

Aguriahana germani (Zett.) (Hom. Auch. Cicadellidae, Typhlocybinae) : breeding and specific feeding behaviour on pine needles

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Objektyp: **Article**

Zeitschrift: **Mitteilungen der Schweizerischen Entomologischen Gesellschaft =
Bulletin de la Société Entomologique Suisse = Journal of the
Swiss Entomological Society**

Band (Jahr): **56 (1983)**

Heft 1-2

PDF erstellt am: **26.04.2024**

Persistenter Link: <https://doi.org/10.5169/seals-402058>

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Aguriahana germari (Zett.) (Hom. Auch. Cicadellidae, Typhlocybinae): breeding and specific feeding behaviour on pine needles

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Aguriahana germari was bred successfully in the laboratory. It is a mesophyll feeder and its habits are illustrated by sections of damaged pine needles and inserted maxillary stylets. The stylets always enter through stomatal openings, probably because the epidermis and hypodermis are cutinised and lignified. Quite exceptional for a mesophyll feeder are the branched stylet-sheaths left at the feeding points. The proximal end of a stylet-sheath forms a flange over the subsidiary cells of a stomatal opening and leads as a funnel intracellularly through the stomatal cavity, often with arms branching to empty mesophyll cells. The endodermis encycling the vascular tissue is not pierced. The beginning of the stylet-sheaths in the stomatal cavities is shown by SEM. Small spherules are embedded in the sheath-lipoprotein. These brochosomes are also lipoprotein and are excreted by the Malpighian tubules.

Aguriahana germari (ZETTERSTEDT, 1840) (Fig. 1) lives on different species of pine trees and has a very broad distribution in North Africa, through the whole of Europe and Russia, at low altitudes to the timberline. By the shape of its genital appendices, this Typhlocybinae can be clearly differentiated from the other palaearctic species living on pines, *A. uncinata* (VILBASTE, 1965) and *A. sichotanus* (ANUFRIEV, 1971), which are only found in eastern Russia.

DWORAKOWSKA (1972) classified some typhlocybinae genera into the genus *Aguriahana*, but VILBASTE (1982) brought *A. germari* back to *Wagneripteryx*, because of the different form of the nymphs.

KIRSCHBAUM (1868) was the first author to mention *A. germari* in Switzerland; HOFMÄNNER (1924 and 1925) gives some biological data about it in the Swiss National Park in the Engadine at an altitude of 2150 m: the adults leave the pine trees in autumn and hibernate under stones and needles. We collected *A. germari* in 25 different places in Switzerland on *Pinus silvestris*, *P. montana*, *P. nigra* and *P. cembra*, but always in very small numbers. In lower regions (400–500 m) we found adults from June to October, at higher altitudes up to the timberline in July, August and September. The overall length of adults found in both low and high regions is practically the same (Table 1) and we registered only one generation per year in all regions.

Observation of *A. germari*'s feeding behaviour in its natural habitat is practically impossible because it occurs in such small numbers and on such tall trees. We therefore started to breed the insect on small pine trees in an air-conditioned chamber, in the same way as we have bred other typhlocybinae species (GÜNTHART, 1971, 1974).

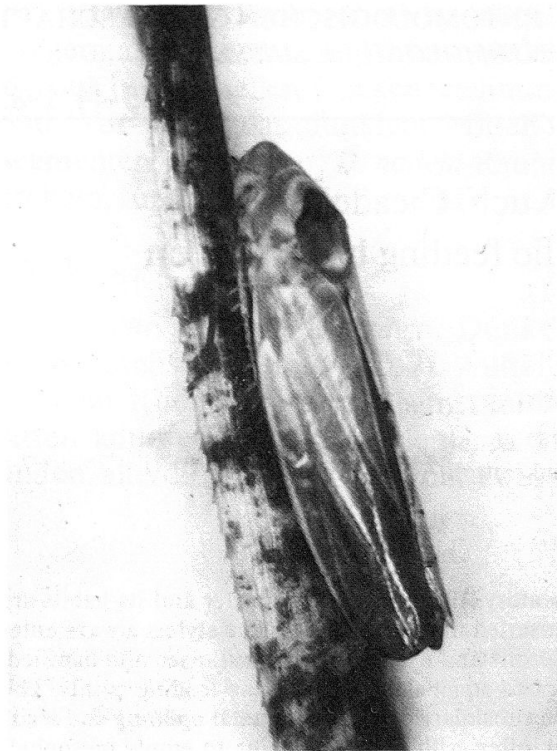


Fig. 1: *Aguriahana germari* on a pine needle with the typical sucking-spots of a mesophyll-feeder.

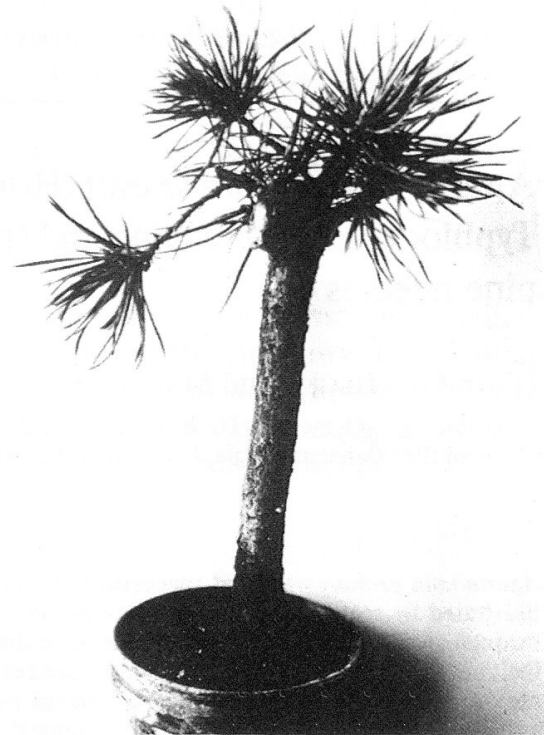


Fig. 2: «Bonsai»-pine tree for breeding *A. germari*.

