National Diagnostic Protocol

Scirtothrips perseae

(Avocado thrips)

NDP 3 V3.1

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Department of Agriculture, Water and the Environment

Street Address 1: 18 Marcus Clarke Street, Canberra City ACT 2601
Street Address 2: 7 London Circuit, Canberra City ACT 2601
Postal Address: GPO Box 858, Canberra City ACT 2601
Switchboard Phone: 02 6272 3933
Web: http://www.agriculture.gov.au

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Purpose

National Diagnostic Protocols (NDPs) are diagnostic protocols for the unambiguous taxonomic identification of plant pests. NDPs:

- are a verified information resource for plant health diagnosticians
- are consistent with ISPM No. 27 – Diagnostic Protocols for Regulated Pests
- provide a nationally consistent approach to the identification of plant pests enabling transparency when comparing diagnostic results between laboratories; and,
- are endorsed by regulatory jurisdictions for use (either within their own facilities or when commissioning from others) in a pest incursion.

Where an International Plant Protection Convention (IPPC) diagnostic protocol exists it should be used in preference to NDPs, unless it is shown that the NDP has improved procedures for Australian conditions. NDPs may contain additional information to aid diagnosis. IPPC protocols are available on the IPPC website:

https://www.ippc.int/core-activities/standards-setting/ispms

Process

NDPs are facilitated and endorsed by the Subcommittee on Plant Health Diagnostics (SPHD). SPHD reports to Plant Health Committee and is Australia's peak technical and policy forum for plant health diagnostics.

NDPs are developed and endorsed according to Reference Standards developed and maintained by SPHD. Current Reference Standards are available at https://www.plantbiosecuritydiagnostics.net.au/initiatives/national-diagnostic-protocols/

NDPs are living documents. They are updated every 5 years or before this time if required (i.e. when new techniques become available).

Document status

This version of the National Diagnostic Protocol (NDP) for *Scirtothrips perseae* is current as at the date contained in the version control box below.

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Further information

Inquiries regarding technical matters relating to this project should be sent to: sphd@agriculture.gov.au

Contents

1 INTRODUCTION

The avocado thrips, *Scirtothrips perseae* Nakahara, is easily recognized as a member of the family Thripidae from the two pairs of long slender wings with their rough surface and long fringes of marginal cilia. However, because of the small body size, scarcely 2 mm long, and the undistinguished yellow colour, satisfactory recognition of an adult as a member of the genus *Scirtothrips* necessitates examination under a microscope. Moreover, recognition of species within this genus requires considerable expertise in the preparation of microscope slide mounts (see Appendix 8.1). Precise identification of *S. perseae* amongst the 100 or more described species of *Scirtothrips* requires that several individuals be well mounted onto microscope slides, and then examined under a good quality (preferably phase-contrast) microscope.

This thrips appears to be fully dependent on the young growing tissues of *Persea americana*. The development period from egg to adult takes between 15 and 21 days, at temperatures between 20 and 30°C, with adult females unlikely to live for more than 10 days (Hoddle, 2002).

The avocado thrips is not known to vector any virus diseases, but feeding by adults and larvae causes damage similar to that caused by other pest species in the genus *Scirtothrips*, on crops such as citrus and mango. Most eggs are laid on partially expanded young leaves, into the basal parts of the lamina, but considerable numbers are also laid into young fruit when these are about 4 cm in length; few eggs are laid into even young petioles (Hoddle, 2002).

1.1 Hosts

Adults of *Scirtothrips perseae* have been collected in small numbers from at least 10 different plant species, but larvae have been found only on avocado trees (Hoddle *et al*., 2002). Thus, for purposes of breeding these thrips appear to be fully dependent on the young growing tissues of *Persea americana*.

2 TAXONOMIC INFORMATION

Correct name: *Scirtothrips perseae* Nakahara, 1997

Five available synonyms:

- *S. aguacatae* Johansen and Mojica-Guzmán, 1998
- *S. kupandae* Johansen and Mojica-Guzmán, 1998
- *S. manihotifloris* Johansen and Mojica-Guzmán, 1998
- *S. tacambarensis* Johansen and Mojica-Guzmán, 1998
- *S. uruapaniensis* Johansen and Mojica-Guzmán, 1998

Animal Kingdom, Phylum Arthropoda, Class Insecta, Order Thysanoptera, Family Thripidae, Subfamily Thripinae

The genus *Scirtothrips* is one of the larger genera of Thripidae, and includes about 100 described species. Although most of these are probably good biological species, the biological validity of 18 species described or recorded from mango flowers remains questionable (Mound & zur Strassen, 2001). This problem was further discussed by Hoddle *et al*. (2008b) who, using both morphological and molecular data, established that the five of the described species listed above all represent *S. perseae* and concluded there was strong support for an avocado-associated group of *Scirtothrips*.

