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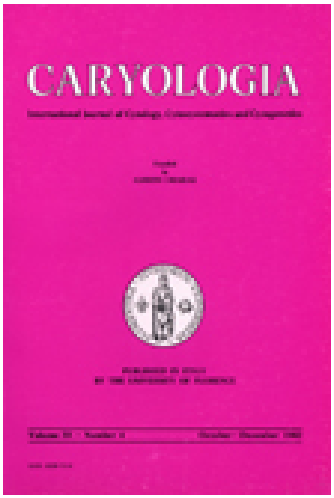

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ULTRASTRUCTURAL OBSERVATIONS ON THE SEPTAL PORE IN *CRONARTIUM FLACCIDUM* (ALB. ET SCHW.) WINT. ALSO IN RELATION TO THE TAXONOMY OF THE UREDINALES

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SUMMARY — The fine structure of the septum and pore in the monokaryotic and dikaryotic vegetative mycelium of *Cronartium flaccidum* was studied. Some ultrastructural observations on the hyphal cell of this fungus were also carried out. The hyphal septa become progressively thinner towards the pore; the septal pore shows none of the swellings and of the structures resembling the parenthesome which characterize the dolipore septum of higher Basidiomycetes. The septal pore of *C. flaccidum* as it as been seen in other Uredinales investigated up now, is a very simple structure morphologically similar to the septal pore of Ascomycetes. Some formations which make up the septal pore apparatus that is amorphous and electron-dense material, differentiated organelle-free cytoplasm and microbodies were observed near the septum and pore in both nuclear phases of the mycelium. It is accepted a relationship between the occurrence of microbodies and the occurrence and function of the dense material at the pore region likely to be associated with eventual migrations of nuclei and other cytoplasmic organelles. The taxonomic importance of the septum and pore fine structure for the systematic assessment of the Uredinales within the Basidiomycetes is discussed.

INTRODUCTION

Researches on the ultrastructure of the septal pore of the Uredinales showed that, although they are Phragmobasidiomycetes, their septa and pores are similar to those typical of the Ascomycetes (EHRLICH and EHRLICH 1963; MOORE 1965; EHRLICH *et al.* 1968; LONGO and NALDINI 1970; LITTLEFIELD and BRACKER 1971; COFFEY *et al.* 1972; JONES 1973). In these early studies, the observations made by some authors (GIESY and DAY 1965; JERSILD *et al.* 1967) concerning a few Holobasidiomycetes were extensively discussed. These authors were of the opinion that dolipore dissolution accompanied by its transformation to a simple pore was possible during the fungus cycle and was correlated to the nuclear stage. Indeed the typical pore of the Ascomycetes, being simpler in structure and perhaps less obstructed was likely to facilitate the dikaryotization process characterized by several and necessary nuclear migrations. If this interpretation had been correct, the pore commonly observed

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in the Uredinales should have been regarded as secondary and not primary in its origin. Further studies and extensive ultrastructural observations showed more and more clearly the ubiquity of this pore typical of the Uredinales and its independence from the various nuclear assessment. For such a pore the authors' descriptions agree about the presence of a « basic structure » (see references in LITTLEFIELD and HEATH 1979) which may be summarized as follows.

The septal pore edge is thin and the pore is associated with electron-opaque formations on either side of the septum. The cytoplasm in the pore region is differentiated to form two relatively organelle-free hemispheres with microbodies visible around the periphery. Furthermore the presence of amorphous electron-opaque material completely occupying the pore rim is an interesting feature often found in the pore (LONGO and NALDINI 1970; LITTLEFIELD and BRACKER 1971; JONES 1973; HARDER 1976; MIMS and GLIDEWELL 1978).

Since the ultrastructural characteristics of the pore are becoming increasingly important from a taxonomic and phylogenetic point of view (MOORE 1978; TU and KIMBROUGH 1978), investigations concerning another Uredinales may be helpful not only in order to supply a more complete description of the pore typical of this order, but also to provide additional evidence for the systematic arrangement of the Uredinales in the Basidiomycetes.

With this in mind the author chose to investigate *Cronartium flaccidum* (Alb. et Schw.) Wint. As regard the genus *Cronartium*, studies had been carried out on the ultrastructure of the septum and pore of *Cronartium ribicola* J. C. Fischer (ROBB *et al.*, 1973) and *Peridermium pini* (Pers.) Lèv. (WALLES 1974) which is considered an endocyclic race of *C. flaccidum*; as revealed by these studies, the above mentioned organisms possess the typical septal pore apparatus of the Uredinales. *C. flaccidum* is a macrocyclic and heteroecious rust which produces its pycnio-aecial stage on some species of the genus *Pinus* and its uredial-telial stage on some Angiosperms (*Vincetoxicum* sp., *Paeonia* sp.). When the monokaryotic stage develops on seedlings about one year old (MORIONDO 1980), the whole cycle is reduced as compared to that developing on adult trees resulting in a shortening of the reproduction stage intervals; furthermore a precocious diagnosis is possible due to the characteristic symptoms appearing on the primary needles. About two months after the infection of the young trees, yellow spots already appear on the primary needles as a consequence of the crowding mycelium development. Later the mycelium spreads to the stem, soon the pycnia appear on it and about nine-ten months after the infection aecia may be found. The acquired information concerning the rust cycle events on young seedlings and the experience deriving from repeated experiments of growth enabled the author to follow up more closely

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the cycle stages and to select the presumably correct times for sampling the monokaryon stage, since much uncertainty still exists about modes and times of dikaryotization. Actually one cannot altogether exclude the possibility of plasmogamy and dikaryotization in other than the protoaecidium, that is at a much earlier stage than protoaecidium itself; as a consequence a certain variability may be expected in the nuclear assessment of the monokaryotic mycelium. Investigations were carried out both on the monokaryotic and dikaryotic mycelium in order to obtain a broad view of the pore structure and its eventual variations correlated with the stages themselves or with the different possible nuclear situations.

