Increased yields from peppermint crops through improved micro nutrient nutrition

A report for the Rural Industries Research and Development Corporation

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August 2005

RIRDC Publication No 05/091
RIRDC Project No UT-37A
Foreword

For some years the total area of peppermint grown in Australia has been declining due to poor oil yields. One aspect of production which has not received detailed attention is plant nutrition. Removal of plant material from fields over many years has depleted micro nutrient levels to a level where severe symptoms of deficiencies are widespread.

This report covers a preliminary survey of nutrient levels in fields in Tasmania which was designed to identify elements which are likely to be in the deficiency range for peppermint. The deficient elements identified were S, Cu, B and Mo. Plants showed responses to these elements when they were applied to soil under controlled conditions. A diagnostic CD was produced to aid in the field identification of deficiency symptoms.

Fertilizer mixtures were prepared based on the preliminary evidence of deficiency and these have been applied in the 2004-2005 season.

This project was funded from RIRDC core funds, which are provided by the Australian Government, together with funds from industry and the University of Tasmania.

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Acknowledgments

I acknowledge the technical assistance provided by David Wilson and Matthew Gregory during the course of this investigation.

I thank RIRDC and Natural Plants Extracts for funding the research and individual peppermint growers for their cooperation and assistance.
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Executive Summary

Peppermint yields in Southern Australia are highly variable and this is impacting on the long term viability of the industry. The average yield is approximately 50 kg/ha, despite attempts to improve management practice. Peppermint is also an important crop to provide the necessary throughput to sustain the viability of field distillation units.

Peppermint yields as high as 140 kg/ha have been achieved in Tasmania using a double cut system (Clark & Menary 1984). In the last 10-15 years there has been a general decline in vigour of crops and a double cut has not been possible.

Observations on crop health during the growing season have indicated that a nutritional problem may exist in peppermint fields. This was further supported by calculations of nutrient, particularly micro nutrient removal each year at harvest and the lack of replacement through standard fertiliser applications.

A preliminary one year research project was undertaken to establish the presence of nutrient disorders in peppermint crops in Tasmania.

Soil and plant analysis surveys were undertaken in all production areas to establish macro and micro nutrient levels. This survey indicated that deficiencies of S, Mo, B & Cu could be occurring. Symptoms expressed were also consistent with those described in the literature for peppermint and other related crops.

To confirm the presence of S, Mo, B and Cu deficiencies, soil was collected from one affected site having all four deficiencies. Peppermint was grown in this soil in pots in the glasshouse. All four nutrients were either present or absent and applied in all combinations one, two, three or four at a time. Responses were obtained with all four nutrients as expressed by leaf symptoms, growth rate and plant analyses.

The field from which soil was collected for the pot trial was used as a test plot for the application of S, Mo and B. This test plot yielded 40% more oil than the surrounding area. This further confirmed the presence of S, Mo & B deficiencies and the leaf symptoms in treated areas were consistent with Cu deficiency.

During the course of the pot trial digital photographs of symptoms were recorded. These were compiled on a CD for distribution to growers. A field day was held to explain the results and the way in which symptoms could be identified in relation to stage of growth. Growers also requested that a new fertilizer mixture be trialled in the coming season and that its composition be based on results of the preliminary trials.

A fertilizer mixture containing granulated forms of micro nutrients was prepared in consultation with a major fertilizer company and made available to peppermint growers.

Whilst the preliminary survey yielded positive results there is still calibration work to be undertaken to prevent toxicities from occurring and to establish soil and plant nutrient levels for optimum growth.
1. Introduction

Peppermint yields in Southern Australia have declined over the past decade to a point at which economic viability is in the balance. The peppermint crop is also an important component in the mix of a steam distilled crops which are required to maintain the economic viability of steam distillation facilities.

Observations on leaf symptoms in the field and calculations of nutrients lost through herb removal indicate that continuous cropping could result in nutrient deficiencies particularly micro nutrients.

Micro nutrient deficiencies have been observed in peppermint namely Fe, Zn, Cu and Mn. (Zeinali et al 2003, Zheljazkov & Warman 2004). These authors observed that responses in peppermint were similar to some vegetable crops such as potato, spinach, tomato and lettuce. Menary (1967) had made a similar observation about the similarly between red beet and peppermint. Further studies in solution culture have demonstrated essentiality for B, Co, A1, Zn and Cu (Bode HR, 1940).

