Germination, Establishment and

Mycorrhizal Synthesis in the Epacrid *Woollsia pungens*

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Summary

Seeds of known provenance of *Woollsia pungens* (Cav.) F. Muell. were collected from a large population growing in the Georges River National Park on two different years and subjected to germination trials, with emergence of the radicle as the measure of successful germination. No dormancy was apparent and we found no specific effects of photoperiod, light intensity, temperature or smoke on percentage germination. We have found that *W. pungens* has a distinct juvenile stage and that there is a transition from this to the adult stage when the seedlings are 12-18 months old. This transition is accompanied by a change in leaf shape. Calcium hypochlorite was found to be an effective surface sterilizing agent for the seed and an axenic culture system was established to grow seedlings and inoculate them with an appropriate mycorrhiza-forming fungus and follow whether mycorrhizal synthesis had occurred and whether it had been beneficial to plant growth. Using this system we inoculated boxes containing seedlings with the endophyte MG60 that had been isolated from *Woollsia pungens* in the wild. We found that plants in boxes that had been inoculated with MG60 developed mycorrhizas. Plants in the boxes that had been inoculated were more vigorous than those in boxes that had not.

Objectives of Programme

To study factors controlling germination, seedling establishment and mycorrhiza formation in epacrids under controlled conditions. *Woollsia pungens* was chosen for specific study (i) because of its importance as a component of native bushland and (ii) the species has horticultural potential, there being a beautiful, rare pink variety. Epacrids are an important component of the native flora in a range of habitats but their biology, especially the biology of their mycorrhizas is poorly understood and they are notoriously difficult to grow. The project was subdivided into the two distinct aims:

(i) To determine what factors control germination and seedling establishment in *Woollsia pungens* and to establish a procedure for routine germination.
(ii) To determine the factors necessary for the establishment of a mycorrhizal relationship in *W. pungens* under axenic conditions and study the development of this relationship.

Introduction

Woollsia pungens is a common and widespread component of dry eucalypt forest and heath on sandstone and coastal dunes throughout Eastern Australia. It often becomes established in open locations after fire and can be viewed as a typical member of the Epacridaceae. Both germination and establishment in members of the Epacridaceae are notoriously difficult to achieve. Seedling germination in some species (e.g. *Lysinema ciliatum*) is known to be promoted by smoke or smoked water but this is not always the case (Gilmour, Epacris study group newsletter 12, p 3, 2001). Our preliminary results showed that germination of *W. pungens* can be promoted by factors other than smoke and this provided an opportunity for an investigation of these factors. These findings should be of relevance to the cultivation of other epacrids.

All epacrids form mycorrhizal relationships. The mycorrhizas are usually of the ericoid type and are similar in appearance to the ericoid mycorrhizas of Northern Hemisphere Ericaceae and it is usually assumed, although without specific evidence, that they will function similarly (see Cairney & Ashford, Tansley Review, New Phytologist, June 2002). Mycorrhizal relationships in Ericaceae are known to promote nitrogen and phosphorus uptake from soil and are fundamental to plant establishment and survival. Australian heath environments, though nutrient poor, are hydrologically diverse and quite different from the typical wet heaths of the Northern Hemisphere. There is a need to clarify the function of epacrid mycorrhizal relationships and for this mycorrhizal plants produced axenically in controlled conditions are required. To date there has been some success in establishing mycorrhizas in culture (see Mclean *et al*. 1998, New Phytol. 139, 589-593) but not in sufficient numbers to determine whether they are beneficial to plant growth. Successful mycorrhizal synthesis in sterile culture would determine whether fungal isolates cultured from hair roots are truly mycorrhizal, and should also create new protocols that may be successful with other species.

