

# final report

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## **MB2007/V04 - MBfP - Rhizobia and Clover**

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**PIRD Objective**

Determine the impact of the nutrients boron and cobalt on the productivity of beef pastures in East Gippsland.

**Outcomes from 2007 PIRD**

Past research indicates that boron deficiency of sub clover results in reduced seed set, plant root development and herbage production.

This PIRD's findings confirm that boron deficiency does impact on seed set, growth and nitrogen fixation of sub clover. Other studies have indicated that when boron deficiency of sub clover occurs, it impacts on root volume and this then reduces the plants ability to gather nutrients and moisture from the soil.

Outcomes here gave similar results as the sub clover in the non boron treatments wilted from moisture stress and failed to respond to potassium when applied at the low rate.

It is highly likely, that boron deficiency plays a role in survival of effective rhizobia in the soil. Where boron deficiency has existed for some time, results indicate that boron combined with sub clover seed and inoculant are all needed together to ensure sub clover growth returns to normal and significant nitrogen fixation occurs.

Boron was found to move across the landscape via stock in urine along with other major nutrients. Such a loss in time can lead to severe boron deficiency developing in parts of paddocks and significantly reduce the pasture productivity of such areas. Because of this, the amount of boron needed to overcome a deficiency will vary depending on the distance from the stock camp.

Water insoluble boron was found to be as effective as the water soluble types in overcoming a deficiency. Its use will significantly lower cost and can also be expected to have a better residual life.

In deficient situations, broadcasting boron fertilizer (2.5kg/ha water soluble) at the same time as sowing inoculated sub clover was not found to impact on seedling germination and survival. Excess boron is known to be toxic to plant growth and its impact at germination time was unknown.

Plant tissue testing and soil testing can both be used to diagnose a deficiency, however with soil tests, the Ca:B ratio is possibly a better guide than the singular boron test.

Once diagnosed, the targeted topdressing of boron to selective areas based on early spring sub clover growth and color was used as a cost effective method to return the pasture to full productivity, but this only applied when effective sub clover rhizobia were present.

Overall, the results indicate that boron deficiency of pasture is hidden when the nitrogen fixing ability of legumes is not functional. When new inoculated sub clover seed plus boron were applied together, pasture production more than doubled.

## **Background**

Old top dressed pastures that had acidified and then limed were identified as under performing compared to more recently developed pastures on 19 beef producing properties across East Gippsland from 1998-2002.

The observed pasture impact was severe nitrogen deficiency caused by the inability of legumes to thrive and fix adequate levels of nitrogen thus enabling vigorous grass growth. Results from PIRD 2002/V04 and PIRD 2004/V09 indicated that the issue was most likely due to a deficiency of boron and cobalt. These nutrients are important for the growth of legumes and nitrogen fixation.

Past legume research has identified these nutrients as likely to be deficient if in marginal supply and then calcium (lime = 30%Ca) is applied to the soil. However the 2004/V09 PIRD was unable to show that these nutrients alone or in combination could improve sub clover growth.

In 2006, soil tests were undertaken to determine the effectiveness of the sub clover rhizobia in some of these problem soils. Testing showed that the sub clover rhizobia present in these soils had poor nitrogen fixing ability compared to the current commercially rhizobia strain.

Because of this, this current PIRD was undertaken to determine if these nutrients in combination with effective sub clover rhizobia could improve sub clover growth.

## **2007 Trials**

Eleven pasture trials were established in autumn 2007. Some were located on know problem sites (Sarsfield, Orbost and Flynn) whilst others were done in conjunction with local producer groups with an interest in 'Why old established pastures were not responding to fertilizer application' (Orbost, Woodside, Omeo, Bairnsdale, Ensay and Delegate).

The objective was to determine if the plant nutrients boron and cobalt could improve sub clover growth after new rhizobia was reintroduced.

