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Seed and seedling responses to inoculation with mycorrhizal fungi and root nodule bacteria: implications for restoration of degraded Mediterranean-type Tuart woodlands Thea So[1], Katinka X. Ruthrof[2] and Bernard Dell[2] ([1]School of Biological Sciences and Biotechnology, Murdoch University, Murdoch, WA 6150, Australia; [2]State Centre of Excellence for Climate Change and Forest and Woodland Health, Murdoch University, Murdoch, WA 6150, Australia).

Key words: direct seeding, mycorrhizal fungi, root nodule bacteria, survival, growth.

Summary

Inoculation with beneficial soil microorganisms has the potential to enhance success of restoration, particularly in harsh Mediterranean-type ecosystems (MTE's). We investigated the effects of microorganisms (mycorrhizal fungi and root nodule bacteria) and planting material (seed and nursery-raised seedlings) on early establishment and growth of two key post-disturbance colonizing species with different life histories, life forms and functional types (*Eucalyptus gomphocephala* and *Acacia saligna*) under field conditions. Establishment and growth was monitored at 13 months, following the first MTE drought period. For *E. gomphocephala*, establishment was higher for seedlings (81%) than for seeding (7.5%). Inoculation with ectomycorrhizal fungal spores was not beneficial. For *A. saligna*, establishment was also higher for seedlings (84%) than for seeding (42.5%). Mycorrhizal fungal inoculum had no effect on establishment or growth. This study has shown that in harsh MTE conditions the use of seedlings is more effective than seeding in degraded woodlands even when attempting to reintroduce key colonizing species. The microorganism treatments tested did not result in significant improvement in establishment or growth.

Introduction

Reforestation of degraded lands can be difficult and expensive (Poopathy *et al.* 2005) as production and planting of nursery-raised seedlings requires substantial labour and capital inputs. Therefore, direct seeding, a low cost method for forest restoration (Engel and Parrotta 2001), may be preferable to planting seedlings. However, establishment and early growth of direct-seeded recruits remains a challenge. Priming with beneficial organisms and other treatments may be useful. To test this, and to compare effectiveness of planting material, we used Tuart (*Eucalyptus gomphocephala* A. DC.) and Orange wattle (*Acacia saligna* Labill. H.L.Wendl.) as they represent different types of symbiotic relationships: non-N_2-fixing and N_2-fixing; ectomycorrhizal and mainly endomycorrhizal woody plants, respectively. They are important species in the revegetation of Tuart woodlands in southwestern Australia.

Methods

Trials were established in Tuart woodland once used for grazing, on sands of the Spearwood Dune System (McArthur and Bettenay 1974) adjacent to Yalgorup National Park (32°42'S, 115°38'E), 80 km south of Perth, Western Australia. The region experiences a Mediterranean climate with hot, dry summers and cool, wet winters (June to August) with an average annual rainfall of 882 mm. Invasive species were controlled sprayed with Glyphosate one week prior to trial establishment.

Two field experiments were established in June 2007. The first investigated the effect of ectomycorrhizal (ECM) fungi and inorganic fertiliser on establishment and growth of Tuart. This was a 2 × 3 factorial experiment consisting of two planting materials (seeds, seedlings) and three treatments in a randomized complete block (RCB) design. In a block there were six plots, three of which were allocated randomly each to seed and seedlings. Each plot was assigned one treatment. Treatment ET1, liquid inoculum of four local ECM fungi (9×10^6 spores, equal mix of *Scleroderma cepa, Pisolithus marmoratus, Laccaria lateritia* and *Amanita eucalypti*) was applied to seedling roots or 0.5 cm beneath the seed. Treatment ET2 was liquid inoculum of the four local ECM fungi plus a 10 g fertiliser tablet (20 % N, 4.4 % P, 8.2 % K, + micronutrients; Langley Australia Pty Ltd), buried at 10 cm, 30 cm from each seedling at three months after planting. The control, ET3, was untreated.

The second experiment examined the effects of N_2-fixing bacteria and mixed mycorrhizal fungi on establishment and growth of Orange wattle. This was a 2 × 8 factorial experiment consisting of two plant materials (seeds, seedlings) and eight treatments in an RCB design. There were 16 plots in each block, eight of which were allocated randomly each to seed and seedlings. Each plot was then assigned one treatment. Treatment AT1, a suspension of crushed nodules collected from young Orange wattle trees from Murdoch University woodland was applied to seedlings in the nursery, one month prior to planting, or immediately beneath the seed at seeding, at the rate of one nodule per seedling or seeding point. Treatment AT2, a sachet of commercial mycorrhizal inoculum (Zadco For Quality Gro PL), was added to the bottom of the hole beneath the root ball or 0.5 cm below the seed. Each sachet contained 21,000 propagules, representing a mixture of *Glomus intraradices, G. mosseae, Laccaria* sp., *Pisolithus* sp. and *Scleroderma* sp.. The other treatments were: AT3= the same fertiliser as in Experiment 1; AT4= crushed nodules + commercial inoculum; AT5= crushed nodules + fertiliser; AT6= crushed nodules + commercial inoculum + fertiliser; AT7= autoclaved sachet of commercial inoculum; and AT8= control (untreated).

