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European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes PM 7/137 (1)

Diagnostics
Diagnostic

PM 7/137 (1) *Thaumatotibia leucotreta*

Specific scope

This standard provides guidance for the identification of *Thaumatotibia leucotreta*.[1]

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

Specific approval and amendment

Approved in 2019–04.

1. Introduction

Thaumatotibia leucotreta (Meyrick), the false codling moth, is an important tortricid pest that is highly polyphagous. Larvae are reported to feed on more than 50 species of plants in 30 different families (van der Geest *et al*., 1991; Brown *et al*., 2008), many of which are important economic crops. In trade destined to the EU it is most often intercepted on *Capsicum* spp., *Citrus* spp., *Solanum melongena* and *Rosa* (Europhyt 2018; van der Straten, NPPO the Netherlands pers. comm. 2018). *Thaumatotibia leucotreta* is reported as being present in 40 African countries and several islands in the Indian Ocean (CPC 2007; EPPO 2007; van der Geest *et al*., 1991) and in Israel (Hamburger *et al*., 2000; EPPO, 2018). Apart from incidental findings probably related to trade (Huisman & Koster, 2000; Svensson, 2002) and a few incursions reported (Gilligan *et al*., 2011; EPPO, 2018), it is not considered established outside of this region. For distribution details, see the EPPO Global database (EPPO, 2018). For host ranges, see Appendix 1 of the "Pest risk analysis for *Thaumatotibia leucotreta*" (EPPO 2013).

This document provides guidance for morphological identification of all life stages of *T. leucotreta* for specimens found in commodities originating from its current area of distribution (Africa south of the Sahara and Israel). For early stages, which cannot be positively identified using morphology only, it provides additional information that can help to identify possible specimens that may be *T. leucotreta*. Appendix 1 provides information to separate larvae of *T. leucotreta* from some other Lepidoptera taxa, specifically those frequently found on the same commodities as *T. leucotreta* or closely related to *T. leucotreta*.

2. Identity

Name: *Thaumatotibia leucotreta* (Meyrick, 1913)
Synonyms: *Cryptophlebia leucotreta* (Meyrick), *Argyroploce leucotreta* (Meyrick), *Thaumatotibia roerigii* Zacher
Taxonomic position: Insecta, Lepidoptera, Tortricidae, Olethreutinae, Grapholitini
EPPO Code: ARGPLE
Phytosanitary categorization: EPPO A2; EU annex I/A1

3. Detection

3.1. General information

Thaumatotibia leucotreta is a polyphagous internal feeder in fruits and, at least in the case of *Rosa*, also in flower buds. For a full list of host plants see EPPO (2013), Appendix 1. Eggs are deposited singly or in small groups on the surface of the fruit or flower bud (on either the petal or the sepal). Although visible with a hand lens they are difficult to detect, since they are small, flat and usually concolorous with the substrate (Figs 1 and 2).

The most likely stage to be detected during inspection of commodities is the larva, whereas in the field the adult stage can also be routinely detected using traps. Larvae can

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

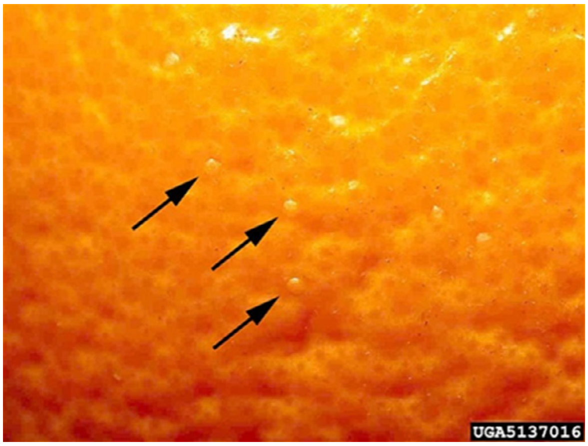

Fig. 1 Eggs on citrus (photo: J.H. Hofmeyr, Citrus Research Int., Bugwood.org).