Apart from the Sarsfield site, clover responses to nutrient treatments were only observed at the Orbost (Honey), Bairnsdale and Omeo sites. At these three sites, the poor clover growth could be explained by the low soil pH and the need for lime application to overcome this problem. No boron responses were observed at these sites, however after lime (Buchan lime 2.5 tonne/ha) is used, the Ca:B ratio would increase Orbost (12.8), Bairnsdale (10) and Omeo (13.5). From the results to date, it could be predicted that these sites would then be highly likely to become boron responsive. (Based on results to date, it is highly likely that boron deficiency will occur when the CaB ratio is > 10)

With regard to the other sites used, inoculated sub clover seed was sown at the sites prior to fertilizer topdressing. Subsequent to this, the clover plants either died out or failed to thrive due to either inoculation problems or herbicide damage. Apart from the Ensay site, long life inoculated seed was used at all sites, but the viability of the rhizobia in the coating was not checked.

## **Sarsfield trials**

Two replicated pasture trials were established at Sarsfield in 2007. A third replicated pasture site looking at boron rates and types was established in 2005 and was also used to generate data.

The soil site details are contained below:

Site	Location	Soil Test No	pH	pH	B	Ca	Ca:B	Likely B	B
			(water)	CaCl <sub>2</sub>	mg/kg	meq/100gm	response	response recorded	
Sarsfield	road	20102112	5.8	4.8	0.31	6.0	19.3	Yes	Yes
Sarsfield	pipeline	20102130	5.9	4.9	0.21	4.0	19	Yes	Yes
Orbost	(Russell)		5.2	4.4	0.5	5.95	11.9	Yes	*
	(Honey)	20448843	5.0	4.4	0.46	3.2	6.9	No	No
	(Adams)	20448842	5.2	4.6	0.39	5.5	14.1	Yes	***
Delegate		20448872	6.5	5.9	0.37	6.0	16.2	Yes	**
Flynn		20448845	5.7	5.2	1.3	7.5	5.7	No	*
Bairnsdale		20448871	5.0	4.6	0.38	2.3	6.0	No	No
Woodside		20448880	4.6	3.8	0.51	2.9	5.6	No	*
Omeo		20102126	5.3	4.5	0.28	2.35	8.3	No	No
Ensay		20089354	5.5	4.7	0.44	3.35	7.6	No	***

- \* = clover nitrogen deficient (long life inoculated seed used at sowing 2007)
- \*\* = clover establishment impacted by sowing technique.
- \*\*\* = clover density and growth severely impacted by herbicide application.

## **Sarsfield trial site information:**

Pipeline Paddock – Split plot design using two replications of untreated pasture compared to two replications of sprayed (Roundup 1.5l/ha) pasture sown to 10kg/ha sub clover (Leura) seed with and without inoculation. The uninoculated seed was treated with fine powdered copper sulphate prior to sowing (100gm to 10kg seed). The drill used for sowing was not cleaned prior to sowing.

Across these bays, four replications of nutrient treatments were applied looking at potassium rate (0,100kg/ha autumn, 100kg/ha autumn and spring – KCl), boron (2.5kg/ha water soluble = ws) and molybdenum (150gm/ha sodium molybdate).

Molybdenum (75gm/ha) and boron (2.5kg/ha water insoluble = wi) were applied to the paddock in 2005. No response to this additional molybdenum application was obtained.

Road Paddock – 2005 trial established to look at boron rates (0,1.5,3,6,12 kg/ha ws) and 0.75kg/ha water sol + 3kg/ha wi. No nutrient responses have been obtained since establishment due to poor clover vigor. In autumn 2007, two replicates were sprayed out with Roundup (1.5l/ha) and seeded (drill above) with 10kg/ha inoculated Leura sub clover seed.

Road Paddock – 2007 trial established to look at water soluble and water insoluble boron applied at 2.5kg/ha across untreated pasture and pasture sprayed and seeded as in the other trials.

Each treatment area was 4x5m. This enabled comparisons to occur using additional boron, cobalt and boron application timing (2x5m areas).

