

Diagnostics¹

Diagnostic

Toxoptera citricidus

Specific scope

This standard describes a diagnostic protocol for *Toxoptera citricidus*.

Specific approval and amendment

Approved in 2006-09.

Introduction

Toxoptera citricidus is a sap-sucking insect in the family *Aphididae* (aphids). The aphid feeds on *Citrus* species and occasionally on other *Rutaceae*. Non-rutaceous plants are not normally suitable hosts of *T. citricidus*, but may be colonized when young and tender citrus foliage is unavailable. *T. citricidus* can cause direct damage to citrus trees by attacking shoots, flower buds and sometimes young fruit but the major impact of *T. citricidus* is due to its transmission of *Citrus tristeza closterovirus* (CTV). Among aphid vectors of CTV, *T. citricidus* is the most efficient (high transmission efficiency, prolific reproduction, dispersal adequately timed with citrus flush cycles to maximize chances of acquiring and transmitting the virus). In particular, it can efficiently transmit the severe strains of CTV causing quick decline and death of citrus trees grafted on sour orange (*Citrus aurantium*). *T. citricidus* is also reported to transmit other diseases such as Citrus vein enation virus, stem-pitting virus, Eureka-seedling virus and bud union decline of citrus. It is also reported as able to transmit mosaic viruses of abaca, pea, yam and zucchini and *chili veinal mottle virus* (*Potyvirus*).

This aphid occurs predominantly in humid tropical regions and is presumed to originate from South-east Asia but it has also spread to areas of Mediterranean climate. It is widespread in Africa south of the Sahara and also present in Morocco and Tunisia, Asia (from India to Japan), Australia, New Zealand, the Pacific Islands and subtropical and warm temperate areas of South America. It has spread to important citrus-growing areas in Central America, the Caribbean and southern USA. Recently, its presence has been detected in the EPPO region,

in Portugal (Madeira in 1994 and mainland in 2004) and Spain (unpublished). Further information can be found in the EPPO datasheet on *Toxoptera citricidus* (EPPO/CABI, 1997) and the Crop Protection Compendium (CABI, 2005).

Identity

Name: *Toxoptera citricidus* (Kirkaldy)

Synonyms: *Toxoptera citricida* (Kirkaldy)

Aphis aeglis (Shinji)

Aphis nigricans (van der Goot)

Aphis tavaresi (del Guercio)

Myzus citricidus (Kirkaldy)

Paratoxoptera argentiniensis (Blanchard)

Note: In the past many records of *T. citricidus* actually refer to *T. aurantii* (Boyer de Fonscolombe), the black citrus aphid, but only rarely does the reverse occur

Taxonomic position: Insecta Hemiptera, Homoptera, Aphididae

EPPO computer code: TOXOCI

Phytosanitary categorization: EPPO A1 list: no. 45, EU Annex designation: II/A1.

Detection

An infestation of *T. citricidus* may be detected on citrus plants by the presence of distorted leaves and impaired shoot growth. On *Citrus* trees, even a few aphids on a young shoot will arrest blossom bud development and cause them to fall. Growth of shoots is greatly impaired and they become distorted; leaves become brittle, wrinkled and curl downwards. Branches may become deformed and leaves shrivelled. Attacked flowers fail to open or do so abortively since the ovaries are deformed. Caution should be shown however, as other aphid species, such

¹The figures in this standard marked 'Web Fig.' are published on the EPPO Website www.eppo.org.

as *Brachycaudus helichrysi* (Kaltenbach), the leaf-curling plum aphid, can also cause distorted leaves. Another sign of an aphid infestation is the presence of honeydew on which black, sooty moulds develop. Ants may also be present, collecting honeydew from the aphids.

Aphids are most likely to be detected if the young growth is inspected. They can also be found by beating foliage over a white surface. Yellow traps (water or sticky) or suction traps can help to monitor populations of alatae, but are only an indication that aphids are present in the area and are not suitable for detecting outbreaks at an early stage.

An infestation of *T. citricidus* may be indicated by the presence of medium-sized, shiny, very dark brown to black aphids. When they are disturbed they may make stridulatory movements with their hind legs, but they do not produce audible sounds, unlike *T. aurantii*, which produces a sound that can be heard up to 45 cm away. Also an indication of the presence of *T. citricidus* in the field, is that when the insect is squeezed onto a white surface, a red colour is obtained. Likewise specimens in alcohol colour the fluid deep red whereas specimens of other *Toxoptera* species do not.