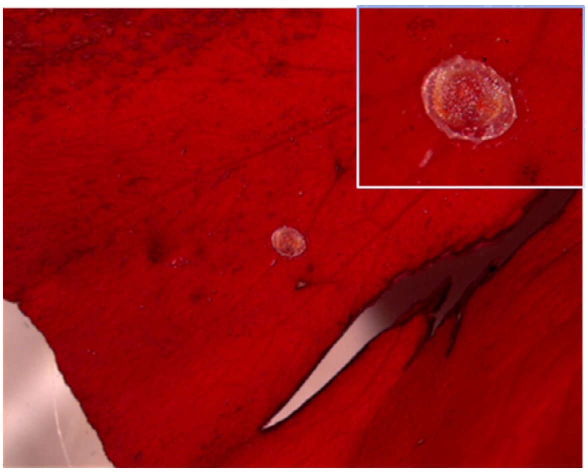

Fig. 2 Egg on rose.

Fig. 3 Entrance hole on circus (photo: P. van der Meijden).

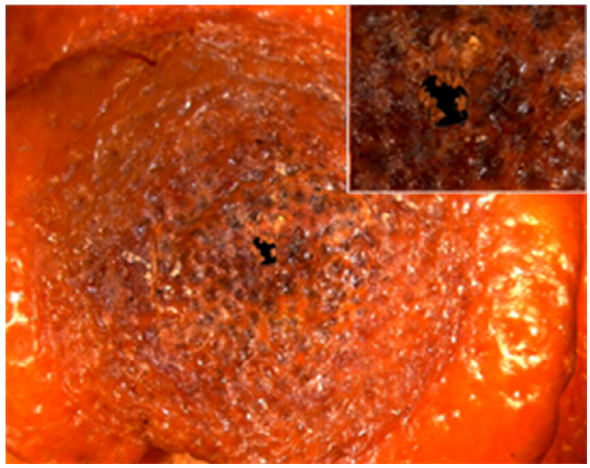

Fig. 4 Entrance hole on circus with frass protruding.

Fig. 5 Symptoms on chili pepper: entrance hole and rotting (photo: KCB, the Netherlands).

be detected visually (with a hand lens) by checking for symptoms and then cutting the fruit or opening the bud. Typically, symptoms are small holes in the fruit or bud where the larva has penetrated or exited the fruit or bud (Figs 3–7). Recent infestations are hard to detect because the only external symptom is a small entrance hole. However, sometimes frass can be found protruding from the hole (Figs 4 and 7). Later, due to fungal or bacterial infections, in many species of fruit (e.g., citrus) a brown blotch may appear around the entrance hole (Figs 3, 4, 5, 8). Cutting of fruit or buds can be performed to randomly sample for larvae. When an infested fruit or bud is cut or opened, abundant frass indicates the presence of larvae (Figs 8–12). Larval damage and behaviour vary depending on the host. In the case of roses, larvae always migrate to the centre of the bud. On soft fruit, larvae tunnel into the pith or feed beneath the surface. On harder fruit, larvae mine in or superficially under the skin. The symptoms listed are not specific for *T. leucotreta* and apply to many other boring insects, especially Lepidoptera. For example, *H. armigera* will cause similar symptoms in *Rosa*. However, Tephritidae infestations in fruit may appear similar but there will be no

Fig. 6 Entrance hole on rose.

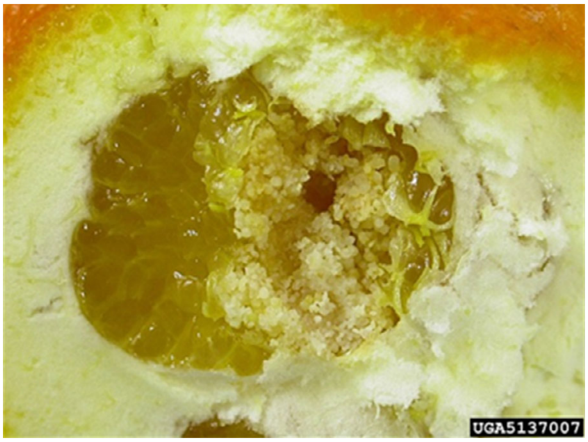

Fig. 9 Citrus with frass under the skin (photo: J.H. Hofmeyr, Citrus Research Int., Bugwood.org).

Fig. 7 Entrance hole on rose with frass protruding.

Fig. 10 Frass and larva in citrus (photo: J.H. Hofmeyr, Citrus Research Int., Bugwood.org).

Fig. 8 Citrus with brown blotch and frass in the fruit.

Fig. 11 Damage in the centre of the rose.

frass. It should be noted that identification based on symptoms only is not possible and actual specimens are required.

Fig. 12 Frass in baby rose.