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MATERIALS AND METHODS

The dikaryotic mycelium of *Cronartium flaccidum* (Alb. et Schw.) Wint. was observed on leaves of *Vincentoxicum hirundinaria* Medicus (= *Vincetoxicum officinale* Moench.) infected in their natural environment. The material was picked in June when the infected leaves show the uredia on the lower epidermis. Small leaf fragments carrying uredosori were employed for fixation. The monokaryon stage was obtained by infecting seedlings of *Pinus pinea* L. with germinating teliospores from leaves of *V. hirundinaria* infected in nature; the leaves were cut in July and inoculation were effected the same day. After two months the pine needles were first collected and pieces were fixed from parts very near the yellow spots. A second sampling, this time from the stem, was taken about seven months after the infection with the purpose of investigating the typical organ involved in the 0-1 stages of the rust; also the choice of the time, intentionally antecedent to the pycnia formation, was not casual since it was aimed at excluding completely spermatization and its effects on the nuclear assessment. Moreover this second sampling and the previous one effected on the primary needles, even if anticipated with respect to the entire monokaryotic stage, could not exclude the possibility of the somatogamic process having taken place and therefore of finding morphologically and functionally dikaryotic nuclear conditions. In any case these different possible nuclear situations could offer the opportunity of studying if and how the pore structure could be affected. Fresh material from the three samples was first observed under the light microscope before fixation to ascertain the actual presence of the mycelium and to determine which spots should be fixed, i.e. the parts with a larger number of crowding hyphae.

Fixation and embedding procedures were as follows: 1) Prefixation in 2.5% glutaraldheyde plus 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 (KARNOVSKY 1965) for one hour at room temperature. After washing rapidly in the same buffer the material was fixed in 2% OsO_4 always in the same buffer for three hours and again washed. After dehydration through the ethanol series, the material was embedded in Epon 812, mixture 3A/7B with 2% of DMP 30 (LUFT 1961). 2) Fixation in 2-4% potassium permanganate in water for 30 min. Dehydration and embedding were the same as at point 1).

The material was sliced with a Reichert OMU 2 ultramicrotome employing glass knives. Sections were stained in an alcoholic solution of uranyl acetate for about one hour (GIBBONS and GRIMSTONE 1960) and in lead citrate for 3-5 min. (VENABLE and COGGESHALL 1965), and then observed with a Philips EM 300 electron microscope at 80 Kv.

RESULTS

The following observations concern the intercellular hyphae of both monokaryon and dikaryon stages as obtained with the previously described fixation procedures. Peculiar differences observed between the two stages and the two procedures have been described.

The longitudinal wall of the hypha is composed of two electron-opaque layers of which the external one is relatively more opaque than the internal one. The boundary between the two layers appears more evident in sections fixed with potassium permanganate (Fig. 1). Both layers show in their thickness dense longitudinal striations clearly visible in some sections (Fig. 2). The plasmalemma, visible inside the hypha, is usually closely contiguous to the wall; an outer layer of amorphous, moderately opaque material, called « capsular sheath » by the majority of the A.A. (see references in LITTLEFIELD and HEATH, 1979), envelops the hypha wall. It may be involved in the adhesion of the hyphae to each other and to the host cells (Fig. 1). (1)

[1] Some ultrastructural observations on the hyphal wall of *Cronartium flaccidum* were reported also in a previous study on the host pathogen-interface of *C. flaccidum* on pine (LONGO, MORIONDO and NALDINI LONGO 1982).

Figs. 1-16: abbreviations.

C = differenziate region of cytoplasm; CL = central lamella; CS = capsular sheath; ER = endoplasmic reticulum; G = glycogen; H = hypha; HCW = host cell wall; L_1 = more electron-dense layer; L_2 = more electron-transparent layer; L = lipid droplets; LW = longitudinal wall; M = microbody; N = nucleus; PB = paramural body; PM = plasma membrane; PR = pore region; S = septum.

Fig. 1. — *Cronartium flaccidum* in *Vincetoxicum hirundinaria* (dikaryotic stage). The boundary between the two layers of different electron-density of the hypha longitudinal wall can be distinguished. Outside these layers is the capsular sheath. Potassium permanganate fixation. × 78,700.

Fig. 2. — *C. flaccidum* in *Pinus pinea* (monokaryotic stage - stem). Longitudinal wall of the hypha; both layers show longitudinal striations in their thickness. Glutaraldehyde-osmium tetroxide fixation. × 66,820.

Fig. 3. — Dikaryotic stage. Two paired nuclei. Glutaraldehyde-osmium tetroxide fixation. × 15,600.

Fig. 4. — Monokaryotic stage (stem). A single nucleus is present. The closely packed lipid droplets fill the hyphal cell almost completely. Glutaraldehyde-osmium tetroxide fixation. × 5,800.

