Identification of thrips species and resistance of *Frankliniella occidentalis* (Thysanoptera: Thripidae) to malathion, spinosad, and bifenthrin in blackberry crops

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Abstract

Mexico is among the most important blackberry producers in the world. In this crop, thrips damage is associated with poor fruit set. The objectives of this study were to identify the main thrips species associated with the flowers of cultivated blackberries, and to determine the resistance level of 13 field-collected populations of western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), to the insecticides malathion, bifenthrin, and spinosad in the states of Michoacán and Jalisco, Mexico. Verification of *F. occidentalis* was conducted by morphology and PCR amplification of the cytochrome oxidase I (COI) partial gene using LCO and HCO primers The susceptibility of adult thrips to insecticides was determined using residual contact exposure on bean (*Phaseolus vulgaris* L.; Fabaceae) leaf sections dipped into aqueous solutions of 8 different concentrations of malathion, bifenthrin, and spinosad. The morphological identification of thrips from 8 different sampling zones confirmed that the main thrips species associated with blackberry flowers was *F. occidentalis*. However, *Retanathrips funestus* (Hood), *Frankliniella insularis* (Franklin), *Frankliniella tolucensis* Watson, *Taenothrips frici* (Uzel), and *Isoneurothrips australis* Bagnall (all Thysanoptera: Thripidae) also were found. Most of the evaluated populations of *F. occidentalis* showed significant differences from the susceptible population in terms of the level of resistance to bifenthrin, malathion, and spinosad. The resistance ratios (RR_{50}) for malathion varied from 36 to 2,458, followed by 4 to 974, and 2 to 248 in bifenthrin and spinosad, respectively. These results suggest the need to establish insecticide resistance management programs, particularly in zones that resulted in the highest levels of insecticide resistance.

Key Words: western flower thrips; berry crops; insecticide resistance

Resumen

México es uno de los productores más importantes de zarzamora del mundo. En este cultivo, el daño de los trips se asocia con una amarre deficiente del fruto. Los objetivos de este estudio fueron identificar las principales especies de trips asociadas a las flores de zarzamora cultivada y determinar el nivel de resistencia de 13 poblaciones de campo del trips occidental de las flores, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), a los insecticidas malatión, bifentrina y spinosad en los estados de Michoacán y Jalisco, México. La verificación de *F. occidentalis* se realizó mediante morfología y amplificación parcial por PCR del gen de la citocromo oxidasa I (COI) utilizando los primeres LCO y HCO en la región COXI. La susceptibilidad de los trips adultos a los insecticidas se determinó utilizando exposición por contacto residual en las secciones de hojas de frijol (*Phaseolus vulgaris* L.; Fabaceae) sumergidas en soluciones acuosas de 8 concentraciones diferentes de malatión, bifentrina y espinosad. La identificación morfológica de 8 diferentes zonas de muestreo confirmó que la principal especies de trips asociadas con las flores de zarzamora fue *F. occidentalis*. Sin embargo, también se encontraron asociadas a las flores las especies *Retanathrips funestus* (Hood), *Frankliniella insularis* (Franklin), *Frankliniella tolucensis* Watson, *Taenothrips frici* (Uzel), *Isoneurothrips australis* Bagnall (todo Thysanoptera: Thripidae). La mayoría de las poblaciones evaluadas de *F. occidentalis* mostraron diferencias significativas con la población susceptible en el nivel de resistencia a bifentrina, malatión y espinosad. La razón de resistencia (RR_{50}) en malatión variaron de 36 a 2,458 seguido de 4 a 974, y de 2 a 248 en bifentrina y spinosad, respectivamente. Estos resultados sugieren la necesidad de establecer programas de manejo de resistencia a estos insecticidas, ene especial en las zonas que resultaron con los más altos niveles de resistencia a los insecticidas.

Palabras Clave: trips oriental de las flores; berries; resistencia a insecticidas

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México is the leading blackberry (*Rubus* sp.; Rosaceae) producer in the world; the states of Michoacán and Jalisco are the most important because of their production area. In 2017, production exceeded 234,820 tons harvested over more than 12,000 ha; more than 96% of this production is exported (SIAP 2017). Thrips associated with flowers are among the most important blackberry pests, resulting in losses that can exceed 70%. This damage is associated with poor fruit set, most likely because of the damage they cause to the flowers, especially to the styles, ovaries, and developing fruits (Rhodes & Liburd 2017), which results in unmarketable fruits.

Thrips are a polyphagous pest of economic importance that feed on and cause damage to various plant species (Castresana et al. 2008; Muchero et al. 2010). There are approximately 5,000 thrips species (Mound 2004). Among them, *Aeolothrips intermedius* Bagnall, *Drepanothrips reuteri* Uzel, *Frankliniella intonsa* (Trybom), *Frankliniella occidentalis* (Pergande), *Thrips angusticeps* Uzel, and *Thrips meridionalis* (Priesner) (all Thysanoptera: Thripidae) have been associated with crops belonging to the Rosaceae family (Mound 2004). Specifically, *F. occidentalis*, *Frankliniella tritici* (Fitch), *Frankliniella fusca* (Hinds), and *Frankliniella bispinosa* (Morgan) (all Thysanoptera: Thripidae) have been associated with blackberries (Northfield et al. 2008; Rhodes & Liburd 2017). Among the most important species in the subtropics, *F. occidentalis* is notable as one of the most abundant and common (Mound 2014; Li et al. 2017). This species has a high reproductive potential and a wide range of hosts, including ornamental crops, garden plants, vegetables, and fruits (Mound 2017). In addition, *F. occidentalis* is a vector of tomato spotted wilt virus in crops, such as tomatoes and chrysanthemums (Badillo-Vargas et al. 2012; Ogada et al. 2013; Margaria et al. 2014). Management strategies for *F. occidentalis* include various tactics, including physical, cultural, genetic, biological, and chemical control methods (Manners et al. 2013; Wu et al. 2018). For chemical control, the range of available compounds and formulations has been limited because of the capacity of *F. occidentalis* to develop resistance (Bielza et al. 2007a). For example, in strawberries, more that 19 sprayings have been reported in Almeria and Murcia, Spain (Bielza 2008). In this regard, different populations of this species have been documented to be resistant to many insecticides in multiple classes, including organochlorines, organophosphates, pyrethroids (Inmaraju et al. 1992; Espinosa et al. 2002), carbamates (Bielza et al. 2009), and spinosyns (Bielza et al. 2007b) in a variety of horticultural crops. For instance, spinosad is among the molecules to which thrips resistance has been detected under greenhouse conditions in various regions, such as Almeria, Spain, where thrips resistance was determined to be of genetic origin (Inmaraju et al. 1992). Other populations collected in roses, carnations, and chrysanthemums from San Diego, California, USA, showed metabolic resistance to bifenthrin, permethrin, methomyl, chlorpyrifos, and abamectin because of an increase in multi-function oxidases (Inmaraju et al. 1992). Additionally, populations in southeastern Spain collected from sweet pepper, tomato, lettuce, artichoke, melon, cucumber, carnation, kidney bean, peach, and plum crops showed resistance to methamidophos, acrinatrine, endosulfan, deltamethrin, and formetanate; however, the resistance type was not determined (Espinosa et al. 2002).

