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SHORT COMMUNICATION

An Assessment of Genetic and Environmental Effects on Sporangial Development in Bracken [*Pteridium aquilinum* (L.) Kuhn] using a Novel Quantitative Method

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A scheme for quantifying sporangial development on *Pteridium* fronds was constructed. Five stages of development were identified, and the percentage cover of each stage was estimated on one pinna of the frond in question. A weighted sum was calculated to give a single quantitative result. The scheme was used to identify developmental differences in bracken plants of four genotypes grown in factorial combinations of two light and two temperature regimes. Both increased temperature and increased light significantly increased the rate of sporangial development. Genotypes were significantly different from each other, and showed significant differences in response to their environment.

Key words: *Pteridium*, bracken, spore, reproduction, sporangia, environment-genotype interaction, fern ecology.

INTRODUCTION

The majority of current bracken spread is by asexual means but the plant does produce varying quantities of spores in some locations in some years (e.g. Braid, 1934; Conway, 1957; Page, 1976; Dyer, 1989). It is not known whether this variation is due to genotypic differences, environmental conditions which vary from year to year and from site to site, or a combination of the two. To determine the stimulus/stimuli to spore production in bracken, it is necessary to have a method by which sporangial development, as an indication of relative spore output, can be assessed in plants under field and experimental conditions. Development is often irregular over the frond, and hence a quantitative, rather than qualitative method is desirable, since qualitative descriptions can be highly subjective, and difficult to test statistically.

Bracken has a marginal sorus that follows the edges of the pinnulets. A double indusium forms to protect the developing sporangia, of which there may be up to five ranks (Conway, 1957). As the sporangia mature, they push through the outermost indusium, and eventually dehisce, releasing the spores. The objective of this work was to design a quantitative scheme whereby the sporangial development on different fronds could be compared over time. Extent and timing of development are likely to be related to eventual spore output since time to produce spores on a single frond is a finite resource, but quantification of this output was not attempted. The developmental scheme was used to identify whether genotype alone determines sporangial development or whether certain environmental conditions are controlling factors, or whether both interact to determine development.

MATERIALS AND METHODS

Bracken frond samples were collected from several different areas in the UK in August and September 1998 and examined for the presence of indusia and sporangia under a Wild M8 stereo microscope.

From fronds examined in the 1998 season, five stages of development were noted: 0, no indusia present; 1, indusia present, but sporangia absent; 2, sporangia present beneath indusia; 3, sporangia present above surface of indusia; 4, dehisced sporangia present. These stages were rarely found uniformly on a frond or pinna, and so an estimation of percentage cover of the sorus of each stage was necessary. One hundred percent cover was considered to be the perimeter of the pinnulets, excluding 0·5 cm at the apex of each (an area lacking indusia in all the fertile specimens examined in 1998). Since a bracken frond can be viewed as a replicating unit, with each pinna representative of the entire frond (Thomson, this volume), the unit to be sampled was standardized as the second lowest pinna.

To obtain the final quantitative measure, a sum weighted in relation to increased development was carried out as follows:

Score = (% cover of stage 0 × 0) + (% cover of stage 1 × 1) + (% cover of stage 2 × 2) + (% cover of stage 3 × 3) + (% cover of stage 4 × 4)

Thus a pinna bearing mature sporangia all along the potential sporangia-bearing area would score 400, while a

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pinna bearing only indusia along the same area would score 100.

To test whether the classification scheme gave meaningful results in an experiment designed to examine the effects of environmental variables, it was implemented in the experiment described below.

