

## The parasitic interaction of *Puccinia gouaniae* (Uredinales)\*

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Investigation of haustoria and haustorial mother cells of *Puccinia gouaniae* revealed some characters new for the rust fungi. Terminal haustorial mother cells were not formed by the intercellular mycelium. In contrast cell wall-bound compartments occurred within the hyphae of the intercellular mycelium. From these compartments haustoria originated. The compartments are designated intrahyphal haustorial mother cells (iHMCs). The iHMCs contained two nuclei which did not migrate into the haustoria and their wall layering was different from that of ordinary HMCs. The septum-like structure of the iHMC wall is discussed. The haustoria of *P. gouaniae* did not exhibit important differences to already described *Puccinia*-D-haustoria.

Keywords: *Puccinia gouaniae*, haustorial mother cell, D-haustorium.

*Puccinia* is the largest genus among the rust fungi and contains some of the most important plant pathogens. Thus plant pathologists have given special attention to *Puccinia* and a considerable amount of the knowledge on the ultrastructure of dikaryotic haustoria of rust fungi comes from investigations of species of that genus (Al Khesraji & Lösel, 1981; Borland & Mims, 1980; Chong & Harder, 1980, 1982; Chong & al., 1981, 1985, 1986; Harder, 1978; Harder & al., 1978; Taylor & Mims, 1991; Wood & Heath, 1986). The species investigated showed an essentially identical haustorial ultrastructure. The investigations, however, have concentrated on only a few, mostly extratropical *Puccinia* species of economic interest.

In the present study the uredial stage of the tropical taxon *Puccinia gouaniae* Holw. was investigated. *P. gouaniae* is a widespread parasite on *Gouania* spp. (Rhamnaceae) throughout the American tropics (Jørstad, 1959). The life cycle of the species comprises pycnia, primary and secondary uredinia and telia (Arthur, 1917). The primary uredinia are accompanied by the pycnia and occur on hypertrophic areas of the host leaves. The secondary uredinia are

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tiny, paraphysate and do not cause hypertrophy or much discolouring of the host.

### Material and methods

The uredial stage (secondary uredia) of *Puccinia gouaniae* Holw. was collected in Costa Rica (Turrialba, at the C.A.T.I.E. property) and Venezuela (Barquisimeto, Parque Nacional Jacumbu, at Laguna El Blanquito) on *Gouania* spp. (Rhamnaceae). The material is stored in the author's herbarium (HeRB 2654 and HeRB A-13) and in the national herbaria of Costa Rica (CR) and Venezuela (VEN), respectively.

Small pieces of the infected leaves were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for several days. After washing 6 times with 0.1 M cacodylate buffer, the material was postfixed for 1 h in 1% aqueous  $\text{OsO}_4$ . Afterwards the specimens were washed three times in distilled water and stained with aqueous uranyl acetate for 1 h. After washing with distilled water (3 $\times$ ) and dehydration in an acetone series (10, 25, 50, 70, 95, 100% three times), samples were embedded in Spurr's resin (Spurr, 1969). The embedded material was sectioned with a Reichert Ultracut E microtome. Ultrathin sections (ca. 85 nm) were collected on formvar-coated copper slot grids and contrasted with lead citrate according to Reynolds (1963). Sections were observed with a Zeiss EM 10.

### Results

#### Intercellular hyphae

Between the cells of the leaf mesophyll very broad, intercellular hyphae of *P. gouaniae* could be observed. The diameter of the hyphae was about 7–12  $\mu\text{m}$ . They were only sparsely branched and septate (Fig. 1). Typical haustorial mother cells (HMCs, often short and inflated cells on short side branches of the intercellular mycelium which give rise to haustoria) could not be observed.

