# Investigating establishment techniques to improve Allocasuarina luehmannii and Eucalyptus microcarpa tube stock survival and growth

**Final Report**

**October 2009**

**Prepared for the Native Vegetation Council by Clinton Sim, PIRSA Forestry**

Government of South Australia
Primary Industries and Resources SA

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## 1. Summary

In July and August 2008 buloke (*Allocasuarina luehmannii*) and grey box (*Eucalyptus microcarpa*) seedlings were established at two sites near Frances, South Australia. A range of different establishment treatments were applied to test the effect of treatment and site on survival and height growth of both species. Each treatment was replicated four times at each site and height measurements recorded after planting. The trees were remeasured for height in June 2009 to determine year one survival and height growth.

The *A. luehmannii* survival was 45% and *E. microcarpa* survival was 64% with significantly higher survival rate at site one for both species. The reason for the difference in the sites was attributed to the difference in texture and water availability of the soil type. There was no significant difference in survival between treatments for both *A. luehmannii* and *E. microcarpa*. The large within treatment variation affected the statistical analysis.

Height growth was a secondary assessment to survival. There was no significant difference in *A. luehmannii* height growth between sites or treatments. The height growth was affected by livestock grazing and the die back and subsequent re-shooting of some *A. luehmannii* limited the number of height growth measurements available for assessment. *E. microcarpa* height was significantly affected by site as height growth was greater at site one where *E. microcarpa* are present in the vicinity but are absent at site two which may indicate that site one is a more suitable site for *E. microcarpa*.

The control treatment of site cultivation and weed control is an acceptable treatment to establish *A. luehmannii* and *E. microcarpa*.

## 2. Aim and Objective

The aim of this research was to identify successful methods for establishing buloke, *(Allocasuarina luehmannii)* and grey box *(Eucalyptus microcarpa)*. This was achieved through evaluating different broad acre establishment treatments on the survival and growth of both species in a replicated trial design at two locations.

## 3. Methodology

### *3.1. Project Location*

Two sites were selected near Frances within the natural distribution of *A. luehmannii* and *E. microcarpa* (Figure 1). This enabled the project to be replicated at both sites. Site one is established located on the Gap Road; approximately 15 km west of Frances (Figure 2). Site two is established on the Bordertown Frances Road; approximately 2 km north of Frances (Figure 2).

The soil group for both sites is deep uniform to gradational soils. The soil unit is deep friable gradational clay loam with properties of neutral to alkaline clay loamy to clayey soil. Within this soil unit there are two soil types; a common soil type that is deep friable gradational clay loam and secondary soil type that is brown or grey cracking clay.

The soil at site one is the common soil type, deep friable gradational clay loam:

- Soil is deep and without obvious texture changes between layers.
- Surface textures range from loamy sands to clay loams.
- There is little if any carbonate in the profile (Hall et al, in prep)

Site two is dominated by the secondary soil, grey cracking clay:

- Soil is clayey throughout.
- Soil cracks to the surface on drying in summer.
- Shear planes (slickensides) occur at depth, indicating significant movement of blocks of clay relative to each other. (Hall et al, in prep)

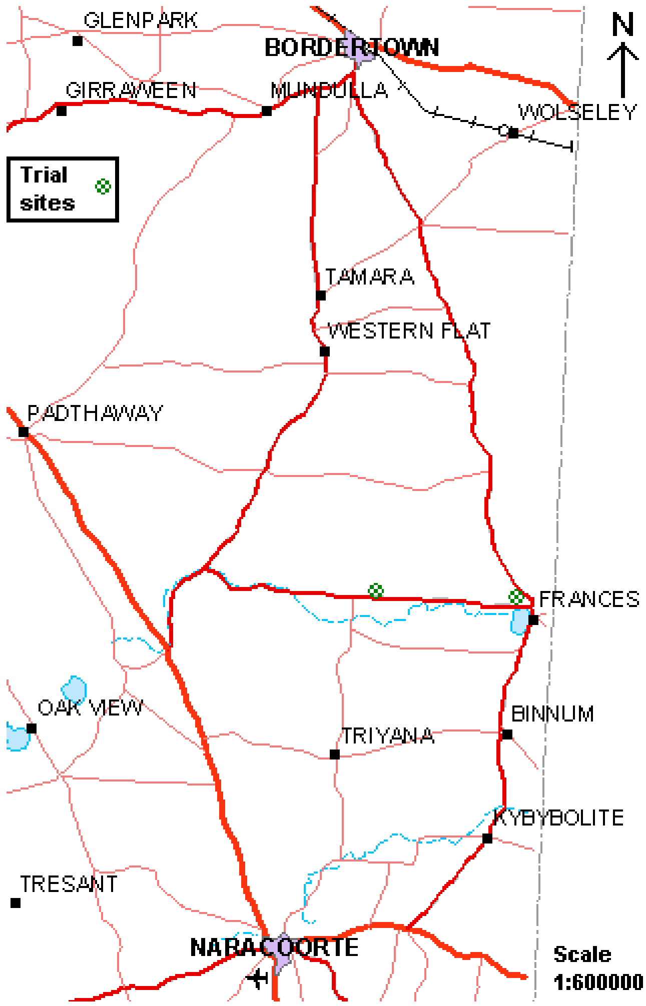


**Figure 1 Location of trial sites at Frances in the Upper South East of South Australia. Scale 1:600,000**

*A. luehmannii* is found naturally in the area along roadsides and in paddocks adjacent to the planted area. *E. microcarpa* is found naturally close to site one along roadsides and adjacent to the planted area in the paddocks. *E. microcarpa* is found within a few kilometres of site two.

### *3.2. Site Layout*

- The site layouts are 24 m wide belts at site one and 18 m wide belts at site two with 6 rows of trees.
- The rows are 3.5m apart at site one and 3m apart at site two, due to the width of the belt.
- There is a one row buffer around the trials.
- Plots are 14 x 14 metres at site one and 13, 13.1 or 13.9 x 12.5 metres at site two.
- Each plot has 20 trees per plot (four rows by five trees). *Note: plot 220 at site two only had 19 trees planted.*
- There are 15 treatments per site that are replicated four times for a total of 120 plots.
- A randomised plot design was used to locate the treatments for the plots.

- Plots are numbered from 101 to 220 (Appendix 3).
- To minimise the effect of a roadside tree overhanging site one, no plot was located beneath it between plot 130 and 131 (Appendix 3).
- There are three contiguous planting areas at each site (Appendix 3).
- The western planting at site one was not long enough to accommodate the entire *E. microcarpa* planting so plot 160 was located in the eastern planting with sufficient buffer from the adjacent *A. luehmannii* plot (Appendix 3).
- The total planted area was 3.2 hectares with 1.8 hectares at site one and 1.4 hectares at site two.


