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Title: Resistance to lambda-cyhalothrin in Spanish field populations of *Ceratitis capitata* and metabolic resistance mediated by P450 in a resistant strain.

Running Title: Resistance to lambda-cyhalothrin in *Ceratitis capitata*.

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Abstract (less than 200 words)

BACKGROUND: The withdrawal of malathion in the European Union in 2009 resulted in a large increase of lambda-cyhalothrin applications for the control of *Ceratitis capitata* in Spanish citrus crops.

RESULTS: Spanish field populations of *C. capitata* have developed resistance to lambda-cyhalothrin (6 to 14-fold), being their LC_{50} values (129 - 287 ppm) higher than the recommended concentration for field treatments (125 ppm). These results contrast with the high susceptibility to lambda-cyhalothrin found in three Tunisian field populations. We have studied the mechanism of resistance in the laboratory selected resistant strain W-1Kλ (205-fold resistance). Bioassays with synergists showed that resistance was almost completely suppressed by the P450 inhibitor PBO. The study of the expression of 53 of the 74 currently annotated P450 genes in the *C. capitata* genome revealed that *CYP6A51* was overexpressed (13-18-fold) in the resistant strain. The W-1Kλ strain showed also high levels of cross-resistance to etofenprox (240-fold) and deltamethrin (150-fold).

CONCLUSION: Field-evolved resistance to lambda-cyhalothrin has been found in *C. capitata*. Metabolic resistance mediated by P450 appears to be the main resistance mechanism in the resistant strain W-1Kλ. The levels of cross-resistance found may compromise the effectiveness of other pyrethroids for the control of this species.

Keywords: (4-6) Fruit fly, pyrethroid, insecticide resistance, P450 overexpresion.

1 INTRODUCTION

One of the key issues to be addressed for the sustainability of current strategies for fruit flies control is the increasing cases of resistance to insecticides. In the last years, resistance to organophosphates and pyrethroids has been reported for field populations of the olive fly, *Bactrocera oleae*, in Greece and Cyprus; the oriental fruit fly, *B. dorsalis*, in Taiwan and mainland China; the melon fly, *B. cucurbitae*, in Taiwan; the peach fruit fly, *B. zonata*, in Pakistan; the lesser pumpkin fly, *Dacus ciliatus*, in Israel; and the Mediterranean fruit fly, *Ceratitis capitata*, in Spain (reviewed by Vontas et al., 2011).

Resistance to malathion was first reported in Spanish field populations of *C. capitata* in 2004-2005, due to the intensive use of this insecticide (Magaña et al., 2007). After the withdrawal of malathion in the European Union in 2009, lambda-cyhalothrin and spinosad have become the most widely used insecticides for the control of this pest in Spanish citrus crops. However, a study by Couso-Ferrer et al. (2011) showed that a field-derived malathion resistant strain (W-4Km) has low to moderate cross-resistance to other organophosphate insecticides (7-16-fold) and to lambda-cyhalothrin (3-fold). Besides, a lambda-cyhalothrin resistant (35-fold) strain (W-1Kλ) was obtained by selecting the W-4Km strain with lambda-cyhalothrin for 12 generations (Couso-Ferrer et al., 2011). This is especially relevant, since the reduced number of insecticides approved for *C. capitata* control, due to European legislation, limits the options of the farmers to use only a reduced number of effective insecticides. Thus, knowledge of the resistance status for these insecticides in field populations and the elucidation of the underlying mechanisms by which insects acquire resistance are essential for devising proactive resistance management strategies that can extend their useful life.

The resistance of the W-4Km strain to malathion has been associated with a mutation G328A in the target acetylcholinesterase (AChE) and to an unknown esterase-mediated mechanism (Magaña et al., 2008). Cross-resistance of the W-4Km

strain to other OPs and the carbamate carbaryl could be explained by the altered AChE. However, other mechanisms might contribute to the cross-resistance found to insecticides that do not target AChE, such as lambda-cyhalothrin. Couso-Ferrer et al. (2011) reported that the esterase inhibitor DEF synergize the activity of lambda-cyhalothrin against the W-1Kλ strain and that the esterase activity in this strain was increased compared to a susceptible strain, suggesting that esterases may be involved in the development of resistance to this insecticide. Metabolic resistance mediated by esterases has been associated with cross-resistance between malathion and pyrethroids in different insect species (Chen and Sun, 1994, Bisset et al., 1997, Heidari et al., 2005). Nevertheless, other resistant mechanisms may be also involved, since the resistance of the W-1Kλ strain to lambda-cyhalothrin was only partially reverted by DEF. In this regard, target site resistance resulting from mutations in the voltage-gated sodium channel (Soderlund and Knipple, 2003; Davies et al., 2008), metabolic resistance mediated by P450 enzymes or glutathione S-transferases (Feyereisen, 2012; Li et al., 2007), and decreased penetration (Liu and Shen, 2003; Lin et al., 2012) have also been reported to play a role in pyrethroid resistance.

In this study we have determined the susceptibility of Spanish field collected populations of *C. capitata* to lambda-cyhalothrin, and compared with that of field collected populations from another country in the Mediterranean area (Tunisia) and with a susceptible laboratory strain. We have also further selected the lambda-cyhalothrin resistant strain W-1Kλ, and tested its susceptibility to the insecticides currently approved for *C. capitata* control in citrus crops in Spain. Finally, we have investigated the mechanism associated to lambda-cyhalothrin resistance in the W-1Kλ strain.

2 MATERIALS AND METHODS

2.1 Field sampling

C. capitata were obtained from infested fruits collected in fruit (citrus, apple and cherimoya) orchards, which had received different insecticide treatments, at five different localities in Spain in 2009 and 2010 (Table 1). The infested fruits were placed in plastic trays inside ventilated containers, both with a layer or vermiculite. They were kept in an environmentally controlled rearing room, at a photoperiod of 16 h light and 8 h dark, and a temperature of 26 ± 3ºC, until pupation occurred. Emerged adults (F0) were daily collected for bioassays.

Pupae obtained from infested fruits collected in 2011 at three different localities in Tunisia (Table 1) were sent to our laboratory in Spain. They were kept as described above to obtain adults (F0) for testing.

2.2 Laboratory strains

The laboratory strain (C) was established at the Instituto Valenciano de Investigaciones Agrarias (Valencia, Spain) from wild *C. capitata* collected at non-treated experimental fields in 2001 and has been maintained in our laboratory without exposure to insecticides at standard conditions (22-25ºC and a photoperiod of 16 h light and 8 h dark).

The lambda-cyhalothrin resistant strain (W-1Kλ) was obtained by laboratory selection performed on the malathion-resistant strain (W-4Km) with increasing concentrations of lambda-cyhalothrin as described in Couso-Ferrer et al. (2011). From generation twelve, the selection pressure is maintained at 1,000 ppm of lambda-cyhalothrin.

2.3 Chemicals

Insecticides tested were lambda-cyhalothrin (Karate Zeon, 100 g liter^{-1} CS, SyngentaAgro S.A., Madrid, Spain), deltamethrin (Decis protech, 15 g liter^{-1}, EW, Bayer Cropscience S.A., Lyon, France), etofenprox (Shark, 300 g liter^{-1}, EC, Sipcam Inagra S.A.,Valencia, Spain), methyl-chlorpyrifos (Reldan*E, 224 g liter^{-1} EC, Dow

Agrosciences Ibérica, Madrid, Spain), malathion (Agromalathion, 500 g liter^{-1} EC, Agrofit S. Coop., Valencia, Spain), spinosad (880g kg^{-1} technical, Dow AgroSciences LLC, Indianapolis, IN) and lufenuron (technical grade, 99.4 g kg^{-1}, Syngenta, Basel, Switzerland). The synergists assayed were piperonyl butoxide (PBO; 90% technical, Aldrich, Milwaukee, WI), S,S,S-tributyl phosphorotrithioate (DEF; 97.2% technical, Chem Service, West Chester, PA), triphenil phosphate (TPP, 98% technical, Fluka, Madrid, Spain), and diethyl maleate (DEM, 97% technical, Aldrich).

