PHILIPP AGRIC SCIENTIST
Vol. 101 No. 3, 271-278
September 2018

ISSN 0031-7454

Morphological, Molecular and Life-History Homogeneity among Local Populations of *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae) in the Philippines

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Chico-chico **in mango fruits, which has been reported only in mango-growing areas in Southern Mindanao, Philippines, has been known to be caused by the feeding of *Scirtothrips dorsalis* in young mango fruits. As *chico-chico* was not observed in other parts of the country, morphological, molecular, and biological studies were conducted to find evidences of variations among the local population of *S. dorsalis* in the Philippines. The local collection sites included Zambales, Bulacan, Laguna, Palawan, and Davao. Discriminant and cluster analysis of the various morphological characters measured showed no distinct differences among the local populations of *S. dorsalis*. In the molecular studies, the sequences from internal transcribed spacer 2 (ITS2) region of nuclear ribosomal DNA (rDNA) of the different local populations used were found to be 100% identical. Larval performance of the local populations collected from Davao and Laguna showed no significant difference in the development time from the first instar up to adult emergence. The total developmental period for Davao and Luzon were 8.86 and 8.88 d, respectively. Both oviposition and feeding were observed in young mango fruits by the tested populations in the field set-up. Further studies to determine population variation of *S. dorsalis* must be conducted on morphometric and molecular analysis using other morphological characters and other gene regions, respectively. Other behavioural studies such as greenhouse and field performance and host preference tests can also be done to verify the results of this study.**

Key Words: chilli thrips, population diversity, internal transcribed spacer 2 region, mango, morphometrics *pavonana, Curcuma longa,* essential oils, *Lantana camara,* topical toxicity, repellency

Abbreviations: HAT – hours after treatment, IGR – insect growth regulator, LC_{50} – concentration that results in 50% mortality, LD – lethal dose, LD_{50} – dose that results in 50% mortality, LRFM – leaf residue film method, RFI – relative feeding index

INTRODUCTION

Mango is the third most important fruit crop of the Philippines after banana and pineapple. In 2014, the 188,100 ha planted to mango produced a volume of 885,000 metric tons (Philippine Statistic Authority 2015).

S. dorsalis has been listed as a pest of mango flowers and shoots (Golez 1997), causing the browning and drying of flowers and the bronzing on the veins and margins of leaves. It was first described in chilli (Hood 1919) and has been one of the economically important insect pests of mango flowers in Taiwan (Lee and Wen 1982) and in Malaysia (Aliakbarpour et al. 2010). Its host range includes more than 100 plant taxa among 40 families such as mango, chilli, onion, mung bean, castor plant, peanut, and pepper, among others (Mound and Palmer 1981).

S. dorsalis is present in most mango-growing areas in the Philippines but no infestation on mango fruits has been reported except in Davao where it is an emerging

pest problem (Medina et al. 2011). Damage to the mango fruit is locally known as *chico-chico* because of the brown scarring produced due to insect feeding. It can also be mistakenly identified as a symptom of scab. *Chico-chico* is second among the causes of the poor quality of harvested mangoes in Buhangin, Davao City (Williams et al. 2009, unpublished). The infestation on mango fruits in Davao raises the question why *S. dorsalis* has only been reported as a major pest in that part of the country. A host shift is not considered as a factor because *S. dorsalis*, being a polyphagous pest, is known to feed on every tender parts of a plant including the young mango fruits. The difference in weather conditions can be a factor but in this case, if it really does trigger thrips outbreak, it must be recorded in all of the mango- growing areas in Mindanao. In addition, the host plant density in an area cannot have any major contribution to infestation since the major mango-producing provinces are in Luzon, particularly in the Pangasinan area. There are also no studies to determine if there are differences in the diversity of the host plants of *S. dorsalis* in different regions in the country. Moreover, mangoes in Davao are considered to be heavily sprayed because mango production is year round. It is unlikely for an insect to prefer a host that is sprayed with several insecticides when there are other host plants available within its reach such as *Mimosa pudica* and Lantana *camara*. These weeds are almost always present near mango orchards. Resistance can play an important role in this thrips problem but this must be supported by data.

