Research Article

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Current situation and forecasting of resistance evolution to lambda-cyhalothrin in Spanish medfly populations

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Abstract

BACKGROUND: The control of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) in Spanish field populations mainly relies on the insecticides lambda-cyhalothrin and spinosad as bait sprays. However, their sustainable used is compromised by the development of lambda-cyhalothrin resistance and the detection of spinosad resistant alleles. In addition, the use of lure-and-kill traps covered with deltamethrin has increased in the last years. It is thus urgent to predict the impact that the combination of both pyrethroids will have in the evolution of lambda-cyhalothrin resistance and how they could be combined with spinosad so as to establish proper resistance management programs.

RESULTS: Toxicity bioassays were performed to analyze the current levels of lambda-cyhalothrin resistance in field populations, proving that it has remained stable in the last decade. An evolutionary model was established to explore the weight of selected parameters in the evolution of lambda-cyhalothrin resistance in *C. capitata* and to forecast resistance development under different resistance management scenarios. Our results highlight the importance of fitness cost and inheritance to fit the experimental results. The analyses predicted that the rotation of lambda-cyhalothrin and spinosad, when deltamethrin traps are also deployed in the field, will slow down the evolution of resistance, especially when cross-resistance between both pyrethroids is considered.

CONCLUSION: Lambda-cyhalothrin resistance has not increased in the last decade, probably due to the alternation of this insecticide with spinosad. Our modelling results indicate that the best option to avoid an increase in lambda-cyhalothrin resistant alleles, considering that deltamethrin use is growing, would be to continue combining their use with spinosad.

Supporting information may be found in the online version of this article.

Keywords: *Ceratitis capitata*; insecticide; resistance monitoring; evolutionary model; resistance management

1 INTRODUCTION

The Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), is one of the main insect pests of fruits, which causes serious economic losses worldwide. The fight against medfly mainly relies on the chemical control.[1,2] At present, lambda-cyhalothrin and spinosad applied as bait sprays, and deltamethrin as lure and kill traps are used for medfly control in Spanish citrus crops.[3] Other strategies such as the sterile insect technique (SIT) are implemented in some areas,[4] but medfly outbreaks require the use of insecticides for a satisfactory management.[5] However, this control strategy is threatened by the development of resistant populations to lambda-cyhalothrin[6] and the detection of spinosad resistant alleles in field individuals.[7] This situation makes urgent the need to implement Insecticide Resistance Management (IRM) strategies to guarantee the sustainability of available insecticides without compromising medfly control.

Theoretical evolutionary models can be used to identify the main factors involved in the evolution and spread of insecticide resistance and to forecast the efficiency of different control strategies,[8] contributing to the decision making in IRM.[9] The evolution of insecticide resistance depends on different factors such as the biology of the insect, the mechanisms and genetics of resistance and the control practices performed in the field.[10] The mechanism of resistance to lambda-cyhalothrin in *C. capitata* has been studied in the resistant strain W-1Kλ, obtained by laboratory selection. Reversion of resistance by the synergist piperonyl butoxide and over-expression of the P450 gene *CYP6A51* was observed in W-1Kλ, suggesting that resistance may be metabolic.[6] Further functional studies confirmed the implication of *CYP6A51* in lambda-cyhalothrin resistance,[11] although high variability in its expression was observed in susceptible field populations.[12]

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The inheritance and fitness cost of lambda-cyhalothrin resistance in *C. capitata* was also studied in the W-1Kλ strain.[13] The results of this work demonstrated that resistance is autosomic, completely dominant and controlled by more than one gene. When the fitness was compared with that of a susceptible strain, it was determined that resistance to lambda-cyhalothrin in W-1Kλ is associated to a lower embryo to pupa viability, a slower developmental time from egg to pupa, an increase in adults' weight and longevity, and a higher α-amylase activity in adult females. However, though useful for the parametrization of evolutionary models, experiments conducted with resistant strains under laboratory conditions may not always reflect all the different scenarios that may occur in field populations.[14] Thus, it is relevant for forecasting purposes to corroborate that the outcomes of the simulation models fit with field experimental results.

To manage insecticide resistance, recommended strategies include rotations (which involve temporal cycles), mosaics (spatial patterns of applications) and mixtures (concomitant use over time and space) of insecticides.[15] Medfly control in citrus crops in Spain during the last decade has mostly relied on two insecticides, lambda-cyhalothrin and spinosad, which are alternated in some orchards along the annual campaign, though in others the same product is used repeatedly. The benefits of the rotation of both insecticides is supported by the results obtained in a simulation study with a medfly multiresistant strain selected in the laboratory, which showed that lambda-cyhalothrin resistance increased when several consecutive treatments with this insecticide were applied, while alternating this insecticide with spinosad was enough to delay the development of resistance.[13] Thus, control practices may be contributing to attenuate the development of resistance and to maintain the efficacy of lambda-cyhalothrin for the control of *C. capitata* in Spain, despite resistant populations were first detected in 2009–2010.[6] However, there is no information regarding the current levels of resistance to lambda-cyhalothrin in field populations that corroborates this hypothesis. Interestingly, the use of bait stations impregnated with the pyrethroid deltamethrin has increased in the last years covering a surface of 12 500 ha in 2018 (V. Dalmau, Servicio de Sanidad Vegetal, Generalitat Valenciana, personal communication). This technique provides a feasible management option when used on low-density or isolated populations, though usually it is necessary to reinforce the control using bait-spray treatments with spinosad and/or lambda-cyhalothrin. The fact that both lambda-cyhalothrin and deltamethrin belong to the same family of structurally related insecticides implies that cross-resistance to both insecticides may occur, and therefore selection pressure over resistant alleles could be multiplied by the combination of both insecticides. The lambda-cyhalothrin resistant strain W-1Kλ has been shown to be more than 100-fold resistant to the pyrethroids deltamethrin and etofenprox.[6] However, further studies are needed to determine if a common or different resistant mechanism may be conferring resistance to both pyrethroids in field populations. In any case, the introduction of this new element has to be considered when establishing IRM strategies.

The goal of this work was to contribute to lambda-cyhalothrin resistance management by analyzing the current situation in field populations and devising how it could evolve in the coming decades based on evolutionary models. With this aim, we have (i) showed that the level of susceptibility to this insecticide in field populations has not changed significantly in the last decade; (ii) explored and identified the parameters (inheritance and fitness cost) that better fit the experimental results; and (iii) forecasted how resistance would evolve in the field under different treatment scenarios, including the combined use of lambda-cyhalothrin with spinosad and/or deltamethrin.

2 MATERIALS AND METHODS

2.1 Field populations of *Ceratitis capitata*

Ceratitis capitata field populations were collected from orchards sited in different localities in the Mediterranean area, in East and South Spain, during the years 2016–2019 (Table 1 and Supplementary Fig. S1). Fruit punctured by *C. capitata* was taken from the field to the laboratory, placed in ventilated plastic boxes (15 × 21 × 28 cm) and kept at controlled conditions (26 ± 2 °C (light) and 22 ± 2 °C (dark); 16: 8 h light: dark). New pupae were harvested and kept in ventilated boxes (12 cm in diameter and 5 cm height). Emerged-adults were provided with water and rearing diet (4: 1 sugar: yeast) *ad libitum*, in an environmentally controlled chamber (Sanyo MLR-350-H, Sanyo, Japan) at 25 ± 1 °C and 16 h light and 8 h dark photoperiod (standard conditions).

2.2 Bioassays

Feeding bioassays were performed with adult flies 3–5 days old with lambda-cyhalothrin (KarateZeon 10% p/v (100 g L^{-1}), Syngenta Limited, Surrey, United Kingdom). Depending on the availability of flies, concentration-response assays using 4–5 concentrations of lambda-cyhalothrin or assays with a unique

Table 1. Spanish field populations of *Ceratitis capitata*

Population	Year	Host	Field treatments*
Sagunt	2016	Citrus	Spinosad and deltamethrin in 2016
Algarrobo Costa	2016	Cherimoya	Non-treated in the last years (experimental field)
Blanca	2016	Citrus	Non-treated in the last year
Alcalà de Xivert	2017	Citrus	Non-treated/Spinosad in 2017 (3X)†
Vinaròs	2017	Citrus	Deltamethrin and lambda-cyhalothrin in 2017 (2X)
Algarrobo Costa	2017	Cherimoya	Non-treated in 2017 (experimental field)
Vila-real	2017	Citrus	Deltamethrin and lambda-cyhalothrin in 2017 (6×)
Rafelguaraf	2019	Loquat	Isolated trees (non-treated)

*The number between brackets refers to the amount of field applications of bait formulations with lambda-cyhalothrin (by ground treatment) or spinosad (by ground or aerial treatments) per year against *C. capitata*. Deltamethrin treatments consisted of bait stations impregnated with this insecticide that were deployed in citrus orchards for at least 3 months during the summer–fall period.
† This population came from two different fields, one that had no insecticide treatment, and another treated with spinosad.

