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European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes PM 7/116 (1)

Diagnostics
Diagnostic

PM 7/116 (1) *Tetranychus evansi*

Specific scope

This standard provides guidance for the identification of *Tetranychus evansi*[1].

Specific approval and amendment

Approved in 2013-09.

Introduction

Tetranychus evansi is a primary pest of Solanaceaous crops, but will also infest a variety of other hosts (Bolland *et al.*, 1998). It originates from South America (Boubou *et al.*, 2011) but has since spread, and is recorded in 39 countries (Migeon & Dorkeld, 2006-2010). In EPPO countries, it is found in the Mediterranean area: Morocco, Algeria, Tunisia, Israel, Jordan, Greece (Crete), Italy, France, Spain (including Canary Islands and Balearic Islands) and Portugal (including Madeira). The first damages where observed in Brazil (Silva, 1954), Argentina (Rossi Simons, 1961), Mauritius (Moutia, 1958; Baker & Pritchard, 1960) and the United States (Wene, 1956). *Tetranychus evansi* is currently not considered as a pest in Southern America, but can be a highly destructive plant pest in Africa (Knapp *et al.*, 2003a; Duverney & Ngueye-Ndiaye, 2005; Fiaboe *et al.*, 2006), Spain (Ferragut & Escudero, 2002) and France (Migeon *et al.*, 2009). Northward dispersion capabilities are limited by winter temperatures for this tropical and non diapausing species (Migeon *et al.*, 2009) but it could occur in glasshouses as is the case for many tropical pests. The spider mite family (Tetranychidae), at present includes 1265 described species (Migeon and Dorkeld, 2006-2010) belonging to more than 70 genera. The genus *Tetranychus* is the third largest of the family with 148 species.

Identity

Name: *Tetranychus evansi* Baker & Pritchard, 1960.
Synonyms: *Tetranychus marianae nec* McGregor, 1950 *pro parte sensu* Silva, 1954; misidentification, corrected by Moraes *et al.* 1987. *Tetranychus takafujii* Ehara & Ohashi 2002, synoymy by Gotoh *et al.*, 2009.
Taxonomic position: Arachnida: Acarina: Prostigmata: Tetranychidae.
Common names: Red tomato spider-mite (English).
EPPO code: TETREV.
Phytosanitary categorization: EPPO A2 list no. 349.

Detection

Due to their minute size (about 0.5–0.6 mm in length for an average female adult) typical of many species of Acari, spider mites can remain undetected until major plant damage occurs.

Mites live on both sides of the leaves with a slight preference for the underside and for the vicinity of the veins. Unlike others injurious *Tetranychus* species, such as *Tetranychus urticae*, this species has a relatively gregarious behaviour. Feeding causes the leaves to become chlorotic. The feeding activity of the mites causes white spots of dead parenchyma cells to appear on both leaf surfaces. Extensive silk webbing is also produced that can 'mummify' the host plant. In very heavy infestations the resultant webbing and persistent feeding activity eventually leads to the death of the host plant.

While high densities are easily detected, low densities and early infestation i.e. the appearance of small and inconspicuous white spots may remain unnoticed or be mistakenly attributed to deficiencies, virus or fungal diseases. Mites can be observed with the naked eye or better with a magnifying glass or stereomicroscope, but detection can be more difficult on very hairy leaves such as aubergines.

Mixed populations of *T. evansi* and other red or white species such as the common two-spotted spider mite *Tetranychus urticae* (Koch) can occur.

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

Photos are presented in the EPPO gallery (http://photos.eppo.int/index.php/album/99-tetranychus-evansi-tetrev-).

Identification

Specific identification requires examination of slide mounted specimens of both an adult male and female, details on the preparation of slides are given in Appendix 1.

Spider mite identification can be difficult and is reliant on a good knowledge of the group and previous experience. It is recommended (at least for a first identification or in case of doubt) that a specialist is consulted for confirmatory diagnosis or a complementary method should be performed (e.g. sequencing). An EPPO Standard PM 7/XX on *DNA barcoding as an identification tool for plant pests* is in preparation. Additional molecular information can be found in the Q-bank Arthropod database (http://www.q-bank.eu/arthropods/).

Morphological characterization

Family Tetranychidae

Colour when alive varies from green to yellow, orange and red.

The gnathosoma has a capsule-like structure, the stylophore, eversible with long slender whiplike chelicerae used for piercing parenchyma cells. Peritremes are simple or anastoming distally, arising from a pair of stigmata near the base of the stylophore. The palps are five segmented. Tarsus I and II usually have duplex setae. The ambulacrum has tenant hairs; the tarsal claws and empodia are either padlike or clawlike; the palpal tibia forms a clawlike complex with the palpal tarsus.