Germination trials

In preliminary experiments seed was germinated without surface sterilization or addition of soil. Seeds were sown directly on to moist facial tissue in polypropylene food boxes (170 x 120 x 50 mm, Universal Wholesalers, Yennora, NSW). The shallow peripheral well around the edge of the bottom of these boxes was a useful reservoir for maintaining moisture without waterlogging. In later trials we used similar lidded plastic boxes but surface sterilized the seed to reduce mould growth. Sterilisation with 2.5 % calcium hypochlorite for 2 hr, followed by 3 rinses with sterile deionised water, was effective in surface sterilizing the seed. After surface sterilization, seed was suspended in 15 ml sterile deionised water for 24 hr with occasional shaking and it was then sown on to moist facial tissue in boxes as described above. We found facial tissue to be a better, more reliable substrate than multiple layers of filter paper, probably because it made better contact with the seed and this improved water uptake. Germination followed a sigmoid curve with a delay of about 15 days after sowing before radicle emergence commenced. By 60 days the curve had leveled off at about 43% germination. Preliminary trials with smoked water, ethylene, ammonia and other dormancy breaking chemicals failed to accelerate germination and there does not appear to be a light or photoperiod requirement. After germination seedlings entered a long juvenile phase with a shoot morphology that is different from that in adult plants (Fig.1). Further details are given in Palmer and Ashford (2004).

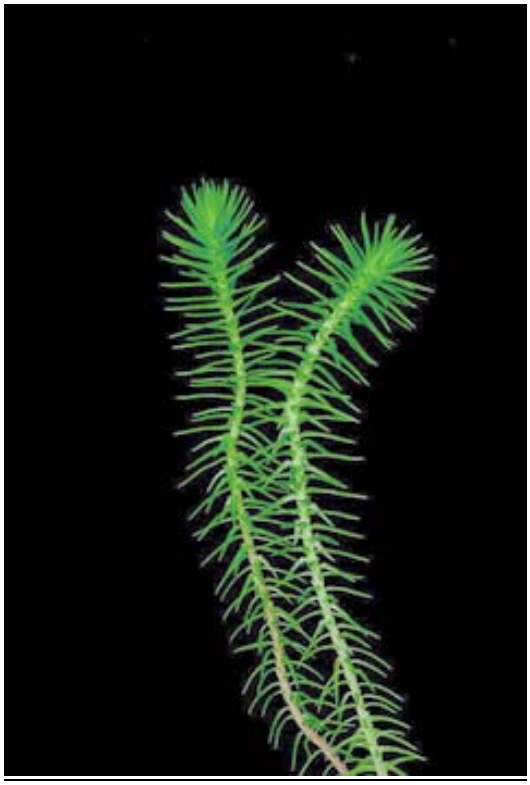

Figure 1. Two one year old seedlings of *W. pungens* in the juvenile stage, with many minute leaves 3-4 mm long. Photo: UNSW Photographic Unit.

Mycorrhizal synthesis

Seedlings that had grown for 2 months under axenic conditions on either agar gel or Phytagel were inoculated with the fungal isolate MG60 and then their roots together with the inoculum were covered with a thin layer of autoclaved soil. MG60 had originally been isolated from a *W. pungens* root system dug up from a natural location (Midgley *et al*. Australian Journal of Botany 50: 559-565, 2002). After 5 months the seedlings were harvested and their hair roots were fixed, stained, and scored for mycorrhizal colonisation. Seedling growth was adversely affected by agar gel (Fig. 2) and mycorrhizas did not form.