It is hypothesized that the population of *S. dorsalis* in Davao is different from that in other mango farms in the country. Studies on this inter-population variation, therefore, will enhance understanding on why this insect became a pest in a specific area only. These studies will aid in designing an appropriate integrated pest management strategy against thrips on mango. The main objective of this study was to quantify and compare variations among populations of *S. dorsalis* in terms of morphological, molecular, and biological parameters.

MATERIALS AND METHODS

The study was conducted at the Insect Ecology Laboratory, Crop Protection Cluster, College of Agriculture and Food Science, University of the Philippianes Los Baños and in San Marcelino, Zambales from January 2012 to April 2015.

Collection of *S. dorsalis*

For the morphological and molecular studies, *S. dorsalis* in mango (Carabao var.) were collected from Davao and provinces from Luzon (Bulacan, Zambales, Laguna, and Palawan). The thrips were placed in 1.5 mL centrifuge tubes by using a fine brush. Specimens were preserved in 70% ethanol (ETOH) and absolute ETOH to prepare them for morphometric and molecular analyses, respectively. Collected insects for molecular studies were stored in the freezer to prevent specimen degradation.

Thrips used in biological studies were from mango in Davao and Laguna and from peanut in Laguna. These thrips were reared in the laboratory and the first generation of insects were used in the experimental set-ups.

Morphometric Analysis of *S. dorsalis* Populations

Thrips were permanently mounted in slides and were identified using the taxonomic characters described by Hoodle and Mound (2003). The length of ocellar setae (OS)1, OS2, OS3, post ocellar setae (POS), head, metanotum, last abdominal segment and abdomen; distances between OS1 and OS2, OS2 and OS3, two posterior ocelli (PO), PO and anterior ocelli (AO), and AO and OS3; and the distance between metanotal median setae and metanotal anterior margin were measured (Fig. 1). To cancel the variation in measurements that can be caused by different body lengths due to nutrition, the ratio of each character with the corresponding insect body region was computed.

Molecular Identification and Genetic Variation Quantification of *S. dorsalis* Populations

DNA was isolated using the Animal and Fungi DNA Preparation Kit (Jena Bioscience GmbH). Each sample contained a single insect that was weighed prior to DNA extraction. To distinguish variation on the local population of *S. dorsalis*, amplification of the extracted DNA was done using the internal transcribed spacer 2 (ITS2) region within the nuclear ribosomal genes using the primers: forward (sdi-16F) 5′GCTTGGATCTGATGGCAAC 3′ and reverse (sdi-20R) 5′ TGTGCACACGAGCCGCTCGC 3′, where the name "sdi" represents *S. dorsalis* ITS2. This primer pair was designed from the ITS2 sequence data of *S. dorsalis* originating from Japan (AB063343). It amplifies a DNA fragment ranging in size from 131 to 135 bp (Farris et al. 2010). PCR amplification was performed in a final volume of 25 µL using 12.5 µL 2X Taq Master Mix (Vivantis Technologies, Inc), 0.75 µL 50 nM MgCl2, 1 µL of each forward (10pM) and reverse (10pM) primer, 1.25 µL nuclease free water and 2.5 µL of DNA template. The PCR condition used in this study included 1 cycle at 94 °C for 3 min followed by 26 cycles at 94 °C for 30 s, 60 °C for

