© 2004 OEPP/EPPO, *Bulletin OEPP/EPPO Bulletin* **34**, 155–157

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

Normes OEPP
EPPO Standards

Diagnostic protocols for regulated pests
Protocoles de diagnostic pour les organismes réglementés

PM 7/35

Organization 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 Diagnostic Protocols for Regulated Pests are intended to be used by National Plant Protection Organizations, in their capacity as bodies responsible for the application of phytosanitary measures to detect and identify the regulated pests of the EPPO and/or European Union lists.

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 diagnostic protocols:

- 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.

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 Diagnostic Protocols for Regulated Pests 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

Nineteen 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) *Cacyreus 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

Several 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 'intercomparison' 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 PM7. They will in future be subject to review by EPPO procedures, on the same terms as other members of the series.

Diagnostic protocols for regulated pests[1]
Protocoles de diagnostic pour les organismes réglementés

Bemisia tabaci

Specific scope

This standard describes a diagnostic protocol for *Bemisia tabaci*.

Specific approval and amendment

This Standard was developed under the EU DIAGPRO Project (SMT 4-CT98-2252) by partnership of contractor laboratories and intercomparison laboratories in European countries. Approved as an EPPO Standard in 2003-09.

Introduction

Bemisia tabaci is a plant sap-sucking insect in the family *Aleyrodidae* of superfamily *Aleyrodoidea* (whiteflies). It is broadly polyphagous, feeding on an estimated 600 plant species. Since the early 1980s, it has caused escalating problems to both field and protected agricultural crops and ornamental plants. Heavy infestations of *B. tabaci* may reduce host vigour and growth, cause chlorosis and uneven ripening, and induce physiological disorders. The larvae produce honeydew on which sooty moulds grow, reducing the photosynthetic capabilities of the plant, resulting in defoliation and stunting. *B. tabaci* is also a vector of over 100 plant viruses in the genera *Begomovirus* (*Geminiviridae*), *Crinivirus* (*Closteroviridae*) and *Carlavirus* or *Ipomovirus* (*Potyviridae*) (Jones, 2003). Begomoviruses are the most numerous of the *B. tabaci*-transmitted viruses and can cause crop yield losses of between 20% and 100% (Brown & Bird, 1992). *B. tabaci* possibly originated in India (Fishpool & Burban, 1994) and as a result of widespread dispersal, particularly during the last 15 years, is now distributed nearly worldwide.

Identity

Name: *Bemisia tabaci* (Gennadius, 1889 – *Aleurodes*).
Synonyms: *Bemisia inconspicua* (Quaintance, 1900 – *Aleurodes*), and many others. A complete list of synonyms and type data is given by Mound & Halsey (1978) and Perring (2001).
Taxonomic position: Insecta: Hemiptera: Homoptera: Sternorrhyncha: Aleyrodoidea: *Aleyrodidae*: *Aleyrodinae*.
Notes on taxonomy and nomenclature: *B. tabaci* was first described in 1889 as a pest of tobacco in Greece, as *Aleurodes tabaci* (Gennadius, 1889). It was subsequently described under numerous names before its morphological variability was recognized (Mound, 1963; Mound & Halsey, 1978; Russell, 1957). The existence of host races or biotypes was proposed in the 1950s to describe distinct populations of *B. tabaci* with specific host associations and virus-vector capabilities. In the mid-1980s, reports emerged of a newly evolved 'B biotype', a highly polyphagous variant that was almost twice as fecund as previously recorded populations (Brown *et al*., 1995). The B biotype has become a major pest of world agriculture and been described as a separate species, *B. argentifolii* Bellows & Perring, based on RAPD-PCR banding patterns, isoelectric focusing electrophoresis, crossing experiments, mating behaviour and morphological evaluation (Bellows *et al*., 1994). It can induce phytotoxic disorders in certain plant species, for example silvering of leaves in *Cucurbita* spp., hence the common name 'silverleaf whitefly'. To date, 41 distinct populations of *B. tabaci* have been characterized using a variety of techniques and 24 of these populations given a specific biotype designation (Perring, 2001). Molecular (Frohlich *et al*., 1999) and allozyme (Brown *et al*., 2000) data from these studies supports the idea that *B. tabaci* is a suite of highly cryptic sibling species that cannot currently be distinguished morphologically. Perring (2001) recently reviewed the species complex and proposed the existence of seven distinct groups, based on comparison of populations from various locations. However, for the purposes of this protocol, the name *B. tabaci* refers to all described variants in the *B. tabaci* species complex.
Bayer computer code: BEMITA (BEMIAR has been used for the B biotype or *B. argentifolii*).
Phytosanitary categorization: EPPO A2 list: no. 178; EU Annex designation: I/A1 (non-European populations); I/B (European populations).

Detection

Whiteflies are usually detected by close examination of the undersides of leaves to search for the tiny yellow/cream, scale-like larval instars. They also occasionally occur on the upper surfaces of the leaves and vary from being widely scattered to

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

forming dense clusters. Shaking the plant may disturb the small white adults, which flutter out and quickly resettle. Adults may also be found on sticky traps placed above infested plants. Samples of larvae should be collected while still attached to the leaves and stored dry or in phials of 70% ethanol for examination in the laboratory.

Infested plants may exhibit a range of symptoms due to direct feeding damage, contamination with honeydew and associated sooty moulds, whitefly-transmitted viruses and phytotoxic responses. There may be one or a combination of the following symptoms: chlorotic spotting, vein yellowing, intervein yellowing, leaf yellowing, yellow blotching of leaves, yellow mosaic of leaves, leaf curling, leaf crumpling, leaf vein thickening, leaf enations, leaf cupping, stem twisting, plant stunting, wilting and leaf loss. Phytotoxic responses such as a severe silvering of courgette and melon leaves usually indicate the presence of a B biotype infestation.

Identification

The taxonomy of the *Aleyrodidae* is based almost entirely on the final (fourth) larval instar or 'puparial stage'. The exuvium is often referred to as the 'pupal case'. With very few exceptions, accurate whitefly identification is only possible from microscopic examination of a slide-mounted puparium or pupal case. The following descriptions in this section all refer to this stage. Slide preparation methods for whitefly puparia are presented in Appendix I. A high power microscope (× 100 to × 400) is required to see all the diagnostic characters. The morphological terminology used here follows that of Bellows *et al*. (1994).

