Chapter (non-refereed)

Pelham, J.; Mason, P.A.. 1981 Nutritional variants of birch. In: Last, F.T.; Gardiner, A.S., (eds.) *Forest and woodland ecology: an account of research being done in ITE.* Cambridge, NERC/Institute of Terrestrial Ecology, 78-81. (ITE Symposium, 8).

17. NUTRITIONAL VARIANTS OF BIRCH

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Insofar as they have been studied, trees, like other plants, vary greatly in their above ground form and behaviour and their responses to a variety of environmental factors. However, comparatively little is known of their response to different soils. Bearing in mind that many non-woody plants differ in their abilities to (i) take up and utilise nutrients and (ii) tolerate toxic concentrations of pollutants, as may be found on sites of dereliction, it would be surprising if comparable differences did not occur among trees. Tree nutrition should not be considered without reference to mycorrhizas, the intimate associations formed when fungi colonize roots sometimes causing gross changes in root morphology, eg the stubby roots developing as a result of colonization by sheathing (ecto-) mycorrhizal fungi. Because these associations have many features in common with root colonization by pathogens and symbionts, such as the nodule bacterium, *Rhizobium trifolii* Dangerd, of legumes, and because the success or failure of the latter is controlled by complementary genetical factors in the plant host and microbial colonizer, it was decided to add an investigation of the genetical factors controlling mycorrhizal formation to broaden a study of tree nutrition. For this work 2 of the 3 British native species of birch (*Betula pendula* Roth. and *B. pubescens* Ehrh.) were chosen because they are (i) widespread and contribute significantly to the landscape particularly of the uplands, (ii) fairly precocious and prolific producers of small seeds with few internal reserves, (iii) readily grown in axenic conditions more particularly as standard surface sterilants easily control the superficial microbes that contaminate the seeds, and (iv) propagated vegetatively without undue difficulty. Initially *Amanita muscaria* (L. ex Fr.) Hooker, the fly agaric, was chosen as the mycorrhizal associate because (i) it was already known to form sheathing mycorrhizas with birches both in 'the field' and controlled laboratory conditions, (ii) it is readily recognised, (iii) it grows adequately on laboratory culture media, and (iv) being a basidiomycete it opened the possibility of exploiting the series of well established techniques of genetic manipulation.

Because of the desirability of working in axenic conditions, seeds collected from birch trees growing on a wide range of sites were surface sterilized with hydrogen peroxide and germinated on water agar before being transferred to slopes of mineral nutrient agar in transparent polystyrene tubes with plastic caps (Pelham & Mason, 1978). The standard nutrient medium contained all the nutrients required for optimum growth as specified for birch by Ingestad (1971). Growth was increased by piercing the caps and plugging the resultant holes with cotton wool but was decreased by increasing the concentration of salts. To minimize variation attributable to genetic segregation in out-bred and variable populations of seedlings, a technique was developed for aseptic vegetative propagation. Single node cuttings, each with a leaf, were taken from seedlings 6-8 weeks old and placed on an agar medium containing minerals, sucrose and naphthalene acetic acid (NAA) so as to accelerate root production. The optimal concentrations of these additives were determined experimentally—none was essential (Table 22). The currently used rooting medium contains the nutrients specified

TABLE 22 Effect of 20 g/l sucrose and 1mg/l naphthalene acetic acid (NAA), singly and combined, on the rooting of birch cuttings when assessed after 17 days cultivation on either water or Ingestad's agar.

Substrate	% rooted	Mean root no. per cutting	Time of root emergence (days)
Water agar	6	0.1	11
+ sucrose	14	0.2	10
+ NAA	86	3.1	7
+ sucrose + NAA	64	3.0	7
Ingested agar	6	0.1	11
+ sucrose	25	0.3	10
+ NAA	93	5.1	6
+ sucrose + NAA	93	6.2	6

TABLE 23 Effects of different amounts of P on the growth of low phosphorus 'tolerant/intolerant' clones of *Betula pubescens* and *B. pendula* *.

