HUMAN HEALTH RISKS FROM FERN SPORES? - A REVIEW

S. E. SIMÁN[1], A. C. POVEY[2] & E. SHEFFIELD[1]
[1]School of Biological Sciences, 3.614 Stopford Building, The University of Manchester, Oxford Road, Manchester M13 9PT, U.K.
[2]School of Epidemiology, Medical School, Stopford Building, The University of Manchester, Oxford Road, Manchester M13 9PT, U.K.

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INTRODUCTION

Ferns do not usually get bad publicity in relation to human health. The most famous exception to this rule is bracken (the genus *Pteridium*), which has been implicated as a health hazard in scientific literature, agricultural publications and British national newspapers (see Fig. 1 and Table 1)*. There are reports relating to adverse health effects of a few other species, but these largely concern livestock. Most cases relate to consumption of green leaves (fronds) or rhizomes, which is undoubtedly most unwise for bracken (see Table 1). Some reports, however, indicate that bracken **spores** may also pose a health risk (see Fig. 2). This has been recognised by the Forestry Commission, who issue their workers with face masks during the sporing season, and the Scout Association (pers. comm.) who discourage their members *"from walking through bracken patches and from using bracken to construct backwoods shelters"*.

In assessing the risks posed by fern spores to human health there are two main aspects to consider. One is whether the spores of any fern species represent a health hazard. The other is to define the extent to which humans are exposed to fern spores, so as to assess the potential risk arising from this exposure. The aim of this review is to examine our current understanding of the potential health risks posed by bracken spores and to consider the extent to which spores of other fern species may present a human health hazard.

PTERIDOPHYTE SPORES AND ALLERGIC REACTIONS

Most reports of ill effects caused by fern spores relate to allergy-like symptoms. Devi *et al.* (1982, 1989) tested skin reactions to whole fern spores and sporangia in rats. All of eight fern species tested produced some kind of reaction. The ornamental species *Adiantum peruvianum, Anemia rotundifolia, Christella parasitica* and *Thelypteris augescens* induced histopathological changes

*In spite of this journal's requirement for plant (and animal) authorities, these have in some places been left out. The reason is that they were not presented in the original papers reviewed and we consider it scientifically indefensible to add them in the absence of assurances of taxonomic accuracy.

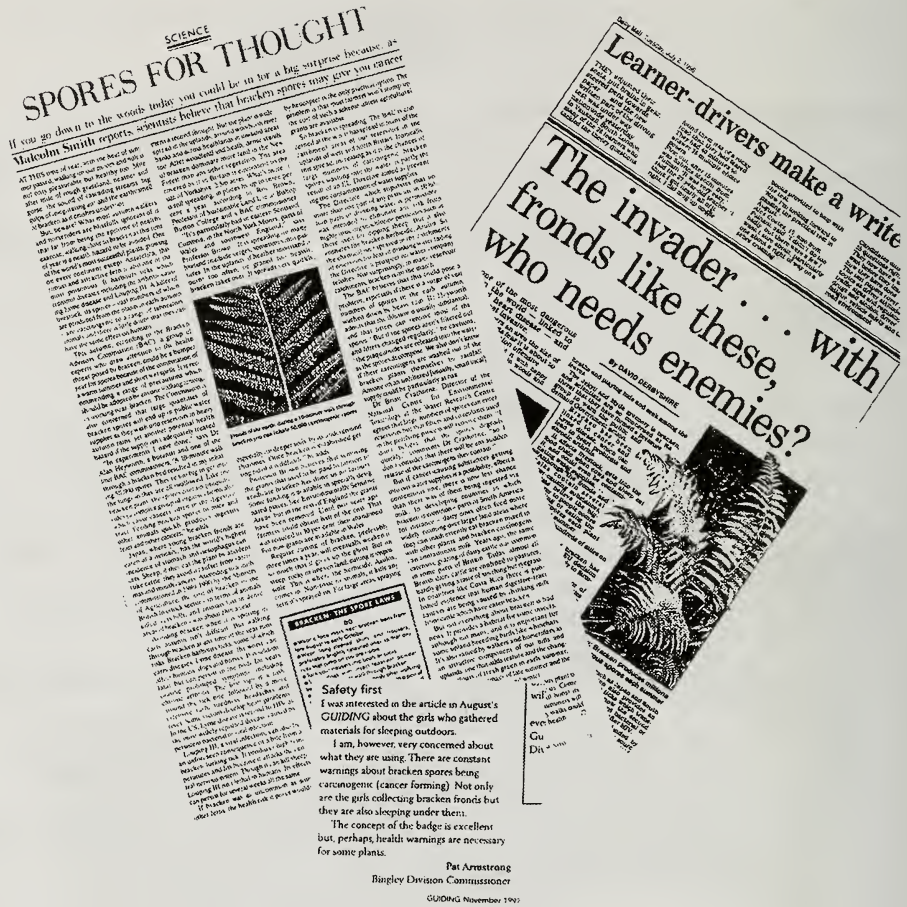
Figure 1. A range of newspapers and magazines have reported the adverse health effects caused by bracken.

