

Normes OEPP EPPO Standards

Diagnostics
Diagnostic

PM 7/52



Organisation Européenne et Méditerranéenne pour la Protection des Plantes
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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) *Cacysus 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

Diaphorina citri

Specific scope

This standard describes a diagnostic protocol for *Diaphorina citri*.

Specific approval and amendment

Approved in 2004-09.

Introduction

Diaphorina citri is confined to *Rutaceae*, occurring on wild hosts as well as on *Citrus*, especially lemon and lime. The main economic importance of *D. citri* is as the vector of the very serious citrus disease (citrus greening) caused by *Liberobacter asiaticum* (EPPO/CABI, 1996). Fourth and fifth instar nymphs, as well as the adults born from these nymphs, are capable of transmitting *L. asiaticum* to citrus. In addition, *D. citri* typically causes defoliation and dieback. Serious damage to growing points can occur, which can lead to dwarfing as well as lack of juice and taste in fruits. Heavy infestations can cause blossom and fruitlet drop. *D. citri* is present throughout Asia, and has been introduced into some South and Central American countries, and to Mauritius and Réunion (Catling, 1970; EPPO/CABI, 1996).

Identity

Name: *Diaphorina citri* Kuwayama

Taxonomic position: *Insecta, Hemiptera, Sternorrhyncha, Psylloidea, Psyllidae*

EPPO computer code: DIAACI

Phytosanitary categorization: EPPO A1 list: no. 37; EU Annex designation: II/A1

Detection

D. citri has three developmental stages: egg, 5 nymphal instars and adult (Web Fig. 1). Plants for planting of citrus material (budwood, grafted trees, rootstock seedlings) can carry eggs and/or nymphs over long distances. The rutaceous plant

Murraya paniculata, frequently used as an ornamental bush or hedge, is one of the best hosts of this psyllid. During inspection of plant material for the presence of *D. citri*, attention should be paid to twisted and stunted growth of young shoots. Sometimes, the leaves are badly curled and they may be covered with honeydew and sooty mould. Heavily infested plants are characterized by the rosette appearance of shoot tips.

The eggs are orange-coloured and almond-shaped, 0.31 (long)–0.15 (wide) mm. Eggs are laid singly inside half-folded leaves of the buds, in leaf axils and other suitable places on the young tender parts of the tree. The nymphs pass through five instars. They are light-yellow to dark-brown, bearing well-developed wing pads. Nymphs will move away when disturbed but normally lead a sedentary existence clustered in groups. Adults are 2.5 mm long with yellowish-brown body and greyish-brown legs. Wings are transparent with white spots or light-brown with a broad, beige, longitudinal band in the centre. Adults are very active and jump on the slightest disturbance.

Identification

A key to the genera of Psylloidea is given by Yang (1984). The genus *Diaphorina* is characterized by the following combination of characters:

- antennae usually very short (0.48 mm), with two apical short setae
- aedeagus with first segment curved
- genal cones flat and broad, not conical in outline and as long as or longer than broad, perfect, apices narrowly rounded or truncate
- forewing broad subapically, rounded at apex and narrow in basal half.

At present, no key is available for the species of *Diaphorina* and identification is done by comparison with the following description by Yang (1984).

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

Adult

(Web Fig. 1): general colour brown. Head (Web Fig. 2A) slightly narrower than thorax. Vertex and genal cones greyish white. Antennae with two basal segments brown, 3rd to 8th segments yellowish with apices of 4th, 6th, 8th and last two segments black. Eyes dark brown. Pronotum ribbon-like, rounded and weakly sinuate anteriorly with two impressed fovea on each lateral side. Mesoscutum brown to dark brown with black markings. Femora dark brown, tibiae and tarsi yellowish white to brown. Abdomen black dorsally and greenish white ventrally. Forewings (Web Fig. 2B) hyaline with scattered maculation, 2.4 times as long as wide, narrow at base, broadest near apex, rounded apically, without distinct pterostigma. Hind wings (Web Fig. 2C) long and slender, 0.9 times as long as forewings, 3 times as long as wide. Male and female genitalia are shown in Web Figs 2 (D and E). Length of body: male 1.53–1.66 mm; female 1.90–2.06 mm; length of antenna: 0.48 mm.