Many *Scirtothrips* species appear to be host specific, including *S. perseae*, but some others are clearly polyphagous. One of these polyphagous and widespread species, *S. dorsalis*, appears to be sufficiently diverse genetically to indicate the possibility that a group of sibling species may be involved (Hoddle *et al*., 2008a).

3 DETECTION

The thrips are not known to survive on any plants other than *Persea* and even on these it requires fresh young growth. Even at low populations, young leaves will show evidence of feeding distortion. However, very small numbers of larvae might be present on young leaves without causing more than superficial damage. Eggs are not known to be laid into mature fruits.

The thrips can cause extensive corky damage to the surface of young avocado fruit (Fig. 1).

Figure 1. *Scirtothrips perseae* damage to avocadoes (Jack Kelly Clark, Regents of the University of California)

As with all species of *Scirtothrips*, the larvae are minute, less than 1 mm long, pale in colour (Fig 2), and will hide in the smallest of cracks. They are not likely to be seen by anyone other than an experienced specialist observer who has been trained to find minute organisms. Visual inspection is thus not likely to be successful, and beating potentially infested leaves gently over a clean plastic tray is the only way in which this thrips are likely to be detected.

Figure 2. *Scirtothrips perseae* adult female (Jack Kelly Clark, Regents of the University of California)

4 IDENTIFICATION

Morphological identification to genus is relatively straightforward using traditional taxonomic methods, however the identification to species is more difficult and requires expert knowledge.

It is recommended that DNA barcoding using the COI region is undertaken on any specimen identified to the genus *Scirtothrips*. The barcoding method is not currently included in this protocol, but links to available journal papers using the methods have been included until the protocol is updated (Section 4.2).

4.1 Morphological identification.

Membership of the genus *Scirtothrips* can be established by presence of the following character states in slide-mounted adult specimens (see Appendix 8.1):

1. Lateral thirds of abdominal tergites covered by dense parallel rows of uniformly ciliate microtrichia (Fig. 3). [This condition also occurs in *Drepanothrips* and *Anascirtothrips*, as well as in the three genera of Thripidae, *Sericothripinae*: *Hydatothrips*, *Neohydatothrips* and *Sericothrips*.]

2. Antennae with eight segments (Fig. 4); two rare species from Australia are known with seven segments. [*Drepanothrips reuteri*, a widespread pest on *Vitis vinifera* leaves, is closely similar to *Scirtothrips* species but has only six antennal segments; *Anascirtothrips* species that live on *Ficus* leaves in the tropics are similar to *Scirtothrips* species but have only seven antennal segments.]

3. Forewing first vein with setal row discontinous, second vein with at least 2 or 3 widely spaced setae (Fig. 5). [*Hydatothrips, Neohydatothrips* and *Sericothrips* species have the forewing first vein setal row continuous, and the second vein with no more than one or two setae, these being near the wing apex.]

4. Abdominal sternites without a posteromarginal fringe of microtrichia. [*Anascirtothrips* species that live on Ficus leaves in the tropics are similar to *Scirtothrips* species but have a distinctive fringe of long microtrichia on the posterior margins of the sternites.]

There are considerable difficulties in distinguishing this species among the 100 species that are listed worldwide in this genus (Mound, 2008), but a modern key is available to the 21 species known from Australia (Hoddle & Mound, 2003). It must be emphasized that there is no single morphological character state by which the avocado thrips can be distinguished. Molecular methods (Hoddle *et al*., 2008; Rugman-Jones *et al*., 2006) are being developed progressively but do not as yet involve sufficient species to provide reliable identifications. Despite these problems, the avocado thrips can be identified positively through presence of the following suite of character states:

1. Abdominal sternites with microtrichia present only laterally (Fig. 6). [The widespread pest species *S. dorsalis* and *S. aurantii* have microtrichia extending fully across the sternites.]

2. Forewing posteromarginal cilia wavy (Fig. 8). [These cilia are straight in the widespread pest species S. dorsalis.]

3. Ocellar setae III close together behind the fore ocellus (Fig. 7). [The pest species *S. dorsalis* and *S. aurantii* have ocellar setae III arising in a more posterior position; similarly, *S. astrictus* that also lives on avocado leaves have ocellar setae III arising between the posterior ocelli.]

