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Evaluation of insecticide efficacy and insecticide adaptive response in Italian populations of *Drosophila suzukii*

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Abstract

Monitoring sensitivity to insecticides is crucial to prevent outbreaks of invasive pests characterized by high reproductive and adaptive potential such as the *Drosophila suzukii* (Matsumura) (Diptera Drosophilidae). The aim of the present study was to investigate the possible appearance of resistance to cyantraniliprole, deltamethrin and spinosad. Field trials on commercial sweet cherry orchards in Northern Italy showed that two out of six strains were not fully controlled using cyantraniliprole and deltamethrin, while spinosad was thoroughly effective. At the bioassay, two populations showed a decrease in deltamethrin and cyantraniliprole susceptibility (LC_{50} values 12.7-21.0 and 3.4-5.8 times higher than those from the untreated populations, respectively). Biochemical analyses revealed that low resistance to the pesticides was associated with high monooxygenase and carboxylesterase activities (range 2.68-4.37-and 1.97-2.73 times higher than in the wild population). A dose-dependent increase in cytochrome P450 monooxygenase *Cyp12d1* and ryanodine receptor gene expression was found when a strain with low resistance to cyantraniliprole in field trials was treated with increasing dosages of the diamide in bioassays. No mutations were detected in voltage-gated sodium channel and ryanodine receptor genes, which accounted for the reduction in pyrethroid and diamide susceptibility in other pests. After 8 generations of selection, starting from a susceptible population, the LC_{50} values of cyantraniliprole and deltamethrin were increased 2.2 and 25.0 fold, respectively, compared with the unselected colony. In contrast, no selection was possible for spinosad. Our study would suggest that spotted wing drosophila, upon continued selective pressure, are more prone to develop low resistance to cyantraniliprole and deltamethrin than spinosad. The adaptive response relies on detoxifying activities of monooxygenases and increased *Cyp12d1* and ryanodine receptor gene expression.

Key words: *Drosophila suzukii*, bioassays, field trials, cyantraniliprole, spinosyns, deltamethrin, molecular markers.

Introduction

The spotted wing Drosophila, *Drosophila suzukii* (Matsumura) (Diptera Drosophilidae), native to Western Asia, is a highly polyphagous invasive pest for fruit crop production worldwide. This pest did not represent any potential menace to agriculture (Kanzawa, 1939) until 2008, when it was detected also in Europe (mostly Italy and Spain) (Calabria *et al.*, 2012; Cini *et al.*, 2012) and North America (Lee *et al.*, 2011), while it reached South America in 2013 (Deprá *et al.*, 2014).

Unlike the vast majority of *Drosophila* species feeding on rotting fruits, *D. suzukii* lays eggs inside ripening fruits, puncturing the fruit skin with its unique saw-like ovipositor (Atallah *et al.*, 2014). Damage is thus caused by larval feeding, resulting in fruit degradation.

This highly polyphagous species is known to develop on several economically important fruit crops, including blackberries, blueberries, cherries, peaches, raspberries, strawberries, grapes, bayberries and kiwis (Kanzawa, 1939; Bolda, *et al.*, 2010; Grassi *et al.*, 2011; Lee *et al.*, 2011; Seljak, 2011; Walsh *et al.*, 2011; Bellamy *et al.*, 2013; Liu *et al.*, 2015). Besides, more than 50 wild host plants have been determined in Europe and North America, providing the pest with a large reservoir of alternative hosts throughout the seasons (Baroffio *et al.*, 2014; Poyet *et al.*, 2014; Lee *et al.*, 2015; Kenis *et al.*, 2016).

Cherry was one of the first crops to suffer from *D. suzukii* attacks when the insect appeared in North America and Europe (Beers *et al.*, 2011; Cini *et al.*, 2012). In Italy, in the first two years of infestation (2010 and 2011), significant damages up to 90% of fruits were observed in late cherry varieties and in orchards located in Trentino and Emilia-Romagna regions (Boselli *et al.*, 2012; Grassi and Palloro, 2012).

Reports about the first pest control strategies against *D. suzukii* infestation on cherry orchards were confirmed in 2018 (Shawer *et al.*, 2018). The results of these repeated field and laboratory tests established the effectiveness of registered insecticides, which were included in pest management strategies (Shawer *et al.*, 2018). The products tested in the last 8-9 years mostly belonged to the families of pyrethroids, organophosphorates or neonicotinoids, already used for the control of another cherry pest, the European fruit fly *Rhagoletis cerasi* (L.) (Diptera Tephritidae). Other insecticides with an improved toxicological profile belonging to the families of spinosoids and diamides have also been employed against *D. suzukii* (Shawer *et al.*, 2018). In many cases, three to six pre-harvest applications of insecticides were used in combination, according to the national authorization for the use of insecticides. However, no significant differences in effectiveness were detected in the tested products, but the timing of application was a key issue for a

successful outcome since the fly control is mostly performed by adulticides (Shawer *et al.*, 2018). Moreover, previous experience revealed that when the strain density was high, the only possibility to limit the attacks by *D. suzukii* was to integrate the chemical approach with other methods, such as the expensive but effective insect-proof nets (Caruso *et al.*, 2017). On the contrary, other techniques did not provide significant improvements (Caruso *et al.*, 2017).

Appropriate agronomical practices of "sanitation", such as the complete removal of dropped and over-ripe fruits, the thinning of vegetation and the cleaning of ground vegetation to reduce shading and humidity in the orchard were crucial (Shawer *et al.*, 2018). Meanwhile, classical biological control techniques are under development. For example, the endemic pupal parasite species *Trichopria drosophilae* (Perkins) (Hymenoptera Diapriidae) may play a relevant role in fly control over wide areas. This approach is mostly based on repeated and preventive releases of *T. drosophilae* over the crop but also around it, to establish a strain of beneficial insects able to hinder *D. suzukii* development and outbreaks, significantly improving the outcome of other control strategies (Rossi Stacconi *et al.*, 2017; 2018; Shawer *et al.*, 2018).

Concerning the most effective insecticides, up to date no standard laboratory data are available on the effects of deltamethrin, spinosad and cyantraniliprole on different *D. suzukii* strains. In this study, the susceptibility to the aforementioned insecticides was evaluated in laboratory conditions based on topic and feeding bioassays. The values of LC_{50} and LC_{90} were estimated for *D. suzukii* populations collected in cherry orchards in Emilia-Romagna Region where different pest management strategies were used. Field trials were also performed to confirm the reduced susceptibility to insecticides observed in laboratory.

