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European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes PM 7/135 (1)

Diagnostics
Diagnostic

PM 7/135 (1) *Zeugodacus cucurbitae*

Specific scope

This Standard describes a diagnostic protocol for *Zeugodacus cucurbitae*.[1]

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

Specific approval and amendment

Approved in 2018–09.

1. Introduction

Zeugodacus cucurbitae is a major pest of Cucurbitaceae, in particular of *Cucumis melo*, *Cucumis sativus*, *Cucurbita melo* and *Momordica charantia*. This species has been recorded on 125 host plants including non-cucurbits (White & Elson-Harris, 1992), some of which are of economic importance, such as Anacardiaceae (*Mangifera indica*, *Anacardium occidentale*), Passifloraceae (*Passiflora edulis*), Rosaceae (*Prunus persica*), Rutaceae (*Citrus sinensis*), Oxalidaceae (*Averrhoa carambola)* and Solanaceae (*Capsicum annuum*, *Capsicum frutescens*, *Solanum lycopersicum*) (White & Elson-Harris, 1992; Vayssières *et al.*, 2007 and De Meyer *et al.*, 2015); however, these hosts are minor and less damage is recorded than on cucurbits (De Meyer *et al.*, 2015).

Zeugodacus cucurbitae is native to Asia. It is present in tropical and sub-tropical areas of Africa, the Indian Ocean islands, Australia (Queensland) and Pacific Islands, including Hawaii. Details on its distribution are available in the EPPO Global Database (EPPO, 2018).

Additional information on the distribution and biology of the pest can be found in EPPO/CABI (1997). It should be noted that the current valid name results from a recent taxonomic revision (Virgilio *et al.*, 2015) and most references to this species in the literature occur under the now junior synonym of *Bactrocera cucurbitae.*

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

2. Identity

Name: *Zeugodacus* (*Zeugodacus*) *cucurbitae* (Coquillett, 1899) (Virgilio *et al.*, 2015).
Synonyms: *Bactrocera* (*Zeugodacus*) *cucurbitae* (Coquillett), *Dacus cucurbitae* Coquillett, *Dacus yuiliensis* Tseng & Chu, *Dacus aureus* Tseng & Chu (Thompson, 1998) and *Chaetodacus cucurbitae* (Coquillett), *Strumeta cucurbitae* (Coquillett) (White & Elson-Harris, 1992).
Taxonomic position: Diptera, Brachycera, Tephritidae, Dacinae, Dacini.
Nomenclature and taxonomy suggested by Fauna Europaea and Virgilio *et al.*, 2015 are used as the reference.
EPPO Code: DACUCU.
Phytosanitary categorization: EPPO A1 List no. 232 – EU IAI.

3. Detection

Fruit flies are mostly detected as larvae in fruits. Holes are visible on the fruits. Eggs might be found inside the fruit at the point where an oviposition puncture is visible on the surface. Larvae will leave the fruits to pupate, and so consequently pupae may also be detected in packaging. The larva is able to jump up to a distance of 15–20 cm (Mir *et al.*, 2014).

Larvae can be reared to the adult stage for species identification. Rearing of larvae is described in White & Elson-Harris (1992). A presumptive diagnosis may be feasible on the third instar (see Section 4.1.1), and molecular tests can also be performed on larvae (see Section 4.2).

If a collected larva is to be preserved, it should be placed in boiling water for a few seconds (until it becomes

immobile) and then either transferred to 70% ethanol for morphological identification or transferred to 95% ethanol for molecular tests.

The males can be attracted with cue lure, and adults collected on traps can be used for identification.

4. Identification

Identification is commonly based on the examination of adult specimens. A protocol for DNA barcoding based on the *COI* gene is described in PM 7/129 *DNA barcoding as an identification tool for a number of regulated pests* (EPPO, 2016) and can be used for all life stages.

4.1. Morphological identification

Morphological examination requires a stereo microscope with a magnification ×10 for external examination of the adult to ×200 for examination of the larvae (for the preparation of the larvae, see part A of Appendix 1) and of the adult female's aculeus (for the preparation of the aculeus, see part B of Appendix 1). A reliable morphological identification to species level can only be made by examination of an adult specimen (either male or female) using the key presented in Table 1. A description of the larvae is also provided and may allow a presumptive diagnosis (see Section 4.1.1). Definitions and illustrations of terms used in this protocol but not specifically defined and illustrated in this protocol can be found in White & Elson-Harris, 1992.