4. Sculpture lines scarcely visible within ocellar triangle (Fig. 7). [Most native Australian species have distinct sculpture lines within the ocellar triangle, as does *S. astrictus* from avocado leaves in Central America.]

5. Head with only two pairs of postocular setae in a row behind the ocelli (Fig. 7).

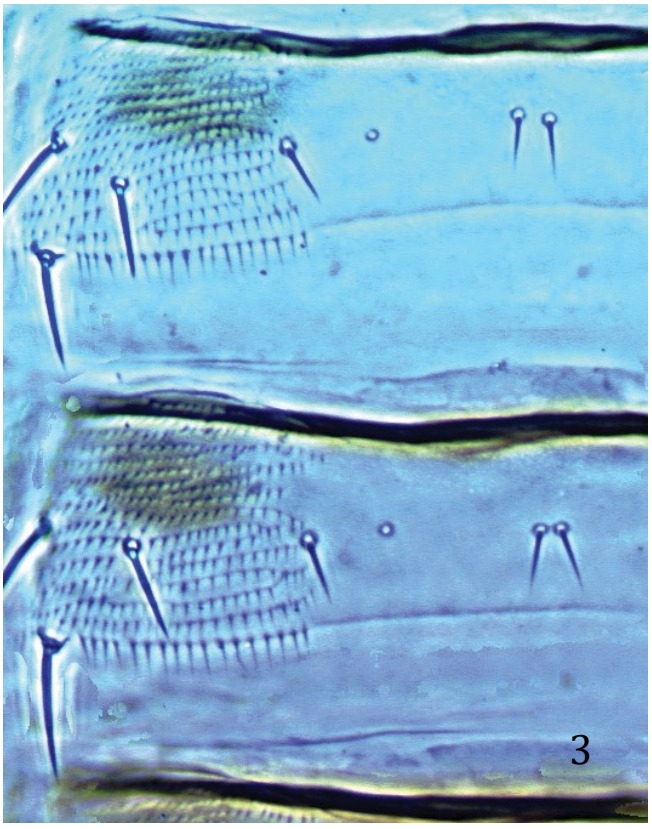
6. Abdominal tergites III–V with median pair of setae arising very close together (Figs 3, 9), the distance between their pores often being less than the diameter of these pores. [In most *Scirtothrips* species these setae are more widely separated, and in some species they are much shorter.]

7. Tergites III–VII with only three discal setae on each lateral microtrichial field (Fig. 3).

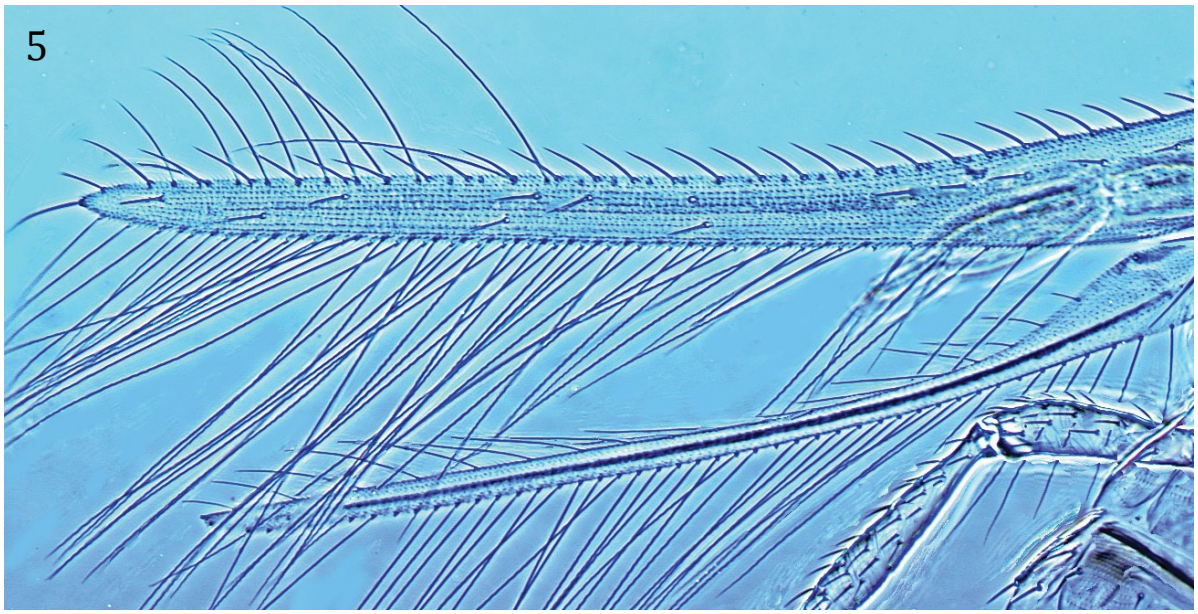
8. Tergites VIII and IX with no microtrichia anteromedially. [Many *Scirtothrips* species have a few microtrichia anteromedially on one or both of these tergites.]

