Journal of Plant Protection Research ISSN 1427-4345

ORIGINAL ARTICLE

Molecular identification, occurrence and distribution of *Thrips palmi*, *Frankliniella intonsa* and *Frankliniella cephalica* (Thysanoptera: Thripidae) on cucurbit crops in Panama

Anovel A. Barba-Alvarado[1†], José N. Jaén-Sanjur[1†], Luis Galipienso[2,3], Laura Elvira-González[2], Luis Rubio[2], José A. Herrera-Vásquez[1*]

[1] Grupo de Investigación de Protección Vegetal (GIPV), Centro de Investigación Agropecuaria Divisa (CIAD), Instituto de Investigación Agropecuaria de Panamá (IDIAP), Ctra. Panamericana, Los Canelos, Santa María, Estafeta de Divisa, 0619 Herrera, Panama

[2] Instituto Valenciano de Investigaciones Agrarias (IVIA), 46113 Moncada, Valencia, Spain

[3] Departamento de Biotecnología, Escuela Técnica Superior de Ingeniería Agronómica y del Medio Natural (ETSIAMN), Universitat Politècnica de València (UPV), Camino de Vera, s/n, 46022 Valencia, Spain

Vol. 60, No. 1: 68–76, 2020

DOI: 10.24425/jppr.2020.132208

Received: August 26, 2019
Accepted: November 18, 2019

*Corresponding address:
joshervs11@gmail.com

†These authors contributed equally to the study

Abstract

The main open-field producer regions of cucurbits (watermelon, squash, melon and cucumber) in Panama (Los Santos, Herrera and Coclé provinces) were surveyed for molecular identification, occurrence and distribution of *Thrips palmi* (the most important pest thrip species on cucurbits in Panama), *Frankliniella intonsa* and *Frankliniella cephalica* during the growing seasons of 2009 to 2013 and 2017 to 2018. Forty plots were surveyed and DNA extracts of 186 thrips (larvae and adults) were analyzed by multiplex PCR, using a set of *T. palmi*-specific primers in combination with a set of insect-universal primers. DNA extracts corresponding to 174 individual thrips (93.5%) rendered both PCR products of expected size with *T. palmi*-specific and insect-universal primers, whereas the remaining DNA extracts corresponding to 12 individual thrips (6.5%) only rendered the product of the expected size with insect-universal primers. Sequencing of those PCR products and BLAST analysis allowed for the identification of *F. intonsa* and *F. cephalica*. *Thrips palmi* was detected in all three provinces, while *F. intonsa* and *F. cephalica* were detected in Herrera and Los Santos provinces. To our knowledge, this is not only the first detection of *F. intonsa* in Panama, but also the first detection of *F. cephalica* in Panamanian cucurbit crops.

Keywords: cucurbits, molecular identification, *Thrips*

Introduction

Cucurbits are one of the most important vegetable crops worldwide and they grow mainly in temperate and tropical regions (Weng and Sun 2011). Among cucurbits, watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai], melon (*Cucumis melo* L.), cucumber (*Cucumis sativus* L.) and squash or pumpkin (*Cucurbita pepo* L., *Cucurbita maxima* Duch. and *Cucurbita moschata* Duch.) are the four most commonly cultivated crops (Weng and Sun 2011). Squash and pumpkin are unique in the sense that they represent several species for the same crop: summer squash is *Cucurbita pepo*, but winter squash may be either *C. pepo*, *C. maxima* or *C. moschata* (Robinson and Decker-Walters 1997; Weng and Sun 2011). In terms of total production (MIDA 2019) watermelon is the most important cucurbit crop in Panama, with a total harvested area of 1,033 ha and a production of 24,576 t in the period 2017–2018, followed by squash (7,471 t), melon (5,283 t) and cucumber (3,123 t).

Thrips (Thysanoptera: Thripidae) are a worldwide problem for cucurbit production and the cause of severe economic losses (Yeh *et al*. 2015). Thrips are found in buds, leaves, fruits and twigs resulting in silvery distortions, stunted growth, feeding scars and color mosaicism (Bethke *et al*. 2014; Yeh *et al*. 2015) (Fig. 1). Direct crop damage from thrips is a consequence of both feeding and oviposition (Childers 1997). Thrips are also vectors of important viruses such as carmovirus, ilarvirus, machlomovirus, orthotospovirus and sobemovirus (Jones 2005; Abudurexiti *et al*. 2019). Orthotospovirus (formerly known as tospovirus) is a virus genus within the family *Tospoviridae* (order *Bunyavirales*) (Abudurexiti *et al*. 2019), which causes many emerging diseases in cucurbits such as watermelon silver mottle, zucchini lethal chlorosis and yellow spot of melon (Iwaki *et al*. 1984; Nakahara and Monteiro 1999; Kato *et al*. 2000). Of the known 1,710 species of Thripidae, 14 of them are currently reported to transmit orthotospoviruses (Riley *et al*. 2011). An annual loss worldwide of over $1 billion is estimated from a single orthotospovirus, *Tomato spotted wilt virus* (TSWV) (Prins and Goldbach 1998).

An updated biological catalogue of the Thripidae of Panama was published in 2012 (Goldarazena *et al*. 2012). From this report, the checklist recorded 246 species (14.4%) of all species of Thripidae reported worldwide (Riley *et al*. 2011). *Thrips palmi*, *Neohydatothrips gracilipes* and four species of *Frankliniella* (*F. bruneri*, *F. kelliae*, *F. shultzei* and *F. williamsi*) were found on cucurbit crops in Panama, but the localities were not specified (Goldarazena *et al*. 2012). In a subsequent study published in 2013, *T. palmi* and *Frankliniella* sp. were found in the cucurbit production provinces of Los Santos, Herrera, Veraguas and Chiriquí (Herrera-Vásquez and Barba-Alvarado 2013). The genus *Frankliniella* can be differentiated from other genera of thrips by some physical characteristics (Smith *et al*. 1997). Due to the similarity between *Frankliniella* species, mainly in the larval stadium and a similar host range, morphological identification is not easy (Przybylska *et al*. 2016). In addition, morphological identification becomes impossible at the species level when thrips are in the immature stage. To distinguish thrip species in each geographic location, it is crucial to implement integrated pest identification and management (Mouden *et al*. 2017). In this sense, molecular detection methods can be used to overcome the limitations of morphological analysis. DNA-based analysis can be applied to identify different thrips without being restricted by its life stages (Liu 2004; Asokan *et al*. 2007; Farris *et al*. 2010; Huang *et al*. 2010; Kobayashi and Hasegawa 2012; Yeh *et al*. 2015).