Among the resistance mechanisms associated with *F. occidentalis*, genetic resistance by modification at the site of action (Espinosa et al. 2002; Bielza 2008), and metabolic resistance because of an increase in the production of multi-function oxidases, also known as cytochrome P-450 monooxygenases (Espinosa et al. 2005), have been reported.

In Mexico, although it is presumed that *F. occidentalis* is among the most important thrips species affecting blackberry flowers, to the best of our knowledge, no information has been published regarding thrips species associated with blackberry flowers, or the resistance of the most common thrips species to the insecticides commonly used with this crop. However, growers have been reporting failures in thrips control, particularly during the spring. Preliminary studies regarding the frequency of use of insecticides for thrips control in Michoacán indicated that malathion, bifenthrin, and spinosad were the most frequently used insecticides in the blackberry-producing regions (Rebollar-Alviter, unpublished information). Although the 3 insecticides belong to different toxicological groups and have different modes of action, their reduced efficacy in the field has been of concern. The objectives of this study were to determine the thrips species associated with cultivated blackberry flowers, and to evaluate the resistance of 13 field-collected populations of *F. occidentalis* to the insecticides malathion, bifenthrin, and spinosad in the states of Michoacán and Jalisco, Mexico.

Materials and Methods

THRIPS SPECIMEN COLLECTION

Thrips for morphological identification and insecticide resistance studies were collected in commercial blackberry plots during the flowering stage from the municipalities of Ziracuaretiro, Los Reyes de Salgado, and Tacámbaro, Mexico, during Sep and Oct of 2016 and Nov of 2017, respectively, in the state of Michoacán. In the state of Jalisco, the samples were collected in Mazamitla during Feb, and in Jocotepec and Zacoalco de Torres during Oct 2017 (Table 1). Thirty blackberry plants were selected randomly in each sampled plot distributed in a zigzag sampling scheme. For each plant, 3 flowers were collected and deposited in plastic containers (11 cm in diam by 17 cm in height) with 5 layers of absorbent paper towels on the bottom; these containers had a 6-cm diam screw cap with an opening covered with organza fabric to allow aeration for the insects to remain alive for molecular identification. In the lab, a set of specimens from 8 selected sampling zones was preserved in 75% ethanol for morphological identification. To perform phylogenetic identification, the thrips were maintained alive in the plastic containers for DNA extraction.

For insecticide resistance studies from the sampling zones, 13 *F. occidentalis* populations were selected from the sampled commercial blackberry fields during the flowering stage, including 9 populations from Michoacán (5 and 4 populations from the municipalities of Ziracuaretiro and Los Reyes de Salgado, respectively), and 4 from Jalisco (2 populations from the municipalities of Mazamitla and 1 each from Jocotepec and Zacoalco de Torres) (Table 1). With the exception of the population M2, which was collected in an organic blackberry plot from Mazamitla, the remaining populations were collected from intensively managed blackberry production plots with a history of continuous use of a range of insecticides, such as spinosad, bifenthrin, and malathion for thrips management. *Frankliniella occidentalis* was selected because it was the most common species of the sampling conducted in the different blackberry production zones. An insecticide-susceptible population from Colegio de Postgraduados, Montecillo, Texcoco, state of Mexico, was used as a reference population in the bioassays. This population was established in 2009 using local field-collected individuals from an experimental plot of potato plants (*Solanum tuberosum* L.; Solanaceae) free of pesticide applications. These individuals have been reared since then on common bean plants (*Phaseolus vulgaris* L.; Fabaceae) cv. 'Flor de Mayo,' under greenhouse conditions (about 25 °C, 60% RH, 12:12 h (L:D), and have never been exposed to insecticides.

Table 1. Source of thrips collection from the flowers of commercial blackberry crops in different areas for the identification and evaluation of resistance to the insecticides malathion, spinosad, and bifenthrin.

Municipality	Location	Population[b]	Collection date
Ziracuaretiro Los Reyes	19.686356°N, 101.237611°W[a]	Z1	23 Sep 2016
	19.422244°N, 101.928658°W[a]	Z2	23 Sep 2016
	19.421643°N, 101.923072°W	Z3	23 Sep 2016
	19.349755°N, 101.928753°W	Z4	23 Sep 2016
	19.385355°N, 101.917974°W	Z5	23 Sep 2016
	19.575181°N, 102.462801°W[a]	R1	8 Oct 2016
	19.613603°N, 102.488224°W[a]	R2	8 Oct 2016
	19.365502°N, 102.295200°W	R3	8 Oct 2016
	19.363253°N, 102.295210°W	R4	8 Oct 2016
Mazamitla	19.570060°N, 103.042621°W[a]	M1	16 Feb 2017
	19.925054°N, 103.064561°W[a]	M2	16 Feb 2017
Jocotepec	20.337366°N, 103.484107°W	J1	5 Oct 2017
Zacoalco	20.220336°N, 103.543769°W	ZT1	5 Oct 2017
Tacámbaro	19.226306°N, 101.480483°W[a]	–	11 Nov 2017
Tacámbaro	19.222212°N, 101.522260°W[a]	–	11 Nov 2017
Texcoco	19.464170°N, 98.908634°W	Susceptible	10 years

[a] A subsample from these locations was used for morphological identification of thrips.

