Acta Bot. Neerl. 27(1), February 1978, p. 27–33.

SUCTION MARKS IN NUTRITION CELLS OF A GALL ON LEAVES OF ACER PSEUDOPLATANUS L., CAUSED BY ERIOPHYES MACRORRHYNCHUS TYPICUS NAL.

T. G. J. SCHMEITS and M. M. A. SASSEN

Botanisch Laboratorium, Katholieke Universiteit, Nijmegen

SUMMARY

Leaf galls on *Acer pseudoplatanus*, caused by *Eriophyes macrorrhynchus*, were investigated in order to demonstrate suction marks in the nutritive tissue. After a special treatment that removed the cell content and the cell wall matrix, small holes with a diameter of approximately 0.35 μm were found in the cellulose skeleton of the cell walls. The holes originate by physical and enzymatical action of the stinging parasite.

1. INTRODUCTION

In the past few years a relative small number of gall studies at the ultrastructural level have been carried out, and in these studies the interest has been focused mainly upon the nutritive tissue. In general there is an agreement concerning the ultra-structure of the protoplasm. Less is known about the actions of the gall-causing animals, mostly Acarina.

KENDALL (1930), for instance, mentioned mites feeding by inserting their chelicerae into cells of the nutritive layer. WESTPHAL (1972) dealt with the suction cones caused by *Cecidophyes psilaspis* Nal. in galls on *Taxus baccata* L.

In the present study an attempt has been undertaken to demonstrate the suction marks of the mite *Eriophyes macrorrhynchus* typicus Nal. in the nutritive layer of its leaf gall on *Acer pseudoplatanus*.

2. MATERIAL AND METHODS

Leaves of *Acer pseudoplatanus* with young galls were collected in May and with full grown galls in September. Small pieces of the leaves with the galls were fixed in 5% glutaraldehyde, buffered with 0.1 M Sörensen-phosphate, pH 7.2, 1 to 2 hours. Post fixation was carried out with 3% $KMnO_4$ or with 2% OsO_4 during 2 hours, after which the objects were dehydrated and embedded in epon 812. Thin sections, made with a Porter & Blum MT2 ultramicrotome, were stained with uranyl acetate (2% in distilled water) and lead citrate (Reynolds). In order to investigate the rostrum of the mite and to measure the

diameter of the chelicerae, galls containing a great number of mites were selected, fixed and embedded as described above. Although in most cases fixation and embedding of the mites were far from optimal, the method was maintained because we were only interested in the harder mouth parts of the mites, which were well preserved.

To demonstrate the cellulose skeleton of cell walls of the nutritive tissue, galls were boiled for 2 hours in a mixture of hydrogen peroxide and acetic acid (1:1), dehydrated and embedded in butyl methacrylate. Sections of about 200 nm thickness on grids, covered with formvar, were brought in amyl acetate for two hours in order to dissolve the butyl methacrylate and were subsequently shadowed with platinum. For examination a Zeiss EM 9 and a Philips EM 201 were used.

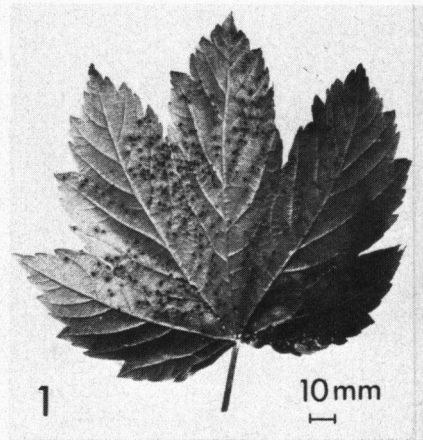
For observation of galls and mites with the scanning electronmicroscope (Jeol JSM-U3), the galls with mites were put into the microscope without any preparation.