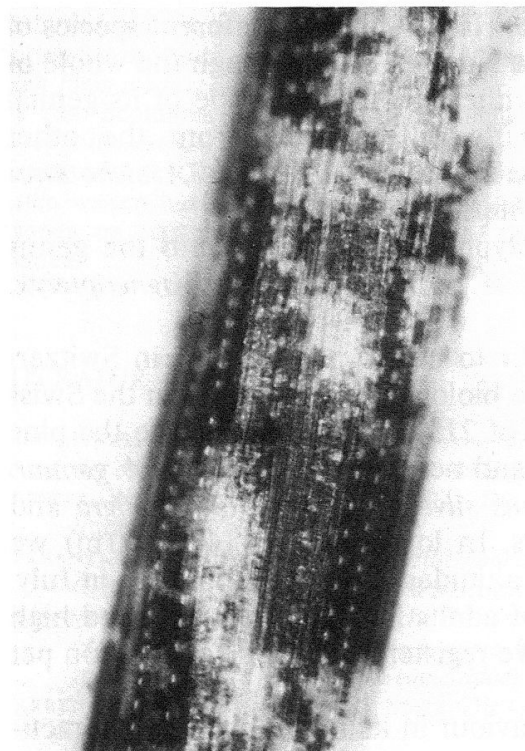


Fig. 3: *A. germari* sucking-spots on pine needle.

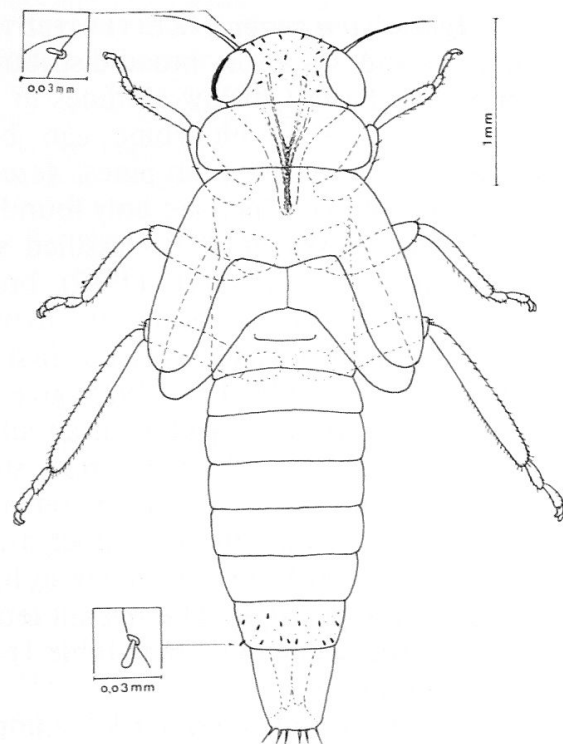


Fig. 4: *A. germari*: last instar nymph. The spines on head, thorax and abdomen are very short (0.015 mm long) and club-shaped (only drawn on head and tergite VIII).

Table 1: Overall length of *A. germari* collected from various altitudes in Switzerland.

	Altitude above sea-level	Overall length in mm		N
		$\bar{x} \pm s$	$\pm s_{\bar{x}}$	
Males	415–1.450 m	4.03 ± 0.09	± 0.03	16
	1.670–2.100 m	4.06 ± 0.11	± 0.04	5
	total	4.04 ± 0.09	± 0.02	21
Females	415–1.450 m	4.31 ± 0.19	± 0.04	9
	1.670–2.100 m	4.30 ± 0.17	± 0.03	23
	total	4.30 ± 0.17	± 0.03	32

Most work on the probing behaviour of Auchenorrhyncha and the location of the target tissue has been reported on leaves. This report describes the feeding behaviour of *A. germari* adapted to the xeromorph anatomy of conifer needles.

METHODS

In mid-September 1980 six *A. germari* females were collected in Dielsdorf and set under a cellophane bag on a 15 cm tall, 10-year-old “Bonsai”-pine tree (Fig. 2). After three weeks at a cool temperature and short daylight period, many sucking-spots were clearly distinguishable on the needles (Fig. 3); these needles were deep-frozen for future feeding-behaviour investigations by the second author.

The adults died during January and the beginning of February 1981 and the small pine tree with the eggs in the needles (Fig. 9) was put in a climatic chamber with 25–27 °C, 75–80% relative humidity and 16 h longday photoperiod. At the beginning of February 1981 the first young larvae hatched (Fig. 4) and during the second half of March the adults of the new generation appeared.

Further damaged needles (and healthy needles) were collected from the pine tree and stored in 4% formalin for light microscopy. Water was then removed with 2-methoxyethanol and the material embedded in paraffin to cut sections of 20 µm which were stained in safranin-fastgreen (FEDER & O'BRIEN, 1968; GERLACH, 1969; KOBLET-GÜNTART, 1975). Needles were freeze-dried for scanning electron microscopy (SEM).

RESULTS

Fig. 5 shows a sector of a section through a healthy pine needle. The vascular bundles in the middle of the needle are surrounded by transfusion-tissue and enclosed by the endodermis, whose cells bear lignified stripes which build a waterproof barrier when the turgor of the needle cells decreases. The space between the endodermis and the heavily cutinised epidermis with the thick-walled hypodermis is filled with mesophyll-cells. These relatively thin-walled cells contain chloroplasts and bear storage substances (starch and oil). Their walls show inward projections (Fig. 6) enlarging considerably the internal cell surface. Resin-canals in the assimilation parenchyma are characteristic for conifers. The resin-filled cavity is surrounded by a layer of secreting cells and a protective sheath of thick-walled lignified cells (Fig. 5). Gas exchange takes place through stomatal openings in the epidermis. The stomatal cavities are controlled by the guard cells sunken under

