

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

Normes OEPP EPPO Standards

Diagnostics
Diagnostic

PM 7/57



Organisation Européenne et Méditerranéenne pour la Protection des Plantes
1, rue Le Nôtre, 75016 Paris, France

Approval

EPPO Standards are approved by EPPO Council. The date of approval appears in each individual standard. In the terms of Article II of the IPPC, EPPO Standards are Regional Standards for the members of EPPO.

Review

EPPO Standards are subject to periodic review and amendment. The next review date for this EPPO Standard is decided by the EPPO Working Party on Phytosanitary Regulations.

Amendment record

Amendments will be issued as necessary, numbered and dated. The dates of amendment appear in each individual standard (as appropriate).

Distribution

EPPO Standards are distributed by the EPPO Secretariat to all EPPO member governments. Copies are available to any interested person under particular conditions upon request to the EPPO Secretariat.

Scope

EPPO Standards on Diagnostics are intended to be used by NPPOs in their capacity as bodies responsible for the application of phytosanitary measures. Standards on diagnostic protocols are concerned with the diagnosis of individual pests and describe different methods which can be used to detect and identify pests of phytosanitary concern for the EPPO region. General Standards on diagnostics are in preparation on: (1) the purpose of diagnostic protocols (which may differ according to the circumstances of their use); and (2) reporting and documentation of diagnoses.

In 1998, EPPO started a new programme to prepare diagnostic protocols for the regulated pests of the EPPO region (including the EU). The work is conducted by the EPPO Panel on Diagnostics and other specialist Panels. The objective of the programme is to develop an internationally agreed diagnostic protocol for each regulated pest. The protocols are based on the many years of experience of EPPO experts. The first drafts are prepared by an assigned expert author(s). They are written according to a 'common format and content of a diagnostic protocol' agreed by the Panel on Diagnostics, modified as necessary to fit individual pests. As a general rule, the protocol recommends a particular means of detection or identification which is considered to have advantages (of reliability, ease of use etc.) over other methods. Other methods may also be mentioned, giving their advantages/disadvantages. If a method not mentioned in the protocol is used, it should be justified.

The following general provisions apply to all EPPO Standards on Diagnostics:

- laboratory tests may involve the use of chemicals or apparatus which present a certain hazard. In all cases, local safety procedures should be strictly followed
- 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
- laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated or that proper positive and negative controls are included.

References

- EPPO/CABI (1996) *Quarantine Pests for Europe*, 2nd edn. CAB International, Wallingford (GB).
- EU (2000) Council Directive 2000/29/EC of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. *Official Journal of the European Communities* L169, 1–112.
- FAO (1997) *International Plant Protection Convention* (new revised text). FAO, Rome (IT).
- IPPC (1993) *Principles of plant quarantine as related to international trade*. ISPM no. 1. IPPC Secretariat, FAO, Rome (IT).
- IPPC (2002) *Glossary of phytosanitary terms*. ISPM no. 5. IPPC Secretariat, FAO, Rome (IT).
- OEPP/EPPO (2003) EPPO Standards PM 1/2(12): EPPO A1 and A2 lists of quarantine pests. *EPPO Standards PM1 General phytosanitary measures*, 5–17. OEPP/EPPO, Paris (FR).

Definitions

Regulated pest: a quarantine pest or regulated non-quarantine pest.
Quarantine pest: a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled.

Outline of requirements

EPPO Standards on Diagnostics provide all the information necessary for a named pest to be detected and positively identified by an expert (i.e. a specialist in entomologist, mycology, virology, bacteriology, etc.). Each protocol begins with some short general information on the pest (its appearance, relationship with other organisms, host range, effects on host, geographical distribution and its identity) and then gives details on the detection, identification, comparison with similar species, requirements for a positive diagnosis, list of institutes or individuals where further information on that organism can be obtained, references (on the diagnosis, detection/extraction method, test methods).

Existing EPPO Standards in this series

Forty-one EPPO standards on diagnostic protocols have already been approved and published. Each standard is

numbered in the style PM 7/4 (1), meaning an EPPO Standard on Phytosanitary Measures (PM), in series no. 7 (Diagnostic Protocols), in this case standard no. 4, first version. The existing standards are:

- PM 7/1 (1) *Ceratocystis fagacearum*. *Bulletin OEPP/EPPO Bulletin* **31**, 41–44
- PM 7/2 (1) *Tobacco ringspot nepovirus*. *Bulletin OEPP/EPPO Bulletin* **31**, 45–51
- PM 7/3 (1) *Thrips palmi*. *Bulletin OEPP/EPPO Bulletin* **31**, 53–60
- PM 7/4 (1) *Bursaphelenchus xylophilus*. *Bulletin OEPP/EPPO Bulletin* **31**, 61–69
- PM 7/5 (1) *Nacobbus aberrans*. *Bulletin OEPP/EPPO Bulletin* **31**, 71–77
- PM 7/6 (1) *Chrysanthemum stunt pospiviroid*. *Bulletin OEPP/EPPO Bulletin* **32**, 245–253
- PM 7/7 (1) *Aleurocanthus spiniferus*. *Bulletin OEPP/EPPO Bulletin* **32**, 255–259
- PM 7/8 (1) *Aleurocanthus woglumi*. *Bulletin OEPP/EPPO Bulletin* **32**, 261–265
- PM 7/9 (1) *Cacoecimorpha pronubana*. *Bulletin OEPP/EPPO Bulletin* **32**, 267–275
- PM 7/10 (1) *Cacysreus marshalli*. *Bulletin OEPP/EPPO Bulletin* **32**, 277–279
- PM 7/11 (1) *Frankliniella occidentalis*. *Bulletin OEPP/EPPO Bulletin* **32**, 281–292
- PM 7/12 (1) *Parasaissetia nigra*. *Bulletin OEPP/EPPO Bulletin* **32**, 293–298
- PM 7/13 (1) *Trogoderma granarium*. *Bulletin OEPP/EPPO Bulletin* **32**, 299–310
- PM 7/14 (1) *Ceratocystis fimbriata* f. sp. *platani*. *Bulletin OEPP/EPPO Bulletin* **33**, 249–256
- PM 7/15 (1) *Ciborinia camelliae*. *Bulletin OEPP/EPPO Bulletin* **33**, 257–264
- PM 7/16 (1) *Fusarium oxysporum* f. sp. *albedinis*. *Bulletin OEPP/EPPO Bulletin* **33**, 265–270
- PM 7/17 (1) *Guignardia citricarpa*. *Bulletin OEPP/EPPO Bulletin* **33**, 271–280
- PM 7/18 (1) *Monilinia fructicola*. *Bulletin OEPP/EPPO Bulletin* **33**, 281–288
- PM 7/19 (1) *Helicoverpa armigera*. *Bulletin OEPP/EPPO Bulletin* **33**, 289–296
- PM 7/20 (1) *Erwinia amylovora*. *Bulletin OEPP/EPPO Bulletin* **34**, 159–172
- PM 7/21 (1) *Ralstonia solanacearum*. *Bulletin OEPP/EPPO Bulletin* **34**, 173–178
- PM 7/22 (1) *Xanthomonas arboricola* pv. *corylina*. *Bulletin OEPP/EPPO Bulletin* **34**, 179–182
- PM 7/23 (1) *Xanthomonas axonopodis* pv. *dieffenbachiae*. *Bulletin OEPP/EPPO Bulletin* **34**, 183–186
- PM 7/24 (1) *Xylella fastidiosa*. *Bulletin OEPP/EPPO Bulletin* **34**, 187–192
- PM 7/25 (1) *Glomerella acutata*. *Bulletin OEPP/EPPO Bulletin* **34**, 193–200
- PM 7/26 (1) *Phytophthora cinnamomi*. *Bulletin OEPP/EPPO Bulletin* **34**, 201–208
- PM 7/27 (1) *Puccinia horiana*. *Bulletin OEPP/EPPO Bulletin* **34**, 209–212
- PM 7/28 (1) *Synchytrium endobioticum*. *Bulletin OEPP/EPPO Bulletin* **34**, 213–218
- PM 7/29 (1) *Tilletia indica*. *Bulletin OEPP/EPPO Bulletin* **34**, 219–228
- PM 7/30 (1) *Beet necrotic yellow vein benyvirus*. *Bulletin OEPP/EPPO Bulletin* **34**, 229–238
- PM 7/31 (1) *Citrus tristeza closterovirus*. *Bulletin OEPP/EPPO Bulletin* **34**, 239–246
- PM 7/32 (1) *Plum pox potyvirus*. *Bulletin OEPP/EPPO Bulletin* **34**, 247–256
- PM 7/33 (1) *Potato spindle tuber pospiviroid*. *Bulletin OEPP/EPPO Bulletin* **34**, 257–270
- PM 7/34 (1) *Tomato spotted wilt tospovirus*. *Bulletin OEPP/EPPO Bulletin* **34**, 271–280
- PM 7/35 (1) *Bemisia tabaci*. *Bulletin OEPP/EPPO Bulletin* **34**, 281–288
- PM 7/36 (1) *Diabrotica virgifera*. *Bulletin OEPP/EPPO Bulletin* **34**, 289–294
- PM 7/37 (1) *Thaumetopoea pityocampa*. *Bulletin OEPP/EPPO Bulletin* **34**, 295–298
- PM 7/38 (1) *Unaspis citri*. *Bulletin OEPP/EPPO Bulletin* **34**, 299–302
- PM 7/39 (1) *Aphelenchoides besseyi*. *Bulletin OEPP/EPPO Bulletin* **34**, 303–308
- PM 7/40 (1) *Globodera rostochiensis* and *Globodera pallida*. *Bulletin OEPP/EPPO Bulletin* **34**, 309–314
- PM 7/41 (1) *Meloidogyne chitwoodi* and *Meloidogyne fallax*. *Bulletin OEPP/EPPO Bulletin* **34**, 315–320