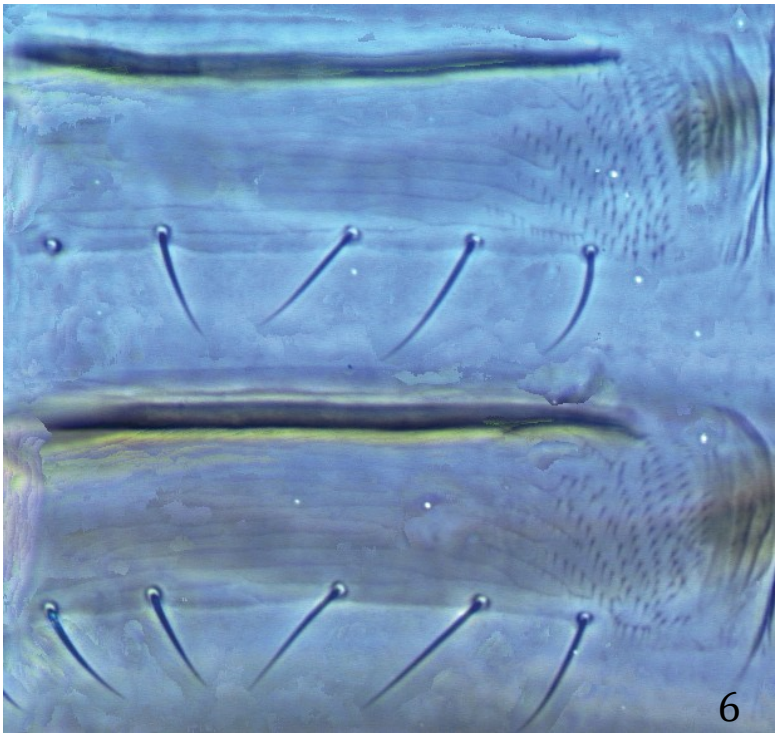
9. Tergites III–VI (often II–VII) with dark antecostal ridge and pair of dark areas anterolaterally (Fig. 9). [Amongst the yellow-bodied *Scirtothrips* species this colour pattern is almost diagnostic.]

10. Male tergite IX posterior angles with pair of stout curved processes (drepanae) extending around segment X (Fig. 10). [This character state is also shared with several other *Scirtothrips* species.