Nineteen species of aphids have been recorded from citrus (Blackman & Eastop, 2000). Some of these cause leaf distortion and like *T. citricidus* are coloured brown to black and mixed colonies of two or more species are common (Halbert & Brown, 1998). All identification must be confirmed by examination of slide mounted specimens in the laboratory. Specimens can be stored in 70–80% alcohol until prepared for slide mounting.

Identification

Some identification features can be seen using a $\times 20$ hand lens or a dissecting microscope, however, for full identification a permanent slide mount (Appendix 1) should be made and retained for reference.

The most comprehensive key to aphids found on citrus is Blackman & Eastop (2000) (Appendix 2).

Identification of aphids is generally based on mature apterous and alate aphids, see Appendix 2 (Blackman & Eastop, 2000) and Appendix 3 (Martin, 1991), as not all larvae stages of immature aphids can be reliably identified.

Identification of larvae

First instar larvae of *Toxoptera* spp. can be identified using the key in Appendix 4 (Martin, 1991). If other instar immatures are present they can be reared to adults for identification.

Identification of adults

Species of the genus *Toxoptera* closely resemble those of the genus *Aphis*, but are easily distinguished from the latter by the presence of a stridulatory apparatus consisting of latero-ventral ridges on the abdomen and peg-like hairs on the hind tibiae, Web Fig. 1 (CSL) and Appendix 2, Web Fig. 2(q) (Blackman & Eastop, 2000).

Identification of *T. citricidus apterae*

T. citricidus apterae are medium-sized aphids, 1.5–2.8 mm long. They are shiny, very dark brown to black. They need to be examined microscopically to observe the very long, fine and erect hairs on the legs and body margins. Siphunculi are as for the alatae but relatively shorter. The cauda is thick and bluntly rounded at the apex.

T. citricidus apterae can be identified using the keys for apterous citrus aphids (Blackman & Eastop, 2000) (Appendix 2), polyphagous tree-dwelling aphids (Blackman & Eastop, 1994), apterous and alate *Toxoptera* spp. (Eastop, 1966), apterous and alate *Toxoptera* spp. (Martin, 1991) (Appendix 3), apterous and alate aphids that are citrus pests in the United States of America (Stoetzel, 1994), apterous and alate citrus aphids (Tao & Tan, 1961).

Identification of *T. citricidus alatae*

T. citricidus alatae are medium-sized aphids, 1.5–2.8 mm long. They are shiny black and can be identified, using a pocket lens, by the wholly black third antennal segment, succeeded by a pale fourth but identification must be confirmed by examining slide mounted specimens, Appendix 3 (Martin, 1991). The median vein of the forewings is normally forked twice. Siphunculi are about 1/6 body length and strongly sculptured, while the cauda is rather bulbously rounded at the apex.

T. citricidus alatae can be identified using the keys apterous and alate *Toxoptera* spp. (Eastop, 1966), apterous and alate *Toxoptera* spp. (Martin, 1991) (Appendix 3), apterous and alate aphids that are citrus pests in the USA (Stoetzel, 1994), apterous and alate citrus aphids (Tao & Tan, 1961).

Of the 19 aphid species reported to feed on citrus, 14 can be keyed out using the key of Blackman & Eastop (2000) (Appendix 2). Six of the 19 aphids are more likely to be confused with *T. citricidus*, namely *Aphis craccivora*, *A. gossypii*, *A. neri*, *A. spiraeicola*, *T. aurantii* and *T. odinae*. All of these six can be keyed out using Blackman and Eastop's key (2000).

There are three other described species of *Toxoptera* which can be compared with *T. citricidus* using the details given below and in Table 1.

Toxoptera aurantii (Boyer de Fonscolombe) is the most widespread and polyphagous of the other *Toxoptera* spp. and is therefore the one most commonly encountered that may be confused with *T. citricidus*. *T. aurantii* can be found in warm temperate, subtropical and tropical areas and under glass elsewhere.

T. odinae (van der Goot) is polyphagous, occurring on various trees and shrubs in Asia, including South-east Asia, and is widespread in Africa south of the Sahara.

T. victoriae was only described by Martin in 1991, from *Zanthoxylum scandens* in Hong Kong. It may occur on other *Zanthoxylum* spp. that are of tree habit. In the key, Appendix 2, it will key out to *T. odinae* but can be separated from the latter species by using the key in Appendix 3. Adults of *T. victoriae* (Martin) are shiny black and immatures are reddish-brown.