Egg

(Web Fig. 3A): light yellow when fresh, and turn bright orange (with two distinct red eye spots) at maturity; length 0.31 mm, width 0.15 mm.

First instar nymph

(Web Fig. 3B): general colour yellow, body elongate; antennae 2-segmented, relative length of each segment about 1 : 5, with 1 sensorium; legs segmented, tibiotarsus with 2 claws; abdomen margin with 7 pairs of sectasetae. Length of body 0.33–0.35 mm; length of antennae 0.06 mm.

Second instar nymph

(Web Fig. 3C): general colour yellow; antennae 2-segmented, relative length of each segment about 1 : 2.8, with 1 sensorium; wing pads developed and separated; tibiotarsus with 1 golf-club-shaped seta; abdomen with stripe like sclerites at base and a large apical plate with margin with 7–8 pairs of sectasetae. Length of body 0.49–0.53 mm; length of antennae 0.08 mm.

Third instar nymph

(Web Fig. 3D): general colour yellow, antennae and abdomen somewhat dark; antennae 2-segmented, relative length of each segment about 1 : 4.5, with 2 sensoria on segment II; wing pads developed and overlapping; tibiotarsus with 1 golfclub-shaped seta; apical plate of abdomen margin with 15–17 pairs of sectasetae. Length of body 0.69–0.72 mm; length of antennae 0.14 mm.

Fourth instar nymph

(Web Fig. 3E): general colour from yellow to yellowish brown. Antennae 3-segmented, relative length of each segment about 1.5 : 1 : 6, with 3 sensoria and two strong spines. Apical plate of

abdomen margin with 27–30 pairs sectasetae. Length of body: 0.98–1.05 mm; length of antenna: 0.19 mm.

Fifth instar nymph

(Web Fig. 3F): body broadly oval, about 1.2 times as long as wide. General colour yellow to yellowish brown, 3rd antennal segment black. All sclerites and wing pads vermiculated or punctuated. Legs relatively short, 4-segmented, tarsus with two claws, a funnel-shaped pulvillus and golf-club-shaped seta. Abdomen with strip-like sclerites at base and a large apical plate, margin with 25–29 pairs sectasetae. Anal opening ventral, seta far away from apex of abdomen. Circum-anal pore ring composed of single row of slit-like pores, enclosing another ring of minute pores. Length of body: 1.45–1.58 mm; length of antenna: 0.27–0.30 mm.

Five other species of *Diaphorina* have been reported from citrus (*D. punctulata* and *D. zebrana* from Swaziland, *D. auberti* and *D. amoena* from the Comoros, and *D. communis* from India; Da Graça, 1991), but these are non-vector species of relatively little importance (Mead, 1977; Hollis, 1987). *D. citri* can be distinguished from *Trioza erytreae* (Del Guercio), the African citrus psyllid or two-spotted citrus psyllid (OEPP/EPPO, 2005), another vector of *Liberobacter* spp. causing greening, by a pair of spots on the base of the abdomen in late stage nymphs in the latter species. In *T. erytreae* the head is black and the forewing is broadest at the middle, unspotted and transparent and the antennae are nearly all black. Advanced nymphs in *Trioza* have 2 basal dark abdominal spots (none in *D. citri*), small wing pads (broad in *D. citri*) and a fringe of white filaments around the whole body, including the head. *D. citri* is closely related to species belonging to the genus *Euphalerus*. Species of this genus differ in the genal cones (head), which are broad and quadrate in *Euphalerus* and thick and perrect with narrowly rounded or truncate apices in *Diaphorina* and in the forewing, which is rounded or somewhat angulate at apex in *Euphalerus* and broad subapically, rounded at apex, narrow at basal half in *Diaphorina* (Yang, 1984).

