EPIPHYTIC EVERNIA PRUNASTRI (L.) ACH. : ULTRASTRUCTURAL FACTS

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SUMMARY. – The insertion of *E. prunastri* thallus in the bark of *Quercus pyrenaica*, and its progression through the plant tissues up to the medulla have been investigated. The material has been processed for light and electron microscopic observations. A dramatical destruction of the bark by hyphae penetration has been observed. Lichen hyphae progress up to the most internal tissues invading the vascular bundles and penetrating into the xylem vessels without an apparent destruction of the cellular integrity at this level.

INTRODUCTION

The fact that epiphytic lichens could be considered as facultative parasites is still debated. This supposition could be related to our observation that branches which support lichens, are extensively defoliated. On this subject OZENDA and CLAUZADE (1970) pointed out that lichen acids could be released into phorophyte cells, as it has been conjectured for both usnic and sekikaic acids from *Ramalina tayloriana* A. Zahlbr.

Even at a structural level, the knowledge about the lichen insertion in the bark and its progression inside the host tissues is excessively uncertain. Recently ESTEVEZ et al. (1980) have reported that a saprophytic *E. prunastri* penetrates into the branches of *Fagus sylvatica* in a way that hyphae are found in the xylem. This penetration shows a radial development and provokes a strong distortion in the peripherical layers of the periderm. LINDAU (1895) and FRY (1926) affirmed that the lichen hyphae do not enter the host cells. Nevertheless, FRANK (1877) and BONNIER (1889) described that the filaments of the algae

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of *Trentepholia* genus are able to perforate both membrane and cell wall of the peridermal cells of the substrate.

In the present paper, both insertion and penetration of *Evernia prunastri* thalli in oak branches have been studied, in order to prove whether there is a real penetration of the hyphae inside the phorophyte cells.

MATERIAL AND METHODS

Living branches of 0.5 cm diameter of *Quercus pyrenaica* with the lichen *Evernia prunastri* (L.) Ach. were collected in Hayedo de Montejo, Madrid (Spain). Branch samples at the insertion point were taken and sections obtained following the method of GRAM and JØRGENSEN (1953), to be examined under the light microscope.

Cylindrical portions of the branches were cut with a surgical knife. They contained along their longitudinal axis, the lichen and its insertion zone in the branch as well as the branch tissues up to the medulla (see fig. 1). These samples were softened in a phenol-acetic mixture (1:1 v/v) for three days. The material

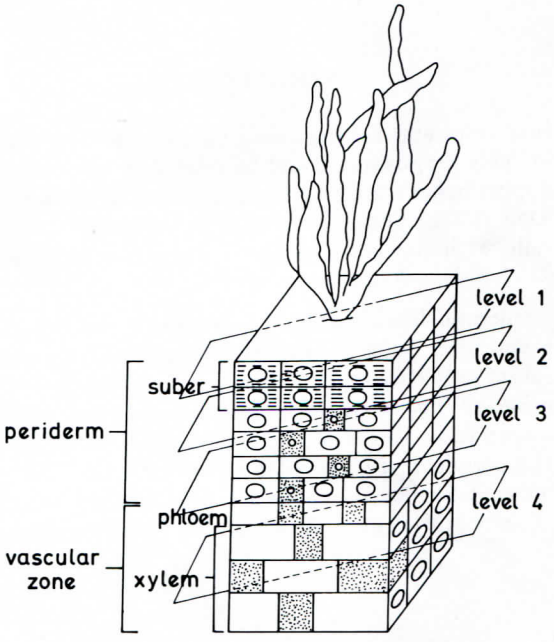

Fig. 1. – Diagram of the samples used for sequential sectioning. Longitudinal-tangential sections were obtained as shown in the diagram.

was then immediately processed for electron microscopy. Fixation and dehydratation were performed as previously described (ASCASO and GALVAN 1976). Embedding was done in Spurr type B (hard) (SPURR 1969) following a two steps procedure : the samples were horizontally introduced into lead-tin capsules (15, 7 and 4 mm) and conveniently embedded in resin. After heating at 70°C for 18 hours, the blocks were removed from the capsules and introduced perpendicularly to the bottom into standard gelatin capsules and the polymerization was prolonged for 24 hours more. Sections (1 μm) obtained with an OmU2 Reichert Ultratome and stained with methylene blue were used for examination under the light microscope. Thin section (600 Å) were post-stained with lead citrate (REYNOLDS 1963) for 18 minutes and examined on a Philips E.M. 300 electron microscope.

