Molecular and Morphological Identification of Banana Thrips in Chantaburi Province, Thailand

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Abstract Thrips are a major pest causing peel damage in banana cv. 'Kluai Khai'. Thrips identification to the species level using only morphological characters is complicated and molecular techniques were also used to accurately confirm identification. Thrips were collected from banana fields in Maung, Tha Mai and Makam districts of Chanthaburi Province. Morphology structure was used to identify distinct species of thrips. Nucleotide sequencing analysis of mitochondrial cytochrome C oxidase subunit I (COI) gene was used to confirm thrips species identification. Morphological and DNA barcoding results indicated *Thrips hawaiiensis* was the most common species in all study areas and cropping systems.

Keywords: DNA Barcoding; *Thrips hawaiiensis*; banana cv. 'Kluai Khai'

Introduction

Chanthaburi Province in the east of Thailand is one of the top five provinces that produce banana cv. 'Kluai Khai'. Thrips are the most important economic pest of banana plantations causing serious damage to fruit. Thrips are tiny, herbivorous insects found in many economic agricultural crops. These insects are the major pest in banana cv. Kluai-Khai causing physical damage to the fruit (Mound, 2005; Mandal *et al*., 2012). They feed by puncturing the outer layer of the flower and fruit of banana tissue and sucking out the cell contents. This results in stippling the fruit surface, reducing the value and rendering these fruits unsuitable for fresh marketing. The presence of thrips causes rejection of bananas for export due to quarantine regulations. Generally, the majority of farmers in Chantaburi Province commonly grow multispecies on a single piece of land. Different economic fruit crops in this mixed cropping system include banana, durian, mangosteen, and rambutan. New agricultural systems are often based on monoculture because this provides high productivity and convenient management. Previous studies showed that mixed cropping systems reducing pests and diseases when compared with single cropping systems (Malezieux *et al*., 2009). Thrips species found in mangosteen are *Scirtothrips dorsalis* and *Scirtothrips oligochaetus* (Poonchaisri, 1992; Ngampongsai, 2006). In rambutan, *Slenothrips rubrocinctus* and *Scirtothrips dorsalis* are common pests. *Scirtothrips dorsalis* is also an important thrips in durian (Astridge, 2001; Astridge, 2006). A study in mangosteen showed that the number of thrips was higher in single crops than in mixed cropping systems (Pankeaw *et al*., 2011). Some thrips species have a broad range of host plants and can emigrate from one crop to other cultivated species or wild species when the host plants are not flowering (Rhodes and Liburd, 2020). Identification of thrips species is important and essential information for developing pest management strategies; however, morphological identification is difficult. Thus, another method is needed to confirm species identification. Here, invasive thrips from banana plantations with different cropping systems were identified using an integrated approach combining morphology and DNA barcode methods.

Materials and methods

Banana thrips collection

Banana inflorescences were randomly collected from seven locations in three main banana production areas including Tha Mai, Maung and Makam districts in Chantaburi Province, Thailand. Banana inflorescences from each location with different cropping systems were put separately into plastic containers with a radius of 25 cm and a height of 40 cm for rearing thrips. Each sample was marked with a different code as follows: TM1 = single-cropping system in Tha Mai District; M2 = single-cropping system in Muang District; M3 = mixed-cropping system with mangosteen in Muang District; MK4 = mixed-cropping system with durian in Makam District; MK5 = single-cropping system in Makam District; TM6 = mixed-cropping system with many crops in Tha Mai District;

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TM7 = mixed-cropping system with papaya in Tha Mai District. A cotton pad soaked with deionized water was placed into each container and the containers were kept in a room at 25-27 ^{o}C.

Identification and morphometric analysis

Adult thrips were isolated from banana inflorescences using a fine brush and put into micro-tubes containing 70% ethanol. Samples were sorted and morphologically identified under a stereomicroscope (Olympus SZX16®). Species identifications were made using keys provided by zur Strassen (2003), Mound and Marullo (1996) and Nakahara (1994). Pictures were taken using an Olympus SZX16® Stereomicroscope.

DNA extraction, amplification and sequencing

Individual living adult thrips were randomly collected from banana inflorescences using a fine pin. Between each sample collection, the pin was cleaned with 5% (v/v) sodium hypochlorite and washed twice with 70% (v/v) ethanol. Three samples were collected from each location and total genomic DNA was extracted from each thrips sample using DNeasy® Blood & Tissue Kit (QIAGEN, Germany) with some modifications.

