Issues in Fern Spore Propagation

Introduction

Ferns differ from flowering plants in that their sexual reproduction is by spore, not seed. Whilst there are some similarities between spore and seed, the biological processes involved in producing a new plant are very different. Ferns are often regarded as having 'two-stage' germination as their gametophyte is a separate entity to their sporophyte (Haufler 1997) (Sakamaki 1999).

Ferns have a number of distinctive features which can be used to help classify them into families. Families often have similar growing conditions, so accurate identification helps identify cultural requirements for species propagation. Germination of fern spore can be considered equivalent to seed germination in seed bearing plants (Edwards and Miller 1972). In fact, a gametophyte is more closely related to a flower in its function than a seed. Figure 1 shows the function of the gametophyte in fern life cycle.

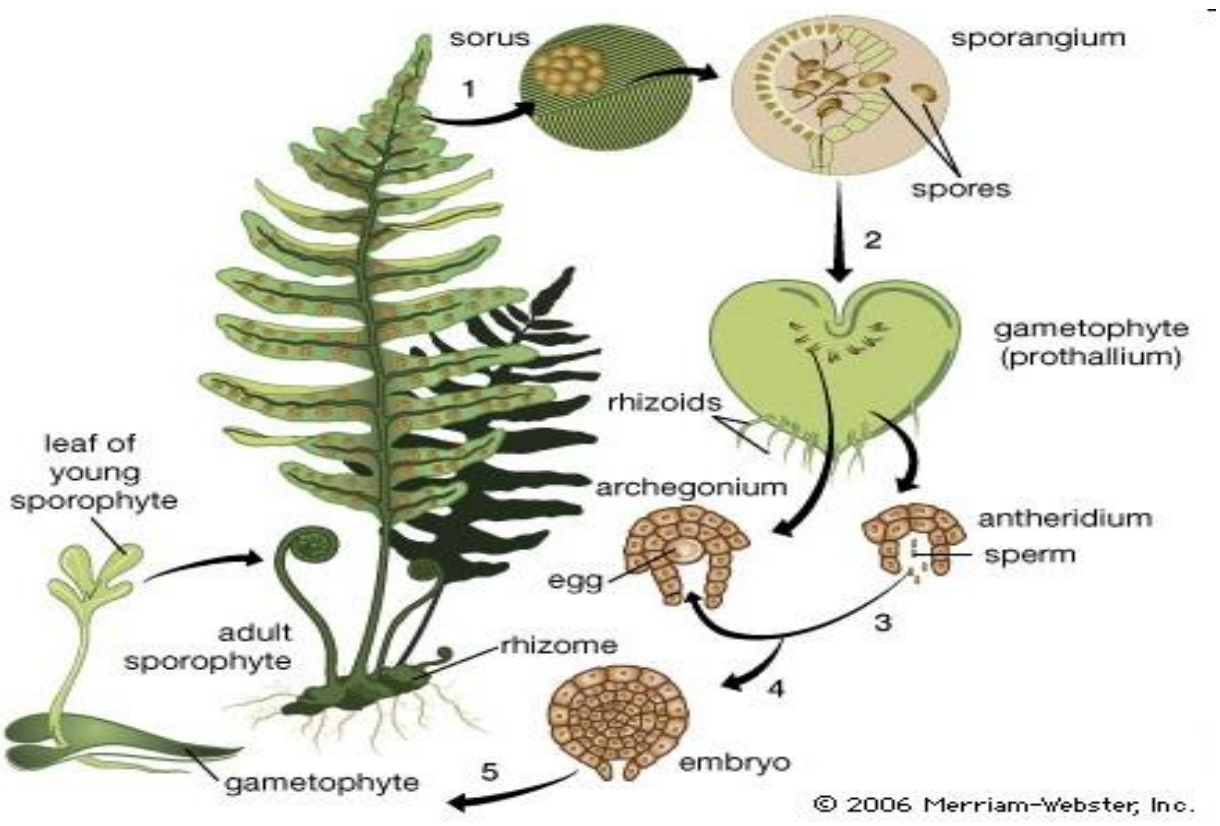


Figure 1: Fern Life Cycle (from Encyclopaedia Britannica 2011)

One way to identify a fern family is by the pattern and development of the sori (spore containing structure). This is most often found on the underside of the frond, but there are exceptions. Sori develop and mature over a period of time, and once ripe, spore is released. The spore release can happen very quickly, or more slowly, depending on the species.

