Vegetative development of *Uromyces appendiculatus* var. *appendiculatus* in *Phaseolus vulgaris*

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The morphology of intercellular and intracellular hyphae derived from basidiospores of *Uromyces appendiculatus* var. *appendiculatus* is described. Light and electron microscopic observations of the bean rust fungus were made on susceptible leaves of *Phaseolus vulgaris* from 3 to 10 days after inoculation. Following egress from invaded epidermal and palisade parenchyma cells, the fungus grew rapidly and developed extensive intercellular mycelium. An amorphous to fibrillar extracellular matrix was deposited between fungal and host cell walls. Intercellular hyphae grew closely appressed to the mesophyll cells and penetrated them to form intracellular hyphae from either a terminal or nonterminal mother cell. Intracellular hyphae were fingerlike, sometimes septate, and generally remained terminal in the invaded mesophyll cell. Occasionally the fungus exited the cell to become an intercellular hypha or an intracellular hypha in an adjacent host cell. The plant rarely exhibited a resistantlike reaction at sites of penetration into mesophyll cells.

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Les auteurs décrivent la morphologie des hyphes intercellulaires et intracellulaires développés à partir de basidiospores d'*Uromyces appendiculatus* var. *appendiculatus*. Des observations en microscopie photonique et électronique du champignon de la rouille du haricot ont été effectuées sur feuilles susceptibles de *Phaseolus vulgaris* de 3 à 10 jours après inoculation. Après sa sortie de cellules du parenchyme palisadique et de l'épiderme, le champignon croît rapidement et développe un mycélium intercellulaire extensif. Une matrice extracellulaire amorphe à fibrillaire se dépose entre les parois cellulaires du champignon et de l'hôte. Les hyphes intercellulaires poussent accolés aux cellules du mésophylle et les pénètrent pour former des hyphes intracellulaires à partir d'une cellule mère terminale ou non terminale. Les hyphes intracellulaires sont digitiformes, quelquefois septés et demeurent généralement terminaux dans la cellule de mésophylle envahie. Occasionnellement, le champignon sort de la cellule pour devenir un hyphe intercellulaire ou un hyphe intracellulaire dans une cellule hôte adjacente. La plante montre rarement une réaction de résistance aux points de pénétration dans les cellules du mésophylle.

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Introduction

Recent studies on basidiospore-derived rust infections have concentrated on the characterization of basidiospore germination, penetration, and host infection (see Littlefield and Heath 1979; Gold and Mendgen 1984; Harder and Chong 1984). Despite several studies on the morphology of intracellular structures derived from basidiospore infections, detailed descriptions of cell to cell and cell to intercellular space growth patterns have only begun to be provided (cf. Gray *et al.* 1983). We describe here the morphological development of intercellular and intracellular hyphae derived from basidiospores of the bean rust fungus, *Uromyces appendiculatus* (Pers.) Unger var. *appendiculatus*, in bean leaf tissue.

Materials and methods

Primary leaves of *Phaseolus vulgaris* L. were inoculated with basidiospores as described by Gold (1983) and Gold and Mendgen (1984). Infected material was collected and prepared for light and transmission electron microscopy (LM and TEM) as outlined in the preceding paper (Gold and Mendgen 1984). However, samples were fixed at later periods after inoculation. For LM, whole leaf samples were taken at 3, 4, 5, 7, and 10 days postinoculation (dpi) and for TEM, at 7 and 10 dpi.