Boron is the one micro nutrient which produces a range of development and physiological effects on peppermint. Fischer & Bussler (1984) have shown that B deficiency influences the development of oil glands and hairs, oil accumulation and vascular bundle development and their extension. It has also been observed that boron is most effective at high concentrations, (Nandi and Chatterjee 1986). Boichenko (1970) has demonstrated field responses in peppermint to applications of boron.

Micro nutrient responses influence oil quality and yield in decreasing order from N, P, K to S (Baird 1957). In recent years the tendency in fertiliser practice is to use high analysis fertilisers which are low in sulphur. Field grown corps in Tasmania often display a bright crimson colour which is characteristic of sulphur deficiency. This observation highlighted the need to include sulphur in the preliminary survey.
2. Objectives

The objectives specified in the original three year grant application were:

- Survey commercial fields, collect samples and analyse soil and plants
- Use established critical levels for related crops to determine extent of deficiency
- Collect analytical data and recommend corrective action

The program was recommended for one year only. This has meant that the scale of stated objectives had to be significantly reduced to obtain preliminary information on the extent of micro nutrient deficiencies in peppermint. Despite this adjustment to the objectives, work was undertaken on a pilot scale to achieve limited information on all three objectives.
3. Materials and methods

3.1 Plant and soil analysis

Nitrogen and sulphur analysis of plant tissue was carried out in a flash combustion Thermo Finnigan, Flash 1112 Series elemental analyser in standard mode. Ignition of 1-2 mg of fine powder or soil dust were ignited in a tin cap in O$_2$ @ 1000°C. The oxide produced was analysed using a thermal conductivity detector.

The metals in plant tissues were analysed as follows:

1-2g of a homogenous sample was weighed into a digestion vessel. 10mL of concentrated (69%) nitric acid was added. The sample was left overnight and then placed on a digestion block and digested at ~95°C for 3 hours.

Samples were then made up to a known volume and run against matrix matched standard solutions via inductively coupled plasma atomic emission spectrophotometer (ICPAES).

The homogenous total elements in soil method were carried out as follows:

A subsample of 1-2g was taken. 5mL of aqua-regia (3:1 conc HCl:HN0$_3$) was added and the samples were digested at ~95°C for 4 hours.

Samples were then made up to a known volume and run against matrix matched standard solutions via ICPAES.

Exchangeable bases were extracted using 1:10 Soil:1M NH$_4$0Ac.

Exchangeable cations were performed as per the Australian Soil and Land Survey Handbook, Rayment and Higginson (1992). Method 15D3.

A subsample of soil was equilibrated with 1M NH$_4$0ac at pH 7.0 for 30 minutes. The suspension was clarified prior to analysis by ICPAES.

P & K in soil were extracted using 1:100 Soil:0.5M bicarbonate.

This is a modification of the bicarbonate procedure by Olsen et al (1954).

A homogenized subsample of 1.0g was equilibrated in 100mL of 0.5M N$_4$HC0$_3$ at pH 8.5 for 16 hours. The suspension was clarified prior to analysis by ICPAES.

PH in soil was measured on a 10g subsample shaken for 1 hour in 50mL of deionised water. The suspension is then tested for pH using a calibrated pH probe.

3.2 Sample collection

In early Spring of 2003 soil samples were collected from fields which were known to exhibit symptoms of decline. The sites selected were Fingal Valley, Derwent Valley and Cressy. Soils were sampled to a depth of 200mm in each field, air dried and passed through a 2 mm sieve. Soils were analysed for macro and micro elements by the methods detailed above.

Plant samples were collected from the same areas in December and March. This represents the early and late stages of the grand period of growth. Nutrient dilution during growth can often be a strong indicator of limiting elements. Shoot tips approx 200mm in length were harvested, dried at 65°C, ground in a stainless steel hammer mill and stored at 2°C until analysed.

Soil was collected from one site which displayed severe symptoms of nutrient deficiency. This soil was sampled to a depth of 200mm. At the same site, samples were taken from the rooting zone of peppermint at two levels viz. 0 – 100mm and 100 – 200mm. This sampling was to access whether there was partitioning of nutrients due to the lack of cultivation and the continuous deposition of organic matter from peppermint herb.
3.3 Glass house pot trial

A pot trial was established in the glasshouse using soil from a peppermint field with severe symptoms of decline. Each pot contained 1.5 kg of air dry soil and the following nutrient treatments were applied.