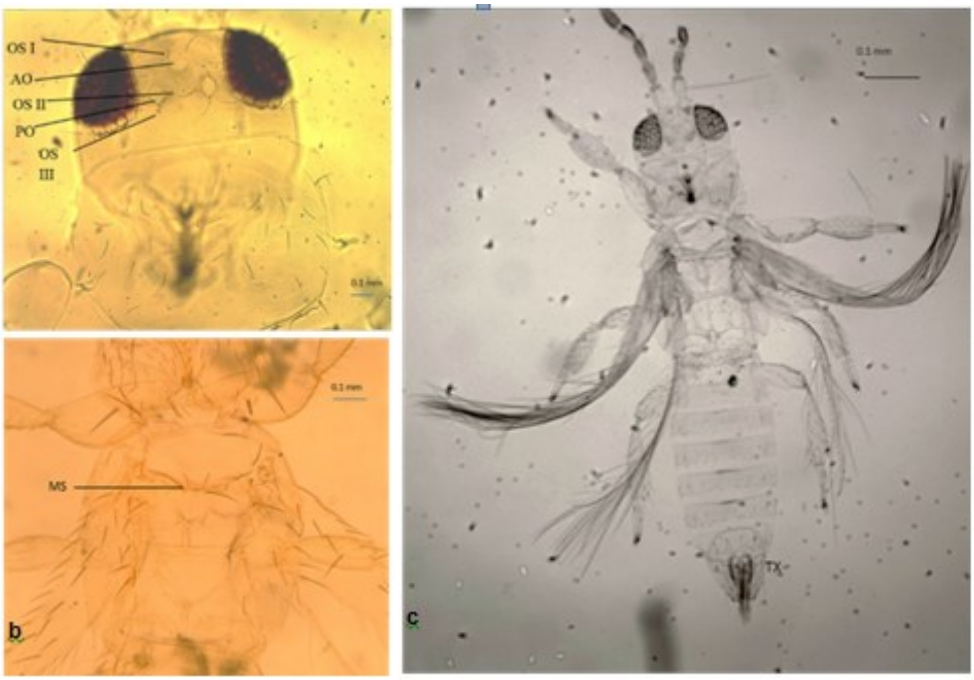

Fig. 1. Slide mounted *S. dorsalis* showing the major parts of the head (a), thorax (b) and (c) abdomen used for morphometrics, respectively. (a–b: 400x). (c: 100x).

10 s, 72 °C for 1 min, and ending with an extension cycle of 72 °C for 10 min. PCR products with precise bands were sent to BGI Tech Solutions Co., Ltd for sequencing.

Biological Studies

Larval Performance of *S. dorsalis* on Peanut

A maximum of 40 thrips per population were placed in peanut seedlings. The sources of laboratory-reared thrips populations were from mango in Davao, mango in Laguna and peanut in Laguna. The developmental period and percentage survival of each population were monitored.

Oviposition and Feeding of *S. dorsalis* in Mango Fruits

This experiment was conducted in a mango orchard in San Marcelino, Zambales. Three treatments were tested: the first treatment used adult thrips from mango in Davao, the second used adult thrips from mango in Laguna and the third did not have any adult thrips at all. The third treatment was included because the very minute size of thrips gave difficulty in finding a net through which it can fit. In addition, the experiment was conducted in the field and we could not control the natural thrips population that may alter the results of this experiment. For the first two treatments, 50 pairs of adult thrips were introduced in mango panicles with 2– 12 fruits each. The panicles were bagged to facilitate the introduction of thrips and not really to contain the thrips inside the panicle. This procedure also did not directly force the thrips to feed or oviposit eggs in the mango panicles. The treatments were replicated thrice with three sub-samples per replicate. After 3 d, the panicles were detached from the tree and the fruits were examined under the microscope for oviposition and feeding damage.

Statistical Analysis

For the morphometrics, the quantitative characters measured were first subjected to stepwise discriminant analysis and then analyzed using canonical discriminant analysis (SAS software 9.1.3). In order to construct the dendogram, Euclidean distances between the local populations were calculated and subjected to cluster analysis using the Minitab software.

For the behavioral studies, data were also analyzed using Statistical Analysis System version 9.1.3. Analysis of variance (ANOVA) was used for the larval performance and significant means were compared and separated using LSD Test. Moreover, paired T-test was used to analyze the data on oviposition and feeding set-up.

RESULTS AND DISCUSSION

Morphometric Analysis of *S. dorsalis* Populations

Stepwise discriminant analysis (SDA) showed that from the original 25 variables, the number was reduced to 14 characters only. The basic idea underlying SDA was that only relevant discriminatory characters were selected and used for the succeeding statistical analysis. The discriminatory descriptors used differed for each local

population, depending on the partial r-squared results. These quantitative characters were subjected to Canonical Discriminant Analysis (CDA) to further analyze the relationship of the five local populations of *S. dorsalis* (Fig. 2). The scatter plot of the entire population subjected to CDA revealed overlaps among the four local populations from Davao, Zambales, Bulacan, and Laguna while a distinct group was formed with the local population from Palawan. The most discriminating character was the length of tergite X followed by the length of metanotum. In addition to this, cluster analysis showed that the Zambales and Davao specimens were most similar while the Palawan population was the least similar to all the groups (Fig. 3).