[b] Population designation used for insecticide resistance studies. No population for insecticide resistance was selected from the Tacámbaro municipality.

MORPHOLOGICAL AND PHYLOGENETIC IDENTIFICATION OF THRIPS

The morphological identification of thrips collected in the different blackberry production areas was based on Jacot-Guillarmod (1974), Moulton (1948), Mound & Marullo (1996), and Mound & Nickle (2009).

Phylogenetic confirmation of *F. occidentalis* was completed on specimens from 11 populations that were selected from the 13 total populations collected for the insecticide resistance studies (Table 1). An additional sample (not included in the insecticide resistance study) from Tacámbaro also was included in this analysis. To conduct the phylogenetic identification, the specimens were identified first by morphology (Cavalleri & Mound 2012), and then were used for DNA extraction and PCR amplification. DNA extraction was conducted using the CTAB 2% method (Doyle 1997) and sodium acetate buffer. DNA quantification was performed using spectrophotometry with a NanoDrop 2000 (Thermo Scientific RMA, Wilmington, Delaware, USA). The cytochrome oxidase I (COI) partial gene was amplified by using conventional PCR using LCO (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO (5'- TAAACTTCAGGGTGACCAAAAAATCA-3') primers (Folmer et al. 1994), which yielded a band of 658 base pairs. The master mix consisted of 7.86 µL of HPLC water, 3 µL of 5X buffer, 0.6 µL of dNTPs, 0.18 µL of LCO-HCO primers, 0.18 µL of *Taq* DNA polymerase, and 3 µL of DNA (60 ng). The thermal conditions were conducted in a C1000 Touch thermal cycler (Bio-Rad, Foster City, California, USA) with an initial denaturation at 94 °C for 1 min prior to the following steps: 5 cycles of 94 °C for 30 s, 45 °C for 1.5 min, 72 °C for 1 min; 35 cycles of 94 °C for 30 s, 57 °C for 1.5 min, and 72 °C for 1 min; and a final extension of 72 °C for 5 min. Electrophoresis was performed on an 1% agarose gel (SeaKem, Lonza, Greenwood, South Carolina, USA), and amplicons were visualized via photodocumentation by using an Infinity system 3026/WL/LC/26 MX X-Press (Vilber Lourmat, Deutschland GmbH, Eberhardzell, Germany).

For DNA sequencing and phylogenetic analysis, the amplicons were cleaned with ExoSAP (Affymetrix, Santa Clara, California, USA), and sequenced in a 3130 DNA 4-capillary Genetic Analyzer (Applied Biosystems, Foster, California, USA). The sequences were assembled using the BioEdit v.7.0.5 software (Hall 1999), and compared with those deposited in the GenBank database with the BLASTN option (Altschul et al. 1997) in the platform from the National Center for Biotechnology Information. The phylogenetic analysis was performed using Bayesian inference with Mr Bayes 3.2 (Ronquist et al. 2012), and a mega file was transformed in the nexus file using the MEGA 7 software (Kumar et al. 2016). Bayesian inference with 1.5 million generations was performed in 4 Monte Carlo chains to obtain a consensus tree. Rapid bootstrapping with 1,000 iterations was implemented using the general time reversible model with an inverse-gamma distribution. The resulting tree was drawn with FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

FIELD POPULATION RESISTANCE TO INSECTICIDES

Thrips Rearing

In the lab, the thrips collected in the field were extracted by gently shaking the flowers on white paper, the insects then separated by their morphological characteristics according to Cavalleri and Mound (2012), and transferred to the rearing containers using a buccal vacuum. Mass rearing of *F. occidentalis* was conducted according to Espinosa et al. (2002). In brief, the thrips were reared on bean pods previously disinfected with 2% sodium hypochlorite for 5 minutes, washed with sterile distilled water, and soaked in a 5% agarose solution. The pods were allowed to dry at room temperature before deposition into the rearing containers. After 5 days, the oviposited pods were transferred to other insect-free containers and maintained in a bioclimatic chamber at 25 ± 2 °C, 50 ± 5% relative humidity, and a 12:12 h (L: D) photoperiod until adult emergence.

Insecticides

Commercial insecticide formulations were used. The products tested were malathion 520 g L^{-1} emulsion concentrate (EC; Lucathion®, Química Lucava Celaya, Guanajuato, Mexico), bifenthrin 100 g kg-1 EC (Talstar®, FMC, Zapopan, Jalisco, Mexico), and spinosad 120 g kg^{-1} suspension concentrate (SpinTor®, Corteva Agriscience, Zapopan, Jalisco, Mexico). These insecticides were selected based on the frequency of their use in the study area based on a previous regional survey (Rebollar-Alviter, unpublished).

Bioassays

For each field-collected population of *F. occidentalis*, the susceptibility of adults to malathion, bifenthrin, and spinosad was determined using the residual contact exposure according to Bielza et al. (2009). Sections of 30 × 5 mm were collected from bean leaves obtained from the middle part of 45-d-old bean plants cv. 'Flor de Mayo,' and were dipped for 10 s in 20 mL of aqueous solution of the insecticides malathion (1,000, 300, 100, 30, 10, 3, 1, 0.3 mg L^{-1}), and bifenthrin and spinosad (100, 30, 10, 3, 1, 0.3, 0.1, 0.03 mg L^{-1}). These concentrations were determined based on a preliminary experiment to estimate the biological response or concentration range that caused between 0 and 100% mortality. Then, intermediate concentrations were included to cover that range. As a control, the bean leaf sections were dipped in distilled water only.

