The material shown was taken from plants in aseptic culture. Each specimen was attached to an SEM stub with quick-drying silver paint and plunged into slushed liquid nitrogen. The slushing chamber was evacuated and the stub transferred to a Cambridge S150 fitted with a cryo stage (Hexland) at −190°C. Superficial ice was removed by sublimation at −80°C and the specimens sputter-coated in a cooled pre-chamber at −190°C. They were then returned to the cryo stage for observation and photography.

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D. THE BIOLOGY OF BRACKEN (*Pteridium aquilinum*)

Isolation and regeneration of *Pteridium* protoplasts

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Protoplast isolation. Protoplasts were isolated from gametophytic tissue using the widely adopted cellulase and pectinase enzymes. Protoplast release per g of tissue increased with gametophyte age. The possibility therefore exists that young *Pteridium* prothalli produce an enzyme-resistant cell wall component, the presence of which decreases with increasing age of the prothallus. This component may take the form of a cuticular layer or a structural change in the cell wall. TEM observations of the wall indicate that there is a change in cell wall structure as the prothalli mature. Huckaby *et al.* (1982) report finding a cellulase-resistant component in outer prothallial cell walls of *Onoclea sensibilis*. Wada and Staehelin (1981) also observed an external waxy cuticle on protonemata of *Adiantum capillus-veneris*.

SEM **of freshly isolated protoplasts.** SEM of freshly isolated protoplasts was performed and comparisons made between freeze-dried (FD) and critical point dried (CPD) and cryo-preserved protoplasts. For biological specimens, it has been found that one of the benefits to be gained from cryopreservation is that the material can be viewed in the SEM without prior fixation. Protoplasts, however, must be suspended in an osmoticum both during and after isolation. On sublimation of a frozen protoplast suspension, the osmoticum therefore remains as crystals, obscuring much of the surface detail. The protoplasts were therefore subjected to a short glutar-aldehyde fixation to allow the osmoticum to be replaced by water. The cryo-treated protoplasts were mounted on membrane filters which absorbed much of the water in the suspensions. The remaining water (ice) was removed by sublimation to expose the protoplasts.

Dehydration of most plant material has been shown to result in shrinkage and also wrinkling and collapse of cell walls. Since protoplasts possess no rigid exterior, the convolutions and wrinkles seen in FD and CPD specimens could result from

dehydration or plasmolysis. Protoplast structure is better preserved during cryo-preservation as the rigours of dehydration are circumvented and less wrinkling is apparent in frozen specimens.

Holes or invaginations have been observed at the surface of many of the protoplasts. These do not appear to be interruptions of the membrane and have been observed by other workers (e.g. Burgess *et al.* 1977). They were not present on protoplasts that were plasmolysed, fixed, then treated with enzymes. The suggestion is advanced that these holes result from enzyme damage caused by the presence of proteases as impurities in the cell wall degrading enzymes.

Protoplast regeneration Protoplasts were cultured on a modification of Moore's medium. The frequency of regeneration was generally low, the greatest percentage occurring with protoplasts isolated from crowded, slow-growing, more juvenile gametophyte cultures prolonged in the antheridial phase (possibly due to A_{Pt} production from the faster growing individuals; see Näf 1979, for review of antheridiogens). These gametophytes show parallels with the ameristic tumerous gametophytes, the protoplasts of which are capable of regeneration (Partanen *et al.* 1980). It seems that the regenerative capacity of *Pteridium* protoplasts is primarily dependent on the physiological condition of the cultures from which they are derived. A build-up of A_{Pt} in the medium is therefore thought to be responsible for the change in cell wall composition which seems to occur as prothalli mature, as mentioned above.

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Curl-tip disease and the potential use of *Phoma aquilina* as an agent of biological control of bracken

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Forty-eight sites for curl-tip disease of bracken have so far been discovered in Great Britain. Three common fungal isolates from diseased tissue are *Phoma aquilina*, *Ascochyta pteridis* and *Septoria* sp. The most virulent isolate, as revealed after inoculations *via* wounds, is *P. aquilina*. Spores of this fungus will cause infection when