- N as NH$_4$NO$_3$ @ 2.0 g/pot
- K&P as KH$_2$PO$_4$ @ 2.0 g/pot
- G as CaSO$_4$ @ 5.0 g/pot
- B as WaB$_4$O$_7$ @ 0.5 g/pot
- Cu as Cu(CH$_3$COO)$_2$ @ 0.25 g/pot
- Mo as Na$_2$MoO$_4$ @ 0.12 g/pot

In the case of S, B, Cu and Mo there were two levels used, nil and the amount detailed above. N, P and K were applied to all pots.

The trial was set out as a $2^4$ factional in a 4 x 4 balanced lattice design, ie. 16 treatments replicated 4 times.

Nutrients were mixed with dry soil on a plastic sheet to ensure even mixing of soil and nutrients. Sand was added to micro nutrients to increase bulk and aid distribution over the soil surface.

Pots were planted with tip cuttings of a single clone. Plant height measurements were taken as a measure of growth and two replications were analysed for macro and micro nutrient levels in plant tissues. Complete analysis was beyond the allocated budget.

Results of this pot trial did not receive statistical analysis as the nature of the survey and objectives did not require other than mean values in order to make qualitative judgements about likely deficiencies encountered in the field.

3.4 Diagnostic CD

Digital photographs were taken of regrowth from the pot trial. These were set up on CD to be used as a diagnostic tool to assist extension staff and growers in the identification of mineral deficiencies in peppermint. It must be stressed that this is a preliminary tool which will be modified when more detailed information is available. Because of the similarity in symptoms between peppermint and red beet potage, previous work on beet (Menary 1967) was included on the CD.
4. Results

The mean analysis of macro elements in soil from 20 affected sites in peppermint production areas are given in Table 1. Element levels when compared with values for agricultural production (Baker and Eldershaw 1993) show that all elements except nitrogen are in adequate supply. Pepperment responses to nitrogen have been demonstrated, Clark and Menary (1984) and have been incorporated into local recommendations.

In Queensland, SIRATRO grass responds to soil S levels above 50ppm. Levels as high as 720ppm have been recorded in the same production area. Since leaves of peppermint show bright crimson colours in affected areas it was decided to include sulphur as an element worthy of study even through a mean soil level of 350ppm was recorded.

Micro nutrient levels for the same set of soils are given in Table 2. The levels recorded would be regarded as adequate for DTPA extractable levels but in this case total elements were measured using strong acid extractant. Based on the small difference because extractable levels recorded in the literature and total levels recorded in affected soil, it was assumed that Cu, B and Mo deficiencies were likely to occur.

Following the soil analysis in late winter, plant samples were collected in early spring and summer of 2004. The results of macro element levels are given in Table 3. In this case all levels of macro elements with the exception of sulphur appear to be adequate. This is based on a comparison with related crops, Weir and Cresswell (1993).

Zeinali (2003) compares mint with some vegetable crops like tomato, potato and lettuce for similarities in macro and micro nutrient levels. Menary (1967) has found a similar set of symptoms associated with decline in red beets in the Lochyer Valley, Queensland. In this case and in the case of other vegetable crops mentioned above the levels for adequate growth exceed the 0.33% recorded in these samples.

Phosphorus levels decreased from 0.32% to 0.22% during the ground period of growth which may be approaching the deficiency range as indicated by the levels in related crops. Ca on the other hand increased from 0.85% to 1.02% which would be expected as plants age. Plant symptoms being expressed as purpling, crimson and chlorosis of foliage were typical of the symptoms in red beets which were associated with N, S, B and Cu deficiencies.

The micro nutrient levels in peppermint for the early spring and mid summer samplings are given in Table 4. The levels which are in the deficiency range when compared with other vegetable crops such as lettuce, potato and beet are B, Cu and Mo.

Following the soil nutrient and plant analysis surveys, it was decided to set out a commercial demonstration trial using Nitrophospka blue containing micro nutrients at one selected site. The crop had previously received 250kg/ha of 0-6-17 and 200kg of Nitram in the Spring. The superphosphate in the mixture contained Mo and sulphur. Nitrophospka blue was applied at 100kg/ha in a strip 40m x 3m in mid December. This area is shown on the CD which forms part of this report.