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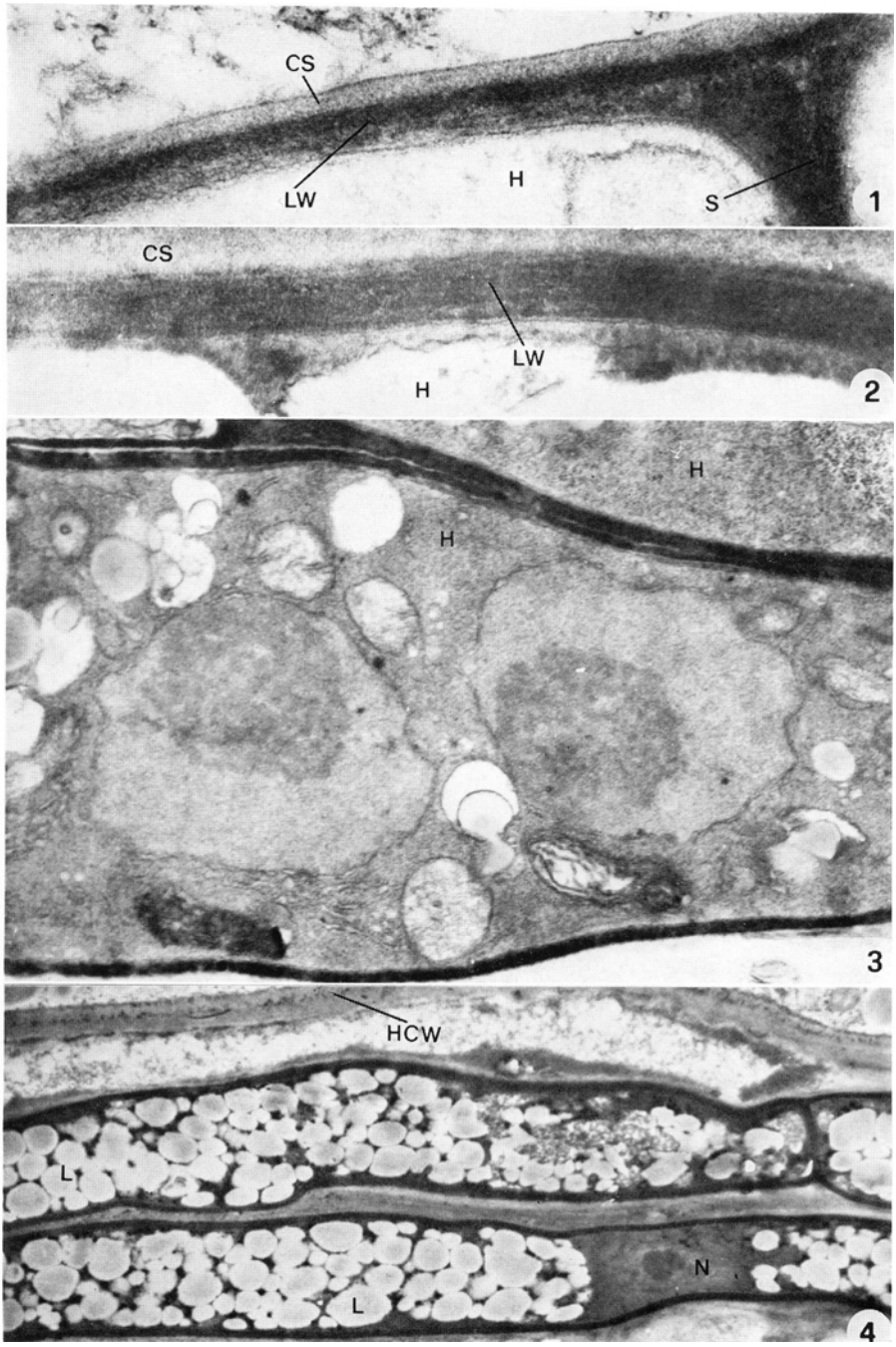

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The fine structure of the cell contents is that of a typical fungal cell. The mycelium observed in leaf sections of *Vincetoxicum hirundinaria* is clearly dikaryotic (Fig. 3), that found in the needle and stem of *Pinus pinea* was always mononucleate (Fig. 4).

Storage products consist of glycogen particles and lipid droplets. Glycogen commonly occurs in the mycelium of both stages and is easily identified because its particles often cluster together forming the typical « rosette » structure (alpha particles) frequently resulting in conspicuously electron-opaque aggregates (Fig. 5). The lipid droplets are particularly numerous in the monokaryotic mycelium, especially in that observed in stem sections of *P. pinea*, where they are so numerous as to fill the cell almost completely hindering the observation of the other cytoplasmic organelles (Fig. 4). The lipid droplets are so closely packed inside the hyphal cell that they no longer maintain their typical round shape. Generally the lipid droplets appear rather electron-transparent suggesting a possible content of saturated fatty acids.

Vacuoles of various morphology and dimensions sometimes fill entire portions of the cell while the cytoplasm is reduced to a narrow strip against the wall (Fig. 5). Such an extreme vacuolation is most usual in intercellular hyphae and is clearly correlated with the differing age of the hyphae or of single hyphal cells.

Mitochondria, rather elongated in shape, have platelike cristae, usually arranged parallel to the mitochondrion long axis, which appear to be typical of the fungal cell.

Lomasomes and plasmalemmasomes have been frequently observed; they are usually referred to as « paramural bodies » (MARCHANT and MOORE 1973) because their origin is not clearly understood. They appear as membranous structures enclosed by the wall and by infoldings of the plasma membrane; occasionally they are free in the cytoplasm. Such structures appear to be an aggregate of spherical, ovoid or rod-shaped vesicles, or concentrically arranged membrane layers (Fig. 6). This variety of structures and arrangements within the cell is obviously a consequence of the plane of the section.

