The Infection of Vascular Tissue of *Crataegus monogyna* by the Monokaryotic Stage of *Gymnosporangium clavariiforme*

L. Larous and D.M. Losel
Institute de Biologie, Universite Ferhat Abbas, Setif 19000, Algerie

Abstract

Larous, L. and D.M. Losel. 1996. The infection of vascular tissue of *Crataegus monogyna* by the monokaryotic stage of *Gymnosporangium clavariiforme*. Arab J. Pl. Prot. 14(1): 57-62.

The fine structure of *Crataegus monogyna* infected with the monokaryotic structures of *Gymnosporangium clavariiforme* was investigated. The distribution of fungal thallus within host tissue, types of infection structures and interactions with host cells have been described with particular reference to vascular system infection. A large nucleus and the formation of a septum at the level of the penetration site is frequently observed. This stage of infection is characterized by the extensive growth of fungal structures in the intercellular spaces of leaves mesophyll, with low frequency of penetration of these cells, and the greater degree of invasion of all types of cells in the host plant vascular system.

Key words: *Gymnosporangium clavariiforme*, ultrastructure, vascular infection.

Introduction

The ultrastructure of rust fungi has been studied extensively, particularly with respect to the uredinial stages of infection (15, 21). It is generally known that obligate parasites, such as rust fungi form specialized type of haustoria in host cells, during the uredinial-telial phases of infection. This type of haustoria (Dikaryotic) is demonstrated to be morphologically different from those produced by spermogonial-aecial stages of infection (11, 12, 22, 24). Most of these investigations have confirmed that the distribution of fungal dikaryotic structures is confined to leaf mesophyll and stem cortex cells. In contrast, recent reports have shown that the rust monokaryotic structures can invade different type of cells in the vascular tissues of host plant (1, 5, 19, 20).

In an attempt to obtain a clearer understanding of physiological and structural relationship of the monokaryotic infection of rust fungi, the distribution and nature of spermogonial-aecial ultrastructures of *G. clavariiforme* were investigated..

Material and Methods

Light and electron microscopy

Naturally infected leaves of *Crataegus monogyna* Jaq. bearing spermogonia and aecia of *G. clavariiforme* were collected from the field. Portions of infected leaves were processed for light and electron microscopy, by the procedure of Woods and Gay (26). The tissue was fixed in 2.5% glutaraldehyde in 0.1 M. sodium cacodylate buffer, pH 7.4, post-fixed in 1% osmium tetroxide in the same buffer, dehydrated in a series of ethanol concentrations and embedded in Araldite. Semi-thin sections for light microscopy were stained with toluidine blue. Ultrathin sections for electron microscopy were cut with a Reichart microtome using a diamond knife, post stained with 2% aqueous uranyl acetate, followed by lead citrate (23), and examined with Phillips 301 electron microscope.

Results

Infection structures

The intercellular spaces of mesophyll become almost completely filled with the intercellular hyphae of the monokaryotic stages of *G. clavariiforme*, but the fungal penetration of these cells is less frequent than it is in the vascular tissue region (Fig 1).

