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# Field observations, laboratory rearing and descriptions of immatures of the planthopper *Hyalesthes obsoletus* (Hemiptera: Cixiidae)

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**Abstract.** The cixiid planthopper *Hyalesthes obsoletus* Signoret is an efficient vector of the stolbur phytoplasma, the cause of various crop diseases. In the field, this monovoltine species feeds on a wide variety of woody and herbaceous plants. It overwinters as larvae on the roots of its host plants. During this study, we collected adults mainly from lavender (*Lavendula angustifolia* Miller), bindweed (*Convolvulus arvensis* L. and *C. sepium* L.), hoary cress (*Cardaria draba* L.), and occasionally from plantain (*Plantago cynops* L.), toadflax (*Linaria striata* L.), bedstraw (*Galium verum* L.), and mountain savory (*Satureia montana* L.). Fertility of field collected females from sites at two different elevations differed significantly. Fertility at 300 m (50.6 eggs per female; N = 28) was more than twice that at 900 m (22.8 eggs per female; N = 19). Only one specimen of the species was found to be parasitized by an undetermined species of Dryinidae (Hymenoptera). *H. obsoletus* was reared in controlled conditions on lavender. Unlike in the field, larvae developed in the laboratory at the base of the host plant and on basal shoots. Egg incubation averaged 7 ± 1.2 weeks (N = 10). Total development time from egg to adult averaged 27 ± 4 weeks (N = 5) on lavender. A morphological description of the five instars is provided. The study was supplemented by scanning electron microscopy. Particular attention was paid to the structure of the wax-plates and the absence of compound eyes in the early larval stages.

## INTRODUCTION

The family Cixiidae includes about 800 species found in all temperate and tropical regions (O'Brien & Wilson, 1985). These planthoppers suck plant sap and are probably phloem-feeders, although some may feed on fungi (Wilson et al., 1994).

The life cycles of few cixiids have been studied in detail. In those few, adult females lay eggs in soil near the base of a host plant. The five larval instars feed at the stem bases or on roots, and recently emerged adults fly to the upper portions of their host plants (which may be different from the natal host plant) (Wilson & Tsai, 1982). In some cave-adapted species the larvae and adults both feed on roots underground (Hoch & Horvath, 1989). The field biology of *Oliarus felis* Kirkaldy (Hacker, 1925) and *Myndus crudus* Van Duzee (Reinert, 1977; Tsai & Kirsch, 1978) have been studied in detail. Larvae of *Bothriocera signoreti* Stål, *Mnemosyne cubana* Stål (Myers, 1929), *Myndus crudus* (Wilson & Tsai, 1982) and *Oecleus borealis* (Wilson et al., 1983) have been described and illustrated.

Eleven species of cixiids are considered pests and four of these have been implicated in transmission of phytoplasma pathogens (Wilson & O'Brien, 1987). Pathogen vectors include *Myndus crudus* Van Duzee and *M. taffini* Bonfils feeding on coconut palm (*Cocos nucifera* L.) (Julia, 1982), *Oliarus atkinsoni* Myers on flax (*Phormium tenax* Forster) (Cumber, 1952), and *Hyalesthes obsoletus* Signoret on various species of plants (Valenta et al., 1961; Sforza, 1998).

The genus *Hyalesthes* Signoret, 1865 (Hemiptera: Cixiidae) includes 34 species belonging to five monophyletic subgroups in the Palaearctic region (Hoch & Remane, 1985; Hoch, 1986). One of these species, *H. obsoletus* Signoret, 1865 occurs throughout the Mediterranean basin up to Germany and Russia (Blattný et al., 1954; Hoch, 1986). It is known to be a vector of stolbur disease of solanaceous plants (Suchov & Vovk, 1948) and of various other crops of economic importance (Valenta et al., 1961; Marchoux et al., 1970). Recently, stolbur phytoplasmas have been identified in numerous plant species (Fos et al., 1992; Daire et al., 1993; Maixner et al., 1995) and the role of *H. obsoletus* as a vector demonstrated, including that of the phytoplasma infesting grapevines (Maixner, 1994; Sforza et al., 1998).

Twenty five plant species have been identified as overwintering hosts (Güclü & Ozbek, 1988; Kovaceski, 1958; Leclant, 1968). In Italy, Germany and France, *H. obsoletus* was captured in different vineyards mainly on bindweed, *Convolvulus arvensis* L. (Vidano et al., 1985; Alma et al., 1987; Maixner et al., 1995; Sforza et al., 1998). In France, the important host plants are bindweed (*Convolvulus arvensis* L.), lavender (*Lavendula angustifolia* Mill.), lavendin (*Lavendula hybrida* Reverchon), and hoary cress (*Cardaria draba* L.) (Leclant, 1968; Fos et al., 1992; Sforza et al., 1998). Adults and immature stages of *H. obsoletus* have been the focus of studies in the Czech Republic (Musil, 1956) and in Turkey (Güclü & Ozbek, 1988). However, in these studies the planthopper was not reared or its larval development surveyed. The

male genitalia of *H. obsoletus* were studied by Musil (1956) and those of the male and female of all species of the genus *Hyalesthes* by Hoch & Remane (1985). Larval stages of cixiid species are difficult to observe in the field, due to their feeding on the roots of their host plants. In the laboratory, the cixiid planthopper, *Myndus crudus* (Van Duzee) has been mass reared in Petri dishes (Tsai et al., 1976) without the use of soil, so permitting observations on larval development.