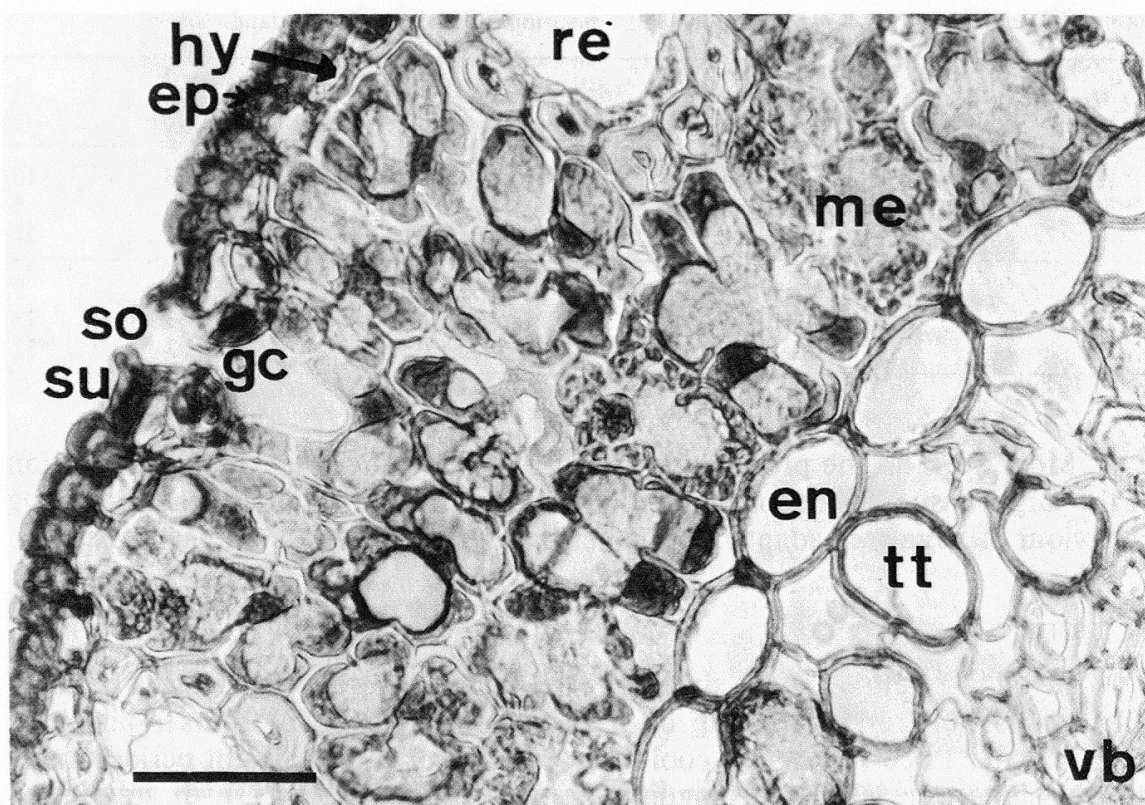


Fig. 5: Sector of a healthy pine needle (*Pinus silvestris*). Scale = 0.5 mm. en = endodermis ep = epidermis gc = guard cell hy = hypodermis mc = mesophyll-cells re = resin canal so = stomatal opening su = subsidiary cell tt = transfusion tissue vb = vascular bundle.

the subsidiary cells. The sub-stomatal cavity is confined by a u-shaped mesophyll-cell. The epidermis and stomatal cavities are lined with a film of wax. With increasing age of the needles (depending on the species) the stomatal cavities are completely filled with wax and/or covered by a cuticular membrane (GÜNTART & WANNER, 1982 a, b).

Chlorotic spots (Fig. 3) on needles where *A. germari* has fed result from empty mesophyll-cells. Sections through damaged cells show that the entire cell content, consisting of cytoplasm, nucleus, chloroplasts and storage substances has been sucked out. If the cell content is not completely emptied it may oxidize and remain as brownish autolysis products. *A. germari* forms stylet-sheaths, which stain red with safranin. Branched stylet-sheaths are common with *A. germari*. They direct the stylet intracellularly to the mesophyll-cells (Fig. 6). The stylets always enter through a stomatal opening and the stomatal cavity. The sheath is produced right from the beginning of the entry of the stylets (Fig. 7) and a waistline may be formed in the sheath by closing guard cells.

The maxillary stylets are partially flexible, they can be moved against each other longitudinally. Fig. 8 shows *in situ* the maxillary stylets of a nymph. They enter through a stomatal opening and lead to a mesophyll-cell. The maxillary stylets are renewed at each moult. The most protruding stylets reach as far as the endodermis-cells but do not pierce them. Puncturing occurs from all needle sides which bear stomatal openings. The rigid epidermis and hypodermis build the first barrier which prevents probing. Resin canals are never pierced and the endodermis hinders the stylets from reaching the vascular bundles. Vascular transport still

