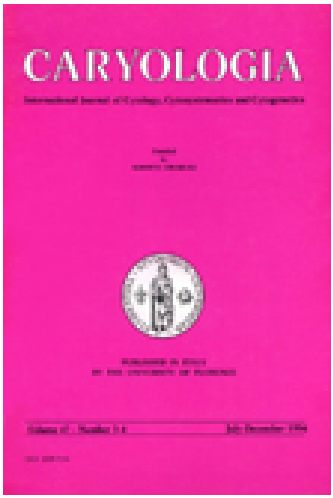

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Penetration and early colonization in basidiospore-derived infection of *Melampsora pulcherrima* (Bub.) Maire on *Mercurialis annua* L.

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SUMMARY — The early phases of basidiospore-derived infection of *Melampsora pulcherrima* (Bub.) Maire on the leaves of *Mercurialis annua* L. were studied by light microscopy, SEM and TEM. The fine morphology of the basidiospore germling penetration and intraepidermal infection structures is discussed in comparison with that of other rusts recently described. The direct penetration through the epidermal cell wall, characteristic of the rust basidiospore-derived germlings, is confirmed. The absence of an extrahyphal matrix around the intraepidermal vesicle and the presence of a collar around the vesicle neck are pointed out.

INTRODUCTION

Melampsora pulcherrima (Bub.) Maire, the «white poplar Mediterranean rust» agent, forms pycnia and aecia on *Mercurialis annua* L. previously infected by the basidiospores produced by teliospores on the leaves of *Populus alba* L. (MAGNANI 1961; MORIONDO *et al.* 1989).

Some epidemiological experiments (NALDINI *et al.* 1993) on the development of the monokaryotic stage of the rust showed that the infection process of *M. pulcherrima* which breaks out later especially on branches and stems, begins through the leaves of the host. However, the way of leaf penetration has never been investigated.

The structural and ultrastructural study of the early phases of infection of *M. pulcherrima* on the leaves of *M. annua* has the following purposes: 1) to help elucidate the still debated problem concerning the rust basidiospore-derived penetration pattern be it direct (through the epidermal cell walls) or indirect (through stomata), in relation to the characteristics of the host tegumental tissues and the presence of stomata on the organ involved in basidiospore germling penetration; 2) to observe the penetration and early colonization of the monokaryotic infection process (intraepidermal structures and thence der-

ived intracellular productions) of *M. pulcherrima* in comparison with the monokaryotic analogous structures of other rusts of which some reports have been published to-date (GOLD and MENDGEN 1984*a*, 1984*b*; GRAY *et al.* 1983; GOLD and MENDGEN 1991; MORIN *et al.* 1992).

MATERIALS AND METHODS

Plant material and inoculation procedures. — Apical shoot leaves of *Mercurialis annua* young plants were inoculated in Petri dishes, either on the upper or lower surface of the blade, with basidiospores falling from germinating teliospores on *Populus alba* leaves adhering on the underside of the dish cover. Inoculations were carried out in a climatic chamber at 15°C with 10,000 lux for 12 hours per day. Small fragments of inoculated leaves were collected 48 hours after inoculation and processed for light and electron microscopy.

Light microscopy. — Collected material was processed according to a PATTON and SPEAR «whole mounts» schedule (1989), modified as follows: clearing with saturated chloral hydrate solution at room temperature for 5 days, staining with lactophenol containing 0.25% aniline blue and 0.25% trypan blue at room temperature for 20hrs, rinsing and mounting with lactophenol. «Whole mounts» were observed and photographed using a Zeiss photomicroscope.

SEM. — The specimens were fixed in 1% OsO_4 in 0.1M phosphate buffer pH 7.2 for 20 hrs at 4°C, dehydrated with a graduated series of acetone, critical point dried; then a few of them were fractured, all were gold sputter coated and observed using a Philips SEM 505.

TEM. — The specimens were fixed in 2.5% glutaraldehyde and 4% paraformaldehyde in 0.1M phosphate buffer, pH 7.2 for 2hrs. During fixation the specimens were observed by light microscopy as «whole mounts» to select the areas showing germinating basidiospores on their epidermal surfaces. Selected fragments were postfixed in 2% OsO_4 in the same buffer for 2 hrs., dehydrated with the ethanol series from 10% to 70% alcool in water, infiltrated in Glicol-Spurr (MORI and TANI 1988) and embedded in Spurr (SPURR 1969). Serial sections were cut with a Reichert OM U3, stained with uranil acetate and lead citrate and observed with a Philips EM 300.

Key to labelling of Figures. — A = Appressorium, AW = Appressorial Wall, B = Basidiospore, C = Collar, E = Extracellular Material, EC = Epidermal Cell, EC-W = Epidermal Cell Wall, EM = Extrahyphal Membrane, EMa = Extrahyphal Matrix, ER = Endoplasmic Reticulum, G = Glycogen, GT = Germ Tube, H = Haustorium, HP = Host Plasma membrane, HW = Host Wall, IH = Infection Hypha, IHW = Infection Hypha Wall, L = Lipid, M = Mitochondrion, N = Vesicle Neck, Nu = Nucleus, NW = Vesicle NeckWall, PC = Palisade Cell, PH = Penetration Hollow, PP = Penetration Peg, PPW = Penetration Peg Wall, V = Vesicle, Va = Vacuole, VW = Vesicle Wall, W = Germ Tube Wall.

