South Australian Murray-Darling Basin Natural Resources Management Board

Angas Bremer Water
Management Committee Inc

MUNDULLA YELLOWS – PRELIMINARY

RED GUM TRIALS

Angas Bremer Land and Water Management Plan

January 2009

1. BACKGROUND

In accordance with the Angas Bremer Water Allocation Plan, license holders are required to nurture and maintain 2 ha of deep rooted perennial vegetation for every 100 ML of water on allocation. Many license holders use the existing remnant vegetation to satisfy this license condition.

With the decline of the health of the red gums (*Eucalyptus Camaldulensus*) license holders where concerned that they would not meet their license obligation of maintaining the red gums and consequently the Angas Bremer Water Management Committee (ABWMC) in collaboration with the South Australian Murray-Darling Basin Natural Resources Management Board (MDB NRM Board) sought the advice of Plant Ecologist, Renate Velzeboer from Department of Environment and Heritage (DEH) to determine a) the cause of decline in tree health and b) advise on what could be done to remediate the situation and improve the heath of the trees.

Based on visual inspection of the trees, the ABWMC and NRM Board were advised that the trees where likely to be suffering from the progressive die back disease Mundulla Yellows (MY). The cause of MY is largely unknown but believed to be related largely to soil properties. It was further advised that the use of iron (Fe) implants can be used to improve the health of the trees in the short term.

The ABWMC resolved to conduct a trial at 3 sites in the Angas Bremer Prescribed Wells Area, with the aim to:

- confirm the diagnosis of MY, and
- test the effectiveness of Fe implants for treatment of the symptoms of MY.

Funding for the project was sought and approved from the MDB NRM Board to undertake the trials. Support was also provided by David Cooney Alexandrina Council, Renate Velzeboer DEH, Barbara Czerniakowski and Ron Walsh Department Primary Industries (DPI) Victoria, Tony Randall and Natalie Wakins Rural Solutions SA, local landholders Brian Meakins and Peter Silver and ABWMC members John Pargeter and John Follett.

1.1 Mundulla Yellows[1]

Mundulla Yellows (MY) is a progressive dieback syndrome affecting a wide range of native plants, such as species of *Eucalyptus, Acacia, Banksia, Melaleuca* and *Allocasuarina*.

MY is caused by a complex interaction of soil properties (texture and parent material): increased alkalinity and salinity, accumulation of bicarbonate, soil compaction and water availability resulting in a deficiency of available iron and other micronutrients in the soil.

Early symptoms of MY include interveinal yellowing of the leaves in the outer parts of a limb or a segment of the crown (early stage). A progression of yellowing occurs towards the main stem or trunk. The yellow leaves may develop red-brown spots and become deformed (medium stage). Late stage symptoms involve epicormic growth from below the affected areas and a progressive dying back of the whole branch. These symptoms spread gradually through the shrub or tree, causing it to die back over several years without recovery.

MY is widespread in the South-East of South Australia and around Adelaide, but has also been reported for Mount Lofty Ranges, Murray-Mallee, Mid-North, Yorke Peninsula and sporadic on Kangaroo Island and Eyre Peninsula. It is generally found in disturbed vegetation, such as near roadsides, paths, urban parklands, car parks and farm paddocks, but also along the edges of and within native vegetation.

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[1] Velzeboer, R. (2007) *Treatment Procedure for trees affected by Mundulla Yells with Iron Implants.* DEH. South Australia.

Researchers from Victoria were able to transform trees with defoliated branches and yellow leaves into trees with healthy, green new growth on the branches through the application of iron into the trunk of the tree through implants or by injection or by feeding it into the soil or sprayed on foliage. Please note that the application of iron is treating the symptoms only, not the cause. The underlying cause for MY is yet unknown. Also, the application of iron generally only lasts for a few growing seasons and is therefore a **short-term** solution only.

Figure 1. Progressive Symptoms of Mundulla Yellows

Source: Velzeboer, R. Department of Environment and Heritage.