Table 1 Comparison with other Toxoptera species

Character	<i>T. citricidus</i>	<i>T. aurantii</i>	<i>T. odinae</i>
Size of apterae and alatae	1.5–2.8 mm	1.1–2.0 mm	1.3–2.4 mm
Colour – apterae	shiny, very dark brown to black	shiny, reddish–brown, brown–black to black	grey–brown to reddish–brown
Colour – immatures	brown	brownish	brown
Colour – alatae	shiny black abdomen	dark brown to black abdomen	reddish–brown to dark brown abdomen
Antennae – apterae	not so conspicuously black–and–white banded	black–and–white banded	pale
Antennae alatae	segment III – black with a pale base segment IV – pale	segment III and IV – white with a dark tip	segment III and IV – pale
Antennal setae – segment III	longer than the basal diameter of the segment	shorter than the basal diameter of the segment	twice as long as the basal diameter of the segment
Forewing – media	normally twice branched	normally once branched	normally twice branched
Forewing – pterostigma	pale	black	pale
Siphunculi	black – longer than cauda	black – longer than cauda	dusky – 3/4 length of cauda
Cauda	black	black	black
Caudal setae – apterae	25–54	9–20	15–18
Caudal setae – alatae	25–40	8–19	15–18
Stridulation	no audible sound to the human ear	an audible sound to human ears	Not known
Preserved specimens	colour fluid deep red (also squashed aphids colour a white surface red)	do not colour fluid or a white surface red	do not colour fluid or a white surface red
Host plants	Rutaceae almost exclusively – only occasionally on members of other plant families	120 + species in more than 10 plant families including the Rutaceae	25 + species in more than 15 plant families including the Rutaceae, although not commonly on <i>Citrus</i> spp.

Reference material

The Natural History Museum, London holds reference specimens which have been identified by UK national specialists. No type material is held there.

Reporting and documentation

Guidance on reporting and documentation is given in EPPO Standard PM 7/77 (1) Documentation and reporting on a diagnosis.

Further information

Further information on this organism can be obtained from Roger Hammon, Pest and Disease Identification Team, Central Science Laboratory, Sand Hutton, York, YO41 1LZ, UK.

Acknowledgements

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Vic Eastop, Roger Blackman and Jon Martin (British Natural History Museum, London, UK) have provided the keys included in this protocol.

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Appendix 1

Permanent microscope-slide preparation of aphids

1. Heat specimens gently, at 70°C, in 70% ethanol for 5–10 min
2. Transfer to 10% KOH and heat until colour just begins to leach out of specimens. To aid maceration the body can be squeezed with fine spatulas and the contents can be gently worked to the rear of the body and squeezed out. If this proves difficult then an incision can be made in one side of the body and the contents squeezed out through this. Caution must be taken to avoid splitting the body
3. Transfer to cold 70% alcohol for at least 10 min
4. Transfer to cold glacial acetic acid for 5 min
5. Reset the heating block to 80°C and heat in chloral phenol until completely cleared
6. Rinse in glacial acetic acid
7. Transfer to fresh cold glacial acetic acid for 5 min
8. Leave in clove oil for at least 10 min
9. Mount aphids on a microscope slide, in Canada balsam, ventral side up with appendages spread.

Appendix 2

Key to aphids recorded on *Citrus* spp. (Blackman & Eastop, 2000)

Fourteen aphid species recorded from citrus are included in the key:

- *Aphis craccivora*, *A. gossypii*, *A. nerii*, *A. spiraecola*
- *Aulacorthum magnoliae*, *A. solani*
- *Brachycaudus helichrysi*
- *Brachyunguis harmalae*
- *Macrosiphum euphorbiae*
- *Myzus persicae*
- *Sinomegoura citricola*
- *Toxoptera aurantii*, *T. citricidus*, *T. odinae*.

[Five other species have been recorded one or more times from citrus but are not keyed here: *Aphis arbuti* Ferrari 1872, *A. fabae*, *Brachycaudus cardui*, *Pterochloroides persicae* and *Rhopalosiphum maidis*].