PCR reactions were used to amplify a portion of mitochondrial cytochrome C oxidase subunit I (COI) gene regions using LCO1490 and HCO2198 primers (Folmer *et al*., 1994). Each PCR amplification reaction in 20 µl volume contained 1 µl of template DNA, 10 µM of each primer, 2 µl of 10X buffer, 2.5 mM $MgCl_2$, 0.25 mM of each dNTP and 5U of TaqDNA polymerase (Fermentas Life Sciences, UK). The reactions were performed in a thermal cycler (Bio-Rad® model T100 Thermo Cycler, USA). Cycling started with an initial 95 ^{o}C for 3 min, followed by 35 cycles of 94 ^{o}C for 30 sec, the appropriate annealing temperature for 30 sec, 72 ^{o}C for 2 min and final extension at 72 ^{o}C for 20 min. The amplified products were resolved on 1% agarose gel and then purified using the ethanol precipitation process. Products were sequenced at Macrogen Inc., Rep. of Korea.

Data analysis

Sequencing data were analyzed using ClustalW2 software (http://www.ebi.ac.uk/Tools/msa/clustalw2) and Blast with the NCBI nucleotide database (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results

Morphological identification

Many living adults, larvae and eggs of thrips were found in all inflorescences from seven different locations in Chantaburi Province, even though all the sampling plantations were routinely sprayed with insecticides (Figure 1). Thrips lays a single kidney-shaped egg in the tissue of inflorescences (Figure 1C-D). No pupae were found in the samples, as most mature larvae normally drop to the ground to pupate.

Taxonomy results in all thrips samples from seven different banana fields in Chanthaburi Province indicated that all specimens in both single-cropping systems (TM1, M2 and MK5) and mixed-cropping systems (M3, MK4, TM6 and TM7) were *Thrips hawaiiensis* (Figure 2) showing the following characters: head wider than length; abdomen brown; thorax and head orange brown; legs yellowish brown; forewings greyish; satae brown. Antennal segments were brown except for segment III which was yellow (Figure 1E). Metanotal sculpture was mainly transverse at the anterior and lateral (Figure 1F).

DNA barcode analysis

DNA sequencing of the COI gene was used to confirm taxonomy results. Alignment results of the COI gene thrips segments from five different areas were blasted to the NCBI database (Figure 3). All DNA samples matched *T. hawaiiensis* voucher TH-92 in cytochrome oxidase subunit 1 (COI gene), partial cds; mitochondrial (identity 98% at 0.0 E-value) indicating that all specimens from different cropping systems were *T. hawaiiensis*.

Thrips are normally difficult to identify, and DNA-based techniques are one option to confirm their morphology. In this study, both morphological and DNA barcode analysis verified that all the samples collected on banana inflorescences from all studied locations represented *T. hawaiiensis.*