The present paper provides new data on the biology of *H. obsoletus* based on field observations and laboratory rearing. Moreover, in order to provide a morphological characterization of this insect vector, this paper presents a description of the immatures and a scanning electron microscopy study of the wax glands.

## MATERIAL AND METHODS

### Field study

Observation and collection of adults of *H. obsoletus* were made once per week from April to October in 1995 and 1996 in France in the Rhône valley, in Burgundy and in North Provence. Detailed field observations and collections of larvae were made five times from October to April. Most of the data were collected at the following sites: Alba-la-Romaine and Lavilledieu (site A) which are at an elevation of 300 m in the South Rhône-Alpes, and Simiane at 900 m (site B) in North Provence.

Adults were swept from vegetation using a sweep net, collected using a D-vac, or captured by yellow sticky traps placed ten meters apart on the ground at the base of host plants. Sticky traps were checked (three traps at site A; six traps at site B) and replaced each week. Specimens were preserved in 70% ethanol. Second to fifth instar larvae were hand-picked from roots of wild plants of bindweed, lavender and hoary cress and transferred to the roots of laboratory-maintained plants of the same species.

Wild females captured either at site A or site B were dissected and the number of eggs in each female recorded.

### Laboratory rearing

Field-collected adults of *H. obsoletus* were placed directly on either bindweed or lavender growing in a 12 cm-diameter pot, covered by a 20 cm high transparent Plexiglas cage. Seedlings of lavender and field-collected pathogen-free bindweed were used in the experiments. Lavender (*Lavandula angustifolia* Miller) was selected because of its slow vegetative development under controlled conditions compared to bindweed and hoary cress.

The soil used in the pots was a mixture of 50% peat and 50% small gravel, ca. 0.5 mm diameter.

Eggs, larvae and adults were kept in an environmental chamber at 23 ± 1°C under a 16L : 8D photoperiod and 80% relative humidity. Field-captured females laid their eggs in controlled conditions at the base of seedlings of lavender or just under the soil surface. First instar larvae were picked up using a small paint brush (No. 2–4), and deposited close to two-month old, 10 cm high lavender seedlings. Larvae were reared in groups of 20–30 per pot. To determine the duration of larval development, individual larvae were placed onto lavender seedlings in separate pots. Observations were made once per week until adult emergence.

Pots were watered every day by pouring water into the saucers in which each pot was standing. Care was taken to prevent overwatering because it increased larval mortality due to drowning. Whenever a plant died, larvae were transferred to another lavender plant.

Data on rearing and duration of larval development were obtained from laboratory cultures of larvae.

### Morphological study

Drawings of eggs and larvae were made with the aid of a stereoscopic microscope (Nikon SMZ-U) and a camera lucida. For morphological studies using scanning electron microscopy (SEM), the insects, adults and larvae, were first cleaned by soaking in chloroform (3 min) and then washing twice in 70% ethanol. Specimens were then dehydrated by placing them in increasing concentrations of alcohol, then critical-point dried and coated with a 65–70 µm gold-palladium film. They were examined using a Jeol JSM 840 scanning electron microscope.

## RESULTS

### Field study

*Hyalesthes obsoletus* is a monovoltine species which overwinters as larvae. In our study, larvae were collected at sites A and B from depths of 2–20 cm in the soil, depending on time of the year and climatic conditions. They were deep in the soil from November to March. We collected larvae on the roots of bindweed and hoary cress at site A and lavender at site B. No first instar larvae were collected. Second and third instars were collected from November to March and are the overwintering instars. Fourth and fifth instars were collected in May and the beginning of June at site A and from the beginning of June at site B. One lavender seedling harboured 37 fourth and fifth instar individuals on its roots. Larvae were active, even those deeply buried during winter. Larvae survived low temperatures; several third instars were still alive after 3 days storage in a refrigerator at 4°C. They were mainly found on axial roots, in small cavities covered by wax produced by their abdominal wax-glands. We observed that the emergence of adults took place in the soil, between 1 to 5 cm below the soil surface in small cavities, as previously described by Suchov & Vovk (1948). The newly emerged adults began their epigean life after leaving the soil by crawling between rocks and earth.

The first adults were captured on 10 June in 1995 and 1996, and the last on 22 August in 1995 and 16 August in 1996 at site A. The population peak occurred on the first week of July in 1995 and at the end of June in 1996. The population peak occurred one month later at site B in 1996. Adults were present for almost ten weeks at both sites.

Gravid females were captured from 17 June until 20 August 1996 at site A. This suggested that copulation must have occurred soon after emergence. Copulating individuals were observed throughout the day.

TABLE 1. Mean number of eggs in the ovaries of *Hyalesthes obsoletus* collected at sites at two different elevations: Site A, 300 m; site B, 900 m.

| | Site A 300 m | Site B 900 m |
|---|---|---|
| No. of gravid females examined | 28 | 19 |
| Mean No. of eggs/female ± SE | 50.6 ± 17.3 | 22.8 ± 6.2 |

Table 1 presents the fertility of collected females at two elevations. The difference in the number of eggs present in females from the two elevations was significant ($t = -6.82$; $df = 45$; $P < 0.005$).