Pupae are not detected easily because, under natural conditions, last instar larvae will exit the fruit or flower and pupate in a silken cocoon hidden in the soil, under leaf litter, or in bark crevices. However, larvae present in a commodity and ready to pupate will pupate regardless of the presence of a natural hiding place. Therefore, pupae can also be associated with commodities and can be found near or in the fruit, flower or packaging material.

In the field and in production, storage, handling and other facilities, adults can most easily be detected with the aid of light traps and/or pheromone traps. Light traps will attract both male and female *T. leucotreta*, as well as many other species of Lepidoptera and other insects. Pheromone traps are more specific and target only male *T. leucotreta*, although related species (other Grapholitini) and sometimes non-related species can be found in *T. leucotreta* pheromone traps.

4. Identification

4.1. Morphological identification

4.1.1. Egg

The egg of *T. leucotreta* is flat and slightly oval (mean width 0.60, mean length 0.77 mm). Eggs are translucent, initially whitish, later becoming orange with the dark head of the larva visible prior to hatching (Daiber, 1979a). Eggs cannot be identified to species using only morphology because they are similar to those of many other species in the subfamily Olethreutinae. Females deposit eggs on the surface of the fruit or flower bud, singly or in small groups, but separated from each other, like many other species of Olethreutinae (Passoa, 2008). This differs from many Tortricinae, which deposit eggs in large clusters with eggs touching or overlapping.

4.1.2. Larva

The larva is a typical lepidopteran larva with the following characteristics:

(1) Distinct head capsule with six pairs of stemmata (ocelli).
(2) Three thorax segments and ten abdominal segments.
(3) Three pairs of thoracic legs ending in a true claw.
(4) Four pairs of abdominal prolegs (on abdominal segments 3–6) and one pair of anal prolegs, all bearing crochets on the ventral face.
(5) Spiracles present on the first thoracic segment and abdominal segments 1–8.
(6) Chewing mouthparts, one pair of antennae and a labial spinneret present.

For an introduction to lepidopteran larval morphology see Gilligan & Passoa (2014).

Early instars of *T. leucotreta* cannot be identified to the species level using morphology only and rearing to later instars or molecular identification is needed for reliable identification. Mid to late instars of *T. leucotreta* can reliably be identified morphologically, *if* found on a known host plant for *T. leucotreta* on which other species of *Thaumatotibia* are not known to occur. If, however, a specimen is found on a plant species that is known to be a host of other African *Thaumatotibia* spp. as well, rearing to adult or molecular identification is recommended for a definitive identification.[2] Timm *et al*. (2007) describe the differences between the larva of *T. leucotreta* and *T. batrachopa*, but the characteristics are difficult to interpret, and no larval characteristics are known that unambiguously distinguish larval *T. leucotreta* from all other species of African *Thaumatotibia*. However, in trade *T. leucotreta* is intercepted frequently on main commodities such as *Citrus, Capsicum* and *Rosa,* while interceptions of other species of *Thaumatotibia* from Africa are unknown so far on these commodities. Note that other species of *Thaumatotibia* occur on other continents (e.g. South-East Asia) whose larvae look very similar to those of *T. leucotreta*.

Appendix 1 provides information to separate larvae of *T. leucotreta* from some other Lepidoptera taxa, specifically those frequently found on the same commodities or closely related to *T. leucotreta*.

4.1.2.1. Early instars (L1/L2). The young larva is whitish with a dark head and dark pinacula, and resembles larvae of many other Tortricidae; it is about 1–2 mm long. First instars cannot be identified reliably to the species level using only morphology. However, from the second instar on it is possible to morphologically identify larvae to subfamily level (see Late instars) with a stereomicroscope, magnification 50× or higher, enabling a conclusion to be made on the possible presence of *T. leucotreta*. The experience of the NPPO-NL in case of findings on Rosa in trade

[2] *T. leucotrata* larvae are regularly detected on imported consignments based on the experience of the diagnostician with previous imported consignments and subject to discussions with the national plant protection organization (NPPO), and rearing may not be required to take phytosanitary action.

is that first instar larvae show a typical behaviour: in the absence of a complete bud, e.g. in a sample consisting of a single petal or sepal, larvae are able to mine on the thicker part of the petal or sepal. A larva detected on Rosa originating from a country where *T. leucotreta* is present or with a previous history of detection of *T. leucotreta* on consignments, that has morphological characters consistent with the subfamily Olethreutinae (see below) and shows the specific mining behaviour is likely to be *T. leucotreta*.