2.4 Bioassays

Feeding bioassays were performed to assess the susceptibility of field populations and laboratory strains to insecticides. The arena for the bioassays consisted of ventilated plastic dishes (89 mm in diameter, 23 mm in height), containing water and rearing diet (4:1:0.1, glass sucrose:hydrolyzed yeast:water) with the appropriate concentration of insecticide. Ten to fifteen adults (3-5 d old) were confined per plastic dish. The adults from the C and W-1Kλ strains were starved for 24 h before treatments. For the Spanish field populations and for the C and W-1Kλ strains four to seven different concentrations which resulted in >0 and <100% mortality were tested, and three-four replicates were performed for each concentration. Discriminating concentrations of 20, 30 and 60 ppm lambda-cyhalothrin was tested on individuals obtained from Tunisian fields (3-4 replicates of 10-13 flies for each concentration). In all cases the control consisted of diet mixed with water. Adult flies were kept in an environmentally controlled chamber during the tests, under the conditions indicated above. Mortality was recorded after 48 h. Flies were considered dead if they were ataxic.

The susceptibility of the C and W-1Kλ strains to lambda-cyhalothrin was also determined by topical application. Adult flies (3-5 d old) were maintained at 4°C for 30 min; thereafter, a 0.5 μl drop of insecticide solution in acetone or acetone alone (used as control) was applied to the dorsal thorax of each fly by using an automatic

microapplicator 900X (Burkard Manufacturing Co., Hertfordshire, United Kingdom). Four replicates per dose (calculated as μg of insecticide per g of fresh weight of insect, assuming an average weight of 10 mg) were performed. After treatment, insects were placed in the ventilated plastic dish containing water and rearing diet. The mortality was recorded after 48 h. To measure the recovery from knock-down after topical application of lambda-cyhalothrin a lower range of doses (0.4-1.0 μg/g) was tested and recorded at 0, 2, 4, 8, 24 and 48 h after treatment.

Sterility bioassays were performed to determine the sterile effect of lufenuron in the W-1Kλ strain. Lufenuron was dissolved in acetone (100 mg of lufenuron in 25 ml of acetone), and the stock solution diluted with acetone to obtain the desired concentrations. Five ml volume of each concentration was added to 10 g of the rearing diet without water and homogenized in a mortar. The final product was left air dry for 1 h in the laboratory. Five mated females (5-7 d old), previously starved for 24 h, were placed in Plexiglas cages (10 by 10 by 10 cm) with a mesh screen on one side. The flies were fed with lufenuron-treated diet dispensed in Eppendorf tips for 24 h and water was dispensed in 3-ml vials with a cloth strip (Ubesol, Valencia, Spain) to prevent flies from drowning. Thereafter, the lufenuron-treated diet was removed, and rearing diet without lufenuron was placed in each cage. Females laid eggs through the mesh screen, and the eggs fell to a plastic container filled with water. One hundred and fifty eggs per cage, laid between 24-48 h after the bait ingestion, were collected with a Pasteur pipette and placed onto three petri dishes with agar gel (3 g liter^{-1}), 50 eggs per petri dish. Three days after the eggs were placed in the dishes, egg hatch was evaluated, by using a stereomicroscope (model MZ75, 40r, Leica Microsystems, Heerbrugg, Switzerland). Eight replicates per concentration (in total, about 1200 eggs per concentration) were performed.

The synergists PBO, DEF, TPP and DEM were diluted in acetone and applied topically to adult flies of the C and W-1Kλ strains, as described above. The applied

doses (0.5 μg PBO, 1 μg DEF, 5 μg of TPP or 1 μg of DEM per insect) showed no mortality on adults from the C strain. Acetone was used as a control. After 2 h, the flies were treated with lambda-cyhalothrin as described in the feeding bioassay.

2.5 RNA extraction, reverse transcription and real-time quantitative PCR of *C. capitata* P450 genes

Total RNA was extracted from groups of 5 adult flies (3-4 d old) using TRIzol® reagent (Molecular Research Center, Cincinnati, USA) following the manufacturer's instructions. The RNA samples were quantified using a spectrophotometer (NanoDrop Technologie Inc) and their integrity confirmed by 1% agarose gel electrophoresis.

First strand cDNA was synthesized from total RNA using iSCRIPT synthesis kit (Invitrogen) following the manufacturer's protocol. The reaction contained 4 μl of 5x iSCRIPT reaction mix, 1μl of iSCRIPT reverse transcriptase, 1μg of RNA, and nuclease free water to a final volume of 20 μl. Each cDNA reverse transcription reaction was performed using the following parameters: 25ºC for 5 min, 42ºC for 30 min and 85ºC for 5 min. The cDNA was stored at -20ºC and diluted to the required concentration for gene expression in nuclease free sterile distilled water.

Real-time quantitative PCR (qPCR) amplification was performed using specific primers for *C. capitata* P450 genes (Table S1). The primers were designed in base to the first assembly of *C. capitata* genome (access given by the USDA-funded Medfly Whole Genome Sequencing Project before automatic annotation). At present, the new assambly Ccap_1.0 has been released to the GenBank database, and accesion numbers are provided for the genes studied in this paper. For some of the genes, two or three different pairs of primers were used. The actin, Rpl tubulin beta-3 and tubulin alpha-1 genes of *C. capitata* (GenBank Accession numbers XM_004527356, XM_004518966, XM_004520879 and XM_004519499, respectively) were included in the qPCR as reference genes, being the actin and Rpl genes selected due to their

stability across samples. The amplification efficiency of each gene was estimated by using the equation: $E=10^{-1/slope}$, where the slope was derived from the plot of amplification critical time (Ct value) versus serially diluted template cDNA.

The qPCR Master mix (15 μl) was composed of 5 μl of cDNA diluted 50-fold, 7.5μl of qPCR Master mix plus for SYBR Green (Eurogentec, Belgium), and 3.6 μM of each gene specific primers. Sterile water (5μl) was used for blank negative controls. All qPCR reactions were performed using the continuous fluorescence detector DNA Engine Opticon 2 (Bio-Rad, Hercules, CA, USA), at the following temperature cycling conditions: 2 min at 50ºC to activate the polymerase, 10 min at 95ºC to denature the samples followed by 40 cycles of 95ºC for 30 s, 60ºC for 30 s, and 72ºC for 30 s. A melting curve was performed after each qPCR in order to verify that the PCR products showed the correct melting temperature (Tm) for the predicted transcript. Amplification of the target and reference genes was made on the same plate to minimize intra-plate variation. Three biological replicates were analyzed for C and W-1Kλ strains and all reactions were run in duplicate to minimize intra-experimental variation.

2.6 Sequentiation of the 5´UTR region of the *CYP6A51* gene

DNA was extracted from adult flies of the C and W-1Kλ strains using the DNA easy blood and tissue kit (Qiagen) following the manufacturer's instructions. The 5´UTR region of the *CYP6A51* gene was amplified from genomic DNA by PCR using the specific primers: F-ACGCGTACGCCTGTTTACTT, R-ATAAGTGCCACGGGTCTGAA, and Amplitaq Gold® (Roche Molecular Systems, Inc.). Thermocycler conditions were: 5 min at 95ºC to denaturate the sample, followed by 35 cycles at 95ºC for 30 s, 60ºC for 30 s, and 72ºC for 30 s. The PCR product was purified using Prep-A-Gene DNA purification kit (Bio-Rad) and sequenced in Eurogentec (France). Sequences of 12 and 14 adult flies from the C and W-1Kλ strains, respectively, were aligned and compared using the MegaAlign program from DNA star (Madison, USA).