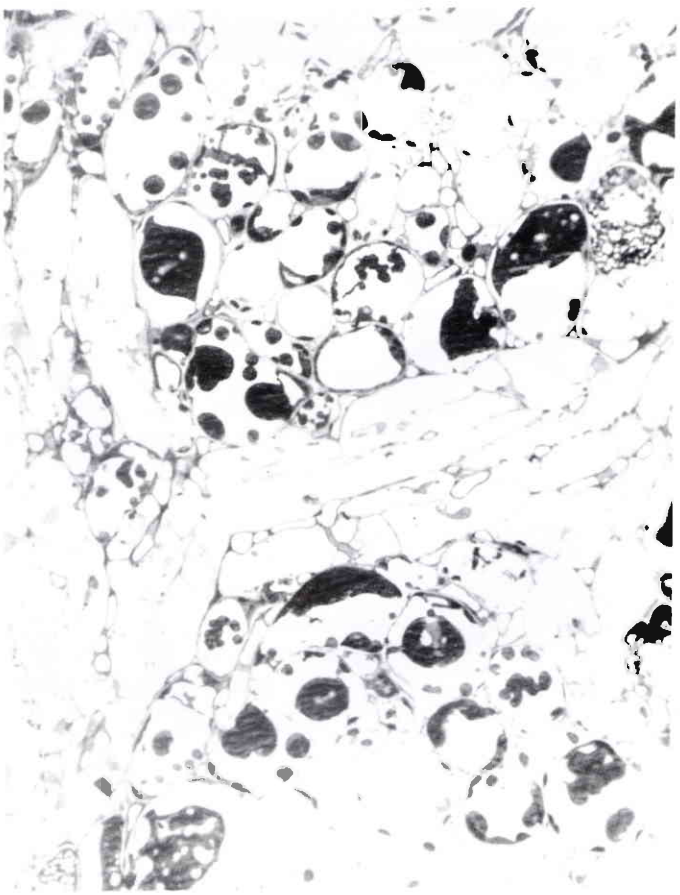

Figure 1. LM micrograph of *C. monogyna* leaf mesophyll infected with the monokaryotic stage of *G. clavariiforme*, showing the dense growth of intercellular hyphae and the relatively infrequent penetration of these cells. 40X.

The intercellular hyphae were often observed partially or completely embedded in the host cell wall. Serial sections from different specimens showed that the nuclei has no nucleoli and contain a variable amount of hetero- and euchromatin similar to that in the filamentous haustoria. Contrasting to the young hyphae, the older ones were vacuolated, with the cytoplasm confined to a layer lining the cell wall (Figs. 2 & 3).

Figure 2. Vacuolated intercellular hyphae (I) in leaf mesophyll, some are partially embedded in host cell wall. Note the oval and elongated shape of the nuclei. 12400X.

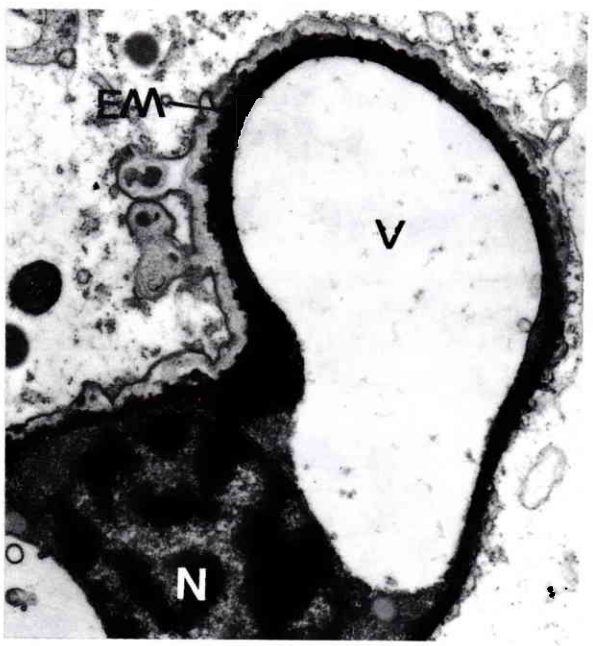

Figure 3. Old monokaryotic haustorium in phloem region surrounded by the extra-haustorial matrix (EM). The host mitochondria, ER, and vesicules are in close association with the haustorium . 18750X.

The spermogonial-aecial stages (monokaryotic) of this fungus are characterized by the existence of the filamentous haustoria, which are irregular in shape, and terminate within the infected host cell. They arise relatively from a small haustorial mother cell (HMC), which is always found appressed to/or embedded completely in host cell wall material (Figs. 4, 5 and 6). The HMC wall appears of the same thickness as that of the filamentous haustoria and much more electron dense than the host wall. A septum separating the HMC from developing haustoria is found repeatedly at the level of host cell wall penetration or proximal to it, which can be either complete or perforated. The pore apparatus of mature perforate septa are associated with numerous microbodies (Fig. 5). The filamentous haustoria are not constricted at the penetration site, and have no clearly differentiated neck region. The neck band, characteristic of dikaryotic haustoria, is absent, they possess one well developed large nucleus (Fig. 4).