A key characteristic of *T. leucotreta* is the shape of the L-pinaculum on the prothorax, which extends under the spiracle (see late instars). If a specimen is a tortricid and has this character, then it belongs to the genera *Thaumatotibia,* or *Cryptophlebia* or the tribe Cochylini (or possibly some other African groups not known as major pests). In L2 larvae, depending on the magnification available, the characteristics described for late instars may be seen. In that case also an L2 stage specimen can be identified using the description below.

4.1.2.2. Late instars (L3–L5). Later instars become orange-pink, turning to dark pink in the last instar, with a medium brown head and thoracic shield; the fully-grown larva is about 7–10 mm long (Fig. 13). The pinacula are well developed and light greyish-brown, as is the anal shield. In addition to the general Lepidoptera larval characteristics described above, *T. leucotreta* larvae exhibit the following diagnostic characteristics (Figs 13–18), which are best observed using a stereomicroscope with magnification 20× or higher. Note that characteristics 7 to 10 are common to (most of the species within) the subfamily Olethreutinae. Characteristic 13 is difficult to see but should be verified if the anal comb is absent. Figure 19 shows the complete setal map for *T. leucotreta*.

Note: Both sides of the larva should be checked; if the setal arrangement appears to be asymmetric (a different number of setae in a group on each side of the larva), usually the higher number is likely to be the correct number.

Fig. 13 Fully grown larva in chilli pepper.

Fig. 14 L-pinaculum on prothorax (photo: Gilligan & Passoa, 2014, LepIntercept).

(7) Prothorax with three L-setae, all on the same pinaculum.
(8) Crochets on ventral prolegs in a full circle (Fig. 17).
(9) Abdominal segment 9 with D2 setae on shared saddle pinaculum (Figs 15, 16).
(10) Abdominal segment 9 with D1 and SD1 setae on the same pinaculum (Figs 15, 16).
(11) L-Pinaculum on prothorax enlarged and extending beneath and beyond the spiracle (Fig. 14). This characteristic will separate it from the larva of many other Tortricidae (see the second paragraph of section 4.1.2.1 Early instars).
(12) Anal comb *normally* present, with 4–10 teeth (occasionally up to 13), but usually with 5–8 teeth (Fig. 18). In some larvae the anal comb is not well developed, and the teeth are reduced in number or length, or the anal comb can be completely absent. If the anal comb is absent, characteristic 13 should be checked.
(13) Abdominal segments with SD2 seta present and anteroventral (in front and below) of SD1 on the same pinaculum (magnification 30× or higher; easiest to see on segments 5–7) (Figs 19 and 20). In *Cryptophlebia* (e.g. *C. peltastica*) SD2 on at least abdominal segments 5–7 is on a separate pinaculum (Fig. 21).

Other characteristics, not unique for *T. leucotreta*, are:

(14) Usually the numbers of SV-setae on abdominal segments A1, 2, 7, 8 and 9 are, respectively, 3, 3, 2, 2 and 1 (Fig. 19) (in *C. peltastica* usually 3, 3, 3, 2, and 2, but some specimens have 3, 3, 2, 2 and 1, like *T. leucotreta*).
(15) SD pinaculum is in front of the spiracle on abdominal segment 8 (Fig. 19).
(16) Abdominal prolegs with 29–48 crochets, irregularly tri-ordinal, slightly shorter and uni-ordinal on the lateral side (Fig. 17).

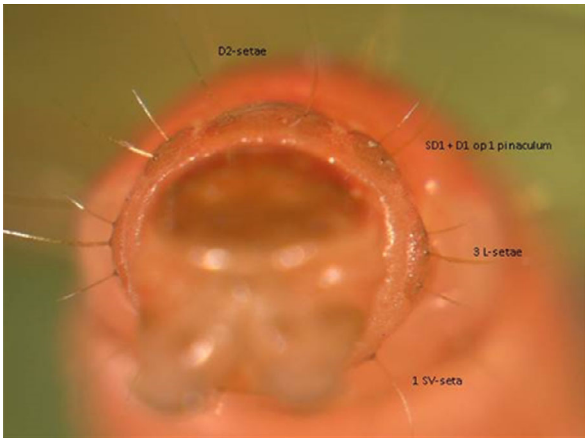

Fig. 15 Abdominal segment 9, caudal view.