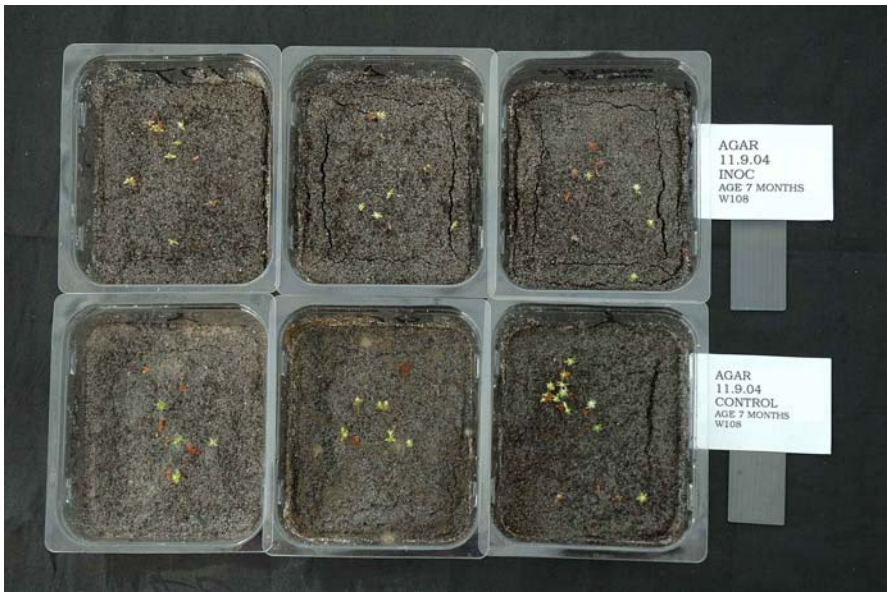

Figure 2. Seedlings of *W. pungens* at the time of harvest showing poor growth on agar, regardless of whether they were inoculated (INOC) or not (CONTROL).

On Phytagel, seedlings appeared more vigorous, although without inoculation their leaves were chlorotic and accumulated a reddish pigment, and their measured growth was not statistically significantly different from those on agar. All the hair root systems of seedlings in inoculated Phytagel culture boxes possessed intracellular fungal hyphae or fungal coils characteristic of ericoid mycorrhizas. These were found in more than half of the hair roots examined from the inoculated culture boxes. The inoculated seedlings on Phytagel had tall shoots with normal leaf colour, and nearly double the number of leaves at harvest as the non-inoculated Phytagel controls (Fig. 3). We have shown that Phytagel covered by natural soil provides a suitable growing medium for *W. pungens* and that inoculation with MG60 results in the formation of typical ericoid mycorrhizal coils with a beneficial effect on seedling growth. Further details may be found in Palmer *et al*. (2007).

Figure 3. Seedlings grown on Phytagel at the time of harvest showing the typical difference in plant vigour between boxes inoculated with MG60 (INOC) and controls (CONTROL) that were not inoculated.

Personnel

Ms Bryony Horton was supported by funds from this project to complete experimentation and analysis both during and immediately after her honours year. She developed an interest in mycorrhizas on Australian plants during the project and has since gone on to pursue a PhD in the mycorrhizal biology of forest trees.

Comment

The aims of this project as set out above have essentially been achieved at an expenditure of about half of what was originally asked for (and granted)* and about double the time originally proposed. The nature of the work involved in the project was such that it took a long time to achieve an experimentally valid outcome. Large numbers of seedlings had to be grown up and maintained until they had developed a root system that had a sufficient number of hair roots for successful inoculation. If boxes became contaminated they had to be thrown away and so replication needed to be high. After inoculation several months were required for mycorrhizal development and for any growth response to become detectable. To continue this project and investigate the development and physiology of mycorrhizal plants would require further funds.

Acknowledgements

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Publications arising from the grant

Palmer JH, Ashford AE (2004). *Woollsia pungens*. Germination and Seedling Growth. Australian Plants 22: 243-252.

Palmer JH, Horton B, Allaway WG, Ashford AE (2007). Growth stimulation of *Woollsia pungens* by a natural ericoid mycorrhizal fungal endophyte. Australasian Mycologist 26: 00-00 (in press).

*The grant to Ashford and Palmer began in 2002/03, and was for two years. On 21/2/07 they were informed erroneously that the grant was for only one year. On 10/6/07 the grantees were informed of our error and offered the second year's payment, but were unable to resume the project, and so declined the offer.

Peter Goodwin, President, Australian Flora Foundation