Adults were collected by sweeping various plants, i.e. lavender, bindweed (*C. arvensis* and *C. sepium* L.), hoary cress, plantain (*Plantago cynops* L.), toadflax (*Linaria striata* L.), bedstraw (*Galium verum* L.), and mountain savory (*Satureia montana* L.). So far only the first three plants have been proved as host plants.

Out of the 2,000 adults captured over two years, only one female collected from Alba-la-Romaine in 1995 was found to be parasitized. A thylacium possibly containing a larva of Dryinidae (Hymenoptera) was found protruding from the middle lateral part of a female abdomen. During the study, we captured two other *Hyalesthes* species, *H. scotti* Ferrari, 1882 and *H. luteipes* Fieber, 1876, both on dogwood (*Cornus sanguinea* L.), lilac (*Syringa vulgaris* L.) and elm (*Ulmus* sp.). Only the latter plant has been reported as a host plant for *H. luteipes* (Hoch & Remane, 1985). In addition, in March 1997, two second instars were found on the roots of *C. sanguinea*. The species could not be identified (no larval identification key available). In 1996, copulation in *H. scotti* was observed; the position of mating adults was as described for *H. obsoletus* (Hoch & Remane, 1985; Sforza & Bourgoin, 1998). Only two females among the hundreds adults of *H. scotti* collected were found to be parasitized. A thylacium was observed protruding from the middle part of the abdomen of two females. The thylacium on *H. scotti* was more flattened and elongated than that observed on *H. obsoletus*.

**Laboratory rearing**

Females collected in the field in the summer of 1996 laid eggs in the laboratory close to the base of lavender seedlings either on the soil surface or 2–3 cm below the surface. Numerous females were found dead in the soil. It was assumed that death had occurred after the laying of eggs. Eggs were always clustered in small groups and averaged 39 ± 7 (N = 6) per group. Several hundred eggs were obtained, but less than fifty planthoppers were reared to the adult stage. Larval development in the laboratory was quite different from that found in the field. Larvae did not live only on the roots. They colonized the base of the plant and the basal parts of stems of lavender and were easily observable. Larvae did not appear to be negatively phototactic as under field conditions, instead they were active and aggregated along the wax plumes deposited on stems. Larvae of the fifth instar, several days before the adult moult, were enclosed separately in a cocoon of wax into which the adults emerged. Unlike in the field where the adult moult occurred in the soil, the cocoons in the laboratory occurred either at the base of a plant or on the basal stems.

An analysis of the duration of the larval instars was completed in spite of high larval mortality (Table 2). Egg incubation averaged 7 ± 1.2 weeks (N = 10). The total development from egg to adult averaged 27 ± 4 weeks (N = 5) on lavender. We began the rearing of *H. obsoletus* from eggs in July 1996. The adults of the first generation appeared in December 1996. The adults of the second generation were obtained in August 1997. Copulation was observed within 10 days of adult emergence. Mass rearing was not possible, due to high mortality.

**Morphological description of immature stages**

SEM study of wax glands

Copious amounts of wax covered the eggs, which made it easy to find them in the ground. The female wax glands (Fig. 1) secrete wax. Each glandular unit is recognizable by its external pore pattern (Figs 1, 2). In adult females, these units are 8–shaped structures almost 3 μm long and 1.8 μm wide. They consist of a central pore surrounded by a circular cuticular ridge, 1 μm in diameter, in a pair of crescent shaped hollows, corresponding to two covered pores (Fig. 2). SEM of the wax plumes secreted by each glandular unit revealed that the plumes had a ladder-shape. The uprights are probably secreted through the modified cuticle of the crescent shaped pores, while the rungs are probably generated by the central pore.

Wax plates are also present in larvae, on abdominal terga VI to VIII, as in many other fulgoromorph larvae (Fig. 3). Glandular units are more complex, flower-like and similar to those already described in another cixiid, *Myndus crudus* by Pope (1985). Each glandular unit consists of a central circular area almost 5 μm in diameter surrounded by a cuticular ridge with 5–8 white pits (Fig. 4). Each pit is externally bordered by a petal-like ornamentation on the internal base of which are a pair of pores, 0.8 μm in diameter (Fig. 4).