It is often difficult to distinguish between third larval instars and puparia until the specimens are slide-mounted, although puparia are generally larger. The antennae of the puparia are straight or gently curved, uniform in width and overlap with the front legs. The antennae of the third instars are strongly curved forming a U shape, broad at the base and apically narrow, and do not overlap with the front legs.

Whitefly puparia are notorious for exhibiting considerable environmentally induced morphological variation. This phenotypic variation is largely dependent on the tactile experience of the first instar before settling to feed, which is determined by leaf surface topography and population density (Neal & Bentz, 1999). Characters such as size, body shape, colour, length of dorsal setae and tubercle development are highly variable. This variation has been studied in detail for *B. tabaci* by numerous authors including Azab *et al*. (1969), Bethke *et al*. (1991), David & Ananthakrishnan (1976), Harakly (1973), Mohanty & Basu (1986), Mound (1963) and Rossell *et al*. (1997). This variation needs to be taken into account during the identification process.

Identification of slide-mounted puparia

Family Aleyrodidae

The family is easily recognizable by the presence of a vasiform orifice, operculum and lingula (see Web Figs 1, 4, 6, 8).

Genus Bemisia

The type-species of *Bemisia* Quaintance & Baker, 1914 is *Aleurodes inconspicua*, a synonym of *B. tabaci*, by original designation. Some 40 species are assigned to the genus *Bemisia* (Martin, 1999) but there is currently no comprehensive key available for their identification. The majority of *Bemisia* species have a limited geographical distribution and host-plant range (Mound & Halsey, 1978) and are unlikely to be encountered in plant trade. *B. tabaci* is the most geographically widespread, polyphagous and economically important species assigned to the genus.

According to Martin (1999), wide phenotypic variation is a particular generic trait of *Bemisia*. The genus may be identified using the following combination of characters:

- cuticle usually completely pale, occasionally brownish pigmentation
- margin irregular crenulate, often modified at caudal and/or tracheal openings at margin to form ill-defined combs of fine teeth, with margin often shallowly indented at these points
- transverse moulting sutures not reaching margin
- medial length of abdominal segment VII less than half that of VI
- vasiform orifice acute-triangular, sometimes laterally sinuous, posteroapically often ill-defined and usually leading into a pronounced caudal furrow; operculum occupying basal half of orifice; head of lingula typically elongate-triangular, finely spinulose, bearing a pair of apical setae, always exposed but included within vasiform orifice
- chaetotaxy and presence/absence of dorsal sculpturing and tubercles may be highly variable within species
- ventrally, caudal and thoracic tracheal folds marked, usually finely stippled.

A simple key to separate *Bemisia* from other plant-pest genera present in Europe and the Mediterranean area is given in Table 1. It is advisable to also refer to Martin *et al*. (2000) for a comprehensive key to the whiteflies of this region and Martin (1987) for a key to common whitefly pests of the World (both works include *B. tabaci*).

Bemisia tabaci

See Web Figs 1–6. In life, the puparium appears translucent, cream to distinctly yellow, without evident adorning wax secretion. Dorsum with thin, transparent wax layer. Size, 0.55–0.87 mm long, 0.35–0.64 mm wide. Shape suboval, often strongly tapered to posterior. When slide-mounted, cuticle evenly pale. Margin finely crenulate, thoracic tracheal opening slightly indented and without tracheal combs; caudal opening may also be slightly indented and without comb. Minute anterior and posterior marginal setae present. Caudal setae long and stout.

Dorsum

Generally smooth. Up to 5 median tubercles and 8 pairs of subdorsal abdominal papillae may be present or tubercles and papillae absent. Small discoidal pores, with associated smaller

Table 1 Key to puparia of some pest genera of *Aleyrodidae* found in Europe and the Mediterranean. For general morphology of a whitefly puparium see Web Fig. 1

1.	With 6 pairs of abdominal compound wax-producing pores, on segments III–VIII, the anterior two pairs much smaller than the posterior four pairs	*Paraleyrodes*
–	Without abdominal compound pores	*2*
2.	Pupal case dark brown to black	3
–	Pupal case colourless, with or without brownish patches	4
3.	Less than half of total length of vasiform orifice occupied by operculum and lingula together	*Acaudaleyrodes*
–	More than half of total length of vasiform orifice occupied by operculum alone or by operculum and lingula together	*Aleurolobus*
4.	Dorsal disc and/or submargin with a pattern of stout, acute or tubiform spines	5
–	Dorsal surface without a pattern of stout spines, although sometimes with a submarginal row of conspicuous hairs or setae, or with a few stout setae on the dorsal disc	6
5.	Dorsal spines tubiform, siphon-like	*Siphoninus*
–	Dorsal spines acute	*Aleurocanthus*
6.	Submargin with a regular row of normally 14 fine, acute setae. Lingula basally bilobed	*Parabemisia*
	Submargin without a regular row of fine, acute setae and lingula not basally bilobed	7
7.	Submargin with a row of papillae and lingula lobulate	*Trialeurodes*
–	Submargin without a row of papillae; lingula not lobulate	8
8.	Thoracic and caudal tracheal openings at margin marked by invaginated pores	*Dialeurodes*
–	Thoracic and caudal tracheal openings at margin marked by combs of differentiated teeth or not marked	9
9.	Wide submarginal area separated from dorsal disc by a suture-like fold	*Aleurothrixus*
–	Submarginal area not separated from dorsal disc by a suture-like fold	10
10.	Lengths of abdominal segments I–VIII similar medially. Vasiform orifice subcordate, hardly longer than wide, sides slightly convex. Caudal furrow present but not pronounced. Margin regularly crenulate	*Aleyrodes*
–	Length of abdominal segments VII much reduced medially. Vasiform orifice triangular, much longer than wide, sides straight to concave. Caudal furrow pronounced	*Bemisia*

porette (often difficult to detect) are aligned in four serially arranged groups. Pores may be duplicated or apparently missing. Longitudinal moulting suture reaching margin; transverse moulting sutures each with obtuse angle halfway to lateral margin, not reaching margin. Vasiform orifice triangular, inset from puparial margin by less than its own length, the orifice leading to a distinct narrow caudal furrow; operculum covering anterior half of orifice; lingula spatulate, with two stout terminal setae, distal portion covered in minute acanthae.