Results

Microscopic observations were initiated at 3 dpi. Following fungal exit from the invaded epidermal and palisade parenchyma cells (cf. Gold and Mendgen 1984), extensive mycelial growth occurred in the intercellular spaces of the mesophyll

tissue (Fig. 1). The intercellular hyphae grew in close contact with palisade and spongy parenchyma cells and entered them at one or more sites to form fingerlike, sometimes septate, intracellular hyphae (Figs. 2–4,6,8,9). An amorphous to fibrillar layer of electron-opaque material (= extracellular matrix, Em) formed regularly between host cell walls and the intercellular hyphae (Figs. 7,10). The Em differed in electron opacity from the cell wall of the fungus, suggesting that they are not identical in composition. Intercellular hyphae were observed to penetrate into mesophyll cells in two different manners: via (i) a distinct terminal (Fig. 2) or (ii) a nonterminal (Figs. 3,4,8) mother cell. Often a distinct mother cell formed and was separated from the intercellular hypha by a septum (Figs. 2,8,9). Penetration into mesophyll cells by the fungus was accompanied by invagination of the host plasma membrane and often by collarlike wall appositions (Fig. 8, inset). The invaginated plasma membrane was designated the extrahyphal membrane (= Eh membrane).

The Eh membrane receded from the fungal cell wall to various degrees. At one extreme, which was typical for the proximal portion of the intracellular hypha, the membrane was so closely appressed to the wall that the extrahyphal matrix (= Eh matrix) was not visible (Figs. 8,9). At the other extreme, the membrane receded up to 0.5 μm from the wall (Figs. 11–13). In such cases, the Eh matrix region could be seen in semithin sections (1–2 μm) with the light microscope. The Eh membrane often appeared undulated, having regions receded from the fungal wall alternating with regions in close contact (Fig. 13). The electron opacity of the Eh matrix was indirectly related to the degree to which the Eh membrane was receded from the fungal wall. The highly receded regions appeared

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ABBREVIATIONS USED: Ap, appressorium; c, collar; *ch*, chloroplast; Cw, host cell wall; E, epidermal cell; Eh, extrahyphal membrane; Ehm, extrahyphal matrix; Em, extracellular matrix; Fpm, fungal plasma membrane; G, germ tube; Gb, Golgi body; H, intracellular hypha; Hc, host cytoplasm; Mc, mother cell; Hn, host nucleus; *hnu*, host nucleolus; Hv, host vacuole; Hw, hyphal wall; Ih, intercellular hypha; Is, intercellular space; L, lipid body; *m*, mitochondrion; N, fungal nucleus; Pc, palisade parenchyma cell; Pm, host plasma membrane; S, septum; Sc, spongy parenchyma cell; T, tonoplast; V, fungal vacuole.

FIGS. 1–6. Light microscopy (LM) (differential interference contrast) of basidiospore-derived infection in fixed and cleared whole leaf samples. Fig. 1. (4 dpi) Following growth from epidermal and palisade cells into the intercellular space, extensive mycelium developed in the mesophyll. $\times 680$. Fig. 2. (4 dpi) Terminal, fingerlike intracellular hypha. Note terminal mother cell (arrow). $\times 1180$. Fig. 3. (4 dpi) The fingerlike intracellular hypha arose from a nonterminal mother cell. $\times 1100$. Fig. 4. (4 dpi) Intercellular hypha with two intracellular hyphae that formed as side branches. $\times 1070$. Fig. 5. (4 dpi) Transcellular penetration site (arrow) showing an intracellular hypha between two palisade cells. $\times 1590$. Fig. 6. (4 dpi) Intracellular hypha with septum. $\times 1520$.

electron lucent with little or no visible substructure (Fig. 13). On the other hand, less receded areas appeared increasingly electron opaque with an amorphous or granular substructure (Figs. 12,13).

Intracellular hyphae had a full complement of organelles and structures as described for basidiospores (cf. Gold and Mendgen 1984). However, no microbodies were observed in the present study. Intracellular hyphae were often closely associated with the host cell nucleus in palisade and spongy parenchyma cells (Figs. 11,14). Once in a spongy mesophyll cell, the intracellular hypha then commonly remained terminal in the invaded cell (Figs. 2–4,9). However, the fungus occasionally grew into an adjacent host cell (Figs. 5,14,15) or grew out of the cell to become an intercellular hypha (Fig. 16).