Early symptoms Interveinal yellowing Late stage symptoms

2. METHODOLOGY

The following section describes the method used to select suitable sites to undertake the treatment trails. It further describes methods used for obtaining soil and foliage samples and details the procedure for inserting implants.

2.1 Site Selection

At each site trees displayed symptom of Mundulla Yellow, i.e. progressive die back and interveinal chlorosis (leaf yellowing).

Three sites were selected these included:

- Site 1. Newmans Horse Radish Farm
- Site 2. Silvers Lucerne Farm
- Site 3. Wellington Road

At each site one control tree and two treatment trees were selected. The control tree was not treated however soil and foliage was analysed. Of the two treatment trees, soil and foliage was analysed from treatment tree 1 and foliage was analysed from treatment tree 2.

The purpose of only doing foliage analysis on treatment tree 2 was because leaf foliage sampling is less expensive than soil sampling, it is easier to collect samples and it may provide the necessary information required to determine the presence of Mundulla Yellows and also to later determine the effectiveness of the treatments.

Each of the three site selected had different land use, both Sites 1 and 2 were used for horticultural production of 1) horse radish and 2) lucerne cropping respectively. Both Sites 1 and 2 were subject to different soil management techniques to suit the different crop types. Site 3 was located on the road side. The surrounding land was not used for any horticultural purpose.

2.2 Soil Sampling and Field Classification

Field classification was undertaken by Rural Solutions SA. The classification site was located between the control tree and treatment tree 1 and it was assumed that the soil sampled was representative of the soil at each particular site. The field classification included colour, textural class, EC and pH. Results are displayed in Appendix 1.

In addition to the field classification, two soil samples were collected from each site. Samples were collected from the surface soil that 0 - 10 cm, and sub-surface soil which is from 10 – 30 cm depth.

Samples were obtaining using a hand operated auger. A total of 3 samples were collected from the base of each control tree and treatment tree 1. Samples were bulked (mixed together), double bagged and labelled.

Approximate 5 kilograms of bulk soils were obtained for each surface and sub surface sample. The labelled bags were stored in a cool dry area with bags open until posted to State Chemical Laboratories, Werribee Victoria. Only 1.5 kg of each of the 6 samples was sent to Victoria. The remainder was stored in case further sample analysis is required.

Soils are analysed for pH, electrical conductivity (EC) and ion concentrations. Results are displayed in Appendix 2.

2.3 Foliage Sampling

Foliage samples were collected from all three sites and from each tree, i.e. the control tree and each of the two treatment trees.

Samples were collected using an extendable arm pruning tool. Approximately 200 grams of leaves were collected from each tree. Leaves were bagged and labelled and stored in a refrigerator at a temperature of between 4 – 6^{o}C with bags open to prevent sweating.

Samples where then packed in polystyrene foam packing boxes and ice and sent to the State Chemical Laboratories, Werribee Victoria via express post.

Foliage samples were analysed for total nutrient concentrations. Results are displayed in Table 2.

2.4 Required Materials for Treatment with Iron Implants[2]

The materials required to treat the affected trees are as follows:

- Iron implants = MEDICAP® FE PROFESSIONAL SUPER, ½ inch Diameter implants
  MEDICAP® FE can be obtained from:

Milton Edwards	or	Paul Barber
Boldscape Distributors		Arborcura
0439 655 355		0405 341 015
info@boldscape.com.au		arborcura@iinet.net.au
- Flexible tape measure
- Metal skewer
- Marker pen
- Rechargeable drill, using a sharp spiral drill bit of 1/2”.
- Flat end punch or dowel rod

[2] Velzeboer, R. (2007) *Treatment Procedure for trees affected by Mundulla Yells with Iron Implants.* DEH. South Australia.