RESULTS

Behaviour of basidiospore germlings and intracellular infection structures.

The germ tube produced by basidiospores on the upper and lower epidermis of *Mercurialis annua* leaf blade generally grows no more than 5-6 μm but sometimes it reaches nearly 20 μm, reaching (with the same probability) any point of an epidermal cell periclinal wall or the anticlinal walls of two contiguous cells. In any case the penetration occurs directly through the epidermal cell wall (Figs. 1, 2, 3, 4, 18, 19, 20, 21). The same direct penetration occurs through the walls of stomatal subsidiary and, sometimes, guard cells (Figs. 1, 21).

The completely expanded intracellular infection structure shows proximally a roundish vesicle connected to the epidermal cell wall by a thin neck and distally a hypha like portion, infection hypha, which is formed by two parts, one wider the other thinner and longer. Septa are present between the two parts of the infection hypha (Figs. 1, 2, 3, 4, 26, 27). The infection structure grows widely within the penetrated epidermal cell. In many cases, it runs along the cell wall and then comes back toward the penetration point after having made a round angle (Figs. 5, 6, 26, 27).

The fully developed infection structure generally forms some branches originating in succession from the distal part of vesicle near the septum (Figs. 2, 3, 4), then from the wider part, and finally from the thinner terminal one of the infection hypha (Figs. 6, 7). The branches extend toward the anticlinal cell wall transcellularly growing into contiguous epidermal cells (Figs. 5, 6, 7), or to the inner periclinal wall of the penetrated epidermal cell from which they then grow outward. Particularly, the transcellular growths forming terminal intracellular structures (haustoria) into contiguous epidermal cells appear in a larger number in lower leaf epidermis level infections. These epidermal transcellular growths are followed, as occurrence and in time, by the exiting of the infection hyphae through the periclinal walls of the epidermal cells, which form intercellular hyphae in spongy parenchyma (Figs. 9, 22).

These hyphae then produce haustoria in the spongy cells (Figs. 8, 9, 10, 11, 12, 13). The transcellular growths into contiguous epidermal cells appear in smaller numbers in upper leaf epidermis level infections, where numerous transcellular growths from the epidermal cells which form haustoria in palisade cells were observed (Figs. 6, 14, 15, 16, 17). The still young haustoria produced either in the epidermal or in the spongy and palisade parenchyma cells, even if they have a relative limited development in the host cells, show a similar shape to that of the infection hypha, with a wider proximal and a quite distinct thinner distal part. Such intracellular structures were not observed coming out of the host cells.

Ultrastructural aspects.

During germination and early stages of germ tube penetration, basidiospores show dense cytoplasm, a few small vacuoles, many small lipid bodies, numerous globoid mitochondria placed for the most part near the basidiospore wall, endoplasmic reticulum, glycogen particles, and nucleus with chromatin patches (Fig. 29). The basidiospore germ tube wall appears continuous with the thin homogenously electron transparent basidiospore wall. It becomes progressively thicker and more electron dense reaching twice the thickness of the basidiospore wall, showing three layers of which the middle is an electron trasparent one (Fig. 30). An appressorium is formed by the end of the basidiospore germ tube: it appears as a slightly swollen undifferentiated terminal portion of the germ tube (Figs. 19, 20, 21, 23).

An extracellular material, fibrouslike in SEM (Figs. 20, 23), amorphous and electron transparent (Fig. 31) is present around the base of the appressorium at the hollow containing the penetration pore. This material also extends along the germ tube wall which adheres to the epidermal cell walls; sometimes it envelops the whole appressorium and germ tube (Figs. 23, 29).

The appressorial wall, which is everywhere as thick as the germ tube wall, is notably thinner where it adheres to the epidermal cell wall around the