Understanding requirements for spore germination - spore dormancy, identification of green spore, subterranean spore etc - is essential prior to sowing. Once understood, propagation by spore is a rewarding process with ferns able to be raised in large quantities. However, it takes time and patience, with the period from spore sowing to realising mature plants ready for sale often taking two years or more (Goudey 1985).

Spore Collection/Parent Material

Freshness and cleanliness are the two most important factors when collecting propagating material. Fresh spore germinates faster and at a higher rate than older material. The usual method of collecting spore is to identify a fertile frond on the plant to be propagated which is either already releasing spore, or shortly about to (Fliflet 1961). A 10x hand lens can be used when examining plants to see whether spore is mature (Goudey 1985).

A frond which is too young to release its spore will have whitish-green sori. Collecting this material is pointless as spore will not develop once removed from the plant. A frond which has already released its spore will show ragged openings, and is often brown. If there is no other material available, this is sometimes worth considering. In the mechanism of spore release, the annulus does not release all of the spore material, generally leaving a few grains of spore behind. ‘Scraping’ the spore with a sharp knife can help obtain a few grains of spore where none other is available (Hoshizaki and Moran 2001).

The best spore comes from the healthiest, most vigorous plants and is generally the easiest to germinate. Spore is collected by placing the fertile frond face down on a piece of paper, which is then covered and placed in a paper bag. Plastic bags should not be used for spore collection as damp plant material may lead to mould developing. The paper bag is placed in a warm, dry location, and the spore releases over the course of 24 – 48 hours

(Hoshizaki and Moran 2001). The name of the fern and date collected should be recorded (Goudey 1985).

Fern spore has the consistency of fine dust, and varies in colour from white, pale to rich yellow, green, brown and black, depending on which species is being propagated. The frond also sheds other material while releasing spore, including hair and spore casings. Any indusium covering the sori will either fall off or wither during spore release (Hoshizaki and Moran 2001).

The organic material is generally fairly harmless, but can be removed by tilting the collection paper – it slides off first, leaving almost pure spore behind. If there is serious risk of contamination from other sources e.g. spore from fungi and other fern species (found in surrounding air and on the surface of the frond) then the spore must be cleaned prior to sowing (Hoshizaki and Moran 2001).

Cleaning of spore can be done in two ways: if the frond has yet to commence spore release, it can be washed in a mild (10%) bleach solution with a little detergent as an emulsifier, and left to dry in a pre-sterilised paper bag, so that clean spore/plant material is the only material released; Alternatively, the spore itself can be washed in a weak (10%) bleach solution, with a little detergent as an emulsifier, and dried on pre-sterilised blotting paper in an enclosed space. Most growers do not sterilize spores before sowing (Hoshizaki and Moran 2001).

Chlorophyllous spore

Some spore is chlorophyllous, or ‘green’, meaning that it continues to photosynthesise after being shed by the parent plant. This effectively shortens the life span of the spore. Green spores have an average life span of 48 days, while non-green spores live for about 3 years. Research into storage to extend the viability period of green spore is continuing (Raghavan 1989).

Chlorophyllous spore tends to germinate faster than non-chlorophyllous spore, possibly due to photosynthetically active pigments allowing growth to commence quickly on finding suitable germination conditions. Green spores also have higher water content than non-green spores, so their life span may be increased by avoiding desiccation through water loss (Raghavan 1989).

Spore Storage

Spore viability varies considerably among species and is predicated on the presence of chlorophyll. Some non-chlorophyllous spore will live for up to 130 years if stored correctly.

Spores maintained under wet conditions have shown a greater ability to germinate than those maintained under dry conditions (Lindsay *et al.* 1992). Wet storage at 5ºC is optimal for spore storage since it maintains high viability, minimises bacterial and fungal contamination and prevents germination in the dark (Quintanilla *et al.* 2002). However, even under desirable storage conditions, a decline in viability is seen with increasing spore age. The addition of sucrose to the germination medium can improve the germination of fern species stored dry at 4ºC, but the size of the effect lessens over time (Raghavan 1989).

Fern spore may remain dormant until a special condition is met. The enormous quantity of spores produced by a fern provides an effective method for dispersal into space, while an enforced period of rest ensures their dispersal in time. Breaking spore dormancy requires hormones and light, but the extent of these are not fully understood (Raghavan 1989). Appendix 1 provides a summary of viability and spore germination time for a variety of fern species (both chlorophyllous and non-green) (Lloyd and Klekowski 1970).