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concentration of 125 ppm of lambda-cyhalothrin (recommended for field treatments) were performed. The insecticide was diluted with water, and then mixed with rearing diet (1 insecticide dilution: 9 rearing diet w/w). Two-four replicates of 10–15 flies per concentration were performed, and water was mixed with rearing diet for the non-treated controls. During the assays, flies were kept at standard conditions into ventilated plastic dishes (89 mm in diameter and 23 mm in height). Mortality after 48 h was recorded and flies were considered dead if they were ataxic.

The synergist piperonyl butoxide (PBO; 90% technical, Aldrich, Milwaukee, WI) was diluted in acetone and applied topically to adult flies. Flies (3–5 days old) were anesthetized with CO_2 and treated with a 0.5 μL drop of PBO solution in acetone or only acetone (used as control) on the dorsal thorax by using an automatic microapplicator 900× (Burkard Manufacturing Co., Hertfordshire, United Kingdom). The dose applied (0.5 μg PBO per insect) showed no mortality on adults. Three to four replicates of 10–15 adults were performed. After PBO treatment, insects were placed in ventilated plastic dishes containing water and rearing diet with lambda-cyhalothrin as previously described. The mortality was recorded after 48 h.

2.3 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 (Supplementary Fig. S2(a)). We analyzed 20 flies from the populations of Blanca (2016), Vinaròs (2017) and Rafelguaraf (2019) that survived to the exposure to pyrethroids. Whole body DNA was extracted and PCR was performed in a volume of 50 μL using 0.04 U of AmpliTaq Gold DNA Polymerase (Thermo Fisher Scientific, Austin, USA), 10x PCR Buffer II, 1.5 mM MgCl2, 0.2 mM dNTPs (Thermo Fisher Scientific, Austin, USA), 100–200 ng of template, and 0.6 μM of the oligonucleotides NaCh899_F (5'-TCGAGTTTTTAAACTTGCCAAA) and NaCh932_R (5'-TTTCCGAACAGTTGCATTCC) for region 'A', Kdr_F (5'-TCGTTTTTCGTGTGCTATGC) and Kdr_R (5'-CCAGGCTTTAAAACGCGATA) for region 'B', NaCh1528_F (5'-AAGCAACCAATCCGTGAAAC) and NacCh1575_R (5'-TCGGTCTAGGAATGGCTTTT) for region 'C' (Supplementary Fig. S2(b)]. PCR conditions were as follows: an initial denaturation step at 95 °C for 5 min; 35 cycles of 95 °C for 30 s, 60 °C for 30 s and 72 °C for 40 s; and a final step of 72 °C for 7 min for fully extension.

2.4 Evolutionary model of lambda-cyhalothrin resistance

An evolutionary model was established to explore the weight of selected parameters in the evolution of lambda-cyhalothrin resistance in *C. capitata* and to forecast resistance development under different resistance management scenarios. It was assumed that the population was panmictic with discrete generations, and that the effective population size was large enough to rule out genetic drift effects. These assumptions are based on the high levels of gene flow found among Spanish *C. capitata* populations,[16] which seem to maintain high effective number of individuals in populations throughout the year due to a wide range of fruits available.[17] Parameters and scenarios tested in the simulation come from experimental data and educated guesses, and are presented in Table 2.

Different types of inheritance of lambda-cyhalothrin resistance were considered: monogenic/polygenic and dominant/co-dominant/recessive. In all cases it was assumed that the relationship between allele and genotype frequencies was determined by Mendelian segregation. For the monogenic model, the simulation considered three different genotypes (XY = *(SS)*, *(RR)* and *(SR)*), in which *S* was a susceptible allele and *R* a resistant allele (Table 2(a)). For the polygenic model, the case of two genes contributing to resistance and nine different genotypes was modeled (XY = *(R1R1 R2R2)*, *(R1R1 R2S2)*, *(R1R1 S2S2)*, *(R1S1 R2R2)*, *(R1S1 R2S2)*, *(R1S1 S2S2)*, *(S1S1 R2R2)*, *(S1S1 R2S2)*, *(S1S1 S2S2)*), in which *S1* and *S2* were the two different susceptible alleles, and *R1* and *R2* the resistant alleles (Table 2(b)).

The frequency of resistant alleles was estimated from susceptibility analyses performed with field populations collected during the years 2016–2019 by reanalyzing the data from Arouri *et al.* (2015)[6] for the period 2009–2010 and assuming monogenic/polygenic and dominant/co-dominant/recessive resistance (see section 3.2 and Table 3).

Expected mortality to field treatments of lambda-cyhalothrin (125 ppm in bait sprays) was estimated from experimental data with the lambda-cyhalothrin resistant strain W-1Kλ and the susceptible strain C.[6,13] For the monogenic model, the expected mortality to lambda-cyhalothrin was defined as $s(\lambda)_{SS} = 1$ (Table 2(a)), as wild-type homozygous die when exposed to field concentrations of lambda-cyhalothrin. However, this concentration has different effects on homozygous and heterozygous individuals for the resistant allele depending on if lambda-cyhalothrin resistance is considered to be dominant ($s(\lambda)_{RR} = s(\lambda)_{SR} = 0$), codominant ($s(\lambda)_{RR} = 0$; $s(\lambda)_{SR} = 0.5$) or recessive ($s(\lambda)_{RR} = 0$; $s(\lambda)_{SR} = 1$). For the polygenic dominant model, we considered that two resistant alleles (*R1* and *R2*) are required, in homozygosis or heterozygosis, to confer resistance, since the expected mortality under these assumptions fits the observed mortality in parents, F1, F2 and backcrosses in the reanalysis of the inheritance data from Guillem-Amat *et al* (2020)[13] for the W-1Kλ strain (Supplementary Table S1). Thus, the expected mortality was defined as $s(\lambda)_{XY} = 0$ for four of the genotypes (*R1R1 R2R2*, *R1R1 R2S2*, *R1S1 R2R2*, *R1S1 R2S2*), and $s(\lambda)_{XY} = 1$ for the rest of the genotypes (Table 2(b)). For the polygenic co-dominant and recessive models, the expected mortality was estimated considering that at least one resistant allele or both resistant alleles of each gene, respectively, need to be present to confer a resistant phenotype (Table 2(b)).

Arouri *et al.* (2015)[6] showed that W-1Kλ has no cross-resistance with spinosad, and therefore the expected mortality for all genotypes when exposed to field treatments of spinosad (260 ppm in bait sprays) was considered as $s(s)_{XY} = 1$, for both the monogenic (Table 2(a)) and the polygenic model (Table 2(b)).

When considering the expected mortality to lure-and-kill traps coated with deltamethrin, three different hypothetical scenarios were established under the monogenic model (Table 2(a)). The existence of cross-resistance between lambda-cyhalothrin and deltamethrin (MO1), as observed for the resistant laboratory strain W-1Kλ.[6] In this case, the expected mortality for *SS* individuals would be $s(d)_{SS} = 0.9$, as it was observed that not all the individuals from the susceptible laboratory strain C reach the traps and die.[18] The absence of cross-resistance between lambda-cyhalothrin and deltamethrin in field populations (MO2), in which all the genotypes would be expected to be as sensitive as C strain ($s(d)_{SS} = s(d)_{RR} = s(d)_{SR} = 0.9$). The intermediate situation with partial cross-resistance (MO3), in which the average mortality to

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Table 2. Parameters used in the evolutionary model for lambda-cyhalothrin resistance in *Ceratitis capitata* considering: (a) monogenic inheritance; and (b) polygenic inheritance (two genes). Scenarios of insecticide treatments tested: (c) lambda-cyhalothrin and spinosad; (d) lambda-cyhalothrin and deltamethrin; and (e) lambda-cyhalothrin, spinosad and deltamethrin

Parameter	Definition
XY	Genotypes (*SS*), (*RR*) and (*SR*) for the monogenic model; and *(R1R1 R2R2)*, *(R1R1 R2S2)*, *(R1R1 S2S2)*, *(R1S1 R2R2)*, *(R1S1 R2S2)*, *(R1S1 S2S2)*, *(S1S1 R2R2)*, *(S1S1 R2S2) and (S1S1 S2S2)* for the polygenic model
w_{XY}	Fitness cost of genotype *XY*; $0 \leq w_{XY} \leq 1$. Five different hypothetical settings of fitness cost values (SC1-5) were tested.
$s(i)_{XY}$	Expected mortality to insecticide i (i = lambda-cyhalothrin (λ), spinosad (s) or deltamethrin (d) of genotype *XY*); $0 \leq s(i)_{XY} \leq 1$. The following hypothetical scenarios were established for deltamethrin: the existence of cross-resistance with lambda-cyhalothrin (MO1); the absence of cross-resistance with lambda-cyhalothrin (MO2); and the intermediate situation with partial cross-resistance, in which the heterozygous were considered as sensible as the resistant genotypes (MO3.1), the susceptible genotypes (MO3.3) or the mean value between them (MO3.2).
$e(i)$	Exposure to insecticide (i), understood as the percentage of insects in the population contacting the insecticide, $e(i) = \{(0.2), (0.5), (0.8)\}$
IF_{XY}	Initial frequency of genotype *XY* used to calculate the predicted rate of resistance evolution