Family descriptions, terms explanations and key to the genera can be found in major works (Jeppson *et al.*, 1975; Gutierrez, 1985b; Meyer, 1987; Baker & Tuttle, 1994; Bolland *et al.*, 1998).

Genus Tetranychus

Identification up to genus level can be done by examination of the empodium and setae pattern.

- The empodium does not bear tenent hair and is split distally in three pairs of hairs;
- The idiosoma bears 13 pairs of dorsal setae (prodorsum 3 and opisthosoma 10);
- One pair of para-anal setae and two pairs of anal setae are present.

Species Tetranychus evansi

Tetranychid mites develop through five stages: egg, larva, protonymph, deutonymph and adult. Active stages alternate with quiescent ones: protochrysalis, deutochrysalis and teleiochrysalis.

Egg. The eggs of *T. evansi* are indistinguishable from other spider mite eggs, they are rounded, orange hyaline to whitish, becoming grey before hatching.

Larvae. Larval stage is hexapodal i.e. has three pairs of legs.

Nymph. The two nymphal stages i.e. the protonymph and deutonymph like the adults have four pairs of legs. Nymphal stages can be paler than or the same colour as the adults i.e. varying from orange to brick-red or dark red.

Adults. There are no fully comprehensive keys to all the known species of the genus *Tetranychus*. Some regional works can be useful: Baker & Tuttle (1994) for North-America and Meyer (1987) for Africa. Flechtmann & Knihinicki (2002) give a key to majors groups of the genus based on females.

Tetranychus evansi belong to the sub-genus *Tetranychus s. str.* and to the *desertorum* group that is characterized by Tarsus I having all 4 proximal tactile setae in line with proximal pair of duplex setae (3a & 3b). Within this group, the shape of the male aedeagus is the most important character used to discriminate species (Figs 5 and 6). Meyer's work on the spider mites of Africa is the most useful as it contains many of the tropical species encountered on Solanaceae, but can be difficult, particularly for the non-specialist.

Because *T. evansi* can be difficult to separate from other morphologically similar species, it has been misidentified as *T. marianae* (in particular in Brazil, Argentina and USA), or as *Tetranychus piercei* in Taiwan and synonymized with *T. takafujii* in Japan.

Morphological characters

Female. Body 500–600 µm long and 280–360 µm wide. From orange to brick-red or dark red; legs pale orange. Idiosoma with 13 pairs of dorsal setae (prodorsum 3 and opisthosoma 10) (Fig. 1), venter with 1 pair of para-anal setae and 2 pairs of anal setae (Fig. 1). Dorsohysterosomal striae longitudinal between setae e1 and between setae f1; forming a diamond shaped pattern between these two pairs of setae (Fig. 2). Peritremes hooked (Fig. 2). Tibia I with 9 tactile setae and 1 sensory seta (Fig. 3). Tarsus I with all proximal 4 tactile setae in line with proximal pair of duplex setae (Fig. 3). All empodia with 3 pairs of proximoventral hairs; empodium I with minute dorsal claw (Fig. 4c).

Male. Body 400–470 µm long and 220–290 µm wide. From yellowish pale orange to orange; legs pale orange. Tibia I with 9 tactile setae and four sensory setae. Tarsus I with 2 proximal sensory setae and 4 tactile setae just proximal to first pair of duplex setae. Empodium I with mediodorsal spur and pair of proximoventral spurs (Fig. 4a). Empodium II with mediodorsal spur as empodium I and proximoventral appendages varying from spurs as empodium I, long claws and hairs (Fig. 4). Aedeagus as Fig. 5.

Identification requires the slide preparation of both males and females specimens when possible in correct position

