THE GROWTH OF OUTPLANTED EUCALYPT SEEDLINGS AFTER NURSERY INOCULATION WITH ECTOMYCORRHIZAL FUNGUS

Report of a demonstration trial in western Sydney from 1994–1996

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It used to puzzle me that nursery inoculation of *Eucalyptus* species with ectomycorrhizal fungi was being vigorously investigated in Western Australia (*e.g.* Thomson *et al.* 1996) but in the eastern States, as far as I could tell, there was little activity in this field even though in the east there would be greater numbers of tree-propagating users (*e.g.* Greening Australia, Landcare, the Roads and Traffic Authority, various mine site rehabilitators, not to mention Green Olympics organisers in a hurry to revegetate Homebush Bay). In early 1994, I approached Michael Adams in the Sydney office of Greening Australia with a proposal to set up a public demonstration of the benefits of ectomycorrhizal inoculation as a first step in raising the profile of eucalypt seedling inoculation in New South Wales. As a result, we agreed to collaborate in conducting a low-budget demonstration trial; the School of Biological Science would contribute the fungal inoculum and mycological knowledge and Greening Australia the plant-raising expertise and facilities at its nursery near Blacktown and the outplanting sites in Horsely Park Corridor. This article reports on what was done and what happened.

Seedlings of three *Eucalyptus* species (*E. tereticornis*, forest red gum; *E. moluccana*, grey box; *E. eugenioides*, thin leaved stringy bark) were raised in Greening Australia's nursery in the Nurragingy Reserve at Doonside in western Sydney. At six weeks, when the seedlings were transferred from flats to tubes, they were inoculated with mycelia of *Pisolithus tinctorius* (Pers.) Coker & Couch. The isolate was No. 021 in Dr A.E. Ashford's collection of fungi at the University of New South Wales. As a precaution against the possible loss of mycorrhiza-forming ability during storage, the fungus was passed through one cycle of aseptic mycorrhiza formation in *E. tereticornis* in the laboratory about three weeks before it was to be used in the field trial. The mycelium inoculum consisted of 10 × 10 × 4 mm blocks cut from the margin of three week old cultures on modified Melin-Norkrans Medium (Marx 1969). About four hours before the inoculation on 9 March 1994, 120 agar blocks were cut out of the petri dish cultures and washed in five changes of 500 ml distilled quality water on a rotary shaker over a period of three hours to remove residual glucose. The blocks were then transported to the nursery at Doonside in a cooler.

When seedlings were transferred from the vermiculite germination medium to the peat and river sand growth medium in 60 × 160 mm tubes they were inoculated by placing a block of inoculum in contact with the roots of each seedling. Twenty seedlings of each eucalypt species were inoculated in this way. Another 20 seedlings of each species were transferred to tubes without receiving an inoculum and served as controls. All the seedlings were thereafter managed in the same manner as other tube stock in the nursery.

On 6 and 13 October 1994, the tube stock was planted out with the labour of LEAP scheme workers; 15 to 20-year-old youths employd under the Landcare and Environment Action Program. The out planting was done at two sites in the Horsely Park Corridor, a part of the Open Space Corridors scheme managed by the Deparment of Urban Affairs and Planning. Both sites were on a heavy clay soil over Wianamatta shale. Site 1 was a flat, grassy area with a soak at one end which included a part of the *E. tereticornis* block, mostly controls. Some *E. amplifolia* about 3 m in height were growing close to the other end of the trial, adjacent to the *E. eugenioides* block. These trees appeared to interfere with the trial: four plants amongst the nearby *E. eugenioides* controls grew noticeably faster than the others and a *Pisolithus*-like fruitbody was collected from an ant mound close by. Site 2 lay towards the bottom of a heavily grassed , untimbered, sloping paddock about 2 km east of Site 1.

The trial design was the same at each site. It consisted of three blocks in a line, each block containing 40 seedlings of one species of eucalypt, either *E. tereticornis*, *E. moluccana* or *E. eugenioides*, in that order. The 40 plants in each block were arranged in four rows of 10 plants, the rows, and the individual plants in the rows, were about 2 m apart. Two rows contained only uninoculated plants and the adjacent two rows contained only inoculated plants. This arrangement would make it easy to see any treatment effects. A statistically rigorous design in which the

seedlings were randomised would have made recognition of such effects impossible and was therefore inappropriate to the aims of the trial. The sites were mown before planting but no fertiliser was applied. The young plants were watered only at planting and weeds were not controlled. Neither were the roots of control or inoculated seedlings inspected at any time to establish the presence, absence or extent of ectomycorrhizal infections.