(a)

				Expected mortality ($s(i)_{XY}$)								
	Initial frequency IF(*XY*).			Lambda-cyhalothrin (125 ppm)			Spinosad	Deltamethrin (MagnedMed).				
Genotype	Dominant	Co-dominant	Recessive	Dominant	Co-dominant	Recessive	(260 ppm)	(MO1)	(MO2)	(MO3.1)	(MO3.2)	(MO3.3)
RR	0.12	0.25	0.44	0	0	0	1	0	0.9	0.4	0.4	0.4
RS	0.44	0.50	0.44	0	0.5	1	1	0	0.9	0.4	0.65	0.9
SS	0.44	0.25	0.12	1	1	1	1	0.9	0.9	0.9	0.9	0.9

	Fitness cost (w_{XY})				
Genotype	SC1	SC2	SC3	SC4	SC5
RR	0.6	0.4	0.2	0.1	0.05
RS	0.3	0.2	0.1	0.05	0.025
SS	0	0	0	0	0

(b)

				Expected mortality ($s(i)_{XY}$)								
	Initial frequency IF(*XY*)			Lambda-cyhalothrin (125 ppm)			Spinosad	Deltamethrin (MagnedMed)				
Genotype	Dominant	Co-dominant	Recessive	Dominant	Co-dominant	Recessive	(260 ppm)	(MO1)	(MO2)	(MO3.1)	(MO3.2)	(MO3.3)
R1R1 R2R2	0.063	0.100	0.440	0	0	0	1	0	0.9	0.4	0.4	0.4
R1R1 R2S2	0.126	0.146	0.074	0	0.5	1	1	0	0.9	0.4	0.8	0.9
R1R1 S2S2	0.062	0.045	0.037	1	1	1	1	0.9	0.9	0.9	0.9	0.9
R1S1 R2R2	0.126	0.146	0.075	0	0.5	1	1	0	0.9	0.4	0.8	0.9
R1S1 R2S2	0.252	0.291	0.149	0	0.5	1	1	0	0.9	0.4	0.65	0.9
R1S1 S2S2	0.124	0.091	0.075	1	1	1	1	0.9	0.9	0.9	0.9	0.9
S1S1 R2R2	0.062	0.045	0.037	1	1	1	1	0.9	0.9	0.9	0.9	0.9

Table 2. Continued

(b)

	Initial frequency IF(*XY*)			Expected mortality ($s(i)_{XY}$)								
				Lambda-cyhalothrin (125 ppm)			Spinosad	Deltamethrin (MagnedMed)				
Genotype	Dominant	Co-dominant	Recessive	Dominant	Co-dominant	Recessive	(260 ppm)	(MO1)	(MO2)	(MO3.1)	(MO3.2)	(MO3.3)
S1S1 R2S2	0.124	0.091	0.075	1	1	1	1	0.9	0.9	0.9	0.9	0.9
S1S1 S2S2	0.062	0.045	0.037	1	1	1	1	0.9	0.9	0.9	0.9	0.9

	Fitness cost (w_{XY})				
Genotype	SC1	SC2	SC3·	SC4	SC5
R1R1 R2R2	0.6	0.4	0.2	0.1	0.05
R1R1 R2S2	0.45	0.3	0.15	0.075	0.0375
R1R1 S2S2	0.3	0.2	0.1	0.05	0.025
R1S1 R2R2	0.45	0.3	0.15	0.075	0.0375
R1S1 R2S2	0.3	0.2	0.1	0.05	0.025
R1S1 S2S2	0.15	0.1	0.05	0.025	0.0125
S1S1 R2R2	0.3	0.2	0.1	0.05	0.025
S1S1 R2S2	0.15	0.1	0.05	0.025	0.0125
S1S1 S2S2	0	0	0	0	0

(c)

Treatment	G1	G2	G3	G4	G5	G6
T1.C	-	-	-	-	-	-
T1.1	-	-	-	-	λ	-
T1.2	-	-	-	s	λ	s
T1.3	-	-	-	s,s	λ,s	s,s
T1.4	-	-	-	λ	s	λ
T1.5	-	-	-	λ,s	s,s	λ,s
T1.6	-	-	-	λ	λ	λ
T1.7	-	-	-	λ,s	λ,s	λ,s
T1.8	-	-	-	λ,λ	λ,λ	λ,λ

(d)

Treatment	G1	G2	G3	G4	G5	G6
T2.C	-	-	-	-	-	-
T2.1	-	-	-	-	λ	-
T2.2	-	-	-	d	λ/d	d
T2.3				λ	-	λ
T2.4	-	-	-	λ/d	d	λ/d

Table 2. Continued

(d)							
Treatment	G1	G2	G3	G4	G5	G6
T2.5	-	-	-	λ	λ	λ
T2.6	-	-	-	λ/d	λ/d	λ/d
T2.7	-	-	-	λ,λ	λ,λ	λ,λ
T2.8				(λ,λ)/d	(λ,λ)/d	(λ,λ)/d

(e)						
Treatment	G1	G2	G3	G4	G5	G6
T3.C	-	-	-	-	-	-
T3.1	-	-	-	λ/d	d	s/d
T3.2	-	-	-	s/d	λ/d	s/d
T3.3				λ/d	s/d	λ/d
T3.4	-	-	-	(s,λ)/d	(s,λ)/d	(s,λ)/d

(-), no insecticide; (λ), a lambda-cyhalothrin bait spray treatment; (s), a spinosad bait spray treatment; (d), deltamethrin treatment with lure and kill trap; (/), treatments coexist; (,), successive treatments (one first, and then the other).

MagnetMed traps coated with deltamethrin obtained for field populations[18] was assumed for the resistant homozygous ($s(d)_{RR} = 0.4$), and for the heterozygous it was considered the possibility of being as sensible as the *RR* ($s(d)_{SR} = 0.4$; MO3.1), as sensible as the *SS* ($s(d)_{SR} = 0.9$; MO3.3), or the mean value between both ($s(d)_{SR} = 0.65$; MO3.2). For the polygenic model, three equivalent scenarios were considered (Table 2(b)). Scenario MO1 considered the existence of cross-resistance between lambda-cyhalothrin and deltamethrin, in which at least one resistant allele of each gene needs to be present to confer a resistant phenotype. Scenario MO2 established the absence of cross-resistance between both pyrethroids. Scenario MO3 established partial cross-resistance, in which at least one resistant allele of each gene with synergistic (MO3.1) or additive (MO3.2) effect, or both resistant alleles of each gene (MO3.3) need to be present to confer a resistant phenotype.

Five different hypothetical settings of fitness cost values (being w_{XY} the fitness cost or selection coefficient of genotype *XY*) were tested to determine which better fitted the current trends in field resistance development. It was considered that, by definition, the wild-type has no fitness cost ($w_{SS} = 0$ or $w_{S1S1S2S2} = 0$). For the remaining genotypes, in the monogenic model, we simulated the effect in the evolution of resistance of a range of fitness cost values (from 0.4 to 0.05 for *RR*, and from 0.2 to 0.025 for *SR*) (Table 2(a)). To evaluate the fitness of genotypes under the polygenic model, it was considered that each resistant allele has a fitness cost and that it is additive, so as the fitness cost of the genotype would be equal to the amount of the fitness cost of all *R* alleles carried (Table 2(b)). Those settings of fitness cost values that proved to better explain the experimental data from field populations were selected for further forecasting of resistance development.

Treatments for the control of *C. capitata* in citrus crops in the area of study (Spanish Mediterranean region) are normally performed at generations G4-G6 every year. During this period, farmers normally treat between 1–6 times with lambda-cyhalothrin and/or spinosad as bait sprays, depending on the level of the attack. Besides, these treatments can be combined with the presence of lure-and-kill traps coated with deltamethrin that remained in the fields during the last three generations. Different scenarios of insecticide treatment strategies, that include the use of lambda-cyhalothrin, were then tested to analyze their contribution to the development of resistance: (i) lambda-cyhalothrin alone or alternated with spinosad (Table 2(c)); (ii) lambda-cyhalothrin alone or in combination with deltamethrin traps (Table 2(d)); and (iii) alternation of lambda-cyhalothrin and spinosad in combination with deltamethrin traps (Table 2(e)).