- | | |
|--|--------------------------------|
| 1. Antennal tubercles weakly developed (Web Fig. 1a–c) | 2 |
| Antennal tubercles well-developed (Web Fig. 1d–f) | 10 |
| 2. Terminal process a little shorter than base of last antennal segment (Web Fig. 2a).
Siphunculi (Web Fig. 2b) much shorter than cauda (Web Fig. 2f) | <i>Brachyunguis harmalae</i> |
| Terminal process much longer than base of last antennal segment. Siphunculi shorter or longer than cauda (e.g. Web Fig. 2c–e, i–o, t–w) | 3 |
| 3. Cauda helmet-shaped in dorsal view, not longer than its width at base (Web Fig. 2g) | <i>Brachycaudus helichrysi</i> |
| Cauda tongue-shaped or triangular in dorsal view, longer than its basal width (Web Fig. 2f, h) | 4 |
| 4. Dorsal abdomen with an extensive black patch (Web Fig. 2i) | <i>Aphis craccivora</i> |
| Dorsal abdomen without an extensive black patch | 5 |
| 5. Siphunculi much shorter than cauda (Web Fig. 2j) | <i>Toxoptera odinae</i> |
| Siphunculi longer than, or at least as long as, cauda (Web Fig. 2k–o) | 6 |
| 6. Terminal process of antenna more than 3.5 times longer than base of last segment. | 7 |
| Cauda with at least 10 hairs | |
| Terminal process of antenna less than 3.5 times longer than base of last segment. Cauda usually with less than 10 hairs | 9 |
| 7. Cauda with usually more than 20 hairs (Web Fig. 2l). Hairs on antennal segment III longer than diameter of this segment at its base (Web Fig. 2p). Thoracic segments often partly sclerotized | <i>Toxoptera citricidus</i> |
| Cauda with usually less than 20 hairs. Hairs on antennal segment III often shorter than diameter of this segment at its base. Thoracic tergites usually unsclerotized | 8 |
| 8. Siphunculi less than 1.5 times longer than cauda (Web Fig. 2k). Stridulatory apparatus present (Web Fig. 2q) | <i>Toxoptera aurantii</i> |
| Siphunculi more than 1.5 times longer than cauda (Web Fig. 2m). Stridulatory apparatus absent | <i>Aphis nerii</i> |

- | | |
|--|--------------------------------|
| 9. Cauda paler than siphunculi, with 4–7 hairs (Web Fig. 2o). Femoral hairs all rather short, less than width of femur at base (Web Fig. 2r) | <i>Aphis gossypii</i> |
| Cauda as dark as siphunculi, with 6–12 hairs (Web Fig. 2n). Some femoral hairs long and fine, exceeding width of femur at its base (Web Fig. 2) | <i>Aphis spiraeicola</i> |
| 10. Inner faces of antennal tubercles convergent in dorsal view (Web Fig. 1f) | <i>Myzus persicae</i> |
| Inner faces of antennal tubercles parallel or divergent (Web Fig. 1d, e) | 11 |
| 11. Siphunculi a little shorter than the dark cauda (Web Fig. 2d, t) | <i>Sinomegoura citricicola</i> |
| Siphunculi much longer than cauda (Web Fig. 2u–w) | 12 |
| 12. Head, legs and antennae mainly dark. Femora basally pale but with distal 0.5–0.75 black. Siphunculi slightly swollen over distal 0.7 of length (Web Fig. 2e, u). Cauda with a constriction | <i>Aulacorthum magnoliae</i> |
| Head, legs and antennae mainly pale. Siphunculi tapering or parallel over most of length (Web Fig. 2v, w). Cauda without any constriction | 13 |
| 13. Inner faces of antennal tubercles parallel (Web Fig. 1e). Siphunculi without any polygonal reticulation (Web Fig. 2v). Cauda only 0.1–0.125 of body length | <i>Aulacorthum solani</i> |
| Inner faces of antennal tubercles divergent (Web Fig. 1d). Siphunculi with a subapical zone of polygonal reticulation (Web Fig. 2w). Cauda longer, 0.14–0.2 of body length | <i>Macrosiphum euphorbiae</i> |

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Appendix 3

Key to *Toxoptera* species, adult apterae and alatae (Martin, 1991)

This key is designed for both apterae and alatae. Although adult alatae of *T. victoriae* (Martin) sp. n. have not been seen, the presence of the key characters in the fourth instar alatoid nymphs indicates that alatae will be readily recognisable from characters also present in apterae. With the variability of many ratios (e.g. siphunculus length: cauda length) such measurements are used as secondary recognition characters in this key, and are simplified for ease of use. Mondal *et al.* (1976) can be consulted for details of measurements of the three major *Toxoptera* species, based upon Indian material.