Structures conforming to the morphological definition of typical « microbodies » are most common. They are single membrane-bound and contain an

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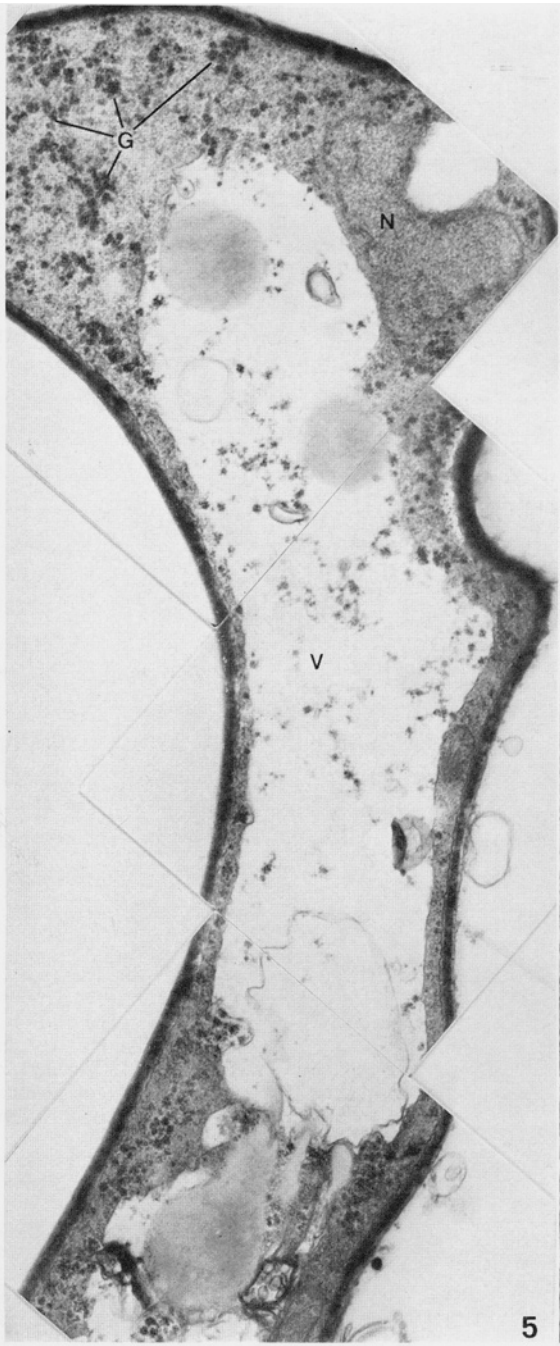
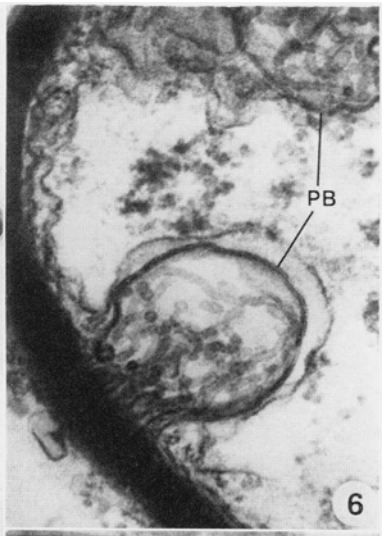
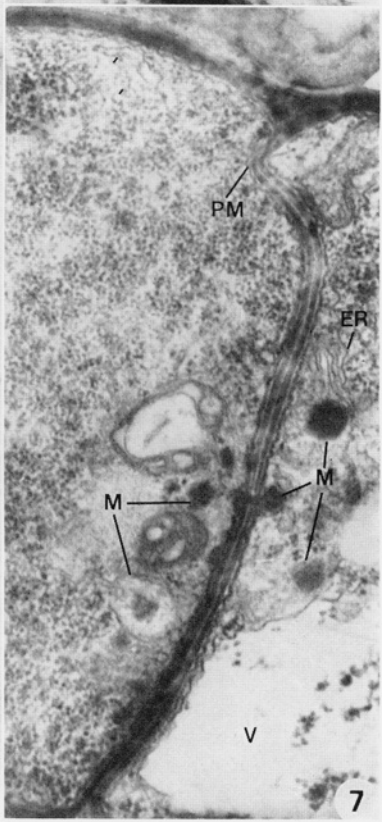
Fig. 5. — Dikaryotic stage (only one nucleus is visible). Vacuolation in a portion of a hyphal cell. Note the glycogen particles. Glutaldehyde-osmium fixation. × 14,850.
Fig. 6. — Dikaryotic stage. Paramural bodies: note the different shape of the membrane structures. Glutaraldehyde-osmium tetroxide fixation. × 49,000.
Fig. 7. — Dikaryotic stage. Median section through the septal pore. The three-layered septum is ticker near the longitudinal wall and thinner towards the pore. Note the crystal-containing microbodies at the pore region: one of them is right at the pore opening. Glutaraldehyde-osmium fixation. × 26,730.







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electron-dense pseudocrystalline formation within a granular matrix. Most commonly they are found associated with the septum and the pore apparatus (Fig. 7). They are more or less closely associated with cisternae of endoplasmic reticulum.

The septum separating the hyphal cells is perforated in its central region by a pore often associated with particular structures. In median sections of the pore this septum appears to be thicker near the longitudinal walls from which it originates and it becomes progressively and uniformly thinner towards the pore ending with a more or less rounded edge (Fig. 7). In the septum two electron-opaque layers separated by an electron-transparent central lamella can be distinguished (Figs. 7, 8, 9). Each electron-opaque layer derives from an invagination of the longitudinal wall inner layer (Figs. 7, 8, 9); these layers are not uniformly electron-opaque and sometimes a more electron-transparent layer can be discerned within the septal thickness towards the cell cytoplasm (Fig. 9). The central lamella, very thin and electron-transparent, extends from the median region of the longitudinal wall (Figs. 8, 9) to the plasmalemma adhering to the septum next to the pore (Figs. 7, 12). The plasmalemma of the two cells adheres more or less closely to the septum and extends uninterrupted through the pore (Figs. 7, 12). In peripheral sections including the pore region we can observe the whole profile of the septum and see that its thickness decreases gradually approaching this region; this is due to the gradual thinning of the electron-opaque layers while the central lamella remains unchanged (Figs. 8, 9). In this connection it is noteworthy to point out that the mycelium observed in stem sections of *P. pinea* showed no gradual and uniform thinning of the septum which was characteristically found in all the other cases. In these septa the thickness of the wall layers remains unchanged until a short distance from the pore region and it decreases abruptly approaching this region (Fig. 10). The reason for this peculiarity found uniquely in this monokaryotic mycelium is still unknown.