Though inherited adaptation to chemical exposure in *D. suzukii* is still poorly investigated, studies conducted in other phytophagous suggest that it may evolve mainly through two distinct mechanisms: the alteration of target sites inducing insensitivity to the insecticide (target-site resistance) and/or increased metabolism of the insecticide (metabolic-based resistance) involving three large enzyme families, the cytochrome P450 monooxygenases (MFO), glutathione S-transferases (GST) and carboxy/cholinesterases (EST) (Li *et al*., 2007; Panini *et al.*, 2016).

Metabolic resistance was inspected by Green *et al*. (2019) in *Drosophila melanogaster* Meigen adults by measuring detoxifying activities through biochemical in vitro assays and following variations in the expression of an ortholog gene cluster (P450 dependent monooxygenases *Cyp12d1*) involved in adaptation to diamides. For target site resistance, the presence of mutated alleles found in pyretroid insensitive *D. melanogaster* (Powell, 1997) and in diamide resistant *Plutella xilostella* (L.) (Guo *et al*., 2014) was verified by sequencing homologous gene regions coding for α-subunit of voltage-activated sodium channel (*Nav*) and ryanodine receptor (*RyR*).

Furthermore, variations in the expression of ryanodine receptor (*RyR*) were examined after adult exposure to cyantraniliprole since, in Lepidoptera, changes in transcriptional activity were detected following diamide challenge (Lin *et al.*, 2013; Liu *et al*., 2015). The study aimed to provide updated baselines for the most widely applied insecticides against *D. suzukii* to be used as a reference for future assessments and to test molecular and biochemical markers that reveal an adaptive response to cyantraniliprole and deltamethrin in *D. suzukii*.

Materials and methods

Pest populations collections

Six *D. suzukii* populations were established in the laboratory from adults captured in different cherry orchards in the province of Modena, Emilia-Romagna Region (Northern Italy) (table 1). The study began in summer 2014 in cherry orchards by a direct sampling of damaged fruits with strong depositions of *D. suzukii* eggs and continued until 2016.

Table 1. Orchards studied and corresponding populations of *D. suzukii* established, and the laboratory and field tests performed (*).

Population acronyms	Location (province)	Coordinates	Year	Orchard conditions pest control strategies	Bioassay	Enzymatic assay	Field trials
BIO	Bologna (BO)	44°30'50.0"N 11°24'26.0"E	2014	Susceptible control (never received any insecticide application)	*	*	
BON	Vignola (MO)	44°27'59.4"N 10°59'19.0"E	2014	Cherry orchard heavily infested, poor insecticide spray program	*	*	
MO2	Vignola (MO)	44°28'49.0"N 11°01'34.4"E	2014	Cherry orchard heavily infested, high insecticide spray program (strain suspected of having become less susceptible)	*	*	
MIX	Vignola (MO)	44°29'08.5"N 11°00'27.5"E	2015	Mixture of strains from infested cherry orchards, high insecticide spray program	*	*	
QUA	Vignola (MO)	44°29'08.5"N 11°00'27.5"E	2016	Cherry orchard extremely infested, high insecticide spray program (strain suspected of having become less susceptible)	*	*	*
TUG	Piumazzo (MO)	44°33'01.2"N 11°04'08.7"E	2016	Cherry orchard extremely infested, high insecticide spray program (strain suspected of having become less susceptible)	*	*	*

The collected cherries were placed in trays inside individual cages (BugDorm-2120 Insect Rearing Tent 60 × 60 × 60 cm, MegaView Science Co., Ltd. Taichung, Taiwan) to prevent mixing of adults. The cages were immediately brought to the laboratory and kept at 23 °C with long photoperiod (L-D:18-6) for insect rearing. The *D. suzukii* populations involved in this study were named according to the harvest site (table 1) and chosen in a cherry orchard with very strong infestation and either low or high insecticide spray program, therefore suspected of having become less susceptible to the commonly used insecticides. Once sampled from fields, all populations have been reared in laboratory for two generations before bioassays. *D. suzukii* larvae and adults were constantly provided with water and artificial diet (Dalton *et al*., 2011) that served both as a food source and oviposition medium. The reference population was a laboratory colony (BIO) established from field population of *D. suzukii* previously collected in wild area and reared for more than one year in the laboratory as described above.

Insecticides

Bioassays of five active ingredients (AI) were performed using formulated insecticide products: Decis evo (deltamethrin 25g/L); Rogor (dimethoate 400g/L); Laser (spinosad 480g/L); Delegate (spinetoram 250g/Kg); Exirel (cyantraniliprole 100g/L).

LC_{50} and LC_{90} bioassay

Adults of each established population of *D. suzukii* (table 1) were exposed to the tested insecticides by contact and feeding. An amount of 0.75 mL of a 5% sucrose : 1% agar (w/v) solution was poured in each well of a 24-well clear plate (Costar® Corning, NY 14831 USA). An amount of 20 µL of each tested compound dissolved in 90% (v/v) ethanol was uniformly applied on the surface of the sucrose-agar base in each well. A typical plate contained one control (solvent only) and five increasing concentrations of insecticide in consecutive wells, each concentration tested in four replicates on the same plate. The concentrations chosen were previously established to cause an adult mortality between 5 and 95%. About ten adult flies, 4-5 day old, previously anesthetized with CO_2, were transferred to each well and the plates were sealed and incubated under standard conditions (23 °C, 70-75% humidity). Each experiment was repeated 3-4 times to provide independent biological replicates. Mortality and other visible effects were assessed up to 48 hours after insecticide applications.

Enzymatic assay

Adult flies 4-5 day old were sampled from insect rearing chambers and used for crude homogenates preparation from whole insect or for abdomen dissection. For carboxylesterases (EST) and glutathione-S-transferases (GST), thirty whole flies were individually homogenate in 200 µl Na (0.1 M, pH 7.5) and K (0.05 M, pH 7.0) phosphate buffer respectively, containing 0.1% Triton-X, using a manual grinder. The homogenate was centrifuged at 10,000 g for 10 minutes at 4 °C and the supernatant used as enzyme source. The carboxylesterase assay was performed using 4-nitrophenyl-acetate (4-NPA) as a substrate (Van Leeuwen *et al*., 2005): 50 µl of homogenate (buffer only in negative controls) were added to 130 µl 0.1 M sodium buffer (pH 7.5) equilibrated at room temperature in a 96-well microplate (Thermo Fisher Scientific Inc., Waltham, MA, USA). All experiments were performed in triplicate. The reaction was started by adding 20 µl 3mM 4-NPA solution (10% acetone v/v in buffer) and the rate of 4-nitrophenol formation was monitored at 405 nm in a Fluostar Optima microtiter plate (BMG Labtech, Ortenberg, Germany) read at 30 °C every 10 seconds for a 2 minutes incubation time. A standard curve of 4-nitrophenol in sodium phosphate buffer (0.1 M, pH 7.5) was used to convert initial slopes (V_{max}) into specific activity after normalization to total protein content (Bradford, 1976). For GST activity, 50 µl of homogenate was added to each microplate well containing 50 µl of potassium buffer (0.05 M, pH 7.0) and 100 µl 12 mM reduced glutathione. The change in absorbance was observed at 340 nm, for 5 minutes at 25 °C after adding 100 µl of 1.2 mM 1-chloro-2,4-dinitrobenzene in ethanol (1% v/v). Specific activity was calculated by correcting for protein content as above, using 9.6 mM-1 cm^{-1} as extinction coefficient. All experiments were performed in triplicate.