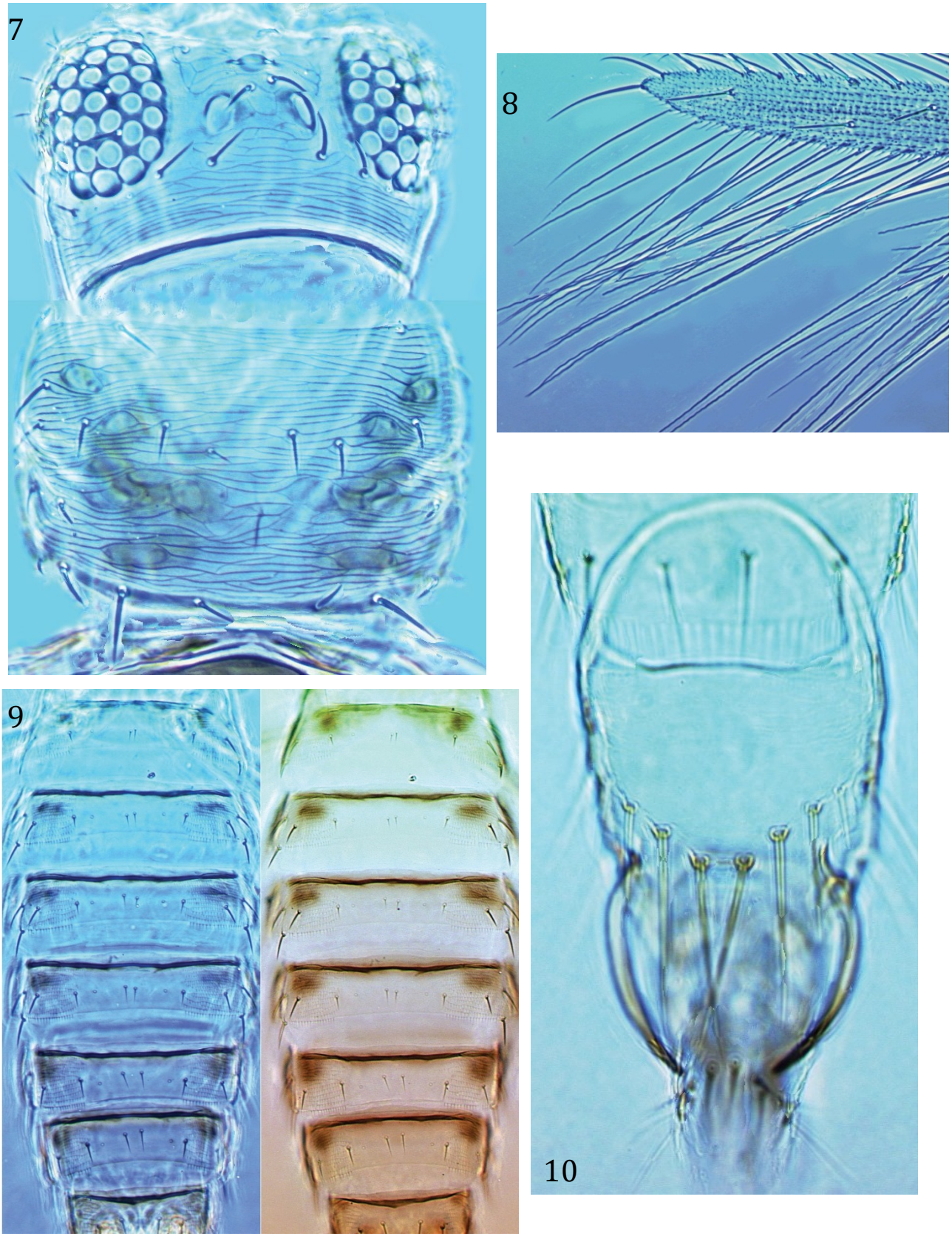


Figures 3–4. *Scirtothrops perseae*: 3, tergites V–VI; 4, antenna;





Figures 5–6. *Scirtothrops perseae*: 5, fore wing and hind wing; 6, sternites IV–V.



Figures 7–10. *Scirtothrops perseae*: 7, head and pronotum; 8, fore wing cilia; 9, female abdomen (phase contrast and bright field); 10, male tergite VIII–IX.

4.2 Molecular identification.

Rugman-Jones *et al.* (2006) developed a molecular identification key for pest species of *Scirtothrips* using the internal transcribed spacer regions 1 and 2 (ITS1 and ITS2) of nuclear ribosomal DNA.

Since then, studies on thrips have demonstrated that DNA barcoding using the COI region is effective in identification of thrips species (Hoddle *et al.* 2008a, Rebith *et al.* 2014). Both these authors have deposited vouchered specimens in collection with the associated information in Genbank.

This protocol will be updated with this information, however in the interim the methods are available on-line (accessed 17.8.2020).

Rebith *et al.* (2014):

https://bioone.org/journals/Florida-Entomologist/volume-97/issue-4/024.097.0407/DNA-Barcoding-and-Elucidation-of-Cryptic-Diversity-in-Thrips-Thysanoptera/10.1653/024.097.0407.full

Hoddle *et al.* (2008a):

https://academic.oup.com/aesa/article/101/3/491/8449

5 CONTACTS FOR FURTHER INFORMATION

The most comprehensive collection of *Scirtothrips* species belongs to the United States Museum of Natural History, Washington, whose collections of Thysanoptera are in the care of the United States Department of Agriculture, Beltsville, Maryland.

The Natural History Museum, London, the Senckenberg Museum, Frankfurt, and the Australian National Insect Collection, Canberra, also hold extensive collections of species in this genus.

The pest species itself is well-known to workers in only a few countries, particularly USA (California, Washington DC) and Australia (Canberra); however, it is probably also known to quarantine entomologists in England (York) and Netherlands (Wageningen).