**Figure 2 Location of the two trial sites near Frances. Image source nrmMAPS, 2008 aerial photograph. Scale 1:75,000**

### *3.3. Site cultivation*

- Both sites were prepared by ripping followed by offset discing or harrowing.
- Site one with the deep gradational clay loam soil was ripped early in 2008. Rip lines were driven over to remove air holes and then ploughed with an offset disc to level rip lines and break up the clods.
- Site two has grey cracking clay and could not be ripped until opening rains enabled a double pass rip. The rip lines were driven over to remove air holes and harrowed to break up the clods leaving a slight depression for water collection into the rip lines.

### *3.4. Weed Control*

- Weeds were sprayed with herbicide in late spring 2007 to minimise weed loading in the autumn.
- A further preplant spray was applied in June 2008, which was a combination of knockdown and residual herbicide to provide weed control into the spring.

### 3.5. Planting

- All *A. luehmannii* seedlings were grown in 300 cubic centre metre (cc) tube stock at two nurseries. All *E. microcarpa* seedlings were grown at one nursery in 300 cc tube stock, except for the Hiko treatment, which were planted in V93 Hiko trays that are 93 cc. 300 cc tube stock were used because previous trials indicated that *A. luehmannii* survived better in tube stock than seedlings from Hiko trays on farmed sites (Smith, 1998). The sites were planted at the end of July through to mid August 2008, after the withholding period for the residual herbicide had concluded and sufficient rain had fallen to wet the soil profile.

### 3.6. Treatments

Table 1 details the ten treatments used in the trial. Nine treatments were applied to *A. luehmannii* and six to *E. microcarpa*. The Inoculation treatment was only applied to *A. luehmannii* and the Hiko treatment was only applied to *E. microcarpa.*

**Table 1 Treatment descriptions and identification of which treatment was applied to each species**

| | Species treatment is relevant to | |
|---|---|---|
| **Treatment type** | *A. luehmannii* | *E. microcarpa* |
| Control | ✓ | ✓ |
| Gypsum | ✓ | ✓ |
| Fertiliser | ✓ | ✓ |
| AquaStore™ | ✓ | ✓ |
| Treemaxx™ | ✓ | ✓ |
| Inoculate | ✓ | |
| Inoculate & Fertiliser | ✓ | |
| Inoculate & AquaStore™ | ✓ | |
| Inoculate & Treemaxx™. | ✓ | |
| Hiko | | ✓ |

- The site cultivation and weed control treatment served as the control treatment.
- The gypsum treatment was selected because the soil testing at both sites indicated the dispersive nature that were moderately to highly sodic. Dispersed clays can block soil pores and reduce the permeability to water and act as cement that hardens the soil when it dries (Frost & Orr, 2006). On these soils with high clay content, gypsum (calcium sulphate), improves soil aeration, soil structure and water permeability, which facilitates root growth. The calcium binds to the clay particles binding two clay particles at once, causing them to clump together. Gypsum can alleviates sodic soils, which have high levels of sodium salts because the sulphate combines with the sodium ions that the calcium displaces and the sodium is then flushed through the profile in the soil water (Frost &Orr, 2006; Gypsum Australia, 2008; SARDI, 2009). Gypsum would probably be beneficial in improving soil structure, water permeability and allow more rainfall to enter the soil thereby improve the water holding capacity.
- The gypsum was applied at 60g per seedling to equate to 5 tonnes of gypsum per hectare, which is the recommended rate for these types of soils. Toole et al. (2009) recommends applying gypsum at a rate of 5 t/ha for highly sodic and sodic alkaline soils, as gypsum is less effective when soils have a high pH. The soil at both sites is moderately to highly sodic and is neutral to slightly alkaline, therefore this rate was selected.
- The water retaining agent, AquaStore™, is an amorphous siliceous earth and a selected cross linked super absorbent polymer. It can absorb up to 150 times its own

weight in water. It is non-toxic, bio-degradable and will last for up to at least 2 years in the soil. The water storage capability of AquaStore™ was expected to provide the seedlings with moisture during water deficit in the summer. 60 g of AquaStore™ was mixed with 10 litres of water an hour or more prior to application so the Aquastore™ absorbed the water. 500 ml of hydrated Aquastore™ was applied to each planting hole, which equates to a rate of 3 g per seedling.

- The fertiliser treatment was included to identify whether providing nutrients would stimulate root growth and thus survival and growth and to also evaluate fertiliser separately from the fertiliser component of the Treemaxx™ treatment. In a trial of *Casuarina cunninghamiana* a fertiliser effect was evident in improved growth responses to nitrogen and phosphorous fertilisers when the site had been ripped and weeds controlled (Flux, 1998). The fertiliser treatment was a 20g dose of two 10 g Typhoon™ tablets buried approximately 10 cm deep and 5-10 cm from the seedling. Typhoon™ tablets slowly release nutrients over 12 months and have the following chemical analysis.

| | |
|---|---|
| TOTAL NITROGEN | 20.00% w/w |
| Nitrogen (N) as ammonium | 1.60% w/w |
| Nitrogen (N) as ureaform | 18.40% w/w |
| TOTAL PHOSPHORUS | 4.40% w/w |
| Phosphorus (P) as water soluble | 3.04% w/w |
| Phosphorus (P) as citrate soluble | 0.85% w/w |
| Phosphorus (P) as citrate insoluble | 0.51% w/w |
| POTASSIUM (K) as sulphate | 8.20% w/w |
| Calcium (Ca) as phosphate | 4.00% w/w |
| Sulphur (S) as sulphate | 6.00% w/w |
| Magnesium (Mg) as oxide | 0.20% w/w |
| Copper (Cu) as sulphate | 0.03% w/w |
| Zinc (Zn) as oxide | 0.50% w/w |
| Iron (Fe) as sulphate | 0.33% w/w |
| Manganese (Mn) as sulphate | 0.16% w/w |
| Molybdenum (Mo) as molybdate | 0.01% w/w |
| Boron (B) as tetraborate | 0.01% w/w |

- The product, Treemaxx™, contained a water retaining agent and a fertiliser. This offered the potential benefit of providing nutrients to stimulate root growth and retain excess moisture in the profile for periods of moisture stress. This would be more efficient than two separate applications of Aquastore™ and Fertiliser. Treemaxx™ was applied at 50g per seedling according to the product instructions. Treemaxx™ is specially formulated with the following chemical analysis.

| | |
|---|---|
| Nitrogen (N) | 4.71% w/w |
| Phosphorus (P) | 2.52% w/w |
| Potassium (K) | 2.96% w/w |
| Calcium (Ca) | 11.35% w/w |
| Sulphur (S) | 6.2% w/w |
| Magnesium (Mg) | 0.323% w/w |
| Copper (Cu) | 0.185% w/w |
| Zinc (Zn) | 0.46% w/w |
| Iron (Fe) | 0.35% w/w |
| Manganese (Mn) | 0.234% w/w |

**Figure 3 Planting and tree guarding at site one.**

- *Allocasuarina* species have soil associations with actinomycete bacteria *Frankia* spp. that fix nitrogen for the host (Flux, 1998; Wheeler et al 2008). Inoculating *Allocasuarina* with *Frankia* bacteria where it is absent can significantly increase planting success and growth (Thrall et al, 2001). Where mycorrhizae fungi is absent from a site inoculation of *Allocasuarina* has been reported be particularly beneficial to facilitate the uptake of nutrients and increase water availability (Torrey, 1983; Thoen et al, 1990; Flux, 1998). Arbuscular mycorrhizae or ectomycorrhizae fungi could also improve the success of *Frankia* symbiosis and together enhance nitrogen fixation and soil nutrient and water uptake (He & Critchley, 2008). Inoculation of transplanted seedlings with soil collected from around the base of nodulated actinorhizal plants has been documented (Carr et al, 2009).

