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European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes

Diagnostics
Diagnostic

PM 7/3 (3) *Thrips palmi*

Specific scope

This Standard describes a diagnostic protocol for *Thrips palmi*.[1]

This Standard should be used in conjunction with PM 7/76 *Use of EPPO diagnostic protocols*.

Specific approval and amendment

First approved in 2000–09. First revision 2005–09. The EPPO protocol was revised on 2018–09, on the basis of the IPPC diagnostic protocol adopted in 2010 (Appendix 1 to ISPM 27). Although this Standard differs in terms of format and provides a new option for molecular tests, it is consistent with the content of the IPPC Standard. It also incorporates literature published since the adoption of the IPPC protocol.

1. Introduction

Thrips palmi Karny (Thysanoptera: Thripidae) is a polyphagous plant pest, especially of species in the families Cucurbitaceae and Solanaceae. It appears to have originated in Southern Asia and to have spread from there during the latter part of the twentieth century. It has been recorded throughout Asia and is widespread throughout the Pacific and the Caribbean. It has been recorded locally in North, Central and South America and Africa. In Europe, it is regularly intercepted on cut flowers and fruit and vegetables (see the EPPO Reporting Service). The few outbreaks that occurred in the Netherlands (1998), the UK (2000) and Germany (2014) have been eradicated (EPPO, 2018). For more general information about *T. palmi*, see EPPO/CABI (1997) or Murai (2002); online pest data sheets are also available from the Pests and Diseases Image Library (PaDIL, 2007) and EPPO (EPPO, 2008).

The species causes economic damage to plant crops, both as a direct result of its feeding activity and from its ability to vector tospoviruses such as *Groundnut bud necrosis virus*, *Melon yellow spot virus* and *Watermelon silver mottle virus*. It is extremely polyphagous and has been recorded from more than 36 plant families. It is an outdoor pest of, amongst others, *Benincasa hispida*, *Capsicum annuum*, *Citrullus lanatus*, *Cucumis melo*, *Cucumis sativus*, *Cucurbita* spp., *Glycine max*, *Gossypium* spp., *Helianthus annuus*, *Nicotiana tabacum*, *Phaseolus vulgaris*, *Pisum sativum*, *Sesamum indicum*, *Solanum melongena*, *Solanum tuberosum* and *Vigna unguiculat*a. In glasshouses, economically important hosts are *C. annuum*, *Chrysanthemum* spp., *C. sativus*, *Cyclamen* spp., *Ficus* spp., Orchidaceae and *S. melongena*. The thrips may be carried on plants for planting, cut flowers and fruits of host species, as well as on or associated with packing material and in soil.

Thrips palmi is almost entirely yellow in colour (Figs 1–3), and its identification is hampered by both its small size (1.0–1.3 mm) and its great similarity to certain other yellow or predominantly yellow species of *Thrips*.

2. Identity

Name: *Thrips palmi* Karny, 1925
Synonyms: *Thrips clarus* Moulton, 1928; *Thrips leucadophilus* Priesner, 1936; *Thrips gossypicola* Ramakrishna & Margabandhu, 1939; *Chloethrips aureus* Ananthakrishnan & Jagadish, 1967; *Thrips gracilis* Ananthakrishnan & Jagadish, 1968
Taxonomic position: Insecta, Thysanoptera, Terebrantia, Thripidae
EPPO Code: THRIPL
Phytosanitary categorization: EPPO A1 List no. 175; EU Annex designation I/A1

[1]Use of brand names of chemicals or equipment in these EPPO Standards implies no approval of them to the exclusion of others that may also be suitable.

Fig. 1 *Thrips palmi*: female (left) and male. Scale bar = 500 µm = 0.5 mm. (Photo: A. J. M. Loomans, PPS, Wageningen, the Netherlands.)

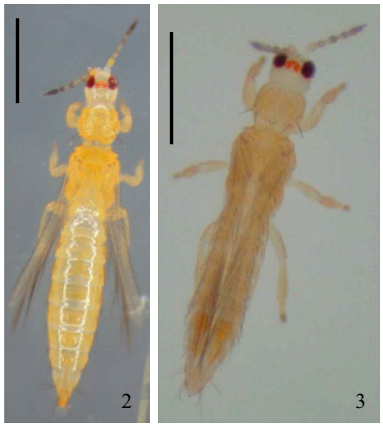

Fig. 2–3 *Thrips palmi*: female (left) and male (right). Scale bar: 300 lm. (Photo: W. Zijlstra, 2 PPS, Wageningen, the Netherlands.)

3. Detection

Thrips palmi may be found in different locations depending on the life stages present.

Eggs	In the leaf, flower and fruit tissue
Larva I	On the leaves, flowers and fruits
Larva II	On the leaves, flowers and fruits
Pupa I	In the soil, packing cases and growing medium
Pupa II	In the soil, packing cases and growing medium
Adult	On the leaves, flowers and fruits

On plant material, *T. palmi* may potentially be found on most above-ground parts of the plant; the parts of the plant infested can differ according to variables such as the host and the characteristics of each separate *T. palmi* population.

During visual examination of plant material for the presence of *T. palmi*, attention must be paid to silvery feeding scars on the leaf surfaces of host plants, especially alongside the midrib and the veins. Heavily infested plants are often characterized by a silvered or bronzed appearance of the leaves, stunted leaves and terminals, reduced pigmentation and necrosis of flowers, or scarred and deformed fruits. Detection may be hampered in circumstances such as:

- low-level infestation, which may produce few or no detectable symptoms
- the presence of the eggs within the plant tissue only (e.g. after external treatment which may have removed visible life stages).

Specimens for morphological examination are best collected in a fluid called AGA, which is a mixture of 10 parts of 60% ethanol with 1 part of glycerine and 1 part of acetic acid. If the specimens are to be stored, they should be transferred to 60% ethanol and kept in the dark, preferably in a freezer to prevent loss of colour. However, several laboratories have reported that AGA may act to denature the DNA of the thrips thereby hindering any subsequent molecular work. An alternative is to use 80–95% ethanol as the collecting fluid, as any unmounted specimens may then be used for molecular studies. However, in this case specimens should be stored in the freezer until used or they may prove difficult to slide mount.

Several methods can be used to collect thrips specimens, and the following is modified fromMantel & Vierbergen, 1996):

- Thrips may be individually removed from the plant (leaves, flowers or fruit), and transferred, using a moist, fine brush, to microtubes containing AGA
- Thrips may be beaten from plant parts onto a small plastic tray (e.g. a white tray for dark-coloured specimens or a black tray for light-coloured specimens). In cooler conditions, the thrips usually start walking across the tray rather than flying off, allowing time for the thrips to be picked off with a moist fine brush, whereas in warmer conditions collection has to be done more rapidly as the thrips are likely to fly off much more quickly. The thrips are easily seen on the tray using just a hand lens, but an experienced observer can also see them easily with the naked eye
- Plant parts may be sealed in a plastic bag for 24 h, with a piece of filter paper enclosed to absorb condensation. Most thrips will leave the plant parts and can then be collected from the inside of the bag. Injection of CO_2 into the bags can be used to immobilize the thrips (Frey *et al.*, 1994)
- A Berlese funnel can be used to process plant material such as bulbs, flowers, turf, leaf litter, moss and even dead branches of trees. The funnel contains a sieve on which the plant material is deposited. Beneath the sieve, the bottom of the funnel leads into a receptacle containing 70–96% ethanol. An alternative is to use 10% ethanol plus wetting agent as some workers find that this makes the preparation of good quality microscope slide mounts easier. The funnel is placed under an electric lamp (60 W), and the heat and light will drive most of the thrips present in the plants down towards the receptacle. After an appropriate period (e.g. 8 h for cut flowers), the contents of the receptacle can then be checked under a stereomicroscope
- Thrips may be monitored (winged adults only) using coloured sticky traps or other appropriate methods. The

ability of a colour to attract thrips varies for different thrips species, but blue or white traps are good for *T. palmi*, though yellow traps will also work. For microscope slide preparation and identification, the thrips will have to be removed from the traps using glue-removing fluids such as those based on citrus oils, dichloromethane or a turpentine substitute.