- | | |
|---|---|
| 1. Abdominal segment VIII bearing only 2, occasionally 3, dorsal hairs. Siphunculi almost always longer than cauda. Hairs on antennal segment III short, usually less than twice basal articular diameter of segment (Web Figs 4.11, 4.14) | 2 |
| Abdominal segment VIII bearing 4–12 dorsal hairs (Web Figs 4.2 and 4.10). Siphunculi either subequal to or less than three-quarters of length to cauda. Hairs on antennal segment III long and fine, over twice basal articular diameter of segment (Web Figs 4.8 and 4.9) | 3 |
| 2. Apterae with antennal segments III and IV completely pale (Web Fig. 4.14). Alatae with antennal segment III brown to black (Web Fig. 4.13), and with median veins of forewings normally twice-branched. Siphunculi of both apterae and alatae covered by dense, coarse, imbrications. Cauda usually with more than 25 hairs. Hairs on antennal segment III longer than basal articular diameter of segment | <i>citricidus</i>
(Kirkaldy) |
| Apterae with antennal segments III and IV each with a distinct brown apex (Web Fig. 4.12). Alatae with antennal segment III pale but with a distinct brown apex as in apterae (Web Fig. 4.11), and with median veins of forewings normally only once-branched. Siphunculi less coarsely imbricate. Cauda only rarely with more than 20 hairs, frequently with many less. Hairs on antennal segment III rarely longer than basal articular diameter of segment | <i>aurantii</i>
(Boyer de Fonscolombe) |
| 3. Siphunculi short and conical, less than three-quarters length of cauda (Web Fig. 4.10). Base of last antennal segment usually with 2 hairs (often additionally with a short spine). Normal tibial hairs of all legs appearing similar, long and fine. Pale cuticle of antennal segments III–V similar in shade to other pale parts of body | <i>odinae</i>
(van der Goot) |
| Siphunculi longer and basally broader, length subequal to cauda (Web Fig. 4.2). Base of last antennal segment with 5–7 hairs (Web Fig. 4.1). Tibial hairs of fore and middle pairs of legs noticeably shorter and more spine-like than the fine hairs of hind legs. Pale cuticle of antennal segments III–V markedly paler than any cuticle elsewhere on body | <i>victoriae</i>
(Martin) |

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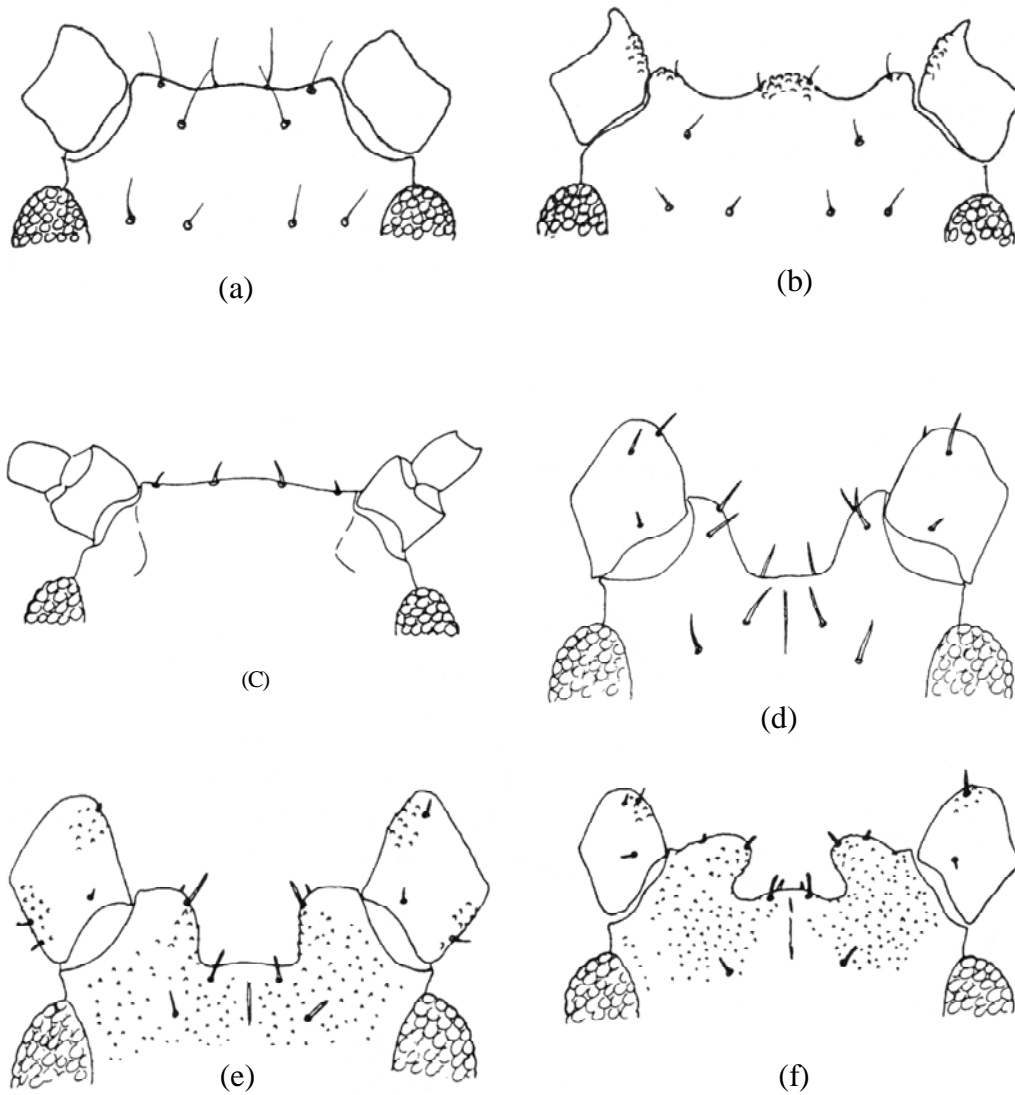
Appendix 4