  Inoculation of the *A. luehmannii* was attempted when the seed was planted in the nursery in November 2007. A total of 6.4 kg of soil was collected from the shallow rooting zone beneath remnant roadside *A. luehmannii* in the Frances region and added to the potting mixture for the tube stock to be inoculated. This was equivalent to 10 g of soil per tube stock to establish inoculated *A. luehmannii*. However due to poor germination and survival of *A. luehmannii* in the nursery, insufficient inoculated tube stock was available. The inoculate treatment had to be applied when the seedlings were planted, which was achieved by applying a measured volume of 50 cc of soil sourced from the shallow rooting zone beneath nearby remnant *A. luehmannii* to each planting hole prior to planting.
- Previous research and experience has indicated that *A. luehmannii* should be grown in larger tube stock of 300cc volume for enhanced survival and growth. There is no

information available whether it is also beneficial for *E. microcarpa*. All *E. microcarpa* seedlings were grown at the one nursery in 300 cc tubes, except for the Hiko treatment, which were planted in V93 Hiko trays.

- When Treemaxx™ or gypsum treatments were applied they were mixed in with soil in the planting hole to avoid burning the seedling roots.
- Treatments were applied at planting except for the fertiliser treatment, which was applied a month later due to delay in availability of the product.

### 3.7. Tree Guards

Ezi guard, core flute, tree guards (450 x 200 mm) were erected around each seedling in the plots and the buffers after planting (Figure 3 and 4).

### 3.8. Measurement Assessments

Initial seedling heights were recorded in September 2008. The following year survival and height measurements were recorded in June 2009.

**Figure 4 Established trial at site two.**

### 3.9. Data analysis

The mean survival and mean positive height growth was assessed for *A. luehmannii* and *E. microcarpa* using a two factor analysis of variance (ANOVA) with replication to distinguish between the two independent variables: site and treatment. The level of significance was set at 0.05. The Tukey test identified which means were significantly different at the 95% confidence limit.

The *A. luehmannii* inoculation treatments were compared with the standard treatments using a single-factor ANOVA with replication to assess the mean survival and mean height growth. The inoculated and standard treatment data was pooled from both sites and across the four treatments (Table 2).

**Table 2 Standard and inoculation treatments for comparison of the effect of inoculation**

| Standard treatment | Inoculation treatment |
|---|---|
| Control | Inoculate |
| Aquastore™ | Inoculate & Aquastore™ |
| Fertiliser | Inoculate & Fertiliser |
| Treemaxx™ | Inoculate & Treemaxx™ |

Positive height growth was assessed rather than final height because, firstly, there was a significant difference in the initial heights of the *A. luehmannii* seedlings between the two sites and a significant difference in the initial heights of the *E. microcarpa* seedlings between treatments. Secondly, a number of *A. luehmannii* seedlings had died back and sprouted from the base and a number of seedlings had been eaten when livestock broke into the trial at site two, which would bias the site effect.

## 4. Results

### 4.1. Survival

The overall *A. luehmannii* survival was 45% and *E. microcarpa* survival was 64%. The ANOVA indicated a significant effect only on *A. luehmannii* survival (Table 3), but the Tukey test, a more sensitive test, could not identify which *A. luehmannii* treatments were significantly different in mean survival. The large variation in mean survival between treatments is highlighted in Figure 5 where one standard deviation for each treatment is indicated by the error bars. All of the error bars overlap verifying that no treatment significantly affects *A. luehmannii* survival. Site had significant effects on *A. luehmannii* and *E. microcarpa* survival (Table 3). Site one had higher mean survival for *A. luehmannii* with 51% survival compared with 38% survival at site two. *E. microcarpa* survival was 76% at site one compared with survival at site two of 51%.

**Table 3 Probabilities of a greater F-value for mean survival at two sites with nine establishment treatments for *A. luehmannii* and six establishment treatments for *E. microcarpa*.**

| Effect | *A. luehmannii* | *E. microcarpa* |
|---|---|---|
| Treatment | 0.007 | 0.06 |
| Site | 0.03 | $9.5 \times 10^{-5}$ |
| Interaction | 0.89 | 0.57 |

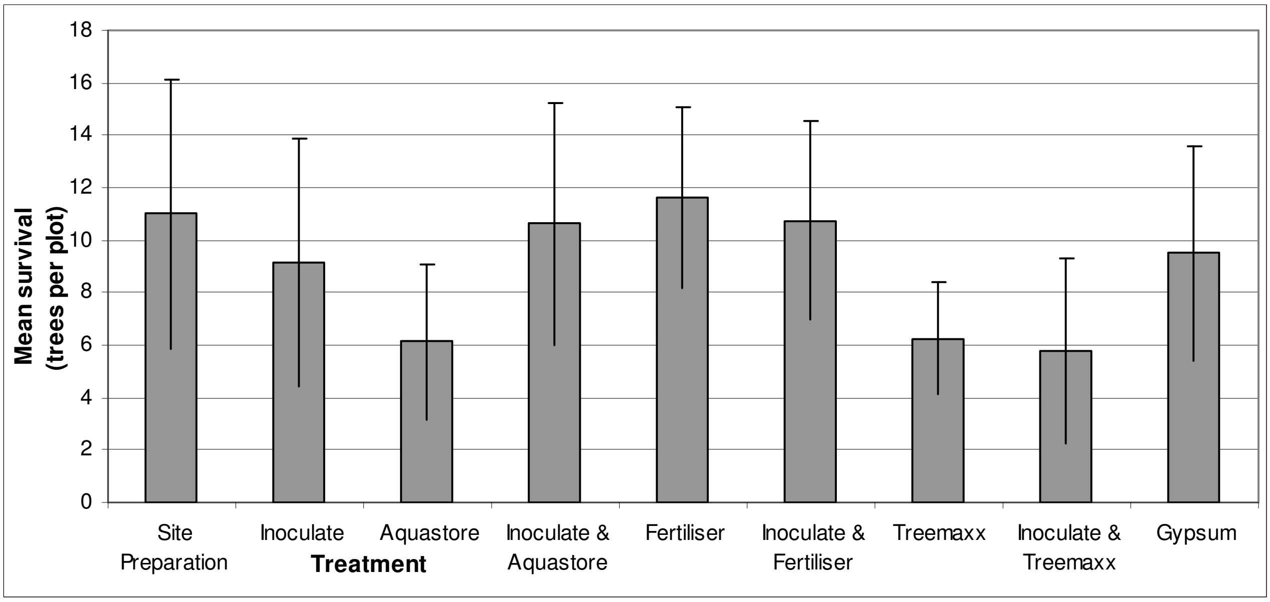


**Figure 5 Mean survival of *A. luehmannii* for the nine treatments with standard deviation error bars**

Observation of survival between the rows at each site indicated that survival is greater in the rows further from the road at site one. There was no difference between rows at site two, as it was not near a road. The mean plot survival between the rows at site one was investigated and a trend exists of increasing mean row survival per plot from the road, row one, to the rows furthest from the road, row three and four. The trend is exhibited in the western block planted with *E. microcarpa* and the middle planted block of *A. luehmannii* but not the eastern planted block of *A. luehmannii* (see Figure 6). There was a significant difference in mean survival between the rows of the western and middle blocks ($P = 5x\ 10^{-5}$). A Tukey test identified that the row beside the road in the western and middle blocks had a significantly lower mean survival compared to row three and four, the rows furthest from the road.