Plant height was adopted as the principal measure for growth. However, at the Spring 1995 assessment stem diameter at 50 mm above soil level was recorded in addition to plant heights. As anticipated, the height and stem diameter values were closely correlated. Growth was measured in February and September 1995 and in February 1996. The accompanying table presents a selection of data that are useful as a basis for discussion: the mean height (cm) of the control plants of each species at each assessment and the effect of inoculation expressed as the percentage growth increment (or reduction) relative to the control value.

Inoculation trial summary

		Mean height of control plants (cm)				Inoculation effect (%)		
Site	Species	Oct. 94	Feb. 95	Sept. 95	Feb. 96	Feb. 95	Sept. 95	Feb. 96
1	*tereticornis*	20	24	27	42	26*	19	12
	moluccana	17	16	22	36	51*	53	50
	eugenioides	16	26	35	72	15	45	26
2	*tereticornis*	20	30	44	101	12	10	-4
	moluccana	17	19	27	59	35*	85	62
	eugenioides	16	25	49	99	35*	10	-1

* Significant at $p < 0.05$

The heights of the control plants show that, not surprisingly, the two site differ in relative fertility. By and large, the trees grew better on site 2 than on site 1. This was especialy true for the uninoculated *E. tereticornis* plants which at site 2 grew to more than twice their height at site 1. *Eucalyptus eugenioides* was the least sensitive of the three species to the different conditions at the two sites.

The table also shows the relative difference between the mean height of the control trees and the inoculated trees, expressed as a percentage of the control. The table shows that the first assessment (Feb. 95). about three months after planting out, the mean height of the inoculated trees of all species at one site or another was significantly greater ($p < 0.05$) than its control. At site 1, inoculated *E. tereticornis* and *E. moluccana* plants were significantly taller than their controls; at site 2 the inoculated plants of *E. moluccana* and *E. eugenioides* were significantly taller than their controls.

Eucalyptus moluccana registered a significant increase at both sites at the first asssessment and, over the next two measurement periods, the inoculated plants of this species showed large growth increments of 50 per cent and more. In contrast, the inoculated plants of the two other species tended to be outyielded by their controls with time after outplanting. In the case of *E. eugenioides* at site 1, the influence of resident ectomycorrhizal fungi from the nearby *E. amplifolia* trees on at least four of the control trees can not be discounted (the growth enhancement at the last assessment at site 1 becomes 62 per cent if the four outsize control plants are ignored).

The results of the trial provide a perhaps fortuitous but nonetheless convincing demonstration that nursery inoculation with an ectomycorrhizal fungus can benefit the growth of outplanted eucalypt seedlings. The success of the demonstration is especially gratifying considering the shoestring budget, the total lack of previous experience in the techniques of preparing and applying the inoculum, the fact that the strain of fungus was untested and that the seedlings were outplanted at the beginning of an extremely dry summer.

The trial was useful from an educational perspective in demonstrating the complexity of manipulating an ectomycorrhizal symbiosis. At Horsely Park, the growth response of the eucalypts to inoculation depended not only

on the species of eucalypt but also on peculiarities of the sites and the length of time after outplanting. That the species and the source of the inoculant fungus are also critical variables is well known from the literature.

It may be argued that the outcome of the demonstration depended more on good luck than good science and because there was no statistical rigour in the trial design and no correlative data on mycorrhizal formation by inoculant and indigenous fungi the trial does not merit serious discussion. The element of luck (mainly in the selection of the strain of *Pisolithus*) can not be denied. But compared to other, similar field experiments, the results of the one reported here stand up well to comparison. For example, some workers in this field consider that a 50 per cent increase such as that seen in inoculated *E. moluccana* is large (Lapayrie *et al.* 1992). In Europe in the last 10 years, variation in the field response of forest trees to ectomycorrhizal inoculation has been very high (Le Tacon *et al.* 1992). from modest increases, to no effect and even growth reduction. In recent eucalypt outplanting experiments in Western Australia (Thomson *et al.* 1996) none out of 12 comparisons at one site and only three out of 12 at another gave statistically significant increases in growth. Albeit the increaes were large (70–100 per cent) but it is quite clear that the use of inoculant ectomycorrhizal fungi is still in an early stage of development.

It goes without saying that I am no longer puzzled why inoculating eucalypts with ectomycorrhizal fungi is not being systematically investigated in New South Wales. Obviously the task needs a sustained, well resourced effort of the sort led by CSIRO in Western Australia, the sort of effort that begs duplication in the small Australian pond. But with the inoculation technology still essentially in its infancy, is there anything that non-industrial tree planters in New South Wales can do?

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