Some of the Standards of the present set result from a different drafting and consultation procedure. They are the output of the DIAGPRO Project of the Commission of the European Union (no. SMT 4-CT98-2252). This project involved four ‘contractor’ diagnostic laboratories (in England, Netherlands, Scotland, Spain) and 50 ‘inter-comparison’ laboratories in many European countries (within and outside the European Union), which were involved in ring-testing the draft protocols. The DIAGPRO project was set up in full knowledge of the parallel activity of the EPPO Working Party on Phytosanitary Regulations in drafting diagnostic protocols, and covered regulated pests which were for that reason not included in the EPPO programme. The DIAGPRO protocols have been approved by the Council of EPPO as EPPO Standards in series PM 7. They will in future be subject to review by EPPO procedures, on the same terms as other members of the series.

Diagnostics¹

Diagnostic

Trioza erytrae

Specific scope

This standard describes a diagnostic protocol for *Trioza erytrae*.

Specific approval and amendment

Approved in 2004-09.

Introduction

Trioza erytrae is a plant sap-sucking hemipteran insect. It is a serious pest of *Citrus* spp., particularly lime (*Citrus aurantiifolia*) and lemon (*Citrus limon*) in eastern and southern Africa (Annecke & Moran, 1982), and also mandarin (*Citrus reticulata*) in the Cameroon (Tamesse & Messi, 2000, 2002). It is the principal vector of the African form of 'citrus greening disease' caused by the bacterium *Liberobacter africanum* (EPPO/CABI, 1997; Tamesse *et al.*, 1999); it is the only vector of this disease in South Africa (van den Berg, 1999). Under experimental conditions, *T. erytrae* can also transmit *L. asiaticum*, which causes the Asian form of the disease (Massonie *et al.*, 1976). *T. erytrae* feeds on several native African plants in the *Rutaceae*, including *Vepris undulata*, *Clausena anisata* and *Fagara capensis* (Annecke & Moran, 1982), and on *Casimiroa edulis* from Central America (Fernandes & Franquinho Aguiar, 2001). *T. erytrae* occurs widely in sub-Saharan Africa and in Madagascar, Mauritius, Réunion, Saint Helena, Saudi Arabia and Yemen (EPPO/CABI, 1998). It has spread throughout Madeira (Portugal) since it was first detected in 1994 and has been found at one locality in Porto Santo Island (Portugal) (EPPO/CABI, 1997; Fernandes & Franquinho Aguiar, 2001). It was first detected in Tenerife and La Gomera (Islas Canarias, Spain) in 2002 (Pérez Padrón & Carnero Hernández, 2002).

Identity

Name: *Trioza erytrae* (Del Guercio)

Synonyms: *Aleurodes erytrae* Del Guercio; *Spanioza erytrae* Del Guercio; *Spanioza eritreae* Del Guercio; *Spanioza erythrae* Del Guercio; *Trioza citri* Laing; *Trioza erythrae* (Del Guercio); *Trioza erytrae* (Del Guercio); *Trioza merwei* Pettey

Taxonomic position: *Insecta: Hemiptera: Sternorrhyncha: Psylloidea: Triozidae*

EPPO computer code: TRIZER

Phytosanitary categorization: EU Annex designation I/A1; EPPO A1; CPPC; OIRSA

Detection

Adult *T. erytrae* are light brown, about 4 mm in length, with large wings and clearly outlined veins. Males are smaller than females and have a blunt tip to the abdomen, the latter ending in a sharp point in females. They fly well, and often jump and fly when disturbed (Annecke & Moran, 1982). When feeding, adults take up a distinctive stance, with the abdomen raised at an angle of about 35° to the feeding surface (Hollis, 1984).

The eggs are yellow or orange, cylindrical, with an upturned, sharp, anterior point. Each egg has a short stalk, which is inserted into the plant tissue. They are laid on leaf margins and along the midribs of young, tender, actively growing foliage (Annecke & Moran, 1982). There are five nymphal instars. The nymphs are dorso-ventrally flattened with a distinct marginal fringe of white, waxy filaments and vary in colour from yellow, olive-green to dark grey. They are largely sedentary and form conspicuous colonies, settling on the underside of young leaves where, after a few days of feeding, they produce distinctive cup-shaped or pit-like, open galls.

T. erytrae can cause severe leaf distortion, curling, stunting, galling and chlorosis. The leaves may also be dusted with faecal pellets. Since *T. erytrae* transmits citrus greening disease, the disease symptoms may indicate the presence of the insect. The disease causes irregular yellow mottling of foliage; the veins are often prominent and yellow. Fruits are under-developed, lopsided, poorly coloured, fail to ripen and may taste bitter (Annecke & Moran, 1982). Affected trees show open growth, stunting, dieback, sparse yellow foliage, severe fruit drop and progressive decline (EPPO/CABI, 1997).

¹The Figures in this Standard marked 'Web Fig.' are published on the EPPO website www.eppo.org.