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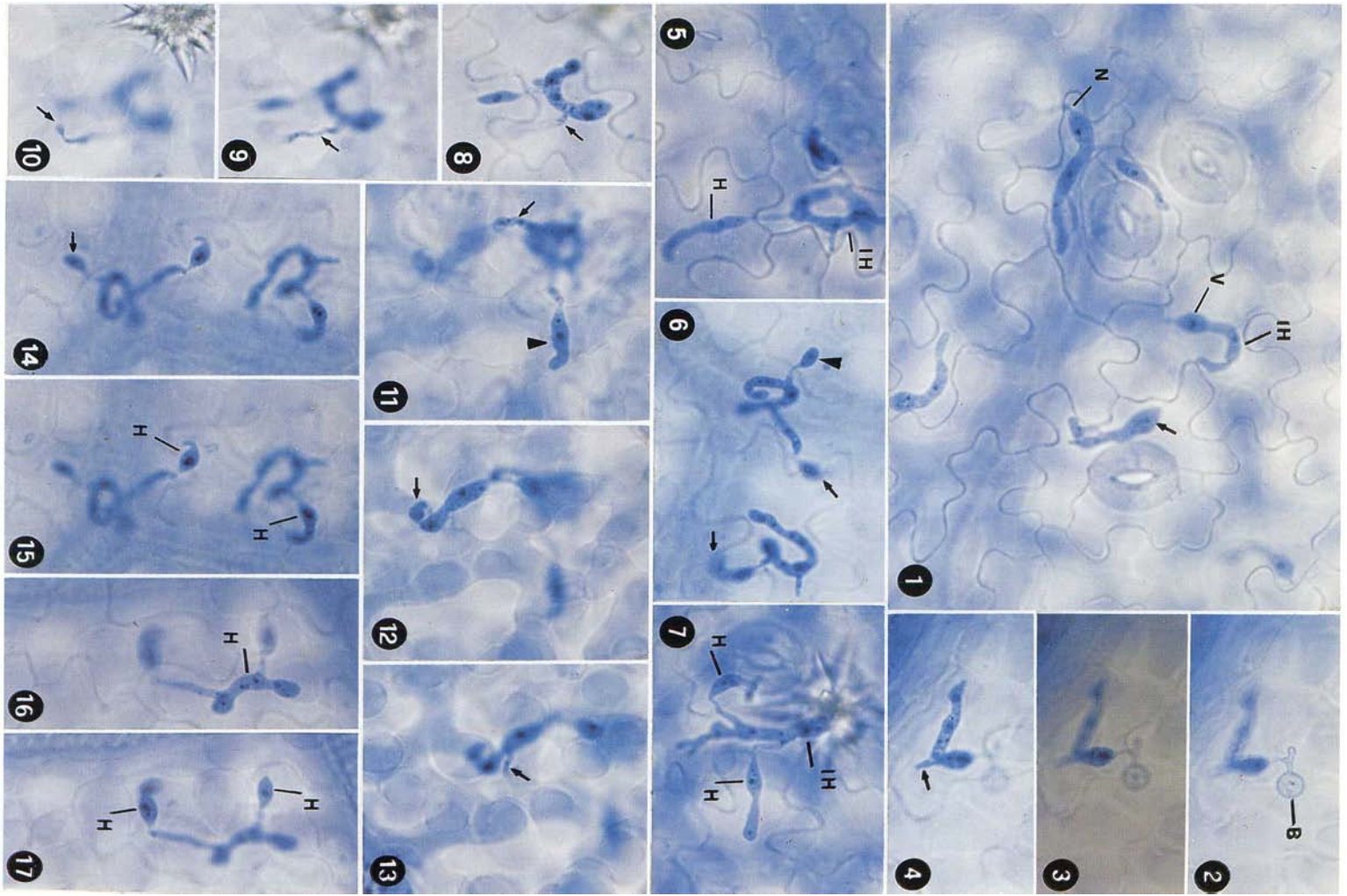
Figs. 1-17. — Light microscopy of *Mercurialis annua* leaves inoculated with basidiospores of *Melampsora pulcherrima*. Whole mounts stained with aniline blue and trypan blue in lactophenol. × 480.
Fig. 1. — Young intracellular infection structures from direct penetration of basidiospore germlings into cells of lower epidermis, one of which penetrated into a stomatal subsidiary cell (arrow). Note their thin neck, roundish vesicle and developing infection hypha.
Figs. 2-4. — Germinated basidiospore and derived infection structure in the upper epidermis in images of three focal planes. Note the beginning of the first branch from the distal part of the vesicle (arrow in Fig. 4).
Fig. 5. — A more developed infection structure in the lower epidermis with its hypha which runs along the cell wall and then comes back toward the penetration point after having made a complete angle. Note the branch transcellularly grown with haustorium in the contiguous epidermal cell.
Fig. 6. — Two infection structures in the upper epidermis running as in Fig. 5. Note in both the first branch with haustorium in the palisade cell (arrows); the structure on the left shows the second branch with an incipient haustorium (arrow-head) in the contiguous epidermal cell.
Fig. 7. — A fully developed infection structure in the lower epidermis with branches formed in succession and two haustoria in the contiguous epidermal cells.
Figs. 8-10. — A fully developed infection structure in the lower epidermis in images of three focal planes. The first branch (arrow in Fig. 8) produced an intercellular hypha (arrow in Fig. 9) and an incipient haustorium (arrow in Fig. 10) in a spongy parenchyma cell.
Figs. 11-13. — A subsequent development of early colonization from an infection structure in the lower epidermis in images of three focal planes. Note: an haustorium (arrow-head in Fig. 11) and an intercellular hypha (arrow in Fig. 11) in the spongy parenchyma; an incipient haustorium (arrow in Fig. 12) in a palisade cell; an intercellular hypha (arrow in Fig. 13) in the palisade parenchyma.
Figs. 14-17. — Early colonization and formation of haustoria from three infection structures in the upper epidermis in images of different focal planes. Note an incipient haustorium in the contiguous epidermal cell (arrow in Fig. 14) and four differently developed haustoria in palisade cells.



penetration pore (Fig. 31). The cytoplasm of the appressorium (when the penetration has occurred and the intracellular infection structure is growing) shows many mitochondria near the appressorial wall, large lipid droplets, and patches of glycogen (Fig. 31).

The diameter of the penetration peg produced by the appressorium (Fig. 24) is ca. 1-1,5 μm outside the epidermal cell wall. In the wall region, where the peg produces a clearly outlined pore, appears notably smaller (0.5 μm) and relatively uniform. The peg wall is not continuous with the wall of the appressorium, but originates «de novo» slightly within the appressorium (Fig. 31). The epidermal cell wall appears more electron dense around the penetration pore, often at the extension of the extracellular adhesive material (Fig. 31). The intraepidermal infection structure originates from the penetration peg.