2.7 Data analysis

Mortality data were used to estimate the concentrations needed to cause 50% mortality (LC_{50}) by probit analysis using the computer program POLO-PC (LeOra Software, 1997), which automatically corrected for control mortality by Abbott´s transformation. For lufenuron, the effective concentration that produces a 50% reduction in fertility (EC_{50}) was calculated. Lethal concentration ratios (LCR) of field populations and resistance ratios (RR) of selected strains were calculated as the LC_{50} value of these populations or strains with respect to the LC_{50} calculated for the control strain in each case. Synergistic ratios (SR) were calculated as the LC_{50} value without synergist with respect to the LC_{50} value with synergist. The LCR, RR and SR ratios were considered significant if the 95% fiducial limit (FL) did not include 1 (Robertson et al., 2007). Mortality data when using discriminating concentrations were subjected to arcsine square root transformation and compared by ANOVA followed by Dunnett´s test.

Analysis of qPCR data was based on the average of three replicates using the comparative C_t method ($2^{-\Delta Ct}$ method) that uses an arithmetic formula to calculate the relative changes in gene expression based on the amplification critical time (C_t) of the real time PCR reactions (Pfaffl, 2001). All the results were analyzed with a program, developed at the INRA Centre de Sophia-Antipolis (France) using the R software (www.r-project.org), that allows normalization of each gene expression level as well as the integration of the technical replicates and amplifications efficiencies and associated errors. Statistical analysis of normalized qPCR data was performed by non-parametric sign-test with R software. The distribution of allelic variants of the 5´UTR region of the *CYP6A51* gene in the C and W-1Kλ strains was compared by Chi-squared test.

3 RESULTS

3.1 Susceptibility of field populations to lambda-cyhalothrin

All Spanish field populations tested, regardless of the insecticide treatment regimes (Table 1), were significantly less susceptible to lambda-cyhalothrin (LC_{50} between 129 and 287 ppm) than the laboratory strain (LC_{50}=20 ppm) (Table 2). The largest lethal concentration ratio (LCR) with respect to the C strain corresponded to the population from Castellsera (14-fold) and the lowest to Sagunto (6-fold). On the contrary, the susceptibility to lambda-cyhalothrin of Tunisian field populations was similar to that of the C strain (Table 3). When a discriminating concentration of 30 ppm of lambda-cyhalothrin was tested, the levels of mortality ranged between 56% and 73% for the three Tunisian field populations, which resulted not significantly different from the C strain (61%). In the case of the polplation form Laazib, two additional concentrations of 20 and 60 ppm were tested, being the results similar to those obtained with the C strain.

3.2 Susceptibility of the W-1Kλ strain to lambda-cyhalothrin and cross-resistance to other insecticides

The susceptibility to lambda-cyhalothrin of the resistant W-1Kλ and the susceptible C strains was assayed by ingestion and topical application (Table 4). In both cases the W-1Kλ strain was significantly more resistant to lambda-cyhalothrin than the C strain, although the resistance ratio (RR) was higher by ingestion (205-fold) than by topical application (4.9-fold). Cross-resistance of the W-1Kλ strain to other pyrethroids, OPs, spinosad and a benzoylphenylurea was tested by ingestion (Table 4). The highest resistant ratios were obtained with other pyrethroid insecticides, being the W-1Kλ strain 150- and 240-fold more resistant than C to deltamethrin and etofenprox, respectively. Differences in susceptibility between both strains were also obtained for the other insecticides tested, being the resistant ratios 3.8-fold for methyl-clorpyrifos, 6.1-fold for malathion, 2.0-fold for spinosad and 5.4-fold for lufeneron.

3.3 Effect of synergists on the toxicity of lambda-cyhalothrin

Adults of the C and W-1Kλ strains were pre-treated with the synergists: PBO (P450 inhibitor); DEF (esterase inhibitor); TPP (inhibitor of aliesterases); and DEM (inhibitor of glutathione S-transferases) (Table 5). The pre-treatment of adults of the W-1Kλ strain with PBO reduced the LC_{50} for lambda cyhalothrin from 3678 to only 72 ppm, which represents a synergistic ratio (SR) of 51. Lambda-cyhalothrin resistance in the W-1Kλ strain was also partially suppressed by DEF (SR = 2.7), but not by TPP (SR = 1.3) or DEM (SR = 1.1). In the C strain, PBO (SR = 1.5), DEF (SR = 0.8), TPP (SR = 1.1) and DEM (SR = 1.3) had no effect on the toxicity of lambda-cyhalothrin.

3.4 Recovery from knock-down effect of lambda-cyhalothrin

All individuals tested from both strains were immediately (0 h) knocked down after topical application with lambda-cyhalothrin at all doses tested (0.4 to 1.0 μg/g fresh weight of insect) (Figure 1). Less than 50% of the flies of the W-1Kλ strain recovered from knock-down 2 hours after the treatment and complete recovery for all individuals was only achieved between 8 and 24 h after treatment for all doses. The recovery of individuals from the C strain progressed slightly slower and some of them die (do not recover after 48 h), especially at the highest dose tested.

3.5 Relative expression of CYP genes in adult flies from C and W-1Kλ strains of *C. capitata*

The expression of genes from the CYP4, CYP6, CYP9 and CYP12 families in adults flies from the W-1Kλ strain were analyzed by qPCR and compared with the expression in the C strain (Table 6). For those genes where two or three different pairs of primers were used, similar relative expression levels were obtained, indicating the consistency of the results. Of the 53 CYP genes tested, only two genes of the CYP6A subfamily with GenBank Accession numbers XM_004534804 (13- to 18.3-fold depending on the

pair of primers used) and XM_004534802 (2.6-fold) showed significantly higher expression levels in the W-1Kλ strain. The first of these two genes has been designated as *CYP6A51* gene by David R. Nelson (http://drnelson.uthsc.edu/biblioB.html#6A). The expression of the second gene was in the limit of the optimal range of detection under our RT-qPCR conditions (Ct values 31,0 in average for the resistant strain) and was not further studied.

The induction of the expresion of *CYP6A51* was analyzed by exposing adults of the C and W-1Kλ strains to a diet containing 20 or 4,000 ppm of lambda-cyhalothrin, respectively. As seen in Figure 2, *CYP6A51* was similarly induced in both the susceptible (2.7-fold) and the resistant (1.6-fold) strains. It is worth to note that differences were again detected in *CYP6A51* expression levels comparing non-treated W-1Kλ and C strains (11.63-fold) in accordance with previous results.

3.6 Sequence of the 5´UTR region of the *CYP6A51* gene

The 5´UTR region comprinsing 484 bp upstream the ATG start codon of the *CYP6A51* gene was sequenced in 14 adult flies of the resistant W-1Kλ strain and 12 adults of the susceptible C strain (Figure 3). Two different sequences were obtained that represent two allelic variants: allele 1 (GenBank Accession number KF305738) and allele 2 (GenBank Accession number KF305739). However, there was not a differential distribution of these two alleles in resistant and susceptible individuals ($P<0.05$, Chi-squared test). From 14 individuals of the W-1Kλ strain analyzed, four were homozygous for allele 1, one was homozygous for allele 2, and nine were heterozygous. From the 12 individuals of the C strain analyzed, two were homozygous for allele 1, five were homozygous for allele 2, and five were heterozygous.