There is no sign of break-down of the invaginated host plasmalemma which surrounds the haustorial body, and is separated from the haustorial wall by the extra-haustorial matrix (EM). This matrix is relatively thin in young haustoria and larger in older ones and consists of two layers differing in chemical composition, the outer layer is more electron lucent than the inner and resembles host cell wall material. The inner contains fibrillar structures projecting outwards from the haustorial cell wall (Fig. 7).

Host response to infection

The collars observed around the intracellular structures of the monokaryotic infections of *G. clavariiforme* are continuous with and indistinguishable from host cell wall material (Figs. 4, 5 and 6). The host endoplasmic reticulum (ER), chloroplasts, and mitochondria are in close association with the extrahaustorial membrane (EHM) (Figs. 3 and 10). As the infection progress, the host cytoplasm becomes less dense in appearance with loss of ribosomes and mitochondria, and decreasing amount of ER.

Vascular infection

The spermogonial-aecial stages of *G. clavariiforme* showed extensive invasion of the conducting system of the infected leaves. Dense intercellular growth of hyphae is observed, not only in the mesophyll but also in the vascular tissue (Fig. 1), and hyphae are frequently observed partially or completely embedded in host wall material (Figs. 8, 9 and 10). Intracellular structures of this rust occur within phloem parenchyma and xylem vessels, where they are sometimes embedded in lignified bands of wall thickening and also in bundle sheath cells (Figs. 8, 9 and 10).

The intracellular hyphae in xylem parenchyma, and phloem parenchyma, appear metabolically active (Fig. 10), although some are largely vacuolated. They contain mitochondria and dense cytoplasm, and are surrounded by the EM, bounded by the invaginated host plasmalemma. In this host cells, the cytoplasm generally remains well supplied with organelles, including chloroplasts, mitochondria and ER. In contrast, the haustoria observed within xylem vissels are generally necrotic in appearance and lack an EHM (Figs. 8 and 9).

Discussion

The filamentous haustoria of *G. clavariiforme*, in most respects, are similar to those described earlier in the spermogonial-aecial stages of other rust fungi (6, 11), and differ from dikaryotic haustoria in the absence of the thickening of the HMC wall at the penetration site, and in having little or no constriction at the neck region and no neck-band. However, *Gymnosporangium haraenum* infecting leaves of Japanese pear does not form haustoria in its monokaryotic stage (18). The HMC wall is continuous and is of uniform thickness with the wall of the haustorium. Harder (14) considered them as haustoria with a primitive level of specialization, in that they lack some of the structural features of more specialized haustoria. However, the monokaryotic haustoria of *Endocronartium harknessii* (16), *Cronartium quercuum* (13), and *Endocronartium pini* (25) are reported to have a constricted neck and expanded body, although lacking a neck-band. The septal pore apparatus is similar to that found in *P. poarum* (3) and *P. punctiformis* (5).