The developing intraepidermal structure (young vesicle), before the first septum forms (Figs. 25, 34), shows a bilayered wall with an outer electron-dense layer and an inner electron-transparent one, a small vacuole, abundant cytoplasm with lipid droplets adjoining tonoplast and mitochondria near the cell wall, and a nucleus behind the vacuole at which level the infection hypha will grow (Fig. 34). The extrahyphal membrane surrounding the developing vesicle adheres to the fungal wall without an interposed extrahyphal matrix (Fig. 35).

The mature intraepidermal vesicle has a very thin neck (ca. 1 μm in diameter) which extends in length for ca. 1.5-2 μm within the host epidermal cell; the neck cytoplasm is poor and the neck wall is much thicker than that of the penetration peg or the vesicle (Fig. 26).

The roundish vesicle is characterized by the presence of a large vacuole and a thin layer of cytoplasm containing lipid droplets, mitochondria and glycogen (Figs. 26, 28).

The wall of the mature vesicle and its relationship with the host plasma membrane (absence of an extrahyphal matrix) (Fig. 36) are similar to those described for the young vesicle.

Figs. 18-25. — Scanning electron microscopy of *Mercurialis annua* leaves inoculated with basidiospores of *Melampsora pulcherrima*.

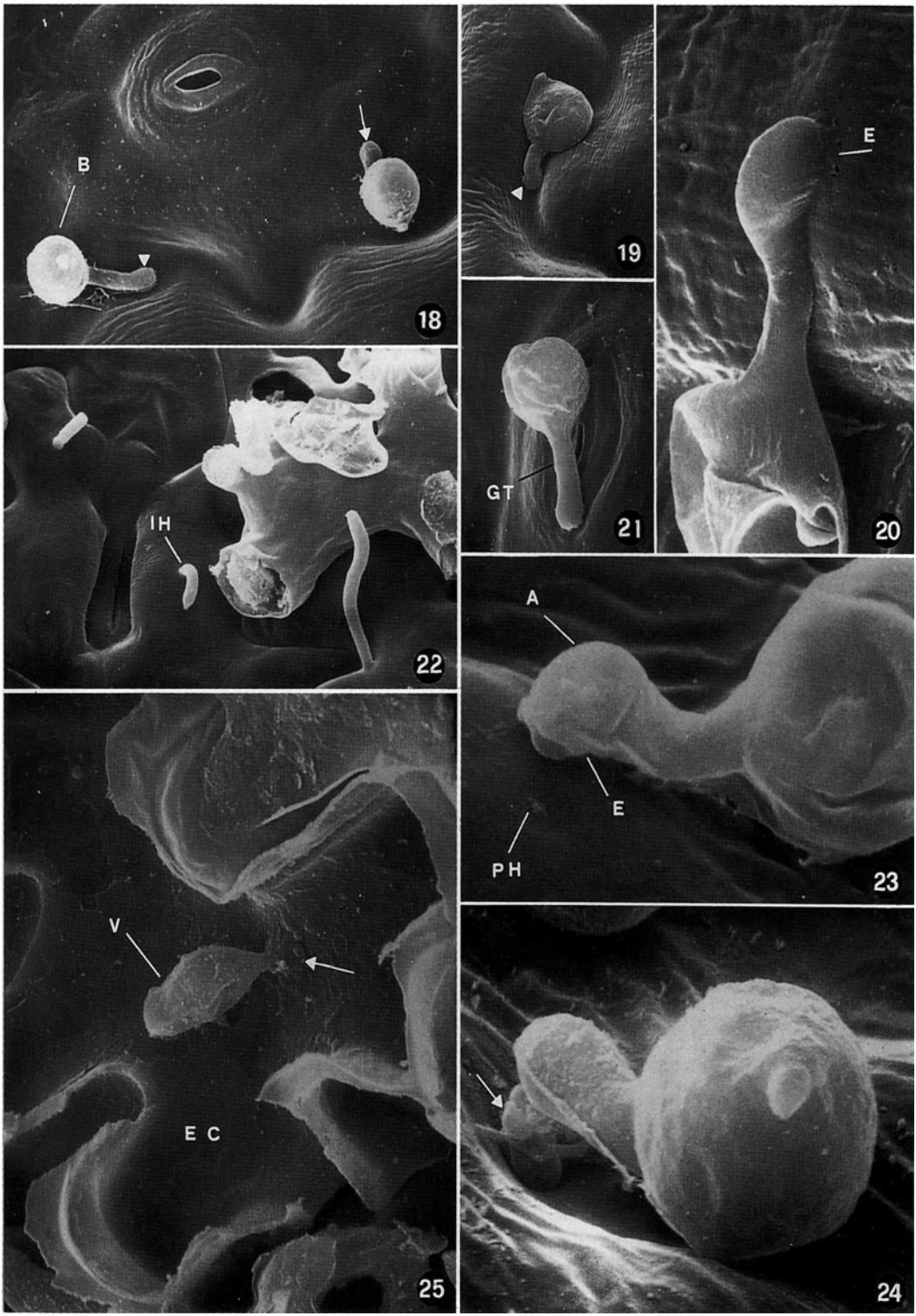
Figs. 18-21. — Germinated basidiospores with short germ tube and appressorium on periclinal (arrow in Fig. 18) and anticlinal (arrow-heads in Figs. 18 and 19) epidermal cell walls. Note the germ tube penetration into a stomatal guard cell (Fig. 21) and fibrouslike extracellular material around the base of the appressorium (Fig. 20). Fig. 18: ×1,230, Fig. 19: ×1,650, Fig. 20: ×4,500, Fig. 21: ×1,900.