Description of immature stages

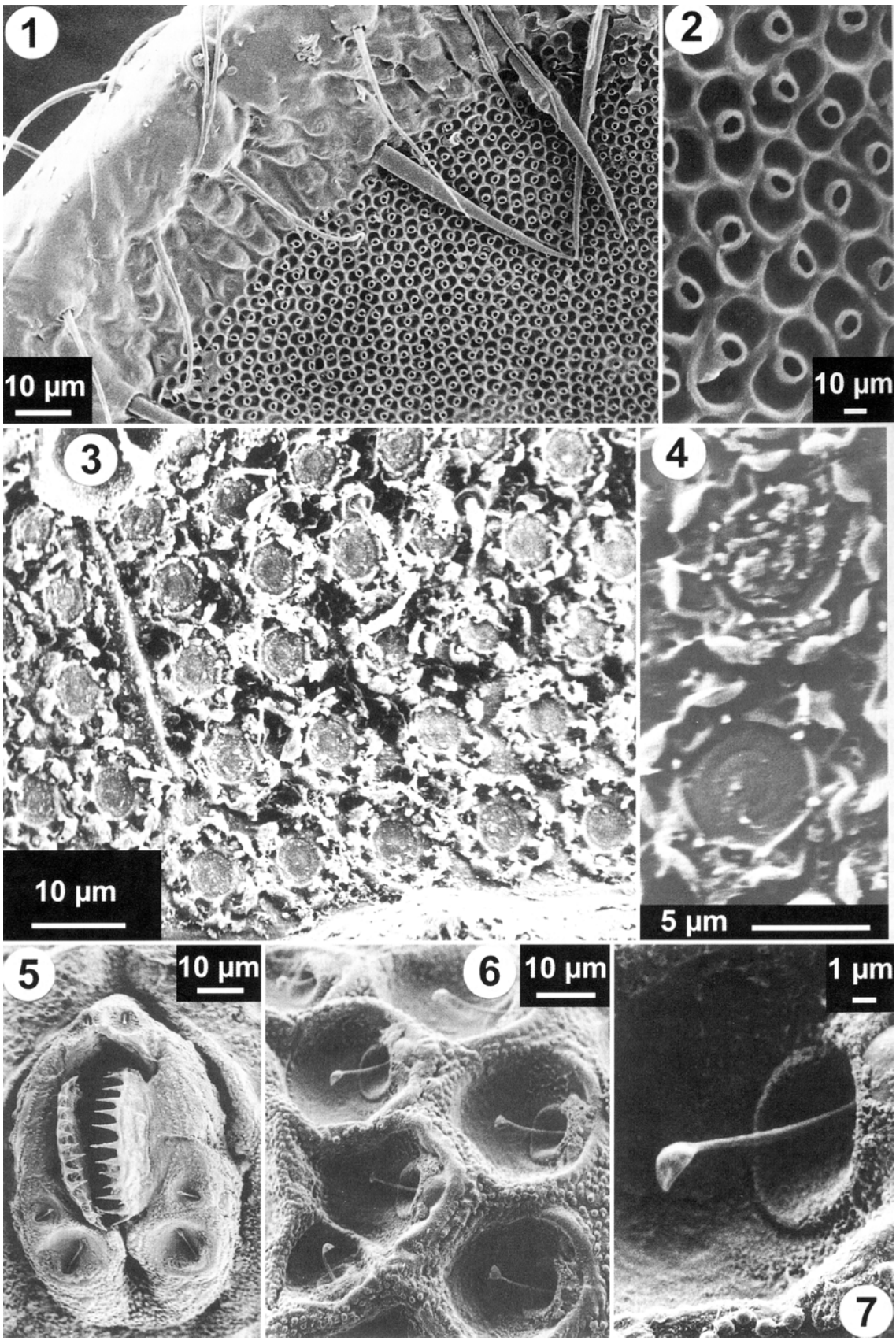
Dimension of eggs and larvae are expressed in millimetres, as mean ± SE. The length of larvae was measured from the tip of the vertex to the tip of the abdomen; their width was measured across the widest part of the body. The thoracic length was measured along the midline from the anterior margin of the pronotum to the posterior margin of the metanotum. Head length was measured from the tip of vertex to the anterior margin of the pronotum; head width was measured between the outer lateral carinae.

**Egg** (Fig. 12) (N = 98). Length 0.53 ± 0.04; width 0.27 ± 0.01. Eggs laid in groups; each egg elongate, oval; white; chorion translucent and covered with wax.