After drying for 15 min in a hood, the bean leaf sections were individually placed in 1.5-mL Eppendorf tubes. Fifteen adult female thrips were placed in each tube using an aspirator. Three replicates, as well as a control, were used per insecticide concentration. The Eppendorf tubes were closed with parafilm and maintained in a vertical position in the bioclimatic chamber as previously mentioned. Mortality was evaluated 24 h after the treatment application; insects that did not show movement were considered dead. Each trial was conducted twice.

Data analysis

Before the data analysis, mortality was corrected in the different concentrations in relation to the observed mortality in the untreated control (Abbott 1925), which did not exceed 10%. A probit analysis was conducted using the Probit procedure of the SAS system (Statistical Analysis System, Cary, North Carolina, USA, vers. 9.3). The median lethal concentrations (LC_{50}) and lethal concentrations for 95% (LC_{95}) values were calculated in mg L^{-1} by pooling the data from 3 repetitions of each experiment (n = 90). The populations were considered significantly different if their confidential limits did not overlap with a confidence level of 95% (Robertson & Preisler 1992). The resistance ratio (RR) for each insecticide was estimated by dividing the LC_{50} value for the field populations by the LC_{50} value for the reference population.

Results

THRIPS SPECIES ASSOCIATED WITH CULTIVATED BLACKBERRIES

Morphological identification of thrips from 8 different collecting zones in the states of Michoacán and Jalisco, Mexico, during 2016 and 2017 indicated that most specimens collected from blackberry flowers were *F. occidentalis*. However, *F. insularis* and *I. australis* also were found in 2 samples from Tacámbaro. In addition, *R. funestus* and *F. tolucensis* also were found in Los Reyes de Salgado and *T. frici* in Tacámbaro, Michoacán and Mazamitla, Jalisco (Table 2).

Phylogenetic analysis confirmed that the 11 specimens from the 13 selected populations used for insecticide resistance studies were *F. occidentalis.* Five of the sequences obtained in this study were deposited in the GenBank database under accession numbers MF993426, MF993428, MF993429, MF993431, and MF993432. They were grouped with sequences belonging to haplotypes described using MtDNA barcode sequences (https://www.ncbi.nlm.nih.gov/nuccore/) (Fig. 1). According to the analysis, samples were clustered in 2 clades corresponding to the haplotypes FCOI-9 and FCOI-3. The 2 haplotype FCOI-9 samples were collected from Los Reyes de Salgado, Michoacán, and Mazamitla, Jalisco. The remaining haplotype FCOI-3 samples were collected from the municipalities of Ziracuaretiro, Tacámbaro, and Los Reyes de Salgado, Michoacán. *Frankliniella occidentalis* was found in all the sampled plots of the different geographic areas producing blackberries in the municipalities of Ziracuaretiro, Los Reyes de Salgado, and Tacámbaro in the state of Michoacán and in Mazamitla, Jalisco, Mexico.

RESISTANCE OF *FRANKLINIELLA OCCIDENTALIS* TO SPINOSAD, BIFENTHRIN, AND MALATHION

For the spinosad insecticide, there were significant differences between 12 of the field populations evaluated and the susceptible population in regard to the estimated LC_{50} values (Table 3). The M2 population corresponding to individuals collected in an organic blackberry plot in Mazamitla, Jalisco, did not show significant differences compared to the susceptible population based on the separation of their confidence limits (Table 3). The Z2 and Z5 populations from the Ziracuaretiro municipality showed the highest LC_{50} values, 1.45 and 2.48 mg L^{-1}, and the RR_{50} values were 145 and 248, respectively. For the municipality of Los Reyes de Salgado, the R4 population showed the highest LC_{50} value (1.20 mg L^{-1}), with an RR_{50} value of 120. The populations collected in the municipalities of Jocotepec (J1) and Zacoalco de Torres (ZT1) produced the lowest RR_{50} values (6 and 7, respectively) after the M2 population, which had an RR_{50} value of 2. For LC_{95}, the Z1 population from Ziracuaretiro, Michoacán, and the M2 population from Mazamitla, Jalisco, did not show significant differences compared to the susceptible population. The RR_{95} was lower than the RR_{50}; in this case, the Z5 populations and R3 were those that showed the highest values, with RR_{95} values of 214 and 159, respectively (Table 2).

Concerning malathion, all of the evaluated populations showed significant differences compared to the susceptible population both at the LC_{50} and LC_{95} levels. The LC_{50} values were within a range of 3.96 to 274.8 mg L^{-1}, with RR_{50} values between 36 for the case of the Z1 population from Ziracuaretiro and 2,498 for the R2 population from Los Reyes de Salgado (Table 4). The obtained LC_{95} values were between 34.23 and

Table 2. Thrips species associated with flowers collected from 8 areas of commercial blackberry plots in Mexico.

Municipality	Location	Collection date	Thrips species identified
Ziracuaretiro	19.686356°N, 101.237611°W	23 Sep 2016	*Frankliniella occidentalis*
Ziracuaretiro	19.422244°N, 101.928658°W	23 Sep 2016	*Frankliniella occidentalis*
Los Reyes de Salgado	19.575181°N, 102.462801°W	8 Oct 2016	*Frankliniella occidentalis*; *Retanathrips funestus*
Los Reyes de Salgado	19.613603°N, 102.488224°W	8 Oct 2016	*Frankliniella occidentalis*; *Frankliniella tolucensis*
Tacámbaro	19.226306°N, 101.480483°W	11 Nov 2017	*Frankliniella occidentalis*; *Tenothrips frici*
Tacámbaro	19.222212°N, 101.522260°W	11 Nov 2017	*Frankliniella occidentalis*; *Frankliniellainsularis*; *Isoneurothrips australis*
Mazamitla	19.570060°N, 103.042621°W	16 Feb 2017	*Frankliniella occidentalis*; *Tenothrips frici*
Mazamitla	19.925054°N, 103.064561°W	16 Feb 2017	*Frankliniella occidentalis*

