Field experiments on the development of fern gametophytes

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I hereby declare that this thesis was composed by myself and the work described herein is my own except where indicated otherwise.

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EDINBURGH 1992

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Abstract

This thesis describes (1) a simple and effective method for growing large numbers of fern gametophytes under field conditions and (2) the application of this method to investigate the sequence and timing of events in the fern life-cycle following spore dispersal or soil disturbance at different times of the year. Long-term field experiments involving *Athyrium filix-femina, Blechnum spicant, Polystichum setiferum* and *Phyllitis scolopendrium* were initiated at 2 sites near Edinburgh in January and October 1988. During these experiments (which continued till January 1990) both sites experienced a series of exceptionally mild Winters. The results for the 4 species were strikingly similar. Spores sown in January germinated in the Spring and gametophytes became sexual in the Summer. Spores sown in October germinated in the Winter and gametophytes became sexual in the following year between mid-Spring and mid-Summer. Germination was synchronous although many spores did not produce gametophytes. Gametophytes quickly became 2-dimensional; no protonemata were observed. Sex organs developed and matured rapidly and their production was continuous. Most populations consisted of male, female and bisexual gametophytes. In general, female gametophytes were larger than bisexual gametophytes and all archegoniate gametophytes were larger than male gametophytes. All archegoniate gametophytes were meristic; all male gametophytes were ameristic. Most populations were predominantly male. Female gametophytes were always more abundant than bisexual gametophytes. All bisexual gametophytes were protandrous. Fertilization was often rapid, often occurred at high frequency and was not seasonal. Polyembryony was rare. All gametophytes from the January sowing survived the Winter of 1988/'89 but male and fertilized archegoniate gametophytes began to die in the Spring or Summer of 1989. No sporelings died. All gametophytes and sporelings from the October sowing survived throughout the experiment. A third (short) field experiment involving *A. filix-femina, B. spicant, P. setiferum, P. scolopendrium, Dryopteris affinis* subsp. *borreri, Dryopteris dilatata, Dryopteris filix-mas* and *Pteridium aquilinum* was initiated in August 1989. Spores of all 8 species germinated more or less immediately but only gametophytes of *P. aquilinum* became sexual before Winter. In one population fertilization occurred before the end of the year.

Contents

Chapter 1

Introduction

Objectives

1. To pioneer a simple experimental approach to facilitate and encourage long-term studies of fern gametophyte development in natural habitats.

2. To use this approach to investigate the sequence and timing of events in the fern life cycle following 'spore dispersal' or 'soil disturbance' at different times of the year.

Background

The Filicales is the largest group of living pteridophytes. There are more than 12,000 species in at least 400 genera (Page, 1979a). Such species diversity and the fact that many of the largest genera of Filicales evolved and radiated concurrently with the angiosperms (Lovis, 1977) belies the popular belief that ferns as a whole are ancient and in decline.

The majority of ferns is homosporous (*Pilularia globulifera* is the only heterosporous species in the British Isles). Like all homosporous pteridophytes, homosporous ferns have a life cycle which involves an alternation of 2 free-living generations; the sporophyte which is large, vascular and long-lived produces the spores; the gametophyte which is tiny, non-vascular and short-lived produces the sex organs [*i.e.* antheridia (♂) and/or archegonia (♀)]. Three types of mating events are possible (Klekowski, 1969; Lloyd, 1974): 1. *inter-gametophytic crossing,* the cross-fertilization of gametophytes derived from the spores of different sporophytes (out-crossing), 2. *inter-gametophytic selfing,* the cross-fertilization of gametophytes derived from the spores of a single sporophyte (which is analogous to selfing in seed plants) and 3. *intra-gametophytic selfing,* the self-fertilization of a bisexual gametophyte (this results in a completely homozygous sporophyte). The term *inter-gametophytic mating* is used to describe the fusion of sperm and egg from different gametophytes if the origin of the gametophytes is uncertain (Klekowski, 1979).

The distribution of the sporophyte is determined by spore dispersal and the establishment and fertilization of the gametophyte. Successful sexual reproduction in a fern thus requires a site where conditions support first the gametophyte, with many features reminiscent of a thalloid liverwort, and then the sporophyte, with some of the characteristics of a terrestrial angiosperm. This dual requirement imposes a restriction on the number of 'safe sites' for each species (Cousens *et al.*, 1985, 1988). The only escape from this restriction is the vegetative spread of perennial sporophytes which can thus invade habitats unsuitable for gametophyte establishment and the asexual propagation of gametophytes by gemmae which can perpetuate species beyond the range of the sporophyte (Farrar, 1967, 1971, 1985; Rumsey *et al.*, 1992).

The first illustrated description of fern spore germination and gametophyte development was published by John Lindsay in 1794 but it was not until around 1850 that the cycle of alternating free-living gametophytes and sporophytes was properly understood (references in Dyer, 1979a). Since then the 'classic' description of the fern life cycle has become firmly established in almost every introductory botanical textbook and most botanists would now profess to 'know the fern life cycle'. However, the classic description and most of the information on gametophytes that has accumulated since [several thousand studies are reviewed by Miller (1968), Nayar and Kaur (1971), Atkinson (1973), Lloyd (1974), Dyer (1979b), Cousens (1988) and Raghavan (1989)] is based almost entirely on observations of material grown under artificial laboratory and greenhouse conditions. In reality, almost nothing is known about the natural fern life-cycle.

Laboratory cultures differ from natural populations in many respects (Cousens *et al.*, 1985; Schneller *et al.*, 1990). They are monocultures of uniform age; they are grown in constant abnormally low light conditions; they are grown at constant abnormally high temperatures; most are grown on artificial arbitrarily defined mineral agar and they are protected from drought, frost, erosion, competitors, herbivores and pathogens. Recent studies have revealed that mineral agar can influence the sexual development of gametophytes (Rubin and Paolillo, 1983; Rubin *et al.*, 1985) and that many brands contain impurities that are toxic to plants (Debergh, 1983; Kordan, 1988).

Despite the obvious need for field studies of fern gametophytes there have been very few *e.g.* Farrar (1967, 1971, 1985), Cousens (1973, 1979, 1981), Farrar and Gooch (1975), Tryon and Vitale (1977), Schneller (1979, 1988), Peck (1980), von Aderkas (1983), Kelly (1987), Cousens *et al.* (1988) and Peck *et al.* (1990). This deficiency is largely attributed to the reluctance of researchers to attempt field studies of natural gametophyte populations, which are difficult, when most species can be easily and quickly grown from spores in the laboratory.

To date, all detailed investigations of fern gametophyte development in nature have involved destructive sampling of natural populations. This approach can be informative, especially while so little is known, but it does have serious limitations. For instance, it yields information about gametophyte populations at only one moment in time; development cannot be followed because natural populations tend to be small and quickly depleted. Moreover, gametophytes are usually difficult or impossible to identify and their ages are always unknown. The environmental conditions with respect to substrate and microhabitat are also unknown and variable between populations. This variation, combined with the inability to devise a standard unbiased sampling procedure, creates serious difficulties when trying to interpret the results.

Destructive sampling on more than one occasion from large semi-natural gametophyte populations (initiated in the field by sowing spores of known origin onto natural substrates) would, at least in theory, be more informative but in practice growth on the chosen substrate cannot be guaranteed *e.g.* Peck (1980) and P. Glaves, Derbyshire College of Higher Education (personal communication). Incidentally, attempts to initiate semi-natural populations of *Equisetum telmateia* have also failed (J.N.B. Milton in Duckett, 1985). Even if semi-natural populations could be grown there would still be a risk of contamination by spores naturally deposited on the substrate either before or after the deliberate deposition. Clearly, an approach allowing greater experimental control is necessary if more meaningful results are to be obtained.

An experimental approach was developed (and tested in the Botany department garden in the Summer of 1987) to overcome the practical difficulties inherent in studies of natural and semi-natural gametophyte populations. In particular, it was

designed to:

1. employ a standard, well defined inoculum (*i.e.* known identity, density and date).
2. use a standard but natural substrate.
3. provide conditions that will support growth (to sporeling stage) and that are as close as possible to natural conditions.
4. ensure abundant replicate material for destructive sampling.
5. allow an easy, standard, non-disruptive method of sampling.
6. enable clear interpretation of results.

The intention was to initiate one large field experiment in the Autumn of 1987 to coincide with the main spore release period and to monitor gametophyte development for 2 years. However, the surprise discovery in 1987 of soil spore banks at the study sites (Lindsay and Dyer, 1990; see Appendix) added a new and exciting dimension to this study. Soil spore banks are of interest because they create opportunities for gametophyte establishment at any time of the year following soil disturbance. Successful gametophyte establishment might result more often from spores that germinate in the Spring, from soil spore banks, than from spores that germinate in the Autumn immediately after dispersal. In view of this possibility, field experiments can, with good reason, be initiated at any time of the year.

Chapter 2

Materials and methods

Selection of species

For practical reasons the main study species had to have epigean gametophytes, had to produce a large number of spores most of which would survive for at least one year in storage, and had to be locally abundant and easily accessible. Several other selection criteria were applied which were not essential for this study but were used to ensure that the species selected were among those most likely to be chosen by other researchers for future field studies. By taking this into consideration, the results obtained in this study ought to have greater scientific value. These additional selection criteria restricted the choice to species that reproduce largely or solely by spores, that have sporophytes which are easily distinguishable from related species, that do not hybridize freely with other local species, that have recognizable spores and that are not exclusively epiphytic or lithophytic.

All native fern species were considered; 4 were chosen. These were *Athyrium filix-femina* (L.) Roth (Woodland Lady Fern), *Blechnum spicant* (L.) Roth (Hard/Ladder Fern), *Phyllitis scolopendrium* (L.) Newm. (Hart's Tongue Fern) and *Polystichum setiferum* (Forsk.) Woynar (Soft Shield Fern).

Selection of sites

Advice on the present local distribution of the four selected species was sought from amateur and professional botanists currently undertaking site surveys for a new flora (*Botany of the Lothians*) which is in preparation. Potential sites were then visited to assess their suitability for this study. An ideal field site was considered to be one where one or more of the selected fern species was abundant and sexually reproducing, close to the laboratory and easily accessible but in a protected area with little or no public access. Sites that had already been surveyed and for which botanical and geological information was available were favoured. Similar site selection criteria were used by Farrar and Gooch (1975), Cousens *et al.* (1985) and Werth and Cousens (1990).

Two field sites were chosen (Figure 2.1). Suitable populations of *A. filix-femina* and *B. spicant* co-exist in Roslin Glen (RG), 11 km south of Edinburgh, inland, near the village of Roslin in Midlothian (N.G.R. NT 27 62). The closest site for suitable populations of both *P. setiferum* and *P. scolopendrium* is Pease Bridge Glen (PBG), 50 km east of Edinburgh/Roslin, near the sea and the village of Cockburnspath in Berwickshire (N.G.R. NT 79 70).

Climatic data

Climatic data (maximum and minimum air temperature and precipitation) for 1988 and 1989 and for the previous 8 years were obtained from the *Met. Office Advisory Service,* Edinburgh. The nearest climatological station to each site is shown in Figure 2.1. Bush House (altitude = 185 m above mean sea level) is 2.5 km west of Roslin Glen (altitude = 100-200 m a.m.s.l). Dunbar (altitude = 25 m a.m.s.l) is 11.5 km north-west of Pease Bridge Glen (altitude = 25-90 m a.m.s.l) and is also on the coast.

Field experiments

There were 3 field experiments (Table 2.1). Experiment 1, the largest, was initiated in January 1988 and ran for 2 years. Experiment 2 was initiated in October 1988 and ran for 15 months. Experiment 3 was initiated in August 1989 and ran for 5 months only.

Additional species

Experiment 3 involved 4 additional species. These were *Dryopteris affinis* subsp. *borreri* (Lowe) Fraser-Jenkins (Common Golden-scaled Male Fern), *Dryopteris dilatata* (Hoffm.) A. Gray (Broad Buckler Fern), *Dryopteris filix-mas* (L.) Schott (Common Male Fern) and *Pteridium aquilinum* (L.) Kuhn (Bracken). These species were not selected using the criteria listed on page 5; they were investigated simply because they grow near *P. setiferum* and *P. scolopendrium* at Pease Bridge Glen and amongst *A. filix-femina* and *B. spicant* at Roslin Glen.

Spore collections

The spores used to initiate Experiment 1 were collected in 1987. The spores used to initiate Experiments 2 and 3 were collected in 1988 (Table 2.2). In each year fronds were collected from approximately 30 individuals of each species and spores were harvested in the laboratory as described by Dyer (1979c). Sporangial debris, insects, *etc.,* were removed by sieving the liberated spores through 4 layers of lens cleaning tissue. Spores were stored in micro-centrifuge tubes at 4°C until required.

Preparing pots

Gametophytes were grown in 500 small plastic pots (5 cm diameter, 5 cm deep; Figure 2.2) which were planted in the ground. Holes were made in the sides of these pots with a soldering iron to ensure that the soil in the pots would receive water [and possibly beneficial micro-organisms (Page, 1979b)] from the surrounding soil. Pots were lined with filter paper (biodegradable) to prevent soil from falling through the holes during transport to the sites. Each pot had a tight-fitting transparent plastic lid (the base of a 5 cm diameter, 2 cm deep, Petri dish). A small ventilation hole was made in the side of each lid with a hot needle to prevent the build-up of potentially harmful gases *e.g.* Carbon dioxide and ethylene (References in Raghavan, 1989). Lids and pots were labelled with waterproof and light stable ink to ensure that they could be reunited if separated in the field (or during transport).

Soil collections

Pots were filled either with soil from Pease Bridge Glen or with soil from Roslin Glen; approximately 20 litres (enough to fill 250 pots) of surface soil (0-10 cm) was collected in small amounts, near natural gametophyte populations, at each site during October and November 1987. At both sites, gametophytes were most abundant on recently exposed mineral soil. The soil collections were thoroughly mixed, passed through a 1 cm aperture riddle, and stored in 2 large containers until required.

Soil analysis

A small sample of each soil mixture was sent to the *Scottish Agricultural Colleges' Central Analytical Laboratory,* Edinburgh, for routine physical and chemical analyses.

Spore bank analysis

Each soil mixture was tested for the presence of viable fern spores by culturing replicate samples for 4 weeks, in the light (photon flux density = $20\mu Em^{-2}s^{-1}$), at *c.*20°C. Large numbers of fern gametophytes (and bryophytes) developed on the surface of these soil samples revealing that large numbers of viable fern spores were present in the soil. Subsequent studies, involving detailed analysis of soil cores extracted from the ground in June 1988, just before the spore release season, confirmed that spore banks are present all year round at Pease Bridge Glen and at Roslin Glen (see Appendix). Re-examination of the spore bank cultures reported in Lindsay and Dyer (1990), once sporophytes had developed, confirmed the presence of *A. filix-femina, B. spicant, P. setiferum, P. scolopendrium* and *Dryopteris* spp., (Dyer and Lindsay, 1992).

Spore bank elimination

Natural but spore-free soil was required for the field experiments. Conventional methods for partial or complete soil sterilization were considered to be unsuitable. Chemical treatments were avoided because of the possibility of unknown and/or toxic residues being left behind. High temperature treatments were avoided because they always alter the structure and chemical composition of the soil. Gamma radiation can be used to sterilize soil without any rise in temperature but this was also avoided because its effects on the chemical composition of soil are still poorly understood (Cawse, 1975). Gametophytes of *Onoclea sensibilis* do not grow on autoclaved soil and develop abnormally on gamma-irradiated soil (Rubin and Paolillo, 1984).

Soil was partially sterilized by a novel low temperature treatment. One week prior to the start of each field experiment the appropriate amount of soil was drenched with water and 'incubated' at room temperature for 72 hours to ensure that the fern spores

in the soil were fully imbibed and physiologically active. The soil was then immersing in liquid Nitrogen (-196°C) for 24 hours and allowed to thaw at room temperature. This was an effective method of killing all resident spores (pteridophyte and bryophyte but probably not microbial) and seeds and was assumed to do less damage to the soil than the alternative treatments. Pots were filled to the brim with the spore-free soil and refrigerated until the spores of known origin were ready to be sown.

Preparing plots

The 3 field experiments were set up in two 1 m^2 plots at each site. These plots were located as close as possible to natural gametophyte populations and out of direct sunlight (to avoid or at least minimize any 'greenhouse' effect). The plots were dug over to a depth of 10 cm in November/December 1987 and all large stones, roots, and bulbs (mostly *Allium ursinum*) were removed to facilitate the planting of pots at later dates. A 1 m^2 sheet of thin absorbent matting (normally used on glasshouse benches), with pre-cut holes for pots, was placed over the surface of each plot and secured with large stones (Figure 2.3). This was intended to prevent recolonization (especially by bryophytes) without interfering with the moisture status of the plot.

Sowing spores

Spores were sown in the laboratory, one species at a time. The appropriate number of pots (for each species and of each soil mixture) were removed from the refrigerator and placed in a random fashion inside a 1 m^2 box drawn on the floor. Spores were then sown from suspension using an aerosol (after Schwabe, 1951). The amount of spores that was required, from each spore collection, to achieve a standard density of approximately 500 viable spores cm^{-2} is given in Table 2.2. Fern spores can be deposited at this density in woodland habitats [Schneller, 1974, 1975, 1979; P. Glaves, Derbyshire College of Higher Education (personal communication)]. The spores were suspended in cold water (a trace of detergent was required to obtain a good suspension) and sown quickly to prevent any from becoming photosensitive. A few Petri dishes containing soil were placed at random among the pots on the floor. These were cultured for 4 weeks, in the light, at 20°C to verify that the desired concentration of viable spores had been achieved. In fact, the numbers of gametophytes (and so presumably the number of viable spores) ranged from 300-500 cm^{-2}.

Immediately after sowing, the lids were fitted and the pots were returned to the refrigerator where they were stored in darkness, usually for less than 24 hours, before being placed in the field. The lids were intended to create a humid environment (essential for the growth of gametophytes), to prevent spores from being washed into the soil and gametophytes from being washed away by rain, and to exclude extraneous moss and fern spores and surface-dwelling herbivores.

Planting pots

At each plot, pots of the different fern species were mixed and planted in a random fashion in a 10 pot x 10 pot arrangement (Figure 2.3). Each pot was separated from its neighbours by at least 5 cm. The plots were watered immediately after planting new pots (in January and October 1988, and August 1989) to help the freshly disturbed soil settle and to ensure good contact between the walls of the pots and the surrounding soil. Watering was not attempted at any other times.

Precautions

Table 2.1 shows the number of pots that were required to obtain the data presented in Chapters 4-6 but it must be emphasized that all plots had extra pots of each species just in case some were lost or damaged. As another precaution, in case gametophytes did not grow at the field sites, 2 plots, identical to those at the field sites (one using soil from Pease Bridge Glen, the other using soil from Roslin Glen), were set up in a secluded shady (but unfortunately very dry) corner of the Botany department garden. These plots were watered as necessary to ensure their survival for 2 years. Fortunately, they were not required.

Maintenance and sampling

Sites were visited at least once a month for sampling and/or general maintenance of plots. More frequent visits were necessary in the Autumns of 1988 and 1989 to remove leaf litter. The sampling dates are shown on the graphs (below the x-axes) in Chapters 4-6. On each of these occasions, one pot of each species (in the appropriate experiment) was removed from each of the 4 plots. Pots to be sampled

were selected in the laboratory before the site visits using plot maps and a random co-ordinate system. Back at the laboratory, pots were placed in moisture retaining plastic boxes and stored in darkness at 4°C until it was convenient to examine them. A few samples were stored under these conditions for almost 2 months without any apparent deterioration. It was assumed that no growth occurred during storage.

Processing specimens

A tiny cluster of gametophytes was carefully but quickly (to avoid dehydration) removed from near the centre of each pot and transferred to water in a Petri dish using watchmakers forceps and a selection of fine dissection needles (made by dipping tungsten wire in molten Sodium Nitrite). This, and most subsequent manipulations required the use of a good dissection microscope (magnification range x10 - x30) and a powerful fibre optic system. Gametophytes were carefully separated under water and as much soil as possible was dislodged from the rhizoids. Additional clusters were treated in the same way until a total of 50-100 gametophytes were obtained. These were then transferred to micro-centrifuge tubes filled with a mixture of chloral hydrate and acetocarmine (recipe in Edwards and Miller, 1972) and incubated at 50°C in a water bath for 2 hours. This stain is excellent for detecting antheridia and archegonia at all stages of development. Some specimens were stored in chloral hydrate-acetocarmine for more than 1 year without any apparent deterioration.

Stained specimens were rinsed in water then examined under water. Gametophytes without sex organs were classed as 'pre-sexual'. Gametophytes with sex organs were classed as 'male', 'female' or 'bisexual' even when the organs were obviously too immature or too old to function. Gametophytes of each sex were placed in separate Petri dishes so that they could be counted more easily. The number of embryos and leaves on sporelings, and general observations on the shape and condition of gametophytes was also recorded.

Ten intact gametophytes of each sex (including pre-sexual) were selected at random for area measurements. If there were less than 10 gametophytes then all intact specimens were measured. The selected gametophytes were carefully mounted in

water on glass slides and sealed under coverslips using thick transparent nail varnish (any attached sporelings were removed first). Areas were determined using a Quantimet 970 Image Analyzing computer (*Cambridge Instruments*, Cambridge) connected to a microscope. An on-screen editing facility enabled rhizoids to be 'removed' before areas were calculated. The programme was written especially for this purpose by Dr. C.E. Jeffree, director of the *Electron Microscopy Unit* at Edinburgh University. Hickok and Schwarz (1986) used a similar image analysis system to measure fern gametophytes. Some mounted specimens were stored for several weeks (at 4°C) before they were measured.

Processing data

The raw area data were processed on the Edinburgh University mainframe computer (EMAS) using 'Minitab' (a widely available statistics package for mainframes and PC's). All graphs were produced on an IBM compatible PC using 'Fig-P' (a scientific graphics package available in the U.K. from *Biosoft Ltd.* Cambridge).

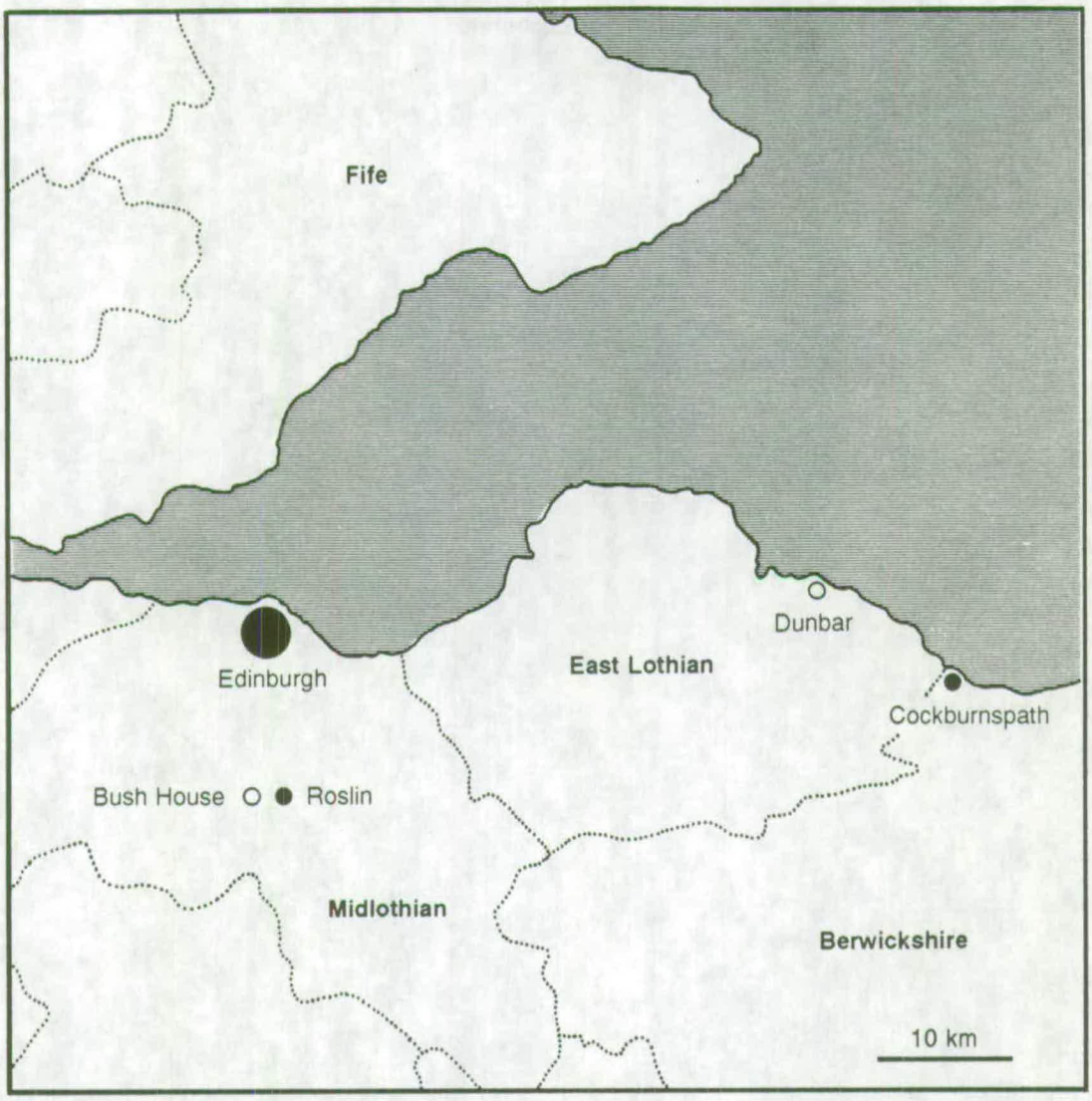


Figure 2.1. Location of field sites (●) and nearest climatological stations (○).

		Experiment 1				Experiment 2				Experiment 3			
Initiated		January 1988				October 1988				August 1989			
Duration		24 months				15 months				5 months			
Site / Soil		PBG		RG		PBG		RG		PBG		RG	
Plot No.		1	2	1	2	1	2	1	2	1	2	1	2
No. of Species		4	4	4	4	4	4	4	4	8	8	8	8
No. of Pots		64	64	60	60	44	44	44	44	16	16	16	16
1988	J	■	■	■	■								
	F	■	■	■	■								
	M	■	■	■	■								
	A	■	■	■	■								
	M	■	■	■	■								
	J	■	■	■	■								
	J	■	■	■	■								
	A	■	■	■	■								
	S	■	■	■	■								
	O					■	■	■	■				
	N	■	■	■	■	■	■	■	■				
	D					■	■	■	■				
1989	J	■	■	■	■	■	■	■	■				
	F			■	■			■	■				
	M	■	■			■	■	■	■				
	A	■	■	■	■	■	■	■	■				
	M					■	■	■	■				
	J	■	■	■	■	■	■	■	■				
	J					■	■	■	■				
	A	■	■			■	■	■	■	■	■	■	■
	S												
	O	■	■			■	■		■	■	■	■	■
	N			■	■			■	■				
	D												
1990	J	■	■	■	■	■	■	■	■	■	■	■	■

Table 2.1. Plan of the field experiments. Shading indicates months in which samples were collected.

	Experiment 1			Experiment 2			Experiment 3	
	Collection site & date	Viability Jan. '88	Amount sown	Collection site & date	Viability Oct. '88	Amount sown	Viability Aug. '89	Amount sown
A. filix-femina	RG Aug. '87	90%	92 mg	RG Sep. '88	90%	92 mg	85%	98 mg
B. spicant	RG Sep. '87	90%	92 mg	RG Sep. '88	90%	92 mg	70%	119 mg
P. setiferum	PBG Aug. '87	40%	208 mg	PBG Jul. '88	30%	277 mg	25%	332 mg
P. scolopendrium	PBG Oct. '87	75%	111 mg	PBG Sep. '88	80%	104 mg	75%	111 mg
D. affinis subsp. *borreri*	-	-	-	RG Sep. '88	-	-	80%	104 mg
D. dilatata	-	-	-	RG Jul. '88	-	-	70%	119 mg
D. filix-mas	-	-	-	RG Aug. '88	-	-	65%	128 mg
P. aquilinum	-	-	-	PBG Sep. '88	-	-	60%	139 mg

Table 2.2. Origin, viability and amount of spores used for the field experiments. The viability (final percentage germination) of each spore collection was determined by culturing samples on mineral agar (See Dyer, 1979c p282 for recipe), in the light (photon flux density = 20 $\mu Em^{-2}s^{-1}$) at 20°C, for 3 weeks. It was estimated [using a 'Sedgewick Rafter' counting cell (*Graticules Ltd.*, Tonbridge)] that there were approximately 60,000 spores in 1 mg of each spore collection.

Figure 2.2. Gametophytes were grown in small plastic pots fitted with transparent plastic lids. This photograph was taken before the need for paper liners was realized.

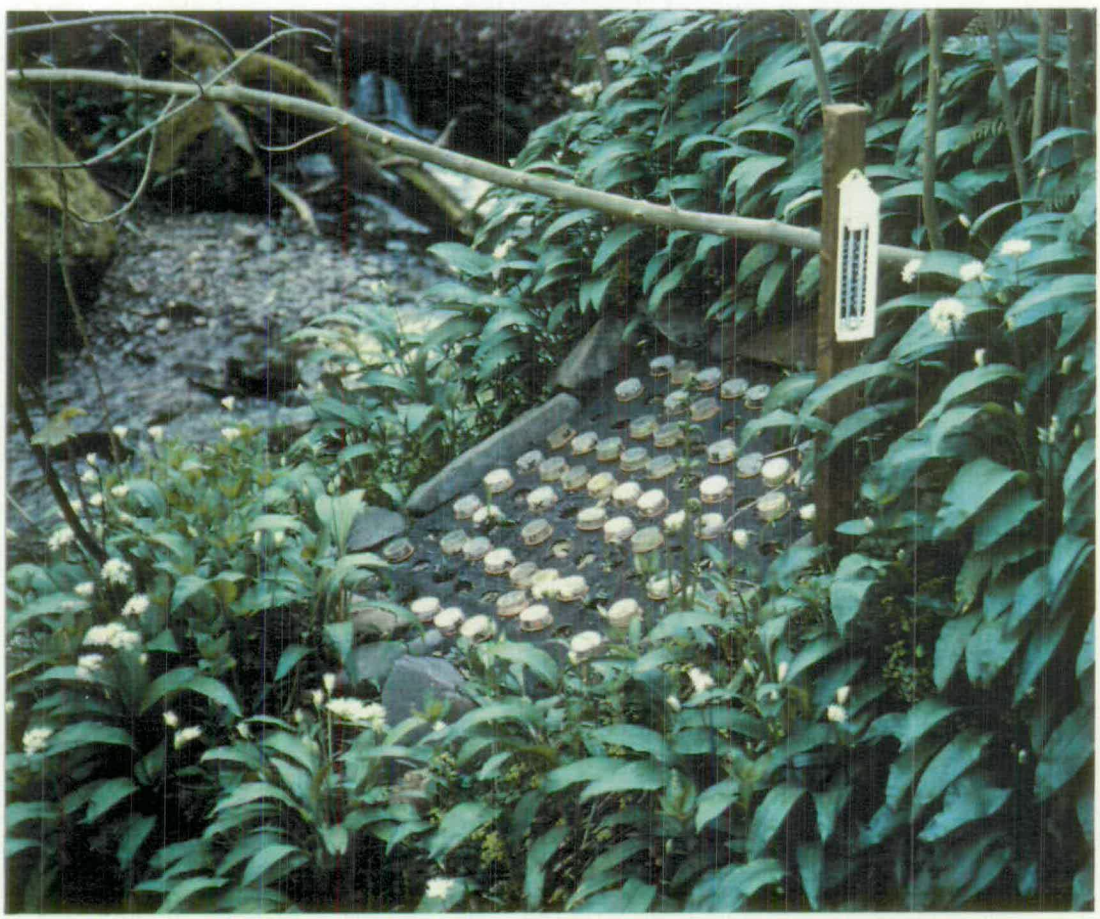


Figure 2.3. One of the 4 experimental plots (PBG.2. May 1989).

Chapter 3

Species

The following species descriptions relate to Table 3.1, Table 3.2 and Figure 3.1. They are based on published information and on personal observations.

Main species

Athyrium filix-femina

A. filix-femina is widespread and abundant throughout most of the British Isles and to an altitude of 850 m. It grows in a wide range of natural and man-made habitats but only on moist acidic soil. *A. filix-femina* is frequently associated with *B. spicant.* At Roslin Glen the fronds emerge in mid-Spring and expansion is complete by early Summer. All fronds are fertile. Most spores are released between mid-August and mid-September. Spore release continues until the first Autumn frost when fronds collapse and then die rapidly. A few spores of *A. filix-femina* can germinate in the dark but most are photoblastic [*i.e.* require light to germinate (Stoutjesdijk, 1972)]. Many can survive in the soil for at least 2 years (Dyer and Lindsay, 1992). Most sporophytes are the products of inter-gametophytic crossing mediated to some extent by a pheromone (antheridiogen) which promotes dioecy. There are 2 other species of *Athyrium* in the British Isles but no hybrids have been reported.

Blechnum spicant

B. spicant is widespread throughout the wetter parts of the British Isles (*i.e.* in western and/or upland areas). It grows in a wide range of habitats from near sea level to mountain tops but only on moist acidic soil. *B. spicant* is frequently associated with *A. filix-femina. B. spicant* is dimorphic. Only the phenology of fertile fronds is shown in Figure 3.1. At Roslin Glen the sterile and fertile fronds emerge at the same time (*contra* Page, 1982) in mid-Spring. Spores are produced in linear sori and most are

released between mid-September and mid-October. Fertile fronds die with the onset of Winter; sterile fronds are Winter-green and begin to die in the Spring shortly before new fronds emerge. All spores of *B. spicant* are photoblastic. Many can survive in the soil for at least 2 years (Dyer and Lindsay, 1992). Most sporophytes are the products of inter-gametophytic crossing mediated to some extent by an antheridiogen. *B. spicant* is the only native member of the genus *Blechnum* in the British Isles.

Polystichum setiferum

P. setiferum grows only at low altitude. It is widespread in western and southern parts of the Britain Isles but is scarce in northern England and rare in Scotland except near the west coast. *P. setiferum* grows only on moist base-rich soil and in Scotland is largely restricted to river-valley woodlands that have high humidity and offer good shelter and shade. *P. setiferum* is frequently associated with *P. scolopendrium.* At Pease Bridge Glen the fronds emerge in mid-Spring and expansion is complete by early Summer. Most fronds are fertile. Spores mature quickly and are released from mid-July to mid-September. Most spores are released in August. Fronds are Winter-green and begin to die in the Spring shortly before new fronds emerge. All spores of *P. setiferum* are photoblastic. Many can survive in the soil for at least 1 year. A laboratory experiment (Lindsay and Dyer, 1989; Lindsay *et al.,* 1992; see Appendix) suggests that some spores might survive much longer. The mating system of *P. setiferum* is not known. *P. setiferum* can hybridize with *Polystichum aculeatum* (Hard Shield Fern) and *Polystichum lonchitis* (Holly Fern).

Phyllitis scolopendrium

P. scolopendrium grows only at low altitude. It is widespread and abundant in western and southern parts of the British Isles but becomes less abundant further east and north. It is rare in central and northern Scotland. *P. scolopendrium* grows in a wide range of natural and man-made habitats on moist base-rich soil or moist base-yielding rocks and walls. In Scotland *P. scolopendrium* is largely restricted to river-valley woodlands that have high humidity and offer good shelter and shade. *P. scolopendrium* is frequently associated with *P. setiferum.* At Pease Bridge Glen the fronds emerge in mid-Spring and expansion is complete by mid-Summer. Most

fronds (even small ones) are fertile. Spores, produced in linear sori, are released throughout Autumn and early Winter. This exceptionally long spore release period results from the fact that the fronds are ever-green and the fact that many spores become trapped deep inside sori and are only released as dehisced sporangia near the surface are lost. Most spores are released in October. All spores of *P. scolopendrium* are photoblastic. Many can survive in the soil for at least 1 year. A laboratory experiment (see Appendix) suggests that some spores might survive much longer. The mating system of *P. scolopendrium* is not known. *P. scolopendrium* can hybridize with *Asplenium adiantum-nigrum* (Black Spleenwort), *Asplenium billotii* (Lanceolate Spleenwort) and *Aplenium trichomanes* subsp. *quadrivalens* (Common Maidenhair Spleenwort).

Additional species

Dryopteris affinis subsp. borreri

D. affinis subsp. *borreri* is apogamous. Archegonia are not produced. Antheridia are produced and although sperm cannot fertilize other gametophytes of the same species they can fertilize archegoniate gametophytes of *D. filix-mas.* Viable spores of *Dryopteris* spp. exist in the soil at Pease Bridge Glen and at Roslin Glen but it has still to be established that at least some are *D. affinis.*

Dryopteris dilatata

The sex expression of *D. dilatata* on mineral agar suggests that it is an out-crossing species (with no antheridiogen) but this has still to be confirmed by genetic analyses and field studies. *D. dilatata* can hybridize with *Dryopteris carthusiana* (Narrow Buckler Fern) and *Dryopteris expansa* (Northern Buckler Fern). Viable spores of *Dryopteris* spp. exist in the soil at Pease Bridge Glen and at Roslin Glen but it has still to be established that at least some are *D. dilatata.*

Dryopteris filix-mas

The sex expression of *D. filix-mas* on mineral agar suggests that it is an out-crossing species (with an antheridiogen) but this has still to be confirmed by genetic analyses and field studies. *D. filix-mas* can hybridize with *D. affinis* agg., *D. carthusiana* and *Dryopteris oreades* (Mountain Male Fern). Viable spores of *Dryopteris* spp. exist in the soil at Pease Bridge Glen and at Roslin Glen but it has still to be established that at least some are *D. filix-mas.*

Pteridium aquilinum

P. aquilinum can reproduce sexually but most of its spread is attributed to enormous rhizomes which grow rapidly and fragment into smaller plants. Some clones are over 1000 years old (Sheffield *et al.,* 1989). Although it produces more spores per frond than any other British species, gametophytes are rarely found in nature. Most sporophytes are the products of inter-gametophytic crossing mediated to some extent by an antheridiogen. Spores of *P. aquilinum* are unusual in that as many as 58% germinate in the dark. A recent study (Lindsay, Sheffield and Dyer, unpublished) has confirmed that spores of *P. aquilinum* survive for only a few months in the soil. *P. aquilinum* is the only species in the genus *Pteridium.*

Sites

In Scotland, ancient deciduous woodland is scarce and in decline. It only exists as small patches in steep-sided river valleys that are unsuitable for cultivation and inaccessible to large grazing animals.

Pease Bridge Glen and Roslin Glen shelter two of the largest remaining fragments of primary mixed oakwoods in south-east Scotland. For this reason, both have been designated Nature Reserves and are presently managed by the Scottish Wildlife Trust (S.W.T.). Both have also been designated Sites of Special Scientific Interest (S.S.S.I.); Pease Bridge Glen for its ancient woodland status and for some locally rare bryophytes and beetles; Roslin Glen for its ancient woodland status and its impressive gorge.

Pease Bridge Glen

Pease Bridge Glen (Figure 3.2a) is a narrow, steep-sided, rocky river valley, 0.5 km from the sea. It is known locally as 'Pease Dean' and it contains the Pease Burn.

The geology of Pease Bridge Glen is diverse and difficult to describe because of large deposits of glacial drift material. The underlying rock which is exposed in many places in and along the sides of the burn is mostly Silurian greywacke and Upper Old Red Sandstone. The greywacke is reputed to be somewhat, but not strongly, base-yielding.

The soil collected in the dean, near natural gametophyte populations, for use in the field experiments, was low in organic matter and was classed after Particle Size Analysis as 'sandy loam' (Table 3.3). It had a circum-neutral pH of 6.1 and compared to most agricultural soils it was rich in Potassium and even richer in Magnesium.

Local records indicate that Pease Bridge Glen has been under native deciduous woodland since at least the middle of the sixteenth century. Today, 13 native tree species are present: *Alnus glutinosa* (Alder), *Betula pubescens* (Birch), *Corylus avellana* (Hazel), *Crataegus monogyna* (Hawthorn), *Fraxinus excelsior* (Ash), *Ilex aquifolium* (Holly), *Prunus avium* (Gean), *Quercus petraea* (Sessile Oak), *Salix caprea* (Great Sallow), *Salix cinerea* (Common Sallow), *Sambucus nigra* (Elder), *Sorbus aucuparia* (Rowan), and *Ulmus glabra* (Wych Elm). There are also 3 introduced tree species: *Aesculus hippocastanum* (Horse-chestnut), *Acer pseudoplatanus* (Sycamore) and *Fagus sylvatica* (Beech). *A. hippocastanum* and *F. sylvatica* are infrequent but *A. pseudoplatanus* has become the dominant tree species in Pease Bridge Glen and its regeneration is being actively discouraged.

On the woodland floor there are over 170 species of flowering plants including many that are indicative of ancient woodland *e.g. Allium ursinum* (Ramsons), *Anemone nemorosa* (Wood Anemone), *Geum urbanum* (Herb Bennet), *Mercurialis perennis* (Dog's Mercury), *Oxalis acetosella* (Wood-sorrel) and *Silene dioica* (Red Campion). *M. perennis* and *A. ursinum* are ubiquitous.

The depth of the dean (up to 25 m), its proximity to the sea, the presence of the burn (2-5 m wide), and the dense tree canopy between Spring and Autumn result in conditions of shelter and humidity that are exceptionally high for eastern Britain. Indeed, conditions in Pease Bridge Glen are so unique that several very rare bryophytes are present. The most notable are *Cololejeunea rossettiana*, *Lejeunea lamacerina* and *Lophocolea fragrans.* These oceanic liverworts are normally restricted to the south-west of Britain and even there *C. rossettiana* and *L. fragrans* are rare (Hill *et al.,* 1991). Pease Bridge Glen is their only known eastern locality.

Conditions in Pease Bridge Glen are also suitable for large populations of *P. setiferum* and *Equisetum telmateia* (Great Horsetail); both species are extremely rare in south-east Scotland.

Table 3.4 lists all the pteridophytes recorded at Pease Bridge Glen since 1985. *A. adiantum-nigrum* and *B. spicant* were not observed during this study. *P. setiferum, P. scolopendrium, A. filix-femina, D. affinis* agg., *D. dilatata,* and *D. filix-mas* are the most common ferns. *P. setiferum* and *P. scolopendrium* are restricted to the steep, base-rich lower slopes near rocky outcrops. *A. filix-femina, Dryopteris* spp. and *P. aquilinum* are most abundant in the acid woodland above.

All the study species produced spores each year from 1986 - 1989. Fern gametophytes and sporelings were frequently observed indicating that at least some species are sexually reproducing at this site.

Pease Bridge Glen receives a large number of visitor but few of them stray from the *Southern Upland Way* long-distance footpath which is high on the east bank, or from a specially constructed walkway high on the west bank. Much of the dean is not visible from these paths and there is no easy access to the burn from them.

The experimental plots were located on the west bank, 10 m apart, and 2 m from the burn. Plot 1 (PBG.1.) was located on level ground. Plot 2 (PBG.2.; Figure 2.3) was located on a slope because there was not another $1m^2$ area of level ground. Both plots were surrounded by *P. setiferum* and *P. scolopendrium* and were within 50 cm of natural gametophyte populations. The soil in both plots was permanently moist.

Roslin Glen

Roslin Glen is an extremely deep gorge, 12.5 km from the sea, containing a 2.5 km section of the River North Esk.

The geology of Roslin Glen is diverse. There are numerous exposed strata and large deposits of glacial drift material. The underlying Carboniferous rock consists of coal seams, red sandstones, limestones, clays, shales and ironstone.

The soil collected in the glen, near natural gametophyte populations, for use in the field experiments, was low in organic matter and was classed, after Particle Size Analysis as 'loam' (Table 3.3). It had an acidic pH of 4.8 and compared to most agricultural soils it was deficient in Calcium and even more deficient in Phosphorous.

Local records indicate that Roslin Glen has been under native deciduous woodland since at least the middle of the eighteenth century. Today, 13 native tree species are present: *A. glutinosa, B. pubescens, C. avellana, C. monogyna, F. excelsior, I. aquifolium, Pinus sylvestris* (Scots Pine), *P. avium*, *Prunus padus* (Bird-Cherry), *Quercus robur* (Common Oak), *S. nigra, S. aucuparia* and *U. glabra.* There are also 6 introduced tree species: *A. hippocastanum, A. pseudoplatanus, F. sylvatica, Larix decidua* (Larch), *Picea abies* (Norway Spuce), and *Tilia* x *vulgaris* (Common Lime). *A. pseudoplatanus* has become the dominant tree species in Roslin Glen and its regeneration is being actively discouraged.

On the woodland floor there are over 200 species of flowering plants including many that are indicative of ancient woodland *e.g. A. ursinum, A. nemorosa, G. urbanum, M. perennis, O. acetosella, S. dioica,* and *Stellaria nemorum* (Wood Chickweed).

The depth of the glen (up to 45 m), the presence of the River North Esk (10-15 m wide), and the dense tree canopy between Spring and Autumn result in an extremely sheltered and humid environment. Consequently, ferns are the most conspicuous feature of this woodland at ground level.

Table 3.4 lists all the pteridophytes recorded at Roslin Glen since 1985. *E. hyemale* and *E. telmateia* are extremely rare in south-east Scotland. *A. adiantum-nigrum, D. carthusiana, P. connectilis, P. scolopendrium, E. fluviatile* and *E. telmateia* were not observed during this study. There is only one plant of *P. setiferum. A. filix-femina, B. spicant, D. affinis* agg., *D. dilatata* and *D. filix-mas* are the most common species.

All the study species, except *P. aquilinum,* produced spores each year from 1986 - 1989; *P. aquilinum* was consistently sterile. Fern gametophytes and sporelings were frequently observed indicating that at least some species are sexually reproducing at this site.

The woodland on the east bank of the River North Esk receives very few visitors. The experimental plots were located in this woodland on the banks of a small stream (1 m wide) which feeds the River North Esk. Plot 1 (RG.1.) was located on the south bank, 10 m from the stream, on level ground. Plot 2 (RG.2.) was located on the north bank 50 m downstream from Plot 1. It was 2 m from the stream and also on level ground (Figure 3.2b). Both plots were surrounded by many ferns (including *A. filix-femina* and *B. spicant*) and were within 1 m of natural gametophyte populations. The soil in plot 1 was permanently moist but the soil in plot 2 was frequently dry.

Climate

Temperature and precipitation : 1980 - 1989

The mean daily, and mean monthly, maximum and minimum air temperatures at Dunbar and Bush House, for the 10 year period, 1980 - 1989, are shown in Figures 3.3 and 3.4. These graphs span a 2 year period so that they can be more easily compared with Figures 3.7 and 3.8.

In an average year, air temperatures at Dunbar (Figure 3.3) would range from -4°C in January to 24°C in July/August; mean daily minima would range from 2°C in January to 11°C in July/August; mean daily maxima would range from 7°C in January to 18°C in July/August. There would be 33 days with frost and frost would occur in

each month from early November to mid-April. Temperatures (except the mean daily, and monthly, maxima in May, June and July) at Bush House would be slightly lower (Figure 3.4). Air temperatures at Bush House would range from -7°C in January to 24°C in July; mean daily minima would range from 0°C in January to 10°C in July; mean daily maxima would range from 5°C in January to 18°C in July. There would be 59 days with frost and frost would occur in each month from mid-October to late April.

The mean monthly precipitation, at Dunbar and Bush House, for the 10 year period, 1980-1989 is shown in Figures 3.5 and 3.6. These bar charts span a 2 year period so that they can be more easily compared with Figures 3.9 and 3.10.

In an average year, Dunbar would receive 594 mm of precipitation (Figure 3.5) and Bush House would receive 933 mm (Figure 3.6). At both sites, most of this precipitation would fall as rain and would be distributed as follows: Dunbar - Spring = 127 mm, Summer = 162 mm, Autumn = 176 mm and Winter = 129 mm; Bush House - Spring = 198 mm, Summer = 222 mm, Autumn = 271 mm and Winter = 242 mm. Very little snow would fall at Dunbar and would lie for only 5 days. More snow would fall at Bush House and would lie for 25 days.

Temperature and precipitation : 1988 and 1989

The mean daily, and monthly, maximum and minimum air temperatures at Dunbar and Bush House, for the 2 year period, January 1988 - January 1990, are shown in Figures 3.7 and 3.8. By comparing these figures with Figures 3.3 and 3.4 it becomes obvious that the Winters 1987/'88, 1988/'89 and 1989/'90 were somewhat atypical.

Winter 1987/'88 was, at the time, the mildest Winter since the mid 1970's with temperatures in January and February as much as 2.5°C above the average for Dunbar and as much as 4.5°C above the average for Bush House. Winter 1988/'89 was even milder and was in fact the mildest Winter in Scotland since the mid 1940's. Temperatures in December and January were as much as 4.5°C above the average for Dunbar and as much as 7°C above the average for Bush House. Winter 1989/'90 was not as mild as the previous Winter but was milder than Winter 1987/'88.

Although December was colder than average, temperatures in January were as much as 3.5°C above the average for Dunbar and as much as 6°C above the average for Bush House.

At Dunbar, there were 18 days with frost in 1988 and 25 days with frost in 1989. The last Spring frost of 1988 was 13 April and the first Autumn frost was 29 October. There was no frost in December 1988 or in January 1989. The last Spring frost of 1989 was 26 April and the first Autumn frost was 23 November. There was no frost in January 1990.

At Bush House, there were 42 days with frost in 1988 and 47 days with frost in 1989. The last Spring frost of 1988 was 28 April and the first Autumn frost was 11 October. There was only one day of frost in December 1988 (-1°C) and one in January 1989 (-0.1°C). The last Spring frost of 1989 was 28 April and the first Autumn frost was 6 November.

Mean daily, and monthly, maximum temperatures in May, June and July 1989, at both sites, were 1-3°C above average.

The monthly precipitation, at Dunbar and Bush House, for the 2 year period, January 1988 - January 1990, is shown in Figures 3.9 and 3.10. These figures should be compared with Figures 3.3 and 3.4.

Despite being flanked by 2 mild Winters, the amount, form, and pattern of precipitation throughout most of 1988, was not unusual. Dunbar received 550 mm; Bush House received 900 mm. Most of this precipitation fell as rain and was distributed as follows: Dunbar - Winter 1987/'88 = 125 mm, Spring = 122 mm, Summer = 185 mm, Autumn = 130 mm and Winter 1988/'89 = 64 mm; Bush House - Winter 1987/'88 = 239 mm, Spring = 196 mm, Summer = 273 mm, Autumn = 226 mm and Winter 1988/'89 = 224 mm. A little snow fell at Dunbar but did not lie. More snow fell at Bush House but lay for only 7 days.

1989 was unusually dry. Dunbar received only 366 mm of precipitation; Bush House received only 715 mm. Most of this precipitation fell as rain and was distributed as

follows: Dunbar - Winter 1988/'89 = 64 mm, Spring = 72 mm, Summer = 121 mm, Autumn = 87 mm, and Winter 1989/'90 = 130 mm; Bush House - Winter 1988/'89 = 224 mm, Spring = 160 mm, Summer = 198 mm, Autumn = 109 mm and Winter 1989/'90 = 398 mm. A little snow fell at Dunbar but as in 1988 did not lie. As usual, more snow fell at Bush House but lay for only 11 days.

Experiments 1-3	*A. filix-femina*	*B. spicant*	*P. setiferum*	*P. scolopendrium*
Common name	Woodland Lady Fern	Hard / Ladder Fern	Soft Shield Fern	Hart's Tongue Fern
Family	Athyriaceae	Blechnaceae	Aspidiaceae	Aspleniaceae
Ploidy level (in Europe)	Diploid (n=40, 2n=80)	Diploid (n=34,2n=68)	Diploid (n=41,2n=82)	Diploid (n=36, 2n=72)
Relative size	Medium - Large	Small - Medium	Medium - Large	Small - Medium
Geographic range in Europe	Northern-Continental	Sub-Atlantic	Mediterranean-Atlantic	Mediterranean-Atlantic
Maximum altitude in British Isles	850 m	1150 m	300 m	500 m
pH preference	Calcifuge	Calcifuge	Calcicole	Calcicole
Primary mode of reproduction	Sexual	Sexual	Sexual	Sexual
Length of fertile frond	70 - 140 cm	32 - 85 cm	56 - 98 cm	10 - 82 cm
Number / length of sori (per frond)	20,000	1800 mm	9,000	2600 mm
Spore production (per frond)	80 Million	5 Million	40 Million	20 Million
Spore size (area)	0.0009 mm^2	0.0017 mm^2	0.0009 mm^2	0.0008 mm^2
Mating system	Largely out-crossing	Largely out-crossing	?	?
Known to hybridize with	0 species	0 species	2 species	3 species
Dark germination	Low (0-3%)	No	No	No
Soil spore bank	Yes	Yes	Yes	Yes
Antheridiogen	Yes	Yes	?	?

Table 3.1. Main study species. Shading indicates personal observations. Other information from Cousens (1973, 1979, 1981), Darrow and Gastony (1982), Jermy *et al.* (1978), Page (1982, 1988), Schneller (1979, 1988) and Soltis and Soltis (1987, 1988).

Experiment 3	*D. affinis*	*D. dilatata*	*D. filix-mas*	*P. aquilinum*
Common name	Golden-scaled Male Fern	Broad Buckler Fern	Common Male Fern	Bracken
Family	Aspidiaceae	Aspidiaceae	Aspidiaceae	Hypolepidaceae
Ploidy level (in Europe)	Triploid (n=123, 2n=123)	Tetraploid (n=82, 2n=164)	Tetraploid (n=82, 2n=164)	Tetraploid (n=52, 2n=104)
Relative size	Medium-Large	Medium-Large	Medium-Large	Extra large
Geographic range in Europe	Atlantic / Sub-Atlantic	Sub-Atlantic	Northern - Continental	Northern - Continental
Maximum altitude in British Isles	1000 m	900 m	900 m	600 m
pH preference	Wide pH tolerance	Wide pH tolerance	Wide pH tolerance	Calcifuge
Primary mode of reproduction	Apomixis	Sexual	Sexual	Asexual
Length of fertile frond	85 - 153 cm	80 -112 cm	80 - 126 cm	100 - 250 cm
Number / length of sori (per frond)	8,000	18,000	9,000	> 5000 mm
Spore production (per frond)	90 Million	40 Million	100 Million	300 Million
Spore size (area)	0.0021 mm^2	0.0018 mm^2	0.0015 mm^2	0.0008 mm^2
Mating system	(Apogamy)	? (Largely out-crossing)	? (Largely out-crossing)	Largely out-crossing
Known to hybridize with	1 species	2 species	3 species	0 species
Dark germination	No	No	No	High (26% - 58%)
Soil spore bank	? (Yes)	? (Yes)	? (Yes)	No
Antheridiogen	?	No	Yes	Yes

Table 3.2. Additional study species. Shading indicates personal observations. Other information from Barker and Willmot (1985), Conway (1949, 1957), Jermy *et al.* (1978), Näf (1958), Näf *et al.* (1975), Page (1982, 1988), Wolf (1986), and Wolf *et al.* (1988).

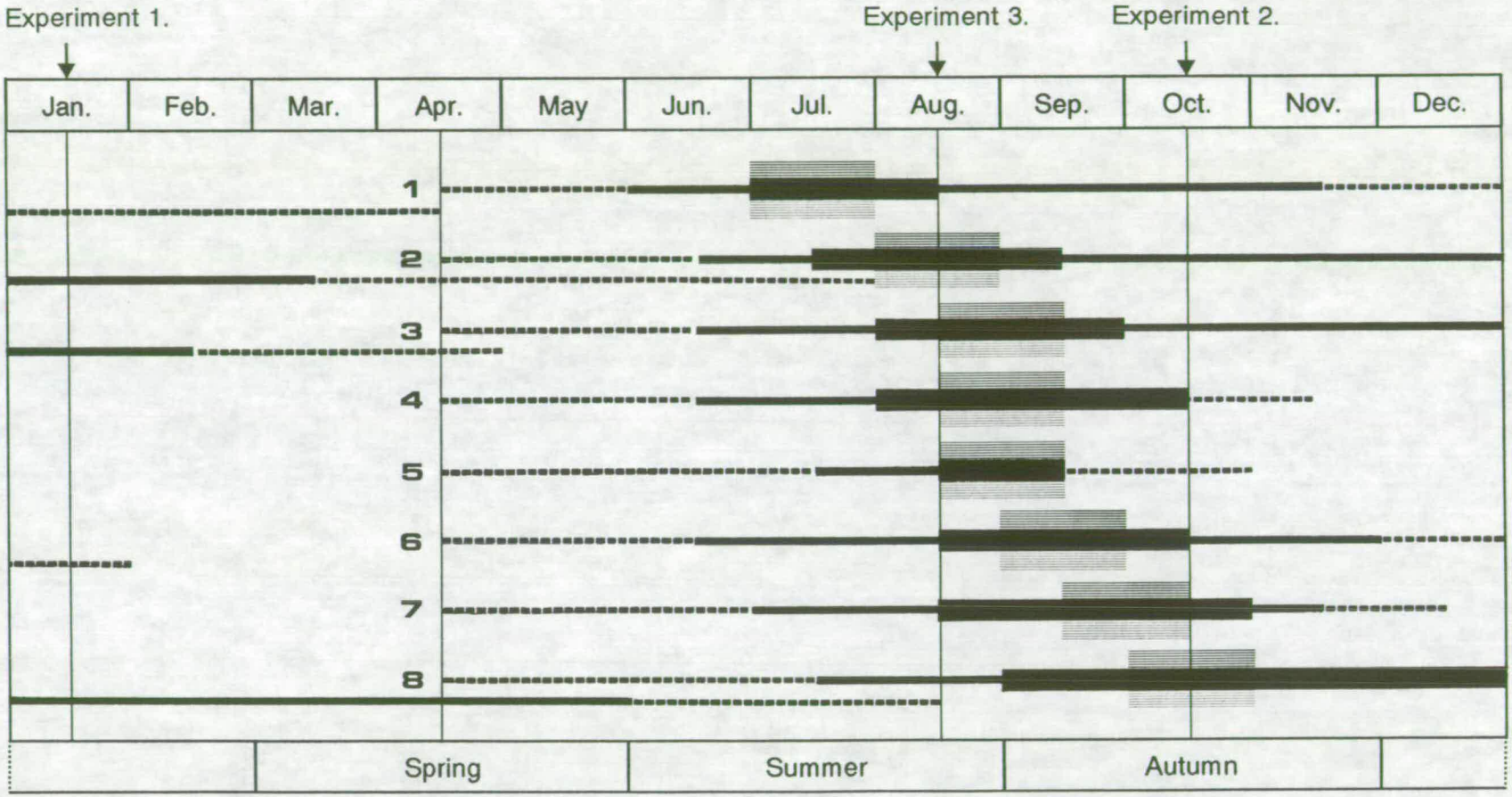
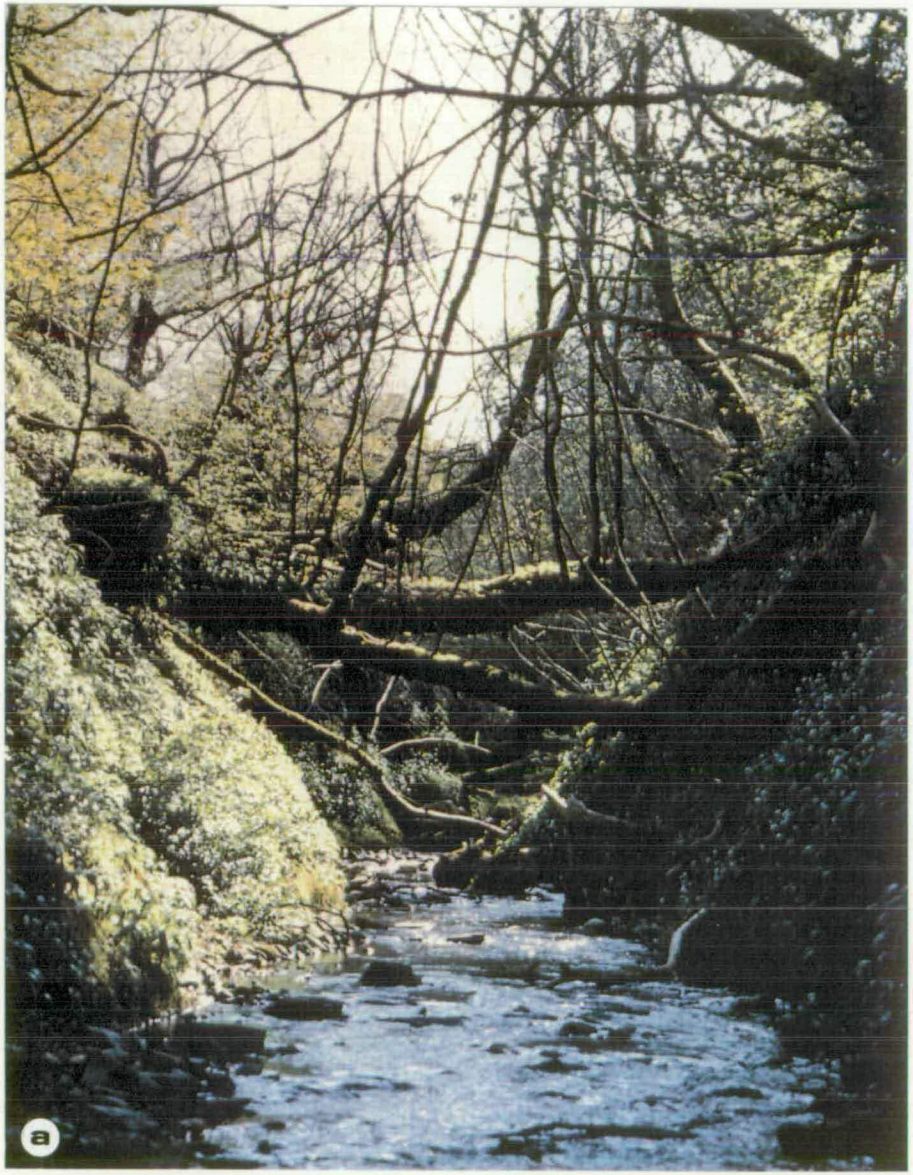


Figure 3.1. 'Phenograms' (after Page, 1982) for sporophylls of **1.** *D. dilatata,* **2.** *P. setiferum,* **3.** *D. filix-mas,* **4.** *A. filix-femina,* **5.** *P. aquilinum,* **6.** *D. affinis* subsp. *borreri,* **7.** *B. spicant,* and **8.** *P. scolopendrium.* They show, the period of frond expansion (first dashed line), the period from complete expansion to spore maturity (first solid line), the spore release period (thick line), the peak spore release period (shaded region), any period after spore release when fronds remain green (second solid line) and the period during which the fronds die (second dashed line). [Personal observations].

Figure 3.2. **a.** Pease Bridge Glen in May 1988. Experimental plots were located on the left bank 25 m upsteam (not visible). **b.** Roslin Glen in June 1988. Plot 2 was located on the level ground to the left of the stream (not visible); plot 1 was located 50 m upstream on the opposite side.


a


b

		PBG	RG
Physical analysis (%)	Organic matter	2.3	2.2
	Coarse sand	26.5	22.9
	Fine sand	25.7	24.4
	Silt	30.4	30.8
	Clay	15.1	19.7
	Textural class	Sandy loam	Loam
	pH	6.1	4.8
Chemical analysis ($mgkg^{-1}$ dry wt.)	Phosphorus	31.0	6.3
	Potassium	285.0	101.0
	Magnesium	383.0	205.0
	Sodium	50.0	35.0
	Calcium	1505.0	597.0
	Total Nitrogen	1900.0	1300.0

Table 3.3. Physical and chemical analyses of the soil used for the field experiments. The values for Phosphorous, Potassium, Magnesium, Sodium and Calcium are estimates of the amounts *available* for plant uptake. Soil from Pease Bridge Glen has a higher pH and a higher nutrient status than soil from Roslin Glen.

Species (Latin name)	PBG	RG	Species (Common Name)
Asplenium adiantum-nigrum L.	+	+	Black Spleenwort
Asplenium trichomanes L. subsp.*quadrivalens* D.E. Meyer emend. Lovis	+	-	Delicate / Common Maidenhair Spleenwort
Athyrium filix-femina (L.) Roth	+	+	Woodland Lady Fern
Blechnum spicant (L.) Roth	+	+	Hard / Ladder Fern
Dryopteris affinis (Lowe) Fraser-Jenkins subsp. *borreri* (Newman) Fraser-Jenkins	+	+	Common Golden-scaled Male Fern
Dryopteris affinis (Lowe) Fraser-Jenkins subsp. *robusta* Oberholzer et von Tavel ex Fraser-Jenkins	+	+	Robust Golden-scaled Male Fern
Dryopteris carthusiana (Vill.) H.P. Fuchs	-	+	Narrow Buckler Fern
Dryopteris dilatata (Hoffm.) A. Gray	+	+	Broad Buckler Fern
Dryopteris expansa (C. Presl) Fraser-Jenkins & Jermy	-	+	Northern Buckler Fern
Dryopteris filix-mas (L.) Schott	+	+	Common Male Fern
Gymnocarpium dryopteris (L.) Newm.	-	+	Woodland Oak Fern
Oreopteris limbosperma (All.) Holub	-	+	Sweet / Lemon-scented Mountain Fern
Phegopteris connectilis (Michx) Watt	-	+	Beech Fern
Phyllitis scolopendrium (L.) Newm.	+	+	Hart's Tongue Fern
Polypodium interjectum Shivas	+	-	Western Polypody
Polypodium vulgare L.	+	+	Common Polypody
Polystichum aculeatum (L.) Roth	+	+	Hard Shield Fern
Polystichum setiferum (Forsk.) Woynar	+	+	Soft Shield Fern
Pteridium aquilinum (L.) Kuhn	+	+	Bracken
Equisetum fluviatile L.	-	+	Water Horsetail
Equisetum hyemale L.	-	+	Dutch Rush / Rough Horsetail
Equisetum palustre L.	-	+	Marsh Horsetail
Equisetum sylvaticum L.	+	+	Wood Horsetail
Equisetum telmateia Ehrh.	+	+	Great Horsetail

Table 3.4. Pteridophytes at Pease Bridge Glen and Roslin Glen (recorded since 1985).

