Treatment of native grass seed with the beneficial fungus Gliocladium virens

Part of the rehabilitation/revegetation program at Kanmantoo Copper Mine in South Australia

By Robin B. Coles MsAgSc Company IXODIA ABN: 42 834 224 149)

8/16/2013

This interim report (p1-2), documents the treatment program, for native grass seed used in the re-vegetation of the Laydown area at Kanmantoo Copper Mine. The second section (p3-13) documents sixteen macro-fungal species recorded in the remnant vegetation, with eight being potentially useful as a source of plant inoculum, e.g. mycorrhizal associations.

1. Native Grass Seed inoculation with Gliocladium virens at the Laydown area Kanmantoo Copper Mine

Introduction:

Native grass seed was harvested from sown trial plots near the mine site. The species included: *Austrodanthonia* sp., *Austrostipa nodosa, Austrostipa blackii, Chloris truncata, Vittadina blackii* and *Themeda* sp.

Dried seed batches of each species were coated with a small seed coat (SSC) emulsion (PolyAG, Biocentral Laboratories Limited), at a rate of 5-6g per kg of seed. This treatment was carried out in a cement mixer rotating for 3-5 mins and formed a sticky coat on the seed surfaces. The beneficial fungus *Gliocladium virens** was then applied as dry spore material (96% conidiospores and 4% chlamydospores) at a rate of 10g per kg of seed. This was then mixed for 3-5 mins to evenly coat the seed wall.

The individual treated seed batches were re-bagged and held at 4°C for 6 days until weather conditions were suitable for planting. The treated seed was hand spread in tilled soil plots measuring 25x25m. Two replicate plots were made for each grass species, with the exception of one trial plot for the limited seed batches of *Austrostipa blackii, Vittadina blackii* and *Themeda* sp. (see figure 1).

The soil in the treated and untreated seed plantings was further tilled, to cover and evenly disperse the seed during germination.

The total area of the treated trial plots was 0.56ha and the untreated plot areas 3.54ha.

Two week after planting, germination had occurred in most of the seeded areas.

Assessments will be made on grass growth rates, grass seed biomass and sap nitrogen levels in the treated and untreated plots. This will determine the beneficial effects of seed treatment with *Glicladium virens.*

**Gliocladium virens* has been trialled as a plant growth promoter and plant disease suppressing agent (Coles, et al, 2005 (1,2). It was successfully trialled on a dry land wheat crop as a seed coat in the Mallee country of South Australia. At harvest yields were increased by 24% (Saunders, 2009 (4).

Wheat and native grasses all belong to the plant group Gramineae. They are monocots that provide cereal foods and stabilise soils. Many grasses are subject to root disease from fungal organisms such as *Rhizoctonia* and *Pythium* which cause damping off and seedling loss. The naturally occurring soil fungus *Gliocladium virens* has been shown to control these diseases and increase plant growth by forming a fungal association with their roots. The grasses benefit by increased plant growth and root disease control. The trials outlined above will determine whether this applies to native grasses.