Four genotypes from two sites were distinguished using isozyme electrophoresis (see Sheffield *et al.*, 1989), and rhizome segments from each were extracted from the soil in late January 1999. Two were genets which have not been recorded sporing in their natural habitat in Glensaugh, Aberdeenshire, UK (A and B respectively in Fig. 1) and two of the genotypes were genets which are known to have spored each year for the last 5 years in their natural environment 550 km south of A and B, in the Manchester area (C and D respectively in Fig. 1). Forty-eight pieces of rhizome, about 20 cm long, from each genotype, were potted individually in a loam based compost/gravel (5:1) mix according to Tyson (1993). Twelve 41 pots from each genotype were subject to each of the four factorial combinations of high and low temperature, and high and low light. 'Low' temperature was a 16/12°C cycle (equivalent to mean summer temperatures experienced by genotypes A and B), and 'high' temperature was a 20/16°C day/night cycle (equivalent to mean summer temperatures experienced by genotypes C and D). 'High light' was a photon flux density of 1000 μmol $m^{-2}s^{-1}$, and 'low light' was 100 μmol $m^{-2}s^{-1}$, equivalent to direct sun, and deep shade, respectively (Hollinger, 1987). A white filter was used below the lights illuminating plants subject to the low light regime. Maximum photon flux density and daytime temperature for both treatments lasted 14 h, darkness and night temperatures lasted 8 h. A 1 h transition occurred between each part of the treatment, during which light and temperature increased or decreased linearly. Humidity and CO_2 concentration in the growth chambers were kept constant. The pots were returned to field capacity three times per week with tap water.

The pots remained in growth rooms under these conditions for 17 weeks, when the experiment was terminated. A score of sporangial development was noted for every plant each week after week 9. Data points shown in Figs 1–3 are mean development scores for each group of 12 replicates. Data were analysed using analysis of variance (ANOVA; Genstat, 1987), and orthogonal linear contrasts (VORTHPOL; Genstat, 1987).

RESULTS AND DISCUSSION

The rhizomes had been extracted during the dormant phase in January (Tyson, 1993) yet gave rise to fronds within 3 weeks of relocation. The morphology of fronds was similar to that of fronds on mature rhizomes in the natural environment. Sporangial development was first noted after 9 weeks, although their maturity suggested that initiation of development had been at least 1 week prior to this. Sporangial initiation was still occurring at the end of the experiment, but as the aim was to evaluate the method, and to compare rates and amounts of development, no attempt was made to determine an end-point of development.

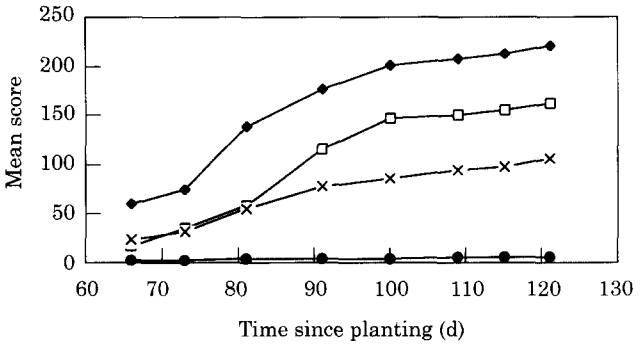

FIG. 1. Mean sporing score, pooled for each genotype [(◆) A; (□) B; (●) C; (×) D] from all treatments, for each sample date. Mean score for genotype A was significantly different from genotypes C and D at all sample dates and after 92 d all genotypes were significantly different according to ANOVA ($P < 0.05$). Standard errors ($n = 12$ for each data point) are not shown; they were less than 40% for A, B and D but for genotype C exceeded 100%. This was to be expected as the data were pooled from treatments which themselves show significant effects on development (see Figs 2 and 3). Orthogonal linear contrasts showed significant differences in the rate of development between all genotypes other than A *vs.* B ($P < 0.05$).

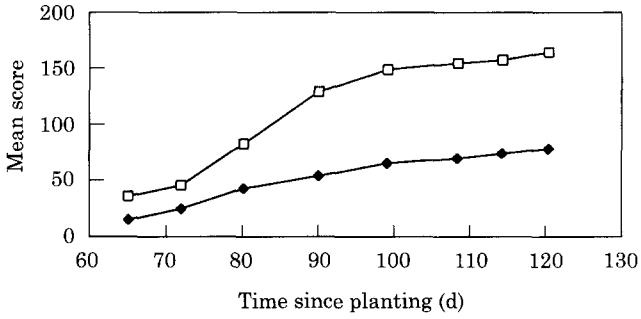

FIG. 2. Mean development score, pooled for all plants in each of two temperature regimes [(□) 20/16°C; (◆) 16/12°C] regardless of genotype or light regime, at each sample date. Standard errors of the means are not shown but were large (less than 31% in all cases), but ANOVA showed significant differences between development in the two temperature regimes at all sample dates ($P < 0.05$). Orthogonal linear contrasts showed significant differences in rate of development between the two temperatures ($P < 0.005$).