Transcellular penetration and cell exit were each observed in TEM. In the first instance, an intracellular hypha was seen in two intact neighboring palisade cells (Fig. 14). The intracellular hypha was seen in serial sections to have penetrated two contiguous host cell walls and grown into the next cell. In response to penetration, the host cell wall became locally thickened and laid down some collar material (Figs. 14,15). An apparent membrane connection between the two host cells was

observed in the penetration pore (Fig. 15). On both sides of the penetrated wall, numerous ribosomes, endoplasmic reticulum, and membrane convolutions were present (Fig. 15). The second instance involved the growth of an intracellular hypha from a vacuolated, but still intact, parenchyma cell into the intercellular space (Fig. 16). In contrast to the above, this exit site appeared to be primarily a result of mechanical forces. This is evidenced by the fact that (i) the ends of host cell wall fibrils appeared jagged and were displaced outward and (ii) a portion of the host cell appeared to have been "dragged" out of the cell and deposited extracellularly.

Golgi bodies were often observed in the host cytoplasm along the Eh membrane in close proximity to penetration and exit sites of host cells (Fig. 8).

On rare occasions the plant exhibited a resistantlike reaction at penetration sites into the epidermis (Fig. 17) or mesophyll cells (Fig. 18). In the former case, the epidermal wall and sometimes a portion of the cytoplasm became extremely electron opaque. The contents of the germ tube and basidiospore appeared disorganized. In mesophyll cells, necrotic intracellular hyphae were observed in vacuolated but still intact cells (Fig. 18). An electron-opaque layer of presumably host

FIGS. 7–9. Transmission electron microscopy (TEM) of intercellular and intracellular fungal development in susceptible bean from 7 to 10 dpi. Fig. 7. Intercellular hypha in cross section showing the extracellular matrix between the hypha and host cell wall. A narrow strip (arrows) of matrix material also extends around the intercellular hypha. $\times 20\,100$. Fig. 8. Branched intercellular hypha in longitudinal section. The left branch is differentiated into a terminal mother cell (note septum, arrows) that penetrated the mesophyll cell and formed a fingerlike intracellular hypha. The right branch has also formed a septum but continued growth intercellularly. A relatively long collar of host wall-like material is present at the penetration site. $\times 10\,000$. Inset. Higher magnification of the site of penetration showing invaginated host plasma membrane (arrow). $\times 20\,000$. Fig. 9. A median section through the penetration site of a mother cell into a mesophyll cell. An extrahyphal matrix is not visible around the intracellular hypha seen in longitudinal section. Loose patches of glycogenlike particles are seen in the intercellular hyphae (arrows). $\times 9150$.

FIGS. 10–13. TEM of intercellular and intracellular fungal development in susceptible bean from 7 to 10 dpi. Fig. 10. Penetration site cut at an oblique angle through the intercellular hyphae, mother cell, and intracellular hyphae. Note extracellular matrix between the walls of host and fungus. $\times 18\,900$. Fig. 11. Cross section of intracellular hyphae in mesophyll cell with (H_1) and without (H_2) a broad, electron-lucent extrahyphal matrix. Observe lobes of the host nucleus around H_2 . $\times 4550$. Fig. 12. Extrahyphal matrix shown at a distal position along the hyphal wall where the extrahyphal membrane receded from the fungal wall. $\times 36\,750$. Fig. 13. Undulated extrahyphal membrane with regions receded from the hyphal wall alternating with regions in close contact (arrows; note denser extrahyphal matrix at those positions). $\times 61\,450$.

material was deposited at the periphery of the disorganized intracellular hyphae.

Macroscopically, no symptoms were visible during the initial 3–4 dpi. After 4–5 days, the first small chlorotic spots (0.5–1.0 mm diameter) appeared. Beginning at ca. 7 dpi, the developing pycnia began to rupture the epidermis. At 8–10 dpi the entire leaf, from upper to lower epidermis, had been colonized by the fungal mycelium. The majority of pycnia were mature and actively producing pycniospores (Fig. 19) at 10 dpi. The pycniospores were deposited in a “honey-dew” exudate at the apex of the pycnia. Pycniospores were commonly ovate to elliptical, smooth walled, hyaline, and measured ca. $6 \times 4 \mu\text{m}$, ranging from 4.8 to 7.6 μm long and from 3.4 to 5.0 μm wide (Fig. 20).