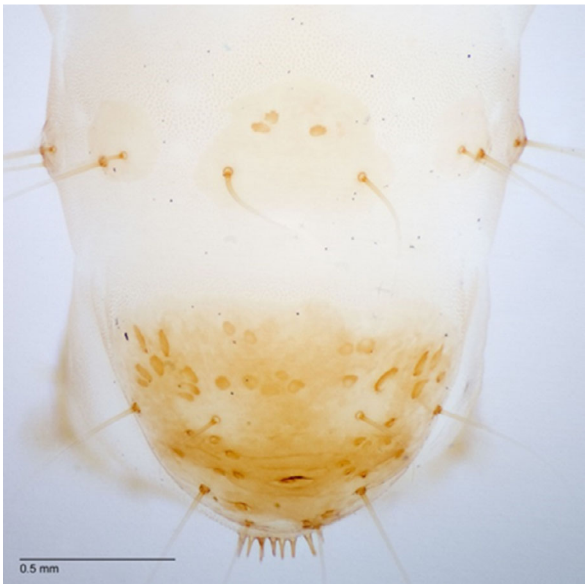

Fig. 16 Abdominal segment 9, dorsal view (photo: Gilligan & Passoa, 2014, LepIntercept).

Fig. 17 Crochets on proleg (ventral view) (photo: Gilligan & Passoa, 2014, LepIntercept).

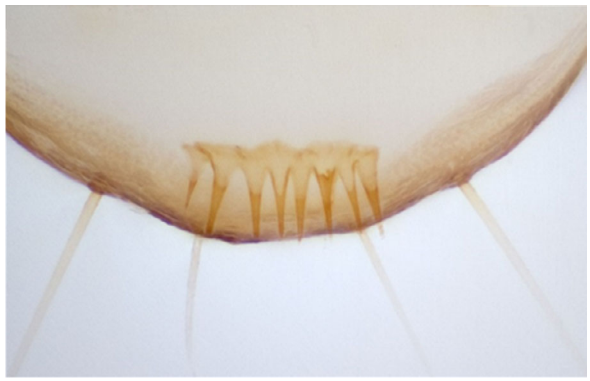

Fig. 18 Anal comb (ventral view) (photo: Gilligan & Passoa, 2014, LepIntercept).

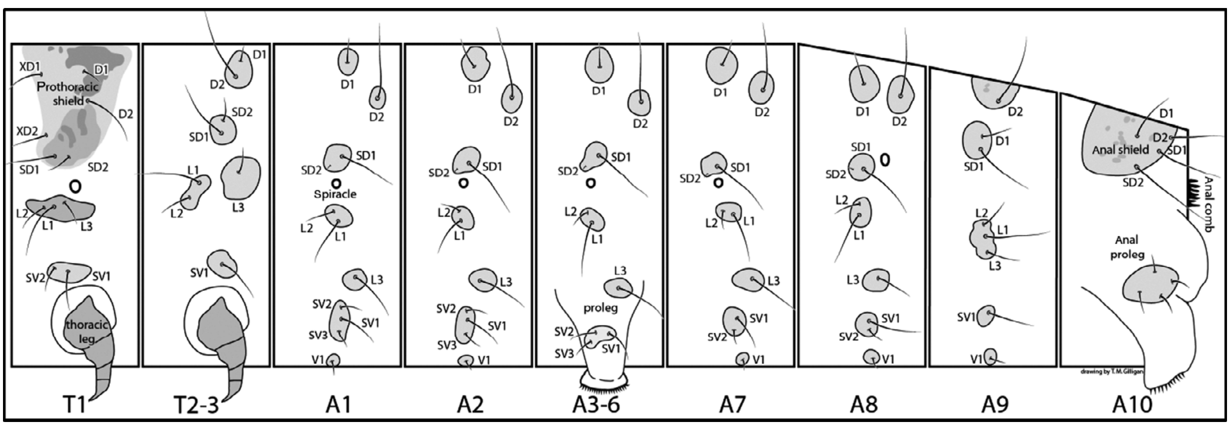

Fig. 19 Setal map (Gilligan & Passoa, 2014, LepIntercept).