There are no recognized methods for extracting thrips pupae from the soil in a quarantine context.

4. Identification

Identification of thrips species by morphological examination is restricted to adult specimens because there are no adequate keys for the identification of eggs, first stage larvae or pupae. A key to the second instar larvae of the Thripidae (Thysanoptera) of the Western Palaearctic region is available (Vierbergen *et al.*, 2010). However, the presence of larvae in samples can give important additional information such as confirming their development on the host plants. The primary method of identification of adult material is from morphological characters. In order to achieve species identification, these should be examined using a high-power microscope (e.g. ×400). Use of this protocol with good-quality slide preparations should allow adult *T. palmi* to be identified with certainty by morphological examination alone.

Molecular tests can be applied to all life stages including the immature stages, for which morphological identification to species is not possible. Additionally, in cases where adult specimens are atypical or damaged, molecular tests may provide further relevant information about their identity. However, the specificity of molecular tests is limited as they have been developed for specific purposes and evaluated against a restricted number of species, using samples from different geographical regions; therefore, such information needs to be carefully interpreted.

4.1. Morphological identification of adult thrips

4.1.1. Preparation of thrips for microscopic examination

For high-power microscopic examination, adult thrips should be mounted on microscope slides. Specimens to be kept in a reference collection are best macerated, dehydrated and mounted in Canada balsam; Mound & Kibby (1998) provide a full description of this process. However, the full slide preparation protocol for archival mounts takes 3 days to complete.

For routine identifications, a water-soluble mountant such as Hoyer's medium (50 mL water, 30 g gum arabic, 200 g chloral hydrate, 20 mL glycerine) is more rapid and relatively inexpensive. One popular method of routine slide preparation is given by Mound & Kibby (1998) and is described below (laboratories may find that other variants work equally well):

Transfer the specimens from the collecting fluid into clean 70% ethanol; if the specimens are reasonably flexible, attempt to spread the legs, wings and antennae using micropins; transfer a single thrips, ventral side uppermost, to a drop of Hoyer's medium on a 13 mm diameter cover slip and use micropins to rearrange the thrips if necessary; gently lower a microscope slide onto the mountant so that the cover slip and mountant adhere to the middle of the slide; invert the slide as soon as the mountant has spread to the edges of the cover slip; label the slide with details including locality, date of collection and host plant; place the slide, cover slip up, into a drying oven at 35–40°C and leave for 6 h before attempting study; leave in the oven for approximately 3 weeks to dry the mountant, before sealing the cover slip with resin or nail varnish.

4.1.2. Identification of the family Thripidae

Thrips palmi belongs to the family Thripidae, which includes more than 2000 species in 276 genera. Species share the characteristics outlined in Table 1.

4.1.3. Identification of the genus Thrips

The genus *Thrips* contains more than 280 species from all parts of the world, though the genus is primarily from the Holarctic region and the Old World tropics. Members of the genus share the characteristics outlined in Table 2.

(A simplified summary of the main characteristics is given in Table 4 and is accompanied by illustrative line drawings and photomicrographs in Figs 4 and 5.)

Identification of the adults can be carried out with keys. Mound & Kibby (1998) provided a key to 14 *Thrips* species of economic importance including *T. palmi*. In addition, a CD-ROM identification aid for thrips is available which includes an identification system for 100 pest species from around the world based on photomicrographs (Moritz *et al.*, 2004).

More comprehensive keys to the genus are available, produced on a regional basis.

Africa	Mound (2010) provides a key to the 34 *Thrips* species that have been reported from the Afrotropical Region (including La Réunion)*
Asia	Bhatti (1980) and Palmer (1992) provide keys for the identification of species of *Thrips* occurring in the Asian tropics
	Mound & Azidah (2009) provide a key to the species of Peninsular Malaysia
Europe	zur Strassen (2003) has produced the most recent comprehensive key to the species of Thysanoptera from Europe including *Thrips* (in German)
North, Central and South America	Nakahara (1994) provides a key for *Thrips* species from the New World. A key to the species of *Thrips* found in Central and South America is given by Mound & Marullo (1996) though only one of these species is native to the region
Oceania	Mound & Masumoto (2005) provide a key to the *Thrips* species of Oceania. (The authors of the paper are aware of the error inadvertently introduced on p. 42 in the section 'Relationships' whereby a characteristic of *T. flavus* Schrank – ocellar setae III close together behind the first ocellus – is attributed to *T. palmi*. The correct information is provided in the *T. palmi* species description immediately above and is illustrated in Fig. 72.)

*This reference is more recent than the 2010 IPPC diagnostic protocol.

Table 1. Family Thripidae – shared characteristics

Body part	Characteristic
Antennae	Seven or eight segments (occasionally six or nine)
	Segments III–IV have emergent sense cones (sensoria)
Forewings (if fully developed)	Usually slender, with two longitudinal veins each bearing a series of setae
Abdomen – female	With a serrated ovipositor, which is turned downwards at the apex
Median sternites – male	With or without glandular areas

Table 2. Genus *Thrips* – shared characteristics, adult specimens

Body part	Characteristic
Body form (female)	Macropterous or micropterous
Antennae	Seven or eight segments
	Segments III–IV with forked emergent sense cones
Ocellar setae	Only two pairs present (pair I absent)
	Pair II shorter (at least no longer) than pair III
Pronotum	Two pairs (rarely one or none) of major posteroangular setae
	Usually three, sometimes four, pairs of posteromarginal setae
Prosternal basantra	No setae present
Forewings	The first vein with variably spaced setal row, second vein with complete setal row
	Clavus with five veinal setae (rarely six)
Metascutum	Median pair of setae at or behind the anterior margin
	Striate or reticulate sculpturing
	Campaniform sensilla (metanotal pores) present or absent
Metasternal furca	Without a spinula
Fore tibia	Apical claw absent
Tarsi	Two-segmented
Abdominal tergites and sternites	Without posteromarginal craspeda (flanges)
Abdominal tergites	Tergites V–VIII with paired ctenidia laterally (combs – each comprising a submarginal row of microtrichia) (occasionally also on IV)
	Tergite VIII: ctenidia posteromesad to the spiracles
Abdominal sternites and pleurotergites	With or without discal (accessory) setae
Abdominal sternites (male)	Abdominal sterna III–VII, or less, each with a glandular area

4.1.4. Identification of Thrips palmi

• Morphological characteristics of *Thrips palmi*

Bhatti (1980), Bournier (1983), Sakimura *et al.* (1986), zur Strassen (1989), Nakahara (1994) and Mound & Masumoto (2005) all provide detailed descriptions of *T. palmi*. Sakimura *et al.* (1986) gave a list of major diagnostic characters to distinguish *T. palmi* from the other known species of the genus *Thrips*; a modified version is presented in Table 3.

Thrips palmi can be reliably separated from all other species of the genus *Thrips* by the possession of all the characters listed in Table 3. Nevertheless, thrips morphology is subject to variation even within a single species and some characters listed here may be subject to occasional slight variation. For instance, antennal coloration or the number of distal setae on the forewing can vary from the most commonly observed states. If the specimen differs with respect to one or more of these character states, then the identification should be checked by reference to an appropriate regional key such as those listed in the section 'Identification of the genus *Thrips*'.