Plants for planting of citrus from infected areas can carry eggs or nymphs, but movement on fruits is unlikely. Late-instar nymphs, as well as the adults derived from these nymphs, are capable of transmitting *L. africanum* to citrus (EPPO/CABI, 1997).

T. erytreae can be confused with *Diaphorina citri*, the principal vector of citrus greening disease in Asia. However, *D. citri* belongs to the family *Psyllidae* and is easily separated from *T. erytreae* using the diagnostic characters given below for the *Triozidae*. The geographical range of the two species did not originally overlap, but they now occur together in Mauritius, Réunion and Saudi Arabia.

Identification

Adult and immature *Psylloidea* require mounting on glass microscope slides and examination under a high-power microscope ($\times 100$ to $\times 400$) in order to see all the diagnostic characters. A method of slide preparation for adult and immature *Psylloidea* is given in Appendix I. The morphological terminology used here follows that of Hollis (1984). *T. erytreae* has been studied in detail by Hollis (1984) in a revision of the Afrotropical *Triozidae* (including a diagnostic key). Most of the taxonomic information presented below has been taken from this major work. All the Figures, except Web Fig. 24, are reproduced from Hollis (1984) with permission of the editor of the *Bulletin of the British Museum (Natural History), Entomology*.

Morphological identification of *T. erytreae* is difficult due to the homogeneity of the species-group to which it belongs (see discussion below). Only adult males can be identified on morphology alone. However, it is important to note that *T. erytreae* is the only trioizid species in the Afrotropical region recorded feeding and galling on *Citrus* and other *Rutaceae* (the immatures produce characteristic cup-shaped, open galls on the under surface of the leaves). Therefore, fifth-instar nymphs and adult females found feeding on *Citrus* material imported from Africa that match the morphological descriptions given below can be identified with confidence as *T. erytreae*. Earlier nymphal trioizid instars found feeding on African *Citrus* can be strongly suspected to be *T. erytreae*.

Family *Triozidae*

Adult *Triozidae* may be separated from all other families of *Psylloidea* by the venation and structure of the forewing (Web Figs 1 and 8): forewing without a costal break; R1 unbranched and pterostigma absent; *M + Cu* stem absent or very short so that *R + M + Cu* stem branches into its component veins at approximately one point; R_s not fused to *M* stem at any point (Hollis, 1984). Fifth instar larvae of most species of *Triozidae* (including *T. erytreae*) may be recognized by the presence of a complete fringe of wax-producing sectaetae (Web Fig. 15). There are 48 genera assigned to the *Triozidae* but many are poorly defined. Hollis (1984) provides a table listing all genera with type-species, number of species, distribution and host plant data.

Genus *Trioza*

There are 389 species assigned to *Trioza* but the genus is in need of revision. It may be separated from other Afrotropical genera using the following suite of characters: medium suture of vertex present and normally complete (Web Fig. 2); enal cones, when present, not constricted basally (Web Fig. 2); propleural suture diagonal, episternum enlarged, epimeron reduced, displaced ventrally and not in contact with lateral margins of pronotum (Web Fig. 6); forewing shape mostly elongate elipsoid and narrowing to a subangular apex, if rounded apex then more than 2–3 times longer than wide (Web Figs 1 and 8); radular areas present only in cells m_1 , m_2 and cu_1 (Web Figs 1 and 8); claval suture reaching hind margin of wing some distance from apex of Cu_{1b} (Web Figs 1 and 8); ventral sense organs of hind femur in median position (Web Fig. 10); basal tarsal segment of hind leg without apical spurs; male proctiger unipartite (Web Figs 14 and 20). There are 53 species of *Trioza* recorded from the Afrotropical Region.

Erytreae-group

T. erytreae is part of a complex of species, all of which are difficult to define morphologically, but which have discrete host plant preferences. Hollis (1984) included 10 species in the *erytreae*-group but there is no single character, which will delimit them from other *Trioza* species, and the grouping may be artificial. The description of *T. erytreae* below serves to define the group. The species, together with their host plants and distribution are listed in Table 1, and the key in Table 2 can be used to separate species within the group. An adult male is required for identification of most species.

Male genitalia characters can be used to separate *T. erytreae* (Web Figs 12 and 13) from *T. gregoryi* (Web Figs 17 and 18), *T. kilimanjarica* (Web Fig. 19), *T. carvalhoi* (Web Figs 21 and 22) and *T. eafra* (Web Fig. 23) but not from the other species in the group. *T. tiliacora* is easily separated as it has setae on all abdominal tergites and a relatively broader forewing; *T. capeneri* has a lower cu_1 cell value and a relatively longer hindwing; *ata* has two pairs of setae and a relatively shorter ultimate rostral segment. It is extremely difficult to separate *T. catlingi* and *T. menispermicola* from *T. erytreae*: in *T. catlingi* the first flagellomere is longer (head width to length of 1st flagellomere 1.06–1.23); in *T. menispermicola* the marginal sectaetae are less dense. However, *T. erytreae* is the only trioizid species in the Afrotropical region recorded feeding on *Citrus* and other *Rutaceae*.