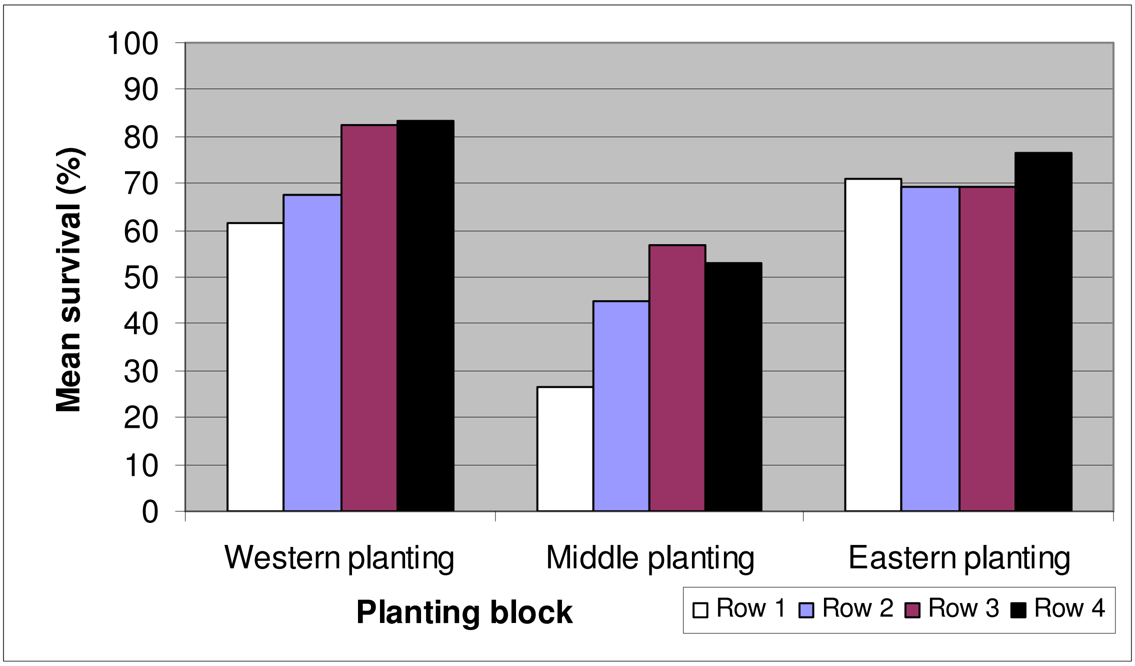


**Figure 6 Site one plot mean survival between the rows of the three planting blocks.**

## *4.2. Height growth*

Treatment had no significant effect on *A. luehmannii* or *E. microcarpa* height growth (Table 4). The *A. luehmannii* Inoculate & Fertiliser treatment, although not significant, did have greater height growth than the other treatments (Figure 7). Site had a significant effect only on *E. microcarpa* height growth (Table 4 & Figure 8). The overall height growth of *E. microcarpa* was 61cm. Site one also had higher mean height growth with 75 cm compared with 47 cm at site two.

**Table 4 Probabilities of a greater F-value for mean height growth at two sites with nine establishment treatments for *A. luehmannii* and six establishment treatments for *E. microcarpa*.**

| Effect | *A. luehmannii* | *E. microcarpa* |
|---|---|---|
| Treatment | 0.06 | 0.37 |
| Site | 0.16 | $2.1 \times 10^{-8}$ |
| Interaction | 0.36 | 0.10 |