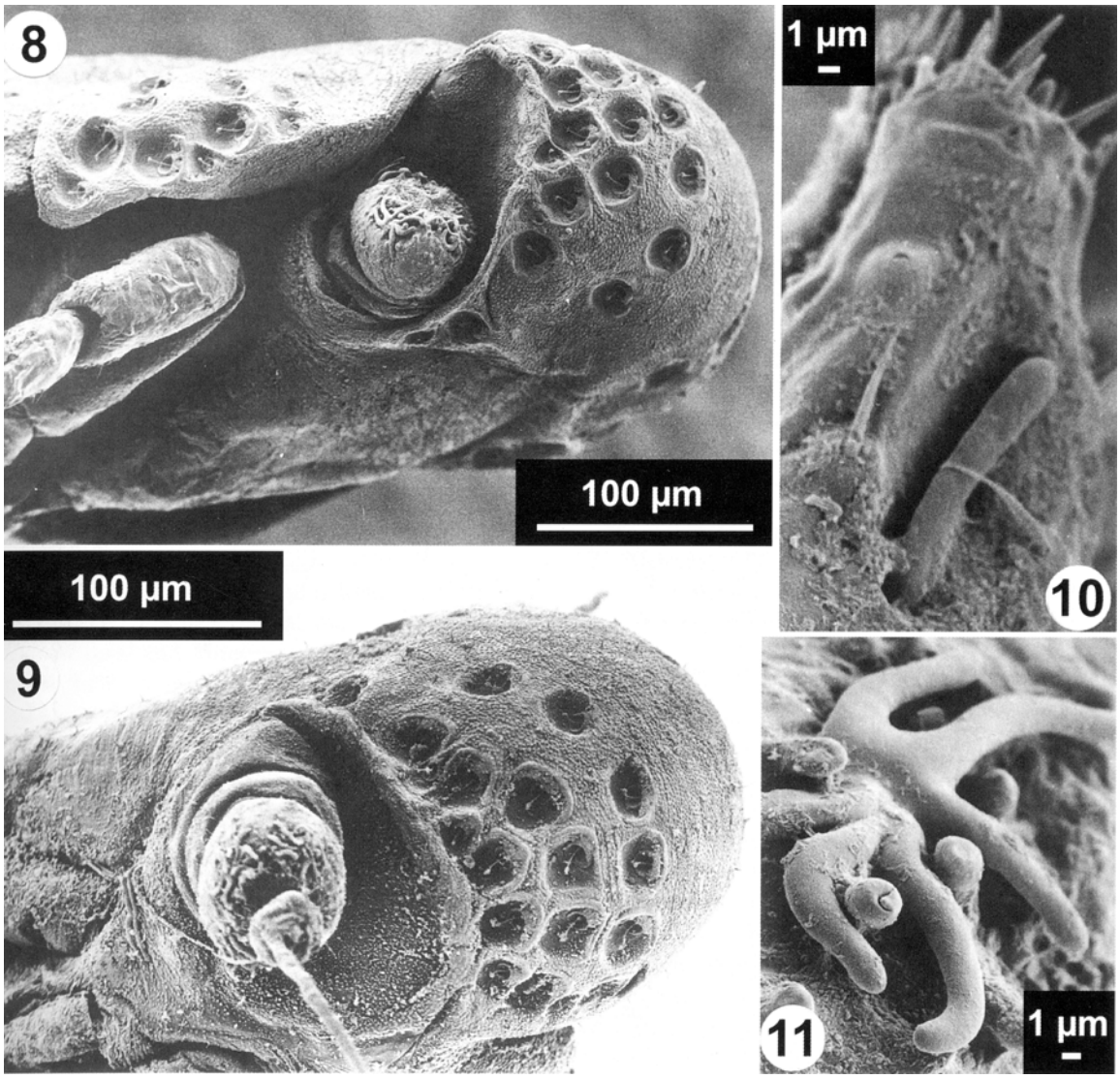
**First instar** (Fig. 13) (N = 21). Length 0.67 ± 0.1; width 0.29 ± 0.7; thoracic length 0.28 ± 0.03; head length

TABLE 2. Duration (in days) and larval mortality of *Hyalesthes obsoletus* under laboratory conditions.

| Larval instar | No. of larvae beginning instar | No. of larvae completing instar | Days | |
|---|---|---|---|---|
| | | | Range | Mean ± SE |
| 1st | 33 | 15 | 13–37 | 15.7 ± 6.22 |
| 2nd | 15 | 1 | 30 | 30 |
| 3rd | 9 | 2 | 24–46 | 35 ± 15.55 |
| 4th | 6 | 3 | 19–22 | 20.3 ± 1.52 |
| 5th | 3 | 3 | 14–28 | 24 ± 7.02 |




Figs 1–7: Stereoscan electron micrographs of *Hyalesthes obsoletus*. 1 – external structure of the wax glands on a wax plate of a female; 2 – details of glandular unit; 3 – wax plate on tergum VII of a fourth instar; 4 – detail of two glandular units; 5 – segment IX of a second instar bearing anal comb; 6 – right lateral view of vertex of a second instar with sensory pits; 7 – detail of a sensory pit.




Figs 8–11: Stereoscan electron micrographs of *Hyalesthes obsoletus*. 8 – right antero-lateral view of a second instar illustrating absence of a compound eye above the antenna; 9 – left anterolateral view of a third instar illustrating absence of compound eye; 10 – left preapical labial sense organ of a second instar; 11 – detail of the plate organ on the antennal pedicel of a second instar.

0.08 ± 0.004; head width 0.20 ± 0.01. Form elongate, subcylindrical, widest across metathorax. Body white with many sensory pits on head, thorax and abdomen; a pit is a crater-like depression, 20 µm in diameter, bearing a clavate hair 10 µm in length, extending from the centre of a circular aperture, 6 µm in diameter (Figs 6–7).