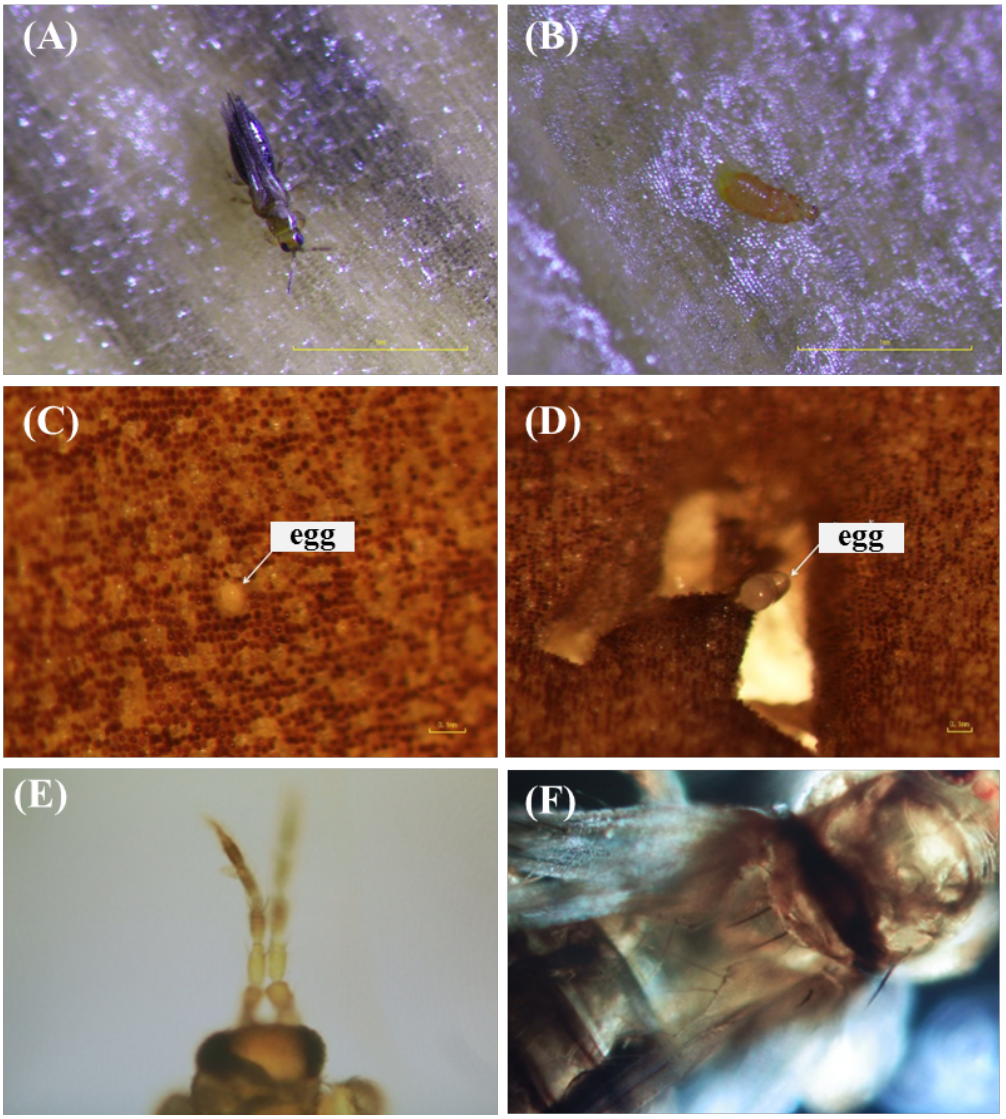

Figure 1. Thrips in banana cv. Kluai-Khai inflorescence (A) adult; (B) larvae; (C) and (D) eggs; (E) antenna; (F) metanotum