Key to *Toxoptera* species, first instar larvae (Martin, 1991)

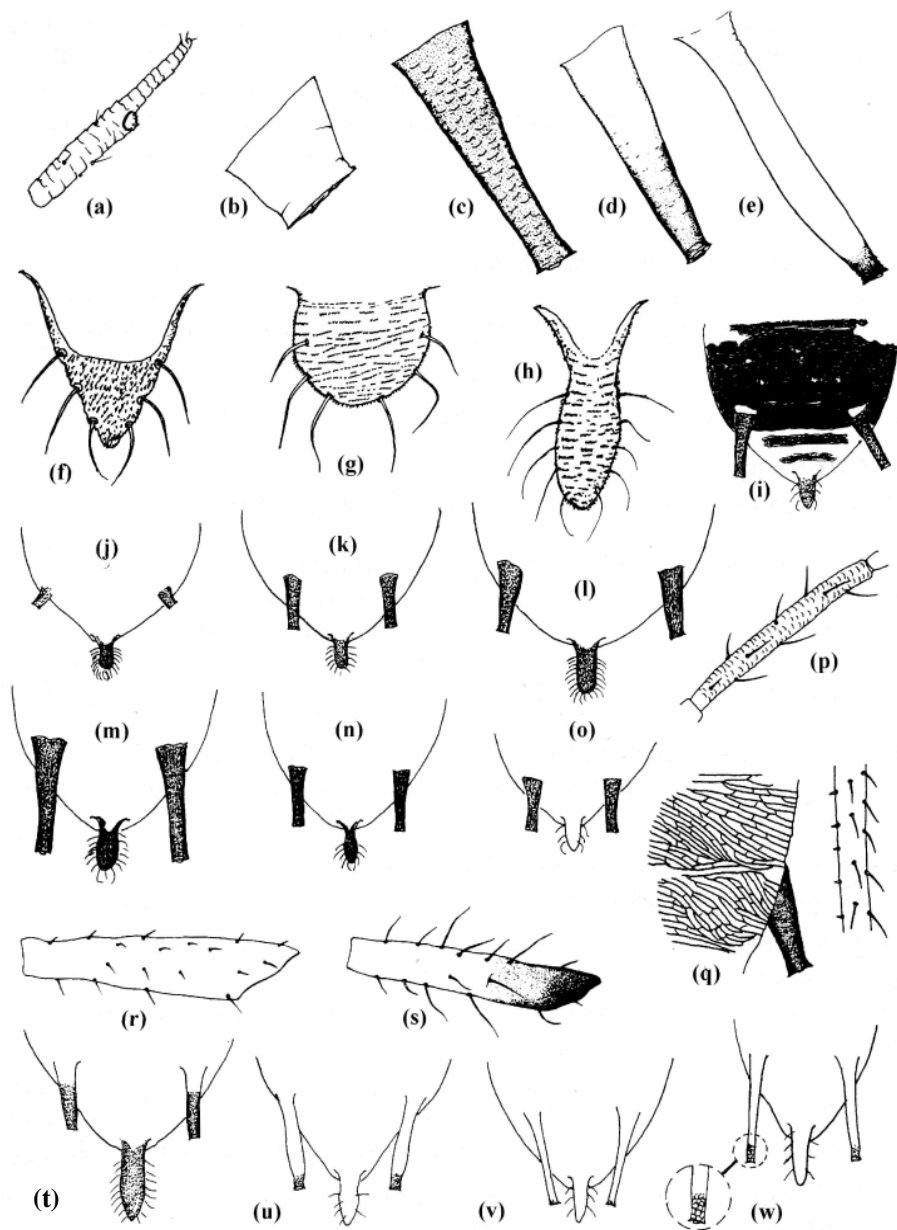
All larval stages of *Toxoptera* spp. are immediately distinguished from those of other genera by the presence of hind tibial pegs. First instar larvae are recognised by the presence of 4-segmented antennae.