Cytochrome P450-dependent monooxygenase (MFO) activity was assessed by measuring 7-ethoxycoumarin-O-deethylation (ECOD) (de Sousa *et al*., 1995). Forty fly abdomens were dissected and individually placed in 96-well black microplates (Corning Inc., NY, USA) together with 100 ml of 0.05 M sodium phosphate buffer, pH 7.2. To completely submerge the abdomens in the wells, microplates were centrifuged at 2000 g for 2 minutes. An amount of 4 µl of 10 mM ECOD in DMSO was added to each well and the reaction incubated for 4 hours at 30 °C, in the dark, with gentle agitation. The reaction was stopped by adding 100 µl of glycine buffer (10^{-4} M) in ethanol (1:1, v:v). Ten similar wells in which glycine buffer was placed prior to incubation were used as negative controls. Fluorescence was read at 380 nm excitation/450 nm emission, before and after the incubation time. A standard curve of 7-hydroxycoumarin (7HC) was used to convert the amount of fluorescence produced in pmol 7HC /min/abdomen.

Field trials

The results of the laboratory tests for the insecticides under investigation in *D. suzukii* obtained in 2015-2016 were validated in summer 2017 by two field trials according to the Good Experimental Practice (GEP) following the EPPO/OEPP guidelines based on the protocol PP 1/281 (https://pp1.eppo.int/standards/PP1-281-1).

The field trials were carried out in two commercial cherry orchards in the Quartieri Farm (QUA) and Tugnetti Farm (TUG). The first field trial was conducted on 18 years old cherry trees of the cultivar “Lapins” in Vignola (Modena, Italy). The second field trial was conducted on 20 year old Sweet Heart cultivar in Piumazzo (Modena, Italy). All tested products, except the untreated control (table 2) were applied at an equivalent water volume of 1000 L ha^{-1} using a backpack mistblower (Stihl SR 420, Andreas Stihl S.p.A., Milan, Italy). Each plot consisted of one old cherry plant, plot size reached approximately

Table 2. Experimental plan of the two field trials.

n°	Active ingredient	Product name	Product rate ml / 100L	Date and timing of applications QUA	TUG
1	Untreated control	-	-	-	-
2	Deltamethrin	Decis Evo	50	1 Jun and 8 Jun 14 and 7 DBH	8 Jun and 15 Jun 14 and 7 DBH
3	Spinosad	Laser	35	1 Jun and 8 Jun 14 and 7 DBH	8 Jun and 15 Jun 14 and 7 DBH
4	Cyantraniliprole	Exirel	75	1 Jun and 8 Jun 14 and 7 DBH	8 Jun and 15 Jun 14 and 7 DBH

DBH, days before harvest.

12 m^2 and treatment size was about 48 m^2. All treatments were applied 14 and 7 days before supposed harvest (7 and 14 DBH) in order to control the *D. suzukii* strain on ripening cherries (standard practice). One hundred ripening cherries were collected in the lower central part of the plot, respectively, one hour before each application and at supposed harvest (BBCH 89). Each ripe cherry was checked under a binocular microscope 1-2 days before incubation at room temperature, calculating the percentage of cherries with eggs and larvae.

Insecticide resistance selection

Selection for resistance to insecticides was performed in the laboratory. Cohorts of adults from a susceptible laboratory colony (LAB) were selected with deltamethrin (DEL) or spinosad (SPIN) or cyantraniliprole (CYA) while another cohort, treated in the same way but without exposure to insecticides, served as unselected control (G0). In the first selection, adults were exposed to LC_{50} of the baseline established for the G0 colony. After 48 hours of exposure to contact and feeding under the conditions described for LC_{50} and LC_{90} bioassay (using a 6 cm petri dish instead of a 24-well plate), surviving adults were transferred to untreated artificial diet and reared in the laboratory under the conditions described above. The concentration of the tree insecticides used to select each subsequent generation was LC_{50} based on the results of bioassays from the previous generation. The number of adults used for each generation varied (300-500), depending on availability. Selection of some generations was deferred because of insufficient number of progeny.

Gene target identification and primer design

Reference sequences for *RyR* and *Cyp12d1* target genes were retrieved by querying annotations in *D. suzukii* genome assemblies: Dsuzukii.v01 from BGI (Genbank accession number GCA_000472105.1) and the Spotted Wing FlyBase (http://spottedwingflybase.org) from UC Davis and Oregon State University. The three *Cyp12d1* gene copies reported in tight linkage in Dsuzukii.v01 assembly (GeneID: 108008502, 108008671 and 108008359) were indeed fused in a unique gene annotation in Spotted Wing FlyBase (accession number DS10_00002643). The most distal copy coded for a *Cyp12d1* (Genbank accession number XP_016927685) with highest identity to *Cyp12d1* orthologs reported in *D. melanogaster* genome, namely, 89.56% amino acid identity with *Cyp12d1-p* (Genbank accession number NP_995812) and 90.14% amino acid identity with *Cyp12d1-d* (Genbank accession number NP_001286304). Putative open reading frames found in *D. suzukii Cyp12d1* genes were aligned using Clustal Omega (Sievers and Higgins, 2014) and a primer pair was designed on a conserved region using Primer3 (Untergasser *et al*., 2012). *Cyp12d1* distal copy was used as a reference (Genbank accession number XM_017072196) and one degenerated position was introduced in each primer to accommodate the remaining two putative paralogs (XM_017072366 and XM_017072565) (Supplemental material table S1). The reference sequences XM_017087623 (Genbank database) and DS10_00006802-RA (Spotted Wing FlyBase) were used to synthesise a primer pair aimed to amplify the 3'end of *RyR* transcripts (Supplemental material table S1) with the intent to avoid putative alternative splicings (Mandal *et al*., 2019) and limit the RNA degradation effect on the analysis of such long transcripts.