4 DISCUSSION

Our results indicate that Spanish field populations of *C. capitata* have developed resistance to lambda-cyhalothrin, being the concentration of lambda-cyhalothrin recommended for field treatments (125 ppm) lower than the LC_{50} values (between 129 and 287 ppm) obtained for populations from different geographical areas. These results contrast with the susceptibility to lambda-cyhalothrin of three different Tunisian field populations, similar to that of the laboratory strain. Other insecticides currently approved for *C. capitata* control in citrus crops in Spain are etofenprox, methyl-clorpyrifos, lufenuron and spinosad. We have tested the susceptibility of the lambda-cyhalothrin resistant strain W-1Kλ to these insecticides, to deltamethrin used against *C. capitata* in other crops, and to malathion that was used in the past for the control of this species. The level of resistance to lambda-cyhalothrin of W-1Kλ increased from 35-fold (Couso-Ferrer et al., 2011) to 205-fold after 24 more generations of selection pressure. The LC_{50} value (4,224 ppm) is 30 times higher than the recommended dose in the field for lambda-cyhalothrin, and similar to the high levels of pyrethroids resistance obtained by selection of laboratory strains of *B. dorsalis* (131-fold for fenvalerate and 125-fold for alphamethrin) (Hsu et al., 2004; Pan et al., 2008). Remarkably, W-1Kλ showed high levels of cross-resistance to the pyrethroids deltamethrin (150-fold) and etofenprox (243-fold). On the contrary, low levels of cross-resistance (3-6-fold) were detected to OPs (methyl-chloropyrifos and malathion), spinosad and lufenuron. These results indicate that the development of resistance to lambda-cyhalothrin in field populations may compromise the effectiveness of other pyrethroids for the control of this species, as already reported for other dipteran species (Sheppard and Joyce, 1992; Liu and Yue, 2000). Thus, the use of insecticides that do not show cross-resistance with lambda-cyhalothrin, such as spinosad, appears more appropriate for those areas where resistance to lambda-cyhalothrin is detected, to avoid failures in controlling *C. capitata*. Nevertheless, it has also been demonstrated the capacity of this species to develop resistance to spinosad by laboratory selection (Couso-Ferrer, 2012).

Three major mechanisms have been involved in resistance to pyrethroids: target site insensitivity, metabolic detoxification, and decreased cuticular penetration of insecticides (Hemingway et al., 2004; Li et al., 2007). Target site resistance is due to a change in the affinity between the insecticide and the binding site on the voltage-gated sodium channel, caused by a single or multiple amino acid substitutions (Soderlund and Knipple, 2003; Davies et al., 2008; Soderlund, 2008). Mutations in this gene have been linked to knock-down resistance, often referred as "kdr", in which resistant insects rapidly recover from the paralysis produced by pyrethroid insecticides and DDT. Both, susceptible C and resistant W-1Kλ flies were knocked down after topical application of sub-lethal dosis of lambda-cyhalothrin, taking several hours for complete recovery, suggesting that kdr resistance mediated by alterations of the target site is not likely to be involved in lambda-cyhalothrin resistance. Pyrethroid resistance mediated by P450s (Liu et al., 2011; Feyereisen, 2012; Riveron et al., 2013), esterases (Dai and Sun, 1984; Gunning et al., 1997), and glutathione-S-transferases (Vontas et al., 2001; Fragoso et al., 2003) have been documented. We have shown that resistance of the W-1Kλ to lambda-cyhalothrin was almost completely suppressed by PBO, indicating that P450 play a very important role in resistance to this insecticide. We also found that the esterase inhibitor DEF partially suppressed the toxicity of lambda-cyhalothrin, as already reported by Couso-Ferrer et al. (2011), who suggested that cross-resistance between malathion and pyrethroids may be associated with increased esterase activity. However, the increase in the resistance to lambda-cyhalothrin during the selection process of the W-1Kλ strain was accompanied with a decline in the resistance to malathion, from 96-fold with respect to C after 12 generations of selection (Couso-Ferrer et al., 2011) to only 6.1-fold in this study, suggesting two independent resistance mechanisms. Decreased cuticular penetration of pyrethroid insecticides has also been found in a number of insect species, such as *B. dorsalis* (Lin et al., 2012), *Musca domestica* (DeVries and Georghiou, 1981), *Spodoptera exigua* (Liu and Shen, 2003) and *Blatella germanica* (Valles et al., 2000). However, selection of the resistant strain

W-1Kλ was performed by ingestion of the insecticide, though occasional contact of the flies with the treated diet may also occur. Besides, the W-1Kλ strain was only 4.9-fold more resistant by topical application, compared with high level of resistance by ingestion (205-fold), making unlikely that resistance has evolved as a result of decreased cuticular penetration.

We provide further evidences for the involvement of *C. capitata* P450s in the resistance to lambda-cyhalothrin by analyzing their expression profiles. P450 genes linked to pyrethroid resistance mostly belong to the CYP4, CYP6 and CYP9 families (Yang et al, 2006; Komagata et al., 2010; Brun-Barale et al., 2010), though other CYP genes such as *CYP325A3* in *Anopheles gambiae* may also be involved in resistance (David et al., 2005). Besides, the up-regulation of genes of the CYP12 family has been shown to confer resistance to DDT (Brandt et al., 2002) and lufenuron (Bogwitz et al., 2005). We have then analyzed by qPCR fifty three P450 genes belonging to the CYP4, CYP6, CYP9 and CYP12 families in *C. capitata*. They represent the 72% (53 of 74) of the *C. capitata* CYP genes from these families currently annotated in Genbank, after the release of the genome of *C. capitata*. Our results showed that *CYP6A51* (GenBank accession number XM_004534804) was overexpressed in the W-1Kλ strain (13-18-fold) when compared to the C strain. Moreover, the expression of *CYP6A51* was induced in both the W-1Kλ (1.6-fold) and C strains (2.7-fold) when adults were treated with concentration of of lambda-cyhalothrin equivalent to their corresponding LC_{50}s, a characteristic of some P450 genes involved in insecticide resistance (Bautista et al., 2007; Liu et al., 2011, Huang et al., 2013). Therefore we hypothesize that *CYP6A51* gene may play a relevant role in the resistance of the W-1Kλ strain to lambda-cyhalothrin by overexpression of a lambda-cyhalothrin-inducible gene. It is well known that insects display an enormous plasticity in their response to insecticide selection, and resistance mediated by P450 can evolve by overexpression of different CYP genes (ffrench-Constant et al., 2004; Scott and Kasai, 2004). Other members of the CYP6A

subfamily reported to be involved in pyrethroid resistance are *CYP6A5v2*, *CYP6A24*, *CYP6A36* and *CYP6A38* in *M. domestica* (Kamiya et al., 2001; Zhu and Liu, 2008; Zhu et al., 2008a,b), *CYP6AA7* in *Culex quinquefasciatus* (Liu et al., 2011), *CYP6AK1* in *A. gambiae* (Müller et al., 2008), *CYP6AE11* in *Helicoverpa armigera* (Brun-Barale et al., 2010), and *CYP6AA3* in *A. minimus* (Rongnoparut et al., 2003). However, the overexpression of a particular P450 does not necessarily need to correlate with insecticide resistance (Komagata et al., 2010), being necessary further work to demonstrate unequivocally the role of *CYP6A51* and the other CYP gene (XM_004534802) in resistance to lambda-cyhalothrin. Finally, we cannot discard the possibility that some of the CYP genes of *C. capitata* not included in this study may also be involved in the resistance mechanisms.

Overexpression of P450 genes in resistant insects may be achieved throught increased transcription by mutations/insertions/deletions in cis-acting promoter sequences or trans-acting regulatory loci, and/or gene amplification mechanisms (Feyereisen, 2012). Scott et al (1999) reported the insertion of a 15 bp fragment, close to the transcription start site (-15 to -29), in the 5´flanking region of *CYP6D1* gene in permethrin resistant strains of *M. domestica*, which was absent in susceptible strains. Likewise, the insertion of transposable elements into the 5´flanking region of the *Cyp6g1* gene has been correlated with increased transcript abundance of this gene in DDT resistant strains of *D. melanogaster* (Daborn et al., 2002; Chung et al., 2007) and *D. simulans* (Schlenke and Begun, 2004). We have sequenced the 5´UTR region of the *CYP6A51* gene of *C. capitata* and found two different alleles, but there was not a differential distribution of these two alleles in resistant and susceptible individuals, suggesting that modifications in the promoter region sequenced (-500 bp from translation start) was not responsible for overexpression of *CYP6A51* gene. Other regulatory mechanisms might then be involved in the overexpression of *CYP6A51*, being necessary further investigations on this issue.