Chaetotaxy

There are 3 or 4 pairs of minute anterior submarginal setae and 5 pairs of minute posterior submarginal setae, the 5th pair may be well developed (these minute setae are often very difficult to detect). There are 6 pairs of dorsal setae, which are highly variable in size and may be asymmetrical. They may all be minute (12 µm) (Web Fig. 2) or very well developed (up to 140 µm) (Web Fig. 3), arising from enlarged bases.

Venter

Thoracic tracheal folds usually with numerous minute spinules; spinules sometimes lacking in individuals with enlarged dorsal setae.

Variation

Phenotypic variation is largely dependent on the tactile experience of the first instar, which is determined by leaf surface topography and population density (Neal & Bentz, 1999). For example, puparia collected from hirsute leaves are often smaller, more pointed posteriorly, show dorsal setal enlargement, have dorsal tubercles and papillae, and often have their outlines indented by stout plant hairs (Web Fig. 3). Puparia collected from glabrous leaves are generally larger, rounded posteriorly, show little or no setal enlargement, have no dorsal tubercles or papillae and are oval (Web Fig. 2). Male puparia are usually slightly smaller than females.

Detailed morphological descriptions and illustrations of puparia are given by Mound (1963), Hill (1969) and Bellows *et al*. (1994) (as *B. argentifolia*). The latter includes a detailed drawing of a syntype specimen of *B. tabaci*.

Possible confusion with similar species

Separation of all life stages of Bemisia tabaci from Trialeurodes vaporariorum

Whiteflies are frequently detected on imported plants in the EU. Apart from *B. tabaci*, the species most commonly encountered is the glasshouse whitefly *Trialeurodes vaporariorum* and it is important for an NPPO to be able to distinguish all life stages of these two species. It is always preferable to make an identification from the puparium stage and those of *B. tabaci* can usually easily be separated from *T. vaporariorum* in the field with a × 20 hand lens. The appearance of the adults and empty

Table 2 Comparison of some morphological and behavioural characters of *Bemisia tabaci* and *Trialeurodes vaporariorum* seen with low magnification (× 20)

	Bemisia tabaci	Trialeurodes vaporariorum
Egg		
Oviposition pattern	Eggs usually scattered or grouped in small clusters; may form semicircles on smooth leaves.	Eggs usually laid in neat circles or semicircles; may be scattered on very hairy leaves.
Colour	Yellowish-white when laid, becoming pale brown. Semitransparent and golden brown after hatching.	Yellowish-white when laid, becoming dark brown to almost black. Smoky black after hatching
Shape after hatching	Often remains erect and maintains shape.	Often flattened or bent double.
Puparium		
Colour	Often distinctly yellow; may be cream. Brown when parasitized.	Usually cream. Black when parasitized.
Shape	Oval or elliptical, often pointed posteriorly. Outline often distorted by plant hairs.	Oval or elliptical, rounded posteriorly. Surrounded by palisade of wax giving a 'pill-box' appearance. Outline not distorted by plant hairs.
Dorsum	1–7 pairs of well developed dorsal setae present; longer on plants with hirsute leaves. Glassy wax rods and submarginal papillae absent.	Dorsal setae absent. Dorsal and submarginal papillae present. Distinct glassy wax rods usually present.
Excretory apparatus	Vasiform orifice triangular and lingula swollen and pointed distally.	Vasiform orifice subcordate and lingula lobed.
Distribution of puparia	Often scattered and density per leaf usually low, except on smooth leaves.	Frequently grouped and the density per leaf high.
Adult		
Colour	Body dark yellow.	Body pale yellow.
Wing shape	Forewings with anterior margin straight.	Forewings with anterior margin curved.
Position at rest	Appears narrower and more pointed posteriorly with the wings held at a sharper angle ('tent-like').	Appears broader and more rounded posteriorly with the wings held more flatly.
Flight pattern	Often direct.	Haphazard.

eggs can also help to indicate which species is present but should not be used in isolation. The main characters that can be used to separate *B. tabaci* from *T. vaporariorum* in the field are listed in Table 2. Characters that can be used to separate all stages, except the egg, when examined under a high power microscope (× 100–400), are listed in Table 3. Hill (1969) gives a more detailed morphological comparison although he does not indicate the range of variation that may be encountered.

Other whiteflies

While *B. tabaci* and *T. vaporariorum* account for the majority of detection of whiteflies in imported consignments, it should not be assumed that these are the only species of *Bemisia* and *Trialeurodes* to be encountered in plant trade as there is the risk of overlooking species such as *B. afer* (Priesner & Hosny) (= *B. hancocki* Corbett), *T. lauri* (Signoret), *T. packardi* (Morrill), *T. ricini* (Misra) and *T. variabilis* (Quaintance).

B. afer has been detected on numerous occasions on imported plant material in the UK, most frequently on fresh vegetables from West Africa and bay (*Laurus nobilis*) plants from Europe. In the puparial stage, *B. afer* is the whitefly species most likely to be confused with *B. tabaci* during phytosanitary inspection. *B. afer* is broadly polyphagous and widespread in the tropics and subtropics and has recently been found breeding under glass and in restricted areas outdoors in the UK (Malumphy, 2003). Mixed populations of *B. afer* and *B. tabaci* have been intercepted on cassava (*Manihot esculenta*) leaves imported from Africa. The puparia of *B. afer* and *B. tabaci* may be separated by comparing the morphological characters listed in Table 4.

Morphological descriptions, illustrations and keys to the puparia of *B. afer* (and *B. tabaci*) are given by Bink-Moenen (1983), Bink-Moenen & Gerling (1992), Mound (1965), Martin (1987, 1999) and Martin *et al.* (2000). *B. afer* shows considerable variation in size, position of the vasiform orifice (Web Fig. 8) with respect to the posterior margin, extent of dorsal sculpturing, length of caudal setae and shape of the lingula. Mound (1965) discussed the phenotypic variation displayed by the puparia of the *B. afer* species group (Web Fig. 7) which according to Martin (1999), is even more complex than that found in *B. tabaci*.