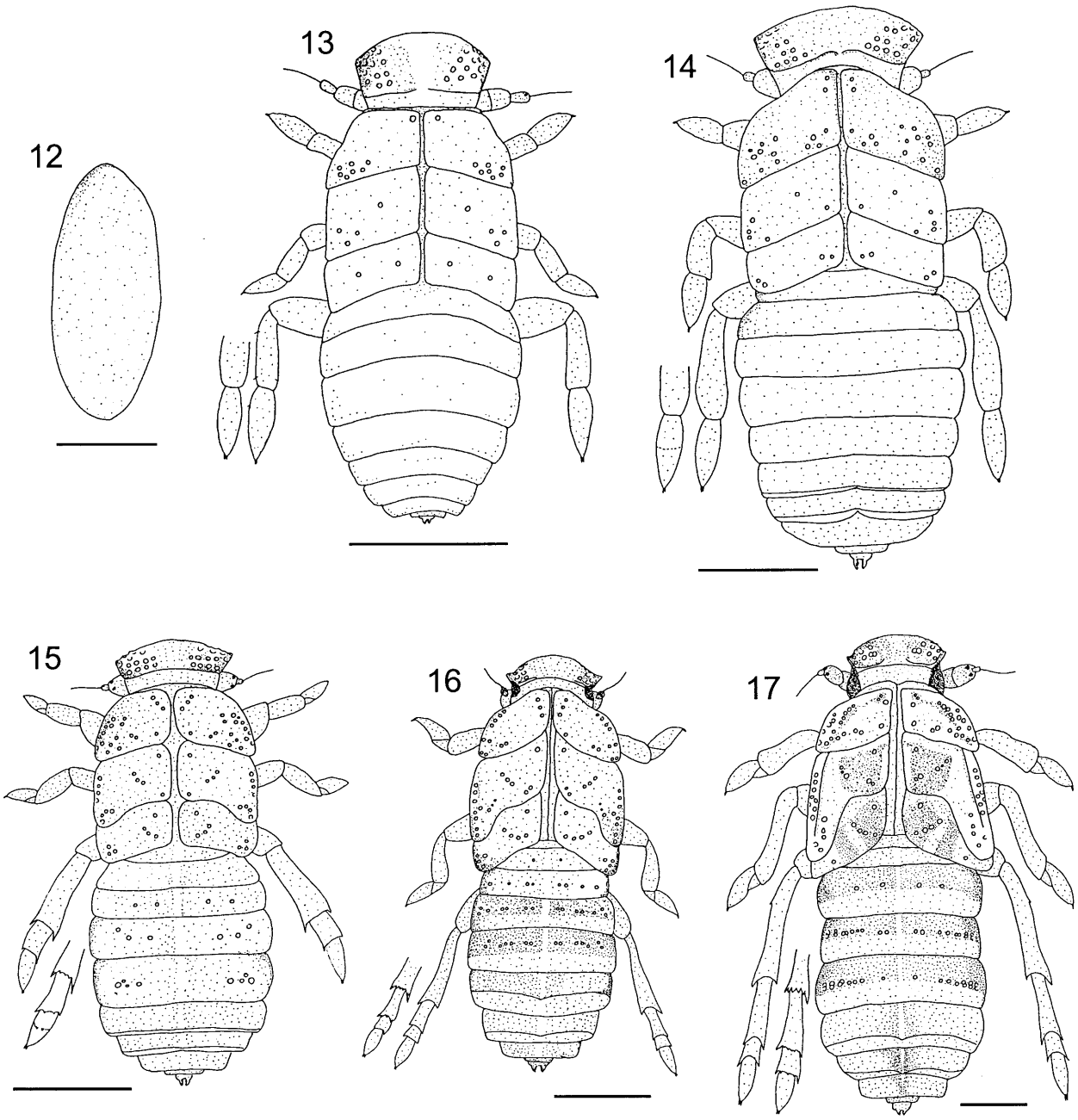
Head white; in dorsal view, sensory pits arranged in three semi-circles on lateral margins of vertex bearing respectively four, four and six pits; vertex approximately twice wider than long.

Compound eyes absent. Antennae three-segmented; scape cylindrical; flagellum thin and elongate and bearing on a basal swelling the Bourgoin's organ and a tubular "second projection" (Shish & Yang, 1996).

Thoracic nota divided by longitudinal middorsal line into three pairs of plates; wingpads not developed; pronotal plates each with eight sensory pits, one on anterior margin near middorsal line and a group of seven on lateral basal part of the plate; mesonotal plate each with four sensory pits, one in median half and three on lateral basal part; metanotal plate with two pits on median half. Metatarsi with one tarsomere subequal to metatibia in length. Apex of metatibia without teeth.

Abdomen 9-segmented; terga semicylindrical; abdomen widest across segments III and IV. Posterior parts of terga VI to VIII bearing wax plates; segment IX surrounding anus.

**Second instar** (Fig. 14) (N = 22). Length 0.99 ± 0.18; width 0.42 ± 0.06; thoracic length 0.38 ± 0.07; head length 0.12 ± 0.03; head width 0.24 ± 0.03. No compound eyes (Fig. 8). Antennal pedicel bearing three plate organs of the "flattened star-shaped" type (Bourgoin & Deiss, 1994) (Figs 8, 11). Basal swelling of flagellum ca. 1/2 × length of pedicel. Distribution of sensory pits on head as in first instar.




Figs 12–17: Egg and immature stages of *Hyalesthes obsoletus*. 12 – egg; 13 – first instar; 14 – second instar; 15 – third instar; 16 – fourth instar; 17 – fifth instar. Scale bar = 0.2 mm (12–14), 0.5 mm (15–17).

Vertex subrectangular, wider than long, anterior margin not differentiated from border with frons, which is convex and rounded in lateral view; in frontal view, lateral carinae extending ventrally to base of antennae. Clypeus narrowing distally, consisting of rectangular basal postclypeus and cylindrical distal anteclypeus. Proboscis three-segmented; segment 2 twice the length of segment 1; segments 2 and 3 subequal. Preapical labial sense organ (Cobben, 1988) long and tubular (Fig. 10).

Pronotal plates each with about 15 sensory pits; mesonotal plates each with 6 pits; metanotal plates each with four pits; wingpads not developed. Metatarsi with one tarsomere bearing a transverse line in middle of plantar surface.

Abdominal segment IX with 4 sensory pits of different sizes (Fig. 5), and surrounding anus; anal combs of cixiid type (Yang & Yeh, 1994), their posterior margin bearing a row of 12–13 teeth of irregular length (Fig. 5).

Otherwise similar to first instar.

