Feeding behaviour of *Eriophyes tiliae tiliae* Pgst. and suction track in the nutritive cells of the galls caused by the mites.

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The feeding mechanism and suction marks of the *Eriophyes tiliae tiliae* have been studied by use of various methods.

The following model has been constructed: the mite deposits spit on the outside of the nutritive cell, thus dissolving the cell wall enzymatically before food is ingested. Subsequently, the chelicera and the other stylets are thrust mechanically into the nutritive cell. Through suction the mite empties the cell. Around the hole in the cell wall a callose cone is formed with embedded spit. This formation as a whole is called suction tracks or suction marks.

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Introduction

Phytophagous mites cause serious damage to their hosts, either directly through their feeding (Ridland 1979) or by transmitting plant pathogens when they feed (Slykhuis 1963, Harris 1981). The only mites reported to transmite plant viruses belong to the Eriophydae. For this reason it is important to acquire knowledge about the feeding mechanism of the eriophyids, regardless of whether an investigation concerns the prevention of direct injuries on crops or greenhouse plants or examines mites as vectors in the biological control of weeds. *Eriophyes tiliae tiliae* causes galls on *Tilia platyphylla*. In previous studies, detailed descriptions of the histology of the gall tissue, the morphology of the mites, and the elements of their feeding organs have been presented (Thomsen 1975, 1976, 1987). The focus in this study is mainly on the feeding mechanism and suction tracks in the nutritive cells.

Material and Methods

Eriophyes tiliae tiliae mites live inside the galls, and the only entrance to them is through a narrow hairy opening on the underside of the leaf. Gall initials and uninfected leaves were treated with auramin O and examined under a fluorescent microscope. Auramin O does not have fluorescence itself but, when combined with lipid-like substance, it becomes an intense fluorescent yellow.

With regard to the selective staining of callose, galls were fixed in 3% glutaraldehyde and embedded in glycol methacrylate (Feder & O'Brien 1968). Serial sections were stained in resorcinol blue (Eschrich & Currier 1964) and examined under a Reichert-Zetopan phase contrast microscope. With regard to the electron microscopic observations of mites and nutritive cells, material was fixed in 3% glutaraldehyde in a 0.1 M phosphate buffer (5c) and postfixed in 2% OSO4 in a 0.1 M phosphate buffer, dehy-

drated in a graded ethanol series, embedded in an epon/araldite mixture (Mollenhauer 1964), and examined under a JEM-T 7 electron microscope.

In order to observe the mites and also to fix the gall tissue it was necessary to cut galls into sections. Only by use of this method was it possible for the fixing liquids to penetrate effectively into the gall tissue. At the same time, however, this intervention implied that the mites were disturbed in their eating position while their mouth parts were in the nutritive cell, with the result that they pulled away. The fixing liquid did not kill the mite immediately, as they can live up to three weeks in gluraraldehyde. Other fixing substances also cause mites to withdraw their mouth parts from the plant cells before they are killed.

Results

Studies of gall initials under a fluorescent microscope showed a substance on the outside of the nutritive cell. This substance was not fluorescent itself but became fluorescent yellow after being treated with auramin O. It was in droplets on the surface of the gall cells and ran together over the slightly submerged anticline cell walls around the original epidermis cells (Fig. 1).

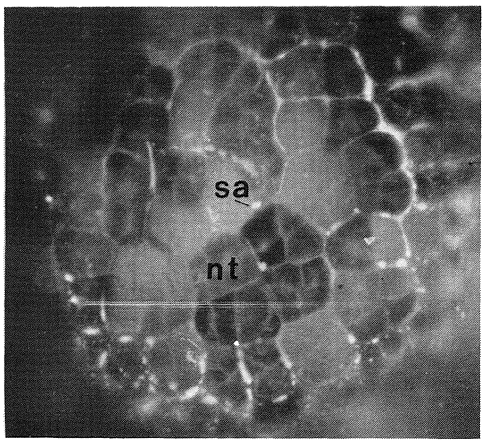

Fig. 1. x 400. Gall initial with droplets of spit from the mite, studied under fluorescent microscope and treated with auramin O.