According to Martin *et al.* (2000), there are only two species of *Bemisia* recorded in Europe and the Mediterranean, *B. afer* and *B. tabaci*. However, there are three other species names available, *B. citricola* Gomez-Menor, *B. ovata* (Goux) and *B. spiraeoides* Mound & Halsey. These three nominal species belong to the *afer* species group and may be separated from *B. tabaci* using the characters given in Table 4.

Table 3 Comparison of morphological characters of slide-mounted *Bemisia tabaci* and *Trialeurodes vaporariorum* seen under high magnification (× 100–400)

	Bemisia tabaci	*Trialeurodes vaporariorum*
1st instar		
Marginal setae	16 pairs.	17 pairs.
Cephalic tubercles	Weakly developed.	Well developed, subrectangular, mesad.
Vasiform orifice	Closed posteriorly.	Open posteriorly.
2nd instar		
Cephalic and 8th abdominal dorsal setae	Usually minute.	Well developed.
Vasiform orifice	Subcordate.	Open posteriorly.
3rd instar		
Marginal crenulations	Irregular.	Uniform.
Vasiform orifice	Subcordate.	Triangular, open posteriorly.
Lingula	Swollen and pointed distally.	Lobed distally.
Puparium	Web Figs 2, 3 and 4(i), 5 and 6	Web Fig. 4(i)i(i)
Submarginal papillae	Absent.	Present.
Marginal crenulations	Irregular.	Uniform.
Vasiform orifice	Subcordate.	Triangular, open posteriorly.
Lingula	Swollen and pointed distally.	Distinctly lobed distally.
Adult		
Upper and lower compound eyes	Connected by a single ommatidium (or very small gap less than the width of an ommatidium).	Separate.
Mesotibia	Opposite tufts of 2–3 stout setae may be present.	Conspicuous opposite tufts (combs) of 4–7 stout setae present.
4th antennal segment	Sensorial cone absent.	Stout sensorial cone near apex.
7th antennal segment	1 sensorial cone present.	2 sensorial cones present; 1 is small, slender and difficult to see.
Aedeagus	Slender with smooth ventral base.	Generally thicker and more robust with spiculate ventral base.
Male collar	Clear.	Pigmented.
Male abdominal dorsal surface	Distinct pores absent.	Distinct pores present.
Female cement gland	Usually distinctly sinuous; without bands and small head.	Not sinuous; with transverse bands (seen under phase contrast); large disc shaped head.
Female abdominal wax plates	Reticulate.	Striated.

Table 4 Comparison of some morphological characters of *Bemisia afer* and *B. tabaci* puparia

Morphological character	*Bemisia tabaci*	*Bemisia afer*
	(Web Figs 2, 3 and 4i, 5 and 6)	(Web Figs 4ii, 7 and 8)
Caudal setae	Always stout and usually as long or longer than vasiform orifice. Little variation between individuals.	Usually less than half the length of the vasiform orifice and often minute; highly variable between individuals.
Vasiform orifice	Slightly longer than the length of the caudal furrow; with straight sides.	Usually shorter than length of the caudal furrow; with sides often distinctly concave.
Lingula	Shorter and slightly wider.	Highly variable, generally longer and narrower.
Dorsal surface	Distinct stippling absent; small tubercles and papillae may be present.	Occasionally with distinct stippling and well developed tubercles and papillae.
Dorsal setae	Up to seven pairs of enlarged, well developed setae present; longer on plants with hirsute leaves.	Highly variable; often minute and difficult to detect but may be well developed.
Dorsal pore/porette pairs	Single pair between median line and first abdominal setae.	Most puparia with two pairs between median line and first abdominal setae; they are often difficult to detect.
Outline	Variable but may be strongly tapered to posterior, particularly on plants with hirsute leaves.	Variable but usually oval and rounded or slightly tapered to posterior.
Colour	Often distinctly yellow.	Highly variable, yellow, cream or almost transparent.

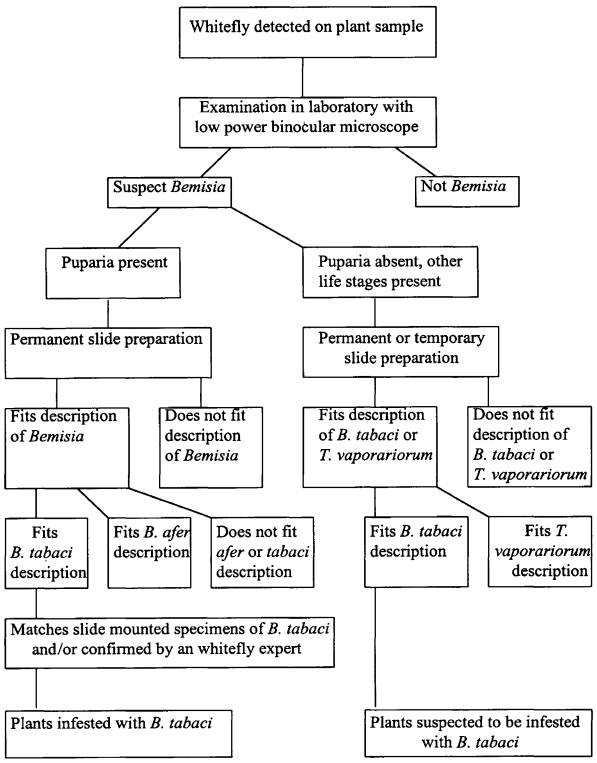

Fig. 9 Decision scheme for the detection and morphological identification of *Bemisia tabaci*.

Parabemisia myricae (Kuwana) is superficially similar to *B. tabaci* but is easily separated by the characters given above for the genus *Bemisia*. It may also be separated by the transverse moulting sutures reaching the margin and the absence of the caudal furrow. In *B. tabaci* the transverse moulting sutures do not reach the margin and the caudal furrow is distinct.

Requirements for a positive diagnosis

This protocol distinguishes *B. tabaci* from the species most likely to be confused with it (*B. afer*) and from the whitefly species most frequently encountered in phytosanitary inspections (*T. vaporariorum*). With experience, it is possible to identify *B. tabaci* puparia with the use of a low power microscope without making a slide preparation.

The requirements for a positive identification vary according to the experience of the diagnostician. Figure 9 provides a general scheme. A good slide preparation of a pupal case is generally needed and the specimen should match the morphological description and illustrations in this protocol. The specimen should be compared with authoritatively identified specimens and/or checked by an experienced whitefly specialist. However, in practice, an experienced diagnostician can identify poorly mounted and damaged specimens and will not need to examine every character described above. Voucher specimens from each interception should be kept for a period in case they require re-examination or further investigation.