As the insecticide treatment is considered to reach only part of the population (when using both bait sprays or lure-and-kill traps), the population was divided into two sub-populations depending on whether or not the insect contacted with the insecticide. For modelling resistance evolution during the period 2009–2010 and 2016–2019, we considered an exposure level ($e(i)$) of 0.5, which is representative of the exposure of the populations to insecticides at regional level. For forecasting lambda-cyhalothrin resistance evolution under different insecticide treatment scenarios, we used three levels of insecticide exposure ($e(i) = 0.2$, 0.5 and 0.8), which represent the variability that may occur among citrus orchards and localities. Note that the putative migration is implicit in the parameter $e(i)$.

The general equations for calculating the relative genotype frequency for the monogenic and polygenic models through generations were the following ones:

$$F_{n+1}(XY) = = \left\{ \begin{array}{c} \{[\sum[F_n(XY)\times F_n(XY)\times F(XY)]]\times(1-w_{XY})\times(1-s(\lambda)_{XY})\times(1-s(s)_{XY})\times(1-s(d)_{XY})\times e(i)\} \\ + \\ \{[\sum[F_n(XY)\times F_n(XY)\times F(XY)]]\times(1-w_{XY})\times(1-e(i))\} \end{array} \right\} / [\sum F_{n+1}(\text{all XY genotypes})]$$

in which,

genotype $(XY) = \{(SS), (RR), (SR)\}$ for the monogenic model; and $(XY) = \{(R1R1\ R2R2), (R1R1\ R2S2), (R1R1\ S2S2), (R1S1\ R2R2), (R1S1\ R2S2), (R1S1\ S2S2), (S1S1\ R2R2), (S1S1\ R2S2), (S1S1\ S2S2)\}$ for the polygenic model

$F_{n+1}(XY)$ = frequency of genotype XY at generation $(n+1)$

$F_n(XY)$ = frequency of genotype XY at generation (n)

$[F_n(XY)\times F_n(XY)]$ = frequency of any cross between genotypes XY and XY

$F(XY)$ = frequency of genotype XY produced by a given cross

w_{XY} = fitness cost of genotype XY; $0 \le w_{XY} \le 1$.

$s(\lambda)_{XY}$, $s(s)_{XY}$, $s(d)_{XY}$ = expected mortality to lambda-cyhalothrin (λ), spinosad (s) and/or deltamethrin (d) of genotype XY; $0 \le s(i)_{XY} \le 1$.

$e(i)$ = exposure to insecticide (i), understood as the percentage of insects in the population contacting the insecticide, $e(i) = \{(0.2), (0.5), (0.8)\}$.

The simulation covered 60 generations (10 years, since *C. capitata* usually has six generations per year in the area of study).

Table 3. Susceptibility to lambda-cyhalothrin of Spanish field populations of *Ceratitis capitata*

Period	Population	Year	n[(1)]	Slope ± S.E.	LC_{50}[(2)] (95% FL)	χ^2	df	RR (95%FL)[(3)]	Expected mortality (%) at 125 ppm[(4)]	Observed mortality (% ± SE) at 125 ppm[(5)]
2009–2010										
	Castellserà	2009			287 (199–470)[(6)]			14 (9–22)#[(6)]	32	
	Llombai	2009			134 (85–199)[(6)]			7 (4–11)#[(6)]	49	
	Almuñecar	2009			144 (82–243)[(6)]			7 (4–13)#[(6)]	47	
	Algarrobo Costa	2009			202 (103–418)[(6)]			10 (2–22)#[(6)]	42	
	Sagunt	2010			129 (99–167)[(6)]			6 (4–9)#[(6)]	50	
	Average/ Total[(7)]		1314	1.05 ± 0.08	162 (134–196)	76.7	94	8 (6–11)#	45	
2016–2019										
	Sagunt	2016	311	0.55 ± 0.18	19 (1.4–44)	14.5*	17	1 (0.1–6)	67	
	Algarrobo Costa	2016	233	1.09 ± 0.18	46 (28–74)	14.7*	14	2 (1.3–4)#	68	
	Blanca	2016	201	1.19 ± 0.24	136 (70–431)	16.4*	10	7 (3–14)#	48	
	Alcalà de Xivert	2017	60							60 ± 6*
	Vinaròs	2017	60							53 ± 5*
	Algarrobo Costa	2017	60							23 ± 2*
	Vila-real	2017	60							45 ± 9*
	Rafelguaraf	2019	440	0.85 ± 0.10	863 (524–1758)	23.2*	18	50 (7–350)#	24	
	Average/ Total[(7)]		1185	0.57 ± 0.07	208 (108–508)	205.51	65	12 (7–21)#	45	45 ± 9§

[(1)] Number of flies considered in the Probit analysis (including non-treated), or number of flies exposed to 125 ppm lambda-cyhalothrin.
[(2)] Lethal concentration 50 (LC_{50}) in ppm of lambda-cyhalothrin in the diet at 48h. Feeding assays performed with Karate Zeon (lambda-cyhalothrin 100 g L^{-1}, CS; SyngentaAgro S.A., Madrid, Spain).
[(3)] Resistance ratio (RR) = LC_{50} (field strain)/LC_{50} (C strain, LC_{50} (95%FL) = 21 (13–29) for 2008-2010 period;[6] 17 (7–35) for 2016–2019 period[13]). The fiducial limits for RR were calculated according to Robertson and Preisler (1992).[20]
[(4)] Expected mortality to 125 ppm of lambda-cyhalothrin, estimated from Probit analysis by Finney transformation.
[(5)] Mortality to 125 ppm of lambda-cyhalothrin (recommended for field treatments).
[(6)] Data from Arouri *et al* (2015).[6]
[(7)] The expected mortality is an average in that period, while LC_{50}, RR and statistic parameters come from a Probit analysis performed with the total amount of individuals tested in each period.
RR is significant ($P < 0.05$) if the 95% FL does not include 1.
*Good fit of the data to the Probit model ($P > 0.05$).
§ Observed mortality is significantly different to mortality of C strain to 100 ppm (98% ± 2%)[13] (ANOVA, Tukey *post hoc*, $P \le 0.05$).

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2.5 Statistics

Susceptibility to lambda-cyhalothrin of *C. capitata* field populations was analyzed using mortality data to estimate LC_{50} values (concentration needed to cause 50% mortality). Probit analysis was performed using the program POLO-PC (LeOra Software14), which corrects samples' mortality by control (non-treated) mortality using Abbott's transformation.[19] Resistance ratios (RR = LC_{50} (field or lab strain)/LC_{50} (C strain)) were considered significant if their 95% fiducial limits did not include 1.[20] Susceptibility to 125 ppm lambda-cyhalothrin (percentage mortality data were previously Arcsin transformed) were compared by ANOVA followed by Tukey *post hoc* test. Data were statistically analyzed with Levene and Shapiro–Wilk tests to check homogeneity and normality, respectively.

3 RESULTS

3.1 Susceptibility to lambda-cyhalothrin in field populations of *Ceratitis capitata*

We tested lambda-cyhalothrin susceptibility of field populations from the Spanish Mediterranean area collected in the period 2016–2019 by concentration-response or 125 ppm concentration (recommended for field treatments) assays (Table 3). Results showed variability in the susceptibility, with: (i) populations highly resistant (Blanca 2016 and Rafelguaraf 2019, with LC_{50} higher than 125 ppm; and Algarrobo Costa 2017 and Vila-real 2017, in which less than 50% of mortality at 125 ppm was observed): (ii) populations with moderate but significantly different levels of resistance compared to the susceptible C strain (Algarrobo Costa 2016 with LC_{50} lower than 125 ppm; and Alcalà de Xivert 2017 and Vinaròs 2017, which had more than 50% of mortality to 125 ppm); and (iii) susceptible populations (Sagunt 2016, which LC_{50} value was not significantly different from the susceptible C strain). On average, the observed mortality at 125 ppm was 45% (varying between 23% and 60%), and the same value was obtained when estimated from concentration-response assays (varying between 24% and 68%). Data from field populations collected in the period 2009–2010[6] were reanalyzed to also estimate the expected mortality at 125 ppm (Table 3). The outcome of this analysis showed that the expected mortality at the concentration used in field treatments varies between 32% and 50% with an average value of 45%. These results indicate that the level of susceptibility to lambda-cyhalothrin in field populations has not changed significantly in the last decade.

The three regions of the VGSC gene that concentrate most of the kdr mutations associated to pyrethroids resistance in insect species (Supplementary Fig. S2, adapted from Dong *et al.*, 2014)[21] were sequenced in flies from Blanca, Vinaròs and Rafelguaraf that survived the treatments with pyrethroids (Table 3). We did not find mutations in any of the 20 individuals analyzed from each population, suggesting that target-site resistance was not present in the three field populations analyzed. We also tested the effect of the synergist PBO on the field population that showed the highest levels of resistance to lambda-cyhalothrin (Rafelguaraf: LC_{50} = 863 ppm, RR = 50, Table 3), with the result that resistance was completely reverted (Rafelguaraf + PBO: LC_{50} = 14.2 ppm, RR = 0.8, n = 238, slope ± SE = 0.78 ± 0.16, χ^2 = 8.2, df = 7).