A simplified summary of the main characteristics is given in Table 4 and is accompanied by illustrative line drawings and photomicrographs (Figs 4 and 5).

• Comparison with similar species (species that are yellow without darker body markings, or predominantly yellow, or sometimes yellow)

For each species listed here, the main character differences by which they may be separated from *T. palmi* are given. If in any doubt, refer to an appropriate regional key such as those listed in section 'Identification of the genus *Thrips*'. These also give details of other *Thrips* species that are not listed below.

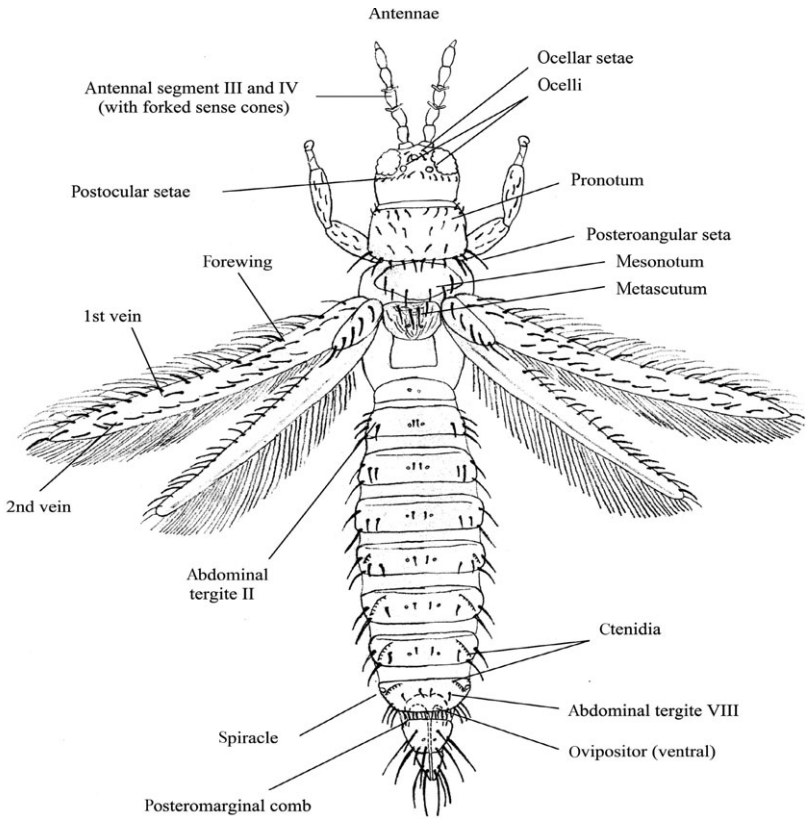

Fig. 4 Location of general characters of *Thrips* (female, dorsal view).

Two Indian species (*Thrips alatus* Bhatti and *Thrips pallidulus* Bagnall) are very similar to *T. palmi*, although little is known about their biology.

Thrips alatus

- antennal segment V uniformly brown
- abdominal tergites III and IV with setae S2 paler and much weaker than S3 in both sexes
- the striate sculpture on the metascutum usually not converging posteriorly
- distribution: India, Malaysia and Nepal.

Thrips pallidulus

- antennal segment IV pale
- sculpture on the metascutum medially reticulate, not striate
- distribution: India.

Three common Palaearctic species (but also with wider distributions) that may be confused with *T. palmi* are *Thrips flavus*, *Thrips nigropilosus* Uzel and *Thrips tabaci* Lindeman.

Table 3. A list of morphological characteristics that collectively distinguish *Thrips palmi* from other species in the genus *Thrips*

	Morphological character
1.	A clear yellow body with no dark areas on the head, thorax or abdomen (slightly thickened blackish body setae); antennal segments I and II pale, III yellow with apex shaded, IV–VII brown but usually with base of IV–V yellow; forewings uniformly slightly shaded, prominent setae dark
2.	Antennae always seven-segmented
3.	Postocular setae II and IV much smaller than remaining setae
4.	Ocellar setae III standing either just outside the ocellar triangle or touching the tangent lines connecting the anterior ocellus and each of the posterior ocelli
5.	Metascutum with sculpture converging posteriorly; median pair of setae behind anterior margin; paired campaniform sensilla present
6.	Forewing first vein with three (occasionally two) distal setae
7.	Abdominal tergite II with four lateral marginal setae
8.	Abdominal tergites III to IV with setae S2 dark and subequal to S3
9.	Abdominal tergite VIII with posteromarginal comb in female complete, in male broadly developed posteriorly
10.	Abdominal tergite IX usually with two pairs of campaniform sensilla (pores)
11.	Abdominal sternites without discal setae or ciliate microtrichia
12.	Abdominal pleurotergites without discal setae
13.	Male: sternites III–VII each with a narrow transverse glandular area

Table 4. Simplified checklists of the diagnostic features for quick recognition: (a) the genus *Thrips*; (b) *Thrips palmi* (see Fig. 4 for the location of the various features)

(a) Specimens can be recognized as *Thrips* by the following combination of characters		
Antenna	With seven or eight distinct segments; segments III and IV with forked sense cones	Figs 5.1, 5.2
Head	With two pairs of ocellar setae (II and III); pair I missing, pair II shorter than pair III	Fig. 5.3
Forewing	First vein – setal row on the first vein continuous or interrupted	Fig. 5.5
Abdominal tergites V–VIII	With paired ctenidia	Fig. 5.6
Abdominal tergite VIII	With ctenidia posteromesad to the spiracles	Fig. 5.6
(b) Specimens can be identified as *Thrips palmi* by the presence of the following characters		
Body colour	Clear yellow body with no dark areas on the head, thorax or abdomen; antennal segments I and II are pale	Figs 1–3
Antennal segment V	Usually yellowish in basal third to half	Fig. 5.1
Antennal segment VI	Length 42–48 µm	Fig. 5.1
Head: ocellar setae pair III	With their bases sited outside the ocellar triangle or touching the tangent lines connecting the anterior ocellus to each of the posterior ocelli	Fig. 5.3
Pronotum	With two pairs of major posteroangular setae	Fig. 5.4
Forewing: first vein	With three (occasionally two) distal setae	Fig. 5.5
Metascutum	With a median pair of setae behind the anterior margin and a pair of campaniform sensilla; with striate sculpture converging posteriorly	Fig. 5.7
Abdominal pleurotergites	Discal setae absent; lines of sculpture without ciliate microtrichia	Fig. 5.8
Abdominal tergite II	With four lateral marginal setae	Fig. 5.9
Abdominal tergites III and IV	S2 almost equal to S3	Fig. 5.10
Abdominal tergite VIII	Female with complete posteromarginal comb; male with posteromarginal comb complete medially	Fig. 5.6
Abdominal tergite IX	With anterior and posterior pairs of campaniform sensilla (pores)	Fig. 5.11
Male: sternites	Transverse glandular areas on sternites III–VII	Fig. 5.12