Discussion

Reference has often been made in ultrastructural studies to an amorphous, electron-opaque deposit (= extracellular matrix,

Em) between the walls of host and fungus (Longo and Naldini Longo 1975; Harder 1978; Littlefield and Heath 1979; Borland and Mims 1980; Pring 1980; Al-Khesraji and Lösel 1981; Khan *et al.* 1982; Longo *et al.* 1982; Gray *et al.* 1983). In the present study an Em was commonly observed at points of contact between the intercellular hyphae or mother cells and the host cell wall. The origin and possible function(s) of this material are uncertain. Many authors have assumed that the Em is of fungal origin (Littlefield and Heath 1979) and aids in the attachment of the mother cell to the host wall (Mendgen 1978; Harder and Chong 1984). Al-Khesraji and Lösel (1981) suggested that the Em is more commonly associated with mycelia derived from basidiospores than urediniospores. However, as evidenced by previous ultrastructural studies (Hardwick *et al.* 1971; Mendgen 1979; Pring 1980) and results presented here, this does not appear to hold for bean rust infections.

The general morphology of intracellular structures derived from basidiospores of the bean rust fungus is similar to that

FIGS. 14–16. TEM of intercellular and intracellular fungal development in susceptible bean from 7 to 10 dpi. Fig. 14. Low magnification of an intracellular hypha traversing the cell walls of two adjacent mesophyll cells (Sc_1 , Sc_2). In other sections the tip of the intracellular hypha was seen at the point marked with a star (*) in Sc_2 . Note the prominent host nucleus and intact appearance of the newly invaded cell (Sc_2). $\times 6750$. Fig. 15. Enlargement of different section of the penetration site shown in Fig. 14. A partially electron-opaque collar of host wall-like material has formed in response to transcellular penetration. Note the membraneous strands associated with the penetration site (arrows). $\times 33\,500$. Fig. 16. Growth of an intracellular hypha into the intercellular space. The mechanism of cell exit appears in this case to be primarily mechanical. Note the jagged ends (arrows) of the outwardly displaced host cell wall and the “dragged” out portions of the host cytoplasm (Hc'). $\times 16\,650$.

FIGS. 17 and 18. TEM of resistantlike host reactions to basidiospore-derived penetration and infection. Fig. 17. (24 hpi) A completely disorganized basidiospore germ tube and appressorium containing highly electron-opaque deposits. The outer host cell wall and a portion of the anticlinal wall are impregnated with a similarly staining electron-opaque material. Note penetration peg (arrow) embedded in the necrotic cell wall. $\times 7550$. Fig. 18. Necrotic intracellular hypha in an apparently intact mesophyll cell. Note layer of electron-opaque material (arrows) bordering the entire intracellular hypha. Also note the disorganized mother cell and necrotic intercellular hypha. $\times 9850$.

FIGS. 19 and 20. LM of sexual structures of the bean rust fungus (ca. 10 dpi). Fig. 19. Mature pycnium. $\times 395$. Fig. 20. Pycniospores. The structure shown at arrow may represent a dislodged pycniosporophore, an abnormal or possibly a germinated pycniospore. $\times 1215$.

reported for other rust fungi at the light (Allen 1930, 1935; Nusbaum 1935) and ultrastructural level (see Littlefield and Heath 1979; Harder and Chong 1984). However, an additional characteristic of the bean rust fungus is the formation of intracellular hyphae that may grow transcellularly or exit the invaded cell. This growth characteristic has been demonstrated in other rust species from temperate regions (Colley 1918; Allen 1930, 1935; Nusbaum 1935; Pady 1935; Gold *et al.* 1979; Littlefield and Heath 1979; Metzler 1980; Longo and Naldini Longo 1982; Gray *et al.* 1983; Gold and Mendgen 1984), but additional research is required to learn more about their occurrence and functional significance. Also similar among intracellular structures derived from basidiospores of several rust fungi is the septum at, or slightly distal to, the point of penetration (Kohn *et al.* 1977; Glidewell and Mims 1979; Littlefield and Heath 1979; Borland and Mims 1980; Metzler 1980; Gray *et al.* 1982, 1983; Longo *et al.* 1982) or just outside the limits of the host cell wall as shown here and by others (Harder 1978; Littlefield and Heath 1979).