Identifications made from stages other than the puparia should be recorded as provisional. Eggs and early instars can be reared to the puparial stage to confirm the identification. In practice, experience of past interceptions, hosts and country of origin, mean that one can strongly suspect nonpuparial stages to be *B. tabaci*.

Report on the diagnosis

A report on execution of the protocol should include:
- results obtained by the recommended procedures
- information and documentation on the origin of the infested material
- a description of the symptoms (including if possible photographs)
- measurements, drawings or photographs of the morphological features required for a positive diagnosis
- an indication of the magnitude of the infestation
- comments, as appropriate, on the certainty or uncertainty of the identification.

Further information

Further information on this organism can be obtained from: Dr C. Malumphy, Central Science Laboratory, Sand Hutton, York YO41 1LZ, UK.

Acknowledgements

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

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Appendix 1 Preparation of immature and adult whiteflies for microscopic examination

Specimens usually need to be macerated, de-waxed, dehydrated, cleared and in some cases, bleached or stained, before mounting on microscope slides. Voucher specimens and those required for future study are mounted in Canada balsam, which Brown (1997) concluded was one of the best mediums for permanent preparations. Specimens that need to be processed rapidly are mounted in Heinz, either directly or by using the temporary method described below. Post-emergence pupal cases and early larval instars are particularly suitable for temporary quick-mounting.

The permanent preparation technique given below is modified from Martin (1987). The procedures are not rigid and can be readily modified to suit particular samples. Specimens are manipulated and mounted on microscope slides with the aid of a binocular dissecting microscope. Heat is supplied, where necessary, by a heating block. Square-based watch glasses and glass slides should be accurately labelled with a waterproof marker throughout the procedure. The permanent preparation technique requires a minimum time of just over 1 h and the temporary technique 20 min.

Materials

Ammonium solution 35%; acid fuchsin stain (0.5 g acid fuchsin; 25 mL 10% HCL; 300 mL distilled water); Canada balsam; chloral phenol (160 g chloral hydrate crystals; 20 mL glucose syrup 50% ww; 160 g phenol crystals); clove oil; distilled water; ethanol 70–95%; glacial acetic acid; glucose syrup; Heinz (10 g Polyvinyl Alcohol (PVA); 40–60 mL distilled water, depending on viscosity required; 10 mL glycerol; 25 mL 1½% phenol solution; 100 g chloral hydrate crystals; 35 mL 85–92% lactic acid); hydrogen peroxide 30-volume; potassium hydroxide (KOH) 10%; xylene.

Procedures

Permanent slide preparation

This method is suitable for larval instars and adults. The best mounts are usually made from 'pupal cases' from which the adults have recently emerged although good results can be obtained from puparia with adequate maceration. Parasitized specimens should be avoided as they are often morphologically atypical. Parasitism can cause the puparium to become melanic, induce morphological variation, damage the puparium with the parasitoid exit hole and obscure diagnostic characters with the black fragments of ecdysed parasitoid larval cuticle. Obtaining good preparations of adults with this method is difficult (but not impossible) due to their fragile nature. Their bodies may collapse during dehydration in glacial acetic acid and the delicate wings are easily damaged.

Gently remove specimens from the leaf surface using a mounted blunt needle taking care not to puncture the specimen. Place about 10 specimens into 70–90% ethanol in a watch glass, cover with a glass square and heat gently to around 80 °C for 5–10 min. Fixation in hot alcohol hardens specimens and makes them less fragile, so they lose fewer setae during mounting. Add a few drops of cold 10% KOH to cool the alcohol. Pipette off the alcohol and KOH using a fine glass teat pipette, taking care not to accidentally suck up the specimens.

Add approximately 1 mL of KOH and heat to around 80 °C for 5–10 min, or until the specimens lose most of their colour. The length of time required varies considerably depending

on the species, body size, wax secretions, how long the specimens have been preserved in alcohol, the particular instar and maturity. Pupal cases require little maceration. Puparia require longer and the process is helped by making a small ventral incision using a mounted needle.

Examine the specimens under a binocular microscope. Where necessary, tease away the wax from the specimens using fine needles. With puparia, expel the liquefied body contents through the ventral incision using two fine spatulas. If the adult is well formed within the puparium it is often necessary to tease the body out. Parasitoid larvae and pupal cases and fungal hyphae are also removed. Parasitoid larvae are retained with the host specimens. Pipette off the excess macerant.

Soak the specimens in about 2 mL of cold distilled water or 70% ethanol for a minimum of 10 min. This rinses out the KOH. Pipette off the liquid. Rinse the specimens in about 2 mL cold glacial acetic acid (this neutralizes any remaining KOH) which is then pipetted off. Add a few drops of liquid chloral phenol, a wax solvent, to the watch glass. Gently heat for 5–10 min, depending on how waxy the specimens are. Waxier specimens require longer. The wax interferes with staining if not adequately removed. Pipette off the chloral phenol. Rinse the specimens in glacial acetic acid to remove the chloral phenol. Pipette off the liquid.

Black puparia require partial bleaching. Rinse specimens with a few drops of 95% ethanol. Decant the ethanol and add a few drops of cold ammonium solution. Add an equal number of drops of hydrogen peroxide 30-Volume and watch the puparia carefully. When the puparia have become pale, decant the bleaching solution. Alternatively the bleaching process may be stopped rapidly by adding a few drops of water-soluble acid. Pale puparia may be stained. Add several drops of glacial acetic acid and a few drops of acid fuchsin stain. Agitate the watch glass so the stain is uniformly mixed. Once the puparia have become a pale pink colour, decant the staining solution. Add fresh glacial acetic acid to the specimens and leave for at least 5 min to dehydrate completely. 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 spatula transfer a single specimen onto a clean glass slide, with the dorsal surface upwards. Parasitoid larvae are usually mounted with their host. Always mount specimens separately unless certain that they are the same species, in which case, mount up to six individuals on the same slide. Space the specimens evenly on the slide to prevent the coverslip from tilting when mounted. With the adults, spread out each body and arrange the appendages. The head should be on its side to see the lateral view of the eyes and the legs should be untangled and pulled away from the body in order to examine the setae arrangements. Absorb excess clove oil with the rolled corner of a tissue. Take care not to leave fibres from the tissue on the slide. Carefully apply a drop of dilute Canada balsam to the specimens on the slide. Rest one edge of a 16 or 18 mm diameter coverslip on the slide holding the opposite edge with a needle. Gently lower the coverslip with the needle onto the droplet of balsam covering the specimen. Take care to ensure that air is excluded and that the meniscus spreads outwards to the edge of the coverslip. Allow the coverslip to settle under its own weight. Label using Bristol board squares before placing in the collection to dry. Drying can take two months or more to complete. When dry scrape off excess balsam that has spread out from beneath the coverslip using a razor blade.