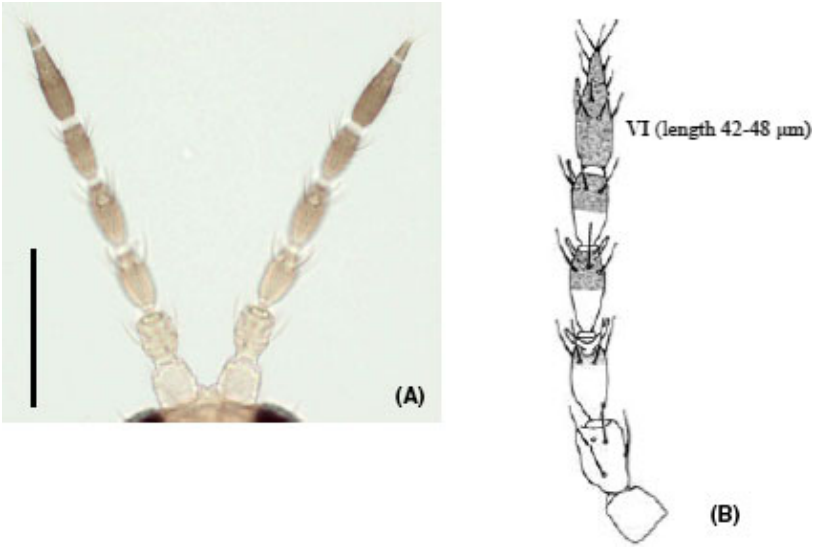

Fig. 5 Figure 5 comprises 12 parts (5.1–5.12) showing the various characters of *Thrips palmi*. (Photos: G. Vierbergen, PPS, Netherlands; figures drawn by S. Kobro, Norwegian Crop Protection Institute, Norway.)
5.1 (a), (b) Antenna: seven segments. Scale bar: 100 µm.

Thrips flavus

- ocellar setae pair III inside the ocellar triangle, just behind the anterior ocellus
- length of antennal segment VI, 54–60 µm (42–48 µm in *T. palmi*)
- lines of sculpture on the metascutum not converging posteriorly
- distribution: common flower thrips throughout Asia and Europe.

Thrips nigropilosus

- usually with dark markings on the thorax and abdomen
- metascutum with irregular reticulations medially (longitudinal striae in *T. palmi*) and no campaniform sensilla
- abdominal tergite II with three lateral marginal setae
- abdominal tergites IV–V with median pair of setae (S1) more than 0.5 times as long as the median length of their tergites (less than 0.3 times in *T. palmi*)

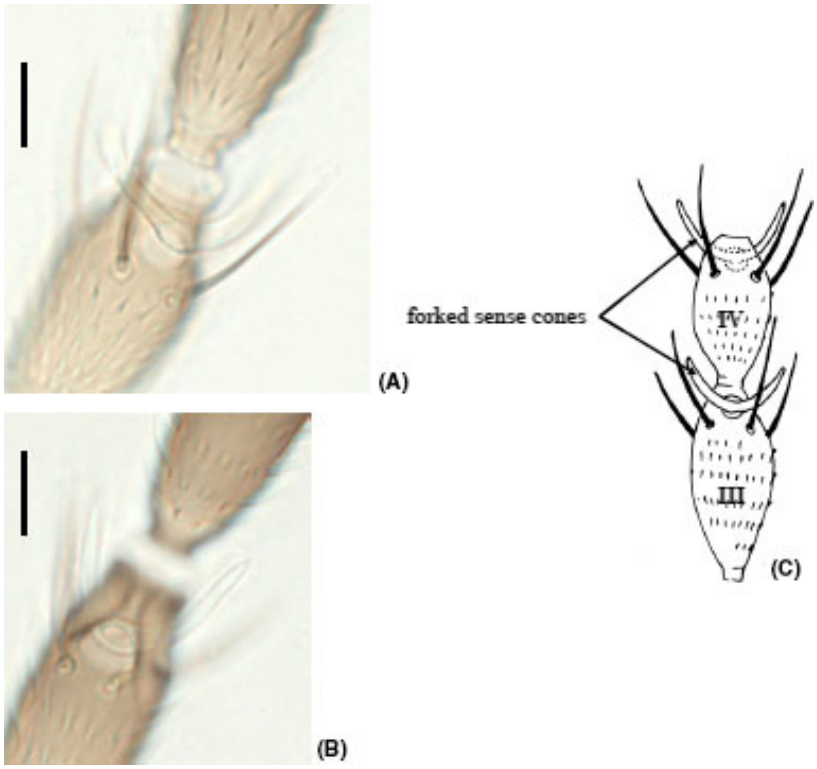

Fig. 5.2 (a)–(c) Antenna, forked sense cones: (a) segment III, dorsal; (b) segment IV, ventral; (c) segment III and IV, dorsal. Scale bars: 10 µm.

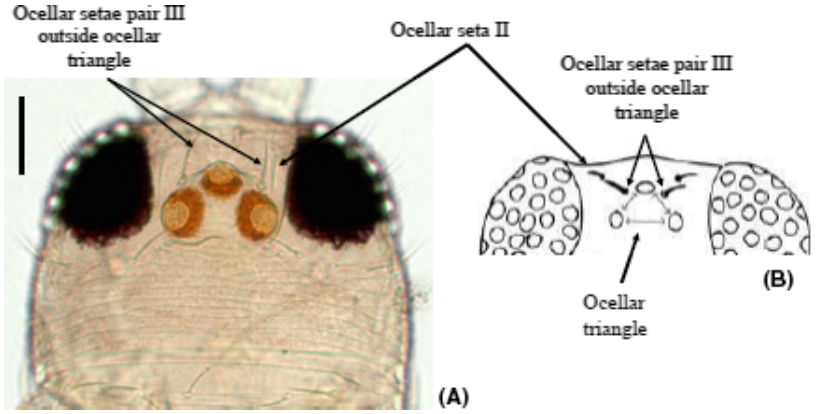

Fig. 5.3 (a), (b) Head with two pairs of ocellar setae (pair I missing). Ocellar setae pair II is situated outside the ocellar triangle. Scale bar: 30 µm.

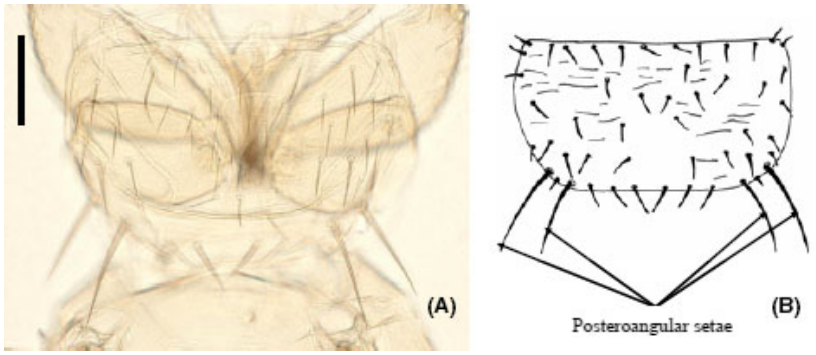

Fig. 5.4 (a), (b) Pronotum, two pairs of major posteroangular setae. Scale bar: 50 µm.

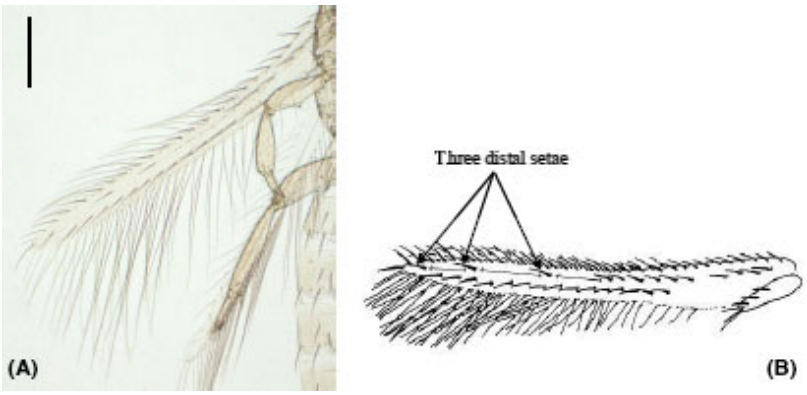

Fig. 5.5 (a), (b) Forewing, first vein – three setae with gaps in the distal half. Scale bar: 100 µm.