Primers for the housekeeping genes arginine kinase (*AK*), TATA binding protein (*TBP*) and α-tubulin (*TUB*) were prepared according to Zhai *et al*. (2014). A primer pair was synthesised to amplify and sequence a *RyR* cDNA fragment harbouring potential sites for missense mutations at the positions G4892 e M4736 (XP_016943112) as occurred for diamide insensitive G4946E and I4790M isoforms in *P. xylostella* (Supplemental material table S1) (Troczka *et al*., 2012; Guo *et al*., 2014). To verify pyrethroids target site resistance in *D. suzukii* strains, transcripts putatively coding for the α subunit of voltage-activated sodium channel (*Nav*) were partially amplified and sequenced, based on reference transcripts (Genbank accession number XM_036821263 and Spotted Wing FlyBase accession number DS10_00008225-RA). Three primers pairs (Supplemental material table S1) were used to encompass regions with potential mutations, as already described in *D. melanogaster Nav* gene (Pittendrigh *et al.*, 1997).

RNA isolation and analysis by Reverse Transcription Polymerase Chain Reaction (RT-PCR)

For expression analysis of *Cyp12d* and *RyR* genes, QUA adults that survived the cyantraniliprole exposure (0, 100, 500 and 1000 mg AI L^{-1}) along with untreated QUA and BIO controls (solvent only) were collected and frozen at −80 °C. Total RNA was extracted from three

biological replicates (6 to 8 insects each) for each tested group by manually homogenization in 500 µL of Tri-Reagent (Sigma Chemical, St. Louis, MO, USA) according to the manufacturer instructions.

The resulting total RNA was resuspended in nuclease-free water and quantified with a spectrophotometer (Thermo Scientific Nanodrop 2000) before integrity control through agarose-formaldehyde gel electrophoresis. Following removal of genomic DNA contamination with the RapidOut DNA Removal kit (Thermo Scientific, Waltham, MA, USA), cDNA was synthesized from 1 µg of purified total RNA using RevertAid first-strand DNA synthesis Kit (Thermo Scientific, Waltham, MA, USA) with random hexamer primers. In parallel a negative control was obtained by omitting the Revert Aid reverse transcriptase from the reaction mixture.

The cDNA was then diluted 1:30 using nuclease-free water, before use in real-time quantitative polymerase chain reaction (RT-qPCR) to amplify target genes (*RyR* and *Cypd12d1*) along with housekeepers [i.e. arginine kinase (*AK*), TATA binding protein (*TBP*) and α-tubulin (*TUB*)]. The amplification reactions were assembled as follows: 5 µl cDNA (8 ng), 0.4 µl of both forward and reverse primers (10 µM) (Supplemental material table S1), 10 µl KAPA SYBR® Fast qPCR Master Mix (2x) (Kapa Biosystems, Wilmington, MA, USA) and 4.2 µL of sterile Milli-Q® water (Merck). RT-qPCR plates were set up on ABI 7500 Fast Real-Time PCR System with one cDNA template and three technical replicates of each biological replicate.

Thermal cycling conditions for gene target amplification were: activation step 3 minutes at 95 °C; 40 cycles of 3 seconds at 95 °C, 30 seconds at 60 °C; fluorescence was collected at the end of each extension step; a final dissociation step cycle was added for melting curve analysis. Fluorescence readings were collected from the qPCR platform and amplification efficiencies for each target gene were calculated using LinReg (Ruijter *et al*., 2009).

For the screening of *Nav* and *RyR* mutations found in *D. melanogaster* (Pittendrigh *et al*., 1997) and in *P. xilostella* respectively (Troczka *et al*., 2012; Guo *et al*., 2014), and potentially also affecting the orthologous genes in *D. suzukii*, total RNA was extracted from field-collected and lab-selected specimens and retrotranscribed as reported above for RT-qPCR. cDNA fragments were amplified in 20 µl reaction mixture including 4 µl 5X Phire Reaction Buffer (Thermo Scientific, Waltham, MA, USA), 0.8 µl of each primer (10 µM) (Supplemental material table S1), 0.4 µl dNTPs (10 mM), 2 µl cDNA and 12 µl of sterile Milli-Q® water (Merck,Darmstadt, Germany) with the following thermal cycler profile: initial denaturation step 30 seconds at 98 °C; 35 cycles of 5 seconds at 98° C, 5 seconds at 58 °C, 15 seconds at 72 °C; final extension 1 minute at 72 °C; run on a Biometra thermocycler Tpersonal (Biometra, Göttingen, Germany). PCR amplification products were purified with spin columns (DNA Clean & Concentrator Kit; Zymo Research, Inc., Irvine, CA, USA) and analysed by Sanger sequencing using the same amplification primers (Eurofins Genomics, Ebersberg, Germany).

Data analysis

The estimates of LC_{50} and LC_{90} for fly mortality and their 95% confidence limits (CL) were obtained using the PoloPlus Version 2.0 (LeOra Software Company, Petaluma, California, USA) based on Finney's Probit analysis (Robertson *et al*., 2017). The differences in responses to insecticides between MO2, MIX, BON, QUA, TUG populations versus BIO populations, were considered not significant if the 95% confidence intervals of LC_{50} or LC_{90} overlapped. Resistance factors were calculated by dividing the LC_{50} and LC_{90} values of MO2, MIX, BON, QUA and TUG by the respective LC_{50} and LC_{90} values of the BIO population.

Results of the field trials were subjected to variance analysis (ANOVA) and means were separated by LSD Fischer test ($p < 0.05$) (STATISTICA 6.0 for Windows; Statsoft Inc., Tulsa, Oklahoma, USA). For each biological replicates, *Cyp12d1* and *RyR* gene expressions were normalised on the geometric mean of multiple housekeepers (Vandesompele *et al*., 2002). Fold changes in target transcripts levels were measured in BIO and QUA populations (the latter exposed to different cyantraniliprole dosages) using QUA untreated strain as reference following the ΔΔCt method with amplification efficiency-correction (Pfaffl, 2001).

Detoxifying activities data prior to statistical analysis were subjected both to Shapiro-Wilkinson's W test and Levene's test to ensure compliance with assumptions of normality and homoscedasticity with the P-value set at 0.05 while Log-transformation of fold changes in gene expression was presumed to fit parametric distributions (Hellemans and Vandesompele, 2011). Log-transformed fold changes in gene expression as well as mean values of detoxifying activities were analysed by one-way ANOVA and Tukey's Honest Significant Difference as post hoc test (STATISTICA 6.0 for Windows; Statsoft Inc., Tulsa, Oklahoma, USA).

Results

LC_{50} and LC_{90} bioassay

The results obtained by PoloPlus analyses for each insecticide are summarized in table 3. The table reports the $LC_{50\text{-}90}$ values, the 95% confidence limits (Robertson *et al*., 2017), the slopes (angular coefficients) and the values of the resistance factor (RF) for each *D. suzukii* population.