Containers

Most nurseries use large trays or nursery flats and cover them with glass, or large pots wrapped in clear plastic bags. Containers are sealed to prevent drying and contamination. Condensation should ideally run down the container sides to avoid any ‘wet spots’, as dripping onto the media may provide a site for fungal contamination (Goudey 1985).

Media

Spore must be sown onto an appropriate medium for germination to occur. The medium should be a fine material such as peat moss or finely ground sphagnum moss as the germinating spore must have ready access to a moist substrate in order to become established. Commercial growers in Australia use a blend of peat moss or its equivalent with either sand, fine gravel or perlite. A Jiffy pot, expanded and spread over a coarser

mix such as seed raising mix is ideal. Spores of most ferns germinate in a slightly acidic or neutral pH range, although this is highly species dependent (Raghavan 1989). Peat is often acidic, so may require the addition of lime to neutralize the media (Goudey 1985).

Sterilisation

Sterilisation of the media is required prior to sowing to avoid contamination by invasive ferns, mosses, fungus and algae as well as harmful insects such as fungus gnats. This is commonly done using boiling water, microwave or steam. The temperature of the media should be raised to approximately 95ºC and kept at that temperature for about 30 minutes. Raising the temperature higher than this should be avoided as it can destroy beneficial bacteria (Goudey 1985).

It is possible to sterilize the medium in the pots. This is done by pouring water through a colander directly into each pot, then covering each container with clear plastic until they have cooled enough for planting. If pouring boiling water into a container, it is essential that the container have drainage holes (Goudey 1985).

Sowing of spore

Spore is added to the surface of the media. For best results the spore should not be applied too thickly, or the spores will not develop properly due to overcrowding. Also prothalli will grow upwards towards the light, instead of flat against the medium and eventually rot. Spore can be suspended in cooled, boiled water, then sprayed onto the surface of the media or tapped in gently with the tip of a knife blade. Because fern spores are easily airborne, they may contaminate other sowings so successive sowings should be avoided (Hoshizaki and Moran 2001). A humid environment, keeping surrounding air still and spraying the work area with a fine mist of water prior to, and after, sowing also minimises contamination.

Once the spore has been sown, the container should be covered immediately with a clean sterilized piece of glass or clear plastic. It should be labeled with details of the fern species, spore source, and date. A dark treatment for up to 14 days after sowing prior to light exposure results in synchronization of development, ensuring gametophytes develop at the same time (Gantt *et al.* 1965).

Gametophytes

Germination

As with seeds, the first step in fern spore germination is imbibition. This allows the spore contents to rehydrate, particularly the storage granules and chromatin. For imbibition and germination, spores must be on a moist surface, with favourable light and temperature conditions, surrounded by an atmosphere at or close to moisture saturation point. Most viable spore will germinate in a few days to a few weeks, depending on the species (Fliflet 1961). In non-green spore, energy for germination comes initially from the hydrolysis of storage proteins to simpler molecules (Raghavan 1989).

After six weeks to three months, a pale green film appears on the surface of the medium. This signals that prothalli are developing, and the container should not be opened until prothalli have developed (Goudey 1985). The prothallium stage occurs as the gametophyte grows and develops its characteristic shape and size. It produces hair like rhizoids which allow it to attach to the surface of the medium and access soil moisture (Fliflet 1961). Figure 2 shows standardized prothalli, with rhizoids and transport systems.

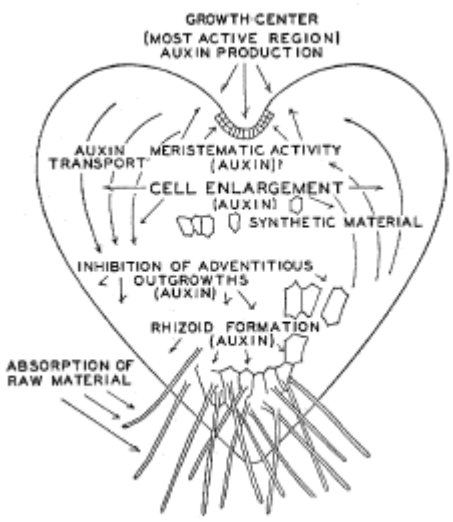


Figure 2: Fern prothalli (from Albaum 1938).