RESULTS

Light microscope observation of the lichen insertion point, in a transversal section of a branch (pl. 1.1), shows a raising of the suberous layers of the bark through which penetrates a compact bundle of hyphae, destroying the phorophyte cells. Sometimes (pl. 1.1), we observed a remarkable separation between cortex (periderm and phloem) and xylem, or the presence of hyphae between two layers of periderm (pl. 1.2).

A study of the lichen insertion in the branches has been made by sectioning the samples sequentially as is shown in fig. 1. Observations at the most external level (level 1 or insertion point) (pl. 1.3) revealed the presence of a dense mass of hyphae surrounding several fragments of suberified cell walls (pl. 1.4). At the next level (level 2), corresponding to the suberous layer of the periderm (pl. 2.1), the hyphae retained their internal structure and the thickness of their cell walls, showing a density similar to those described in the most external level (pl. 2.2). In this section, a spatial relation has been observed between hyphae and cellular fragments which were not so dispersed as those previously described.

A deeper level in the periderm (level 3) (pl. 2.3) presents cells which retain a more complete integrity, showing the penetration of hyphae between the cell walls of two adjoining cells (pl. 2.4).

At the level of vascular bundles (level 4), at its most external zone, hyphae have been observed in both intercellular spaces and inner part of the cells (pl. 3.1). Finally, at the most internal zone (pl. 3.2) hyphae are located inside the xylem elements, leaning against their cell walls (pl. 3.3, 3.4, 3.5 and 4.1). We can observe the penetration of hyphae through the wall of xylem element (pl. 4.2).

DISCUSSION

Before discussing the results, it is important to point out some of the difficulties encountered in embedding the samples. Besides the inherent problems to the lichen material per se, it was necessary to prepare for electron microscopy

part of the branches, which greatly complicated the problem. With the pre-treatment with phenol-acetic mixture a little ultrastructural resolution is lost, but more entire cuts of each zone can be obtained which are interesting to be studied. Pre-treatment, and use of a hard resin (Spurr type B) instead of normally employed one (Spurr type E) are necessary to embed together lichen thallus and branch tissues.

The views obtained with the light microscope reveal a separation between cortex (periderm and phloem) and xylem. This fact can be explained either by a mechanical traction produced in the thallus itself or by the effects of preparation of the samples.

Periderm appears very degraded by the action of hyphae. This observation could be explained as an attack similar to that produced by some parasitic fungi where hydrolytic enzymes play a main role (HALL and WOOD 1973).

The results confirm, at an ultrastructural level, the hypothesis of LINDAU (1895), PORTER (1917) and BRODO (1973), that the hyphae develop through the intracellular spaces in the phorophyte. Nevertheless, our observations support also the suggestion of FRANK (1877) and BONNIER (1889) on a real penetration of hyphae inside the cells. Our electron microscopic pictures show hyphae inside the xylem vessels. From these results and from the observed defoliation of oak branches (running from the insertion point of the lichen to the end of the branch), it may be concluded that the xylem invaded by lichen hyphae is a functioning tissue. This fact would provide a structural basis to OZENDA and CLAUZADE (1970) hypothesis which supposes a release of lichen acids into phorophyte cells. This release into xylem flow would assure acropetal translocation of lichen acids to leaves. The parasitic role of certain epiphytic lichens could so be established at a physiological level, since lichen acids are strong photosynthetic inhibitors (VICENTE and ESTEVEZ 1976) which could lead to defoliation.

ACKNOWLEDGMENTS. – We are grateful to Mr. F. Pinto, Mr. C. Macias and Miss T. Carnota for technical help.