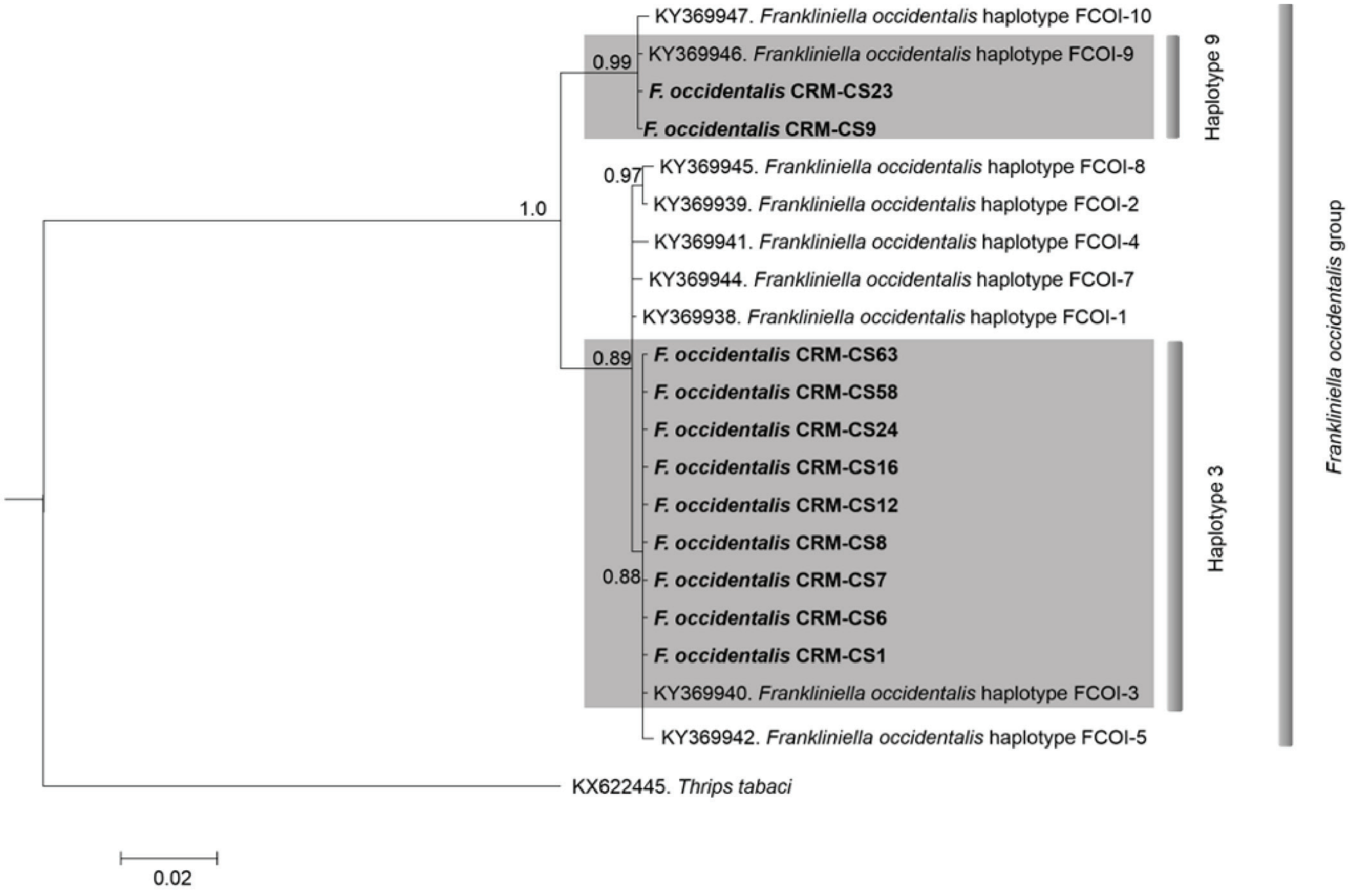

Fig. 1. Phylogenetic consensus tree resulting from Bayesian inference based on COI mitochondrial DNA partial sequences showing the relationship of 11 thrips samples collected from commercial blackberry plots in Michoacán and Jalisco, Mexico. The sample numbers correspond to Ziracuaretiro (24, 58, 8, 7, 6, 1, and 63), Tacámbaro (16), Los Reyes de Salgado (12 and 9), and Mazamitla (23). Reference sequences from the different *Frankliniella occidentalis* haplotypes were downloaded from GenBank.

8,640 mg L^{-1} for the Z1 and Z3 populations, which were both collected in the municipality of Ziracuaretiro; the RR$_{95}$ values were 7 and 1,272, respectively.

Regarding bifenthrin, the LC$_{50}$ of the susceptible population was 0.014 mg L^{-1}, and the 13 field populations evaluated were significantly different from this population based on their confidence limits (Table 5). The LC$_{50}$ ranged between 0.06 mg L^{-1} for the Z1 population and 13.63 mg L^{-1} for the Z3 population, with RR$_{50}$ values of 4 and 974, respectively. The 2 populations of Mazamitla (M1 and M2), together with that of J1 and Zacoalco de Torres ZT1, showed the lowest RR$_{50}$ values

Table 3. Slopes, lethal concentrations (LC$_{50}$, LC$_{95}$) and resistance ratios (RRs) of 13 populations of *Frankliniella occidentalis* collected in blackberry crops from the states of Michoacán and Jalisco, Mexico, and treated with spinosad.