- Hammer
- Flagging tape (optional)
- Disinfectant

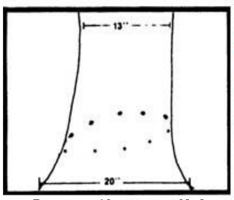
2.5 Method for Inserting Treatments[3]

1. Measure the circumference of the tree with a tape measure around the trunk of the tree at least 1.2 m (4 ft.) above ground.

2. To calculate the number of implants needed
   a. Divide the circumference of the tree by 15 cm (6 INCH) for Medium stage symptoms
   b. Divide the circumference of the tree by 10 cm (4 INCH) for Late stage symptoms
   If in doubt, use larger spacings as not to cause any leaf burning.

3. Measure tree bark thickness
   Place a metal skewer with sharp end against the bark of the tree. Push the skewer into the bark until the skewer penetrates to the wood interface (you will feel the change in resistance). Once the wood interface has been reached, mark the skewer with a marker pen, take the skewer out of the tree bark and measure bark thickness with a tape measure. Repeat this procedure for each hole.

4. Calculate drill hole depth
   The holes need to be deep enough to allow each implant to be recessed just inside the inner bark.
   Drill hole depth = tree bark thickness (see 3) + length of implant
   Mark the drill hole depth on metal skewer.



5. Drill implant holes as follows:
   a. At drill hole depth as calculated in 4
   b. At spacings as indicated in 2a or 2b
   c. Spiralling up and around the trunk base starting at least 15 cm from the soil level
   After drilling each hole, check with the metal skewer if the drill hole depth is sufficient. Remove all drill shavings from each hole.

6. Inserting the implants
   Place the implant into the pre-drilled hole, simply pressing them into the tree trunk. Be sure to press the implants in as far as possible. Using a hammer and flat end punch or dowel rod carefully drive the cartridge into the tree, recessing the large end slightly beneath the bark.
   Do not use a sharp end punch as you may puncture the cartridge.

7. Completion of application process
   The natural sap flow in the tree will systematically absorb the chemical and distribute the iron throughout the tree. The active layer of cambium (which is just below the bark) will soon grow over and close the implant site. The cartridges are to be left inside the tree. Do not remove the previously implanted cartridges

8. Disinfect the drill bit between trees being treated with a spray bottle containing a diluted bleach solution of 1 part of household bleach to 4 parts of water.

[3] Velzeboer, R. (2007) *Treatment Procedure for trees affected by Mundulla Yells with Iron Implants.* DEH. South Australia.

2.6 Notes

- Carefully read the application timing for optimum results
- Always read and follow label directions for implants being used
- Use proper drill bit
- Remove shavings from hole
- Recess cartridge end below the inner bark
- Disinfect the drill bit between trees being treated
- Water thoroughly if weather conditions are dry

Do not
- Enlarge the hole diameter
- Place implant too deep
- Do not use methylated spirit as disinfectant since methylated spirit is flammable
- Repeat implant treatment where tree has not shown the ability to adequately close over the prior treatment.

3. RESULTS

The following section details the results from the soil and leaf analysis and compares the results obtained from this trial with the results of the symptomatic and asymptomatic trees in the previous study by Czerniakowski B, Crnov R, Smith I and Luck JE (2006).

3.1 Soil Analysis

Soil samples analysed at all three sites show moderate to high levels of alkalinity with values of surface and subsoil ranging in pH 7.5 – 8.5. These values are similar to the values found in symptomatic trees in the previous study by Czerniakowski et al. (2006). In asymptomatic trees the pH values were lower and ranged between 5.1 and 6.8.

The EC level for both surface and subsoil were relatively high and ranged from 0.22 – 0.86 dS/m. These values are directly comparable to the values found in symptomatic trees in the previous study which ranged from 0.22 – 0.88 dS/m. In the soil of asymptomatic trees the EC values ranged from 0.08 – 0.17 dS/m and are lower than that of the soil found at symptomatic tree sites.

Soil texture results from the prior study found a higher clay content in surface soil from sites with symptomatic trees. Soil textures of top soil at the three sites included light clay, very fine sandy clay loam (heavy) and fine sandy clay loam.