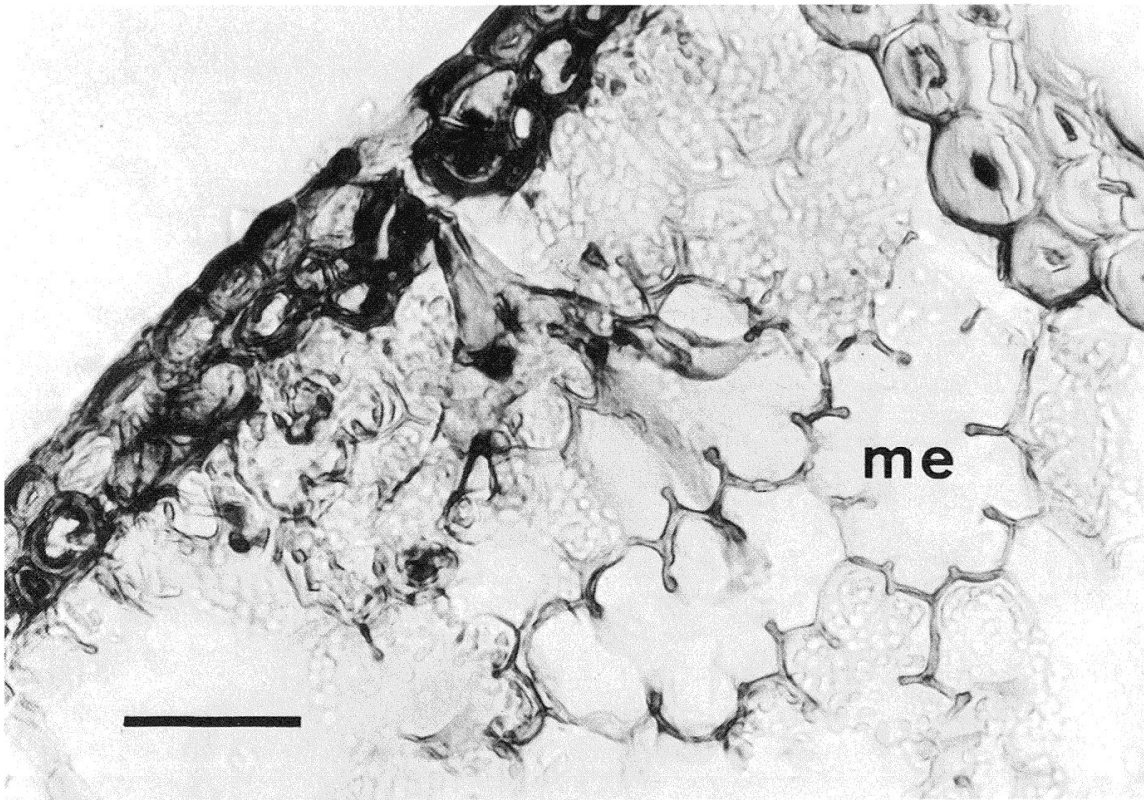


Fig. 6 + 7: Branched stylet-sheath formed by *A. germari* entering through a stomatal opening and leading intracellularly to damaged empty mesophyll-cells. Scale = 0.5 mm.

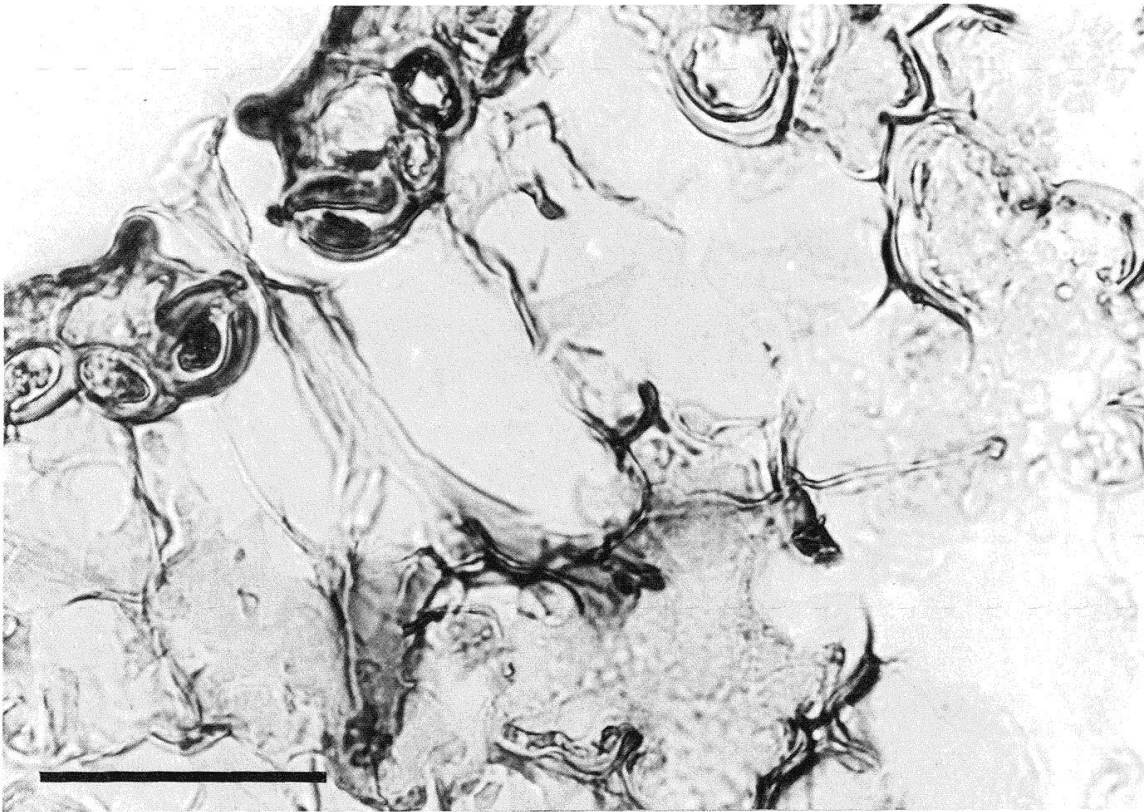


Fig. 7: The beginning of the stylet-sheath lying on the subsidiary cells. Scale = 0.5 mm.