Species *Trioza erytreae*

Morphological description

Adult (Web Figs 2–14): **integument** sparsely covered with short setae; **head** (Web Figs 2, 3 and 6), in profile, almost at 90° to longitudinal axis of body (Web Fig. 6), from above almost as wide as mesoscutum; occipital margin rounded; vertex pentagonal with anterior margin deeply incised by median suture, rounded down to frons, lateral ocelli on outer sides of

Table 1 The *Trioza erytrae* group

| Species | Host plant family | Host plant species | Distribution |
|------------------------------|-------------------|--|--|
| <i>ata</i> Hollis | Salicaceae | <i>Salix safsaf</i> | Angola, Tanzania |
| <i>capeneri</i> Hollis | Araliaceae | <i>Seemannaralia gerrardii</i> | South Africa |
| <i>carvalhoi</i> Hollis | Araliaceae | <i>Cussonia angolensis</i> ; <i>C. paniculata</i> ; <i>C. spicata</i> | Kenya, Angola, South Africa, Swaziland |
| <i>catlingi</i> Hollis | Menispermaceae | <i>Cissampelos torulosa</i> , <i>Stephananis abyssinica</i> | Kenya, Tanzania, South Africa |
| <i>eafra</i> Hollis | Araliaceae | <i>Cussonia spicata</i> | Kenya, Tanzania |
| <i>erytrae</i> (Del Guercio) | Rutaceae | <i>Casimiroa edulis</i> , <i>Clausena anisata</i> , <i>Citrus</i> spp., <i>Fagara capensis</i> , <i>Vepris undulata</i> | Tropical and South Africa, São Tomé, St Helena, Réunion, Madagascar |
| <i>gregoryi</i> Hollis | Unknown | | Nigeria, Burundi, Tanzania |
| <i>kilimanjarica</i> Hollis | Unknown | | Tanzania |
| <i>menispermicola</i> Hollis | Menispermaceae | <i>Cissampelos owariensis</i> , <i>Triclisia macrophylla</i> | Ghana, Nigeria |
| <i>tiliacora</i> Hollis | Menispermaceae | <i>Tiliacora</i> | Tanzania |

Table 2 Key to adult *Trioza erytrae* group (adapted from Hollis, 1984)

| | |
|--|--|
| 1. Forewing membrane with spinules in addition to radular surface | <i>eafra</i> |
| – Forewing membrane devoid of spinules apart from radular areas | 2 |
| 2. All visible abdominal tergites with a transverse row of setae; setae on wing veins twice as long as width of veins | <i>tiliacora</i> |
| – Transverse row of setae present only on first two visible abdominal tergites; setae on wing veins shorter than width of veins | 3 |
| 3. Ratio of head width to length of ultimate rostral segment 4.6 : 1 or more | 4 |
| – Ratio of head width to length of ultimate rostral segment 4.5 : 1 or less | 5 |
| 4. Forewing length less than 2.6 mm in male and 2.9 mm in female; paramere broadening towards apex which is truncate | <i>kilimanjarica</i> |
| – Forewing length more than 3.0 mm in male and 3.3 mm in female; paramere broad medially but narrowing to subacute apex | <i>ata</i> |
| 5. cu_1 cell value not more than 2.4 in male and 2.45 in female; forewing, at most, 1.58 times longer than hindwing | 6 |
| – cu_1 cell value not less than 2.55; forewing, at least 1.59 times longer than hindwing | 7 |
| 6. Male paramere conical, in profile narrowing towards apex which is abcurved; proctiger broader than long due to strong lateral expansions | <i>carvalhoi</i> |
| – Male paramere ovoid, in profile broadening medially then narrowing towards apex; proctiger narrower with less-developed lateral lobes | <i>capeneri</i> |
| 7. Male paramere and aedeagus as in Web Figs 17 and 18 | <i>gregoryi</i> |
| – Male paramere and aedeagus as in Web Figs 12 and 13 | 8 |
| 8. Ratio of head width to length of 1st flagellomere not more than 1.25 : 1 | <i>catlingi</i> |
| – Ratio of head width to length of 1st flagellomere not less than 1.26 : 1 | <i>erytrae</i> and <i>menispermicola</i> |