Cytochemical (Littlefield and Heath 1979; Chong *et al.* 1981) and ultrastructural observations from freeze-substituted materials (D. E. Harder and K. Mendgen, unpublished results) leave little doubt of the physical and chemical existence of the extrahyphal matrix (Eh matrix) and extrahaustorial matrix. As in *Uromyces vignae* Barcl., *Melampsora lini* (Pers.) Lév. (Littlefield and Heath 1979), and *Puccinia poarum* Niels. (Al-Khesraji *et al.* 1980), the matrix around intracellular hyphae of the bean rust fungus was heterogeneous, containing translucent and opaque components. Although the Eh matrix is generally accepted to be real and to contain stainable material (e.g., polysaccharides and protein (Chong *et al.* 1981)), the undulated appearance of the extrahyphal membrane (Eh membrane) is likely an artifact of fixation (Allen *et al.* 1979; D. E. Harder and K. Mendgen, unpublished results). The extreme distention of the Eh membrane and frayed appearance of the electron-opaque material of the Eh matrix seen in the present work as well as in numerous other studies (e.g., Fig. 68, p. 174, in Littlefield and Heath 1979; Fig. 5A in Al-Khesraji *et al.* 1980) support the contention made by Allen *et al.* (1979) that the intracellular structure shrinks from the Eh membrane during fixation.

Walles (1974) reported that a small percentage of intracellular hyphae become necrotic in intact susceptible pine cells invaded by *Peridermium pini* (Pers.) Lév., presumably as a result of "point incompatibility." Similar observations were made in bean rust infections. However, necrotic intracellular hyphae were generally encased by an electron-opaque material and fungal disorganization sometimes extended into the intercellular hypha.

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AL-KHESRAJI, T. O., and D. M. LÖSEL. 1981. The fine structure of haustoria, intracellular hyphae and intercellular hyphae of *Puccinia poarum*. *Physiol. Plant Pathol.* **19**: 301–311.

AL-KHESRAJI, T. O., D. M. LÖSEL, and J. L. GAY. 1980. The infection of vascular tissue in leaves of *Tusilago farfara* L. by

pycnial-aecial stages of *Puccinia poarum* Niel. *Physiol. Plant Pathol.* **17**: 193–197.

ALLEN, R. F. 1930. A cytological study of heterothallism in *Puccinia graminis*. *J. Agric. Res.* **40**: 585–614.

———. 1935. A cytological study of *Puccinia malvacearum* from the sporidium to the teliospore. *J. Agric. Res.* **51**: 801–818.

ALLEN, F. H. E., M. D. COFFEY, and M. C. HEATH. 1979. Plasmolysis of rusted flax: a fine structural study of the host-parasite interface. *Can. J. Bot.* **57**: 1528–1533.

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.

CHONG, J., D. E. HARDER, and R. ROHRINGER. 1981. Ontogeny of mono- and dikaryotic rust haustoria: cytochemical and ultrastructural studies. *Phytopathology*, **71**: 975–983.

COLLEY, R. H. 1918. Parasitism, morphology, and cytology of *Cronartium ribicola*. *J. Agric. Res.* **15**: 619–660.

GLIDEWELL, D. C., and C. W. MIMS. 1979. Ultrastructure of the haustorial apparatus in the rust fungus *Kunklelia nitens*. *Bot. Gaz. (Chicago)*, **140**: 148–152.