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EXPLANATION OF PLATES

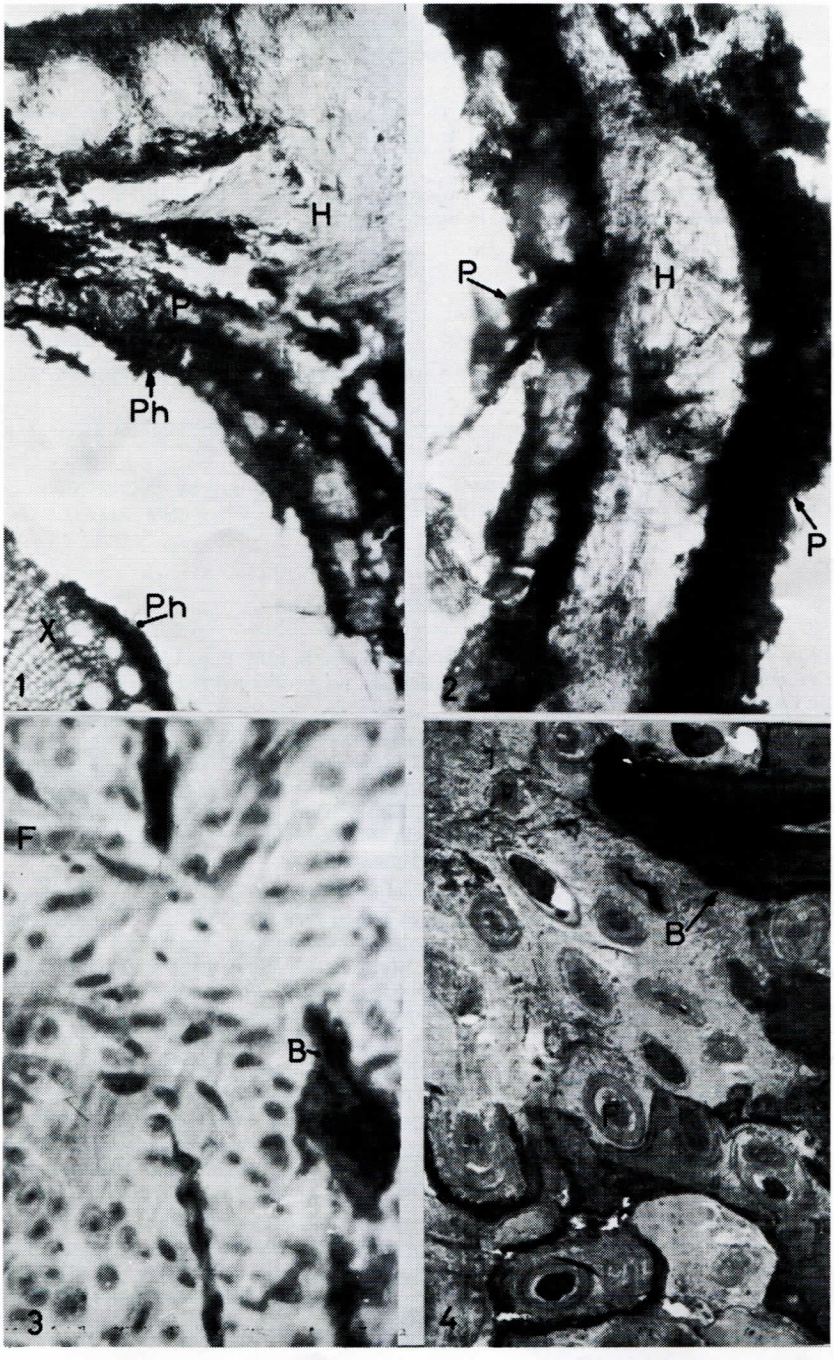
Key to lettering : Ph, phloem; H, hyphae; X, xylem; B, bark; P, periderm; F, fungal cell; sc, suberified cell; cf, cellular fragment; s, sclereids; pc, peridermal cell; pw, peridermal cell wall; vw, vessel element wall.

Pl. 1. – 1. Light micrograph of a transversal section of a branch in the insertion point of *Evernia prunastri* in *Quercus pyrenaica* bark. Gram and Jørgensen's method (x 360) – 2. Hyphae of *E. prunastri* invading the space between two separate layers of oak periderm. Gram and Jørgensen's method (x 540) – 3. Thin section (1 μ) of oak bark from level 1 (see fig. 1) showing a dense mass of fungal cells. Stained with methylene blue (x 3,600) – 4. Electron micrograph of a section from the same level as in 3 (x 5,600).

Pl. 2. – 1. Thin section (1 μ) of oak bark from level 2 (see fig. 1), showing the suberous layer of the periderm and a mass of hyphae penetrating. Stained with methylene blue (x 1,600) – 2. Section for Electron Microscope, from the same level as in 1 (x 5,000) – 3. Thin section (1 μ) of oak bark from level 3 (see fig. 1), showing the most internal layer of the periderm. Stained with methylene blue (x 1,600) – 4. Section for Electron Microscope from the same level as in 3. A collapsed hyphae between two adjacent cell walls (x 13,000).

Pl. 3. – 1. Hyphae found near the walls of two adjacent cells, from the external zone of vascular bundles. Electron microscopic observation (x 8,600) – 2. Thin section (1 μ) from level 4 (see fig. 1), showing the xylem vessels. Stained with methylene blue (x 1,050) – 3. Section for Electron Microscope from the same level as in 2. Longitudinal section of a hyphae located along a wall of xylem element (x 5,000) – 4. and 5. Sections as in 3. Transversal and longitudinal sections of hyphae over a thickening of vessel wall (x 12,500 and 9,100).