Table 1. Fern species whose vegetative tissues (fronds and/or rhizomes) have been reported to induce adverse health effects in various animals.

Species	Adverse health effects	Animals	Reference
Cheilanthes sieberi	acute toxic syndrome	cattle	Clark & Dimmock, 1971
	bovine enzootic haematuria	cattle	Clark & Dimmock, 1971
Dryopteris filix-mas	paralysis	tape-worm	Linnell, 1955
	death	insects	Hartzell, 1947
Notholaena distans	fits of trembling, fatal respiratory paralysis	cattle, goat, sheep	Radeleff, 1964
N. sinuata	fits of trembling, fatal respiratory paralysis	cattle, goat, sheep	Radeleff, 1964
Pteridium aquilinum	urinary bladder tumours, haematuria	guinea pig	Bringuier *et al.*, 1995
	urinary bladder tumours, haematuria	llama	Peauroi *et al.*, 1995
	thiamine deficiency	monogastric animals	Smith & Seawright, 1995
	stomach cancer	human	Evans, 1976; Galpin *et al.*,1990
	apathy, death	pig	Evans *et al.*, 1972
	bright blindness	sheep	Barnett & Watson, 1970
	bovine enzootic haematuria	cattle	Rosenberger & Heeschen, 1960
	decrease in leukocytes and thrombocytes	cattle	Evans *et al.*, 1958
P. sp.	oesophageal carcinoma	human	Kamon & Hirayama, 1975

(i.e. pathological changes detectable via microscopy). The changes started to subside when the exposure was interrupted. In rats exposed to spores of *Acrostichum aureum*, they observed sloughing of the stratum corneum and in those exposed to *Diplazium esculentum*, a slight skin oedema. Exposure to spores of *Abacopteris multilineata* and *Drynaria quercifolia* caused a slight slackening (laxity) of the skin.

Devi *et al.* (1979) used antigens prepared from spores of the ferns *Adiantum peruvianum* Kaulf., *Blechnum occidentale* L., *Cyclosorus parasiticus* (L.) Tardieu and *Microsorium punctatum* (L.) Copel. in clinical allergy tests on 136 patients of


SCIENCE

SPORES FOR THOUGHT

If you go down to the woods today you could be in for a big surprise because, as Malcolm Smith reports, scientists believe that bracken spores may give you cancer

Learner-drivers make a write

The invader . . . with fronds like these, who needs enemies?

By DAVID DERBYSHIRE

Safety first

I was interested in the article in August's *GUIDING* about the girls who gathered materials for sleeping outdoors.

I am, however, very concerned about what they are using. There are constant warnings about bracken spores being carcinogenic (cancer forming). Not only are the girls collecting bracken fronds but they are also sleeping under them.

The concept of the badge is excellent but, perhaps, health warnings are necessary for some plants.