Population	n	Slope ± SE	LC$_{50}$ (95% CL)	LC$_{95}$ (95% CL)	Pr > χ^2	RR$_{50}$	RR$_{95}$
Susceptible	786	0.99 ± 0.07	0.01 (0.01 – 0.02)	0.47 (0.30 – 0.82)	0.96	1	1
Z1	582	1.62 ± 0.16	0.08 (0.06 – 0.10)	0.82 (0.50 – 1.62)	0.81	8	2
Z2	568	1.34 ± 0.13	1.45 (1.08 – 1.92)	24.50 (14.70 – 51.23)	0.90	145	52
Z3	579	1.08 ± 0.12	0.68 (0.49 – 0.99)	22.33 (10.17 – 74.06)	0.93	68	48
Z4	585	0.96 ± 0.09	0.70 (0.50 – 0.98)	36.08 (17.63 – 99.87)	0.97	70	77
Z5	564	1.02 ± 0.09	2.48 (1.78 – 3.48)	100.42 (51.63 – 255.15)	0.92	248	214
R1	639	1.13 ± 0.12	0.28 (0.20 – 0.38)	7.85 (4.21 – 19.56)	0.85	28	17
R2	658	1.42 ± 0.17	0.74 (0.56 – 1.00)	10.47 (5.76 – 26.91)	0.65	74	22
R3	661	0.71 ± 0.10	0.37 (0.19 – 0.61)	74.76 (25.13 – 523.77)	0.97	37	159
R4	668	1.51 ± 0.13	1.20 (0.93 – 1.56)	14.62 (9.29 – 27.09)	0.94	120	31
M1	582	1.00 ± 0.09	0.28 (0.20 – 0.39)	11.94 (6.01 – 31.36)	0.94	28	25
M2	604	1.07 ± 0.09	0.02 (0.01 – 0.03)	0.93 (0.53 – 2.04)	0.91	2	2
J1	533	1.29 ± 0.11	0.70 (0.53 – 0.93)	13.12 (7.84 – 26.37)	0.81	6	3
ZT1	556	1.04 ± 0.10	0.79 (0.55 – 1.09)	30.26 (16.60 – 70.52)	0.65	7	6

n = number of tested thrips; SE = standard error; LC$_{50}$ and LC$_{95}$ = lethal concentrations that caused 50 and 95% insect mortality (mg L^{-1}), respectively; Pr > χ^2 = probability of dose-probit line fitted as a straight line; RR = resistance ratio (LC$_{50}$ field population/LC$_{50}$ susceptible population).

Table 4. Slopes, lethal concentrations (LC_{50}, LC_{95}) and resistance ratios (RRs) of 13 populations of *Frankliniella occidentalis* collected in blackberry crops from the states of Michoacán and Jalisco, Mexico, and treated with malathion.

Population	n	b ± SE	LC_{50} (95% CL)	LC_{95} (95% CL)	Pr > χ^2	RR_{50}	RR_{95}
Susceptible	782	1.01 ± 0.07	0.11 (0.08 – 0.15)	4.73 (2.81 – 9.34)	0.96	1	1
Z1	587	1.76 ± 0.16	3.96 (3.15 – 5.00)	34.23 (23.01 – 58.81)	0.82	36	7
Z2	585	0.96 ± 0.09	71.69 (51.35 – 101.28)	3,692 (1,802 – 10,201)	0.89	652	781
Z3	560	0.94 ± 0.09	158.47 (112.49 – 223.63)	8,640 (4,219 – 24,035)	0.95	1441	1827
Z4	578	1.41 ± 0.11	216.22 (167.71 – 278.96)	3,188 (2,052 – 5,707)	0.84	1966	674
Z5	592	1.31 ± 0.11	88.89 (66.92 – 116.21)	1,596 (1,014 – 2,933)	0.94	808	337
R1	663	1.27 ± 0.11	96.93 (72.72 – 127.52)	1,925 (1,199 – 3,632)	0.87	881	407
R2	674	1.23 ± 0.12	274.80 (204.46 – 365.49)	6,015 (3,492 – 13,015)	0.96	2498	1272
R3	669	1.13 ± 0.12	96.60 (70.85 – 132.26)	2,771 (1,468 – 6,977)	0.91	878	586
R4	676	1.25 ± 0.12	28.57 (21.44 – 37.89)	590.42 (341.57 – 1,288)	0.77	260	125
M1	573	1.17 ± 0.11	30.01 (21.83 – 40.34)	768.42 (459.50 – 1,549)	0.83	273	162
M2	591	1.03 ± 0.09	20.04 (14.67 – 27.58)	777.52 (411.21 – 1,881)	0.82	182	164
J1	531	0.94 ± 0.10	21.32 (13.87 – 30.90)	1,199 (615.85 – 3,147)	0.75	194	253
ZT1	561	0.98 ± 0.12	21.72 (14.79 – 31.08)	1,031 (481.03 – 3,388)	0.73	197	218

n = number of tested thrips; b ± SE = slope, standard error; LC_{50} and LC_{95} = lethal concentrations that caused 50 and 95% insect mortality (mg L^{-1}); Pr > χ^2 = probability of dose-probit line fitted as a straight line; RR_{50} and RR_{95} = resistance ratios.

(11, 9, 6, and 9, respectively). For LC_{95}, the Z1 population had the lowest value (0.93 mg L^{-1}), and the Z3 population had the highest value (711.16 mg L^{-1}); the RR_{95} values were 2 and 1,823, respectively (Table 4).

Discussion

Thrips have been a pest of economic importance during recent years in the blackberry-growing areas in the states of Michoacán and Jalisco, Mexico; thrips interfere with fruit set and result in berry deformation, which directly affects fruit quality, reduces yield, and prevents its marketing, most likely because of the damage they cause to the flowers, especially to the styles, ovaries, and developing fruits (Rhodes & Liburd 2017).