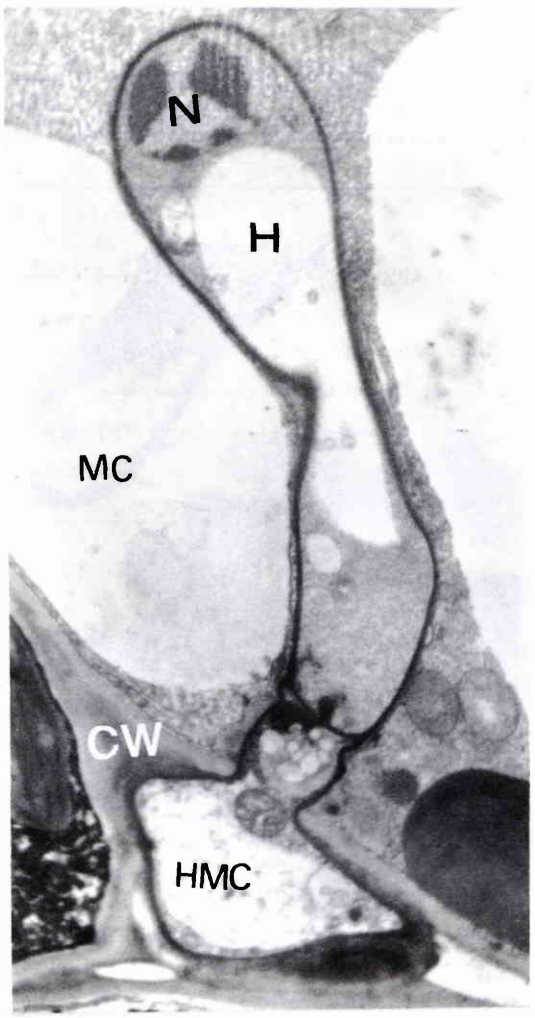

Figure 4. Filamentous haustorium (H) in mesophyll cell (MC), the fungal wall is densely stained and is of uniform thickness, there is no constriction at the penetration site, and the neck lacks a neck-band. Note the localization of the septum and the oval nucleus (N). 10000X.

The haustorial cell content are generally similar to those reported from other rust fungi (15, 21), except for that the nucleus is characterized by a large size and containing a variable amount of hetero-and euchromatin. The absence of nucleoli in both intercellular hyphae and haustoria correspond to previous reports (3, 11).

The formation of collars were frequently linked to the degree of host-pathogen compatibility or to the age of haustorium (9, 21). However, the filamentous haustoria of *G. clavariiforme* is partially encased in material which appears as an extension to the host cell wall, having the same staining properties to it. Similar host wall-like material was found around the monokaryotic haustorium of *P. podophylli* described by Borland and Mims (6), *P. poarum* (27), *P. coronata* (14) and *E. harknessii* (17). The host wall-like material observed here seems to be different in composition from that in dikaryotic infection of rust fungi.

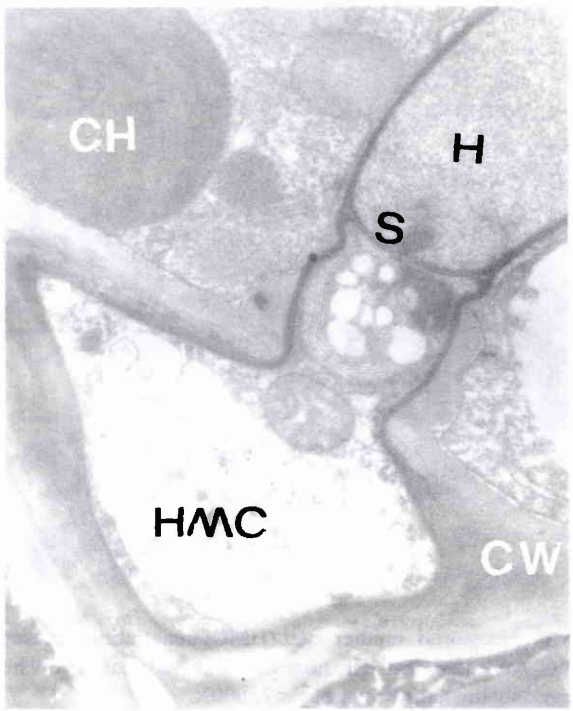

Figure 5. Monokayotic haustorium (H) of *G. clavariiforme* showing a perforate septum (S) proximal to the penetration site, crystal containing microbodies are closely associated with the pore apparatus. 12400X.

The EM had previously been suggested to be of host origin (7, 14, 16), fungal origin (10) or an artifact of preparation (4). Recent cytochemical studies on *Puccinia graminis* (8) and on *P. menthae* (Larous and Losel, unpublished) support the point of view that it is of host origin. The continuity of EHM with the host plasma membrane, commonly accepted for both mono and dikaryotic haustoria.