The gametophyte is the fern equivalent of a flower as it contains the male gamete (antheridia) and female gamete (archegonia). When conditions are appropriate, the antheridia swell and release sperm, which swim to an ovary located in a nearby archegonia. Fertilization then occurs, producing the embryo, and in turn, the sporophyte (or adult plant) (Fliflet 1961).

Abiotic factors

Light

The majority of fern species require light for germination, which is not related to day length (unlike angiosperms). Spore requiring light can be placed under a constant light source – no exposure to dark is required. Light controls most early developmental processes of fern gametophytes. Some fern species have subterranean spore, and will only germinate in darkness e.g. *Botrychium spp.* and *Ophioglossum spp.*, but these species are uncommon in cultivation.

Light frequency *is* important. Light in the near-red spectrum encourages growth, whilst light from the far-red spectrum inhibits it. Blue light may inhibit germination to an extent, but is necessary for normal gametophyte development. The higher the number of hours of light applied per day during the first ten days, the higher the germination percentage (Towill and Ikuma 1975) (de Paula Fiilppini 1998).

Light intensity is also important. Direct sunlight is much too strong for the developing prothalli - ideal light intensity for developing prothalli is between 1,615 and 5,382 lux (Edwards and Miller 1972).

Temperature

The ideal temperature range for spore germination is between 22 – 26°C. The temperature should be maintained to keep the humidity constant around the germinating spore. Variation in temperature can lead to condensation or dry spots forming on surfaces surrounding the prothalli. Condensation may form drips leading to ‘wet spots’ on the media surface which can lead to the development of fungi, algae and other problems and result in destruction of the prothalli (Fliflet 1961).

Lower temperatures can be used to obtain hardier plants (Fliflet 1961) and bottom heat can be applied to spore in cool temperate areas to assist in germination (Goudey 1985) provided the temperature is kept reasonably constant.

Moisture

High media moisture is required for spore to imbibe water. Once imbibed, the spore will split open and commence growth. The developing gametophyte first develops rhizines (thin, hair-like roots) to anchor to the substrate and as a mechanism to obtain moisture and nutrients. Constant moisture is required to ensure these tiny structures can access moisture and avoid drying out. If the medium was thoroughly moistened and the planting covered, watering should be unnecessary after sowing. This notwithstanding, water can be added through the bottom of the container by placing it on a capillary mat or in a shallow container if required (Hoshizaki and Moran 2001).

Once the prothalli have developed, they require misting with water. This provides a film of water which allows the motile sperm to travel to the archegonia (Fliflet 1961). The sperm fertilize the egg, producing a zygote, then an embryo. This then develops into the sporophyte. Potter (Heatons 2011) advises that in their nursery the newly germinated prothalli covered nursery flats are organized on gently sloping benches in a 'mist room' which mists for 10 seconds every 5-10 minutes (depending on the season) until most of the prothalli have 'shot' (developed sporophytes).

Nutrition

Nutrients are required for all processes in the growth and development of the prothallus as well as sporophyte formation (Fernandez *et al.* 1997) (Fernandez *et al.* 1996). Spore is similar to seed, as it contains an initial food supply in the form of protein granules. Germinated spore forming prothalli on the surface of the media develop chloroplasts, and obtain nutrition by photosynthesis. Rhizines both anchor the prothalli to the substrate, and extract nutrition from the media.

Prothalli can benefit in growth and development from regular application of fertilizer. Given the prothalli are very small and thin (a single molecule thick in some cases) half-strength nitrogenous fertilizer should be applied weekly or fortnightly to maximize its effectiveness (Goudey 1985).

The addition of plant hormones or sucrose/sugar/glucose solution may be beneficial in spore germination. Indole acetic acid is found in the meristems of the prothalli, and the addition of a weak solution to the media may be beneficial to the developing

gametophytes (Hoshizaki and Moran 2001). The addition of sugars must be done with some care, as excess sugar is phytotoxic to prothalli and increases the rate of plant tissue ageing. It may also lead to contamination from fungi or algae (White 1971) (Fernandez *et al.* 1999).

Biotic factors

Antheridiogen

Antheridiogen is a plant hormone (pheromone) released by gametophytes which induces the development of male sexual characters in nearby gametophytes. Ayrepetov and Ganger (2009) reported that the percentage of male gametophytes increased with increasing density of gametophytes probably due to the cumulative effect of antheridiogen production. That is, overcrowded prothalli may lead to the absence of archegonia, making fertilization impossible.