Herb samples were harvested from Nitrophospka treated and untreated areas in late January and distilled using bench scale distillation equipment. This comprised a 10L stainless steel vat with glass water cooled cohabitation condenser. The crop which received the Nitrophospka yielded 40% more oil than untreated. Although the trial area was adequately supplied with N,P,K fertiliser, severe symptoms of purpling and chlorosis occurred in the absence of Nitrophospka.

Soil was used from this commercial trial site to undertake a controlled nutrition trial in pots in the glasshouse as previously described. Shoots were measured after 8 weeks of growth and then harvested for chemical analysis. Digital photographs were taken of the regrowth from this trial and these were incorporated in the CD for diagnostic purposes.
The results of the analysis of macro elements excluding S are given in Table 5. The levels were adequate for normal growth by comparison with crops having similar requirements.

Micro elements and S analyses and plant height are given in Table 6. In this case the comparison is made between treatments with and without micro elements and S.

The tissue measurements indicate that these were plant responses to Mo, S, B and Cu. This is further supported by the low levels of S, B, Cu and Mo recorded where these elements were not applied. The tissue levels recorded where nutrients were added are likely to represent luxury consumption since high levels of soil application were used. In any event, these values in conjunction with those recorded in red beet and for vegetables with similar requirements, are being taken as a guide to adequate levels until calibration trials are undertaken.

Peppermint was grown in solution culture and analysed for Mo and N03 content. Symptoms of interveinal chlorosis were present when Mo levels were 4ppm. These leaves also contained free nitrate as measured by diphemylamine in concentrated H2SO4. A Mo level of 20ppm was recorded for the glasshouse trial where Mo was applied. In vegetables, the range from deficient to adequate varies by a factor of 4 to 5. In this case, 20ppm is not unreasonable but is certainly higher than levels recorded in the literature. This high level of Mo will need to be verified by further trials.

Even though Mn and Zn were not applied to the soil used in this experiment mean new levels recorded namely 231 and 46ppm respectively appear to be adequate.
5. Discussion

The soil and plant analysis from commercial peppermint fields revealed deficiencies of S, B, Cu and Mo. All four elements were not deficient at all sites and this was related to pH use of molybdenum/superphosphate and the age of the crop in years. This age factor is related to removal of approximately 3.5 tonnes dry matter per annum which corresponds to a per ha per annum loss of 0.3 kg Mo, 0.6 kg Cu, 1.0 kg of boron and 10.5 kg of S.

Available critical level data for micro nutrients in peppermint herb have been used where possible to aid in the interpretation of deficiencies. This established that Cu and B were likely to be deficient based on field plot samples. The level of sulphur was compared with that of red beets because of the similarity in symptoms and decline expressed in the field.

The pilot trial conducted in the glasshouse was able to demonstrate that deficiency symptoms of Cu, B and Mo and S could be corrected with soil applications of these elements. This was further supported by plant analytical data and plant growth. The levels of nutrients applied may be in excess of those required for optimal growth, ie. the luxury consumption range.

Further trials could determine critical levels in tissue and the fertilizer application rates required to achieve adequate levels without luxury consumption.

In the case of Mo, a solution culture experiment determined that 4ppm in tissue was still in the poverty adjustment range. This was apparent from the chlorotic symptoms displayed and the accumulation of nitrate. At 20ppm, no symptoms were apparent and further work will be required to establish a critical level.
6. Implications

A complex deficiency involving S, Cu, B and Mo has been demonstrated. The pilot scale field test in which molybdate superphosphate and Nitrophospka blue were applied, demonstrated that a 40% increase in oil yield could be achieved. It is likely that greater responses would have occurred if Cu had been included in the fertilizer and if the fertilizer mixture had been applied at the commencement of the grand period of growth. Despite these limitations, the remedial application of fertilizer increased yield by 16 kg/ha amounting to an increased gross margin in excess of $700/ha.
7. Recommendations

A CD was prepared using deficiency symptoms displayed in the field, the glasshouse and a red beet nutrition trial.

A field day was organised with growers to explain the complex nature of the micro nutrient deficiency and how it was detected and corrected.