Soil colour description from both the in-field classification and the laboratory classification described the sites as displaying yellows, greys and browns (refer to Appendix 1 and 2). Yellow, grey and olive colours generally indicate poor soil oxidation usually due to permanent or near permanent water logging (Watkins 2007).

The moisture content in the prior study was found to be higher in symptomatic trees (10 – 21%) than asymptomatic trees (2.1 – 9.3%), and while the moisture content was not analysed as part of this study, the colour of the soils may indicated that the moisture content is typically high.

The exchangeable cations and exchangeable Ca at all three sites where comparable with the results of the previous study and were higher in symptomatic trees soils than asymptomatic tree soils. Results are displayed in Table 1.

Examination of soil micro nutrient levels found lower levels of DTPA extractable Fe when compared to asymptomatic trees and comparable levels to those found in symptomatic sites from the prior study. DTPA extractable Fe ranged from 19 – 48 mg/kg at all three trial sites. In the prior study DTPA extractable Fe of symptomatic tree sites ranged from 8 – 24 g/kg while asymptomatic site values ranged from 99 – 399 mg/kg.

DTPA extractable Mn of the symptomatic sites ranged from 4 – 9 mg/kg. The previous study found that generally the DTPA extractable Mn was lower for symptomatic sites in relation to asymptomatic sites. Generally the results from the symptomatic sites are higher than that of the symptomatic sites of the previous study.

DTPA extractable Boron (B), had an average value of 3.3 mg/kg, this value is similar to that found in the symptomatic trees in the previous study and is greater than that of asymptomatic trees which had an average values of 0.55 mg/kg.

Full soil analysis results are displayed in Appendix 1. The results displayed below only relate to the top 10 cm of soil or surface soil.

Unfortunately further comparison to the previous study was not possible as different techniques were used to analyse ion concentrations which are not directly comparable.

Other observations of the soil properties include:

- With the exception of Site 3, Wellington Road the majority of soils have high Na concentrations ranging from 9 – 19 % and are considered sodic (> 6%) and strongly sodic (>15)
- With the exception of Site 3, Wellington Road the majority of soils have high Cl concentrations ranging from 300 – 500 mg/kg.

High levels of both Na and Cl were found in the prior study to be associated with symptomatic trees.

Table 1. Physical and Chemical Properties of Top Soil (0 – 10 cm depth) of Symtomatic and Asymptomatic Trees

Site Name	State	Texture	pH in Water	EC dS/m	4 Cat. + meq 100/g	Exch. Ca meq 100/g	Exch. Mg meq 100/g	Exch. K meq 100/g	DTPA Fe mg/kg	DTPA Mn mg/kg	B (CaCl2) mg/kg
Symptomatic sites											
Newmans Surface	SA	Light clay	7.7	0.80	34	19	8.7	1.9	35	6	4.7
Silvers Surface	SA	Very fine sandy clay loam (heavy)	7.5	0.86	44	28	9.7	1.9	48	6	6.2
Wellington Road Surface	SA	Fine sandy clay loam	8.1	0.30	32	25	5.3	1.4	22	9	2.6
Asmptomic Sites											
Lowe's	SA	Sandy loam	6.8	0.09	6.7	4.8	1.4	0.2	99	3.3	0.9
Poocher swamp	SA	Sandy loam	6	0.17	17	12	3	1.1	240	10	2.7
You Yangs	VIC	Coarse sandy loam	5.2	0.11	3.1	0.5	1.6	0.3	200	3.7	0.5
Anakie	VIC	Fine sandy clay loam	5.1	0.13	4.6	2.3	1.3	0.4	390	15	0.6

3.2 Foliage analysis

Analysis of foliage nutrient levels found that Cl values generally higher in symptomatic tree foliage ranging from 2.66 – 8.45 g/kg than asymptomatic tree foliage from the prior study with Cl values ranging from 1.23 – 4.99 g/kg. In addition, Na values were again higher in the symptomatic tree in this study (3.14 – 9.05 g/kg) compared with the asymptomatic trees of the prior study (1.7 – 3.9 g/kg). In most cases Mn was lower in the symptomatic tree foliage when compared to the asymptomatic trees of the prior study. These results are consistent with the results from the prior study. Nutrient concentrations from foliage are displayed in Table 2 below, asymptomatic sites are from the previous study conducted by Czeniakowski et al (2005).