Growth characteristic	Species of *Betula*	Low phosphorus 'tolerant' (mg P/l) 26.0	0.4	Low phosphorus 'intolerant' (mg P/l) 26.0	0.4	S.E.
Leaf area (cm^2)	*pendula*	8.8 *(2.17)*	8.0 *(2.08)*	7.9 *(2.07)*	3.5 *(1.25)*	*(±0.213)*
	pubescens	9.9 *(2.29)*	5.9 *(1.77)*	14.7 *(2.69)*	5.2 *(1.66)*	
Stem length (mm)	*pendula*	46.5 *(3.84)*	33.8 *(3.52)*	39.0 *(3.66)*	14.4 *(2.67)*	*(±0.098)*
	pubescens	41.2 *(3.72)*	30.4 *(3.41)*	41.4 *(3.72)*	13.9 *(2.63)*	
Root number	*pendula*	19.5 *(2.97)*	56.4 *(4.03)*	14.4 *(2.67)*	18.6 *(2.92)*	*(±0.328)*
	pubescens	9.4 *(2.24)*	51.6 *(3.94)*	29.8 *(3.40)*	39.3 *(3.67)*	

* For statistical analysis, estimates of growth (x) were transformed to $\log_e x$ (italicised figures).

by Ingestad (1971) supplemented with 0.1 mg/ l NAA and 10 g/l sucrose. Cuttings from the oldest and youngest nodes of a plant respectively grew significantly more and less rapidly than cuttings taken from intermediate nodes, a degree of variation that was acknowledged in experimental randomisations.

To allow experiments to extend beyond 8 weeks, plants have been grown aseptically using containers made by sealing a clear plastic beaker into the lid of a petri dish. A cotton wool plugged hole in the beaker assisted ventilation and the lid was sterilized by gamma irradiation. The modified lids were used to replace the conventional lids over plantlets already growing in petri dishes containing nutrient medium, thus providing more space for aerial growth. These containers and plantlets were grown at room temperature in cabinets lined with aluminium foil and continuously lit with fluorescent strip lights (Plate 11).

Synthesis of mycorrhizas was achieved by growing 10 day old birch seedlings on agar slants containing Ingestad's mineral nutrients plus glucose and thiamine hydrochloride (Mason, 1975). Each seedling was inoculated with a block of Hagem agar (Modess, 1941) culture of selected isolates of *A. muscaria* collected from birch and pine in Britain, India and the USA. The inoculated seedlings were incubated as described above for 6-8 weeks.

1. Results

By modifying the concentrations of different nutrients, it was possible to assess the growth capabilities of different clones. Between-clone differences were detected when 16 clones were grown in media with a decreased concentration of phosphate (P), but not when the standard concentration of phosphate was used. Thus, in an experiment with a pair of selected contrasting clones of each species the aerial parts of "low phosphorus tolerant" clones grew nearly as much with the smaller as with the standard amount of phosphate, whereas "low phosphorus intolerant" clones grew significantly less when phosphate concentrations were decreased (Table 23). In contrast, decreasing the concentration of phosphate increased root growth of "intolerant" plants slightly but greatly stimulated that of "tolerant" plants. Chemical analysis of the tissues of the 16 clones grown at standard and lesser phosphate concentrations showed that, although at low concentrations the amount of P (%) in the tissues was similar, there were considerable differences between clones and even between species when grown at the standard phosphate concentration. Differential responses also occurred when birch cuttings were grown on media with different concentrations of calcium.

The studies of nutrient response were extended to assess effects of 3 different concentrations of phosphate on the establishment of mycorrhizas by 6 isolates of *A. muscaria* (Table 24). Mycorrhizas were only formed by one isolate on a medium with the lowest concentration of phosphate, whereas 4 of the 6 formed mycorrhizas at higher concentrations.