Fig. 22. — Underside of lower epidermis in a fractured leaf: infection hyphae exiting through the inner periclinal walls of epidermis into intercellular spaces of spongy parenchyma. ×1,070.

Fig. 23. — Extracellular material in correspondence with the penetration hollow, wrapping the whole appressorium and germ tube. ×4.540.

Fig. 24. — Appressorium with extracellular material and penetration peg (arrow). ×5,770.

Fig. 25. — Fractured epidermal cell with young vesicle of a developing infection structure. Note the collar of host wall-like material around the vesicle neck (arrow). ×2,170.



The infection hypha (Figs. 37-39) shows a thin bilayered wall, similar to that of vesicle, with a highly electron-dense outer layer (Fig. 38); its cytoplasm is abundant, with many ribosomes and small vacuoles, scattered and multiform mitochondria, few lipid droplets, many and conspicuous patches of glycogen; one large nucleus per cell is present, with a highly electron-dense nuclear membrane, and chromatin bodies (Figs. 26, 38, 39). A moderately electron-dense and finely structured extrahyphal matrix, wider in the parts far from the vesicle, is evident between the infection hypha wall and the extrahyphal membrane (Fig. 37). This membrane appears thicker and more electron-dense than the host plasma membrane; it is closely and deeply undulated and at intervals reaches the fungal wall (Figs. 37, 38).

Host rough endoplasmic reticulum (ER) surrounding the infection structure also runs long distances parallel to the extrahyphal membrane: the inner ER membrane becomes smooth where it closely adjoins the extrahyphal membrane, so that semi-rough ER facing the extrahyphal membrane occurs in continuity with the remainder of host cell rough ER (Fig. 36). The host epidermal cell cytoplasm shows unaltered organelles even when it faces a well-developed infection structure (Figs. 37, 38).

Host wall-like material trapping membrane remnants is frequently present on the inner side of the epidermal cell wall around the pore of the penetration peg (Fig. 32). This material adheres to the proximal part of the vesicle neck like a «collar». Often, the host cell plasma membrane bounding the collar and the wall of the vescicle can be seen to infold between these structures (Fig. 33).

At the level of the transcellular growths of the infection hypha, observed

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Figs. 26-40. — Transmission electron microscopy of *Mercurialis annua* leaves inoculated with *Melampsora pulcherrima*. Glutaraldehyde and Paraformaldehyde, OsO_4, uranil acetate, lead citrate.

Figs. 26-27. — Intraepidermal infection structure at different levels. Note the position of septa (arrows). Thick neck wall, large vacuole and cytoplasm are visible in the vesicle; the infection hypha shows the nucleus with chromatin bodies. Fig. 26: ×7,270, Fig. 27: ×4,350.

Fig. 28. — Particular view of vesicle cytoplasm containing lipid droplets, mitochondria and glycogen. ×26,860.

Fig. 29. — Germinated basidiospore with many lipid bodies, scattered vacuoles, mitochondria at the periphery. Note the extracellular material around the germ tube. ×7,420.

Fig. 30 — Particular view of the thickening wall of the basidiospore germ tube. Note the three wall layers. ×28,640.

Fig. 31. — Appressorium with penetration peg through the epidermal cell wall. Note: the extracellular material connecting the appressorium to the epidermal cell, the thinning of the appressorial wall towards the penetration pore and the peg wall originating slightly within the appressorium. The epidermal cell wall appears more electron dense in correspondence with the extracellular material. ×19,440.

Figs. 32-33. — Collars of host wall-like material around the proximal part of vesicle neck. See membrane remnants in the collar of Fig. 32 and the host plasma membrane infolded between the collar and the vesicle neck wall in Fig. 33. Fig. 32: ×21,250, Fig. 33: ×26,730.

between host epidermal cells and palisade cells, the contiguous host cell walls show an increased electron density in the zone surrounding the passage pore (Fig. 40). The fungal wall is uninterrupted and uniformly thick through the transcellular growth.

Host wall-like «collars» with membrane remmants surround the fungal wall for a short distance both proximally and distally to the transcellular passage. For these distances, the collars appear to adhere to the fungal wall and the «matrix» surrounding the whole of the remainder of both the infection hypha in the epidermal cell and the haustorium in the palisade cell seems to join with the collar material (Fig. 40).

The haustoria in the palisade cells show: a large penetration hole through the host cell wall; a haustorium-infection hypha separating septum in the proximal region immediately beyond the penetration hole; a hypha-like shape without a distinct neck and body; a well developed extrahaustorial matrix (Fig. 40).

