Use of fern spores and gametophytes in toxicity assessments

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Experiments have been carried out using spores and gametophytes of fern taxa and one moss taxon in the assessment of toxic responses to sodium chloride, ozone, and various heavy metal ions. Spores and gametophytes were cultured in Falcon Brand 24- and 96-well tissue culture plates employing Hoagland's Solution under standard conditions of pH, light and temperature. For the NaCl and heavy metal ion experiments, spores and/or gametophytes were exposed to concentration series of these substances. In the ozone experiment, gametophytes were exposed for various lengths of time to a fixed amount of ozone. These various toxicity assessments were based on the measurement of one or more of three stress-affected parameters—percentage spore germination, relative length of rhizoids, and incidence of gametophytic injury.

Spores of *Acrostichum danaefolium*, a mangrove swamp associate, are capable of germination and limited growth at higher salt concentrations (up to 2·5% NaCl) than are the spores of *Osmunda cinnamomea*, *O. claytoniana*, *Onoclea sensibilis*, and *Dryopteris intermedia*, for which germination ceases at approximately 0·625% NaCl. Fourteen-day-old gametophytes of *A. danaefolium* could tolerate 1·25% NaCl for 7 days. However, 2·5% NaCl resulted in their death. Rhizoid length decreased with an increase in salt concentration.

Cellular injury to the gametophytes of *Onoclea sensibilis* in the form of plasmolysis and chlorosis was induced by their exposure to 0·5 ppm of ozone for 15 minutes.

Fern spore germination was shown to be a useful toxicity gauge for different heavy metals tested both singly and in combinations. As a rule, combinations of heavy metals ions showed a synergistic effect, yielding greater toxicities than expected if their individual toxicities were added together. Furthermore, fern spores of different species yielded definite species-specific responses to various heavy metal ions of potential value in elucidating edaphic niche requirements of pteridophytes.

Photocontrol of spore germination in *Polypodium vulgare* L.

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Under continuous white light, spores of *P. vulgare* gave a germination level of 75%. Under monochromatic light, at all wavelengths from blue to far-red, the level of germination did not deviate significantly from 50%. The action spectrum for germination showed red light to have the highest photon efficiency.

The inductive effect of exposure to red light was reversed by subsequent exposure to either blue or far-red light. The effects of alternating red and far-red light showed repeated reversibility but for red and blue light, the reversibility was incomplete; once spores had been exposed to blue light the germination level subsequently inducible by

red light was reduced to 25%, and, thereafter, sequential irradiation with red and blue light gave reversibility only up to this depressed level. However, if the red and blue exposures were separated by a dark period, 75% germination occurred.

The photoreversible effects of red and far-red light, together with the evidence of action spectra for induction and reversal, implicate phytochrome in the control of germination but the effects of blue light are incompatible with an interpretation in terms of phytochrome alone. It is postulated that an uncharacterised pigment absorbing blue light is also involved in the inductive and inhibitory responses but that not all spores contain effective levels of both pigments. Spores containing only phytochrome show characteristic phytochrome-mediated responses; spores containing only the blue light absorbing pigment show only blue light induction of germination; when both pigments are present, the phytochrome response is modified in that the effects of blue and red light are not repeatedly reversible.

Some effects of temperature on germination and protonemal growth in *Asplenium ruta-muraria* and *A. trichomanes*

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The British and European distributions of *Asplenium ruta-muraria* and *A. trichomanes* are very similar (Jalas and Suominen 1972), suggesting that the two species have similar temperature requirements for growth. Laboratory studies have shown, however, that this may not be the case. Germination of *A. ruta-muraria* spores is slower than that of *A. trichomanes* and is virtually non-existent at 15°C (Table 1).

Table 1. Percentage of spores germinating after 20 days. Spores were sown on glass-fibre filter-paper discs moistened with Hoagland's culture solution at constant temperature under white fluorescent tubes with a PAR fluence rate of 40 μmol m^{-2} sec^{-1}. Each figure is the mean of 9 discs

	Temperature (°C)		
	15	20	25
A. ruta-muraria	1	12	30
A. trichomanes	55	80	88

Detailed studies of the distribution of the two species in a small area near Malham Tarn showed that the micro-distribution of the two species differed, *A. trichomanes* being found in deeper crevices (7–28 cm) and *A. ruta-muraria* only in the shallower crevices (1–5 cm). *A. ruta-muraria* also colonises deeper crevices which are south-facing. These results suggest that *A. ruta-muraria* is colonising the warmer crevices, being excluded from the deeper ones by the high temperature requirements of spore germination. Measurement of the temperature of the base of crevices colonised by the two species confirms this conclusion, the mean temperature for *A. ruta-muraria* crevices being more than 6°C higher than that for *A. trichomanes* crevices.