Concerning deltamethrin QUA and TUG populations collected in 2016 have $LC_{50\text{-}90}$ values statistically higher in comparison to all other populations collected in 2014-2015. A value of $LC_{50\text{-}90}$ higher than BIO (but not significant) is observed in MO2, BON and MIX. The resistance factors for LC_{50} (RF_{50}) and for LC_{90} (RF_{90}) are higher than 10 for QUA and TUG. Results for dimethoate bioassay showed that in comparison to all other populations, the populations QUA and TUG have $LC_{50\text{-}90}$ values statistically higher and RF values above ten. No significant differences in values of $LC_{50\text{-}90}$ are detected for spinosad and spinetoram in all populations studied. In contrasts, concerning cyantraniliprole, TUG and QUA populations

Table 3. Bioassay results for each active ingredient in field populations.

Population	Adults	Slope ± SE	LC_{50} † mg AI L^{-1} (95% CL)	RF_{50}	LC_{90} † mg AI L^{-1} (95% CL)	RF_{90}	χ^2(df) ††
Deltamethrin bioassay							
BIO	534	2.10 ± 0.15	0.45 (0.39-0.52)	-	1.84 (1.50-2.37)	-	1.20 (4)
BON	509	2.05 ± 0.16	0.39 (0.26-0.53)	0.9	1.63 (1.08-3.34)	0.9	9.25 (4)
MO2	621	1.85 ± 0.12	0.63 (0.41-0.94)	1.4	3.11 (1.91-6.95)	1.7	17.47 (5)
MIX	657	1.71 ± 0.12	0.43 (0.21-0.88)	1.0	2.40 (1.09-25.34)	1.3	45.71 (5)
QUA	931	1.73 ± 0.09	9.46 (7.93-11.28)	21.0	51.64 (39.85-71.23)	28.1	6.57 (6)
TUG	577	1.18 ± 0.08	5.73 (2.81-0.43)	12.7	69.36 (31.77-304.00)	37.7	25.38 (6)
Dimethoate bioassay							
BIO	501	2.62 ± 0.21	20.61 (15.26-28.46)	-	63.52 (42.25-137.70)	-	15.61 (5)
BON	806	2.26 ± 0.16	31.44 (21.97-51.68)	1.5	115.53 (65.43-396.07)	1.8	29.60 (5)
MO2	543	2.97 ± 0.24	27.25 (22.10-33.64)	1.3	73.53 (54.47-124.91)	1.2	11.21 (5)
MIX	1011	1.95 ± 0.11	24.44 (15.56-37.79)	1.2	110.90 (63.58-360.75)	1.7	41.84 (5)
QUA	378	2.62 ± 0.26	327.42 (278.53-387.12)	15.9	1006.15 (785.08-1424.72)	15.8	0.67 (5)
TUG	539	1.43 ± 0.11	161.24 (83.00-322.43)	7.8	1212.63 (530.56-8035.45)	19.1	27.36 (5)
Spinosad bioassay							
BIO	551	2.19 ± 0.21	11.85 (5.47-20.01)	-	45.50 (25.45-242.20)	-	16.02 (3)
BON	462	1.04 ± 0.23	6.17 (0.79-17.50)	0.5	105.12 (30.90-21369.04)	2.3	24.75 (4)
MO2	501	1.90 ± 0.24	15.44 (8.62-24.83)	1.3	72.50 (39.75-350.83)	1.6	9.95 (3)
MIX	605	1.08 ± 0.18	6.05 (1.33-15.46)	0.5	92.05 (29.68-4847.00)	2.0	28.07 (4)
QUA	429	1.93 ± 0.15	9.60 (6.69-12.87)	0.8	43.99 (30.47-78.05)	1.0	4.88 (4)
TUG	551	2.96 ± 0.14	10.91 (7.88-14.38)	0.9	29.50 (21.13-54.13)	0.6	6.38 (3)
Spinetoram bioassay							
BIO	506	2.56 ± 0.21	9.64 (7.22-12.45)	-	30.52 (21.57-57.67)	-	8.96 (4)
BON	529	3.02 ± 0.23	6.36 (5.17-7.55)	0.7	16.86 (13.74-22.52)	0.6	4.96 (4)
MO2	369	2.79 ± 0.23	6.11 (3.94-8.62)	0.6	17.59 (11.97-36.06)	0.6	6.39 (3)
MIX	723	2.52 ± 0.13	5.69 (5.00-6.37)	0.6	18.30 (15.82-21.94)	0.6	0.79 (3)
QUA	380	1.57 ± 0.16	3.69 (1.21-7.06)	0.4	24.05 (11.85-126.57)	0.8	15.31 (4)
TUG	730	2.10 ± 0.11	5.09 (2.41-8.67)	0.5	20.61 (11.46-87.49)	0.7	28.76 (4)
Cyantraniliprole bioassay							
BIO	504	1.86 ± 0.15	18.11 (12.85-23.48)	-	88.39 (65.64-135.94)	-	4.53 (4)
BON	782	2.03 ± 0.13	20.31 (14.42-26.51)	1.1	86.61 (61.33-152.04)	1.0	15.30 (5)
MO2	882	1.71 ± 0.11	21.41 (14.31-29.44)	1.2	120.02 (75.50-284.46)	1.4	19.44 (5)
MIX	592	1.86 ± 0.16	16.08 (11.30-20.75)	0.9	77.97 (57.14-126.70)	0.9	7.98 (5)
QUA	813	2.08 ± 0.17	105.38 (79.02-145.14)	5.8	433.54 (274.35-999.92)	4.9	29.44 (7)
TUG	570	1.78 ± 0.16	62.43 (50.33-76.66)	3.4	325.03 (236.95-505.63)	3.7	8.57 (7)

AI, active ingredient; SE, standard error; CL, confidence limits; RF, resistance factor estimated as RF = LC_{50} or LC_{90} field population / LC_{50} or LC_{90} BIO population.

† LC values between populations considered significantly different, if the 95% CL do not overlap.

†† A high χ^2 indicates a significant deviation from the probit model, $p < 0.05$.

have $LC_{50\text{-}90}$ values statistically higher than all other populations. The values of RF_{50} and RF_{90} are respectively 5.8 and 4.9 for QUA, and 3.4 and 3.7 for TUG.

Enzymatic activity assay

One-way ANOVA revealed that the mean of all tested enzyme activities were significantly different among the *D. suzukii* populations (ANOVA: df = 5; $F_{MFO} = 112.23$, $F_{GST} = 21.44$, $F_{EST} = 148.3$; $p < 0.001$)

Overall detoxifying enzyme activities in BON and MIX populations were closer to those shown by the reference BIO than QUA and TUG populations (table 4). The latters had higher 7-ethoxycoumarin-O-deethylation (ECOD) activity ratios (4.37 and 2.68 fold respectively) as well as increased level of carboxylesterases (2.73 and 1.97 fold respectively) (table 5). A modest increase (1.61 fold) of monooxygenase cytochrome P450-dependent enzymes (MFO) was also detectable in MO2 population. Levels of glutathione-S-transferases (GST) slightly varied among tested populations, with TUG showing the highest enzyme activity ratio (1.83-fold), followed by MO2 with 1.61-fold change.