Pl. 4. – 1. Sections as in pl. 3,3. Transversal sections of two hyphae, one of them located along a wall of a xylem element, another near the lignified thickening. The primary wall is still present (x 5,600) – 2. Sections for Electron Microscope as above. Section of a hyphae penetrating through a wall of a xylem element (x 7,100).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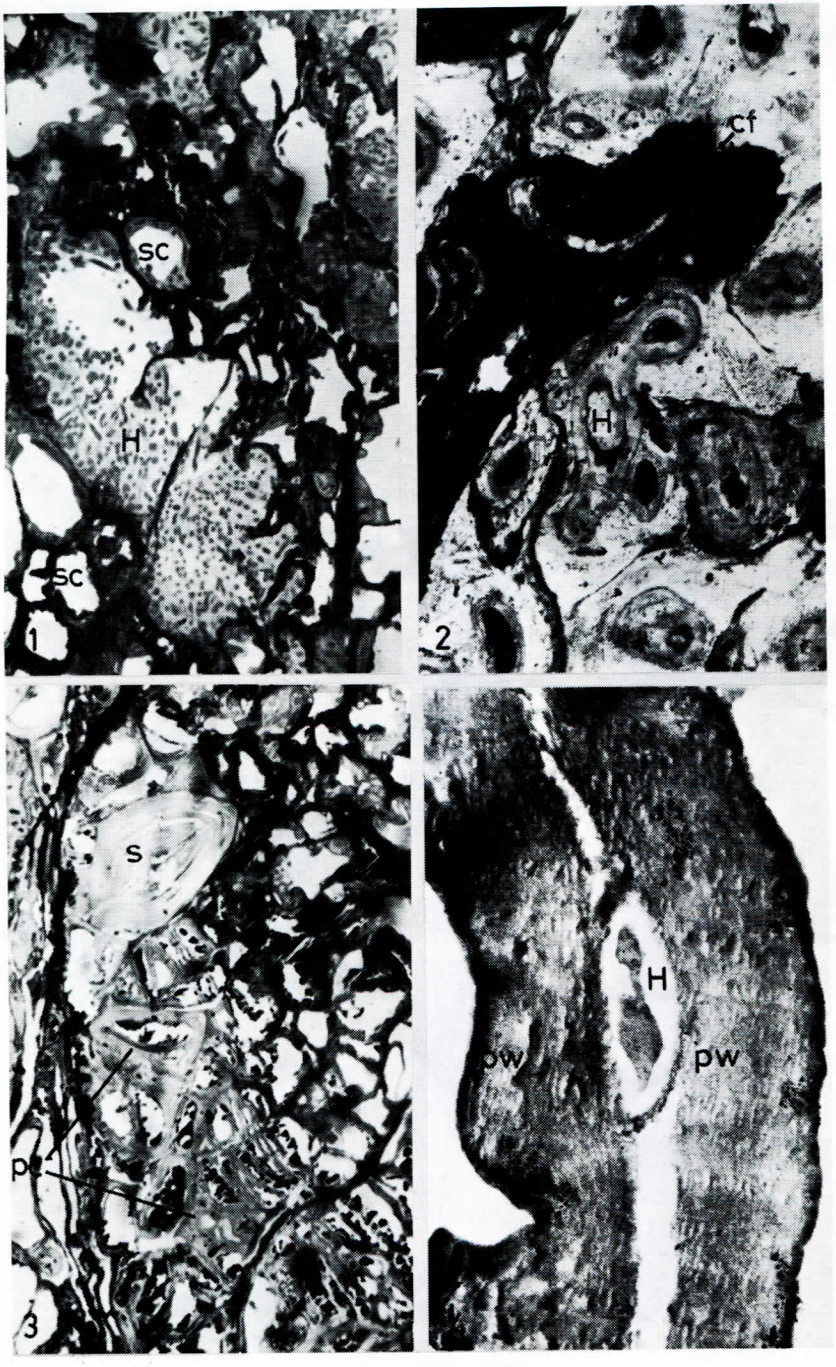
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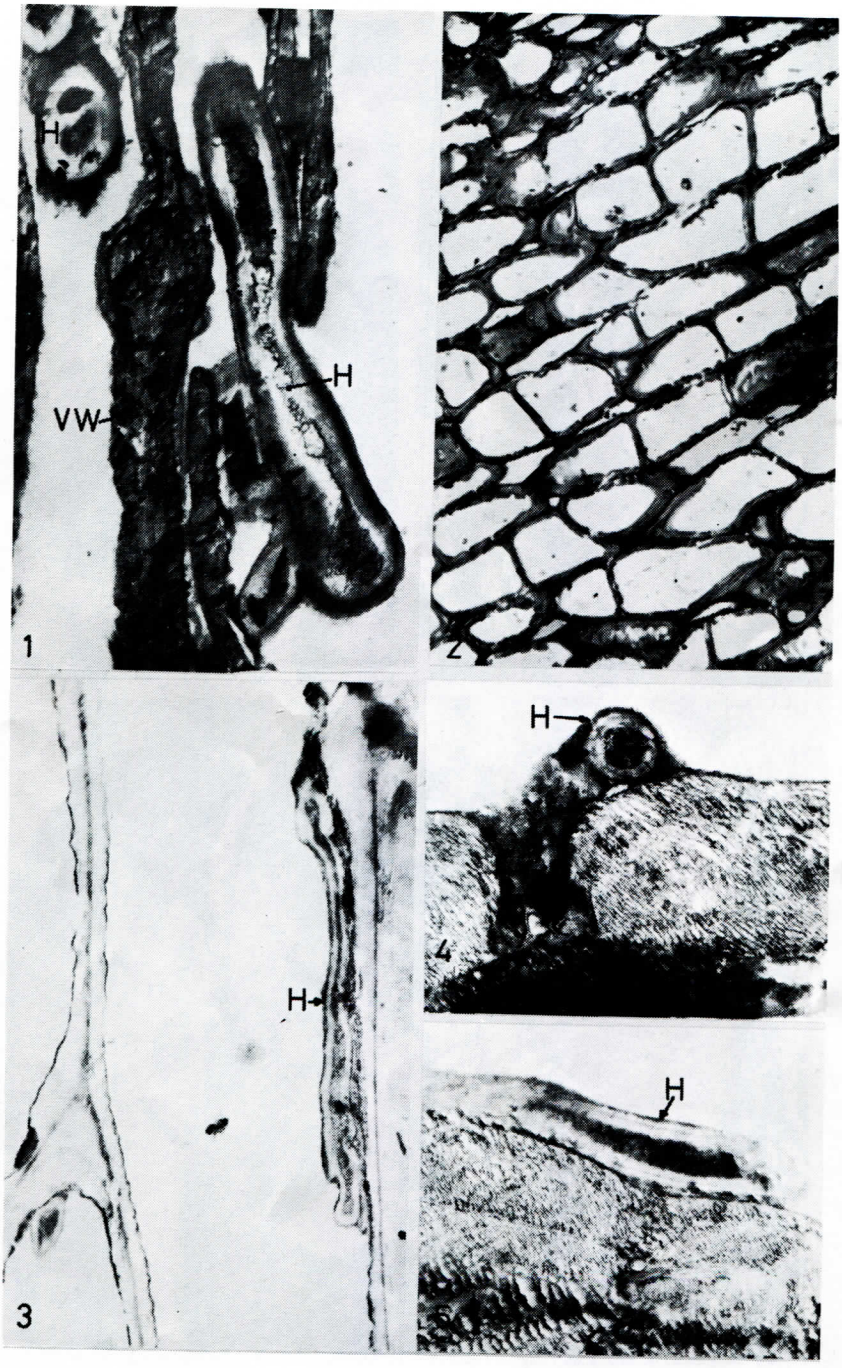

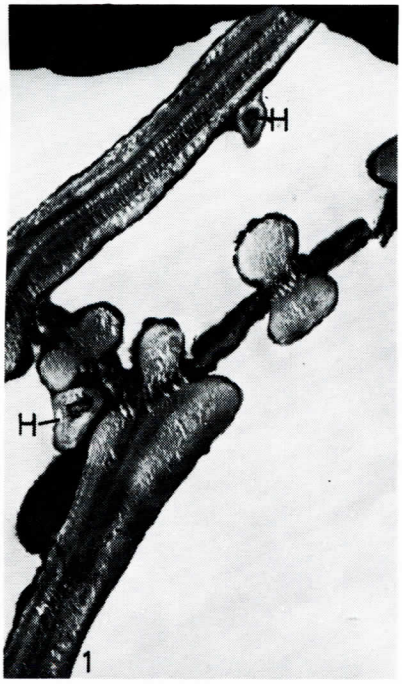
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