FERN SPORE BANKS: IMPLICATIONS FOR GAMETOPHYTE ESTABLISHMENT

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Summary.

Although angiosperm seed banks have been well documented, almost nothing is known about fern spore banks. This paper reviews the published evidence for spore banks and presents new observations made during a wider investigation of gametophyte establishment at two woodland sites near Edinburgh, Scotland. Analysis of soil cores has revealed the existence of large numbers of viable spores, of more than one species, to a depth of at least 35 cm at one site and to at least 95 cm at the other. Moreover, these spore banks are present throughout the year. Additional investigations in other habitats indicate that fern spore banks are widespread. The biological significance of these observations is discussed.

Key words: fern, spore bank, gametophyte establishment.

Resumen.

Al contrario de lo que ocurre con los bancos de semillas de las angiospermas que están bien documentados, prácticamente no se conoce nada acerca de los bancos de esporas de los helechos. Este artículo pasa revista a los conocimientos publicados sobre los bancos de esporas y presenta nuevas observaciones realizadas durante una amplia investigación sobre el desarrollo de los gametófitos en dos zonas forestales próximas a Edimburgo, (Escocia). El análisis de muestras de suelo ha revelado la existencia de grandes cantidades de esporas viables de más de una especie a profundidades de al menos 35 cm en una zona estudiada y al menos a 95 cm en otra. Además, estos bancos de esporas están presentes a lo largo de todo el año. Otras investigaciones adicionales en otros hábitats indican que los bancos de esporas están ampliamente extendidos. Asi mismo, se discute el significado biológico de estas observaciones.

Palabras clave: helecho, banco de esporas, establecimiento de gametófitos.

INTRODUCTION.

Extensive studies since the middle of the nineteenth century have shown that reservoirs of viable seeds exist beneath the soil surface in many habitats. These "seed banks" play a vital role in the survival strategies of some angiosperm species, particularly short-lived colonizers of disturbed ground. However, the possibility that spores might fullfil a similar function in the second largest group of vascular plants, the ferns, has not been properly explored. Although there are several indications that fern "spore banks" might be widespread (Table 1), there is little information in the literature to confirm this or indicate their importance in fern biology. GRIME (1985) considers that fern spore banks are unlikely to have a significant role.

All the published reports of viable fern spores in soil are listed in chronological order in Table 2. In a study of weeds in pineapple plantations in Malaysia, WEE (1974) reported that viable spores of nine fern species greatly outnumbered the angiosperm seeds in the top 15 cm of the soil. STRICKLER & EDGERTON (1976) detected viable spores of *Cystopteris fragilis* in only the top 2cm of soil during an investigation of seed banks in mixed coniferous forest in Oregon, USA. There is a brief reference in an account of a biosystematic investigation on *Athyrium filix-femina* in Europe (SCHNELLER, 1979) to the occurrence in the soil of spores capable of germinating nearly a year after the last period of spore release but the habitat, precise locality and soil depth were not specified. PECK (1980) made similar observations on spores of *Dryopteris goldiana* which had overwintered on the soil surface beneath fertile plants. In a study of bryophyte diaspores in soil, DURING & TER HORST (1983) noted the presence, over a period of twelve months, of at least two unidentified fern species in soil sampled to a depth of 6 cm from chalk grasslands in the Netherlands. In another very similar investigation of bryophyte diaspore banks, DURING *et al.* (1987) discovered at least two species in the top 2 cm of soil from several different habitats in Spain. A detailed investigation of seeds in the top 10 cm of soil from freshwater tidal wetlands on the Delaware River, USA, yielded information on the accompanying spore bank of bryophytes and

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a. Spores of many species require light to trigger germination.

b. Spores of many species remain viable for years when stored under
relatively dry conditions.

c. Large numbers of viable spores exist for many months on the soil surface after dispersal.

d. Spores of species with subterranean gametophytes can enter the soil.

e. Many mosses have spore banks.

Table 1. Indications that fern spore banks might be widespread.