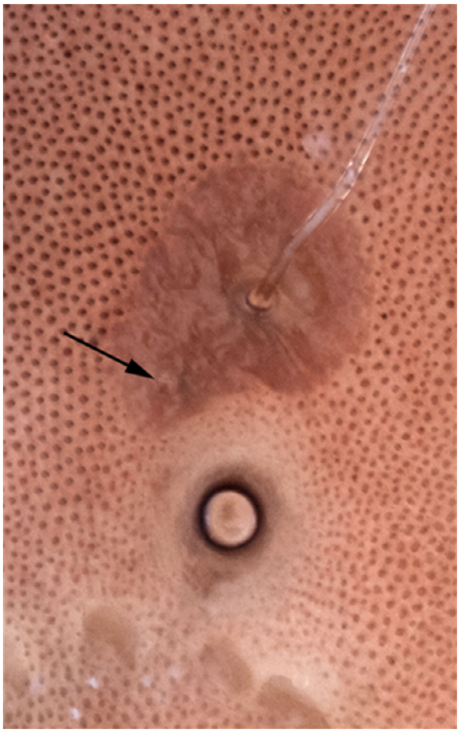

Fig. 20 *Thaumatotibia leucotreta*, abdominal segment 6; SD2 (arrow) on the same pinaculum as SD1 (photo: T. Gilligan, 2018).

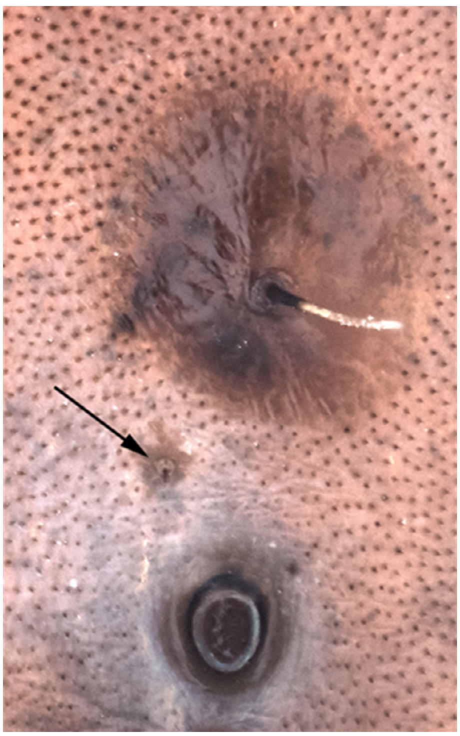

Fig. 21 *Cryptophlebia peltastica*, abdominal segment 6; SD2 pinaculum (arrow) separated from SD1 pinaculum (photo: T. Gilligan, 2018).

(17) L-group on A9 usually trisetose (with all setae usually on the same pinaculum, but L3 can be on a separate pinaculum as well) (Figs 15 and 19).
(18) Skin densely covered with short spinules (magnification 30× or higher) (Figs 17, 20 and 21).
(See also Appendix 1)

Additional information on micro-setae, visible at high magnification in late instars: Setae MD1, MSD1 and MSD2 are present on the second and third thorax segment, and MD1 on the abdominal segments (for a fully-grown larva visible at magnification 30× or higher with MD1 and MV1 best seen at abdominal segment 9). At magnification 40× or higher MV-setae can also be seen on the second and third thorax segment, and on the abdominal segments (difficult to see: for a fully grown larva best seen on A9).

Note: These setae are not included in the setal map (Fig. 19). See, for example, Stehr (1987).

4.1.3. Pupa (Figs. 22 to 24)

The pupa of *T. leucotreta* is a typical tortricid pupa and cannot reliably be identified to species level using only morphology. It can, however, be separated from the pupa of many other Tortricids by the lack of a cremaster. Rearing to adult or molecular techniques are needed for reliable identification. The pupa has the following characteristics (Komai, 1999; Timm *et al*., 2007):

Fig. 22 Female pupa, dorsolateral view.

(1) Medium brown and 7.9–9.8 mm in length.
(2) Two rows of dorsal spines on abdominal segments 2–7, the anterior row consisting of coarse spines and the posterior row of fine spines.
(3) One row of dorsal spines on abdominal segments 8–9 in females and 9 in males.
(4) Abdominal segment 10 bearing dorsally and lateroventrally several pairs of strong projections (Komai, 1999) (spines, slightly bigger than those on A9).
(5) Cremaster absent, but along the anal rise one pair of setae, hooked at the apex.

Fig. 23 Female pupa, abdominal segments 6–10, dorsolateral view.

Fig. 24 Male (top) and female pupa, ventral view (photo: J.H. Hofmeyr, Citrus Research Int., Bugwood.org).