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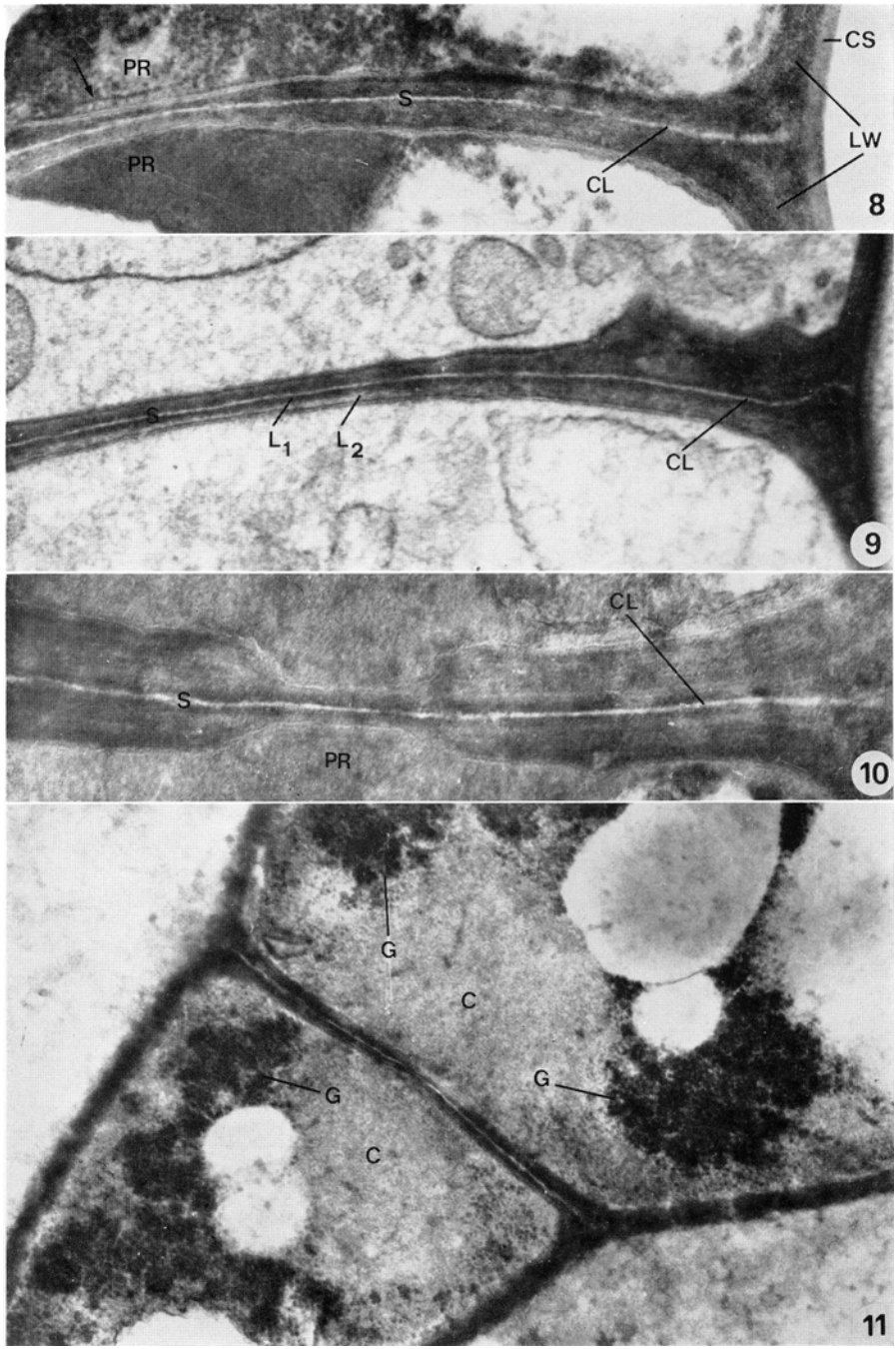
Fig. 8. — Monokaryotic stage (primary needle). Section away from the septal pore. The septum layers originate from an invagination of the longitudinal wall inner layer and are separated by an electron-transparent central lamella originating from the median region of the longitudinal wall. Note the septum thickness which gradually decreases approaching the pore region where electron-dense material is visible (arrow). Glutaraldehyde-osmium fixation. × 51,900.

Fig. 9. — Dikaryotic stage. Section away from the septal pore. Each electron-opaque septum layer is composed of two layers of different electron-density. Potassium permanganate fixation. × 54,450.

Fig. 10. — Monokaryotic stage (stem). Section away from the septal pore. Note the septum thickness which abruptly decreases at the pore region. Glutaraldehyde-osmium tetroxide fixation. × 66,820.

Fig. 11. — Monokaryotic stage (primary needle). Differentiated region of cytoplasm near the pore on either side of the septum. This region is organelle-free and not membrane-bounded. Note the glycogen aggregates. Glutaraldehyde-osmium tetroxide fixation. × 26,730.



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The cytoplasm next to the pore is differentiated from the remaining cytoplasm in that it contains only few organelles. It is usually shaped like a hemisphere and never enclosed by a membrane; this cytoplasmic differentiation can be observed on either side of the septum (Fig. 11), but it may be found also on one side only.