Nutrition appeared to have no influence on this situation (additional nutrition can often influence the development of sexual characters). A large variation in the occurrence and sensitivity of plants to the hormone has been observed in individuals, fern species and populations (Prada *et al.* 2008). The operation of an antheridiogen system is an important consideration when sowing spore, and care should be taken not to sow the spore too thickly.

Asexual reproduction (Apogamy)

Variations on the spore reproductive process exist, and depend on the individual fern species. Common, easy to grow ferns are often apogamous. They can generally produce sporophytes faster than other species as they do not require sexual organs or the energy for sexual reproduction, and produce sporophytes without. Ferns from drier climates also use this form of asexual reproduction, as it avoids the requirement for a film of moisture, necessary for motile sperm.

Conditions causing apogamy in species which would otherwise reproduce sexually include lack of sufficient moisture, insufficient nutrition leading to reduced vitality of gametophytes, and changes in light intensity. The best apogamous growth can be induced by incorporating 2.5% sucrose into the media, and illuminating with white light, blue

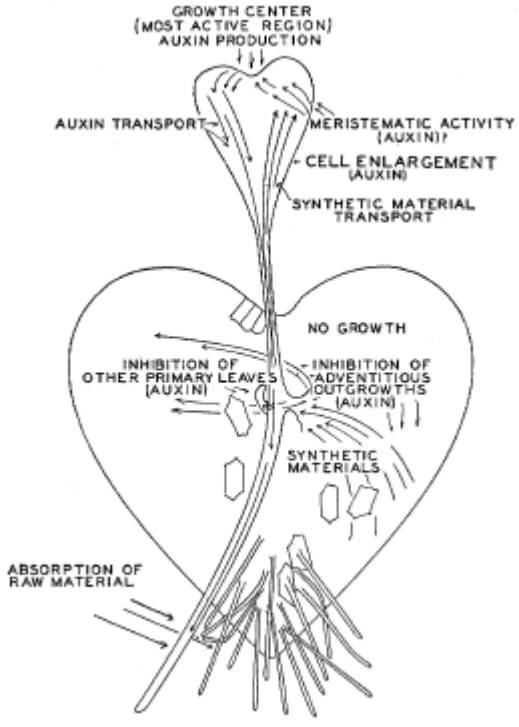
light or far-red light (Whittier and Steeves 1960, Whittier 1964b, Whittier and Pratt 1971).

Ethylene

Ethylene regulates spore germination. It inhibits dark germination (Edwards and Miller 1972) and limits overcrowding and competition among prothalli. Ethylene enables the differentiation of prothalli into sporophytes, by establishing a physiological state in the prothalli, but requires sugar to do so (Elmore and Whittier 1975a) (Raghaven 1989). The effect of ethylene on germination of fern spores is the opposite of its effect on seed germination in higher plants.

Sporophytes

Sakamaki (1999) has suggested that the most efficient strategy for fern spore reproduction is that gametophytes form sporophytes as quickly as possible. This is dependent on gametophytes achieving the critical size needed for sporophyte production. If the gametophytes are too small, archegonia will not form. And if gametophytes have grown too large, sporophytes will not form. Figure 3 shows a prothallus with developing sporophyte.



Sakamaki (1999) also noted that higher productivity in gametophytes before fertilization led to a higher growth rate in young sporophytes, although the embryo takes some days to develop after fertilization. Growth of the embryo and sporophyte is supported by photosynthetic activity of gametophytes, which is affected by light and other environmental factors.

Figure 3: Prothalli with developing sporophyte (from Albaum 1938).

The sporophyte is entirely dependent on the energy produced by the gametophyte until it emerges, and for approximately ten days afterwards. After this point, the sporophyte begins to photosynthesise independently. The gametophyte continues to support the sporophyte and produce its photosynthate until the first leaf of the sporophyte has fully expanded. About 20 days after emergence, the prothallium stops growing, and the supportive role of the gametophytes ceases (Sakamaki 1999).

Abiotic factors

Light

Fliflet (1961) states that the best light conditions for developing sporophytes are between 5,382 and 12,967 lux. The ideal light requirements are species dependent, with some ferns preferring a low light level, while others prefer more. Note that sporophytes can generally tolerate a higher light level than gametophytes.