Figures 3.3 and **3.4.** Mean daily, and mean monthly, maximum and minimum air temperatures at Dunbar (Figure 3.3) and Bush House (Figure 3.4) for the 10 year period, 1980 - 1989; mean daily minimum (●), mean daily maximum (■), mean monthly minimum (○) and mean monthly maximum (□). Compare with Figures 3.7 and 3.8.

Figures 3.5 and **3.6.** Mean monthly precipitation at Dunbar (Figure 3.5) and Bush House (Figure 3.6) for the 10 year period, 1980 - 1989. Compare with Figures 3.9 and 3.10.

Figures 3.7 and **3.8.** Mean daily, and monthly, maximum and minimum air temperatures at Dunbar (Figure 3.7) and Bush House (Figure 3.8) for the 2 year period, January 1988 - January 1990; mean daily minimun (●), mean daily maximum (■), monthly minimum (○) and monthly maximum (□). Compare with Figures 3.3 and 3.4.

Figures 3.9 and **3.10.** Monthly precipitation at Dunbar (Figure 3.9) and Bush House (Figure 3.10) for the 2 year period, January 1988 - January 1990. Compare with Figures 3.5 and 3.6.

Figures 3.3 and **3.4.** Mean daily, and mean monthly, maximum and minimum air temperatures at Dunbar (Figure 3.3) and Bush House (Figure 3.4) for the 10 year period, 1980 - 1989; mean daily minimum (●), mean daily maximum (■), mean monthly minimum (○) and mean monthly maximum (□). Compare with Figures 3.7 and 3.8.

Figure 3.3

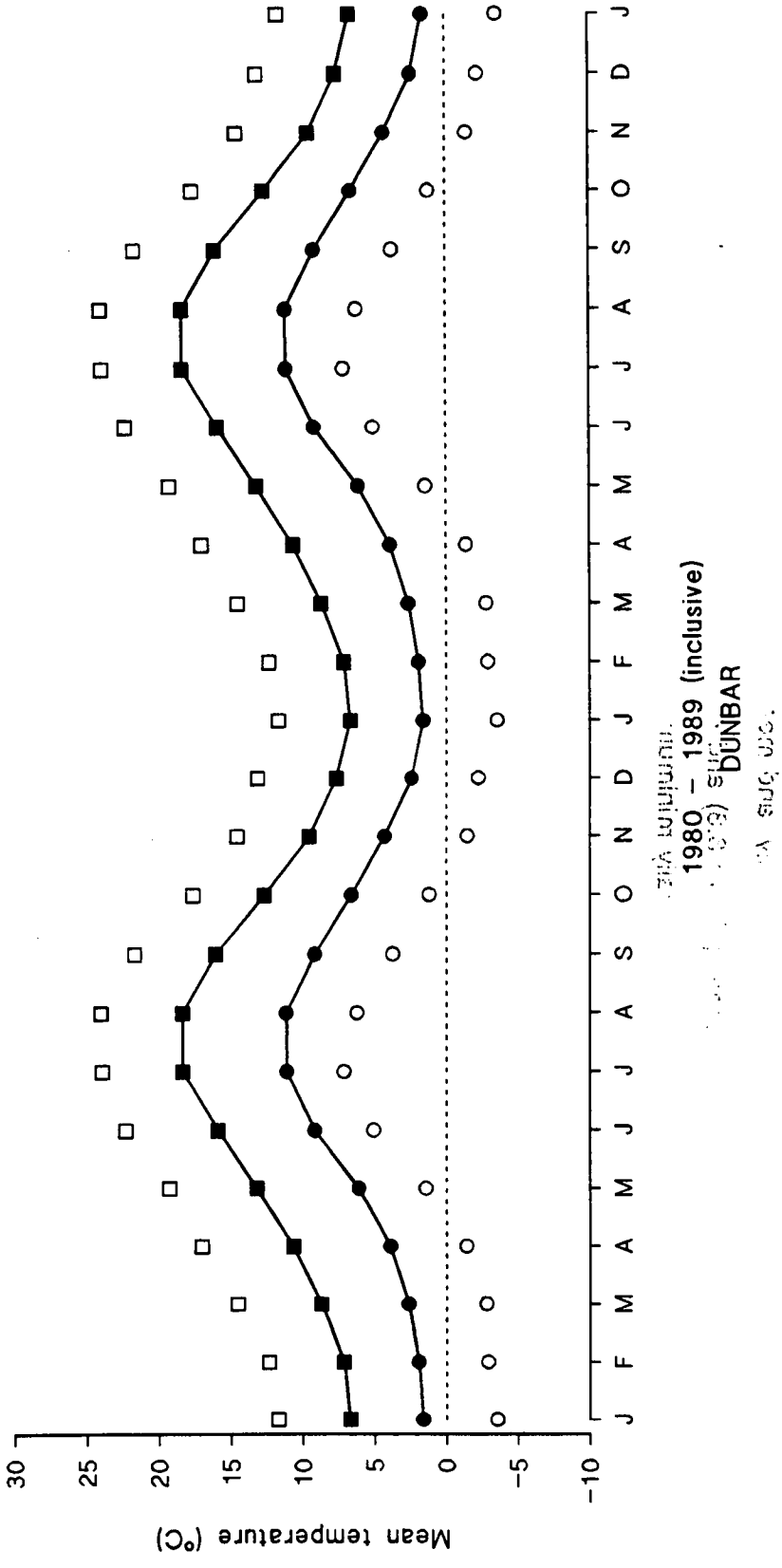
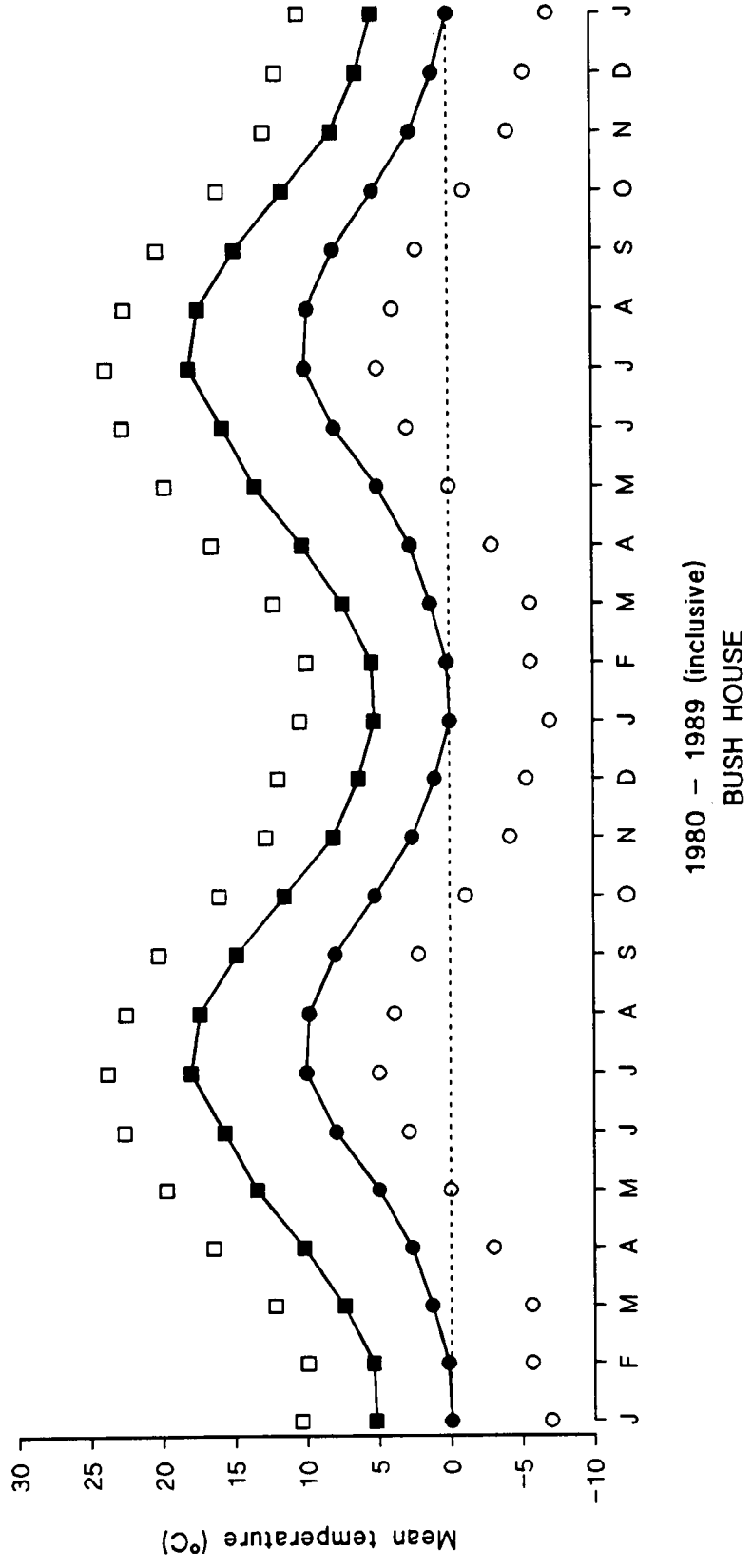

Mean temperature (°C)
30
25
20
15
10
5
0
−5
−10
J F M A M J J A S O N D J F M A M J J A S O N D J
1980 – 1989 (inclusive)
DUNBAR

Figure 3.4


Mean temperature (°C)
30
25
20
15
10
5
0
-5
-10
J F M A M J J A S O N D J F M A M J J A S O N D J
1980 – 1989 (inclusive)
BUSH HOUSE

Figures 3.5 and **3.6.** Mean monthly precipitation at Dunbar (Figure 3.5) and Bush House (Figure 3.6) for the 10 year period, 1980 - 1989. Compare with Figures 3.9 and 3.10.

Figure 3.5

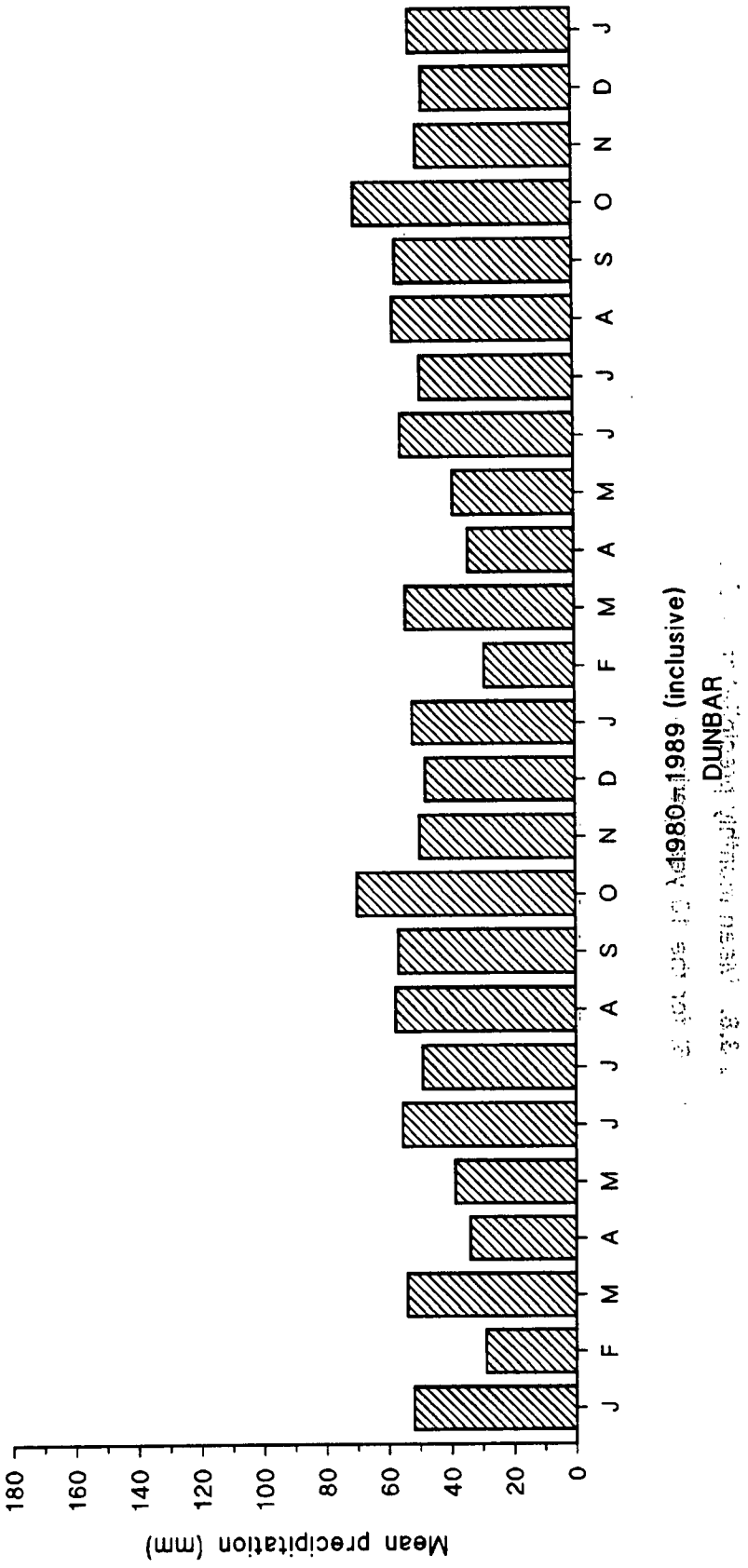
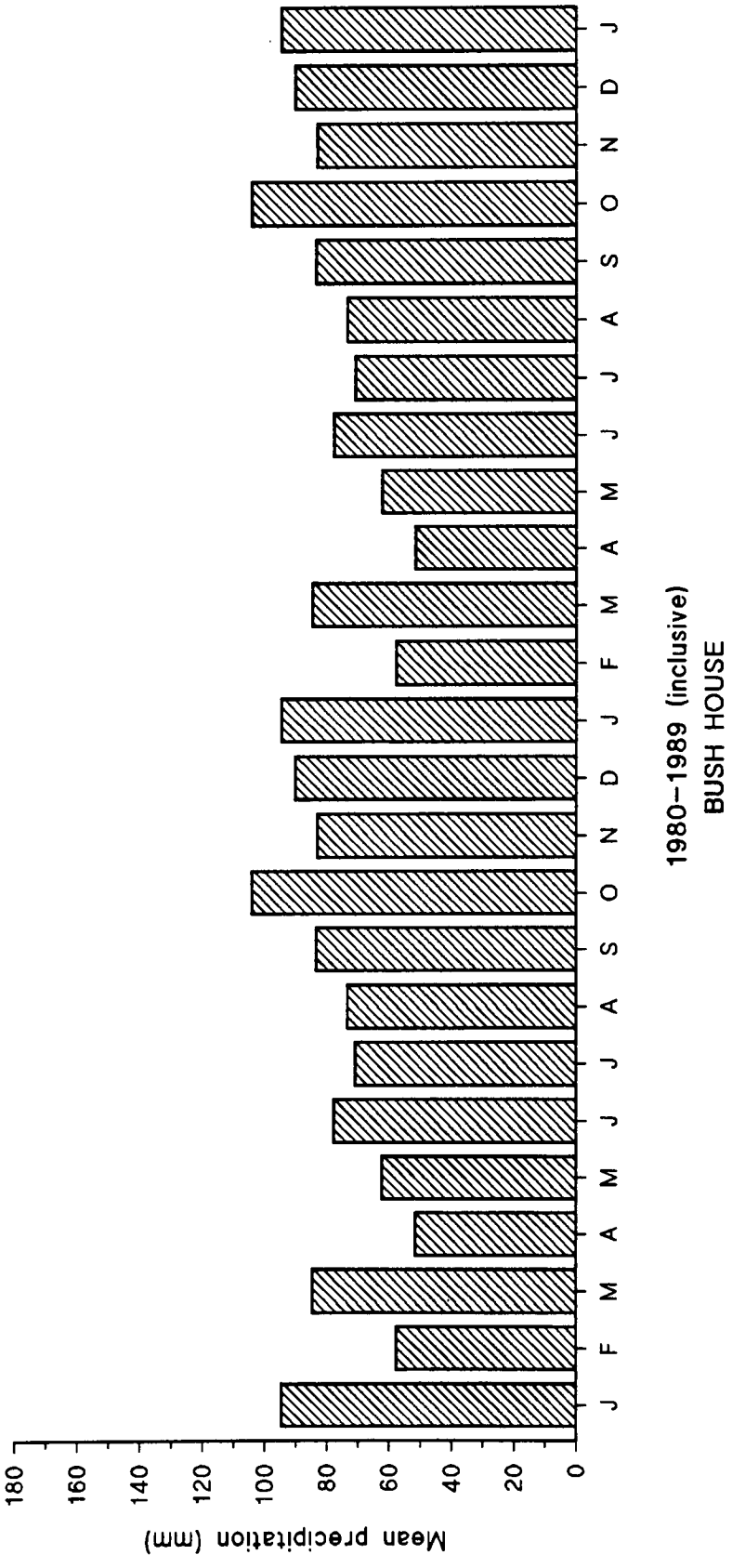
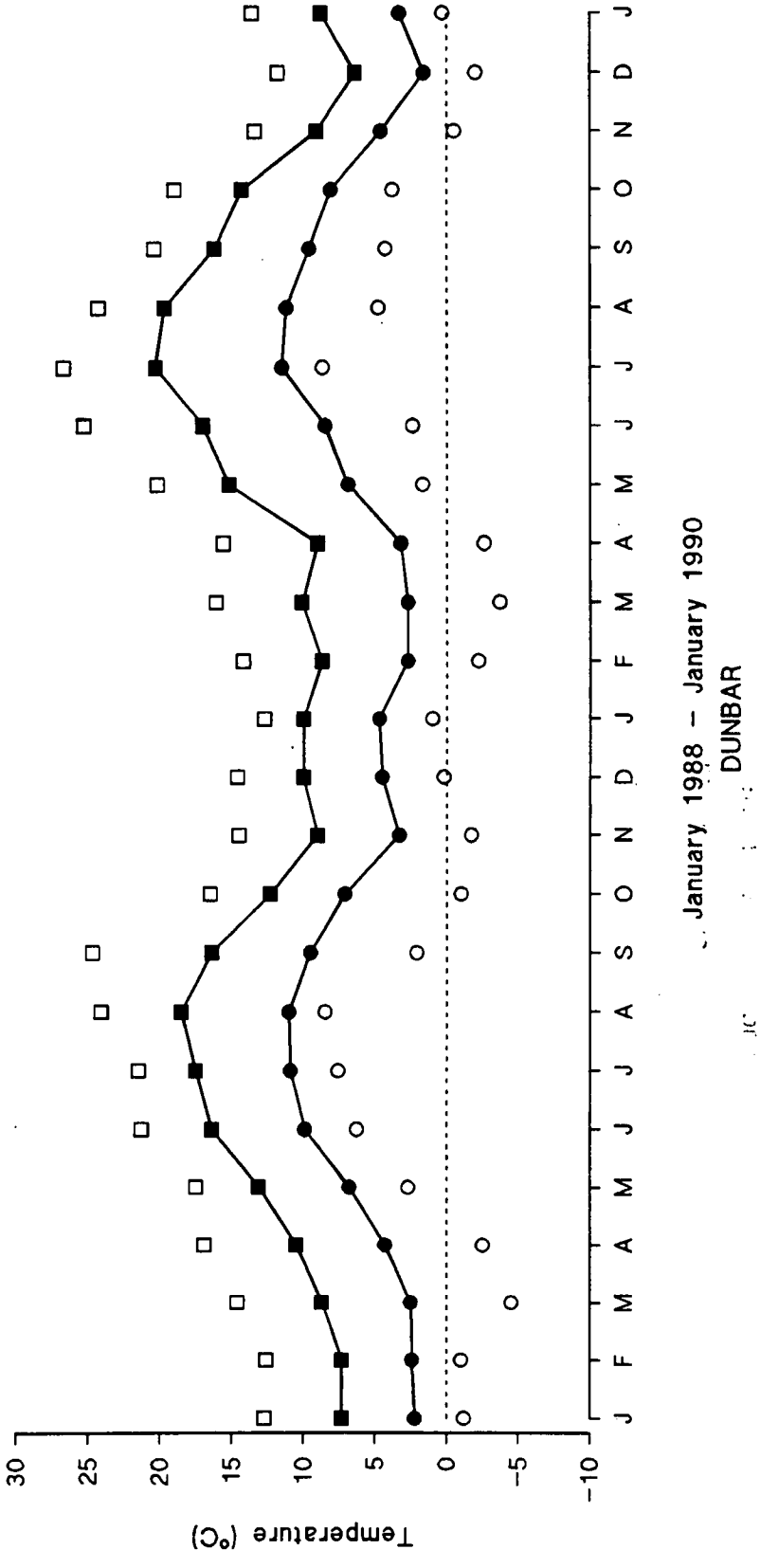

Mean precipitation (mm)
0
20
40
60
80
100
120
140
160
180
J F M A M J J A S O N D J F M A M J J A S O N D J
1980-1989 (inclusive)
DUNBAR

Figure 3.6


Mean precipitation (mm)
180
160
140
120
100
80
60
40
20
0
J F M A M J J A S O N D J F M A M J J A S O N D J
1980–1989 (inclusive)
BUSH HOUSE

Figures 3.7 and **3.8.** Mean daily, and monthly, maximum and minimum air temperatures at Dunbar (Figure 3.7) and Bush House (Figure 3.8) for the 2 year period, January 1988 - January 1990; mean daily minimun (●), mean daily maximum (■), monthly minimum (○) and monthly maximum (□). Compare with Figures 3.3 and 3.4.

Figure 3.7


Temperature (°C)
30
25
20
15
10
5
0
-5
-10
J F M A M J J A S O N D J F M A M J J A S O N D J
January 1988 – January 1990
DUNBAR

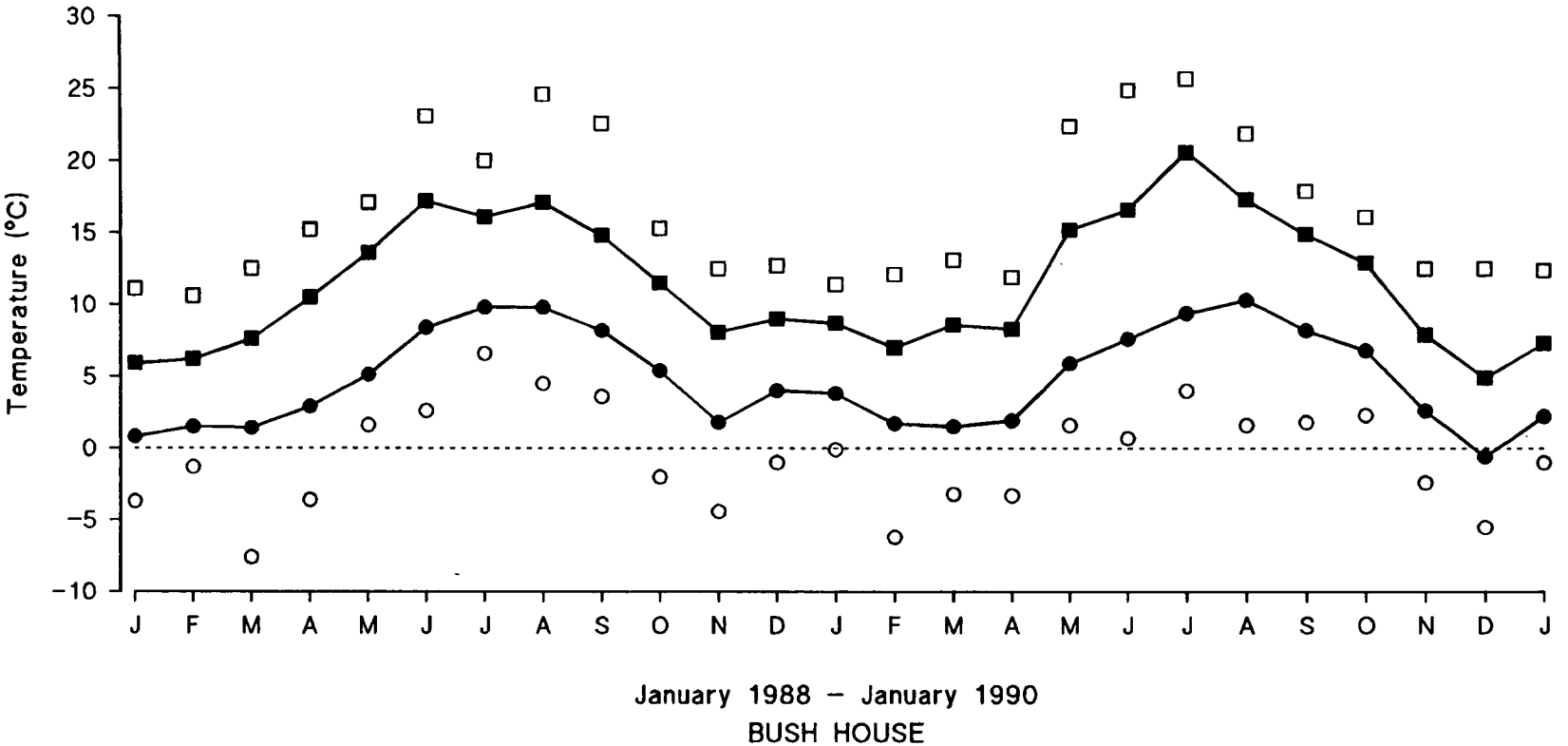

Temperature (°C)
30
25
20
15
10
5
0
−5
−10
J F M A M J J A S O N D J F M A M J J A S O N D J
January 1988 – January 1990
BUSH HOUSE

Figure 3.8

Figures 3.9 and **3.10.** Monthly precipitation at Dunbar (Figure 3.9) and Bush House (Figure 3.10) for the 2 year period, January 1988 - January 1990. Compare with Figures 3.5 and 3.6.

Figure 3.9

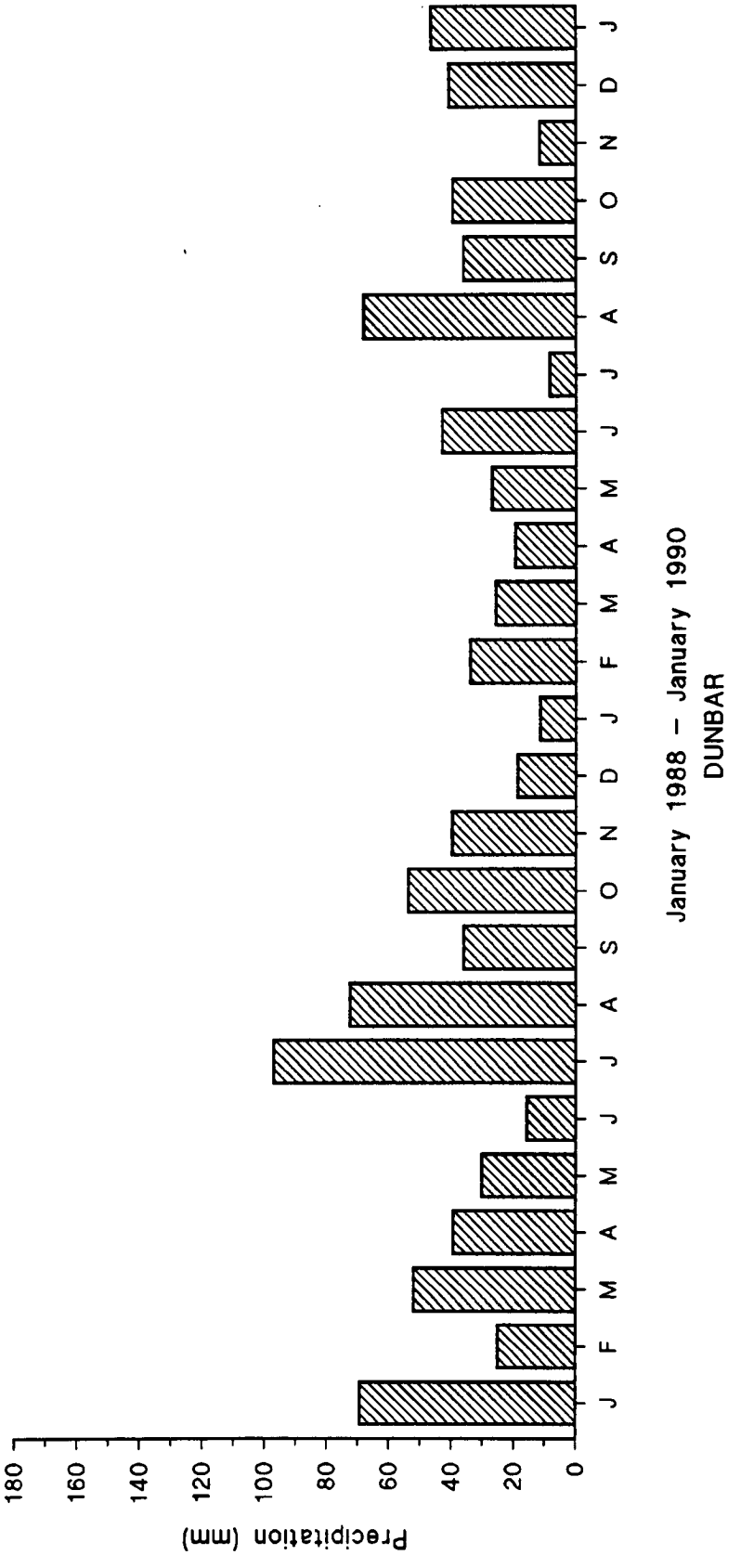
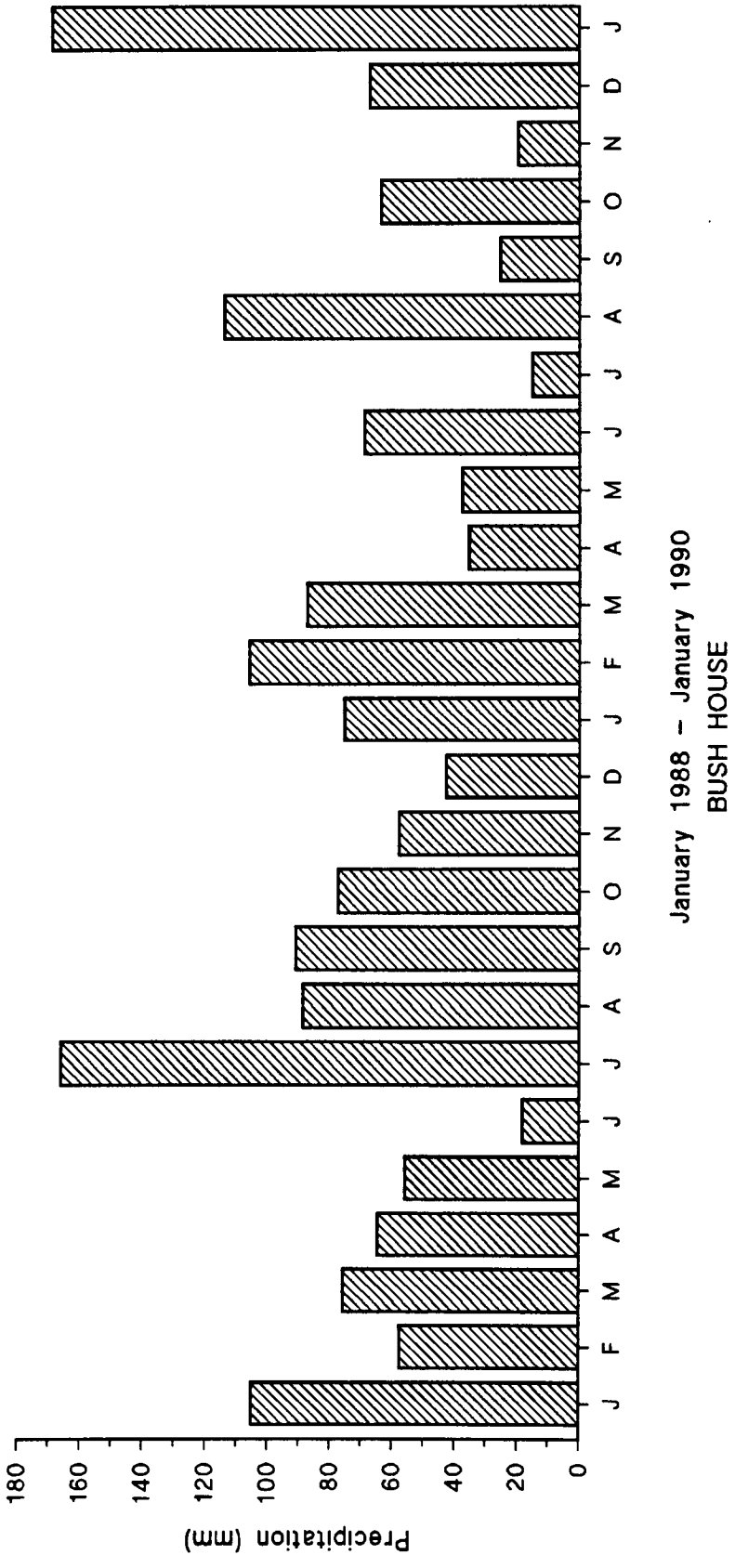

Precipitation (mm)
0
20
40
60
80
100
120
140
160
180
J F M A M J J A S O N D J F M A M J J A S O N D J
January 1988 – January 1990
DUNBAR

Figure 3.10


Precipitation (mm)
180
160
140
120
100
80
60
40
20
0
J F M A M J J A S O N D J F M A M J J A S O N D J
January 1988 - January 1990
BUSH HOUSE

Chapter 4

Results of Experiment 1

Introduction

Soil spore banks are of interest because they create opportunities for gametophyte establishment at any time of the year following soil disturbance. Successful gametophyte establishment might result more often from spores that germinate in the Spring, from soil spore banks, than from spores that germinate in the Autumn immediately after dispersal.

Experiment 1 was initiated in January 1988, several months after the main spore release season (Figure 3.1), to investigate the events in the fern life-cycle following 'soil disturbance' in Winter.

Pease Bridge Glen

The following text relates to Table 4.1 and Figures 4.1 - 4.8.

Germination

Spores were sown in January 1988. Tiny gametophytes (mean areas: 0.0045 mm^2 - 0.0089 mm^2) of *A. filix-femina, P. setiferum* and *P. scolopendrium* were first detected, in both plots, in April 1988. The largest gametophytes (comprising 3-4 chlorocytes) were already 2-dimensional. Gametophytes of *B. spicant* were first detected, in both plots, in May 1988. Most of these were also 2-dimensional. Within each species there was no evidence of staggered germination (Figures 4.1b - 4.8b). The numbers of gametophytes in the pots were not accurately determined but densities were estimated to range from approximately 50-250 gametophytes cm^{-2}.

Sex expression

Most populations were pre-sexual for only 1-2 months. Antheridia or antheridia and archegonia first appeared in most populations (including both populations of *B. spicant*) in June or July 1988. Sexual development in 2 populations, *P. setiferum* [2] and *P. scolopendrium* [2], was a little slower; male, female and bisexual gametophytes first appeared in these in August 1988. Most antheridia were already mature when they were first detected.

The sexual transition periods were surprisingly short; 80-100% of gametophytes in all populations were sexual by July or August 1988; all gametophytes in all populations were sexual by November 1988.

The sexual composition of all populations remained relatively constant from near the end of the sexual transition periods to the Spring or early Summer of 1989 (see 'equilibrium periods' in Table 4.1; Figures 4.1a - 4.8a). *P. scolopendrium* [1] had an approximately equal number of male and archegoniate gametophytes but all the other populations were predominantly (*i.e.* 55-90%) male.

Bisexual gametophytes were detected in all populations but most archegoniate gametophytes were female. All bisexual gametophytes were protandrous.

Size and shape

In general, in all populations, female gametophytes were larger than bisexual gametophytes and all archegoniate gametophytes were considerably larger than male gametophytes. All archegoniate gametophytes were meristic (somewhat heart-shaped); all male gametophytes were ameristic (spathulate when young, various shapes when older but never heart-shaped). The shape of pre-sexual gametophytes was not recorded. In all populations the first archegoniate gametophytes were considerably larger than the largest pre-sexual gametophytes in the sample 1 month before (Figures 4.1b - 4.8b).

Fertilization

Fertilization occurred in all populations and was not restricted to any particular period of the year. Embryos first appeared as early as July 1988 (less than 1 month after the first appearance of archegonia *e.g. A. filix-femina* [1] and *P. scolopendrium* [1]) and as late as January 1989 (5 months after the first appearance of archegonia *e.g. B. spicant* [2]).

By January 1989 (1 year after sowing) the majority of archegoniate gametophytes in most populations and all archegoniate gametophytes in *A. filix-femina* [1] and *P. scolopendrium* [1] had been fertilized. Only 1% of archegoniate gametophytes in *B. spicant* [2] had been fertilized but 74% had been fertilized in *B. spicant* [1]. By the end of the second year all archegoniate gametophytes in all populations, including *B. spicant* [2], had been fertilized. Polyembryony was not observed.

Mortality

All gametophytes survived the Winter of 1988/'89. Male gametophytes in all populations began to die in the Spring of 1989. Decomposition was rapid and their decline was noticed by the increasingly frequent absence of male gametophytes from samples rather than by the presence of dead or dying gametophytes. Some archegoniate gametophytes attached to sporelings also began to die during late Spring or early Summer. Their death was more easily observed. Between the Spring or Summer of 1989 (see Figures 4.1a - 4.8a for exact time in each population) and the end of the experiment, all male and nearly all archegoniate gametophytes died. During this period all populations changed from being composed entirely of gametophytes to being composed almost entirely of established sporelings. Curiously, only archegoniate gametophytes attached to sporelings died; archegoniate gametophytes bearing embryos and unfertilized archegoniate gametophytes (although very few of these remained at this stage) did not. Some of the unfertilized gametophytes developed one or more lobes resembling new archegoniate gametophytes. One 'daughter' gametophyte on each lobed gametophyte was fertilized during the second half of 1989 by the few remaining functional males (polyembryony was not observed). Lobed gametophytes were most frequent in *B. spicant* [2] but there were a few similar individuals in all populations.

Sporelings of *B. spicant, P. setiferum* and *P. scolopendrium* were Winter-green but the larger leaves of *A. filix-femina* died during the Winter of 1989/'90. Despite the death of older leaves new ones continued to emerge. No sporelings died.

Maximum development

The largest gametophyte in most populations was female but in *P. setiferum* [1] and *P. scolopendrium* [1] it was a bisexual. The largest gametophyte observed at this site, during this experiment, was a female in *B. spicant* [1] which had an area of 114.12 mm^2 (not illustrated in Figure 4.3b because it is off the scale). The most advanced sporophyte in each population in January 1990 had 5-9 leaves.

Roslin Glen

The following text relates to Table 4.2 and Figures 4.9 - 4.16.

Germination

Spores were sown in January 1988. Tiny gametophytes (mean areas: 0.0040 mm^2 - 0.0065 mm^2) of *A. filix-femina, P. setiferum* and *P. scolopendrium* were first detected, in both plots, in April 1988. The largest gametophytes (comprising 3 chlorocytes) were already 2-dimensional. Gametophytes of *B. spicant* were first detected, in both plots, in May 1988. Some of these were also 2-dimensional. Within in each species there was no evidence of staggered germination (Figures 4.9b - 4.16b). The numbers of gametophytes in the pots were not accurately determined but densities were estimated to range from approximately 50-250 gametophytes cm^{-2}.

Sex expression

Both populations of *A. filix-femina* and *B. spicant* were pre-sexual for 1 month or less; antheridia appeared in June or July 1988 before archegonia. Both populations (but especially those in plot 2) of *P. setiferum* and *P. scolopendrium* were pre-sexual for much longer. In all populations most antheridia were already mature when they were first detected.

The sexual transition periods in Plot 1 were surprisingly short; the sexual transition periods in plot 2 were much longer. Indeed, all gametophytes in plot 1 were sexual by November 1988, whereas not all gametophytes in plot 2 were sexual until June 1989.

The sexual composition of all populations remained relatively constant from near the end of the sexual transition periods to late Spring or early Summer of 1989 (see 'equilibrium periods' in Table 4.2; Figures 4.9a - 4.16a). All populations were predominantly (*i.e.* 75-90%) male.

Bisexual gametophytes were detected in all populations but most archegoniate gametophytes were female. All bisexual gametophytes were protandrous.

Size and shape

In general, in all populations, female gametophytes were larger than bisexual gametophytes and all archegoniate gametophytes were considerably larger than male gametophytes. All archegoniate gametophytes were meristic (somewhat heart-shaped); all male gametophytes were ameristic (spathulate when young, various shapes when older but never heart-shaped). The shape of pre-sexual gametophytes was not recorded. In all populations the first archegoniate gametophytes were considerably larger than the largest pre-sexual gametophytes in the sample 1 month before (Figures 4.9b - 4.16b).

Fertilization

Fertilization occurred in all populations and was not restricted to any particular period of the year. In plot 1 embryos first developed in *A. filix-femina, B. spicant* and *P. setiferum* in September 1988 but embryos did not appear in *P. scolopendrium* until almost a year later. No embryos appeared in plot 2 in the first year; embryos first developed in *A. filix-femina, B. spicant* and *P. setiferum* in June 1989 and embryos did not appear in *P. scolopendrium* until the Winter of 1989/'90.

By January 1990 (2 years after sowing), nearly all archegoniate gametophytes in all populations (except *P. scolopendrium* [2]) had been fertilized. Polyembryony was not observed.

Mortality

All gametophytes survived the winter of 1988/'89. Male gametophytes in *P. scolopendrium* [1] began to die between April and June 1989. Fertilized archegoniate gametophytes in this population and many male and fertilized archegoniate gametophytes in all the other populations died sometime after June 1989 (Figures 4.9a - 4.16a) but the precise times are not known because no samples were collected for 5 months between June and November 1989. By November 1989, 6 of the 8 populations had almost no male gametophytes. The two remaining populations *B. spicant* [2] and *P. scolopendrium* [2] still had many healthy and fertile male gametophytes. Also by November, most gametophytes attached to sporelings in most populations were in poor condition. However, all sporelings still had gametophytes attached. This was also true in January 1990 (at the end of the experiment) for most populations but there were many established sporelings in *A. filix-femina* [1] and *B. spicant* [1]. Unfertilized archegoniate gametophytes always remained healthy. Many of these developed one or more lobes resembling new archegoniate gametophytes (observed in November 1989 and January 1990). All populations in plot 2 had some gametophytes like this but only one population (*P. setiferum*) in plot 1 had any. None of these lobed gametophytes were fertilized.

Sporelings of *B. spicant, P. setiferum* and *P. scolopendrium* were Winter-green but the larger leaves of *A. filix-femina* died during the Winter of 1989/'90. Despite the death of older leaves new ones continued to emerge. No sporelings died.

Maximum development

The largest gametophyte in all populations was female. The largest gametophyte observed at this site, during this experiment, was a female in *P. setiferum* [2] which had an area of 40.53 mm^2. The most advanced sporophyte in each population in January 1990 had 2-6 leaves.

Summary

The results for *A. filix-femina, B. spicant, P. setiferum* and *P. scolopendrium* were strikingly similar.

At both sites, gametophytes of all 4 species appeared in the Spring but at a lower density than in the laboratory (see page 9). There was no staggered germination and no obvious protonemal phase (Raghavan, 1989).

The majority of gametophytes became sexual in the first Summer; transition periods were often surprisingly short; most antheridia and some archegonia were mature when they were first detected. The sexual composition of all populations remained relatively constant from near the end of the sexual transition period to the Spring or Summer of the following year. During this equilibrium period most populations were predominantly male (and to a greater degree at Roslin Glen than at Pease Bridge Glen). Bisexual gametophytes were detected in all populations but most archegoniate gametophytes were female. All bisexual gametophytes were protandrous.

In general, female gametophytes were larger than bisexual gametophytes and all archegoniate gametophytes were considerably larger than male gametophytes. All archegoniate gametophytes were meristic; all male gametophytes were ameristic. Curiously, the first archegoniate gametophytes in all populations were considerably larger than the largest pre-sexual gametophytes in the sample 1 month before.

Fertilization occurred in all populations and was not seasonal (embryos continued to appear throughout the Winters of 1988/'89 and 1989/'90). In some populations embryos appeared less than 1 month after the first appearance of archegonia. By the end of the experiment (*i.e.* after 2 years) all archegoniate gametophytes at Pease Bridge Glen and most archegoniate gametophytes at Roslin Glen had been fertilized. Polyembryony was not observed.

All gametophytes survived for at least 1 year (and through the Winter of 1988/'89). Male and fertilized archegoniate gametophytes began to die in the Spring or Summer of 1989. Unfertilized archegoniate gametophytes did not die (some of these

developed lobes, bearing new batches of archegonia, during the last 6 months of the experiment). No sporelings died.

In terms of the rates of early (*i.e.* pre-sexual) gametophyte development, the rates at which populations became sexual, the percentages of archegoniate gametophytes at equilibrium, the percentages of fertilization and the rates of fertilization and leaf production, all 4 species grew better at Pease Bridge Glen than at Roslin Glen. Moreover, all 4 species grew as well in PBG.1. as in PBG.2. At Roslin Glen all 4 species grew much better in plot 1 than in plot 2.

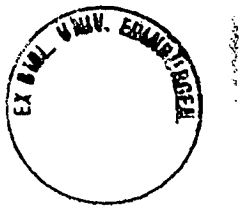


Table 4.1. Results of Experiment 1 at Pease Bridge Glen. Shading indicates the species that were on alien soil.

Experiment 1 : Pease Bridge Glen	*A. filix-femina*		*B. spicant*		*P. setiferum*		*P. scolopendrium*	
Plot	PBG.1.	PBG.2.	PBG.1.	PBG.2.	PBG.1.	PBG.2.	PBG.1.	PBG.2.
See Figure	4.1	4.2	4.3	4.4	4.5	4.6	4.7	4.8
Spores sown	Jan.'88	Jan.'88	Jan.'88	Jan.'88	Jan.'88	Jan.'88	Jan.'88	Jan.'88
Germination	Apr.'88	Apr.'88	May '88	May '88	Apr.'88	Apr.'88	Apr.'88	Apr.'88
Pre-sexual period (months)	1	1	< 1	1	1	3	2	3
Size of last sterile gametophytes (mm^2)	0.37	0.93	0.60	0.86	0.22	0.28	0.26	1.37
First male gametophytes	Jun.'88	Jun.'88	Jun.'88	Jul.'88	Jun.'88	Aug.'88	Jul '88	Aug.'88
Size of first male gametophytes (mm^2)	0.14	0.12	0.16	0.40	0.31	1.11	0.56	0.64
First female gametophytes	Jun.'88	Jul.'88	Jun.'88	Aug.'88	Jul.'88	Aug.'88	Jul.'88	Aug.'88
Size of first female gametophytes (mm^2)	0.79	1.64	0.52	1.58	5.10	6.67	2.75	1.51
First bisexual gametophytes	Jul.'88	Aug.'88	Jul.'88	Aug.'88	Jul.'88	Aug.'88	Jul.'88	Aug.'88
Size of first bisexual gametophytes (mm^2)	5.07	2.26	1.37	1.49	4.45	4.34	1.79	1.46
All gametophytes sexual by	Aug.'88	Nov.'88	Aug.'88	Sep.'88	Jul.'88	Aug.'88	Aug.'88	Nov.'88
Transition period (months)	3	6	3	3	2	1	2	4
Equilibrium period (months)	*c.*8	*c.*8	*c.*6	*c.*7	*c.*8	*c.*5	*c.*11	*c.*7
% of population male at equilibrium	*c.*65	*c.*65	*c.*60	*c.*70	*c.*55	*c.*90	*c.*50	*c.*60
% of population archegoniate at equilibrium	*c.*35	*c.*35	*c.*40	*c.*30	*c.*45	*c.*10	*c.*50	*c.*40
First embryo	Jul.'88	Sep.'88	Sep.'88	Jan.'89	Aug.'88	Nov.'88	Jul.'88	Nov.'88
% of archegoniates fertilized in 1st year	100	95	74	1	95	50	100	67
% of archegoniates fertilized by Jan.'90	100	100	100	100	100	100	100	100
Largest gametophyte by Jan.'90 (mm^2) (sex)	37.78 ♀	39.28 ♀	114.12 ♀	41.45 ♀	33.69 ♂	39.80 ♀	22.40 ♂	56.55 ♀
Most advanced sporeling by Jan.'90 (leaves)	7	6	6	5	9	9	5	5

Table 4.2. Results of Experiment 1 at Roslin Glen. Shading indicates the species that were on alien soil.

Experiment 1 : Roslin Glen	*A. filix-femina*		*B. spicant*		*P. setiferum*		*P. scolopendrium*	
Plot	RG.1.	RG.2.	RG.1.	RG.2.	RG.1	RG.2.	RG.1	RG.2.
See Figure	4.9	4.10	4.11	4.12	4.13	4.14	4.15	4.16
Spores sown	Jan.'88	Jan.'88	Jan.'88	Jan.'88	Jan.'88	Jan.'88	Jan.'88	Jan.'88
Germination	Apr.'88	Apr.'88	May '88	May '88	Apr.'88	Apr.'88	Apr.'88	Apr.'88
Pre-sexual period (months)	1	1	< 1	1	3	7	4	5
Size of last sterile gametophytes (mm^2)	0.29	0.93	0.53	1.46	0.92	0.80	0.83	0.40
First male gametophytes	Jun.'88	Jun.'88	Jun.'88	Jul.'88	Aug.'88	Jan.'89	Sep.'88	Nov.'88
Size of first male gametophytes (mm^2)	0.28	0.53	0.23	0.24	0.61	0.95	0.34	0.82
First female gametophytes	Jul.'88	Aug.'88	Aug.'88	Feb.'89	Aug.'88	Feb.'89	Sep.'88	Jun.'89
Size of first female gametophytes (mm^2)	0.72	2.40	1.06	2.81	1.92	2.32	3.50	2.85
First bisexual gametophytes	Aug.'88	Aug.'88	Aug.'88	Apr.'89	Aug.'88	Apr.'89	Sep.'88	Jun.'89
Size of first bisexual gametophytes (mm^2)	1.48	1.70	1.01	3.46	2.24	4.39	2.30	2.54
All gametophytes sexual by	Aug.'88	Jan.'89	Sep.'88	Jun.'89	Sep.'88	Apr.'89	Nov.'88	Jun.'89
Transition period (months)	3	8	4	12	2	5	3	9
Equilibrium period (months)	*c.*9	*c.*8	*c.*10	*c.*2	*c.*10	*c.*2	*c.*5	*c.*1
% of population male at equilibrium	*c.*80	*c.*90	*c.*75	*c.*85	*c.*80	*c.*80	*c.*80	*c.*85
% of population archegoniate at equilibrium	*c.*20	*c.*10	*c.*25	*c.*15	*c.*20	*c.*20	*c.*20	*c.*15
First embryo	Sep.'88	Jun.'89	Sep.'88	Jun.'89	Sep.'88	Jun.'89	Nov.'89	Jan.'90
% of archegoniates fertilized in 1st year	95	0	75	0	48	0	0	0
% of archegoniates fertilized by Jan.'90	98	86	96	76	97	79	94	35
Largest gametophyte by Jan.'90 (mm^2) (sex)	32.61 ♀	20.79 ♀	16.51 ♀	12.38 ♀	38.25 ♀	40.53 ♀	15.67 ♀	3.67 ♀
Most advanced sporeling by Jan.'90 (leaves)	6	3	5	3	6	2	3	2

Figures 4.1 - 4.16. Sex expression, fertilization and size of gametophytes in each population during Experiment 1. Note:

1. Sampling dates are shown below the x-axes.

2. Symbols: spores or pre-sexual gametophytes (○), male gametophytes ( ), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

3. It has been necessary to use a log scale on the y-axes of Figures 4.1b - 4.16b to accommodate the largest and smallest values. Log scales are somewhat misleading for larger values; big differences in area can be represented by small differences in the position of symbols. Range bars: spores or pre-sexual gametophytes (2.0 mm), male gametophytes (1.0 mm), female gametophytes (1.5 mm) and bisexual gametophytes (0.5 mm).

4. Identification of sterile, male, and archegoniate gametophytes was easy and the values presented for these are accurate. Sub-dividing archegoniate gametophytes into 'females' and 'bisexuals' was more difficult because soil particles adhering to the rhizoids often obscured the rhizoid region where antheridia are usually located. Consequently, some gametophytes scored as female might have been bisexual and the values presented for these sex categories should probably be regarded as overestimates and underestimates respectively.

5. The vertical line in Figures 4.1a - 4.16a represents the point at which some gametophytes began to die. Thereafter an increasing number of gametophytes were either in poor condition or had perished and it became impossible to select a representative sample for area measurements. Established sporelings were scored as 'fertilized archegoniate gametophytes' even though the gametophytes were no longer present.

Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Figure 4.1a

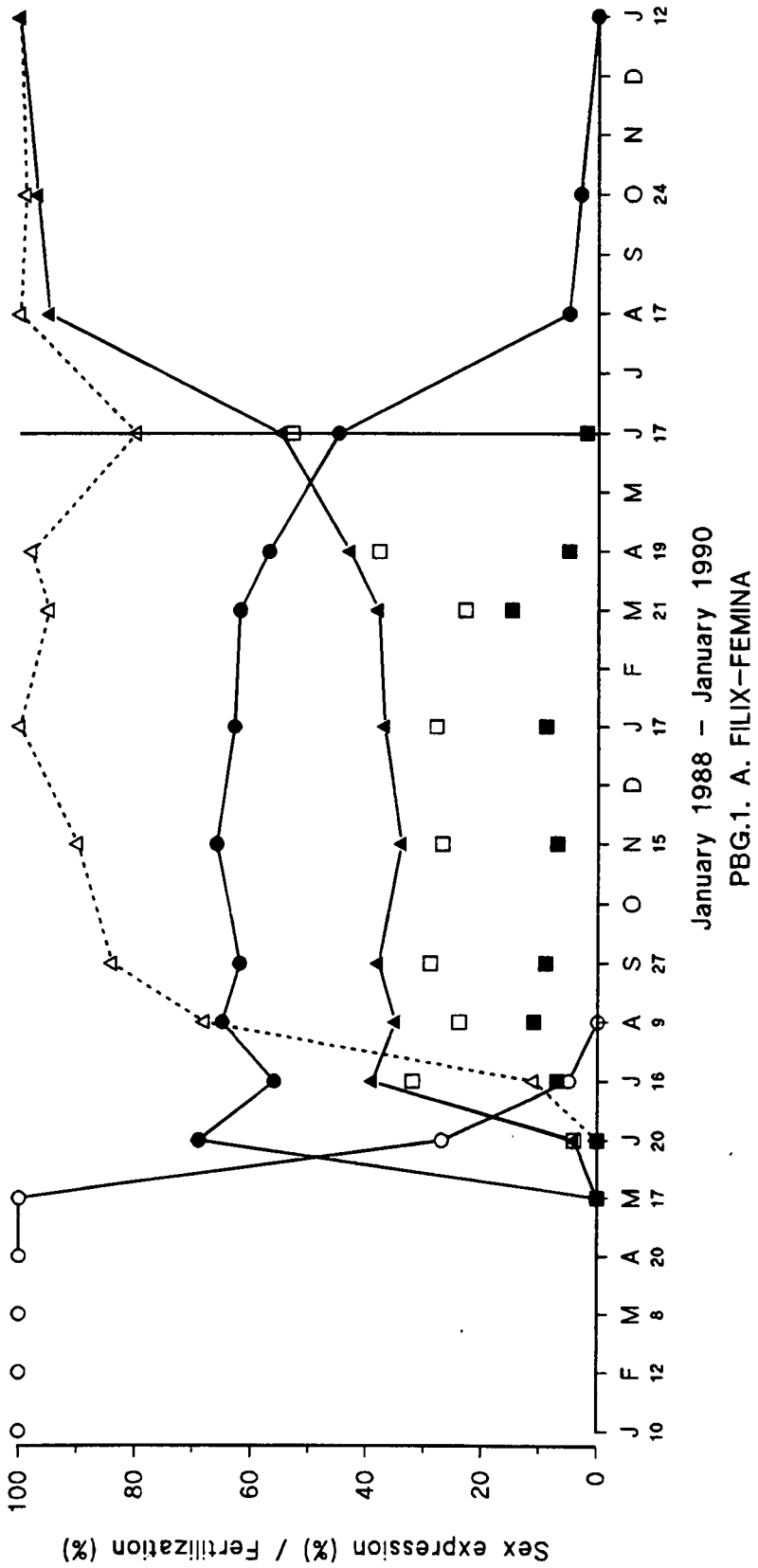
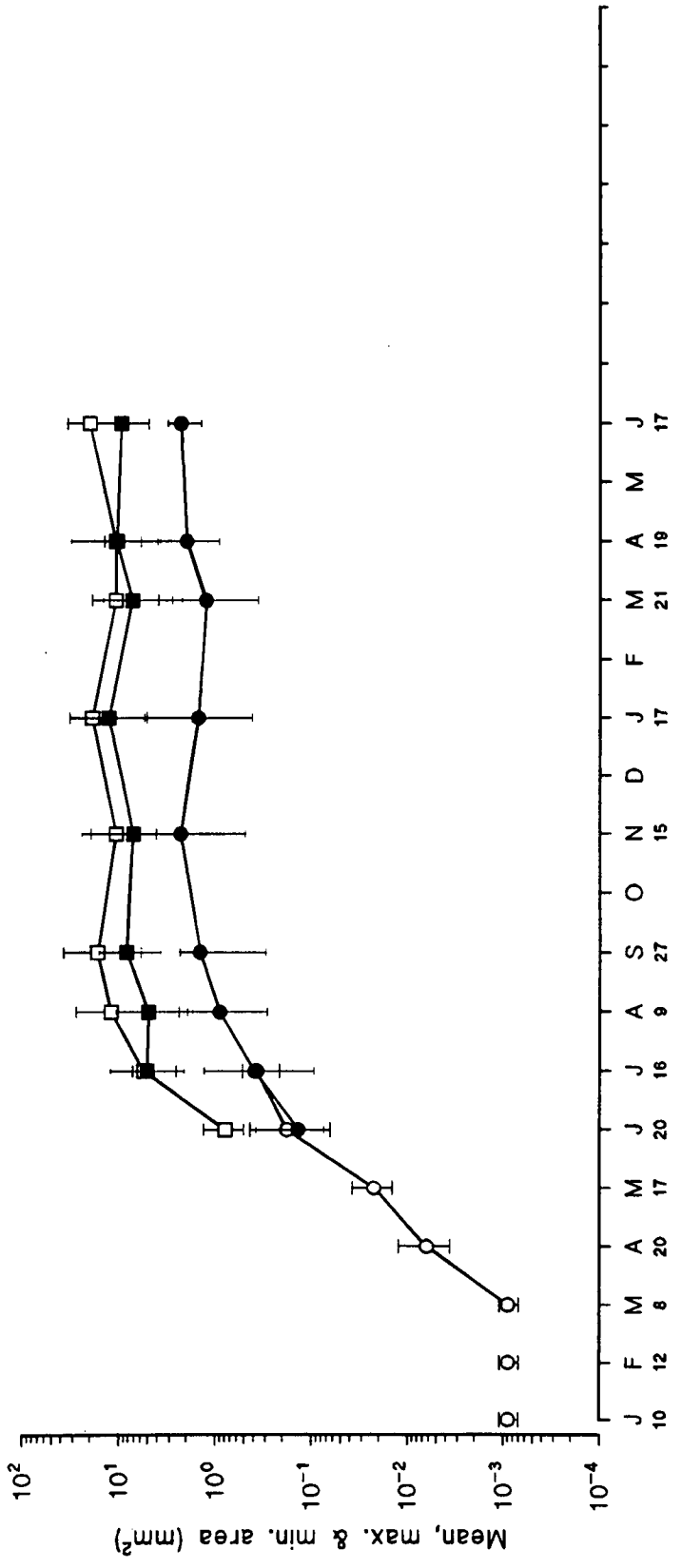

Sex expression (%) / Fertilization (%)
0
20
40
60
80
100
J F M A M J J A S O N D J J A S O N D J
10 12 8 20 17 20 16 9 27 15 17 21 19 17 17 24 17 12
January 1988 – January 1990
PBG.1. A. FILIX-FEMINA

Figure 4.1b


Mean, max. & min. area (mm²)
10²
10¹
10⁰
10⁻¹
10⁻²
10⁻³
10⁻⁴
J F M A M J J A S O N D J F M A M J
10 12 8 20 17 20 16 9 27 15 17 21 19 17
January 1988 – June 1989
PBG.1. A. FILIX-FEMINA

Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Figure 4.2a

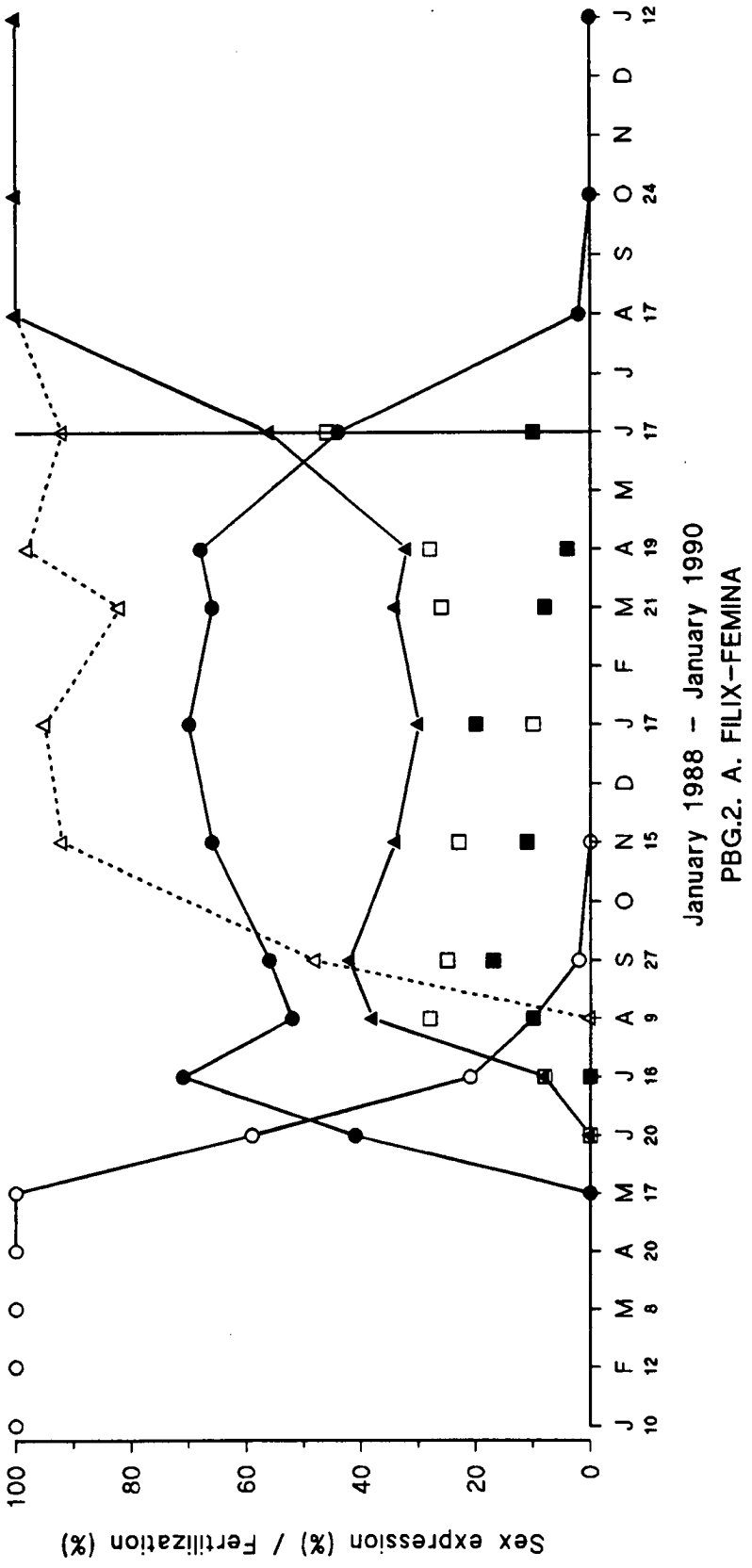
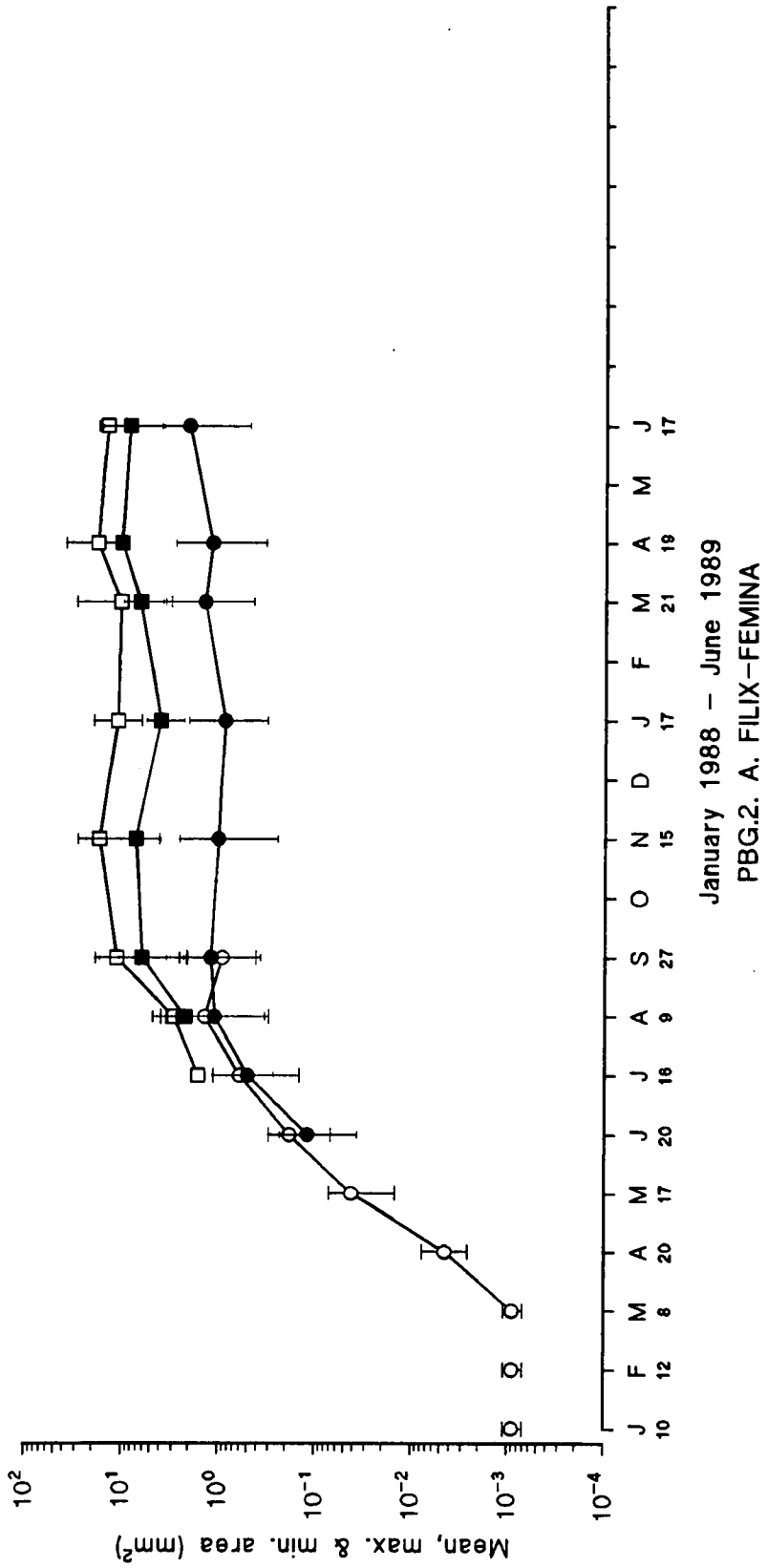