To mimic woodland restoration activities, seedlings of Tuart were raised for six months before planting. Orange wattle seedlings, raised from seed collected in the region in 2002, were grown in composted pine bark, peat and perlite. For both experiments, each plot contained 25 planting holes with a spacing of 1 × 1 m. Each experiment contained three replicated blocks. Seedlings were planted using a Pottiputki. For direct seeding, a trowel was used to make seeding points, 4 × 4 × 1 cm, in which ca. 3 seeds were dispersed and covered with soil to a depth of ca. 0.2 cm. No irrigation was applied to the site at planting. At three months, direct seeding plants were thinned to one plant per point to prevent competition, and weeds were removed manually within 30 cm of each point.

We assessed seedling establishment at 4, 6 and 13 months and height at 13 months, but we only report the last assessment. Seedling establishment was calculated based on the number of planting holes with a seedling as percentage of the total planting points per plot. Data were analysed using SPSS Version 15.0 and univariate Analysis of Variance was applied. Duncan's Multiple Range Test was used to detect differences among means at $p \leq 0.05$. Percentage data were arc-sin square root transformed prior to statistical analysis. Untransformed data are shown in Table 1.

Results

Experiment 1: at 13 months, 81% of the nursery-raised Tuart seedlings had survived compared to 8% seedling establishing from direct seeding (Table 1). Nursery-raised seedlings were more than double the height of those from direct seeding. The interaction between the two main factors was not significant.

Table 1. Number and height of Tuart (Experiment 1) and Orange wattle (Experiment 2) seedlings at 13 months and effects of treatments. Values are replicated block means (n = 3) ± S.E. In a column, means followed by the same letters are not significantly different at $\alpha = 0.05$.

Tuart			**Orange wattle**		
	Establishment (%)	Height (cm)		Establishment (%)	Height (cm)
Treatment			*Treatment*		
ET1:Ectomycorrhizal fungi	46.0±17.4	57.1±12.2	AT1:Crushed root nodules	60.0±12.6ab	71.7±12.7abc
ET2:Ectomycorrhizal fungi+ inorganic fertiliser	44.7±18.2	54.6±15.6	AT2:Commercial mycorrhizal inoculum	50.7±12.9b	52.1±11.7c
ET3:Control	42.7±14.6	49.1±10.0	AT3:Inorganic fertiliser	78.7±7.1a	89.3±14.4a
			AT4:Crushed root nodules+ commercial mycorrhizal inoculum	67.3±6.9ab	76.8±19.1abc
			AT5:Crushed root nodules + inorganic fertiliser	77.3±7.8a	76.2±10.8ab
			AT6:Crushed root	60.7±7.9b	84.2±11.5a

			nodules+ commercial mycorrhizal inoculum + inorganic fertiliser		
			AT7:Autoclaved commercial mycorrhizal inoculum	56.0±15.5b	73.2±18.1abc
			AT8:Control	55.3±11.6b	59.4±12.5bc
Plant material			*Plant material*		
Seed	7.6±2.0b	33.1±4.5b	Seed	42.5±4.2b	48.4±3.7b
Seedling	81.3±3.1a	74.1±9.1a	Seedling	84.0±1.8a	97.3±5.7a

Analysis of variance

Species	**Tuart**					**Orange wattle**				
Source	d.f.	F	Sig.	F	Sig.	d.f.	F	Sig.	F	Sig.
Block	2	0.826	0.465	8.142	0.008	2	0.038	0.963	1.416	0.258
Treatment(T)	2	0.221	0.805	0.395	0.683	7	3.685	0.005	2.740	0.025
Plant material (Pm)	1	241.777	0.000	29.431	0.000	1	120.57	0.000	69.146	0.000
T × Pm	2	1.775	0.219	0.885	0.443	7	2.132	0.070	1.073	0.405
Error	10					30				

Experiment 2: establishment at 13 months was significantly affected by treatments with fertiliser application (AT3), with or without a nodule suspension (AT5) resulting in improved seedling establishment over the control by 42 and 40%, respectively. Treatments with added fertiliser, with or without microbial inocula (AT3 and AT6) improved height by 50% and 42%, respectively. The microorganism treatments did not result in significant improvement in establishment or growth. Establishment and growth of nursery-raised seedlings were twice those of plants from seeding.

Discussion

Mycorrhizal fungi added in this experiment were ineffective in promoting establishment or growth. This may be due to the added fungi not colonizing host plants and not effective in promoting nutrient uptake and growth, or sufficient propagules of native mycorrhizal fungi were already present. It was not possible to identify which of these explanations is the major contributing factor, because mycorrhizal formation was not assessed. While previous work has demonstrated that *Glomus mosseae* is effective for Orange wattle (Benbrahim and Ismaili 2002), it is not known if it formed effective associations in this experiment.

Establishment of nursery-raised seedlings was superior to direct seeding, with seedling numbers being double for the Orange wattle and 11 times for Tuart. Our finding is in accordance with field experiments in eucalypt woodlands (Standish *et al.* 2008; Ruthrof *et al.* 2010). The significant differences in establishment between the two plant materials may be explained in part by the rapid drying of the soil profile as the dry season progresses and the capacity of the shoot to allocate resources for root growth into the soil profiles to access water. The lack of available soil nutrients and water (McArthur and Bettenay 1974) during the dry season discourage root development, and this, in combination with high temperatures in summer, could have led to desiccation of the direct-seeded seedlings.

Implications for management

Reforestation of degraded lands can be difficult and expensive. This study has shown that in degraded woodlands with a harsh Mediterranean-type environment, the use of seedlings is more effective than seeding even when attempting to reintroduce key colonizing species. The microorganism treatments did not result in significant improvement in establishment or growth.

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