- | | |
|--|---|
| 1. Hairs on apical part of antennal segment III shorter than maximum diameter of segment. | 2 |
| Siphunculi as long as, or longer than, maximum width | |
| Hairs on apical part of antennal segment III twice maximum diameter of segment, or longer. | 3 |
| Siphunculi normally shorter than maximum width | |
| 2. Siphunculi noticeably widest at base, about as long as maximum width distinct spinules scattered over surface. Body larger, 0.65–0.80 mm in length | <i>citricidus</i> (Kirkaldy) |
| Siphunculi almost parallel-sided, often rather longer than maximum width surface spinules may be present, but less distinct. Body smaller, 0.45–0.65 mm in length | <i>aurantii</i>
(Boyer de Fonscolombe) |
| 3. Base at last (fourth) antennal segment with only 2 hairs. Abdominal segment VIII normally with only 2 dorsal hairs. Sides of siphunculi pigmented but mostly smooth. At least some hairs on fore and middle tibiae longer than median diameter of segment | <i>odinae</i> (van der Goot) |
| Base of last antennal segment with up to 5 hairs. Abdominal segment VIII with 4 dorsal hairs. Sides of siphunculi pigmented and roughened. Length of hairs on fore and middle tibiae maximally up to median diameter of segment | <i>victoriae</i> Martin |