3.2 Modelling of lambda-cyhalothrin resistance evolution

To construct the evolutionary model, we first estimated the frequency of the resistant and susceptible alleles and genotypes in field populations and explored which values of selected parameters (inheritance and fitness cost) better fit with the stability in the levels of resistance currently observed in Spanish field populations with respect to those observed when first detected. We performed this analysis at regional level (Spanish Mediterranean area), assuming that spatial heterogeneity was minimal because the high levels of gene flow among populations[16] and the use of the same insecticides (lambda-cyhalothrin and spinosad) in the studied area during the last decade.

The mortality data at field concentrations during the periods 2009–2010 and 2016–2019 were pooled to estimate the frequency of the resistant and susceptible alleles and genotypes during the last decade, since there were no differences in the mean susceptibility between these two periods. This yielded the initial frequencies of genotypes *SS*, *SR* and RR under the consideration that the inheritance of resistance is monogenic and dominant, co-dominant or recessive (Table 2(a)). To compare the dynamics of the monogenic and polygenic models, we have to establish a direct relationship between the initial values of both models. To this purpose, the initial frequency found in the monogenic model for sensitive and resistant genotypes; namely, F (sensitive) = 0.44 and F (resistant) = 0.56, was distributed between sensitive and resistant genotypes according to their frequencies under the polygenic (with two genes contributing to resistance) model (Table 2(b)). For the case where resistance was assumed dominant, the expected mortality value (0.44) was distributed among the sensitive genotypes following their frequencies according to a panmictic population: (*S1S1 S2S2*) = (1/16), (*S1S1 S2R2*) = (2/16), (*S1S1 R2R2*) = (1/16), (*S1R1 S2S2*) = (2/16) and (*R1R1 S2S2*) = (1/16). Therefore, (0.44) was distributed among these five sensitive genotypes, resulting in the following initial frequencies: (*S1S1 S2S2*) = 0.062, (*S1S1 S2R2*) = 0.124, (*S1S1 R2R2*) = 0.062, (*S1R1 S2S2*) = 0.124 and (*R1R1 S2S2*) = 0.062. The resistance value (0.56) was also distributed among the resistant genotypes following their frequencies according to a panmictic population: (*R1R1 R2R2*) = (1/16), (*R1R1 S2R2*) = (2/16), (*S1R1 R2R2*) = (2/16) and (*S1R1 S2R2*) = (4/16). Therefore, 0.56 was distributed among these four resistant genotypes, resulting in the following initial frequencies: (*R1R1 R2R2*) = 0.063, (*R1R1 S2R2*) = 0.126, (*S1R1 R2R2*) = 0.126, and (*S1R1 S2R2*) = 0.252. The same approach was followed to estimate the initial frequencies under the assumption of co-dominant or recessive inheritance.

We then explored which values of fitness cost better fitted with the stability found in the resistant levels to lambda-cyhalothrin in field populations, assuming a dominant/co-dominant/recessive and monogenic/polygenic model. We modelled eight different scenarios of insecticide treatments strategies that include the use of lambda-cyhalothrin, alone or in combination with spinosad, the main strategies to control medflies in Spanish citrus crops in the last decade (Table 2(c)). For each simulation, we tested several fitness cost values (Table 2(a) and (b)) and considered that 50% of the flies in a population were exposed to the insecticide in each treatment (*e(i)* = 0.5). We found that under the assumption of dominant inheritance, there was a set of values of fitness cost (SC3) in which resistant alleles and genotypes initially increase (T1.4, T1.6, T1.7 and T1.8, in which the use of lambda-cyhalothrin was higher) or decrease (T1.1, T1.2, T1.3 and T1.5), but tend to stable levels after a number of generations for both, monogenic and polygenic models (Fig. 1). However, resistant alleles and genotypes tend to disappear in most of the treatment combinations for the highest values of fitness cost evaluated (SC1 and SC2), while they tend to fixate with the lowest values of fitness cost (SC4 and SC5). Remarkably, all of the combinations of fitness cost

and insecticide treatment scenarios tested, under the assumption of recessive inheritance, yielded unstable outcomes that led to the fixation of the resistant or the susceptible alleles, for both the monogenic and polygenic models (Supplementary Fig. S3). An intermediate situation was obtained under the assumption of co-dominant inheritance, in which stable allelic frequency levels were reached at intermediate values of fitness cost (SC3 and SC4), though for a reduced number of insecticide treatment scenarios (Supplementary Fig. S4). Thus, we decided to select dominant inheritance and SC3 fitness cost, which better fits with the experimental results obtained with field populations, to forecast lambda-cyhalothrin resistance evolution. Since either the monogenic or the polygenic models were compatible with these experimental results, both models were contemplated in our forecasting scenarios. It may be other potential solutions (sets of parametric values) that may result in stable gene frequencies that match our experimental field observations. However, we have focused our model parameterization efforts on those factors that we consider key in model predictions and restricted the scenarios tested to current agronomic practices, which we consider is more valuable for forecasting purposes.

3.3 Forecasting lambda-cyhalothrin resistance evolution under different insecticide treatment scenarios

For forecasting lambda-cyhalothrin resistance evolution, we consider those treatment scenarios when deltamethrin traps are also deployed in the field, in combination with lambda-cyhalothrin or with lambda-cyhalothrin and spinosad. Although deltamethrin use is growing, its adoption is variable within the studied area, which may originate spatial heterogeneity in the development of resistance. Thus, we used three levels of exposure for the three insecticides (0.2, 0.5 and 0.8), which represent the variability that may occur among citrus orchards and localities and modelled the expected consequences of the combined use of both pyrethroids depending on the degree of cross-resistance between them.

We first modelled the case in which lambda-cyhalothrin treatments and deltamethrin traps are combined (scenarios T2.2, T2.4, T2.6 and T2.8) and compared them with the use of only lambda-cyhalothrin treatments (scenarios T2.1, T2.3, T2.5 and T2.7) (Table 2(d)). We found that, for the most common case of medium exposure (e(i) = 0.5), resistant alleles tend to get stable in the population with F(R) between 0.2 and 0.95, under the assumption of either the monogenic or the polygenic model (Fig. 2). The frequency of resistant alleles when both insecticides are combined, with respect to the use of only lambda-cyhalothrin, is expected to diminish in the case of absence of cross-resistance (MO2) and to increase in case of complete (MO1) and partial (MO3.1, MO3.2 and MO3.3) cross-resistance. The higher increases of resistant alleles occurred when two or more treatments of lambda-cyhalothrin are combined with deltamethrin traps, cross resistance is partial, and heterozygous individuals are susceptible to deltamethrin (MO3.3). Resistant alleles would get fixed under this scenario in a polygenic model, but they would not completely displace S alleles in the 60 generations frame of the monogenic model prediction. Resistant alleles would also tend to get fixed in the population when lambda-cyhalothrin and deltamethrin are combined (T2.2, T2.4, T2.6 and T2.8), insecticide exposure is high (e(i) = 0.8) and cross-resistance is partial (MO3.2 and MO3.3 for the polygenic model and MO3.3 for the monogenic model) (Supplementary Fig. S5). On the other hand, resistant alleles would be eliminated from the population in the case of low