Contact addresses for Thysanoptera collections and specialists:

1. United States National Museum of Natural History, PO Box 37012, Smithsonian Institute, Washington D.C., 20013–7012 U.S.A.
2. The Natural History Museum, Cromwell Road, London SW7 5BD, England.
3. Senckenberg Forschungsinstitut und Naturmuseum, Senckenberganlage 25, 60325 Frankfurt, Germany.
4. Dr Mark Hoddle, Department of Entomology, University of California, Riverside, CA 92521, U.S.A.
5. Dr Stan Diffie, Department of Entomology, University of Georgia, P.O. Box 748, Tifton, GA 31793 U.S.A.
6. Dr Dom Collins, Pest and Disease Identification Team, Central Science Laboratory, Sand Hutton, York YO41 1LZ, England.
7. G. Vierbergen, Plant Protection Service, Section of Entomology, P.O. Box 9102, 6700 HC Wageningen, The Netherlands
8. Dr Laurence Mound, c/o CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601.

Australian expertise:

Lixin Eow, Research Scientist (Entomology) | Agriculture Victoria Research Division|Agriculture Victoria. Email: lixin.eow@agriculture.vic.gov.au

Luke Watson, entomologist. Department of Agriculture & Water Resources. Email: Luke.Watson@awe.gov.au

Desley Tree. Email: treefamily@bigpond.com

Dr Laurence Mound https://people.csiro.au/M/L/Laurence-Mound.aspx

6 ACKNOWLEDGEMENTS

This protocol was developed from a document written by Laurence Mound and reviewed by Greg Baker SARDI, Luke Watson DAFF and Li Xin Eow, Agriculture Victoria.

7 REFERENCES

Hoddle, M.S. 2002. Oviposition preferences of *Scirtothrips perseae* Nakahara (Thysanoptera: Thripidae) in southern California avocado orchards. *Pan-Pacific Entomologist* 78: 177–183.

Hoddle, M.S., Mound, L.A. 2003. The genus *Scirtothrips* in Australia (Insecta, Thysanoptera, Thripidae*). Zootaxa* 268: 1–40.

Hoddle, M.S., Heraty, J.M., Rugman-Jones, P.F., Mound, L.A. & Stouthamer, R. 2008a. Relationships among species of *Scirtothrips* (Thysanoptera: Thripidae, Thripinae) using molecular and morphological data. *Annals of the Entomological Society of America* 101: 491–500.

Hoddle, M.S., Mound, L.A., Rugman-Jones, P.F. & Stouthamer, R. 2008b. Synonymy of five *Scirtothrips* species (Thysanoptera: Thripidae) described from Avocados (*Persea americana*) in Mexico. *Florida Entomologist* 91: 16–21.

Mound, L.A. 2008. Thysanoptera (Thrips) of the World — a checklist. https://www.ento.csiro.au/thysanoptera/worldthrips.php (accessed 17.8.2020)

Mound, L.A., zur Strassen, R. 2001. The genus *Scirtothrips* (Thysanoptera: Thripidae) in Mexico: a critique of the review by Johansen & Mojica-Guzmán (1998). *Folia Entomologica Mexicana* 40: 133–142.

Rebijith, K. B., Asokan, R., Krishna, V., Ranjitha, H. H., Krishna Kumar, N. K., Ramamurthy, V. V. 2014. DNA Barcoding and Elucidation of Cryptic Diversity in Thrips (Thysanoptera). *Florida Entomologist*, *97*(4), 1328–1347. https://doi.org/10.1653/024.097.0407

Rugman-Jones, P.F., Hoddle, M.S., Mound, L.A. & Stouthamer, R. 2006. A molecular identification key for pest species of *Scirtothrips* (Thysanoptera: Thripidae). *Journal of Economic Entomology* 99: 1813–1819.

Useful references:

https://keys.lucidcentral.org/keys/v3/thrips_of_california_2019/the_key/key/california_thysanoptera_2019/Media/Html/entities/scirtothrips_perseae.htm

8 APPENDICES

8.1 Techniques for preparing micro-slides of thrips

For routine identifications, a temporary slide using a water soluble mountant is often all that is required, particularly for pale adult specimens or larvae. However with darker specimens, it may be necessary to use the more permanent mount to ensure they are cleared enough to be studied.

The second method (8.1.2.) is traditionally chosen for thrips study as often maceration is necessary for effective diagnosis.

8.1.1 Slide preparation for routine identifications

The following slide mounting method uses a water-soluble mountant, such as Hoyers (which has to be prepared and not available commercially), or the CMC-10 , a High Viscosity Colourless Mountant (commercially available from Masters Co. Inc. 890 Lively Blvd. Wood Dale, IL 60191, (630) 238–9292, https://clombardi5.wixsite.com/mysite/specialty-products). This method is rapid and thus relatively inexpensive.