The extensive growth of the monokaryotic stages of *G. clavariiforme* in the intercellular spaces of the leaf mesophyll, accompanied by low frequency of penetration of cells in these tissues and the grater degree of invasion of the vascular system, correspond with the observation on the infection by *P. poarum* of *Tussulago farfara* (1). The above

observation, together with those of a series of ultrastructural studies by Harder (14) Al-Khesraji *et al.* (2), Baka and Losel (5) and Larous and Losel (19, 20), point to the conclusion that invasion of the vascular system is frequent in primary, or spermogonial-aecial stages of rust infection but absent in the secondary, uredinial-telial phases of infection. As in the systems described in the above studies, the cytoplasm in vascular tissue infected by *G. clavariiforme* remains of relatively healthy ultrastructural appearance. The seemingly greater tolerance to infection by cells in the vascular region than in the mesophyll may be a consequence of their continuing supply of nutrients and would be consistent with the view that the adverse effect of infection on mesophyll cells result mainly from nutrient depletion by the pathogen.

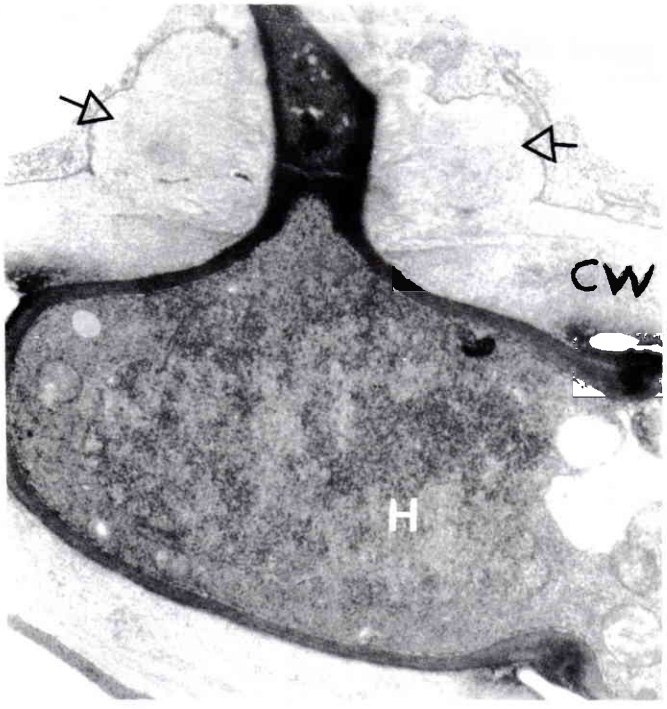

Figure 6. Haustorial mother cell (HMC) penetrating a mesophyll cell of *C. monogyna* leaf. The host wall-like material (arrow heads) is surrounding the haustorium neck. 25000X.

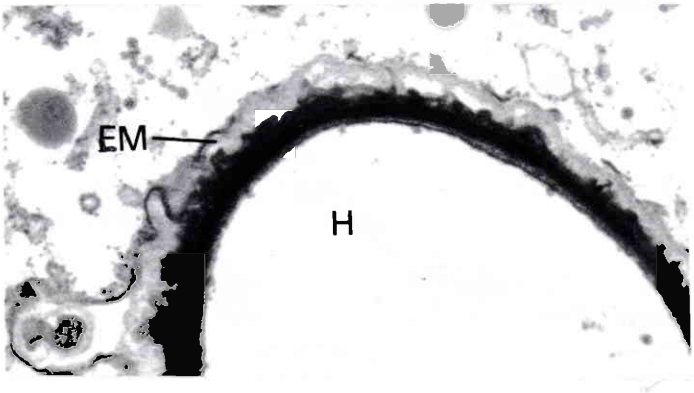

Figure 7. A part of a haustorium showing electron dense extra-haustorial matrix (EM) surrounding the haustorial body and is composed of two different layers. The inner layer is more electron dense than the outer, enclosed by the extra-haustorial membrane (EHM). 32500X.