raised tubercles, a shallow concavity present on either side of median suture; median ocellus not visible in dorsal view; frons completely covered by genae in anterior view; genal cones well developed, elongate conical with rounded apices (Web Fig. 2); antennal flagellum (Web Fig. 5) 2.08–2.81 times longer than head width, head width to length of 1st flagellum in male 1.26–1.70, in female 1.30–1.82; a single rhinarium present subapically on flagellomeres 2, 4, 6 and 7, apical flagellomere with a long pointed seta and a truncate seta apically; clypeus with a pair of setae (Web Fig. 3), ultimate rostral segment with two pairs of setae (Web Fig. 4); **thorax** (Web Figs 6 and 7), strongly arched; pronotum just visible from above, in profile strongly rounded down behind occiput. Mesopraescutum (Web Fig. 7) about as wide as long, its anterior margin strongly arcuate in dorsal view, in profile strongly down curved to pronotum; **forewing** (Web Fig. 8), hyaline, elongate oval and narrowing to a rounded rectangular apex, 2.79–3.09 times longer than wide, radular areas elongate triangular, remainder of membrane devoid of spinules; veins bearing short setae, *R* branch acutangular, *M* branch distal to *Rs-Cu_{1a}* line, *Cu* stem 2.75–4.20 times longer than *Cu_{1b}*, *m₁* cell value 1.10–1.38, *cu₁*

cell value 2.56–3.71; forewing 1.59–1.82 times longer than hindwing, costal margin of hindwing with up to two setae proximal to costal break, setae distal to costal break clearly divided into two groups; **hind leg** (Web Figs 9 and 10), coxa with a well-defined meracanthus and without anterior lobe; tibia with a moderately developed basal spine, with one outer and three (rarely two) inner apical spurs; **abdomen** (Web Figs 11–14), with setae on tergites 2 and 3 in male, and 3 and 4 in female; male proctiger (Web Fig. 14) with a laterally expanded basal part and a very short and narrow apical part; paramere as in Web Fig. 12; apical segment of aedeagus simple (Web Fig. 13); female genital segment (Web Fig. 11) short, conical, subgenital plate with a ventral bulge, ventral valves of ovipositor weakly serrate apically; **measurements** (mm) – aximum width of head, male 0.37–0.40, female 0.38–0.46; length of antennal flagellum, male 0.85–1.10, female 0.83–1.10; length of ultimate rostral segment, male 0.09–0.10, female 0.09–0.11; length of forewing, male 2.40–2.96, female 2.61–3.46; length of hind tibia, male 0.50–0.62, female 0.48–0.62.

Fifth instar larva (Web Figs 15 and 16): dorsal surface outline oval, about 1.5 times longer than wide (Web Fig. 15).

Antenna with 4–5 flagellomeres. Cephalothorax separate from rest of thorax, which is entire. Forewing pad about 0.8 mm long, humeral lobe extending forward beyond anterior margin of eye. Caudal plate about 0.65 times as long as wide, anus ventral and distinct from posterior margin of abdomen, anus and pore ring as in Web Fig. 16. Truncate tubular sectasetae forming a dense, entire marginal fringe, postocular seta absent, sectasetae absent from dorsum.

Reporting and documentation

Guidance on reporting and documentation is given in EPPO Standard PM7/– (in preparation).

Further information

Further information on this organism can be obtained from: J. Martin, Natural History Museum, London SW7 (UK).

Acknowledgements

This protocol was originally drafted by C. Malumphy, Central Science Laboratory, York (GB).

References

- Annecke DP & Moran VC (1982) *Insects and Mites of Cultivated Plants in South Africa*. Butterworth, Durban (ZA).
- EPPO/CABI (1997) *Trioza erytreae* and Citrus greening bacterium. *Quarantine Pests for Europe*, 2nd edn, pp. 547–550 and pp. 971–976. CAB International, Wallingford (GB).
- EPPO/CABI (1998) Map no. 151 *Trioza erytreae*. *Distribution Maps of Quarantine Pests for Europe*. CAB International, Wallingford (GB).
- Fernandes A & Franquinho Aguiar AM (2001) Development of quarantine pests *Toxoptera citricida* and *Trioza erytreae* in the Archipelago of Madeira. *Boletín de Sanidad Vegetal, Plagas* **27**, 51–58.
- Hollis D (1984) Afrotropical jumping plant lice of the family Triozidae (Homoptera: Psylloidea). *Bulletin of the British Museum (Natural History), Entomology* **49**, 1–102.
- Massonie G, Garnier M & Bové JM (1976) Transmission of Indian citrus decline by *Trioza erytreae*, the vector of South African greening. *Proceedings of the Seventh Conference of the International Organization of Citrus Virologists*, pp. 18–20. University of California, Riverside (US).
- Pérez Padrón F & Carnero Hernández A (2002) [Presence of *Trioza erytreae*, the African citrus psyllid, on the island of Tenerife.]. *Revista Granja* **9**, 54–57 (in Spanish).
- Tamesse JL & Messi J (2000) Réceptivité à *Trioza erytreae* de variétés d'agrumes au Cameroun. *Fruits* **55**, 389–400.
- Tamesse JL & Messi J (2002) Incidence de *Trioza erytreae*, psylle vecteur du greening, sur la sensibilité des plantules d'agrumes dans une pépinière au Cameroun. *Insect Science and its Application* **22**, 97–103.
- Tamesse JL, Messi J, Nguyen TX & Quilici S (1999) Présence de *Trioza erytreae*, le psylle des agrumes, dans les principales zones éocoimatiques du Cameroun. *Fruits* **54**, 311–321.
- van den Berg M (1999) Measures to reduce citrus psylla populations and the spread of greening disease. *Nelropika Bulletin* **303**, 5–6.
- Williams DJ & Watson G (1988) *The Scale Insects of the Tropical South Pacific Region. Part 1. The Armoured Scales (Diaspididae)*. CAB International, Wallingford (GB).