#### Rhizobia x Boron Interaction.

Because of the trial design, a rhizobia x boron interaction could not be determined at the pipeline paddock trial site. This was due to the treatments used. Here the untreated pasture was compared to replicated sprayed treatments sown to inoculated and uninoculated Leura sub clover seed.

The response that occurred could have been due to the introduced clover cultivar, rhizobia introduction or a combination of both these inputs. The impact of the spray treatment on releasing nitrogen from the dead plant material, or removing the kikuyu were unlikely to have affected outcomes as the same response occurred to all sowing treatment sites regardless of grass and weed composition.

Hormone herbicide was used at all sites to remove capeweed. Its impact was to halve the growth of sub clover compared to unsprayed over all treatments.

#### Sowing treatments.

All three trial sites only gave a positive response to boron application when used in conjunction with lime coated and inoculated Leura sub clover seed. Without this sowing treatment, no effective responses to nutrient treatments were obtained.

In the Pipeline site where sub seed was sown, both the inoculated and uninoculated treatments grew well compared to untreated. Only a small response occurred as a result of the inoculation treatment where both areas were seeded and this outcome was a surprise as visually both treatments appeared similar. As the seed drill used for sowing was not decontaminated prior to sowing, it is possible the uninoculated seed was contaminated by rhizobia residues in the contractors drill.

Pipeline site: (October 1st harvest)

	Untreated	Sub seed	Sub seed + inoculant
Sub clover %	52	72	73
CloverYield (kg DM/ha)	555	985	1185

Yield lsd 5% = 192

#### Potassium x Boron response.

Before the pipeline site was established, soil test data indicated that the area was grossly potassium deficient (50ppm) even though the paddock has had annual inputs of potassium for some 30 years at rates based on trial data done on similar soils in the 1960's (Potassium for Victorian Pastures, 1986, p97). Because of the low potassium level, a split dressing of potassium was used as a treatment.

Pipeline site (October 1<sup>st</sup> Harvest)

Sub Clover Yield (kgDM/ha)

	Untreated	Sub + inoculant
P+S+Mo	504	901
P+S+Mo+K1	534	1030
P+S+Mo+K2	634	1323*
P+S+Mo+K1+B	564	1182*
P+S+Mo+K2+B	666	1740*

Isd 1% = 288 inoculant treatment

Isd 5%=138 nutrient treatment

The trial data indicates a nil response to nutrient treatment in the untreated areas apart from the highest rate. Where seed and inoculant were used, a significant response to potassium was obtained, but only at the higher rate of potassium and where potassium was applied with boron.

No additional yield improvements were observed where additional boron and cobalt treatments were applied to half the P+S+Mo+K2+B treatment area.

This data does provide an explanation as to why the potassium levels are so low in this soil. With an annual application rate half that of the K1 treatment, a plant response would not be occurring. Thus the applied potassium would most likely be leaching past the plant root zone and resulting in the very low soil potassium levels.

#### Boron rate response.

This trial was established in 2005 and until 2007 no results have been obtained until inoculated sub clover seed was sown across two of its replications.

No nutrient responses have been recorded to nutrient treatment in the replications that are unsown. The site itself is the area described below in 'Pasture data for Road Paddock 2005 – lower slope'

Trial results (September 26<sup>th</sup> harvest – sown area)

Treatments Soil B	Yield (kg DM/ha)	<b>Sub Clover</b>		Tissue B (mg/kg)
		Seed (kg/ha)		
Nil	631	30	13	0.33
Boron 1.5kg/ha ws	714	22	17	0.33
Boron 3kg/ha ws	829	96	19	0.40
Boron 6kg/ha ws	1048	104	27	0.45
Boron 12kg/ha ws	723	94	95	0.96
Boron 0.75kg/ha ws + Boron 3 kg/ha wi	843	84	27	0.51

(w = water, s = soluble, i = insoluble)

yield Isd 5% = 151

Herbage, seed, tissue and soil data indicate that the maximum response occurred at 6kg/ha ws. A similar result was obtained from the mix of water soluble and water

insoluble treatments. The cost of this latter treatment would be around a third the water soluble treatment and may have better residual impacts.