These droplets did not appear on unaffected pieces of leaf. In addition, a change in colour, which arose in the cause of 2-5 minutes, indicated that the permeability of the gall initials treated with auramain O was different from that of the common epidermis cells on the leaf. In contrast to the normal cells, the gall cells absorbed the auramin O.

During examination of the galls under an electron microscope, an osmophilic substance was found on the surface of the nutritive cells. As shown in Fig. 2, this substance penetrated into the cuticula of the nutritive cell and the outmost part of the cellulose wall. Fig. 2 also illustrates that the structure of the wall was neither destroyed nor pressed together, but the cuticula and cellulose seem to have dissolved.

Even though none of the mites were fixed while their mouth parts were in the nutritive cells, tracks of their feeding were found in the galls. The term »suction tracks« or »suction marks« does not only comprise the hole in the cell wall but also the changes which occurred in the part of the wall around the hole. Under a phase contrast microscope the suction track looks like a cobolt blue cone with a dark column as a result of callose reaction with the dye resorcinol blue. A series of sections showed that normally there was only one track in every cell. The attacked cells appeared to be structured differently from the other nutritive cells (Thomsen 1975). They were often almost empty; without cytoplasma or nuclei, and sometimes completely fouled up. The series of sections also showed that the suction tracks were often grouped in cells that were close to one another. Under the electron microscope the suction tracks appear as in Fig. 3. Here it can be seen that the course of the microfibrills in the cellulose wall was only slightly disturbed. It can also be seen that the cone was inside the original cell wall and outside the plasmalemma which was in close contact with the cellulose wall. The callose seems grainy and contains dark areas.

The dark column in Fig. 3 reaches out to the cuticula, which demonstrates that the

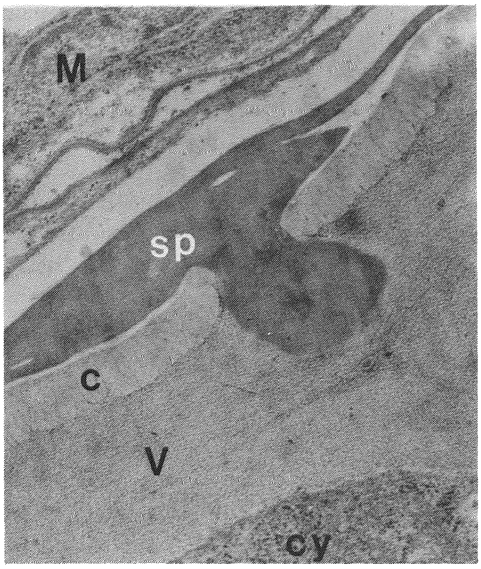

Fig. 2. x 18,000. Nutritive cell examined under electron microscope. Spit from the mite penetrated the cuticula and cell wall of the nutritive cell. Microfibrills in the cell wall are not destroyed or pressed together.

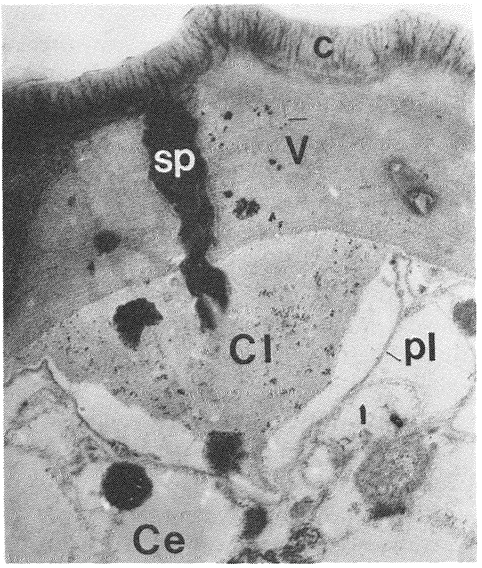

Fig. 3. x 12,000. Suction track in nutritive cell. The track consists of a dark column as well as a callose cone on the inside of the wall of the nutritive cell. Plasmalemma can be seen round the cone, while most of the cytoplasma is sucked away.

section was very close to the center of the suction track. On the other hand, the dark column does not reach the point of callose cone on any of the suction tracks examined. Likewise, studies in which the electron microscope was used showed that the cytoplasma and nuclei in the attacked cells had degenerated.

