (Guillem-Amat et al. 2020b, 2022). However, care should be taken when combining deltamethrin traps and lambda-cyhalothrin sprays, since our results indicate that different scenarios of cross-resistance between these two pyrethroids may occur for Medfly populations in the area of study. Guillem-Amat et al. (2022) simulated the evolution of lambda-cyhalothrin resistance under different cross-resistance scenarios with deltamethrin and concluded that the efficacy of both pyrethroids would be seriously compromised under the assumption of cross-resistance, even when partial. Thus, farmers should avoid the overuse of both pyrethroids in the same fields for long periods of time, as there is a risk of cross-resistance between them. In any case, these IRM strategies must be reinforced and harmonized with other control strategies implemented at present, such as the sterile insect technique that provides an effective Medfly control in spring (Juan-Blasco et al. 2014), contributing to reduce the population levels along the year.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Consent for publication Not applicable for that section.

Ethics approval Not applicable for that section.

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