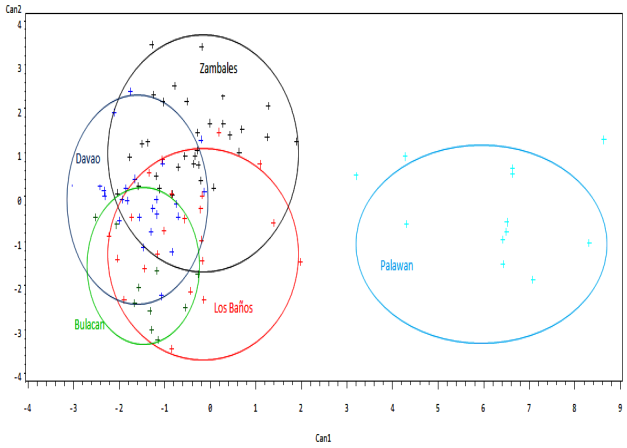

Fig. 2. Groupings of *S. dorsalis* local populations based on their computed canonical coefficients.

Morphometrics is one of the modern methods in identifying minute insect species, e.g., thrips. Sometimes the specimens are very similar such that only the complex combination of characters, both qualitative and quantitative, may distinguish them as observed by Gikonyo et al. (2017). In thrips, the long or short setae may be treated as qualitative, but for an inexperienced person, it may be difficult to determine which characteristic a given thrips has (Mehle and Trdan 2012). The statistical methods for taxonomic studies, and in particular principal component analysis, have been shown to be useful for differentiating thrips. Principal component analysis compares sample groups (taxa or specimens), taking into consideration the value of their characters. Although the morphometric results are subject to measurement errors, these data could play a large role in the discrimination of similar species, e.g., *T. atratus* and *T. montanus* (Kucharczyk and Kucharczyk 2009).

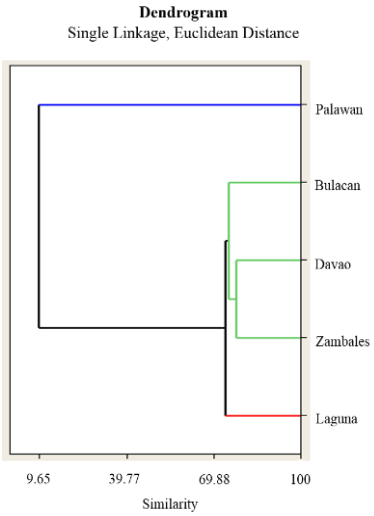

Fig. 3. Cluster analysis of different local populations of *S. dorsalis*.

Although morphometrics has been found to be reliable in discriminating thrips species, results from the series of statistical analyses of the morphological data from different local populations of *S. dorsalis* revealed that there was no distinct morphological character of thrips that can separate one local population from the other. The canonical discriminant analysis of the morphometric data of *S. dorsalis'* local populations revealed a high probability of them being misclassified with each other. The probability of misclassification of Palawan population with those from Bulacan was only 8.33% but still it showed similarity with the later population.

Table 1. Mean number of developmental period of the three local populations of *S. dorsalis* reared on peanut seedlings.

Place of Collection/ Host	N	Duration (No. of Days) Mean ± SEM				
		Larva 1	Larva 2	Pre-Pupa	Pupa	Total
Davao/mango	40	1.89 ± 0.05 a	2.64 ± 0.09 a	2.13 ± 0.09 a	2.31 ± 0.07 a	8.86 ± 0.12 a
Laguna/mango	30	1.65 ± 0.09 b	2.62 ± 0.09 a	1.75 ± 0.10 b	2.65 ± 0.09 a	8.88 ± 0.21 a
Laguna/peanut	25	1.33 ± 0.10 c	2.40 ± 0.10 a	1.25 ± 0.09 c	2.26 ± 0.13 a	7.21 ± 0.21 b

Means within a column followed by a common letter are not significantly different by LSD.

Table 2. Percent survivorship of three local populations of *S. dorsalis* per developmental stage.