Sex expression (%) / Fertilization (%)
0
20
40
60
80
100
J F M A M J J A S O N D J J F M A M J J A S O N D J
10 12 8 20 17 20 16 9 27 15 17 21 19 17 17 24 17 12
January 1988 - January 1990
PBG.2. A. FILIX-FEMINA

Figure 4.2b


Mean, max. & min. area (mm²)
10²
10¹
10⁰
10⁻¹
10⁻²
10⁻³
10⁻⁴
J F M A M J J A S O N D J F M A M J
10 12 8 20 17 20 16 9 27 15 17 21 19 17
January 1988 – June 1989
PBG.2. A. FILIX–FEMINA

Symbols: spores or pre-sexual gametophytes (○), male gametophytes ( ), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Figure 4.3a

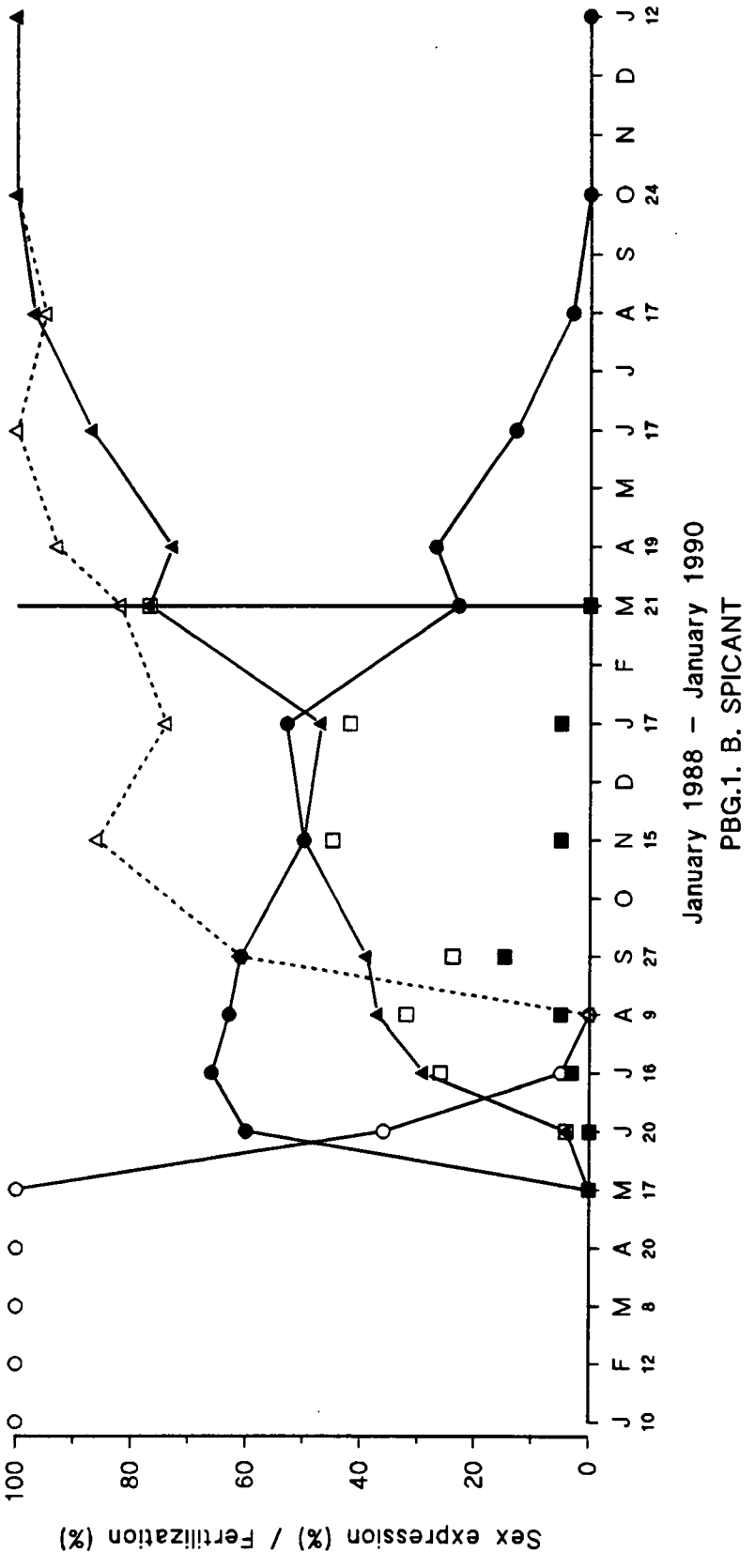
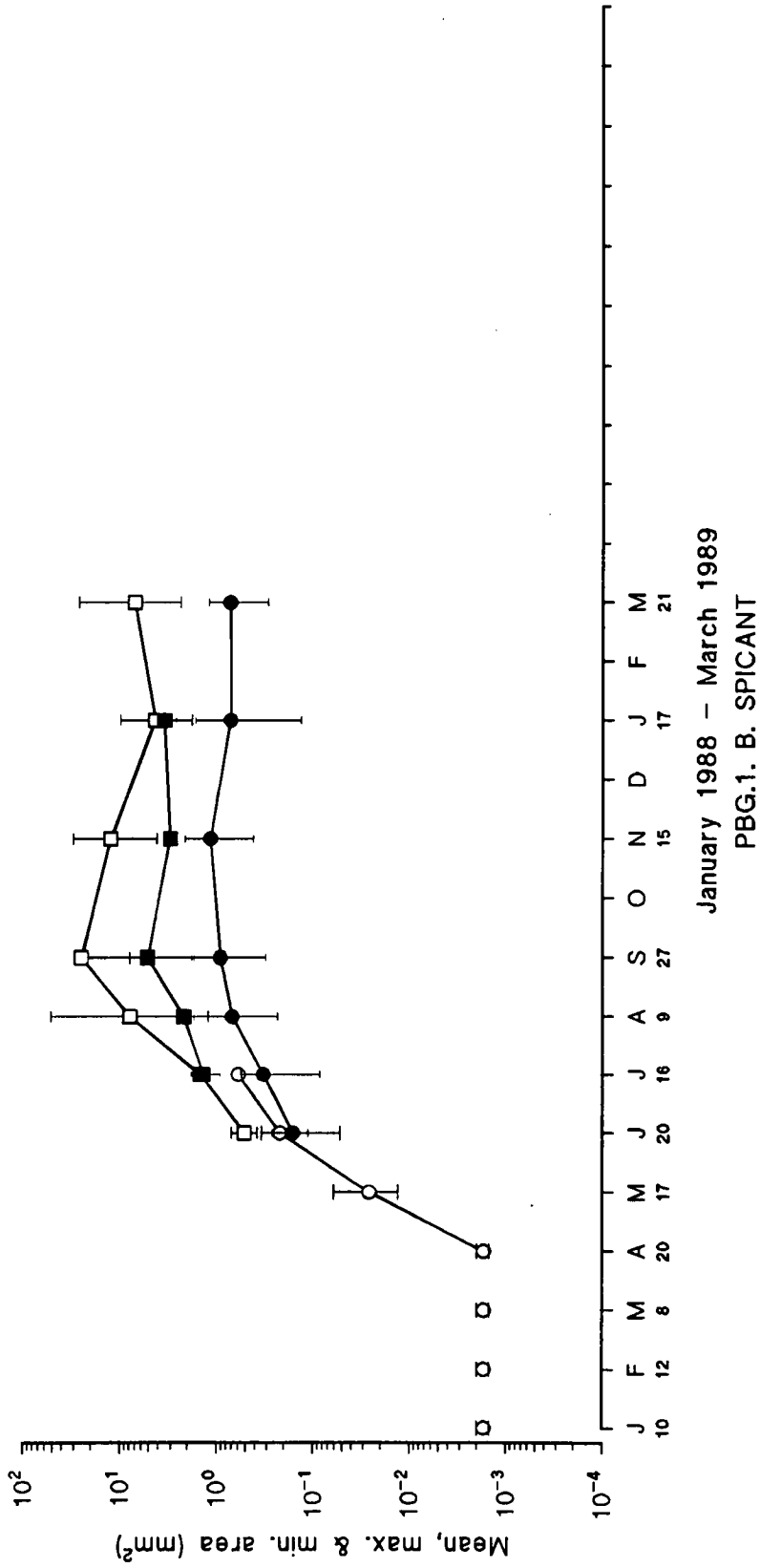
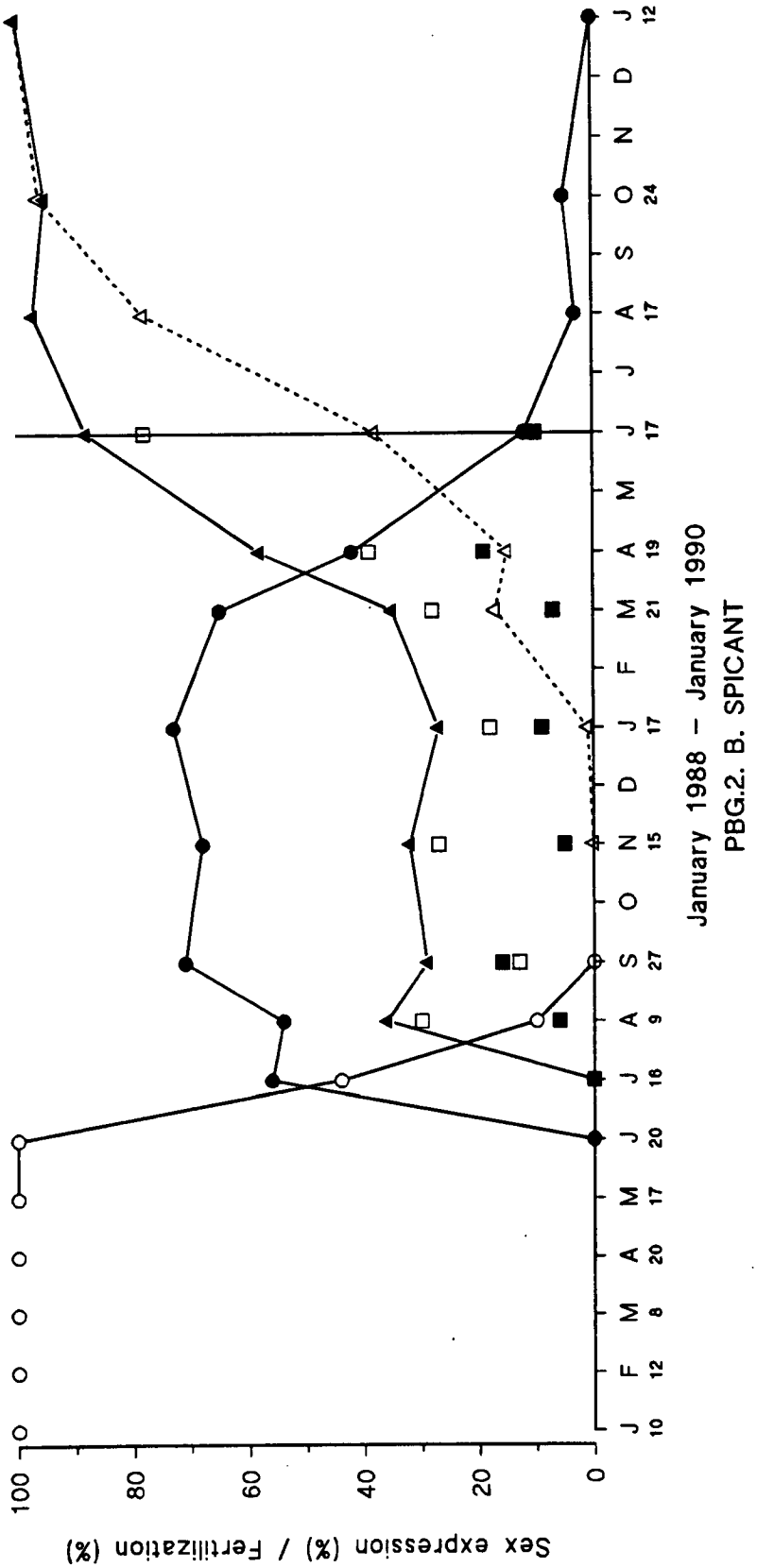

Sex expression (%) / Fertilization (%)
0
20
40
60
80
100
J F M A M J J A S O N D J J F M A M J J A S O N D J
10 12 8 20 17 20 16 9 27 15 17 21 19 17 17 24 17 12
January 1988 – January 1990
PBG.1. B. SPICANT

Figure 4.3b


Mean, max. & min. area (mm²)
10²
10¹
10⁰
10⁻¹
10⁻²
10⁻³
10⁻⁴
J F M A M J J A S O N D J F M
10 12 8 20 17 20 16 9 27 15 17 21
January 1988 – March 1989
PBG.1. B. SPICANT

Symbols: spores or pre-sexual gametophytes (○), male gametophytes ( ), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Figure 4.4a


Sex expression (%) / Fertilization (%)
0
20
40
60
80
100
J F M A M J J A S O N D J J F M A M J J A S O N D J
10 12 8 20 17 20 16 9 27 15 4 17 21 19 17 17 24 17 12
January 1988 – January 1990
PBG.2. B. SPICANT

Mean, max. & min. area (mm²)
10²
10¹
10⁰
10⁻¹
10⁻²
10⁻³
10⁻⁴
J F M A M J J A S O N D J F M A M J
10 12 8 20 17 20 16 9 27 15 17 21 19 17
January 1988 – June 1989
PBG.2. B. SPICANT

Figure 4.4b

Symbols: spores or pre-sexual gametophytes (○), male gametophytes ( ), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

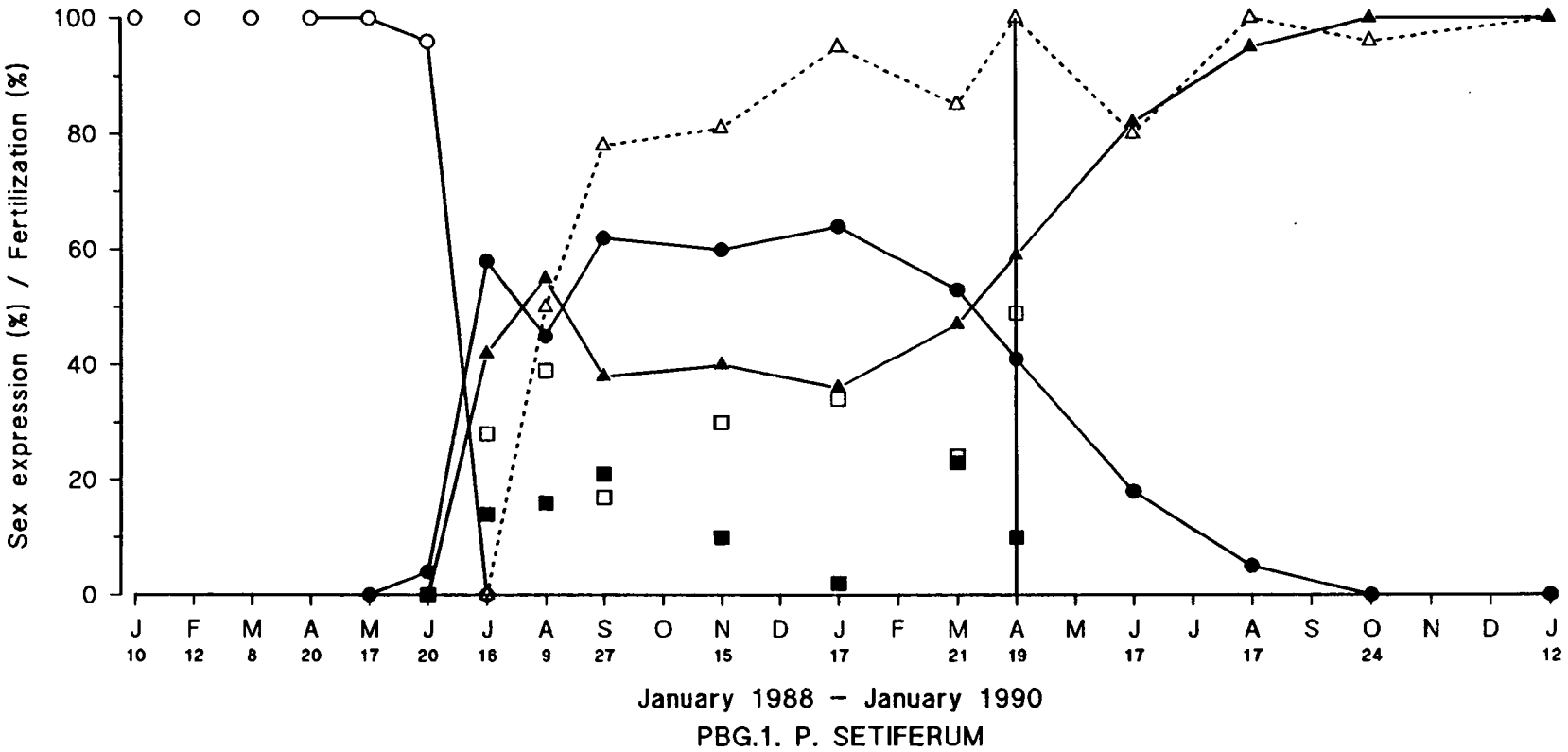
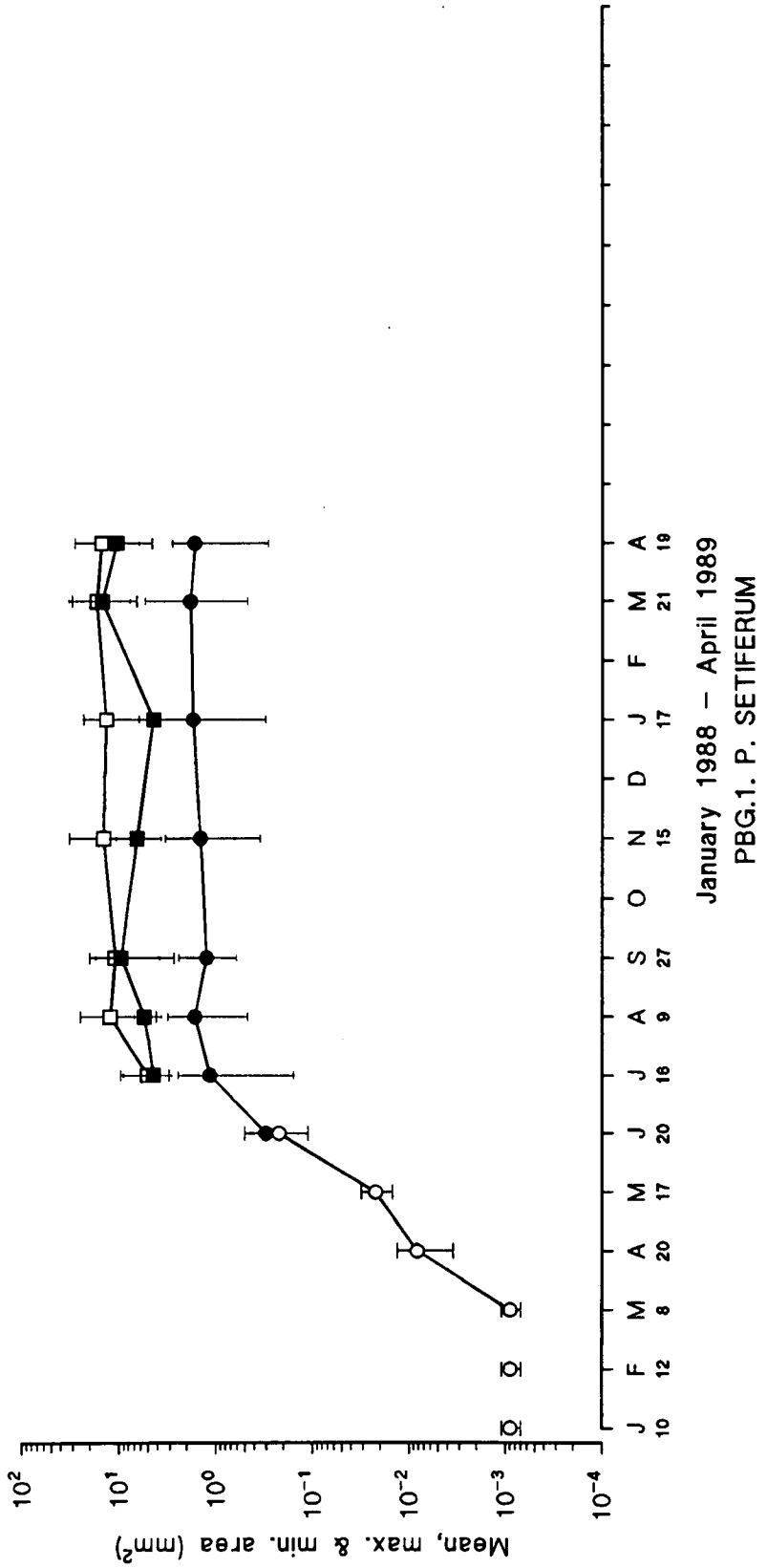

Sex expression (%) / Fertilization (%)
100
80
60
40
20
0
J F M A M J J A S O N D J F M A M J J A S O N D J
10 12 8 20 17 20 16 9 27 15 17 21 19 17 17 24 12
January 1988 – January 1990
PBG.1. P. SETIFERUM

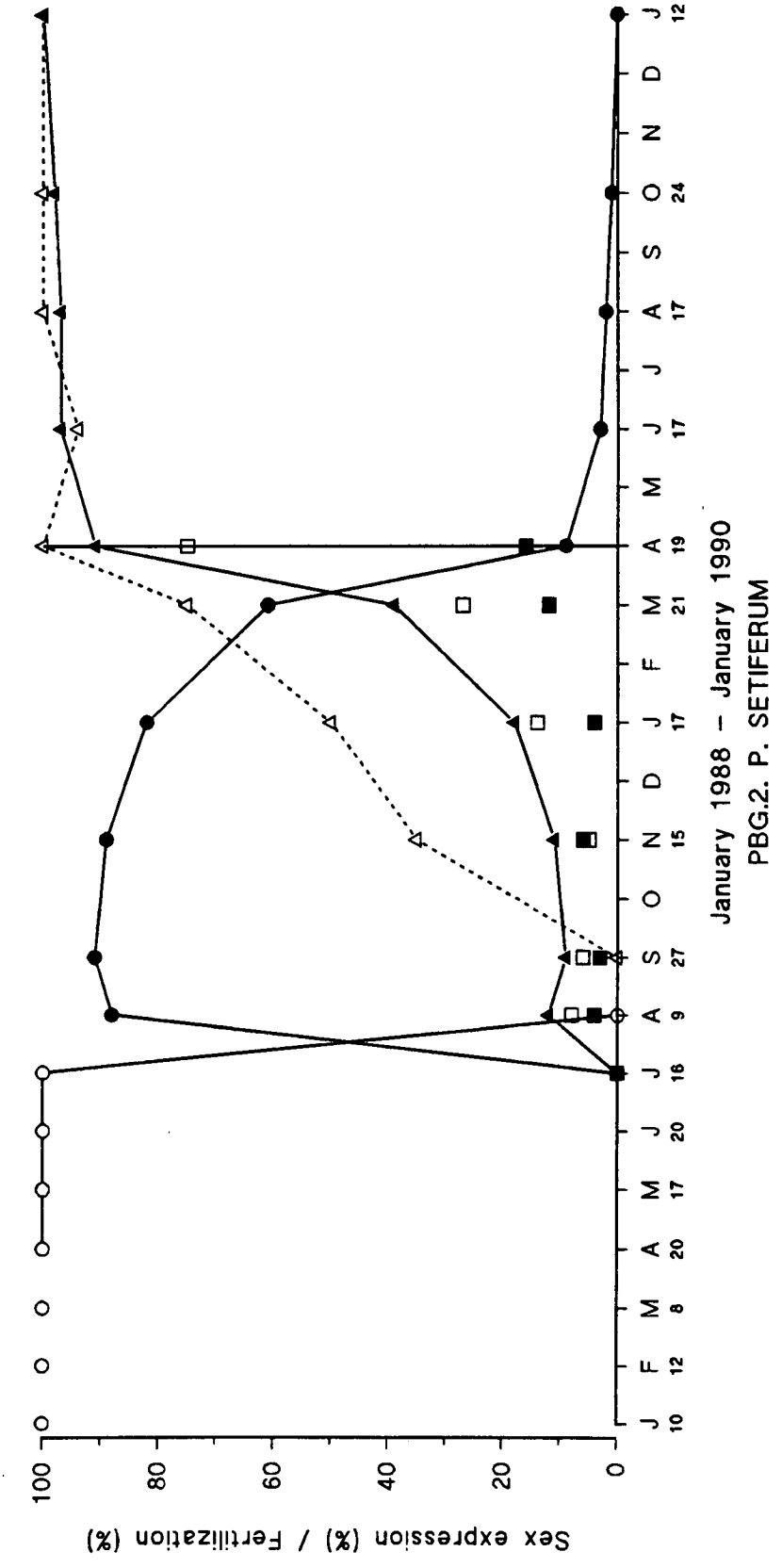
Figure 4.5a

Figure 4.5b


Mean, max. & min. area (mm²)
10²
10¹
10⁰
10⁻¹
10⁻²
10⁻³
10⁻⁴
J F M A M J J A S O N D J F M A
10 12 8 20 17 20 16 9 27 15 17 21 18
January 1988 – April 1989
PBG.1. P. SETIFERUM

Symbols: spores or pre-sexual gametophytes (○), male gametophytes ( ), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Figure 4.6a


Sex expression (%) / Fertilization (%)
0
20
40
60
80
100
J F M A M J J A S O N D J J A S O N D J F M A M J J A S O N D J
10 12 8 20 17 20 16 9 27 15 17 21 19 17 17 24 17 12
January 1988 – January 1990
PBG.2. P. SETIFERUM

Mean, max. & min. area (mm²)
10²
10¹
10⁰
10⁻¹
10⁻²
10⁻³
10⁻⁴
J F M A M J J A S O N D J F M A
10 12 8 20 17 20 16 9 27 15 17 21 19
January 1988 – April 1989
PBG.2. P. SETIFERUM

Figure 4.6b

Symbols: spores or pre-sexual gametophytes (○), male gametophytes ( ), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Figure 4.7a

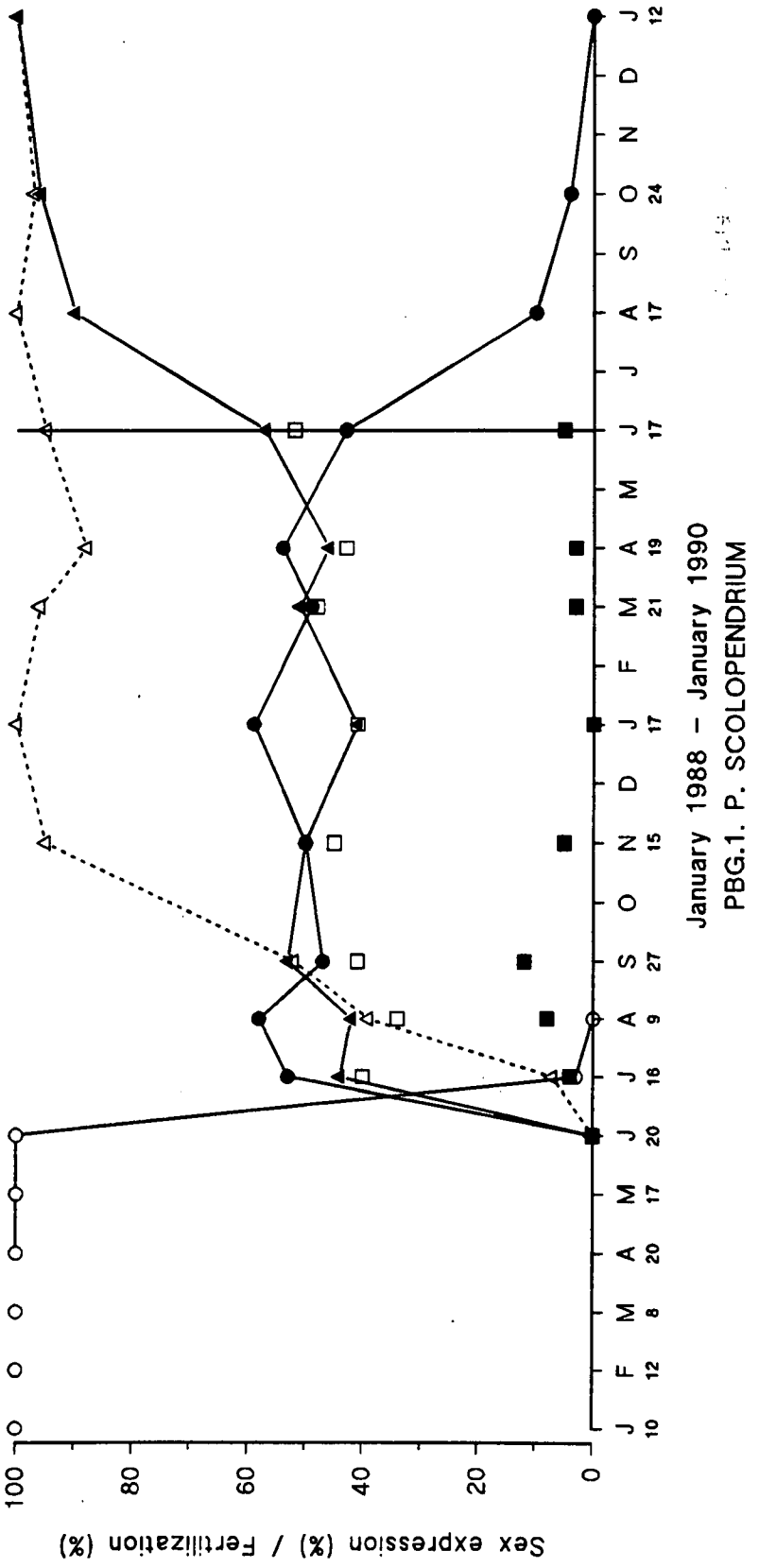
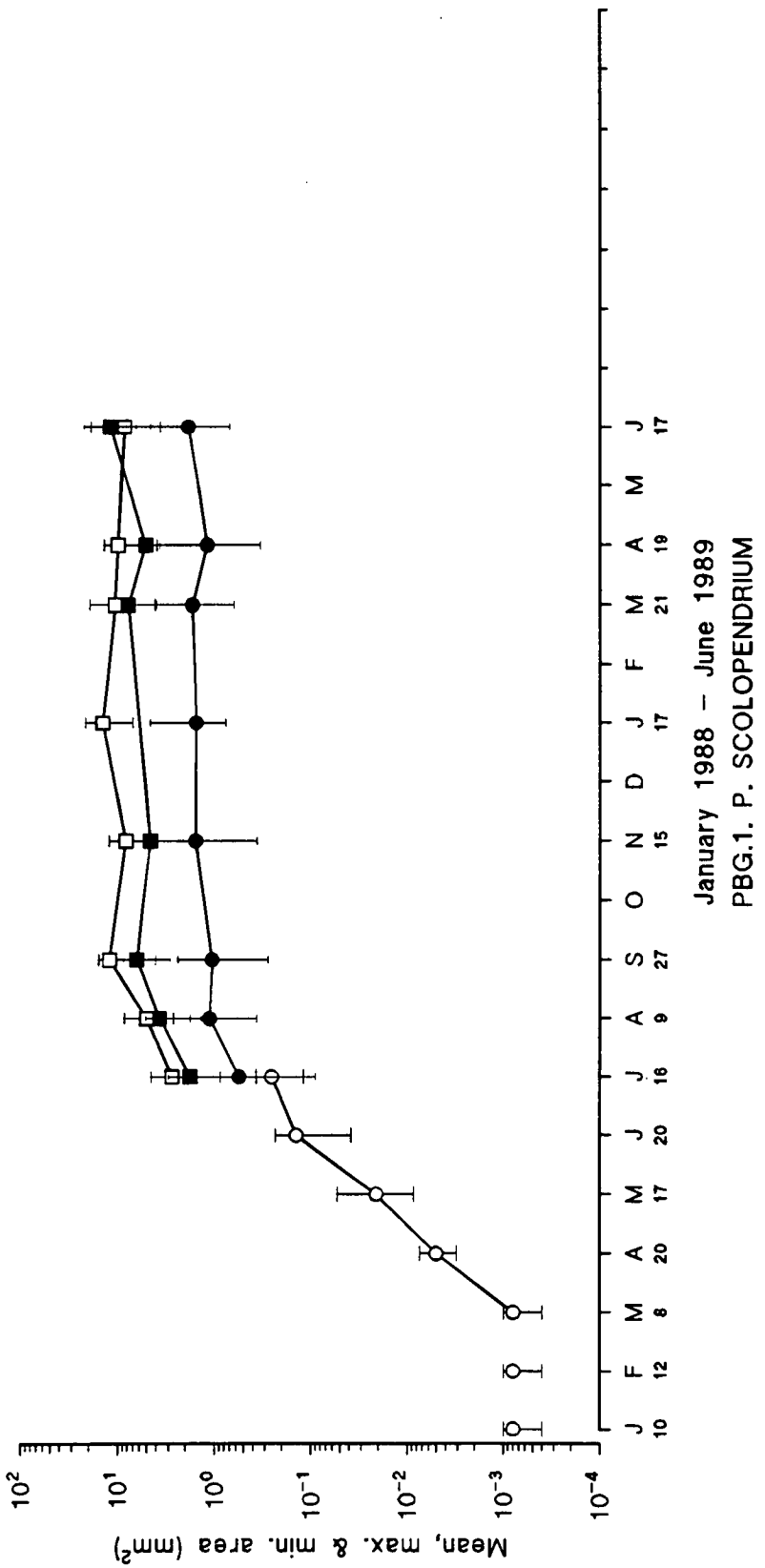
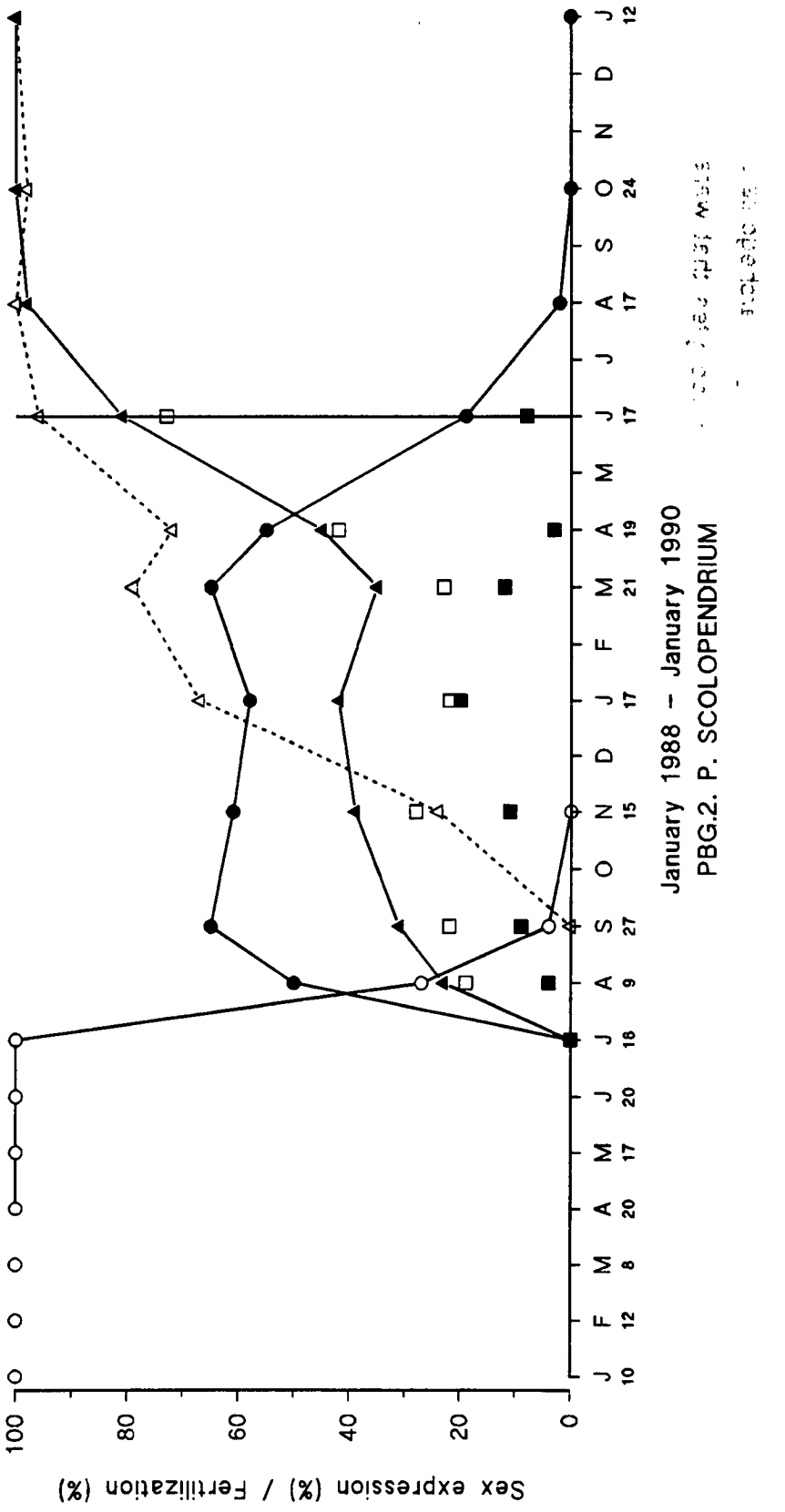

Sex expression (%) / Fertilization (%)
100
80
60
40
20
0
J F M A M J J A S O N D J J F M A M J J A S O N D J
10 12 8 20 17 20 16 9 27 15 17 21 19 17 17 24 17 12
January 1988 – January 1990
PBG.1. P. SCOLOPENDRIUM

Figure 4.7b


Mean, max. & min. area (mm²)
10²
10¹
10⁰
10⁻¹
10⁻²
10⁻³
10⁻⁴
J F M A M J J A S O N D J F M A M J
10 12 8 20 17 20 16 9 27 15 17 21 19 17
January 1988 – June 1989
PBG.1. P. SCOLOPENDRIUM

Symbols: spores or pre-sexual gametophytes (○), male gametophytes ( ), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Figure 4.8a


Sex expression (%) / Fertilization (%)
0
20
40
60
80
100
J F M A M J J A S O N D J J F M A M J J A S O N D J
10 12 8 20 17 20 16 9 27 15 17 21 19 17 17 24 17 12
January 1988 – January 1990
PBG.2. P. SCOLOPENDRIUM

Mean, max. & min. area (mm²)
10²
10¹
10⁰
10⁻¹
10⁻²
10⁻³
10⁻⁴
J 10
F 12
M 8
A 20
M 17
J 20
J 16
A 9
S 27
O
N 15
D
J 17
F
M 21
A 19
M
J 17
January 1988 – June 1989
PBG.2. P. SCOLOPENDRIUM

Figure 4.8b

Symbols: spores or pre-sexual gametophytes (○), male gametophytes ( ), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Figure 4.9a

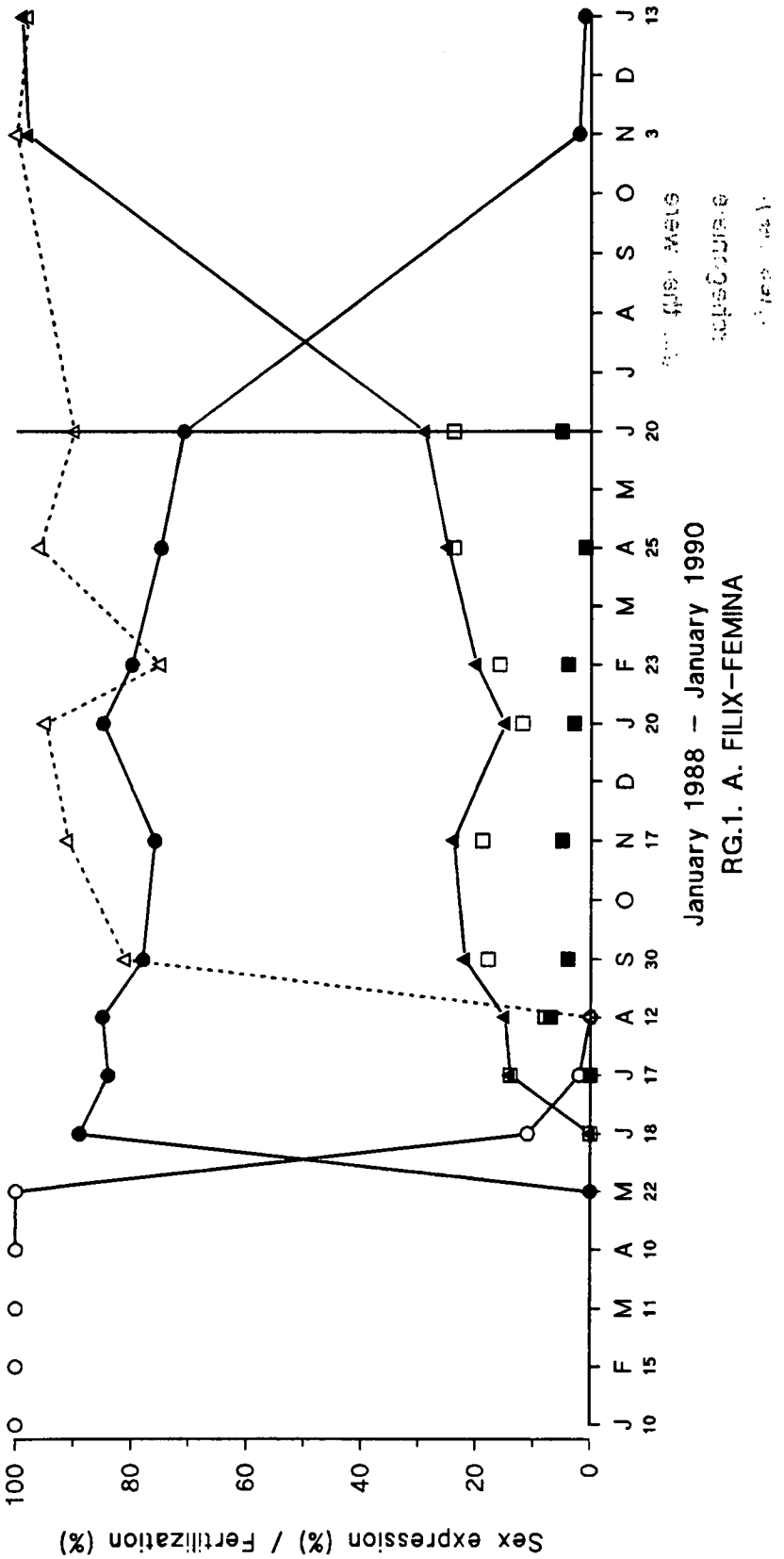
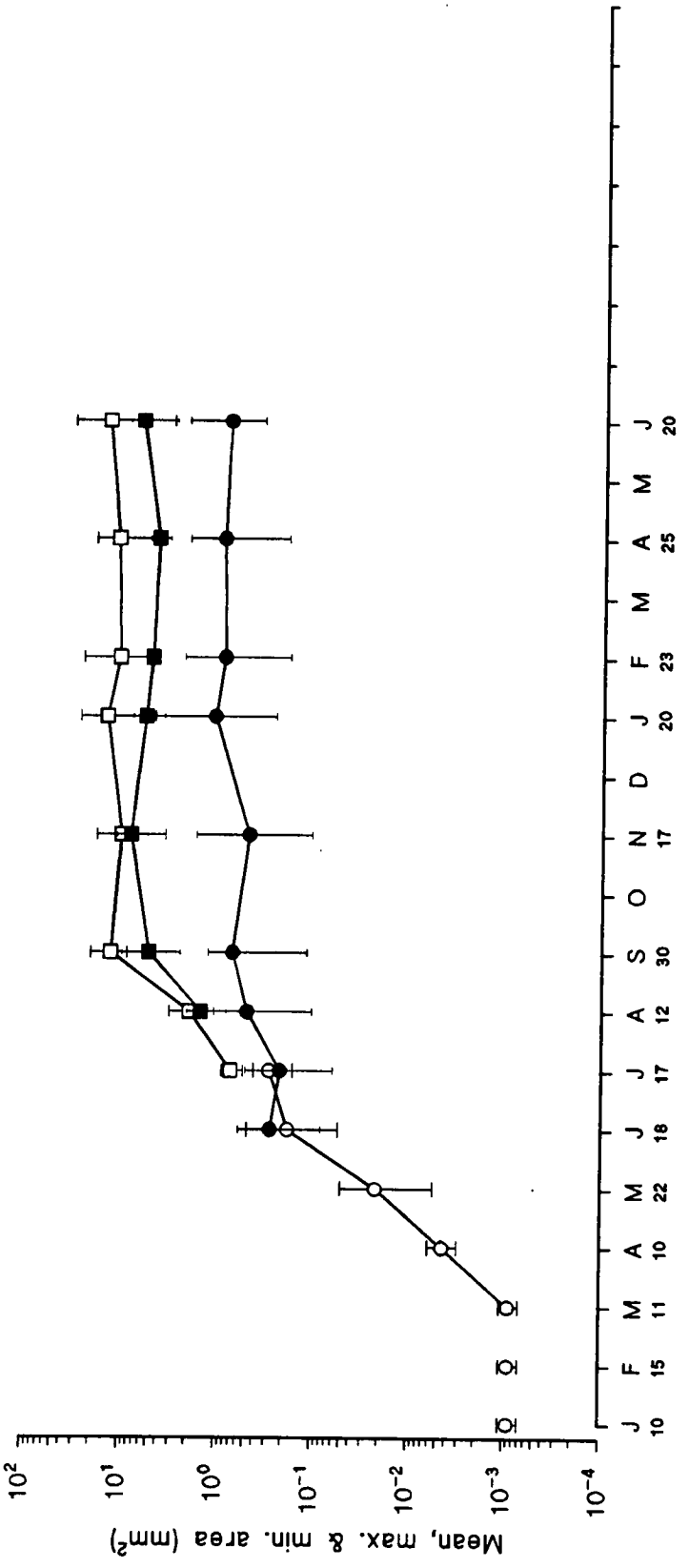

Sex expression (%) / Fertilization (%)
0
20
40
60
80
100
J F M A M J J A S O N D J J A S O N D J
10 15 11 10 22 18 17 12 30 17 20 23 25 20 3 13
January 1988 – January 1990
RG.1. A. FILIX-FEMINA

Figure 4.9b


Mean, max. & min. area (mm²)
10²
10¹
10⁰
10⁻¹
10⁻²
10⁻³
10⁻⁴
J F M A M J J A S O N D J F M A M J
10 15 11 10 22 18 17 12 30 17 20 23 25 20
January 1988 – June 1989
RG.1. A. FILIX-FEMINA

Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Figure 4.10a

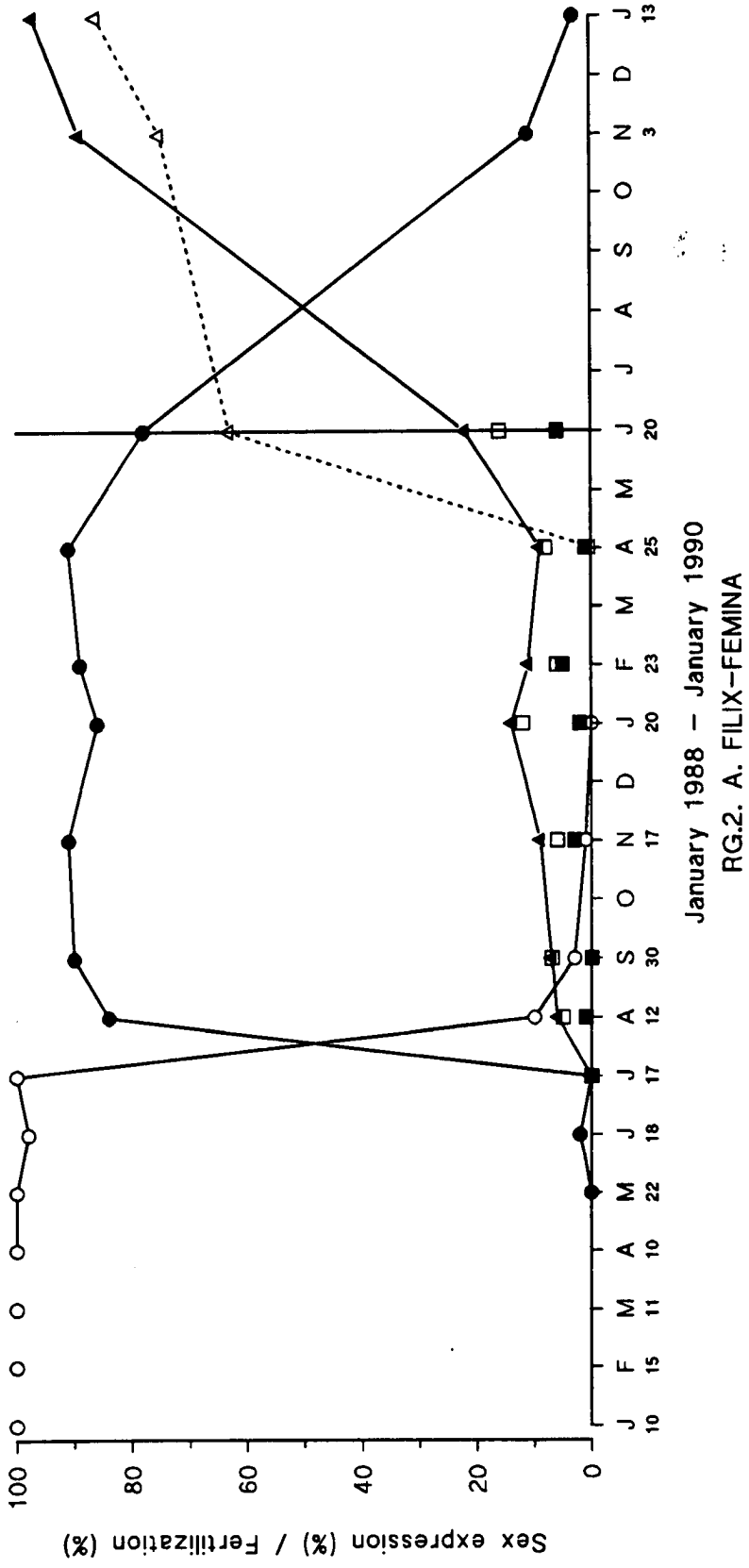
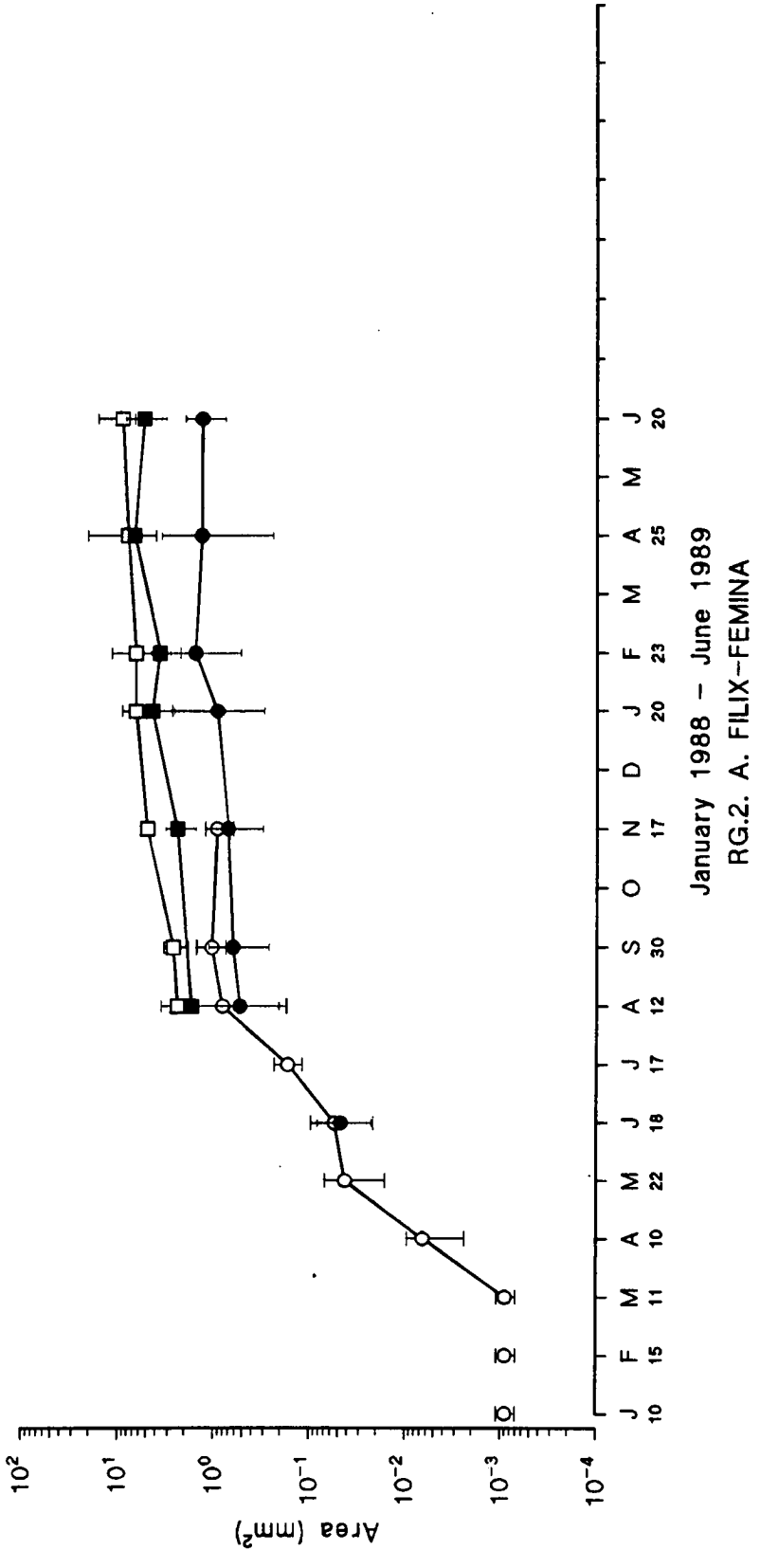

Sex expression (%) / Fertilization (%)
100
80
60
40
20
0
J F M A M J J A S O N D J J F M A M J J A S O N D J
10 15 11 10 22 18 17 12 30 17 20 23 25 20 3 13
January 1988 – January 1990
RG.2. A. FILIX-FEMINA

Figure 4.10b


Area (mm²)
10²
10¹
10⁰
10⁻¹
10⁻²
10⁻³
10⁻⁴
J F M A M J J A S O N D J F M A M J
10 15 11 10 22 18 17 12 30 17 20 23 25 20
January 1988 – June 1989
RG.2. A. FILIX-FEMINA

Symbols: spores or pre-sexual gametophytes (○), male gametophytes ( ), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Figure 4.11a

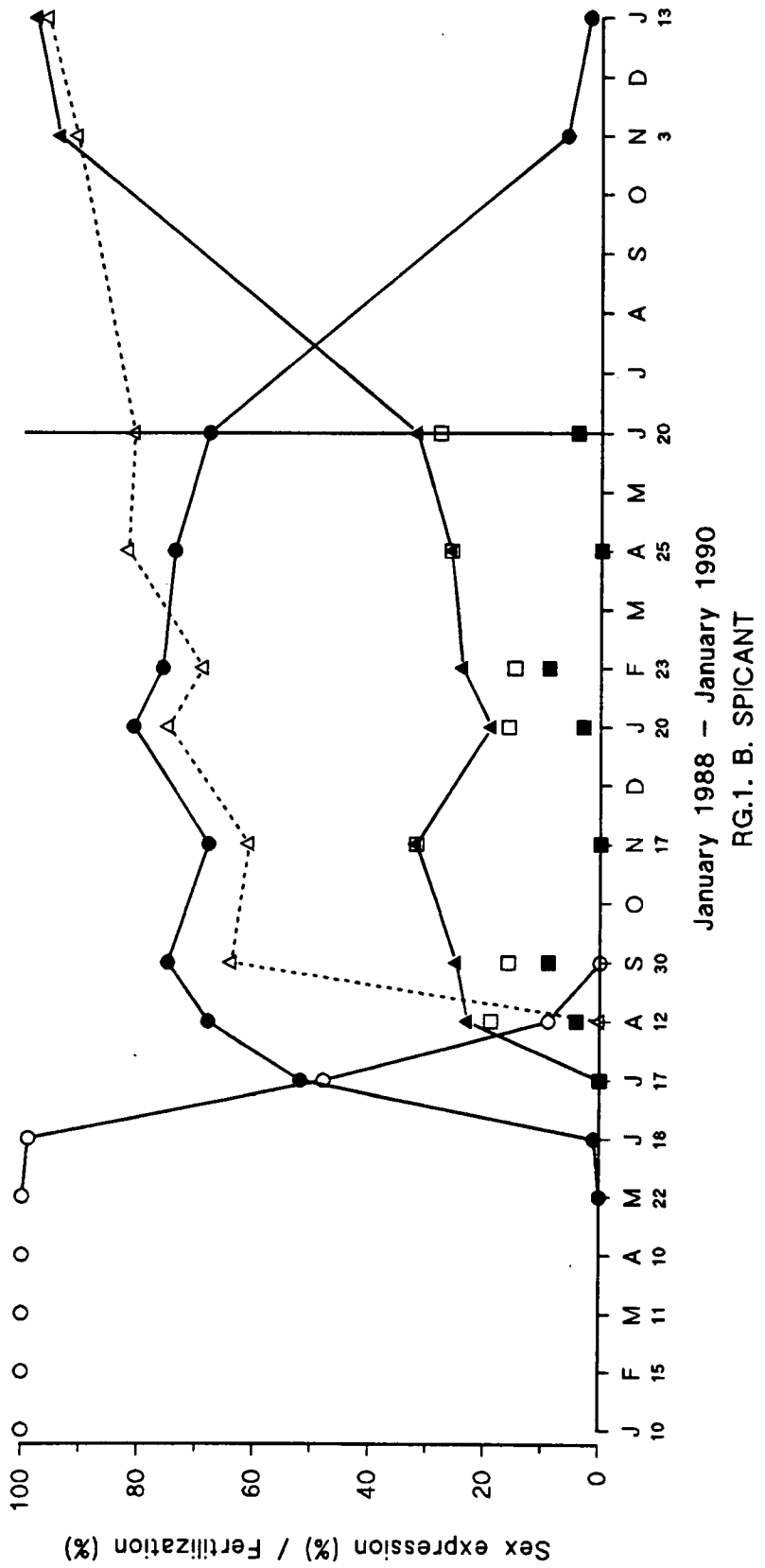
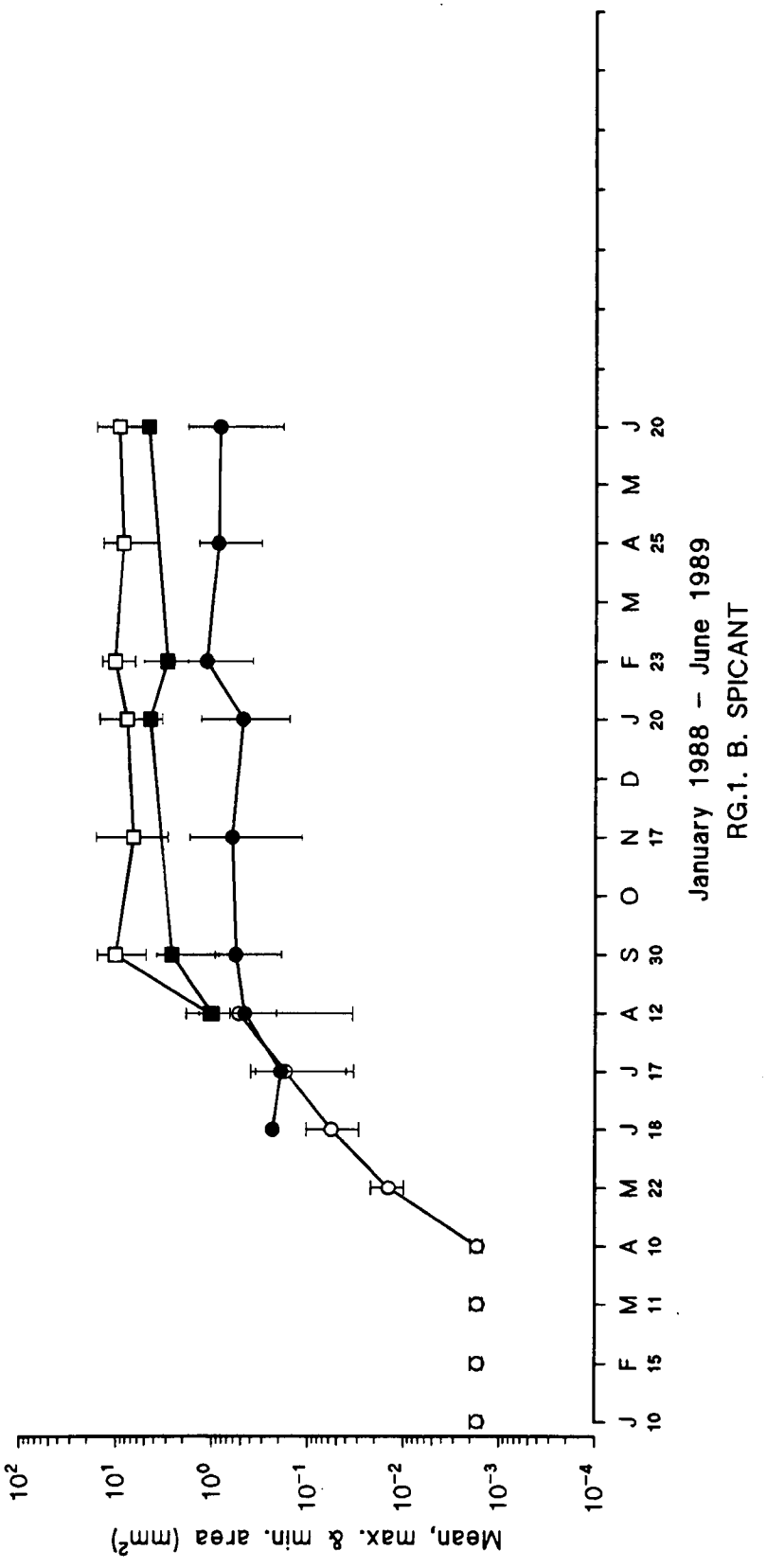

Sex expression (%) / Fertilization (%)
0
20
40
60
80
100
J F M A M J J A S O N D J J A S O N D J
10 15 11 10 22 18 17 12 30 17 20 23 20 25 3 20 13
January 1988 – January 1990
RG.1. B. SPICANT

Figure 4.11b

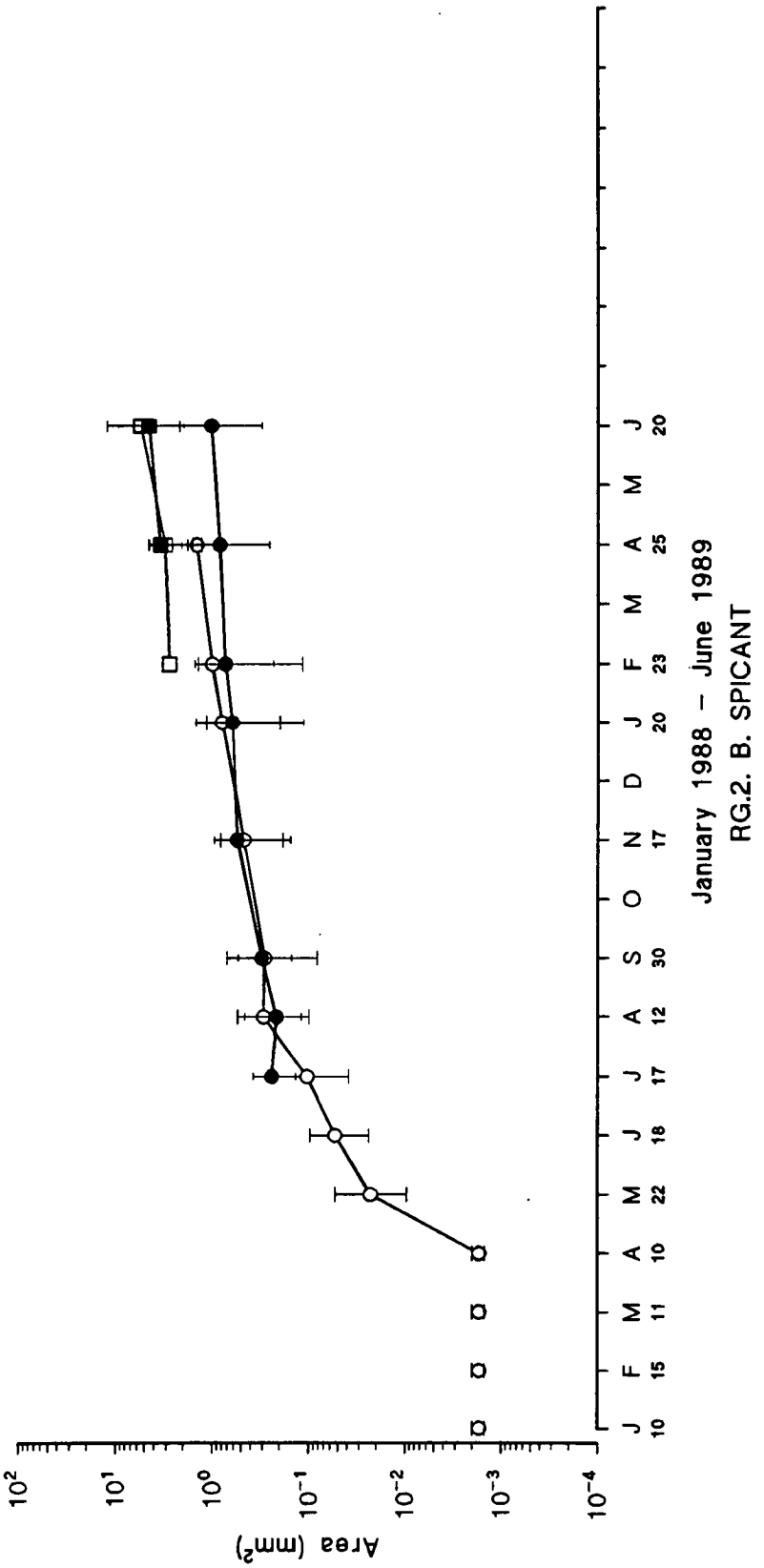

Mean, max. & min. area (mm²)
10²
10¹
10⁰
10⁻¹
10⁻²
10⁻³
10⁻⁴
J F M A M J J A S O N D J F M A M J
10 15 11 10 22 18 17 12 30 17 20 23 25 20
January 1988 – June 1989
RG.1. B. SPICANT