1. Remove the specimens from the collecting fluid into clean 70% alcohol and attempt to open the wings and straighten the antennae using micro-pins (see below).

2. Place a drop of Hoyers Mountant or CMC10 Mountant onto a cover slip (13 mm circle, No. 1). Place a thrips into this drop, ventral side uppermost, and gently lower a slide onto the drop. Invert the slide as soon as the mountant has spread sufficiently.

3. Place immediately into an oven, or onto a hot-plate, at about 40–50°C. Leave for a few hours before attempting to study. Alternatively warm over a spirit lamp.

4. When the mountant is dry, ring with nail varnish and label appropriately (see below).

Hoyers Medium:

Ingredients:

Gum Arabic 15g
Chloral Hydrate 75g
Distilled Water 25ml
Glycerine 5ml

NB: Chloral hydrate is a poison and in some countries a controlled substance.

Procedure:

1) Add gum to distilled water and mix well at room temperature. Let mixture stand for 1-2 days or until completely dissolved.
2) Add chloral hydrate and let mixture stand for a day or until completely dissolved.
3) Then add glycerine to mixture and mix well. Let the mixture stand until sediments settle.
4) Filter mixture through glass wool using the funnel and vacuum system. Repeat filter process if necessary. Store in airtight and amber colored container.

8.1.2 Slide preparation for archiving and taxonomic study

To reveal fine details of body sculpture and minute setae, specimens need to be macerated gently. A few specimens may be prepared without maceration to preserve the natural colouration.

Tools:

Specimens can be manipulated with fine micro-pins, mounted in sealing wax on match sticks. A simple lifting tool can be made from a small loop of fine wire. The best dishes are 'excavated glass blocks',15 mm high, 40 mm square with an excavation of about 5 ml volume.

Maceration

Maceration removes body contents by soaking specimens in a weak NaOH solution. Treatment overnight in about 2% solution seems optimum, but black specimens require longer (even several days). Maceration of thrips should always be carried out at room temperature, in contrast to techniques used for preparation of aphid and coccid specimens.

1. Place thrips into clean water in an excavated block; it is best if the specimens float with their wings on the surface. Leave for 1 hour.
2. Add to the water an equal volume of 5% NaOH solution and leave overnight.
3. Transfer the specimens from NaOH solution to water for a few hours, using a needle or wire loop. Then transfer the specimens into 60% ethanol for storage or further treatment.
4. Replace the 60% alcohol with 70% alcohol and leave for about 1 hour.
5. Replace with 80% alcohol and leave for 20 minutes.
6. Replace with 95% alcohol and leave for 10 minutes.
7. Replace with absolute alcohol and leave for 5 minutes.
8. Replace with fresh absolute alcohol and leave for another 5 minutes.
9. Transfer to clove oil and leave until fully clear.

Mounting

Prepare a small mounting block by fixing to the centre of a microscope slide a 2 mm deep layer of 25 mm square card, and cover this with plastic tape to provide a clean surface.

1. Place a clean 13 mm diameter cover slip onto the mounting block; put a drop of Canada Balsam onto the centre of the cover slip and into this place one thrips specimen ventral side uppermost.
2. Spread the legs and wings, and straighten the antennae by pressing on the basal segments with a fine needle.
3. Invert a clean microscope slide and lower it firmly but gently onto the specimen in balsam on the cover slip. As soon as the surfaces touch, re-invert the slide with the coverslip adhering.

Sometimes it helps to place a small drop of balsam in the centre of the slide before touching the balsam on the cover slip.

4. Place the slide onto a hot-plate at once, at about 50°C, to drive off the xylene as quickly as possible. Dry the slides in an oven at about 50°C.

Labelling

With the head of the thrips directed toward you, the right-hand label should indicate the host plant, followed by the country (in capital letters) and then the locality and date, with collector's name (and code number). The left-hand label should indicate the sex, morph and genus and species names of the thrips.

9 DIAGNOSTICS PROCEDURES TO SUPPORT SURVEILLANCE

9.1 Introduction

This section outlines a few essential steps in thrips collection to support surveillance and diagnostic work. The aim is to successfully sample the suspect thrips, and to store and package the sampled materials appropriately so that these arrive at the testing laboratory in adequately good condition for the purpose of diagnosis.

9.2 Collecting thrips samples

Two of the most relevant thrips collecting methods are elaborated here –A) direct hand collection using the beating technique, and B) collecting the plant materials directly. Where required, both approaches can be carried out together to ensure the targeted thrips are found.