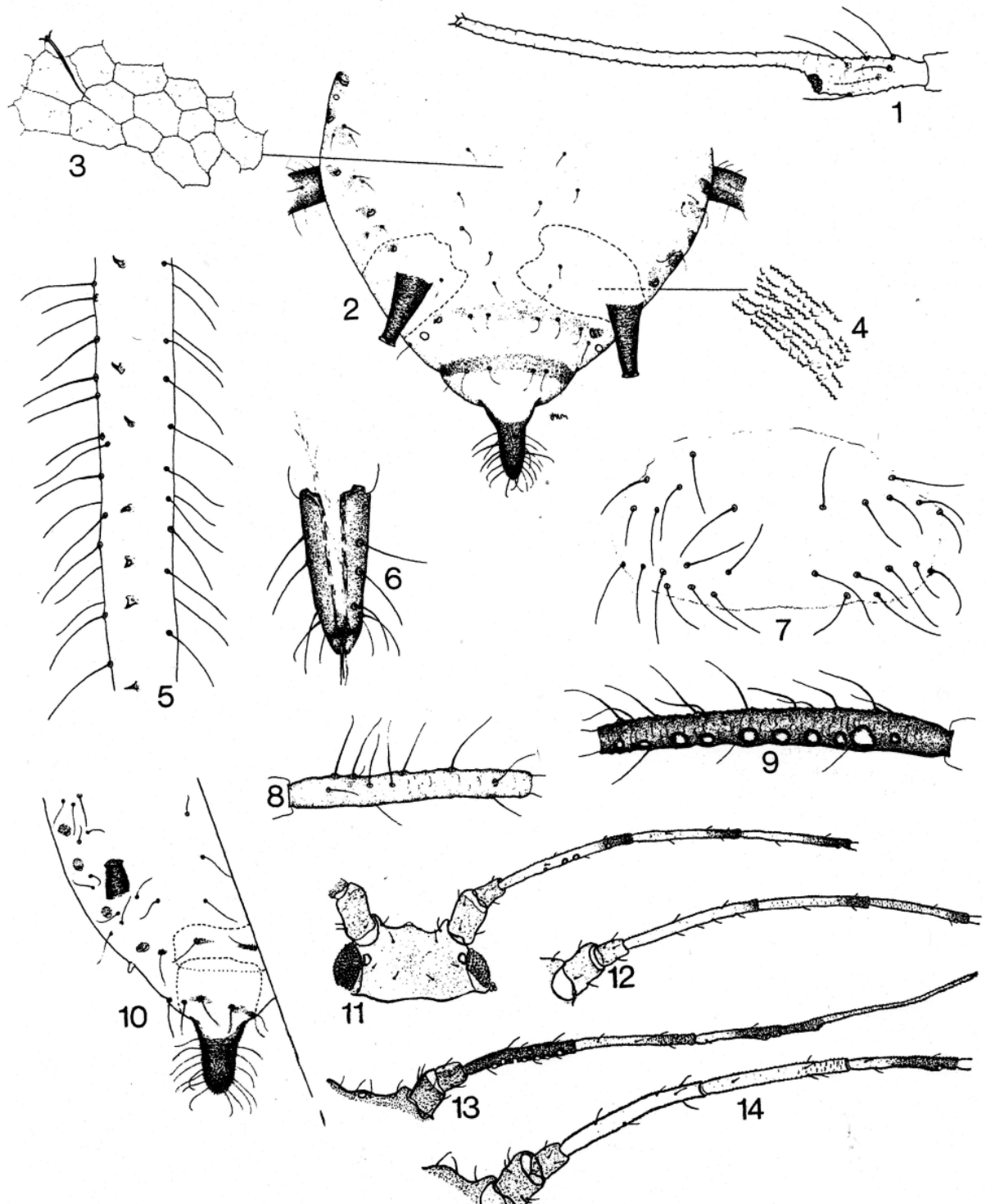
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Web Fig. 1 Dorsal views of heads of various Aphidinae, to show differences in shape of head and degree of development of the antennal tubercles. (a) – (c) Weakly developed or undeveloped: (a) *Aphis fabae*; (b) *Rhopalosiphum padi*; (c) *Pseudaphis abyssinica*. (d) – (f) Well-developed: (d) inner faces divergent – *Macrosiphum euphorbiae*; (e) inner faces parallel – *Aulacorthum solani*; (f) inner faces convergent – *Myzus persicae*.



Web Fig. 2. Citrus aphids. (a) Last antennal segment of *Brachyunguis harmalae*, (b)-(e) siphunculi of (b) *B. harmalae*, (c) *Aphis spiraecola*, (d) *Sinomegoura citricola*, (e) *Aulacorthum magnoliae*, (f) cauda of *Brachyunguis harmalae*; (g) same of *Brachycaudus helichrysi*, (h) same of *A. spiraecola*, (i)-(o) ends of abdomens of (i) *Aphis craccivora*, (j) *Toxoptera odinae*, (k) *T. aurantii*, (l) *T. citricidus*, (m) *Aphis nerii*, (n) *A. spiraecola*, (o) *A. gossypii*, (p) antennal segment III of *T. citricidus*, (q) stridulatory apparatus of *T. aurantii*, (r) hind femur of *A. gossypii*, (s) same of *A. spiraecola*, (t)-(w) end of abdomens of (t) *Sinomegoura citricola*, (u) *Aulacorthum magnoliae*, (v) *Aulacorthum solani*, (w) *Macrosiphum euphorbiae*.



Web Figs 1-7. *Toxoptera victoriae* sp. n., aptera: (1) antennal segment VI; (2) abdomen, dorsum (extent of ventral stridulatory roughening indicated by dashed line); (3) detail of dorso-median abdominal cuticle; (4) detail of ventro-marginal abdominal stridulatory cuticle; (5) section of posterior tibia with modified stridulatory pegs; (6) ultimate rostral segment; (7) subgenital plate.

Web Figs 8-10. *Toxoptera odinae*: (8) aptera, antennal segment III; (9) alata, antennal segment III; (10) abdomen, dorsal.

Web Figs 11-12. *Toxoptera aurantii*: (11) alata, head and antennal segments I-V; (12) aptera, antennal segments I-V.

Web Figs 13-14. *Toxoptera citricidus*: (13) alata, antenna; (14) aptera, antennal segments I-V.