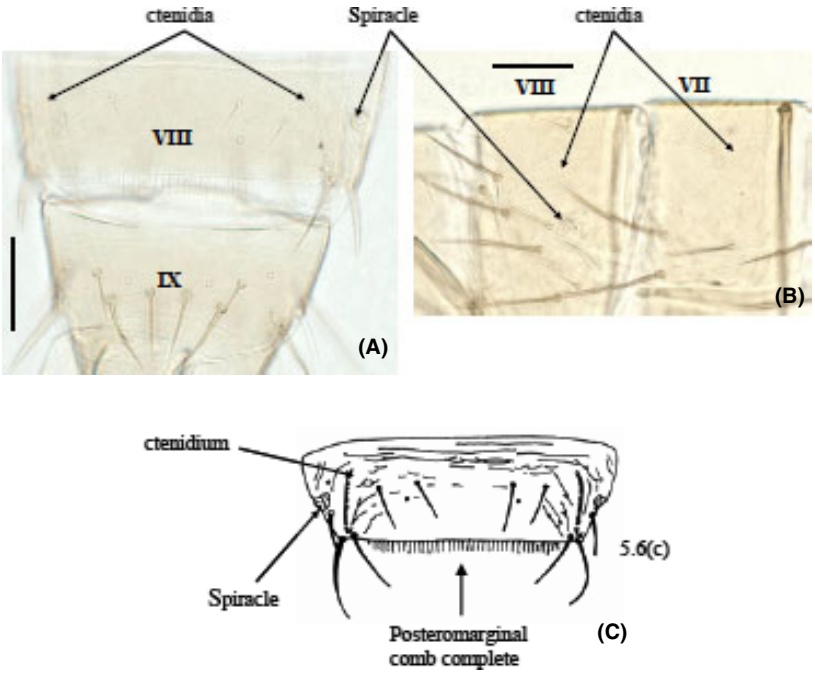

Fig. 5.6 (a)–(c) Abdominal tergite VIII: ctenidia posteromesad to the spiracle; posteromarginal comb complete. (a) Male, tergite VIII and IX, dorsal, comb complete medially. (b) Female, tergite VII and VIII, lateral. (c) Female, tergite VIII, dorsal, comb complete, three pairs distal setae. Scale bars: 30 µm.

- distribution: common leaf-feeding species, sometimes a pest of plants in the family Compositae; Asia, East Africa, Europe, North America and Oceania.

Thrips tabaci

- highly variable in coloration, but usually with more or less brown or greyish markings
- all postocular setae subequal in length
- metascutum with irregular longitudinal reticulations, usually with small internal wrinkles medially, and no campaniform sensilla
- forewing first vein usually with four (occasionally between two or six) distal setae
- abdominal tergite II with three lateral marginal setae
- abdominal tergite IX with posterior pair of campaniform sensilla only
- abdominal pleurotergites with numerous ciliate microtrichia arising from lines of sculpture
- male: narrow transverse glandular area on abdominal sternites III–V only
- distribution: polyphagous pest with a worldwide distribution.

Two further species, one Palaearctic (*Thrips alni* Uzel) and one European (*Thrips urticae* Fabricius), are less commonly encountered but may be confused with *T. palmi*. Females of *T. alni* are particularly similar in morphology to those of *T. palmi*.

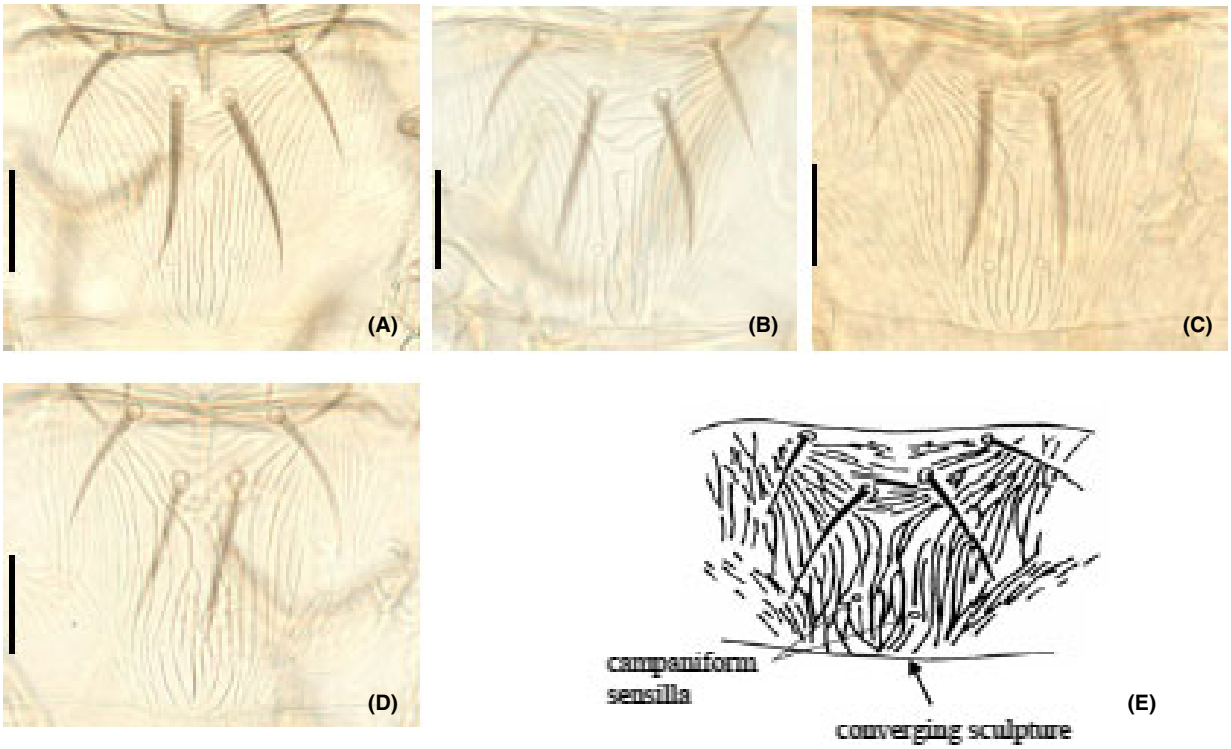

Fig. 5.7 (a)–(e) Metascutum, variation in sculpture; campaniform sensilla. Scale bars: 20 µm.

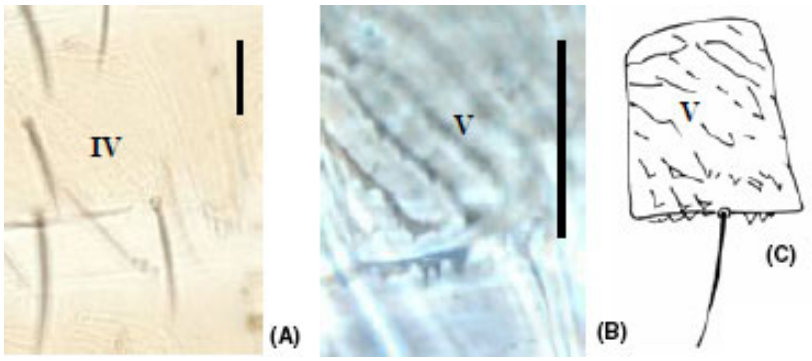

Fig. 5.8 (a)–(c) Abdominal pleurotergites IV and V, ciliate microtrichia and discal setae absent: (a) bright field; (b) phase contrast; (c) complete tergite. Scale bars: 20 µm.