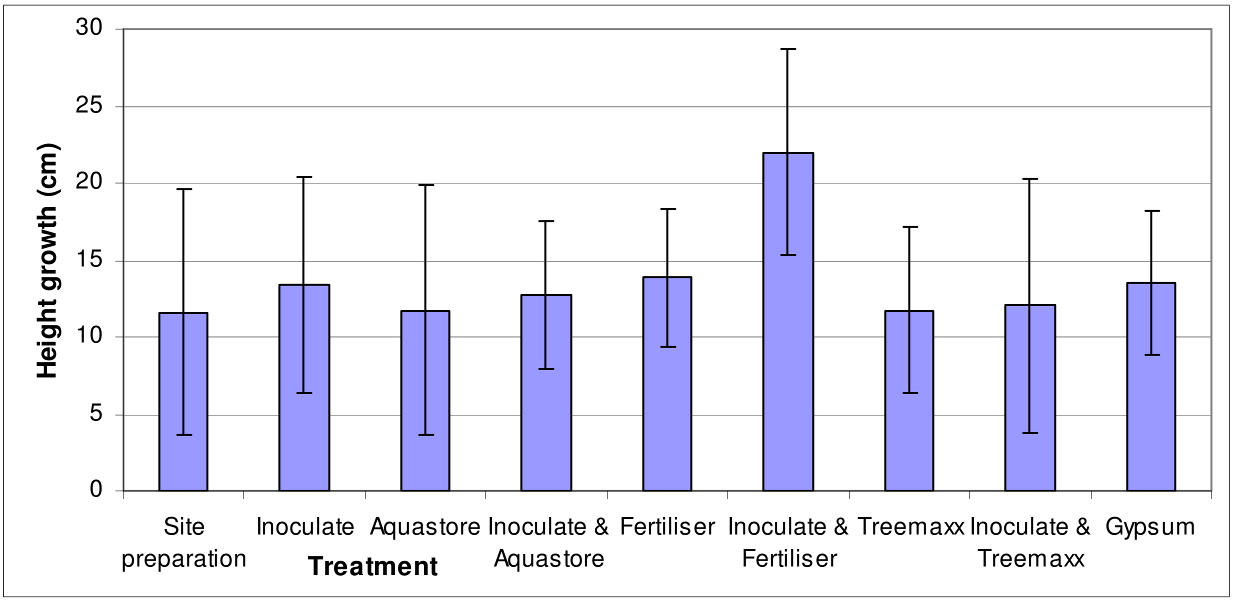


**Figure 7 *A. luehmannii* mean plot height growth for the different treatments with standard deviation error bars**

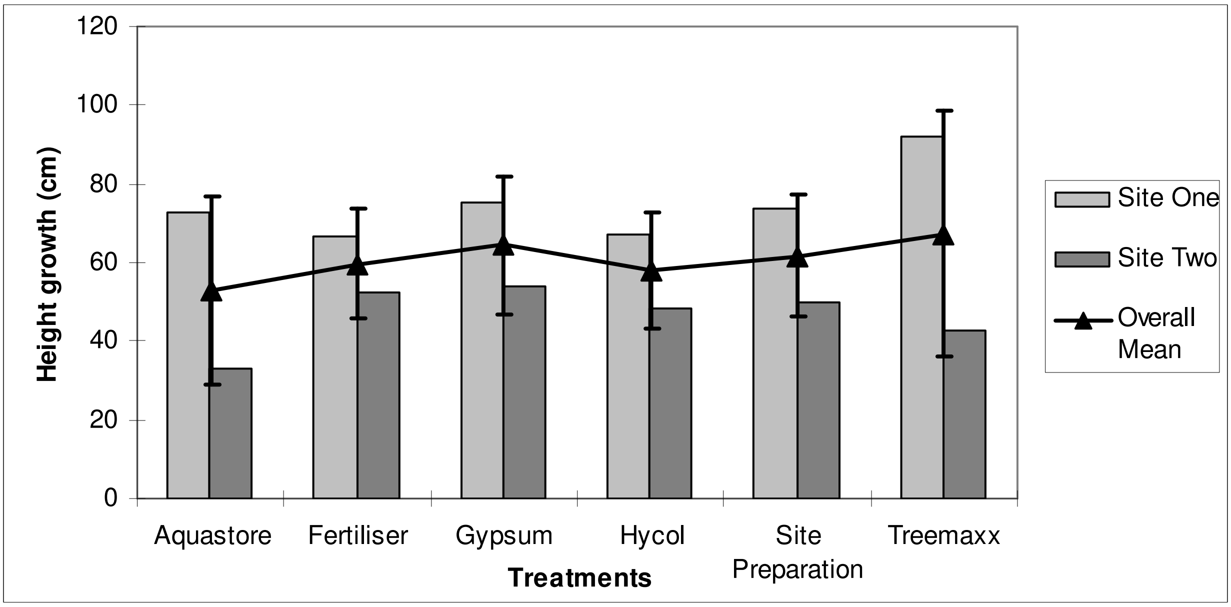


**Figure 8 *E. microcarpa* mean plot height growth for the different treatments at the two sites and the overall mean for each treatment with standard deviations displayed**

### 4.3. Inoculation

Inoculation had no significant effect on *A. luehmannii* survival or height growth (Table 5). Although there was no significant effect on height growth, there was a trend of increased height growth in all treatments when inoculated. The improvement in height growth is in contrast to survival where seedlings that were not inoculated had predominantly better survival.

**Table 5 Probabilities of a greater F-value for *A. luehmannii* mean survival and height growth comparing inoculation and standard treatment.**

| Effect | *A. luehmannii* survival | *A. luehmannii* height growth |
|---|---|---|
| Treatment | 0.77 | 0.12 |

## 5. Discussion

### 5.1. Treatments

**Survival**

The treatments for both species could not be ranked because there is no treatment that is significantly better than another treatment regardless of site due to the large variation in survival between the plots. The lack of any difference between *A. luehmannii* treatments corresponds with the results of the Wimmera Farm Tree Improvement Group who found no difference between the best bet treatment using weed mat, fertiliser tablet and water retaining gel and the control (Smith, 1998). Flux (1998) recommended ripping early, breaking clods in the rip lines to remove air pockets and maintaining a weed free site prior to planting *A. luehmannii.* The results for the control treatment; site cultivation and weed control, indicate that basic site cultivation and weed control is sufficient to maximise survival and growth of *A. luehmannii* and *E. microcarpa* in the first year and additional treatments are not beneficial.

The extra effort and cost of applying the treatments trialled cannot be justified because there was no significant improvement in survival. Furthermore, some treatments performed poorer than the control. Despite the lack of statistical significance Aquastore™ and Treemaxx™ would not be recommended when planting *A. luehmannii* and Aquastore™ would not be recommended when planting *E. microcarpa*. Both Aquastore™ and Treemaxx™ contain a water retaining agent that may not have improved water availability because of the strong retention of water characteristic of soils with high clay percentages (Smith, 1998; McLaren & Cameron, 1996). As the soil dried, the clay particles may have scavenged the moisture stored in the products making this unavailable for the seedlings negating any benefit of the agent retaining the water.

*A. luehmannii* seedlings had to be sourced from two nurseries as extreme summer heat reduced the number of seedlings available from the original nursery. The planting stock differed between the nurseries by growth stage and root development. To avoid the impact of this variation in planting stock the same number of seedlings from each nursery was used in each plot.

The *A. luehmannii* seedlings supplied from one nursery were not well developed. They were small with limited foliage and corresponding small root mass. Although the roots were predominantly fine white roots ideal for replanting, there were too few to hold the planting medium together when removed from the tube and planted. This increased the risk of damage to the roots, which could have affected survival and ability to grow rapidly. Damaged roots may not have been able to grow sufficiently after planting to source water and nutrients resulting in death during the dry summer.

The *A. luehmannii* seedlings from the other nursery were very tall with a lot of foliage and had a solid mass of roots. This indicated that these seedlings were old stock, predominantly two years old. The aged root system was brown and quite bound with few fine white growing tips. This could have impacted on the ability of the roots to grow sufficiently to provide enough resources for the excessive foliage. A number of these seedlings died back and sprouted from the base to cope with the lack of water available from the small number of actively growing roots.

The Hiko *E. microcarpa* treatment had a lower mean survival than the control in tube stock. The Hiko seedlings were 6.7 cm at planting, which was significantly smaller ($P= 8.7 \times 10^{-10}$) than the tube stock seedlings at 15.3 cm.

PIRSA Forestry recommends that seedlings should ideally be 20 cm in height and no taller than 30 cm. The leaves should be healthy, but hardened off with a minimum of five pairs for eucalypt seedlings. The stems should be strong enough to support new growth with a minimum diameter of 2.3mm. The root system needs to be fibrous, well distributed with good vertical growth.

Overall *A. luehmannii* survival might have been higher if the seedlings were of better quality. There is a need to source and plant only one year old planting stock that has a vigorous actively growing root system with a matching healthy amount of foliage. The lower survival of the Hiko treatment seedlings highlights that seedlings should meet minimum standards at planting to maximise survival. It is recommended that *E. microcarpa* seedlings meet the recommended specifications regardless of what size tray or tube stock is used.

The mean survival of *A. luehmannii* in treatments combined with inoculation was not significantly different from the same treatments without inoculation. The treatments without inoculation tended to have slightly higher mean survival than the corresponding treatment with inoculation (Figure 5); however the opposite is true for Aquastore™ and Inoculate & Aquastore™. Although not significant there was a large variance and Inoculate and Aquastore™ treatment had higher survival than Aquastore™. The soil differences at plot location may account for the higher survival of the Inoculate & Aquastore™ as two plots were located in the eastern planting of site one with comparatively better soil properties than two Aquastore™ treatment plots at site two that were located in an area that was low lying and prone to water logging.