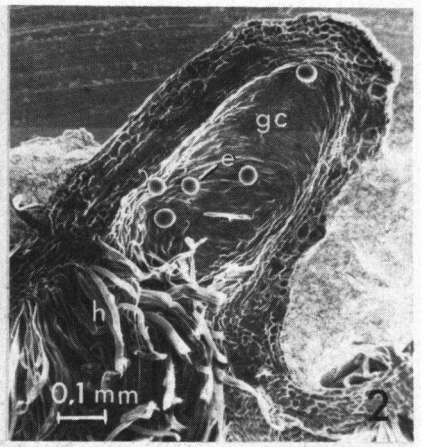
3. RESULTS

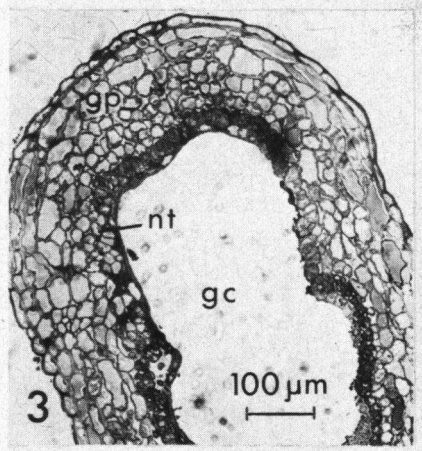
The leaf galls from *Acer pseudoplatanus* caused by *Eriophyes macrorrhynchus* are oblong and about 1 to 3 mm high (*fig. 1*). They are situated on the upper side of the leaf (DOCTERS VAN LEEUWEN 1957; WOLL 1954) and open toward the lower side of the leaf. The opening is almost closed by bundles of hairs (*fig. 2*). The first eggs, occasionally accompanied by a mite, were seen not earlier than one and a half weeks after the gall originates (*fig. 2*). In the course of the next months the number of eggs and mites gradually increases. *Fig. 3* shows a part of a longitudinal section of the gall with the nutritive tissue and the gall parenchyma. In the young gall the nutritive tissue has a meristematic appearance (*fig. 4*). The nuclei are relatively large, the cytoplasm dense and the vacuoles are small and not frequently seen. In contrast, the older galls are crowded with mites and show a totally changed nutritive tissue (*fig. 5*). Several

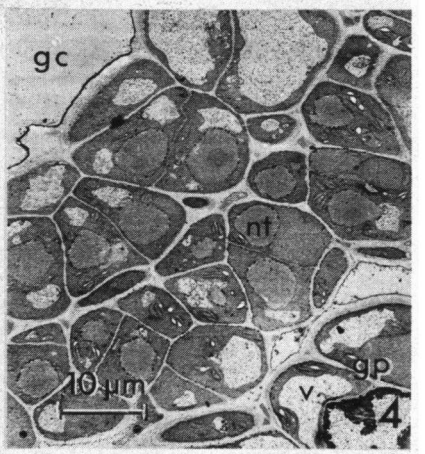
Key to labelling	e = egg	mp = mouthparts
ch = chelicerae	gc = gall chamber	nt = nutritive tissue
cs = cellulose skeleton	gp = gall parenchyma	r = rostrum
cu = cuticle	h = hair	v = vacuole

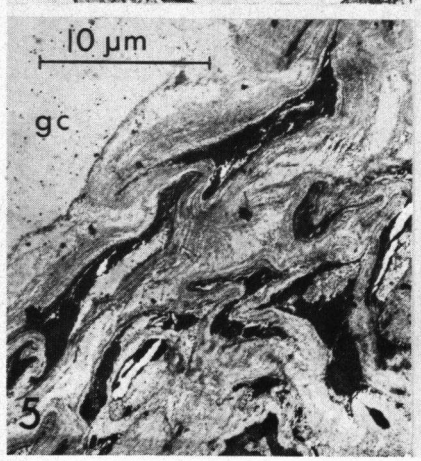
Fig. 1. Upper side of the leaf of *Acer pseudoplatanus* covered with galls. 1/3 ×
Fig. 2. A gall in longitudinal section. Gall chamber (gc) with eggs (e) and hairs (h) at the opening. 63 ×
Fig. 3. Longitudinal section of upper part of a gall with gall parenchyma (gp) and nutritive tissue (nt). 180 ×
Fig. 4. Inner part of a young gall with nutritive tissue (nt) and a few cells of the parenchyma tissue (gp) with large vacuoles (v). 1075 ×
Fig. 5. Part of an old gall with nutritive tissue. The cells are colllapsed and only the cell walls are left. 2280 ×
Fig. 6. Part of the mite *Eriophyes macrorrhynchus* with legs and rostrum (r). 720 ×