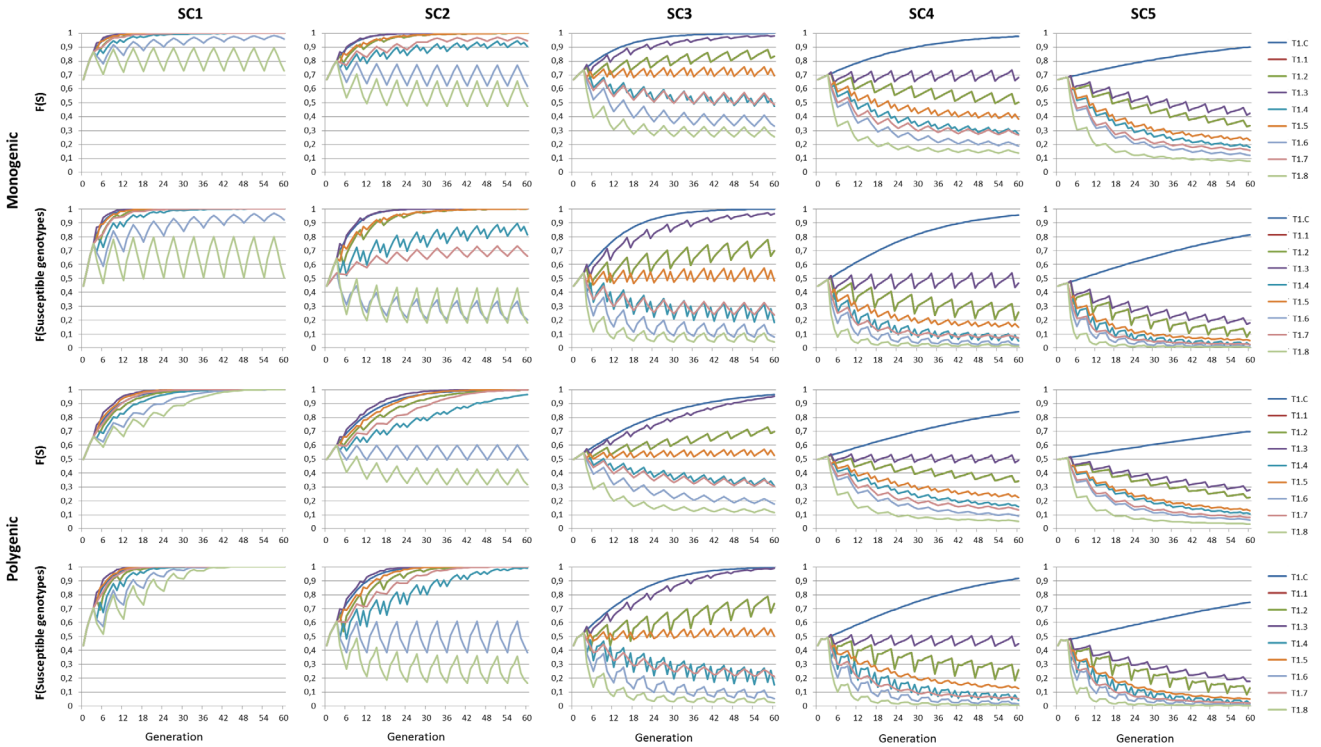

Figure 1. Predicted evolution of lambda-cyhalothrin susceptible allele F(S) and susceptible genotypes F(*Susceptible genotypes*) frequencies in a series of scenarios (T1.C-T1.8, Table 2(c)) when lambda-cyhalothrin and spinosad treatments alone or in combination are applied in the field and reach 50% of *Ceratitis capitata* individuals (e(i) = 0.5). Five different scenarios of fitness cost (SC1–SC5, Table 2(a)) were analyzed assuming dominant lambda-cyhalothrin resistance and both a monogenic or a polygenic model. The insecticide treatments T1.1 and T1.2 overlap under all scenarios tested.

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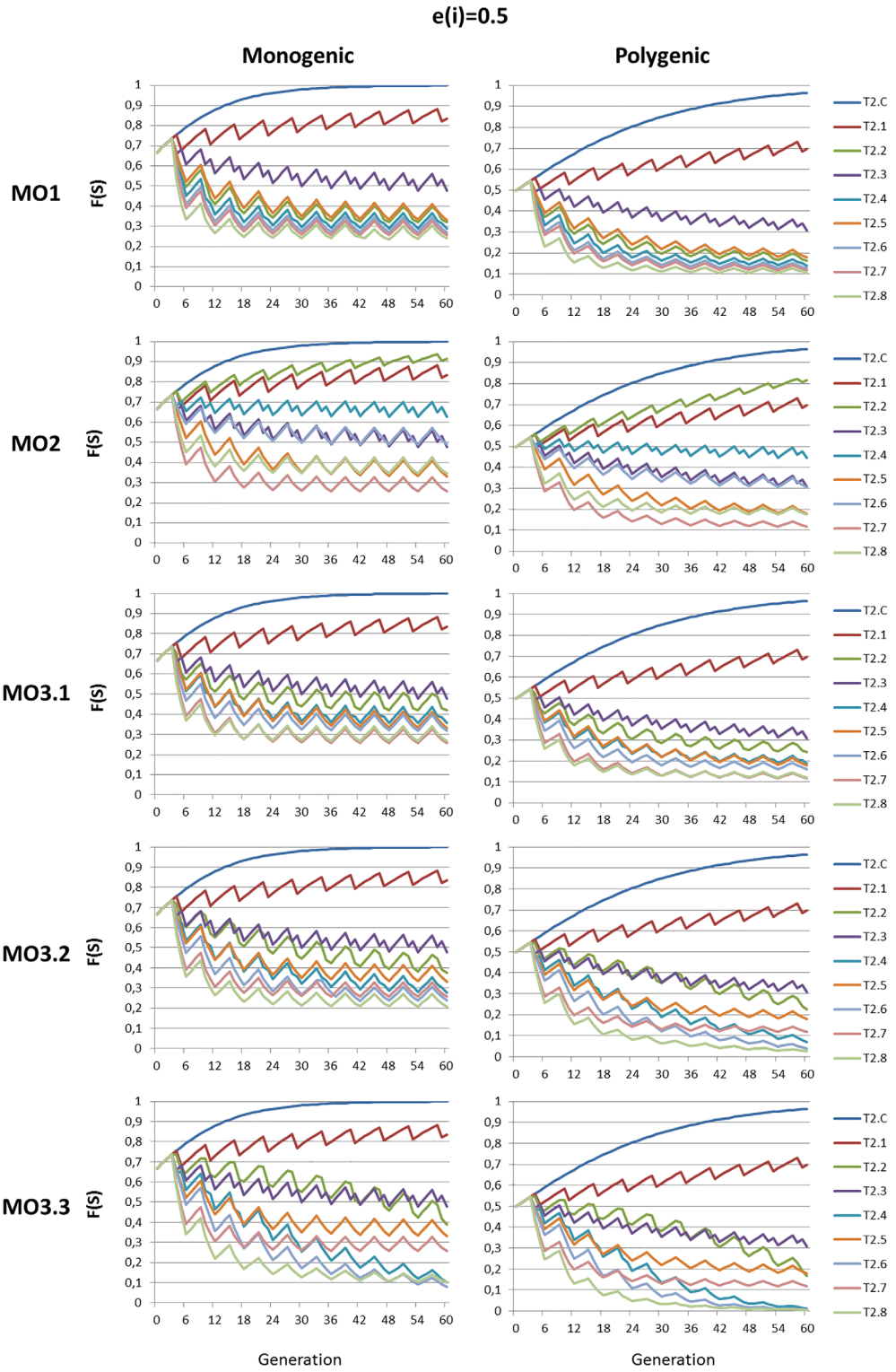

Figure 2. Predicted evolution of lambda-cyhalothrin susceptible allele frequency F(*S*) in a series of scenarios (T2.C-T2.8, Table 2(d)) when lambda-cyhalothrin treatments alone or in combination with lure-and-kill traps coated with deltamethrin are used in the field and reach 50% of *Ceratitis capitata* individuals (e(*i*) = 0.5). Five different expected mortalities (MO1-MO3.3, Table 2(a)) for deltamethrin exposure were considered in both a monogenic and a polygenic model.

exposure (e(*i*) = 0.2) and monogenic model when applying only one or two treatments of lambda-cyhalothrin (T2.1 and T2.3), non-depending on the degree of cross-resistance (MO1-MO3.3), and when applying one, two or three treatments of lambda-cyhalothrin and deltamethrin (T2.2, T2.4 and T2.6) in case of absence of cross-resistance (MO2) or when cross-resistance is partial (MO3.2 and MO3.3) (Supplementary Fig. S5). The same trends were forecasted for the polygenic