In recent years, there has been an increasing spread of thrips in Panama (Herrera-Vásquez and Barba-Alvarado 2013), possibly as a result of the movement of infested host plants (Vierbergen 1995; Cannon *et al*. 2007). In this work, we surveyed 35 localities of the Los Santos, Herrera and Coclé provinces which are the three main cucurbit production regions of Panama by multiplex PCR (Lin *et al*. 2003; Yeh *et al*. 2015), DNA sequencing and phylogenetic analysis to study and identify the occurrence and distribution of *T. palmi*, *Frankliniella intonsa* and *F. cephalica* on cucurbit crops.

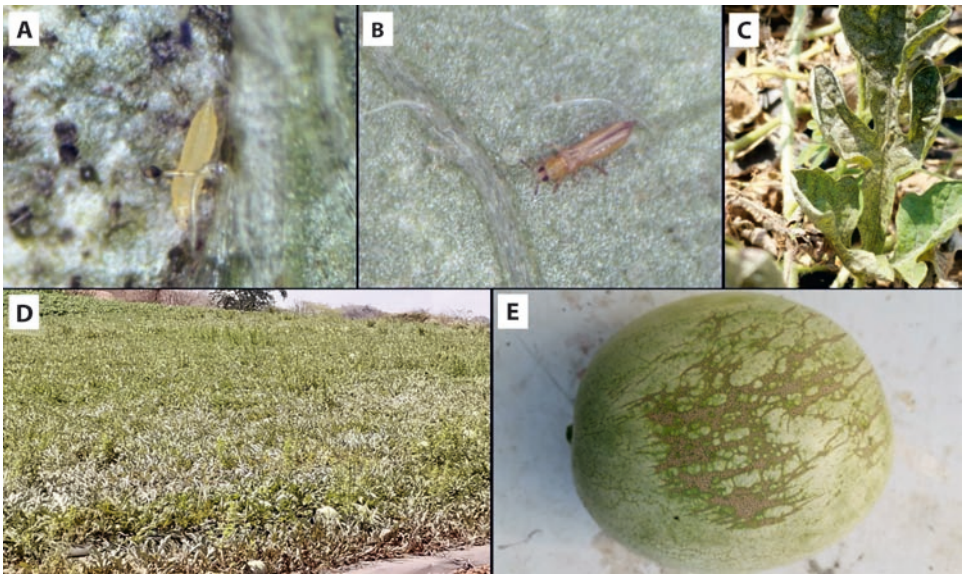

Fig. 1. Thrip larva (A) and adult (B), distortion and silverleaf (C), feeding scars on the fruit surface (D) and watermelon plot severely affected by thrips (E)

Materials and Methods

Survey and sampling

Surveys were conducted in cucurbit open fields in three provinces (Los Santos, Herrera and Coclé, with a production of 2,552, 4,692 and 2,584 t, respectively) which are considered to be the main cucurbit-growing regions in Panama (MIDA 2019). Surveys covered all relevant cucurbit production areas of these provinces in the growing seasons (December to April) from 2009 to 2013 and 2017 to 2018 (Fig. 2; Table 1). Forty cucurbit plots located at an altitudinal range of 10–152 meters above sea level (MASL) in 35 localities were surveyed. Each plot belonged to different localities, except plots 21 and 34 which were in Los Hatillos locality and 26 and 35–38 which were in El Barrero locality, both in the province of Herrera. The exact location of each plot was geo-referenced using a hand-held Global Positioning System (GPS) unit (Garmin, Taipei County, Taiwan) (Table 1). A base map of Panama was geo-referenced in a coordinate system and digitized using Google Earth Pro (V. 7.1 for Windows 10), while the image of the map was edited in the Paint 3D program (V. 1703 for Windows 10) (Fig. 2). Thrips were collected from watermelon, squash, melon and cucumber plants by the whole bud collection sampling technique (Moura *et al.* 2003; Bacci *et al.* 2008). This technique consisted of bagging a single bud followed by its detachment from the plants. The sampling unit consisted of taking a bud from the apical third of the plant canopy, which is the best colonization site for thrips. In each plot, buds of five different plants were randomly collected and mixed to make one composite sample and brought to the laboratory for evaluation. The time spent for taking each sample and moving from one sample to another was ~75 s. In most cases, five thrip samples (larvae and adults) were collected in each plot and they were preserved in 95% ethanol at room temperature until analysis (Yeh *et al.* 2015).

DNA extraction

Total DNA was extracted from whole individual thrip samples (larvae and adults) (Huang *et al.* 2010) following a modified Chelex-100 method (Walsh *et al.* 1991; De la Rúa *et al.* 2006). Briefly, it consisted of crushing the insect with a micropestle against the bottom of a 1.5 ml tube containing 20 µl of stirred Chelex-100 5% solution (Bio-Rad, California, USA). Then, the homogenate was incubated at 56°C for 15 min and at 99°C for 3 min and centrifuged at 14,000 rpm for 10 min. The upper aqueous supernatant (15 µl) was collected and stored in a new tube at –20°C until it was used as a template for the multiplex PCR amplification.