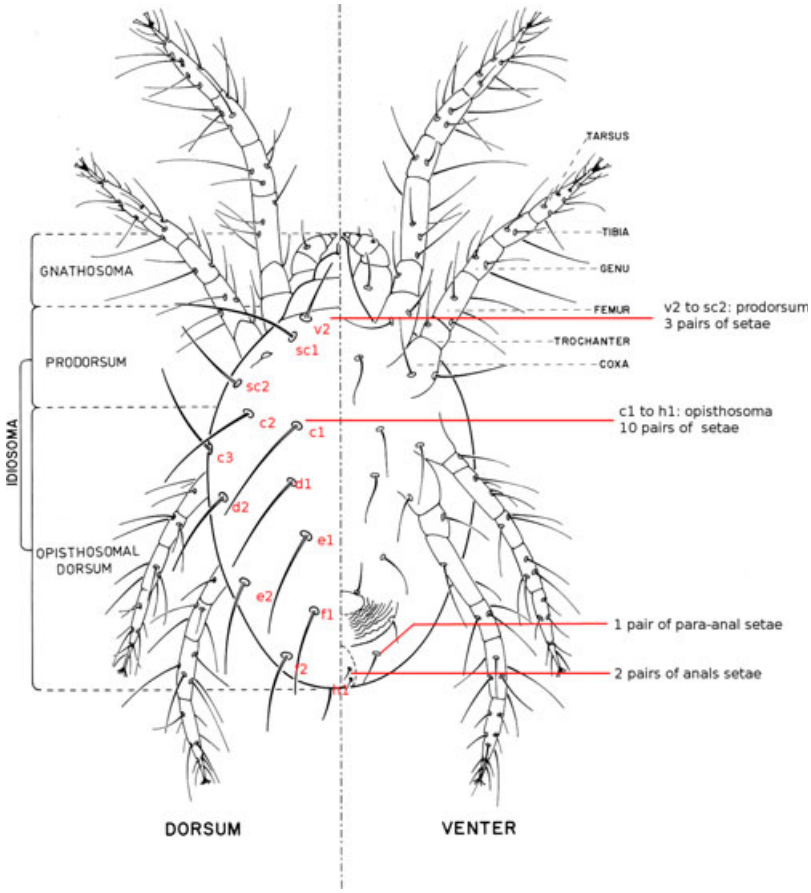

Fig. 1 *Tetranychus sp.*: dorsoventral aspect of the female showing the body setae. The characteristics of the genus are indicated in red (modified from Gutierrez, 1985).

(see Appendix 1). Genus identification can be made using Bolland *et al.*, 1998. Specific identification is made by the examination of the female, confirming the attribution to the *desertorum* group, ensuring that the main characters of this group have been seen (diamond pattern, tarsus I aligned setae). Specific determination is completed by the lateral examination of the male aedeagus which should exactly match the drawings. The use of Figs 1-5 drawings and the match of all the above characters allow the separation of *T. evansi* from closely related species. *Tetranychus evansi* is separated from the other species belonging to the desertorum group encountered in Europe by the shape of the aedeagus, as represented in Fig. 6.

Reporting and documentation

Guidance on reporting and documentation is given in EPPO Standard PM7/77 (1) *Documentation and reporting on a diagnosis*.

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 to consult this database as additional information may be available there (e.g. more detailed information on analytical specificity, full validation reports, etc.).

Further information

Further information on this organism can be obtained from:

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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.

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.

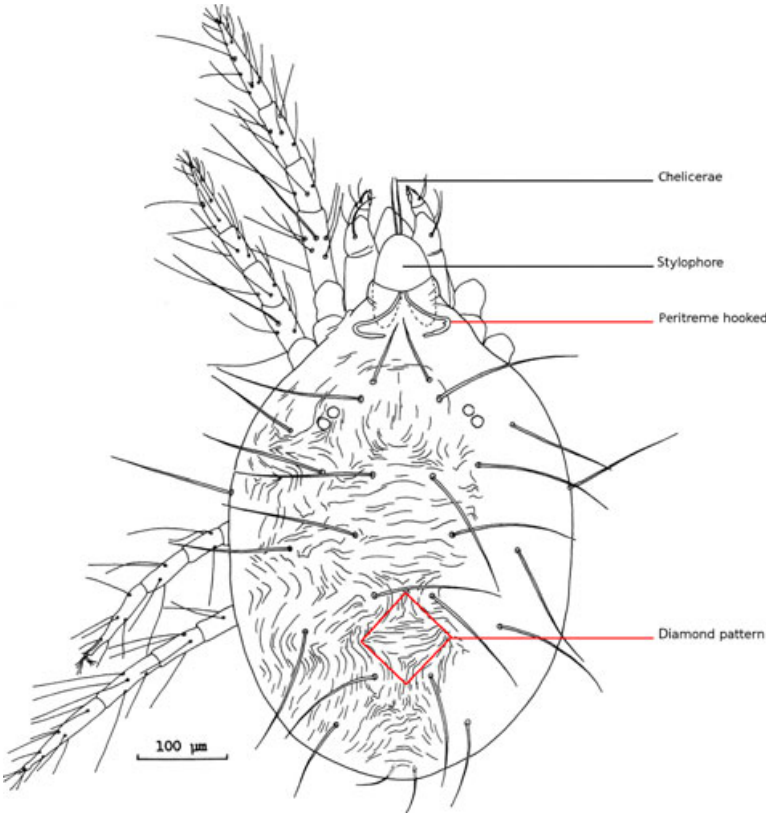

Fig. 2 *Tetranychus evansi*: dorsal aspect of the female - Japan. Characteristics of the species are indicated in red (modified from Ehara & Ohashi, 2002).