Moisture

The root system of a fern must have access to oxygen and water. While some ferns will tolerate saturation (e.g. *Osmunda spp.*) most dislike constantly wet roots. Young sporophytes have small root systems, which need a constant source of moisture to ensuring their water requirements are met. As a result, they are often potted into mixtures containing high quantities of peat moss (Heatons 2011).

Growers find it preferable to apply water to the soil surface and not the foliage, as wet foliage is susceptible to disease. Also, moisture levels should be varied as constant dampness can cause roots to rot, especially during cooler months (Goudey 1985). Potter (Heatons 2011) advised that at their nursery, trays of sporophytes are placed into a humid area adjacent to the misting area (once shot) for 2-3 days to stabilize, just prior to pricking out.

Nutrition

Young sporophytes (sporelings) use their root systems to make nutrients in the soil available to the plant. Sporelings will respond to the use of fertilizers from an early nearly in age. Because the plants and root systems are so small, weak liquid solutions are recommended (about half strength is ideal). Seaweed based fertilizers are preferred as

they can be applied every two to four weeks and will not harm juvenile plants (Jones 1987).

Biotic factors

Auxins

As the prothallium stops growing, auxins formerly used by its apical regions to promote growth are transported to the primary leaf, and leaf metabolism and growth become more active. Auxin is then produced in the primary leaf of the young sporophyte, which both prevents the outgrowth of other leaves and inhibits any adventitious growth from the prothallium (Albaum 1938). Roots, buds and stems differ in their sensitivity to auxins, with roots being more sensitive, and shoots least sensitive, although their reactions to the hormone are all basically the same (Thimann 1937).

Hardening off

Young sporophytes should be hardened off prior to pricking out to acclimatise them to lower humidity levels. This is done by exposing them incrementally by gradually removing their covering/protection over two to three weeks. Once the young ferns have been hardened off, their growth rate usually increases, and after six to eight week they will be ready to be pricked out (Hoshizaki and Moran 2001).

Potted transplants are initially protected in frames under glass or plastic coverings. Once established they must be hardened. This is achieved by lifting the glass or plastic covering a little more each day to allow the foliage to adapt to drier air and higher light intensity. The hardening process may take three to six weeks.

Transplantation

Most growers wait until there is an even coverage of sporophytes before transplanting. The young ferns can be transplanted at almost any time, but they are easier to handle once they have reached about 2.5cm high. Commercial growers transplant them into tubes, generally containing peat moss blended with perlite, clean gravel or polystyrene beads. It is important that the transplant mix not contain any organic material, as this can injure the young ferns (Goudey 1985).

Potter (Heatons 2011) advised that their ferns are pricked out into a clump, rather than an individual plant (except for tree ferns and some *Blechnum spp.*). This lessens the likelihood of transplant shock, and maximizes their visual appeal to buyers (particularly *Adiantum spp.*). A clump may consist of 8 – 20 plants at a time. Their tubes contain 70% peat moss, which is pH adjusted to a neutral value (or slightly acidic for Adiantum spp.)

Plants are then watered and briefly returned to a humid environment to help them recover from the shock and root damage caused by pricking out. Heaton's nursery uses a capillary mat for watering, which is watered every 2-3 days. This is to avoid the plants getting water on their foliage.

Pests/Diseases

Despite sterilization, contamination of fern cultures may occur. The most common of these are algae, fungi and moss, which enter the environment by airborne spore (Fliflet 1961). Some insect pests are also relatively common.

Algae are harmful to young prothalli. It may appear as a black or grey slime on the media surface in spore containers. It spreads and either crowds out or covers the developing prothalli. Once contamination has occurred, it is difficult to stop. Clumps of algae can be removed as they appear, and/or remaining prothalli can be replanted on a fresh, sterilized soil medium.

Grey mould (botrytis) is common on young developing prothalli, and causes rot just above the surface level of the media. Prothalli may appear limp, soft and darker green than usual. Unless dealt with quickly, this can spread rapidly over the surface and destroying the germinating spore. Moulds in spore containers are generally associated with over-crowding of prothalli, and can be avoided by planting spores very thinly. If necessary, clumps of prothalli can be pricked out into fresh, clean containers to reduce problems associated with overcrowding, and provide the gametophytes with more room to grow (Goudey 1985).

