1991
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ISSN 0260-6925

Bangor Occasional Paper No. 20

CULTIVATION AND USE OF BRYOPHYTES AS EXPERIMENTAL MATERIAL

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February 1991

Cultivation and use of bryophytes as experimental material.

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SUMMARY

Bryophytes can be grown successfully if kept moist, supplied with nutrients, and out of direct sunlight. They remain greener on peat than on sand. However, difficulties were encountered when attempting to grow mosses and liverworts in an unshaded glasshouse, in spring and summer. Even spraying hourly with water did not prevent scorching and desiccation.

Growth can be measured using a variety of techniques; height measurement and shoot elongation from thread markers proved the most reliable.

INTRODUCTION

Bryophytes are acknowledged to be difficult subjects for experimental work, primarily due to problems in both cultivating and maintaining them, (B.G.Bell, pers.comm.). This paper outlines methods which were found to be successful during the preparation of bryophytes for an experiment, in which they were to be grown for several months in a glasshouse.

Preliminary studies were carried out to assess suitable growth media, methods of cultivation and means for marking and measuring to evaluate growth. Using the information gained from these trials, experimental material was placed in an unshaded glasshouse for the main experiment. The practical use, in a glasshouse, of the various marking and measuring methods was assessed. The responses of the species used, to this hostile environment were investigated.

MATERIALS AND METHODS

Bryophyte material (10 mosses and 4 liverworts) was collected from 5 sites within 15 miles of Bangor (Table 1).

1 Preliminary trials.

Most bryophytes are found in sheltered, moist habitats. Those workers who have successfully cultivated mosses and liverworts have maintained them in a humid atmosphere, and supplied nutrients. Therefore, material was kept moist and cool, and "potted on" within 2 to 3 days of collection, to prevent desiccation.

Plants were grown in 3" pots, covered with polythene sheeting, and kept in a shady, unheated room. Stocks of all species were grown on 50:50 mix of acid washed sand and Irish moss peat. Distilled water was applied daily as a mist spray, and 1/10th concentration Long Ashton solution added once per week.

(a) Growth media.

Material of 9 species (5 mosses and 4 liverworts, Table 2) was grown on either acid washed sand or Irish moss peat. For branched species (e.g. Pleurozium schreberi) single stems were inserted into the medium, leaving a constant length of shoot exposed. The tufted species (e.g. Dicranum scoparium) were treated similarly, except that small groups of stems were used. Approximately even-sized pieces of liverworts and epiphytic mosses (e.g. Hypnum cupressiforme), still attached to the substrate, were firmly pushed onto the sand or peat.

Visual assessments of plant health were made throughout the trial. Three species were considered suitable for growth measurements, Pleurozium schreberi, Dicranum scoparium, and

Racomitrium lanuginosum. Stems were measured, (using a calliper gauge), when initially planted on the 2 growth media, and again after 10 weeks, (Table 2).

(b) Methods of marking and measuring bryophyte growth.

Various methods of marking and assessing bryophyte growth, both experimentally and in the field, are described in the literature. These range from simply measuring shoot length from the substrate to the use of markers, either on the substrate (Pitkin,1975) or tied to shoots at known distance from the apex (Longton & Greene,1979). Preliminary trials of all three methods were conducted, and then adapted for use on the plant material in the main experiment.

2 Glasshouse experiment.

In most cases, experimental material had been potted on during August and September 1988, and kept moist until transfer to the glasshouse in late October 1988. The bryophytes were grown on Irish moss peat in 3" pots. The glasshouse was ventilated with charcoal filtered air; the flow rate set to change the atmosphere completely 1.5 times per minute. There was no shading, supplementary heating or lighting. Plants were sprayed with de-ionised water at least 3 times per day. During the exceptionally hot summer period this was increased to once per hour, during daylight hours. Nutrients were supplied weekly, initially as 1/10th concentration Long Ashton solution; from 1st February 1989 this was lowered to 1/100th concentration. The liverworts and, the very delicate moss, Hookeria lucens were covered with polythene sheeting overnight,(1700-0900), to help maintain high humidity around the plants.

Growth measurements were made, before transfer to the glasshouse, and at intervals throughout the study; the method used varied according to growth form. All liverworts, Hookeria lucens, Plagiomnium undulatum and Pogonatum urnigerum were measured for maximum height and width. Prostrate, branched mosses, (Hypnum cupressifome, Isothecium myosuroides and Thuidium tamariscinum), were measured from plastic markers in the pot to the shoot tip. Maximum height was recorded for upright growing species, (Dicranum scoparium, Pleurozium schreberi, Polytrichum formosum and Racomitrium lanuginosum). For Polytrichum formosum, the numbers of shoots per pot were also counted and categorised as (a) old, (b) old with new growth, (c) small new shoots (< 1 cm.) and (d) larger new shoots (> 1 cm.).