4.1.4. Adult

Adults of *T. leucotreta* are sexually dimorphic, differing in size and hindwing characteristics (Figs 25 and 26). The wingspan of males is 15–16 mm and of females is 19–20 mm. Male forewings are triangular with an acute apex, while female forewings are more elongated with a rounded apex (Figs 27 and 28). The forewing of both sexes has the same basic pattern, as described below:

(1) Small white dot near the end of the discal cell.
(2) Patch of raised, usually rust or orange coloured, scales near the middle of the wing.
(3) Usually distinct ("question mark like") band with a pattern of dark scales along the termen.
(4) Semicircular band of dark scales in the middle of the costa (not obvious in all specimens).

Specific characteristics of the male are:

Fig. 25 Male adult (photo: Gilligan et al., 2011).

Fig. 26 Female adult (photo: Gilligan et al., 2011).

Fig. 27 Male adult, left wings: pattern forewing indistinct.

(5) The hindwing is narrow and has a semicircular pocket of opalescent scales at the lower margin (Fig. 29). This pocket of scales will separate males of *T. leucotreta* from all other species of African (and North American) tortricids. It will also separate them from all tortricids from other continents, although males of some other *Thaumatotibia, Cryptophlebia* and *Archiphlebia* spp. (e.g. from Australia) also have pockets with scent scales (Horak & Komai, 2016), but the structure is different from that of *T. leucotreta*.

Fig. 28 Female adult, left wings.

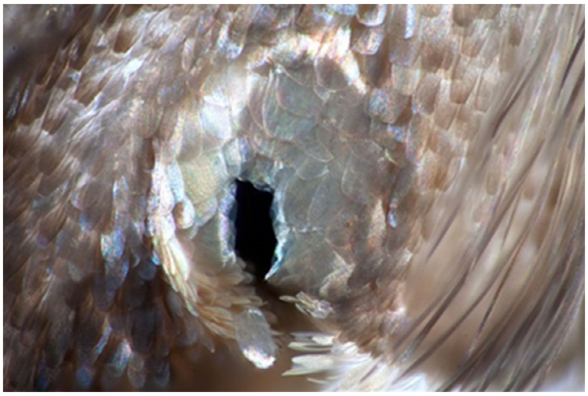

Fig. 29 Male hindwing, scent pocket in detail (photo: Gilligan et al., 2011).

Fig. 30 Male, hindleg with tufts of modified scales (photo: Gilligan et al., 2011).

(6) The tibia of the hind leg has tufts of modified scales (Fig. 30) and an enlargement of the inner apical spur. Males lack a forewing costal fold. However, these characteristics are also present in males of some other African species of *Thaumatotibia* and *Cryptophlebia* (Komai, 1999; Gilligan & Epstein, 2012).

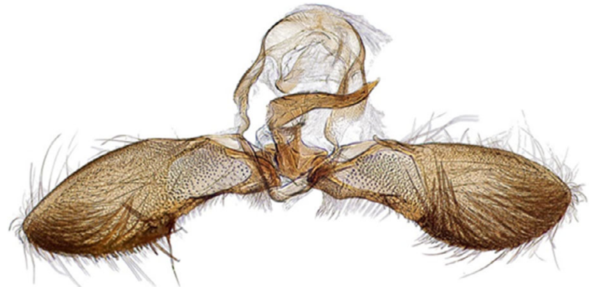

Fig. 31 Male genitalia (photo: Gilligan et al., 2011).

Fig. 32 Female genitalia (photo: Gilligan et al., 2011).

In some specimens the above described characteristics of the forewing can be faint or absent (e.g. the small white dot) (Fig. 27). For females, dissection of the genitalia or molecular techniques is recommended to confirm identity.

Male genitalia (Fig. 31) are characterized by a rounded tegumen lacking an uncus or socii, large rounded valvae and a tapered aedeagus that is upcurved distally. Female genitalia (Fig. 32) are characterized by a semicircular sterigma, narrow ductus bursae and large rounded corpus bursae with a pair of thorn-shaped signa (Gilligan & Epstein, 2012).

4.2. Molecular identification

No specific molecular test has been developed to identify *T. leucotreta*. Several studies have used segments of COI to identify *T. leucotreta* using standard DNA barcoding procedures:

Timm *et al*. (2007, 2008) separated *T. leucotreta* from several other tortricid pests in South Africa.

Mazza *et al*. (2014) identified *T. leucotreta* larvae intercepted in Italy.

Xu *et al*. (2015) identified *T. leucotreta* larvae intercepted in China (Fig. 30–32).