Habitat(s)	Locality	Species	Depth (cm)	Reference
Pineapple fields	West Malaysia	Blechnum indicum Burm., Dicranopteris linearis Und., Histiopteris incisa J.Sm., Lygodium scandens Sw., Nephrolepis biserrata Schott, Pityrogramma calomelanos Link, Pteridium esculentum Nakai, Stenochlaena palustris Bedd., One other species (not identified)	0–15	Wee (1974)
Coniferous forests	Eastern Oregon, USA	Cystopteris fragilis (L.) Bernh.	0–2	Strickler & Edgerton (1976)
Not specified	Europe	Athyrium filix-femina (L.) Roth	Not specified	Schneller (1979)
Deciduous woodland	Central Iowa, USA	Dryopteris goldiana (Hooker) A.Gray	Surface	Peck (1980)
Chalk grasslands	The Netherlands	At least 2 species (not identified)	0–6	During & ter Horst (1983)
Coastal shrubland Deciduous woodland	Barcelona, Spain	At least 2 species (not identified)	0–2	During et al. (1987)
Tidal marsh	Delaware River, USA	Athyrium filix-femina L. var. angustum (Small) Rydb., Dennstaedtia punctilobula (Michx.) Moore, Dryopteris spp., Onoclea sensibilis L., Thelypteris palustris Schott, Woodwardia areolata (L.) Moore, Woodwardia virginica (L.) Smith	0–10	Leck & Simpson (1987)
Chalk grasslands Grazed pasture Deciduous woodland	The Netherlands	At least 2 species (not identified)	0–1	van Tooren & During (1988)
Forests	Switzerland	Athyrium filix-femina (L.) Roth, Dryopteris spp.	0–65	Schneller (1988)

Table 2. Published reports of viable fern spores in soil.

pteridophytes which was published separately (LECK & SIMPSON, 1987). Although *Onoclea sensibilis* was the largest component of the spore bank, a total of seven species was recorded. VAN TOOREN & DURING (1988) found viable spores of at least two unidentified fern species in the top 1 cm of soil from several habitats in the Netherlands and discovered that some fern spores retain their viability after passing through the guts of earthworms. Recently, in a short account of spore bank studies at four forest sites in Zwitzerland, SCHNELLER (1988) showed that soil taken from within populations of fertile sporophytes shortly after spore release, contained viable spores of the locally dominant ferns to a depth of at least 65 cm, with the majority in the first 10 to 15 cm.

Although SCHNELLER identified some of the possible implications of these observations, current knowledge of spore banks is too fragmentary to permit their contribution to the reproductive strategies of ferns to be fully understood. Much more information is needed on the ecological distribution, identity, movement, longevity and potential for establishment of buried spores. Wherever possible, investigations should be conducted throughout the year as part of a broader enquiry into the biology of particular species and especially into the dispersal of spores and the establishment of gametophytes in the wild.

Most of the observations presented here derive from studies commenced in 1987 as part of a wider investigation of gametophyte establishment in four species native to Scotland.

MATERIALS AND METHODS.

The four species chosen for this study were:*Athyrium filix-femina* (L.) Roth, *Blechnum spicant* (L.) Roth, *Polystichum setiferum* (Forsk.) Woynar, and *Phyllitis scolopendrium* (L.) Newm. *A. filix-femina* and *B. spicant* have a northerly distribution and a preference for acidic soils. In contrast, *P. setiferum* and *P. scolopendrium* have a southerly distribution and a preference for calcareous soils. Suitable populations of *A. filix-femina* and *B. spicant* occur near each other at Roslin Glen Wildlife Reserve, near Roslin, 6 miles south of Edinburgh. Pease Bridge Glen, near Cockburnspath, 45 miles east of Roslin, is the nearest site with suitable populations of *P. setiferum* and *P. scolopendrium*. Both study sites are areas of mixed deciduous woodland in small river valleys where ferns are abundant and sexually reproducing.

The possible existence of viable fern spores in the soil was investigated using a simple technique based on that used by FURNESS & HALL (1981). Using a 9 cm diameter corer, cores of soil were removed from the ground near mature sporophytes and transferred directly to polythene bags to prevent contamination by air-borne spores. In the laboratory, the cores were chopped into 5 cm strata from the centre of which smaller cores were taken as subsamples in a further attempt to minimize contamination. Finally two replicate subsamples from each stratum were separately sealed in small plastic petri dishes (diameter = 5 cm, area = $c.20$ cm^2) and cultured in a growth chamber at 20° C + 2 C° under continuous illumination (irradiance = 20 uEm^2 s) provided by four 30 Watt "Warm White" fluorescent tubes. The samples were kept moist during the culture period by adding sterile distilled water to the petri dishes when necessary. After approximately 8 weeks, the presence of fern gametophytes was determined with a dissecting microscope. The total number of visible prothalli was recorded, distinguishing between those with trichomes and those without. In these investigations, no attempt was made to identify the gametophytes further.