Symbols: spores or pre-sexual gametophytes (○), male gametophytes ( ), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Sex expression (%) / Fertilization (%)
100
80
60
40
20
0
J 10
F 15
M 11
A 10
M 22
J 18
J 17
A 12
S 30
O
N 17
D
J 20
F 23
M
A 25
M
J 20
J
A
S
O
N 3
D
J 13
January 1988 – January 1990
RG.2. B. SPICANT

Figure 4.12a

Figure 4.12b


Area (mm²)
10²
10¹
10⁰
10⁻¹
10⁻²
10⁻³
10⁻⁴
J F M A M J J A S O N D J F M A M J
10 15 11 10 22 18 17 12 30 17 20 23 25 20
January 1988 – June 1989
RG.2. B. SPICANT

Symbols: spores or pre-sexual gametophytes (○), male gametophytes ( ), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Figure 4.13a

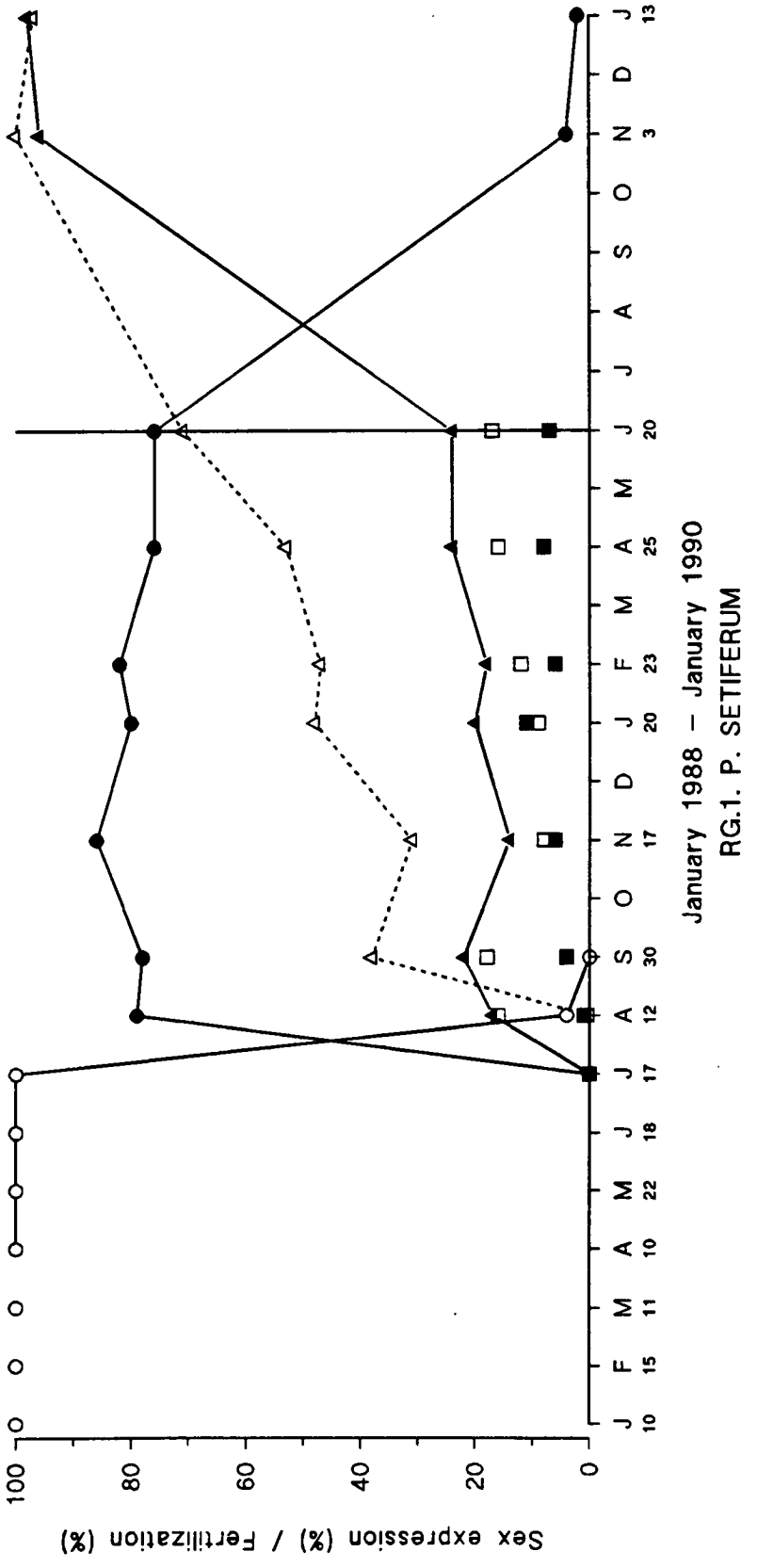
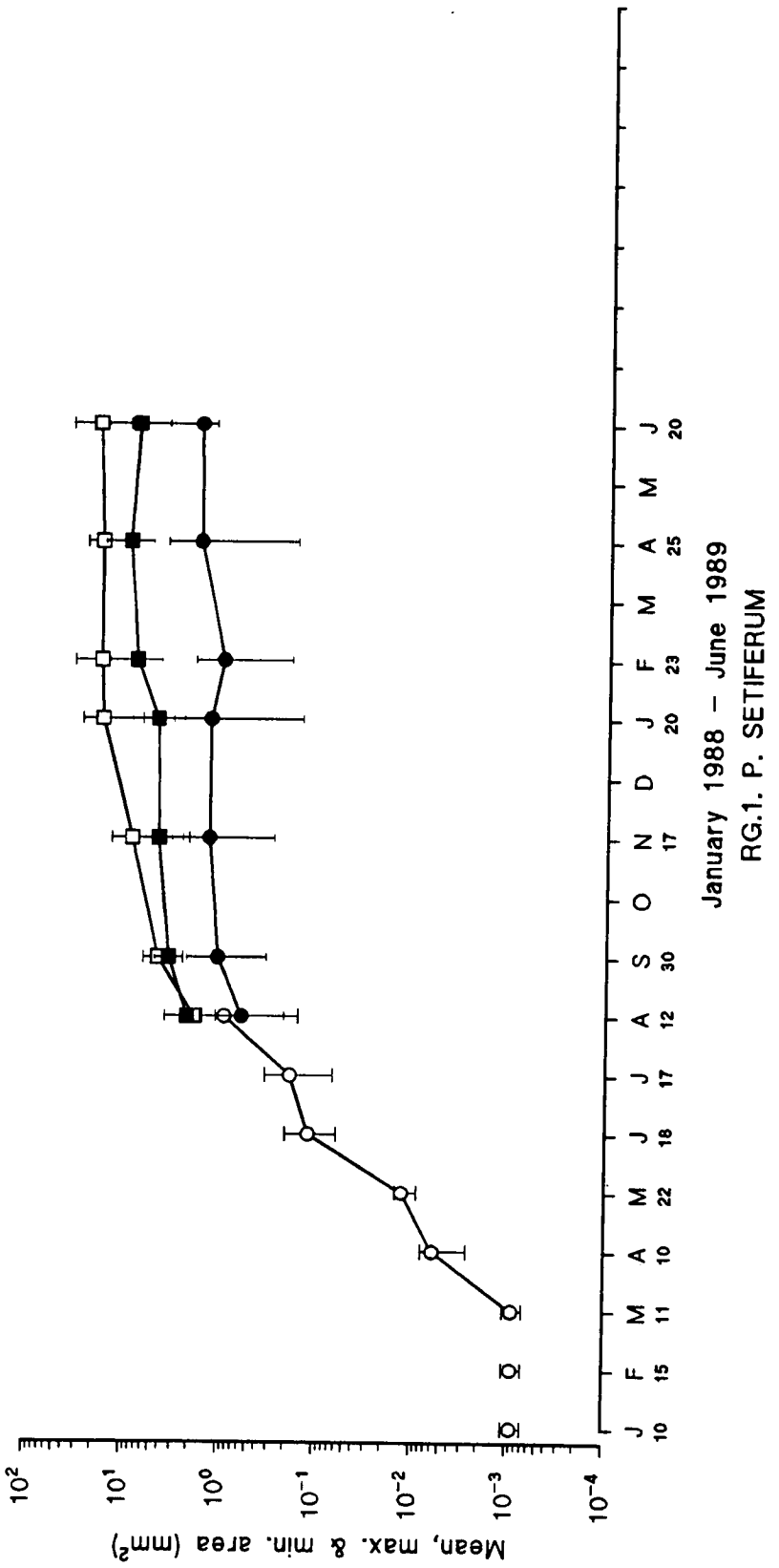

Sex expression (%) / Fertilization (%)
0
20
40
60
80
100
J F M A M J J A S O N D J J A S O N D J F M A M J J A S O N D J
10 15 0 0 0 11 10 22 18 17 12 30 17 20 23 20 25 3 20 13
January 1988 – January 1990
RG.1. P. SETIFERUM

Figure 4.13b


Mean, max. & min. area (mm²)
10²
10¹
10⁰
10⁻¹
10⁻²
10⁻³
10⁻⁴
J F M A M J J A S O N D J F M A M J
10 15 11 10 22 18 17 12 30 17 20 23 25 20
January 1988 – June 1989
RG.1. P. SETIFERUM

Symbols: spores or pre-sexual gametophytes (○), male gametophytes ( ), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Figure 4.14a

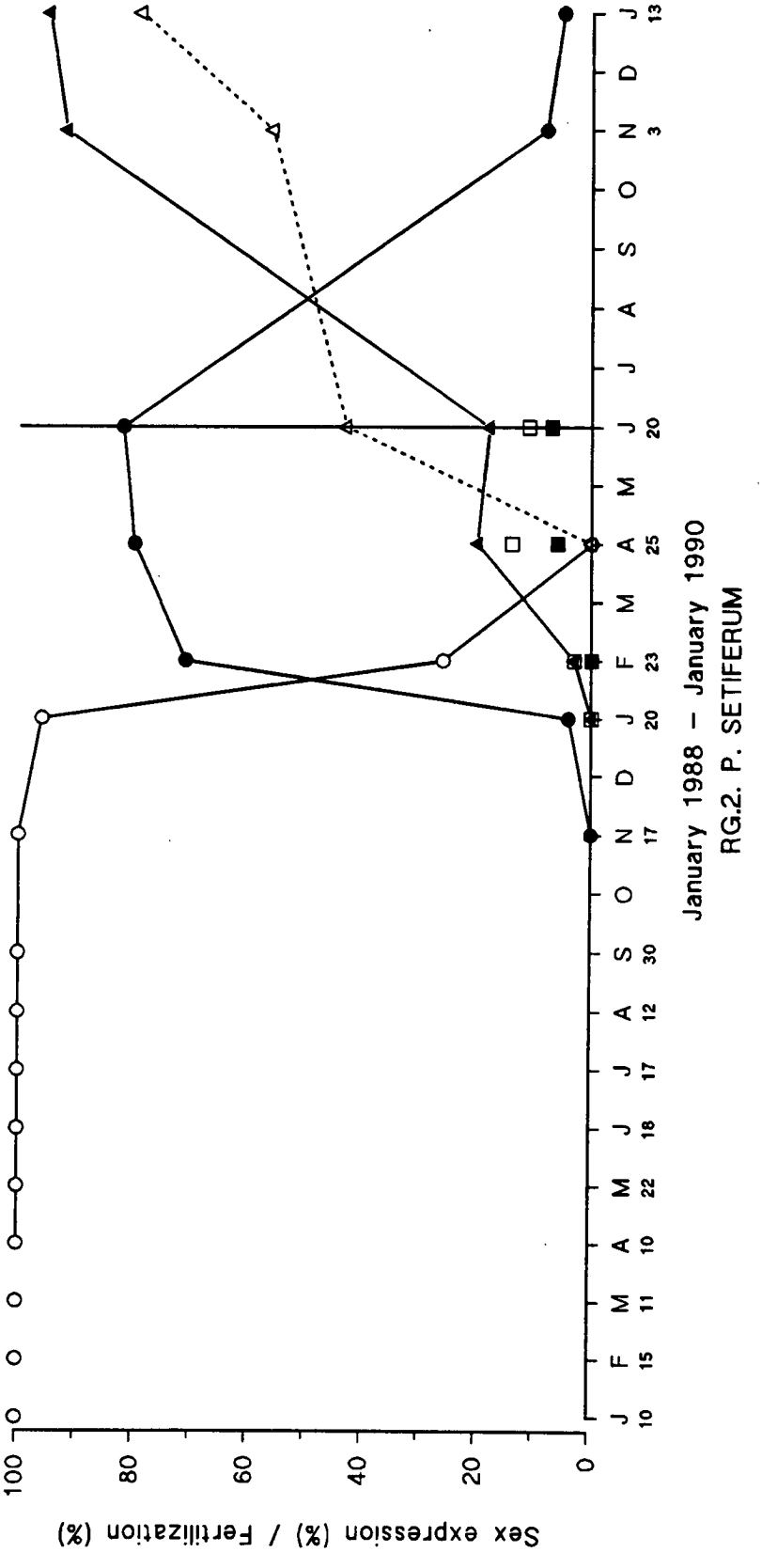
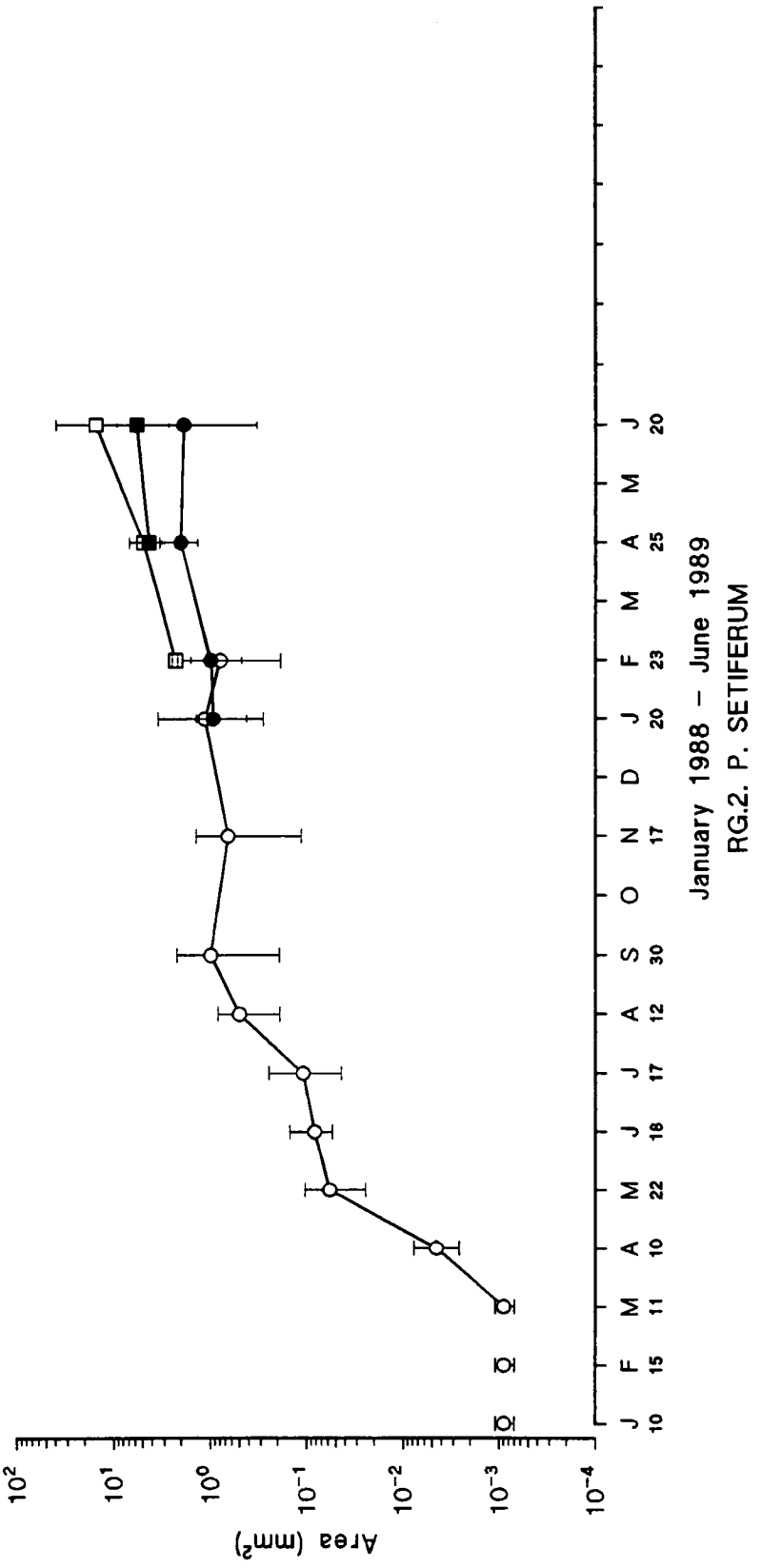

Sex expression (%) / Fertilization (%)
0
20
40
60
80
100
J F M A M J J A S O N D J J A S O N D J
10 15 11 10 22 18 17 12 30 17 20 23 25 20 3 13
January 1988 – January 1990
RG.2. P. SETIFERUM

Figure 4.14b


Area (mm²)
10²
10¹
10⁰
10⁻¹
10⁻²
10⁻³
10⁻⁴
J F M A M J J A S O N D J F M A M J
10 15 11 10 22 18 17 12 30 17 20 23 25 20
January 1988 – June 1989
RG.2. P. SETIFERUM

Symbols: spores or pre-sexual gametophytes (○), male gametophytes ( ), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Figure 4.15a

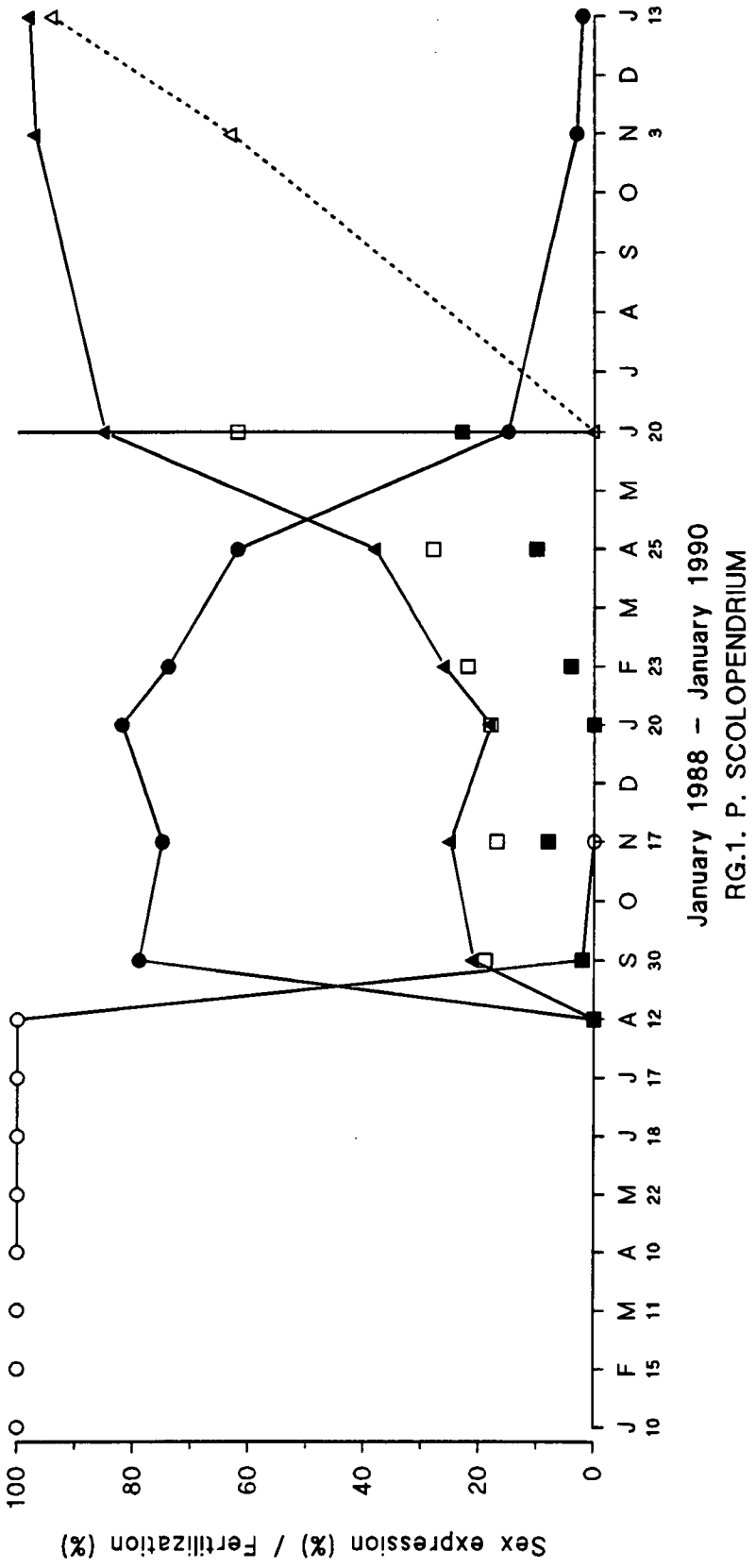
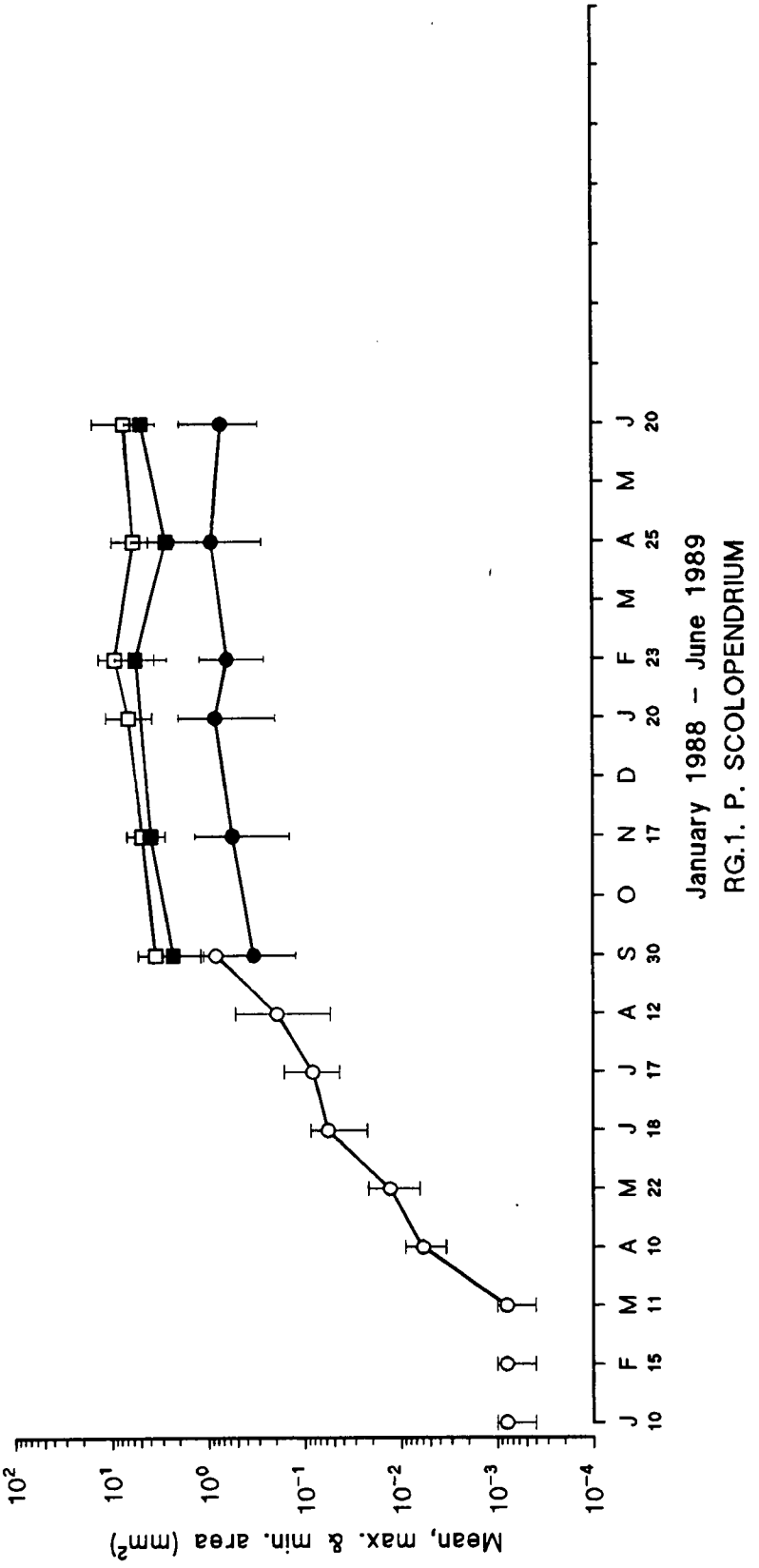
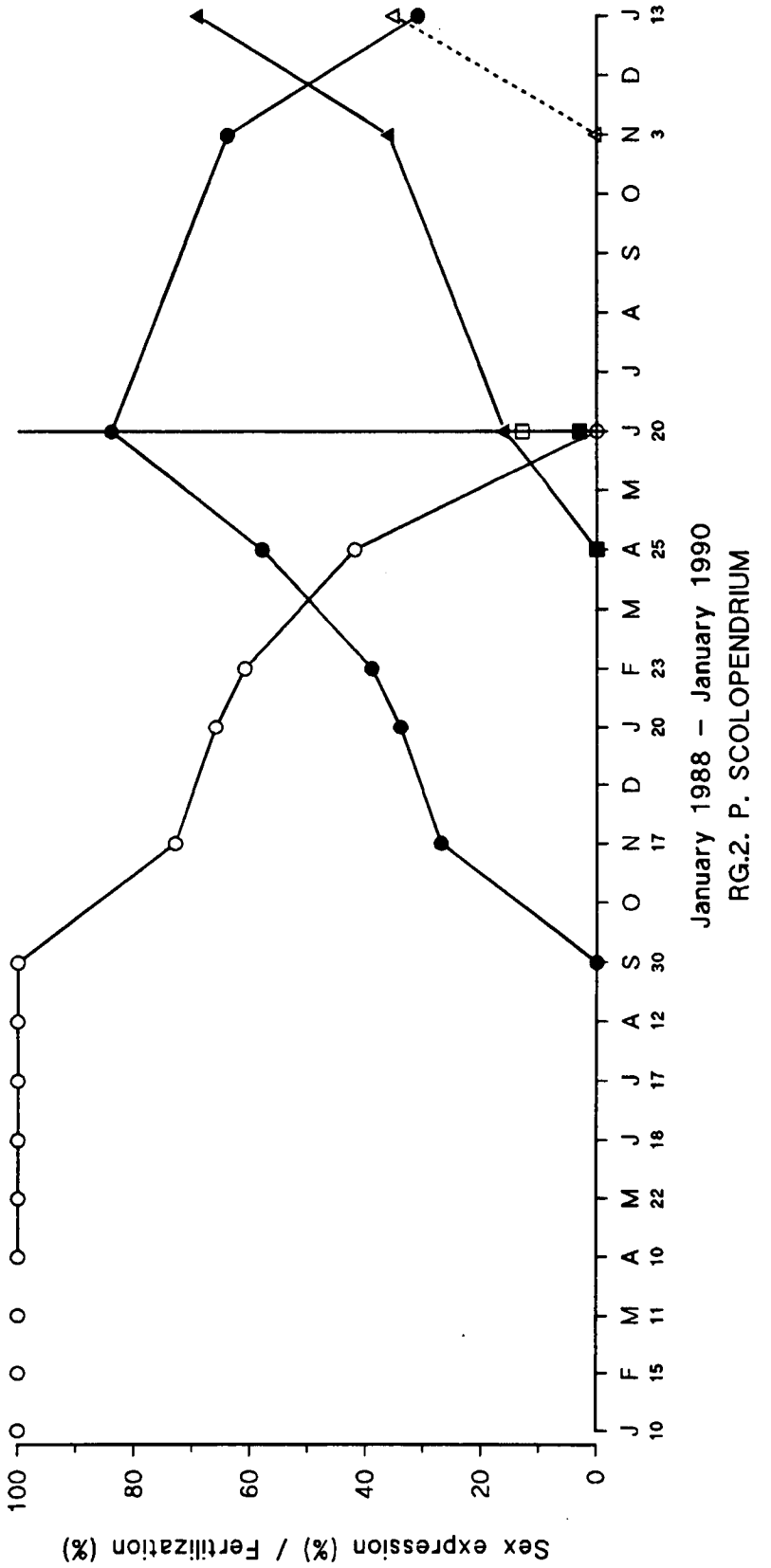

Sex expression (%) / Fertilization (%)
0
20
40
60
80
100
J F M A M J J A S O N D J J F M A M J J A S O N D J
10 15 11 10 22 18 17 12 30 17 20 23 25 20 3 13
January 1988 – January 1990
RG.1. P. SCOLOPENDRIUM

Figure 4.15b


Mean, max. & min. area (mm²)
10²
10¹
10⁰
10⁻¹
10⁻²
10⁻³
10⁻⁴
J F M A M J J A S O N D J F M A M J
10 15 11 10 22 18 17 12 30 17 20 23 25 20
January 1988 – June 1989
RG.1. P. SCOLOPENDRIUM

Symbols: spores or pre-sexual gametophytes (○), male gametophytes ( ), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Figure 4.16a


Sex expression (%) / Fertilization (%)
0
20
40
60
80
100
J F M A M J J A S O N D J J A S O N D J
10 15 11 10 22 18 17 12 30 17 20 23 25 20 3 13
January 1988 – January 1990
RG.2. P. SCOLOPENDRIUM

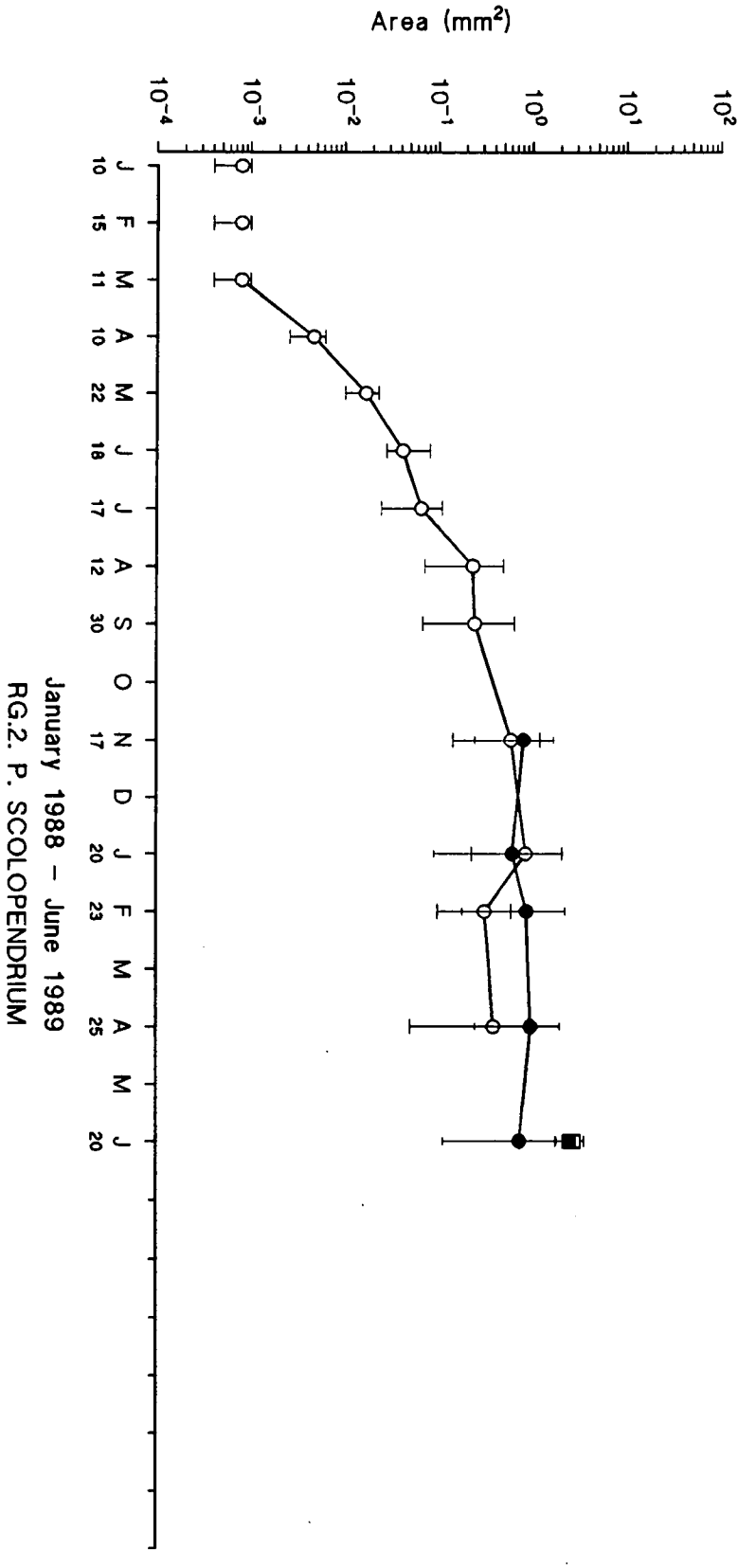

Area (mm²)
10⁻⁴
10⁻³
10⁻²
10⁻¹
10⁰
10¹
10²
J F M A M J J A S O N D J F M A M J
10 15 11 10 22 18 17 12 30 17 20 23 25 20
January 1988 – June 1989
RG.2. P. SCOLOPENDRIUM

Figure 4.16b

Chapter 5

Results of Experiment 2

Introduction

The most logical time to initiate field experiments on the development of fern gametophytes is of course during the peak spore release period of each species. The peak spore release period for *P. setiferum* was August, for *A. filix-femina* it was mid-August to mid-September, for *B. spicant* it was mid-September to mid-October and for *P. scolopendrium* it was October (Figure 3.1). Clearly, there is no ideal date to initiate a field experiment involving (and requiring spores of) these 4 species. To reduce this problem, 2 field experiments were conducted.

The first experiment was initiated in October 1988, near the end of the main spore release season, and was more appropriate for *B. spicant* and *P. scolopendrium* than for *P. setiferum* and *A. filix-femina*. The second experiment was initiated in August 1989, near the beginning of the main spore release season and was more appropriate for *P. setiferum* and *A. filix-femina* than for *B. spicant* and *P. scolopendrium.*

The results of the experiment initiated in October 1988 are presented in this chapter; the results of the experiment initiated in August 1989 are presented in chapter 6.

Pease Bridge Glen

The following text relates to Table 5.1 and Figures 5.1 - 5.8.

Germination

Spores were sown in October 1988. Tiny gametophytes (mean area: 0.0017 mm^{-2} - 0.0079 mm^{-2}) of *A. filix-femina, P. setiferum* and *P. scolopendrium* were first detected, in both plots, in January 1989. The largest gametophytes [comprising 3 chlorocytes (Dyer, 1979c)] were already 2-dimensional. Gametophytes of *B. spicant* were first detected in early

March 1989 but note that no samples were collected in February when gametophytes were probably present. Within each species there was no evidence of staggered germination (Figures 5.1b - 5.8b). The numbers of gametophytes in the pots were not accurately determined but densities were estimated to range from approximately 50-200 gametophytes cm^{-2}.

Sex expression

Most populations were pre-sexual for 3 months. Antheridia or antheridia and archegonia first appeared in May or June 1988. Most antheridia were already mature when they were first detected.

The sexual transition periods were surprisingly short; 80-100% of gametophytes in all populations were sexual by June 1988; all gametophytes in all populations were sexual by July 1988.

The sexual composition of all populations remained relatively constant from near the end of the sexual transition periods to the end of the experiment in January 1990 (see 'equilibrium periods' in Table 5.1 and Figures 5.1a - 5.8a). Five populations consisted of approximately equal numbers of male and archegoniate gametophytes. Three populations, *P. setiferum* [1 & 2] and *P. scolopendrium* [2], were predominantly (*i.e.* 60-70%) male.

Most archegoniate gametophytes were female. Bisexual gametophytes were detected in all populations except in *B. spicant* [2]. All bisexual gametophytes were protandrous.

Size and shape

In general, in all populations, female gametophytes were larger than bisexual gametophytes and all archegoniate gametophytes were considerably larger than male gametophytes. All archegoniate gametophytes were meristic (heart-shaped); all male gametophytes were ameristic (spathulate when young, various shapes when older but never heart-shaped). The shape of pre-sexual gametophytes was not recorded. In

all populations the first archegoniate gametophytes were considerably larger than the largest pre-sexual gametophytes in the sample 1 month before (Figures 5.1b - 5.8b).

Fertilization

Fertilization occurred in all populations throughout the Summer and Autumn of 1989 and the Winter of 1989/'90. Embryos first appeared in most populations only 1-2 months after the first appearance of archegonia. Fertilization was particularly fast in *A. filix-femina* [2]; almost one third of archegoniate gametophytes, when they were first detected, had embryos.

By October 1989 (one year after sowing) the majority of archegoniate gametophytes in most populations and all archegoniate gametophytes in *A. filix-femina* [1] had been fertilized. Only in *B. spicant* [1] and *P. scolopendrium* [2] were most still unfertilized but by the end of the experiment (January 1990) the majority of archegoniate gametophytes in these populations also had been fertilized.

Polyembryony was observed twice. In October 1989, there was one female gametophyte in *P. setiferum* [2] with 2 embryos and in January 1990, there was one female in *B. spicant* [2] with 2 sporelings attached.

Mortality

At the end of the experiment all gametophytes, even those with sporophytes attached, were healthy and intact; no gametophytes died during this experiment. There were no lobed gametophytes (*cf.* Experiment 1). Sporelings of *B. spicant, P. setiferum* and *P. scolopendrium* were Winter-green but the larger leaves of *A. filix-femina* died during the Winter of 1989/'90. Despite the death of older leaves new ones continued to emerge. No sporelings died.

Maximum development

The largest gametophyte in all populations was female. The largest gametophyte observed at this site, during this experiment, was a female in B. spicant [2] which had

an area of 93.29 mm^2. The most advanced sporophyte in each population in January 1990 had 2-5 leaves.

Roslin Glen

The following text relates to Table 5.2 and Figures 5.9 - 5.16.

Germination

Spores were sown in October 1988. Tiny gametophytes (mean area: 0.0027 mm^{-2} - 0.0060 mm^{-2}) of all 4 species were first detected, in both plots, in January 1989. The largest gametophytes (comprising 3 chlorocytes) were already 2-dimensional. There was no evidence of staggered germination (Figures 5.9b - 5.16b). The numbers of gametophytes in the pots were not accurately determined but densities were estimated to range from approximately 50-200 gametophytes cm^{-2}.

Sex expression

Most populations were pre-sexual for 4 months. Antheridia or antheridia and archegonia first appeared in these, in June 1989. *P. scolopendrium* [2] was pre-sexual for at least 6 months. Antheridia were first observed in this population in November 1989 but note that no samples were examined between July and November.

80-100% of gametophytes in all populations in plot 1 were sexual by July 1989 but sexual transition in all populations in plot 2 was much slower. In fact, it was not even possible to determine the sexual transition periods for *P. setiferum* and *P. scolopendrium* in Plot 2 because not all gametophytes had become sexual by the end of the experiment.

The sexual composition of most populations remained relatively constant once all (or nearly all) gametophytes had become sexual (see 'equilibrium periods' in Table 5.2 and Figure 5.9a - 5.16a). All populations (including those that did not reach equilibrium by January 1990) were predominantly (*i.e.* 65-90%) male.

Most archegoniate gametophytes were female. A few bisexual gametophytes were detected in all populations except in *A. filix-femina* [2] and *P. setiferum* [2]. All bisexual gametophytes were protandrous.

Size and shape

In general, in all populations, female gametophytes were larger than bisexual gametophytes and all archegoniate gametophytes were considerably larger than male gametophytes. All archegoniate gametophytes were meristic (heart-shaped); all male gametophytes were ameristic (spathulate when young, various shapes when older but never heart-shaped). The shape of pre-sexual gametophytes was not recorded. In all populations the first archegoniate gametophytes were considerably larger than the largest pre-sexual gametophytes in the sample 1 month before (Figures 5.9b - 5.16b).

Fertilization

By October 1989 (one year after sowing) only 3 populations (all in plot 1) had evidence of fertilization. However, by the end of the experiment (January 1990) 7 populations contained at least some sporelings and/or embryos and in *A. filix-femina* [1] all archegoniate gametophytes had been fertilized. There was no fertilization in *P. scolopendrium* [2]. Polyembryony was not observed.

Mortality

At the end of the experiment all gametophytes, even those with sporophytes attached, were healthy and intact; no gametophytes died during this experiment. There were no lobed gametophytes (*cf.* Experiment 1). All sporelings, including those of *A. filix-femina* were Winter-green. No sporelings died.

Maximum development

The largest gametophyte in all populations was female. The largest gametophyte observed at this site, during this experiment, was a female in P. setiferum [1] which had an area of 40.23 mm^2. The most advanced sporophyte in each population in January 1990 was an embryo or a 1-2 leaved sporeling.

Summary

In this experiment, as in Experiment 1, the results for *A. filix-femina, B. spicant, P. setiferum* and *P. scolopendrium* were strikingly similar.

At both sites gametophytes of all 4 species appeared in the Winter but at a lower density than in the laboratory (see page 9). There was no staggered germination and no obvious protonemal phase (Raghavan, 1989).

Most gametophytes became sexual between mid-Spring and mid-Summer; transition periods were often surprisingly short; most antheridia and some archegonia were mature when they were first detected. The sexual composition of those populations which reached equilibrium remained relatively constant to the end of the experiment (January 1990). Three populations at Pease Bridge Glen and all populations at Roslin Glen were predominantly male. The other 5 populations (at Pease Bridge Glen) consisted of approximately equal numbers of male and archegoniate gametophytes. Most archegoniate gametophytes were female. Bisexual gametophytes were detected in all but 3 populations. All bisexual gametophytes were protandrous.

In general, female gametophytes were larger than bisexual gametophytes and all archegoniate gametophytes were considerably larger than male gametophytes. All archegoniate gametophytes were meristic; all male gametophytes were ameristic. Curiously, the first archegoniate gametophytes in all populations were considerably larger than the largest pre-sexual gametophytes in the sample 1 month before.

Fertilization occurred in all populations and was not seasonal (embryos appeared throughout the Summer and Autumn of 1989 and the Winter of 1989/'90). Embryos first appeared in many populations only 1-2 months after the first appearance of archegonia. In one population many archegoniate gametophytes, when they were first detected, had already been fertilized. By the end of the experiment most archegoniate gametophytes at Pease Bridge Glen had been fertilized but most archegoniate gametophytes at Roslin Glen had still to be fertilized. Polyembryony was not observed.

No gametophytes died during this experiment (*i.e.* all gametophytes survived for 1 year). No sporelings died either.

In terms of the rates of early (*i.e.* pre-sexual) gametophyte development, the rates at which populations became sexual, the percentages of archegoniate gametophytes at equilibrium, the percentages of fertilization and the rates of fertilization and leaf production, all 4 species grew better at Pease Bridge Glen than at Roslin Glen. Moreover, all 4 species grew as well in PBG.1. as in PBG.2. At Roslin Glen all 4 species grew much better in plot 1 than in plot 2.

Table 5.1. Results of Experiment 2 at Pease Bridge Glen. Shading indicates the species that were on alien soil.

Experiment 2 : Pease Bridge Glen	*A. filix-femina*		*B. spicant*		*P. setiferum*		*P. scolopendrium*	
Plot	PBG.1.	PBG.2.	PBG.1.	PBG.2.	PBG.1.	PBG.2.	PBG.1.	PBG.2.
See Figure	5.1	5.2	5.3	5.4	5.5	5.6	5.7	5.8
Spores sown	Oct.'88	Oct.'88	Oct.'88	Oct.'88	Oct.'88	Oct.'88	Oct.'88	Oct.'88
Germination	Jan.'89	Jan.'89	Mar.'89	Mar.'89	Jan.'89	Jan.'89	Jan.'89	Jan.'89
Pre-sexual period (months)	2	3	3	3	3	3	3	4
Size of last sterile gametophytes (mm^2)	0.11	0.09	0.20	0.29	0.27	0.23	0.08	0.30
First male gametophytes	May '89	May '89	Jun.'89	Jun.'89	May '89	May '89	May '89	Jun.'89
Size of first male gametophytes (mm^2)	0.08	0.06	0.18	0.24	0.53	0.28	0.08	0.39
First female gametophytes	May '89	Jun.'89	Jun.'89	Jun.'89	Jun.'89	Jun.'89	Jun.'89	Jun.'89
Size of first female gametophytes (mm^2)	0.78	2.15	0.92	0.86	3.00	1.50	1.26	1.55
First bisexual gametophytes	Jul.'89	Jun.'89	Aug.'89	-	Jun.'89	Jun.'89	Jul.'89	Aug.'89
Size of first bisexual gametophytes (mm^2)	3.97	1.85	3.14	-	2.27	1.06	3.48	5.73
All gametophytes sexual by	Jun.'89	Jul.'89	Jul.'89	Jul.'89	Jun.'89	Jun.'89	Jun.'89	Jul.'89
Transition period (months)	3	3	2	2	2	2	2	2
Equilibrium period (months)	> 7	> 5	> 6	> 6	> 7	> 6	> 5	> 7
% of population male at equilibrium	*c.*50	*c.*50	*c.*50	*c.*50	*c.*60	*c.*75	*c.*50	*c.*70
% of population archegoniate at equilibrium	*c.*50	*c.*50	*c.*50	*c.*50	*c.*40	*c.*25	*c.*50	*c.*30
First embryo	Jul.'89	Jun.'89	Aug.'89	Jul.'89	Jul.'89	Jul.'89	Jul.'89	Aug.'89
% of archegoniates fertilized in 1st year	100	68	43	70	81	66	90	32
% of archegoniates fertilized by Jan.'90	93	82	66	88	97	89	79	59
Largest gametophyte by Jan.'90 (mm^2) (sex)	51.28 ♀	42.31 ♀	59.24 ♀	93.29 ♀	25.84 ♀	24.07 ♀	67.67 ♀	23.30 ♀
Most advanced sporeling by Jan.'90 (leaves)	5	4	3	3	4	3	4	2

Table 5.2. Results of Experiment 2 at Roslin Glen. Shading indicates the species that were on alien soil.

Experiment 2 : Roslin Glen	*A. filix-femina*		*B. spicant*		*P. setiferum*		*P. scolopendrium*	
Plot	RG.1.	RG.2.	RG.1.	RG.2.	RG.1	RG.2.	RG.1	RG.2.
See Figure	5.9	5.10	5.11	5.12	5.13	5.14	5.15	5.16
Spores sown	Oct.'88	Oct.'88	Oct.'88	Oct.'88	Oct.'88	Oct.'88	Oct.'88	Oct.'88
Germination	Jan.'89	Jan.'89	Jan.'89	Jan.'89	Jan.'89	Jan.'89	Jan.'89	Jan.'89
Pre-sexual period (months)	4	4	4	4	4	4	4	6
Size of last sterile gametophytes (mm^2)	0.45	1.40	0.42	0.63	0.71	> 0.90	1.58	> 0.59
First male gametophytes	Jun.'89	Jun.'89	Jun.'89	Jun.'89	Jun.'89	Jun.'89	Jun.'89	Nov.'89
Size of first male gametophytes (mm^2)	0.31	0.21	0.22	0.33	0.34	0.60	0.16	0.62
First female gametophytes	Jun.'89	Jul.'89	Jun.'89	Jul.'89	Jul.'89	Jul.'89	Jul.'89	Jan.'90
Size of first female gametophytes (mm^2)	1.17	2.37	0.63	1.83	1.96	1.56	1.28	2.74
First bisexual gametophytes	Nov.'89	-	Jul.'89	Jul.'89	Jun.'89	-	Nov.'89	Jan.'90
Size of first bisexual gametophytes (mm^2)	3.07	-	1.54	1.63	1.63	-	4.10	2.89
All gametophytes sexual by	Jul.'89	?	Nov.'89	Jan.'90	Nov.'89	?	Jan.'90	?
Transition period (months)	2	> 8	6	8	6	> 8	8	> 6
Equilibrium period (months)	> 6	> 2	> 4	> 6	> 6	?	> 2	?
% of population male at equilibrium	*c.*65	*c.*90	*c.*70	*c.*75	*c.*85	*c.*90	*c.*70	*c.*75
% of population archegoniate at equilibrium	*c.*35	*c.*10	*c.*30	*c.*25	*c.*15	*c.*5	*c.*30	*c.*5
First embryo	Jul.'89	Jan.'90	Nov.'89	Jan.'90	Nov.'89	Jan.'90	Jan.'90	-
% of archegoniates fertilized in 1st year	*c.*55	0	*c.*25	0	*c.*15	0	0	0
% of archegoniates fertilized by Jan.'90	100	36	69	50	66	17	13	0
Largest gametophyte by Jan.'90 (mm^2) (sex)	30.28 ♀	9.18 ♀	20.05 ♀	16.44 ♀	40.23 ♀	6.00 ♀	26.70 ♀	5.99 ♀
Most advanced sporeling by Jan.'90 (leaves)	2	Embryo	2	1	2	Embryo	Embryo	-

Figures 5.1 - 5.16. Sex expression, fertilization and size of gametophytes in each population during Experiment 2. Note:

1. Sampling dates are shown below the x-axes.

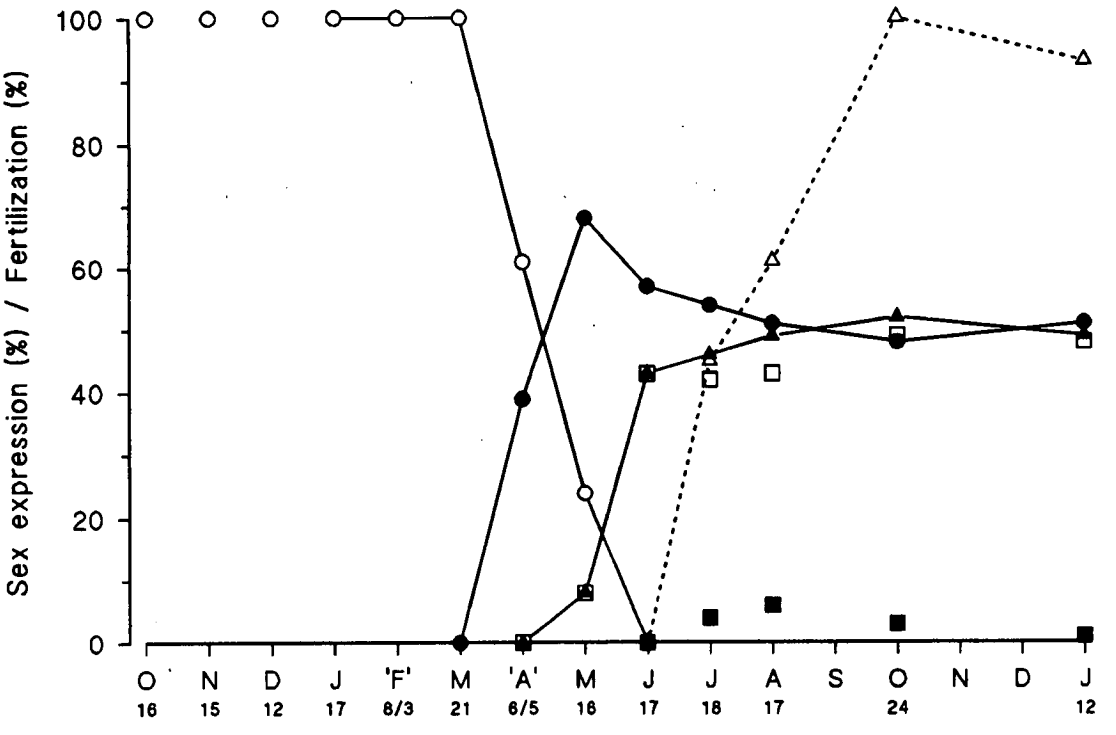
2. Symbols: spores or pre-sexual gametophytes (○), male gametophytes ( ), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

3. It has been necessary to use a log scale on the y-axes of Figures 5.1b - 5.16b to accommodate the largest and smallest values. Log scales are somewhat misleading for larger values; big differences in area can be represented by small differences in the position of symbols. Range bars: spores or pre-sexual gametophytes (2.0 mm), male gametophytes(1.0 mm), female gametophytes (1.5 mm) and bisexual gametophytes (0.5 mm).

4. Identification of sterile, male, and archegoniate gametophytes was easy and the values presented for these are accurate. Sub-dividing archegoniate gametophytes into 'females' and 'bisexuals' was more difficult because soil particles adhering to the rhizoids often obscured the rhizoid region where antheridia are usually located. Consequently, some gametophytes scored as female might have been bisexual and the values presented for these sex categories should probably be regarded as overestimates and underestimates respectively.

Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

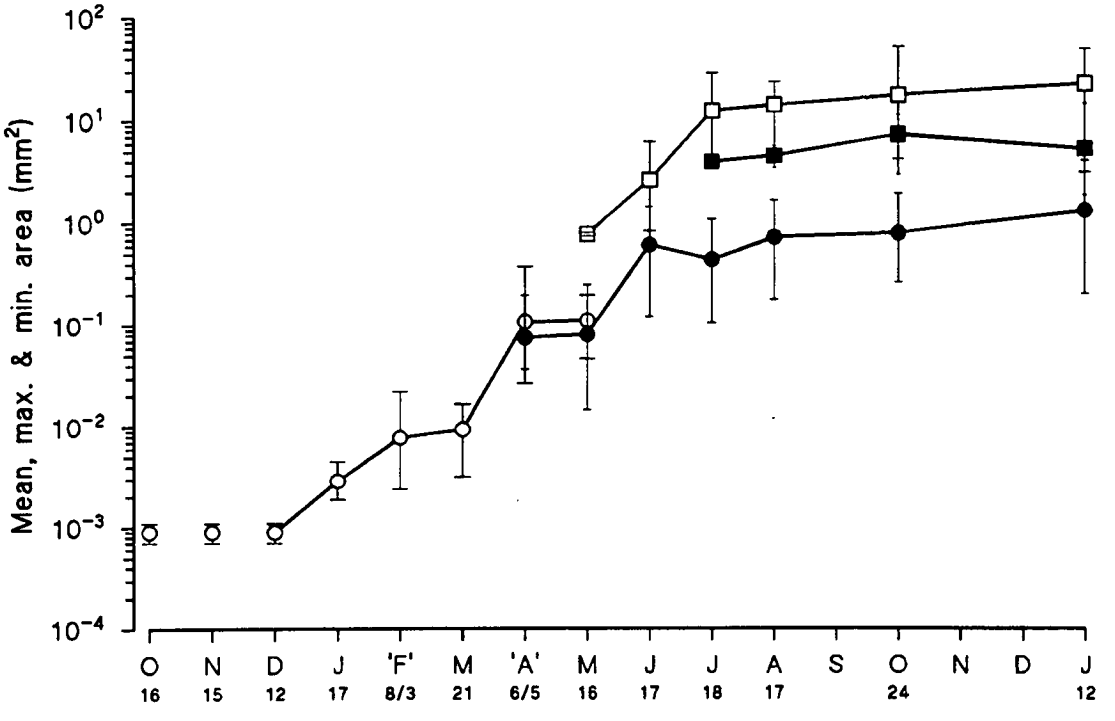
Figure 5.1a



October 1988 – January 1990

PBG.1. A. FILIX-FEMINA

Figure 5.1b

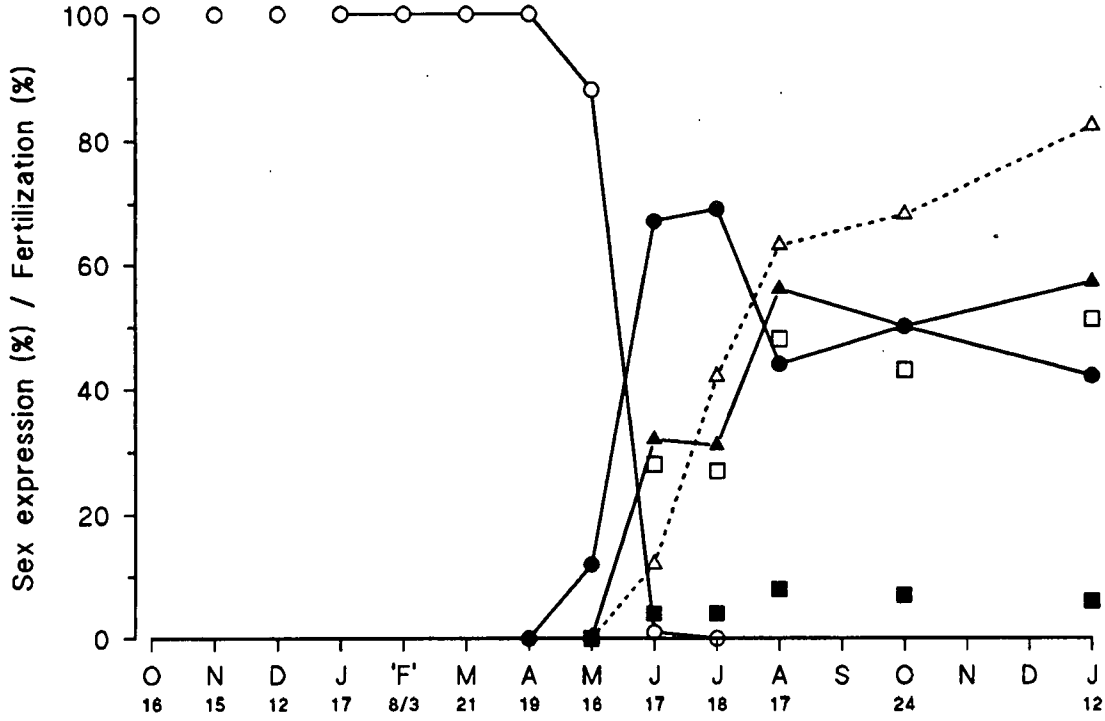


October 1988 – January 1990

PBG.1. A. FILIX-FEMINA

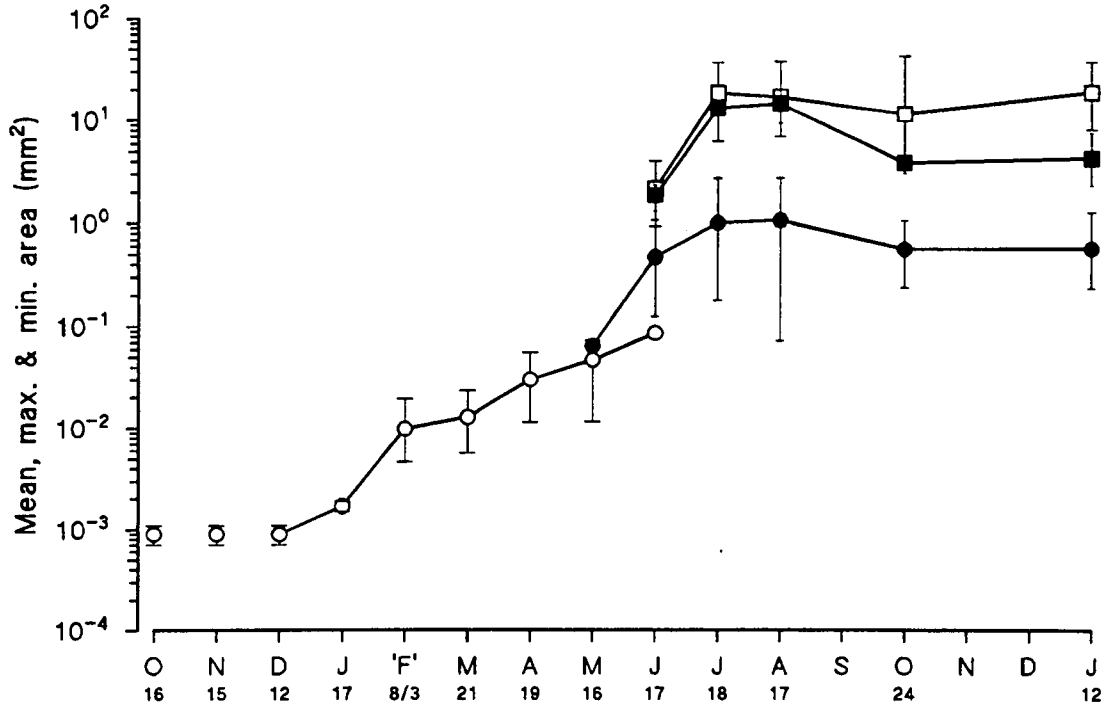
Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Figure 5.2a



October 1988 – January 1990
PBG.2. A. FILIX–FEMINA

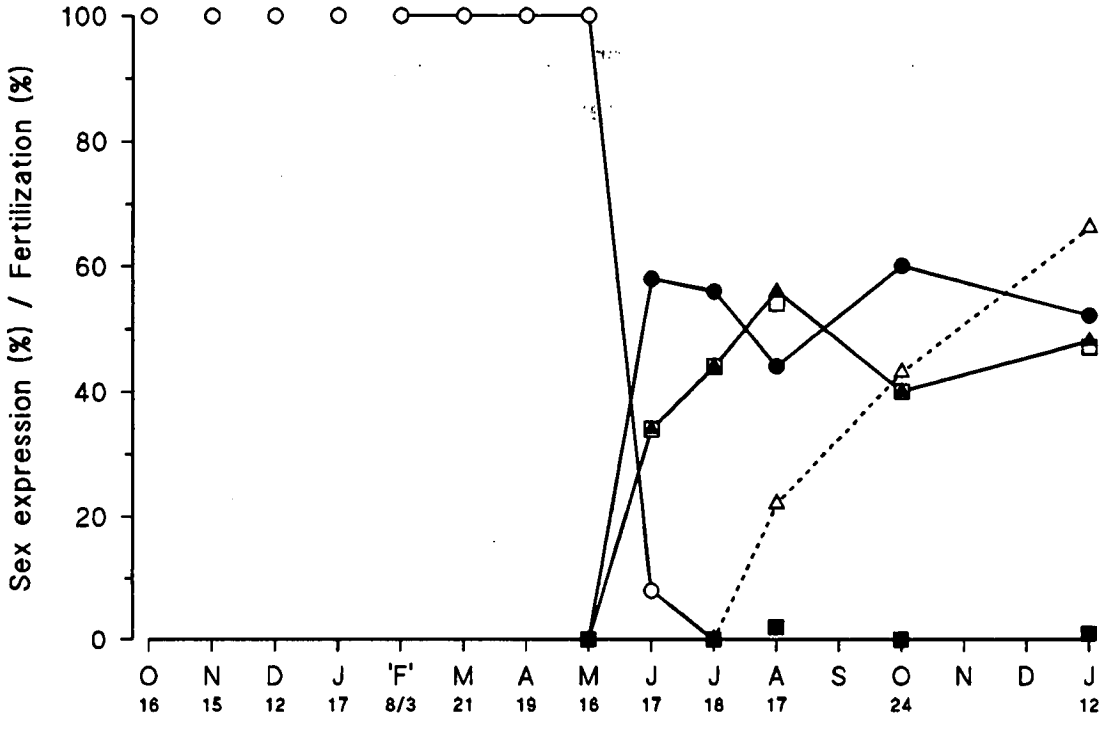
Figure 5.2b



October 1988 – January 1990
PBG.2. A. FILIX–FEMINA

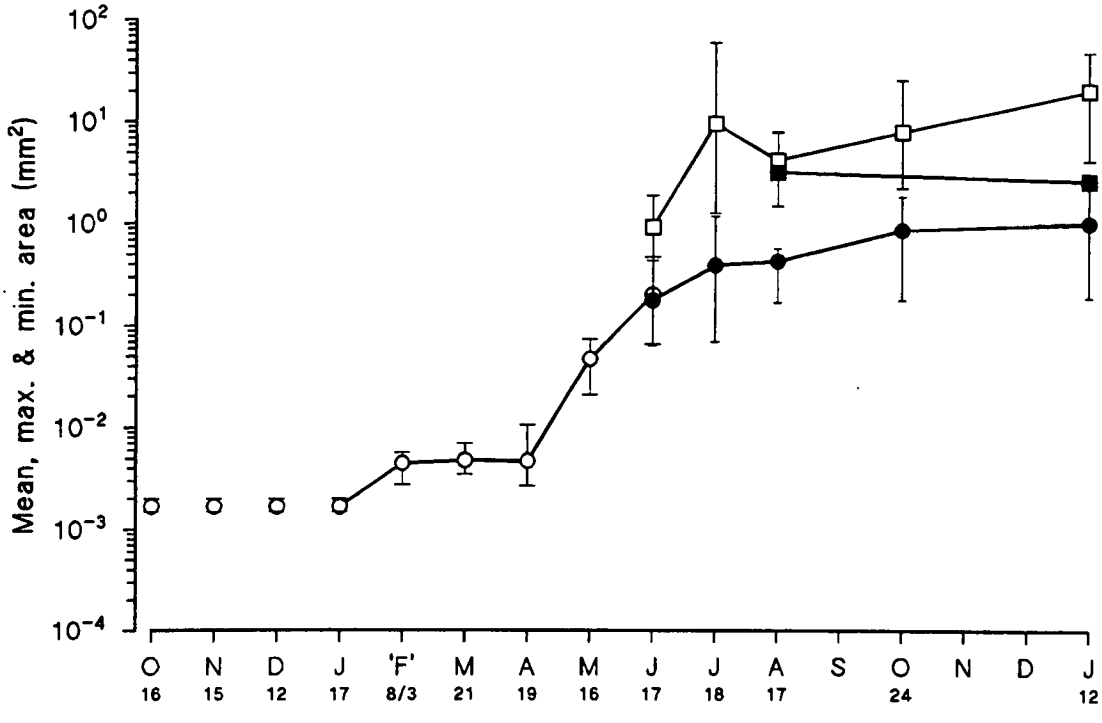
Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Figure 5.3a