At the higher rate (12kg/ha), toxicity occurred (leaf edge burn in clover plants), thus the yield result is poorer due to clover growth suppression.

This data tends to indicate that the water soluble vs water insoluble boron treatments have similar impacts on sub clover growth.

Whilst the seed data was generated from two replications, the trend indicates that the nil and 1.5kg/ha B treatments generated around one third the seed levels of the other treatments.

#### Effectiveness of Boron fertilizer types

A new trial was established in 2007 to determine the effectiveness of water soluble and water insoluble products and check the impact of using boron fertilizer at sub clover sowing time. The rate used was 2.5kg/ha boron as water soluble (Granubor) and water insoluble (Illexite). This site is located next to the 2005 established boron rates trial. Information from the 2005 trial indicates that the rate used is around half needed to get maximum yield at this location.

Trial results (September 26<sup>th</sup> harvest)

Treatments	Sub Clover Yield (kg DM/ha)	
	Untreated	Seed + inoculant
Nil	531	615
Boron 2.5kg/ha ws	421	876
Boron 2.5kg/ha wi	471	952

(w = water, s = soluble, i = insoluble)    Isd 5% = 72 (inoculant)    Isd 5% = 102 (nutrient)

Again, the impact of applying nutrient treatment to inoculated seed is demonstrated. A significant boron response has occurred, however there is no difference between types.

#### Germinating sub clover seed x boron application.

Immediately after sowing inoculated sub clover seed on 28/3/07, boron fertilizer at 2.5kg/ha (water soluble and water insoluble) was broadcaste applied. Six weeks later, germination counts were done comparing the boron treated areas to untreated.

Average sub clover seedling numbers/10cms.

No boron	3.4
Boron (water soluble)	3.7
Boron (water insoluble)	3.2

These results indicate that the boron treatments did not impact on sub clover germination at this boron responsive site.

Boron x grazing management interactions.

Boron was shown to move toward the stock camp in the 2005 data obtained from the paddock containing the 'Road Paddock' trials.

The 2005 data shown below indicates the impact on spring pasture composition and yield measured from the lower slope, mid slope and at the stock camp. The interval between each measure taken is 80m.

**Pasture data for Road Paddock 2005**

Location	Location	Distance metres * = location	Yield kgDM/ha	Bot Comp %					
				Sub cl	Cluster clover	Capeweed	Grass Silver	Grass Barley	Bare
Lower slope	Trial site	0*	1335	30	50	0	10		10
		80**	3400	30	30	20	10	10	
Upper slope	Stock camp	160***	5375	5	0	0		95	

**Soil tests data** (\* indicates the results for each pasture spot above).

Location	pH (H2O)	pH CaCl2	Organic Carbon %	N (NO3)	P Olsen	K Avail	S KCL	<b>B</b> Mg/kg	CEC	Al	Ca meq/100gm	Mg	Na	K	Ca:B ratio
*	5.2	4.5	2.3	3.9	12	59	3.7	<b>0.45</b>	5.94	0.05	5	0.69	0.05	0.15	11.1
**	6.1	5.2	2.7	7.4	15	206	4.4	<b>0.77</b>	8.68	0.03	7	1	0.13	0.53	9
***	6.1	5.2	3.4	23	32	506	8.5	<b>0.9</b>	9.38	0.03	6	1.92	0.13	1.3	6.6