This method of analyzing soil cores has disadvantages. For instance, it only reveals the number of viable spores on or near the soil surface that are exposed to light and subsequently germinate. It is not possible to deduce from these values the total number of viable spores in the soil samples. In addition, nothing is learnt about the number and identity of non-viable spores in the soil. This information is clearly essential if spore banks are to be defined accurately.

In future, detailed information on the total number of spores, their identity and distribution might be obtained more rapidly by extracting viable and non-viable spores directly from soil (Furness & Hall, 1981) and subsequently identifying them using a light microscope. Percentage viability could then be determined by culturing these spores on mineral agar.

OBSERVATIONS.

This investigation is still in its early stages and only preliminary results are available. However, it is already possible to recognize several important characteristics of fern spore banks.

Fern spore banks are widespread.

Viable fern spores have been found in every soil core collected at the two main study sites. Unpublished observations at other sites have revealed spore banks in the soil on open hillsides and in pastures, arable fields and urban parks in Scotland and in the soils of forests and abandoned fields in North Carolina, USA. These observations, taken in conjunction with the limited information in the literature, are clear indications that fern spore banks are widespread both geographically and ecologically. Further studies are underway to define more accurately the extent of their distribution.

Fern spore banks are found to a considerable depth.

Viable fern spores have been repeatedly found at depths of 20-30 cm. On two occasions, viable spores were found 95 cm below the surface. It is likely that viable spores might exist even deeper in suitable soils but practical difficulties were encountered when trying to obtain soil samples one metre or more below the surface.

Typically, the number of viable spores producing gametophytes on the surface of the cultured soil declines as the sampling depth increases (Figure 1). This might be simply due to a reduction with depth in the total number of spores present because of their restricted downward movement in the soil. However, it is also conceivable that the proportion of spores that are viable might decline with increasing depth. For instance, there is likely to be a loss of viability with age and the age of spores might increase with depth as successive annual depositions move downwards through the soil. In addition, the inherent longevity of spores might decline with depth because certain types of spores, for example small ones with less stored reserves, move further in the soil. The viability of more deeply buried spores might also be adversely affected by increasing anaerobiosis or accumulating phytotoxic or allelopathic substances.

Another intriguing observation is that the gametophytes appearing on the deeper soil samples develop more slowly than those on soil samples collected from nearer the surface. Again the reasons are not yet known. Slower development might be a precursor of spore death caused by one or more of the factors suggested above. It is known that spores stored for several years in herbaria or laboratories germinate more slowly than fresh ones (WINDHAM et al., 1986). It might also be significant that smaller spores develop more slowly, at least initially, than larger spores of the same species (SCHEDLBAUER, 1976; DYER, unpublished observations). A further possibility, in view of the fact that the spores are cultured on the soil from which they were sampled, is that soil taken from below the surface limits the rate of development through nutritional deficiency or some other inadequacy.

Further investigations are in progress to establish which of these explanations account for our observations.

Fern spore banks are present from one spore release period to the next.

Analysis of soil cores at the end of July, just before a new crop of spores was released, showed that substantial numbers of viable spores were still present in the soil at all depths (Figure 1). Clearly, some spores can survive in the soil for at least one year. Despite reports that spores of some species can survive several decades when stored under relatively dry conditions (SUSSMAN, 1965; LLOYD & KLEKOWSKI, 1970; WINDHAM et al., 1986) it has not yet been established that spores can survive for more than one year in the soil, where they are likely to be partially or

fully imbibed. However, laboratory experiments have now shown that the viability of imbibed spores of *A. filix-femina, B. spicant, P. setiferum* and *P. scolopendrium* does not decline during the first 8 months of storage in darkness at 20 C. Other long term storage experiments currently in progress will yield additional information about the longevity of imbibed spores.

