Assessments of native plant establishment after inoculation with the beneficial fungus with *Gliocladium virens* in the lay down area of Hillgrove Resources copper mine

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Native Grass Seed inoculation with *Gliocladium virens* at the Laydown area and plant establishment assessments at 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 1 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.

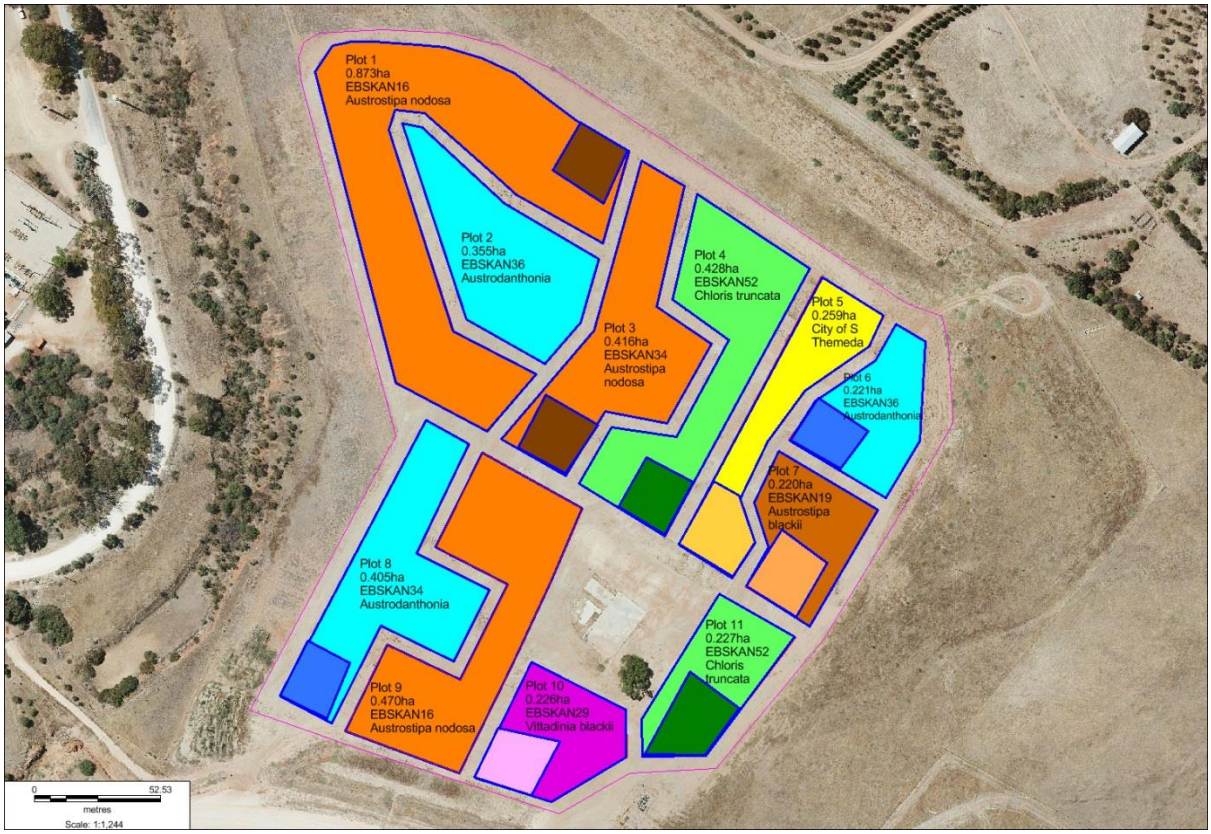

Figure 1. Laydown area Kanmantoo mine site, smaller squares and polygons show the inoculated seed areas with 2 m buffers

Methods

Native seed inoculated with *Gliocladium virens* and un-inoculated seed batches were evenly hand spread over the cultivated plots on the 9th and 10th of July after sufficient rain had moistened the soil. The entire site was then lightly harrowed to evenly distribute and cover the seed with approximately 1-2 cm of soil (see figure 1).

After an exceptionally wet winter plant assessments were made for each plot by counting the number of seedlings and mature plants in 100 half metre random squares in both inoculated and un-inoculated plots (see appendix)

On the 19/06/2014 *Vittadina blackii* and *Austrostipa nodosa* replicate plots were assessed for plants numbers/metre square and establishment. On the 26/8/2014 replicate plots for *Austrodanthonia* were assessed for the same values. The total number of plants in the 100 randomly distributed half metre squares plots was summed and the average plant numbers/metre square metre (m sq) was calculated for each plot (see figure 2).

Figure 2. Half metre square quadrat overlying seedlings in established *Austrodanthonia* plot.