Field trials

The results obtained in the first trial (table 6) show that the infestation appeared only in the untreated control with a low percentage of larval infestation (5.5%). There was a significant difference only between the active ingredients and the untreated control. In the treated plots, no fruits with *D. suzukii* larvae were observed.

The results of the second field trial (table 6) show that the insecticide applications were performed during a high infestation by *D. suzukii*. The untreated control showed a high percentage (18.5%) of fruits infested by larvae. This

Table 4. Enzyme activity level as mean (± 95% confidence limits) of cytochrome P450-dependent monooxygenases (MFO), glutathione-S-transferases (GST) and carboxylesterases (EST) in field populations, collected in wild area (BIO) or subjected to integrated pest management.

Population	MFO† mean (n) (95% CL)	R	EST† mean (n) (95% CL)	R	GST† mean (n) (95% CL)	R
BIO	115.44 (40) a (83.41-147.46)	1.00	34.08 (30) ab (30.03-38.13)	1.00	46.48 (30) ad (39.70-53.27)	1.00
BON	129.78 (40) a (112.21-148.63)	1.12	32.27(30) a (29.58-34.96)	0.94	45.25 (30) a (37.11-53.38)	0.97
MO2	185.98 (40) b (160.62-211.33)	1.61	41.17 (30) b (37.88-44.46)	1.21	74.71 (30) bc (65.28-84.13)	1.61
MIX	127.25 (40) ab (103.25-151.24)	1.10	30.68 (30) a (27.23-34.13)	0.90	61.71 (30) bd (51.63- 71.79)	1.33
QUA	504.89 (40) c (467.63-542.14)	4.37	93.22 (30) c (87.96-98.48)	2.73	38.28 (30) a (33.83- 42.73)	0.82
TUG	309.16 (40) d (269.28-542.14)	2.68	67.14 (30) d (61.74-72.53)	1.97	85.11 (30) c (77.79- 92.43)	1.83

n, number of tested specimens; CL, confidence limits; R: enzyme activity ratio using BIO population as a reference.
† Enzyme activities reported as follows: MFO activity: pmol 7HC/min/abdomen; EST activity: nmol 4-nitrophenol/min/mg protein; GST activity: nmol GS-DNB/min/mg protein; mean values that share a superscript are not significantly different ($p < 0.05$, Tukey's HSD test).

Table 5. Results for dose-response bioassays using different active ingredient in unselected (LAB) and selected populations using deltamethrin (DEL), cyantraniliprole (CYA) and spinosad (SPIN).

Population	N	Slope ± SE	LC_{50} † mg AI L^{-1} (95% CL)	RF_{50}	LC_{90} † mg AI L^{-1} (95% CL)	RF_{90}	LC_{99} mg AI L^{-1} (95% CL)	RF_{99}	χ^2(df) ††
Deltamethrin selection									
LAB	215	1.44 ± 0.19	0.10 (0.05-0.15)	-	0.78 (0.52-1.35)		4.13 (2.17-11.42)	-	1.78 (4)
DEL	101	2.32 ± 0.54	2.55 (1.87-3.53)	25.0	9.08 (5.66-29.39)	15.6	59.26 (18.97-394.84)	14.3	3.50 (4)
Cyantraniliprole selection									
LAB	161	1.38 ± 0.20	14.52 (3.41-40.75)	-	122.15 (42.77-718.18)		693.12 (136.33-207776)	-	11.10 (4)
CYA	333	1.62 ± 0.17	32.41 (16.97-79.91)	2.2	200.03 (80.77-659.57)	1.6	881.93 (220.77-107927)	1.2	14.01 (4)
Spinosad selection									
LAB	342	1.87 ± 0.19	10.54 (6.56-15.07)	-	50.82 (32.99-106.26)		183.24 (91.60-701.42)	-	5.65 (4)
SPIN	333	1.83 ± 0.19	11.09 (8.58-13.94)	1.05	55.32 (40.84-83.74)	1.08	204.99 (126.05-417.43)	1.11	1.94 (4)

AI, active ingredient; SE, standard error; CL, confidence limits; RF, resistance factor.
† LC values between populations considered significantly different, if the 95% CL do not overlap.
†† A high χ^2 indicates a significant deviation from the probit model, $p < 0.05$

Table 6. Results of the field trials as mean of *D. suzukii* eggs and larvae infestation on 100 fruits.

n°	Active ingredient	Mean nr eggs/100 fruits		Mean nr larvae/100 fruits	
First field trial					
1	Untreated control	2.5	ns	5.5	a
2	Deltametrin	0.75	ns	0.5	b
3	Spinosad	1.5	ns	0	b
4	Cyantraniliprole	2	ns	0.25	b
Second field trial					
1	Untreated control	0.75	ns	18.5	a
2	Deltametrin	1.25	ns	8.75	ab
3	Spinosad	1.5	ns	0.75	b
4	Cyantraniliprole	3.5	ns	7.25	b

nr, number; ns, not significant. Means followed by a different letter were significantly different $p < 0.05$, LSD Fischer test.