#### Intracellular haustorial mother cells

Within the intercellular hyphae irregularly globular to ellipsoid areas could be observed which were delimited by a cell wall. A single cell of the intercellular hyphae contained up to three of these intracellular compartments, but in most cases only one or two could be found (Fig. 1). Each of the cell wall-bound areas contained two

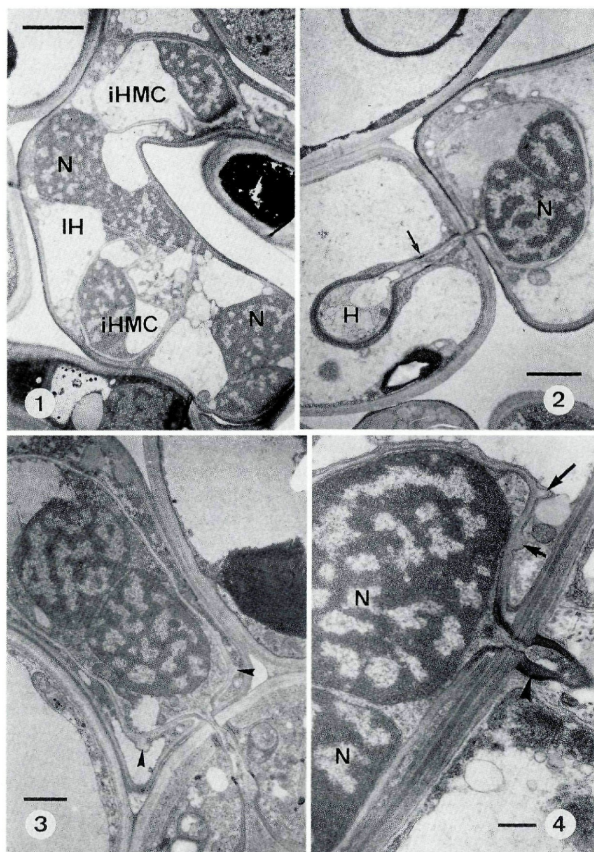


Fig. 1. - Intercellular hypha (IH) containing two intracellular haustorial mother cells (iHMC). The nuclei (N) are visible within the cells of the IH. - Scale bar: 3  $\mu$ m.

Fig. 2. - Section through a intercellular hypha lying adjacent to a host cell. The iHMC gives rise to a haustorium (H). The nuclei (N) remain in the iHMC. A neckband is formed at about half of the length of the neck (arrow). - Scale bar: 2  $\mu$ m.

Fig. 3. - iHMC and haustorial neck. The wall of the iHMC is slightly thickened around the penetration site and shows some wart-like thickenings (arrowheads). - Scale bar: 1  $\mu$ m.

Fig. 4. - Detail of iHMC and the penetration site. The huge nuclei (N) fill most of the lumen of the iHMC. The wall of the iHMC bears some wart-like thickenings (arrows). The base of the haustorial neck (arrowhead) is thickened. - Scale bar: 0.5  $\mu$ m.

nuclei. Where the intercellular hyphae were in contact with the host cells, the intracellular compartments were attached to the walls of the hyphae and gave rise to small, stalked haustoria with a bulb-shaped to allantoid haustorial body (Figs. 2, 3). Thus the intracellular compartments appear to be haustorial mother cells, and they are therefore referred to as 'intracellular haustorial mother cells' (iHMC). The nuclei remained in the iHMCs where they lay closely together in front of the penetration peg (Figs. 2-4). Only in one case had the nuclei migrated into the haustorium and were observed in the haustorial body. The intercellular hyphae which contained iHMCs were vigorous as revealed by their well preserved nuclei and cell organelles (Figs. 1, 3).