The mouth parts of *Eriophyes tiliae tiliae* have been described in detail (Thomsen 1987). These include the following elements: one pair of pedipalps, one pair of chelicers, one pair of auxiliary stylets, an oral stylet, a stylet sheath which is open dorsally, and pharynx. Fig. 4 shows a section of certain mouth elements. The distal part of the pedipalps were transformed into a plate which in the eating position can be brought up to the epidermis cells.

Discussion

The feeding behaviour of the Eriophyoidea has been investigated by various researchers.

Most models have been made on the assumption that the mouth parts enter into the plant cell mechanically, in that the pedipalps are placed on the surface of the cell

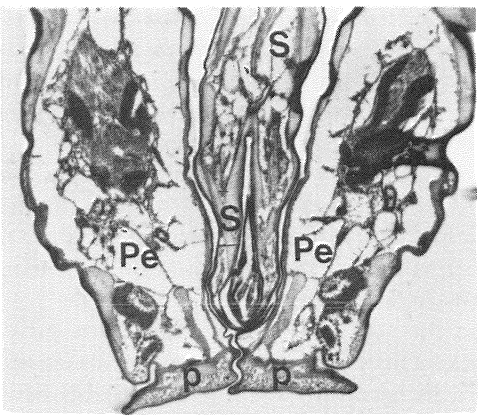

Fig. 4. x 6,200. Longitudinal section through the mouth parts of *Eriophyes tiliae tiliae*. The plate at the distal end of the pedipalps and the stylet sheath are shown.

and then compressed while the other mouth parts cut or drill into the cell wall (Shevtchenko & Silvere 1968, Krantz 1973, McCoy & Albrigo 1975, Nuzzaci 1976, Keifer 1959, Hislop 1976).

Other researchers think that an enzymatic dissolution of the cell wall takes place in connection with the consumption of food (Schmeits & Sassen 1978). Corresponding enzymatic dissolution of the cell walls is also known from infection by parasitic fungi (McKeen 1974, Zeyen & Bushnell 1979, Politis 1976) or from observations that insects secrete spit containing enzymes when they penetrate the plant tissue (Hori 1974, Miles 1965).

In this study a substance was detected on the surface of the nutritive cells by use of several methods of investigations. It is assumed that it was the same substance that was found via these various methods and that the substance was spit secreted by *Eriophyes tiliae tiliae*.

Treatment of the spit with auramin O demonstrated that the permeability of the cells in the gall initials, which had been exposed to mite spit, changed so that they could absorb the fluorescent substance in contrast to the common leaf epidermis cells which were not affected by the mite spit.

Furthermore, the examinations in which the electron microscope was used demonstrated that the spit penetrated the plant cell from the outside. The fact that the substance did not deform the structure in the adjacent part of the cell wall indicates that the penetration took place enzymatically.

Comparison of the dimension of the stylet-formed mouth parts (Fig. 4) with the dimensions of the suction track (Fig. 3) shows that the mouth parts are broader than the dark column which represents the hole in the cell wall. If the mouth parts penetrated the cell wall by mechanical means only, the structure would most probably have been permanently deformed, but this was not the case. It is therefore likely that an enzymatic dissolution of the cell wall preceded the entry of the mouth parts. It is conceivable that the enzymatic dissolution made the cell wall become more plastic so that the mouth parts could penetrate more easily. Another possibility is that the enzymatically formed hole has the same width as the mouth parts, thus allowing them to enter directly, and that the cell wall, after the mite has withdrawn the mouth parts and no longer fills out the hole, collapses. The visible column in the suction track becomes smaller than the mouth parts.