DISCUSSION

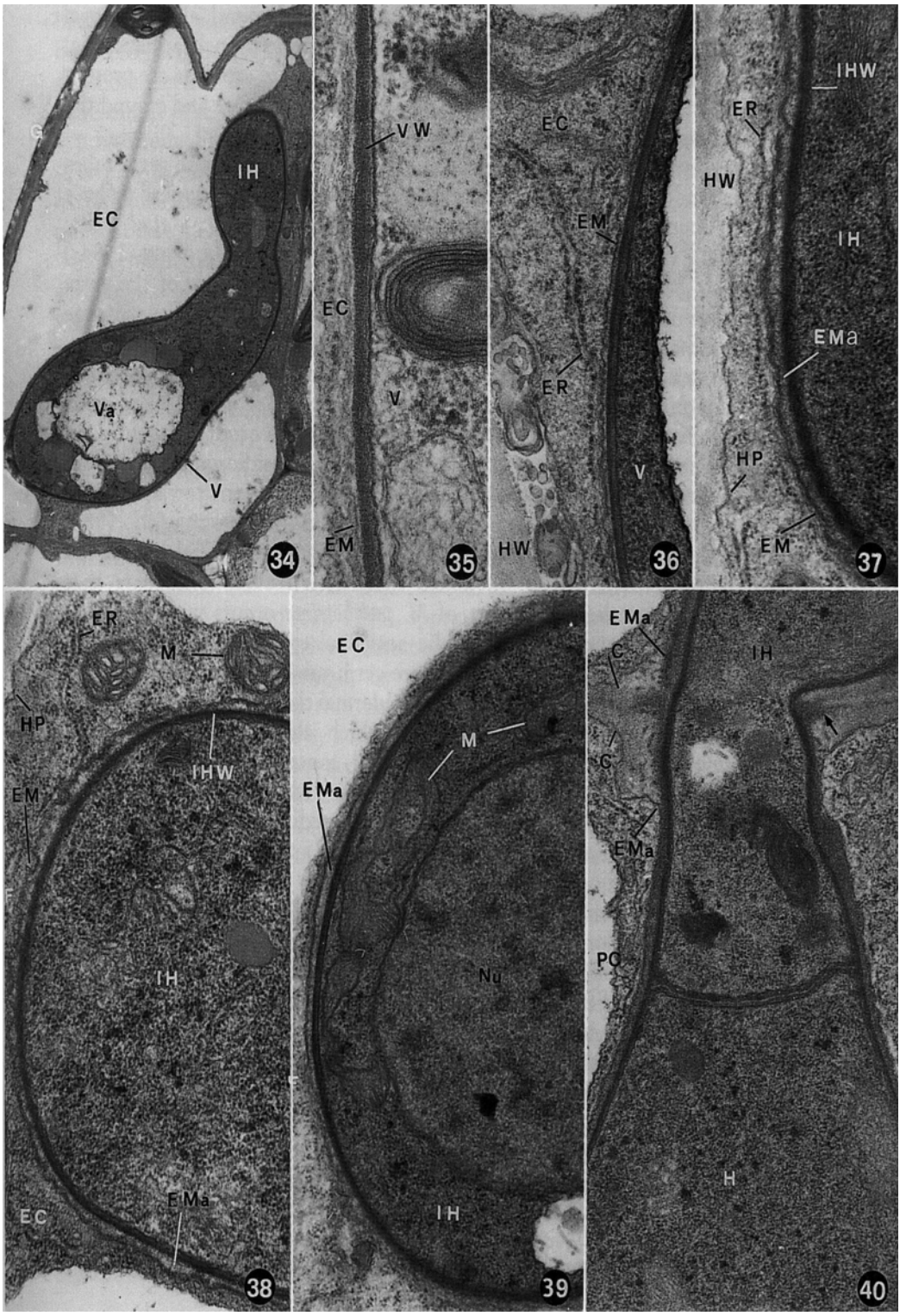
Some of the most significant aspects related to the basidiospore germling penetration and the infection structures of *Melampsora pulcherrima* on *Mercurialis annua* leaves are the following: a) short randomly growing germ tube with a swollen terminal portion which constitutes an undifferentiated appressorium (germ tube and appressorium adhering to the epidermal cell wall with an extracellular material); b) direct penetration by a thin peg from the appressorium through the epidermal cell wall producing an intracellular infection structure formed by a vesicle and a septate hypha; the branches of the septate hypha exit from epidermal cells, producing either intercellular hyphae in the mesophyll or directly terminal hypha-like haustoria in the contiguous epidermal cells and the underlying mesophyll cells; c) a «matrix» is present between the

Fig. 34. — Developing infection structure, still without septa, with vacuole in young vesicle and abundant cytoplasm in the region where the infection hypha is growing. ×4,620.

Figs. 35-36. — Particular views of young (Fig. 35) and mature (Fig. 36) vesicle; the extrahyphal membrane adheres to the fungal wall without an interposed matrix. In Fig. 36 the semi-rough ER facing the extrahyphal membrane occurs in continuity with host cell rough ER. Fig. 35: ×57,550, Fig. 36: ×36,300.

Figs. 37-39. — Particular views of an infection hypha. The extrahyphal matrix is evident between the fungal wall and the extrahyphal membrane: see the extrahyphal membrane in comparison with host plasma membrane (Figs. 37-38). Cytoplasm and nucleus of the fungal cell and unaltered host organelles are visible. Fig. 37: ×41,580, Fig. 38: ×30,500, Fig. 39: ×26,730.

Fig. 40. — Particular view: infection hypha transcellularly grown and proximal region of the haustorium formed in palisade cell. Note the electron density of the contiguous host cell walls at the transcellular passage (arrow); the collars and matrices merge at both sides of the host walls. ×25,240.



invaginated host plasma membrane and fungus wall around all the fungal intracellular structures (infection hypha, infection hypha branches, haustoria) but not around the intraepidermal vesicle; d) host wall-like «collars» are present around the intraepidermal vesicle necks at the penetration pore and around the rust structures at the point of the transcellular growth.