In December 1988, 15 extra pots fully filled with Pleurozium schreberi and 15 of Dicranum scoparium, were added. Thread markers were tied to randomly selected shoots. Growth from marker to shoot tip was measured, immediately after transfer to the glasshouse and at intervals throughout the experiment. From January this technique was extended to all the other mosses, except Hookeria lucens, (considered so fragile that attaching markers would damage plants).

RESULTS

1 Preliminary trials of growth media.

After 10 weeks, all plants of all 9 species had survived on both 100% acid washed sand and 100% peat. Plants on peat were slightly greener than those on sand. Growth measurements showed that Pleurozium schreberi, Dicranum scoparium and Racomitrium lanuginosum had all increased in size, on both media, (Table 2).

Peat was chosen for the glasshouse experiment as (a) plants remained greener on peat, (b) the pH (4.5) suited all the species to be used and (c) it would retain more moisture.

2 Glasshouse experiment; visual assessments.

All species, except Polytrichum formosum, showed signs of desiccation soon after transfer to the glasshouse. Vigilant spraying with de-ionised water enabled all species to survive the winter period, with at least 50% of the material green. However, as temperatures rose in spring and summer, all except Polytrichum formosum became increasingly damaged by scorching and desiccation, despite hourly moistening. The pots full of Pleurozium schreberi and Dicranum scoparium were much less affected than pots with smaller amounts of plant material; these, and Pogonatum urnigerum remained 60% green until March-April. However, by May these plants were yellowing, and most of the others were 80% brown. Polytrichum formosum plants had remained green with slight browning of leaf tips, and produced much new growth.

3 Growth measurements.

(a) Maximum height and/or width.

In preliminary trials, this method was used successfully on both mosses and liverworts. It proved less useful in the glasshouse, where die-back and regrowth of plants caused measurements to fluctuate, (Tables 3 & 4). However, it could still be a useful technique for those species not suitable for attachment of thread markers.

(b) Plastic markers.

This method was also successfully used, on mosses and liverworts, in the preliminary trials; measurable growth occurred beyond the markers. Unfortunately, it too proved impractical when employed in the glasshouse. Many markers were dislodged or lost, due to rain drips from the roof, and by movement of pots, during measuring and when covering with polythene sheeting, (Tables 3 & 4).

(c) Thread markers.

Accurate growth measurements were consistently obtained using this method. It also allowed the use of full pots of moss, helping to alleviate desiccation, (Tables 3 & 4).

Most species of mosses and all the liverworts grew very poorly in the glasshouse, (Tables 3 & 4;. Polytrichum formosum increased in height over the first 13 weeks of the glasshouse experiment, and produced new growth in spring (Table 5).

DISCUSSION

Preliminary trials showed that bryophytes can be successfully grown, for several months, on sand or peat; if kept out of direct sunlight, moist and supplied with nutrients. Under these conditions, health and growth can be assessed (a) visually, (b) using height and width measurements and (c) measuring increase in width, or shoot extension, from markers. Thus, if only simple cultivation and/or growth assessments are required, bryophytes can be kept on peat, under polythene, in a shady room and regularly moistened and supplied with nutrients. As these requirements are easily met, bryophtyes can be suitable experimental subjects.

As expected, the glasshouse environment proved too hostile to maintain bryophytes, in spite of regular mist spraying. All species except Polytrichum formosum suffered scorching and desiccation. The leaves of Polytrichum formosum often became adpressed, but recovered soon after spraying. This species has a rhizome and a water conductance system, and was thus less prone to desiccation than the other species.The spring and summer of 1989 were exceptionally hot and temperatures in the glasshouse were often over 30°C for prolonged periods. During a cooler summer or in autumn and winter, it would probably be feasible to grow mosses (but not liverworts) successfully in a shaded glasshouse.

Simple height measurements, for upright growing species, and the use of thread markers to assess shoot elongation, proved the most reliable methods of assessing growth.

ACKNOWLEDGMENTS

The work was part of a project funded by the Nature Conservancy Council. Mr.T.Blackstock and Dr.M.O.Hill advised on selection of species. Dr.S.Woodin and Professor J.Lee provided helpful discussion throughout.

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Table 1.

Species used in the glasshouse study, and details of collection sites.

SPECIES	COLLECTION SITE	GRID REFERENCE
Mosses:-		
Dicranum scoparium Hedw.	Stabilised slate waste tip near Bethesda, slate waste.	SH 620660
Hookeria lucens (Hedw.)Sm.	Banks of river Ogwen, near Bethesda, shady, humid area under mature trees.	SH 608687
Hypnum cupressiforme agg.	Stabilised slate waste tip near Bethesda, tree bark.	SH 620660
Isothecium myosuroides Brid.	Stabilised slate waste tip near Bethesda, tree bark.	SH 620660
Plagiomnium undulatum(Hedw.)Kop.	Wooded road verge, near Llanddona. Soil.	SH 558800
Pleurozium schreberi (Brid.) Mitt.	Stabilised slate waste tip near Bethesda, slate waste.	SH 620660
Pogonatum urnigerum (Hedw.) P.Beauv.	Exposed boulders in stone wall in road verge, Llanddona.	SH 564795
Polytrichum formosum Hedw.	Experimental pots at I.T.E. Bangor.	
Racomitrium lanuginosum (Hedw.) Brid.	Stabilised slate waste tip near Bethesda, slate waste.	SH 620660
Thuidium tamariscinum (Hedw.) Br.Eur.	Wooded road verge, near Llanddona. Soil.	SH 558800