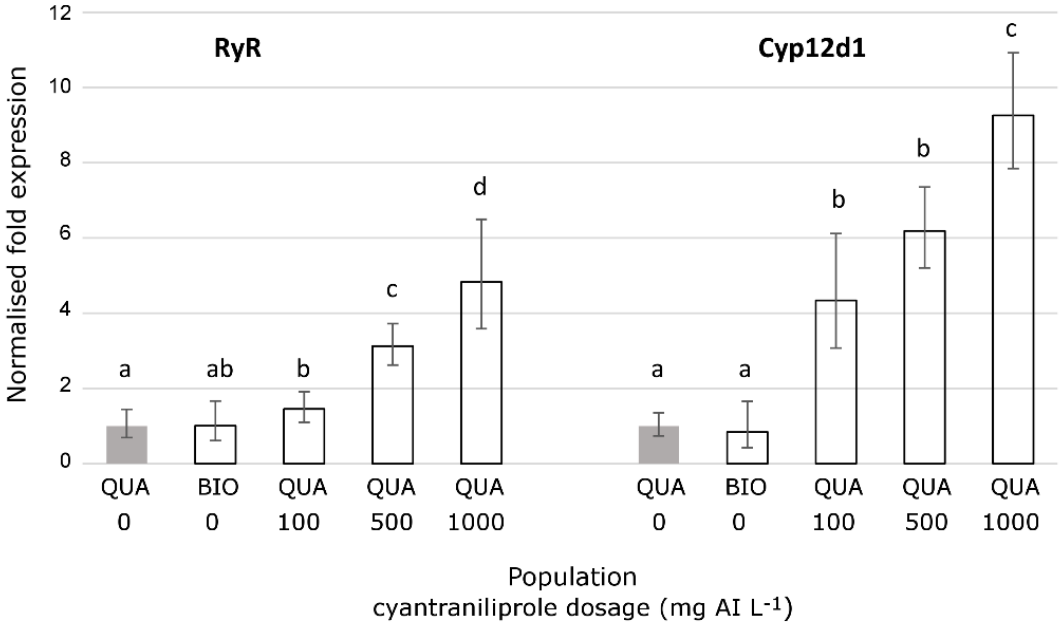

Figure 1. Effect of cyantraniliprole exposure on ryanodine receptor (*RyR*) and P450 *Cyp12d1* gene expression in QUA treated and BIO populations using QUA untreated as reference (grey bar chart). Bars represent the fold change mean ± 95% confidence interval) of target gene expression from three biological replicates (n = 6-8 specimens each). Different letters denote statistically significant differences in fold change across cyantraniliprole doses and populations for at least $p < 0.05$ using Tukey's HSD post hoc test.

high level of infestation allowed to detect significant differences among the active ingredients tested. Moderate activity was observed for deltamethrin and for cyantraniliprole, but not for spinosad.

Insecticide resistance selection

The results indicate that the toxicity to adults of *D. suzukii* of cyantraniliprole and especially deltamethrin was significantly decreased as a result of selection for resistance in the laboratory, while no difference was observed for spinosad (table 6). The level of resistance against deltamethrin was higher than the resistance against cyantraniliprole after the same number of selected generations. After 8 generations of selection, the LC_{50} values of cyantraniliprole and deltamethrin for *D. suzukii* adults from CYA and DEL populations were increased 2.2 and 25.0 fold, respectively, compared with the unselected LAB colony.

Cyp12d1 and *RyR* gene expression and mutation screening of *Nav* and *RyR* genes

Expressions of both target genes differed among treated and untreated populations ($ANOVA_{Cyp12d1}$: df = 4; F = 153.03; $p < 0.001$ and $ANOVA_{RyR}$: df = 4; F = 79.41; $p < 0.001$). Difference between the mean of all possible pairs using Tukey's showed that QUA untreated and BIO populations displayed the same transcript level for *RyR* ($p_{adj} = 0.646$) and *Cyp12d1* ($p_{adj} = 0.546$) genes (figure 1). Exposure to increasing concentrations of cyantraniliprole in QUA adults elicited a positive mRNA overexpression as compared to untreated controls. For the *RyR* gene the upregulation was significant at 500 and 1000 mg AI L^{-1} (3.1 fold and 4.8 fold respectively; $p_{adj} < 0.001$ ANOVA with Tukey's post-hoc test). The slight increase in *RyR* gene expression at 100 mg AI L^{-1} (1.4 fold) was not enough to clearly distinguish QUA treated from BIO population (figure 1). The insecticide application also positively affects the *Cyp12d1* gene expression in a dosage-dependent manner as compared to QUA controls: 4.3, 6.2 and 9.5 fold increases after challenging insects with 100, 500 and 1000 mg AI L^{-1} of cyantraniliprole, respectively ($p_{adj} < 0.001$ ANOVA with Tukey's post-hoc test) (figure 1).

The sequencing of fragments of the *Nav* and *RyR* cDNAs in field-collected and in laboratory selected populations did not reveal any of the mutations affecting amino acid positions previously associated to pyrethroid resistance in *D. melanogaster* (Pittendrigh *et al*., 1997) or affecting diamide sensitivity in *P. xylostella* such as G4946E/V and I4790M (Troczka *et al*., 2012; Guo *et al*., 2014) at the homologous positions G4892 and M4736 of *D. suzukii RyR* (Genbank accession number XP_016943112) (Supplemental material figure S1).

Discussion and conclusions

Assessment of resistance risk through in-field and laboratory tests is challenging since it could be affected by several factors such as population genetic background, mode of action (MoA) of the examined AI, previously applied selective pressures (i.e., pesticide management history, dosage and application timing of different AI, and adopted experimental procedures). Hence collecting

data from various scenarios is a crucial aspect for a more robust risk assessment, especially for rapidly spreading alien pests such as *D. suzukii*, where the genetic variability could be reduced by the founder effect (Fraimout *et al*., 2017).

In Italy, ten years after the first reports of infestation in cherry cultivations, the chemical control strategies of *D. suzukii* are widely established but limited to the combination of a small number of AI. The current pest management of *D. suzukii* includes pyrethroids as well as AI with relatively new MoA such as spynosoids and diamides. Several factors in the Italian scenario favours an increase in the selection exerted on the phytophagus, such as reduced insecticide portfolio, limited periods for treatments to respect pre-harvest interval constraints and overpopulated orchards in small districts devoted to cherry cultivation. Furthermore, the need to protect early and late varieties of cherry trees, coexisting alongside each other, prolongs the exposure of *D. suzukii* to sublethal doses of insecticides endowed with a certain photostability and discreet persistence, like deltamethrin. A slow adaptation that does not significantly affect the population size is more likely in *D. suzukii* since it possesses the highest number of genes among Drosophilids that regulate the metabolic response to xenobiotics (Nguyen *et al*., 2016).

This event might have apparently happened in the USA, during the last two years, where limited pest control in the field was accompanied by a reduced bioassay response to spinosad along with cross-resistance towards pyrethroid cypermethrin or organophosphoric malathion as well (Mishra *et al*., 2018; Gress and Zalom, 2019).

Therefore it is advisable, after inspecting the actual level of sensitivity to the applied AI, to evaluate the risk of resistance development through both field trials and molecular tests. The results from field trials and bioassays presented here revealed that all five tested populations had similar susceptibility to spinosad and spinetoram when compared with BIO control population. In contrast, TUG and, even more, QUA populations were less susceptible to deltamethrin, dimethoate and, to a lesser extent, to cyantraniliprole. Biochemical analyses indicated that this insecticide low resistance was associated with high levels of monooxygenases and carboxylesterases. Furthermore selection experiments under laboratory conditions showed that a susceptible population of *D. suzukii* was more prone to develop a reduced sensitivity to the pyrethroid than to the diamide and spinosoids.

To get an insight into the molecular markers associated with adaptive response in *D. suzukii*, different resistance mechanisms have been explored in the field strains. Reduced susceptibility to class II pyrethroids and anthranilic diamides has been associated both to alterations in the sequence and in the expression of genes coding for the insecticide target as well as to the up-regulation of detoxifying metabolic pathways (McDonnell *et al*., 2012; Liu *et al*., 2015; Duneau *et al*., 2018; Kim *et al.*, 2018; Scott, 2019).