In conclusion, resistance to lambda-cyhalothrin has been found for the first time in field populations of *C. capitata*, and metabolic resistance mediated by P450 appears to be the main resistance mechanism in the resistant strain W-1Kλ. We have also found that resistance to lambda-cyhalothrin confers high levels of cross-resistance to other pyrethroids currently approved against *C. capitata* in citrus (etofenprox) or other (deltamethrin) crops. These findings come on top of the previously reported case of resistance to malathion (Magaña et al., 2007, 2008), that was shown to confer moderate levels of cross-resistance to other OPs (Couso-Ferrer et al., 2011), reducing further the number of insecticides that can be effectively used for the control of *C. capitata*. Appropriate resistance management strategies based on the alternation of insecticides with different modes of action, and their combination with other control methods, must then be implemented to avoid the maintenance of positive selection favoring the evolution of resistance in the field.

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Table 1. Location, year of sampling, host and insecticides used against *C. capitata* in the fruit orchards where infested fruits were collected.

Country	Locality	Year	Host	Insecticide treatments
Spain	Castellsera (Lleida)	2009	Apple	deltamethrin in 2008; methyl-chlorpyrifos and deltamethrin in 2009
	Sagunto (Valencia)	2010	Citrus	spinosad in 2009 and 2010
	Llombay (Valencia)	2009	Citrus	malathion in 2007, lambda-cyhalothrin in 2008, and spinosad and lambda-cyhalothrin in 2009
	Almuñecar (Granada)	2009	Cherimoya	non-treated in 2009
	Algarrobo Costa (Malaga)	2009	Cherimoya	non-treated in 2009
Tunisia	Korbous (Nabeul)	2011	Citrus	dimethoate in 2011
	Laazib (Bizerte)	2011	Citrus	dimethoate in 2011
	Jadaida (Mannouba)	2011	Pear	methidathion in 2011

Table 2. Susceptibility to lambda-cyhalothrin in field collected populations and a laboratory strain of *C. capitata*.

Population	n	Slope ± S.E.	LC_{50}[a] (95% FL)	χ^2	d.f.	LCR (LC_{50})[b] (95% FL)
Laboratory (C)	263	2.08 ± 0.40	20 (12 -28)	22.6[#]	14	1
Castellsera	180	1.28 ± 0.22	287 (199-470)	11.2[#]	14	14 (9-22)*
Sagunto	336	1.42 ± 0.19	129 (99-167)	7.9[#]	22	6 (4-9)*
Llombay	282	0.90 ± 0.15	134 (85-199)	13.6[#]	22	7 (4-11)*
Almuñécar	229	1.02 ± 0.19	144 (82-243)	15.4[#]	18	7 (4-13)*
Algarrobo Costa	129	1.01 ± 0.21	202 (103-418)	6.8[#]	10	10 (2-22)*

[a] Lethal concentration (LC_{50}) expressed in ppm of lambda-cyhalothrin in the diet.

[b] Lethal concentration ratio (LCR) at LC_{50} level of each population with respect to the laboratory strain (C).

[#] Good fit of the data to the probit model (P>0.05).

* LCR is significant (P<0.05) if 95% fiducial limits does not include 1.

Table 3. Susceptibility to lambda-cyhalothrin of field collected populations for Tunisia and a laboratory strain of *C. capitata*.

	Mortality (%) ± SE [a]		
Population	20 ppm	30 ppm	60 ppm
Korbous		66 ± 6	
Laazib	43 ± 12	56 ± 7	72 ± 3
Jdaida		73 ± 8	
Laboratory (C)	46 ± 5	61 ± 7	71 ± 8

[a] lambda-cyhalothrin added to the diet (3-4 replicates of 10-13 flies, n=30-48).

Mortality was not significantly different from laboratory C strain at any of the concentrations tested ($P<0.05$, Dunnett´s test, using arcsine square root transformation of mortality data).

Table 4. Susceptibility of the susceptible (C) and the lambda-cyhalothrin resistant (W-1Kλ) strains of *C. capitata* to different insecticides

Insecticide	Assay	Strain	N	Slope ± S.E.	LC_{50} [a] (95% FL)	χ^2	d.f.	RR [b] (95% FL)
Lambda-cyhalothrin	Feeding	C	263	2.08 ± 0.40	20 (12 -28)	22.6 [#]	14	
		W-1Kλ	312	1.21 ± 0.22	4224 (2980-6945)	7.5 [#]	18	205 (120-349)*
	Topical	C	336	1.97 ± 0.32	1.4 (1.1-1.7)	8.0 [#]	22	
		W-1Kλ	336	2.40 ± 0.31	6.8 (5.8-8.2)	10.2 [#]	22	4.9 (2.6-5.4)*
Deltamethrin	Feeding	C	341	1.11 ± 0.20	7.8 (3.8-11.7)	7.7 [#]	22	
		W-1Kλ	426	0.68 ± 0.17	1177 (685-3631)	11.7 [#]	23	150 (53-426)*
Etofenprox	Feeding	C	262	2.35 ± 0.37	43 (34-53)	3.6 [#]	14	
		W-1Kλ	246	1.39 ± 0.24	10397 (7690-14451)	14.6 [#]	22	243 (163-361)*
Methyl-chlorpyrifos	Feeding	C [c]	345	3.79 ± 0.44	4.6 (3.9-5.2)	6.6 [#]	14	
		W-1Kλ	241	1.79 ± 0.31	17 (11-24)	6.9 [#]	14	3.8 (2.4-5.9)*
Malathion	Feeding	C	363	3.07 ± 0.45	19 (15-22)	11.9 [#]	22	
		W-1Kλ	206	1.49 ± 0.53	122 (43-170)	9.5 [#]	10	6.1 (3.1-13)*
Spinosad	Feeding	C [c]	305	4.44 ± 1.01	0.6 (0.5-0.6)	12.2 [#]	18	
		W-1Kλ	257	3.91 ± 0.60	1.1 (1.0-1.3)	4.7 [#]	14	2.0 (1.7-2.3)*
Lufenuron	Feeding	C [c]	1250	4.53 ± 0.52	9.0 (6.2-10.7)	90	18	
		W-1Kλ	3598	2.14 ± 0.12	48 (39-55)	262	44	5.4 (4.7-6.2)*

[a] Lethal concentration (LC_{50}) expressed in ppm of insecticide in the diet for the feeding bioassays and as μg of insecticide per g fresh weight for topical assays. For lufenuron, the EC_{50} that produced a 50% reduction in fertility was calculated.

[b] Resistance ratio (RR) = LC_{50} (resistant W-1Kλ strain) / LC_{50} (susceptible C strain) is significant (* P<0.05) if 95% fiducial limits does not include 1.

[c] Data for the susceptible C strain from Couso-Ferrer et al. (2011).

[#] Good fit of the data to the probit model (P>0.05).

Table 5. Effect of synergists on the toxicity of lambda-cyhalothrin to the susceptible (C) and the resistant (W-1Kλ) strains of *C. capitata*.

Strain	Insecticide	Synergist	n	Slope ± S.E.	LC_{50}[a] (95% FL)	χ^2	d.f.	SR[b] (95% FL)
C	Lambda-cyhalothrin	-[c]	263	2.08 ± 0.40	20 (12 -28)	22.6[#]	14	
		+PBO	290	2.15 ± 0.36	14 (9-18)	14.9[#]	18	1.5 (0.9-2.3)
		+DEF[d]	288	2.01 ± 0.28	23 (15-33)	20.3[#]	14	0.8 (0.5-1.2)
		+TPP	242	2.21 ± 0.41	18 (13-22)	9.9[#]	14	1.1 (0.8-1.7)
		+DEM	246	1.88 ± 0.39	16 (10-23)	14.0[#]	14	1.3 (0.8-2.1)
W-1Kλ	Lambda-cyhalothrin	-	254	1.36 ± 0.32	3678 (2355-5437)	11.6[#]	14	
		+PBO	296	1.14 ± 0.22	72 (46-111)	10.9[#]	18	51 (27-96)*
		+DEF	308	1.11 ± 0.22	1376 (800-2021)	14.2[#]	18	2.7 (1.4-5.1)*
		+TPP	384	1.87 ± 0.29	2905 (2205-3795)	23.5[#]	22	1.3 (0.8-2.1)
		+DEM	296	1.49 ± 0.22	3386 (2064-4715)	8.4[#]	18	1.1 (0.6-1.9)

[a] Concentrations expressed in ppm of insecticide in the diet.