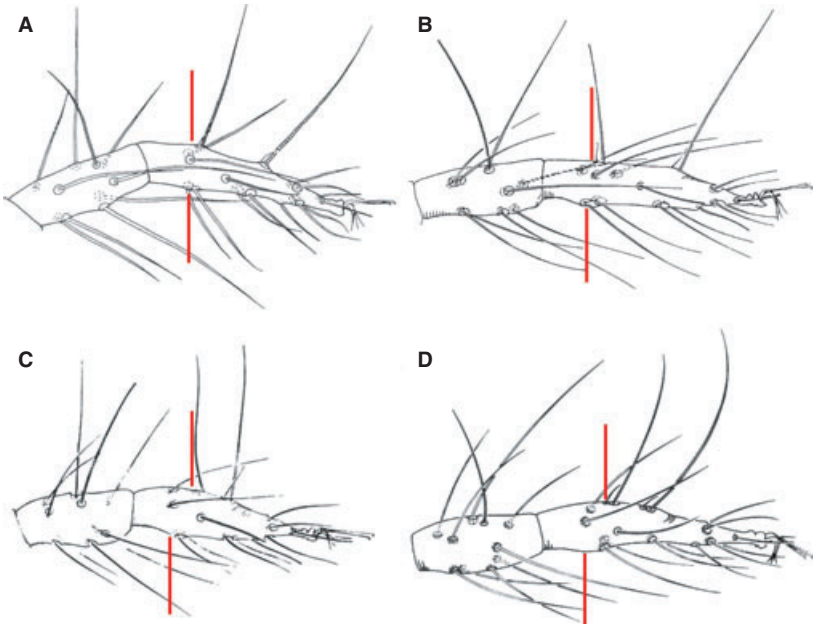

Fig. 3 *Tetranychus spp.* female tibia and tarsus I: variation of setae pattern on tarsus I. (A) *T. evansi* from Japan; (B) *T. evansi* from Mauritius; (C) *T. marianae*; (D) *T. piercei*. Red line top indicates proximal duplex setae, red line bottom indicates proximal tactile setae. (A) and (B) all proximal 4 tactile setae in line with proximal pair of duplex setae. (C) and (D) proximal pair of duplex setae distad of proximal tactile setae. (A) from Ehara & Ohashi, 2002; (B) from Baker & Pritchard, 1960; (C) from Pritchard & Baker 1955; (D) from Guttierez & Bolland, 1979).

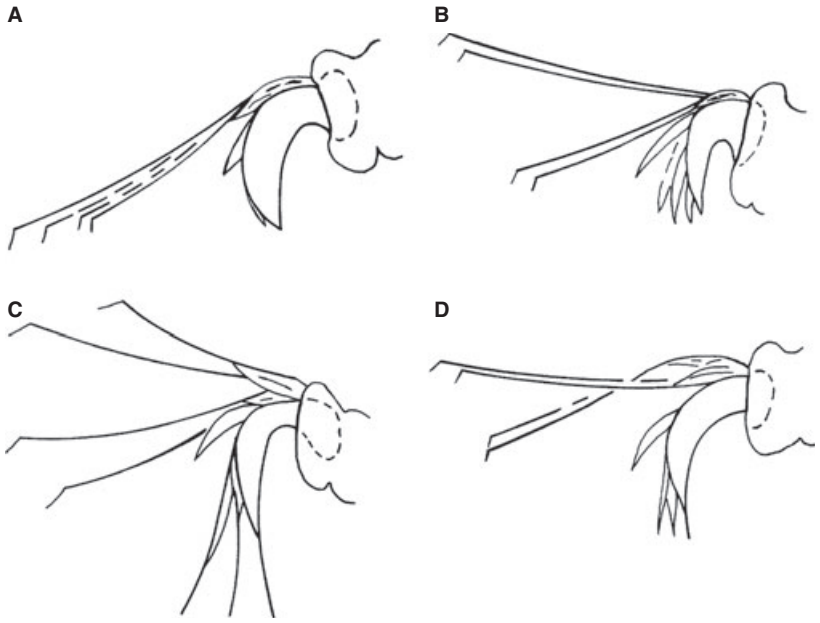

Fig. 4 *Tetranychus evansi* male: empodium II. (A–D) variation of the empodium of leg II in the species. Two differents types may be encountered on the same specimen (from Gotoh *et al.*, 2009). (4A) also corresponds to male empodium I. (4C) also corresponds to female empodium I.