October 1988 – January 1990
PBG.1. B. SPICANT

Figure 5.3b



October 1988 – January 1990
PBG.1. B. SPICANT

Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△)

Figure 5.4a

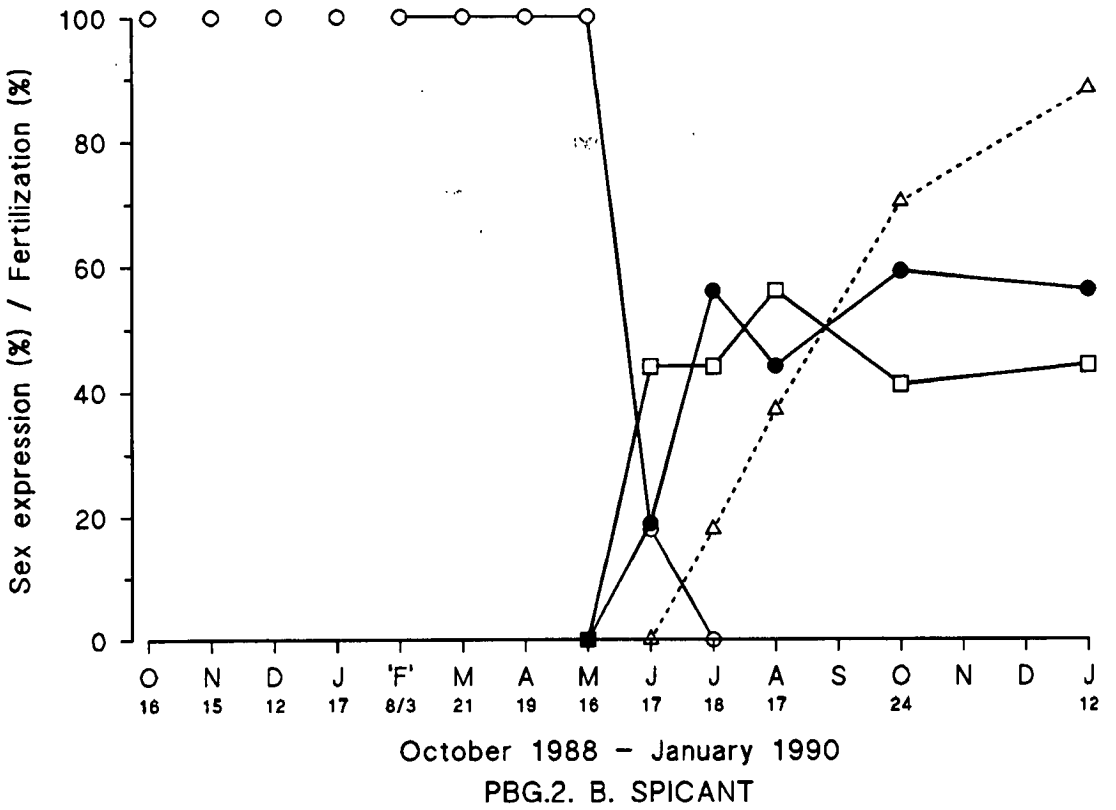
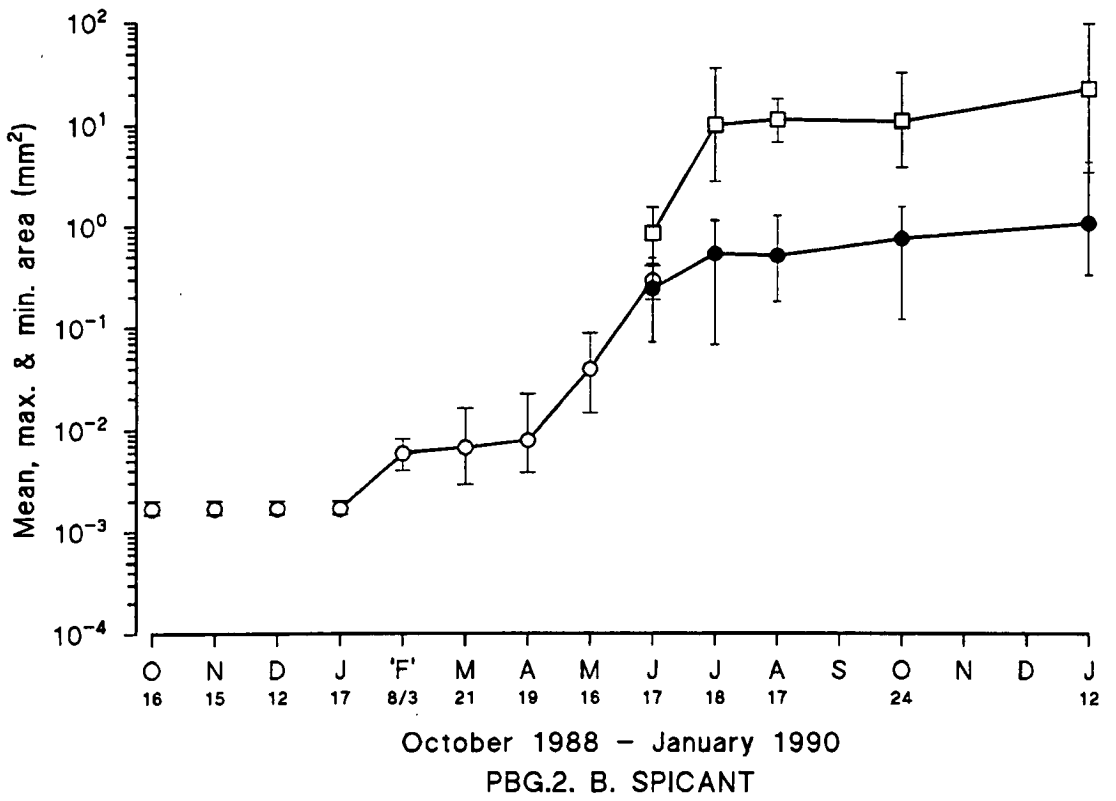
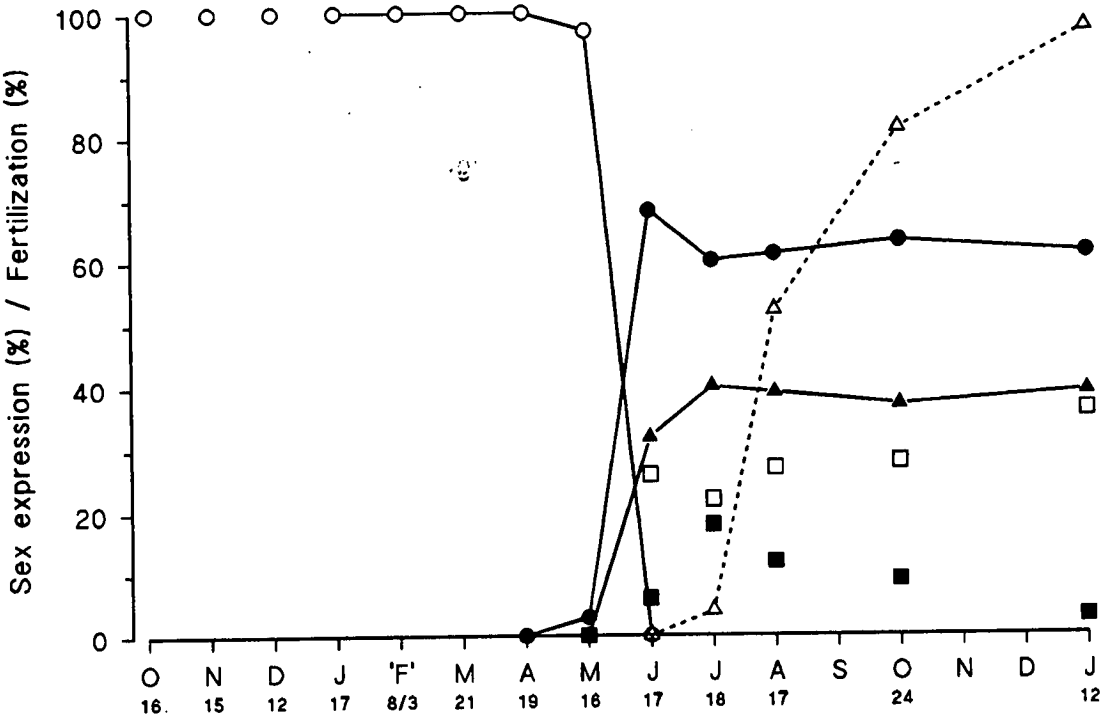


Figure 5.4b



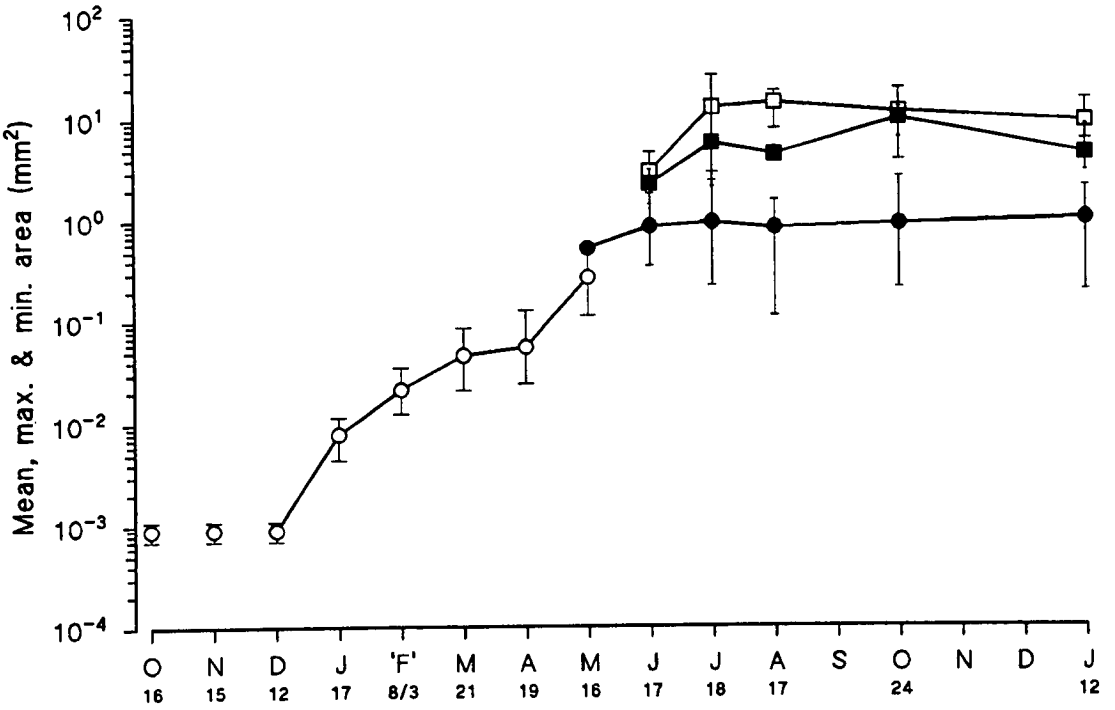
Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Figure 5.5a



October 1988 – January 1990
PBG.1. P. SETIFERUM

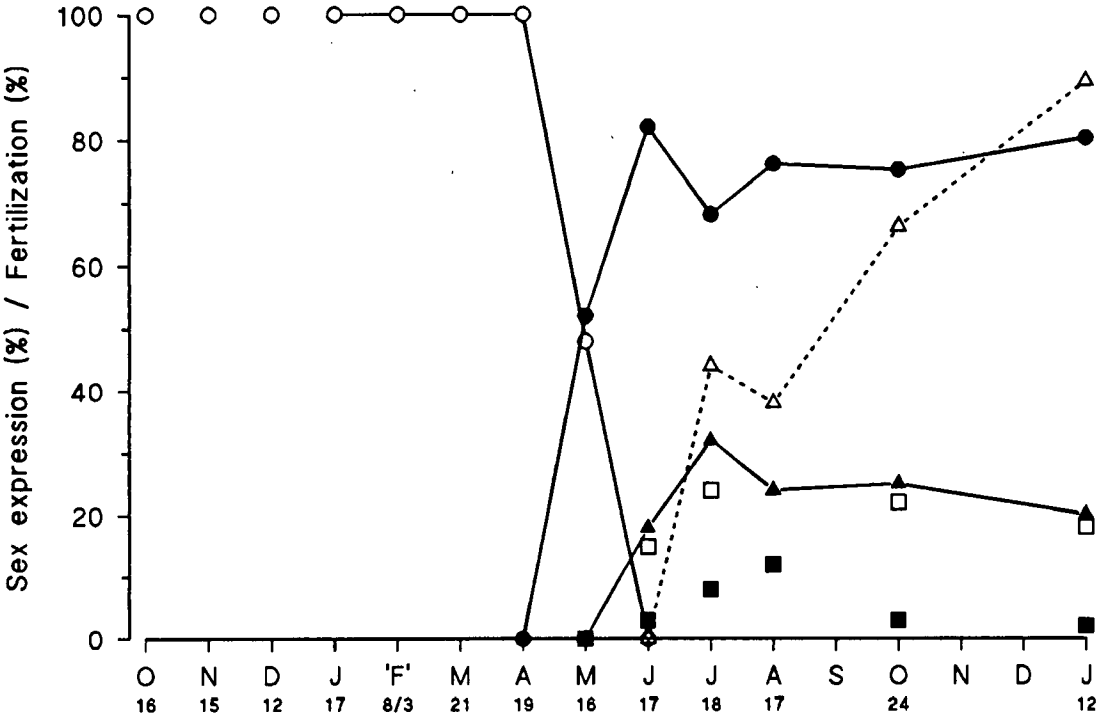
Figure 5.5b



October 1988 – January 1990
PBG.1. P. SETIFERUM

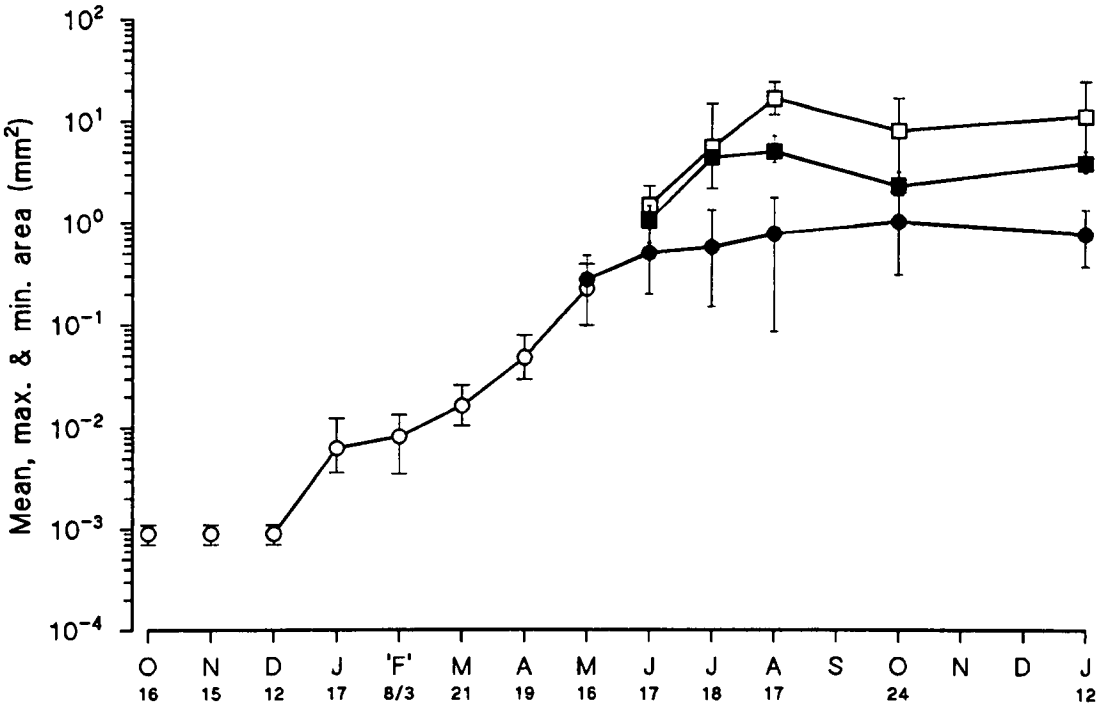
Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Figure 5.6a



October 1988 – January 1990
PBG.2. P. SETIFERUM

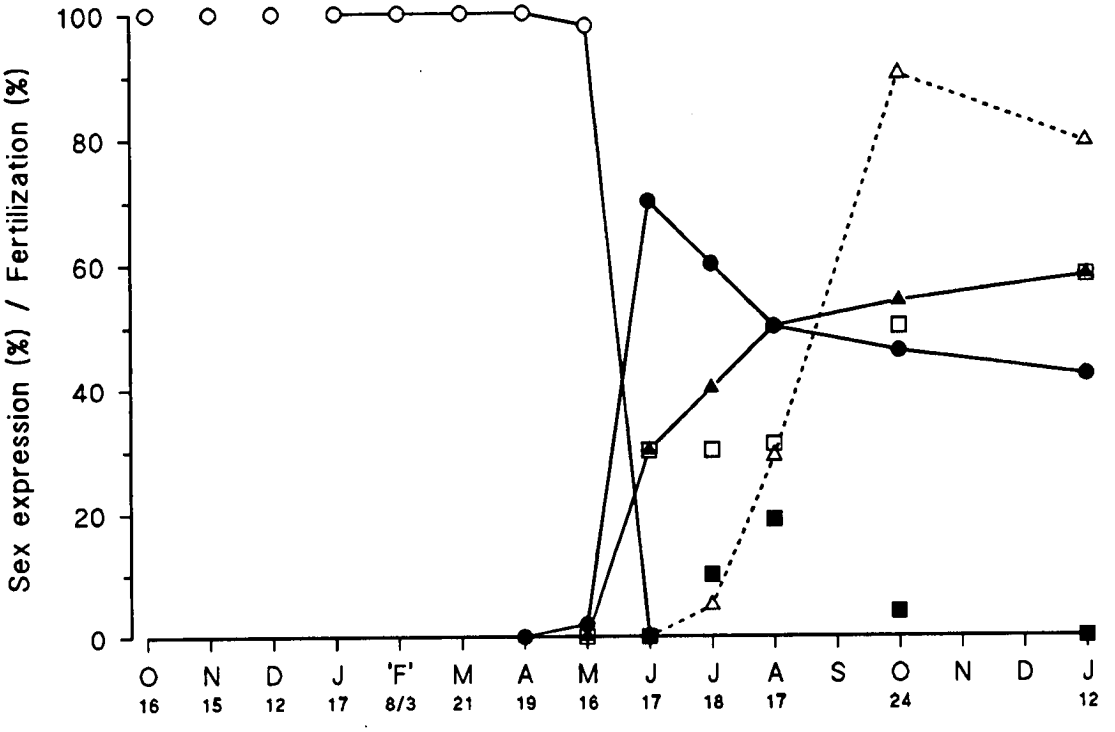
Figure 5.6b



October 1988 – January 1990
PBG.2. P. SETIFERUM

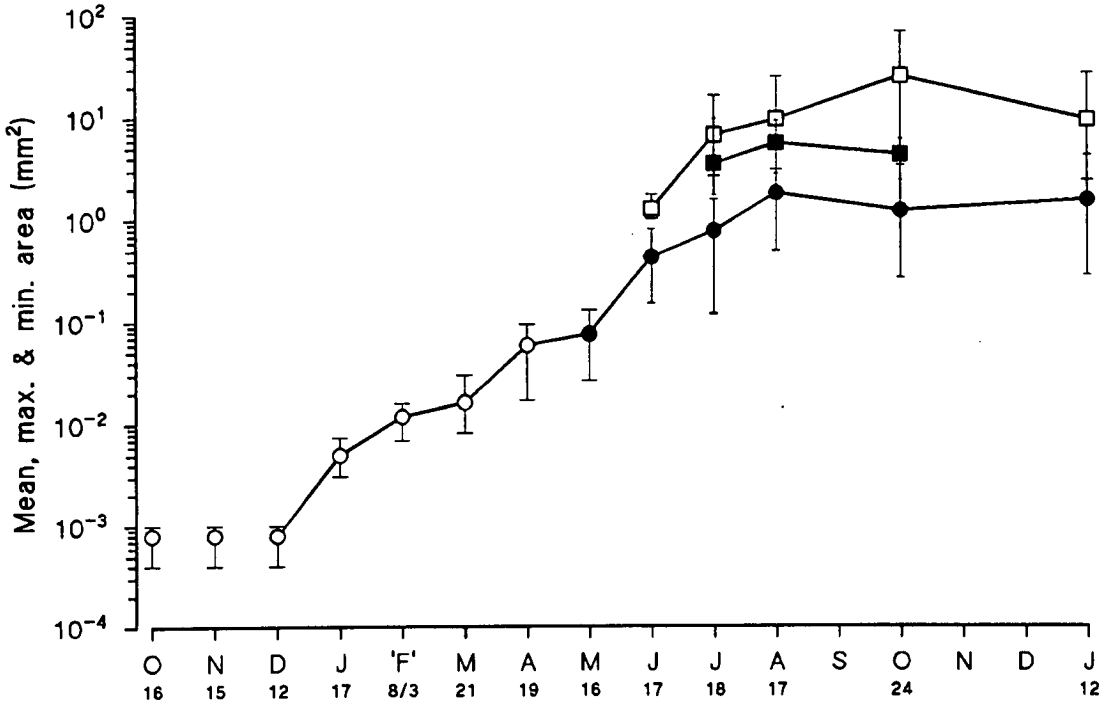
Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Figure 5.7a



October 1988 – January 1990
PBG.1. P. SCOLOPENDRIUM

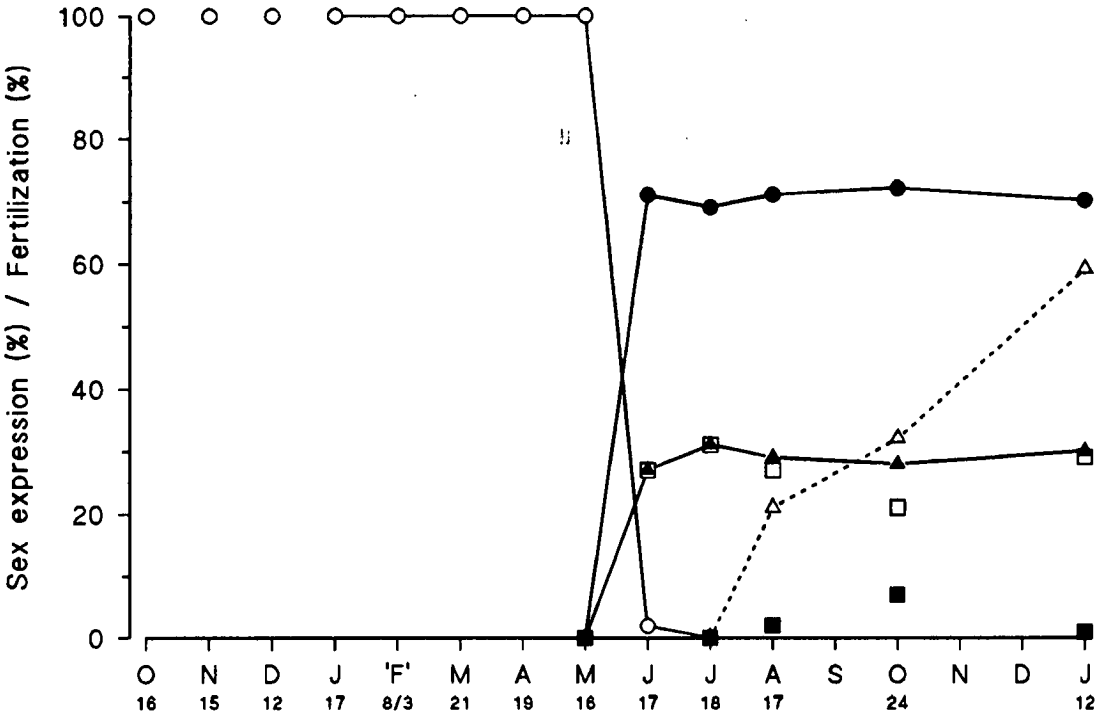
Figure 5.7b



October 1988 – January 1990
PBG.1. P. SCOLOPENDRIUM

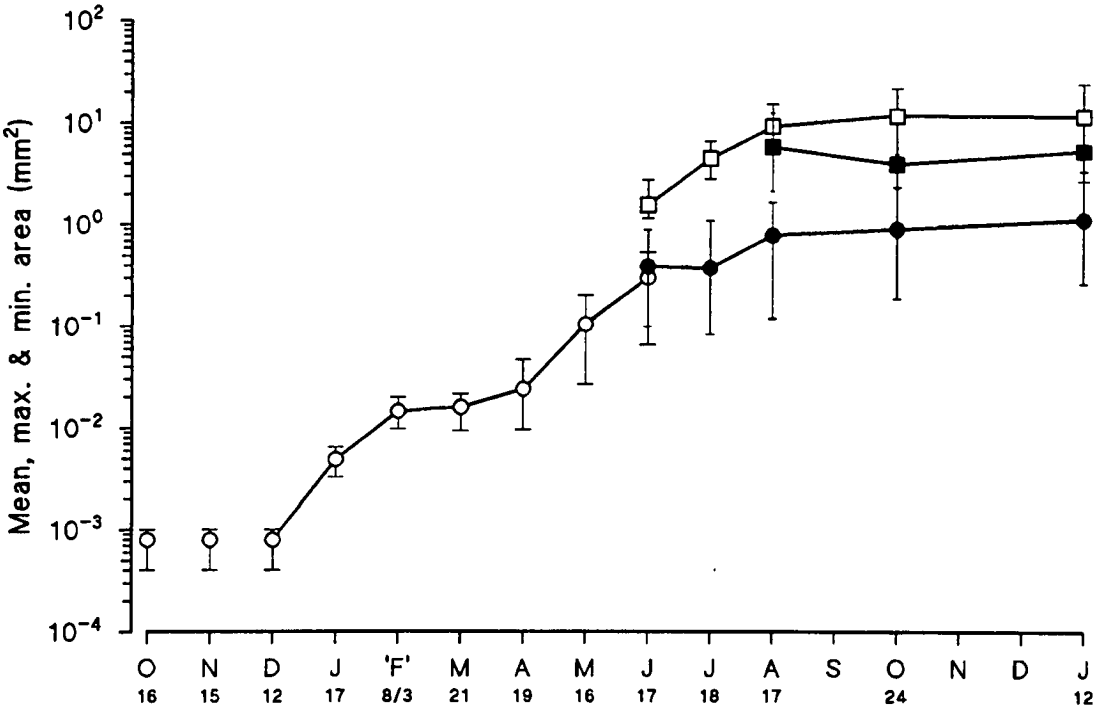
Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Figure 5.8a



October 1988 – January 1990
PBG.2. P. SCOLOPENDRIUM

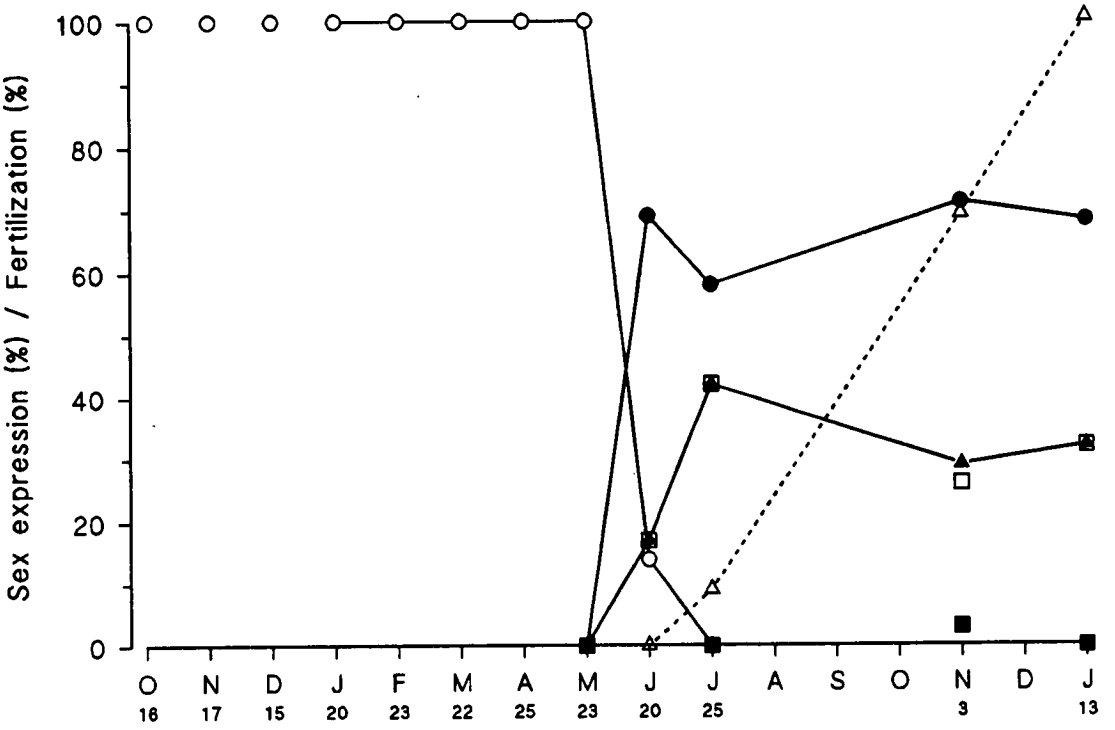
Figure 5.8b



October 1988 – January 1990
PBG.2. P. SCOLOPENDRIUM

Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

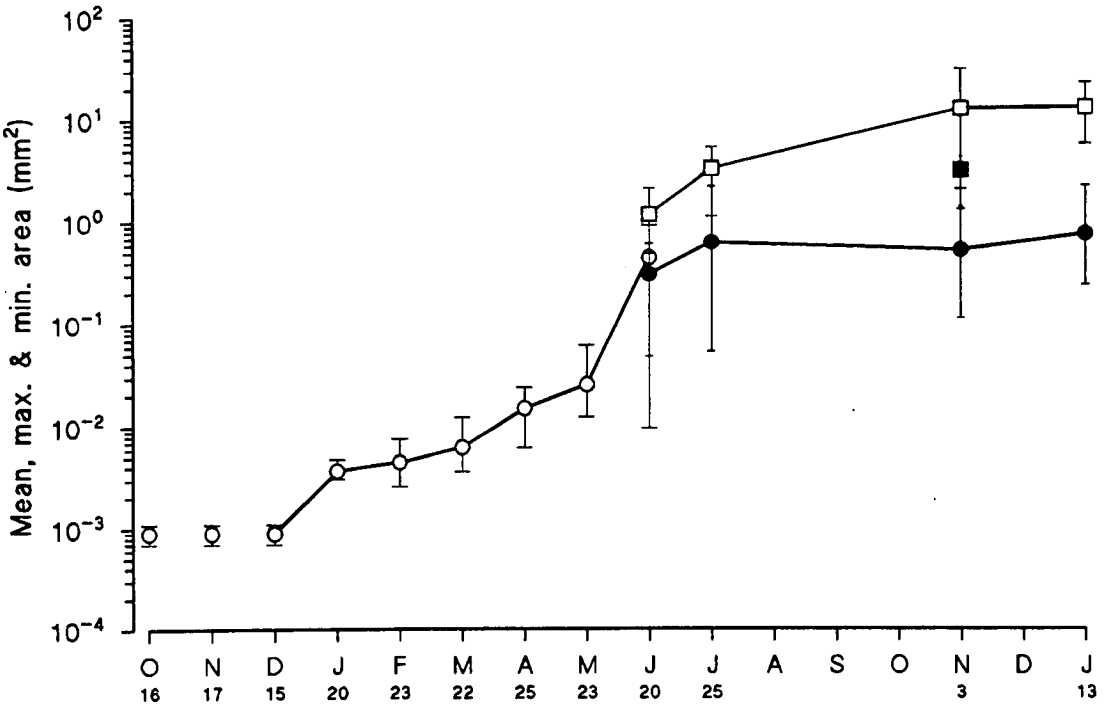
Figure 5.9a



October 1988 – January 1990

RG.1. A. FILIX-FEMINA

Figure 5.9b

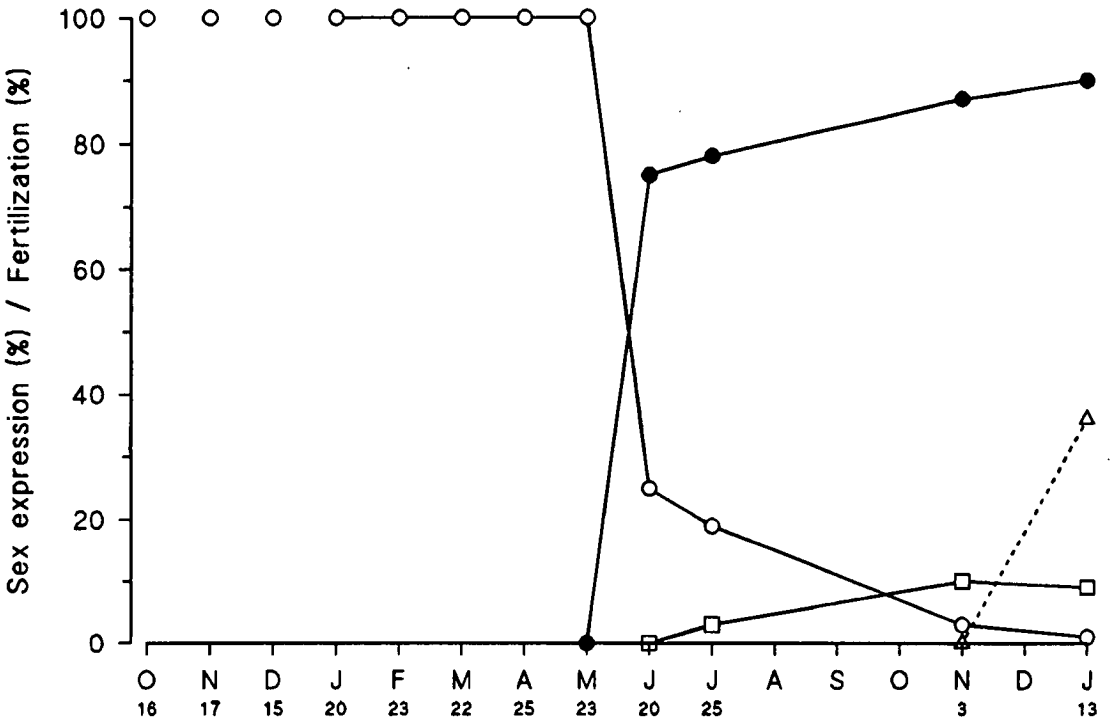


October 1988 – January 1990

RG.1. A. FILIX-FEMINA

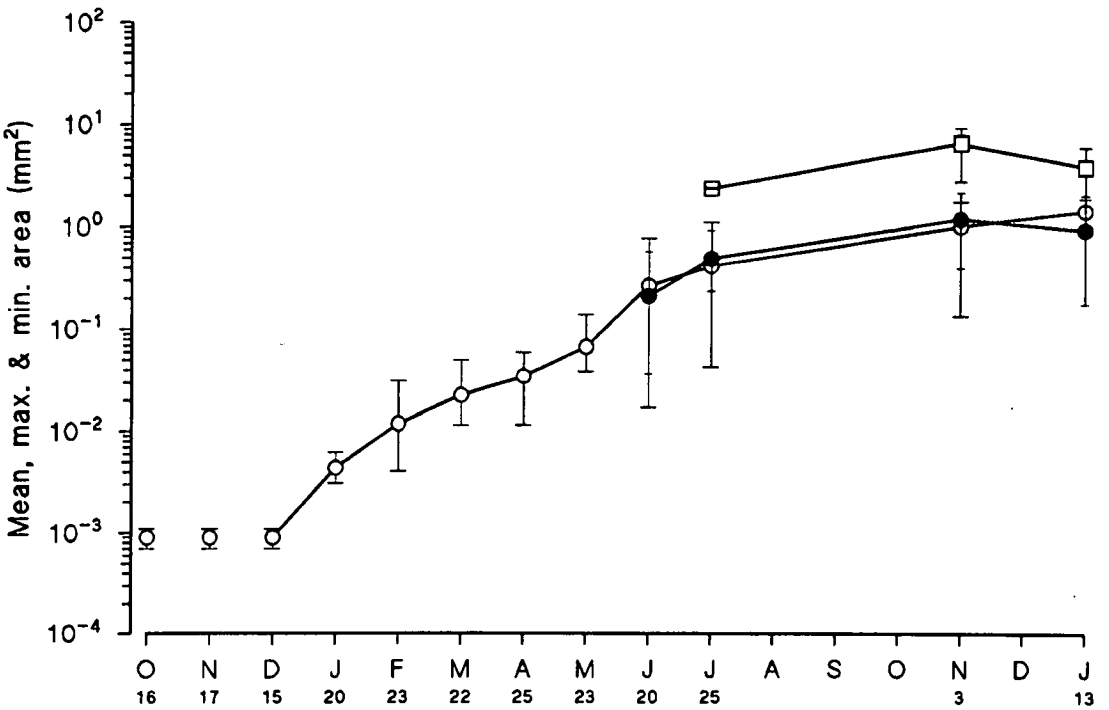
Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Figure 5.10a



October 1988 – January 1990
RG.2. A. FILIX-FEMINA

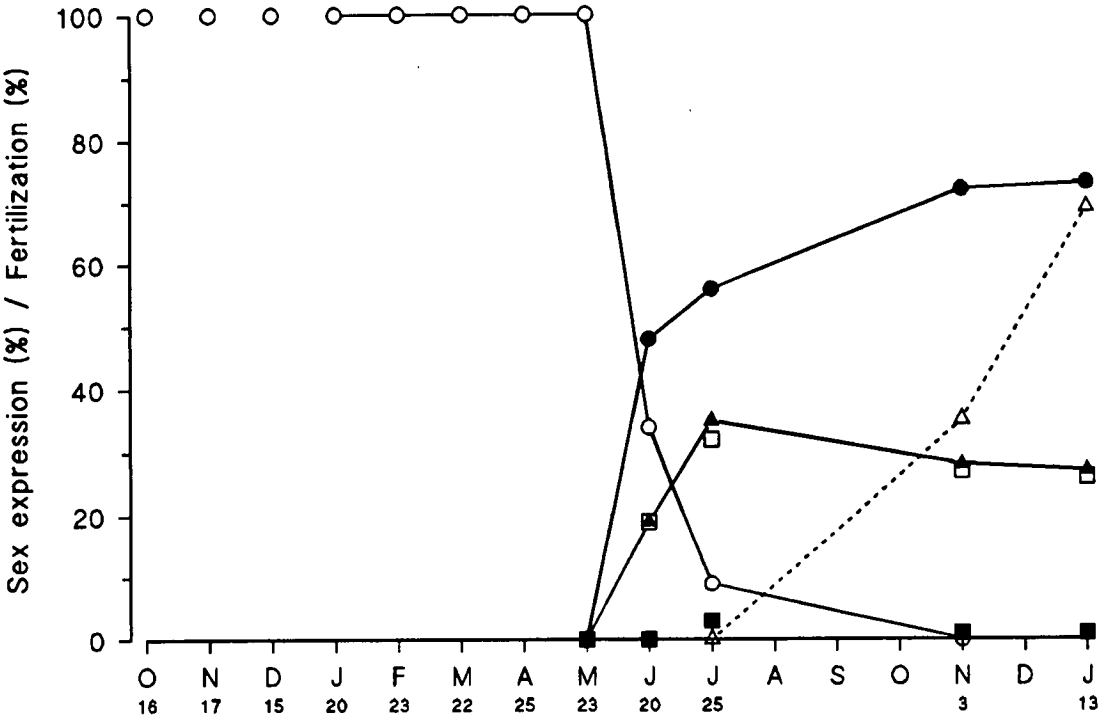
Figure 5.10b



October 1988 – January 1990
RG.2. A. FILIX-FEMINA

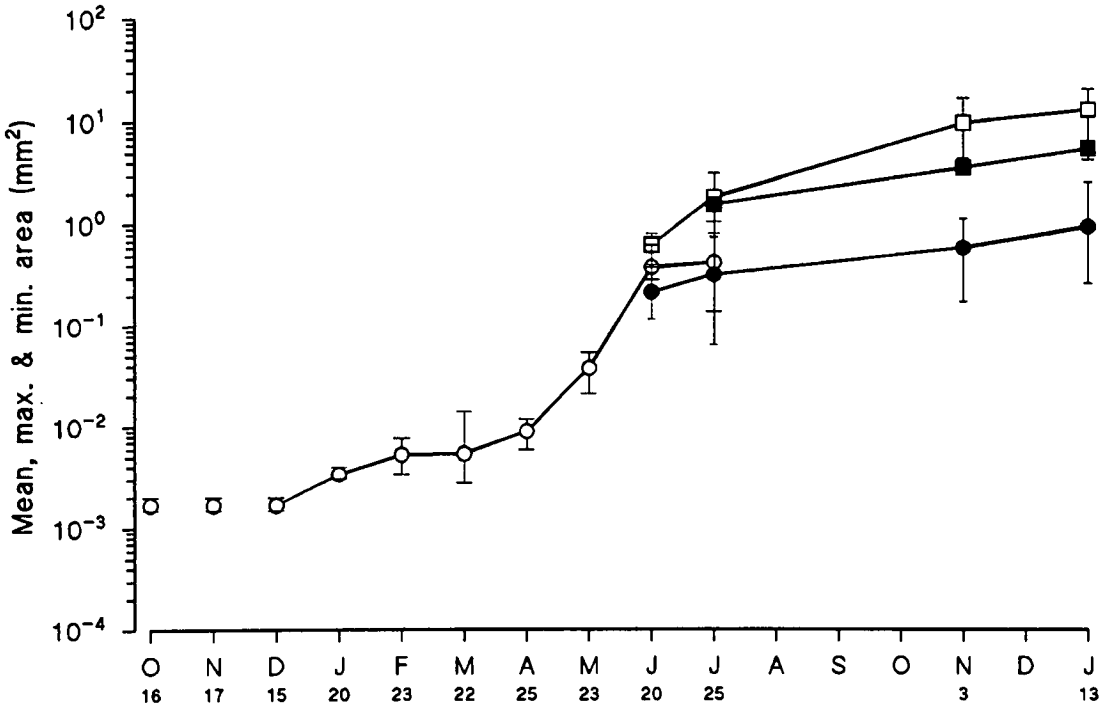
Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Figure 5.11a



October 1988 – January 1990
RG.1. B. SPICANT

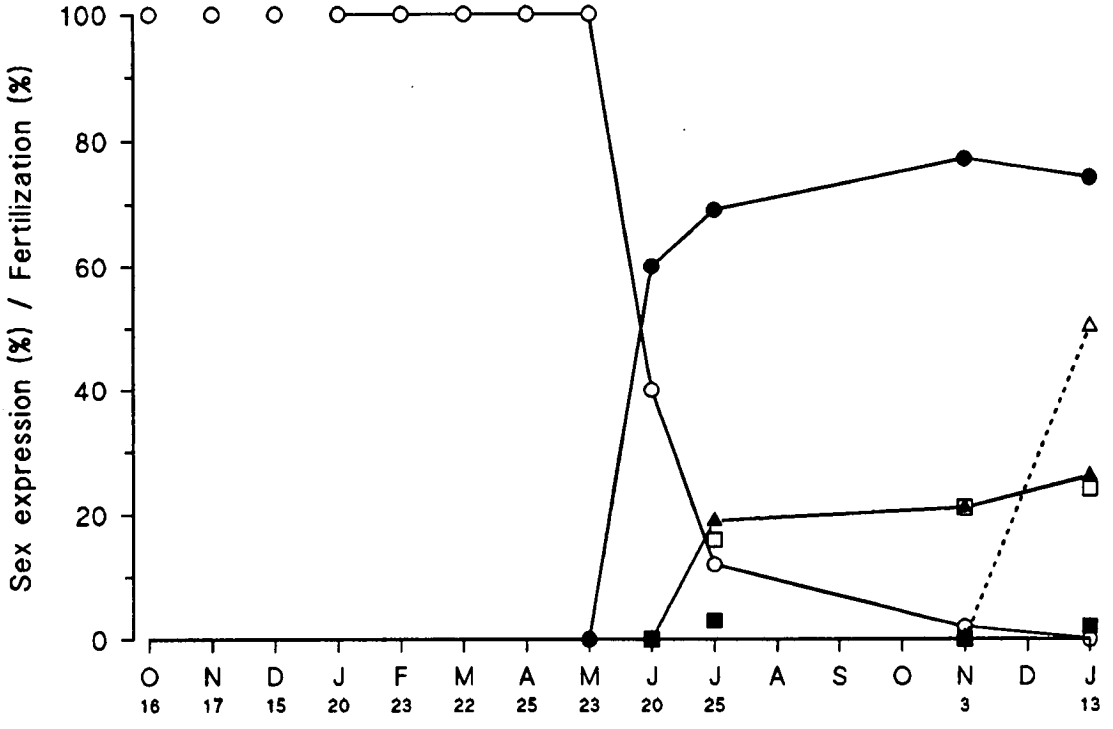
Figure 5.11b



October 1988 – January 1990
RG.1. B. SPICANT

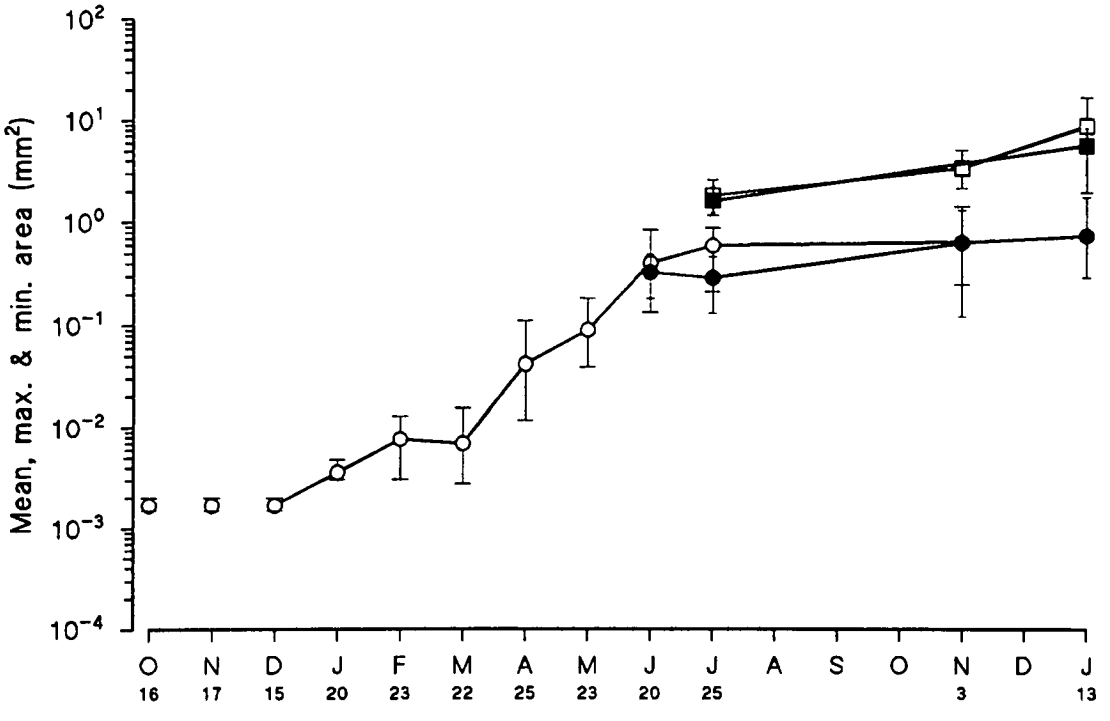
Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Figure 5.12a



October 1988 – January 1990
RG.2. B. SPICANT

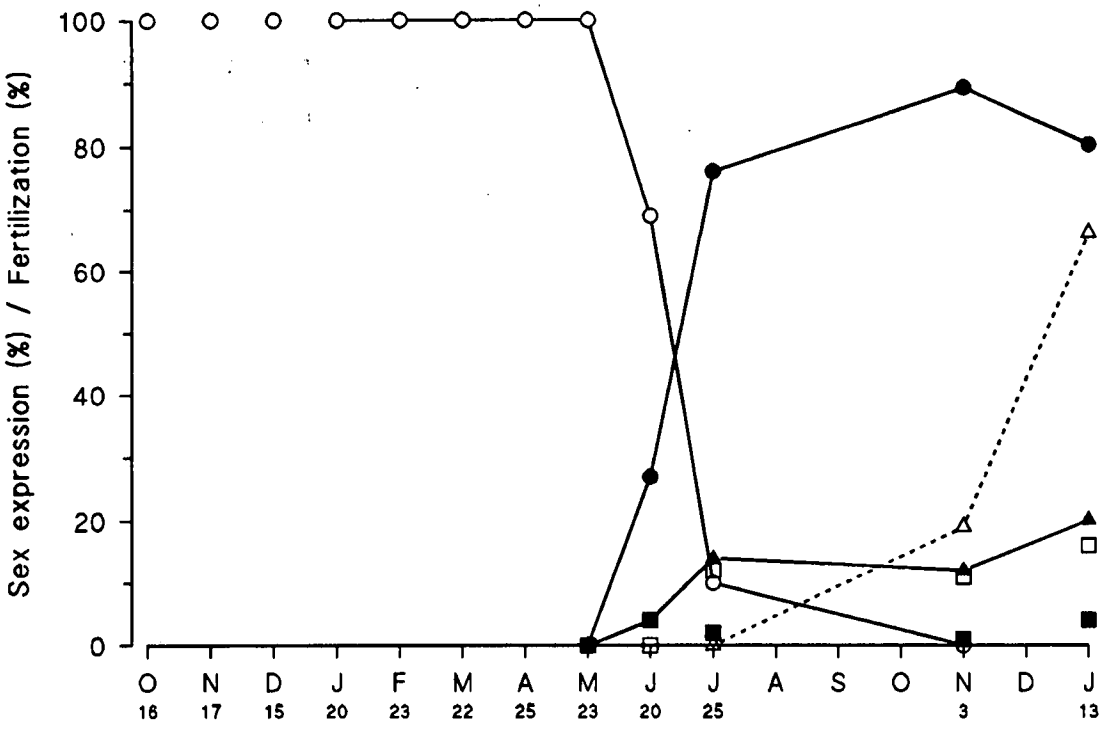
Figure 5.12b



October 1988 – January 1990
RG.2. B. SPICANT

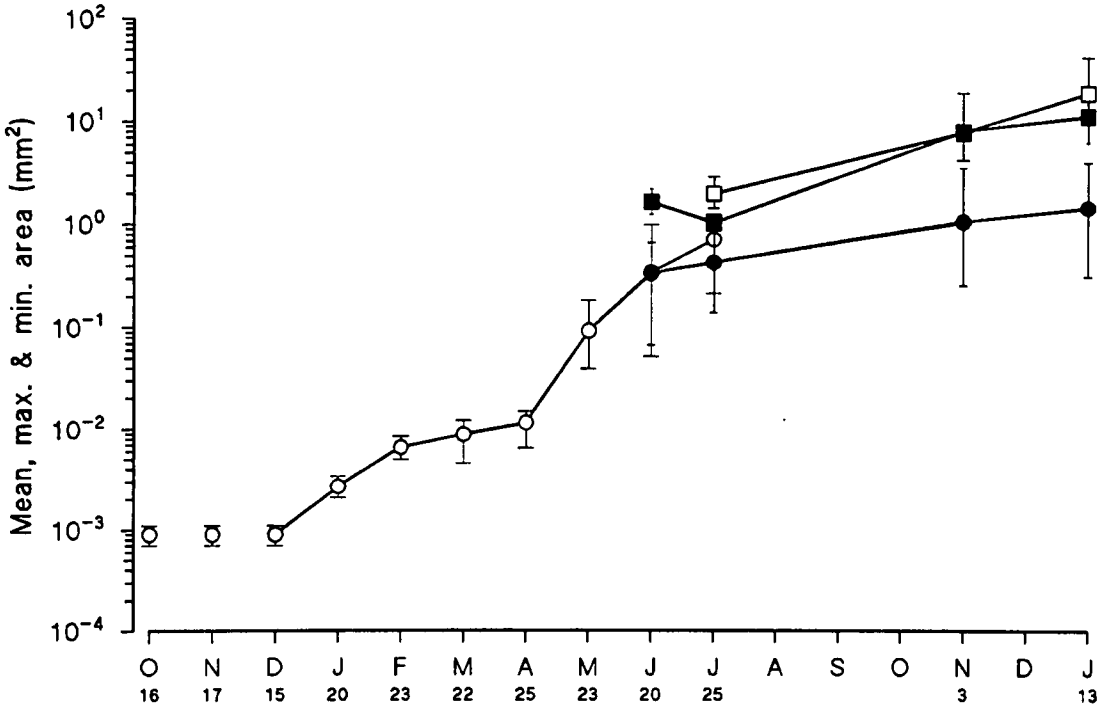
Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Figure 5.13a



October 1988 – January 1990
RG.1. P. SETIFERUM

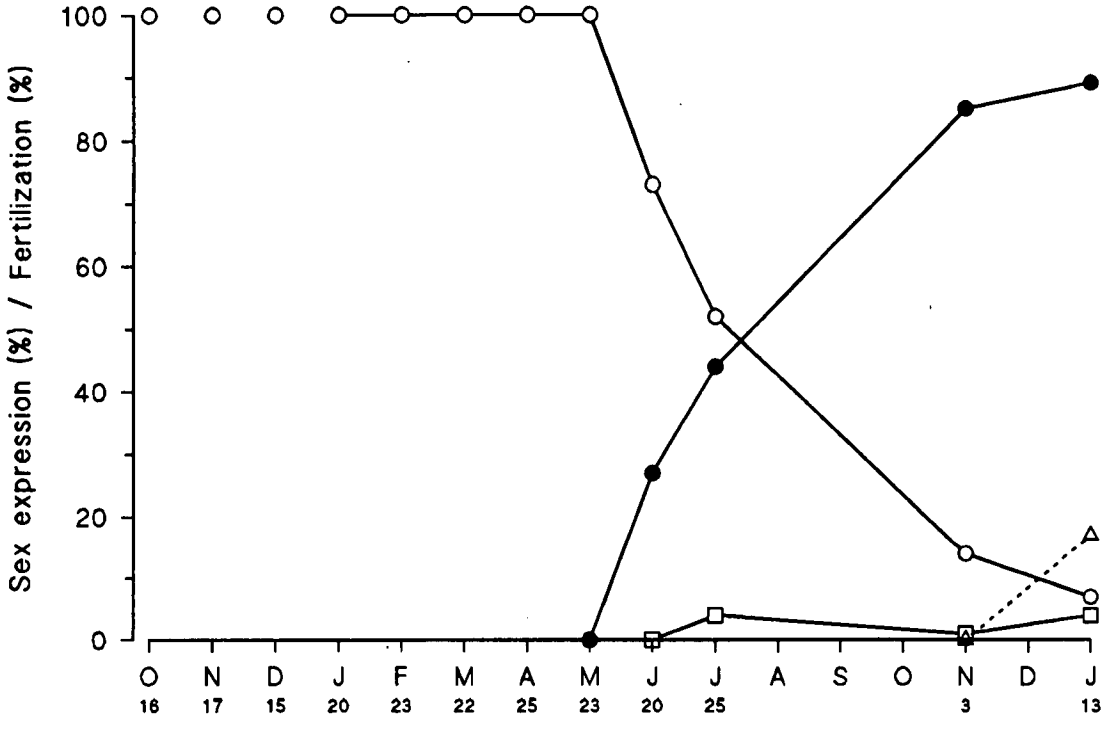
Figure 5.13b



October 1988 – January 1990
RG.1. P. SETIFERUM

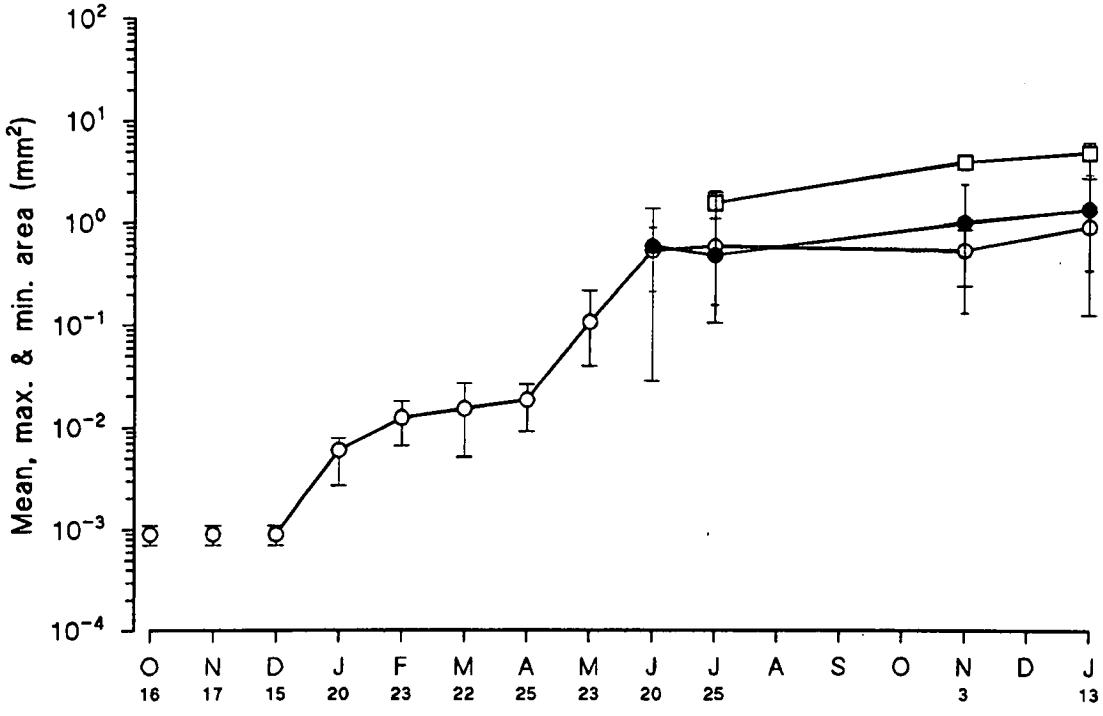
Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Figure 5.14a



October 1988 – January 1990
RG.2. P. SETIFERUM

Figure 5.14b



October 1988 – January 1990
RG.2. P. SETIFERUM

Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Figure 5.15a

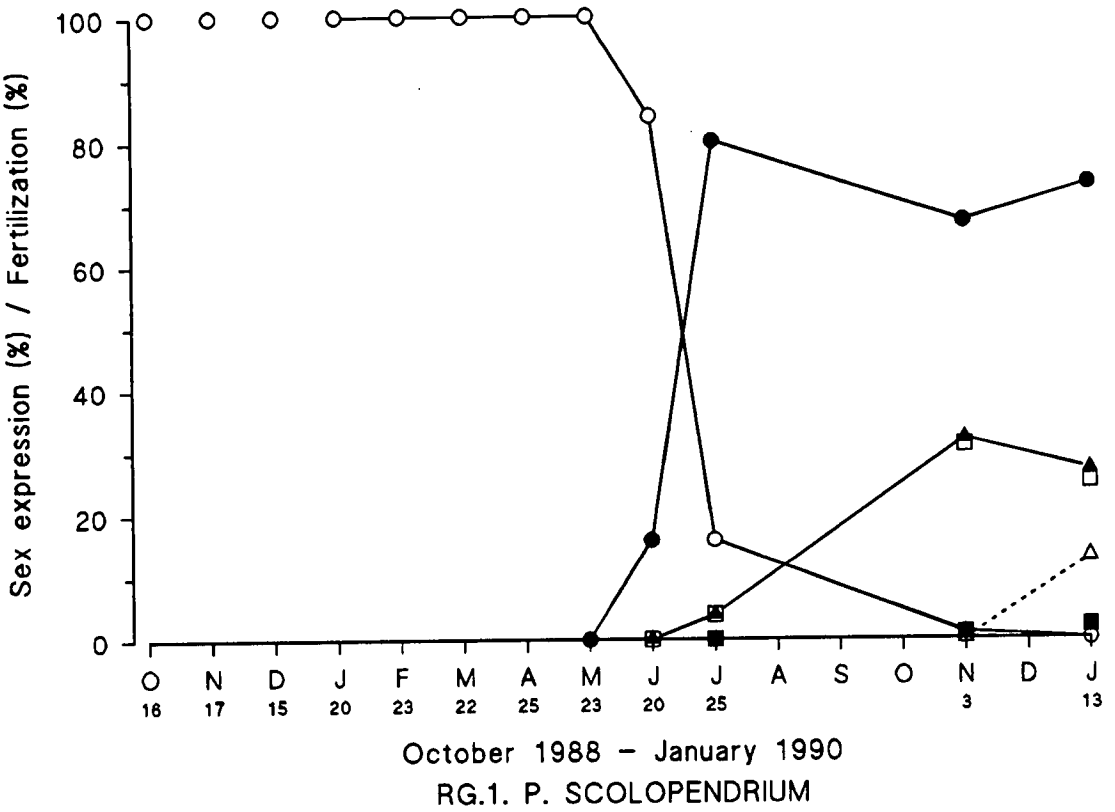
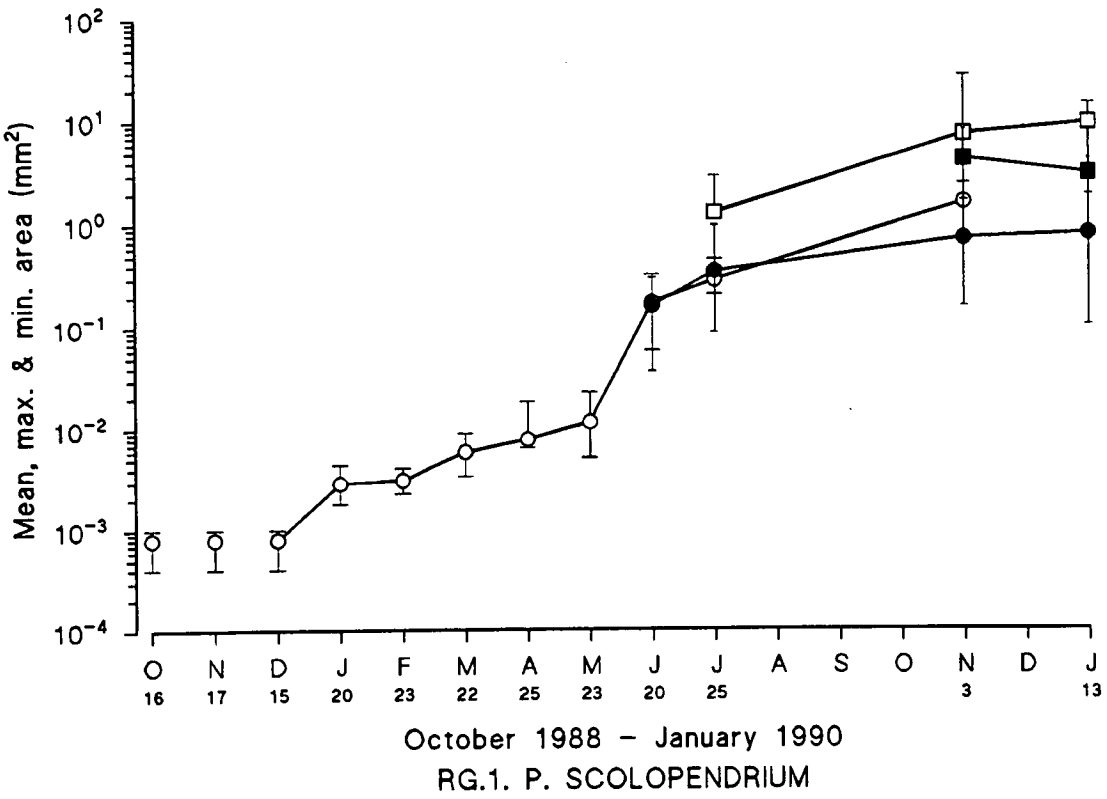
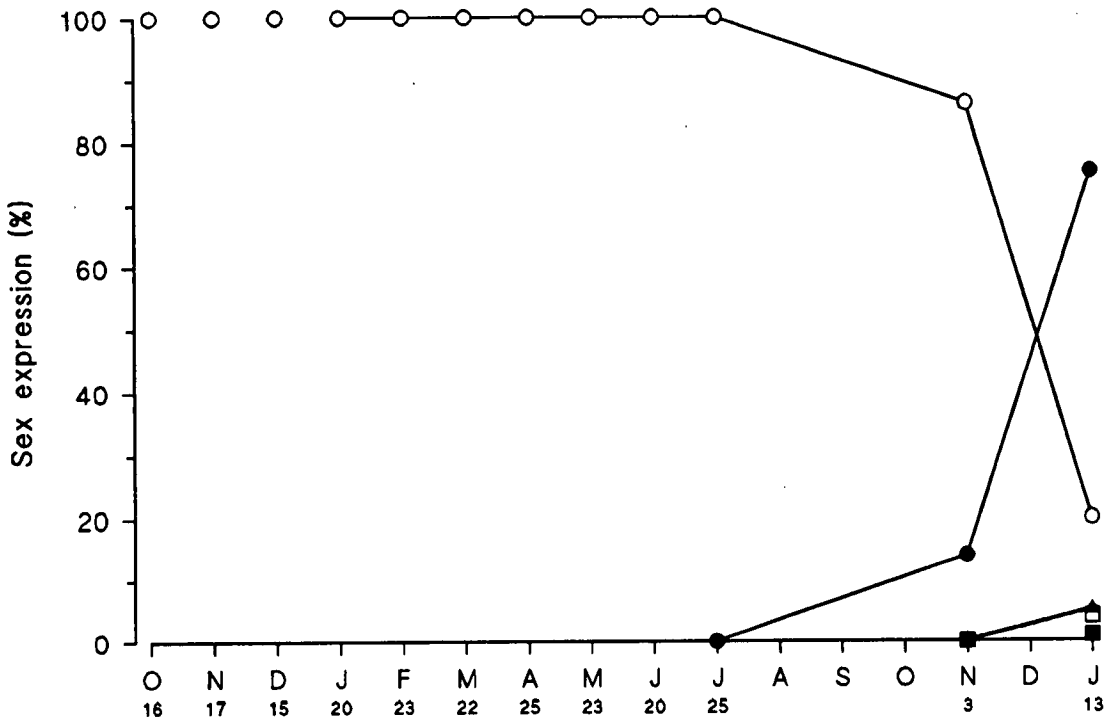


Figure 5.15b



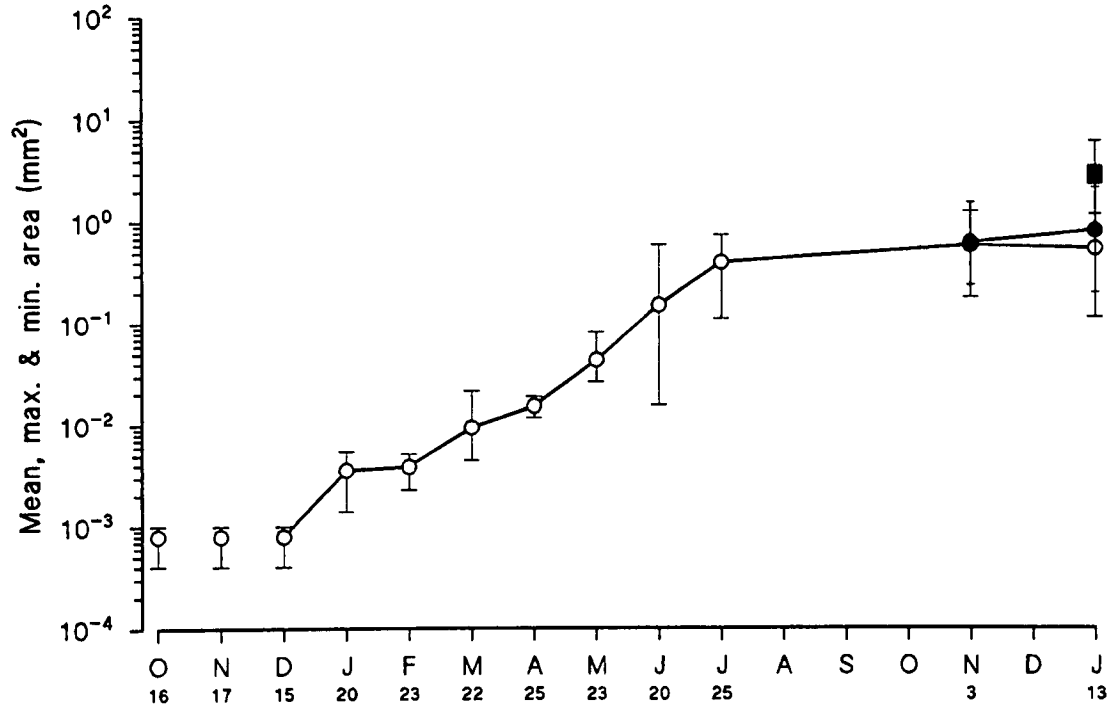
Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Figure 5.16a



October 1988 – January 1990
RG.2. P. SCOLOPENDRIUM

Figure 5.16b



October 1988 – January 1990
RG.2. P. SCOLOPENDRIUM

Chapter 6

Results of Experiment 3

Introduction

In the previous experiment, spores sown in October 1988, near the end of the main spore release season, did not germinate until the following year. Experiment 3 was initiated in August 1989 to discover if spores 'dispersed' (or 'exhumed') near the beginning of the main spore release season can develop into gametophytes before Winter.