Pat Armstrong
Bingley Division Commissioner

GUIDING November 1997

Figure 2. Possible health effects suffered as a result of inhaling bracken spores have been highlighted in several papers and magazines. (Both of the illustrated reports herein depicted species other than bracken to illustrate the accounts!)

various ages and with different allergy backgrounds. A strong allergic reaction was induced by *A. peruvianum* and *M. punctatum* in 3-5% of the subjects.

Geller-Bernstein *et al.* (1987) reported positive skin test reactions to aqueous fern spore extracts in a group of patients suffering from hay-fever. Seven common household ferns were tested. *Dryopteris setigera* gave the strongest reaction. *Adiantum capillus-veneris, Cyrtomium falcatum* and *Pteris vittata* induced slightly milder reactions. *Nephrolepis exaltata, Platycerium bifurcatum* and *Polypodium glaucum* did not give any detectable reactions. Similar results were obtained by Bunnag *et al.* (1989) when spore extracts of *Achrostichum aureum* L. were used in skin tests and nasal provocation tests. The tests were carried out on patients suffering allergic health problems and on non-allergic patients. Of the former group, 60-70% reacted to the fern spore extracts. Only 15-20% of the latter group did so.

In 1978, Hausen & Schulz reported contact dermatitis caused by *Arachniodes adiantiformis* (G.Forst.) Tindale in a florist. The dermatitis was traced to an allergen which was not specifically ascribed to the spores, but was found present in the plant only during the period of sporogenesis. At that time it was present in the fronds, sporangia and spores. Kobayashi (1980) reported a case of asthma in a dental technician caused by exposure to spores of *Lycopodium*.

In addition to the published literature we have been given anecdotal reports of adverse health effects of fern spores, such as skin rashes in horticulturists working with ferns in glasshouses (Hoshizaki, pers. comm.) and respiratory problems (e.g. constricted breathing and sneezing) and watering of the eyes in fern spore collectors and herbarium curators (Barrington, pers. comm.; Camus, pers. comm.).

DNA-DAMAGING POTENTIAL AND CARCINOGENICITY OF PTERIDOPHYTE SPORES

The DNA-damaging potential of *Pteridium* spores was reported by Povey *et al.* (1995, 1996). They showed that the DNA of mice fed spores or an aqueous extract of fronds from *Pteridium aquilinum* developed adducts, i.e. covalent modifications of the normal bases present in the DNA (O'Connor & Margison, 1990), in the gastrointestinal tract within 6-24 hours of the treatment. The adducts were detected by the ^{32}P-postlabeling assay (Gupta & Randerath, 1988). Preliminary studies of three other homosporous ferns, *Anemia phyllitidis* (L.) Sw., *Pteris vittata* L. and *Sadleria pallida* Hook. & Arn. indicate that their spores also induce DNA adducts when fed to mice (Winston, 1998). Research in our laboratory is currently extending these studies.

Evans (1984) showed that spores of *Pteridium aquilinum* caused leukaemia, gastric and mammary tumours in laboratory mice. Fifty mice were force-fed 0.2 g bracken spores on ten occasions and another 50 were used as controls. The animals in the control group were all alive after a year, whereas 39% of the animals fed bracken spores had developed tumours and died. Villalobos-Salazar (1995) reported lung and mammary tumours in mice caused by intake of *Pteridium aquilinum* spores. Sixty Swiss albino mice (30 of each gender) were fed a diet containing whole bracken spores for 48 weeks. Another 120 animals (60 of each gender) were used as controls and fed a basic grain diet. Mammary tumours developed in 26.6% of the female mice fed a bracken spore-containing diet, compared to 3% among the female controls, a difference that was statistically significant. Villalobos-Salazar (1995) also reported enlargement of the thymus, lower bodyweight and shorter life-span in the mice fed bracken spores compared with the controls. Spores of other ferns and pteridophytes have received less attention than those of bracken, but it is clear that *Lycopodium* spores can cause granuloma (e.g. Antopol, 1933; Erb, 1935).

THE CARCINOGENIC CONSTITUENT(S?) OF BRACKEN FERN

The convincing evidence that bracken spores are carcinogenic suggests that there is at least one carcinogenic compound present in the spores and it is possible that it would be the same compound as in the rest of the plant.