Plant species precluded from the sampling were *Themeda* sp and *Austrostipa blackii* because of low seed viability and only one replicate, *Chloris truncata* was not assessed because establishment was poor in the two replicates.

Results

Vittadinia blackii established rapidly and was flowering by May 2014. Transect counts were made at this time, however, uneven distribution of seedlings was noted and the first transect assessment method was unsuccessful. The second assessment method using plant counts in 100 random half metre squares was considered more accurate and was the most reliable. Plant establishment for the inoculated seed was 71.6 plants/ m sq and the un-inoculated had a higher value of 79.4 plants/ m sq.

In contrast *Austrodanthonia* (EBSKAN36) inoculated seed plots with two replicates both had higher numbers of plants e.g. (59.6 and 51.6 plants/sq m) and the un-inoculated control plots (53 and 39.9 plants/m sq). This approximated a 16.2% plant increase in establishment for the inoculated seed.

The *Austrostipa nodosa* (EBSKAN16) inoculated seed plots with two replicates had higher numbers of plants e.g. (12.4 and 13.4 plants /sq m) with uni-inoculated controls (8.1 and 11.5 plants/m sq) This approximated a 23.7% plant increase in establishment for the inoculated seed (see Table 1).

Plant species	No. of plants/sq m, seed inoculated with *Gliocladium virens*	No. of plants/sq m, no seed inoculation Control
Vittadinia blackii (EBSKAN29)	71.6 Rep 1 (one trial plot)	79.4 Rep 1 (one trial plot)
Austrodanthonia (EBSKAN36)	59.3 Rep 1 51.6 Rep 2 =16.2% establishment increase	53 Rep 1 39.9 Rep 2
Austrostipa nodosa (EBSKAN16)	12.4 Rep 1 13.3 Rep 2 =23.7% establishment increase	8.1 Rep 1 11.5 Rep 2
Austrostipa blackii (EBSKAN19)	N/A	N/A
Chloris truncata (EBSKAN52)	N/A	N/A
Themeda (City of S)	N/A	N/A

Table 1. Native plant establishment trialling seed inoculated with *Gliocladium virens*

Discussion

The reduction of plant number of *Vittadinia blackii* (EBSKAN29) in the inoculated plots was possibly due to the use of the wrong inoculum or the uneven establishment pattern of seedlings on the edge of the trial site. This single plot on the south western outlying area was considered unrepresentative because of uneven wetting and weed invasion from the surrounding buffer and embankments (see figure 1). A better suited inoculum for this plant species could be *Pisolithilus* species (the horse dung puff ball).

Austrodanthonia (EBSKAN36) species showed an antagonist or allopathic property to weeds and precluded other plant species from establishing in the seeded plots. This was particularly evident on the 2 metre buffer edges of the seeded plot (see figure 3).