Experiments 1 and 2 revealed no major differences between *A. filix-femina, B. spicant, P. setiferum* and *P. scolopendrium. D. affinis, D. dilatata, D. filix-mas* and *P. aquilinum* were incorporated into this experiment in a final attempt to discover if major differences exist between species. Their spore release periods are shown in Figure 3.1.

Pease Bridge Glen

The following text relates to Table 6.1 and Figures 6.1 - 6.16

Germination

Spores were sown in August 1989. No samples were collected in September but gametophytes of all 8 species were detected, in both plots, in October 1989. These gametophytes were much larger [mean areas: 0.03 mm^2 (sterile) - 1.49 mm^2 (female)] than the first gametophytes detected in Experiments 1 and 2, suggesting that germination had occurred soon after spore 'dispersal'. All the gametophytes were 2-dimensional and there was no evidence that they had developed from protonemata. There was also no evidence of staggered germination (Figures 6.1b - 6.16b). The numbers of gametophytes in the pots were not accurately determined but densities were estimated to range from approximately 100-300 gametophytes cm^{-2}.

Sex expression

In this experiment the pre-sexual, sexual transition, and equilibrium periods are not known.

All populations except those of *A. filix-femina* and *P. aquilinum* were pre-sexual throughout the experiment. Male gametophytes were detected in both populations of *A. filix-femina* in January 1990; all the antheridia were immature suggesting that they had only appeared recently. Both populations of *P. aquilinum* were sexual by October 1989; a few male gametophytes (with mature antheridia) were observed in *P. aquilinum* [2]; *P. aquilinum* [1] consisted entirely of male, female and bisexual gametophytes. This suggests that spores germinated soon after they were sown and that both populations were pre-sexual for only a few weeks. Almost all gametophytes in *P. aquilinum* [2] were sexual by January 1990.

The sexual composition of neither population of *P. aquilinum* had reached equilibrium by the end of the experiment. Nevertheless there were (and at equilibrium there would have been) approximately equal numbers of male and archegoniate gametophytes in *P. aquilinum* [1]. *P. aquilinum* [2] would have remained predominantly male.

Bisexual gametophytes were detected in both populations but most archegoniate gametophytes were female. All bisexual gametophytes were protandrous.

Size and shape

In general, female gametophytes were larger than bisexual gametophytes and all archegoniate gametophytes were considerably larger than male gametophytes. All archegoniate gametophytes were meristic (heart-shaped); all male gametophytes were ameristic (spathulate). The shape of pre-sexual gametophytes was not recorded. In both populations of *P. aquilinum* the first archegoniate gametophytes were considerably larger than the largest pre-sexual gametophytes in the sample 1 month before (Figures 6.15b - 6.16b).

Fertilization

A few embryos had developed in *P. aquilinum* [1] by January 1990; fertilization must have occurred at the end of Autumn 1989 and/or during the Winter of 1989/'90. Polyembryony was not observed.

Mortality

At the end of the experiment all gametophytes were healthy and intact; no gametophytes died during this experiment.

Maximum development

The largest gametophyte in both populations of *P. aquilinum* was female. The largest gametophyte observed at this site, during this experiment, was a female in *P. aquilinum* [1] which had an area of 5.69 mm^2. The most advanced sporophyte was an embryo.

Roslin Glen

The following text relates to Table 6.2 and Figures 6.17 - 6.32.

Germination

Spores were sown in August 1988. No samples were collected in September but gametophytes of all 8 species were detected, in both plots, in October 1989. These gametophytes were much larger [mean areas: 0.01 mm^2 (sterile) - 0.06 mm^2 (sterile)] than the first gametophytes detected in Experiments 1 and 2, suggesting that germination had occurred soon after spore 'dispersal'. All the gametophytes were 2-dimensional and there was no evidence that they had developed from protonemata. There was also no evidence of staggered germination (Figures 6.17b - 6.32b). The numbers of gametophyte in the pots were not accurately determined but densities were estimated to range from approximately 100-300 gametophytes cm^{-2}.

Sex expression

In this experiment the pre-sexual, sexual transition, and equilibrium periods are not known.

All populations except those of *P. aquilinum* were pre-sexual throughout the experiment. Male gametophytes (with mature antheridia) were first detected in both populations of *P. aquilinum* in January 1990 but note that no samples were collected in November and December 1989. No archegoniate gametophytes were observed in this experiment.

Shape

All male gametophytes were ameristic (spathulate). The shape of pre-sexual gametophytes was not recorded.

Fertilization

Not applicable.

Mortality

At the end of the experiment all gametophytes were healthy and intact; no gametophytes died during this experiment.

Maximum development

The largest gametophyte in both populations of *P. aquilinum* was male. The largest gametophyte observed at this site, during this experiment, was a pre-sexual in *A. filix-femina* [1] which had an area of 0.28 mm^{-2}.

Summary

The results for *A. filix-femina, B. spicant, P. setiferum, P. scolopendrium, D. affinis, D. dilatata* and *D. filix-mas* were strikingly similar; the results for *P. aquilinum* were strikingly different.

At both sites spores of all 8 species germinated soon after 'dispersal'. There was no staggered germination and no obvious protonemal phase. Gametophyte densities were lower than in the laboratory (see page 9).

Only gametophytes of *P. aquilinum* became sexual before Winter. Bisexual gametophytes were detected but most archegoniate gametophytes were female. All bisexual gametophytes were protandrous.

In general, female gametophytes were larger than bisexual gametophytes and all archegoniate gametophytes were considerably larger than male gametophytes. All archegoniate gametophytes were meristic; all male gametophytes were ameristic.

Fertilization occurred in one population of *P. aquilinum* at the end of Autumn 1989 and/or during the Winter of 1989/'90.

No gametophytes died during this experiment (*i.e.* all gametophytes survived for 5 months).

In terms of the rates of early (*i.e.* pre-sexual) gametophyte development, the rates at which populations became sexual and the rates of fertilization, all 8 species grew better at Pease Bridge Glen than at Roslin Glen.

Table 6.1. Results of Experiment 3 at Pease Bridge Glen. Shading indicates the species that were grown on alien soil.

Experiment 3 : Pease Bridge Glen	*A. filix-femina*		*B. spicant*		*P. setiferum*		*P. scolopendrium*	
Plot	PBG.1.	PBG.2.	PBG.1.	PBG.2.	PBG.1.	PBG.2.	PBG.1.	PBG.2.
See Figure	6.1	6.2	6.3	6.4	6.5	6.6	6.7	6.8
Spores sown	Aug.'89	Aug.'89	Aug.'89	Aug.'89	Aug.'89	Aug.'89	Aug.'89	Aug.'89
Germination	?	?	?	?	?	?	?	?
Pre-sexual period (months)	2	2	> 5	> 5	> 5	> 5	> 5	> 5
Size of last sterile gametophytes (mm^2)	> 0.55	> 0.37	> 0.21	> 0.13	> 0.23	> 0.35	> 0.20	> 0.25
First male gametophytes	Jan.'90	Jan.'90	-	-	-	-	-	-
Size of first male gametophytes (mm^2)	0.47	0.28	-	-	-	-	-	-
First female gametophytes	-	-	-	-	-	-	-	-
Size of first female gametophytes (mm^2)	-	-	-	-	-	-	-	-
First bisexual gametophytes	-	-	-	-	-	-	-	-
Size of first bisexual gametophytes (mm^2)	-	-	-	-	-	-	-	-
All gametophytes sexual by	-	-	-	-	-	-	-	-
Transition period (months)	-	-	-	-	-	-	-	-
Equilibrium period (months)	-	-	-	-	-	-	-	-
% of population male at equilibrium	-	-	-	-	-	-	-	-
% of population archegoniate at equilibrium	-	-	-	-	-	-	-	-
First embryo	-	-	-	-	-	-	-	-
% of archegoniates fertilized by Jan.'90	-	-	-	-	-	-	-	-
Largest gametophyte by Jan.'90 (mm^2) (sex)	1.46 p-s	0.69 p-s	0.43 p-s	0.25 p-s	0.35 p-s	0.74 p-s	0.32 p-s	0.46 p-s
Most advanced sporeling by Jan.'90 (leaves)	-	-	-	-	-	-	-	-

Table 6.1. (Continued).

Experiment 3 : Pease Bridge Glen	*D. affinis*		*D. dilatata*		*D. filix-mas*		*P. aquilinum*	
Plot	PBG.1.	PBG.2.	PBG.1.	PBG.2.	PBG.1.	PBG.2.	PBG.1.	PBG.2.
See Figure	6.9	6.10	6.11	6.12	6.13	6.14	6.15	6.16
Spores sown	Aug.'89	Aug.'89	Aug.'89	Aug.'89	Aug.'89	Aug.'89	Aug.'89	Aug.'89
Germination	?	?	?	?	?	?	?	?
Pre-sexual period (months)	> 5	> 5	> 5	> 5	> 5	> 5	< 1	< 1
Size of last sterile gametophytes (mm^2)	> 0.47	> 0.42	> 0.42	> 0.16	> 0.45	> 0.18	< 0.38	> 0.21
First male gametophytes	-	-	-	-	-	-	Oct.'89	Oct.'89
Size of first male gametophytes (mm^2)	-	-	-	-	-	-	0.38	0.05
First female gametophytes	-	-	-	-	-	-	Oct.'89	Jan.'90
Size of first female gametophytes (mm^2)	-	-	-	-	-	-	1.49	1.13
First bisexual gametophytes	-	-	-	-	-	-	Oct.'89	Jan.'90
Size of first bisexual gametophytes (mm^2)	-	-	-	-	-	-	1.43	0.83
All gametophytes sexual by	-	-	-	-	-	-	Oct.'89	Feb.'90?
Transition period (months)	-	-	-	-	-	-	< 2	> 4
Equilibrium period (months)	-	-	-	-	-	-	?	?
% of population male at equilibrium	-	-	-	-	-	-	*c.*55	*c.*90
% of population archegoniate at equilibrium	-	-	-	-	-	-	*c.*45	*c.*10
First embryo	-	-	-	-	-	-	Jan.'90	-
% of archegoniates fertilized by Jan.'90	-	-	-	-	-	-	*c.*15	0
Largest gametophyte by Jan.'90 (mm^2) (sex)	0.99 p-s	0.81 p-s	1.00 p-s	0.37 p-s	0.96 p-s	0.50 p-s	5.69 ♀	1.67 ♀
Most advanced sporeling by Jan.'90 (leaves)	-	-	-	-	-	-	Embryo	-

Table 6.2. Results of Experiment 3 at Roslin Glen. Shading indicates the species that were grown on alien soil.

Experiment 3 : Roslin Glen	*A. filix-femina*		*B. spicant*		*P. setiferum*		*P. scolopendrium*	
Plot	RG.1.	RG.2.	RG.1.	RG.2.	RG.1	RG.2.	RG.1	RG.2.
See Figure	6.17	6.18	6.19	6.20	6.21	6.22	6.23	6.24
Spores sown	Aug.'89	Aug.'89	Aug.'89	Aug.'89	Aug.'89	Aug.'89	Aug.'89	Aug.'89
Germination	?	?	?	?	?	?	?	?
Pre-sexual period (months)	> 5	> 5	> 5	> 5	> 5	> 5	> 5	> 5
Size of last sterile gametophytes (mm^2)	> 0.13	> 0.18	> 0.06	> 0.11	> 0.15	> 0.11	> 0.08	> 0.12
First male gametophytes	-	-	-	-	-	-	-	-
Size of first male gametophytes (mm^2)	-	-	-	-	-	-	-	-
First female gametophytes	-	-	-	-	-	-	-	-
Size of first female gametophytes (mm^2)	-	-	-	-	-	-	-	-
First bisexual gametophytes	-	-	-	-	-	-	-	-
Size of first bisexual gametophytes (mm^2)	-	-	-	-	-	-	-	-
All gametophytes sexual by	-	-	-	-	-	-	-	-
Transition period (months)	-	-	-	-	-	-	-	-
Equilibrium period (months)	-	-	-	-	-	-	-	-
% of population male at equilibrium	-	-	-	-	-	-	-	-
% of population archegoniate at equilibrium	-	-	-	-	-	-	-	-
First embryo	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-
% of archegoniates fertilized by Jan.'90	-	-	-	-	-	-	-	-
Largest gametophyte by Jan.'90 (mm^2) (sex)	0.28 p-s	0.27 p-s	0.15 p-s	0.23 p-s	0.21 p-s	0.14 p-s	0.11 p-s	0.16 p-s
Most advanced sporeling by Jan.'90 (leaves)	-	-	-	-	-	-	-	-

Table 6.2. (Continued).

Experiment 3 : Roslin Glen	*D. affinis*		*D. dilatata*		*D. filix-mas*		*P. aquilinum*	
Plot	RG.1.	RG.2.	RG.1.	RG.2.	RG.1.	RG.2.	RG.1.	RG.2.
See Figure	6.25	6.26	6.27	6.28	6.29	6.30	6.31	6.32
Spores sown	Aug.'89	Aug.'89	Aug.'89	Aug.'89	Aug.'89	Aug.'89	Aug.'89	Aug.'89
Germination	?	?	?	?	?	?	?	?
Pre-sexual period (months)	> 5	> 5	> 5	> 5	> 5	> 5	*c.*2	*c.*2
Size of last sterile gametophytes (mm^2)	> 0.12	> 0.19	> 0.10	> 0.13	> 0.20	> 0.15	> 0.12	> 0.08
First male gametophytes	-	-	-	-	-	-	Jan.'90	Jan.'90
Size of first male gametophytes (mm^2)	-	-	-	-	-	-	0.11	0.10
First female gametophytes	-	-	-	-	-	-	-	-
Size of first female gametophytes (mm^2)	-	-	-	-	-	-	-	-
First bisexual gametophytes	-	-	-	-	-	-	-	-
Size of first bisexual gametophytes (mm^2)	-	-	-	-	-	-	-	-
All gametophytes sexual by	-	-	-	-	-	-	-	-
Transition period (months)	-	-	-	-	-	-	-	-
Equilibrium period (months)	-	-	-	-	-	-	-	-
% of population male at equilibrium	-	-	-	-	-	-	-	-
% of population archegoniate at equilibrium	-	-	-	-	-	-	-	-
First embryo	-	-	-	-	-	-	-	-
% of archegoniates fertilized by Jan.'90	-	-	-	-	-	-	-	-
Largest gametophyte by Jan.'90 (mm^2) (sex)	0.16 p-s	0.26 p-s	0.23 p-s	0.19 p-s	0.26 p-s	0.22 p-s	0.22 ♂	0.15 ♂
Most advanced sporeling by Jan.'90 (leaves)	-	-	-	-	-	-	-	-

Figures 6.1 - 6.32. Sex expression, fertilization and size of gametophytes in each population during Experiment 3. Note:

1. Sampling dates are shown below the x-axes.

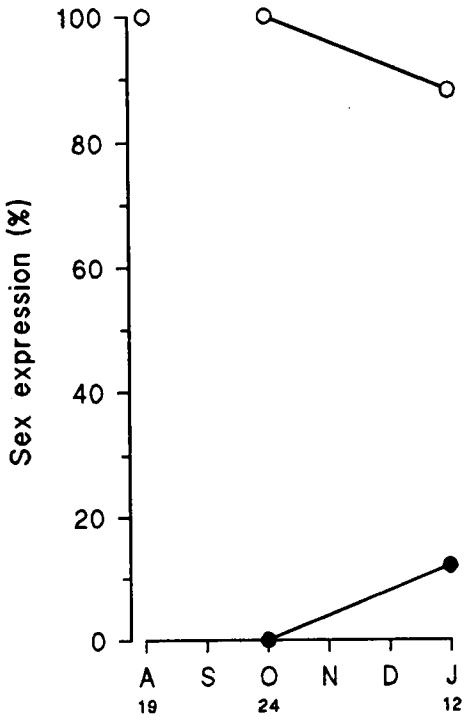
2. Symbols: spores or pre-sexual gametophytes (○), male gametophytes ( ), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

3. It has been necessary to use a log scale on the y-axes of Figures 6.1b - 6.32b to accommodate the largest and smallest values. Log scales are somewhat misleading for larger values; big differences in area can be represented by small differences in the position of symbols. Range bars: spores or pre-sexual gametophytes (2.0 mm), male gametophytes(1.0 mm), female gametophytes (1.5 mm) and bisexual gametophytes (0.5 mm).

4. Identification of sterile, male, and archegoniate gametophytes was easy and the values presented for these are accurate. Sub-dividing archegoniate gametophytes into 'females' and 'bisexuals' was more difficult because soil particles adhering to the rhizoids often obscured the rhizoid region where antheridia are usually located. Consequently, some gametophytes scored as female might have been bisexual and the values presented for these sex categories should probably be regarded as overestimates and underestimates respectively.

Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

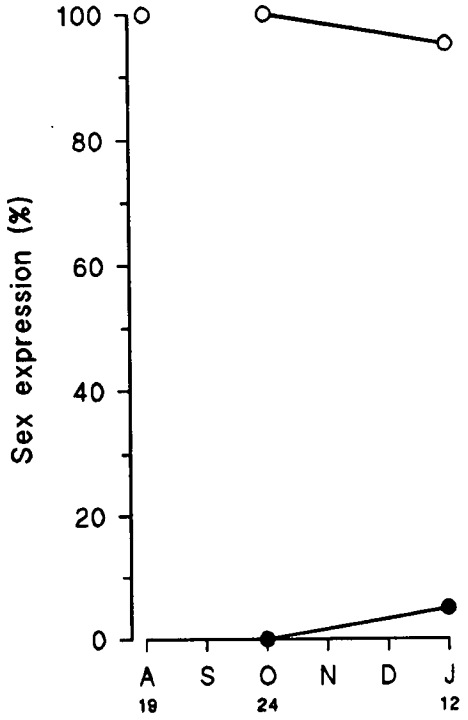
Fig. 6.1a



Aug.'89 – Jan.'90

PBG.1. A. FILIX-FEMINA

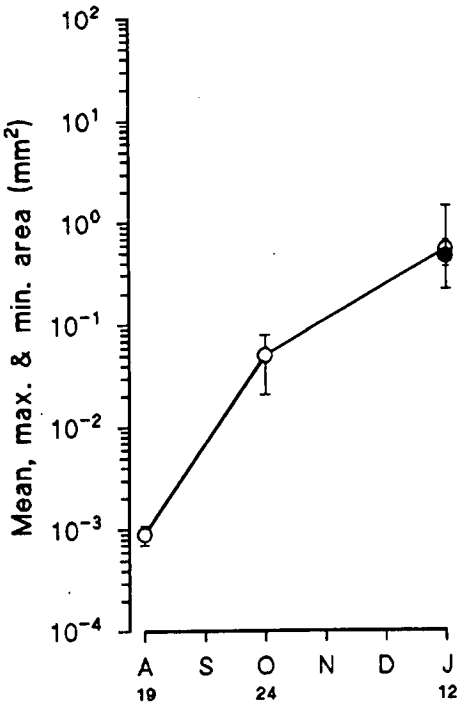
Fig. 6.2a



Aug.'89 – Jan.'90

PBG.2. A. FILIX-FEMINA

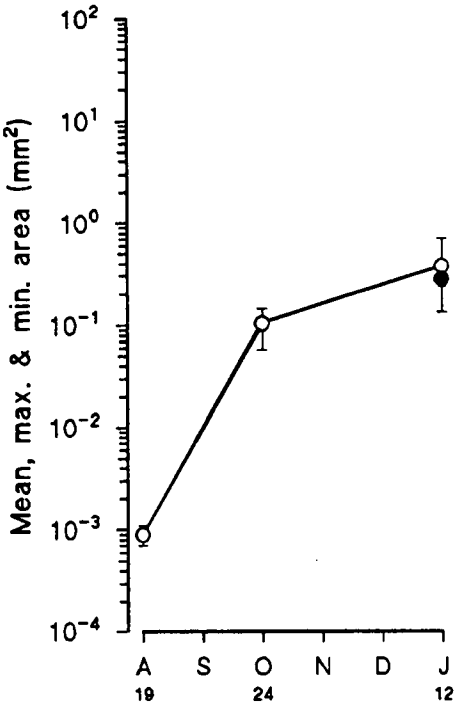
Fig. 6.1b



Aug.'89 – Jan.'90

PBG.1. A. FILIX-FEMINA

Fig. 6.2b

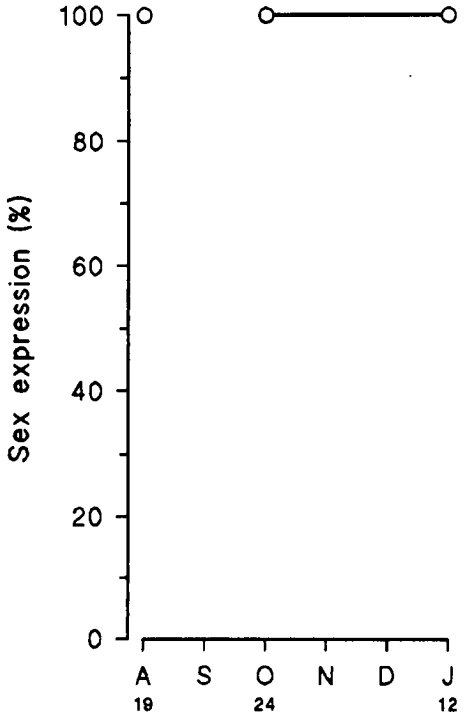


Aug.'89 – Jan.'90

PBG.2. A. FILIX-FEMINA

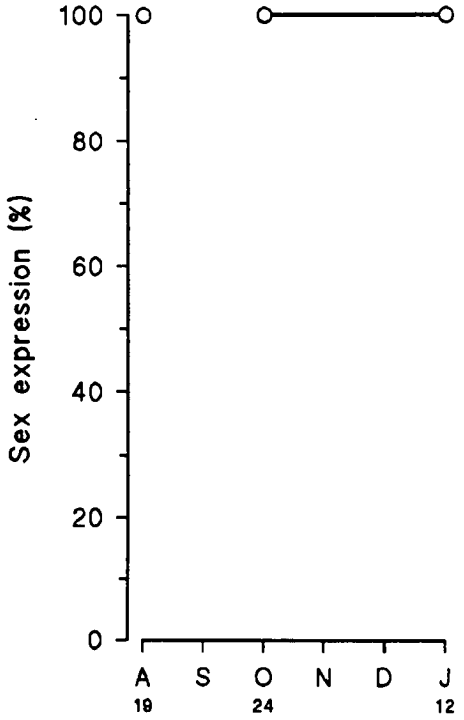
Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Fig. 6.3a



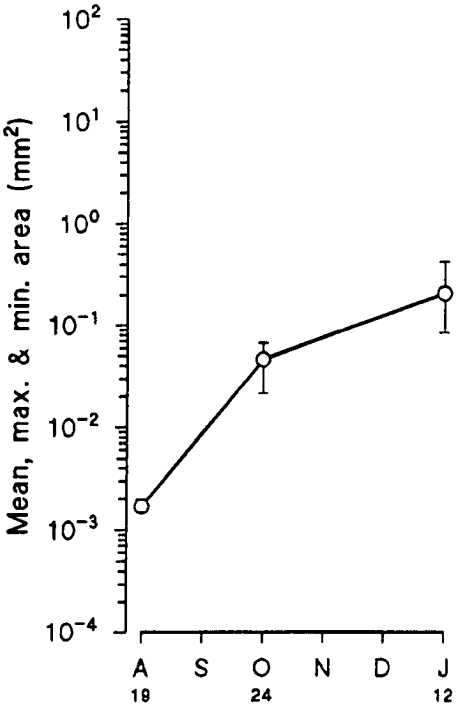
Aug.'89 – Jan.'90
PBG.1. B. SPICANT

Fig. 6.4a



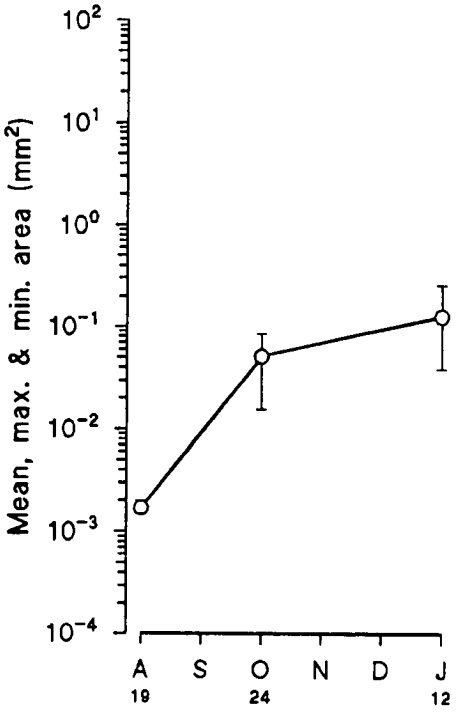
Aug.'89 – Jan.'90
PBG.2. B. SPICANT

Fig. 6.3b



Aug.'89 – Jan.'90
PBG.1. B. SPICANT

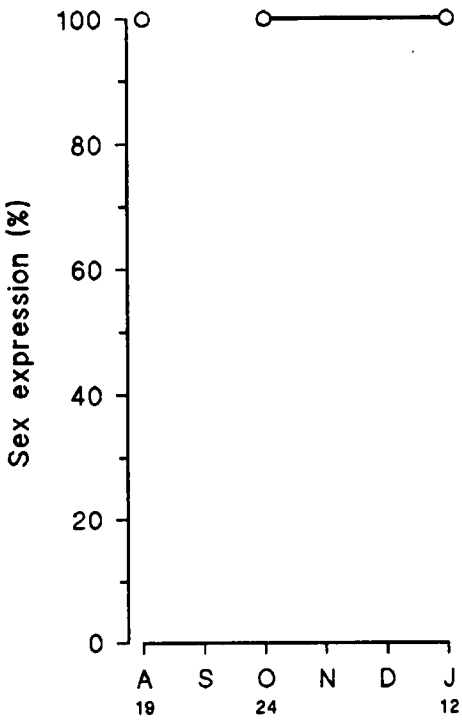
Fig. 6.4b



Aug.'89 – Jan.'90
PBG.2. B. SPICANT

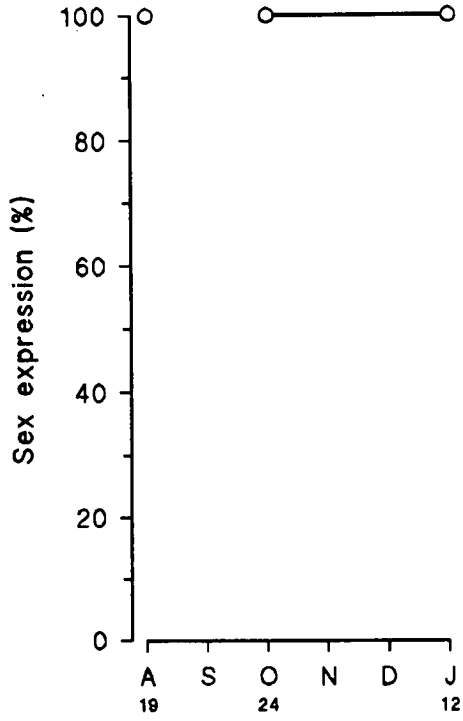
Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Fig. 6.5a



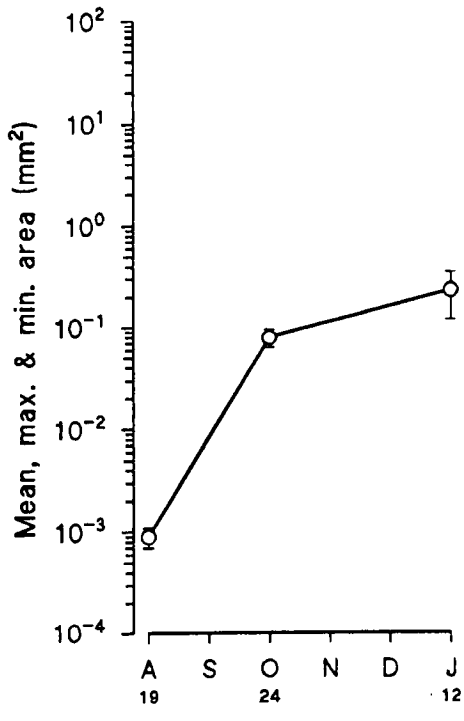
Aug.'89 – Jan.'90
PBG.1. P. SETIFERUM

Fig. 6.6a



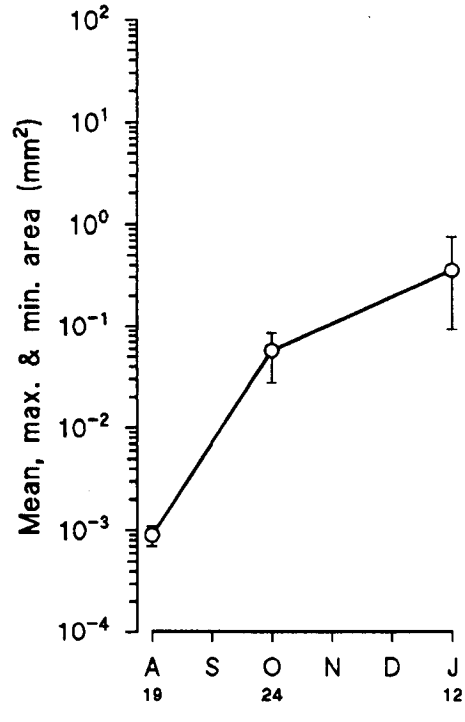
Aug.'89 – Jan.'90
PBG.2. P. SETIFERUM

Fig. 6.5b



Aug.'89 – Jan.'90
PBG.1. P. SETIFERUM

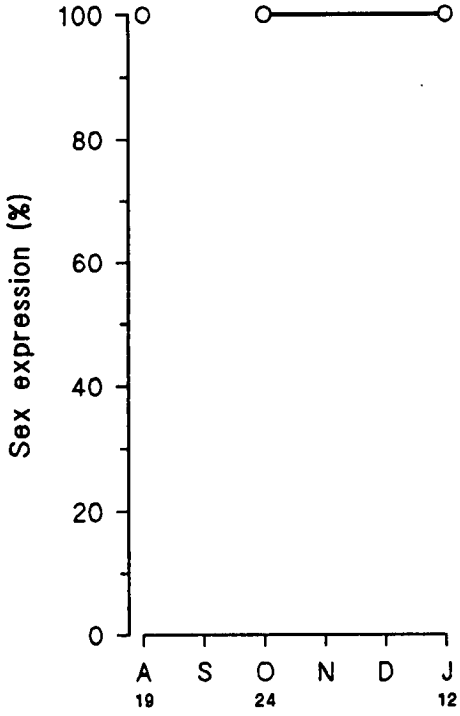
Fig. 6.6b



Aug.'89 – Jan.'90
PBG.2. P. SETIFERUM

Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

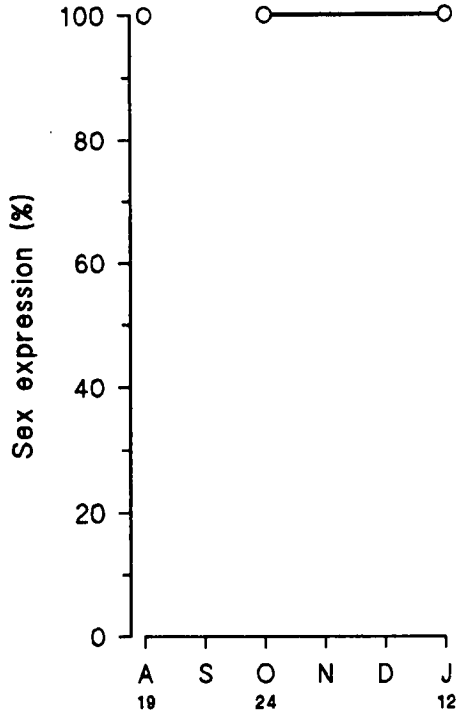
Fig. 6.7a



Aug.'89 – Jan.'90

PBG.1. P. SCOLOPENDRIUM

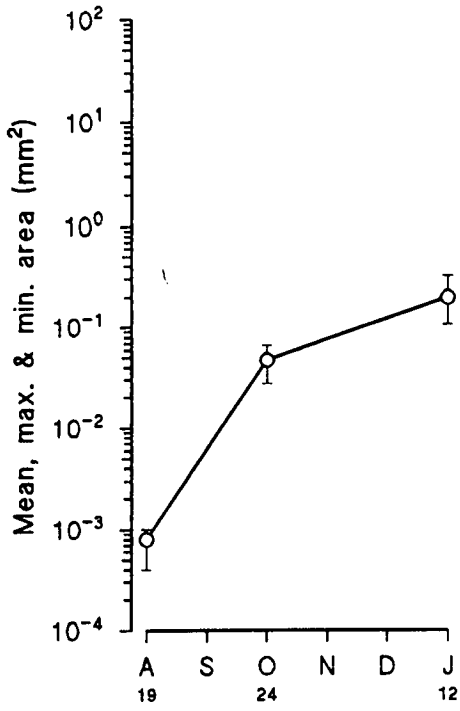
Fig. 6.8a



Aug.'89 – Jan.'90

PBG.2. P. SCOLOPENDRIUM

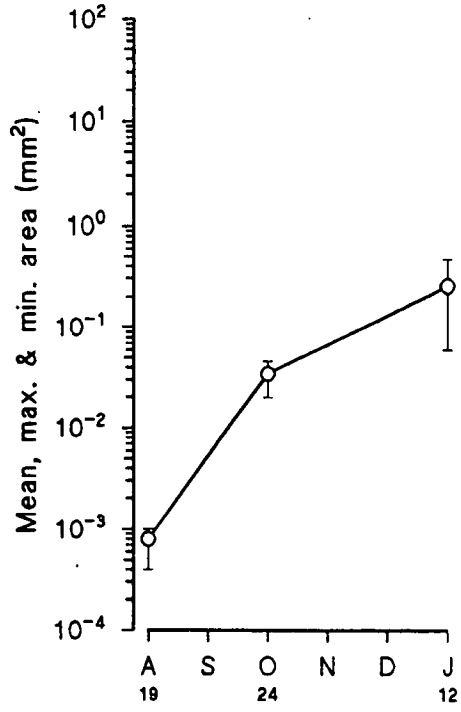
Fig. 6.7b



Aug.'89 – Jan.'90

PBG.1. P. SCOLOPENDRIUM

Fig. 6.8b

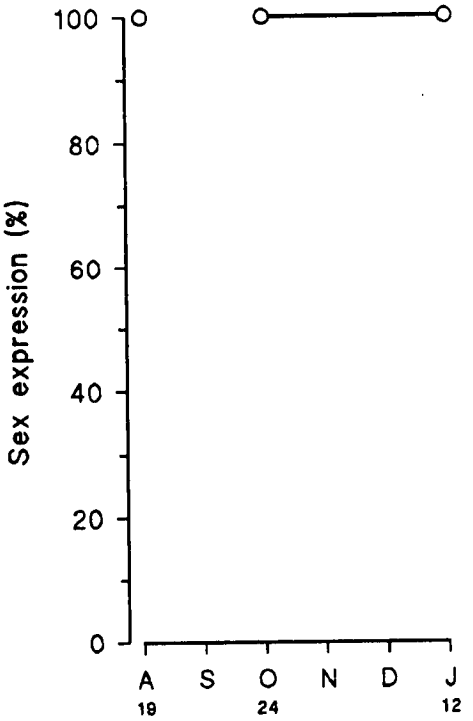


Aug.'89 – Jan.'90

PBG.2. P. SCOLOPENDRIUM

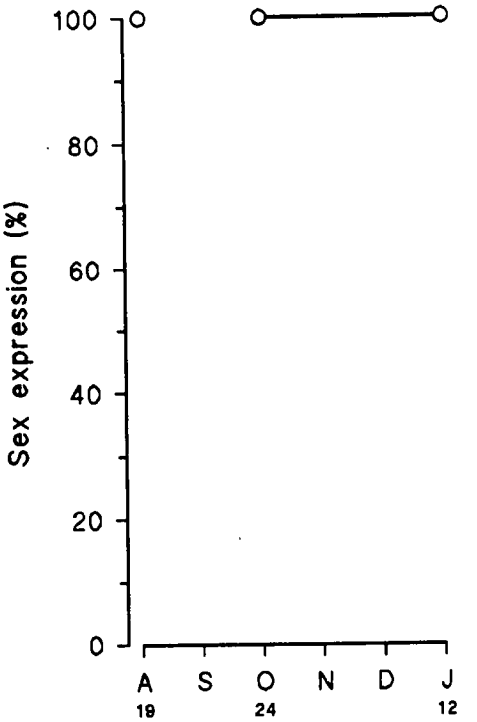
Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Fig. 6.9a



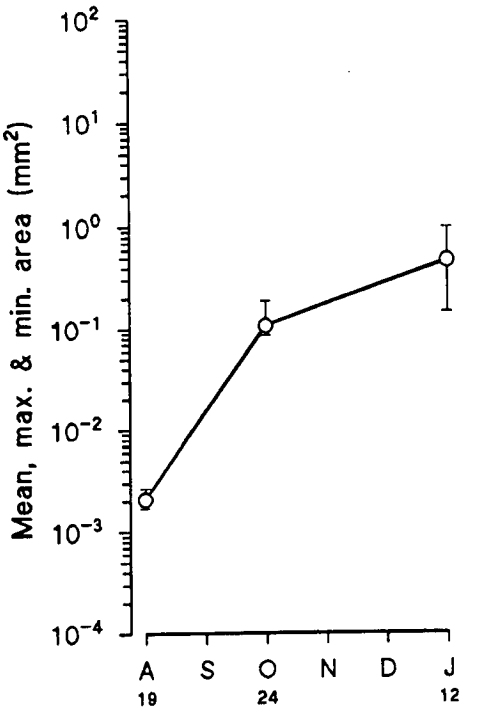
Aug.'89 – Jan.'90
PBG.1. D. AFFINIS

Fig. 6.10a



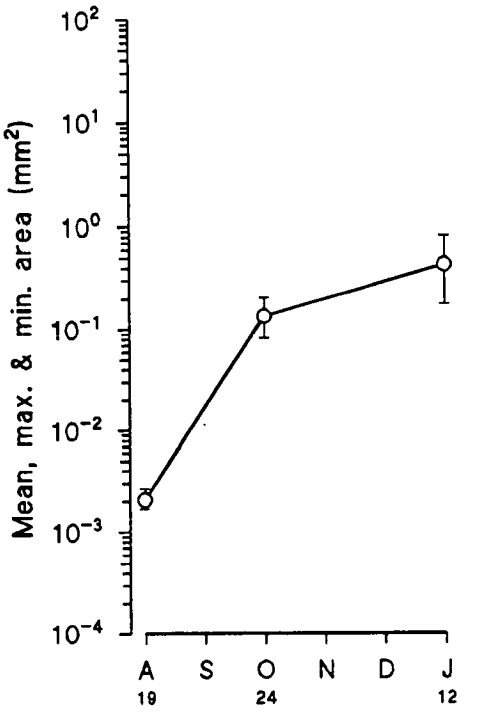
Aug.'89 – Jan.'90
PBG.2. D. AFFINIS

Fig. 6.9b



Aug.'89 – Jan.'90
PBG.1. D. AFFINIS

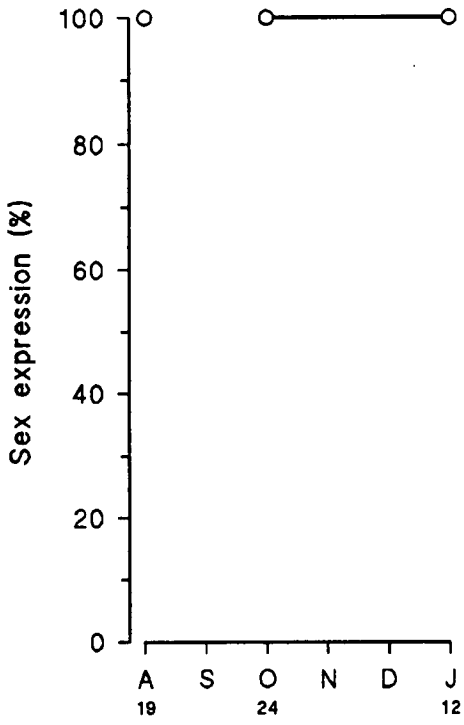
Fig. 6.10b



Aug.'89 – Jan.'90
PBG.2. D. AFFINIS

Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

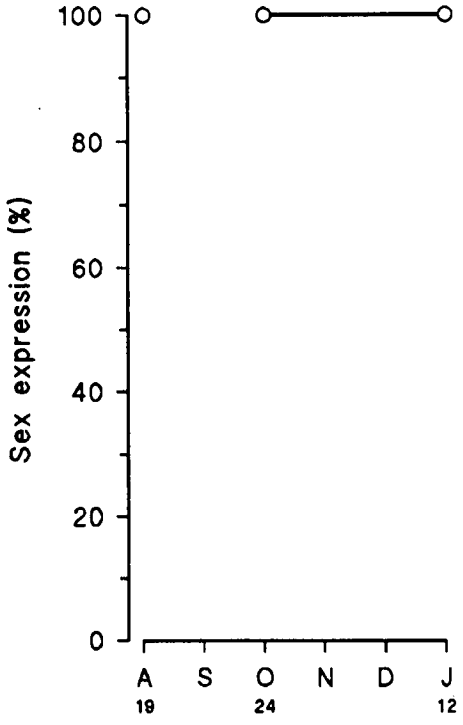
Fig. 6.11a



Aug.'89 – Jan.'90

PBG.1. D. DILATATA

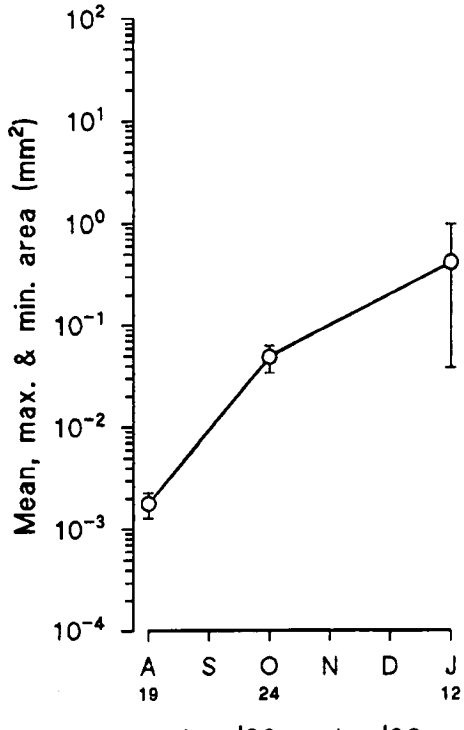
Fig. 6.12a



Aug.'89 – Jan.'90

PBG.2. D. DILATATA

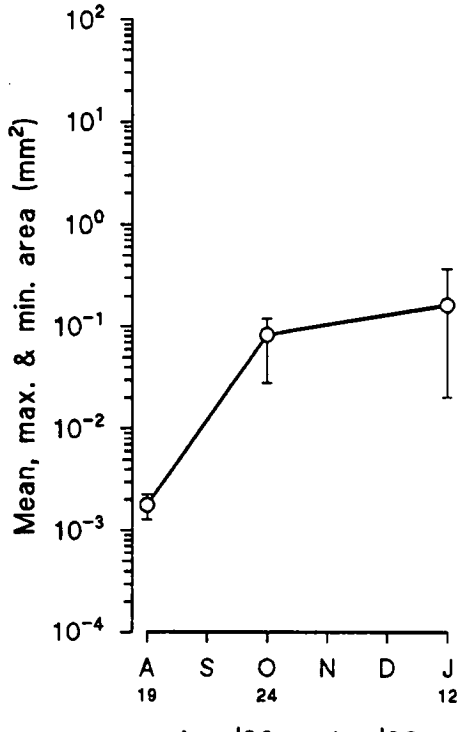
Fig. 6.11b



Aug.'89 – Jan.'90

PBG.1. D. DILATATA

Fig. 6.12b

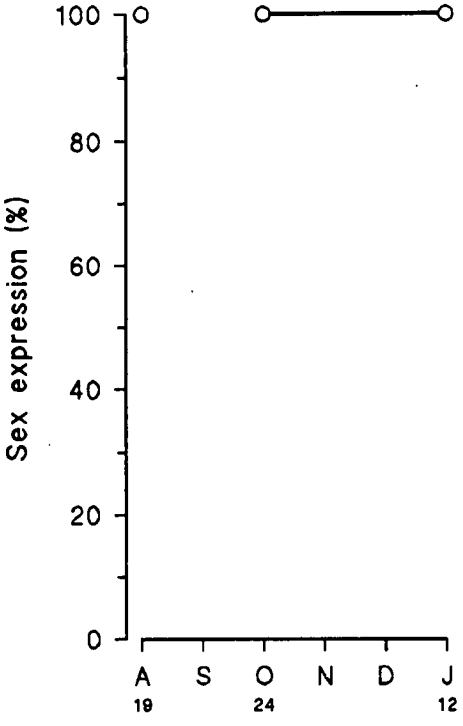


Aug.'89 – Jan.'90

PBG.2. D. DILATATA

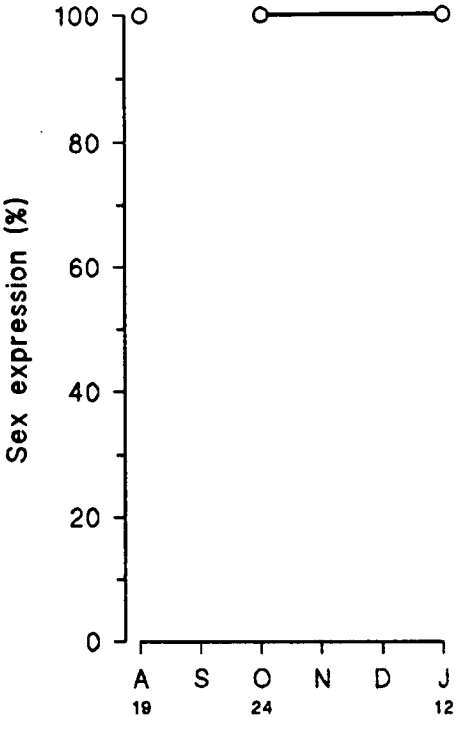
Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Fig. 6.13a



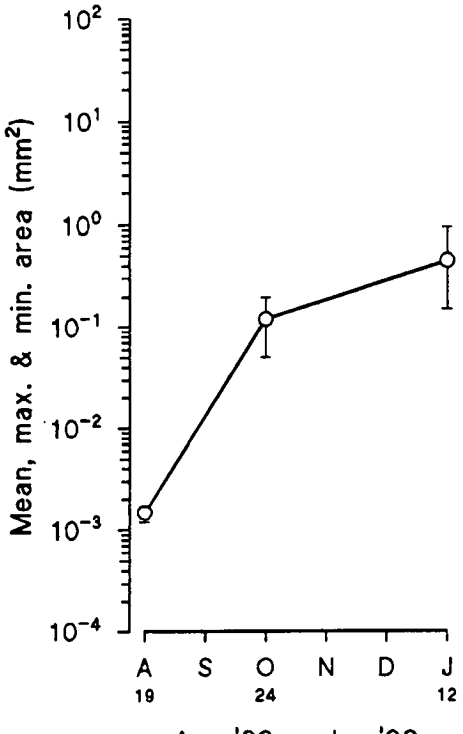
Aug.'89 – Jan.'90
PBG.1. D. FILIX–MAS

Fig. 6.14a



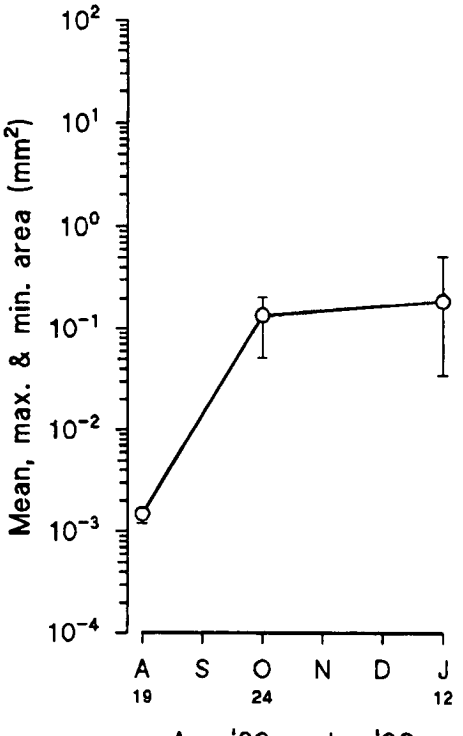
Aug.'89 – Jan.'90
PBG.2. D. FILIX–MAS

Fig. 6.13b



Aug.'89 – Jan.'90
PBG.1. D. FILIX–MAS

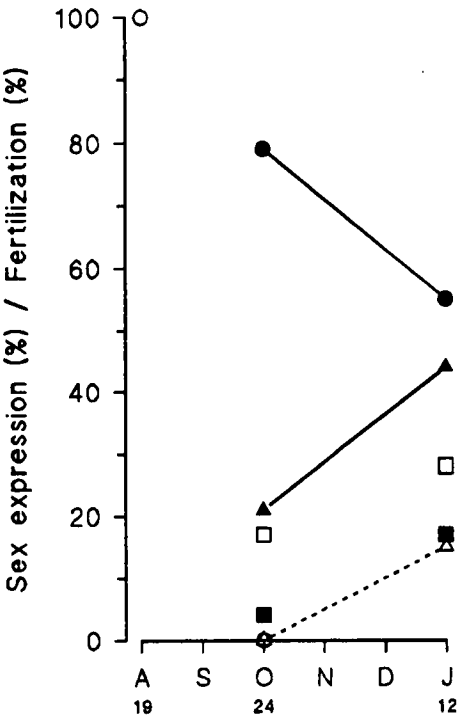
Fig. 6.14b



Aug.'89 – Jan.'90
PBG.2. D. FILIX–MAS

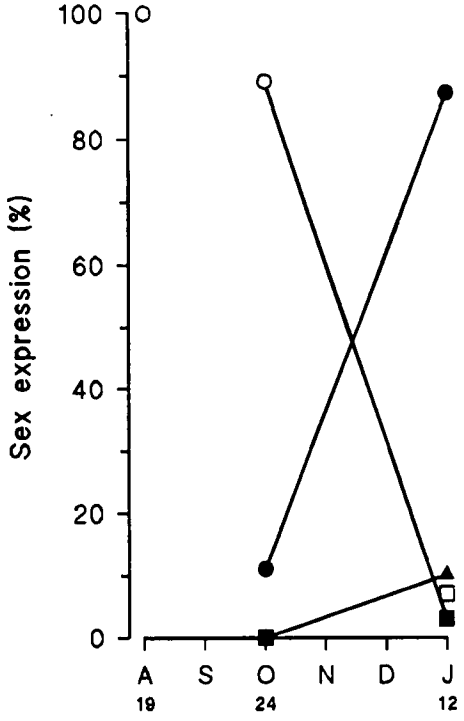
Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Fig. 6.15a



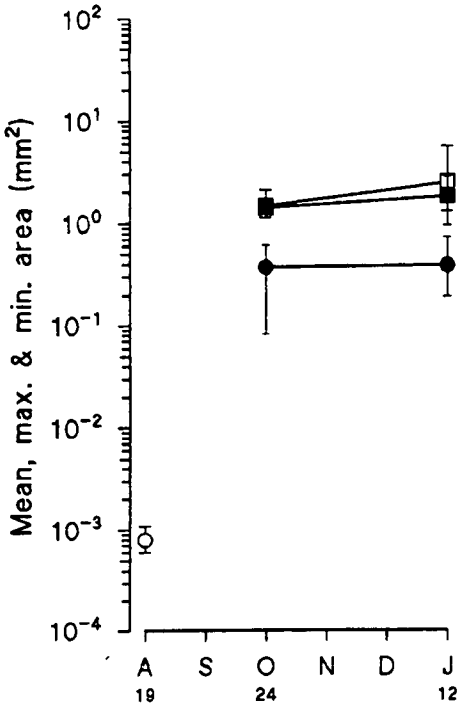
Aug.'89 – Jan.'90
PBG.1. P. AQUILINUM

Fig. 6.16a



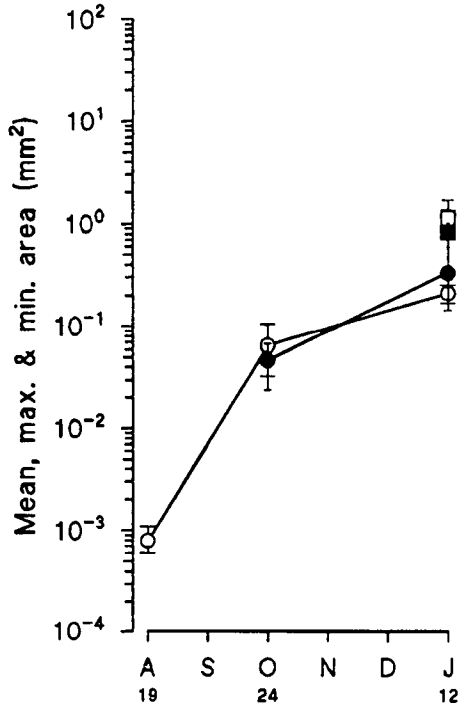
Aug.'89 – Jan.'90
PBG.2. P. AQUILINUM

Fig. 6.15b



Aug.'89 – Jan.'90
PBG.1. P. AQUILINUM

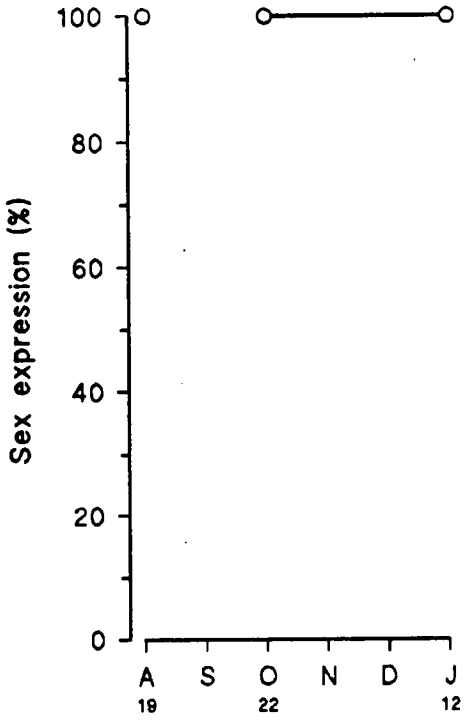
Fig. 6.16b



Aug.'89 – Jan.'90
PBG.2. P. AQUILINUM

Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

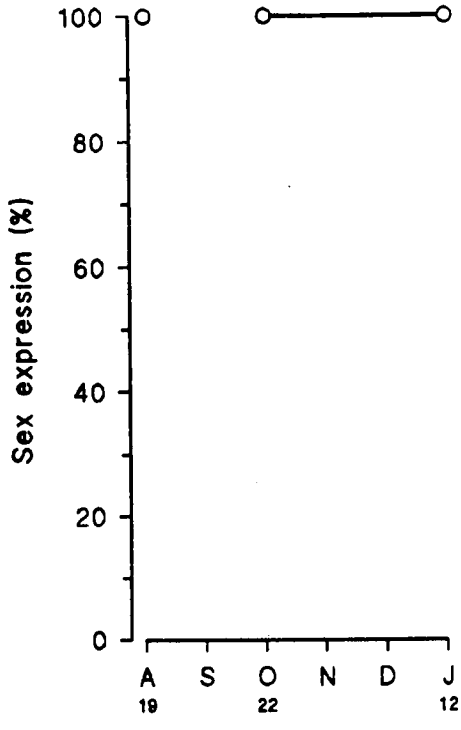
Fig. 6.17a



Aug.'89 – Jan.'90

RG.1. A. FILIX-FEMINA

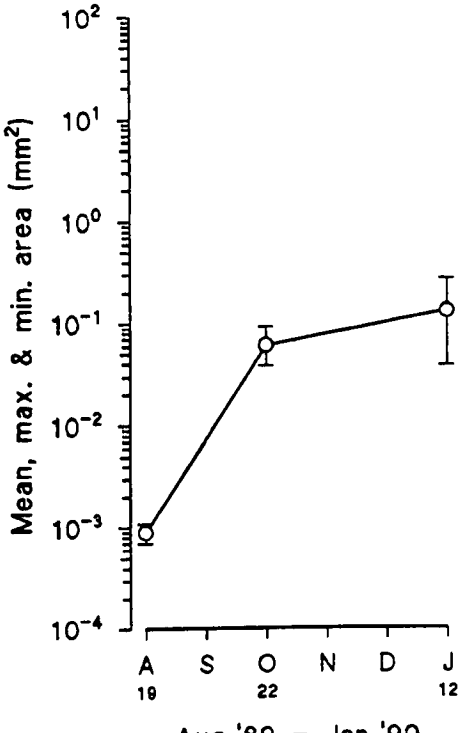
Fig. 6.18a



Aug.'89 – Jan.'90

RG.2. A. FILIX-FEMINA

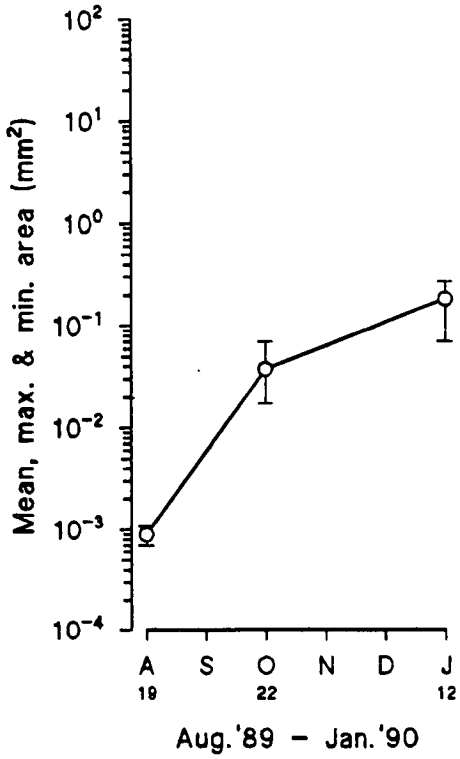
Fig. 6.17b



Aug.'89 – Jan.'90

RG.1. A. FILIX-FEMINA

Fig. 6.18b

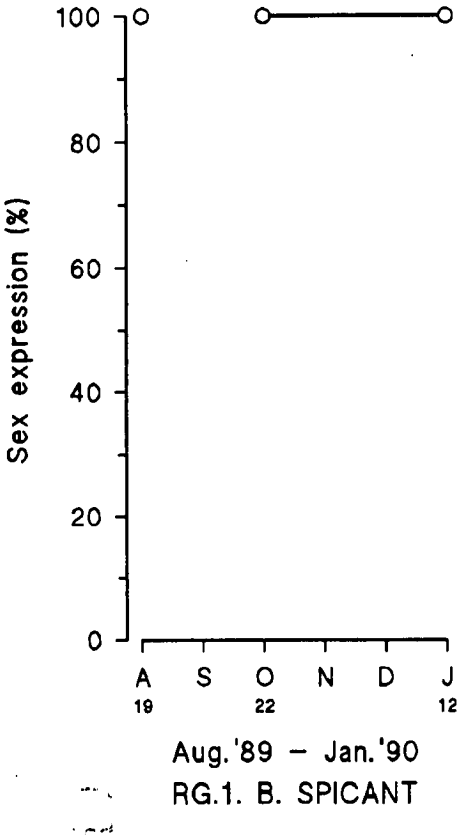


Aug.'89 – Jan.'90

RG.2. A. FILIX-FEMINA

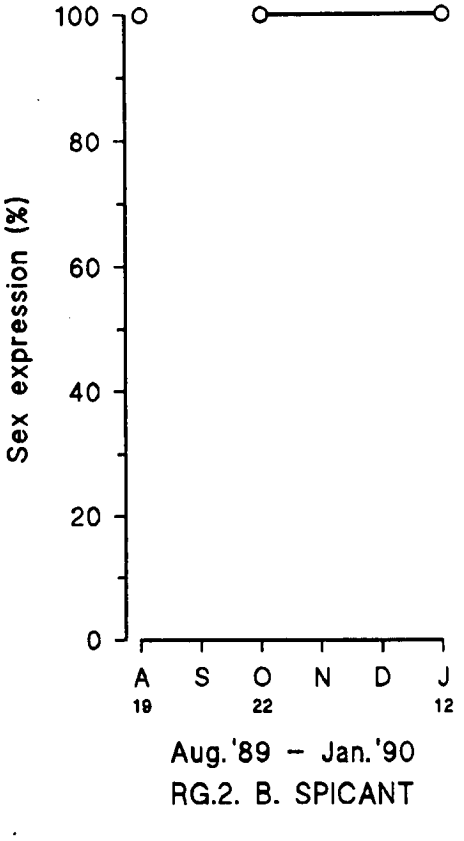
Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Fig. 6.19a