There has been some argument about which compound(s) in *Pteridium* tissues possess the DNA damaging and carcinogenic property. Evans & Osman (1974) suggested that the carcinogenesis was partly due to shikimic acid. This was based on a study in which 9 out of 14 mice treated with 1-100 mg shikimic acid developed cancerous or precancerous lesions within 70 weeks. However, Hirono *et al.* (1977) in a similar study, in which twelve rats (six of each gender) were fed a diet of 0.1% shikimic acid for 142 days, did not find any evidence to support the carcinogenic property of shikimic acid. The dose of shikimic acid per animal was more than twice that contained in an amount of bracken sufficient to induce tumours in 100% of animals in feeding experiments (Hirono *et al.*, 1977). El-Mofty *et al.* (1987) suggested that α-ecdysone was the carcinogenic compound of *Pteridium.* This compound is a natural constituent of *Pteridium aquilinum* (Kaplanis *et al.*, 1967) and has been reported to induce neoplastic lesions in the toad *Bufo regularis* (El-Mofty *et al.*, 1987). Quercetin and kaempferol have also been suggested to be the carcinogens of *Pteridium* (see Smith & Seawright, 1995). The carcinogenicity of quercetin has been investigated several times (see Ito, 1992) and though the results are somewhat controversial, they are mostly negative. Moreover, quercetin is a common compound in various fruits and vegetables and it has even been discussed as a possible anti-cancer drug (see Ito, 1992). Kaempferol is present in *Pteridium* and has been shown to be mutagenic, but there is doubt about its carcinogenic effects (see Smith & Seawright, 1995). Shikimic acid, quercetin and kaempferol have all been designated "*unclassifiable as to carcinogenicity to humans*" by the IARC (IARC, 1983; IARC, 1987). According to the IARC, there is limited evidence to suggest quercetin is carcinogenic to experimental animals, but inadequate evidence for shikimic acid and kaempferol.

In 1983, two research groups independently isolated and characterised a norsesquiterpene glucoside (van der Hoeven *et al.*, 1983; Niwa *et al.*, 1983) from *Pteridium aquilinum.* van der Hoeven *et al.* (1983) also confirmed the ability of this compound to cause mutations in *Salmonella* bacteria. The compound, named ptaquiloside, is unstable under both acid and alkaline conditions (Agnew & Lauren, 1991). In an alkaline environment it generates a dienone which can act as a very strong alkylating agent (Ojika *et al.*, 1987). This dienone, also known as activated ptaquiloside, has recently been reported to cause carcinomas in rats (Shahin *et al.*, 1998). Ptaquiloside has been reported to withstand boiling quite well (Hirono *et al.*, 1978), but this property is not generally accepted. According to Oelrichs *et al.* (1995) temperatures above 40 °C during isolation of ptaquiloside decrease the final yield of the compound. It has been shown that ptaquiloside treatment of thymocytes (cultured thymus cells) in vitro induces DNA adducts that are detectable by the ^{32}P-postlabeling assay (Smith & Seawright, 1995).

Ptaquiloside is ready soluble in water (Ojika *et al.*, 1987). This property lends support to the concerns about drinking water from sources close to *Pteridium*-infested areas (Wells & McNally, 1995) and milk from cows grazing in such areas (Alonso-Amelot *et al.*, 1996; Alonso-Amelot, 1997). So far ptaquiloside has only been found in one other fern species: *Cheilanthes sieberi* (Smith *et al.*, 1989), in which it was present in all tissues except the spores. We have not found any reports on attempts to extract and identify ptaquiloside from *Pteridium* spores.