[b] Synergistic ratio (SR) at LC_{50} of lambda-cyhalothrin with respect to lambda-cyhalothrin plus the synergist in the same strain. The fiducial limits for SR were calculated according to Robertson et al. (2007). SR is significant (* $P<0.05$) if 95% fiducial limits does not include 1.

[c] Results from a bioassay also showed in Table 4

[d] Data from Couso-Ferrer et al. (2011).

[#] Good fit of the data to the probit model ($P>0.05$).

Table 6. Relative expression of CYP4, CYP6, CYP9 and CYP12 genes in adult flies from C and W-1Kλ strains of *C. capitata* using qPCR

C. capitata CYP genes		Mean expression ± S.E.		
CYP Family	GenBank Accession number	C	W-1Kλ	*P*-value
CYP4	XM_004520608	1.420 ± 0.392	1 ± 0.290	0.41
	XM_004536131	1.096 ± 0.368	1 ± 0.276	0.86
	XM_004534556	1 ± 0.294	1.289 ± 0.191	0.38
	XM_004521289	1 ± 0.417	1.073 ± 0.201	0.67
	XM_004518404	1.029 ± 0.445	1 ± 0.201	0.80
	XM_004518377	1.014 ± 0.375	1 ± 0.236	0.91
	XM_004518376	1.124 ± 0.386	1 ± 0.080	0.97
	XM_004534558	1 ± 0.487	1.939 ± 0.287	0.15
	XM_004534809	1 ± 0.199	1.284 ± 0.102	0.28
	XM_004518403[#]	1.426 ± 0.402	1 ± 0.181	0.37
		1.426 ± 0.402	1 ± 0.181	0.37
	XM_004521003	1.201 ± 0.399	1 ± 0.217	0.75
	XM_004521002	2.145 ± 0.591	1 ± 0.251	0.12
	XM_004529469	1 ± 0.324	1.459 ± 0.105	0.29
	XM_004526004	1 ± 0.526	1.142 ± 0.296	0.61
	XM_004526003	1 ± 0.583	1.248 ± 0.269	0.45
CYP6	XM_004520247	1 ± 0.187	1.637 ± 0.301	0.12
	XM_004534543	1 ± 0.294	1.247 ± 0.184	0.42
	XM_004534542	1 ± 0.369	1.225 ± 0.181	0.46
	XM_004534544[#]	1 ± 0.467	1.493 ± 0.221	0.27
		1 ± 0.407	1.661 ± 0.246	0.19
		1 ± 0.329	2.104 ± 0.311	0.13
	XM_004534798	1 ± 0.488	1.640 ± 0.243	0.24
	XM_004534800	1 ± 0.417	1.687 ± 0.408	0.32
	XM_004534796	1 ± 0.646	1.640 ± 0.243	0.24
	XM_004534549	1 ± 0.523	1.553 ± 0.230	0.26
	XM_004519454[#]	1 ± 0.297	1.066 ± 0.158	0.71
		1 ± 0.197	1.448 ± 0.238	0.22
	XM_004534803	1.197 ± 0.356	1 ± 0.281	0.67
	XM_004534802	1 ± 0.318	2.578 ± 0.381	0.04*
	XM_004534545	1 ± 0.527	1.263 ± 0.187	0.41
	XM_004534799	1 ± 0.468	2.039 ± 0.182	0.24
	XM_004534548	1.019 ± 0.312	1 ± 0.159	0.90
	XM_004534546	1.268 ± 0.626	1 ± 0.518	0.83

	XM_004534547	1.171 ± 0.585	1 ± 0.512	0.78
	XM_004534804[a#]	1 ± 0.377	18.301 ±2.70	0.001*
		1± 0.218	13.047±1.611	0.008*
		1± 0.383	13.833±1.709	0.003*
	XM_004537716	1 ± 0.288	1.973 ± 0.222	0.06
	XM_004523207	1.294 ± 0.458	1 ± 0.199	0.68
	XM_004536996	1 ± 0.382	1.043 ± 0.101	0.67
	XM_004535568	1 ± 0.403	1.046 ± 0.159	0.68
	XM_004535606	1 ± 0.297	1.122 ± 0.166	0.61
	XM_004522908	1.168 ± 0.415	1 ± 0.240	0.82
	XM_004522819[#]	1.194 ± 0.411	1 ± 0.296	0.74
		1.766 ± 0.487	1 ± 0.080	0.13
	XM_004522816	1 ± 0.441	1.145 ± 0.180	0.54
CYP 9	XM_004526336	1.118 ± 0.382	1 ± 0.209	0.90
	XM_004526487	1 ± 0.307	1.267 ± 0.187	0.40
	XM_004526337	1 ± 0.447	1.082 ± 0.245	0.69
	XM_004526488	1.009 ± 0.414	1 ± 0.283	0.93
CYP 12	XM_004521275	1 ± 0.526	1.130 ± 0.305	0.64
	XM_004521276	1 ±0.562	2.522 ± 0.373	0.11
	XM_004521170[#]	1.092 ± 0.374	1 ± 0.258	0.90
		1.003 ± 0.372	1 ± 0.109	0.76
	XM_004536491[#]	1 ± 0.430	1.291 ± 0.191	0.41
		1 ± 0.566	1.195 ± 0.301	0.53
	XM_004520781	1 ± 0.353	1.600 ± 0.125	0.26
	XM_004520782	1 ± 0.438	2.022 ± 0.299	0.11
	XM_004520689[#]	1.068 ± 0.403	1 ± 0.258	0.99
		1.062 ± 0.467	1 ± 0.369	0.20
		1 ± 0.108	1.340 ± 0.204	0.99
	XM_004520677	1.163 ± 0.347	1 ± 0.269	0.92
	XM_004521171	1 ± 0.329	1.675 ± 0.248	0.15

[a] This gene has been designated as *CYP6A51* by David R. Nelson (http://drnelson.uthsc.edu/biblioB.html#6A).
[#] Two or three different pairs of primers were used for these genes. The order in the Table is the same than in Table S1.
* Significantly different from C (Sign test, p≤0.05)