GOLD, R. E. 1983. Activation and pattern of teliospore germination in *Uromyces appendiculatus* var. *appendiculatus* and basidiospore infection of *Phaseolus vulgaris*. Doctoral dissertation; Universität Konstanz, Konstanz, Federal Republic of Germany.

GOLD, R. E., L. J. LITTLEFIELD, and G. D. STATLER. 1979. Ultrastructure of the pycnial and aecial stages of *Puccinia recondita*. *Can. J. Bot.* **57**: 74–86.

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**: This issue.

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.

———. 1983. Ultrastructure of the infection and early colonization of *Pinus taeda* by *Cronartium quercuum* formae speciales *fusiforme*. *Mycologia*, **75**: 117–130.

HARDER, D. E. 1978. Comparative ultrastructure of the haustoria in uredial and pycnial infections of *Puccinia coronata avenae*. *Can. J. Bot.* **56**: 214–224.

HARDER, D. E., and J. CHONG. 1984. Structure and physiology of haustoria. In *The cereal rusts*. Vol. 1. Edited by W. R. Bushnell and A. P. Roelfs. Academic Press, New York. pp. 431–476.

HARDWICK, N. V., A. D. GREENWOOD, and R. K. S. WOOD. 1971. The fine structure of *Uromyces appendiculatus* in *Phaseolus vulgaris*. *Can. J. Bot.* **49**: 383–390.

KHAN, S. R., J. W. KIMBROUGH, and P. G. WEBB. 1982. The fine structure of septa and haustoria of *Cronartium quercuum* formae speciales *fusiforme* on *Quercus rubra*. *Mycologia*, **74**: 809–819.

KOHNO, M., H. ISHIZAKI, and H. KUNOH. 1977. Cytological studies on rust fungi. VI. Fine structure of infection process of *Kuehneola japonica* (Diet.) Dietel. *Mycopathologia*, **61**: 35–41.

LITTLEFIELD, L. J., and M. C. HEATH. 1979. Ultrastructure of rust fungi. Academic Press, New York.

LONGO, N., and B. NALDINI LONGO. 1975. Observations on the fine structure of the haustorium of *Melampsora pinitorqua* (A. Br.) Rostr. in cells of *Pinus pinea* L. *Caryologia*, **28**: 389–405.

———. 1982. Behaviour of the monokaryotic haustorium of *Melampsora pinitorqua* (A. Br.) Rostr. Preliminary note. *Caryologia*, **35**: 471–476.

LONGO, N., F. MORIONDO, and B. NALDINI LONGO. 1982. Ultrastructural observations on the host-pathogen interface in infections of *Cronartium flaccidum* on pine. *Caryologia*, **35**: 307–326.

MENDGEN, K. 1978. Attachment of bean rust cell wall material to host and non-host plant tissue. *Arch. Microbiol.* **119**: 113–117.

———. 1979. Microautoradiographic studies on host-parasite interactions. II. The exchange of ³H-lysine between *Uromyces phaseoli* and *Phaseolus vulgaris*. *Arch. Microbiol.* **123**: 129–135.

- METZLER, B. 1980. Ontogenie von *Gymnosporangium fuscum* DC. (Uredinales) in der Haplophase und damit verbundene Wirt-Parasit-Beziehungen. Diplomarbeit, Universität Tübingen, Tübingen, Federal Republic of Germany.
- NUSBAUM, C. J. 1935. A cytological study of the resistance of apple varieties to *Gymnosporangium juniperi-virginianae*. J. Agric. Res. **51**: 573–596.
- PADY, S. M. 1935. The role of intracellular mycelium in systemic infections of *Rubus* with the orange-rust. Mycologia, **27**: 618–637.
- PRING, R. J. 1980. A fine structural study of the infection of leaves of *Phaseolus vulgaris* by uredospores of *Uromyces phaseoli*. Physiol. Plant Pathol. **17**: 269–276.
- WALLES, B. 1974. Ultrastructure of the rust fungus *Peridermium pini* (Pers.) Lév. Stud. For. Suec. **122**: 1–30.