HUMAN EXPOSURE TO FERN SPORES

Though measurements of human exposure to airborne biological particles are common (see e.g. Lacey & Dutkiewicz, 1994; Anon., 1996), data on fern spores are very scarce. Fern spores are usually either grouped with pollen or not measured. Extant data concerning fern spores are dominated by bracken studies conducted in out-door environments. Two types of methods have been used in these studies: personal and static air sampling. A few studies have been carried out by people using personal samplers. These instruments are designed to draw in air at a rate comparable to human breathing and to deposit any particulate material in the air on to a filter which can be examined under a microscope to determine the amount of the material potentially inhaled. Such studies suggest that a person walking through a stand of bracken **which is sporing** may inhale around 50,000 spores in 10 minutes (Smith, 1996). This corresponds to an air spore concentration of roughly 800 spores l^{-1} (calculated on a tidal volume of 0.5 l and a respiratory rate of 12 times min^{-1} (Guyton, 1987)).

Other studies have involved static air sampling in various locations. A short-term air sampler placed in a fertile bracken stand in Wales during the sporulation season recorded 800 spores l^{-1} air over some days (Povey *et al.*, 1995). A long-term air sampler placed 20 cm from a bracken stand throughout the sporulation season often recorded a daily mean of 750 spores m^{-3} air (equivalent to 0.75 spores l^{-1}). Most of the spores were caught in the morning and very few at night (Lacey & McCartney, 1994). In an on-going ten-year study a long-term volumetric spore trap has been placed on a roof-top 21 m above the ground in Edinburgh, UK. The air concentration of bracken spores has at no time exceeded 2 spores m^{-3} (equivalent to 0.002 spores l^{-1}). The closest sporing bracken stands are 1.6 and 5.5 km away (Caulton *et al.*, 1995; Caulton, pers. comm.). There are closer bracken stands, but they are infertile. Hence chronic exposure can occur in the U.K. in areas remote from bracken stands, but the very low dose suggests that this is unlikely to pose a human health risk. Elsewhere this may be different: e.g. in Bangkok, Bunnag *et al.* (1989) performed air sampling over a year with a Rotorod trap. The trap collects airborne particles on quickly rotating U-shaped arms coated with a sticky film. Fern spores were found to be the third most common airborne propagule in that area, occurring with a frequency of 17 %. Only pollen of sedges (23 %) and grasses (20 %) was more frequent.

It appears from the above mentioned studies that the collected spore load diminishes drastically with increasing distance from the spore source. This agrees with the results of studies designed to measure dispersal distance of fern spores. Nearly all (95%) trapped spores of an 8 m high tree fern were detected within 10 m of the plant (Conant, 1978) and 90% of trapped *Dryopteris dilatata* spores were deposited within 3 metres of the sporophyte in natural conditions (Glaves, 1991). From studies of spore dispersal patterns in *Osmunda* and *Dryopteris* Raynor *et al.* (1976) concluded that most spores settled within a short distance from the source and that only a few were carried further away. There are, however, reports of fern spores having travelled very long distances. Eight years after the formation of the volcanic island of Surtsey a *Cystopteris fragilis* plant was found growing in the bare soil of the island (Fridriksson, 1975). This island is at least 20 km from the nearest possible source of spores. The Hawaiian islands are even further from possible sources and boast a rich and genetically diverse fern flora (e.g. Sheffield *et al.*, 1995). For more examples of long-distance spore dispersal in ferns, see Tryon (1986).

The problem, shared by all spore dispersal studies mentioned above, is that an unknown fraction of the total spore output is not trapped at all. Studies of moss spore dispersal distances (e.g. Stoneburger *et al.*, 1992) lend support to the contention of Conant (pers. comm.) that the trapped spores represent a very small proportion of the total. There have been too few studies on frond fertility or spore output to make generalisations about spore loads in air realistic.

Figure 3. The under-side of a sterile bracken frond shows no signs of sporangia along the pinnule edges. Inset shows indusial flap with no sporangia beneath.