A more comprehensive guide of collecting and preparing thrips for study is available online on Thripswiki for further reference: https://thrips.info/wiki/Collecting_and_preparing_thrips_for_study (Thrips Wiki 2013, accessed December 2019).

A) Direct hand collection using the beating technique.

Direct hand collection using a beating tray is appropriate for collecting thrips from all forms of plant materials, either potted or in the field. This method is particularly effective in cases where thrips are suspected to be present on a particular plant, as it allows the collector to observe which part of the plant the thrips are found, and furthermore ensures actual thrips specimens have been sampled directly from the host plants for diagnostic purposes. If hand collection is not feasible, see method B – collecting and submitting plant materials.

Ideally, choose a sunny morning to early afternoon to attend field sites. Collecting thrips is more challenging when the condition is windy, or wet from dew or rain.

You will need (Fig. 11):

- A fine paint brush.
- A white tray. White plastic or melamine serving trays are good options as they are sturdy and easy to hold with one hand.
- A beating tool. A handheld garden shovel is a good option as it is of good length and weight and can be washed between hosts or sites.
- Rubber sealed cap microtubes containing ethanol of at least 70%. If molecular work is intended, use at least 80% to absolute ethanol.
- A pencil and paper labels, for recording collection details.
- Large zip-lock bags, to hold plant materials if hand collection is not feasible (see method B).
- A magnifying hand lens (optional).

To collect thrips, place a clean beating tray below the plant material, tap the plant firmly two to three times with the beating tool. Debris and insects will fall onto the tray, from which thrips can be spotted and picked up with the fine brush (tip dampen with ethanol) into the collection tube containing ethanol. Whenever possible, sample multiple individuals from the same host to obtain a good series of adult specimens. Collection information can be written on a label with a pencil and wedged inside the collection tube or can be submitted separately.

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Figure 11: Tools for collecting thrips and demonstration of the beating technique: Collecting tray, fine paint brush, rubber sealed cap microtubes, hand lens, small garden trowel (Images source: Thrips Wiki 2013)

B) Collecting and submitting plant materials.

If direct hand collection is not feasible, plant materials can be sampled and then promptly transferred to the diagnostic laboratory or a facility with dissecting microscope for detailed examination of the presence of thrips. The downside of this method is that the suspect thrips may not be present on the sampled plant parts, so it is useful to submit multiple samples to increase the chances of finding the targeted insects.

For larger plants, select entire terminal section containing florets, young fruits, young and old leaves, especially symptomatic parts. Small potted plants can be submitted as whole. It is also important to communicate with the testing laboratory about intention to send in entire or large amount of plant materials.

The plant materials would need to be packaged in a way to prevent live insects from escaping whilst in transit. A method is to place the plant materials in a plastic container, then in a large zip-lock bag.

You will need:

- Zip-lock bags and plastic containers appropriate to the size of the plant materials.
- Pruning shears.

Submitting samples to the testing laboratory.

The collected thrips samples in microtubes, and the plant parts may be packaged based on the recommended packaging methods in the CRC Plant Biosecurity published guide: How to send samples for diagnosis in Australia: Plant disease and insect Identification. CRC Plant Biosecurity (Hall, 2011).

Essential information accompanying the samples submitted for diagnosis should include:

- Location/origin. Give as much information about the origin of the sample as possible (e.g. state, suburb; or, intercepted at location X from plant imported from country Y).
- Host, or the type of materials from which the insects were found.
- Date of collection.
- Reason for submission: whether there is a suspect species to rule out, or if a general diagnostic is required.

9.3 In Field Tests

No in-field rapid molecular diagnostic tests are currently available for the *Scirtothrips perseae*.

Due to its small size (often <1mm), *Scirtothrips* species are hard to detect and triage by visual inspection. The best way to find *Scirtothrips* would be direct hand collection via the beating technique, or by carefully sampling plant materials, specifically targeting the young growing section of the plant to include stem, leaves and young fruit. Both approaches are outlined in Section 9.2.

9.4 Laboratory Tests

No molecular tests are listed in the current NDP, however links to barcoding methods are included.

Morphological identification of this species can be performed by a diagnostician based on the methods outlined in the Section 4.

A list of experts and institutions who may be consulted for the confirmatory diagnosis of a suspect positive of this species are listed in the Section 5.

9.5 Acknowledgements

This section was developed by Li Xin Eow (Agriculture Victoria) and revised in 2021.

9.6 References

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