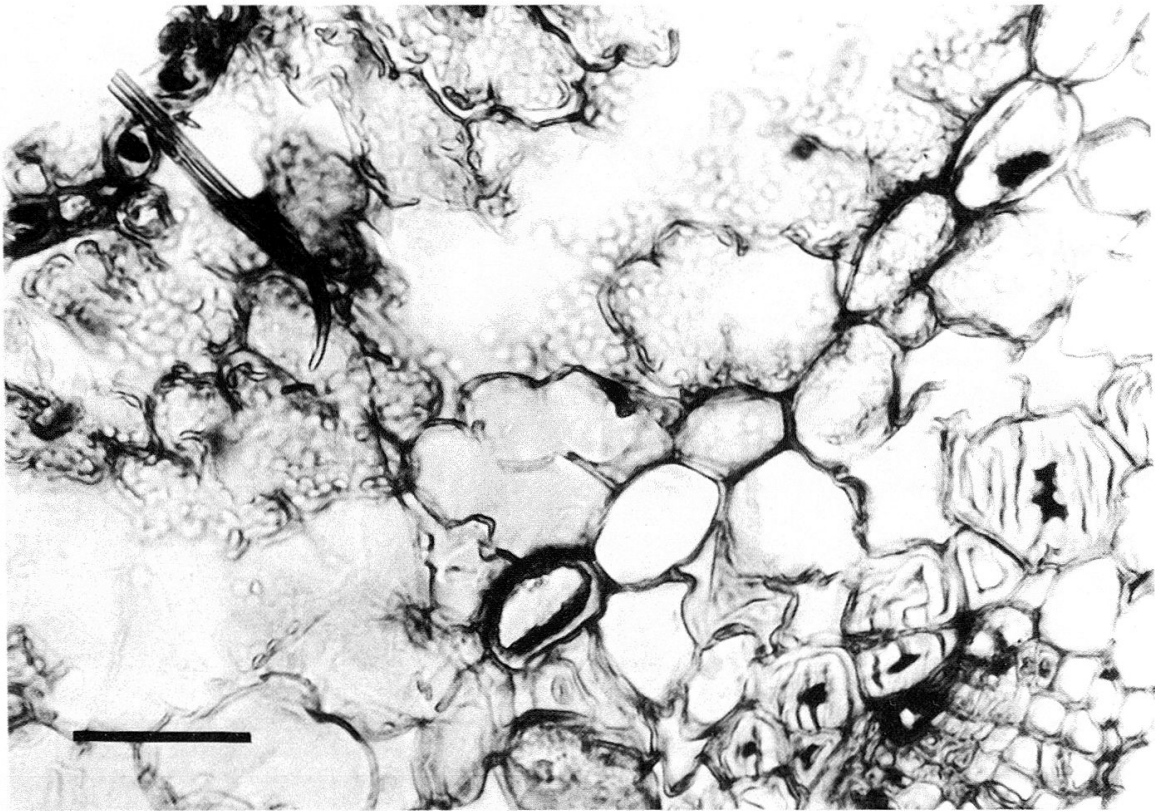


Fig. 8: Maxillary stylets of an *A. germari* nymph piercing through a stomatal opening to the mesophyll. Scale = 0.5 mm.

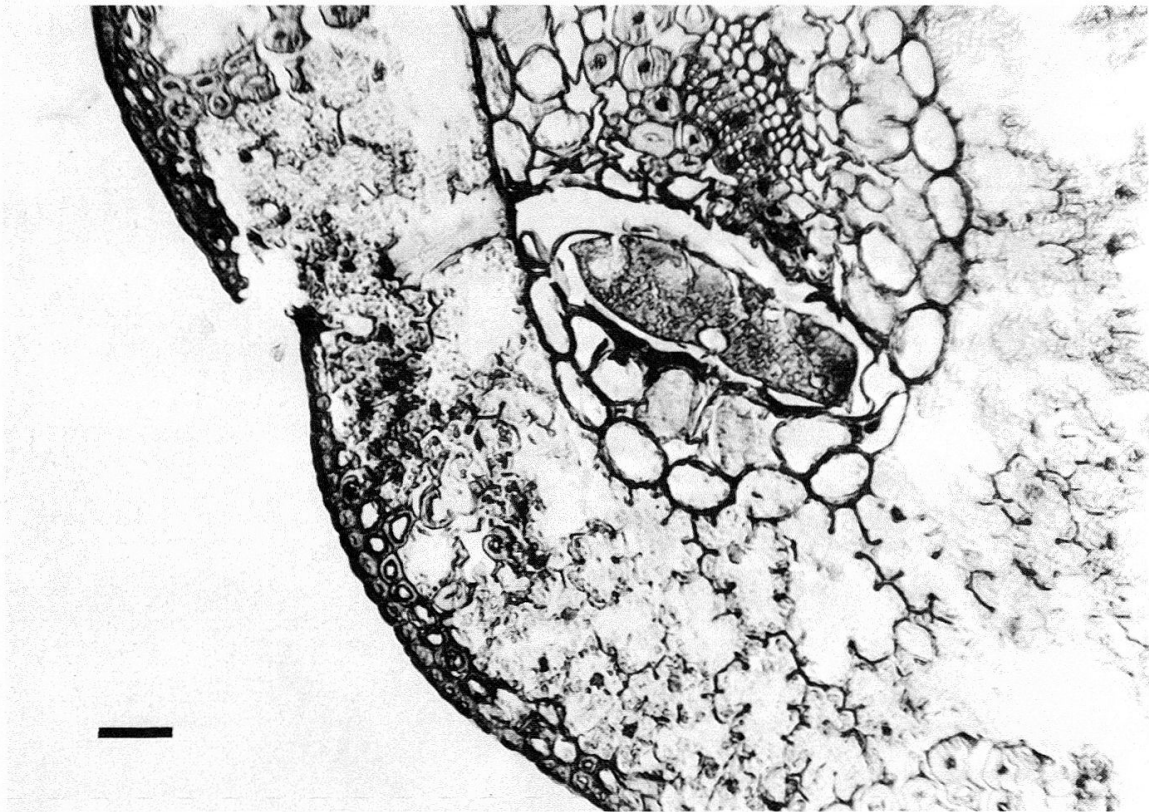


Fig. 9: *A. germari* embryo situated inside the endodermis. Scale = 0.5 mm.

functions, even in heavily sucked needles and the damage caused to needles by *A. germari* is always limited compared to that of phloem-sucking leafhoppers on leaves.

On the other hand, the oviposition is not restricted by rigid cells. The ovipositor can penetrate all tissues and eggs may lie in the mesophyll as well as inside the endodermis. Fig. 9 shows an embryo close to a vascular bundle.

This part of the investigations was presented at the Fourth Auchenorrhyncha Meeting in Tvärminne, Finland (GÜNTART & GÜNTART, 1981).

