STUDIES IN THE MORPHOLOGY OF FUNGAL SPORES I:

The teliospores of Puccinia prostii and Nyssopsora echinata

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This series of papers is planned to examine the final and developmental stages of fungal spores especially those of the rust fungi. The taxonomy of such obligate parasites as the rusts and smut fungi is based largely on spore morphology—for in many there is little else to describe and quantify. The spores of these fungi are complex cells or cell-groups often with distinctive layering, well-developed pores or surface patternings of the cell-wall. While these features have been used for long they have not been more closely examined particularly in regard to their development. Indeed, although he did not specifically discuss surface patterning in detail, the seventy-year old studies by Sappin-Trouffy (1897) are still perhaps the most illuminating in suggesting mode of development of some rust spores. The first studies of this series will concentrate on developmental features of the spores of the rust fungi—lamination, pores, surface spines and warts.

Puccinia prostii and *Nyssopsora echinata* have somewhat unusual teliospores—with relatively long, pointed spines. Many years ago, Lamb (1934) described the development of the spines of *Puccinia prostii* as due to crumpling and contraction of the wall with consequent spine extension. Savile (1954) has more recently given a very similar description of spine development for *Puccinia podophylli*.

MATERIAL AND METHOD

Herbarium material of *Puccinia prostii* was used at first. The spores were infiltrated so far as possible with 'Epon'—and spun down to the base of gelatine capsules in a centrifuge before polymerisation. Sections about 900Å thick were cut on an LKB Ultrotome with a glass knife. The sections were mounted on ordinary glass slides and stained with Heidenhain haematoxylin or acid fuchsin according to normal schedules. After dehydration the sections were mounted in balsam. As the refractive index of balsam is near that of 'Epon' there is no need to attempt removal of the 'Epon'. This method gives reasonably good sections for light microscopy. The main problem is the failure of the embedding medium to infiltrate the spores.

For electron microscopy the same embedding schedule was followed but failure of infiltration makes satisfactory sectioning almost impossible. Although fresh material of the two species under discussion has not been studied extensively, it is now known that one of the main barriers to successful electron microscope studies of heavily walled fungal spores is difficulty in achieving sufficient infiltration of the embedding medium—even using spores freshly-fixed from the living state.

Progressive acetolysis (treatment with conc. suphuric acid in acetic anhydride at 98°C) has also been used. Spores will withstand this drastic treatment for up to thirty minutes—the main effect is progressive removal of the

wall of the spores from without inwards. The details of the method as used for pollen grains has been fully described by Erdtman (1960).

The most revealing method used in the present study was the simplest—mounting freshly collected crushed sori of *Puccinia prostii* and *Nyssopsora echinata* in lactophenol-methylene-blue. The *Puccinia prostii* was collected on *Tulipa sylvestris* in the Royal Botanic Garden, Edinburgh, the *Nyssopsora echinata* on *Meum athamanticum* in Glen Lyon, Perthshire, Scotland.

Puccinia prostii

The teliospores of *Puccinia prostii* develop in conspicuous black sori on the leaves. The mature spores are two-celled with up to twenty conspicuous spines up to 10μm long on each cell. With light microscopy the wall and spines show no laminar structure and the spines appear solid and without a lumen (Pl. 9, a). Sections of the walls of spores at this stage examined by light microscopy confirm this structure. The developmental stages of the teliospores mounted fresh in lactophenol-methylene blue are more revealing. At the earlier stages of development the spores are hyaline and smooth in outline (Pl. 9, b); the wall is very thin and shows no sign of the massive thickening of the mature spores. When the two-celled spore is about 25 μm in diameter, fifteen to twenty light-coloured growing points appear evenly spaced over the surface of the spore (Pl. 9, c). From these points grow out tapering sacs up to 7–10 μm long.

These tapering sacs are thin-walled and hyaline (Pl. 9, d). Until they have completed growth, no thickening of the spore wall and deposition of major secondary thickening of the spine wall is observable. The cytoplasm of the spore cell also fills the lumen of the spine (Pl. 9, d). During this development of the spine sacs the cell itself increases in size—from 25μm at initiation of the sacs to 35–40μm diameter at their completion. Once the spine-sac formation is completed the thick dark-coloured secondary wall is quickly laid down. Its deposition takes place within the original, thin hyaline primary wall and it is laid down rapidly and simultaneously both between and within the spine sacs. The sacs are filled so that they become solid and their main structure is continuous with the main wall of the spore. The secondary wall which is up to 4μm thick is brown in colour. At first it is permeable to methylene-blue so that the cytoplasm of the spores stain rapidly on immersion in the stain but as maturity progresses there is an abrupt change to impermeability. This change is not connected with any morphological event so far as present observations go. No germ pores were observed at any time during development and they are not present at maturity.