4.1.1. Larvae

A key for the third-instar larvae is available in White & Elson-Harris (1992). This key allows identification to the genus level, but not discrimination between different species. It should be noted that in this key *Z. cucurbitae* is referred to as *Dacus cucurbitae.*

Examination of the third-instar larvae in combination with knowledge about the origin and the host, as well as the evidence provided by previously identified specimens from earlier and similar consignments, may allow a presumptive diagnosis (Balmes & Mouttet, 2017).

4.1.1.1. Description of a Tephritid larvae after Smith (1989) and Stehr (1991).

- Body cylindrical and rounded with a small tapering head, 3 thoracic and 8 abdominal segments (Fig. 1);
- Head without sclerotization but with the cephalopharyngeal skeleton partially visible by transparency (Fig. 2);
- Anterior spiracle in a lateral position on each side of the first thoracic segment (Fig. 3);
- Posterior spiracle on the surface of the last segment of the abdomen, unpigmented and without spine or lobe;
- Two posterior spiracles with 3 spiracular openings or slits, arranging more or less parallel to each other (Fig. 4).

4.1.1.2. Partial description of third larval instar of Zeugodacus (after White & Elson-Harris, 1992; Carroll et al., 2004 and Mir et al., 2014).

Size: medium to large, length 8.3–11 mm, width 1.5–2.7 mm;

Head: antenna with 2 segments. Oral ridges present with 17–23 rows of moderately long, uniform, bluntly rounded teeth. Accessory plates present and numerous;

Cephalopharyngeal skeleton: mouthhook with a small and delicate preapical tooth (Fig. 5); dental sclerite present; and parastomal bars elongate, free from hypopharyngeal sclerite;

Anterior spiracles: elevated, with 16–20 tubules in a single uniform row (Fig. 6);

Thoracic and abdominal segments: anterior portion of T1 with an encircling, broad band of spinules which dorsally and laterally form small plates 7–10 rows deep, becoming

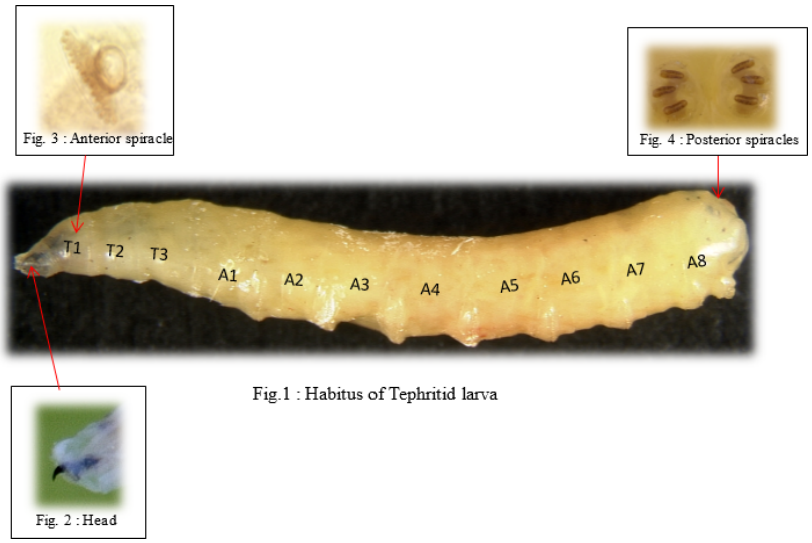

Fig.1 : Habitus of Tephritid larva

Fig. 1–4 1. Habitus of tephritid larva. 2. Head. 3. Anterior spiracles. 4. Posterior spiracles.

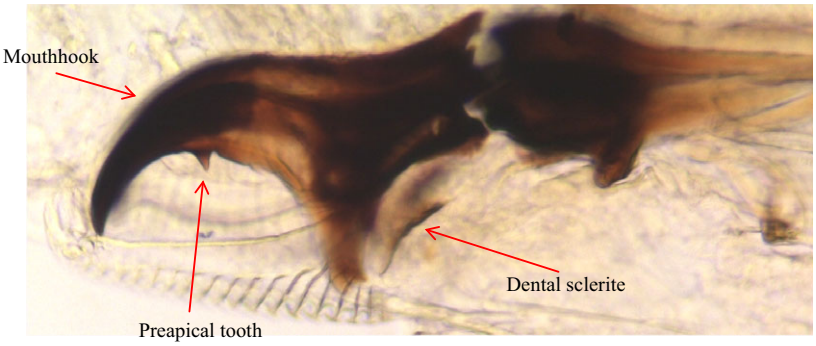

Fig. 5 Part of cephalopharyngeal skeleton.