At the optimum concentration (6.5 ppm P) both seed-lot and fungal isolate determined the number and branching of mycorrhizas. Moreover in an experiment in which 4 isolates were applied to 4 seed-lots, one isolate collected from beneath a spruce tree formed more mycorrhizas with 3 of the birch seed-lots than did either of the birch isolates (Table 25). The performance of the fourth isolate

TABLE 24 Effects of 3 phosphorus concentrations on the development of mycorrhizas when seedlings of *Betula pendula* (ex Scotland) were inoculated with 6 isolates of *Amanita muscaria.*

Cultures of *A. muscaria*		Presence (+) or absence (–) of mycorrhizas on *B. pendula* growing in media with different P concentrations		
Isolate	Origin	3.25 ppm	6.5 ppm	26 ppm
1	Britain *(Betula)*	–	+	+
2	Britain *(Betula)*	+	+	+
3	Britain *(Pinus)*	–	+	+
4	USA *(Pinus)*	–	–	–
5	USA *(Pinus)*	–	+	+
6	USA *(Pinus)*	Not tested	+	–

TABLE 25 Interacting effects of 4 isolates of *Amanita muscaria* and 4 seed-lots of *Betula pendula* on numbers of mycorrhizas on roots of seedlings.

Cultures of *A. muscaria*		Seed-lots of *B. pendula*			
Isolate	Origin	Q11	V21	W 5	W 2
7	Britain *(Betula)*	6.2	5.4	10.0	15.4
8	Britain *(Betula)*	14.2	14.8	20.6	18.4
9	Britain *(Picea)*	26.0	14.6	23.8	19.6
10	India *(Pinus)*	19.8	20.2	12.6	12.8

from beneath *Pinus pseudostrobus* Lindl. in southern India also indicates the ability of isolates of *A. muscaria* to infect other tree species.

Although the tubes used in these experiments severely limited the size of birch plants produced, *A. muscaria* considerably stimulated root growth in many experiments, the amount of increase being clearly dependent upon both seed-lot and isolate used. For example, 3 isolates each stimulated the numbers and total lengths of roots as compared with the uninoculated control, but to differing degrees (Table 26).

Inoculation with *A. muscaria* was also associated with significantly increased stem diameters in the colonized birch seedlings (Table 27). Inspection of transverse sections of stems under the microscope revealed that, in addition to altering the widths of the different tissues, inoculation also altered their fine structure (Mason *et al.*, 1977).

The most conspicuous changes in inoculated plants were (i) the accelerated development of bark and (ii) the stimulation of larger, more rounded cortical cells with intercellular spaces, differences that might enable mycorrhizal seedlings to be better "equipped" when planted in "stress conditions" of waterlogging or drought.

From the work done to date, it seems that the response within a species to added nutrients is variable. The value of this inherent variation in fitting a particular tree to a site and for increasing yield has yet to be determined, as has its inheritance and combining ability in breeding programmes. In the future, when the genetics of mycorrhizal systems are better known, it may be deemed necessary for the tree breeder to select, breed and multiply the fungal isolate best suited to the trees being bred. An extension of this work to some of the currently popular economic tree species and their associated fungi is being considered.

TABLE 26 Effects of 3 isolates of *Amanita muscaria* on the development (n) of roots of seedlings of *Betula pendula* (ex Scotland). Data were transformed to log (n + 1) for analyses; detransformed values in brackets.

		Growth of roots of *B. pendula*		
	Inoculation treatments	Number of roots per seedling	Total root length per seedling (mm)	Mean length per root (mm)
Isolates of *A. muscaria*	1	4.70 *(111)*	5.48 *(254)*	1.11 *(2.23)*
	2	4.48 *(91)*	5.43 *(241)*	1.28 *(2.61)*
	3	3.89 *(65)*	4.79 *(178)*	1.27 *(2.57)*
	Uninoculated control	3.60 *(46)*	4.29 *(105)*	1.13 *(2.11)*
Least significant difference (P = 0.05)		0.145	0.122	0.061

TABLE 27 Effect of inoculating roots of *Betula pendula* with the mycorrhizal fungus *Amanita muscaria* on the width of the different stem tissues.

Treatment	Width of stem tissues (μm)			
	Epidermis/bark	Cortex	Xylem/pith	Total
Uninoculated control	16	100	514	629
Inoculated with:				
isolate 11	100 *(530)*	121 *(22)*	543 *(6)*	765 *(22)*
isolate 12	85 *(430)*	138 *(38)*	604 *(18)*	827 *(31)*

Measurements were made on 3 sections of each replicate seedling. Figures in brackets refer to percentage increases.

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