Electron micrographs of the wall of teliospores of herbarium specimens of *Puccinia prostii* confirm some of these findings (Pl. 10, e). The spine consists essentially of two layers, an outer layer about 400Å thick, corresponding to the hyaline wall of the spore initial and the solid inner secondary wall which is relatively thick, rather electron-dense and not obviously differentiated into layers.

Nyssopsora echinata

Nyssopsora echinata has three-celled teliospores with 25–30 spines 10–15μm long on each spore.

The apex of the spines is sometimes slightly forked. Essentially the same

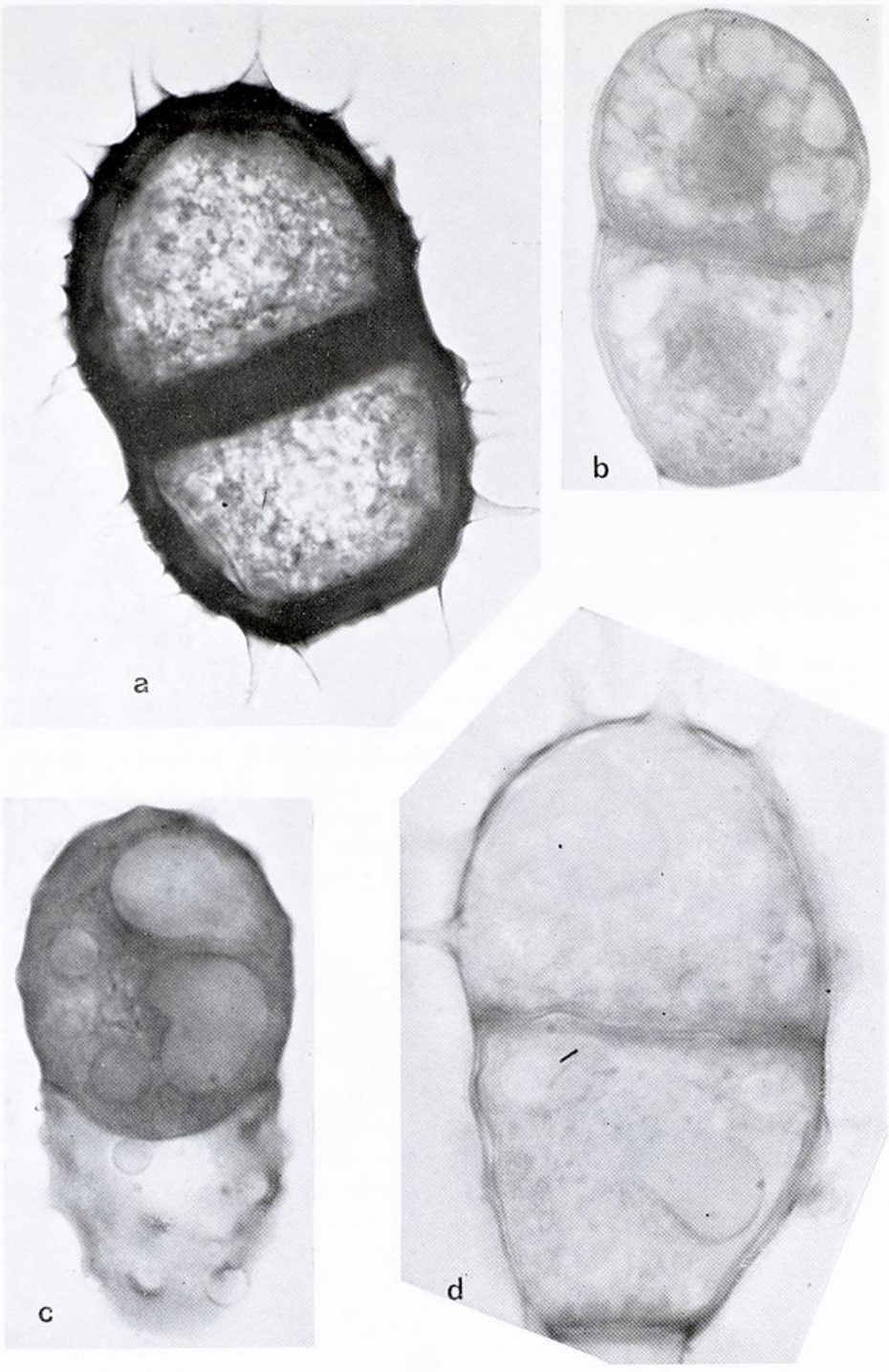
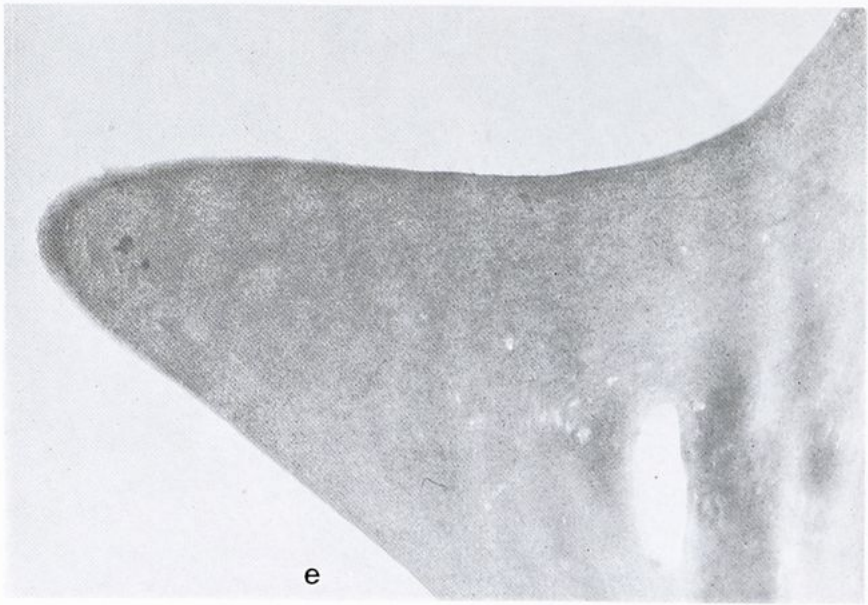
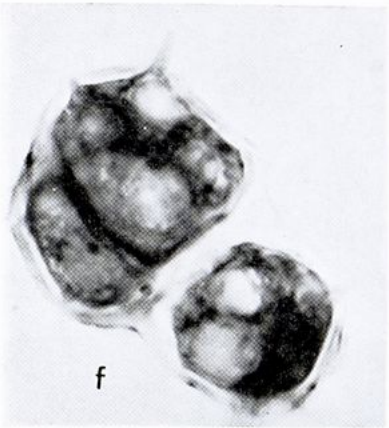