Structures of various shape and dimensions, made of amorphous electron-opaque material, may be observed frequently in the pore region (Figs. 8, 12, 13, 14). They usually look like dark bands on both sides of the septum and, for a short distance, on both sides of the pore too ending in close contact with the plasmalemma. In median and peripheral sections of the pore, these bands show clearly a more or less conspicuous infolding at the pore opening, becoming then approximately parallel to the septum at the sides of the opening. In such cases the two bands enclose an area which corresponds approximately to the pore edge and shows the same electron opacity as the cytoplasm surrounding the bands (Fig. 12); in other instances this area becomes uniformly electron-dense and produces structures associated entirely with the pore (Fig. 13). All these structures are not membrane-bounded (Fig. 12, 13). The most conspicuous and frequent organelles directly associated with the pore region are, as mentioned before, the « microbodies » which show no constant pattern in their arrangement. They have been observed around the periphery of the « differentiated cytoplasm », in the cytoplasmic portion close to the pore presumably associated with the dense structures just described and in the septum (Figs. 7, 14). Only in one case was a microbody seen right at the pore opening which appeared then to be characteristically free from any other type of structure (Fig. 7).

In median section of the pore no other organelle was ever noted and least of all nuclei suggesting with their position a possible passage through the pore.

The mycelium observed in sections of material fixed with potassium

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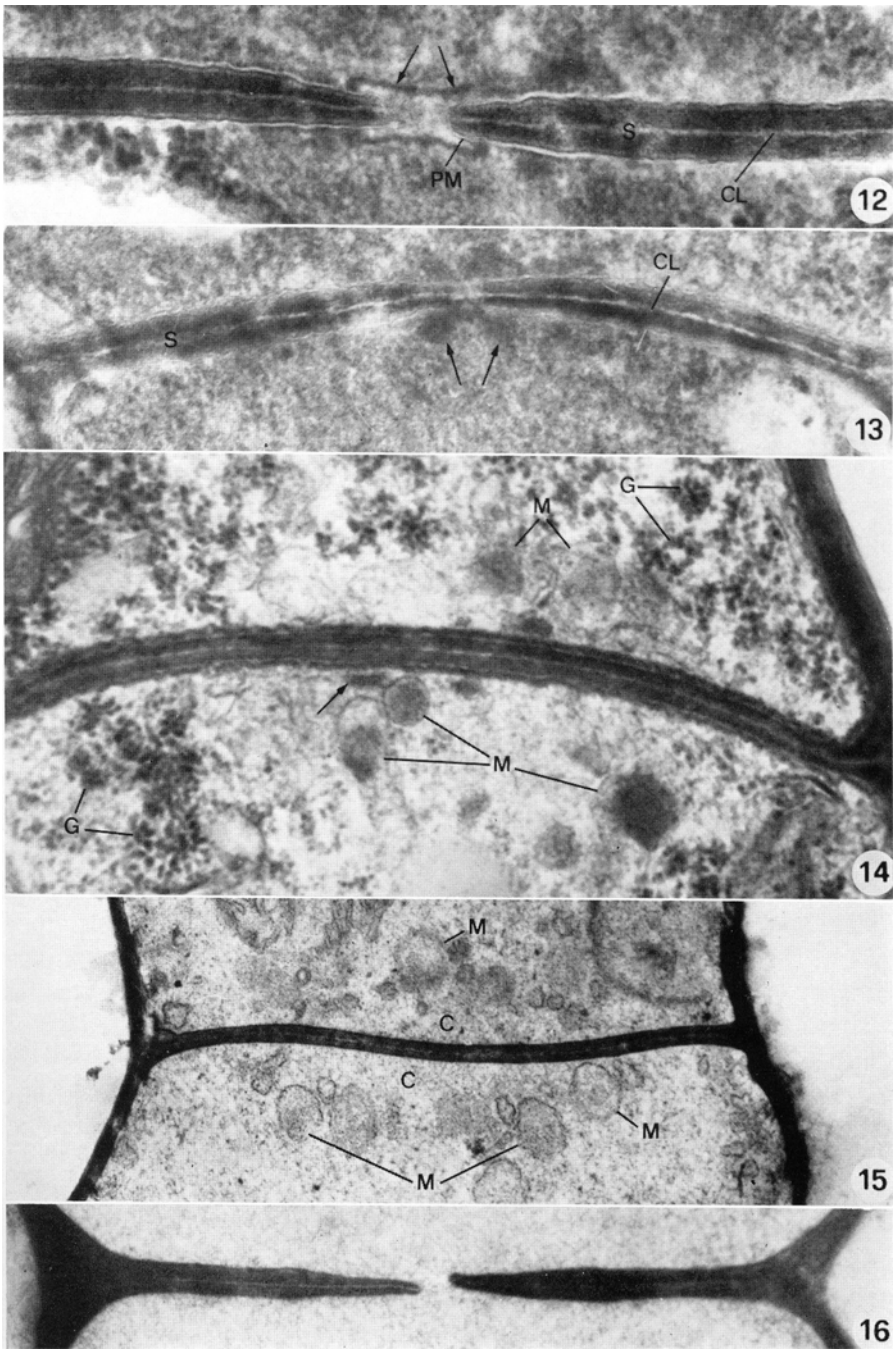
Fig. 12. — Monokaryotic stage (primary needle). Median section through the septal pore showing the amorphous electron-dense bands (arrows) at the pore region. The bands are not membrane-bounded. The plasma membrane bounding the septum extends uninterrupted through the pore. Glutaraldehyde-osmium fixation. × 66,820.

Fig. 13. — Monokaryotic stage (primary needle). The area enclosed by the osmiophilic bands (arrows) appears uniformly electron-dense. Glutaraldehyde-osmium fixation. × 51,970.

Fig. 14. — Dikaryotic stage. Section away from the septal pore. Numerous crystal-containing microbodies in the septum and at the pore region are visible; note their proximity to the electron-dense structures (arrow) associated with the pore region. Glutaraldehyde-osmium fixation. × 46,200.