The very limited development of *M. pulcherrima* basidiospore germ tubes and their random behaviour on the surface of the host organ appear to be a commonly found aspect of basidiospore-derived germ tubes (GRAY *et al.* 1983; HOPKIN *et al.* 1988; MORIN *et al.* 1992), although GOLD and MENDGEN (1984*a*) in *Uromyces appendiculatus* var. *appendiculatus* report a tendency of the germ tube to grow along the host epidermal cell junction, probably due to some contact or chemical responses (WYNN and STAPLES 1981).

The appressorium formation and the direct penetration through any point of the epidermal cell wall comprising the subsidiary and stomatal guard cells, which are observed in *M. pulcherrima*, do not appear to occur frequently. In fact several authors maintain it is more probable that basidiospore germ tubes penetrate through the epidermal areas near cell wall junctions towards which germ tube growth occurs (GOLD and MENDGEN 1984*a*; HOPKIN *et al.* 1988; MORIN *et al.* 1992). From the number of penetration cases calculated on the observed «whole mounts» of *M. annua* inoculated leaves, it appears that basidiospore germ tube penetration of *M. pulcherrima* occurs with the same probability through the periclinal or the anticlinal epidermal cell walls. The differences in the behaviour of basidiospore germ tubes reported for different rust species may be attributed to the host epidermal tissue characteristics, thus reconfirming the hypothesis according to which also in the basidiospore-derived (monokarion) direct penetration some contact and/or chemical response occurs (DESPREZ-LOUSTEAU and LE MENN 1989; GOLD and MENDGEN 1991), even if this would be less specific than in urediniospore-derived (dikarion) indirect penetration through stomata (HOCH and STAPLES 1991).

Appressorium not separated from the remaining germ tube by septa but appearing as a lightly enlarged germ tube end, as described in *M. pulcherrima*, is commonly reported for basidiospore germlings (GOLD and MENDGEN 1984*a*; MIMS and RICHARDSON 1989; MORIN *et al.* 1992). Such a type of structure is very different from differentiated urediniospore-derived appressorium which produces indirect penetration (STAPLES and MACHO 1984).

The extracellular material present especially around the appressorium, but also extending to the greater part of the basidiospore germ tube, is considered to be an exudate characteristic of developing basidiospore germlings (GOLD and MENDGEN 1991) and is generally associated with the fungal strcutures involved in the direct penetration process (NICHOLSON and EPSTEIN 1991); its roles would be not only the attachment and protection of the appressorium (see GOLD and MENDGEN 1984*a*; MORIN *et al.* 1992), but also the reservoir for penetration enzymes (GOLD and MENDGEN 1984*a*). The latter role too appears

likely for *M. pulcherrima* because its penetration peg produces a clear-cut bordered pore, probably due to enzymatic activity. Moreover, the extension of the more electron dense zone in the penetrated host epidermal cell wall at the penetration pore corresponds to that of the extracellular material around the appressorium. Finally, a function in host recognition (see appressoria of *Erysiphe pisi*, KUNOH *et al.* 1991) it is not to be excluded for this material, even if extracellular material is also found around basidiospore germlings developing on artificial membranes (MIMS and RICHARDSON 1989).

Typically, as in *M. pulcherrima*, the penetration peg wall of basidiospore germlings is not continuous with that of the appressorium. Indeed, the appressorial wall becomes thinner towards the point where the penetration peg is produced, whereas the peg wall originates «de novo» from slightly within the appressorium (METZLER 1982; GOLD and MENDGEN 1984*a*; GRAY *et al.* 1983). The «appressorial ring» (METZLER 1982), the particular collar-like zone, adjacent to which the peg wall may form, which is considered to have a role in the direct penetration (GOLD and MENDGEN 1991), was not found in *M. pulcherrima*.

The basidiospore germling penetration peg, which in other rusts is very thin through the epicuticular wax and cuticle, and generally enlarges within the penetrated epidermal cell wall (METZLER 1982; GOLD and MENDGEN 1984*a*; GRAY *et al.* 1983; LONGO *et al.* 1991), appears rather uniform in *M. pulcherrima*.

The basidiospore-derived intracellular infection structure is generally formed, as in *M. pulcherrima*, by a vesicle-like portion connected to the penetration peg through a thin neck without septa, and a hypha-like portion separated from the vesicle by a septum, the septate branches of which originate successively from vesicle towards the distal part of the hypha and grow into the intercellular spaces and the contiguous host cells (GOLD and MENDGEN 1984*a*; DESPREZ-LOUSTEAU and LE MENN 1989; HEATH 1989; MORIN *et al.* 1992). Diversely, in the intracellular infection structure of *Cronartium quercuum* f. sp. *fusiforme* (GRAY *et al.* 1983) the vesicle and the hyphal portion are both relatively extended in length and without branches. Moreover, owing to the presence of a septum at the vesicle neck, GRAY *et al.* (1983) compared this structure to the dikaryotic substomatal vesicle typically separated from the appressorium by a septum. Ultrastructural observations on the intracellular infection structures particularly on the intraepidermal vesicles are few. However, it can be noted that while GRAY *et al.* (1983) in *C. quercuum*, f. sp. *fusiforme* reported a «sheath» surrounding the whole of infection structure and the intracellular hyphae, both in *M. pulcherrima* and in *U. appendiculatus* var. *appendiculatus* (GOLD and MENDGEN 1984*a*), the vesicle lacks the «matrix», i.e. the host-parasite interfacies pre-eminent structure typically present in all rust intracellular productions (HARDER 1989). For this aspect it might be right to compare the intraepidermal vesicle of these rusts with the intercellular substomatal dikaryotic vesicle (LITTLEFIELD and HEATH 1979). On the other hand,