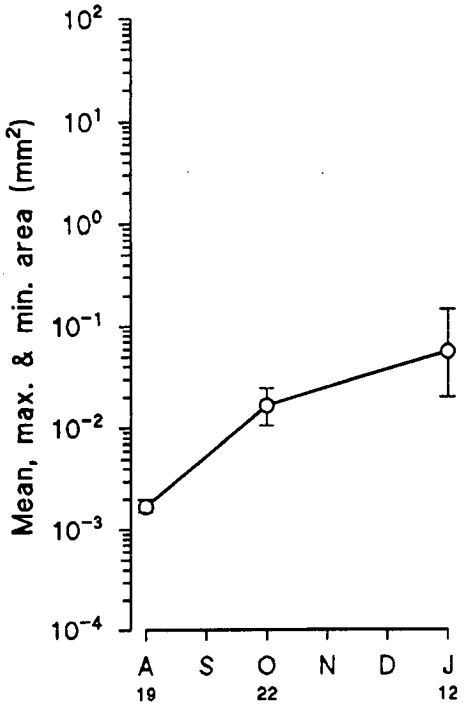
Aug.'89 – Jan.'90
RG.1. B. SPICANT

Fig. 6.20a



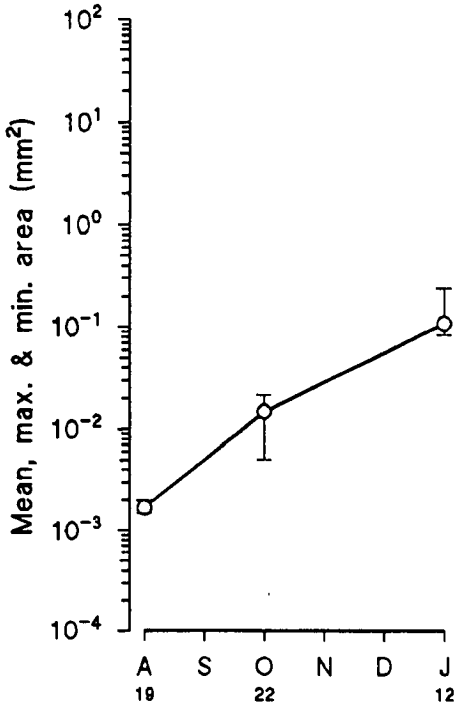
Aug.'89 – Jan.'90
RG.2. B. SPICANT

Fig. 6.19b



Aug.'89 – Jan.'90
RG.1. B. SPICANT

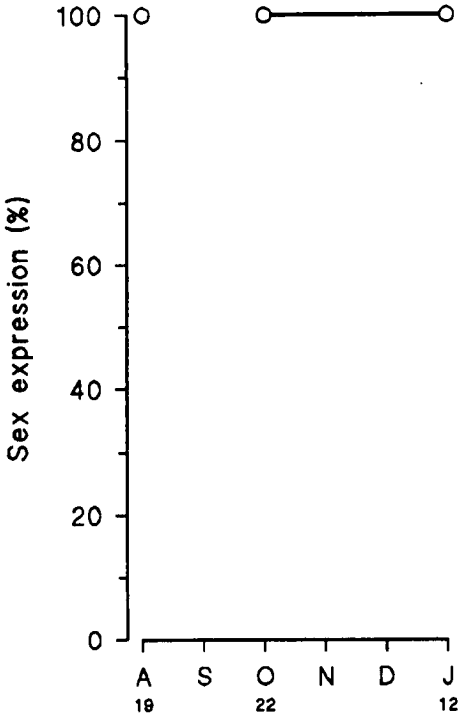
Fig. 6.20b



Aug.'89 – Jan.'90
RG.2. B. SPICANT

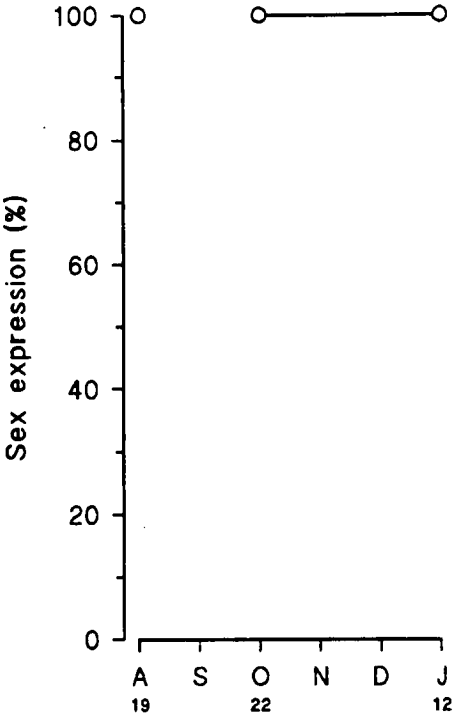
Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Fig. 6.21a



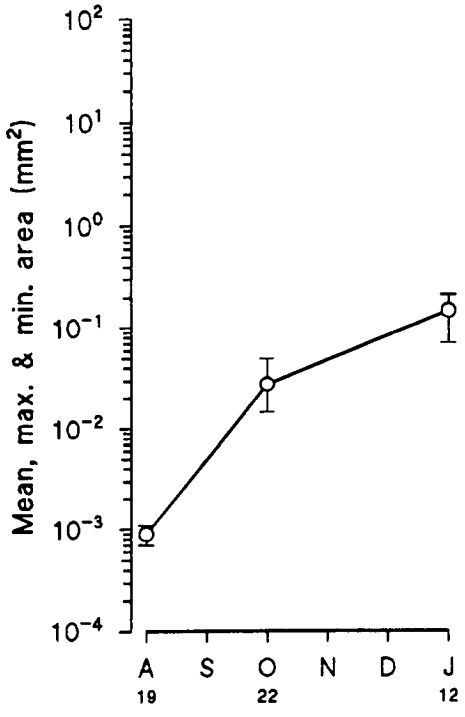
Aug.'89 – Jan.'90
RG.1. P. SETIFERUM

Fig. 6.22a



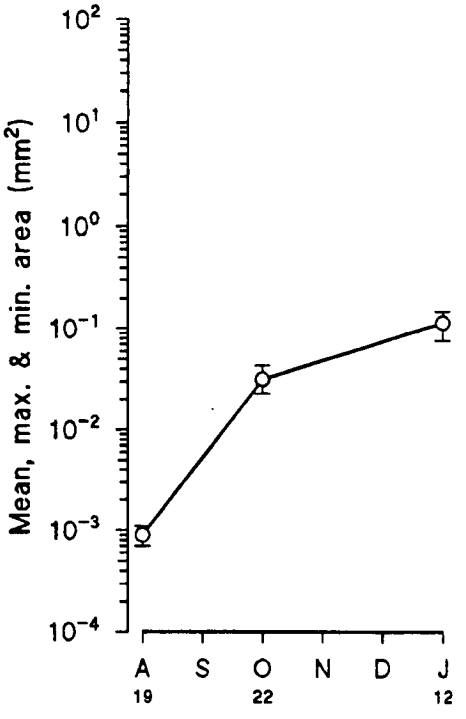
Aug.'89 – Jan.'90
RG.2. P. SETIFERUM

Fig. 6.21b



Aug.'89 – Jan.'90
RG.1. P. SETIFERUM

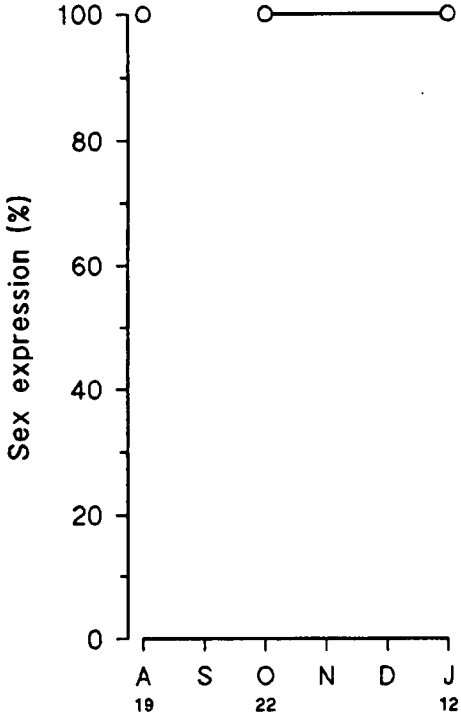
Fig. 6.22b



Aug.'89 – Jan.'90
RG.2. P. SETIFERUM

Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

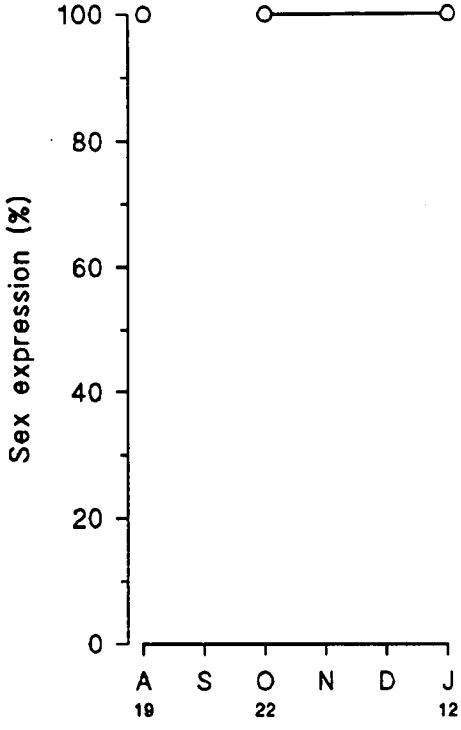
Fig. 6.23a



Aug.'89 – Jan.'90

RG.1. P. SCOLOPENDRIUM

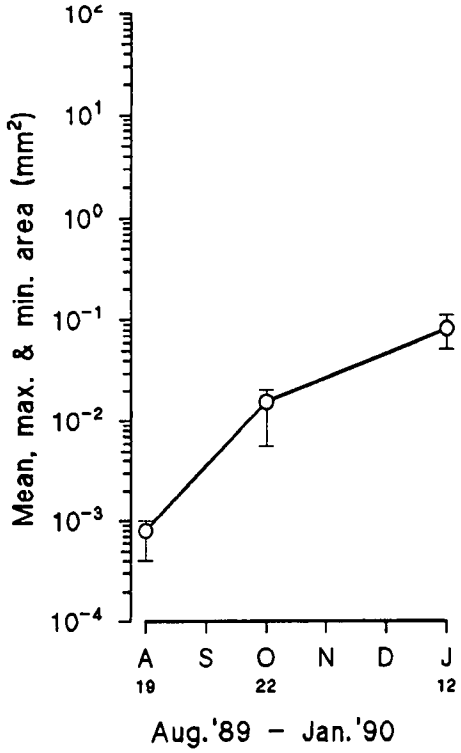
Fig. 6.24a



Aug.'89 – Jan.'90

RG.2. P. SCOLOPENDRIUM

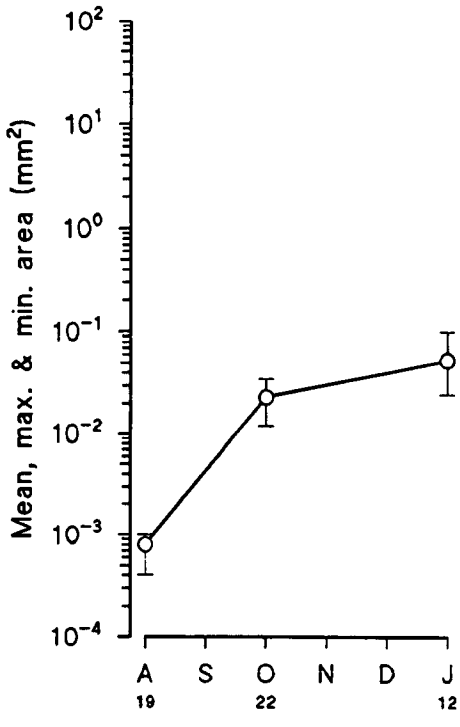
Fig. 6.23b



Aug.'89 – Jan.'90

RG.1. P. SCOLOPENDRIUM

Fig. 6.24b

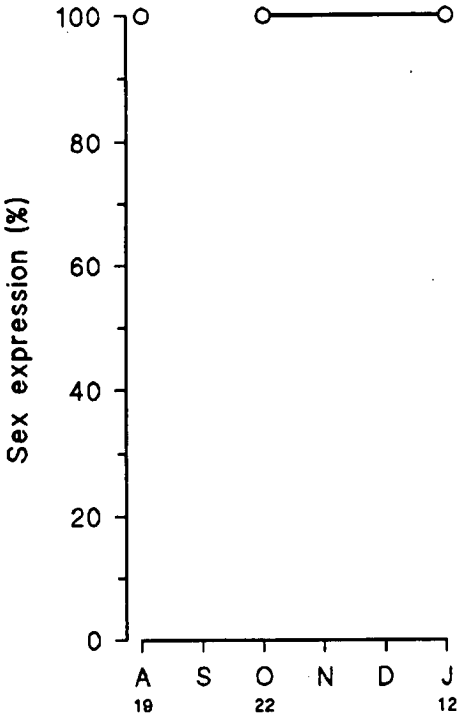


Aug.'89 – Jan.'90

RG.2. P. SCOLOPENDRIUM

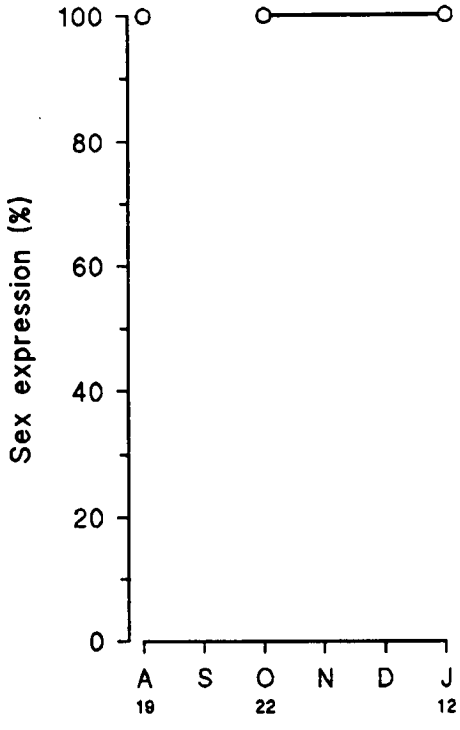
Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Fig. 6.25a



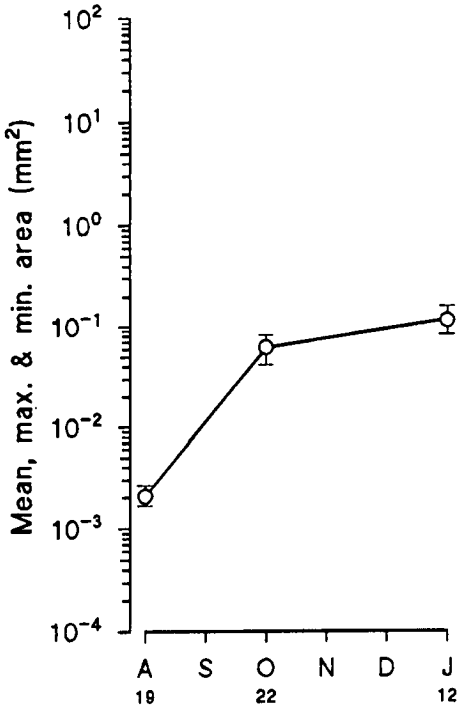
Aug.'89 – Jan.'90
RG.1. D. AFFINIS

Fig. 6.26a



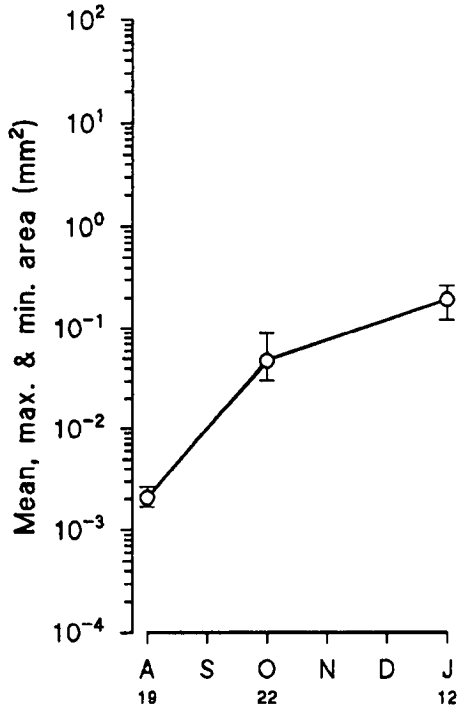
Aug.'89 – Jan.'90
RG.2. D. AFFINIS

Fig. 6.25b



Aug.'89 – Jan.'90
RG.1. D. AFFINIS

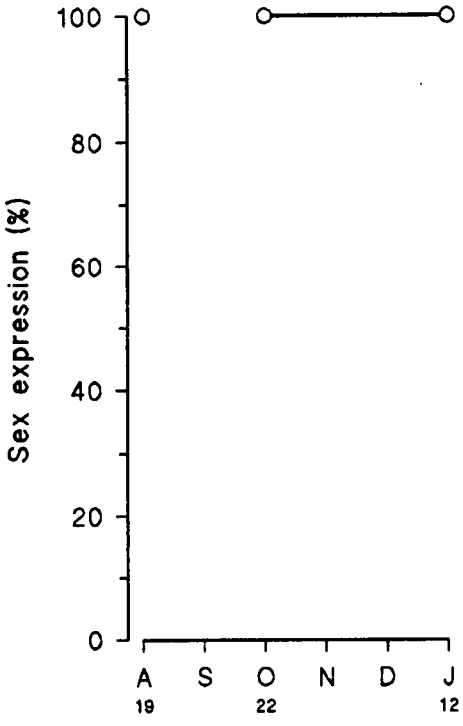
Fig. 6.26b



Aug.'89 – Jan.'90
RG.2. D. AFFINIS

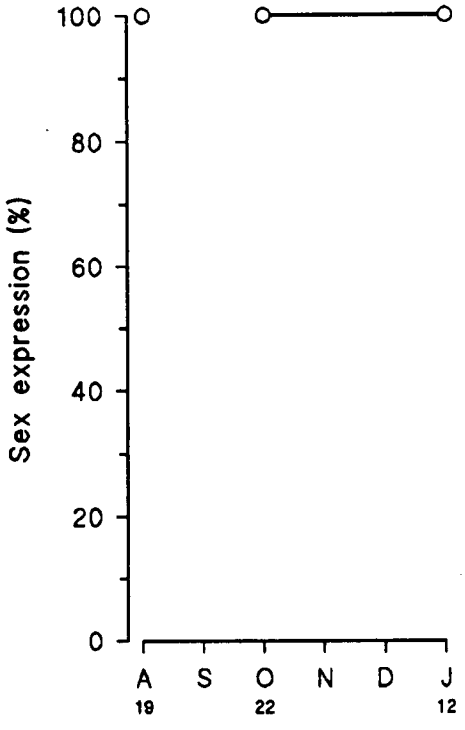
Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Fig. 6.27a



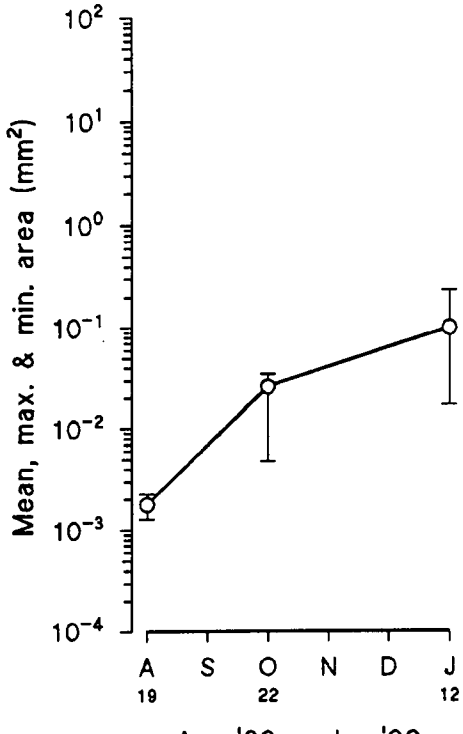
Aug.'89 – Jan.'90
RG.1. D. DILATATA

Fig. 6.28a



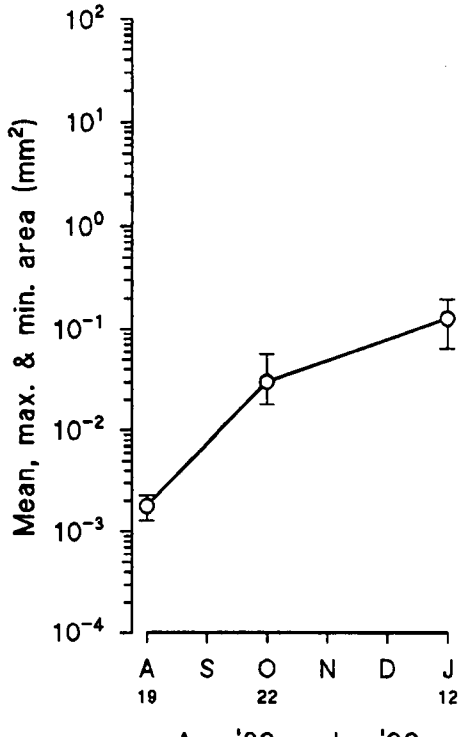
Aug.'89 – Jan.'90
RG.2. D. DILATATA

Fig. 6.27b



Aug.'89 – Jan.'90
RG.1. D. DILATATA

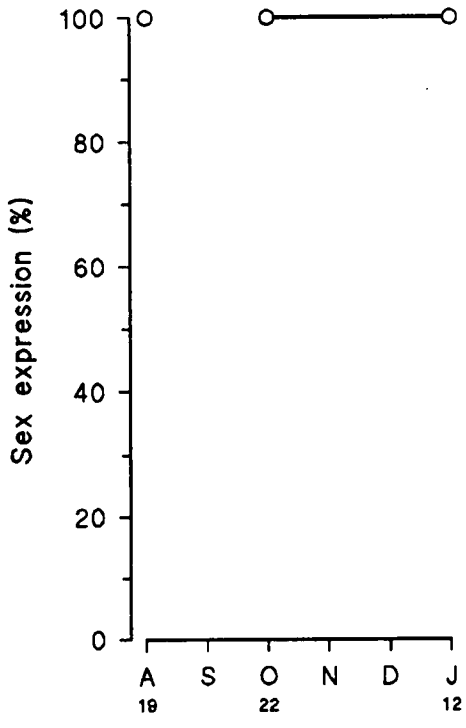
Fig. 6.28b



Aug.'89 – Jan.'90
RG.2. D. DILATATA

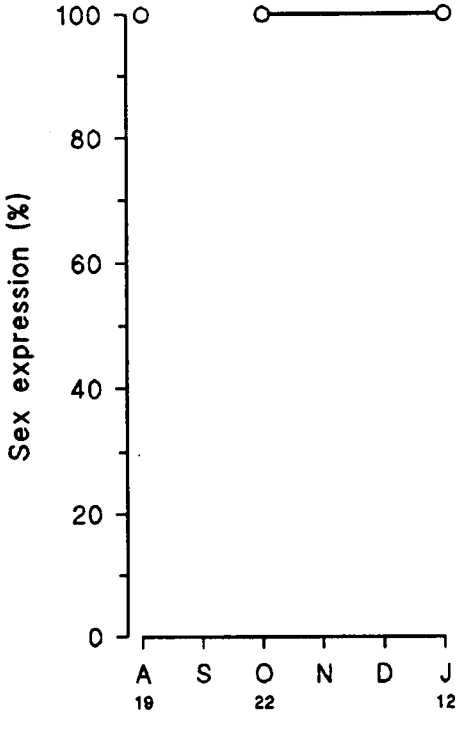
Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Fig. 6.29a



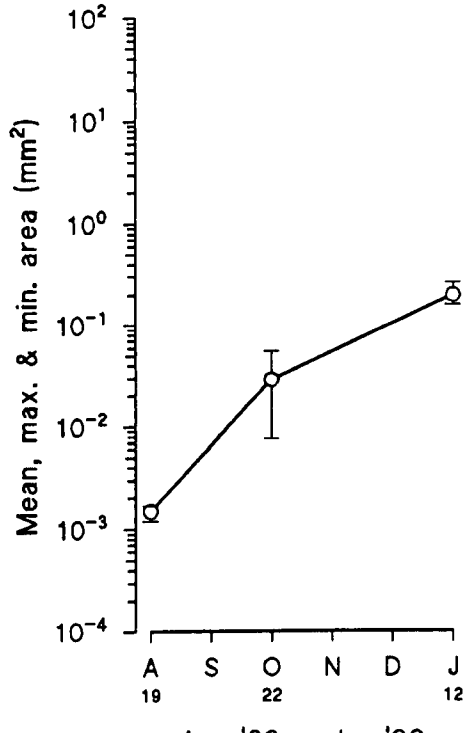
Aug.'89 – Jan.'90
RG.1. D. FILIX–MAS

Fig. 6.30a



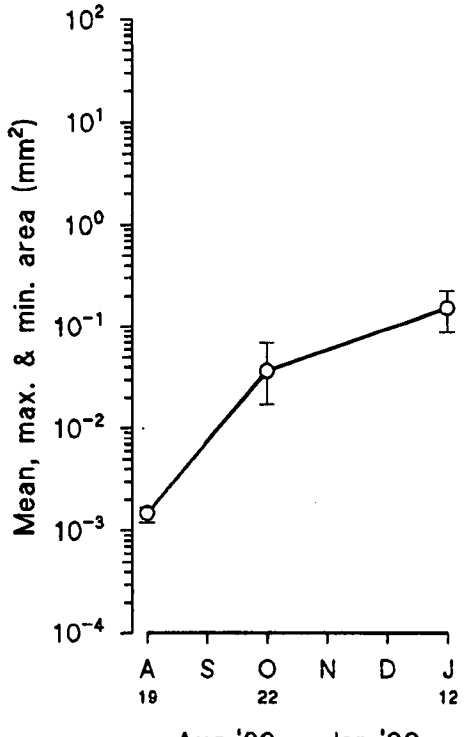
Aug.'89 – Jan.'90
RG.2. D. FILIX–MAS

Fig. 6.29b



Aug.'89 – Jan.'90
RG.1. D. FILIX–MAS

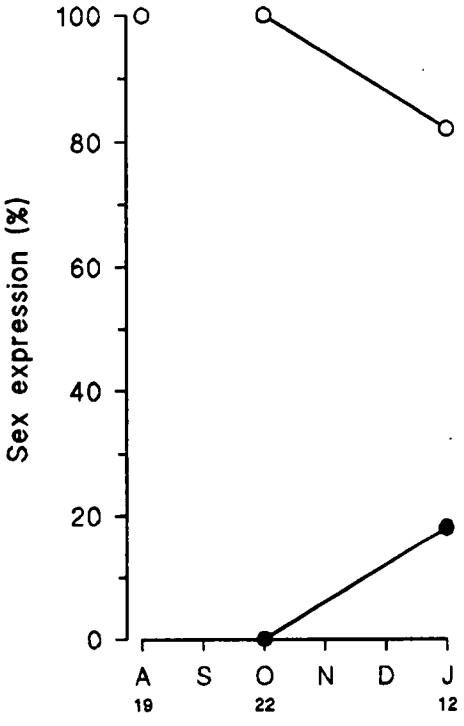
Fig. 6.30b



Aug.'89 – Jan.'90
RG.2. D. FILIX–MAS

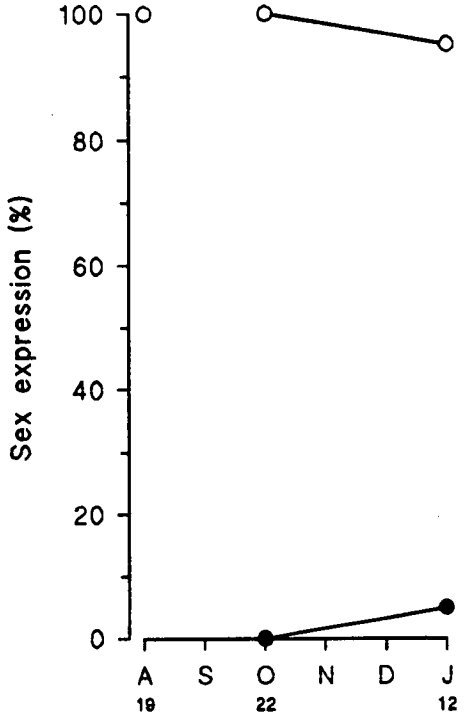
Symbols: spores or pre-sexual gametophytes (○), male gametophytes (●), female gametophytes (□), bisexual gametophytes (■), archegoniate gametophytes (▲) and percentage of archegoniate gametophytes that were fertilized (△).

Fig. 6.31a



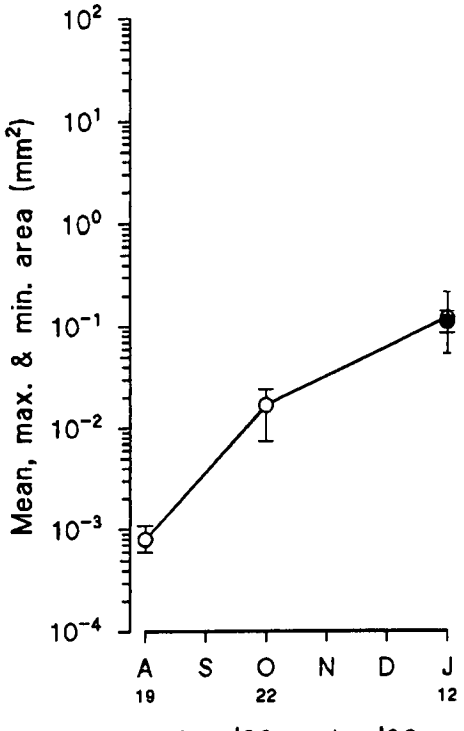
Aug.'89 – Jan.'90
RG.1. P. AQUILINUM

Fig. 6.32a



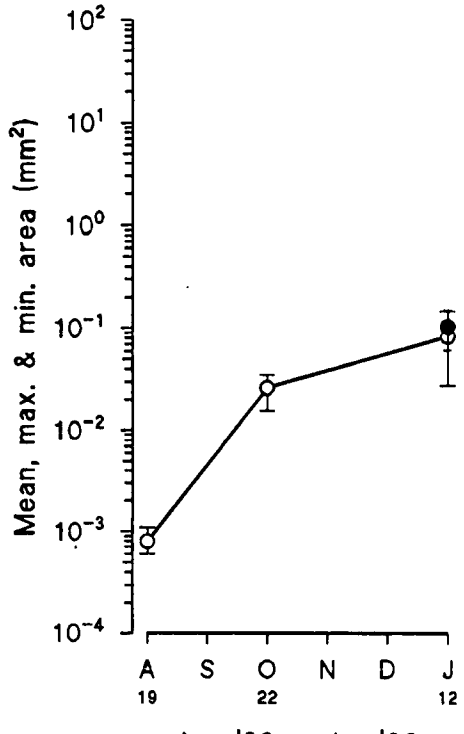
Aug.'89 – Jan.'90
RG.2. P. AQUILINUM

Fig. 6.31b



Aug.'89 – Jan.'90
RG.1. P. AQUILINUM

Fig. 6.32b



Aug.'89 – Jan.'90
RG.2. P. AQUILINUM

Chapter 7

Discussion

Experimental approach

The field experiments were simple but effective. Large numbers of gametophytes were obtained and most populations produced sporelings. Watering was not necessary.

Detailed examination of the gametophytes has yielded new and interesting information about *when* and *how* gametophyte populations develop in the wild if fundamental growth requirements are satisfied. The experiments were not designed to yield information on *why* gametophytes grow *where* in the wild.

Transparent plastic lids were fitted on the pots to create 'safe sites' for gametophyte and subsequently sporeling establishment. These safe sites were artificially created but the conditions within them [particularly humidity, temperature (assuming no 'greenhouse' effect), light, soil type and soil moisture] were probably not significantly different from the conditions experienced by natural gametophyte populations in safe sites nearby. Of course, the experimental populations were artificial in some respects; they were monocultures of uniform age and they were protected from erosion, competitors and surface dwelling herbivores.

The experimental plots had to be large enough to provide material for study on a more or less monthly basis for 2 years but they also had to be as small as possible to minimize micro-habitat differences between pots (and to be inconspicuous). To prevent the plots from exceeding $1 m^2$ only enough pots for one of each species to be removed on each sampling date were planted in the field. Ideally, each plot should have contained enough pots for replicate samples. The solution, in future experiments, would be either to use smaller pots so that at least twice as many could be planted in the same area or to use the same number of pots and sample less frequently with replication.

The results of the field experiments are interpreted using air temperature data recorded at climatological stations near the field sites. This is not ideal; conditions within natural habitats can be very different from those recorded at nearby climatological stations (Peck, 1980). Moreover, temperatures at 1.25 m above the ground (the conventional height for recording air temperature) can be very different from those at ground level where, of course, gametophytes grow. Ideally, computerized *Data Loggers* should have been left in the field throughout this study to monitor the micro-climate in each plot at ground level. *Data Loggers* are currently being used to monitor the micro-climate of sites occupied by gametophytes of *Trichomanes speciosum* [F. Rumsey and E. Sheffield, Manchester University (personal communication)].

Results

In all 3 experiments spores germinated simultaneously at Pease Bridge Glen and at Roslin Glen suggesting that a critical daylength and/or temperature was required for germination.

Spores of *A. filix-femina, B. spicant, P. setiferum* and *P. scolopendrium* germinated in Winter (Experiment 2), Spring (Experiment 1), and Summer (Experiment 3), and in each experiment there was no staggered germination. These observations indicate that there is no photoperiodic control of spore germination in these species (*cf.* Eakle, 1975; references in Dyer and Lindsay, 1992). There is insufficient information to assess whether or not daylength controls spore germination in *D. affinis, D. dilatata, D. filix-mas* and *P. aquilinum*.

In Experiment 3, spores of 8 species were sown in August, near the beginning of the main spore release season, and it appears that they germinated more or less immediately. This is hardly surprising; July and August are the warmest months of the year.

In Experiment 2, most spores germinated in the middle of Winter. This is contrary to expectation but it must be remembered that temperatures in December 1988 and January 1989 were well above average and were in fact more typical of temperatures

not normally experienced until April or May. This suggests that if this field experiment had been conducted in a more typical year, the spores would not have germinated until the Spring. This, in turn, implies that in south-east Scotland spores released near the end of the main spore release season (October), or exhumed during Winter, do not normally germinate until the following Spring. This theory receives support from the results of Experiment 1 where even with the mild Winter of 1987/'88 spores sown in January did not germinate until April or May. It is worth noting that gametophytes which began their development in Winter did not produce sporophytes much earlier than gametophytes which began their development in the following Spring.

Although the results of Experiment 2 might be atypical for the study species at Pease Bridge Glen and Roslin Glen, they should not be regarded as misleading. This experiment has demonstrated that spores of these species can and do germinate in Winter, albeit rarely, in south-east Scotland. Moreover, Winter germination might even be a common phenomenon in more southern parts of the British Isles where, of course, *P. setiferum* and *P. scolopendrium* are more abundant.

The results of all 3 experiments suggest that in a typical year in south-east Scotland spores of *A. filix-femina, B. spicant, P. setiferum* and *P. scolopendrium* would not germinate before April or after September (*i.e.* temperatures would be inadequate for germination for 6 months of the year). It is surely no coincidence that sporophytes of these species also remain dormant until April.

In each field experiment, many viable spores did not produce gametophytes. Spore 'loss' in a completely natural situation could easily be explained by the fact that many spores are washed into the soil by rain (Lindsay and Dyer, 1990; see Appendix) or are eaten *e.g.* by *Collembola, Myriopoda, Dermaptera, and Trichoniscidae* (Schneller, 1975, 1979). However, in the field experiments the spores were protected from rain and surface dwelling herbivores. Nevertheless, water droplets formed by condensation on the plastic lids might have washed some spores into the soil.

Fewer gametophytes developed in Experiments 1 and 2 than in Experiment 3. In Experiments 1 and 2 there was an interval of at least 3 months before temperatures

were suitable for germination. During that time many spores might have been washed into the soil by water droplets formed by condensation. Moreover, germinating spores in Experiments 1 and 2 might have been killed by sub-zero temperatures.

Light transmitted through a forest canopy contains very little radiation in the wavelengths 400-700 nm and is relatively rich in far red and near infrared. Stoutjesdijk (1972) discovered that while many seeds cannot germinate under a leaf canopy spores of *D. filix-mas* and *P. vulgare* can. Experiment 3 confirms Stoutjesdijk's results for *D. filix-mas* and proves that spores of 7 other fern species can also germinate in woodland habitats while the canopy is closed.

Many introductory botanical textbooks state and/or imply by illustration (*e.g.* Grounds, 1979; Raven *et al.,* 1986) that all fern gametophytes develop from filamentous protonemata. However, no protonemata were observed in this study; gametophytes became 2-dimensional after the second or third cell division and the first chlorocytes were isodiametrical. This suggests that protonemata do not feature as often, or as prominently, in the *real* fern life cycle as they do in laboratory cultures.

Most of the experimental populations consisted of male, female and bisexual gametophytes [*i.e.* they were trigametophytic (Klekowski, 1969)]. Female gametophytes were larger than bisexual gametophytes and all archegoniate gametophytes were considerably larger than male gametophytes. This correlation between size and sex expression also exists in natural populations *e.g.* Tryon and Vitale (1977), Cousens (1979, 1981), Cousens *et al.* (1988) and Hamilton (1988).

In several species [including *A. filix-femina* (Schneller, 1979, 1988), *B. spicant* (Cousens, 1973, 1979) and *P. aquilinum* (Näf, 1958, 1979)] the differentiation of gametophyte populations into 3 sexual types (male, female and bisexual), each of a different size, is mediated by antheridiogens. The results of this study support the previous reports of antheridiogens in *A. filix-femina, B. spicant* and *P. aquilinum* and suggest, for the first time, that sexual development in *P. setiferum* and *P. scolopendrium* is also mediated by antheridiogens. Experiment 3 was terminated too early to assess whether or not antheridiogens control sex expression in *D. affinis, D. dilatata* and *D. filix-mas* (but see Chapter 3).

Antheridiogens are pheromones secreted by large meristic gametophytes which induce the formation of antheridia on young gametophytes. Antheridiogens are active at very low concentrations, have high chemical and biological stability and are water soluble (Näf et *al.,* 1975; Schneller *et al.,* 1990). Consequently, they can act rapidly at distances of up to 15 cm through soil and up to 30 cm through agar (Schraudolf, 1985; Schraudolf in Schneller, 1988). The following paragraph, based on Näf (1958, 1979) and Schraudolf (1985), summarizes the accepted model for the action of antheridiogen in laboratory cultures.

In any population of gametophytes, individuals develop at different rates. The most rapidly developing individuals become insensitive to antheridiogen a few days after becoming heart-shaped and before they have started to produce the pheromone (or before they have produced it at an effective concentration). As a result, they are destined to become female gametophytes (all meristic gametophytes produce archegonia sooner or later). Even before archegonial initials are visible (approximately 1 week after becoming heart-shaped) these pre-female gametophytes begin to secrete antheridiogen. Consequently, the less rapidly developing gametophytes, which are heart-shaped but are not yet insensitive to antheridiogen, develop antheridia. These heart-shaped male gametophytes will, within a few days, become insensitive to antheridiogen and also become antheridiogen producers. Within 1-2 weeks they too will develop archegonia (and so become bisexual gametophytes). The slowest-growing gametophytes (those that are not yet heart-shaped) respond, 2-3 days after antheridiogen is first detected, by producing large numbers of antheridia. Antheridium formation in the growing region of these juvenile gametophytes interferes with the organization of the meristem that is necessary for transition to the heart-shape. Consequently, male gametophytes remain ameristic, small, sensitive to antheridiogen and incapable of producing archegonia.

The results of this study suggest (a) that such a sequence of events occurred in the experimental populations and (b) that antheridiogens act rapidly in the field as well as in culture.

Regrettably, no attempt was made to distinguish pre-sexual gametophytes that were heart-shaped from pre-sexual gametophytes that were not. Presumably, the largest

pre-sexual gametophytes in the samples one month before antheridia first appeared were heart-shaped and secreting or preparing to secrete antheridiogen. If this was true, then the antheridiogens must have acted rapidly because within 1 month some gametophytes (up to 90%) in all populations had developed mature antheridia. In almost half of the populations in this study, male and archegoniate gametophytes were first detected in the same sample. More frequent sampling would probably have revealed that even in these populations antheridia developed first.

In all populations, the first archegoniate gametophytes were considerably larger (up to 33 x) than the largest pre-sexual (putative pre-archegoniate) gametophytes in the sample 1 month before. Näf (1979) made a similar observation and suggested that the acquisition by a pre-archegoniate gametophyte of a highly organized meristem results in accelerated growth. Male gametophytes grow more slowly, not simply because they fail to develop highly organized meristems but also because much of their growth potential is diverted from the formation of vegetative cells to the formation of antheridial cells (Näf 1979).

Laboratory experiments have shown that antheridiogens can induce photoblastic spores of some species to germinate, and produce protonemata bearing antheridia, in the dark. These experiments and field observations by Schneller (1988) suggest that antheridiogens are important in nature for recruiting male gametophytes from soil spore banks. Earlier, it was suggested that a large number of spores in the field experiments had been washed into the soil before they could germinate. However, if antheridiogens were operating in the experimental populations then many of these 'lost' spores still ought to have germinated. Since no etiolated gametophytes were detected, it seems more likely that the spores which did not produce gametophytes had died and were not simply dormant in the soil.

Archegonia first appeared in most populations in the Summer, less than 1 month after the first appearance of antheridia (in accordance with the 'antheridiogen model'). In 3 populations (1 each of *B. spicant, P. setiferum* and *P. scolopendrium*) the first archegonia appeared in Winter. Archegonia at all stages of development were detected in Spring and Autumn. These observations indicate that the production of archegonia in *A. filix-femina, B. spicant, P. setiferum* and *P. scolopendrium* is not

under photoperiodic control. There is no evidence yet that archegonial production in any fern gametophyte is controlled by daylength but this is largely because the subject has not been investigated. Many thalloid liverworts which resemble fern gametophytes and grow in similar micro-habitats require long days for the production of sex organs (Hartman and Jenkins, 1984).

In many populations embryos appeared less than 1 month after the first appearance of archegonia. This demonstrates that, like antheridia, archegonia mature rapidly in the field.

All populations were predominantly male. Female gametophytes were always more abundant than bisexual gametophytes. All bisexual gametophytes were completely dichogamous; those with mature antheridia always bore immature archegonia; those with mature archegonia always bore old and empty antheridia. These observations indicate that all the sporophytes observed during this study were the products of inter-gametophytic mating. This is contrary to the popular belief (based on the prevalence of bisexual gametophytes in laboratory cultures) that most sporophytes are the products of intra-gametophytic selfing, but is compatible with the results of recent electrophoretic studies which prove that inter-gametophytic crossing is the norm in many natural populations *e.g.* Haufler and Soltis (1984), Wolf (1986), Soltis and Soltis (1987, 1988, 1990) and Wolf *et al.* (1988).

Fertilization occurred throughout the year (including Winter) confirming that mature antheridia and archegonia were always present. It is conceivable that if the experimental populations had not experienced mild Winters the production and fertilization of gametes might have been more seasonal. Cousens *et al.* (1988) observed seasonally produced bands of antheridia on gametophytes of *Lorinseria areolata*.

The sexual composition of all populations in Experiments 1 and 2 remained relatively constant for periods of up to 10 months. Such apparent 'equilibrium periods' were probably artifacts created by growing the artificial populations in somewhat protected environments and resulting from the fact that no gametophytes were dying (all gametophytes survived for at least 1 year) and none were being recruited. In natural

populations gametophyte mortality can be extremely high (Cousens 1973, 1981; Peck, 1980; von Aderkas, 1983; Kelly, 1987; Cousens *et al.,* 1988; Peck *et al.,* 1990) and there are probably numerous opportunities for gametophytes to be recruited either from spores deposited into existing populations or from spores in the soil under populations (Schneller, 1979, 1988; Schneller *et al.,* 1990).

The lack of gametophyte mortality resulted in extremely dense populations ranging from 50-300 gametophytes cm^{-2}. From the limited information in the literature, it appears that densities in nature rarely exceed 75 gametophytes cm^{-2} (references in Cousens, 1988). In future experiments spores should be sown at lower density to create more realistic gametophyte populations.

All 8 species grew better at Pease Bridge Glen than at Roslin Glen. This was despite the fact that at Pease Bridge Glen gametophytes (and sporelings) of *A. filix-femina, B. spicant, D. affinis, D. dilatata, D. filix-mas* and *P. aquilinum* were grown on soil on which mature sporophytes of these species do not grow naturally. Additional laboratory and field experiments would be required to determine whether it was the higher nutrient status of the soil or some other feature of the habitat at Pease Bridge Glen (*e.g.* the slightly higher temperatures) that promoted this better growth. At Roslin Glen there was little evidence to suggest that growth of *P. setiferum* and *P. scolopendrium* (on alien soil) was any poorer than that of *A. filix-femina* and *B. spicant* (on native soil). These observations indicate that gametophytes of *A. filix-femina, B. spicant, P. setiferum, P. scolopendrium, D. affinis, D. dilatata, D. filix-mas* and *P. aquilinum* can readily grow and fulfil their function in habitats that are unsuitable for further sporophyte development. Consequently, these species could be more widespread than the distribution of their sporophytes suggests.

At Roslin Glen all species grew much better in plot 1 than in plot 2. Doubtless this was because the soil in plot 1 was permanently moist whereas the soil in plot 2 was frequently dry. Gametophytes developed slowly in plot 2 but not abnormally; embryos appeared eventually. All populations in RG.2. had a greater percentage of male gametophytes than all populations in RG.1. Conditions unfavourable for rapid growth, even in the absence of antheridiogen, are frequently correlated with a preponderance of male gametophytes (references in Miller, 1968). This would also

explain why all populations at Roslin Glen had a slightly greater percentage of male gametophytes than all populations at Pease Bridge Glen.

Experiment 3 was short but particularly informative. It demonstrated that germination can occur in the same year as dispersal but that gametophytes of most species do not become sexual until the following year (presumably in the Spring and assuming that they survive the Winter). This experiment also revealed (by the fortuitous inclusion of *P. aquilinum*) that there can be major phenological differences between species. Unlike all the other species, gametophytes of *P. aquilinum* became sexual before Winter and in one population fertilization occurred before the end of the year. This confirms Conway's inkling that spores of *P. aquilinum* germinate immediately after dispersal and that under good conditions, fertilization can occur before Winter (Conway, 1957). Incidentally, Experiment 2 confirmed her other suspicion that spores released as late as mid-October would be unable to germinate before Winter. This remarkable ability of *P. aquilinum* to produce sporelings before Winter is probably a survival strategy related to the fact that spores of this species cannot survive overwinter [Lindsay, Sheffield and Dyer (unpublished)].

Conclusion and future work

This study has demonstrated that field *experiments* on the development of fern gametophytes are possible. It has also provided a timescale for events in the fern life-cycle in south-east Scotland.

The results for the 4 main species and for 3 of the additional species were strikingly similar. In view of this, future phenological studies should concentrate on fewer species and greater replication. Field experiments should also be conducted on a smaller scale so that more research time can be allocated to complementary laboratory experiments. For example, in this study, there was an obvious need for laboratory experiments to discover the minimum and optimum temperatures for spore germination; to examine the possibility that meristic gametophytes of *P. setiferum* and *P. scolopendrium* produce antheridiogen; to examine the possibility that photoperiod does not control sexual development in *A. filix-femina*, *B. spicant*, *P. setiferum* or *P. scolopendrium* and to establish the reason why all 8 species grew better at Pease Bridge Glen than at Roslin Glen.

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Appendix

Appendix

Contents

1. LINDSAY, S. and DYER, A.F. (1990). Fern spore banks: implications for gametophyte establishment. *In: Taxonomía, Biogeografía y Conservación de Pteridófitos* (Editor: Rita, J.). Societat d'Historia Natural de les Illes Balears - Institut Menorquí d'Estudis, Palma de Mallorca, Spain, pp. 243-253.

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4. Supplement to Lindsay, Williams and Dyer (1992), (Unpublished).

Fern spore banks: implications for gametophyte establishment

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Abstract

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 30 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.

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 may fulfil 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.

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.

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 and Edgerton (1976) detected viable spores of *Cystopteris fragilis* in only the top 2 cm of soil during an investigation of seed banks in mixed coniferous forests 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 and 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 pteridophytes which was published separately (Leck and Simpson, 1987). Although *Onoclea sensibilis* was the largest component of the spore bank, a total of seven species was recorded. Van Tooren and During (1988) found viable spores of at least two unidentified fern species in the top 1 cm of soil

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.

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 Switzerland, 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, 11 km south of Edinburgh. Pease Bridge Glen, near Cockburnspath, 50 km 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 and 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 (photon flux density = 20$\mu Em^{-2}s^{-1}$) 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 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 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.

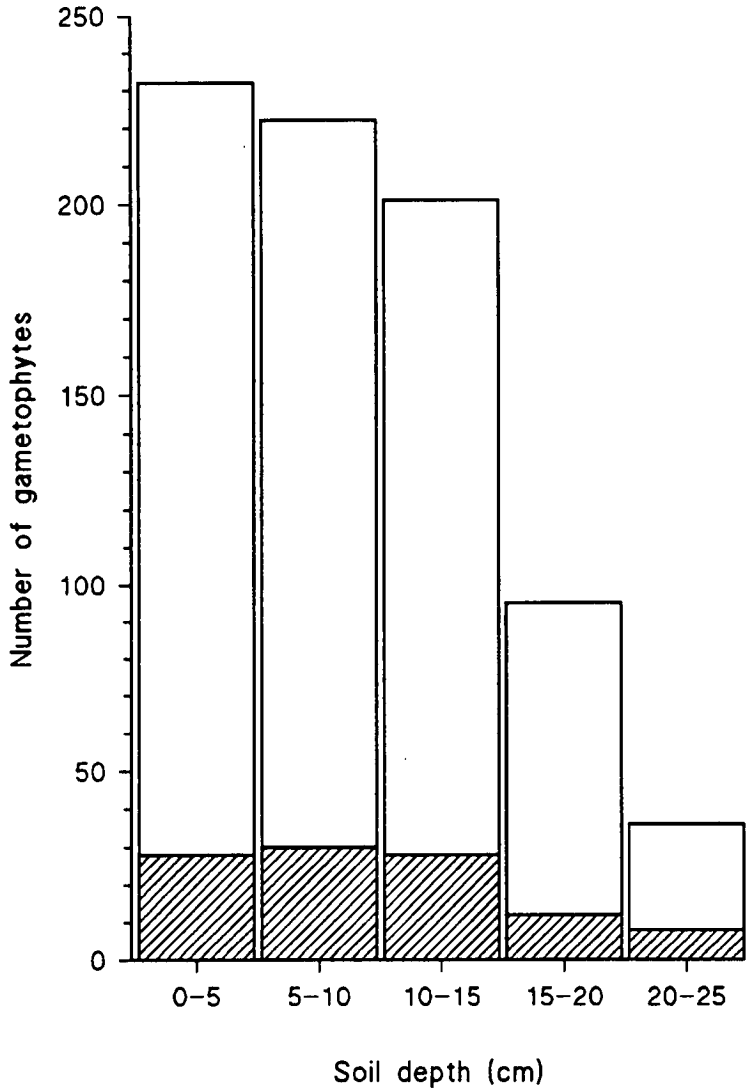

Figure 1. A typical distribution of viable fern spores in soil.

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 cm^2, 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 June 1988, a few weeks before spore release.

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 progess 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 collected at the end of June, just before a new crop of spores was released, showed that substantial numbers of viable spores were still present in the soil at all sampled 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 and 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 gametophtye 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).

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.

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 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

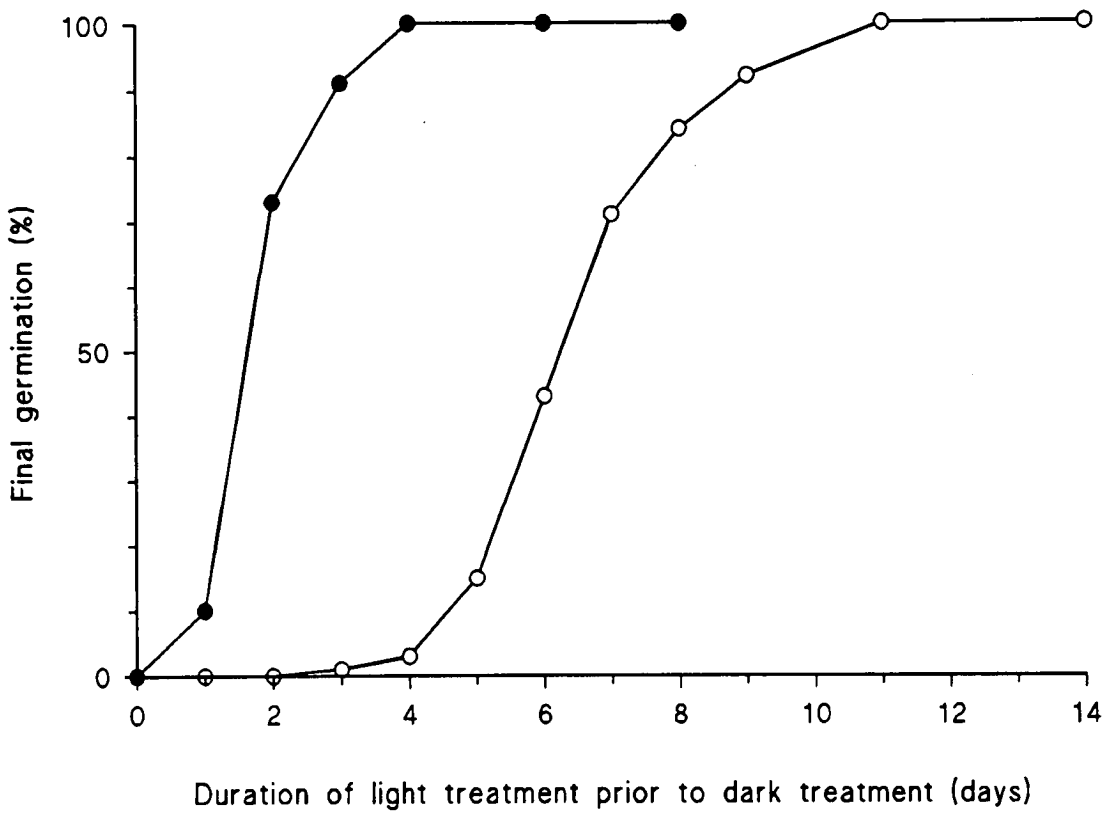

Figure 2. The time required for light to trigger germination of spores of *A. filix-femina* (●) and *B. spicant* (○) at 15°C.

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 (photon flux density = $20\mu Em^{-2}s^{-1}$). 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 spore do not normally germinate in darkness but they will if germinartion 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.

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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 gametophtyes 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

We would like to thank the Scottish Wildlife Trust (S.W.T.) for permission to conduct field work in Roslin Glen Wildlife Reserve and both the Forestry Commission and the Nature Conservancy Council (N.C.C.) for permission to work in Pease Bridge Glen. We also thank the Science and Engineering Research Council (S.E.R.C.) for financial support.

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WOOLLEY, J.T. and STOLLER, E.W. (1978). Light penetration and light-induced seed germination in soil. *Plant Physiology,* 61: 597-600.

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.

KOMAROVA, T.A. (1987). The spore regeneration of ferns in fresh burns of the South Sikhote-Alin. *Botanicheskii Zhurnal-Moskva,* 72: 215-220.

DUCKETT, J.G. and CLYMO, R.S. (1988). Regeneration of bog liverworts. *New Phytologist,* 110: 119-127.

HAMILTON, R.G. (1988). The significance of spore banks in natural populations of *Athyrium pycnocarpon* and *Athyrium thelypterioides. American Fern Journal,* 78: 96-104.

The effect of imbibition and temperature on the longevity of fern spores

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Abstract

There are several reports describing the ability of fern spores, of certain species, to survive remarkably long periods of storage (up to 100 years!) under relatively dry conditions (1). Most of these reports derive from studies of herbarium specimens. A comparative study of the survival potential of different species, housed in more than one herbarium, is not possible because storage conditions are not standardized and are rarely fully documented.

There is no published information on the survival and potential longevity of imbibed fern spores. However, such information is now particularly important following recent discoveries of large reservoirs of viable spores in the soil in many habitats (2).

For these reasons, we are conducting an experiment in the laboratory to compare the longevity of four species, stored either dry or imbibed, at two temperatures.

The species chosen for this study were: *Athyrium filix-femina* (L.) Roth, *Blechnum spicant* (L.) Roth, *Polystichum setiferum* (Forsk.) Woynar and *Phyllitis scolopendrium* (L.) Newm. Spores of these species will germinate only if imbibed and exposed to light.

Imbibition was achieved by sowing dry spores onto mineral agar in compartmentalized Petri dishes. These Petri dishes were sealed and stored in complete darkness at 4°C or at 20°C. Vials containing dry spores were stored under the same conditions. Percentage germination (viability) of dry and imbibed spores was determined every 2 months by culturing samples on mineral agar, in the light, at 20°C for 3 weeks.

The results obtained in the first year of storage are summarized in the table below:

Mean percentage change in germination after 12 months

	4°C		20°C	
Species	Dry	Imbibed	Dry	Imbibed
A. filix femina	+5	+3	-72*	-11
B. spicant	-11	+3	-97*	+1
P. setiferum	-16	+2	-100*	+3
P. scolopendrium	+17	+4	-96*	+4

* Statistically significant changes ($P<0.05$)

These results show that the longevity of imbibed spores of *A. filix-femina*, *B. spicant*, *P. setiferum* and *P. scolopendrium* is equal to, or greater than, the longevity of dry spores stored at the same temperature. This is contrary to the popular belief that fern spore viability is retained best under dry conditions, but is in agreement with reports that seeds of some angiosperms survive longer when fully imbibed (3).

This study suggests that spores, of at least some species, might survive even longer in the soil than on herbarium specimens. Consequently, some fern spore banks could be extremely long-lived and have important implications for population genetics and conservation (2). However, microbial activity in soil and fluctuations in temperature might play an important role in limiting spore longevity under natural conditions.

Our experiment provides the first evidence that imbibed fern spores can survive longer than dry spores under certain conditions.

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1. WINDHAM, M.D., WOLF, P.G. and RANKER, T.A. (1986). *American Fern Journal*, 76: 141-148.
2. LINDSAY, S. and DYER, A.F. *In: Taxonomía, Biogeografía y Conservación de Pteridófitos*. Proceedings of the symposium held in Menorca, October 27-30, 1988 (In press).
3. VILLIERS, T.A. (1974). *Plant Physiology*, 53: 875-878.

Wet storage of fern spores: unconventional but far more effective!

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Introduction

Although many pteridologists (including the BPS and AFS Spore Exchange organizers) store fern spores air-dry at room temperature, it was until recently believed that the viability of most spores was best retained under very dry and cold conditions (*e.g.* Scheuerlein *et al.*, 1989 and references in Dyer, 1979, page 258). However, the recent discoveries of viable fern spores in soil many months after spore dispersal (reviewed by Lindsay and Dyer, 1990), imply that fern spores can survive for long periods in a hydrated state. This apparent anomaly prompted a comparative study of the survival at room temperature (approximately 20°C) of spores stored air-dry with that of spores stored fully hydrated.

Materials and methods

Five species, from different genera, were selected for this study: *Todea barbara* (L.) Moore (Crape/King Fern), *Athyrium filix-femina* (L.) Roth (Woodland Lady Fern), *Blechnum spicant* (L.) Roth (Hard/Ladder Fern), *Polystichum setiferum* (Forsk.) Woynar (Soft Shield Fern) and *Phyllitis scolopendrium* (L.) Newm. (Hart's Tongue Fern).

T. barbara, a native of South Africa and Australasia, belongs to the Osmundaceae. Like all species in this family, including the British native, *Osmunda regalis* (L.), *T. barbara* produces 'green' (*i.e.* chlorophyllous) spores which cannot be stored for long by conventional methods. The other four species are common in Britain. They, like the majority of ferns, produce 'non-green' (*i.e.* non-chlorophyllous) spores which are

more amenable to storage. Various aspects of chlorophyllous and non-chlorophyllous spores are described by Lloyd and Klekowski (1970).

Spores were fully hydrated by sowing them onto mineral agar (See Dyer 1979, page 282 for recipe) in sterile plastic Petri dishes. Petri dishes were sealed with 'Parafilm-M'; a self-sealing waterproof film manufactured by *American Can Company* (Greenwich, Connecticut) and frequently used in laboratories to prevent water loss and shrinkage of agar. The fully hydrated spores were prevented from germinating by storing the Petri dishes in complete darkness (spores of most fern species require light to germinate and so darkness enforces dormancy). Air-dry spores were stored alongside in vials also in complete darkness. *T. barbara* was stored for almost 5 months. The other species were stored for 2 years.

At regular intervals during storage, spores were tested for their ability to germinate by culturing samples on mineral agar, in the light (photon flux density = $20\mu Em^{-2}s^{-1}$), at 20°C. Germination was carefully monitored by recording percentage germination at regular intervals during culture. Final percentage germination (= 'viability') was scored after 21 days by which time there was no further significant increase in germination.

Results

The results are presented in Figures 1-9; no data on germination rates were obtained for *T. barbara*. Each point represents the mean of at least two replicates.

Air-dry spores of all 5 species deteriorated markedly during storage, as revealed by a progressive loss of viability (Figures 1-5) and an increase in the time required to germinate (Figures 6a, 7a, 8a and 9a).

Surprisingly, fully hydrated 'green' spores deteriorated much more slowly than those stored air-dry (Figure 1). Even more striking results were obtained for fully hydrated 'non-green' spores. Their ability to germinate [*i.e.* viability (Figures 2-5) and rate (Figures 6b, 7b, 8b, and 9b)] was unchanged even after 2 years of storage at room temperature!

Figure 1

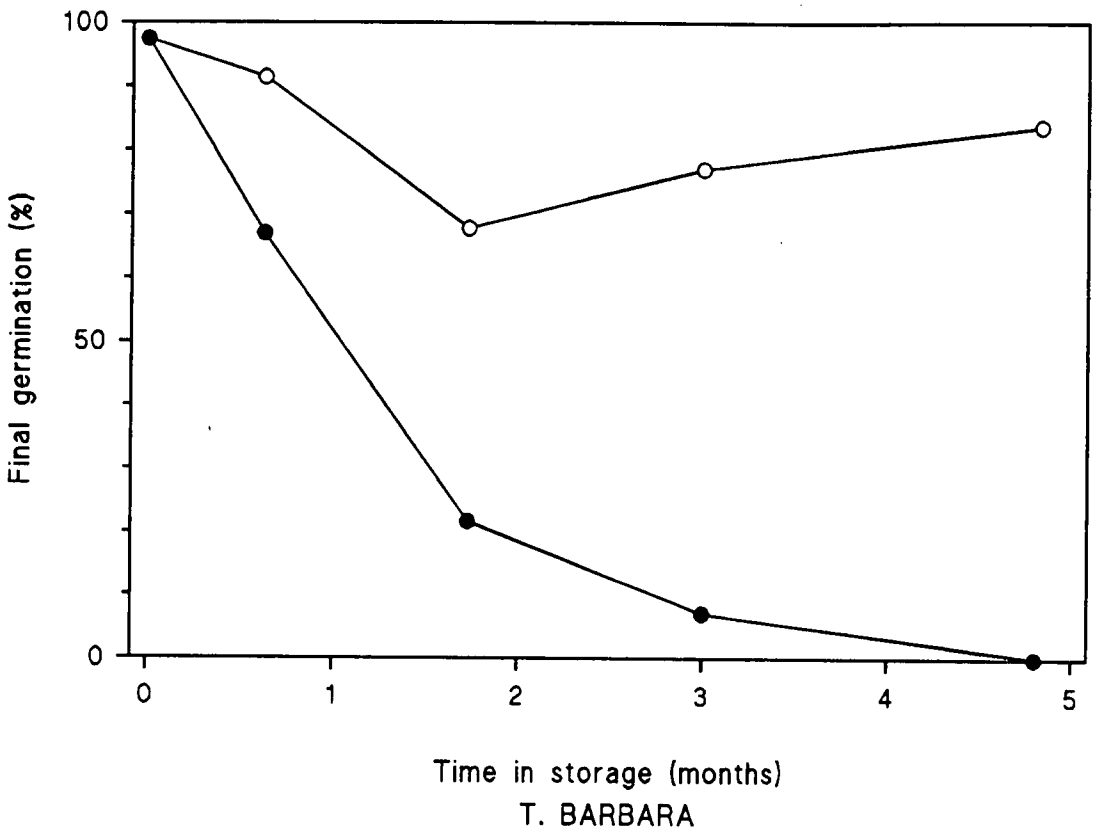
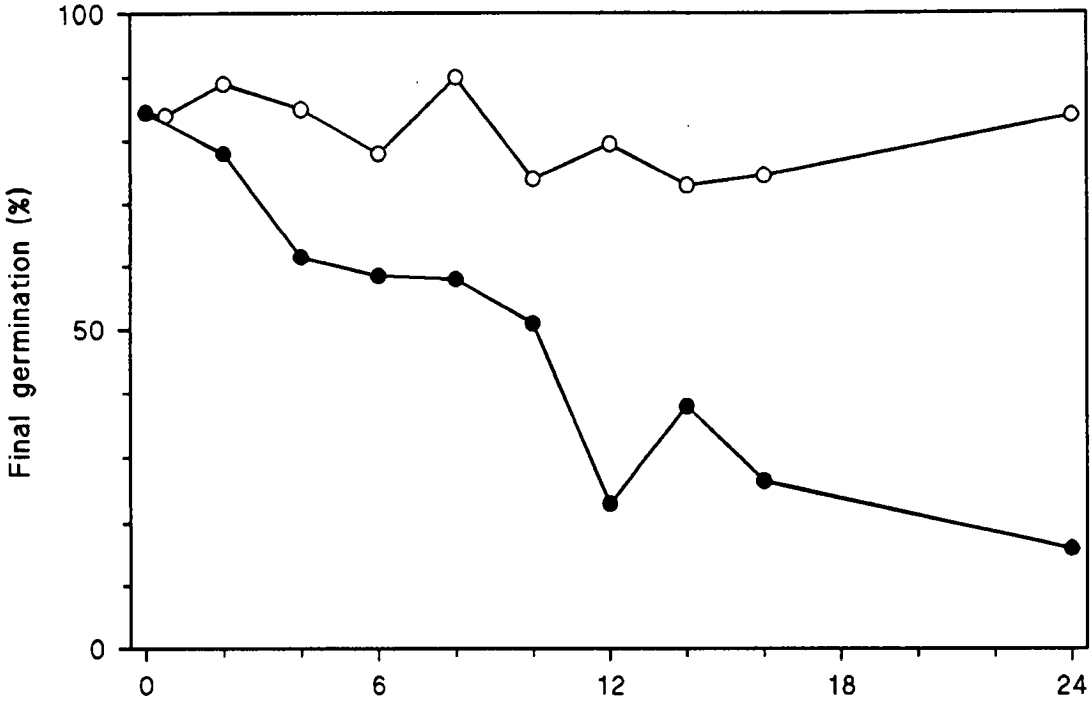


Figure 1. Final percent germination after 21 days culture (='viability') of 'green' spores of *T. barbara* stored at *c.*20°C either air-dry (●) or fully hydrated (○) for periods of up to nearly 5 months. Dry spores died within 5 months; most wet spores remained viable.