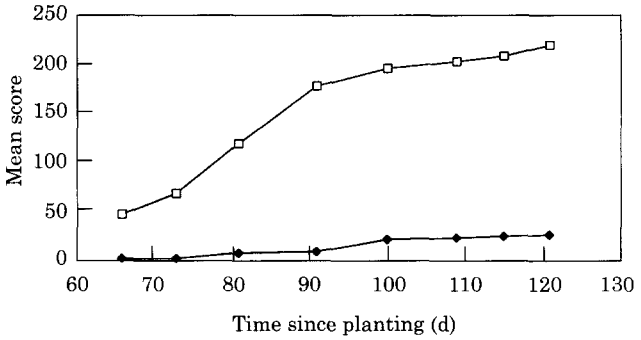

FIG. 3. Mean development score, pooled for all plants in each of two photon flux density regimes [(□) 1000 μmol m^{-2} s^{-1}; (◆) 100 μmol $m^{-2}s^{-1}$] regardless of genotype or temperature regime, at each sample date. Standard errors of the means are not shown but were large (less than 82% in low light, and less than 20% at high light). Despite this, ANOVA gave significant differences ($P < 0.001$) between data points on all sample dates. Orthogonal linear contrasts showed significant differences in rate of development between the two light regimes ($P < 0.0005$).

The score for sporangial development showed differences between genotypes both in the final measurement (ANOVA $P \leqslant 0{\cdot}002$), and in the rate of change ($P < 0{\cdot}05$), though the difference between genotypes A and B was not statistically significant (see Fig. 1). This suggested that genotype may have an important influence upon sporangial development, although the influence of previous conditions at each site cannot be discounted unless a second series of experiments with the same potted rhizomes of these genotypes grown in the same environment give similar results.

Temperature had highly significant effects (see Fig. 2). A higher temperature resulted in higher mean development scores at all sampling times ($P < 0{\cdot}045$ for each sample date). The sporing development index increased significantly more quickly at the higher temperature ($P = 0{\cdot}005$). Temperature therefore appears to have a role in controlling sporangial development, with high temperatures promoting the process, as suggested by Sheffield (1996).

High light also promoted sporangial development (see Fig. 3). The higher photon flux density gave higher development scores at every stage of sampling, with the different light conditions giving significantly different scores at all times ($P = 0{\cdot}001$). Sporing development proceeded significantly faster in the high light ($P = 0{\cdot}0005$) than in the low. The general trend of increased light and temperature acting to promote sporangial development held for each genotype, although the magnitude of the response varied.

These results were obtained from bracken plants grown in experimental conditions but do indicate that the technique for quantifying sporangial development described here could be a useful tool with which to compare *Pteridium* fronds. It provides a mechanism to give unbiased, quantitative comparisons rather than the highly subjective qualitative data collected by previous authors. Environmental conditions do clearly exert some control over sporing behaviour in bracken, as photon flux density and temperature both control the rate and extent of sporangial development of plants in cultivation. Development also appears to depend upon genotype. Collectively these data refute the suggestion of Sheffield (1996) that sporulation may be triggered by rhizome disturbance alone.

The implications of the results of this study are that plants in the natural environment that experience the same or similar environmental conditions will not necessarily respond in the same way, making generalizations about sporing in a particular season difficult. Since environmental conditions vary both within and between stands of bracken as well as within and between seasons, it is also likely that there will be patchy distribution of sporangial development, unless threshold conditions, which have not yet been fully defined, are met for each frond.

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