**Plant tissue data** (\* indicates the result for each pasture spot above).

Location	N %	P %	K %	S %	Ca %	Mg %	Na %	Mn mg/kg	Fe mg/kg	Cu mg/kg	Zn mg/kg	Mo mg/kg	<b>B</b> mg/kg	Co mg/kg
Range	3.2-5.5	.25-.5	1.2-2.5	.25-.4	.8-2.5	.15-.5	0-0.5	25-300	50-65	5-30	15-50	0.5-1	<b>25-100</b>	>0.1
*	2.9	0.2	0.85	0.29	2.2	0.31	0.2	62	95	7.2	27	0.32	<b>37</b>	0.13
**	3.1	0.2	1.3	0.31	2.3	0.31	0.2	90	74	4.8	27	0.2	<b>59</b>	0.08
***	3.3	0.19	1.5	0.35	1.8	0.34	0.1	92	83	5.7	43	0.14	<b>480</b> (toxic)	0.17



These results indicate that nitrogen, phosphorus, potassium, sulphur, magnesium and boron are all being shifted toward the stock camp and as a consequence are generating inefficiencies in plant production.

Whilst fertilizer addition to specific areas could overcome this issue, more effective stock management would be far more economical. In the case of the paddock itself, it has been subject to a rotationally grazing for over 20 years, however this has not resolved the problem.

All adjoining paddocks are of similar size (7ha) and have had the same grazing management and fertilizer history, and all were sown to a sub clover based pastures over 30 years ago.

One of these paddocks that had a similar stock camp at the top of the slope had a fence changed three years ago, and this placed a shelter belt of *Pinus radiata* in the base of the paddock. The sheep now prefer to camp in the shelter belt rather than at the top of the slope. The impact on the pasture and soil after this time is shown below.

Data obtained from this paddock in 2007 is presented to indicates how the soil nutrient distribution has altered due to the grazing impacts of the shelter belt.

Location up slope	pH	pH	Organi	N	P	K	S	B	CEC	Al	Ca	Mg	Na	K	Ca:B ratio
	(H2O)	CaCl2	c Carbon %	NO3	Olse n	Avail	KCL	meq/100gm							
Base	5.7	4.9	2.3	8.5	11	140	10	<b>0.38</b>	6.92	0.10	5.5	0.91	0.05	0.36	14.4
+50m	5.7	4.8	2.6	9.9	16	130	5.6	<b>0.58</b>	7.08	0.10	5.5	1.1	0.04	0.34	9.4
+100m	5.8	5.0	3.0	10	15	230	7.7	<b>0.57</b>	8.23	0.10	6.0	1.5	0.05	0.58	10.5
+150m	6.0	5.3	3.4	10	27	280	12	<b>0.65</b>	9.38	0.10	6.5	2.0	0.07	0.71	10
+200m	6.5	5.8	3.4	11	20	240	9.7	<b>0.53</b>	6.94	0.10	4.7	1.6	0.02	0.62	8.8

Whilst the nitrogen, phosphorus, potassium and magnesium levels are still higher up the slope, based on the more even pasture composition across the paddock, the overall pasture has higher quality and quantity of herbage more evenly distributed.

The botanical composition at the time of sampling was estimated to be 40% sub clover, 10% silver grass and 50% barley grass at each soil sampling site. The barley grass is displaced by kikuyu in the late spring period.

#### Boron and sub clover seed set.

Past studies have indicated that boron deficiency impacts on sub clover seed set. Trends from the boron rates trial above indicate that seed level are increased three fold where boron is applied at adequate rates (2.5kg/ha +).

In relation to the pipeline trial, seed levels indicated no significant response to boron treatment. (No boron – 86kg/ha, boron – 100kg/ha). This result was likely due to boron being applied to the paddock in 2005 – rate used was 2.5kg/ha wis boron.

### **Extension**

Six producer meetings were addressed during 2007 and covered information generated from this and past PIRD's. The meetings were at Delegate, Bairnsdale, Orbost, Mansfield, Sale (BIA conference) and Nimmitabel.

Further to this, a full report on the findings will be presented to the Grasslands Society of South Eastern Australia during their annual conference in August 2008.

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Report Prepared (February 2008) by Leo Hamilton. (Project Supervisor)