Identification of *Thrips palmi* by multiplex PCR

For identification of *T. palmi,* a multiplex PCR method based on a combination of one *T. palmi*-specific primer pair Tpal2F/Tpal2R (Yeh *et al.* 2015) and one insect-universal primer pair 28Sg/28Sh (Lin *et al.* 2003) was used (Table 2). *Thrips palmi*-specific primer pair Tpal2F/Tpal2R can produce a 304-bp amplicon from the internal transcribed spacer 1 (ITS1) region of *T. palmi* genome; the insect-universal primer pair 28Sg/28Sh can produce a 520-bp amplicon from insect genomic DNA encoding for 28S

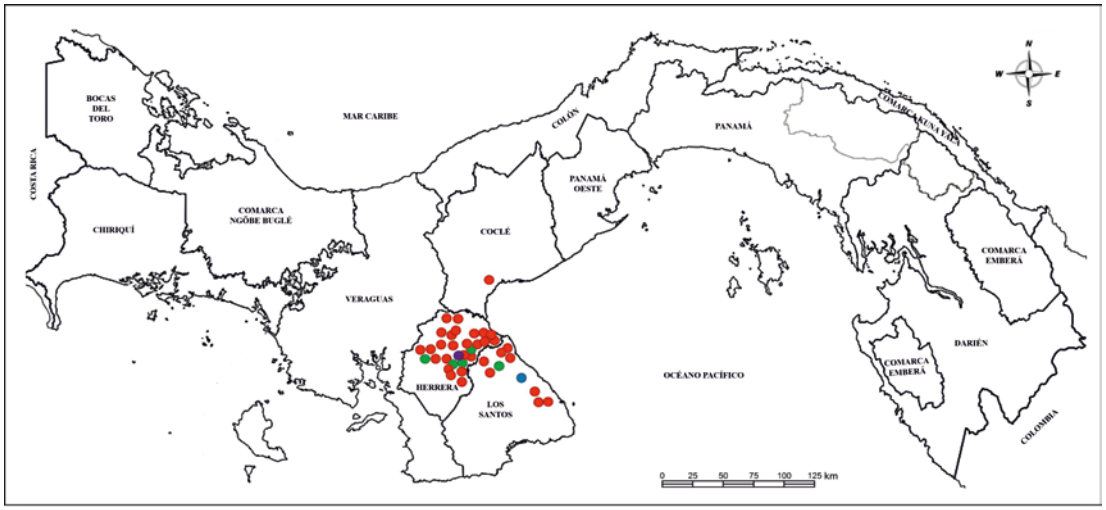

Fig. 2. Map of Panama showing the provinces surveyed (Los Santos, Herrera and Coclé) and distribution of thrip species. *Thrips palmi* (indicated with a red point), *T. palmi* + *Frankliniella cephalica* (indicated with a blue point), *T. palmi* + *Frankliniella intonsa* (indicated with a green point), and *F. intonsa* + *F. cephalica* (indicated with a purple point) are shown in all the provinces surveyed indicated above

Table 1. Results of the surveys performed between 2009 and 2018 showing the location of surveyed plots, host, number of collected thrips and name of species which belonged

Year	Province	Locality	Coordinates N W	Altitude[a]	Host	No. *Thrips*	*Thrips palmi*	*Frankliniella intonsa*	*Frankliniella cephalica*
2009	Herrera	Plot 1: El Higuerón	7°46´54´´80°40´00´´	10	Watermelon	5	5	0	0
		Plot 2: Ocú	7°56´24´´80°46´48´´	118	Melon	5	5	0	0
	Los Santos	Plot 3: Chumajal	7°49´00´´80°19´60´´	106	Watermelon	5	5[b]	0	0
		Plot 4: Santo Domingo	7°44´54´´80°13´15´´	22	Melon	3	3[b]	0	0
		Plot 5: Tres Quebradas	7°51´48´´80°23´27´´	80	Melon	3	2	0	1[c]
		Plot 6: El Guásimo	7°49´09´´80°31´59´´	35	Squash	4	4	0	0
2010	Herrera	Plot 7: Llano Grande	7°58´23´´80°42´32´´	73	Watermelon	3	3	0	0
		Plot 8: La Trinidad	7°52´22´´80°33´51´´	71	Watermelon	5	5	0	0
		Plot 9: Potuga	8°03´44´´80°37´19´´	34	Watermelon	5	5	0	0
		Plot 10: La Arena	7°57´50´´80°28´57´´	20	Watermelon	5	5	0	0
		Plot 11: El Chorro	7°53´34´´80°45´09´´	107	Watermelon	5	5	0	0
		Plot 12: El Ciruelito	7°53´55´´80°40´15´´	15	Watermelon	5	5	0	0
		Plot 13: El Calabazal	7°59´12´´80°42´42´´	56	Melon	5	5	0	0
		Plot 14: El Jazmín	7°57´00´´80°34´60´´	35	Melon	5	4	1[c]	0
	Los Santos	Plot 15: La Villa de Los Santos	7°56´26´´80°24´17´´	16	Watermelon	4	4	0	0
		Plot 16: Las Palmitas	7°45´56´´80°16´16´´	49	Squash	3	3	0	0
2011	Herrera	Plot 17: La Chilonga	7°57´14´´80°25´51´´	15	Watermelon	4	4	0	0
		Plot 18: La Arenita	7°52´22´´80°33´51´´	71	Watermelon	5	4	1[c]	0
		Plot 19: Señales	7°55´03´´80°47´09´´	131	Watermelon	5	4	1[c]	0
		Plot 20: Santa María	8°07´00´´80°40´00´´	10	Watermelon	5	5	0	0
		Plot 21: Los Hatillos	7°54´24´´80°36´46´´	80	Melon	5	5	0	0
2012	Herrera	Plot 22: Llano de la Cruz	7°57´15´´80°38´27´´	49	Cucumber	5	5[b]	0	0
2013	Herrera	Plot 23: El Vasco	7°54´17´´80°36´33´´	72	Watermelon	5	5	0	0
		Plot 24: Las Garzas	8°07´00´´80°40´00´´	10	Watermelon	5	5	0	0
		Plot 25: Guayabito	7°54´54´´80°49´26´´	152	Watermelon	5	5	0	0
		Plot 26: El Barrero	8°01´02´´80°38´07´´	25	Watermelon	5	5	0	0
		Plot 27: La Flora	7°57´00´´80°28´60´´	22	Watermelon	4	4	0	0
		Plot 28: Las Cabras	7°53´07´´80° 34´37´´	48	Melon	5	5	0	0
		Plot 29: Los Chicharrones	7°58´12´´80°26´49´´	39	Melon	5	5	0	0
		Plot 30: San Pedro	7°57´50´´80°28´57´´	20	Melon	5	5	0	0
		Plot 31: El Torno	8°07´10´´80°37´05´´	26	Squash	5	5[b]	0	0
	Los Santos	Plot 32: El Ejido	7°55´00´´80°22´60´´	35	Watermelon	4	4	0	0
		Plot 33: Chupaito	7°47´51´´80°34´19´´	82	Watermelon	5	5	0	0
		Plot 34: Los Hatillos	7°43´48´´80°32´24´´	92	Watermelon	5	5	0	0
2017	Herrera	Plot 35: El Barrero	7°55´54´´80°33´24´´	27	Watermelon	4	0	2[c]	2[c]
		Plot 36: El Barrero	7°55´46´´80°31´43´´	30	Watermelon	5	2	3[c]	0
		Plot 37: El Barrero	7°57´26´´80°32´53´´	32	Watermelon	5	5	0	0
		Plot 38: El Barrero	7°56´12´´80°33´13´´	31	Watermelon	5	5	0	0
2018	Los Santos	Plot 39: Agua Buena	7°50´26´´80°24´12´´	40	Watermelon	5	4	1[c]	0
	Coclé	Plot 40: El Olivo	8°26´46´´80°31´57´´	32	Watermelon	5	5	0	0
Total						186	174	9	3