Table 2. Nutrient concentrations from foliage analysis

Site Name	Cl g/kg	N g/kg	P g/kg	K g/kg	Ca g/kg	Mg g/kg	Na g/kg	Fe mg/kg	Mn mg/kg	Zn mg/kg	Al mg/kg	B mg/kg
Symtomatic												
Newmans Control	6.95	12.8	0.835	5.45	13.6	2.11	9.05	130	25.3	22.1	180	32.9
Newmans Treatment 1	4.21	13.7	0.999	2.83	19.2	2.43	3.72	112	209	29.8	159	61.6
Newmans Treatment 2	5.35	13.9	1.04	4.71	16.2	2.43	4.36	86.2	67.8	22.6	110	47.5
Silvers Control	7.08	16.9	1.78	6.45	13.6	2.86	5.97	122	47.8	26.3	160	36.3
Silvers Treatment 1	7.69	14.5	1.11	5.69	10.5	2.2	5.62	115	31.8	17.5	154	30.4
Silvers Treatment 2	2.66	15	1.33	5.03	19.5	3.38	3.14	168	48	27.1	235	96.3
Wellington Road Control	5.19	11.5	1.03	2.92	33.9	3.31	3.44	102	41.1	67.2	145	83.7
Wellington Road Treatment 1	8.45	15.3	1.35	5.54	20	2.7	5.46	74.2	98.3	29.4	93.5	50
Wellington Road Treatment 2	6.67	15.6	1.11	3.7	20	3.46	5.02	109	31	33.4	138	45.2
Asymtomatic												
Lowe's	4.99	13.85	1.3	7.3	9.9	3.2	3.9	117.5	61.2	30.9	175	66.4
Poocher swamp	4.62	14.82	1.3	8.6	9	2.4	3.1	105.89	86.21	25.86	159.1	58.42
You Yangs	1.23	12.93	1	5.8	13.1	2.7	1.7	101	1255.7	18.98	171.5	65.63
Anakie	2.00	13.43	0.7	4.5	13.8	1.7	2.4	100.98	835.25	28.5	159.3	64.4

Analysis of Fe in foliage shows no diversion from that of symptomatic tree foliage to asymptomatic tree foliage. This was also reported in the previous study and explained due to a phenomenon called “the chlorosis paradox” where chlorotic leaves have a higher Fe concentration than green leaves (Alhendawi et al. 1997; Kosengarten et al. 2001; Kosengarten et al. 2004, Lucena 2000; cited by Czerniakowski et al. 2005).

In summary, the results of the foliage analysis of symptomatic trees were consistent with the results of symptomatic trees from the previous study particularly with respect to Cl, Na, Mn and Fe.

3.3 Visual Pre and Post Treatment Analysis

Photographs of each site and tree where taken prior to inserting the iron implants on 31 August 2007 and again on the 14 January, 12 August and 13 November 2008. The purpose of the photographs was to see how the trial trees responded to the treatments. The results are displayed below and summarised in Table 3.

Figure 1a (August 2007) and 1b (January 2008). Site 1 Newmans control (no treatment)
Slight decline in health of control tree evident in Figure 1b by less foliage displayed on left hand side.

1c (August 2008) and 1d (November 2008).
By November 2008, foliage on left hand side has not improved.

Figure 2a (August 2007) and 2b (January 2008). Site 1. Newmans Treatment 1 comparison of pre and post treatment
Slight improvement in tree health evident Figure 2b which displays dark green foliage denser than that displayed prior to treatment.