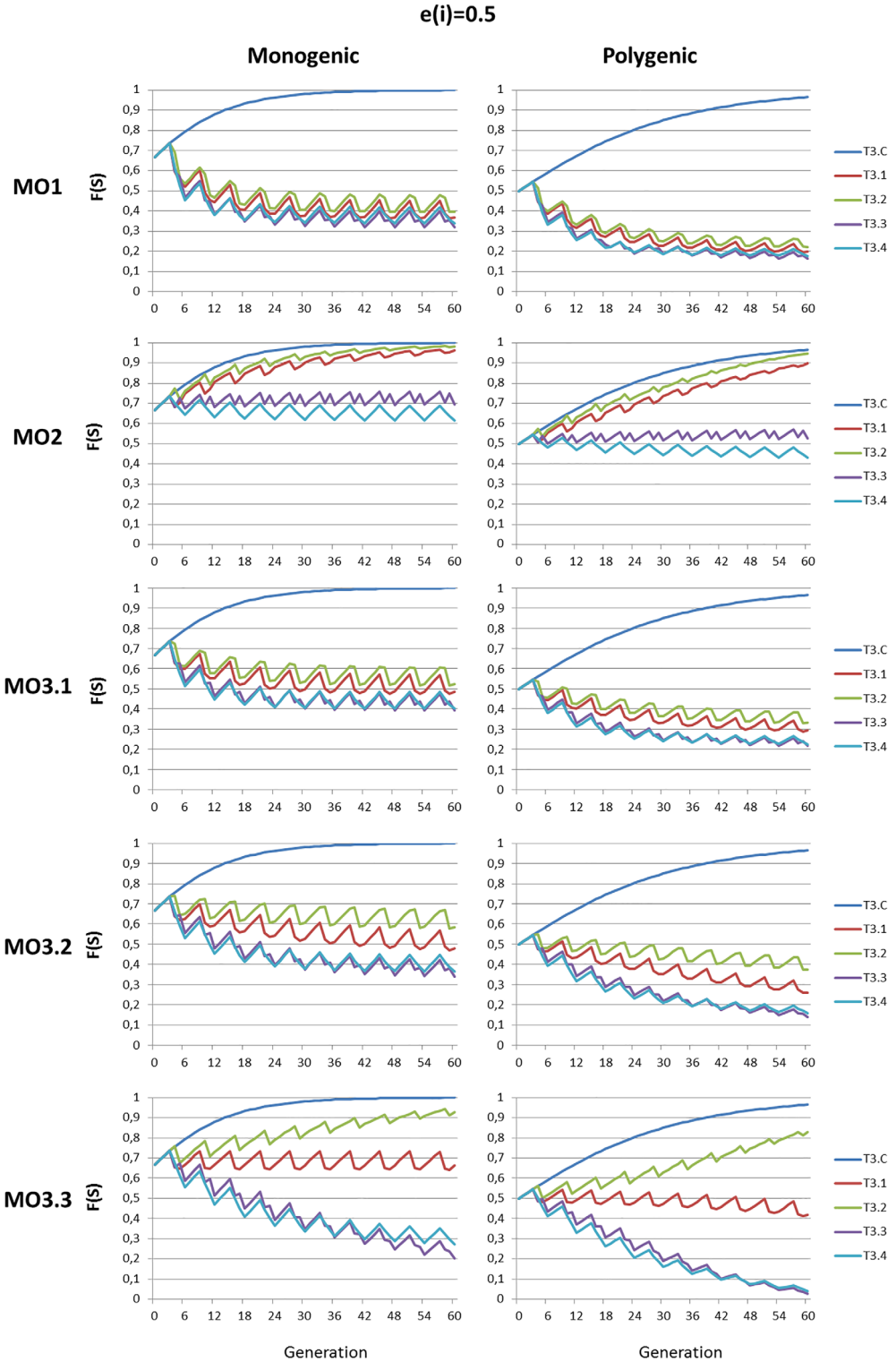

Figure 3. Predicted evolution of lambda-cyhalothrin susceptible allele frequency F(*S*) in a series of scenarios (T3.C-T3.4, Table 2(e)) when lambda-cyhalothrin and spinosad treatments are combined with lure-and-kill traps coated with deltamethrin and reach 50% of *Ceratitis capitata* field individuals (e(*i*) = 0.5). Five different expected mortalities (MO1-MO3.3, Table 2(a)) for deltamethrin exposure were considered in both a monogenic and a polygenic model.

model in the case of low exposure (e(*i*) = 0.2), but resistant alleles were not completely eliminated from the population in the 60 generations frame of the prediction (Supplementary Fig. S5).

We then modelled the expected effect of rotating lambda-cyhalothrin and spinosad, when deltamethrin traps are also deployed in the field (Table 2(e)). We found that, in general, for the case of medium exposure (e(*i*) = 0.5): (i) resistant alleles tend

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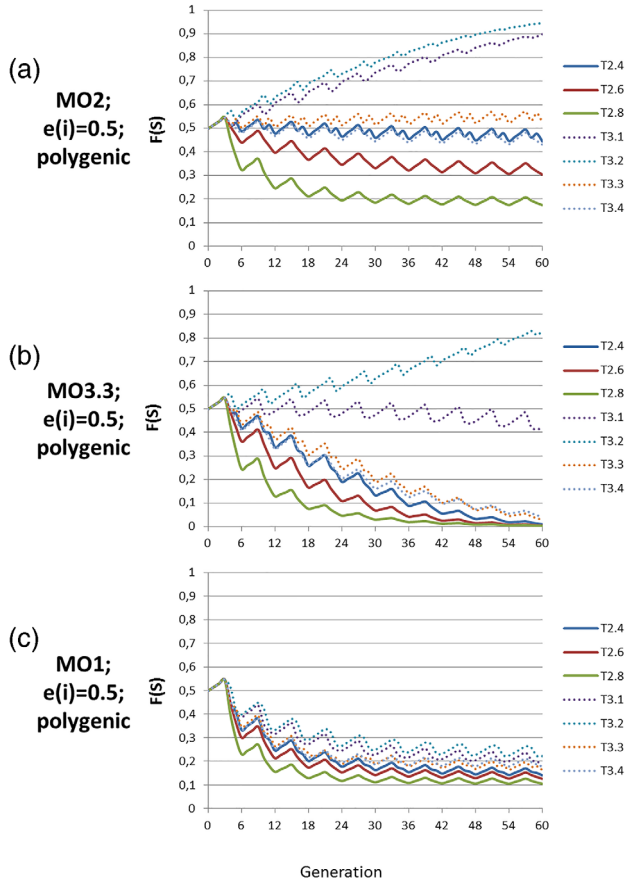

Figure 4. Predicted evolution of lambda-cyhalothrin susceptible allele frequency F(*S*) in selected scenarios when lambda-cyhalothrin treatments in combination with lure-and-kill traps coated with deltamethrin are applied (T2.4, T2.6 and T2.8, Table 2(d)) (continuous lines), or when lambda-cyhalothrin and spinosad treatments are combined with deltamethrin traps (T3.1, T3.2, T3.3 and T3.4, Table 2(e)) (spotted lines). It is considered that 50% of *Ceratitis capitata* field individuals are reached by the insecticide (e(*i*) = 0.5) and that resistance is polygenic. Three different expected mortalities for deltamethrin exposure were considered: (a) MO2; (b) MO3.3; and (c) MO1 (Table 2(a)).

to get stable in the population; (ii) the levels of resistant alleles under the polygenic model are slightly higher than under the monogenic model; and iii) there was an enrichment in susceptible alleles compared to the scenarios in which only lambda-cyhalothrin and deltamethrin traps are used (Fig. 3). The expected attenuation of resistance levels by the combined use of the three insecticides was especially remarkable in the case of no-cross resistance between both pyrethroids (MO2). The model predicts that the frequency of resistant alleles will not increase after two treatments with lambda-cyhalothrin and one with spinosad (T3.3), or three treatments with lambda-cyhalothrin and three with spinosad (T3.4), or even will be eliminated with one treatment with lambda-cyhalothrin and one (T3.1) or two (T3.2) with spinosad. However, an increase in resistant alleles is expected with three or more treatments with lambda-cyhalothrin (T2.6 and T2.8) and the resistant alleles are only maintained with two treatments with lambda-cyhalothrin (T2.4) (Fig. 4(a)). Likewise, when lambda-cyhalothrin is rotated with spinosad, the expected fixation of resistant alleles by the combined use of lambda-cyhalothrin and deltamethrin (T2.4, T2.6 and T2.8) if cross-resistance is partial and heterozygous individuals are susceptible to deltamethrin (MO3.3), will be delayed (T3.3 and T3.4), hold (T3.1) or even reversed (T3.2) (Fig. 4(b)). When cross-resistance was considered (MO1), a reduction in the frequency of resistant alleles was also forecasted for the combined use of the three insecticides (T3.1, T3.2, T3.3 and T3.4) with respect to only the two pyrethroids (T2.4, T2.6 and T2.8), though the reduction was less marked (Fig. 4(c)). A similar trend in the enrichment of susceptible alleles is expected for the cases of low (e(*i*) = 0.2) and high (e(*i*) = 0.8) exposure for both the monogenic and the polygenic models (Supplementary Fig. S6). Under low exposure conditions, resistant alleles will tend to disappear from the population, except for the case of cross-resistance (MO1) (Supplementary Fig. S6). However, resistant alleles will still get fixed, though with some delay, if high exposure to the insecticides happens (e(*i*) = 0.8) and cross-resistance is partial (MO3.3 for both the monogenic and the polygenic models, and MO3.2 for the polygenic model), independently of the treatment applied (Supplementary Fig. S6).

4 DISCUSSION

In the present work, we have shown that field resistance of *C. capitata* to lambda-cyhalothrin has remained stable during the last decade in the Spanish populations analyzed, without showing a remarkable increase or decrease with respect to the resistance levels observed when first detected in 2009–2010.[6] This result is noteworthy, as the available insecticides for the control of this pest in citrus crops are restricted to a few compounds, mainly lambda-cyhalothrin and spinosad as bait sprays, and deltamethrin as lure-and-kill traps. Thus, an increase in the levels of resistance to lambda-cyhalothrin could seriously compromise the fight against this pest. Field populations remain susceptible to spinosad, but the potential of *C. capitata* to develop resistance to this insecticide has already been proved in the laboratory,[22] and spinosad resistant alleles have been found in field populations at low frequencies.[7] Modeling performed by our group predicted that, if the fitness cost of field resistant individuals is equivalent to that estimated for resistant laboratory strains, the resistant alleles would rapidly decline over time resulting in the disappearance of spinosad resistant individuals from the field populations.[7] Nonetheless, if field-evolved spinosad resistance is associated with a low fitness cost, resistant individuals would be expected to rise in the field. A recent study did not reveal significant levels of resistance of field populations to deltamethrin and spinosad in Greece.[12] However, Guillem-Amat (2019)[18] reported that MagnetMed traps coated with deltamethrin do not provide a complete control for all Spanish field populations tested. Altogether, IRM strategies aimed to overcoming resistance to lambda-cyhalothrin and preventing the development of resistance to spinosad and deltamethrin are required.