A number of factors may have affected the inoculation treatment effect on *A. luehmannii* survival. *Frankia* actinomycete bacteria were not expected to have an affect on survival in the first season where water availability would be critical, as it fixes nitrogen (Carr et al 2009). Micorrhizal fungi can potentially assist with water availability (Torrey, 1983), but the soil used to inoculate the seedlings was not tested to determine if it contained any micorrhizal fungi. If mycorrhizal fungi was present Thoen et al (1990) has shown that six week old *A. luehmannii* germinants inoculated with mycorrhizal fungi and grown under optimal conditions in a laboratory produced associations in two months. However in the field the conditions were very different so that if there was a suitable mycorrhizal fungus in the introduced soil the conditions may not have been conducive or it may not have been able to establish an association in time to improve seedling during the water stress period. Additionally, there may already have been mycorrhizal fungi present in the soil that were able to established associations with the non inoculated seedlings. Assessment of seedlings would be the only way to assess if any relationships had formed in both deliberately inoculate and non- inoculated seedlings. Although there are risks of spreading diseases in nursery (Carr et al, 2009) and there were difficulties in this trial with seedling numbers, inoculation of seedlings in the nursery of known micorrhizal fungi and *Frankia* symbiotic with *A. luehmannii* will ensure that the association is established prior to planting and maximises the possible benefit in the field.

**Height growth**

There was no treatment that produced significantly better *A. luehmannii* height growth. There were two factors that limited the statistical analysis of the *A. luehmannii* height growth: the combined effect of livestock grazing on seedlings in site two and seedlings dying back, due to lack of rain, and sprouting from the base after the heavy rains in December. The reduced the number of height growth measurements for each plot increased the variance and thereby reduced the strength of the statistical analysis of the means. This could have contributed to the lack of significance in height growth between treatments or falsely identifying the Inoculate & Fertiliser treatment as producing the greatest *A. luehmannii* height growth

*E. microcarpa* treatments did not produced significantly different height growth. The height growth across all treatments was very similar, even though the Hiko treatment seedlings were significantly smaller ($P = 8.7 \times 10^{-10}$) and only 6.7 cm at planting compared with 15.3 cm for the other treatments. The use of the height growth as the assessment measure took into account the difference in initial seedling height between treatments. Although Hiko seedlings will produce suitable height growth that is comparable to tube stock, the lower survival of the Hiko seedlings in this trial is the restriction of this treatment.

Inoculation does not significantly improve *A. luehmannii* survival or height growth. There is a trend that inoculated treatments have a greater mean height growth than their non-inoculated pair (Figure 7) and is particularly noticeable in the Fertiliser treatment pair. As stated above the restricted number of measurements may have contributed to the lack of significance or produced a distorted positive trend for inoculation. Furthermore, there is no certainty that the soil used to inoculate the *A. luehmannii* had the actinomycete bacteria *Frankia* or micorrhizal fungi or the actual amount each seedling and plot received was equal. Carr et al (2009) stated that the results of soil transfer are variable depending on the amount of inoculum present. The results indicate that there is possibly something in the soil that is improving height growth. Other research where *Casuarinas* were inoculated with a *Frankia* strain resulted in a spectacular increase in tree heights of 50 to 200% (Carr et al, 2009) and *Casuarina cunninghamiana* inoculated with *Frankia* strains recorded height increases in 14 months after planting of 50% to 70% in comparison with non-inoculated seedlings (Reddel, 1988). Although the results are not significant, they warrant further research into *Frankia* bacteria or micorrhizal fungi inoculation in the nursery or at planting and the effect on *A. luehmannii* height growth.

The large variation in survival and height growth of *A. luehmannii* and *E. microcarpa* between treatments affected the statistical ability to determine significant difference between treatments at 95% confidence limits. The random location of the plots at each site and restriction of only four plots per treatment did not sufficiently reduce the error. The site specific soil differences may have had the greatest influence on the survival and height growth of the plots than the treatments. Further trials should involve greater replication to reduce variation, which may in turn return clearer results. Smaller plots could be used, as space is an issue, although too small a sample size reduces the quality of the plot data and may result in no effective gain in statistical significance.

It must be noted that height growth is secondary to the survival. It is most important to have seedlings to firstly survive and we assessed height growth as it would have been useful to distinguish between treatments with similar survival.

### 5.2. Site

The rainfall immediately after planting until December and in the first two months of 2009 was lower than the mean for these months and may have affected the survival rate of both species at both sites (Figure 9). 64 mm of rain fell on 13 December ensuring seedlings of both species survived and may have allowed those *A. luehmannii* that had died back to re-shoot at this point and survive through to measurement. This highlights that climatic events will have a large bearing on the results of establishment of trees in this environment. To maximise survival, good establishment practice of soil cultivation and weed control will maximise the availability of moisture.

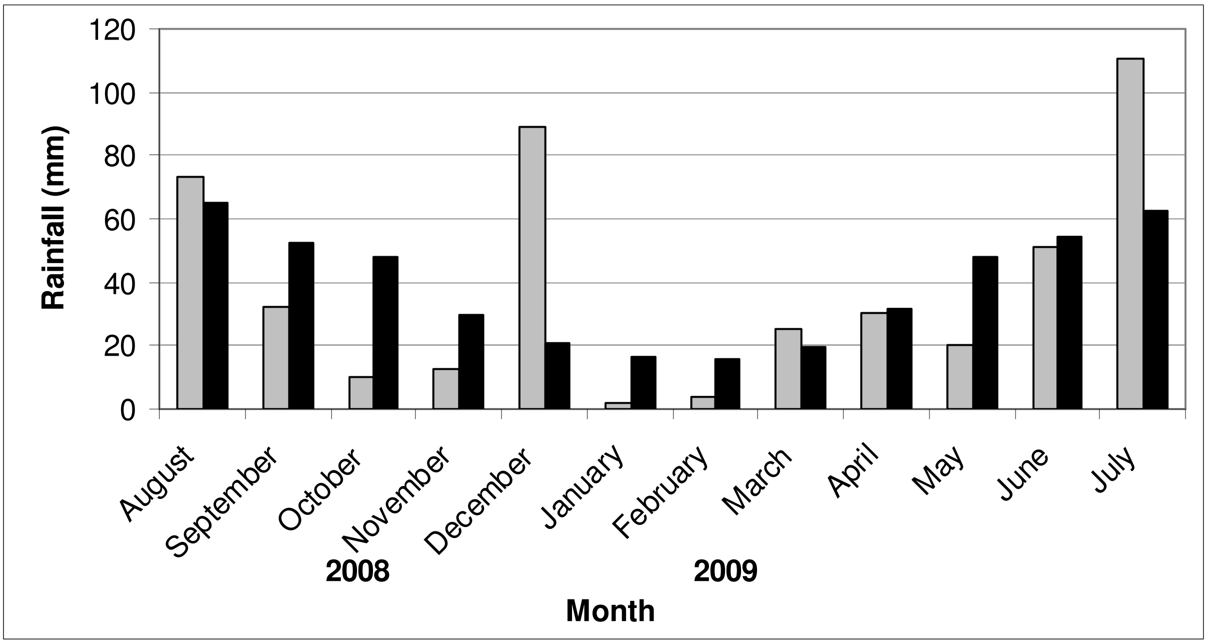


**Figure 9 Monthly rainfall at Frances meteorological station (026007) (grey bars) from planting in August 2008 until first year measurement in July 2009 compared to the median rainfall (black bars).**

Site one had better *A. luehmannii* survival. This was due to the high mean survival of 73% in the eastern block of site one compared to the survival at site two (Table 6). The significantly higher survival in the eastern block ($P= 2.2x10^{-5}$) is likely due to better soil structure. The texture of the soil in the eastern block is loamier than the other blocks resulting in higher water availability. This distinction in soil type was emphasised with observed difference in the weed spectrum between blocks.