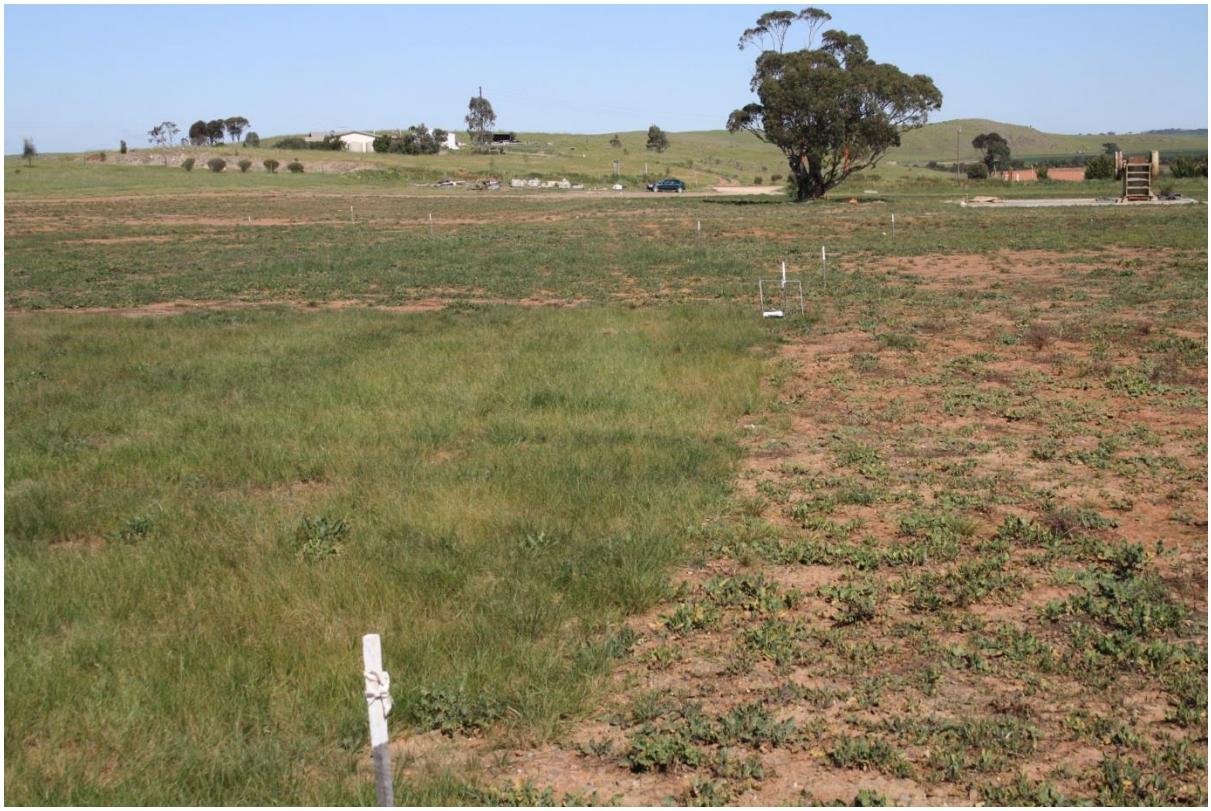

Figure 3. Inhibition of weed at the buffer zone and within seeded plots of *Austrodanthonia* species (EBSKAN36).

A visual difference in the greenness of the inoculated and un-inoculated *Austrodanthonia* species (EBSKAN36) was recorded on the 26/08/2014. Plant colour in the inoculated plots was more intense and a darker green than plants in the un-inoculated plots (see figure 4). The uptake of more nitrogen (in the form of nitrate) through the roots in plants treated with *Gliocladium virens* was recorded in wheat and onions (Shane Phillips pers. comm., 2009)

Figure 4. Foreground *Austrodanthonia* plot treated with *Gliocladium virens* showing darker green colouration than the yellowing untreated control plot to the left of tree guards.

Conclusion

Austrodanthonia (EBSKAN36) had a 16.2% establishment increase after seed inoculation with the beneficial fungus *Gliocladium virens.* *Austrostipa nodosa* (EBSKAN16) had a 23.7% establishment increase after seed inoculation. Future assessments in October and November 2014 on seed yields will provide additional data.

Reference

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

Appendix

Vittadina blackii seed inoculated with Gliocladium virens						plant count/0.5m sq		19/06/2014	Rep 1
1	1	0	9	1	1	0	0	1	1
0	2	0	0	0	0	0	0	0	7
0	0	2	0	0	11	0	3	1	2
0	0	4	2	13	15	0	18	5	2
2	1	4	0	5	6	14	4	1	2
3	0	5	4	6	2	0	17	2	7
0	1	3	14	1	4	1	1	6	38
0	2	0	20	0	3	4	0	6	7
2	7	8	3	2	0	8	0	3	0
1	1	9	5	4	0	0	7	0	0
9	15	35	57	32	42	27	50	25	66
								358	
								71.6plants/m sq	

Vittadinia blackii seed un-oculated with Gliocladium virens						plant count/0.5m sq		19/06/2014	Rep 1
9	3	0	0	1	0	0	0	13	0
0	0	2	0	0	2	1	3	0	7
0	6	4	1	5	7	0	8	8	9
1	3	9	0	7	1	0	13	1	0
0	2	6	22	1	3	0	1	0	0
4	3	3	2	15	15	4	2	0	1
16	9	2	5	16	1	12	7	3	0
7	4	1	4	2	5	3	4	0	0
0	0	1	1	5	12	10	7	7	8
0	11	1	0	3	0	12	10	0	0
37	41	29	35	55	46	42	55	32	25
								397	
								79.4plants/m sq	

Austrostipa nodosa inoculated with Gliocladium virens						plant count/0.5m sq		19/06/2014	Rep 1
10	3	11	10	2	6	9	8	5	6
10	0	11	12	4	3	5	5	11	2
9	0	4	7	8	6	5	6	8	10
12	0	3	10	9	9	6	6	4	7
7	1	2	11	3	11	7	8	7	7
6	0	2	9	3	6	6	10	8	12
11	0	2	7	2	8	7	13	4	5
6	0	4	6	4	6	3	11	6	7
0	1	2	7	6	9	5	10	8	8
0	9	9	7	2	9	4	8	7	9
71	14	50	86	43	73	57	85	68	73
								620	
								12.4plants/m sq	