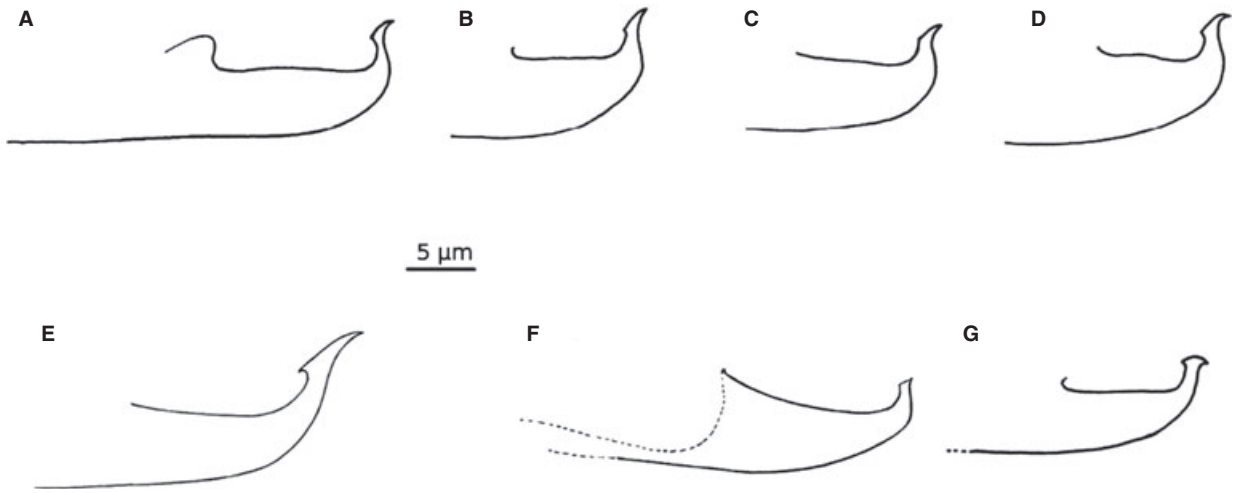

Fig. 5 *Tetranychus spp*, males aedeagus. (A–D) variation of the shape of the aedeagus of *T. evansi*; (A) from Mauritius, (B–D) from Japan; (E) *T. marianae*; (F) *T. piercei;* (G) *T. urticae*. [(A) from Baker & Pritchard, 1960; (B–D) from Ehara and Ohashi, 2002; (E) from Pritchard and Baker 1955; (F) from Guttierez and Bolland, 1979 (G) *T. urticae,* from Pritchard and Baker 1955)].

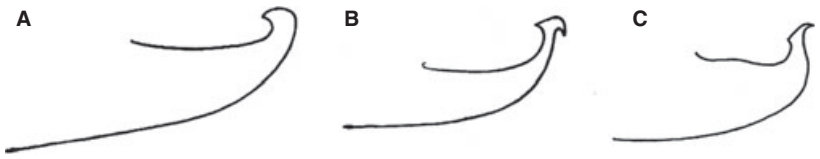

Fig. 6 Aedeagus of the *desertorum* group species found in Europe. (A) *Tetranychus ludeni*; (B) *Tetranychus desertorum*; (C) *Tetranychus evansi*.

Acknowledgements

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Appendix 1 – Preparation of specimens

Specific identification requires examination of slide mounted adult males and females. Hoyer medium (see Table 1) is traditionally used but it contains toxic chloral-hydrate.

Table 1 Hoyer's medium

Distilled H_20	50 mL
Arabic gum (acacia)	30 g
Choral hydrate	200 g
Glycerin	16 mL

Use of clearing and mounting directly for non-permanent slides in lactic acid is convenient for a rapid identification. Clearing should last 24 h at room temperature or few hours at approximately 40°C. Use a cavity ground in slide as described by Gutierrez (1985a). The cavity is made with a small tungsten carbide grindstone mounted on a drill and should be about 3 mm diameter. The use of such slides allows the orientation of the specimen in any direction inside the cavity, by sliding the cover glass back and forth. Females are examined dorso-ventrally and males laterally to observe the shape of the aedeagus. Sealing with Euparal allows the preparation to be kept for 2 or 3 years.

Dissolve Arabic gum completely in the distilled H_2O. Then, and only then, completely dissolve in Choral hydrate, then add glycerin and mix well. Medium may be diluted when needed with small amounts of distilled H_2O. Before using Hoyer's medium, let it stand undisturbed for several days in order to clarify. Store mixture at 4°C.