Morphological studies indicated that *F. occidentalis* was the principal thrips species associated with blackberry flowers. However, *R. funestus*, *F. insularis*, *F. tolucencis*, *T. frici*, and *I. australis* also were found. *Retanathrips funestus* has been reported previously in Mexico (Mound & Nickle 2009) and Panama in tropical trees (Tiliaceae, Maranthaceae, Rubiaceae, and Bignoniaceae) (Goldarazena et al. 2012), whereas *F. insularis* has been associated with raspberry (*Rubus idaeus* L.; Rosaceae) flowers in Mexico State (Sánchez et al. 2001) and moringa (*Moringa oleifera* Lam.; Moringaceae) flowers in southeastern Mexico (López-Guillén et al. 2018). This latter species has been reported causing puncture marks and patches to rose flowers (*Rosa* sp.; Rosaceae) in Brazil (Bezerra et al. 2016). *Frankliniella tolucensis* was described for the first time in plants of the genus *Eryngium* (Apiaceae) in the Nevado de Toluca volcano in the city of Toluca, state of Mexico (Watson 1942), but it has not been documented as a crop pest. *Taenothrips frici*, known as Mediterranean dandelion thrips (*Taraxacum* sp.; Asteraceae), has been associated with plants of the Asteraceae family, principally weeds such as *Hypochaeris* sp., and *Centaura* sp., but has not been observed causing damage in crops, and although its distribution is worldwide, it inhabits mainly warm temperate areas of Central and South America (Mound & Marullo 1996). No previous reports that confirm this species as a pest in *Rubus* sp. have been published. *Isoneurothrips australis* is associated principally with *Eucalyptus* sp. (Myrtaceae), *Crataegus* sp. (Rosaceae), *Rubus* sp., and other hosts such as oranges (*Citrus sinensis*

Table 5. Slopes, lethal concentrations (LC_{50}, LC_{95}) and resistance ratios (RRs) of 13 populations of *Frankliniella occidentalis* collected in blackberry crops from the states of Michoacán and Jalisco, Mexico, and treated with bifenthrin.

Population	n	b ± SE	LC_{50} (95% CL)	LC_{95} (95% CL)	Pr > χ^2	RR_{50}	RR_{95}
Susceptible	799	1.14 ± 0.07	0.014 (0.010 – 0.017)	0.39 (0.26 – 0.65)	0.71	1	1
Z1	582	1.37 ± 0.13	0.06 (0.04 – 0.08)	0.93 (0.58 – 1.80)	0.84	4	2
Z2	583	1.33 ± 0.13	7.53 (5.57 – 9.96)	129.70 (78.78 – 263.88)	0.83	538	333
Z3	615	0.95 ± 0.09	13.63 (9.62 – 19.15)	711.16 (357.52 – 1,889)	0.84	974	1,823
Z4	581	0.93 ± 0.09	2.07 (1.45 – 2.96)	123.62 (58.36 – 361.99)	0.88	148	317
Z5	583	1.00 ± 0.09	1.17 (0.83 – 1.61)	51.41 (27.42 – 123.99)	0.96	84	132
R1	672	1.04 ± 0.09	0.71 (0.51 – 0.97)	26.94 (14.28 – 64.79)	0.91	51	69
R2	680	1.27 ± 0.12	0.88 (0.66 – 1.17)	17.20 (10.17 – 36.02)	0.98	63	44
R3	670	1.23 ± 0.12	0.84 (0.63 – 1.12)	18.08 (10.40 – 39.74)	0.91	60	46
R4	669	1.17 ± 0.12	1.01 (0.75 – 1.37)	25.53 (14.02 – 60.33)	0.84	72	65
M1	589	1.07 ± 0.10	0.16 (0.11 – 0.21)	5.43 (2.95 – 12.61)	0.89	11	14
M2	599	1.25 ± 0.11	0.12 (0.09 – 0.16)	2.47 (1.52 – 4.75)	0.95	9	6
J1	522	1.45 ± 0.14	0.67 (0.50 – 0.89)	9.18 (5.75 – 17.66)	0.75	6	2
ZT1	535	1.07 ± 0.10	1.00 (0.71 – 1.39)	34.87 (19.22 – 80.21)	0.79	9	7

n = number of tested thrips; b ± SE = slope, standard error; LC_{50} and LC_{95} = lethal concentrations that caused 50 and 95% insect mortality (mg L^{-1}); Pr > χ^2 = probability of dose-probit line fitted as a straight line; RR_{50} and RR_{95} = resistance ratio.

L.; Rutaceae) and roses (*Rosa* sp.). It is reported in California and Hawaii, USA (Nickle 2008). All the species found in flowers of cultivated blackberry are reported for the first time on this crop in Mexico.

Regarding *F. occidentalis*, the principal thrips species found associated with blackberry flowers, phylogenetic studies confirmed the morphological studies and showed 2 haplotypes (FCOI-9 and FCOI-3) in the populations selected for insecticide tests. The determination of 2 haplotypes of *F. occidentalis* identified using molecular methods suggests possible genetic variation in the species populations, and represents the first report of both haplotypes in cultivated blackberries in Mexico. Interestingly, 5 additional thrips species were found associated with blackberry flowers and, to our knowledge, also represent new records for this crop in Mexico.

Frankliniella occidentalis previously has been reported as a pest associated with avocado (*Persea americana* Mill.; Lauraceae) crops in Mexico (Herron & James 2005). This crop is widely grown in the same regions as the blackberry crops sampled in the present study; movement from 1 host to the other is highly likely.

The results from the insecticide resistance studies showed that malathion had the highest values of LC_{50}, LC_{95}, and resistance ratios, particularly in the municipality of Los Reyes de Salgado, followed by Ziracuaretiro, Michoacán. This insecticide has been in use for more than 25 yr in blackberry crops in Mexico. The results reported here for malathion are similar to those found in *F. occidentalis* populations collected in apple, pepper, cotton, lettuce, peach, carnation, strawberry, and tomato crops in Australia, with an RR_{50} from 1.8 to 2.8. Malathion is among the insecticides with the longest history of use in the world (Herron & James 2005). However, the existence of different *F. occidentalis* populations that showed no resistance to malathion has been documented, despite the intensity of the malathion applications and the time that this insecticide has been on the market. These previous reports suggest that the environment and the geographic areas where these populations were collected might have influenced the results because the resistant genes were maintained at a low frequency due to the high rate of immigration of susceptible individuals (Dagh & Tung 2007).