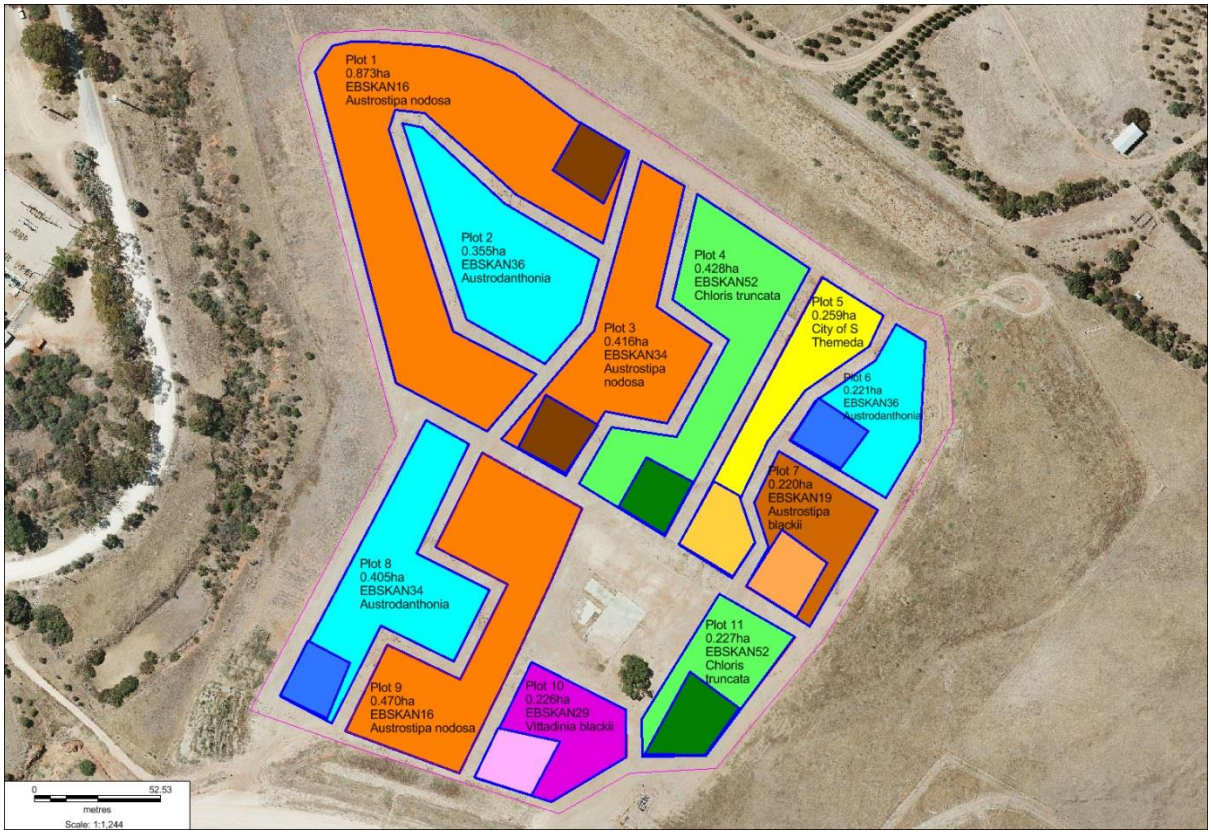

Figure 1. Laydown area Kanmantoo mine site, smaller squares and polygons show the inoculated seed areas

2. Macro-fungal species (Mushrooms, toadstools, puffballs and bracket fungi) recorded in the remnant vegetation at Kanmantoo Copper Mine.

A source of beneficial woody plant inoculum, e.g. mycorrhiza

On the 25 July a fungal survey was carried out in the remnant vegetation stand of *Eucalyptus odorata* (Peppermint Box), *Allocasurina* sp. (Sheoaks). A smaller survey was made in a revegetated stand of *Eucalyptus camaldulensis* (River Red Gum).

The survey documents some of macro-fungi found in the Kanmantoo Copper Mine site in July 2013, and provides a list of species suitable for a source of mycorrhizal inoculum for woody plant species.

Fungal species found in the survey were assigned GPS co-ordinates UTM, (see table 1) and photographically recorded (see figures F1-F16*). The genera of the macro-fungi were determined as near as possible using mycological texts. Spore print colour and microscopic spore sizes were recorded at 400x magnification. Identified fungi were assigned a mycorrhizal rating (yes/no, table 1) according to mycological texts. The species were dried at 26°C and stored in labelled envelopes.

The area surveyed was approximately 10 ha and sixteen different fungi species were recorded, eight are probable mycorrhizal associates. These groups may provide a useful source of seed/seedling mycorrhizal inoculum for *Eucalyptus odorata* and *Eucalyptus camaldulensis* and many other native plant species.

*One fungus *Pisolithilus* sp., (F16) was recorded in early autumn (March 2013) near a plantation of *Eucalyptus camaldulensis.* It was evident these native puff-balls had formed a mycorrhizal association with the lateral roots of the tree species (Bougher et. al., 1990(3). The puff-ball spores were harvested from several kilograms of dried material using a sieve and bucket and stored for future inoculum use.