For a positive diagnosis, the characters listed in this section should be present. A positive identification is possible on adult females and advanced larval instars. Microscope-slide preparation of stages of *D. citri* is strongly advised to allow proper identification (see Appendix I in OEPP/EPPO, 2005).

Reference material

Kuwayama (1908); Trans. Sapporo Nat. Hist. Soc., 2: 160, pl. III, Fig. 16 (deposition types: five syntypes and 10 non-type specimens in the Hokkaido University Insect collection). The specimen should preferably be compared with other slide-mounted specimens, which have been identified by a specialist.

Reporting and documentation

Guidelines on reporting and documentation are given in EPPO Standard PM7/– (in preparation).

Further information

Further information on this organism can be obtained from: H. Stigter, Plant Protection Service, Section of Entomology, PO Box 9102, 6700 HC Wageningen (Netherlands).

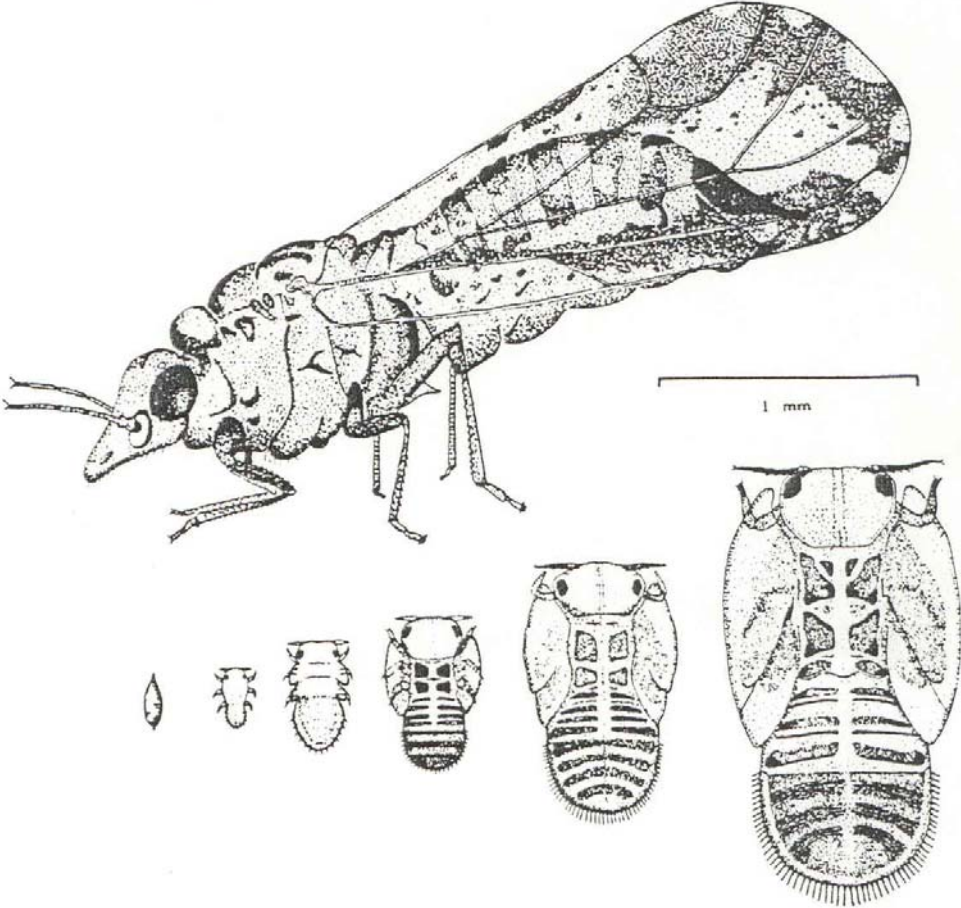
Acknowledgements

This protocol was originally drafted by H. Stigter, Plant Protection Service, Wageningen (NL).