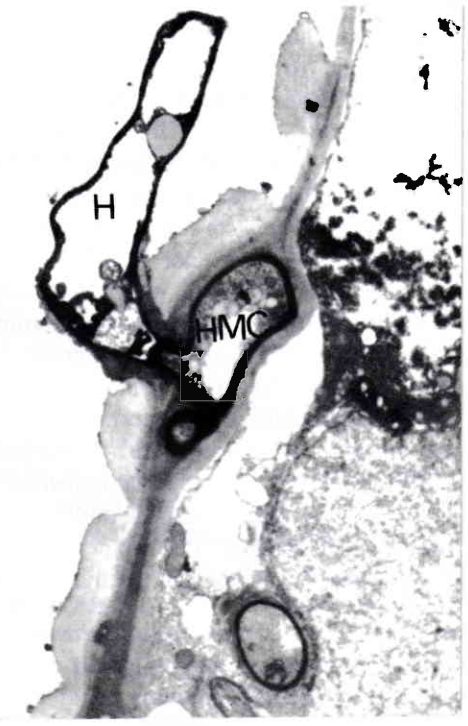

Figure 8. Necrotic, filamentous haustorium (H) of *G. clavariiforme* in xylem vessl (XV) of the *C. monogyna* leaf. The haustorium mother cell (HMC) is embedded in xylem vessel wall. 7500X.

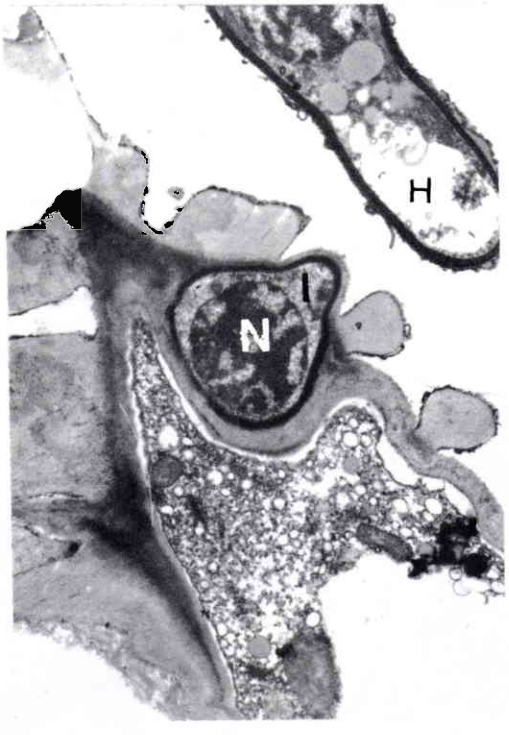

Figure 9. The spermogonial-aecial stages of *G. clavariiforme* infecting *C. monogyna* leaf vascular tissue showing a necrotic haustorium (H) in xylem vessel (XV) contains vesicular bodies, vacuoles and lipid droplets. Note also intercellular hyphae (I) embedded in xylem vessel wall with a large nucleus (N). 7500X.