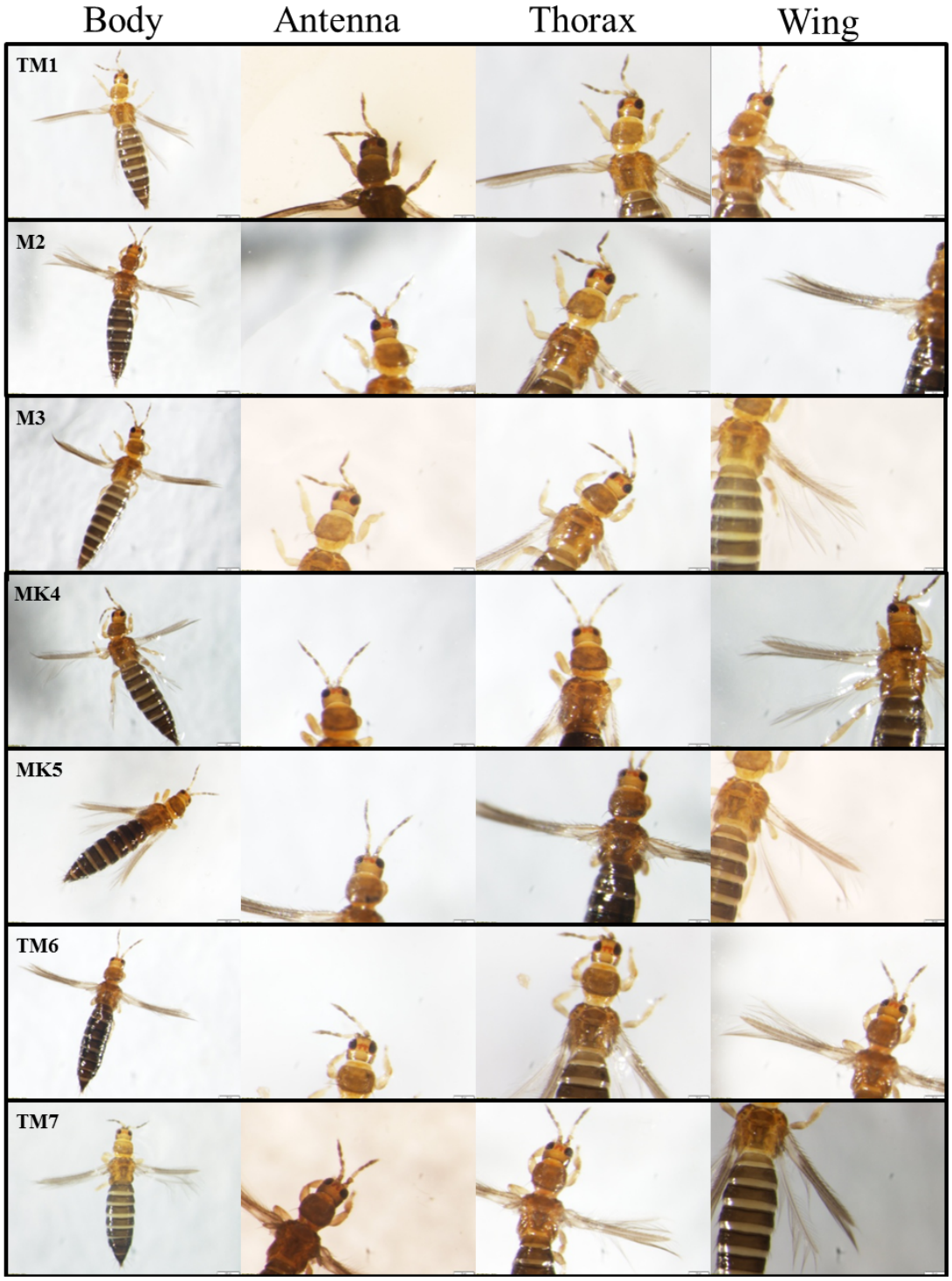

Figure 2. Morphological characters of thrips from seven field collections. (TM1, M2, and MK5 =single crop; M3, MK4, TM6 and TM7 = mixed crops).

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A    CTACA-GTG-ATATTTATTTGTTGGATTTGTGATCACTGATTAATAAAGACTTTCATTAAGAATAATCATTCGACTAAACTTACGAACATCAATAAAACTTTATGTAAGAAACGATCAAT 118
C    --ACA-TTATATTTTTATTTTTGGGATTT-TGATCAG-GATTAATAG-GACTTTCATTAAGAATAATCATTCGACTAAACTTACGAACATCAATAAAACTTTATGTAAGAAACGATCAAT 114
B    ---CATATGTAT-TGGATTTATTGGATTT--GATCAG-GATTAATAG-GACTTTCATTAAGAATAATCATTCGACTAAACTTACGAACATCAATAAAACTTTATGTAAGAAACGATCAAT 112
D    ---CA-ATATAT-TTTATTT-TTGGATTT-TGATCAG-GATTAATAG-GACTTTCATTAAGAATAATCATTCGACTAAACTTACGAACATCAATAAAACTTTATGTAAGAAACGATCAAT 111
E    -------------------------------------------------------TTAAGAATAATCATTCGACTAAACTTACGAACATCAATAAAACTTTATGTAAGAAACGATCAAT  64
                                                            ************************************************************

A    TTTATAATTCAATCGTAACAGCACACGCATTTATTATAATTTTTTTTACAGTAATACCAATCATAATTGGAGGATTTGGTAACTGATTAGTTCCACTAATATTAGGAGCACCAGACATAG 238
C    TTTATAATTCAATCGTAACAGCACACGCATTTATTATAATTTTTTTTACAGTAATACCAATCATAATTGGAGGATTTGGTAACTGATTAGTTCCACTAATATTAGGAGCACCAGACATAG 234
B    TTTATAATTCAATCGTAACAGCACACGCATTTATTATAATTTTTTTTACAGTAATACCAATCATAATTGGAGGATTTGGTAACTGATTAGTTCCACTAATATTAGGAGCACCAGACATAG 232
D    TTTATAATTCAATCGTAACAGCACACGCATTTATTATAATTTTTTTTACAGTAATACCAATCATAATTGGAGGATTTGGTAACTGATTAGTTCCACTAATATTAGGAGCACCAGACATAG 231
E    TTTATAATTCAATCGTAACAGCACACGCATTTATTATAATTTTTTTTACAGTAATACCAATCATAATTGGAGGATTTGGTAACTGATTAGTTCCACTAATATTAGGAGCACCAGACATAG 184
     ******************************************************************************************************************