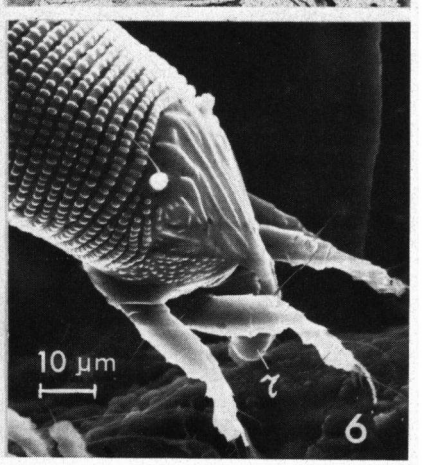

1
10mm


gc
e
h
0.1 mm
2


gp
nt
gc
100 µm
3


gc
nt
gp
v
10 µm
4


10 µm
gc
5


10 µm
6

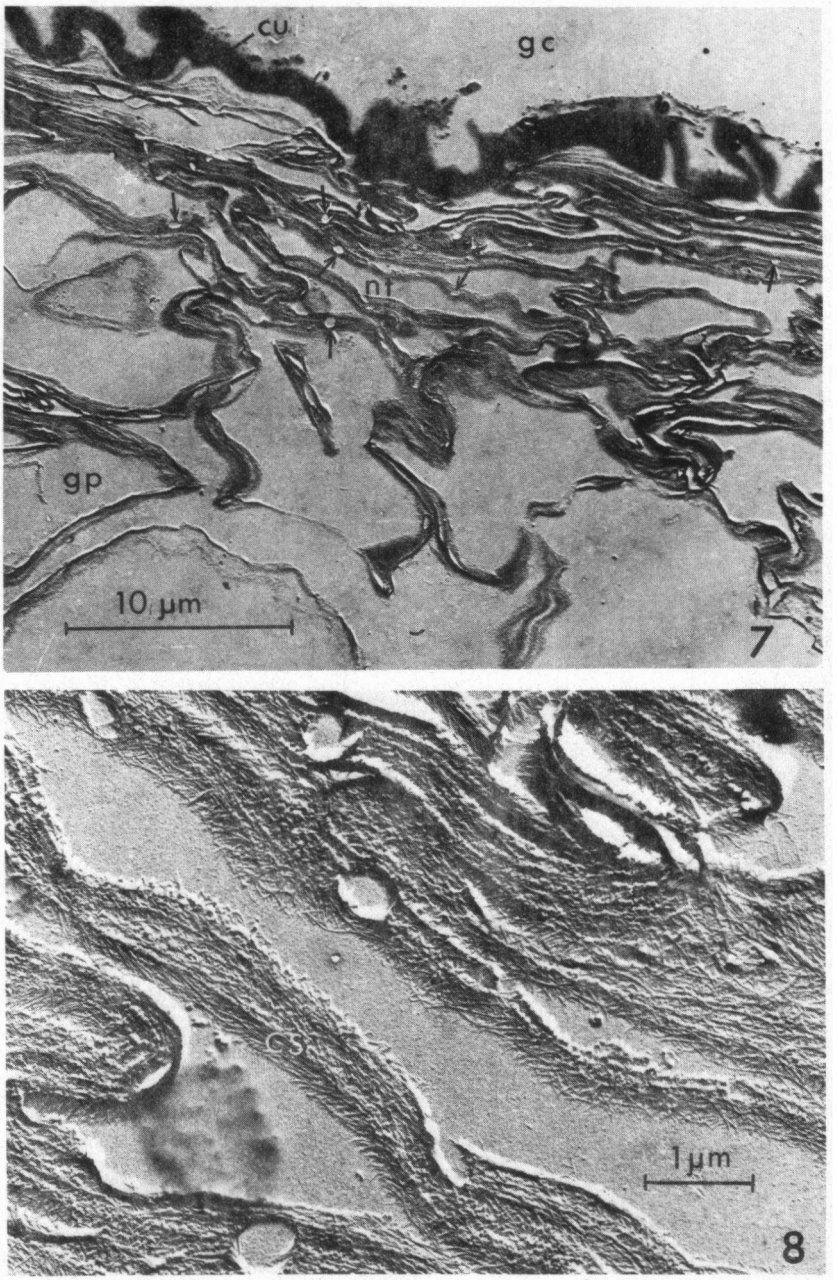


Fig. 7. Inner part of gall tissue after treatment with hydrogen peroxide and acetic acid. In the part of the tissue near the gall chamber (gc) numerous holes (arrows) are visible in the cell walls. 3055 ×

Fig. 8. Detail of *figure 7* with cellulose skeleton (cs) and holes. 14400 ×

cell layers of the nutritive tissue next to the gall chamber are fully destroyed and in fact only the densely packed cell walls are left. Between the compressed cell walls the remainder of the cytoplasm is seen as homogeneous electron dense material. Obviously the cells are emptied by sucking of the mites, but no suction marks could be found in thin sections of these cell walls.