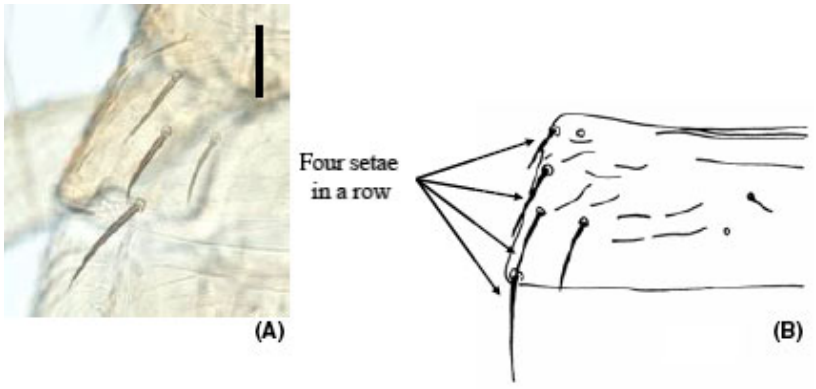

Fig. 5.9 (a), (b) Abdominal tergite II, four lateral marginal setae. Scale bar: 20 µm.

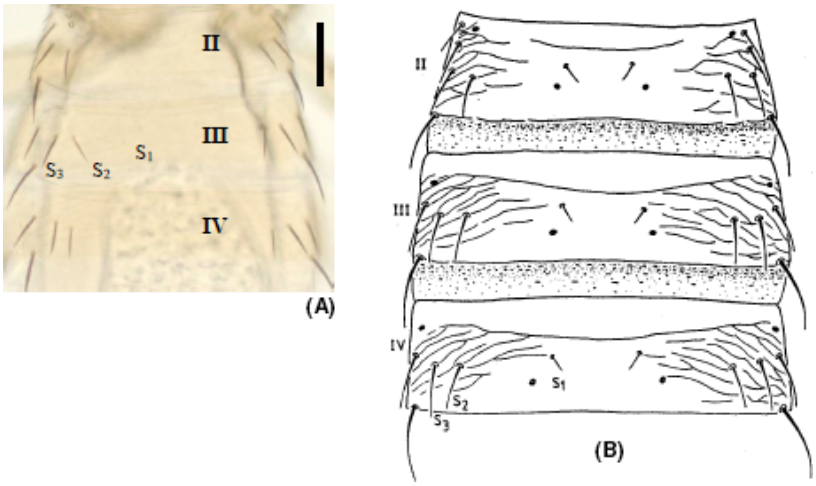

Fig. 5.10 (a), (b) Tergites II–IV, female, setae S2 about the same size as setae S3 (5.10b from zur Strassen, 1989). Scale bar: 50 µm.

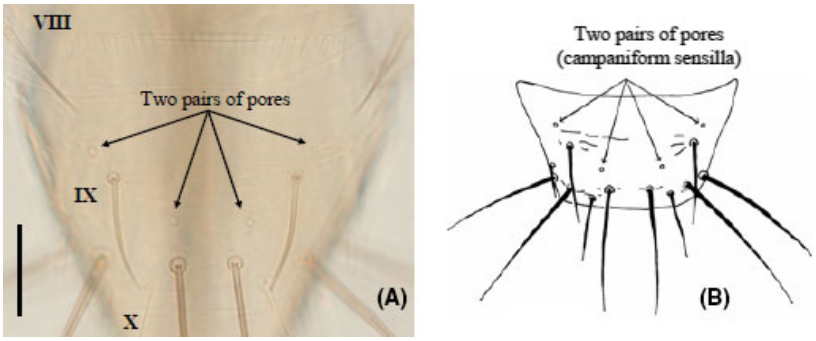

Fig. 5.11 (a), (b) Abdominal tergite IX (dorsal), two pairs of campaniform sensilla. Scale bar: 30 µm.

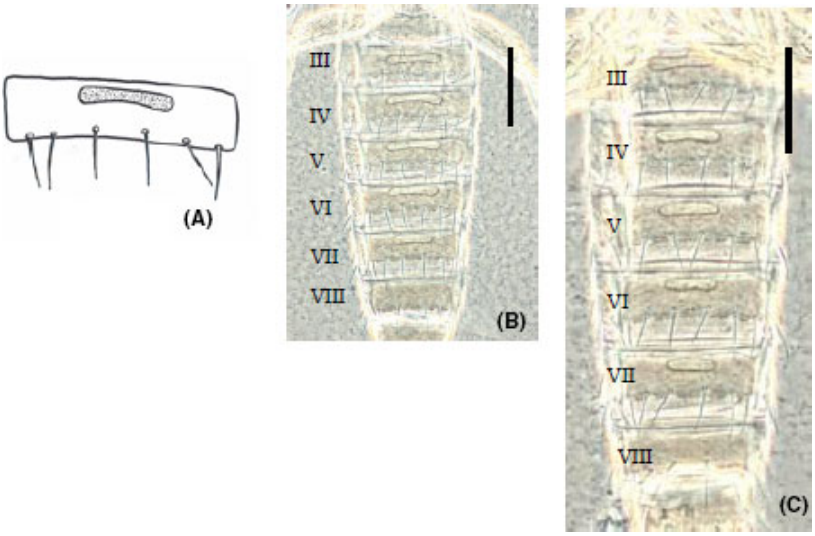

Fig. 5.12 (a)–(c) Male glandular areas (showing variation): (a) sternite V; (b), (c) sternites III–VIII, phase contrast. Scale bars: 100 µm.

Thrips alni

- antennal segment V uniformly brown
- abdominal tergites II–V with setae S2 pale
- abdominal tergite V with seta S2 much weaker than seta S3 (these setae are subequal in *T. palmi*)
- abdominal tergite VIII with seta S1 subequal to seta S2 (S1 is much weaker than S2 in *T. palmi*)
- male: abdominal sternites III–VI each with a small oval glandular area
- distribution: restricted to the leaves of *Alnus*, *Betula*, *Salix*; Europe, Siberia and Mongolia.

Thrips urticae

- pronotum with a pair of setae on the anterior margin almost twice as long as any of the discal setae (usually more than 30 μm; not so in *T. palmi*, all less than 25 μm)
- metascutum with longitudinal reticulations medially
- abdominal tergites usually with a grey area medially
- abdominal tergite IX with posterior pair of campaniform sensilla only
- distribution: restricted to *Urtica dioica*; Europe.

4.2. Molecular tests for identifying *Thrips palmi*

Four molecular tests that can be used to support a morphological identification of *T. palmi* are included in the IPPC protocol. The specificity of each test is also described. This indicates the thrips species against which each test was evaluated and the original use for which the test was designed. A CD-ROM identification system is also available that includes molecular data for thrips species (Moritz *et al.*, 2004). Considering the specific limitations of molecular methods, a negative molecular test result does not exclude the possibility of positive identification by morphological methods.

The test used in EPPO plant pest diagnostic laboratories is the test developed by Kox *et al.* (2005). This test is described in full in Appendix 1. Other tests are described in ISPM 27 (DP1) Appendix 3 (IPPC, 2010).

A loop-mediated isothermal amplification (LAMP) test has also been developed and is described in Appendix 2.