It is important to note that while fertility is the rule for mature individuals of most ferns, infertility is frequent in bracken and, in most years and/or some locations, the majority of fronds are completely sterile (e.g. Conway, 1957; Dyer, 1989). Although a single fertile frond may indeed be capable of releasing up to 300,000,000 spores (Conway, 1952) and large stands may occasionally serve as a vast source of spores, the overwhelming majority of fronds and indeed stands of bracken studied in Scotland and the north of England during the autumn of 1998 were completely sterile (Wynn & Sheffield, pers. obs.). Infertility appears to be the most usual condition for these bracken stands, so it is little wonder that research has not revealed a link between acid soils and leukaemia clusters (Trotter, 1990). The study in question made the assumption that bracken could be mapped to acid soils and hypothesised that therefore the prevalence of leukaemia would be higher in those areas. There are no empirical data supporting the assumption that bracken has a preference for acid soils and the assumption that all bracken stands are a source of spores is untenable. It remains to be seen whether adverse health effects can be linked to proximity to **sporing** bracken stands.

Research is underway in our laboratory to attempt to determine the environmental and genetic factors that govern sporing behaviour of *Pteridium.* In the meantime we suggest that most of the media hype and public fear surrounding bracken stands is unwarranted. Simple inspection of the underside of fronds quickly distinguishes fertile from infertile material, as is shown in Figures 3 and 4, and where fronds are infertile, there is no cause for alarm or need for humans to take precautions against airborne contamination by this species.

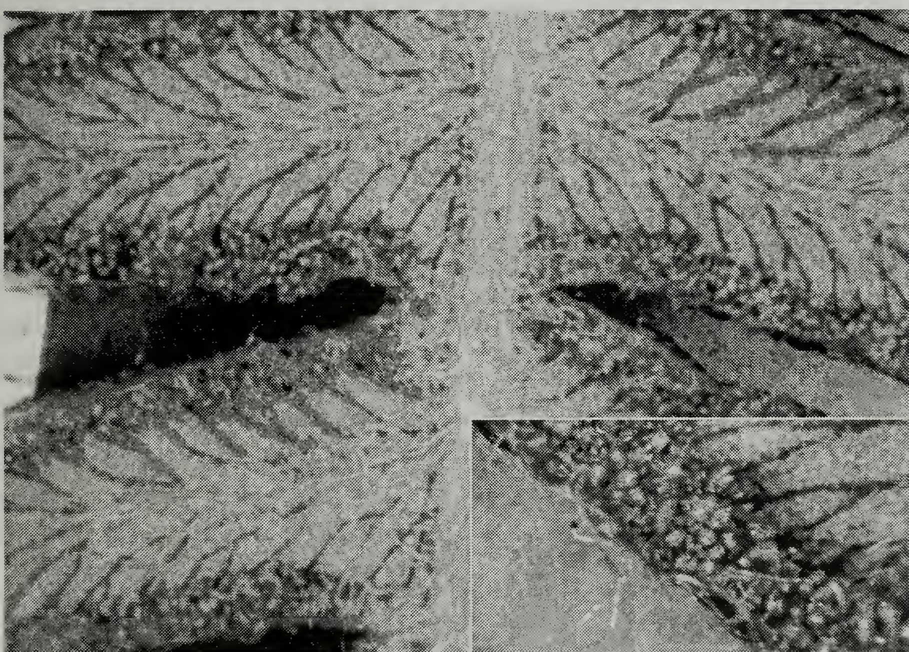


Figure 4. Dense clusters of sporangia line the edges of the under-side of a fertile bracken frond. Inset shows numerous sporangia, which would be visible to the naked eye. Individual spores would be revealed by a hand lens.

Only very limited pilot data are available for air concentrations and potential inhalation rates of fern spores in indoor environments, such as plant nurseries and conservatories (Winston, 1998). The steady increase in the popularity of ferns as ornamental plants (Gress, 1996) has prompted us to expand and extend these pilot studies. Until such time as we are able to publish our findings we **would** advise caution when handling fertile fronds or fern spores. Potential risks from inhalation are easy to avoid by the simple, quick and cheap application of a face mask such as used by industrial workers to avoid inhaling particles (see Fig. 5).

Figure 5. It is advisable (and easy) to take some precautions when handling fertile fronds or fern spores. In the background, one of the many makes of face masks suitable for these cases.

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