Following the presentation, it was recommended that a trial fertilizer blend should be formulated for use in the coming season. Fertilizer companies were contacted to obtain a fertiliser mixture in granular form which would guarantee an even distribution of micro nutrients on the production areas. The following mixture was made available to growers:

- 25% ammonium sulphate
- 25% su-perfect (superphosphate) containing 0.4% Mo
- 1% zinc hydrate
- 2.5% copper granules
- 6.5% boron as granular
- 40% potassium sulphate

Since soil pH’s were generally above 6.5 and since en-sure could not be mixed with Cu, it was decided to use ammonium sulphate as the source of nitrogen on this occasion. This will be reviewed when field responses have been assessed during the coming season.
8. Bibliography


## 9. Appendix

### Table 1
Mean levels of macro nutrients in soils from production areas

<table>
<thead>
<tr>
<th>Analyte</th>
<th>pH</th>
<th>P (ppm)</th>
<th>Total N (%)</th>
<th>Ca (ppm)</th>
<th>K (ppm)</th>
<th>Mg (ppm)</th>
<th>S (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levels</td>
<td>5.9</td>
<td>8.0</td>
<td>0.17</td>
<td>4770</td>
<td>1416</td>
<td>853</td>
<td>350</td>
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</tbody>
</table>

### Table 2
Mean levels of micro nutrients in soils from production areas

<table>
<thead>
<tr>
<th>Analyte</th>
<th>B (ppm)</th>
<th>Cu (ppm)</th>
<th>Mo (ppm)</th>
<th>Zn (ppm)</th>
<th>Mn (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Units</td>
<td>3</td>
<td>5</td>
<td>0.5</td>
<td>49</td>
<td>495</td>
</tr>
</tbody>
</table>

### Table 3
Mean levels of macro nutrients in plants from production areas at two sampling dates

<table>
<thead>
<tr>
<th>Analyte</th>
<th>N (%)</th>
<th>P (%)</th>
<th>K (%)</th>
<th>Ca (%)</th>
<th>Mg (%)</th>
<th>S (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>December</td>
<td>2.7</td>
<td>0.32</td>
<td>1.92</td>
<td>0.85</td>
<td>0.48</td>
<td>0.31</td>
</tr>
<tr>
<td>March</td>
<td>2.06</td>
<td>0.22</td>
<td>1.07</td>
<td>1.02</td>
<td>0.59</td>
<td>0.35</td>
</tr>
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</table>
### Table 4

Mean levels of micro nutrients in plants from production areas at two sampling dates

<table>
<thead>
<tr>
<th>Analyte</th>
<th>B</th>
<th>Cu</th>
<th>Mo</th>
<th>Mn</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Units (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>December</td>
<td>24</td>
<td>9.3</td>
<td>&lt; 1</td>
<td>55</td>
<td>25</td>
</tr>
<tr>
<td>March</td>
<td>16</td>
<td>7.2</td>
<td>1.4</td>
<td>80</td>
<td>25</td>
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</table>

### Table 5

Mean levels of macro nutrients in plants from pot trials

<table>
<thead>
<tr>
<th>Analyte</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Units (%)</td>
<td>1.81</td>
<td>0.27</td>
<td>2.9</td>
<td>0.71</td>
<td>0.34</td>
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</tbody>
</table>

### Table 6

Mean levels of micro nutrients in treated and untreated plants from pot trials

<table>
<thead>
<tr>
<th>Analyte</th>
<th>-S</th>
<th>+S</th>
<th>-B</th>
<th>+B</th>
<th>-Cu</th>
<th>+Cu</th>
<th>-Mo</th>
<th>+Mo</th>
<th>Mn</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Units</td>
<td>%</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
</tr>
<tr>
<td>Levels</td>
<td>0.16</td>
<td>0.5</td>
<td>20</td>
<td>29</td>
<td>10</td>
<td>15</td>
<td>1.8</td>
<td>20</td>
<td>231</td>
<td>46</td>
</tr>
</tbody>
</table>

### Table 7

Mean plant height of peppermint in presence and absence of elements

<table>
<thead>
<tr>
<th>Analyte</th>
<th>-S</th>
<th>+S</th>
<th>-B</th>
<th>+B</th>
<th>-Cu</th>
<th>+Cu</th>
<th>-Mo</th>
<th>+Mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (mm)</td>
<td>459</td>
<td>481</td>
<td>433</td>
<td>465</td>
<td>424</td>
<td>484</td>
<td>426</td>
<td>462</td>
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