Figures 2-5. Final percentage germination after 21 days culture (='viability') of 'non-green' spores stored at *c.*20°C either air-dry (●) or fully hydrated (○) for periods of up to 2 years. Figure 2: *A. filix-femina*, Figure 3: *B. spicant*, Figure 4: *P. setiferum*, and Figure 5: *P. scolopendrium*. After 2 years storage, few dry spores of *A. filix-femina* remained viable and all dry spores of the other 3 species were dead. For wet spores of all species, there was no loss of viability.

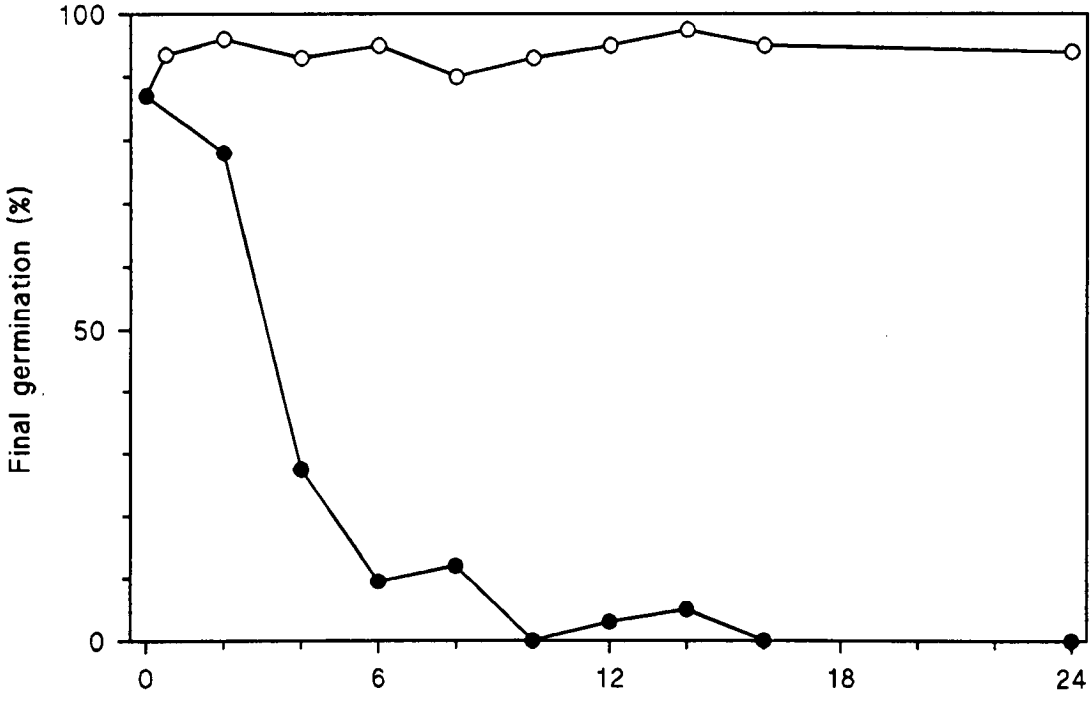
Figures 6-9. Germination curves for 'non-green' spores stored at *c.*20°C either air-dry (Figures 6a, 7a, 8a and 9a) or fully hydrated (Figures 6b, 7b, 8b and 9b) for 0 (●), 2 (△), 4 (■), 8 (○), 12 (▲) and 24 (□) months. Figure 6: *A. filix-femina*, Figure 7: *B. spicant*, Figure 8: *P. setiferum* and Figure 9: *P. scolopendrium*. For dry spores, germination became slower with increasing time in storage but for wet spores there was no change in the rate of germination even after 2 years.

Figure 2



A. FILIX-FEMINA

Figure 3



B. SPICANT

Figure 4

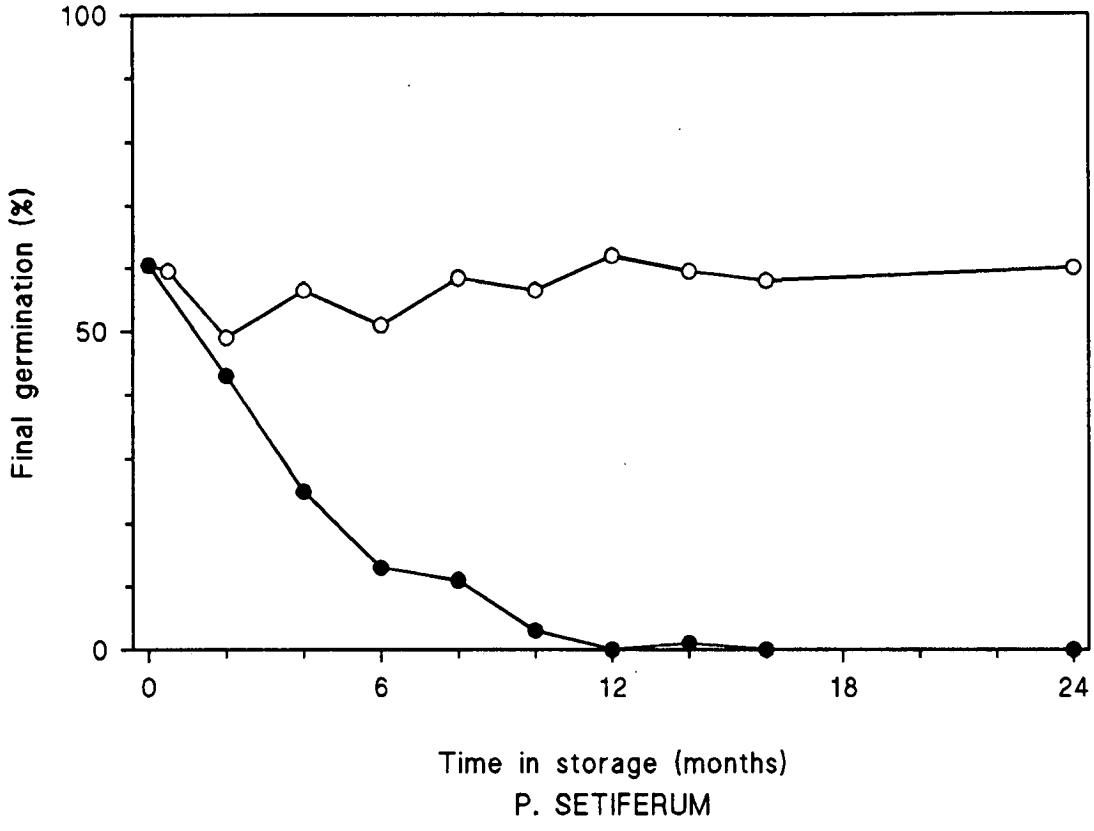


Figure 5

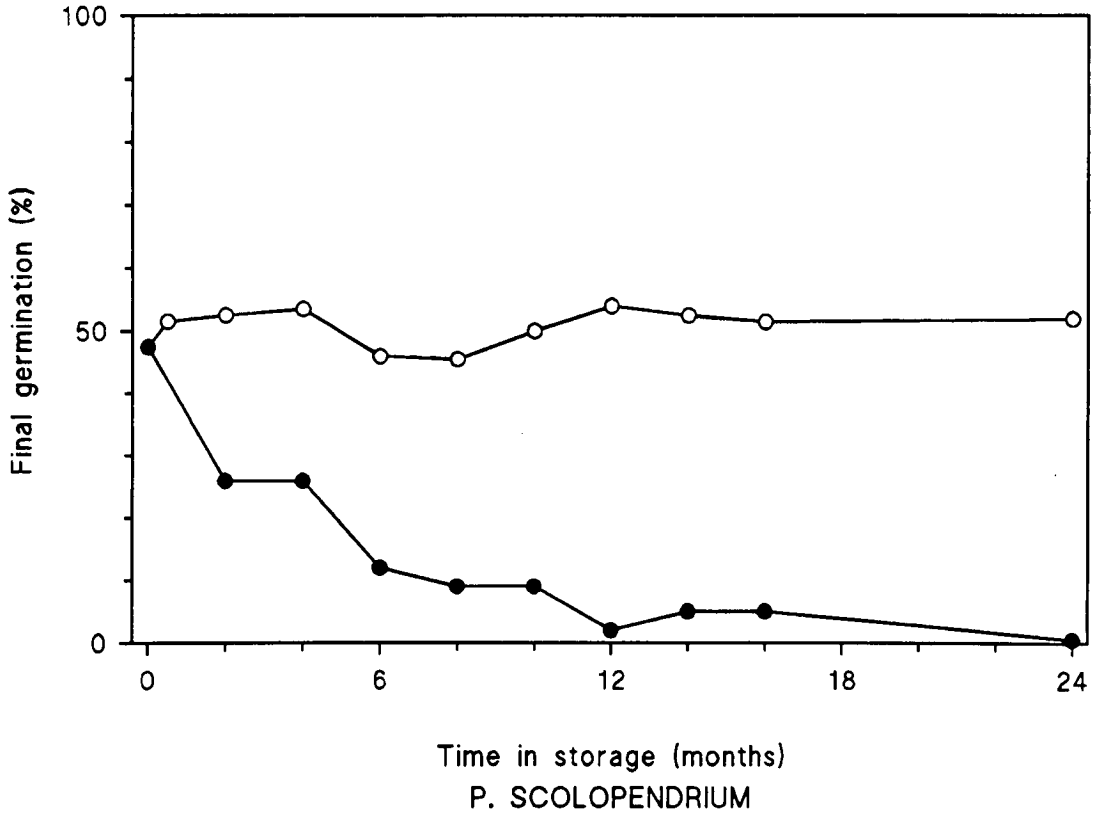
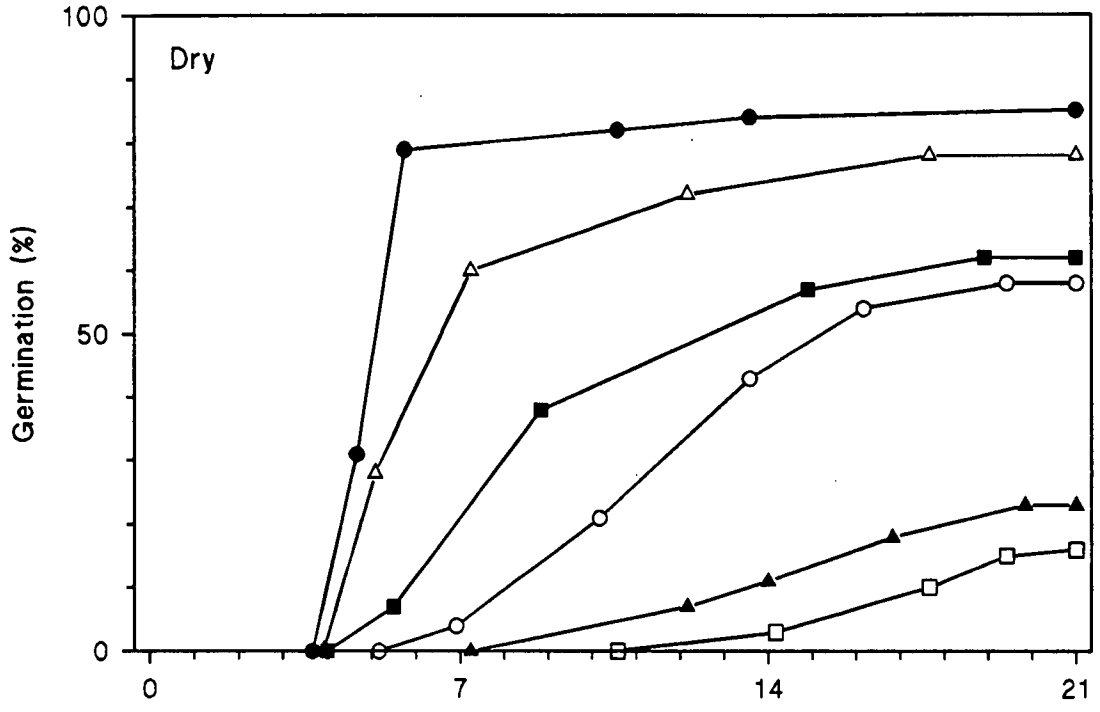
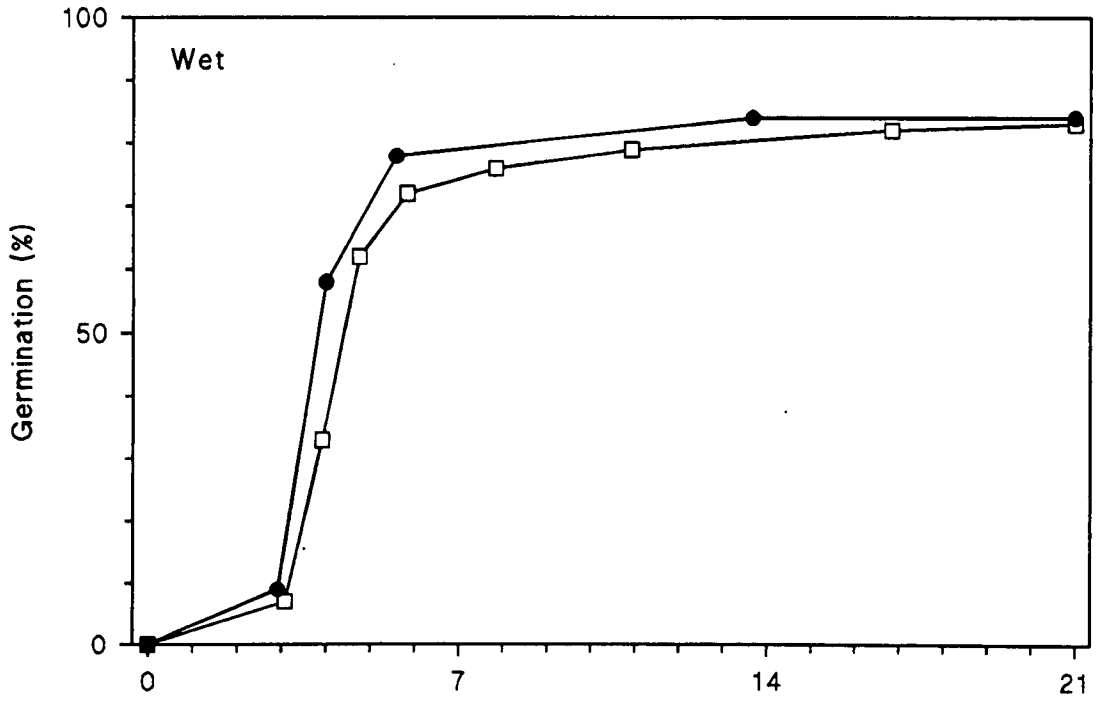


Figure 6a



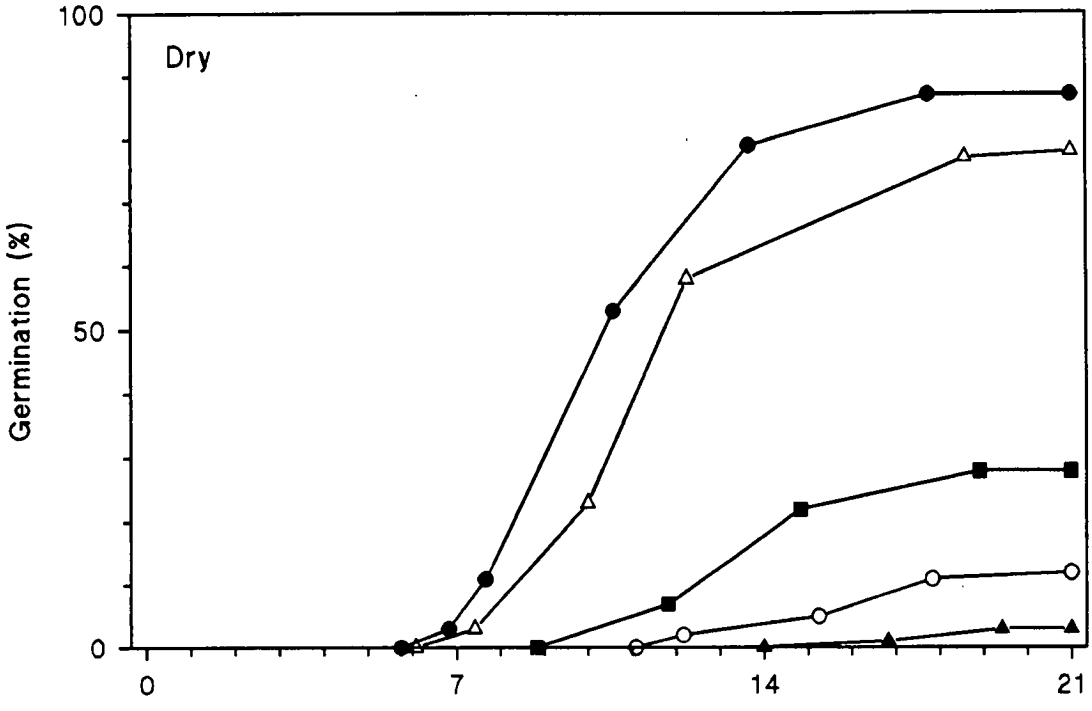
A. FILIX-FEMINA

Figure 6b



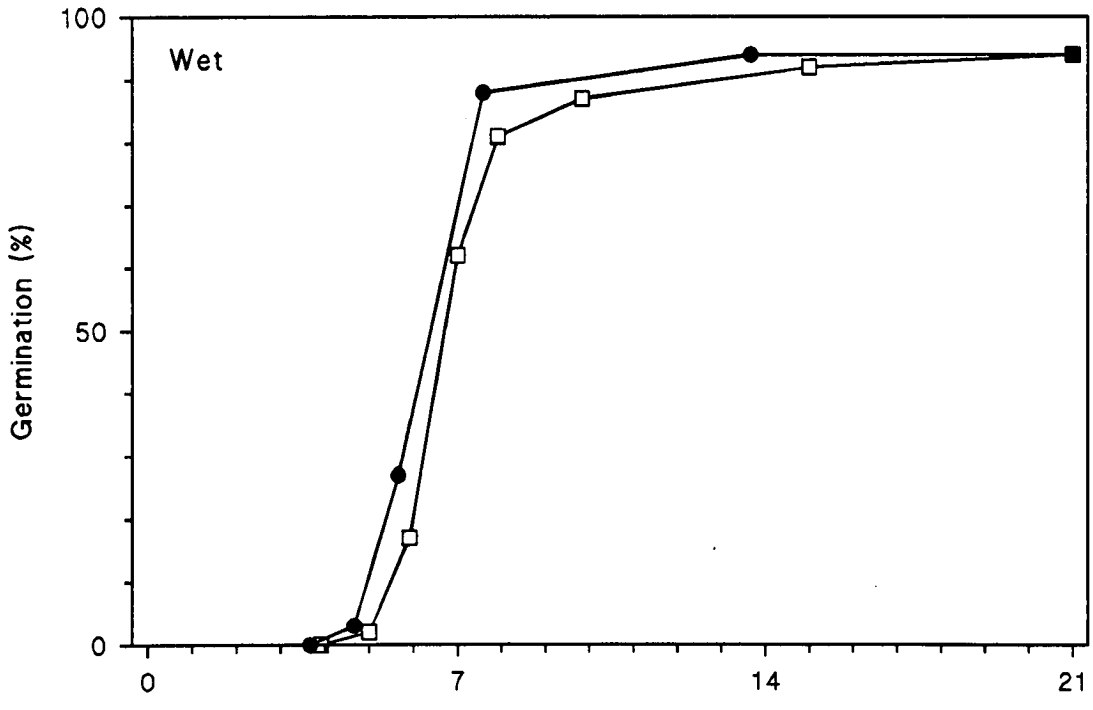
A. FILIX-FEMINA

Figure 7a



B. SPICANT

Figure 7b



B. SPICANT

Figure 8a

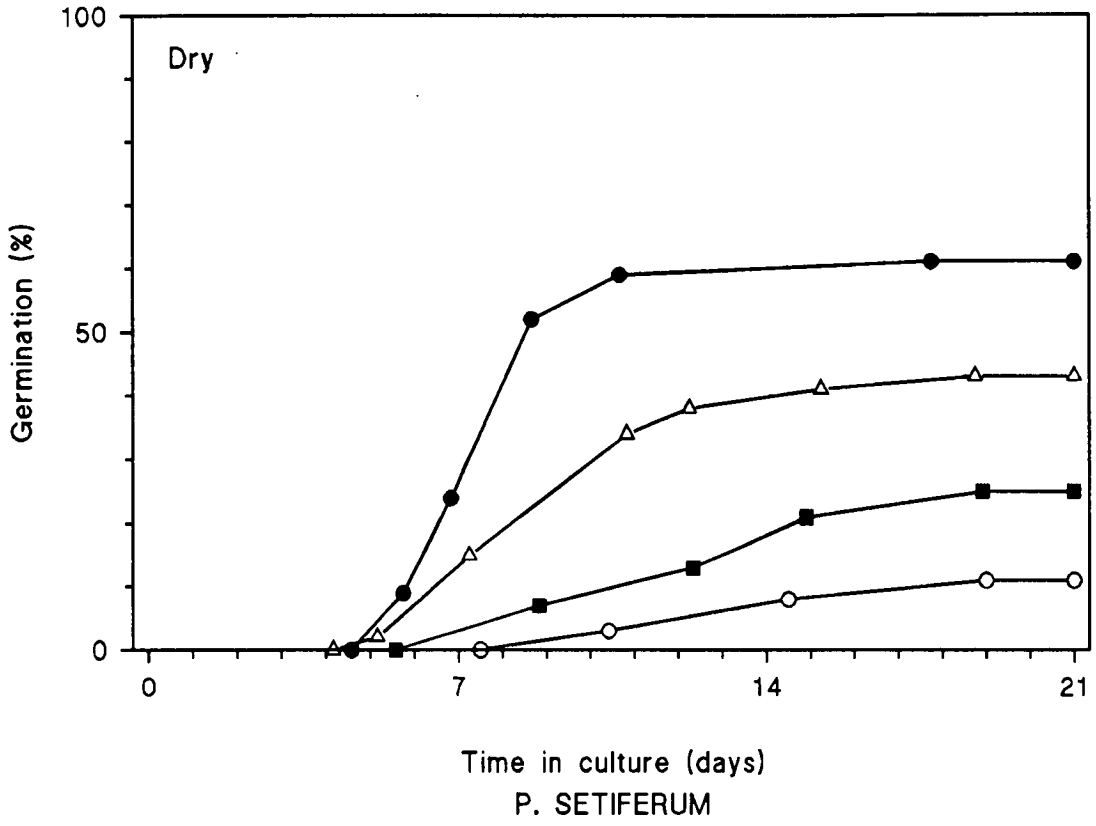


Figure 8b

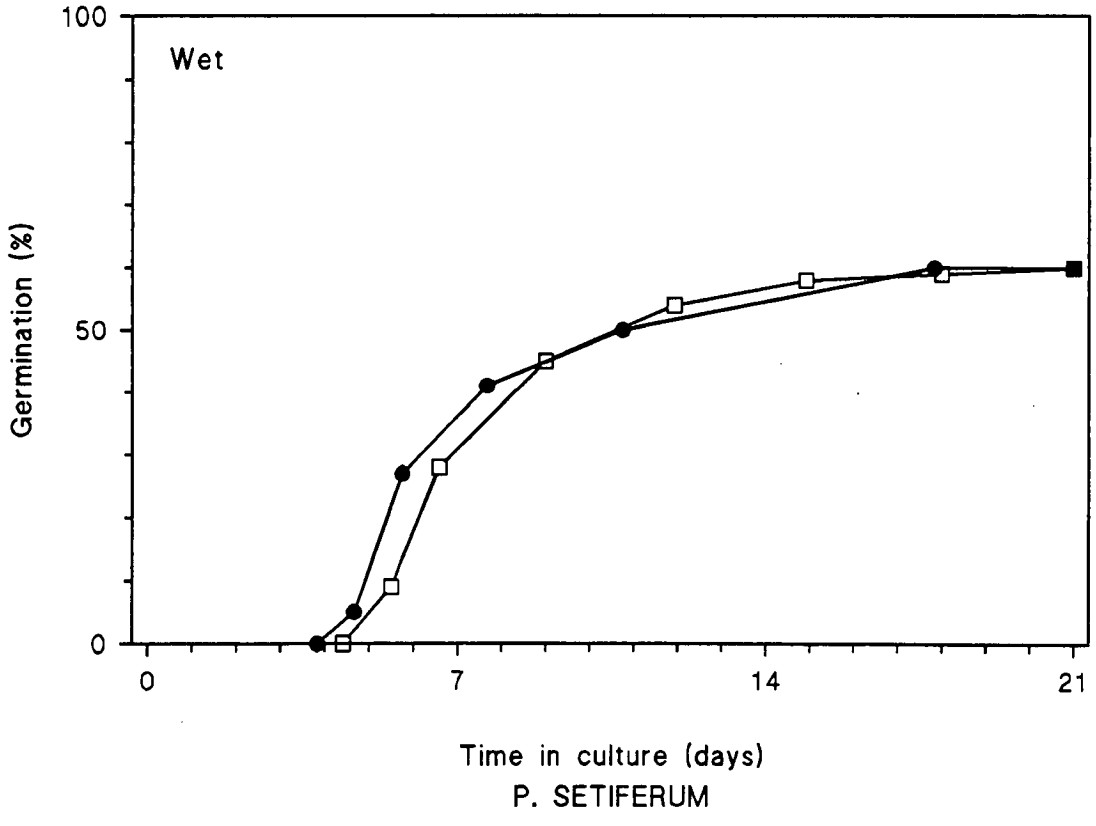


Figure 9a

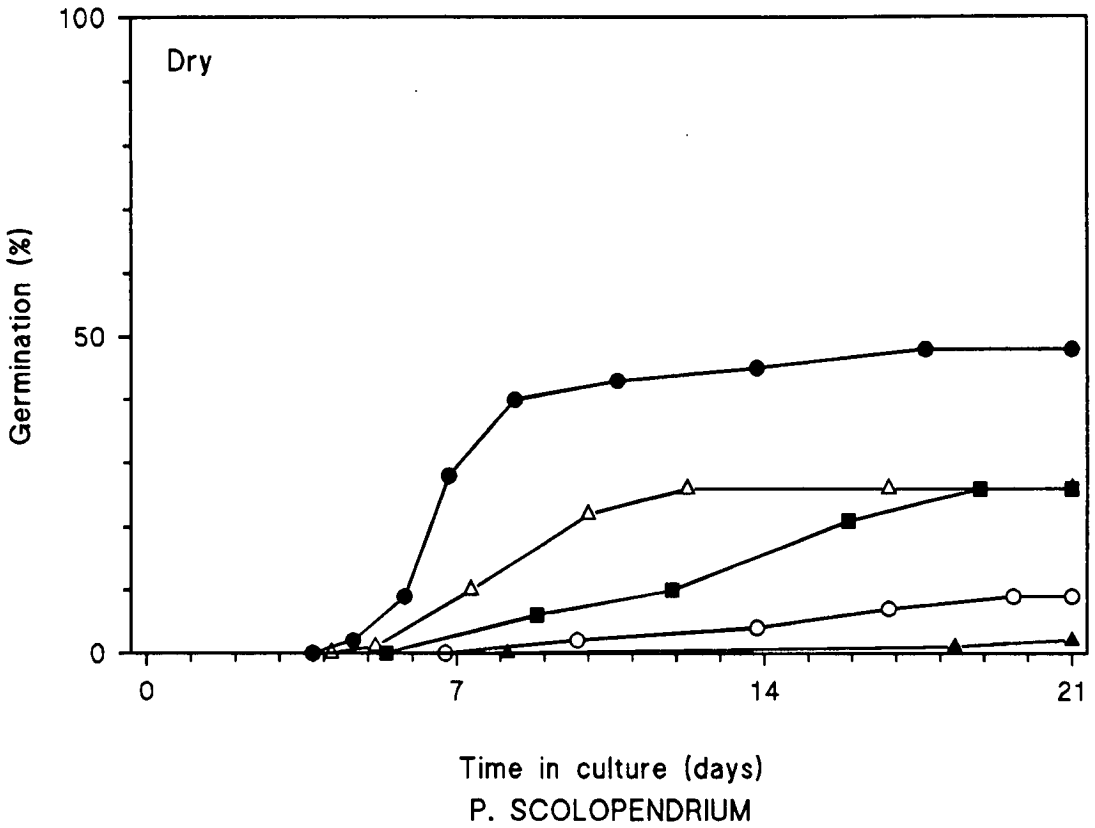
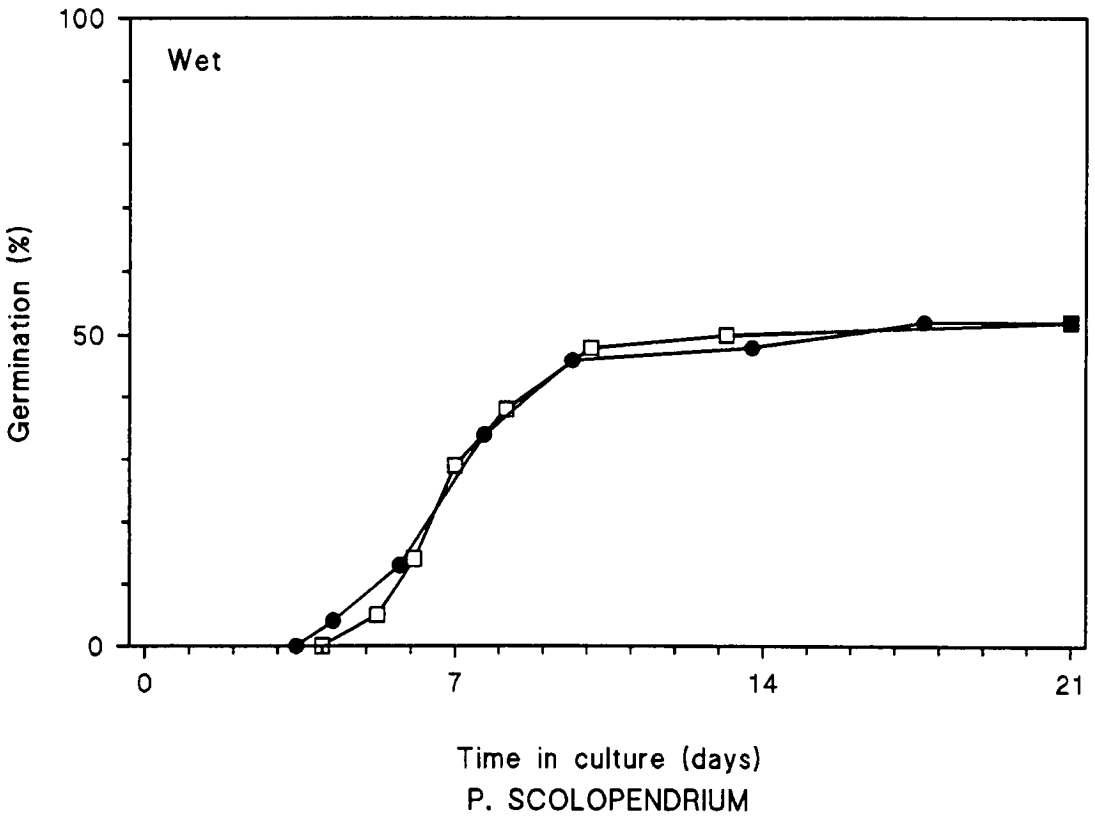


Figure 9b



Discussion

Our observations on 5 unrelated species suggest that this response of spores to hydration might be widespread among ferns. Furthermore, a recent report that spores of *Psilotum nudum* (L.) Pal. Beauv. also survive longer if stored on mineral agar (Whittier, 1990), reveals that this effect is not restricted to ferns. It is therefore surprising that it has not been previously detected in any pteridophyte.

In contrast, it has been recognized for almost 40 years (Toole and Toole, 1953) that seeds of some angiosperms (*e.g.* Brussels sprout, Lettuce, Onion) will survive longer if stored fully hydrated in aerobic conditions. It is now known that this is due to the activation, in fully hydrated seeds, of mechanisms which continuously repair or replace old and damaged cellular components (including chromosomes) and thereby counteract the normal deteriorative process of ageing (Villiers, 1974, 1975; Villiers and Edgcumbe, 1975). In dry seeds, the damage associated with ageing simply accumulates, making germination difficult (*i.e.* slow) and ultimately impossible (*i.e.* death).

Our results are easily explained if similar 'turnover' mechanisms operate in fully hydrated fern spores.

It was once thought that wet storage techniques would have a major role to play in the genetic conservation of flowering plants but, in practice, such techniques are now rarely used in seed gene banks because of the technical difficulties of maintaining seeds in a simultaneously fully hydrated, aerobic, and dormant condition (Roberts, 1989). The success and simplicity of the experiment described here clearly indicates that fern spores are much better suited for wet storage techniques. Further investigation will show whether our technique can be simplified, for example by storing spores in vials of oxygenated sterile water or liquid mineral media, without reducing its effectiveness.

Conclusion

Wet storage of fern spores deserves further study as an alternative, more natural, and more effective method for long-term storage especially a) for 'green' spores, b) when a high degree of genetic stability is required and c) where low temperature facilities are not available.

Acknowledgements

We thank Dr. C.N. Page for permission to collect spores of *T. barbara* at the Royal Botanic Garden, Edinburgh, and the Scottish Wildlife Trust (S.W.T.) for permission to collect spores of the other species at two Nature Reserves near Edinburgh. S. Lindsay would also like to thank the Science and Engineering Research Council (S.E.R.C.) for financial support.

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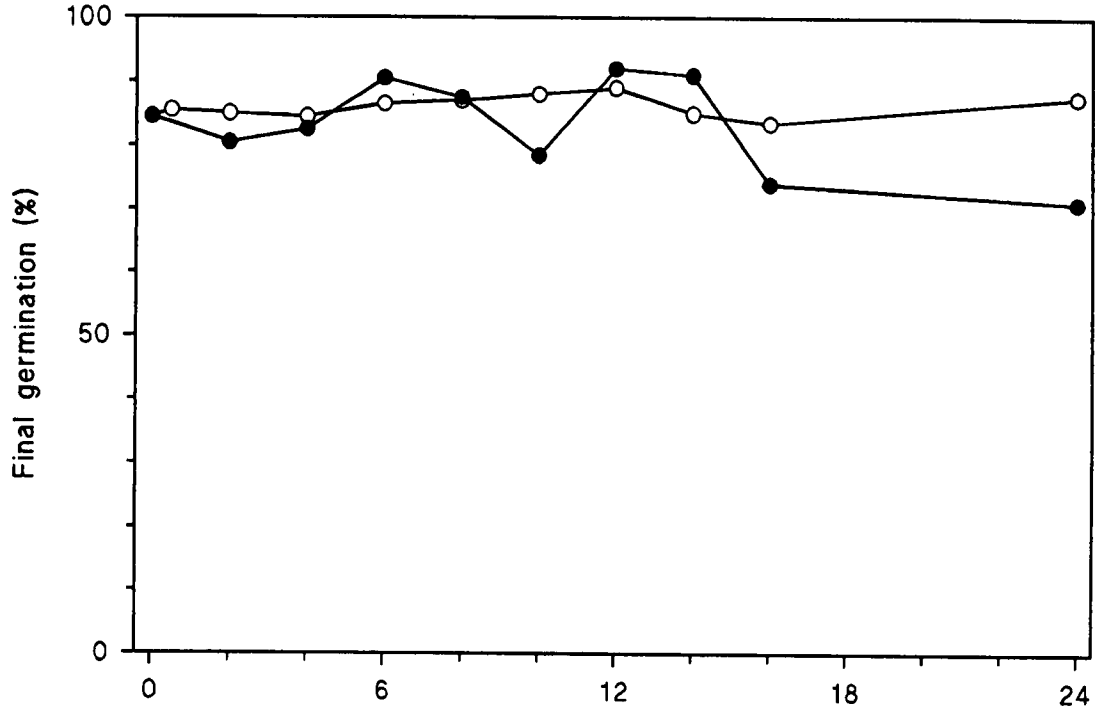
Supplement to Lindsay, Williams and Dyer (1992)

In 1989, Lindsay and Dyer published interim results of a survival experiment in which spores of *Athyrium filix-femina,* (L.) Roth, *Blechnum spicant,* (L.) Roth, *Polystichum setiferum* (Forsk.) Woynar and *Phyllitis scolopendrium* (L.) Newm. were being stored either air-dry or fully hydrated at 4°C or at 20°C [Appendix (2)]. The final results for storage at 20°C were published [with comparable data for *Todea barbara* (Williams and Dyer)] in Lindsay, Williams and Dyer (1992) [Appendix (3)]. The final results for storage at 4°C have not been published. They are presented here (Figures 1-8) in the same format as in Lindsay, Williams and Dyer (1992). Air-dry spores of all 4 species deteriorated during storage at 4°C but more slowly than those stored at 20°C. As at 20°C, fully hydrated spores did not deteriorate.

Figures 1-4. Final percentage germination after 21 days culture (='viability') of 'non-green' spores stored at *c.*4°C either air-dry (●) or fully hydrated (○) for periods of up to 2 years. Figure 1: *A. filix-femina*, Figure 2: *B. spicant*, Figure 3: *P. setiferum*, and Figure 4: *P. scolopendrium*. Many air-dry spores of *B. spicant* and some air-dry spores of the other 3 species died within 2 years. For wet spores of all species there was no loss of viability. Compare with Figures 2-5 in Lindsay, Williams and Dyer (1992).

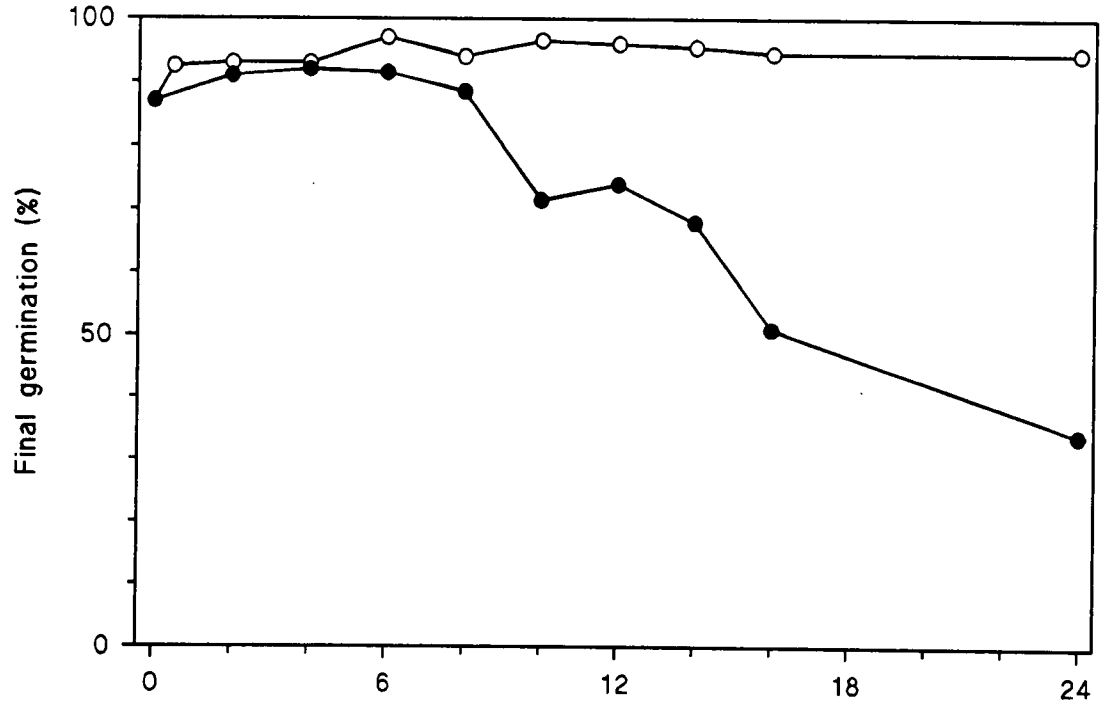
Figures 5-8. Germination curves for 'non-green' spores stored at *c.*4°C either air-dry (Figures 5a, 6a, 7a and 8a) or fully hydrated (Figures 5b, 6b, 7b and 8b) for 0 ( ), 12 (▲) and 24 (□) months. Figure 5: *A. filix-femina*, Figure 6: *B. spicant*, Figure 7: *P. setiferum* and Figure 8: *P. scolopendrium*. For dry spores, germination became slower with increasing time in storage but for wet spores there was no change in the rate of germination even after 2 years. Compare with Figures 6-9 in Lindsay, Williams and Dyer, 1992.

Figure 1



A. FILIX-FEMINA

Figure 2



B. SPICANT

Figure 3

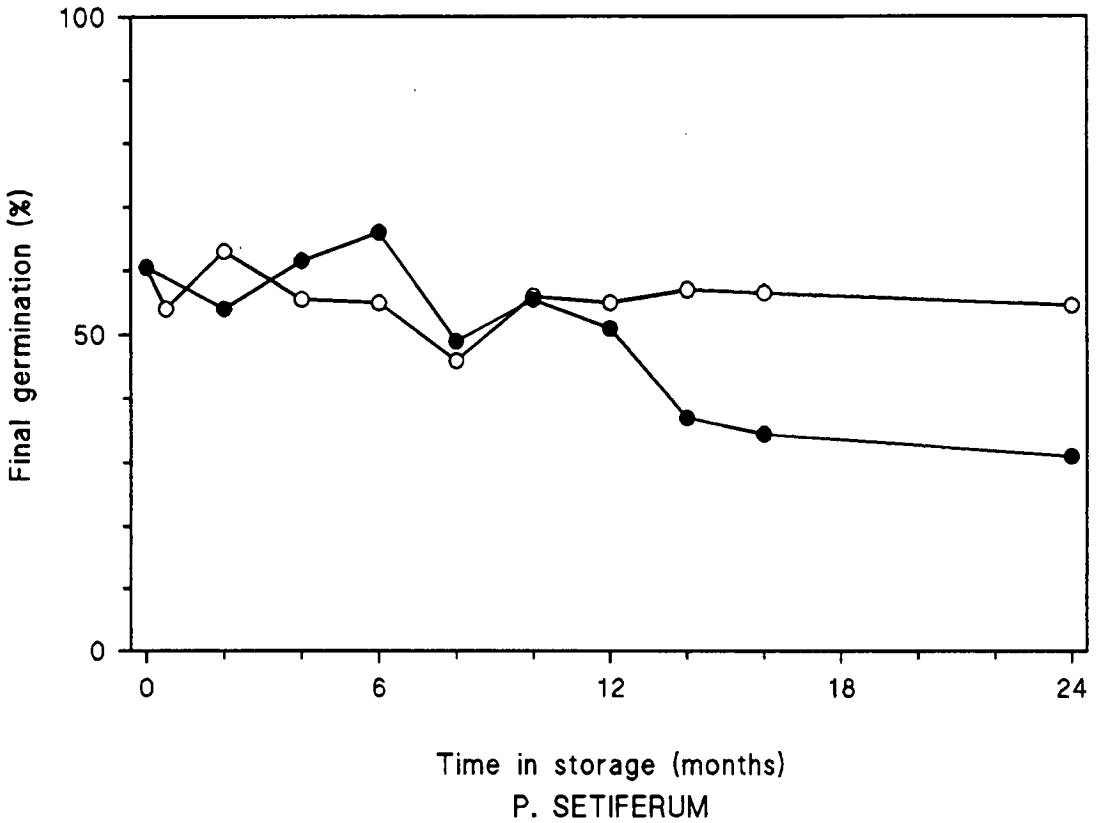


Figure 4

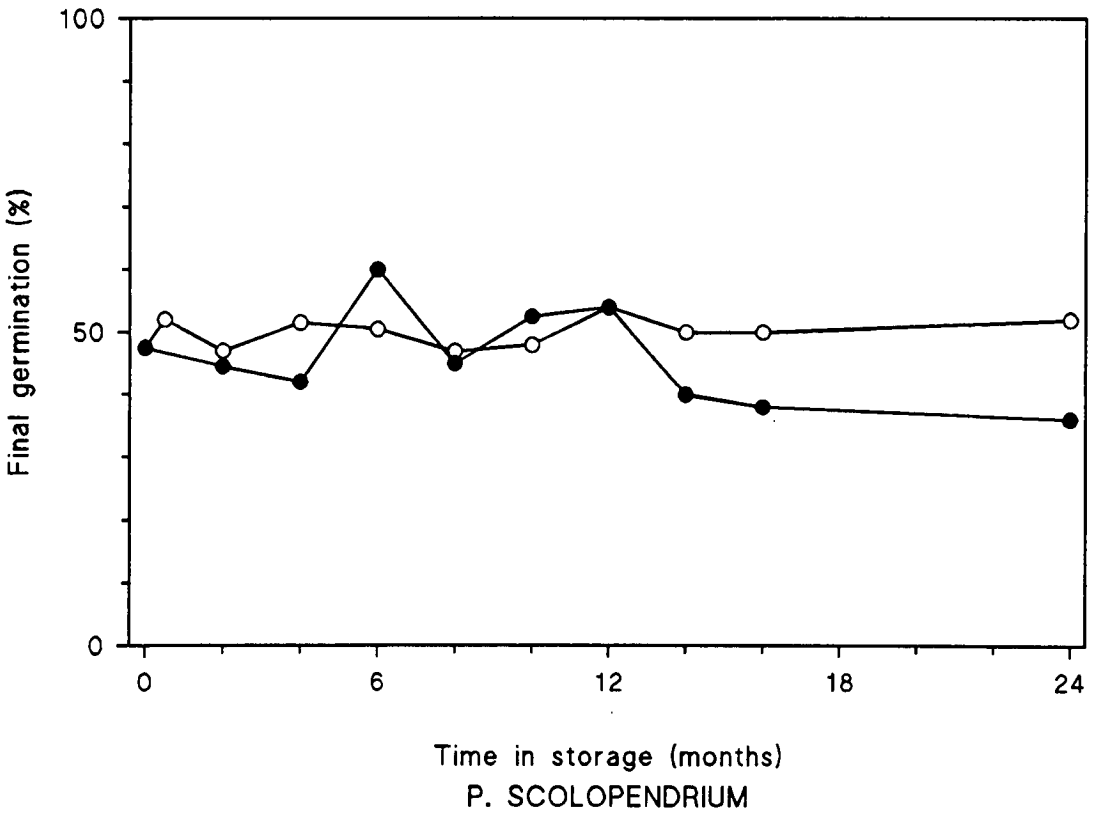
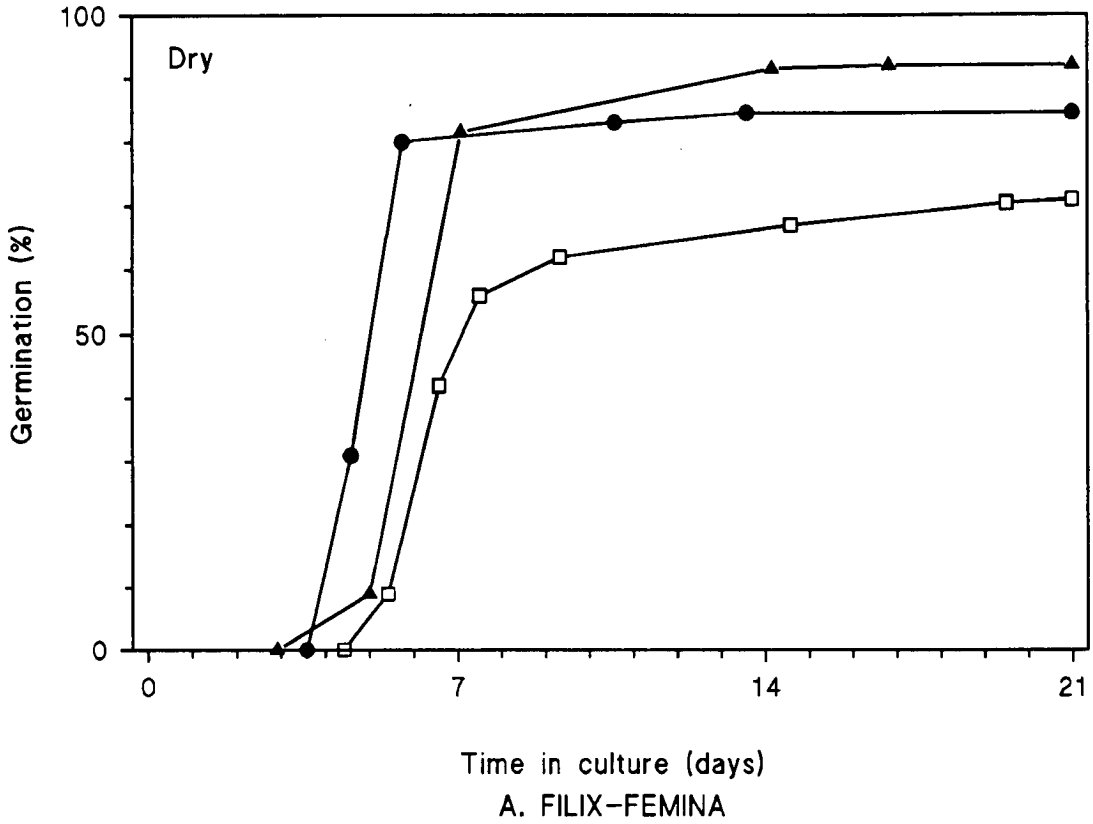
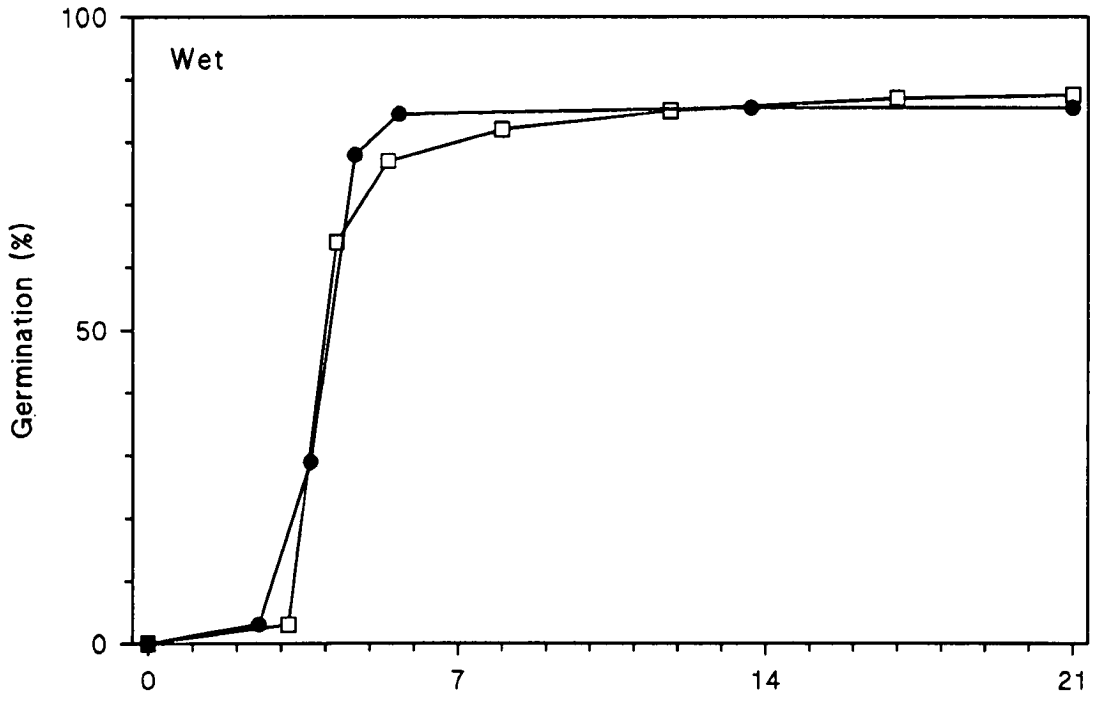


Figure 5a



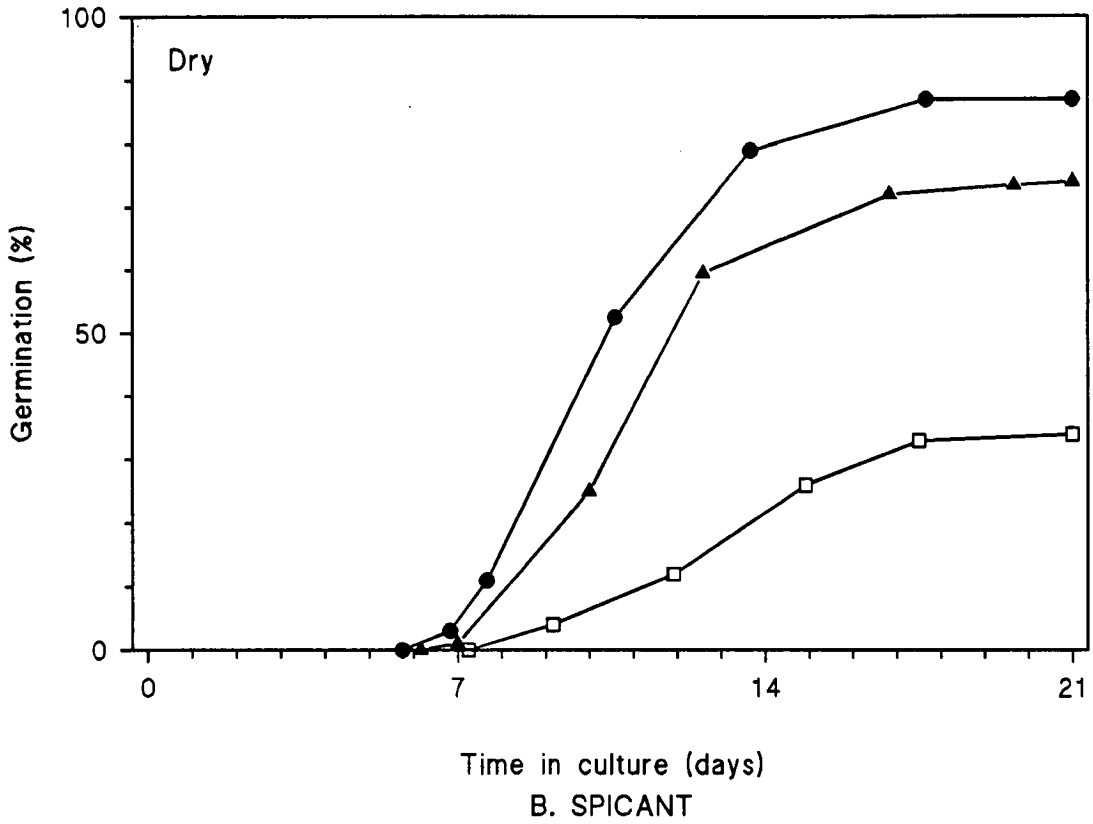
A. FILIX-FEMINA

Figure 5b



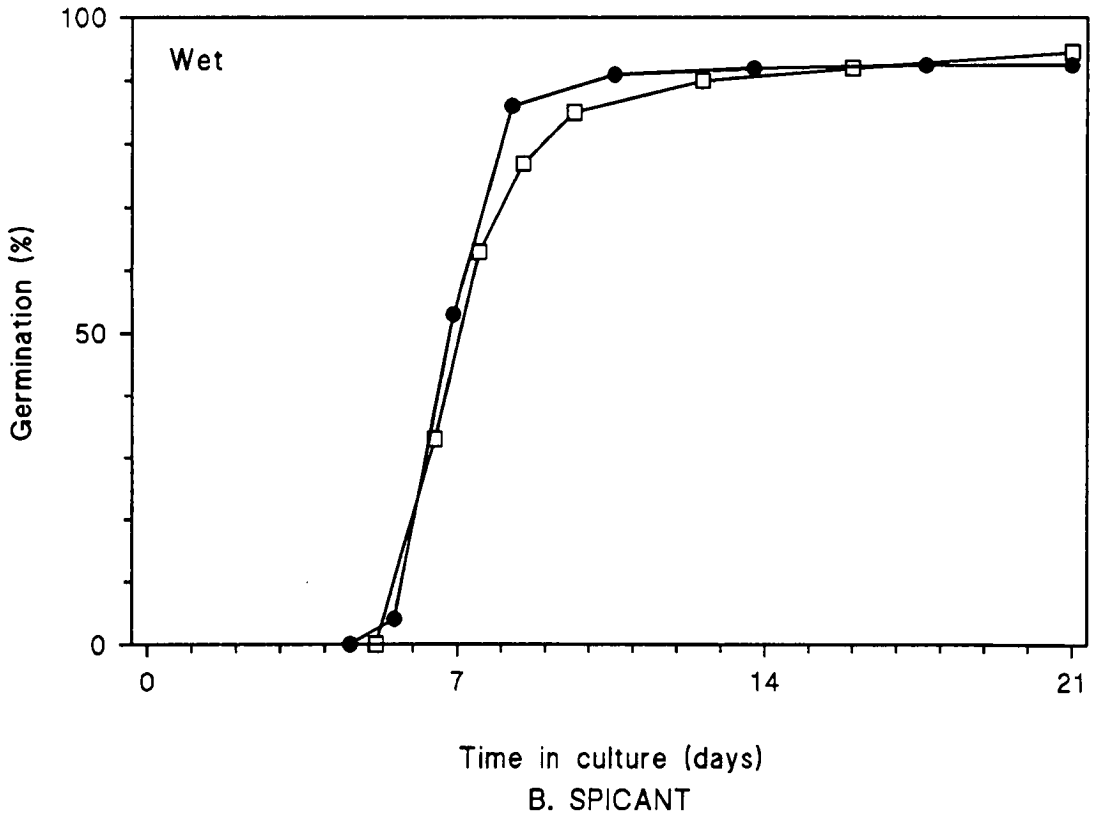
A. FILIX-FEMINA

Figure 6a



B. SPICANT

Figure 6b



B. SPICANT

Figure 7a

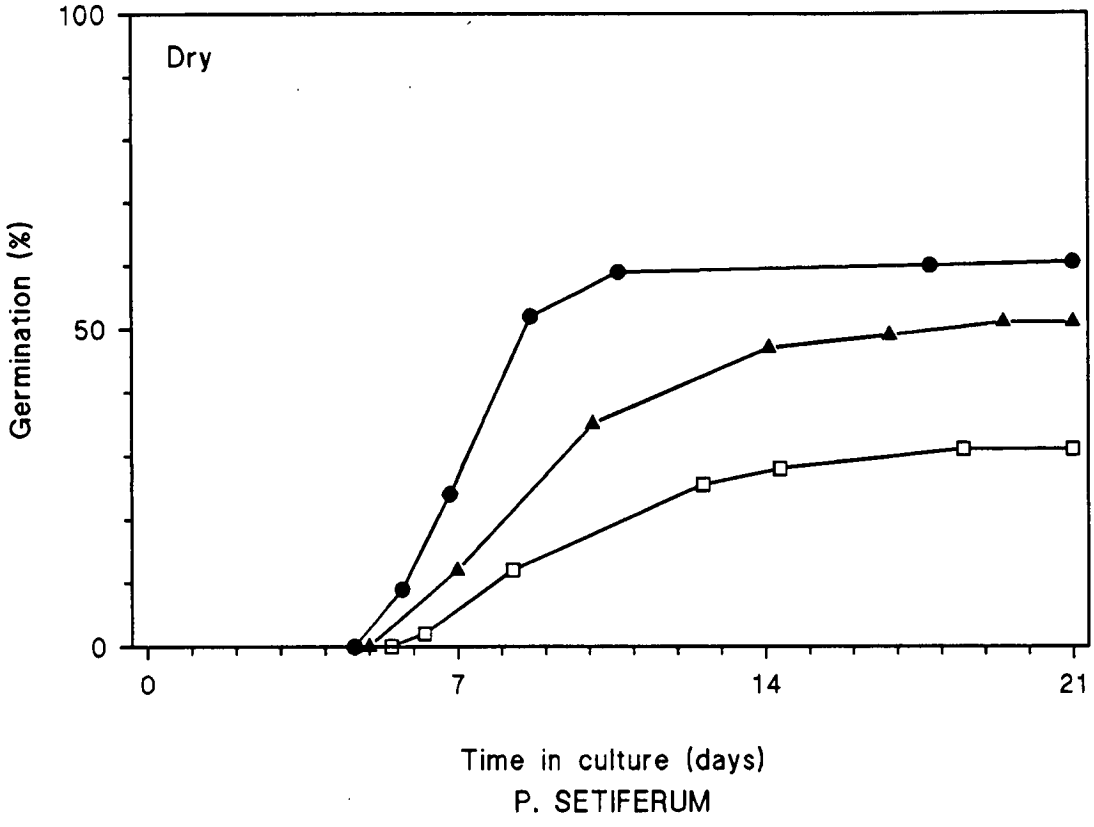


Figure 7b

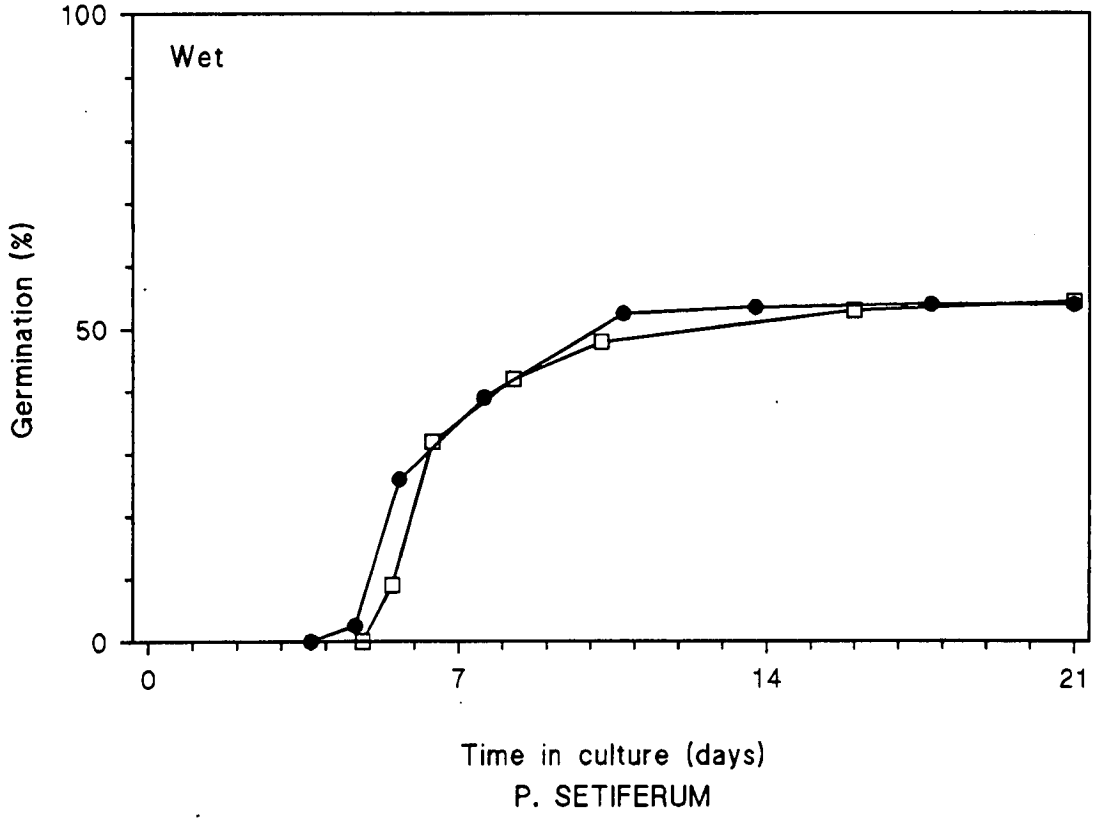


Figure 8a

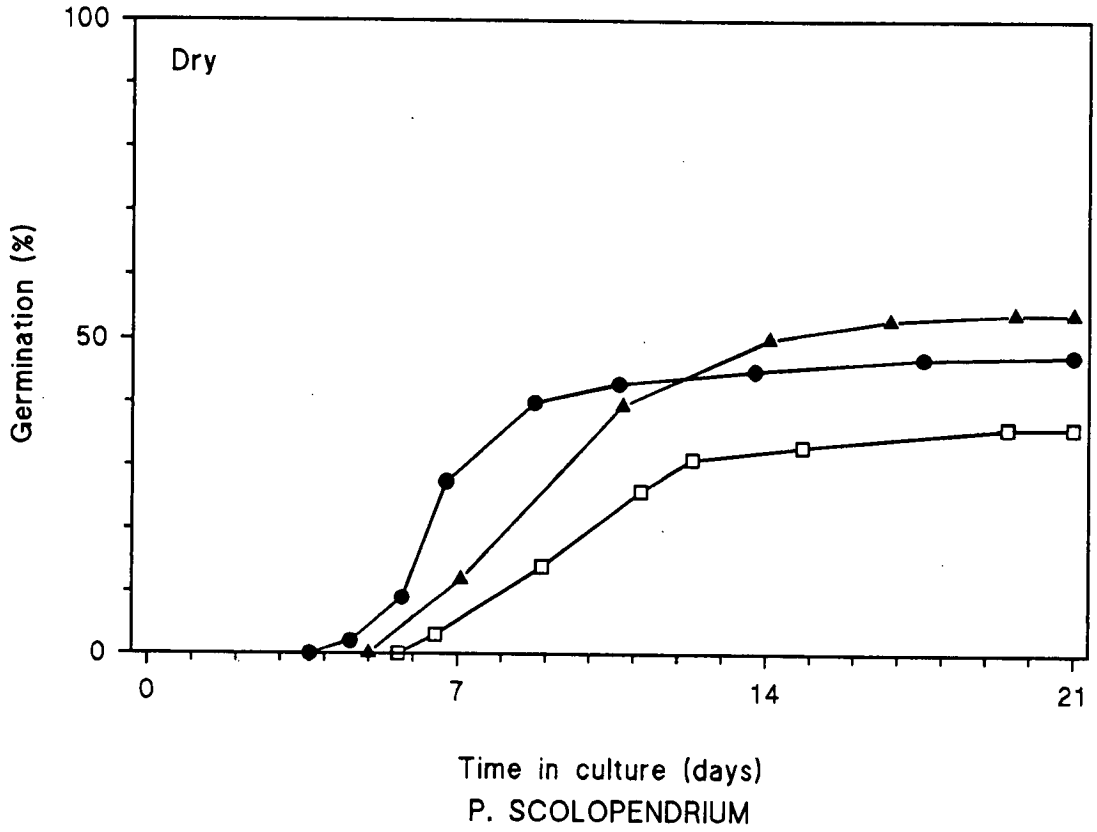


Figure 8b