**Third instar** (Fig. 15) (N = 17). Length 1.48 ± 0.22; width 0.55 ± 0.03; thoracic length 0.53 ± 0.05; head length 0.13 ± 0.02; head width 0.30 ± 0.04.

Ocular structure represented by three poorly developed, red circular facets; no compound eyes (Fig. 9). Basal swelling of flagellum ca. 2/5 × length of pedicel. Mesonotal wingpads weakly developed, covering metanotal wingpads laterally at base. Pronotal plates each bearing about 20 sensory pits in three rows, 14 pits along lateral

margin, three pits near posterior margin and three pits on anterior margin. Mesonotal plates each with about 13 sensory pits, eight pits near lateral margin, three pits on middle half and two pits near middorsal line. Metanotal plates each with seven pits.

Metatibia with apex bearing a transverse row of five spines, two lateral ones much more elongate. Metatarsi with two tarsomeres; tarsomere 1 subequal in length to tarsomere 2; tarsomere 2 partially subdivided and bearing two weak spines in middle of plantar surface.

Otherwise similar to second instar.

**Fourth instar** (Fig. 16) (N = 27). Length 2.17 ± 0.28; width 0.83 ± 0.11; thoracic length 0.82 ± 0.10; head length 0.16 ± 0.03; head width 0.46 ± 0.03. Body white with a few light brown markings on thoracic plates and dorsal aspect of abdomen.

Compound eyes represented by fewer than ten poorly developed, red circular facets. Basal swelling of flagellum ca. 1/4× length of pedicel.

Pronotum with each plate subquadrate, with about 14 sensory pits, six pits near lateral margin, five pits near posterior margin and three pits near middorsal line. Mesonotal plates each with about 17 sensory pits, 10 obscure pits near lateral margin, three pits in median half, and three pits near middorsal line. Metanotal plates each with nine sensory pits, four pits near lateral margin, four pits near posterior margin, and one pit near anterior margin. Pronotal median length subequal to that of mesonotum. Each mesonotal plate bearing a wingpad covering lateral half of metanotal wingpad.

Terga VI, VII and VIII bearing each at least eight sensory pits between wax plate area; pits always along a middle transverse row, either singly or in groups of two or three.

Metatibia with apex bearing a transverse row of 5 black-tipped spines apically on plantar surface, outer spine ca. 4× length of the others; metatarsi with three tarsomeres; tarsomere 1 with 4 weak spines and subequal in length to tarsomere 3; tarsomere 2 ca. 1/2× length of tarsomere 1.

Otherwise similar to third instar.

**Fifth instar** (Fig. 17) (N = 41). Length 3.01 ± 0.56; width 1.20 ± 0.23; thoracic length 1.15 ± 0.12; head length 0.19 ± 0.04; head width 0.62 ± 0.06. Bulbous portion of flagellum ca. 1/4× length of pedicel.

Red compound eyes. Thoracic nota infused with brown. Pronotum with each plate subrectangular, anterior margin slightly sinuate, with about 20 pits on each plate. Mesonotal median length ca. 1.5–2× that of pronotum; each plate bearing an elongate lobate wingpad extending nearly to the apex of the metanotal wingpad. Each plate of mesonotum bearing about 21 pits in three rows with 12 pits on lateral outer margin, three pits on posterior part of wingpad, a row of five pits on median part of each plate, and one pit on upper part of the lateral inner margin. Part of metanotum not covered by the wingpad of mesonotum bearing 6 pits.

Abdomen 9-segmented with anterior parts of terga light brown. Each tergum with the following number of pits on either side of midline in dorsal view: Tergum III with two pits, tergum IV with 6 to 11 pits, tergum V with 8 to 10 pits.

Metatibia with a row of six black-tipped spines apically on plantar surface, outer spine ca. 3× length of others. Metatarsi with three tarsomeres; length of tarsomere 1 subequal to that of tarsomere 3; tarsomere 1 with transverse row of 6 black-tipped spines; tarsomere 1 ca. 2–3× length of tarsomere 2; tarsomere 2 with five black-tipped spines.

Otherwise similar to fourth instar.

**Key to larval instars**

1 Metatarsi with three tarsomeres (Figs 16, 17) . . . . . 2
— Metatarsi with fewer than three tarsomeres (Figs 13–15) 3
2 Mesonotal wingpad extending nearly to apex of metanotal wingpad (Fig. 17) . . . . . fifth instar
— Mesonotal wingpad covering lateral half of metanotal wingpad (Fig. 16) . . . . . fourth instar
3 Metatarsi with two tarsomeres, tarsomere 2 partially subdivided, bearing two weak spines in middle of plantar surface; mesothoracic wingpad weakly developed (Fig. 15) . . . . . third instar
— Metatarsi with 1 tarsomere, mesothoracic plates without wingpads (Figs 13, 14) . . . . . 4
4 Body length >0.75 mm (Fig. 14) . . . . . second instar
— Body length <075 mm (Fig. 13) . . . . . first instar

## CONCLUSIONS AND DISCUSSION

This study revealed new data on the biology of *Hyalesthes obsoletus*, which is an important phytoplasma vector in Europe. For the first time, we succeeded in rearing this insect, which showed a surprising and interesting behaviour under laboratory conditions. Moreover, we proposed a diagnosis and a key to larval instars useful for studies in entomology and plant pathology.