PLATE 9. *Puccinia prostii* teliospores: a, mature teliospore, × 1200; b–d, developmental stages, fresh material in lactophenol-methylene-blue, × 1400; b, smooth young spore with thin primary wall; c, spore with spine initials showing as growing points on the wall; d. stage of fully developed spine sacs with cytoplasm in sacs continuous with that of spores, the wall still unthickened.





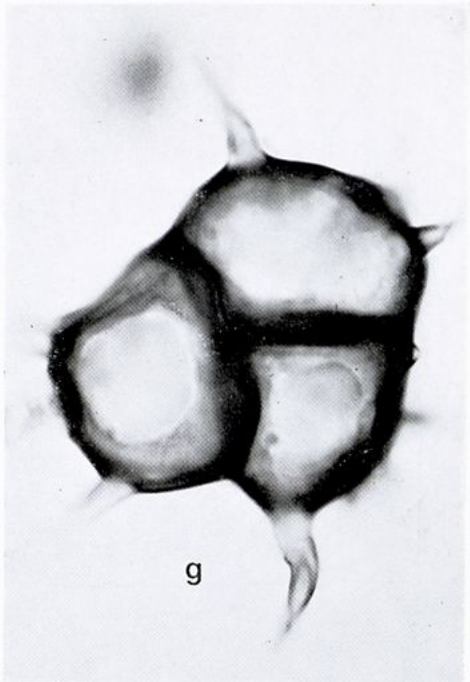


PLATE 10. *Puccinia prostii:* e, base of mature spine, outer primary wall and undifferentiated secondary wall, electron micrograph, × 21,000.
f–g, *Nyssopsora echinata* teliospores, × 1600: f, young spore with spine sacs; g, partially acetolysed spore with primary wall persistent.

course of development of spines as in *Puccinia prostii* has been followed in this species—the outgrowth of spine sacs from a thin-walled spore initial (Pl. 10, f) and then later the deposition of the thick dark secondary wall within the primary wall. However, treatment by acetolysis for ten minutes of mature teliospores of *N. echinata* provided added confirmation of the structure of the mature spines. The apex of the spines erodes first in this treatment and the inner secondary wall breaks down somewhat more quickly than the primary wall so that the primary wall is often left as a frill round the eroding secondary wall (Pl. 10, g). This confirms the continued presence of the primary wall as a structural entity after the deposition of the secondary wall.

Discussion

The central problem of the ornamentation which eventually comes to appear on the surface of spores is the mode of development. This paper demonstrates one way in which large spines may develop—by the outgrowth of spine sacs and their subsequent infilling by secondary wall. This is shown here to apply to *Puccinia prostii* and *Nyssopsora echinata.* The same method probably also accounts for the large apical spines on the coronate teliospores of *Puccinia coronata* and its allies; but it is probably a relatively uncommon method of spine development.

Attention was first focussed on *P. prostii* by the description of spore development by Lamb (1934) and by Savile (1954). This present account totally fails to confirm their account of spine development by spore shrinkage. The spines develop as a result of the outgrowth of growing points while the spores are still enlarging.

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