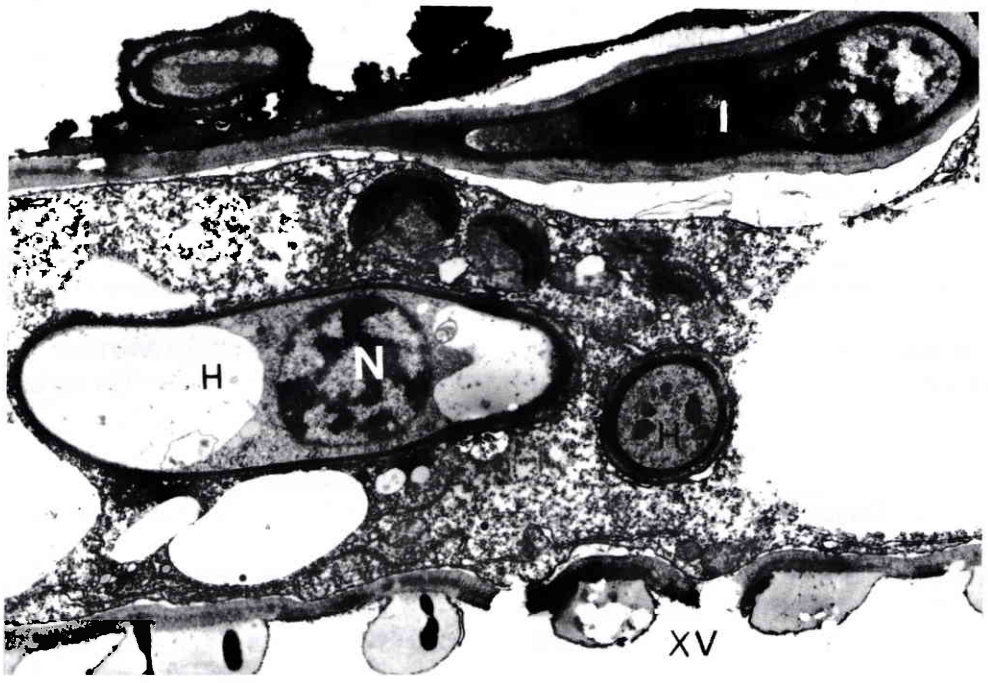

Figure 10. A haustorium of *G. clavariiforme* with extra-haustorial matrix bounded by the invaginated host plasma membrane, in xylem parenchyma cell (XP) adjacent to xylem vessel (XV) of *C. monogyna*. An intercellular hyphae (I) is embedded completely in host wall material. Note the organelle-rich cytoplasm of both host and fungal cells. 10000X.

الملخص

لعروس، العربي و د.م. لوزل. 1996. الإصابة الوعائية لنسج الزعرور البري *Crataegus monogyna* بالطور أحادي النواة لفطر الصدأ *Gymnosporangium clavariiforme*. مجلة وقاية النبات العربية. 14(1): 57-62.

تم التعرض في هذه الدراسة إلى البنية الدقيقة للطور أحادي النواة لفطر الصدأ *Gymnosporangium clavariiforme*، المتطفل على نبات الزعرور البري *Grataegus monogyna*، حيث تم وصف توزع التراكيب الفطرية ومدى تداخلها مع نسيج النبات العائل، مع التركيز على الإصابة الوعائية. وقد تبين بأن الطور أحادي النواة لهذا الفطر ينمو بغزارة في المسافات البينية لخلايا الميزوفيل، دون التوغل بداخلها بدرجة كبيرة، إلا أنه ذو قدرة كبيرة على إصابة جميع الأنواع الخلوية للحزمة الوعائية.

كلمات مفتاحية: *Gymnosporangium clavariiforme*، بُنية دقيقة، إصابة وعائية.

References

1. **Al-Khesraji, T.O. and D.M. Losel.** 1980. Intercellular structures of *Puccinia poarum* on its alternate hosts. Trans Br. Mycol. Soc., 75:397-411.
2. **Al-Khesraji, T.O., D.M. Losel and J. Gay.** 1980. The infection of vascular tissue in leaves of *Tussilago farfara* L. by the pycnial-aecial stages of *Puccinia poarum* Niel. Physiol. Mol. Plant Pathol. 17:193-197.
3. **Al-Khesraji, T.O. and D.M. Losel.** 1981. The fine structure of haustoria, intracellular hyphae and intercellular hyphae of *Puccinia poarum*. Physiol. Mol. Plant Pathol. 9:301-311.
4. **Allen, F.H.E., M.D. Coffey and M.C. Heath.** 1979. Plasmolysis of rusted flax: A fine structural study of host-pathogen interfaces. Can. J. Bot. 57:1528-1533.
5. **Baka, Z.A.M. and D.M. Losel.** 1992. Ultrastructure of the thistle rust, *Puccinia punctiformis*. Mycol. Res. 96:81-88.
6. **Borland, J. and C.W. Mims.** 1980. An ultrastructural comparison of the aecial and telial haustoria of the autoecious rust, *Puccinia podophylli*. Mycologia 72:767-774.
7. **Chong, J., D.E. Harder and R. Rohringer.** 1981. Ontogeny of mono and dikaryotic haustoria: Cytochemical and ultrastructural studies. Phytopathology 71:975-983.
8. **Chong, J., D.E. Harder and R. Rohringer.** 1986. Cytochemical studies on *Puccinia graminis* f. sp. *tritici* in a compatible wheat host. 1. Walls of intercellular hyphal cells and haustorium mother cells. Can. J. Bot. 63:1713-1724.
9. **Chong, J. and D.E. Harder.** 1982. Ultrastructure of haustorium development in *Puccinia coronata avenae*: some host responses. Phytopathology 72:1527-1533.