Table S1. Oligonucleotide primers used for qPCR of *C. capitata* CYP genes

C. capitata CYP genes			Primer sequence		
CYP Family	GenBank Accession number	Product pb	Forward	Reverse	Efficiency %
CYP4	XM_004520608	106	GTATTGGCAACCGATTTGCT	GTGGCATTGAACGAGGTCTT	106
	XM_004536131	143	TGGGTTTCGACAATGCTACA	TGCAAGTGCGTCTTGTTTTC	102
	XM_004534556	138	TTCACACTCTCTCGCCACAC	GCTGAATCAGTGCCGAAAAT	110
	XM_004521289	138	CCAAACCCGAACAGTTCAAT	CCGCCTTCTCTTCGAGTATG	106
	XM_004518404	87	GTCAAATATGGCCGCCTCTA	GAGAAAAATAAGCGCGTTGC	98
	XM_004518377	145	GGGCGAGTACAAGACTTTGG	CTCACGCTCAAACACCTCAA	93
	XM_004518376	133	TGCAAGAGACTGCCGTTATG	GCCATGCGCTTCTTACTACC	98
	XM_004534558	148	CCGGCTAATTGCCTCTCTCT	GCGATCCTTAAATCGCTCAG	116
	XM_004534809	140	CACGTCATTTTGTTGGCTTG	CTAAGTCCGCTCTCGCAAAC	115
	XM_004518403[#]	116	ATTATCGATCGTCGGTCAGC	GATTGCAACAGCACATCCAG	96
		123	ACGTACTGTGCTGGTGCAAG	CGCCTTTTAAGCCCCATATT	93
	XM_004521003	142	CTCGCCTCTGGTCCATACAT	GTCGCTTCGATACCTTTTCG	105
	XM_004521002	140	CGTTGATCGGTGTTGTTTTG	CGAACAGCCCAATACCAAAT	108
	XM_004529469	135	CTTGATGGTCATTCGGCTTT	TGCTGTGGAGCAGAGTATGG	110
	XM_004526004	102	CAACGGAGTGGAGGTCATCT	CTGAGACGGCGTTAGGTGTT	104
	XM_004526003	106	GCCAGGTTACTCTCCACCTG	TGTCGTTCGAATGGATGAAA	103
CYP6	XM_004520247	138	CCATTGGAACGCTGTTCTTT	AGGGATCACGTATGGTCTGC	101
	XM_004534543	104	ACTACCGCATTTGGTTCGTC	TGGCAATAACGGGTATGGTT	114
	XM_004534542	136	TGATGAGCATGTGTGGGATT	AATCCGACGTCCCATTACAC	102
	XM_004534544[#]	89	CTGCGCGTTCGGTATAGATT	GGCGCTGATCTACAAACACA	102
		114	CAACCCCGAGGAGTTCAAT	AAACGCAGGCCAATACAGTT	110

	142	TCGTCACCGCTCTAACACTG	AAAGTGCGCGTCTTCAGAAT	101
XM_004534798	119	CAGTCATTGCCATCGCTCTA	TGTTTTCGTCACGCCATTTA	109
XM_004534800	122	TGCCATACTTGGGGCATTAT	TGCGATGTACTTCCGTCAAA	111
XM_004534796	134	CCCTTTCTTTCTTCGCACAG	TTGGTATGAGCACGGTGGTA	105
XM_004534549	87	ACGTCGCCACTTCAGCTACT	GTACCGATGCCGCTCATATT	99
XM_004519454[#]	143	ATTTGGTCGTTCGATCTTCG	TACGCACGGCATTGATAAAG	110
	143	ACGCTTCGAGCCTGATATTG	ATTTGTAGTCCCGCAGCAAA	113
XM_004534803	142	CACTCACCGGTCACCTCTTT	CAAGCATGCACCAACTCACT	92
XM_004534802	113	CTTCCTCGGTTTGCTTATCG	CCGCCAGTATAATGGGCTTA	102
XM_004534545	124	ATCCACTCACCGGTCAACTC	CCCACACGACACACAGTAGG	113
XM_004534799	142	AAGCTGATGAAGTCGGAGGA	GATGCTGTGCCAGTTCGTAA	97
XM_004534548	102	GACGGAAGGCATTAATCGAA	TACGCACTGCACACACATTC	99
XM_004534546	137	GCCGATGAGTTAGTGGATGC	ATTGGCTTCTGCAATGTGTG	110
XM_004534547	107	GAAATTGCAAAGTCGCCATC	CTATGAGCGCAACGCCTATC	110
XM_004534804[#]	149	CCCGATCCAGAAAAGTTTGA[##]	TATGTGAGACCGACCAACGA	112
	160	GCTCGTGCTCAGTGTTACGA	CTTTGTAGTGCGCCATTCCT	93
	130	CGGAATACTTCCCTGATCCA	TATGTGAGACCGACCAACGA	95
XM_004537716	149	GGCGCGCAATATACAAGTTC	TGAGCTTTCCAGTGGAGAAAA	105
XM_004523207	139	AGCCGGTAGTGAGACCACAT	AAGAGGATCACTGGGCTTCA	95
XM_004536996	137	TCCTTTCATGGGGCTCTTTA	CTGCTCACACATCGTTGCTT	100
XM_004535568	147	TCCACACAGTGGATTCCAAG	CCTTTTCCAACACCTCAGGA	101
XM_004535606	148	CTAGGTTGGCGTGAAGAAGC	TGTCGTCGGCACTAATTTCA	92
XM_004522908	148	TGGCGCGCAGTATAGAAGTA	AGCTTGCCAGTGGAGAAAAG	94
XM_004522819[#]	150	GATGCGTTGTCATGGTGAAG	AAAGCGTGGAAGACGAGGTA	113
	94	TCCTACCGGAGAACAAGCAT	AGCGTTCCCCAATACAGTTG	98

	XM_004522816	148	CGCTATGATGCAGACGAAAA	ACGCACCACATTCAGGTGTA	93
CYP 9	XM_004526336	96	GAGGCAAGCCCTTGTCATAC	TAAACCCAGCAAGGTTCCAC	105
	XM_004526487	101	GCGACAATGTGTCATGAGCTG	GACAAGGGCTTGCCTCCA	99
	XM_004526337	105	CAAGATCGAACGTTCTGCAA	CTTCGAGGTACCAGCCTGAC	93
	XM_004526488	109	GGAAGGAGCGAGGTCTGTAG	CCGGTGCATTACATGAAAAA	106
CYP 12	XM_004521275	147	ACTCAGCCTGGCAAAGAATC	CCAGTGGAAACGGGATACAT	116
	XM_004521276	88	ATCCATGCTGGGAGGTAAGA	GTTGTGGTATTCGCCTACGG	118
	XM_004521170[#]	128	CAAGGAACGCGTGGACTTAT	TGACGGCAGTACGAAAGTTG	106
		115	GGAGGGTCGGAAATATCACA	CGCTAGGGTGACCTCCAAT	107
	XM_004536491[#]	149	CAAGCGAACGCACGTTACTA	CCAAAACCGAAAGGCAAATA	105
		108	AGCAGTGGAGCTCATTTCGT	GCTCCACAAGCTCCTGATTC	113
	XM_004520781	141	ACATAATGCCCGATCCAAGA	GCACAATATCCTTGGGCAAT	97
	XM_004520782	125	ACAGGTCGCGATCTAGTGCT	CGTTCGGGCAAATATTCATT	102
	XM_004520689[#]	89	ACTATTTGGCCAGTGCGTTT	AAACCGGTAACACCGTGAAA	113
		144	GGTACCCAAAGGTGTTGGTG	GGATTGTGCTTTTGGGAGAC	96
		85	AATGTGGGCTTTCGAAACTG	CGCCATAGCATTGGATTTTT	107
	XM_004520677	111	GAAACTGGCGCAGATCTTGT	ACGTGGACGTGGAAAGAAAA	107
	XM_004521171	129	TTCGTGCCATAGCAGAGATG	GTCCTTCGTTGCGGAAAATA	104

[#] Two or three different pairs of primers were used for these genes.