Austrostipa nodosa uninoculated plant count/0.5m sq 19/06/2014 Rep 1

0	5	10	0	6	2	8	5	0	0
3	8	0	8	5	3	9	11	7	1
3	4	11	9	7	7	2	4	2	0
4	3	2	5	2	7	11	1	4	0
5	0	6	1	1	2	7	2	2	3
9	0	1	4	8	4	7	7	4	6
3	0	9	7	2	4	4	5	5	2
3	0	0	2	2	4	8	3	5	3
4	0	4	6	3	9	7	2	2	3
2	2	6	2	3	6	7	0	6	2
36	22	49	44	39	48	70	40	37	20

405

8.1plants/m sq

Austrostipa nodosa inoculated with Gliocladium virens plant count/0.5m sq 19/06/2014 Rep 2

7	5	6	9	6	4	5	6	9	8
8	5	7	9	8	4	5	8	8	9
6	5	6	9	7	5	8	8	6	5
10	7	9	7	7	5	7	6	7	8
6	6	7	7	8	5	7	5	6	6
3	7	6	6	7	8	9	7	8	6
11	6	6	8	6	5	6	4	9	6
8	7	6	11	10	8	6	6	8	6
9	5	8	5	3	10	5	7	5	6
5	7	7	4	3	6	5	7	7	6
73	60	68	75	65	60	63	64	73	66

667

13.3plants/m sq

Austrostipa nodosa uninoculated plant count/0.5m sq 19/06/2014 Rep 2

3	5	4	6	7	7	7	4	5	4
6	7	7	6	3	6	6	5	5	6
9	6	6	6	7	5	5	4	6	6
8	6	5	5	6	4	8	4	7	4
4	6	5	5	6	5	4	4	7	5
8	9	5	6	7	5	4	5	8	5
9	7	5	6	5	6	5	5	4	5
9	2	6	6	6	8	6	8	6	4
6	8	6	6	6	8	5	4	6	6
5	7	7	2	7	4		5	13	6
67	63	56	54	60	58	50	48	67	51

574

11.5plants/m sq

Austrodanthonia seed inoculated with Gliocladium virens count made on 26/8/14 Rep 1

35	28	27	25	34	42	21	35	35	35
27	25	27	26	34	25	25	30	30	35

22	35	24	40	28	33	28	42	39	25
33	26	29	27	28	38	40	29	27	30
17	35	33	40	26	20	35	24	35	25
23	34	24	30	28	24	39	32	32	30
29	25	27	30	34	24	40	20	35	35
25	24	26	31	27	24	38	28	30	34
28	25	23	35	36	31	36	28	24	20
24	22	26	28	33	35	37	30	26	23
263	279	266	312	308	296	339	298	313	292

2966

59.3 plants/m sq

Austrodanthonia seed un-inoculated count made on 26/8/14 plants/0.5m sq

26	23	25	30	18	25	36	25	22	26
40	28	24	30	26	20	23	23	29	32
38	30	25	25	19	28	16	24	33	35
38	17	21	10	22	25	18	27	31	28
30	34	25	22	25	25	25	27	39	25
28	36	25	34	20	30	17	20	24	34
33	36	28	19	20	25	36	23	29	21
20	25	49	17	19	26	27	20	30	19
26	35	35	23	28	27	25	18	32	27
23	26	36	25	25	27	42	22	28	12
302	290	293	235	222	258	265	229	297	259

2650

53 plants/m sq

Rep

Austrodanthonia seed inoculated with Gliocladium virens count made on 26/8/14 plants/0.5m sq 2

35	38	25	20	30	28	30	25	27	21
29	22	20	20	35	19	31	20	29	19
34	23	23	33	32	20	27	23	26	15
40	25	33	38	30	18	17	27	17	21
25	26	28	33	32	20	20	28	19	21
33	25	39	20	28	14	25	24	22	16
30	30	38	28	29	18	20	26	18	15
30	38	29	13	27	27	18	25	18	17
36	26	31	22	28	27	19	29	30	21
36	24	26	34	27	23	36	27	22	18
328	277	292	261	298	214	243	254	228	184

2579

51.6plants/m sq

Autrodanthonia seed un-inoculated count made on the 26/08/2014 plants/0.5m sq Rep 2

21	15	19	20	13	28	16	20	25	22
26	15	17	22	15	29	18	18	26	19
15	18	28	23	13	20	25	15	20	21
16	17	27	19	11	21	19	21	23	22
18	18	23	20	14	18	18	25	26	27

15	20	19	21	25	15	15	18	28	23
18	16	18	18	18	20	18	18	23	16
19	17	20	12	23	20	17	17	20	15
23	20	22	12	19	22	25	15	20	15
18	15	23	14	20	21	18	20	20	18
189	171	216	181	171	214	189	187	231	198
								1947	
								39.9plants/m sq	