The mean height growth of *A. luehmannii* at site two was significantly less than site one. This result is bias because of the missing height measurements due to the browsing of a large number of seedlings at site two. The browsing of seedlings introduces uncertainty into the result, for if these seedlings had not been browsed then they may have increased the mean seedling height at site two and therefore there may not have been a significant difference. This result is not conclusive and should be ignored.

**Table 6 Mean plot survival and variance for each block of *A. luehmannii* planted at the two sites.**

| *Groups* | *Count* | *Sum* | *Average* | *Survival* | *Variance* |
|---|---|---|---|---|---|
| Site one eastern block | 10 | 146 | 14.6 | 73% | 10.3 |
| Site one central block | 26 | 226 | 8.7 | 43% | 13.8 |
| Site two southern block | 18 | 145 | 8.1 | 40% | 20.2 |
| Site two northern block | 18 | 129 | 7.2 | 36% | 8.3 |

The difference in *E. microcarpa* survival and height growth between the sites may be better attributed to vegetation-soil associations. Site one had remnant *E. microcarpa* in the paddock and along the roadside and had a gradational clay loam soil. Although site two has *A. luehmannii* trees present, there were no *E. microcarpa* trees close by on this grey cracking clay soil. This site was 'Buloke (*A. luehmannii*) woodland' and was not known for previously supporting *E. microcarpa* (Johnson, 2005). Therefore the soil at this site is likely to be less suitable for *E. microcarpa*, hence survival and height growth was effected.

The difference between the two sites in survival for both species and height growth for *E. microcarpa* can be attributed to the difference in the clay content of the soils. These soils are dominated by clay, but the clay content of the soil at site two was higher than site one. The effect of too much clay in the texture results in poorer structure and reduced water availability (McLaren & Cameron, 1996). There are fewer pores of a suitable size that store plant available moisture and more soil water is held tightly in smaller pores by the large surface area and charge of the clay particles (McLaren & Cameron, 1996). A good balance of loam at site one, especially in the eastern block, ensured that these seedlings had a larger amount of plant available water in the soil than site two.

The high clay content and cracking nature of these soils leads to greater soil moisture loss during summer. When there is a high proportion of clay in this soil type there is a propensity for the large cracks to open up at the surface as the soil dries over summer (McLaren & Cameron, 1996). This cracking increases soil moisture loss reducing height growth and increasing the likelihood of seedling deaths. The cracks also produce shear planes that severe roots reducing the area of soil that seedlings can access moisture (McLaren & Cameron, 1996). Under low rainfall conditions that was experienced after planting the seedlings would have been under high water stress leading to reduced height growth and mortality in the grey cracking clay at site two and central block at site one.

The difference in row survival may have been caused by the proximity to the road and roadside fence. There may be products in the road base material that washes off the road onto this area that is impacting on soil properties. The road was a limestone sourced white metal road for a long period and a bitumen surface now overlays this. The run off will have included calcium carbonate from the limestone material that has increased the pH of the high clay content soil at the western and central blocks adjacent to the road. There may also be products from vehicle exhaust, pollution – such as engine oil, and more recently the products in the bitumen that may affect the soil in these blocks reducing seedling survival adjacent to the road. Soil tests would be needed to confirm this.

# 6. Conclusions

The major factor of difference in survival of both species between sites and in height growth of *E. microcarpa* between sites was the difference in soil type. Furthermore, variations in soil within sites had a significant impact on survival. This was particularly noticeable at site one compared to the more uniform site two. The loamier soil had greater survival and *E. microcarpa* height growth than the higher clay percentage soil because the soil structure is better, water holding capacity is greater and there is less propensity for cracking in summer reducing moisture stress of seedling. The improved survival and height growth due to the difference in site rather than treatment indicates that the affect of soil type is greater than any of the treatments trialled.

There was no significant difference in survival and height growth between the treatments for both species. Therefore the control treatment of site cultivation and weed control alone is recommended for establishing *A. luehmannii* and *E. microcarpa*. Treatments that contain water storage crystals are not recommended for similar heavy clay soils due to poor survival results especially for *A. luehmannii*. Further testing of establishment practices should include greater replication and plot design to account for soil variation.

Site cultivation and weed control alone is a good method for establishing both *A. luehmannii* and *E. microcarpa*. This treatment was the easiest and lowest cost treatment applied in this trial. This result highlights the importance of good weed control to maximise the availability of moisture for the seedling and the preparation of the soil to ensure the seedling roots were able to access moisture from the greatest amount of soil.

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# 8. Appendices

## *8.1. Appendix 1*

### Activities at each site

| Date | Activity |
| --- | --- |
| 09/11/2007 | Assess layout of trial and GPS both sites, collect inoculated soil; |
| 27/02/2008 | Test soil and assess ripping at site one and assess soil at site two; |
| 13/03/2008 | Assess discing at site one; |
| 28/04/2008 | Check fencing at site one and soil for ripping at site two; |
| 17/06/2008 | Provide spray prescription and herbicide for both sites and assess ripping at site two; |
| 24/07/2008 | Dropped off tree guards and stakes at both sites; |
| 25/07/2008 | Pegged out trial plots at both sites; |
| 31/07/2008 | Planting, treatment application and tree guarding site one; |
| 01/08/2008 | Planting, treatment application and tree guarding site one; |
| 04/08/2008 | Planting, treatment application and tree guarding site one; |
| 05/08/2008 | Planting, treatment application and tree guarding site one; |
| 13/08/2008 | Planting, treatment application and tree guarding both sites; |
| 14/08/2008 | Planting, treatment application and tree guarding site two; |
| 15/08/2008 | Planting, treatment application and tree guarding site two; |
| 21/08/2008 | Planting, treatment application and tree guarding site two; |
| 22/08/2008 | Planting, treatment application and tree guarding site two; |
| 08/09/2008 | Completed measurements at site one. |
| 17/09/2008 | Sprayed edge of trial where weed control was insufficient adjacent to the buffer at site two; |
| 19/09/2008 | Completed measurements at site two. |
| 26/09/2008 | Applied fertiliser treatment with tablet inserted in soil adjacent to seedlings at both sites. |
| 12/11/2008 | Site visit inspected the weed control and *A. luehmannii* and *E. microcarpa* survival at both sites. There were very few weeds at both sites and acceptable survival for a dry spring. |
| 13/03/2009 | Site visit inspected the weed control and *A. luehmannii* and *E. microcarpa* survival at both sites. Residual weed control was effective due to two months of minimal rain. Survival was approximately 50%. |
| 05/06/2009 | Measured all of the plots at the site one weed loading had increased considerably. |
| 12/06/2009 | Measured all of the plots at the site two the weed loading had increased but was not as prominent as the site one possibly due to livestock having entered and grazed the weeds and some trees in the trial. |
| 06/08/2009 | Field day held and both sites visited. |
| 12/08/2009 | Applied second year weed control at site one as one metre spots around all trees. |
| 18/08/2009 | Applied second year weed control at site two as one metre spots around most of the trees. |
| 19/08/2009 | Completed the second year weed control at site two. |