[a]The altitude corresponds to meters above sea level (MASL)

[b]For *Thrips palmi* four individuals were sequenced (one individual/host/locality)

[c]For *Frankliniella intonsa* and *F. cephalica* all individual were sequenced

Table 2. Set of primers used for molecular identification of *Thrips palmi*, *Frankliniella intonsa* and *F. cephalica*

Thrips	Primer name	Sequence (5′–3′)	Amplicon size [bp]	Genomic region	Reference
T. palmi	Tpal2F[a]	GGGTGCCTGTTCTCCAAAA	304	ITS1	Yeh *et al*. 2015
	Tpal2R[b]	CGCCTTCGAAGAACTTGGAA			
F. intonsa	28Sg[a]	AGTTTGACTGGGGCGGTACA	520	28S rDNA	Lin *et al*. 2003
	28Sh[b]	CTTAGAGGCGTTCAGGCATAA			
F. cephalica	Fcep1F[a]	ATTTCGCGTCGAAGCAACGG	461	ITS1	Yeh *et al*. 2015
	Fcep1R[b]	ATCGGTCCGTTCCGTTCAAC			

[a]Forward primers
[b]Reverse primers

rDNA (Yeh *et al*. 2015). All multiplex PCRs were performed with final concentrations of 1× PCR buffer (containing 200 mM Tris-HCl pH 8.4, 500 mM KCl), 1.5 mM $MgCl_2$, 0.2 mM dNTP mix, 0.5 µM for each primer, 0.5 U of *Taq* DNA polymerase (Invitrogen, California, USA), 1 µl of total DNA and sterile PCR water to make up the volume to 25 µl. DNA amplifications were performed in a Mastercycler Ep gradient thermocycler (Eppendorf, Hamburg, Germany) programed for a 2-min initial denaturation at 94°C, followed by 35 cycles of denaturation at 94°C for 40 s, annealing at 50°C for 50 s and extension at 72°C for 1 min; a final extension at 72°C for 10 min was introduced to finish incomplete PCR fragments, followed by cooling at 4°C until samples were recovered. A negative control tube, containing sterile PCR water as template, was incubated during each amplification experiment under the same multiplex PCR conditions. PCR-amplified products were separated by electrophoresis on 1.2% agarose gel in 1× TAE buffer (40 mM Tris-acetate and 1 mM EDTA at pH 8.0) and visualized by GelRed® (Biotium, California, USA) staining. Fragment sizes were determined by comparison with a 1 kb DNA Ladder Plus (Thermo Fisher Scientific, Massachusetts, USA).

Sequence analysis of thrips

To confirm the identity of *T. palmi*, four PCR products (corresponding to four individual thrip samples) obtained with the *T. palmi*-specific primers Tpal2F/Tpal2R from insects collected from watermelon, squash, melon and cucumber were purified using a QIAquick PCR Purification Kit (Qiagen, California, USA) and sequenced in both directions with *T. palmi*-specific primers Tpal2F/Tpal2R using a Big Dye Terminator V. 3.0 Cycle Sequencing kit in a ABI 3130 XL capillary sequencer (Applied Biosystems, California, USA). Nine of 12 PCR products that were amplified only with the insect-universal primer set 28Sg/28Sh, were purified and sequenced as described above, but with the insect-universal primer set 28Sg/28Sh. *Frankliniella cephalica*-specific primer pair Fcep1F/Fcep1R from the ITS1 region of *F. cephalica* genome (Yeh *et al*. 2015) (Table 2) were used to sequence this thrip species (three PCR products, corresponding to three individual thrip samples) as described above. In this case, we used *F. cephalica*-specific primer pair Fcep1F/Fcep1R and not the insect-universal primer set 28Sg/28Sh, because in the GenBank database from the National Center for Biotechnology Information (NCBI) (http://ncbi.nlm.nih.gov/) there were no sequences from the 28S rDNA for *F. cephalica*. The nucleotide sequences were compared by Basic Local Alignments Search Tool (BLAST) (Altschul *et al*. 1997) with sequences producing significant alignments which are available from the GenBank database. Sequence alignments were obtained with CLUSTALW implemented in MEGA (Molecular Evolutionary Genetics Analysis) version 10.0.5 (Kumar *et al*. 2018). Phylogenetic relationships were inferred for *T. palmi* with the Maximum-Likelihood method implemented in MEGA by using the nucleotide substitution model that best fitted the sequence data (Jukes-Cantor model) and 500 bootstrap replicates to estimate the statistical significance of each node. Phylogenetic inference was not possible for *F. intonsa* and *F. cephalica* since only one sequence for each thrip species was in the GenBank database.