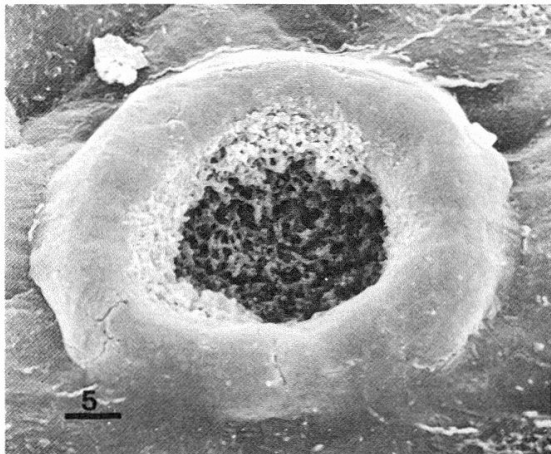


Fig. 10: Surface of a *Pinus silvestris* needle covered with partially crystalline wax. The subsidiary cells build a ring around the stomatal cavity (SEM). Scale-number = μm .

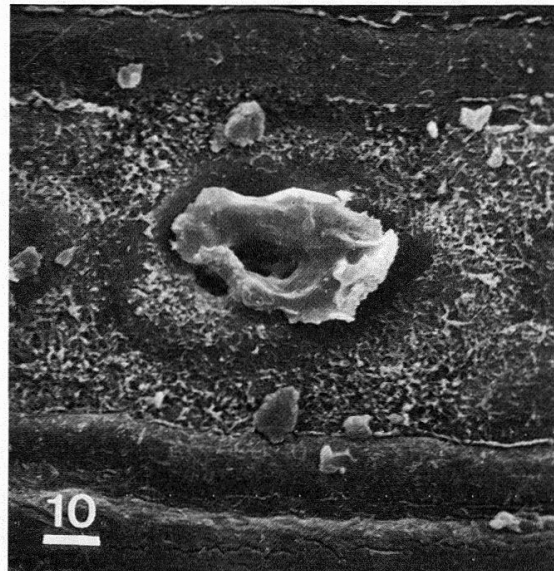


Fig. 11: Pine needle with a stylt-sheath leading through a stomatal opening (SEM). Scale-number = μm .

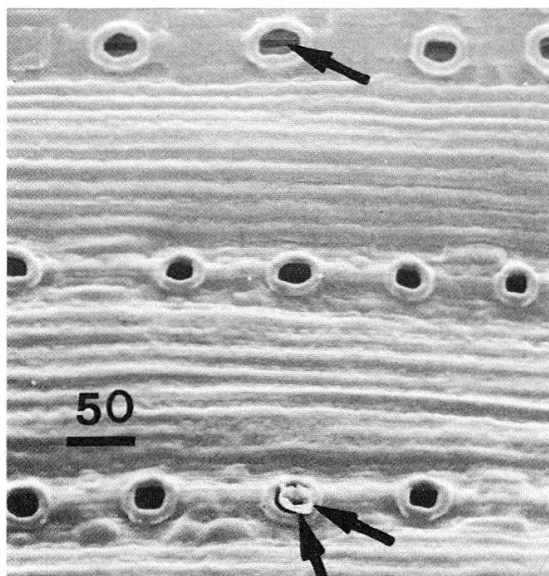


Fig. 12: Pine needle with the wax removed (SEM). Scale-number = μm .

→ slit controlled by the guard cells
⇒ proximal end of a stylt-sheath.

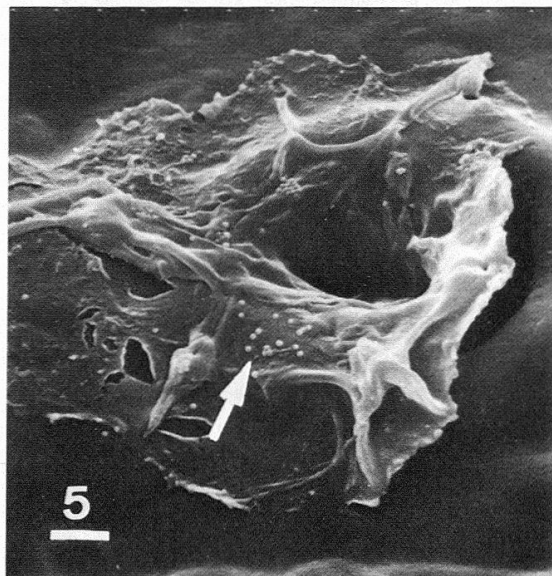


Fig. 13: Entrance of a stylt-sheath (enlarged, SEM)

→ brochosomes. Scale-number = μm .

The surface of the needles is covered by a partially crystalline wax layer (Fig. 10). After *A. germari* has fed on a needle some of the stomatal openings are obstructed by a kind of plug (Fig. 11). Initially this was interpreted as an accessory wax-formation similar to a wound-callus which may originate in wounded tissue. However, on needles where the wax has been removed by chloroform, the guard cells appear underneath the rampart formed by the subsidiary cells, but the plugs are still present (Fig. 12). Thus the material must be different from waxy substances; in fact the proximal end of the stylet-sheath-tube is being observed (Fig. 13).