A protocol for DNA barcoding based on *COI* is described in Appendix 1 of PM 7/129 (1) *DNA barcoding as an identification tool for a number of regulated pests: DNA barcoding arthropods* (2016) and allows the identification of *T. palmi.* Sequences are available in Q-bank (http://www.q-bank.eu/arthropods/).

5. Reference material

Slides may be available for loan from:

Netherlands Food and Consumer Product Safety Authority, National Plant Protection Organization, PO Box 9102, 6700 HC Wageningen, the Netherlands

ILVO, Plant, Crop Protection, Burg. Van Gansberghelaan 96, 9820 Merelbeke, Belgium

ANSES – Laboratoire de la santé des végétaux, Station de Montpellier, Campus International de Baillarguet, 34988 Montferrier-Sur-Lez Cedex, France

6. Reporting and Documentation

Guidelines on reporting and documentation are given in EPPO Standard PM 7/77 (1) *Documentation and reporting on a diagnosis.*

7. Performance criteria

When performance criteria are available, these are provided with the description of the test. Validation data is also available in the EPPO Database on Diagnostic Expertise (http://dc.eppo.int), and it is recommended that this database is consulted as additional information may be available there (e.g. more detailed information on analytical specificity, full validation reports, etc.).

8. Further information

Further information on this organism can be obtained from:

Vierbergen G. Entomology Section, National Reference Laboratory, Plant Protection Service, PO Box 9102, 6700 HC Wageningen, Netherlands. Telephone: +31 317 496824; e-mail: g.vierbergen@nvwa.nl; fax: +31 317 423977.

9. Feedback on this diagnostic protocol

If you have any feedback concerning this diagnostic protocol, or any of the tests included, or if you can provide additional validation data for tests included in this protocol that you wish to share please contact diagnostics@eppo.int.

10. Protocol revision

An annual review process is in place to identify the need for revision of diagnostic protocols. Protocols identified as needing revision are marked as such on the EPPO website.

When errata and corrigenda are in press, this will also be marked on the website.

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Appendix 1 – *COI* sequence-based real-time PCR test for *Thrips palmi*

1. General information

1.1 This test was adapted from Kox *et al.* (2005). The primers described below have been adjusted for greater sensitivity since the original publication.

1.2 The targeted gene is the mitochondrial cytochrome oxidase I (*COI*) gene.

1.3 Amplicon size 148 bp.

1.4 Forward primer: Tpalmi 139F* (5′-TCA TGC TGG AAT TTC AGT AGA TTT AAC-3′), sequence (orientation 5′–3′);
reverse primer: Tpalmi 286R* (5′-TCA CAC RAA TAA TCT TAG TTT TTC TCT TG-3′);
TaqMan probe: TpP (6-FAM 5′-TAG CTG GGG TAT CCT CAA-3′ MGB).

1.5 The test was performed on either the ABI Prism 7700 or the ABI 7900HT Sequence Detection System (Applied Biosystems).

1.6 Software and settings (automatic or manual) for data analysis.

2. Methods

2.1. Nucleic acid extraction and purification

DNA can be extracted from adults, pupae or larvae using the High Pure PCR Template Preparation Kit

(Roche) according to the manufacturer's instructions in the mammalian tissue protocol. Before DNA extraction, each specimen should be ground in 100 µL of lysis buffer using a micro pestle. After grinding, another 100 µL of lysis buffer is added to reach the 200 µL mentioned in the kit instructions. At the end of the protocol the DNA should be eluted in 50 µL (instead of 200 µL) pre-warmed (70°C) elution buffer (10 mm HCl, pH 8.5).

Laboratories may find that alternative extraction techniques work equally well [see ISPM 27 Diagnostic Protocols for Regulated Pests DP 1: Thrips palmi (FAO, 2015)]. Several recent papers have described non-destructive techniques for extracting DNA from thrips, which have the advantage that after DNA extraction has been completed a cleared specimen remains available for slide mounting (e.g. Rugman-Jones *et al.*, 2006; Mound & Morris, 2007).

2.2. Real-time polymerase chain reaction

2.2.1. Master mix.

Reagent	Working concentration	Volume per reaction (µL)	Final concentration
Molecular-grade water†	N.A.	To make up to 24 µL	N.A.
2× TaqMan Universal Master Mix (Applied Biosystems)	–	12.5	–
Forward Primer (Tpalmi 139F*)	10 µM	2.25	0.9 µM
Reverse Primer (Tpalmi 286R*)	10 µM	2.25	0.9 µM
TaqMan Probe 1 (TpP)	10 µM	0.25	0.1 µM
Subtotal		24	
DNA dilution		1	
Total		25	

†Molecular-grade water should preferably be used or prepared, purified (deionized or distilled), sterile (autoclaved or 0.22 µm filtered) and nuclease-free water.

2.2.2. PCR conditions: 10 min at 95°C, then 40 cycles of 15 s at 95°C and 1 min at 60°C.

3. Essential procedural information

3.1. Controls

For a reliable test result to be obtained, the following (external) controls should be included for each series of nucleic acid extraction and amplification of the target organism and target nucleic acid, respectively:

- Negative isolation control (NIC) to monitor contamination during nucleic acid extraction: nucleic acid extraction and subsequent amplification in clean extraction buffer
- Positive isolation control (PIC) to ensure that nucleic acid of sufficient quantity and quality is isolated: nucleic acid extraction and subsequent amplification of the target
- Negative amplification control (NAC) to rule out false positives due to contamination during the preparation of the reaction mix: amplification of molecular-grade water that was used to prepare the reaction mix
- Positive amplification control (PAC) to monitor the efficiency of the amplification: amplification of nucleic acid of the target organism. This can include nucleic acid extracted from the target organism, whole-genome amplified DNA or a synthetic control (e.g. cloned PCR product).

As alternative to (or in addition to) the external positive controls (PIC and PAC), internal positive controls (IPC) can be used to monitor each individual sample separately. Positive internal controls can either be genes present in the matrix DNA (i.e. universal genes such as *COI* or *18S*) or added to the DNA solutions.

Alternative internal positive controls can include:

- Specific amplification or co-amplification of endogenous nucleic acid, using conserved primers that amplify conserved non-pest target nucleic acid that is also present in the sample (e.g. eukaryotic 18S rDNA)
- amplification of samples spiked with exogenous nucleic (control sequence) acid that has no relation to the target nucleic acid (e.g. synthetic internal amplification controls) or amplification of a duplicate sample spiked with the target nucleic acid.

Other possible controls

- Inhibition control (IC) to monitor inhibitory effects introduced by the nucleic acid extract: the same matrix spiked with nucleic acid from the target organism.

3.2. Interpretation of results

In order to assign results the following criteria should be followed:

Verification of the controls

- The PIC and PAC (as well as IC and IPC as applicable) amplification curves should be exponential
- NIC and NAC should give no amplification.

When these conditions are met:

- A test will be considered positive if it produces an exponential amplification curve
- A test will be considered negative if it does not produce an amplification curve or if it produces a curve which is not exponential
- Tests should be repeated if any contradictory or unclear results are obtained.

4. Performance criteria available

Performance criteria are as provided in Kox *et al.* (2005) with additional information from ISPM 27 Appendix 1 (IPPC, 2010).

4.1 Analytical sensitivity data

From Kox *et al.* (2005): a 10 000-fold dilution of *T. palmi* DNA (corresponding to 1/1000th of an adult thrips) could be detected in all cases.