A    CATTCCCACGATTAAACAATATAAGATTCTGACTTTTACCACCATCATTAACACTTTTAATTATAGGACTTATAAAAGAAGGAGCAGGAACAGGATGAACAGTATATCCACCTTTATCAA 358
C    CATTCCCACGATTAAACAATATAAGATTCTGACTTTTACCACCATCATTAACACTTTTAATTATAGGACTTATAAAAGAAGGAGCAGGAACAGGATGAACAGTATATCCACCTTTATCAA 354
B    CATTCCCACGATTAAACAATATAAGATTCTGACTTTTACCACCATCATTAACACTTTTAATTATAGGACTTATAAAAGAAGGAGCAGGAACAGGATGAACAGTATATCCACCTTTATCAA 352
D    CATTCCCACGATTAAACAATATAAGATTCTGACTTTTACCACCATCATTAACACTTTTAATTATAGGACTTATAAAAGAAGGAGCAGGAACAGGATGAACAGTATATCCACCTTTATCAA 351
E    CATTCCCACGATTAAACAATATAAGATTCTGACTTTTACCACCATCATTAACACTTTTAATTATAGGACTTATAAAAGAAGGAGCAGGAACAGGATGAACAGTATATCCACCTTTATCAA 304
     ******************************************************************************************************************

A    CTTTTTATCATTCAGGAAACTCCGTAGATCTAACAATTTTTTCCCTTCATTTAGCAGGTATTTCATCTATTTTAGGAGCACTAAATTTTATTACTACAATTATTAATTTAAAAGCCAAAA 478
C    CTTTTTATCATTCAGGAAACTCCGTAGATCTAACAATTTTTTCCCTTCATTTAGCAGGTATTTCATCTATTTTAGGAGCACTAAATTTTATTACTACAATTATTAATTTAAAAGCCAAAA 474
B    CTTTTTATCATTCAGGAAACTCCGTAGATCTAACAATTTTTTCCCTTCATTTAGCAGGTATTTCATCTATTTTAGGAGCACTAAATTTTATTACTACAATTATTAATTTAAAAGCCAAAA 472
D    CTTTTTATCATTCAGGAAACTCCGTAGATCTAACAATTTTTTCCCTTCATTTAGCAGGTATTTCATCTATTTTAGGAGCACTAAATTTTATTACTACAATTATTAATTTAAAAGCCAAAA 471
E    CTTTTTATCATTCAGGAAACTCCGTAGATCTAACAATTTTTTCCCTTCATTTAGCAGGTATTTCATCTATTTTAGGAGCACTAAATTTTATTACTACAATTATTAATTTAAAAGCCAAAA 424
     ******************************************************************************************************************

A    AAATTTCAACAGAAAAAATTAGATTATTTGTTTGATCAGTTATATTAACAGCAATTTTACTTCTTTTATCATTACCAGTTTTAGCAGGAGCTATCACCATACTTTTAACAGATCGAAATT 598
C    AAATTTCAACAGAAAAAATTAGATTATTTGTTTGATCAGTTATATTAACAGCAATTTTACTTCTTTTATCATTACCAGTTTTAGCAGGAGCTATCACCATACTTTTAACAGATCGAAATT 594
B    AAATTTCAACAGAAAAAATTAGATTATTTGTTTGATCAGTTATATTAACAGCAATTTTACTTCTTTTATCATTACCAGTTTTAGCAGGAGCTATCACCATACTTTTAACAGATCGAAATT 592
D    AAATTTCAACAGAAAAAATTAGATTATTTGTTTGATCAGTTATATTAACAGCAATTTTACTTCTTTTATCATTACCAGTTTTAGCAGGAGCTATCACCATACTTTTAACAGATCGAAATT 591
E    AAATTTCAACAGAAAAAATTAGATTATTTGTTTGATCAGTTATATTAACAGCAATTTTACTTCTTTTATCATTACCAGTTTTAGCAGGAGCTATCACCATACTTTTAACAGATCGAAATT 544
     ******************************************************************************************************************