discontinuous rows ventrally; T2 with smaller, stouter spinules, forming 5–7 discontinuous rows around anterior portion of segment; T3 similar to T2, but reduced to 4–6 rows. A8 (see Fig. 1) with pigmented transverse line (mature larvae only); tubercles and sensilla well defined;
Anal area: anal lobes plainly visible, but not strongly protuberant; simple;
Posterior spiracles: spiracular slits large, about 3 times as long as broad; spiracular hairs long, fine and often branched in apical half; number of dorsal and ventral spiracular processes 6–12; number of lateral spiracular processes 4–6.

4.1.2. Adults
4.1.2.1. Description of the adult (after Munro, 1984; Carroll et al., 2002; White, 2006 and Drew & Romig, 2013).
Medium size species (8–10 mm long for 14–16.9 mm wingspan); male smaller than female; predominantly orange-brown (Fig. 7);
Head (see Fig. 8). Structure: antenna longer than face; first flagellomere elongated, rounded apically; arista longer than first flagellomere, bare or with short rays. Chaetotaxy: ocellar seta absent or minute, setula-like; post-ocellar absent. Frontal setae: 2–3 pairs. Orbital setae: 1 pair; orbital seta reclined, acuminated. Coloration: face yellow with moderate dark round spots in each antennal furrow;
Thorax: (see Figs 9 & 10). Chaetotaxy: anterior notopleural seta present; scutum with prescutellar acrostichal and anterior supra-alar setae present (rarely absent); basal scutellar seta absent (rarely present); scutellum with one pair of apical scutellar setae present. Coloration: scutum predominantly red-brown, with medial and lateral yellow post-sutural vitta (Fig. 9); post-pronotal lobe yellow; notopleural callus yellow; lateroterga with single xanthine (area of bright yellow to orange colour) across anatergite and katatergite (Fig. 10); scutellum entirely yellow (except for basal dark margin);

Fig. 7 Habitus female.

Fig. 6 Anterior spiracle.

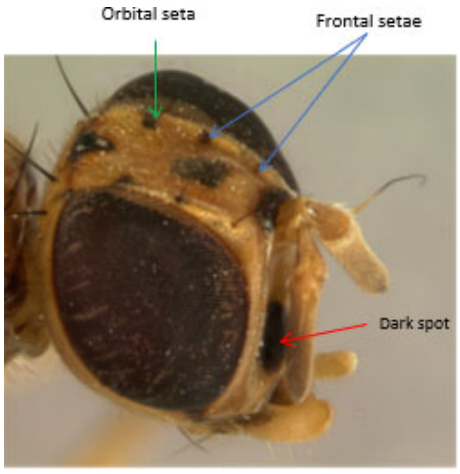

Fig. 8 Head.

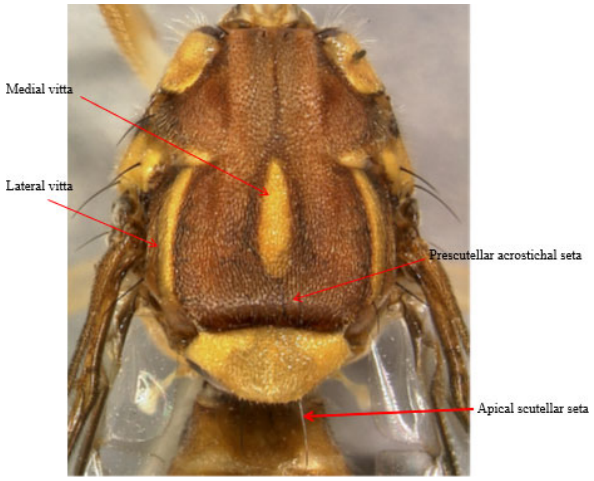

Fig. 9 Thorax dorsal view.

