THE MORPHOLOGY OF FUNGAL SPORES: MILESINA BLECHNI

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ABSTRACT. Ultramicroscope examination of the uredospores of *Milesina blechni* shows that they develop by the same general stages as those of other rust fungi. The spore wall has no pores and the spores have an irregular coating of sticky material which probaby aids dispersal. The significance of these observations relative to the position of the genus *Milesina* is discussed.

Milesina (*Milesia*) is an interesting genus of some thirty-four species of the rust fungi. It is wholly restricted to ferns in the diplont stages; all the aecidia known occur on species of *Abies*. For a number of reasons, its simple hyaline uredospores, relatively undifferentiated teliospores and its hosts (members of the Pteridophyta and Gymnosperms) it has been considered a 'primitive' rust. Only the uredospores are reported on in this study. The uredospore sori are so unlike most other rusts, at least of the temperate regions, that they have been classified in non-rust genera on several occasions. The uredosorus is sub-stomatal with a well developed dome-shaped peridium which releases the spores through an apical pore. The spores emerge in a rather irregular, almost pure white, tendril. Anyone who lifts the uredospores off with a needle can observe that the spores are slightly adherent as though some sticky substance is present on their surface.This stickiness presumably aids dispersal by insects. Compared with other rust uredospores those of *Milesina* appear relatively simple by light microscopy. The wall is thin, more or less hyaline and no germ pores are visible. In some species the surface is quite smooth but most have rather widely scattered small spines. These spines have have always seemed rather simple structurally and not as clearly differentiated from the spore wall as for example the spines of *Puccinia* or *Phragmidium*. Faull (1932) wrote an excellent monograph of the genus in which the discrimination of the species rests largely on the size and frequency of the ornamentation of the uredospore wall.

MATERIALS AND METHODS

Milesina blechni (Syd.) Syd. is a most convenient rust for study in that its uredosori are active over long periods. The material for these studies was collected on *Blechnum spicant* (L.) Roth from Roslin Glen, Midlothian, Scotland, in October. The uredosori were fixed in 2% aqueous potassium permangenate for two hours at room temperature. Fixed sori were dehydrated in a series of ethanol, passed through propylene oxide and embedded in Spurr, low viscosity medium. Sections were stained in lead citrate according to Venable & Coggeshall's (1965) procedure. Single stage carbon replicas were prepared by Pegler & Young's (1971) routine.

Observations

Light microscopy of *Milesina* uredospores, suggests that they are apparently rather simple in structure. The spines appear undifferentiated from the spore wall and no pores are visible. Single stage carbon surface replicas (plate 6A) rather confirm this picture. No pore structures are detectable and the spines are simple without even the basal ring which is present in the uredospores of many other rust genera. No 'sticky material' is observable on the spore surface, unless it is represented by faint wrinkle markings on the surface.

A section including both immature thin-walled non-spiny spores and relatively mature thick-walled and spiny spores (plate 6B) shows a sharp contrast between the two. This abruptness may be due to the intermediate stages of development proceeding rather rapidly. Certainly this characteristic makes investigation of spine and wall formation more difficult as intermediate stages of development are rare. A striking feature of this view of a sorus are the many patches of amorphous material between the mature spores. Another micrograph (plate 6C) through the periphery of a sorus shows the spores in relation to the smooth-walled relatively small peridial cells (plate 6C, pc).

The general sequence of events in wall development are primary wall formation with concomitant development of spines within it, followed by deposition of a secondary wall—the main spore wall—and exposure of the spines by removal of the surrounding primary wall. At an early stage when the primary wall is still 270 μm thick the fusion of vesicles to the plasmalemma is a conspicuous feature. This is usually interpreted as transport from the cytoplasm and release to the wall of metabolites for wall growth. No predetermining features of spine location were observed in *M. blechni* due perhaps to the few intermediates between spineless and spine-bearing cells. Young spines grow in invaginated pits with an associated patch of endoplasmic reticulum immediately within the plasmalemma (plate 7B, s & er). In more heavily stained preparations (plate 7C) the possible internal complexity of a spine and associated primary wall is evident. The irregular outline of the spine and the basal banding together with irregular banding in the wall suggests the incorporation of different materials at this time. The deposition of further material on the spine surface also appears probable. The adjoining spore (plate 7C, x) has already formed its denser secondary wall and the primary wall has largely disappeared. On almost mature spores (plate 7) very little primary wall remains, only a thin layer (plate 7D, pw) 80 μm thick especially evident over the spines. The electron dense secondary wall predominates. The secondary wall is clearly intractable in the ultramicrotome; the appearance of microstructure within it is here regarded as artefact. The amorphous 'sticky material' is most obvious where adjoining spores are held apart by their spines.