Place of Collection/ Host	Larva 1		Larva 2		Prepupa		Pupa	Larva-Adult
	N		N		N			
Davao/mango	40	88.00	35	85.71	30	97.00	29	100.00	72.50
Laguna/mango	30	87.00	26	80.77	21	95.00	20	85.00	56.67
Laguna/peanut	25	84.00	21	95.24	20	100.00	20	100.00	80.00

Molecular Analysis of *S. dorsalis* Populations

All samples from different local populations of *S. dorsalis* were 100% identical with the *S. dorsalis* ITS2 partial sequences of Farris et al. (2010) (*Scirtothrips dorsalis* isolate X070910-001 internal transcribes spacer 2, partial sequence). The query covers the sequences submitted were all 98% which shows a high degree of overlap between the reference sequences in the NCBI database. Alignment using the ClustalW of the MEGA 5.2 software yielded a pairwise distance of 0.000 which demonstrates that no transition/ transversion substitution/s occurred in the local population sequences.

In the attempt to find molecular variations among local populations of *S. dorsalis* in the Philippines, the internal transcribed spacer 2 of the rDNA was used since this is a rapidly evolving region and has been widely used for the identification of closely related species (Fritz et al. 1994). It means that there was a high possibility that this particular gene can differentiate one species from another. In the study of Rugman Jones et al. (2006), molecular data using ITS1 and ITS2 regions suggested that Indian and South African specimens of *S. dorsalis* are indeed not the same species. The level of variation, in the sizes and sequences of the two regions between *S. dorsalis* specimens from India and South Africa, was too great to be recognized as different host races. Also, Alvarez and Hoy (2002) used the noncoding ribosomal region (ITS2) in separating populations of the parasitoid *Ageniaspis citricola* (Hymenoptera: Encyrtidae) from different geographic areas (Australia and Taiwan). Intra- and inter-individual variations in ITS2 sequence and length were present in the three *Ageniaspis* populations. Intra-individual sequence variation was sometimes greater than between individuals in each *Ageniaspis* population.

The consistent results that were obtained from the sequence identity indicated that no difference was found among the local populations using the ITS2 gene. This means that the populations of *S. dorsalis* in the different mango-growing areas in the Philippines were definitely the same species and that there were other factors that must be investigated to know why the particular population in Davao infests mango fruits.

Larval Performance of *S. dorsalis* on Peanut

The means of total duration of development of *S. dorsalis* from Davao mango and Laguna mango did not differ (8.86 and 8.88 d, respectively). On the other hand, *S. dorsalis* collected from Laguna peanut had a significantly different duration of 7.21 d only. Significant differences among the populations evaluated were obtained when analyzed for each stage of development. For the first instar larva, the duration of development is longest in the Davao mango population (1.89 d), followed by Laguna mango (1.65 d) and then Laguna peanut (1.33 d). No difference was observed during the second larval stage. For the pre-pupal stage, the same trend with larva 1 was observed, with the Davao population having the longest duration of development of 2.13 d for completion while the Laguna population of *S. dorsalis* took 1.75 d to complete this particular stage of development (Table 1).

The Davao mango thrips population had 72.50 % survivorship from the first instar larva up to the adult stage while the Laguna mango thrips population had only a 56.67 % survivorship. The survivorship of *S. dorsalis* from Davao mango translates to 29 adults out of the 40 first larval instars observed in the experiment. On the other hand, 17 out of 30 thrips from Laguna mango survived up to the adult stage. The Laguna peanut population had the highest survivorship rate of 80% (Table 2).

Larval performance of different populations of *S. dorsalis* is one of the insect characteristics considered in the effort to shed light on why only a particular local population of *S. dorsalis* from Davao inflicted a vast damage on mango fruits. Larvae from the laid eggs on fruits are considered destructive because of their feeding damage. Mango has been reported as one of the main hosts of *S. dorsalis* (Centre for Agriculture and Biosciences International 2012). It is assumed that the prolonged

encounter of this pest in mango gave it the opportunity to respond to the plant traits, physically and chemically, which leads to positive response in their population growth. Strong host-plant driven selection has been linked with population differentiation and local adaptation of insects on common host plants (Bossart and Scriber 1995).