Key components of an effective IRM strategy are the early detection of resistance and the reduction of selection pressure directed towards a particular insecticide, which favors the increase of resistant alleles in the populations.[23,24] Our modelling results suggest that the use of both lambda-cyhalothrin and spinosad during the last decade has been crucial to avoid an increase in the levels of resistance to lambda-cyhalothrin in field populations, and may explain why lambda-cyhalothrin continues being efficiently used for the control of *C. capitata*. A simulation study with a multi-resistant strain selected in the laboratory further supports this, since it was shown that the alternation of lambda-cyhalothrin with spinosad was enough to delay the development of resistance to lambda-cyhalothrin.[13] The most likely explanation for the efficacy of alternating lambda-

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cyhalothrin with spinosad is the lack of cross resistance between these two insecticides, since they belong to different chemical families and it has been shown that laboratory resistant strains to lambda-cyhalothrin, W-1Kλ,[6] and to spinosad, JW-100 s,[17] do not show cross-resistance to spinosad or lambda-cyhalothrin, respectively. In addition, the typology of commercial citrus orchards in the area of study, mostly small and adjacent patched fields, and the high mobility of this species between fields and host fruits,[25] favored that populations were exposed to both insecticides in the same or different generations, even in those cases in which a unique insecticide is applied in a particular field. Finally, high levels of gene flow reported for this species among Spanish populations[16] may have been a factor to explain why lambda-cyhalothrin resistance was already widely spread in Spain when first detected,[6] but may have also contributed to avoid the fixation of resistant alleles in particular areas.

Our theoretical model was also aimed at assessing the influence of main factors in resistance evolution for which there is still a degree of uncertainty. The importance of fitness cost in determining optimal resistance management strategies has already been highlighted.[26] We have explored which values of fitness cost better fit with the level of resistance to lambda-cyhalothrin observed in field populations in the last decade, which resulted 0.2 for *RR* and 0.1 for *RS* genotypes under the assumption of dominant inheritance for both the monogenic and polygenic models. These values are lower than those estimated for the fitness cost associated with genotypes carrying spinosad resistant alleles in *C. capitata* (0.4 for *RR* and 0.2 for *RS*), obtained in a study of their stability when in competition with individuals carrying the wild-type alleles under laboratory conditions.[7] These fitness cost values may be related to the trade-offs in life-history and behavioral traits reported, respectively, for lambda-cyhalothrin[13] and spinosad resistance,[27] but the distinct methodologies used for their estimation does not allow direct comparisons. Another aspect that may have an impact on the evolution of resistance is its type of inheritance. Pyrethroids resistance caused by alteration of the target site VGSC usually has an incompletely recessive inheritance pattern.[28] On the contrary, the case of metabolic resistance is variable, with examples that go from recessive[29] to dominant inheritance.[30] We analyzed if individuals from field populations resistant to lambda-cyhalothrin had resistance-associated mutations in the VGSC gene, which cause insensitivity to pyrethroids. Interestingly, 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,[21] suggesting that target-site resistance was not present in the field populations analyzed. Nevertheless, though the presence of mutations in other regions of the VGSC gene is unlikely, it cannot be discarded. We have also demonstrated that lambda-cyhalothrin resistance was reverted by PBO in the field population that showed the highest levels of resistance, as reported in the W-1Kλ strain,[6] which suggest that metabolic resistance mediated by P450 detoxification enzymes may be also involved in this particular population. However, further studies are needed to test if field resistance to lambda-cyhalothrin has the same molecular mechanism than the W-1Kλ strain. In any case, our experimental data support the results of the modelling analysis, since we have found that no stable outcomes are predicted under the assumption of recessive inheritance. Although resistance in the selected laboratory strain W-1Kλ was proved to be polygenic,[13] there is no evidence about lambda-cyhalothrin resistance inheritance in field populations. Our models forecast that the development of resistance would be similar independently of assuming a monogenic or a polygenic inheritance, with a slight increase in the frequency of resistant alleles under the polygenic model in all scenarios tested.

The treatment scenarios for medfly control in citrus crops in Spain have become more versatile in recent years due to the possibility to use lure-and-kill traps coated with deltamethrin, whose deployment is expected to increase in the future. This provides an additional insecticide which can be useful for resistance management proposes, but since lambda-cyhalothrin and deltamethrin are both pyrethroids, there is the possibility that cross-resistance occurs between them. Indeed, the laboratory lambda-cyhalothrin resistant strain W-1Kλ showed high levels of cross-resistance to deltamethrin by ingestion.[6] Our model predicts that, if cross-resistance (MO1) also occurs in field populations, the combined use of lambda-cyhalothrin sprays and deltamethrin traps is expected to increase the frequency of resistant alleles. Susceptible alleles were not predicted to be completely removed from the populations in any of the treatment scenarios, levels of exposure and inheritance models tested. Besides, the increase in resistant alleles could be partially reversed when spinosad is incorporated in rotation with lambda-cyhalothrin in fields where deltamethrin traps are also deployed. Thus, according to our model, the combination of dominant inheritance, in which the susceptible alleles are preserved in the heterozygotes, and the biological cost of resistance will avoid the fixation of resistant alleles, which will tend to stabilize in the population after a number of generations. Their frequency after they reach a steady-state, assuming cross-resistance, would then depend on the number of treatments with lambda-cyhalothrin and on the rotation or not with spinosad. However, preliminary studies suggest that susceptibility of medfly field populations to lambda-cyhalothrin and deltamethrin may not be always linked (unpublished results). Our models predict that in the absence of cross-resistance between both pyrethroids (MO2) or in the case of partial cross-resistance when the heterozygous are as susceptible to deltamethrin as the resistant homozygous (MO3.1), the frequency of resistant alleles would decrease or reach also a stable state, respectively. In both cases, deltamethrin penalizes S alleles relatively less than R alleles compared to lambda-cyhalothrin, which results in reduced selection against S alleles and a lower R allele equilibrium. However, if cross-resistance is partial and the heterozygous are as susceptible to deltamethrin as the susceptible homozygous (MO3.3) or have a medium susceptibility level between the susceptible and the resistant homozygous (MO3.2), the combined use of lambda-cyhalothrin sprays and deltamethrin traps would seriously compromise the maintenance of an equilibrium between susceptible and resistant alleles. Indeed, resistant alleles will be expected to get fixed in the population under certain conditions: MO3.3 for both the monogenic and the polygenic models at high exposure (e(i) = 0.8) and for the polygenic model at medium exposure (e(i) = 0.5); and MO3.2 at high exposure (e(i) = 0.8) for the polygenic model. When spinosad is rotated with lambda-cyhalothrin sprays in the same fields where deltamethrin traps are deployed, this effect can be retarded, but resistant alleles will still get fixed. This occurs because under the modalities MO3.2 and MO3.3 of partial cross-resistance the selection of genotype *RR* by deltamethrin is higher than that of the genotype *RS*, tending to eliminate susceptible alleles from the populations, whereas under the modality MO3.1 of partial cross-resistance and under total cross-resistance both the genotypes *RR* and *RS* are equally selected maintaining susceptible alleles in the populations. Current guidance for IRM establishes that insecticides should be applied at the proper dose/concentration rate to make sure that

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heterozygous individuals are killed and, therefore, that resistant alleles are removed from the population.[31] Thus, care should be taken to ensure that the lure-and-kill traps coated with deltamethrin that are deployed in the field must contain an insecticide concentration able to kill heterozygous individuals.

This work suggests that current treatment strategies that combine the use of lambda-cyhalothrin and spinosad are expected to continue being valuable for the sustainability of lambda-cyhalothrin. However, the lack of knowledge about cross-resistance between lambda-cyhalothrin and deltamethrin in field populations highlights the need to avoid the overuse of both pyrethroids in the same fields for long periods of time and to rotate with an insecticide from a different chemical family as spinosad. However, it is important to keep in mind that the use of spinosad also needs to be managed, as a previous work already demonstrated that an excessive use of spinosad without an appropriate IRM strategy to reduce the selection pressure over resistant alleles could contribute to the development of spinosad resistance in field populations.[7] In addition, the harmonization of insecticide treatments with other control methods as cultural practices or SIT[4] would contribute to a more rational and sustainable use of these chemical products.

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AUTHOR CONTRIBUTIONS

FO and LS conceived the study. FO, LS and AGA participated in the design of the experiments and the interpretation of the results. AGA, LS, ELE and JCS performed the experiments. AGA and FO wrote the first draft of the manuscript. All authors read, corrected and approved the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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