In field populations, mutations conferring insecticide resistance may occur before or after the exposure to the pesticide and then selected as adaptive response. Resistance to both anthranilic and phthalic diamides was mainly documented in Lepidoptera species, where target-site mutations caused amino acid replacements in transmembrane domains of the insect *RyR*s conferring a significant reduction in insecticide susceptibility (Troczka *et al.,* 2012; Guo *et al*., 2014; Steinbach *et al*., 2015; Roditakis *et al*., 2017; Yao *et al*., 2017).

The glycine residue at position 4946 replaced by glutamic acid (or valine) in the diamide insensitive *P. xylostella RyR*, is evolutionarily conserved in *D. melanogaster* and *D. suzukii* orthologs, though genome editing showed that this glycine is under more functional constraints in Drosophilids than in Lepidoptera (Douris *et al*., 2017). Furthermore, the insensitive allele I4790M in *P. xilostella* naturally occurs in wild type *D. melanogaster* and *D. suzukii RyR*.

The reverse substitution M4790I introduced by genome editing in *D. melan*ogaster *RyR* increase the susceptibility to flubendiamide and chlorantraniliprole and in less extend to cyantraniliprole (Douris *et al*., 2017). Concerning the *D. suzukii* strains included in this study, the sequencing of cDNA fragments from the *RyR* C-terminal coding region, comprising the positions (G4946E and I4746M) mutated in *P. xylostella*, did not reveal sequence differences in-field collected or lab-selected populations. In any case, the presence of further missense mutations in other regions of *RyR* gene in these populations could not be ruled out *a priori*. Along with modifications of key amino acids for diamide binding, changes in *RyR* expression have also been associated with reduced susceptibility to diamide insecticides.

The adaptive response in Lepidoptera consists, more often, in an up-regulation of *RyR* gene expression (Sun *et al*., 2012; Qi *et al*., 2014; Yang *et al*., 2014; Liu *et al*., 2015; Peng *et al*., 2017; Wang *et al*., 2018) and less frequently in a decrease of the *RyR* transcript level (Lin *et al*., 2013; Gong *et al.*, 2014). The up-regulation of *RyR* gene expression was also detected in head dissected from *D. melanogaster* specimens survived to both moderate and high selective chlorantraniliprole pressure (Kim *et al*., 2018). In QUA population, the exposure to sublethal doses of cyantraniliprole did not trigger changes in the *RyR* expression at the whole body level, while a significant transcriptional up-regulation was detectable only after challenging with insecticide doses beyond the LC_{90}. The lack of a transcriptional response to lower doses might suggest the existence of a QUA subpopulation less susceptible to cyantraniliprole with a constitutive *RyR* overexpression.

The role of different detoxifying enzymes in diamide resistance is more controversial since, being encoded by multigene families, they are involved in many biological responses other than pesticide adaptation (Nauen and Steinbach, 2016).

Comparative genomics on 14 *Drosophila* species showed that in *D. suzukii* genome, probably due to the fresh fruit feeding preferences of larvae, gene duplications and positive selection have occurred, leading to the expansion of gene clades linked to detoxifying activities (Rane *et al*., 2019). Low and high resistance to chlorantraniliprole (2.1 and 21.3 resistant ratio) in lab-selected strains of *D. melanogaster* was associated with increased esterase activities (especially in head) and

low levels of GSTs (Kim *et al*., 2018). QUA and TUG populations, which were less susceptible to field pest-control, had indeed higher levels of monooxygenase and carboxylesterase activities, suggesting their involvement in reducing the susceptibility to deltamethrin and cyantraniliprole in field trials.

These phenotypes probably resulted from local selection since strains collected from treated fields shared similar pest-control strategies. Low variability in the overall genetic background along with a short history of diamide exposure of *D. suzukii* populations could foster an adaptive response, which relays on the versatility of multigene families, which govern the detoxifying pathways. In this case, the appearance and the stabilization missense nucleotide polymorphisms are favoured by their redundancy in copy number.

Accordingly, genome and transcriptome wide association studies conducted on a reference panel of *D. melanogaster* inbred lines showed that reduction in chlorantraniliprole susceptibility was linked to an increased *Cyp12d1* gene copy number, which positively affected transcription levels. Furthermore, transgenic over-expression of *Cyp12d1* gene reduced susceptibility to both chlorantraniliprole and cyantraniliprole (Green *et al.*, 2019).

As a matter of fact, when QUA specimens were exposed to cyantraniliprole, survivors displayed an increased expression of *Cyp12d1* gene, suggesting that a transcriptional response could be induced under strong selective pressure in this population. The *Cyp12d1* gene copy number has not been estimated in QUA population so either cis (gene amplification or mutation) and trans (mutation in regulatory elements) mechanisms could be responsible for *Cyp12d1* over-expression. Many naturally occurring mutations in sodium voltage channels happen (Dong *et al*., 2014; Scott, 2019) in evolutionarily conserved pyrethroid binding sites, and account for the reduction in pyrethroid sensitivity in different pest species other than Drosophilids. Indeed 9 of these common amino acid replacements occurred in *D. melanogaster Nav* gene after mutagenesis with ethyl methanesulfonate (EMS) (Pittendrigh *et al*., 1997).

However genome-wide association studies performed on 205 inbred fly lines derived from field populations collected worldwide, after pyrethroid introduction into the market, detected none of the artificially induced mutations with EMS (Battlay *et al*., 2018; Duneau *et al*., 2018). Duneau *et al.* (2018) concluded that reduced sensitivity to deltamethrin in field conditions due to target-site resistance is not common in *D. melanogaster*. Likewise, field-collected and lab-selected strains of *D. suzukii* analysed in this study, did not carry mutations EMS-induced in *D. melanogasters* in their *Nav* gene. However, presence of missense SNPs in alternative positions of *Nav* gene cannot be ruled out.

Furthermore, Duneau and coworkers (2018) showed that most of the variation in resistance to deltamethrin among the inbred fly lines of *D. melanogaster* appeared associated with a single locus, harbouring putative paralog *Cyp6a23* and *Cypa17* genes. Even if the role of the putative ortholog gene in *D. suzukii* was not investigated in this study, populations with the highest level of monoxygenases (TUG and QUA) had a reduced susceptibility to deltamethrin in bioassays. Overall, the results suggest that an initial adaptive response of *D. suzukii* to treatment with deltamethrin and cyantraniliprole is based primarily on the detoxifying activity of monooxygenases and carboxylesterases. However, this adaptive response does not affect the susceptibility to spynosins. Increased transcription of the *RyR* and *Cyp12d1* genes may help to counteract exposure to cyantraniliprole.

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