Results

Identification of *Thrips palmi* by multiplex PCR

In the surveys conducted during the growing seasons from 2009 to 2013 and from 2017 to 2018, 186 individual thrip samples (larvae and adults) were collected from watermelon, squash, melon and cucumber from 40 plots in different regions of Panama (Fig. 2). The results of these surveys are summarized in Table 1. Identification of *T. palmi* was realized by multiplex PCR with the *T. palmi*-specific primers Tpal2F/Tpal2R which amplified a fragment of the ITS1 region. A total

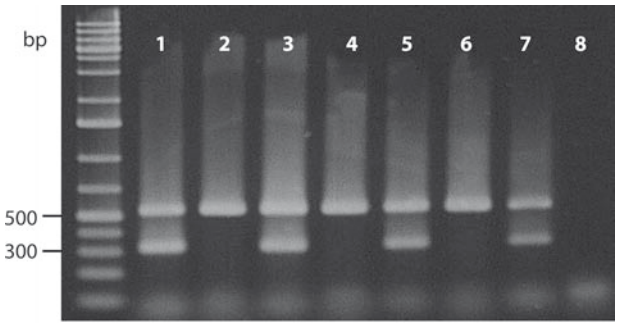

Fig. 3. Multiplex PCR with ITS1-specific primer set of *Thrips palmi* (Tpal2F/Tpal2R) and 28S rDNA-universal primer set of insects (28Sg/28Sh). Lanes 1, 3, 5 and 7, *T. palmi* from watermelon, squash, melon and cucumber, respectively; Lanes 2 and 4, *Frankliniella intonsa* from watermelon and melon, respectively; Lane 6, *Frankliniella cephalica* from watermelon. Lane 8, negative control (sterile PCR water). The first lane is 1 kb DNA ladder (Thermo Fisher Scientific, Massachusetts, USA)

of 174 thrip samples (93.5% of all individuals collected) showed the PCR product of expected size (304-bp) on agarose gel electrophoresis, suggesting that they were *T. palmi* (Fig. 3). This assay was effective in identifying *T. palmi* regardless of its life stage. Thirty-nine plots (97.5% of all plots surveyed) were infested with *T. palmi*, and it was found in all survey years, localities and hosts (Table 1). Production of the 520-bp PCR production by the insect-universal primer set 28Sg/28Sh in all tested samples ensured a qualitative control for the multiplex PCR assay (Fig. 3). In addition, 12 thrip samples (6.5% of all individuals collected) showed only the expected 520-bp PCR product generated by the insect-universal primer set 28Sg/28Sh, suggesting that samples corresponded to other thrip species which differed from *T. palmi* (Fig. 3). No amplification products were observed when water controls were used as template in the multiplex PCR assays (Fig. 3).

Sequence analysis of *Thrips palmi*, *Frankliniella intonsa* and *Frankliniella cephalica*

A total of 16 PCR products were sequenced. Four of them were *T. palmi*-specific primers Tpal2F/Tpal2R amplicons; nine of them were obtained from the insect-universal primer set 28Sg/28Sh amplicons; and three of them were *F. cephalica*-specific primers Fcep1F/Fcep1R amplicons. BLAST analysis of nucleotide sequences revealed that those obtained with *T. palmi*-specific primers Tpal2F/Tpal2R corresponded to this species, whereas sequences obtained with the insect-universal primer set 28Sg/28Sh and *F. cephalica*-specific primers Fcep1F/Fcep1R corresponded to *F. intonsa* (nine sequences) and *F. cephalica* (three sequences), respectively. *Thrips palmi* sequences were obtained from individuals collected from watermelon, squash, melon and cucumber from the localities of Chumajal and Santo Domingo, in the Los Santos province, and Llano de la Cruz and El Torno, in the Herrera province, in 2009 and in the period 2012–2013 (Table 1). *Frankliniella intonsa* sequences were obtained from individuals collected from watermelon and melon crops from the localities of El Jazmín, La Arenita, Señales and El Barrero (Herrera province), and Agua Buena (Los Santos province), in the periods 2010–2011 and 2017–2018 (Table 1). *Frankliniella cephalica* sequences were obtained from individuals collected from melon and watermelon crops from the locality of Tres Quebradas (Los Santos province) and El Barrero (Herrera province), in 2009 and 2017 (Table 1). The nucleotide sequence for different parts of the ITS1 region of *T. palmi* and *F. cephalica* and 28 rDNA of *F. intonsa* was determined from all the cucurbit species examined (Table 1). In all cases, the obtained DNA sequences were as expected. The sequences obtained in this work for each thrip species were compared with those published in the GenBank database. Nucleotide sequence identity among individuals within *T. palmi*, *F. intonsa* and *F. cephalica* was 100% in the genome zone studied. Thus, only one partial sequence of each species was deposited in GenBank database (*T. palmi*, accession number MN129041; *F. intonsa*, accession number MN128614; *F. cephalica*, accession number MN129042).