After treating the galls with hydrogen peroxide and acetic acid, which removes the cell contents and the matrix of the cell wall, a network of cellulose microfibrils is visible. In these cell walls (*figs. 7* and *8*) a number of circular holes are shown, with an average diameter of 0.35 μm. The holes are only found in cells having collapsed after sucking by mites. The distance of the holes from the gall chamber is not more than approximately 10 μm. If these holes are made by the mouthparts of the mites, we should find that these mouth parts are smaller than the diameter of the holes. The rostrum of the mites (*figs. 6* and *9*) measures about 15 μm in length and about 8 μm in dia-

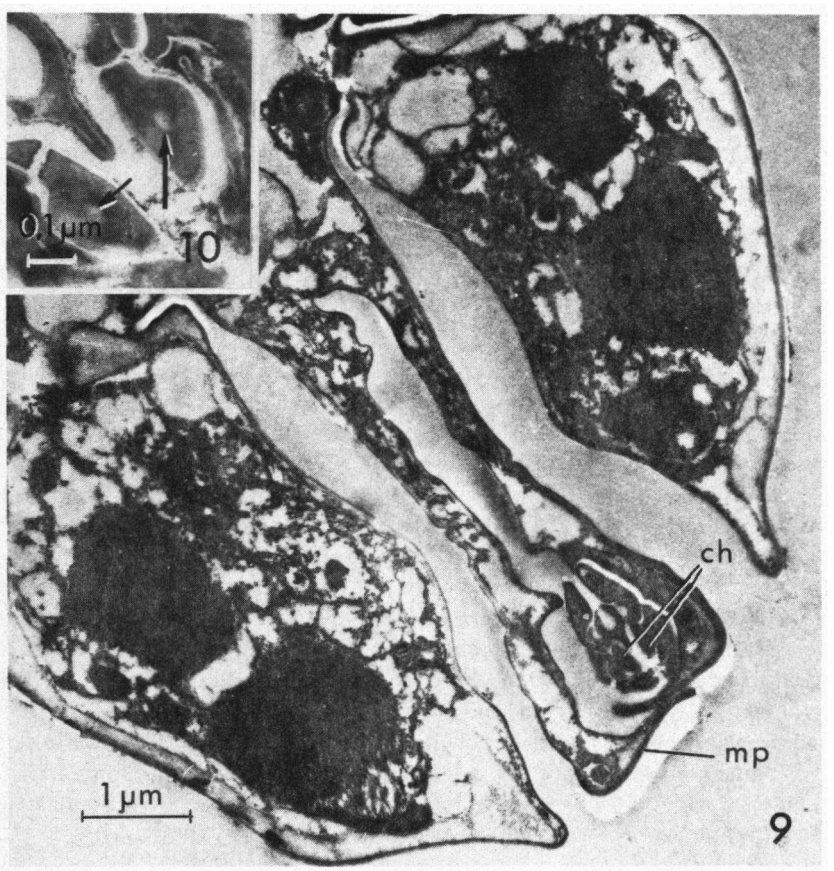


Fig. 9. Transverse section through the rostrum of the mite. 14660 ×
Fig. 10. Detail of chelicerae (ch) with canals (arrows). 60000 ×