Discussion

The most interesting feature of this study is undoubtedly the confirmation that the rather unusual uredospores of *M. blechni* and hence of probably all *Milesinas* follow a wall and spine developmental pattern similar to that of other rust uredospores. It might have been thought that the relatively simple

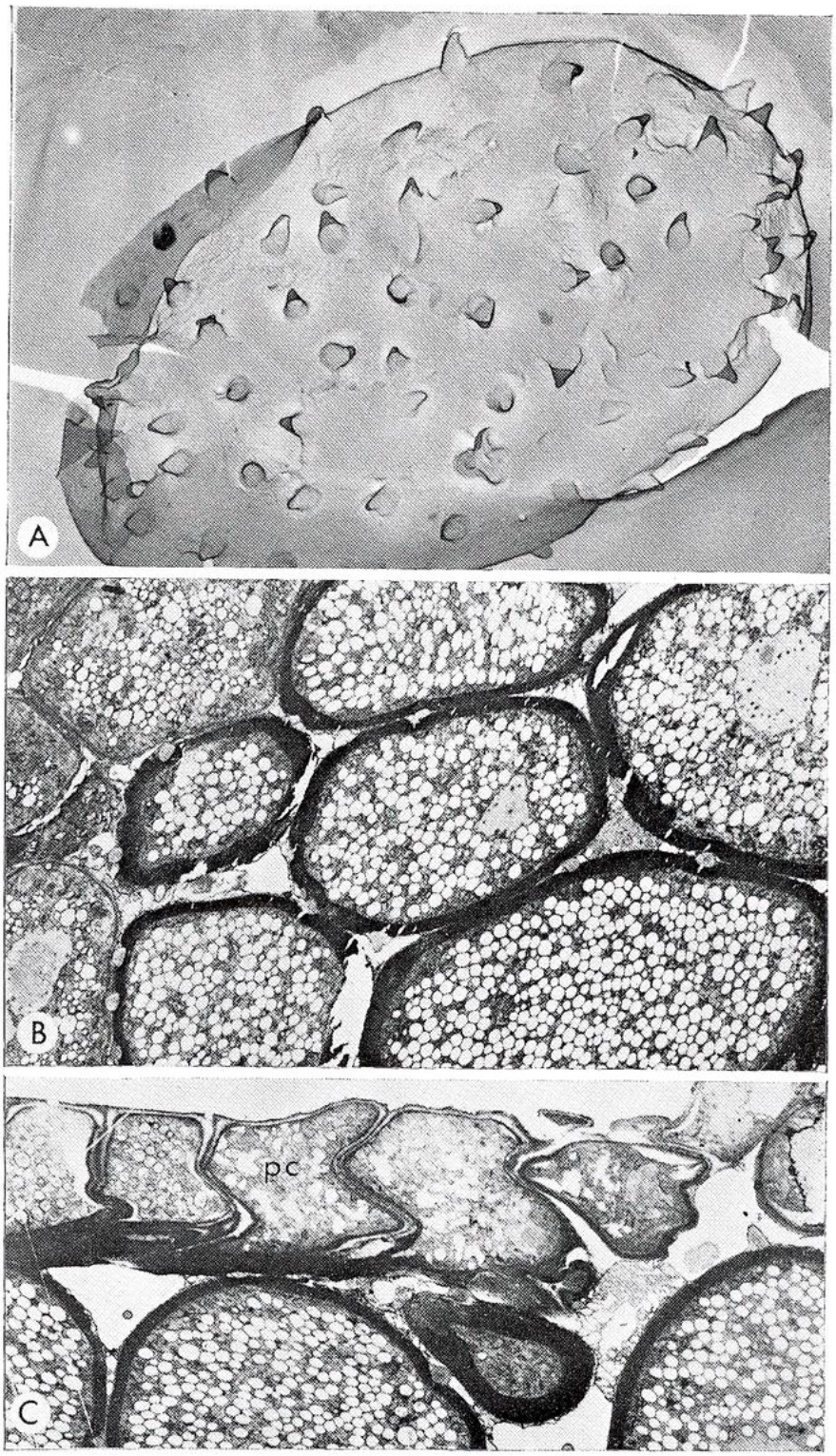

PLATE 6. *Milesina blechni*: A, single stage carbon replica of a single mature uredospore; B, longitudinal section through a uredosorus, from basal cells to sub-mature spores; C, section of lateral portion of uredosorus, mature uredospores and peridium. pc, peridial cells.