References:

(1) Coles RB, Wicks TJ and Hall BH (2005) *Gliocladium virens*: a fungal parasite of *Alternaria radicina*. In Proceedings of Australasian Plant Pathology Society; 15th Biennial Conference Geelong, September p159.

(2) Coles RB, Wicks TJ and Hall BH (2005) Control of *Alternaria radicina* infested carrot seed with the mycoparasite *Gliocladium virens*. In proceedings of Australasian Plant Pathology Society; 15th Biennial Conference Geelong, September p163.

(3) Bougher NL, Grove TS and N. Malajczuk N (1990) Growth and phosphorus acquisition of karri (*Eucalyptus diversicolor* F. Muell.) seedlings inoculated with ectomycorrhizal fungi in relation to phosphorus supply. New Phytologist. 114 (1) p77-85.

(4) Saunders R (2009) Agronomy Matters, News Letter p2

Table 1. ***Macro-fungi recorded at Kanmantoo Mine Site in Remnant Vegetation stands of Eucalyptus odorata, Allocasurina sp. and Eucalyptus camaldulensis plantings.***

Fungi **GPS (UTM)**	**Name**	**Mycorrhizal**	**Habitat**
F1. 0318096 6115338	Tricholoma sp. or Leptiota sp.	Yes No	On the ground amongst moss and E. odorata
F2a. (upper surface) F2b (lower surface) 0318030 6115311	Clityocybe sp. or Clitopilus sp.	? ?	On the ground amongst grass
F3. (left) F4. (middle) F5. (right) 0318021 6115342	(left) Laccaria sp. (middle) Entoloma sp. (right) Cortinarius sp.	? ? Yes	On the ground amongst mosses and native grass. In E. odorata stand
F6a. (upper surface) F6b. (lower surface) 0318013 6115358	Sternum sp. (Bracket fungus)	No	On rotting E. odorata wood
F7. 0318142 6115501	Cortinarius sp.	Yes	On ground in E. odorata stand
F8. 0318310 6115628	Tricholoma sp. or Entoloma sp.	Yes No	Amongst moss and grass near a rock outcrop
F9. 0318362 6115679	Lycoperdon sp. or Disciceda sp.	No	On ground in Allocasurina stand in sandy soil.
F10. 0318329 6115403	Agaricus sp. (Field mushroom)	No	On ground in E. odorata stand

F11. 0318329 6115403	Collybia sp.	?	On ground in E. odorata stand
F12. 0318042 6115347	Possibly a Russula sp. (Very young specimen)	Yes? If Russula sp.	On ground in E. odorata stand
F13. 0317942 6115333	Mycena sp.?	No.	On ground in E. camaldulensis planting
F14. 0317920 6115327	Scleroderma sp.	Yes	On ground in E. camaldulensis planting
F15. 0317920 6115327	Hydnangium sp.	Yes	On ground in E. camaldulensis planting
F16. 0317920 6115327	Pisolithilus sp. (horse dung puff ball).	Yes	On ground in E. camaldulensis planting

Figures F1-F16.

Figure 2. *Eucalyptus odorata* remnant forest at Kanmantoo Copper Mine. July 2013

F1 Tricholoma sp. or Leptiota sp

F2a. (upper surface) Clityocybe sp.or Clitopilus sp.

F2b (under surface left) Clityocybe sp. or Clitopilus sp.

F3. (left), F4. (middle), F5.(right): F3. Laccaria sp (left). F4.Entoloma sp. (middle), F5. Cortinarius sp. (right).

F6a. (upper surface) Sternum sp. (Bracket fungus)

F6b. (lower surface) Sternum sp. (Bracket fungus)

F7. Cortinarius sp.

F8. Tricholoma sp. or Entoloma sp.

F9. Lycoperdon sp. or Disciceda sp.

F10. Agaricus sp. (Field mushroom)

F11. Collybia sp.

F 12. Possibly a Russula sp. (Very young specimen)

F13. Mycena sp.?

F14. Scleroderma sp.

F15. Hydnangium sp.

F16. Pisolithilus sp.