meter. *Figure 9* shows a transverse section of a rostrum. At the frontside of this section we discern a cross section of the so called chelicerae which are the responsible organs for puncturing the plant cells. On a transverse section (*figs. 9* and *10*) the chelicerae consist of two triangular bodies, with a base of 0.328 μm and a height of about 0.163 μm (*fig. 10*). After staining with $KMnO_4$ and OsO_4 they appear electron dense with an electron opaque spot in the center. This must be a canal-like structure which traverses the length of the chelicerae. In view of the measured values of the chelicerae (about 0.35 μm in diam.), it is obvious that the above mentioned holes in the cell walls of the nutritive tissue must be the result of puncturing actions by mites. In young galls, without mites, no such holes were found after the hydrogen peroxide and acetic acid treatment.

4. DISCUSSION

Some aspects of the gall discussed here were investigated earlier by WOLL (1954) with the use of the light microscope but no suction marks of the mites were found. With the help of the electron microscope ROHFRITSCH (1971) and GAILHOFER-DENGG (1972) noticed a peculiar structure near the gall chamber. WESTPHAL (1972) mentions the more or less spongy thickenings of the cell walls at the point of parasite action, the so called suction cones. These thickenings are thought to be a reaction of the living cell to the puncture of the parasite. Most probably polysaccharides, particularly callose, should be produced to heal the wounds. Neither Westphal nor we could find holes in the walls of nutritive tissue as far as regards the thin sections of epon-embedded gall tissue. It is possible that the holes are plugged by cell wall material after puncturing, but in the leaf gall of *Acer pseudoplatanus* plugging is by no means an adequate reaction since the cells die soon after being punctured. In our opinion the holes are passively filled with matrix material immediately after sucking because we consider the matrix a semifluid. In contrast, the cellulose microfibrils are not replaced and therefore the holes are visible after the treatment with hydrogen peroxide and acetic acid. The edges of the holes are smooth and therefore we think that the microfibrils are not merely pushed aside by mechanical forces, but that hydrolytic enzymes are playing a role. According to Lukoschus (pers. communication) the mites inject digestive enzymes into the cell prior to absorption through the chelicerae. The canals traversing the chelicerae may play an important role in this action.

Because of a limiting length of the chelicerae, the mites cannot reach through more than one cell layer from the gall chamber. After this layer is collapsed only the cell wall is left and the distance to the next cell layer is diminished with the consequence that this new layer can be attacked by the parasite.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to Mr. A. W. Dicke and Mrs. A. M. C. Wolters-Arts for their assistance during this investigation.

REFERENCES

DOCTERS VAN LEEUWEN, W. M. (1957): *Gallenboek*, Zutphen.

GAILHOFER-DENGG, E. (1972): Membranstrukturen in Cytoplasma und in der Vakuole der Gallenzellen von Aceria (Eriophyes) macrorrhyncha macroorrhyncha (Nal.) auf Acer campestre L. *Protoplasma* **75**: 19–35.

KENDALL, J. (1930): The structure and development of certain Eriophyid galls. *Z. Parasitenkunde* **2**: 478–501.

ROHFRITSCH, O. (1971): Infrastructure des cellules du tissu nourricier de la galle de Geocrypta galii H. Lw. sur Galium mollugo. *C. R. Aacad. Sci. Paris* **272**: 76–78.

WESPHAL, E. (1972): Observations ultrastructurales et histochimiques sur les cônes de succion du Cecidophyes psilaspis Nal., Acarien cécidogène des bourgeons de l'If. *C. R. Acad. Sci. Paris* **274**: 893–896.

WOLL, E. (1954): Untersuchungen über die cytologische Differenzierung einiger Pflanzengallen. *Planta* **43**: 477–494.