A    TAAACACTTCTTTTTTTGATCCTTGGAGGAGGGTGGAGACCACAGTC--T----------------- 646
C    TAAACACTTCTTTTTTTGATCCA-AGAGGAGG--TGAGACCACAGTC--TATACCAACAACCATTT- 655
B    TAAACACTTCTTTTTTTGATCCA-AGAGGAGG-TG-AGACCACAGTC--TATAC------------- 641
D    TAAACACTTCTTTTTTTGATCCA-AGAGGAGG-TGGAGACC-CAGTTCGTATACGCAGCAACGCTTT 655
E    TAAACACTTCTTTTTTT-------------------------------------------------- 561
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Figure 3. Alignment sequencing results of thrips in banana cv. Klaui-Khai field (A=single-cropping system; B=single-cropping system; C=mixed-cropping system with mangosteen; D=mixed-cropping system with many crops; E=mixed –cropping system with papaya).

Discussion

All specimens were collected from conventional plantations with regular pesticide use. Collected adults and larva were weak, suggesting that application of insecticides reduced survival. However, thrips are always found in banana inflorescences even after spraying with insecticide. This thrips species is highly polyphagous and feeds in a wide range of hosts (Hill, 1987).

Morphological identification results showed that the species collected from banana fields in all single- and mixed-cropping systems was *T. hawaiiensis*. Morphological identification of thrips is difficult due to close similarities between species (Mound and Marullo, 1996). Previous studies showed that COI sequencing was effective for thrips species identification (Timm, 2008; Glover *et al*., 2010). Here, DNA sequences of the COI gene proved useful for species identification, and sequence analysis confirmed morphological identification. *T. hawaiiensis* is found in a wide variety of tropical flowers, fruits and seed crops within the Oriental and Oceanic Regions to the Caribbean Basin, from Japan and Hawaii to New Zealand and the Tuamotu Islands (Palmer and Wetton, 1987). Thrips species are major pests of apples, pears, mangoes, coffee, citrus, passion fruit, and bananas (Abraham *et al*., 1970; Ananthakrishnan, 1971; Swaine and Corcoran, 1975; Srivastava and Bhullar, 1980; Lee and Wen, 1982; Palmer and Wetton, 1987). In Taiwan, *T. hawaiiensis* is a major pest of banana crops (Tsai *et al*., 1992). Incidence of thrips damage on banana was first recorded in 1967 (Tang, 1974). Eggs are normally found inside the epidermis of banana fingers and cause black pimples to develop on the fruit skin, reducing market value (Cheng *et al*., 1980). *T. hawaiiensis* has been recorded in 141 species of host plant (Chang, 1995) but it is not a virus vector (Goldarazena, 2011). In Thailand, thrips adults and nymphs cause direct damage to many fruit crops including banana, significantly reducing fresh market quality.

Morphological identification with subsequent barcode analysis showed that the species responsible for major damage in banana fruit in the Chantaburi area was *T. hawaiiensis*. In mixed-cropping systems, bananas were not the preferred hosts of thrips from other cultivated plant species in the systems.

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References

Abraham, E.V., Padmanabhan, M.D. Mohandoss, A., and Gunasekharan, C.R. (1970). Record of some insects of economic importance on the hill crops in Tamil Nadu. Madras Agricultural Journal 57: 718-722.

Astridge, D. (2001). Insect fauna surveys on rambutan, durian and mangosteen in North Queensland. Technical Bulletin Department of Primary Industry and Fisheries, Northern Territory of Australia, 75-79.

Astridge, D. (2006). Rambutan. Development of integrated pest management. Pest monitoring and insecticide screening. Publication No. 05/187. Rural Industries Research and Development Corporation, 50 pp.

Ananthakrishnan, T.N. (1971). Thrips (Thysanoptera) in agriculture and forestry diagnosis, bionomics and control. Journal of Scientific and Industrial Research 30: 113-146.

Chang, N.T. (1995). Major pest thrips in Taiwan. Thrips biology and management: proceedings of the 1993 International Conference on Thysanoptera: 105-108.

Cheng, E.Y., Hwang, M.T., Ruaan, J.C., and Tsai, T.C. (1980). Activity and spatial distribution of *Thrips hawaiiensis* Morgan in banana plantation. Bulletin of Taiwan Banana Research Institute 1: 26-31.

Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294-299.

Glover, R.H., Collins, D.W., Walsh, K., and Boonham, H. (2010). Assessment of loci for DNA barcoding in the genus Thrips (Thysanoptera: Thripidae). Molecular Ecology Resources 10: 51-59.

Goldarazena, A. (2011). First record of *Thrips hawaiiensis* (Morgan, 1913) (Thysanoptera: Thripidae), an Asian pest thrips in Spain. Bulletin OEPP/EPPO (Organisation Europeene et Mediterraneene pour la Protection des Plantes/European and Mediterranean Plant Protection Organization) 41(2): 170-173.

Hill, D.S. (1987). Agricultural insect pests of temperate regions and their control. Cambridge University Press. 647 p.

Lee, H.S. and Wen, H.C. (1982). Seasonal occurrence of and injury caused by the thrips and their control on mangoes. Plant Protection Bulletin Taiwan 24: 179-187.

Malezieux, E., Crozat, Y., Dupraz, C., Laurans, M., Makowski, D., Ozier-Lafontaine, H., Rapidel, B., Tourdonnet, S. de, and Valantin-Morison, M. (2009). Mixing plant species in cropping systems: concepts, tools and models. A review. Agronomy for Sustainable Development 29: 43-62.

Mandal, B., Jain, R.K., Krishnareddy, M., Krishna Kumar, N.K., Ravi, K.S., and Pappu, H.R. (2012). Emerging problems of Tospoviruses (Bunyaviridae) and their management in the Indian Subcontinent. Plant Disease 96: 468-479.

Mound, L.A. and Marullo, R. (1996). The thrips of Central and South America: an introduction (Insecta: Thysanoptera). Memoirs on Entomology International 6: 487.

Mound, L.A. Thysanoptera: diversity and interactions. (2005). Annual Review of Entomology 50: 247-269.

Nakahara, S. (1994). The genus Thrips Linnaeus (Thysanoptera: Thripidae) of the new world. USDA Technical Bulletin No. 1822. 133 pp.

Ngampongsai, A. (2006). Thrip outbreaks and damage on mangosteen in southern Thailand and control measures. Final report. Department of Pest management, Faculty of Natural Resources, Prince of Songkla University. Songkhla. [online] http://www.natres.psu.ac.th/e-book/Complete.pdf. (in Thai).

Pankeaw, K., Ngampongsai, A., Permkam, S., and Rukadee, O. (2011). Abundance and distribution of thrips (Thysanoptera: Thripidae) in mangosteen (*Garcinia mangostana* L.) grown in single- and mixed-cropping systems. Songklanakarin Journal of Science and Technology 33: 263-269.

Palmer, J.M. and Wetton, M.N. (1987). Amorphometric analysis of the *Thrips hawaiiensis* (Morgan) species-group (Thysanoptera: Thripidae). Bulletin of Entomological Research 77: 397-406.

Poonchaisri, S. (1992). Thrips in fruit trees. Division of Entomology and Zoology, Department of Agriculture. Bangkok. (in Thai).

Rhodes, E.M. and Liburd, O.E. (2020). Flower thrips (Thysanoptera: Thripidae) dispersal from alternate hosts into southern highbush blueberry (Ericales: Ericaceae) plantings. Florida Entomologist 94: 311-320.

Srivastava, S. and Bhullar, J.S. (1980). Thrips – a new record as a pest of citrus blossoms in Himachal Pradesh. Current Science 49: 747.

Swaine, G. and Corcoran, R.J. (1975). Banana flower thrips and its relationship to corky scab damage of Cavendish bananas in south-east Queensland. Queensl. Journal of Animal Science 32: 79-89.

Tang, C.C. (1974). The ecology study on banana thrips in Taiwan. Research Bulletin of National Science Institute 7: 371-383.

Timm, A.E., Stiller, M., and Frey, J.E. (2008). A molecular identification key for economically important thrips species (Thysanoptera: Thripidae) in southern Africa. African Entomology 16: 68-75.

Tsai, Y.P., M.T. Hwang and H.P. Chen. (1992). Occurrence and damage of Thrips hawaiiensis in banana orchards. Chinese Journal of Entomology 12: 231-237.

Zur Strassen, R. (2003). Die terebranten Thysanopteren Europas und des Mittelmeer-Gebietes. Die Tierwelt Deutschlands 74: 1-277.