Fern spore banks consist of more than one species.

In almost every case, even when cores are taken immediately beneath sporing fronds, the appearance of some gametophytes with trichomes and some without, indicates that at least two species are present. Gametophytes differing in trichome characteristics were sometimes observed, indicating that there were more than two species present, but accurate identification to species using gametophyte morphology is difficult and was not attempted. However, gametophytes can be identified further if necessary. Gametophytes can be cultured longer and those which produce sporophytes can be identified on the basis of sporeling morphology. Alternatively, starch gel electrophoresis, although destructive and expensive, could be used to discriminate between species with morphologically indistinguishable gametophytes (SOLTIS *et al*, 1983; Kelly & Cousens, 1985).
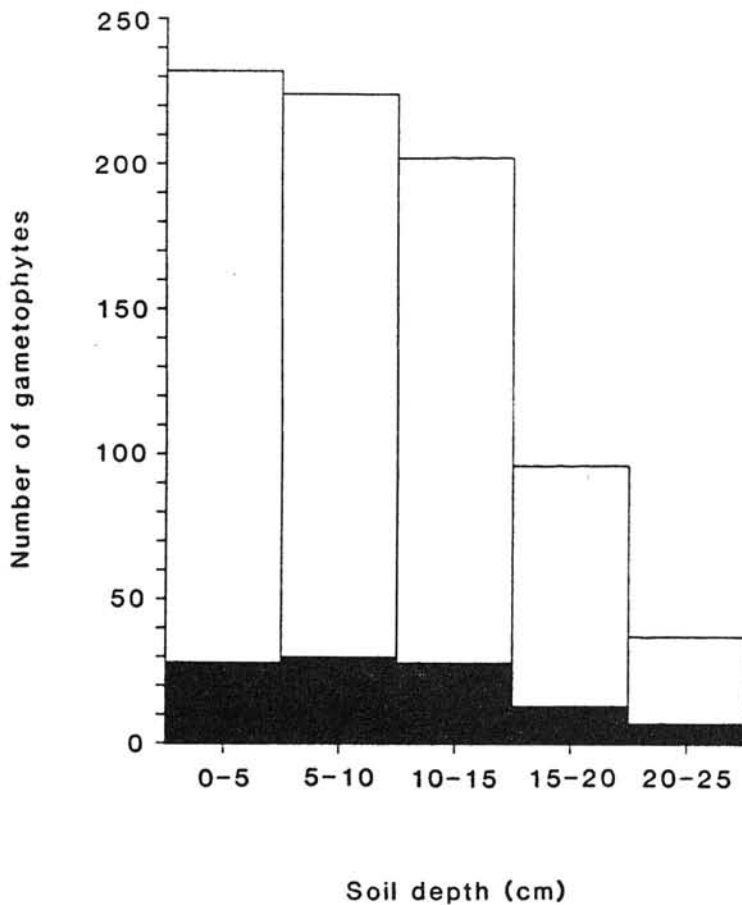

Soil depth (cm)

Fig. 1. A typical distribution of viable fern spores in soil.

Relative estimates of the number of viable spores at various depths were obtained by culturing soil samples, each with a surface area of approximately 20 cm², and counting the gametophytes produced. Gametophytes with trichomes, were distinguished from those without.

The results shown here were obtained by analyzing a soil core collected from Roslin Glen Wildlife Reserve in July 1988, a few weeks before spores release.

IMPLICATIONS.

Clearly, much more information is required to fully understand the role of fern spore banks. Nevertheless, it is possible to speculate on the biological significance of our observations and some other observations reported in the literature.

Spore bank formation.

There are three main ways in which spores could become buried in the soil: by the deposition of soil or humus above them; by percolation, the passive transport of spores by water; or by animal activity. Whatever the process, it is tempting to believe that the viable fern spores found buried to a depth of 95 cm are very old, having reached this level over many years. However, this conclusion is premature while so little is known about the method(s) or rate of movement of spores in soil.