Small spherules embedded in the sheath-material, known as "brochosomes" and enlarged in Fig. 14, are the visiting cards left by *A. germari*. The shape of these spherules and their presence all over the surface of many leafhoppers has been reported with SEM by the first author (GÜNTHART, 1977): the spherules are $0.436 \pm 0.044 \mu\text{m}$ in diameter, most of them have 12 pentagons and 20 hexagons (resembling a soccer ball). Very occasionally smaller ($0.33 \mu\text{m}$ with 12 pentagons and 4 hexagons) or larger ($0.56 \mu\text{m}$ with 12 pentagons and 30 hexagons) spherules can be found. Literature offering further explanations on these spherules: COCHRANE found the first spherules in 1948 with the electron microscope on some leafhoppers, but published only in 1952 (TULLOCH *et al.*, 1952). WILDE & COCHRANE (1957) named them "brochosomes". DAY & BRIGGS (1958), SMITH *et al.* (1960) and GOURANTON *et al.* (1967) also published on brochosomes. Brochosomes are formed in a special part of the Malpighian tubules and secreted in the lumen. They are composed of lipoproteins (insoluble in chloroform or hexanol, GÜNTHART, 1977). Brochosomes have been verified for many different species of Auchenorrhyncha (Table 2), but never in other orders or suborders of insects. They are a particular form of excretion of nitrogen (WIGGELSWORTH, 1972). STOREY & NICHOLS (1937) observed that *Cicadulina mbila* (NAUDÉ) has two different excretory products: the normal excrements which are a transparent liquid ejected from the anal tube and known as "honey-dew"; a SEM-photograph

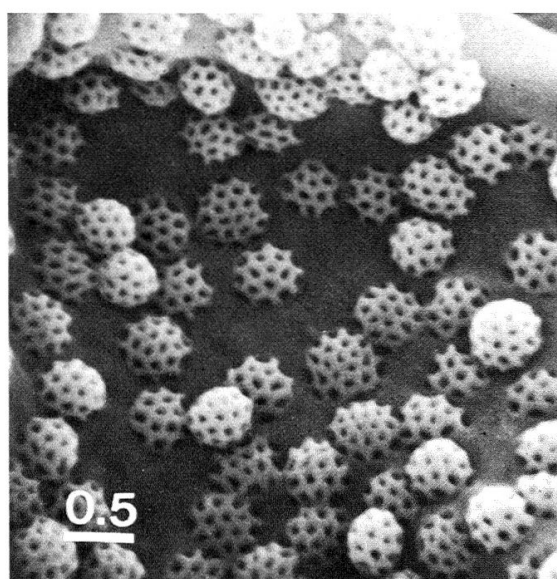


Fig. 14: Brochosomes enlarged (SEM). Scale-number = μm .

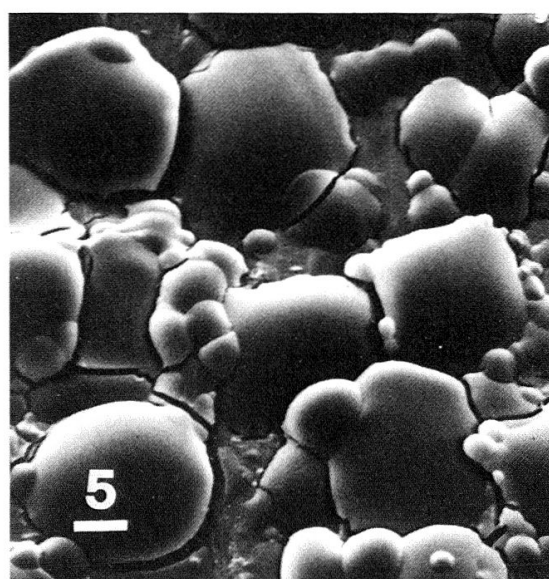


Fig. 15: Excrement-droplets of *Zygina rhamni* FERRARI, directly deposited on the metallic SEM-supports (SEM). Scale-number = μm .

Table 2: Brochosomes in Hom. Auch.-species and verifying authors.

Genus, species	Family	Subfamily	Author
<i>Centrotus cornutus</i> (L)	Membracidae	Centrotinae	Gouranton et al., 1967
<i>Austroagallia torrida</i> Evans	Cicadellidae	Agalliinae	Day et al., 1958
<i>Cicadella viridis</i> (L)	Cicadellidae	Cicadellinae	Gouranton et al., 1967
<i>Aquriahana germari</i> (Zett.)	Cicadellidae	Typhlocybinae	Günthardt H. & M., this paper
<i>Dikraneura absenta</i> De L.	Cicadellidae	Typhlocybinae	Wilde et al., 1957
<i>Eupteryx melissae</i> Curtis	Cicadellidae	Typhlocybinae	Günthardt, 1977
<i>Fagocyba douglasi</i> (Edw.)	Cicadellidae	Typhlocybinae	Gouranton et al., 1967
<i>Flammigeroidia angusta</i> (Leth.)	Cicadellidae	Typhlocybinae	Günthardt, 1977
<i>Flammigeroidia flammigera</i> (Fourcroy)	Cicadellidae	Typhlocybinae	Günthardt, 1977
<i>Flammigeroidia rhamnicola</i> (Horvath)	Cicadellidae	Typhlocybinae	Günthardt, 1977
<i>Flammigeroidia schneideri</i> Günthardt	Cicadellidae	Typhlocybinae	Günthardt, 1977
<i>Flammigeroidia tiliae</i> (Fall.)	Cicadellidae	Typhlocybinae	Günthardt, 1977
<i>Zygina rhamni</i> Ferrari	Cicadellidae	Typhlocybinae	Günthardt, 1977
<i>Cicadulina bimaculata</i> Evans	Cicadellidae	Deltocephalinae	Day et al., 1958
<i>Circulifer tenellus</i> Baker	Cicadellidae	Deltocephalinae	Wilde et al., 1957
<i>Euscelis incisus</i> (Kirschb.)	Cicadellidae	Deltocephalinae	Gouranton et al., 1967
<i>Macrosteles fascifrons</i> Stal	Cicadellidae	Deltocephalinae	Wilde et al., 1957
<i>Macrosteles fascifrons</i> Stal	Cicadellidae	Deltocephalinae	Smith et al., 1960
<i>Nephotettix virescens</i> (Dist.)	Cicadellidae	Deltocephalinae	Günthardt, 1977
<i>Orosius argentatus</i> (Evans)	Cicadellidae	Deltocephalinae	Day et al., 1958
<i>Eurymeloides</i> sp.	?		Day et al., 1958

of this excrement proves that it does not contain brochosomes (Fig. 15). The second excretory product is a viscid, opaque drop, which appears about twice daily at the anus, is picked up by the hind legs and spread over the whole body to dry; it is the content of the Malpighian tubules which contains the brochosomes. A small number of brochosomes may become loose and distributed in the air. According to NEVILLE & SMITH (1970) some were caught near a nuclear power station in England and recorded as "unknown airborne organisms".