The phylogenetic analysis of the ITS1 region of the sequence of *T. palmi* from Panama showed that it was grouped with reference sequences of *T. palmi* from Taiwan and Japan (Fig. 4). Interestingly, the Taiwanese sequence KM877306 was more similar to the Panamanian sequence than to other sequences of *T. palmi* from Taiwan.

Discussion

The identification of thrip species provides the basis for efficient pest management. Morphological identification of different species, mainly of those belonging to genus *Frankinella*, is very difficult or sometimes it can be impossible to obtain a definite identification if individuals are in an immature stage. Here, we performed a successful assay based on a multiplex PCR and DNA sequencing to identify different thrip species in larval and adult stages infesting cucurbit crops in Panama.

Thrips palmi is a thrip species which has spread from Florida to Venezuela and it is a serious threat in the Caribbean region (Goldarazena *et al.* 2012). Our results show that *Thrips palmi* is more widespread in Panama than previously reported (Goldarazena *et al.* 2012; Herrera-Vásquez and Barba-Alvarado 2013)

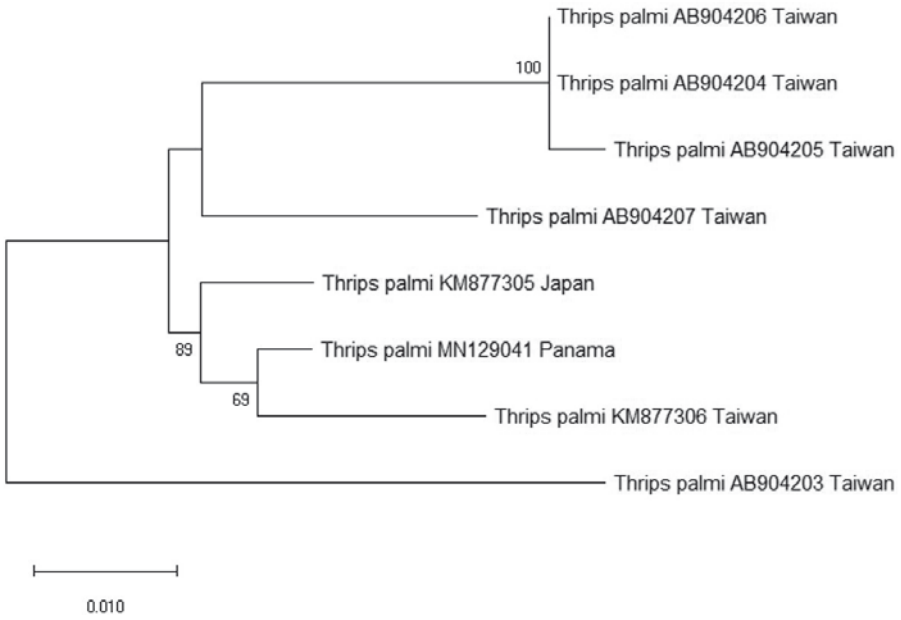

Fig. 4. Unrooted Maximun-likelihood phylogenetic tree of the ITS1 region of *Thrips palmi*. Bootstrap values >50 are shown in the nodes. Branch lengths are proportional to the genetic distances

and it affected all cucurbit crops studied (watermelon, squash, melon and cucumber) in the three surveyed provinces of Los Santos, Herrera and Coclé during all survey years. *T. palmi* was identified in commercial cucurbit open field plots, located at an altitudinal range of 10–152 MASL. Until this study, *T. palmi* had been reported in Panama below only 100 MASL in the Herrera, Los Santos, Veraguas and Chiriquí provinces (Herrera-Vásquez and Barba-Alvarado 2013). So, to our knowledge, this is not only the first detection of *T. palmi* over 100 MASL, but also the first detection of this species in the Coclé province.

We found that the incidence of *T. palmi* was higher (81.8–100%) in the period 2009–2013 and 2018 than the incidence of 2017 (63.2%). Watermelon, squash, melon and cucumber crops were surveyed from 2009 to 2013 and 2018, whereas in 2017 only watermelon was surveyed. Thus, these deviations could be due to the experimental design, with a greater number of crops surveyed (2009–2013 and 2018) and of survey sites in the present study, resulting in an increase in the identification of *T. palmi*.

In addition to *T. palmi*, two different thrip species such as *F. intonsa* and *F. cephalica*, were recently identified in a few individuals. *Frankliniella cephalica* has been previously found infesting a wide range of flowers and weeds, but not in cucurbits in Panama (Goldarazena *et al*. 2012). So, to our knowledge, this is the first detection of *F. intonsa* in Panama and the first detection of *F. cephalica* infesting cucurbit crops in this country. Both species 'might be' widespread in the Los Santos and Herrera provinces, except for Coclé, probably due to the limited number of samples collected from this province. A larger number of samples should be tested in further studies in order to assess the presence/absence of these thrip species in cucurbit crops in Coclé province. The temporal distribution of *F. intonsa* was higher (2010–2011 and 2017–2018) than *F. cephalica* (2009 and 2017). Additionally, these thrip species overlap geographically and temporally, and could be present in the same plot and host. Different species of thrips can occur in the same location and on the same plant (Gao *et al*. 2019). *Frankliniella intonsa* has been previously reported in several countries of Europe, Asia, Africa, North America and Oceania (CABI/EPPO 1999). *Frankliniella cephalica* is present in the Caribbean as well as in most of the mainland countries between Mexico and Colombia (Mound and Marullo 1996). The spread of thrips through international trade of ornamental plants has been documented (Perrings *et al*. 2005) and is probably how *F. intonsa* entered Panama. The economic importance of thrips comes from the direct damage it causes while feeding on plant tissue (Barba and Suris 2015), and the indirect damage caused by virus transmission (Riley *et al*. 2011). *Thrips palmi, F. intonsa* and *F. cephalica* are very efficient in the transmission of several orthotospoviruses (Riley *et al*. 2011) although these have not been reported yet in Panama (Herrera-Vásquez *et al*. 2013).