4.2 Analytical specificity data

Analytical specificity was evaluated with 15 populations of *Thrips palmi* from different locations around the world and 23 other species of thrips, including 11 belonging to the genus *Thrips* (*T. alliorum* (Priesner), *T. alni*, *T. angusticeps* Uzel, *T. fuscipennis* Haliday, *T. latiareus* Vierbergen, *T. major*, *T. minutissimus*, *T. parvispinus* (Karny), *T. tabaci*, *T. urticae*, *T. vulgatissimus*). These were predominantly, but not exclusively, European species. A number of specimens from India identified as *T. palmi* on the basis of their morphology (Asokan *et al.*, 2007) did not produce a positive result using this test and have *COI* sequences that have 2–3 mismatches with the TaqMan probe and 3 and 2–3 mismatches with forward and reverse primers, respectively, in this test. These sequences are deposited in GenBank. The taxonomic or phylogenetic significance of this sequence differentiation currently remains unclear.

4.3 Data on repeatability is not available (see below)

4.4 Data on reproducibility is not available (see below)

4.5 Other information

The test has been performed 26 times between 2013 and 2016, and when comparing the results with the positive amplification controls it can be derived that the test is 100% repeatable and reproducible.

Appendix 2 – Loop-mediated isothermal amplification (LAMP) test

1. General information

1.1 The LAMP test described in the following section is performed to detect *Thrips palmi.*

1.2 The test was developed by Blaser *et al.* (2018) and can be used for on-site detection as well as for screening method in laboratories. The test is based on a quick DNA extraction protocol and can be completed within 1 h.

1.3 The test targets a part of the mitochondrial gene cytochrome oxidase I (*COI*).

1.4 The test is available in a kit format including the primers from OptiGene Ltd.

1.5 The test was successfully validated using the GenieII (OptiGene) and 7500 Real-Time PCR System (Applied Biosystems). GenieII can be applied for on-site detection.

1.6 GenieII software (OptiGene) as well as 7500 Real-Time PCR System software was applied during the validation. However, it is also possible to use alternative real-time fluorescence acquisition software.

2. Methods

2.1. Nucleic acid extraction and purification

The test can be performed after a quick chemical DNA extraction step using 0.6 M potassium hydroxide (KOH) as described in the following section. Afterwards, the DNA extract can be used directly without any purification process.

2.1.1 Suspected insect material (larvae, adults) is transferred into a tube containing 30 µL of 0.6 M KOH and heated up for 5 min at 95°C. The heating step can be performed using any conventional heat block (e.g. Eppendorf Thermomixer comfort) or with GenieII (OptiGene) for on-site detection. The resulting DNA extract is used directly for the following LAMP reaction.

2.1.2 Extracts of total nucleic acids can be stored at 4°C for immediate use or at −20°C for later use.

2.2. LAMP

2.2.1 Ready-to-use kits are commercially available to conduct the test (e.g. OptiGene, cat. no. ISO-001LNL).

2.2.2 Master mix

Reagent	Working concentration	Volume per reaction (µL)	Final concentration
Molecular-grade water*	N.A.	5	N.A.
Lyse n' Lamp	N.A.	15	N.A.
Isothermal Master Mix (OptiGene, Cat. No. ISO-001LNL)Primer mix (OptiGene)	10×	2.5	1×**
Subtotal		22.5	
DNA extract		2.5	
Total		25	

*Molecular-grade water should preferably be used, or prepared, purified (deionized or distilled), sterile (autoclaved or 0.22 µm filtered) and nuclease-free water.

**Final concentration of primers: 0.2 µM F3 and B3 primers, 2 µM FIP and BIP primers and 1 µM F-loop and B-loop primers.

2.2.3 LAMP amplification conditions: 65°C for 45 min; melting curve analysis: 98–75°C, 0.05°C s^{-1}.

3. Essential procedural information

3.1. Controls

For a reliable test result to be obtained, the following (external) controls should be included for each series of nucleic acid extraction and amplification of the target organism and target nucleic acid, respectively:

- Negative isolation control (NIC) to monitor contamination during nucleic acid extraction: nucleic acid extraction and subsequent amplification in clean extraction buffer

- Positive isolation control (PIC) to ensure that nucleic acid of sufficient quantity and quality is isolated: nucleic acid extraction and subsequent amplification of the target organism
- Negative amplification control (NAC) to rule out false positives due to contamination during the preparation of the reaction mix: amplification of molecular-grade water that was used to prepare the reaction mix
- Positive amplification control (PAC) to monitor the efficiency of the amplification. This can include nucleic acid extracted from the target organism, amplified *T. palmi* DNA containing the mitochondrial *COI* gene or a synthetic control (e.g. cloned PCR product).

As alternative to (or in addition to) the external positive controls (PIC and PAC), internal positive controls (IPC) can be used to monitor each individual sample separately. Positive internal controls can either be genes present in the matrix DNA (i.e. universal genes such as *COI* or *18S*) or added to the DNA solutions.

Alternative internal positive controls can include:

- Specific amplification or co-amplification of endogenous nucleic acid, using conserved primers that amplify conserved non-pest target nucleic acid that is also present in the sample (e.g. eukaryotic 18S rDNA)
- Amplification of samples spiked with exogenous nucleic (control sequence) acid that has no relation to the target nucleic acid (e.g. synthetic internal amplification controls) or amplification of a duplicate sample spiked with the target nucleic acid.

3.2. Interpretation of results:

Verification of controls:

- NAC (and if relevant NIC) should produce no fluorescence
- The PAC (and if relevant PIC, IPC) amplification curve should be exponential. The PAC Tm (melting temperature) should be between 78 and 84°C. A similar Tm range is expected when analyses are performed on any other device, but this needs to be verified.

When these conditions are met:

- A test will be considered positive if it produces a positive reaction as defined for the PAC (see above)
- A test will be considered negative if it produces no fluorescence
- Test should be repeated if any contradictory or unclear results are obtained.

4. Performance criteria available

Validation was performed on the adult stage (Blaser *et al.*, 2018).

4.1. Analytical sensitivity data

One adult.

4.2. Analytical specificity

The LAMP test is specific to *T. palmi*. *In silico* analyses of closely related insect species indicated no significant homology of target sequences. Results were confirmed by *in vitro* experiments.

Inclusivity was evaluated with *T. palmi* samples from the Dominican Republic, India, Indonesia, Malaysia, Pakistan, Sri Lanka, Thailand and Vietnam.

Exclusivity was evaluated with *Cephalothrips monilicornis*, *Frankliniella intonsa*, *Frankliniella occidentalis*, *Haplothrips* sp., *Scirtothrips aurantii*, *Scirtothrips dorsalis*, *Thrips parvispinus* and *Thrips tabaci*.

4.3. Data on repeatability, diagnostic sensitivity and diagnostic specificity (evaluated on bot Genie II and 7500 Real-Time system)

A total of 104 suspected *T. palmi* samples were analysed using the described LAMP method. Results were cross-validated using the barcoding approach by sequencing a part of the mitochondrial *COI* gene as described by Herbert *et al.* (2003).

Results:

Result	Number
True positive	82
True negative	24
False positive	0
False negative	2

- Diagnostic sensitivity: 98.7%
- Diagnostic specificity: 100%.

4.4. Data on reproducibility

Validation of the LAMP test described in Section 4.3 was performed at two separate locations under different conditions. Early validations were done under laboratory conditions by the Molecular Diagnostics, Genomics and Bioinformatics Research Group of Agroscope. The second part of the validation was performed under on-site conditions in the office of the NPPO at Zurich Airport.

4.5. Other data

LAMP primers were designed by studying available *T. palmi COI* sequences from NCBI GenBank, as well as sequences generated by the import diagnostic process of insect quarantine organisms in Switzerland.

In the first half of 2018, the test and the extraction have been validated in *Thrips* larvae detected on imported consignments at Zurich Airport.