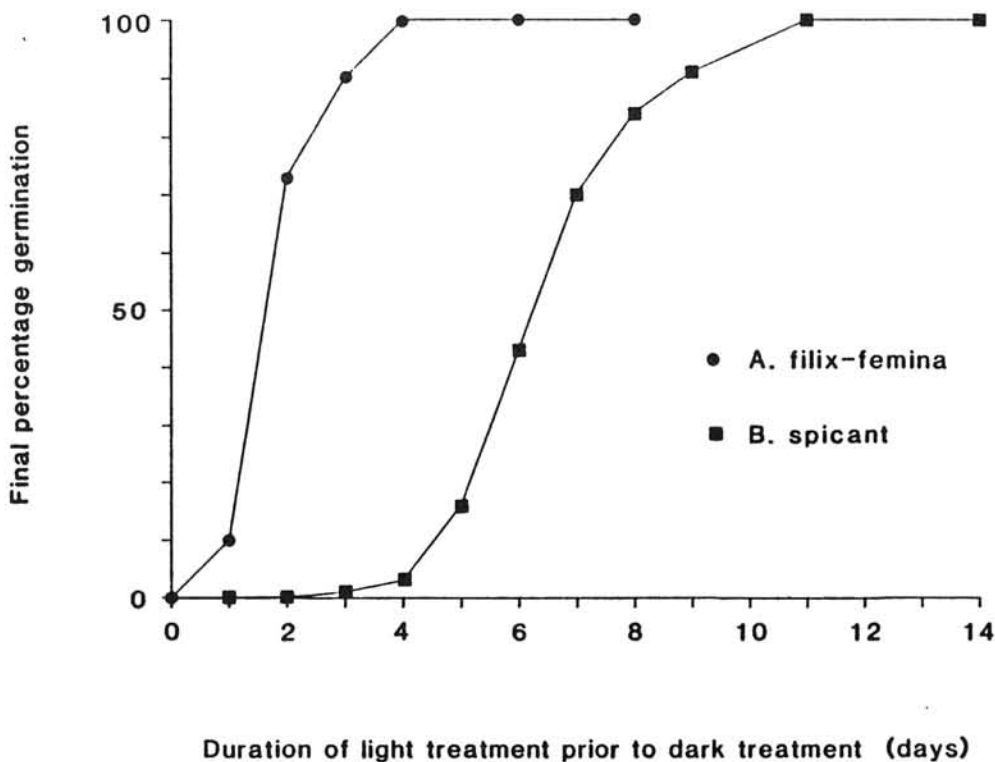Duration of light treatment prior to dark treatment (days)

Fig. 2. The time required for light to trigger germination of spores of *A. filix-femina* and *B. spicant* at 15°C.

The spores were sown on mineral agar in small petri dishes and placed in a growth chamber providing a constant temperature of 15°C and continuous illumination (irradiance = $20\mu Em^{-2}s^{-1}$). One petri dish of each species was removed from the growth chamber every 24 hours for the next 14 days and cultured for another 20 days at the same temperature but in complete darkness. Percentage germination was determined at the end of the dark treatment.

Photoblastic spores do not normally germinate in darkness but they will if germination has already been initiated by light. Accordingly, any germination observed in these experiments must have been triggered by the light treatment received prior to the dark treatment.

There is no evidence that fern spores have an inherent dormancy when released. Indeed, most fern spores will germinate as soon as they receive adequate moisture and light and experience a suitable temperature. Darkness can enforce dormancy on photoblastic spores but only if germination has not already been initiated by light. These observations suggest that if spores are to remain dormant in nature, they must either settle on a surface where there is insufficient moisture for imbibition and/or inadequate light to trigger germination, or they must enter the dark recesses of the soil before germination is initiated. Laboratory experiments conducted at 15°C have shown that 50% of spores of *B. spicant* become photosensitive and will germinate, even in subsequent darkness, after receiving moisture and light for approximately 6 days. The time required to trigger germination of 50% of spores of *A. filix-femina*, under the same experimental conditions is less than 2 days (Figure 2). These experiments imply that the initial movement of spores into soil must be rapid. The depth to which light can penetrate soil depends on the soil type and its physical state (WOOLLEY and STOLLER, 1978). Thus, while some spores might experience total darkness within a few millimetres of the soil surface, others must reach a depth of a few centimetres before they can escape from light and contribute to a spore bank.