Thrips are difficult to control. In the framework of integrated pest management (IPM) programs, multiple complementary tactics are necessary, including monitoring, cultural, physical and mechanical measures, host plant resistance, biological control and semiochemicals, along with the judicious use of pesticides. In order to achieve successful control, strategies

should be tailored to fit the requirements of different production systems. Controlling pests is not, and has never been, a trivial issue. The basic question remains as to how to achieve consistent longterm control. Most importantly, there remains the need for transdisciplinary approaches integrating different practices for control of thrips (Bethke *et al*. 2014; Mouden *et al*. 2017).

Acknowledgements

This research was funded by the Secretaría Nacional de Ciencia, Tecnología e Innovación (SENACYT; grant 2017-4-ITE16-R2-014) of Panama. The SENACYT, through the National Research System (SNI) (grant 151-2018), supported the research activities of J.A.H.V., who participated with A.B. in a training stage in the virology laboratory of IVIA, where this research was partially carried out under L.G.'s supervision. We thank the technical personnel of the Dirección Nacional de Sanidad Vegetal of the Ministerio de Desarrollo Agropecuario (DNSV MIDA, Panama) and the IDIAP, as well as the Panamanian cucurbit farmers who helped with sample collection.

References

Abudurexiti A., Adkins S., Alioto D., Alkhovsky S.V., Avšič-Županc T., Ballinger M.J., Bente D.A., Beer M., Bergeron É., Blair, C.D., *et al*. 2019. Taxonomy of the order Bunyavirales: update 2019. Archives of Virology 164 (7): 1949–1965. DOI: 10.1007/s00705-019-04253-6

Altschul S.F., Madden T.L., Schäffer A.A., Zhang J., Zhang Z., Miller W., Lipman D.J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25 (17): 3389–3402. DOI: 10.1093/nar/25.17.3389

Asokan R., Krishna-Kumar N.K., Kumar V., Ranganath H.R. 2007. Molecular differences in the mitochondrial cytochrome oxidase I (mtCOI) gene and development of a species-specific marker for onion thrips, *Thrips tabaci* Lindeman, and melon thrips, *T. palmi* Karny (Thysanoptera: Thripidae), vectors of tospoviruses (Bunyaviridae). Bulletin of Entomological Research 97 (5): 461–470. DOI: 10.1017/S0007485307005147

Bacci L., Picanço M.C., Moura M.F., Semeão A.A., Fernandes F.L., Morais E.G.F. 2008. Sampling plan for thrips (Thysanoptera: Thripidae) on cucumber. Neotropical Entomology 37 (5): 582–590. DOI: 10.1590/S1519-566X2008000500014

Barba A., Suris M. 2015. Presencia de *Thrips palmi* Karny (Thysanoptera: Thripidae) en arvenses asociadas al cultivo de la sandía para la región de Azuero, Panamá. Revista de Protección Vegetal 30 (3): 171. Available on: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S1010-27522015000300002 [Accessed: August 21, 2019]

Bethke J.A., Dreistadt S.H., Varela L.G. 2014. Pest Notes: Thrips. Oakland: University of California, Agriculture and Natural Resources, Publication 7429. Available on: http://ipm.ucanr.edu/PDF/PESTNOTES/pnthrips.pdf [Accessed: October 11, 2019]

CABI/EPPO. 1999. *Frankliniella intonsa* (thrips, flower). Available on: https://cabi.org/isc/datasheet/24423#toDistributionMaps [Accessed: August 21, 2019]

Cannon R.J.C., Matthews L., Collins D.W. 2007. A review of the pest status and control options for *Thrips palmi*. Crop Protection 26 (8): 1089–1098. DOI: 10.1016/j.cropro.2006.10.023

Childers C.C. 1997. Feeding and oviposition injuries to plants. p. 505–537. In: "Thrips as Crop Pests" (T. Lewis, ed.). CAB International, Wallingford, UK, 736 pp.

De la Rúa P., Simón B., Cifuentes D., Martinez-Mora C., Cenis J.L. 2006. New insights into the mitochondrial phylogeny of the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) in the Mediterranean Basin. Journal of Zoological Systematics and Evolutionary Research 44 (1): 25–33. DOI: 10.1111/j.1439-0469.2005.00336.x

Farris R.E., Ruiz-Arce R., Ciomperlik M., Vasquez J.D., Deleón R. 2010. Development of a ribosomal DNA ITS2 marker for the identification of the thrips, *Scirtothrips dorsalis*. The Journal of Insect Science 10 (1): 26. DOI: 10.1673/031.010.2601

Gao Y.F., Gong Y.J., Cao L.J., Chen J.C., Gao Y.L., Mirab-balou M., Chen M., Hoffmann A.A., Wei S.J. 2019. Geographical and interspecific variation in susceptibility of three common thrips species to the insecticide, spinetoram. Journal of Pest Science. DOI: 10.1007/s10340-019-01128-2

Goldarazena A., Gattesco F., Atencio R., Korytowski C. 2012. An updated checklist of the Thysanoptera of Panama with comments on host associations. Check List 8 (6): 1232–1247. DOI: 10.15560/8.6.1232

Herrera-Vásquez J.A., Barba-Alvarado A.A. 2013. Identificación de *Thrips palmi* karny (Thysanoptera: Thripidae) en cultivos de cucurbitáceas en Panamá. Agronomía Mesoamericana 24 (1): 47–55. Available on: https://www.redalyc.org/articulo.oa?id=43726204003 [Accessed: August 21, 2019]

Herrera-Vásquez J.A., Córdoba-Sellés M.C., Cebrián M.C., Font-San-Ambrosio M.I., Alfaro-Fernández A., Jordá C. 2013. Viruses of cucurbits in Panama. Journal of Plant Pathology 95 (2): 435–440. DOI: 10.4454/JPP.V95I2.03