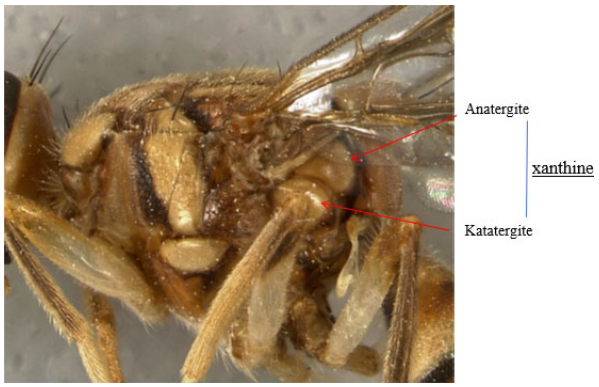

Fig. 10 Thorax lateral view.

Fig. 11 Abdomen (female).

Abdomen: tergites I–V separated (or all tergites separated); medial T-shaped mark present (Fig. 11); male with a pecten on tergite III (Fig. 12);
Aculeus: apex pointed, length 1.7 mm (Fig. 13);
Legs: coxae brownish; femora bicoloured, pale basally, red-brown on apical 1/3; tibiae and tarsi brown; metatarsi yellow;

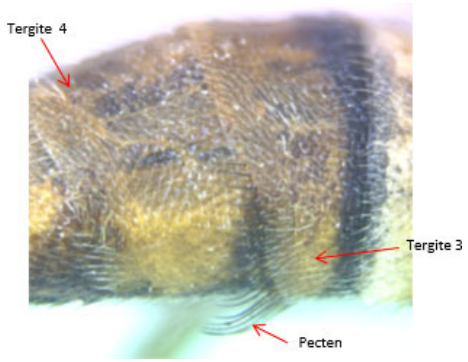

Fig. 12 Abdomen (male) with pecten.

Wing (Fig. 14): length about 6–7 mm; wing pattern dark-brown, characteristic; costal band complete, fairly deep, extending below vein R_{2+3} or to vein R_{4+5} before wing apex, apically distinctly expanded into a spot reaching about mid-depth of cell r_{4+5}; cross-band on cross-vein dm–cu; anal band present, reaching nearly to wing margin along cell *cup* extension; basal cells bc and c without extensive covering of microtrichia; cell bm without microtrichia.

4.1.2.2. Key to adults

For identification of the family Tephritidae see Oosterbroek, 2006.

4.2. Molecular methods – sequencing

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 *Z. cucurbitae*. Sequences are available in different databases those in Q-bank are curated (http://www.q-bank.eu/arthropods/). For African species, sequences are available in http://projects.bebif.be/fruitfly/index.html.

5. Reference material

Links to specimens are available in Q-bank (http://www.q-bank.eu/arthropods/) and http://projects.bebif.be/fruitfly/index.html

6. Reporting and documentation

Guidelines on reporting and documentation are given in EPPO Standard PM 7/77 *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 consultation of this database is recommended as additional information may be available there

Fig. 13 Aculeus + apex of aculeus.