2c (August 2008) and 2d (November 2008).
No further improvement in tree health evident.

Figure 3a (August 2007) and 3b (January 2008). Site 1 Newmans Treatment 2 comparison pre and post treatment

No response evident. Deep cracking in treated bough, not visible in photograph.

3c (August 2008) and 3d (November 2008).

Still no response evident. Possible signs of decline by November 2008.

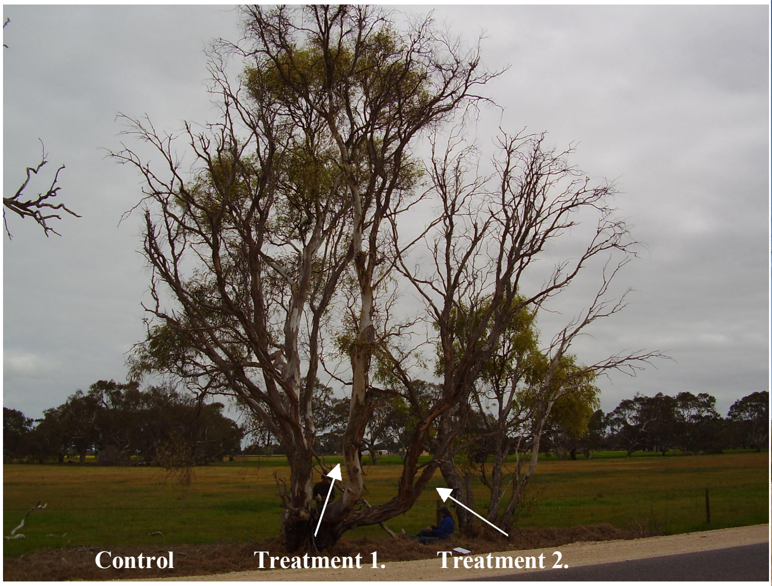
Figure 4a (August 2007) and 4b (January 2008). Site 2 Silvers Control (no treatment) and Treatment 1 pre and post treatment
Clearly visible die back of Control tree evident in Figure 4b. Very slight improvement in treatment tree.

4c (August 2008) and 4d (November 2008).
Little change in Control tree. No evidence of new growth in Treatment Tree 1 since January 2008.

Figure 5a and 5b. Site 2 Silvers Treatment 2 pre and post treatment.
Very slight improvement, requires further monitoring.

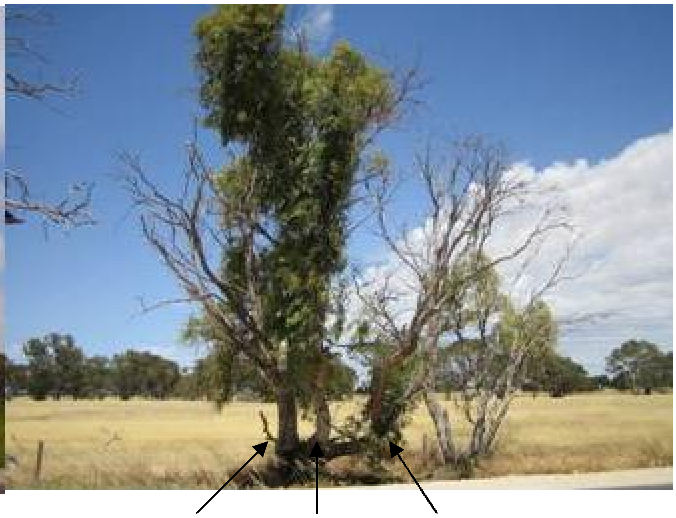
5c (August 2008) and 5d (November 2008).
No further signs of improvement evident.

Figure 6a and 6b. Site 3 Wellington Road Control and Treatment 1 and 2 pre and post treatment.
Substantial improvement in treatment branch 1 as evident by Figure 6b, no response in treatment 2.