## 8.2. Appendix 2

### Seedlings

#### Eucaleuca Nursery, Naracoorte

- Initial request for seedlings, established 27/09/2007
- Soil collected for inoculated seedlings 9/10/2007
- *A. luehmannii* planted 11/10/2007
- Final numbers confirmed 16/11/2007
- *E. microcarpa* planted in December
- Inspection 17/06/2008 indicated that numbers of *A. luehmannii* would fall short, with only 1360 tube stock of the original 2210. A minimum of 850 further were required.
- Collected seedlings as needed on 31/07/2008, 04/08/2008, 15/08/2008

#### The Wool Factory nursery, Wail

- Requested 1000 *A. luehmannii* seedlings with sterile potting mix on 18/06/2008.
- Tube stock transported to Naracoorte with certificate for cross border transport and collected on 31/07/2008.

## 8.3. Appendix 3

### Maps of plot layout and treatment at each site

Plot layouts for each site can be found on the following page.

**Site one *A. luehmannii* and *E. microcarpa* trial site**

N

320 metres **Western planting** ***Eucalyptus microcarpa*, grey box**

Buffer surrounding plots; Row: Buffer, 1, 2, 3, 4, Buffer

| Plot | | 159 | 158 | 157 | 156 | 155 | 154 | 153 | 152 | 151 | 150 | 149 | 148 | 147 | 146 | 145 | 144 | 143 | 142 | 141 | 140 | 139 | 138 | 137 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | | H | A | F | T | G | S | G | S | T | T | A | F | S | A | G | H | T | H | S | G | H | F | A |

380 metres **Middle planting** ***Allocasuarina luehmannii*, buloke**

Buffer surrounding plots; Row: 1, 2, 3, 4

| Plot | 136 | 135 | 134 | 133 | 132 | 131 | | 130 | 129 | 128 | 127 | 126 | 125 | 124 | 123 | 122 | 121 | 120 | 119 | 118 | 117 | 116 | 115 | 114 | 113 | 112 | 111 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | A | S | IA | A | A | IT | | I | IA | IF | T | T | F | IT | G | F | I | IT | G | S | G | G | A | T | IF | I | F |

160 metres **Eastern planting** ***Allocasuarina luehmannii*, buloke**

Buffer surrounding plots; Row: Buffer, 1, 2, 3, 4, Buffer

| Plot | 160 | | 110 | 109 | 108 | 107 | 106 | 105 | 104 | 103 | 102 | 101 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | F | | S | IF | F | IA | IF | IA | IT | T | I | S |

Treatment

| | Treatment |
|---|---|
| S | Site cultivation and weed control only |
| G | Gypsum |
| H | Hiko planting stock (*E. microcarpa* only) |
| T | Treemaxx™ |
| A | Aquastore™ |
| F | Fertiliser tablet |
| I | Inoculation (*A. luehmannii* only) |
| IT | Inoculation with Treemaxx™ (*A. luehmannii* only) |
| IA | Inoculation with Aquastore™ (*A. luehmannii* only) |
| IF | Inoculation with Fertiliser tablet (*A. luehmannii* only) |

Plots 14 x 14m
Plots 4 rows wide and 5 trees long
One row buffer surrounding the trial

4/11/2009

**Site two *A. luehmannii* and *E. microcarpa* trial site**

N

| Plot | Treatment |
|---|---|
| 220 | I |
| 219 | T |
| 218 | IA |
| 217 | F |
| 216 | IF |
| 215 | S |
| 214 | I |
| 213 | S |
| 212 | IT |
| 211 | G |
| 210 | T |
| 209 | I |
| 208 | A |
| 207 | T |
| 206 | IA |
| 205 | A |
| 204 | F |
| 203 | G |

Buffer surrounding all sides

Row: Buffer 4 3 2 1 Buffer

**Northern planting**

***Allocasuarina luehmannii,* buloke**

270 metres long

Plots 12 x 13.0m
Plots 4 rows wide and 5 trees long
One row buffer surrounding the trial

| Treatment | |
|---|---|
| S | Site cultivation and weed control only |
| G | Gypsum |
| H | Hiko planting stock (*E. microcarpa* only) |
| T | Treemaxx™ |
| A | Aquastore™ |
| F | Fertiliser tablet |
| I | Inoculation (*A. luehmannii* only) |
| IT | Inoculation with Treemaxx™ (*A. luehmannii* only) |
| IA | Inoculation with Aquastore™ (*A. luehmannii* only) |
| IF | Inoculation with Fertiliser tablet (*A. luehmannii* only) |

| Plot | Treatment |
|---|---|
| 202 | F |
| 201 | T |
| 200 | T |
| 199 | H |
| 198 | S |
| 197 | H |
| 196 | H |
| 195 | S |
| 194 | G |
| 193 | A |
| 192 | F |
| 191 | F |
| 190 | S |
| 189 | A |
| 188 | T |
| 187 | S |
| 186 | H |
| 185 | T |
| 184 | F |
| 183 | G |
| 182 | A |
| 181 | G |
| 180 | G |
| 179 | A |

Buffer surrounding all sides

Row: Buffer 4 3 2 1 Buffer

**Middle planting**

***Eucalyptus microcarpa,* grey box**

355 metres long

Plots 12 x 13.1 m
Plots 4 rows wide and 5 trees long
One row buffer surrounding the trial

Plots 12 x 13.9m
Plots 4 rows wide and 5 trees long
One row buffer surrounding the trial

**Site two *A. luehmannii* and *E. microcarpa* trial site**

N

**Eastern planting** ***A. luehmannii*, Buloke** 265 metres long

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | | | | | | Row |
| | Buffer | | | | | | | | | | | | | | | | | | Buffer | Buffer |
| | | | | | | | | | | | | | | | | | | | | 4 |
| Plot | | 178 | 177 | 176 | 175 | 174 | 173 | 172 | 171 | 170 | 169 | 168 | 167 | 166 | 165 | 164 | 163 | 162 | 161 | 3 |
| Treatment | | F | IA | IF | S | G | IF | G | IT | IF | S | IA | T | I | F | IT | IT | A | A | 2 |
| | | | | | | | | | | | | | | | | | | | | 1 |
| | | | | | | | | | | | | | | | Buffer | | | | | Buffer |