The larval performance of the local populations of *S. dorsalis* from Davao and Laguna showed no difference in the mean duration of their developmental period. This result implies that both populations had the same ability to complete their life cycle in a given suitable host plant. However, it is important to note that thrips in Davao mangoes had a consistently higher survivorship rate than the Laguna mango population. Consequently, higher damage in the host can be inflicted by the Davao population given that there was a higher population turn out in its life cycle. This result must be investigated further by using mango as host.

This particular study showed that a difference in the larval survivorship can occur when a suitable host is provided even if no morphological and molecular variations were obtained from the local populations of *S. dorsalis* tested. In finding variation in local populations of a certain species, selection might operate on traits affecting performance of different host plant species, migratory ability, thermal tolerance, and resistance to natural enemies (Brower et al. 1995). Mangoes are continuously produced in Mindanao, particularly in Davao all year round while the bulk of the output in Luzon was during March to May (Briones 2013). This practice of mango growers in Davao translates to a continuous source of mango host for thrips. Thrips cannot utilize mango in Luzon all year round because of the seasonal presence of the mango shoots and flowers.

Oviposition and Feeding of *S. dorsalis* in Mango Fruits

Local populations of *S. dorsalis* from Davao and Luzon exhibited oviposition and feeding in young mango fruits. The mean numbers of eggs laid in fruits are 6.00 and 1.67 eggs for Davao and Luzon populations, respectively (Table 3). Even if these means were not significantly different using independent t-test, the mean number of eggs laid by thrips from Davao was higher when compared with the Luzon population. Feeding of *S. dorsalis* was also evaluated after 3 d from the field set up. The feeding damage symptoms included silvering of the leaf surface, brown frass markings on the fruits, grey to black markings on fruits, often forming a distinct ring of scarred tissue around the apex, and fruit distortion (European and Mediterranean Plant Protection Organization Standards 2005). Feeding has also been observed in both local populations tested. No oviposition and feeding of *S. dorsalis* were found in untreated mango panicles. This means that the natural population of thrips in the experimental site did not interfere with the results of the experiment.

A low feeding incidence was observed in the set-up even if a minimum of 50 pairs of adults were released in every mango panicle. Thrips, especially the adults, can feed on a wide range of food because they are more mobile than those at the immature stages. This can be one of the reasons of the low incidence of feeding observed in the field set-up.

As expected, the local population of *S. dorsalis* collected from Davao has been observed to feed and breed on mango fruits. The other local populations of *S. dorsalis* studied also fed and laid eggs in mango fruit. This supported the results of the morphological and molecular studies which showed no distinct variations among the local populations of *S. dorsalis* collected from various mango-growing areas in the Philippines.

CONCLUSION AND RECOMMENDATIONS

Morphological and molecular analysis did not show evidences of variations among populations of *S. dorsalis* collected from Davao, Zambales, Bulacan, Laguna, and

Table 3. Mean number of eggs laid by *S. dorsalis* in mango fruit panicles.

Treatment	Mean	T	P
Davao population Luzon population	6.00 1.67	0.92 ns	0.3735
Davao population Untreated	6.00 0.00	0.76 ns	0.4571
Luzon population Untreated	1.67 0.00	1.20 ns	0.2478

ns – not significant

Palawan. The biological parameters measured in this study showed no variations among the populations tested except for the higher survivorship of the Davao population in the laboratory.

It is recommended that other quantitative characters such as ovipositor length, abdominal microtrichial length, and other important characters be subjected to morphometrics to further determine intraspecific variations in local populations of *Scirtothrips dorsalis*. More gene regions such as the internal transcribed spacer (ITS) 1 region of the ribosomal DNA and the cytochrome b (cytb) from the greater number of samples and different areas in the country need further investigation. Studies on larval performance of thrips in mango under greenhouse and field conditions must be conducted to verify the results of the laboratory set-up. Host preference studies on the three different mango parts which *S. dorsalis* utilizes such as shoots, flowers, and fruits, must also be done to further analyze the host selection behavior of the species.

ACKNOWLEDGMENTS

The authors would like to thank the Department of Science and Technology- Accelarated Science and Technology Human Resource Development Program and the Australian Centre for International Agricultural Research for funding this research. Significant contributions from Drs. Barbara L. Caoili, Pio A. Javier and Aurora M. Baltazar also aided the implementation of this study.

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