Huang K.S., Lee S.E., Yeh Y., Shen G.S., Mei E., Chang C.M. 2010. Taqman real-time quantitative PCR for identification of western flower thrip (*Frankliniella occidentalis*) for plant quarantine. Biology Letters 6 (4): 555–557. DOI: 10.1098/rsbl.2009.1060

Iwaki M., Honda Y., Hanada K., Tochibara H., Yonaha T., Hokama K., Yokoyama T. 1984. Silver mottle disease of watermelon caused by *Tomato spotted wilt virus*. Plant Disease 68 (11): 1006–1008. DOI: 10.1094/PD-68-1006

Jones D.R. 2005. Plant viruses transmitted by thrips. European Journal of Plant Pathology 113 (2): 119–157. DOI: 10.1007/s10658-005-2334-1

Kato K., Handa K., Kameya-Iwaki M. 2000. *Melon yellow spot virus*: a distinct species of the genus *Tospovirus* isolated from melon. Phytopathology 90 (4): 422–426. DOI: 10.1094/PHYTO.2000.90.4.422

Kobayashi K., Hasegawa E. 2012. Discrimination of reproductive forms of *Thrips tabaci* (Thysanoptera: Thripidae) by PCR with sequence specific primers. Journal of Economic Entomology 105 (2): 555–559. DOI: 10.1603/ec11320

Kumar S., Stecher G., Li M., Knyaz C., Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Molecular Biology and Evolution 35 (6): 1547–1549. DOI: 10.1093/molbev/msy096

Lin J.S., Wang C.L., Yeh W.B. 2003. Molecular identification of multiplex-PCR and PCR-RFLP for the quarantine pest, *Frankliniella occidentalis* (Pergande). Formosan Entomology 23: 353–366. Available on: http://entsocjournal.yabee.com.tw/AlldataPos/JournalPos/Vol23/No4/TESFE.2003031.PDF [Accessed: August 21, 2019]

Liu Y.C. 2004. Molecular identification of a plant quarantine pest (*Frankliniella occidentalis*) by one-tube nested PCR targeting ribosomal DNA internal transcribed spacer regions. Plant Protection Bulletin 46 (1): 27–46 Available on: https://www.cabdirect.org/cabdirect/abstract/20043070815 [Accessed: August 21, 2019]

MIDA. 2019. Ministerio de Desarrollo Agropecuario. Dirección de Agricultura, Unidad de Planificación, cierre agrí-

cola 2016–2017. Available on: https://mida.gob.pa/upload/documentos/2017-2018cierre.pdf [Accessed: August 21, 2019]

Mouden S., Sarmiento K.F., Klinkhamer P.G.L., Leiss K.A. 2017. Integrated pest management in western flower thrips: past, present and future. Pest Management Science 73 (5): 813–822. DOI: 10.1002/ps.4531

Mound L.A., Marullo R. 1996. The thrips of Central and South America: an introduction (Insecta: Thysanoptera). Memoirs on Entomology, International, Vol. 6, Florida, USA, 488 pp.

Moura M.F., Picanço M.C., Silva E.M., Guedes R.N.C., Pereira J.L. 2003. Plano de amostragem do biótipo B de *Bemisia tabaci* na cultura do pepino. Pesquisa Agropecuária Brasileira 38 (12): 1357–1363. DOI: 10.1590/S0100-204X2003001200001

Nakahara S., Monteiro R.C. 1999. *Frankliniella zucchini* (Thysanoptera: Thripidae), a new species and vector of *Tospovirus* in Brazil. Proceedings of the Entomological Society of Washington 101 (2): 290–294.

Perrings C., Dehnen-Schmutz K., Touza J., Williamson M. 2005. How to manage biological invasions under globalization. Trends in Ecology and Evolution 20 (5): 212–215. DOI: 10.1016/j.tree.2005.02.011

Prins M., Goldbach R. 1998. The emerging problem of tospovirus infection and nonconventional methods of control. Trends in Microbiology 6 (1): 31–35. DOI: 10.1016/S0966-842X(97)01173-6

Przybylska A., Fiedler Ż., Obrępalska-Stęplowska A. 2016. PCR--RFLP method to distinguish *Frankliniella occidentalis, Frankliniella intonsa, Frankliniella pallida* and *Frankliniella tenuicornis*. Journal of Plant Protection Research 56 (1): 60–66. DOI: 10.1515/jppr-2016-0009

Riley D.G., Joseph S.V., Srinivasan R., Diffie S. 2011. Thrips vectors of tospoviruses. Journal of Integrated Pest Management 2 (1): 1–10. DOI: 10.1603/IPM10020

Robinson R.W., Decker-Walters D.S. 1997. Cucurbits (Crop Production Science in Horticulture). CAB International, Wallingford, UK, 240 pp.

Smith I.M., McNamara D.G., Scott P.R., Holderness M. 1997. *Frankliniella occidentalis*. p. 267–272. In: "Quarantine Pests for Europe". CAB International, Wallingford, UK, 1425 pp.

Vierbergen G. 1995. International movement, detection and quarantine of Thysanoptera pests. p. 119–132. In: "Thrips Biology and Management" (B.L. Parker, M. Skinner, T. Lewis, eds.). Springer, Massachusetts, USA, 636 pp.

Walsh P.S., Metzger D.A., Higuchi R. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 10 (4): 506–512. DOI: 10.2144/000114018

Weng Y., Sun Z. 2011. Major cucurbits crops. p. 1–16. In: "Genetics, Genomics and Breeding of Cucurbits" (Y.H. Wang, T.K. Behera, C. Kole, eds.). CRC Press, Florida, USA, 425 pp.

Yeh W.B., Tseng M.J., Chang N.T., Wu S.Y. 2015. Agronomically important thrips: development of species-specific primers in multiplex PCR and microarray assay using internal transcribed spacer 1 (ITS1) sequences for identification. Bulletin of Entomological Research 105 (1): 52–59. DOI: 10.1017/S000748531400073X