Only one parasitized specimen of *H. obsoletus* was collected. Parasitism of this cixiid planthopper has not been reported previously. The parasitoid was not captured but it was very probably a member of the family Dryinidae (Hymenoptera). This low level of parasitism is unlikely to regulate field populations of this cixiid. Nevertheless, laboratory investigations could help to propose a biological control of the species.

Previous studies showed that during copulation, male and female are positioned back to back (Hoch & Remane, 1985) and copulation lasts for approximately 40 min (Sforza & Bourgoin, 1998). Our field and laboratory observations revealed that copulation occurred less than 10 days after emergence. In addition, the number of eggs laid varied depending on the two sites at which the females lived. Moreau & Leclant (1973) observed a similar fertility per female at a location similar to site B, and Musil (1956) reported an average of 60 eggs per female at an unspecified site. We suggest that either elevation, or climatic conditions, or the effect of host plant determine these differences. Although it was reported that eggs are normally laid on the soil surface close to host plants, we never found eggs at our experimental sites.

The aim of the rearing was to obtain healthy adults of *H. obsoletus* for use in transmission trials of stolbur phy-

toplasma. In recent studies, stolbur phytoplasma was detected in insects and plants using PCR (Maixner et al., 1995; Sforza et al., 1998). In France, it is known that stolbur phytoplasma causes many diseases of tomato and grapevine, and is suspected of being the pathogenic agent in yellow decline of lavendin (Cousin & Moreau, 1991; Fos et al., 1992; Daire et al., 1993). Except in the latter disease, the role of *H. obsoletus* as a vector was demonstrated by the typical yellowing of inoculated plants and the positive detection of stolbur in inoculated plants (Fos et al., 1992; Sforza et al., 1998). However, the duration of feeding access, latent period and feeding transmission by *H. obsoletus* are still unknown. Experimentally reared pathogen-free insects could be used to investigate the transmission process in countries where *H. obsoletus* is known or suspected to be a vector. As an aid for such studies, we present a key to the larval instars together with a precise diagnosis. This study reveals that it is possible to perform studies with larval instars. The diagnosis does not permit the identification of field-collected specimens to species, but as far as we know, no key to immature cixiids exists. A morphological approach is sometimes insufficient for distinguishing a complex of young larvae belonging to different species or different genera, as *Hyalesthes* or *Pentastiridius* Kirshbaum. A molecular approach using RAPDs (Random Amplified Polymorphic DNAs), as already demonstrated for the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Sonvico et al., 1996), may be useful for distinguishing species. The larval development under controlled conditions took about half as long as under natural conditions. The differences may be due to a "consecutive dormancy" (Witsack, 1987), induced in the field by unfavourable conditions. The state of quiescence is due to a restriction of development or a reduction in metabolism. Under controlled conditions, the absence of quiescence could explain the unexpected epigean life on basal stems, which made it easier to observe the behaviour of the young stages. The conditions used were not optimum for mass rearing of *H. obsoletus*, although favorable for the mass rearing of Cicadellidae species, e.g. *Euscelidius variegatus* Kirschbaum (Caudwell & Larrue, 1977) and *Psammotettix alienus* Dahlbom (unpubl. data). It is likely that the humidity and the phenological stage of the host plant were unsuitable for a mass rearing and the production of three generations per year.

The absence of compound eyes in the early larval instars is noteworthy. This was observed by Suchov & Vovk (1948) in "early stages" and confirmed by this study. The "absence of compound eyes" in first to third larvae is an apomorphy. However, this character, considered as a potential synapomorphy for the Cixiidae, should be used with caution as precise information on its occurrence within the family is lacking, even if it seems a common character (Hoch and Asche, pers. com.). Moreover, the primary homology of such a character is not certain as it might be an adaptation to radicicolous life. Even if a radicicolous life is the ancestral condition for cixiids (Bourgoin, 1997a), we cannot exclude that the absence of compound eyes in the early instars might have evolved several times independently (homoplasy) among the different cixiid lineages. In addition, as 73% of cavernicolous Fulgoromorpha are Cixiidae (Hoch, 1994), we may suggest that radicicolous way of life for cixiids is an evolutionary transition to epigean way of life.

A good example of such homoplasious reduction is the absence of compound eyes in the adults of various cixiid and meenoplid species, which have adapted to a cavernicolous way of life (Hoch & Horvath, 1989; Hoch, 1994). Further data on the larval stages and taxa concerned and on larval ethoecology will be necessary before this character can be used for phylogenetic purposes.

Wax plates have very diverse forms and only a few have been described in Cixiidae (Pope, 1985), Lophopidae (Liang, 1997), and Meenoplidae-Kinnaridae (Bourgoin, 1997b). However, wax plates are known to be present in species belonging to several fulgoromorph families (Yang & Yeh, 1994). The role of wax is not well known. In the case of larvae, wax deposits along the roots and in the galleries where the larvae live would prevent them from drowning when water infiltrates the soil. Females might cover eggs with waxy secretions to protect them against predators or parasites (Mason et al., 1989).

*H. obsoletus* is a vector of pathogenic agent and has been shown to be an efficient vector of a wide range of plant diseases. If it proves possible to mass rear *H. obsoletus* it could become a model species among Fulgoromorpha for studying the transmission of stolbur phytoplasma which is a ubiquitous pathogenic agent.

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