[##] This forward primer contains a mismatch C/T at base 18 due to a mistake in the nucleic sequence of the previous assembly to Ccap_1.0. Experiments performed with the other two pair of primers designed for this gene confirmed that this mismatch did not affect qPCR results

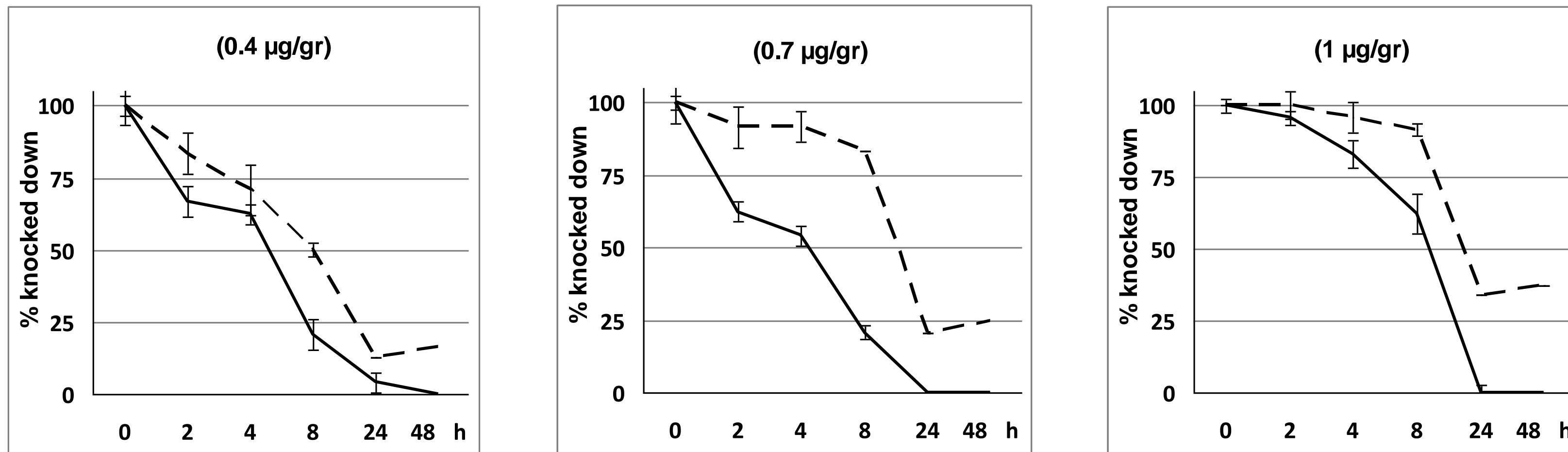

Figure 1. Knock-down recovery of susceptible (C - - -) and resistant (W-1Kλ ——) *C. capitata* flies treated topically with and lambda-cyhalothrin (µg/g fresh weight of fly). Error bars account for standard errors.

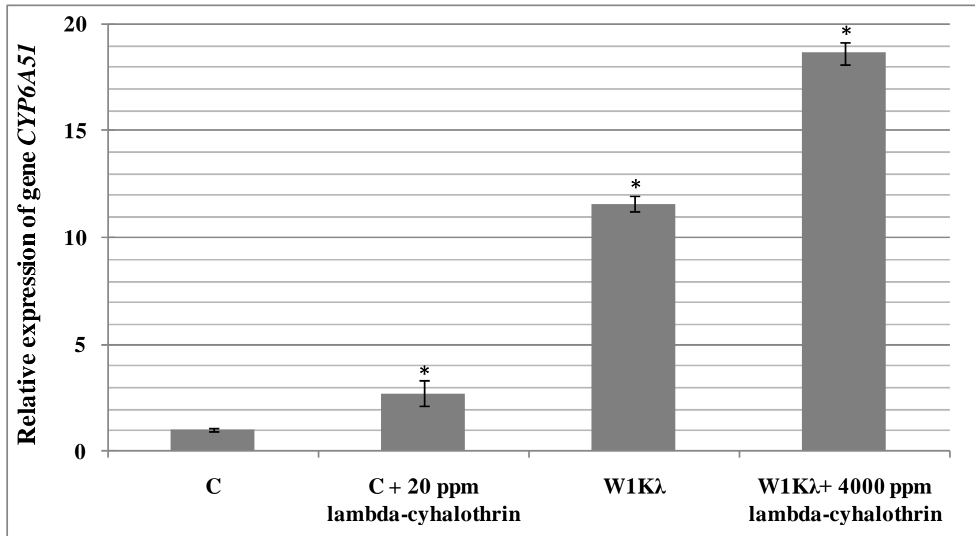

Figure 2. Relative expression of *CYP6A51* gene in treated (with lambda-cyhalothrin) and untreated adults of susceptible (C) and resistant (W-1Kλ) strains of *C. capitata.* Error bars account for standard errors. * Significantly different from C (Sign test, $p \leq 0.05$).

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Allele 1 CTGAAGTCCAGCACTCGTAATGGTGATACTTCAAAAAATGTAAATATAATGTATTCATTG 60
Allele 2 CTGAAGTCCAGCACTCGTAATGGTGATACTTCAAAAAATGTAAATATAATGTATTCACTG 60
         ********************************************************* **

Allele 1 ATATTTACATTTTATTATTTTTTATAAAGACCGCCCAAAGTACGACGGAAATTTGTTTAC 120
Allele 2 ATATTTACATTTTATTATTTTTTATAAAGACCGCCCAAAGTACGACGGAAATTTGTTTAC 120
         ************************************************************

Allele 1 TAGCTCATAATATGAAAGAAATATGTATACTTGTATTATAGTGTCTGAGCTGTTTTCTGC 180
Allele 2 TAGCTCATAATATGAAAGAAATATGTATACTTGTATTATAGTGTCTGAGCTGTTTTCTGC 180
         ************************************************************

Allele 1 TGAATCATTCGCTGAGAGAAATAACACCATGAACATAAAAAAATTTAAAAAGCACAAAAC 240
Allele 2 TGAATCATTCGCTGAGAGAAATAACACCACGAACACAAAAAAATTTAAAAAGCACAAAAC 240
         **************************** ***** ************************

Allele 1 ATATAACTCGTACATATCTATAAGAAGCAGACAAAACCAATGCGATAACATTTTGACAAT 300
Allele 2 ATATAACTCGTACATATTTATAAGAAGCAGACAAAACCAATGCGATAACATTTTGACAAT 300
         ***************** ******************************************

Allele 1 GACTTGAAGAGTTCGCTAGACAACAGAGAGCCTGTTCTTTTCTACAAGAAATTCGCCTAT 360
Allele 2 GACTTGAAGAGTTCGCTAGACAGCAGAGAGCCTGTTCTTTTCTACAAGAAATTCGCCTAT 360
         ********************** *************************************

Allele 1 AAGTAGCACACAAATCGATGGGTAGATTGTAGTTATATATTTTTAGCGTTTACAAGAGGT 420
Allele 2 AAGTAGCATACAATTCGATGGGTAGATTGTAGTTATATTTTTTTAGCGTTTACAAGAGGT 420
         ******** **** ************************ *********************

Allele 1 TTAGAATTCTAAGTAAAGAGATTTTCTTCGAATAAATATAAGCAATGAGCGTGTTTCTGG 480
Allele 2 TTAGAATTCTAAGTAAAGAGATTTTCTTCGAATAAATATAAGCAATGAGCGTGTTTCTGG 480
         ************************************************************

Allele 1 CTTTGCTCGTGCTCAGTGTTACGATCTTTGGGTTATTCCTCAAGTACCGTCATGGTTTTT 540
Allele 2 CTTTGCTCGTGCTCAGTGTTACGATCTTTGGGTTATTCCTCAAGTACCGTCATGGTTTTT 540
         ************************************************************

Allele 1 GGCAACGACGCGGCATACCACATGAAGTCCCCAGCTTTCCCATGGGCGATTTTAAGGAAT 600
Allele 2 GGCAACGACGCGGCATACCACATGAAGTCCCCAGCTTTCCCATGGGCGATTTTAAGGAAT 600
         ************************************************************

Allele 1 CATCCCCATTTGCCGGCATGTTTCTAGTCGGCGCACTACAAAGGGGTTCTTTGAGATAAT 660
Allele 2 CAGCCCCATTTGCCGGCATGTTTCTAGTCGGCGCACTACAAAGGGGTTCTTTGAGATAAT 660
         ** *********************************************************

Allele 1 CGGGCCTATATATAAGAAATACAAGGGCA 689
Allele 2 CGGGCCTATATATAAGAAATACAAGGGCA 689
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Figure 3. Alignment of the two alleles found for the 5´UTR region of the *CYP6A51* gene of *C. capitata*. Sequence alignment using ClustalW2 (EMBL). Allele 1 (GenBank Accession number KF305738), Allele 2 (GenBank Accession number KF305739). Asterisks represent identities between the two alleles. ATG indicates the start codon of the *CYP6A51* gene.