Fig. 15. — Dikaryotic stage. Section away from the septal pore. Pore region showing the differentiated portion of cytoplasm bounded by microbodies containing hardly visible electron-dense inclusions. Potassium permanganate fixation. × 21,050.

Fig. 16. — Dikaryotic stage. Median section through the pore with no apparatus visible: the pore looks like a plain opening. Potassium permanganate fixation. × 32,670.



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permanganate showed none of those peculiar structures, at the pore region, which had been observed in material fixed with OsO_4. Only occasionally was it possible to see the portion of « differentiated cytoplasm » (mentioned above) and some microbodies containing basely visible electron-dense inclusions (Fig. 15).

Therefore in most cases the pore looked like a plain opening with a thinning edge (Fig. 16).

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DISCUSSION

From the results above reported, it can be said that the septum and septal pore of *C. flaccidum* correspond, from a morphological point of view, to the typical pore and septum as described by the latest literature (see references in LITTLEFIELD and HEATH 1979) dealing with the order Uredinales and particularly with the genus *Cronartium* (ROBB *et al.* 1973; WALLES 1974).

During the monokaryotic and dikaryotic stages the mycelium septa of this rust appear to be more or less gradually thinning towards the pore ending with a slightly rounded profile. Also this septal pore shows none of the swellings which characterize the dolipore septum of Basidiomycetes in general and of the majority of Phragmobasidiomycetes; further a structure of any kind associated with the pore itself and resembling the parenthesome was never found. Therefore it can be said that septum and pore of *C. flaccidum*, as it has been seen in other Uredinales investigated up now, are very simple structures similar, in this respect, to the septum and pore of Ascomycetes.

The septa of *C. flaccidum* are clearly made of three layers. MOORE (1978) regards this three-layered septum as a peculiarity of Basidiomycetes, while HUNSLEY and GOODAY (1974) report the occurrence of this characteristic septum in *Neurospora crassa* Emerson (Ascomycetes) with the supporting evidence of other authors' studies. More study should be devoted to this problem because, as suggested by MOORE (1978), if this ultrastructural peculiarity is exclusive to Basidiomycetes, it could become a useful taxonomic criterium notably in those mycelia lacking for some reason the main characteristics of the class. As regard the possibility of each electron-dense layer being composed of two layers of different electron-opacity, as revealed in our micrographs, to our knowledge no similar reports were found in literature.

The cytoplasmic area next to the pore, devoid of other organelles but usually containing microbodies, is the most salient ultrastructural peculiarity of many Uredinales (EHRLICH *et al.* 1968; COFFEY *et al.* 1972; ROBB *et al.* 1973; HEATH and HEATH 1975; WALLES 1974). In the dikaryotic mycelium of *Puccinia graminis* Pers. f. sp. *tritici* Erikss et Henn, *P. recondita* Rob. ex Desm. f. sp. *tritici* (EHRLICH *et al.* 1968), *P. helianthi* Schw. and *Melampsora*

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lini (Ehrenb.) Lèv. (COFFEY *et al.* 1972), in contrast to what has been observed in the monokaryotic mycelium of *Cronartium ribicola* J. C. Fischer (ROBB *et al.* 1973) and in the monokaryotic and dikaryotic mycelium of *C. flaccidum* this cytoplasmic area appears to be surrounded, at least partially, by a membrane. This structural characteristic, according to EHRLICH *et al.* (1968), could be regarded as one of the morphological aspects which make the pore of the Uredinales resemble the complex structure of the dolipore. Therefore, according to these authors, the function of such cytoplasmic area is to plug the pore preventing the intercellular migration of nuclei and of other cytoplasmic organelles in the hypha; in the higher Basidiomycetes this action, in order to maintain the functional diploidy, is performed by the dolipore (MOORE and MCALEAR 1962; MOORE 1965). On the grounds of this hypothesis, namely the functional analogy between the septal pore apparatus of the rusts and the dolipore-septum of the higher Basidiomycetes (EHRLICH *et al.* 1968), LITTLEFIELD and HEATH (1979) suggest that these two types of equally complex septum pore apparata have presumably followed two independent phylogenetic lines of evolution.

The electron-dense and amorphous material observed at pore region is commonly found in the vegetative mycelium of the rusts (see references in LITTLEFIELD and HEATH 1979) and was observed in the septum separating the haustorial mother cell from the vegetative mycelium (HEATH and HEATH 1975). This material is frequently shaped like dark bands, usually called « diaphragms » (ROBB *et al.* 1973; LITTLEFIELD and HEATH 1979), enclosing a certain pore region. Sometimes the whole region enclosed by the diaphragms appears electron-dense and WALLES (1974) explained such structures, in the mycelium of *Peridermium pini* (Pers.) Lèv., as Woronin bodies plugging the pore. Since the Woronin bodies are generally considered specific to Ascomycetes (BRACKER 1967) and furthermore they are membrane bounded (BRACKER 1967; MCKEEN 1971; WERGIN 1973), these structures, in *C. flaccidum*, should be regarded as mere deposits of dense material presumably plugging the pore. The majority among the authors are of the opinion that the pore occlusion is due to the compact, electron-dense formation and that the « diaphragms » should be regarded as a portion of the pore basic structure.