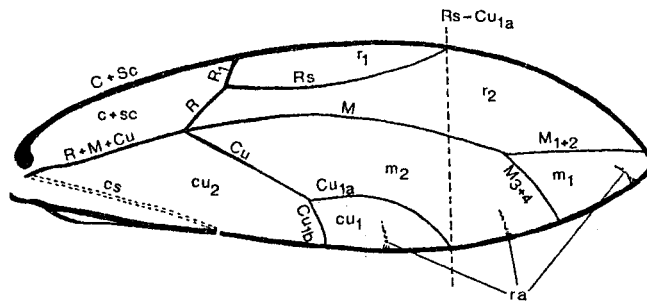
Appendix I

Permanent microscope slide preparation of jumping plant lice

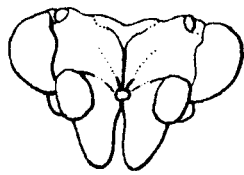
Williams & Watson (1988) describe the following method for the slide preparation of diaspidids.

Place specimens into approximately 2 mL of 70% ethanol in a watch glass, cover with a glass square and heat gently to simmering point for a few minutes. Pipette off the excess alcohol. Add approximately 2 mL of 10% potassium hydroxide (KOH) and heat to simmering point for approximately 5–10 min, or until the specimens lose most of their body colour (sclerotic patterning is retained). Pipette off the excess KOH. Soak the specimens in about 2 mL of cold distilled water or 70% ethanol for a minimum of 10 min. Pipette off the liquid. Rinse the specimens in about 2 mL cold glacial acetic acid. Pipette off the liquid. Add a few drops of chloral phenol (100 g chloral hydrate; 20 mL glucose syrup 50% w/w; 160 g phenol), a wax solvent. Gently heat for a minimum of 15 min until the specimens have cleared. The length of time required depends upon how waxy the specimens are. Carbol xylene may alternatively be used.

Dissect adult specimens carefully into the following separate parts: head, including antennae; prothorax with forelegs, dorsal mesothorax with forewings; dorsal metathorax with hindwings; abdomen; hind legs; venter of metathorax; venter of mesothorax with middle legs; clypeus with rostrum. Dissection of immature specimens is unnecessary. Pipette off the chloral phenol. Rinse the specimens with fresh glacial acetic acid. Pipette off the liquid. Add fresh glacial acetic acid to the specimens and leave for 5 min. This is again pipetted off. Add a few drops of clove oil, enough to allow the specimens to float freely, and leave for at least 10 min while the specimens clear. Using a fine brush, transfer a single dissected adult or several immature specimens to a clean glass slide. The constituent parts of the adult should be arranged in the pattern shown in Web Fig. 24, to aid quick scanning of the slide when comparing specimens. The abdomen should be mounted laterally. Some immature specimens should be mounted dorsal surface uppermost, others with the ventral surface uppermost. Absorb excess clove oil with the rolled corner of a tissue. Apply a tiny drop of dilute Canada balsam to the specimens on the slide. Arrange the specimens and leave for 5 min partially to dry, so that they are anchored in position. Add a second drop of Canada balsam so that there is sufficient depth of mountant to prevent distortion of the specimen (particularly the adult head and genitalia). Apply coverslip. Label and place in a drying oven for approximately two months.



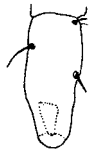
Web Fig.1. Triozidae forewing structures, showing vein and cell nomenclature



Web Fig. 2. *Trioza erytrae*, head anterior view



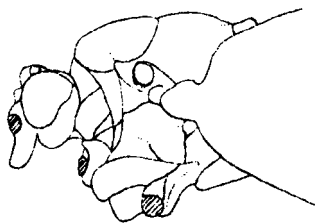
Web Fig. 3. *Trioza erytrae*, clypeus lateral view



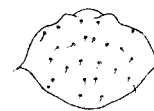
Web Fig. 4. *Trioza erytrae*, ultimate rostral segment



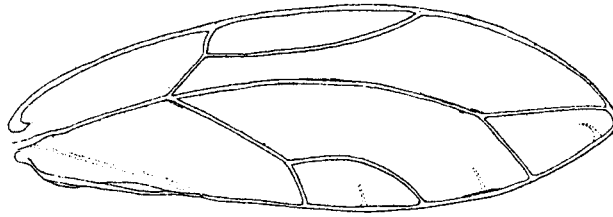
Web Fig. 5. *Trioza erytrae*, antenna flagellum