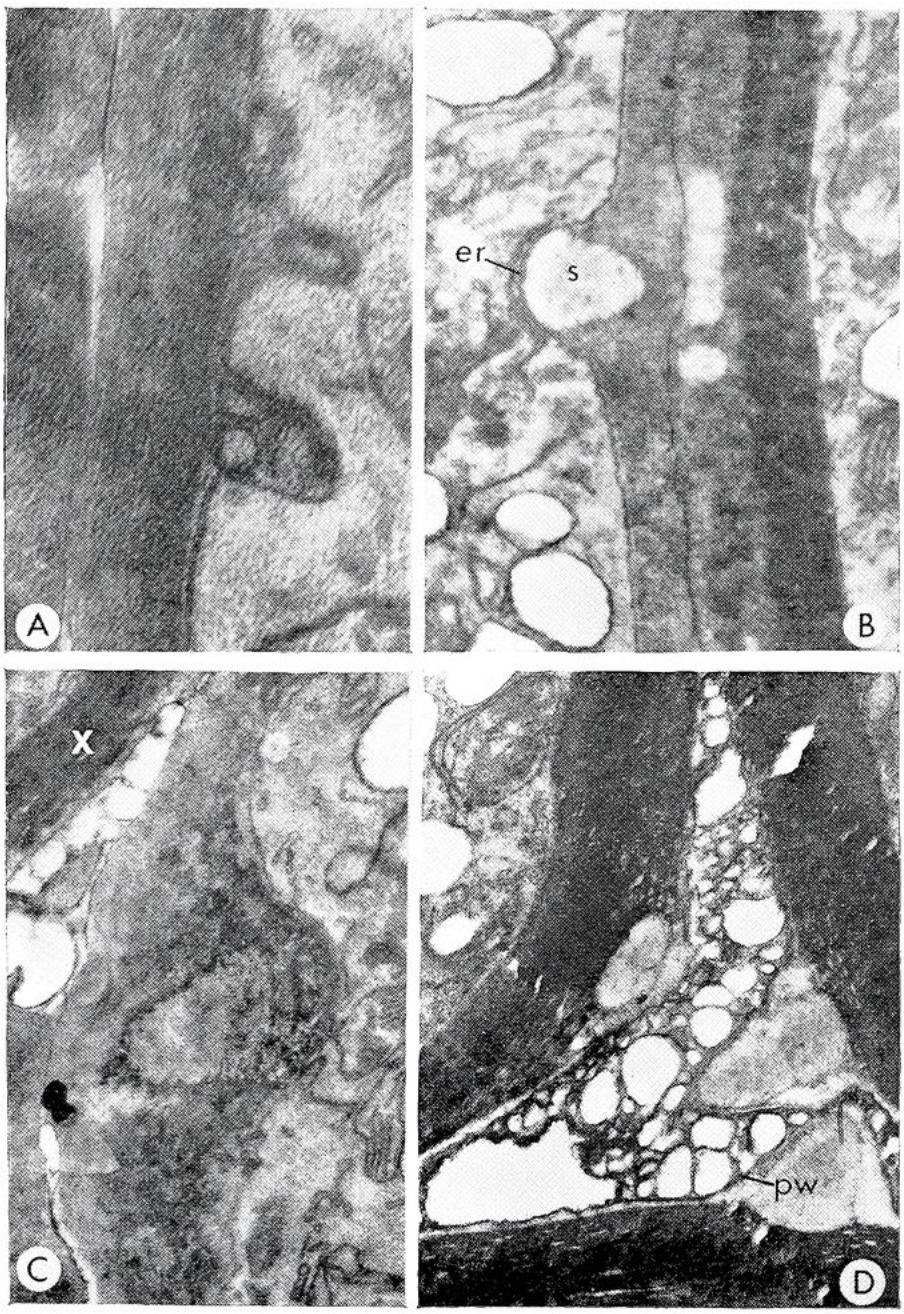

PLATE 7. *Milesina blechni*: A, section of young primary wall with vesicles fusing with plasmalemma; B, primary wall with developing spine in a pit invaginated in cytoplasm; C, primary wall with developing spine, wall and spine with some sub-structure; D, parts of three adjoining almost mature spores, primary walls degraded, spines projecting from dark secondary wall, vesicular sticky material conspicuous in space between the spores. er, endoplasmic reticulum; s, spine; x, secondary wall of spore; pw, primary wall.

spines on rather thin walls could have had a different course of development—perhaps pointing back to some ancestor of the rusts—but this is not so. The invaginated pits, the development of spines within a primary wall which is then lost to leave the spines projecting from an inner secondary wall is the same process as in *Puccinia* (Thomas & Isaac, 1967), *Phragmidium* (Henderson & Prentice, in press) and many other rusts. But there is no evidence of pore formation in the walls and in that respect the spores are simple. It is noteworthy also that the sticky material between the spores does not arise from the breakdown of the primary wall, it seems to be between the thin-walled spores at very early stages of their development.

References

FAULL, J. H. (1932). Taxonomy and geographical distribution of the genus Milesia. *Contrib. Arnold Arb.* 2:1–138.

PEGLER, D. M. & YOUNG, T. W. K. (1971). Basidiospore morphology in the Agaricales. *Beih. Nova Hedwigia* 35:1–210.

THOMAS, P. L. & ISAAC, P. K., (1967). The development of echinulation of wheat stem rust. *Can. Journ. Bot.* 45:287–289.

VENABLE, J. H. & COGGESHALL, R. (1965). Simplified lead citrate stain for use in electron microscopy. *Journ. Cell Biology* 25:407–409.