At this moment the Barcode of Life Data Systems (BOLD v3) database has over 50 specimens with barcodes representing a variety of haplotypes. Thus, DNA barcoding should be reliable to identify all life stages of *T. leucotreta* to the species level. A protocol for DNA barcoding based on COI is described in Appendix 1 of PM 7/129 DNA barcoding as an identification tool for a number of regulated pests: DNA barcoding Arthropods (EPPO, 2016) and can support the identification of *T. leucotreta*.

5. Reference material

Reference material for adults, larvae and pupae can be obtained from A. Loomans, Netherlands Food and Consumers Product Safety Authority (a.j.m.loomans@nvwa.nl).

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 are 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 the authors.

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.

Acknowledgements

This diagnostic protocol was originally drafted by M.J. van der Straten, Netherlands Food and Consumers Product Safety Authority, Ministry of Agriculture, Nature and Food Quality, National Reference Centre NPPO-NL, Wageningen, Steve Passoa, National Lepidoptera Specialist, United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA/APHIS/PPQ), Columbus, Ohio, and Todd Gilligan, United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA/APHIS/PPQ), Center for Plant Health Science and Technology. All photos are protected by copyright. Unless stated otherwise photos were taken by M.J. van der Straten, National Reference Centre NPPO-NL, Ministry of Agriculture, Nature and Food Quality. Specials thanks to the inspectors of the KCB, the Netherlands, who detected so many specimens of *T. leucotreta* at import inspections; without them far less knowledge of the presence and behaviour of the species in trade would be available.

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Appendix – Table of characteristics that separate larvae of *Thaumatotibia* from some other Lepidoptera taxa

This table gives characteristics that separate larvae of Thaumatotibia from some other Lepidoptera taxa, specifically closely related taxa and taxa frequently found on the same commodities as T. leucotreta. Per group a few characteristics are given that suffice to separate larvae of other species from those of Thaumatotibia. Characteristics not given for a certain group/species are irrelevant. Identification of a larva suspected to be T. leucotreta must be accomplished with the full description given in the main document! Note: Always check both sides of the specimen: there can be differences between the two sides, specifi-cally the number of setae. Count the highest number.

Taxon	Pairs of ventral prolegs	Arrangement of crochets on ventral prolegs	Anal comb present	Number of L-setae on T1	Position of SD1 and D1 on A9	L-pinaculum on T1	Position of SD2 and SD1 on A5-7
Geometridae	1	Mesoseries	No				
Noctuidae: Plusiinae	2	Mesoseries	No	2			
Noctuidae sensu stricto (most)*	4	Mesoseries	No	2			
Pyralidae†	4	Usually circle or (mesopen)ellipse	No	2			
Crambidae‡	4	See Pyralidae	No	2		*	
*Phyllocnistis citrella***	3		No	2			
Prays citri	4	Circle (uni-ordinal)	No	3	On separate pinaculum		
Tortricinae††	4	Circle	Yes or No	3	On separate pinaculum		
Lobesia spp.	4	Circle	Yes or No	3	On shared pinaculum	Not extending under the spiracle	SD2 absent
Grapholita spp.	4	Circle	Yes or No	3	On shared pinaculum	Not extending under the spiracle	SD1 and SD2 on separate pinaculum
Cryptophlebia spp.	4	Circle	No	3	On shared pinaculum	Extending under the spiracle	SD1 and SD2 on separate pinaculum
Thaumatotibia spp.	4	Circle (tri-ordinal, irregular)	Yes *	3	On shared pinaculum	Extending under the spiracle	SD1 and SD2 on shared pinaculum

*For example, *Helicoverpa armigera* and *Spodoptera* spp.

†For example, *Ectomyelois ceratoniae*, *Cryptoblabes gnidiella* and other Phycitinae. Many species of Phycitinae have a sclerotized ring around SD1 on A8.

‡For example, *Duponchelia fovealis*, *Leucinodes* spp. (Note: In *Leucinodes* the L-pinaculum also extends under the spiracle, but it has a different shape to that in *T. leucotreta*.)

***Phyllocnistis citrella* is a leafminer, which can also mine the skin of the fruit.

††For example, *'Tortrix' dinota*, *Choristoneura occidentalis* and *Epichoristodes acerbella*.

‡‡Note: Also in *Thaumatotibia* incidentally the anal comb is absent; more often it is present but poorly developed. Check the position of SD1 and SD2 on A7.