We have frequently associated the pore region and the above described dense formations to the microbodies. The occurrence of similar organelles more or less associated with the pore and the septum of the other Uredinales has been reported by several authors (EHRLICH *et al.* 1968; COFFEY *et al.* 1972; LITTLEFIELD and BRACKER 1971; JONES 1973; ROBB *et al.* 1973; HEATH and HEATH 1975; WALLES 1974). As regard their morphology, according to the literature concerning rust microbodies (see references in LITTLEFIELD and HEATH 1979), the microbody paracristal inclusions seen by

the author in the mycelium of *C. flaccidum* is a characteristic feature of the microbodies found in the proximity of the septum and pore of the vegetative mycelium hyphae. The microbody biochemical activity in the septum is unclear. During the present study the absence of an evident and specific relationship between microbodies and lipid droplets or other organelles made it impossible for the author to suggest a plausible hypothesis about the microbody function. Moreover no information is available about the enzyme contents of the microbodies investigated in this study since the specific tests have not been made; however catalase, which is indicated as omnipresent in microbodies (MAXWELL *et al.* 1977), is probably present. In actual fact, it can be said that microbodies occur in the proximity of dense structures (of indetermined chemical composition), regardless of their shape and the mycelium nuclear phase; also, accepting the actual occurrence of a relationship between such structures and the microbodies, a particular biochemical activity explaining their association with the septal pore can be postulated. Other authors too associate microbody function with origin of dense structures in the pore (JONES 1973; HEATH and HEATH 1975).

CONCLUSION

This study has shown that both monokaryotic and dikaryotic hyphal cells of *C. flaccidum* have septa and pores which, similar in both nuclear phases, appear to be structurally very simple. The absence in this rust of the dolipore septum represents additional evidence confirming the hypothesis, suggested by EHRLICH *et al.* (1968), that the type of septum and pore found in the Uredinales investigated may be considered intermediate between the simple pore, typical of the Ascomycetes, and the dolipore typical of the higher Basidiomycetes. In this study no certain displacement of cytoplasmic organelles was observed and far less any intercellular migration of nuclei revealing an indubitable connection between the behaviour of the pore apparatus and such migrations. In our endeavour to explain their function, we can only suggest that the electron-dense formations observed at the pore region are characterized, in both nuclear phases of the mycelium, by manifest variations in structure so that a swift change from a simple « diaphragm » to a uniformly dense aggregate structure and viceversa is possible. If we accept the hypothesis according to which the electron-dense formations produce the pore occlusion while the « diaphragms » do not hamper intercellular migrations, we can explain the rapid variation from one structure to the other by postulating a tendency to preserve, during a length of time in a certain stage, the nuclear situation actually present. This tendency has already been reported with respect to the dolipore function in higher Basidiomycetes during the dikaryotic phase (see discussion)

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and has been suggested by EHRLICH *et al.* (1968) with respect to the pore of two Uredinales during both nuclear phases.

Additional studies will improve our knowledge of the pore apparatus structure and function in the Uredinales; moreover they will supply additional evidence for the systematic assessment of this order within the Basidiomycetes and settle any question about the evolutionary line followed by Basidiomycetes. Recent studies (TU and KIMBROUGH 1978) in fact have emphasized the taxonomic importance of the septum and pore although other outstanding features such as presence or absence of fruit-bodies and basidial septation still retain their importance. Uredinales and Ustilaginales were initially classed under the Hemibasidiomycetes because they are without fruit-bodies (AINSWORTH 1966). Later TALBOT (1968) and AINSWORTH *et al.* (1973) grouped this order under the Teliomycetes stressing the importance, in contrast with other Phragmobasidiomycetes, of the presence of telia instead of typical basidiocarps; the same authors rank the Teliomycetes as a class of the subdivision Basidiomycotina. Also, the ultrastructural observations reported that the Teliomycetes species have the same pore structure (TU and KIMBROUGH 1978). In view of this fact there is a trend to group within the Teliomycetes other species of Basidiomycetes possessing the same kind of septum and pore as the Uredinales, although they do not belong to the same order. In fact the genus *Septobasidium* which was indicated to be related to the Uredinales, having a transversely septate basidium, is regarded as closer to the Teliomycetes (TU and KIMBROUGH 1978) after the observations made by DYKSTRA (1974) who showed that pore and septum in this genus are similar to those of the Uredinales. According to KHAN and KIMBROUGH (1980), the genus *Eocronartium* (Auricolariales) also is to be related to the Teliomycetes having the same characteristics as the genus *Septobasidium*, i.e. a trasversely septate basidium and a septum and pore like those of the Uredinales. The same authors are of the opinion that the affinity between the pore of *Eocronartium* and that typical of the Uredinales adds evidence to the hypothesis, already advanced by ATKINSON (1902) on morphological ground, that this genus, one of the most primitive among the Auricolariales, is one of the ancestors of the modern Uredinales in general and of the genus *Cronartium* in particular. All the above observations emphasize the importance of the ultrastructural characteristics of the septum and the pore. MOORE too (1978) underlines the taxonomic importance of this ultrastructural feature; as regards the fruit-body, he believes that its absence in the Septobasidiales and Uredinales is to be considered a sign of their evolution. On the basis of this assumption, he outlines a classification pattern in which the Uredinales are the most highly evolved order among the Basidiomycetes. In this event two hypothesis are possible, either the rust simple pore is the outcome of a simplification process of the dolipore or, as suggested

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by LITTLEFIELD and HEATH (1979), the dolipore and the simple pore, equally complex, have followed two independent lines of evolution. On the other hand TU and KIMBROUGH (1978) suggest that the sequence of the evolutionary steps in the whole Basidiomycetes group may be closely correlated with the structure of the septal pore both simple and dolipore. Also, remembering that the typical pore of the Uredinales is similar to that of Ascomycetes and this in turn is similar to that of Red Algae, they conclude saying that: « in our concept of the evolution of the septal pore structure in relation to the phylogeny of Basidiomycetes, *Puccinia* has the most primitive basidiomycetous septum » and that « perhaps the Uredinales are the most primitive group of fungi in the Basidiomycetes ». The above reasons account for the uncertainty in the systematic settlement of the Uredinales within the Basidiomycetes.

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