Temporary slide preparation

Specimens may be mounted directly into Heinz. This is particularly useful for small specimens with little wax, for example, first larval instars. However, better results are usually obtained by using the following method which is suitable for larval instars and adults. Only an outline is given as the details are the same as those above.

Place specimens in 70–90% ethanol in a watch glass; cover with a glass square and heat gently to around 80 °C for 5–10 min. Pipette off the alcohol. Simmer specimens in 10% KOH for approximately 5–10 min, or until the specimens lose most of their body colour. Examine the specimens under a binocular microscope. If necessary, expel the body contents by making a incision and pumping the liquefied body contents out, using two fine spatulas. Pipette off the excess KOH. Soak the specimens in cold distilled water or 70% ethanol for two minutes. Pipette off the liquid. Rinse the specimens in fresh 70% ethanol for 5 min. Mount the specimens in Heinz on a glass microscope slide.

Fig. 1. General morphology of a whitefly puparium

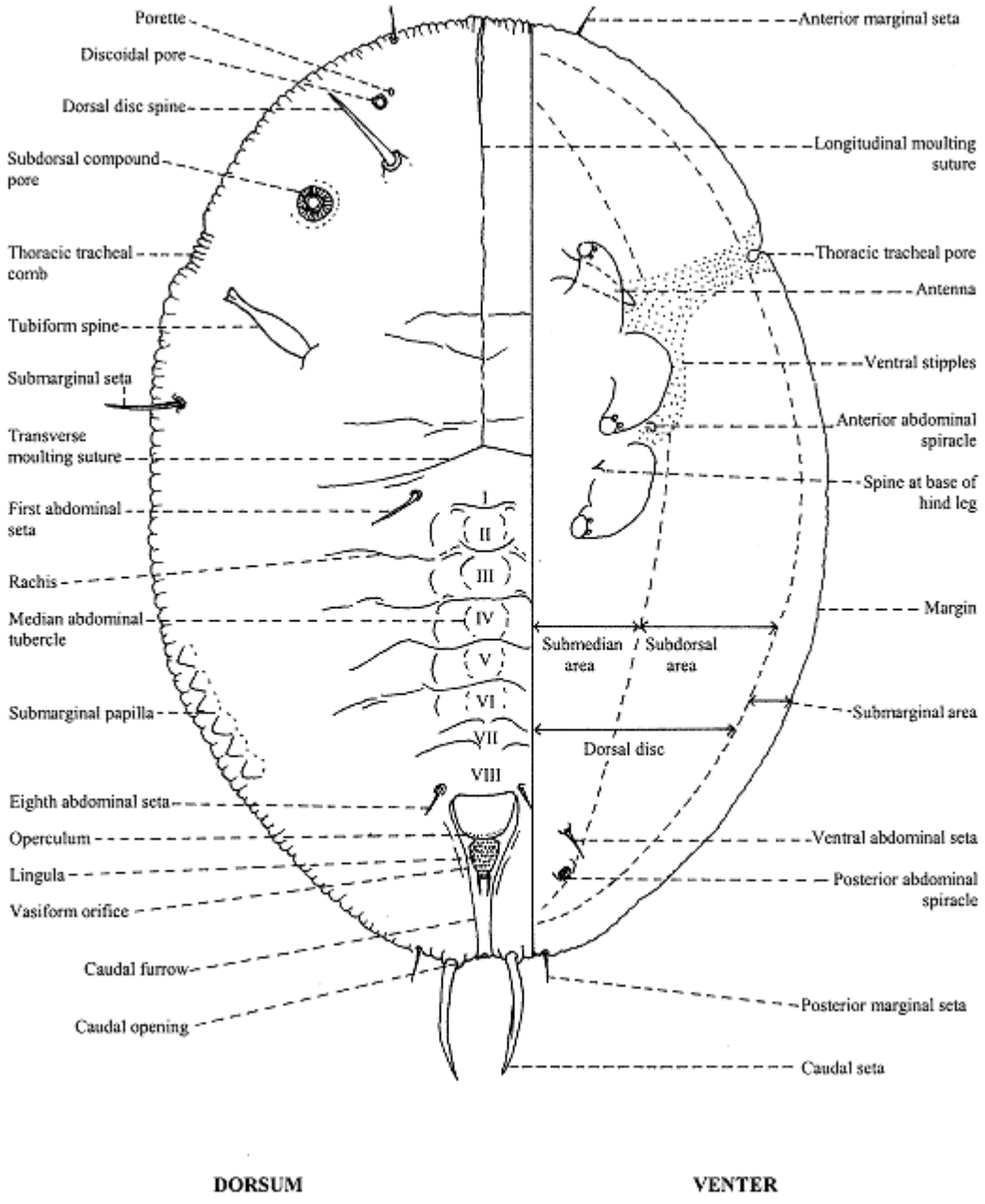


Fig. 2. *Bemisia tabaci*, puparium ex *Solidaster*, Kenya (glabrous, smooth leaf). The dorsum is illustrated on the left and ventrum on the right.