10. **Chou, C.K.** 1970. An electron microscope study of host penetration and early stages of haustorium formation of *Peronospora parasitica* on cabbage cotyledons. Ann. Bot. 34:189-204.
11. **Glidwell, D.C. and C.W. Mims.** 1979. Ultrastructure of haustorial apparatus in the rust fungus *Kunkelia nitens*. Bot. Gaz. 140:148-152.
12. **Gold, R.E. and K. Mendgen.** 1984. Cytology of basidiospore germination, penetration, and early colonization of *Phaseolus vulgaris* by *Uromyces appendiculatus* var *appendiculatus*. Can. J. Bot. 62:1989-2002.
13. **Gray, D.J., H.V. Amerson and C.G. Van Dyke.** 1982. An ultrastructural comparison of monokaryotic and dikaryotic haustoria formed by the fusiform rust fungus *Cronartium quercuum* f. sp. *fusiforme*. Can. J. Bot. 60:2914-2922.
14. **Harder, D.E.** 1978. Comparative ultrastructure of the haustoria in uredial and pycnial infections of *Puccinia coronata avenae*. Can. J. Bot. 56:214-224.
15. **Harder, D.E.** 1984. Developmental ultrastructure of hyphae and spores, pp. 333-373. In: The cereal rusts. Vol. 1. W.R. Bushnell and A.P. Roelfs (eds.). Academic Press, New York.
16. **Hopkin, A.A. and J. Reid.** 1988a. Cytological studies of the M-haustoria of *Endocronartium harknessii:* Morphology and ontogeny. Can. J. Bot. 66:974-988.
17. **Hopkin, A.A. and J. Reid.** 1988b. Host responses in susceptible hard pine tissue infected with *Endocronartium harknessii*. Can. J. Bot. 66:2511-2517.
18. **Kohno, M., H. Ishazaki and H. Kunoh.** 1976. Cytological studies on rust fungi: 4. Intracellular hyphae of *Gymnosporangium haraenum* in cells of Japanese pear leaves. Ann. Phytopathol. Soc. Japan, 42:417-423.
19. **Larous, L. and D.M. Losel.** 1993a. Vascular infection by *Puccinia menthae* and other rust fungi. Mycol. Res. 97:409-414.
20. **Larous, L. and D.M. Losel.** 1993b. Strategies of pathogenicity in monokaryotic and dikaryotic phases of rust fungi, with special reference to vascular infection. Mycol. Res. 97:415-420.
21. **Littlefield, L.J. and M.C. Heath.** 1979. Ultrastructure of rust fungi. Academic Press, New York.
22. **Longo, N. and B.N. Bruscaglioni.** 1986. Ultrastructural observations on the dikaryotic haustorium of *Cronartium flaccidum* in *Vincetoxicum medicus*. Caryologia 39:51-64.
23. **Reynolds, E.S. 1963.** The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. Journal of Cell Biology 17:208-212.
24. **Rijkenberg, F.H.J. and S.J. Truter.** 1973. Haustoria and intracellular hyphae in the rusts. Phytopathology 63:281-286.
25. **Walles, B. 1974.** Ultrastructure of the rust fungus *Peridermium pini*. Studie Forestalia Suecica 122:1-30.
26. **Woods, A.M. and J.L. Gay.** 1983. Evidence for a neckband delimiting structural and physiological regions of the host plasma membrane associated with haustoria of *Albugo candida*. Physiol. and Mol. Plant Pathol. 23:73-88.
27. **Woods, A.M. and J.L. Gay. 1987.** The interface between haustoria of *Puccinia poarum* (Monokaryon) and *Tussilago farfara*. Physiol. Mol. Plant Pathol. 30:167-185.