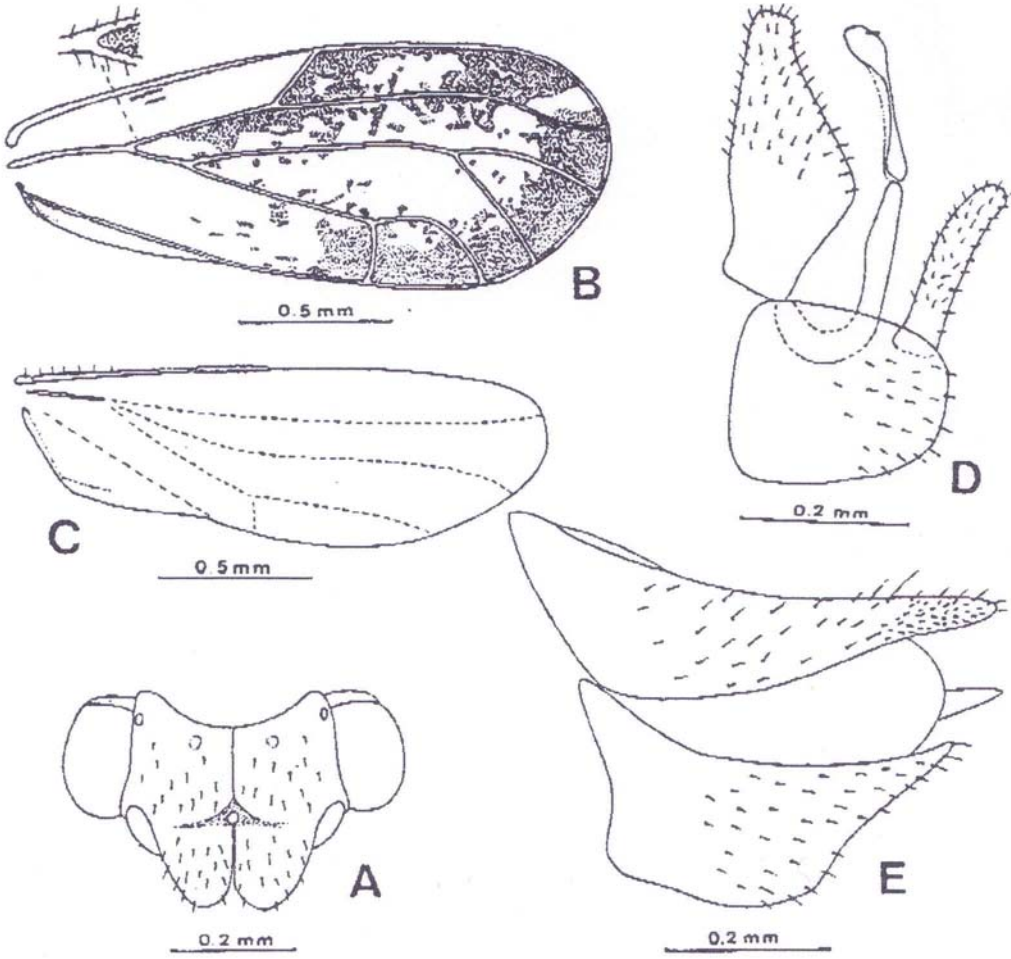
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Web Fig. 1: Developmental stages of *Diaphorina citri* (from Catling, 1970); top: adult, bottom: egg (left) and nymphal instars.



Web Fig. 2: Characteristics of adult *Diaphorina citri* **A.** Head, **B.** Forewing, **C.** Hind wing, **D.** Male genitalia, **E.** Female genitalia (from Yang, 1984).



Web Fig. 3: Characteristics of juvenile *Diaphorina citri* **A.** Egg, **B.** First instar nymph, **C.** Second instar nymph, **D.** Third instar nymph, **E.** Fourth instar nymph, **F.** Fifth instar nymph, **G.** Apex of hind leg, **H.** Antenna, **I.** Circum-anal pore ring (from Yang, 1984)