Web Fig. 6. *Trioza erytrae*, head and thorax, lateral view



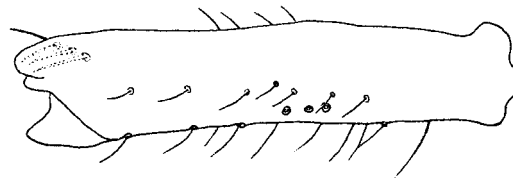
Web Fig. 7. *Trioza erytrae*, mesopraescutum, dorsal view



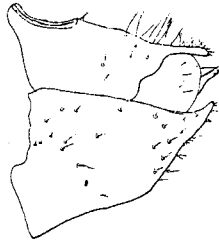
Web Fig. 8. *Trioza erytreae*, forewing



Web Fig. 9. *Trioza erytreae*, apex of hind tibia



Web Fig. 10. *Trioza erytreae*, hind tibia, posteroventral view



Web Fig. 11. *Trioza erytreae*, female genital plate



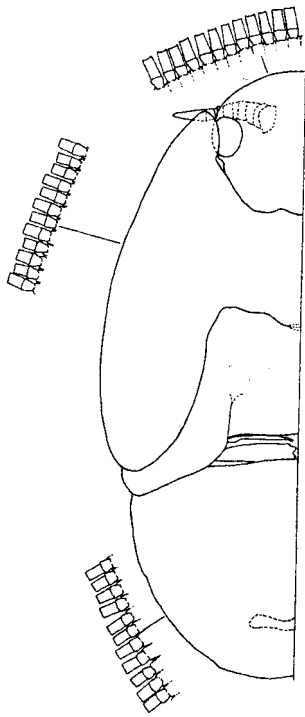
Web Fig. 12. *Trioza erytreae*, paramere



Web Fig. 13. *Trioza erytreae*, apical segment of aedeagus



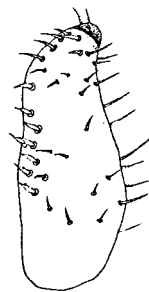
Web Fig. 14. *Trioza erytreae*, proctiger lateral view



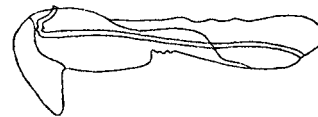
Web Fig. 15. *Trioza erytrae*, fifth-larva instar



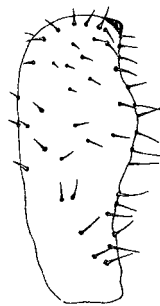
Web Fig. 16. *Trioza erytrae*, anal-pore area



Web Fig. 17. *Trioza gregoryi*, paramere



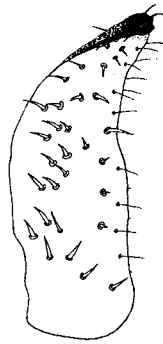
Web Fig. 18. *Trioza gregoryi*, apical segment of aedeagus



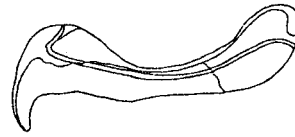
Web Fig. 19. *Trioza kilimanjarica*, paramere



Web Fig. 20. *Trioza carvalhoi*, female genital plate



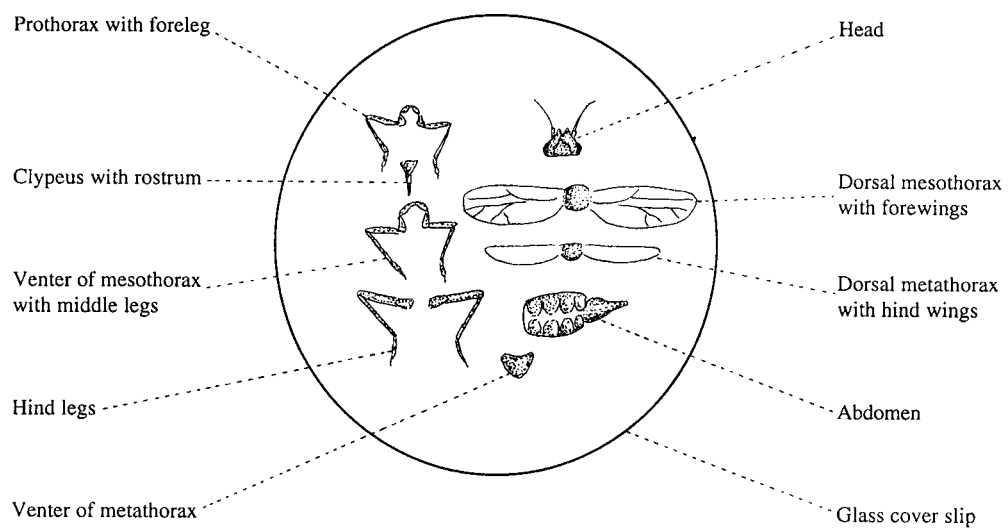
Web Fig. 21. *Trioza carvalhoi*, paramere



Web Fig. 22. *Trioza carvalhoi*, apical segment of aedeagus



Web Fig. 23. *Trioza eafra*, paramere



Web Fig. 24. Arrangement of dissected psyllid adult on a microscope slide