Mosses and liverworts may spread and choke prothalli and young ferns. They are difficult to treat with chemicals, which are likely to kill the developing ferns as well as the pest. Whilst young developing ferns can tolerate less light than mosses and algae, it is

best to remove them from cultures. This can be done by tweezers or with a knife blade, and must be completed prior to the appearance of fruiting bodies (Goudey 1985).

The larval stage of fungus gnats can be fatal to prothalli. The larvae eat prothalli (especially rhizines), although they mostly live on organic matter in the soil. Their excretions also cause a black mould which will also destroy the prothalli as well (Goudey 1985). They are best controlled using biological controls such as beneficial nematodes or predatory mites (Setting up a control program for fungus gnat, 2011).

Leaf nematodes may sometimes attack prothalli and sporelings. These pests can be disastrous to small plants, causing brown streaks on prothalli and fronds and eventually spreading and killing entire patches of tissue. Spore pots containing leaf nematodes should be destroyed (by burning) (Jones 1987).

Prevention is better than cure. Best results are achieved by properly sterilizing the soil before sowing and properly sealing containers as chemical treatments can often burn or even kill the tender foliage of prothalli and sporelings (Goudey 1985). Cultural methods, such as removal and destruction of infected material, replanting into clean media, and avoiding reuse of contaminated material can help. Commercially available fungicides can help with fungal contamination – commonly used preparations include Rovral Aquaflow (Heatons 2011), Fongarid, and weak potassium permanganate solutions. Carbaryl (Goudey 1985) and Confidor are also helpful in controlling pests.

Conclusion

Growing ferns from spore is interesting and challenging. It requires patience and an understanding of the cultural requirements of ferns in general, as well as individual species to ensure the best chance of success. There is a great deal of published material available, mostly relating to the structure and development of the gametophyte, although primarily based on laboratory culture. But little practical research is available on growth and development of sporophytes and their care. This is problematic for the fern grower wishing to apply recent scientific advances to a more practical environment. More work in this area would provide a better understanding of abiotic and biotic factors in all stages of fern propagation than currently exist. Research to help manage pest and disease

problems would assist growers by decreasing time to market and optimizing the production of healthy ferns in plentiful quantities.

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Appendix 1

TABLE 1. *Summary of spore germination and viability in ferns.*

Species	Spore color[a]	Days to germination	Length of viability (days[b])	Source
Equisetaceae				
Equisetum arvense L.	G	1	10–24	Okada 1929
E. hyemale L.	G	1	16	L.[c]
E. telmateia Ehrh.	G	1	12	L.
E. sp.	G	½	———	Campbell 1905
Marattiaceae				
Marattia sambucina Bl.	NG	16	———	Stokey 1942
Osmundaceae				
Osmunda banksiaefolia (Presl) Kuhn	G	3	ca. 10	L.
O. cinnamomea L.	G	1–2	———	Campbell 1892
O. cinnamomea L.	G	3	43–54	Okada 1929
O. claytoniana L.	G	1–2	———	Campbell 1892
O. japonica Thunb.	G	1–2	23–43	Okada 1929
O. regalis L.	G	1	150–210	Gerhardt 1927
O. regalis var. spectabilis (Willd.) A. Gray	G	2	Under 240	L.
Plagiogyriaceae				
Plagiogyria glauca (Bl.) Mett.	?	180–210	———	Stokey and Atkinson 1956b
P. semicordata (Presl) C. Chr.	?	ca. 30	Over 1.5 yrs	Stokey and Atkinson 1956b
Gleicheniaceae				
4 genera, 10 species	NG	16–21	150–180	Stokey 1950
Dipteridaceae				
Dipteris conjugata Reinw.	NG?	ca. 14	Few	Stokey 1945
Cheiropleuriaceae				
Cheiropleuria bicuspis (Bl.) Presl	NG?	16	———	Atkinson and Stokey 1954
Grammitidaceae				
3 genera, 26 species	G	Within sporangium	Few	Stokey and Atkinson 1958
Cochlidium sp.	G	———	Under 45	L.
Grammitis jungermannioides (Kl.) Ching	G	———	Under 45	L.
G. sp.	G	———	Under 45	L.
Xiphopteris sp.	G	———	Under 45	L.
Polypodiaceae				
Christiopteris tricuspis (Hook.) C. Chr.	G	1	Under 60	Nayar 1967 L.
Marginariopsis wiesbaurii (Sod.) C. Chr.	G	"on frond"	Under 90	L.
Polypodium californicum Kaulf.	NG	9	Over 1.5 yrs	L.
Schizaeaceae				
Anemia phyllitidis (L.) Sw.	NG	10	———	Life 1907
Lygodium palmatum (Bernh.) Sw.	NG	4–6	Over 300	Rogers 1923
Anemia sp.	NG	10	14 yrs	Mickel 1962
Adiantaceae				
Pellaea sp.	NG	———	34 yrs	Mickel 1962
Cheilanthes mysurensis Wall.	NG	———	8.5 yrs	Wright 1909
Pityrogramma calomelanos (L.) Link	NG	10	———	Life 1907
Pteris quadriaurita Retz.	NG	5	———	L.
Loxsomaceae				
Loxsoma cunninghami R. Br.	NG	10–12	Over 365	Stokey and Atkinson 1956a
Loxsomopsis costaricensis C. Chr.	NG	10–14	ca. 90	Stokey and Atkinson 1956a
Dennstaedtiaceae				
Pteridium aquilinum (L.) Kuhn	NG	———	1.3 yrs	Conway 1953
Cyatheaceae				
15 species	NG	———	Over 1.3 yrs	Stokey 1930
Dicksonia antarctica Labill.	NG	———	22 yrs	Anony. 1910
D. apiifolia Sw.	NG	8	Over 365	Life 1907
Lophosoria quadripinnata (Gmel.) C. Chr.	NG	11–12	———	Life 1907
Thyrsopteris elegans Kunze	NG	4–7	Over 270	Stokey 1930

