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# *ACACIA* SPECIES USED FOR REVEGETATION IN SOUTH-EASTERN AUSTRALIA REQUIRE MORE THAN ONE MULTI-STRAIN RHIZOBIAL INOCULANT

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Revegetation of degraded land requires either nursery production of tube stock for out-planting or successful establishment of plants after direct seeding, using a range of native species including *Acacia* spp. Inoculation of *Acacia* spp. with effective rhizobia benefits plant establishment and growth in the nursery and the field (Brockwell et al., 1999; Thrall et al., 2005). *Acacia* species exhibit strain specificity for effective nitrogen fixation (Burdon et al., 1999) and the use of a multi-strain inoculant is a practical method for ensuring effective nodulation over a broad host range. In this study, *Acacia* species commonly used for revegetation were inoculated with a multi-strain product in a commercial nursery trial. The trial aimed to determine: (i) whether there was strain selection by the host for nodulation; and (ii) whether the inoculant strains were competitive for nodulation in the presence of a background soil population of *Acacia* rhizobia.

Seeds of 15 *Acacia* species were sown into pasteurised commercial growth medium inoculated with Wattle Grow™ (Becker Underwood Pty Ltd, Somersby, New South Wales, Australia), containing four strains of *Bradyrhizobium* (CPBR1, CPBR2, CPBR3 and CPBR6). There were two inoculation treatments for each species. In the first treatment, Wattle Grow was mixed into the growth medium to give $2.33 \times 10^5$ inoculant rhizobia $g^{-1}$ medium. In the second treatment, soil taken from beneath a natural stand of *Acacia acinacea*, as well as Wattle Grow, was mixed into the medium to give $2.27 \times 10^4$ soil rhizobia $g^{-1}$ plus $2.22 \times 10^5$ inoculant rhizobia $g^{-1}$. Plants were assessed for shoot dry matter, extent of nodulation, and nodule occupancy by inoculant rhizobia and other strains determined by RAPD PCR fingerprinting of nodule isolates (Ballard et al., 2004). Data were analysed by ANOVA and, where F values were significant ($P < 0.05$), LSD was used as the *post hoc* test ($P < 0.05$).

For *A. dealbata*, *A. genistifolia*, *A. implexa*, *A. mearnsii* and *A. rubida*, 76–100% of plants inoculated with Wattle Grow became nodulated, with no change ($P > 0.05$)

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observed when soil rhizobia were added to the growth medium. Nodules of each species were occupied by all four inoculant strains and also by other strains originating from the introduced soil population and from contamination (inevitable under commercial nursery conditions). The only evidence for inoculant strain selection was seen for *A. dealbata*, where strains CPBR2 and CPBR6 dominated nodules in the Wattle Grow treatment (60% and 24% nodule occupancy, respectively) and in the Wattle Grow plus soil treatment (35% and 44% nodule occupancy, respectively). There was no difference in shoot dry matter ($P > 0.05$) between the two treatments for any species. These data indicate that Wattle Grow is an effective multi-strain inoculant for these species.

In contrast, *A. salicina*, *A. buxifolia*, *A. decora* and *A. verniciflua* failed to nodulate fully with Wattle Grow despite the large population of rhizobia ($2.33 \times 10^5$ $g^{-1}$ medium) in the growth medium. This was apparent in increased nodule scores ($P < 0.05$) for all species when the soil rhizobia were added. For *A. salicina*, *A. buxifolia* and *A. decora*, increases in nodule score with added soil rhizobia corresponded with increased ($P < 0.05$) shoot dry matter. It is not clear whether the increase in nodulation and shoot dry matter is due to the increase in inoculum level with added soil or to minor changes in nodule occupancy by the inoculant strains and other strains. These species may benefit from an alternative more effective multi-strain inoculant.

*A. iteaphylla*, *A. acinacea*, *A. pycnantha* and *A. flexifolia* nodulated preferentially with strains other than the four inoculant strains. In the Wattle Grow treatment, nodule occupancy by other strains (presumably contaminants) ranged from 45% to 90% and, when soil rhizobia were added, nodule occupancy by other strains ranged from 63–90%. Not surprisingly, there was no difference ($P > 0.05$) in nodulation parameters or shoot dry weight between inoculation treatments. These species may benefit from the development of an alternative multi-strain inoculant.

*A. doratoxylon* failed to nodulate properly when inoculated with Wattle Grow (3% plants nodulated; nodule score 0.01 out of 5). Addition of soil rhizobia improved ($P < 0.05$) nodulation, but it remained suboptimal (52% plants nodulated; nodule score 0.62 out of 5), and there was no increase in shoot dry matter ($P > 0.05$). Nodule occupancy in the Wattle Grow plus soil treatment was 5% for CPBR2, 5% for CPBR6 and 90% for other strains (no isolates were obtained for the Wattle Grow treatment). These data show that *A. doratoxylon* has different strain requirements from the other species and may benefit from identification and incorporation of a specific *A. doratoxylon* strain in a multi-strain inoculant.

## References

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