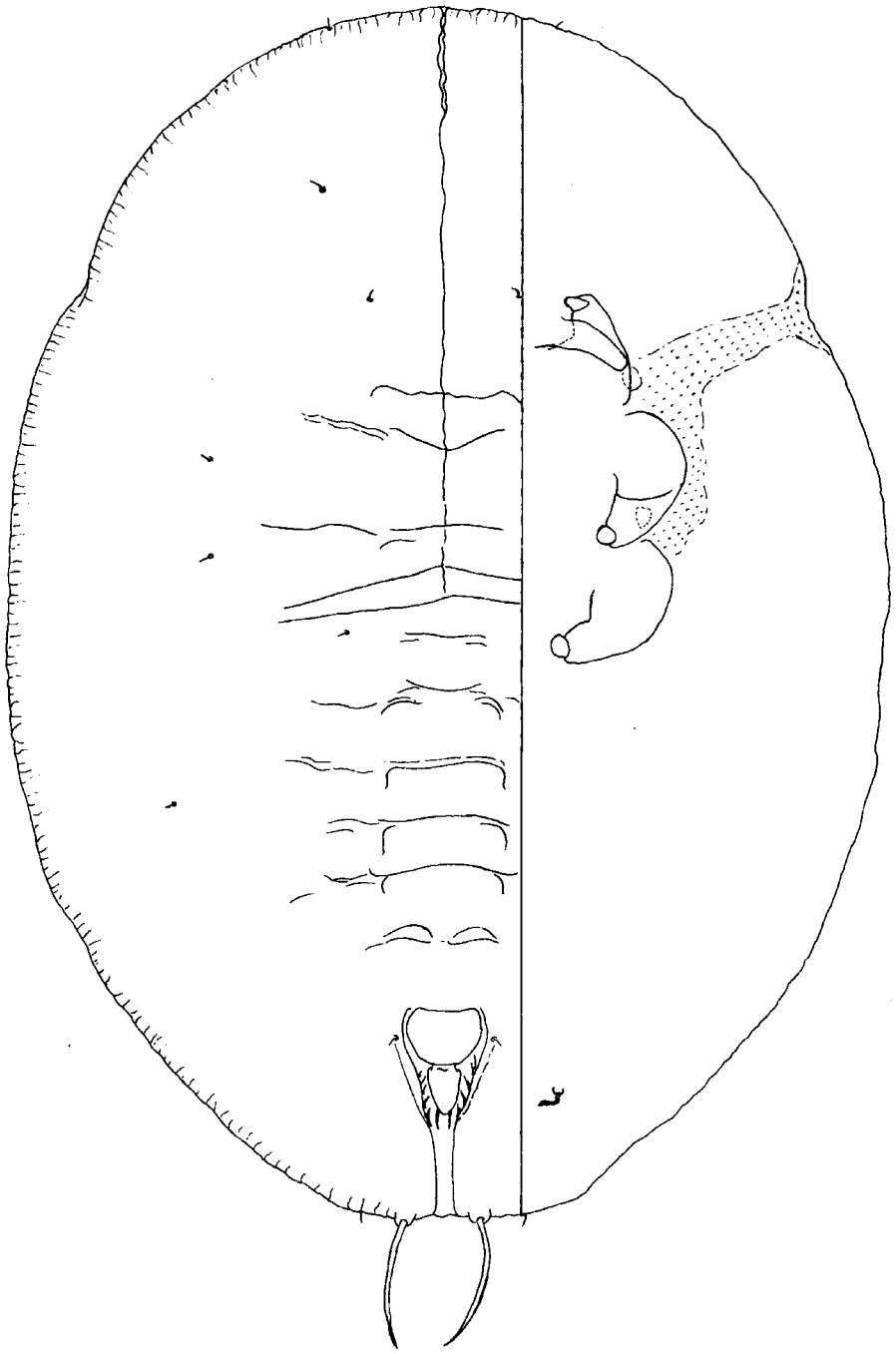


Fig. 3. *Bemisia tabaci*, puparium ex *Viola*, France (hirsute leaf). The dorsal setae are enlarged, dorsal tubercles and papillae are present and plant hairs have indented the margin.

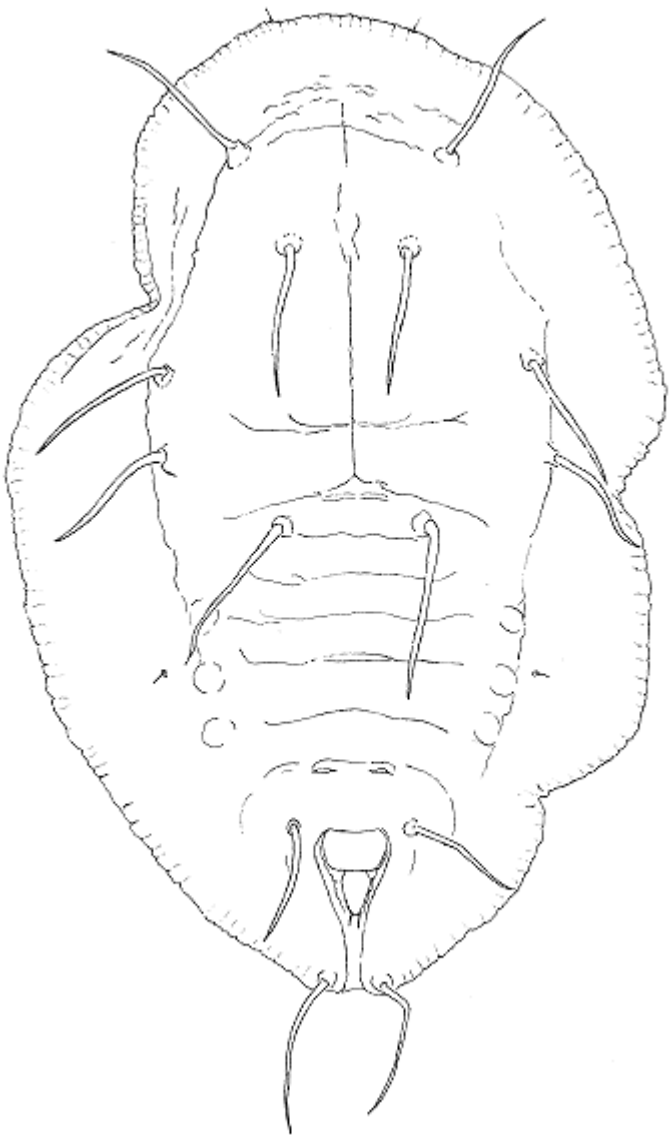
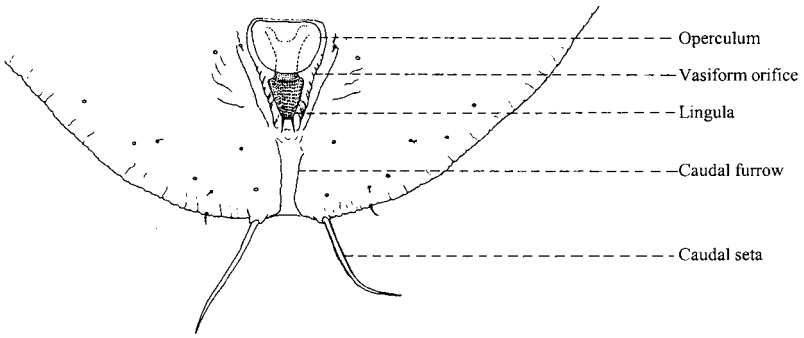
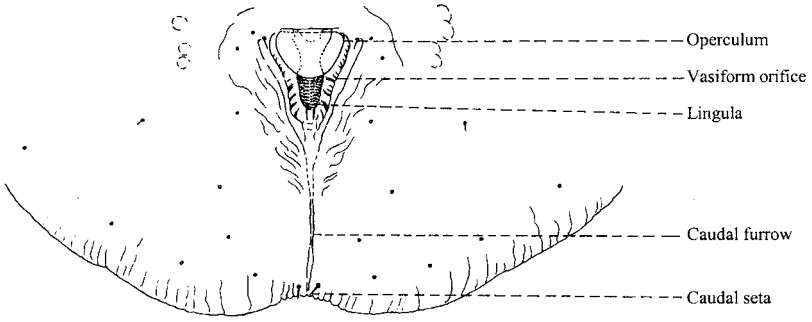