Species	Spore color[a]	Days to germination	Length of viability (days[b])	Source
Hymenophyllaceae				
Hymenophyllum, 7 spp.	G	Few hours	———	Stokey 1940
H. javanicum Spr.	G	———	19	Stokey 1940
Mecodium sanguinolentum (Forst.) Presl	G	Within sporangium	Short	Atkinson 1960
M. scabrum (A. Rich.) Copl.	G	Within sporangium	Short	Atkinson 1960
Trichomanes maximum Bl.	G	Under 2	6	Stokey 1940
T. schmidianum Zenk.	G	1–2	———	Stokey 1940
Aspleniaceae				
Asplenium aethiopicum (Burm.) Bech.	NG	15–20	———	Braithwaite 1964
A. friesiorum C. Chr.	NG	16	———	L.
A. serra Langsd. ex Fisch.	NG	———	48 yrs	Fischer 1911
Diellia falcata Brack.	NG	———	Over 2 yrs 10 mo	Wagner 1952
Blechnaceae				
Blechnum gibbum (Labill.) Mett.	NG	4–5	———	L.
B. glandulosum Link	NG	6	———	L.
B. nudum (Lag.) Luerss.	G	2–4	ca. 90	Stone 1961
Stenochlaena palustris (Burm.) Bedd.	NG	7–8	Over 1.5 yrs	Stokey and Atkinson 1952
Woodwardia orientalis Sw.	NG	(2) 5	200	Okada 1929
Aspidiaceae				
Athyrium goeringianum (Kunze) Moore	NG	10	———	L.
A. thelypteroides Desv.	NG	9	———	L.
Currania oyamensis (Bak.) Copl.	NG	9	———	L.
Diplazium sp.	NG	6	———	L.
Dryopteris viridescens O. Ktze.	?	(1) 2?	30–100	Okada 1929
Matteuccia orientalis Trev.	G	2	———	L.
M. struthiopteris (L.) Tod.	G	5	150	Okada 1929
M. struthiopteris	G	2	Under 1 yr	L.
Onoclea sensibilis L.	G	(13 hrs) 1	ca. 1 yr	L.
Onocleopsis hintonii Ball.	G	3	120–150	L.
Marsileaceae (sporocarps)				
Marsilea fournieri C. Chr.	NG	Under 24 hrs	Over 61.5 yrs	Allsopp 1952
M. vestita Hook.	NG	Under 24 hrs	Over 68 yrs	Allsopp 1952
Parkeriaceae				
Ceratopteris sp.	NG	4–5	5 yrs	Klekowski (pers. comm.)

[a] Key: NG=non-green, G=green.
[b] Unless otherwise noted.
[c] L.=Data published in this paper by the author.

(from Lloyd and Klekowski 1970)