Except in situations where there is rapid soil deposition or leaf fall, the recruitment of spores into spore banks within hours or days of deposition is most likely to result from percolation. The rate of percolation is probably influenced by spore size, shape and degree of surface sculpturing and this might in turn result in different species being represented at different depths in the soil. Percolation of spores could be extremely rapid if spores are washed into channels in the soil left by decayed roots or burrowing animals. In some habitats, transport by the animals themselves might be the major cause of spore movement within the spore bank and in certain soils, the activity of earthworms could be particularly important. VAN TOOREN & DURING (1988) report that some fern spores retain their viability after passing through the guts of earthworms and other investigations have shown that earthworms can transport pollen grains through a vertical distance of 55 cm in 6 weeks (WALCH et al. 1970). These observations strongly suggest that earthworms could be responsible not only for downward movement of fern spores in soil but also for upward movement, returning spores to the surface where conditions might be suitable for germination and gametophyte establishment.

Dark germination.

Laboratory experiments have shown that antheridiogens produced by gametophytes of some species can induce photoblastic spores to germinate in the dark. For instance, an antheridiogen produced by cultured prothalli of *Athyrium filix-femina* triggered germination of spores and resulted in the development of dwarf males of that species 1 cm below the soil surface (SCHNELLER, 1988). An antheridiogen of *Anemia phyllitis* is reported to have had a similar effect on spores of that species as much as 15 cm below the soil surface (see SCHRAUDOLF in SCHNELLER, 1988). These, and other observations (NÄF, 1979; SCHNELLER, 1979) suggest that antheridiogens might be important in nature for recruiting male-fertile gametophytes from spores that are not exposed to light. This is certainly an intriguing possibility but it has still to be established that antheridiogens do function like this in nature.

Colonization.

Viable spores can exist in the soil from one spore release period to the next. This suggests that even in a seasonal climate, where spore release is restricted to a few months of the year, there is a potential at any time of the year, for gametophyte establishment following soil disturbance. For instance, successful gametophyte establishment might take place in the Spring, as well as, or even instead of, the Autumn. Soil disturbance, such as that caused by wind-throw of trees, erosion by water or animal activity, will encourage gametophyte establishment, not only by exposing spores to light but also by providing a bare substrate which many gametophytes appear to prefer. In addition, spores in the soil will be protected from many of the hazards present on the surface and soil disturbance following an above-ground catastrophe such as fire could result in rapid re-colonization by species represented in the spore bank.

Accumulation.

A long-lived spore bank will accumulate deposited spores from year to year. This will increase the chances of colonization of fern species which are rare or distant. Accumulation of spores in the soil is likely to be particularly important for peripheral or disjunct populations where conditions suitable for spore production and/or gametophyte establishment might be infrequent. The concentration of spores in the soil, amongst other factors, might indirectly influence the breeding systems of gametophytes. For instance, as the number of viable spores in the soil increases, then so too will the opportunity for inter-gametophytic mating between gametophytes of the same species, including those from different sporophyte populations or even from different generations.

Hybridization.

A spore bank consisting of two or more species has the potential to initiate mixed gametophyte colonies and some of these might produce hybrid zygotes. Obviously, for hybridization to occur, not only must the participating species be closely related but the gametophytes of these species must have similar ecological requirements. Where only some of the species in a mixed spore bank are capable of establishing on the soil surface, opportunities for hybridization might be restricted.

Conservation.

It is conceivable that a long-lived spore bank could conserve a larger gene pool than is present in the sporophyte population on the surface. Recruitment from these spore banks could re-introduce alleles that have been eliminated by selection. Moreover, long-lived spore banks might even provide a means of re-establishing native populations at sites where they are thought to have become extinct.

CONCLUSION.

It is now evident that reservoirs of viable fern spores do exist throughout the year beneath the soil surface in many temperate habitats. Although most spores in the soil undoubtedly die, fern spore banks can have important implications for gametophyte establishment in some species.

Further studies are underway to confirm the importance of spore banks in the biology of temperate ferns and to compare their role with that of angiosperm seed banks.

ACKNOWLEDGEMENTS.

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Note added in proof

Since completing this article, we have discovered four other published reports of viable fern spores in soil. These are listed below in chronological order:

CLYMO, R.S. and DUCKETT, J.G. (1986). Regeneration of Sphagnum. *New Phytologist*, 102: 589-614.

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