Fig. 4. Vasiform orifice: i, *Bemisia tabaci*; ii, *Bemisia afer*; iii, *Trialeurodes vaporariorum.*

i.



ii.



iii.

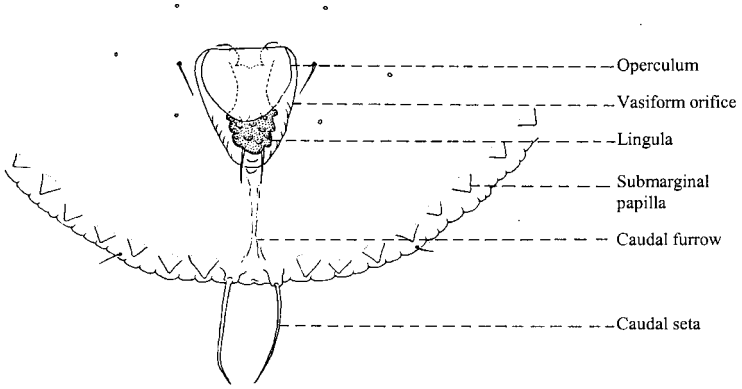


Fig. 5. *Bemisia tabaci*, pupal case ex *Euphorbia pulcherrima* (hirsute leaf), UK

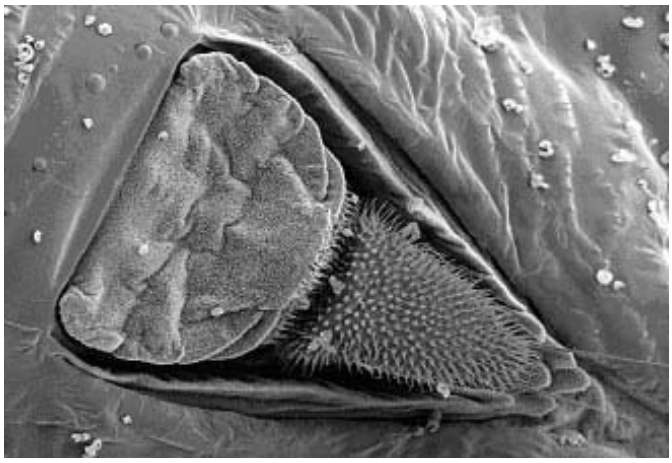


Fig. 6. *Bemisia tabaci*, vasiform orifice, operculum and lingula

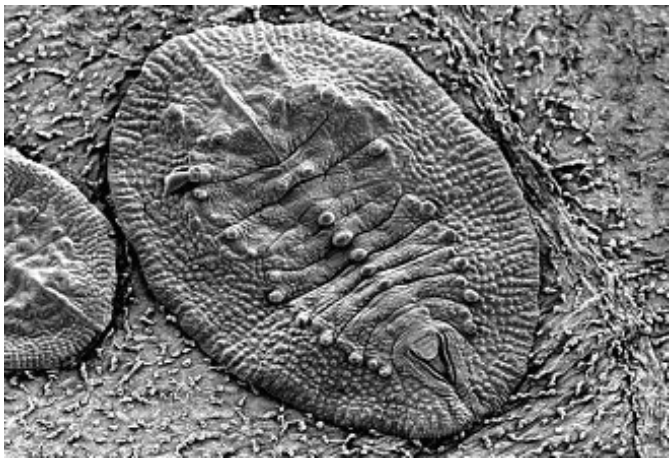


Fig. 7. *Bemisia afer*, puparium ex *Manihot esculenta* (glabrous leaf), Uganda

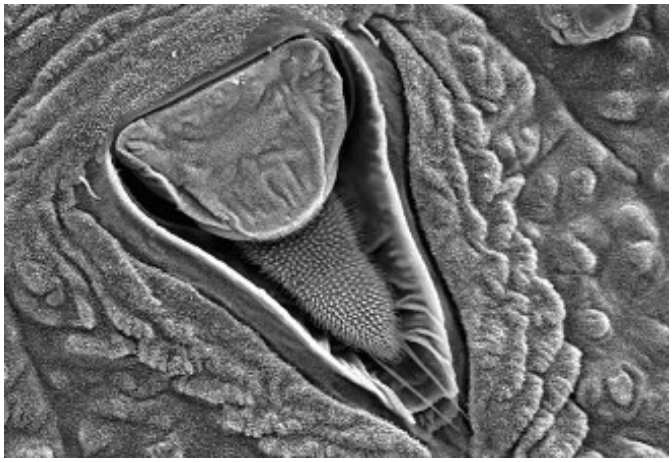


Fig. 8. *Bemisia afer*, vasiform orifice, operculum and lingula