the presence of the «matrix» around the infection hypha (distally to the vesicle) and hyphal branches, as in *M. pulcherrima*, is reported also by GOLD and MENDGEN (1984*a*) for *U. appendiculatus* var. *appendiculatus*. Therefore, it appears interesting to research the analogous morphological and functional aspects of the intracellular and intercellular infection structures respectively from basidiospore- and urediniospore-derived infection processes.

The transcellular behaviour of the intraepidermal infection hypha and its branches, which was observed for *M. pulcherrima* especially in the contiguous epidermal cells of the leaf, but also in the mesophyll and particularly in the palisade cells, was recently described for *U. appendiculatus* on *Phaseolus vulgaris* (GOLD and MENDGEN 1984*a*), *U. vignae* on *Vigna unguiculata* (HEATH 1989) and *Puccinia xanthii* on *Xanthium occidentale* (MORIN *et al.* 1992). This behaviour was not reported in *Endocronartium harknessii* on *Pinus contorta* (HOPKIN *et al.* 1988) and seldom occurs in the contiguous epidermal cells in *C. fusiforme* f. sp. *fusiforme* on *Pinus taeda* (GRAY *et al.* 1983). It is interesting to note that for these two rusts the observations were made on organs (cotyledons and epicotyls) histologically different from the dorsiventral leaves colonized by the other three rusts. Owing to the fact that in *M. pulcherrima* the infection hypha transcellular growth was shown in the epidermis and from the upper epidermis to the palisade cells, but not from the lower epidermis to the spongy parenchyma cells, it can be presumed that such a behaviour is affected by the host tissue type and particularly by the amount and extension of the intercellular spaces below the epidermis.

Appositions of host wall-like material similar to those which are reported at the point of cell penetration of the intracellular rust structures also in the compatible interactions (LITTLEFIELD and HEATH 1979) were observed in *M. pulcherrima*. These appositions were present not only at the level of the infection hypha transcellular growth but also at the penetration pore around the proximal portion of the vesicle neck. The wall appositions at the level of the infection hypha transcellular growth are reported also by other authors (GRAY *et al.* 1983; GOLD and MENDGEN 1984*a*, 1984*b*); those around the neck vesicle, observed only for *Uromyces* sp. under light microscopy by GOLD and MENDGEN (1984*a*) and HEATH (1989), were not described with TEM in the previous works. Two characteristic aspects of «type 1 collars» (LITTLEFIELD and HEATH 1979) i.e. the double-folded host plasma membrane interposed between the host wall-like material and the fungal wall, as well as the presence of membrane remnants within such appositions, make the wall apposition of the vesicle neck ultrastructurally similar to (a) those at level of the transcellular growth above described and (b) to the «collars» of the older haustoria of *M. pulcherrima* (LONGO, unpublished).

In *M. pulcherrima* the intracellular structures, produced by infection hypha both transcellularly in the contiguous epidermal and palisade cells and through the intercellular spaces in the spongy parenchyma cells, are terminal

unlike the infection hypha (therefore they are here defined «haustoria» according to BUSHNELL (1972) as well as all intracellular structures, even monokaryon ones, of older rust infections (LITTLEFIELD and HEATH 1979; BUSHNELL and ROELFS 1984). Even though many intracellular structures of this work should be considered still growing because of the short time that has elapsed since infection, they all were considered to be terminal according to previous observations on similar but fully developed structures (LONGO, unpublished). Terminal intracellular structures produced during the early stages of colonization by basidiospore-derived mycelium were reported also by MORIN *et al.* 1992; GRAY *et al.* 1983; HEATH 1989. On the contrary LONGO *et al.* (1988, 1991) for *M. pinitorqua* and GOLD and MENDGEN (1984*a*, 1984*b*) for *U. appendiculatus* showed that many intracellular structures which derive directly from the infection hypha exited in turn from the invaded host cell (therefore they were defined «intracellular hyphae»): however, in these rusts, such behaviour is presumibly characteristic only of the early stages of infection.

Since the ultrastructure of host-rust interfacies at the level of the «intracellular hyphae» has not to date been sufficiently studied with cytochemical techniques, it is still unclear if some differences in the efficiency of host-parasite interaction correspond to a different behaviour of intracellular structures in the invaded host cells, i.e. terminal or not terminal.

In conclusion, from structural and ultrastructural observations carried out on *M. pulcherrima* the behaviour and type of penetration characteristic of basidiospore-derived germlings were confirmed on the leaves of *M. annua*, for which an indirect penetration might be thought more probable because of the large number of stomata on the epidermis of the lower blade.

Moreover, two interesting aspects of the fine morphology of the monokaryotic infection structure, little studied also in other rusts, resulted from this work: a) the absence of an extrahyphal matrix around the intraepidermal vesicle which appeared for this similar to an intercellular structure; b) the presence of a collar of host wall-like material around the vesicle neck.

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