Of the 3 evaluated insecticides, spinosad was the last insecticide to enter the market worldwide, having been registered in 1999 (Thompson et al. 2000); however, in some countries like Mexico, the insecticide was registered in 1997 (Osorio et al. 2008). Because of its high efficacy against thrips, commercial availability, and affordable cost, spinosad has been used widely in the blackberry-producing states of Michoacán and Jalisco in Mexico. In these areas, spinosad has been applied up to 9 times in 6 mo (A. Rebollar-Alviter, unpublished data) without considering label recommendations, which state that its use should be limited to a maximum of 3 applications per crop cycle (Bielza et al. 2007a). This application pattern could have resulted in populations with a high RR_{50}, such as the Z5 population from the municipality of Ziracuaretiro, Michoacán, with an RR_{50} of 248. Additionally, spinetoram, another spinosyn insecticide widely used in blackberry crops and that is in the same chemical family as spinosad, is recommended for the control of the spotted wing drosophila *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), increasing the selection pressure even more on thrips, although they are not the target pest for these sprayings. In greenhouses in Spain, where more than 10 spinosad applications were conducted per crop cycle, populations with an $RR_{50} > 13{,}500$ in Almeria and RR > 3,682 in Murcia were found after 2 yr of use (Bielza et al. 2007b). In contrast, in populations of thrips in Beijing, China, lower resistance ratios were documented because of lower selection pressure, where an RR_{50} of 35.38 and 80.80 were obtained in laboratory and field populations, respectively (Wang et al. 2011).

The resistance of *F. occidentalis* to spinosad is associated with a modification of the target site by the mutation G275E, resulting in a replacement of the glycine (G) amino acid in susceptible insects by glutamic acid (E) in resistant insects (Puinenan et al. 2013). This change occurs in the nicotinic receptor of the acetylcholine of a nucleotide in the sequence encoding the alpha 6 subunit (Foα6) of the nicotinic receptor of the acetylcholine. The same point mutation was reported in *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) and *Plutella xylostella* L. (Lepidoptera: Plutellidae) with the spinosad insecticide (Rinkevich et al. 2010; Hsu et al. 2012).

Our results with bifenthrin indicated that the RR_{50} ranged from 4 to 974 in the different evaluated thrips populations. For *F. occidentalis* populations collected in greenhouses in San Diego and Santa Barbara, California, USA, resistance ratios were reported to be between 70 and 106, and between 142 and 275, respectively (Inmaraju et al. 1992). In our study, the highest RR_{50} obtained was estimated at 974 for the Z3 population. Thrips populations from San Diego and Santa Barbara, California, USA, were subjected to applications with synergists, such as piperonyl butoxide, which showed that the compound synergized the effect of bifenthrin, causing greater pest mortality and indicating that the mixed-function oxidase enzymes were the primary resistance mechanism. Although the resistance type was not determined in this study, it was concluded that for permethrin, another pyrethroid insecticide, the resistance was of a metabolic type due to the increase in the production of mixed-function oxidase enzymes (Inmaraju et al. 1992). In New South Wales and Queensland, Australia, RR_{50} values were found to be between 23 and 61 for field populations, but the *F. occidentalis* resistance type towards bifenthrin was not determined (Thalavaisundaram et al. 2008).

The 3 insecticides evaluated in this study also are registered in Mexico for the control of *D. suzukii*, an invasive pest that affects blackberry crops (Burrack et al. 2013), further increasing the selection pressure on the thrips. In the production system, insecticide applications start at the beginning of the blooming period and continue for at least 2 mo, because as the fruits ripen, they become susceptible to *D. suzukii* infestation. In addition, at the same time, the pressure of the thrips population is high because of the overlapping of the blooming period, fruit development, and ripening of the berries on the same plants. In addition, most blackberry producers have sections of the crop in different phonological stages of development to harvest near the peaks of the best blackberry prices in the market; that is, the cropping system is linked to the market dynamics. This growing system permits the pest populations to continually develop throughout the yr in subtropical blackberry production systems, such as that in Mexico.

The *F. occidentalis* resistance represents an obstacle to the success of crop production systems and pest management over the long term. This condition requires the implementation of insecticide resistance management (IRM) plans, either by moderation, saturation, or multiple attacks according to the action modes of the insecticide (Georghiou 1994; Wu et al. 2018). Our results suggest the need to integrate the 3 types of resistance management strategies, beginning with the temporary suspension of insecticides that showed high resistance ratios (Osorio et al. 2008).

Following this step, rotation with other pesticides that have a different action mode (Broughton & Herron 2009) should occur, and nonchemical tactics should be considered, given the previous applications and the use of action thresholds to extend the efficacy of the insecticide (Zhao et al. 2002; Thalavaisundaram et al. 2008; Broughton & Herron 2009; Gao et al. 2012), as has been documented in India for *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in cotton crops (Aggarwal et al. 2006). Similarly, successful cases of insecticide resistance management for *F. occidentalis* management have been reported in cucumber crops in the USA (Reitz et al. 2003) and southern Spain (Sánchez et al. 2000), where nonchemical tactics played a fundamental role, and the delayed rotation of insecticides and reversed resistance development thus retained a high frequency of susceptible genes in the populations. For populations of *F. occidentalis* resistant to spinosad, acrinatrina, methiocarb, and formetanate collected in crops of Murcia and Almeria, Spain, cyantraniliprole was proposed as a new valuable tool for the control of thrips (Bielza & Guillen 2014). The use of enzyme inhibitors (synergists) also is recommended to increase the insecticidal effect on *F. occidentalis* populations that showed metabolic-type resistance (Wang et al. 2014).

This study confirms that the most frequently occurring thrips species associated with cultivated blackberries in the states of Michoacán and Jalisco was *F. occidentalis* (haplotypes FCOI-9 and FCOI-3). In addition, *F. insularis* and *I. australis* also were found in 2 samples from Tacámbaro. In Los Reyes de Salgado, *R. funestus* and *F. tolucensis* also were found. *Taenothrips frici* was found in Tacámbaro, Michoacán, and Mazamitla, Jalisco. At all the sampled sites, *F. occidentalis* was the most common species, and *F. occidentalis* showed different levels of resistance to malathion, spinosad, and bifenthrin. These results suggest there is an increased level of resistance to the evaluated insecticides in some of the sampled populations, and provide a basis for the design of a resistance management program linked to an integrated regional management program for thrips in blackberry crops.

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