Formation of callose in connection with injuries or attacks by parasites on the plant cell are well known phenomena (Lipetz 1970, Nims, Halliwell & Rosberg 1967, Currier 1957). Callose cones at the suction tracks from eriophyid mites have also been described (Westphal 1973, 1975, Westphal, Bronner & Le Ret 1980). Because of the rapidity of formation and destruction of the callose, and because of the fact that one has got to work with long fixation time, the process can scarcely be followed under an electron microscope.

Nims, Halliwell & Rosberg (1967) have demonstrated on live tobacco cells how callose is formed in cells by the use of microneedles. The callose was formed in 15 minutes, and the superposition could take place while the needle was in the cell.

It must be assumed that at the formation of suction tracks by *Eriophyes tiliae tiliae*, the basis of the callose cone is formed while the mite still has its mouth parts in the cell. At this time, the plasmalemma is intact and in contact with the cell wall, and the period for the formation of the callose (Nims, Halliwell & Rosberg 1967) corresponds to the time it takes for the mite to feed (Orlob 1966).

When the mite withdraws, the plasmalemma regenerates and the formation of callose continues around and below the callose that was formed first. The dark column of spit in the callose cone is hereby embedded.

Comparison of the suction tracks of *Eriophyes tiliae tiliae* galls with the suction tracks in the galls which Westphal (1973, 1975) examined shows that the suction

tracks in *Eriophyes tiliae tiliae* galls are more simply constructed, in that they only consist of callose, while the suction tracks in the galls examined by Westphal are more intricate, in that the callose is surrounded by deposits of cellulose and pectin. In the latter case the cells are not destroyed by a single suction, and the cytoplasma contains even more elements which make further reparation of the suction hole possible.

Conclusion

By summing up the observations above one can construct the following model of the working principle of feeding behaviour in *Eriophyes tiliae tiliae*. The mites place spit on the surface of the gall cells. This spit contains enzymes which dissolve the cuticula of the cell wall and a large part of the cellulose wall. By feeling at the surface of the nutritive tissue the mite locates this dissolved area. The pedipalps are pushed toward the cell and contracted. During this contraction the other mouth parts are pushed directly into the hole. The plasmalemma is punctured and the cell juice is sucked up. During ingestion, spit can be secreted into the nutritive cells. The mite sucks out almost all the contents of the cell in 10-20 minutes and withdraws its mouth parts. It can then find a new perforated cell nearby under the layer of spit.

Sammenfatning

Fødeoptagelse hos *Eriophyes tiliae tiliae*, som forårsager galler på *Tilia platyphylla*, er blevet undersøgt med forskellige metoder.

Følgende model for arbejdsprincippet ved fødeoptagelse er opstillet: Miden anbringer spyt på overfladen af næringsvævscellerne i gallen. Dette spyt indeholder enzymer, der opløser cellevæggens cuticula og en stor del af cellulosevæggen. Miden finder derpå ved at føle på overfladen et sådant opløst område, pedipalperne stemmes mod cellen og trækkes sammen, og derved føres de øvrige mundelementer ind i hullet. Plasmalemma punkteres, og cellesaften kan suges op. Under fødeoptagelsen kan der udskilles spyt inde i næringsvævscellen. Miden tømmer næsten cellen for indhold på 10-20 minutter og trækker sine munddele tilbage. Herefter kan den finde en ny, perforeret celle lige i nærheden under spytlaget. Omkring hullet i cellevæggen dannes en callosekegle med indlejret spyt. Hele dannelsen benævnes sugespor.

Explanation of symbols

c = cuticula; Ce = sucked-out cytoplasma; Cl = callose cone; cy = cytoplasma with organells; M = mite; nt = nutritive cells; p = plate, Pe = pedipalp; pl = plasmalemma; S = stylet sheath; sa = spit coloured with auramin O; sp = spit; V = cellulose wall with microfibrills.

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