6c (August 2008) and 6d (November 2008).
Some of the new leaves on treatment branch 1 showing signs of yellowing in August 2008. Leaves remain on Treatment branch 1 in November and yellowing is less evident. No change in Treatment branch 2. Control branch also has more leaves than in August 2007.

Control Treatment 1 Treatment 2



Control Treatment 1 Treatment 2

Table 3. Summary of visual results following treatment with Fe implants

Site	***Comments***		
	January 2008	***August 2008***	***November 2008***
Newmans Control	Very slight decline in health of control evident on left hand side	No change	No change
Newmans Treatment 1	Slight increase in foliage and deep green colour, decrease in yellowing of foliage evident. No closure over of implants.	No further improvement	No further improvement
Newmans Treatment 2	No response, no new growth, deep cracks in bark and bough evident approximately 5 cm above location of implants	No change	Possible further decline
Silvers Control	Die back of branches evident	No further change	No further change
Silvers Treatment 1	Slight increase in foliage main truck, mainly epicormic growth, no closure over implants evident.	No further change	Some evidence of decline
Silvers Treatment 2	Very slight increase in foliage main trunk. No closure over implants evident.	No further improvement	No further improvement
Wellington Road Control	No visible change	Some new growth	No further improvement
Wellington Road Treatment 1	Prolific epicormic growth, leaves deep green in colour. No closure over implants evident	New leaves showing signs of yellowing.	Less yellowing evident
Wellington Road Treatment 2.	No new growth. No closure over implants evident.	No change	No change

4 CONCLUSIONS AND RECOMMENDATIONS

Based on the comparison of results with those from the study undertaken by Czerniakowski et al. (2006) the initial diagnosis of MY as being the primary cause of the decline in tree health is supported by the both the soil and foliage properties.

Unfortunately not all the results were able to be directly compared to the previous study by Czerniakowski et al. (2006) which analysed ion concentrations in aqueous soil extract and reported results in mg/L. Our trial results were reported in meq/100 g and although a conversion factor can be applied the results are not comparable as the different techniques used in the analysis give different results (Walsh 2004).

Initially the trees treated with Fe implants showed slight improvement in most cases. The fact that the new growth was deep green in colour rather than showing signs of yellowing suggests that the treatment had been effective in reducing the symptoms of the disease. Further observation and follow up leaf analysis was recommended.

At the end of 2008 the results of the iron treatment on the trees still appeared to be mixed. The trees that had responded earlier had maintained their new growth but were not showing any renewed signs of vigour. One treated branch of a tree that had responded positively began to show signs of the disease in the new growth. Those that had not shown an early response with new growth continued to decline and show symptoms of the condition. Unfortunately the weather during winter and spring had not been conducive to a lot of new spring growth.

As only a couple of trees appeared to be responding positively to the treatment, it was decided to delay any further foliage and soil testing until the trees displayed better signs of recovery. Further photo monitoring of the trees will continue.

With such a small sample size and the mixed results so far it is currently difficult to determine at this time whether this simple and inexpensive procedure is effective for improving tree health in the Angas Bremer area.

5 REFERENCES

Czerniakowski B, Crnov R, Smith I and Luck JE (2006). Soil properties associated with the tree decline ‘Mundulla Yellows’. *Journal of Plant Soil* 285: 197 – 206

Walsh, M. 2008, *Telephone Conversation,* Personal Communications, 21 January, Victoria

Watkins, N. 2007 *Mundulla Yellows Soil Profile Descriptions Langhorne Creek.* Rural Solutions SA, South Australia.

6 FURTHER READING

Czerniakowski B, Crnov R, Smith I and Luck JE (2006). Soil properties associated with the tree decline ‘Mundulla Yellows’. *Journal of Plant Soil* 285: 197 – 206.

Luck JE, Crnov R, Czerniakowski B, Smith I and Moran JR (2006). Investigating the presence of biotic agents associated with Mundulla Yellows. *Journal of Plant Disease* 90: 404 – 410.