#### Walls of iHMCs

The walls of the iHMC were thinner than those of the intercellular hyphae (Figs. 2-4). The part of the wall of the iHMC surrounding the penetration site was sometimes slightly thickened, and in most cases distant, wart-like pegs were formed towards the lumen of the intercellular hyphae (Figs. 3, 4). Directly at the penetration site an inner layer of the wall of the iHMC stained more densely than the remaining wall (Figs. 4, 5). The cell walls of the intercellular hyphae were layered, and at least three layers could be discerned. The outermost layer showed a homogeneous structure and was electron-dense. It was separated from the broad, innermost layer by a moderately electron-dense layer (Fig. 5). The innermost layer had a slightly fibrous appearance and was more electron-translucent than the outer ones. At the penetration site the walls of the intercellular hyphae and the host cells were penetrated. The inner part of the innermost wall layer of the intercellular hyphae sloughed, bent back and surrounded the iHMC at least basally (Figs. 4, 5). Between this wall layer and the more electron-dense inner wall layer of the iHMCs around the penetration site an electron-translucent, thin lamella occurred (Fig. 5). Further away from the penetration site the lamella disappeared and it was not possible to distinguish different wall layers of the iHMCs. The penetration channels were markedly constricted where the wall layers of the intercellular hyphae were penetrated (Figs. 4, 5) and became even narrower within the walls of the host cells (Fig. 5). The fungal wall of the penetration peg was extremely thin; it was continuous with the wall of the iHMC (Figs. 4, 5) and stained electron-densely, comparable to the inner wall layer of the iHMC at the penetration site (Fig. 5).

The haustoria consisted of a slender, tall neck and a haustorial body of various but mostly oblong rounded shape (Fig. 2). The

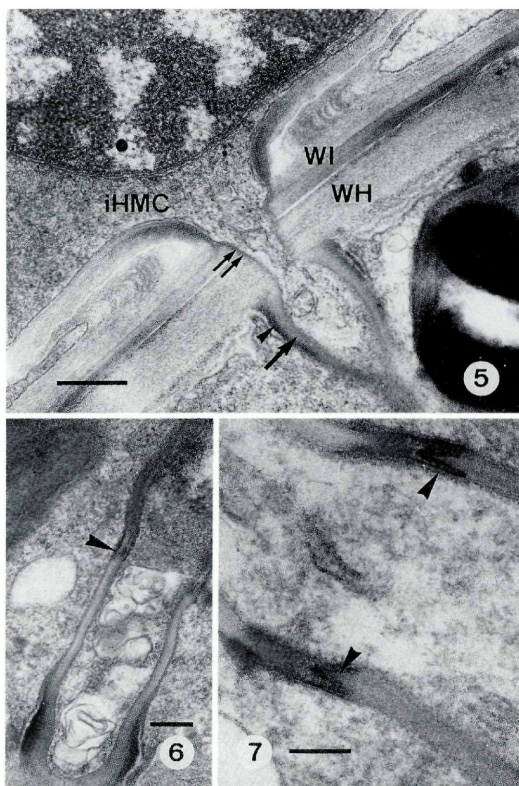


Fig. 5. – Penetration peg. The wall (WI) of the intercellular hypha and the host cell wall (WH) are penetrated by the roughly wedge-shaped penetration peg. The fungal wall of the penetration peg is electron-dense and extremely thin (double arrow). It is continuous with the electron-dense wall layer of the iHMC at the penetration site. The wall of the haustorial neck is thickened basally and shows a darker staining outer and a less electron-dense inner layer (arrowhead and arrow). – Scale bar: 0.3  $\mu\text{m}$ .

Fig. 6. – Haustorial neck. The wall of the neck becomes thinner towards the neckband (arrowhead). The more densely staining outer layer tapers off, and the neck wall stains homogeneously up to the neckband. Distal to the neckband the wall of the neck stains more electron-densely. – Scale bar: 0.2  $\mu\text{m}$ .