DISCUSSION

Typhlocybinae can be monophagous or oligophagous and live mainly on trees, shrubs, herbs or grasses. Only 4 species of Typhlocybinae: *Empoasca vitis* (GÖTHE) (= *flavescens* F.), *E. fabae* (HARRIS), *E. libyca* (DE BERGEVIN) and *Amrasca biguttula biguttula* (ISHIDA) (= *Empoasca devastans* DISTANT) are known to feed on vascular tissue, but 52 species on parenchyma (SMITH & POOS, 1931, DAY *et al.*, 1952, POLLARD, 1968, NAITO, 1976, CLARIDGE & WILSON, 1976, SOHI, 1976 and 1977). Stylet-sheath formation is reduced in this group (KOBLET-GÜNTHART, 1975). There are several published photomicrographs of stylet-tracks: *Eupteryx aurata* (L.) and *Hauptidia maroccana* (MELICHAR) on potato (SMITH, 1926), *Empoasca vitis* and *E. libyca* on vine (VIDANO, 1963), *Empoasca decipiens* (PAOLI) and *Eupteryx atropunctata* (GOEZE) on *Vicia faba* (GÜNTHART & WANNER, 1981). Cicadellidae groups other than Typhlocybinae mainly feed on vascular tissue (PORT, 1978).

Aguriahana germari is a typical typhlocybinae species in its choice of target tissue. This is however the first investigation on a leafhopper which exclusively enters through stomatal openings. The aphid *Elatobium abietinum* WALKER on *Picea abies* has also been observed to probe through stomatal openings (MAKSYMOW, 1981). Some further species which suck on needles must be investigated to support the opinion that the xeromorph nature of needles builds barriers which can not normally be pierced with the mouth-parts – only the ovipositor is strong enough.

Branched stylet-sheaths are common with Fulgoromorpha, whereas Typhlocybinae normally secrete incoherent traces of saliva. *A. germari* is an exception in its building of normal and branched stylet-sheaths.

Two-year-old needles are more densely covered with wax than one-year-old needles (GÜNTHART & WANNER, 1982b). *A. germari* does not select to feed on either old or young needles, as do other Typhlocybinae. Younger needles are not necessarily preferred and probing is indifferent to the wax obstructing the stomatal openings.

The beginning of the stylet-sheaths left by *A. germari* is often enlarged, building a funnel. The same features observed by SEM and lightmicroscopy at the proximal end of the stylet-sheaths were reported as “flanges” for other sheath-forming Auchenorrhyncha.

Even when the wax is removed from the needles by means of chloroform, the stylet-sheaths and little brochosomes embedded in their flanges still remain (Fig. 13 & 14). Stylet-sheaths are mainly protein and probably contain about 10% phospholipid (MILES, 1968) and due to this chemical nature they are insoluble (as also the brochosomes). Brochosomes adhering to the mouthparts of *A. germari* are shed together with the saliva and are left, like a visiting card on the pine needle, sunken in the salivary-sheath material.

ACKNOWLEDGEMENTS

The investigations of the second author were supported by Professor Dr. H. WANNER and the “Schweizerischer Nationalfonds für wissenschaftliche Forschung”. SEM-photographs were taken by U. JAUCH and Dr. V. E. COLOMBO. We are grateful to Dr. R. MAAG Ltd. for the technical installations (air-conditioned chamber, etc.) and special thanks are due to Dr. Ernest GÜNTHART for his assistance in this respect.

ZUSAMMENFASSUNG

Aguriahana germari (ZETTERSTEDT, 1840), (Hom. Auch. Cicadellidae, Typhlocybinae): Zucht und Eigenart der Nahrungsaufnahme an Föhrennadeln – Die Untersuchung zeigt deutlich, dass die Art der Nahrungsaufnahme von *A. germari*, welche in der Natur stets in sehr kleiner Zahl auftritt, nur Dank einer erfolgreichen Zucht auf «Bonsai»-Föhren abgeklärt werden konnte, weil dadurch genügend lebendes Material zur Beobachtung und Bearbeitung zur Verfügung stand.

Die Nahrungsaufnahme von *A. germari* ist in mikroskopischen Schnitten durch beschädigte Föhrennadeln mit eingestochenen Saugborsten illustriert. *A. germari* ist ein Mesophyllsauger, die Saugborsten dringen stets durch die Spaltöffnungen ein, wahrscheinlich weil die Epidermis und die Hypodermis stark kutinisiert und lignifiziert sind. Unerwartet für einen Mesophyllsauger sind die verzweigten Speichelscheiden in den Saugflecken. Das äussere Ende der Speichelscheide bildet einen Trichter über die Nebenzellen der Spaltöffnung. Die Speichelscheide bildet einen Kanal durch die vertiefte Spaltöffnung und intrazellulär, meist verzweigt durch das beschädigte, leergesaugte Mesophyll. Die Endodermis, welche die Leitbündel umgibt, ist nicht beschädigt (höchstens durch den Ovipositor zur Eiablage). Der Anfang der Speichelscheiden in den Spaltöffnungen wird in REM-Photos

(Raster-Elektronen-Mikroskop) gezeigt. Eingebettet im äusseren Trichter der Speichelscheide sind in den REM-Photos submikroskopische «Kügelchen», die Brochosomen, sichtbar. Die Brochosomen sind eine Ausscheidung aus den Malpighischen Schläuchen und bestehen wie die Speichelscheiden aus Lipoproteinen. Die Brochosomen stellen eine besondere Art der Stickstoffexkretion bei vielen (siehe Tab. 2), vermutlich bei allen Auchenorrhyncha dar.

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(erhalten am 20.9.1982)