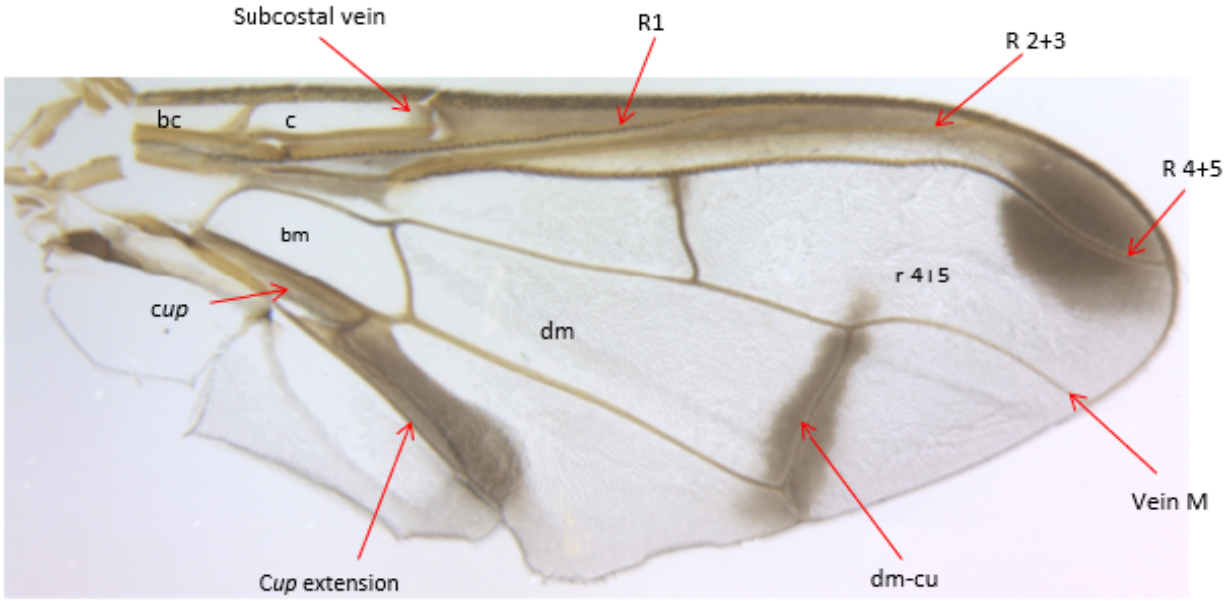

Fig. 14 Wing.

Table 1. Simplified key for the identification of the adult of *Zeugodacus cucurbitae* (after White & Elson-Harris, 1992; White, 2006) and separation from the most commonly related species detected at import (for a complete key see White, 2006)

1	Subcostal vein abruptly bent and dorsal side of vein R1 with setulae (Fig. 14)	Tephritidae 2
	Subcostal vein not abruptly bent or dorsal side of vein R1 lacks setulae	Other families
2	Cell *cup* very narrow, about half the width of cell bm (Fig. 14). Abdominal tergite 1 + 2 not longer than broad (Fig. 11)	3
	Cell *cup* broad, more than half the width of cell bm	Other Tephritidae
3	Abdominal segment not fused (Fig. 11)	4
	Abdominal segment fused	Genus *Dacus*
4	Scutum with prescutellar acrostichal setae (Fig. 9) (rarely absent). Scutellum with 1 pair of scutellar setae	5
	Scutum without prescutellar acrostichal setae. Scutellum with 1 or 2 pairs of scutellar setae	Other species
5	Laterotergite xanthine present across anatergite and katatergite (area of bright yellow to orange colour) (Fig. 10)	6
	Laterotergite xanthine confined to katatergite or absent	Other species
6	Scutum with medial *and* lateral yellow or orange vittae (Fig. 9). Face with a dark spot in each antennal furrow (Fig. 8). Wing with brownish marking along cross-vein dm–cu (Fig. 14)	*Zeugodacus cucurbitae*
	Scutum without medial *and* lateral yellow vittae	Other species

(e.g. more detailed information on analytical specificity, full validation reports, etc.).

8. Further information

Further information on this organism can be obtained from:

V. Balmès. ANSES – LSV – Unité d'Entomologie et Plantes Invasives, 755 avenue du campus d'Agropolis CS30016, 34988 Montferrier sur Lez, France. E-mail: valerie.balmes@anses.fr

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 protocol was originally drafted by V. Balmès. ANSES – LSV – Unité d'Entomologie et Plantes Invasives, CBGP 755 avenue du campus d'Agropolis CS30016, 34988 Montferrier sur Lez, France. E-mail: valerie.balmes@anses.fr

It was reviewed by the Panel on Diagnostics in Entomology.

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Appendix 1

A: preparation of larvae for observation using a stereo microscope and compound microscope with ×100 magnification (Balmes & Mouttet, 2017)

(1) Cut the anterior part of the larva with fine scissors or pins and place it in a 10% potassium solution for 1 h at room temperature or 15–20 min at between 60°C and 80°C;
(2) Put the larva in distilled water and flatten the body contents by gentle pressure with a spatula (use a mandrel with flattened fishing thread);
(3) Transfer the larva into clean distilled water for several minutes;
(4) The larva can be mounted on a slide in a drop of glycerol with a cover slip or prepared for permanent mounting.

B: preparation of aculeus for examination using stereo microscope and compound microscope with ×200 or ×400 magnification

(1) Break off the abdomen of the female and place it in a 10% potassium solution for 1 h at room temperature or 20–30 min at between 60°C and 80°C;
(2) When the abdominal sclerites are smooth enough, remove them leaving only the aculeus. Use a pin to separate the aculeus and take care to not damage the tip of the aculeus;
(3) Transfer the aculeus to distilled water for several minutes and mount on a glass in a drop of glycerol with a cover slip.