Liverworts:-

Plagiochila porelloides (Torrey ex Nees) Lindenb.	Banks of river Ogwen, near Bethesda. Shady, humid area, under mature trees.	SH 608687
Plagiochila spinulosa(Dicks.)Dum.	" " " " " " " "	
Scapania gracilis Lindb.	" " " " " " " "	
Scapania nemorea (L.) Grolle	" " " " " " " "	

Plant names according to Smith, 1978 (mosses) and Watson, 1981 (liverworts).

Table 2.

Bryophytes grown on Irish moss peat or sand, for 10 weeks.

SPECIES	INITIAL SHOOT LENGTH (in centimetres) ON PEAT AND SAND	LENGTH AFTER 10 WEEKS PEAT	SAND
Dicranum scoparium	4.0	4.5	5.5
Pleurozium schreberi	7.0	10.0	10.0
Racomitrium lanuginosum	2.0	3.5	2.8

Species used for peat:sand comparison, not measured:-
Hypnum cupressiforme
Isothecium myosuroides
Plagiochila porelloides
Plagiochila spinulosa
Scapania gracilis
Scapania nemoreum

Table 3.

Growth of mosses in the glasshouse.

SPECIES	TIME	MEASUREMENT	(cm.)
Dicranum scoparium	10/88-1/89 (11 weeks)	Height increase	-0.19
	12/88-1/89 (4 weeks)	Shoot elongation	-0.03
	1/89-4/89 (12 weeks)	Shoot elongation	-0.03
Hookeria lucens	10/88-1/89 (11 weeks)	Height increase	-1.30
		Diam.increase	-2.96
	1/89-4/89 (12 weeks)	Height increase	+0.18
Hypnum cupressiforme	10/88-1/89 (11 weeks)		+0.17
		Shoot	+0.01
	1/89-4/89 (12 weeks)	elongation	+0.18
Isothecium myusuroides	10/88-1/89 (11 weeks)		+0.06
		Shoot	+0.03
	1/89-4/89 (12 weeks)	elongation	+0.22
			+0.38
Plagiomnium undulatum	11/88-1/89 (8 weeks)	Height increase	+0.60
		Diam.increase	+2.54
	1/89-4/89 (12 weeks)	Height increase	-0.41
		Diam.increase	-3.56
		Shoot elongation	+0.19
Pleurozium schreberi	10/88-1/89 (11 weeks)	Height increase	-0.02
	12/88-1/89 (4 weeks)	Shoot elongation	+0.04
	1/89-4/89 (12 weeks)	Shoot elongation	+0.15
Pogonatum urnigerum	11/88-1/89 (8 weeks)	Height increase	+0.41
		Diam.increase	+2.01
	1/89-4/89 (12 weeks)	Height increase	+0.37
		Diam.increase	+1.60
		Shoot elongation	+0.19

Polytrichum formosum	10/88-1/89 (13 weeks)	Height increase	+0.26
	1/89-3/89 (8 weeks)	Height increase	+0.17
		Shoot elongation	+0.01
Racomitrium lanuginosum	10/88-1/89 (11 weeks)	Height increase	-0.02
	1/89-4/89 (12 weeks)	Height increase	-0.19
		Shoot elongation	+0.20
Thuidium tamariscinum	11/88-1/89 (8 weeks)	Shoot elongation	+0.34
	1/89-4/89 (12 weeks)		+0.26

Table 4.

Growth of liverworts in the glasshouse.

SPECIES	TIME	GROWTH MEASUREMENT MAX.HT. (cm.)	MAX.WIDTH (cm.)
Plagiochila porelloides	11/88-1/89 (8 weeks)	+ 0.07	+ 1.25
	1/89-4/89 (12 weeks)	- 0.08	+ 0.86
Plagiochila spinulosa	10/88-1/89 (11 weeks)	- 0.44	+ 0.10
	1/89-4/89 (12 weeks)	+ 0.19	+ 0.09
Scapania gracilis	10/88-1/89 (11 weeks)	- 0.03	+ 0.55
	1/89-4/89 (12 weeks)	+ 0.14	+ 0.23
Scapania nemoreum	10/88-1/89 (11 weeks)	+ 0.04	+ 0.38
	1/89-4/89 (12 weeks)	+ 0.05	+ 0.10

Table 5.

Numbers of shoots of *Polytrichum formosum*.

TIME IN GLASSHOUSE	SHOOT TYPE	NUMBER OF SHOOTS
4/4/89	Old shoots	22.1
4/4/89	New shoots(> 1 cm.)	44.8
4/4/89	New shoots(< 1 cm.)	6.6
2/5/89	Old shoots with new growth	6.9
19/5/89	Old shoots with new growth	7.5