Fig. 7. – Haustorial neck and neckband. At the neckband the fungal wall is slightly constricted. Apically (on the right hand) the neckband shows a more densely staining structure (arrowheads) which is more or less V-shaped in section. – Scale bar: 0.1  $\mu\text{m}$ .

haustorial wall was conspicuously two-layered and thickened at the base of the neck (Figs. 4–6). The inner, less electron-dense layer continued towards the haustorial body and became only slightly thinner (Fig. 6). The outer, darker staining layer tapered off towards the neckband (Figs. 5, 6). The neckband formed at about half of the neck length (Figs. 2, 6) and was more or less spool-shaped in section. At the neckband the fungal wall stained more densely along the fungus and host plasmalemma, but also in the centre of the neckband areas of higher electron-density could be observed (Fig. 7). Sometimes a collar was formed by the infested host cell, but it was never observed to encase more than the basal part of the neck.

### Discussion

Haustorial mother cells are generally described as cells which occur terminally on intercellular hyphae or as terminal cells of short side branches of the intercellular hyphae. HMCs are usually short, rounded and often inflated (e. g. Gray & al., 1982; Heath & Heath, 1975; Khan & al., 1982; Mims & al., 1989; Rice, 1927). A lack of ordinary HMCs and a formation of intracellular HMCs as in *P. gouaniae* has never been described for other *Puccinia* spp. or the rust fungi. The character seems to be constant in *P. gouaniae*, as it could be observed in material from Costa Rica and Venezuela. The described parasitic behaviour may be a unique feature of *P. gouaniae*, but it could also have a wider distribution in the genus *Puccinia*, as there remain thousands of species to be examined.

Ordinary HMCs possess more wall layers than other cells of the intercellular mycelium (Chong & al., 1986). Normally the wall of a HMC is thickened at the penetration site, and already in conventionally fixed specimens a showy layer of higher electron-density can be observed within the wall (Borland & Mims, 1980; Harder, 1978; Harder, 1989 and references therein; Littlefield & Bracker, 1972; Rijkenberg & Truter, 1973). Similarly, there is a densely staining wall layer in *P. gouaniae* iHMCs around the penetration site. The layering of the walls of iHMCs is different, however. In contrast to normal HMCs it is the inner layer of the wall of the iHMC which stains more densely, and material of this layer seems to form the fungal wall in the penetration peg.

Unfortunately no earlier developmental stages of iHMCs could be found. One can only speculate, therefore, about the ontogeny of the iHMCs. The structure of the wall of mature iHMCs at the penetration site is similar to that of septa of rust fungi and other basidiomycetes (Harder, 1984; Bourett & McLaughlin, 1986; Kleven & McLaughlin,

1989). As in ordinary septa, the wall of the iHMC appears to be continuous with an inner layer of the original hypha. In iHMCs the 'septum' appears to be cleaved longitudinally, however, and additional, dark-staining material seems to be deposited at the inner side of the 'septal' halves around the penetration site. This material merges with the material of the 'septal' wall farther from the penetration point. One may thus assume that development of iHMCs comprises a  $\pm$  cup-shaped, septum-like structure that grows into the intercellular hypha. The structure finally closes to form a more or less globoid compartment which includes two nuclei. In other basidiomycetes formation of complete septa may be a matter of only a few minutes (Lü & McLaughlin, 1991; Orlovich & Ashford, 1994). The putative septal nature of iHMCs could mean that iHMC formation is an equally fast process. This could explain why initial stages of iHMC formation could not be found in the fixed material.

Mature haustoria of rust fungi are generally found to be dikaryotic, as the nuclei and most of the cell organelles pass from the HMC into the haustorium (Littlefield & Heath, 1979). The HMCs remain highly vacuolised and almost empty (Heath & Heath, 1975). *P. gouaniae* differs by binucleate HMCs and anucleate haustoria. To my knowledge rust haustoria whose nuclei remain in the HMCs have so far not been reported. 'Dikaryotic' haustoria containing only one nucleus were observed by Zimmer (1970) in *Puccinia carthami* and in *Puccinia poarum* by Al Khesraji & Lösel (1981); these haustoria were probably aberrant. Except for the lacking nuclei, the haustoria of *P. gouaniae* were similar to D-haustoria of other rust fungi (Harder & Chong, 1984; Harder & Chong, 1991 and references therein).

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