Increased Yields from Peppermint Crops through Improved Micro nutrient Nutrition (Stage 2)
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Foreword

For some years the total area of peppermint grown in Australia has been declining with poor oil yields a contributing factor. 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 follows on from a preliminary study which identified elements which were in the deficiency range for peppermint. The deficient elements identified were sulfur, copper, boron and molybdenum.

Fertilizer mixtures were prepared based on the preliminary evidence of deficiency and these were applied in a single season. Critical levels for each nutrient are reported as are the measured responses to applied nutrients in terms of oil yield and chemical composition.

This project was funded from industry revenue which was matched by funds provided by the Australian Government.

This report, an addition to RIRDC’s diverse range of over 2100 research publications, forms part of our Essential Oils and Plant Extracts R&D program, which aims to provide the knowledge and skills base for industry to provide high, consistent and known qualities in their essential oils and plant extracts products that respond to market opportunities and enhance profitability.

Most of our publications are available for viewing, downloading or purchasing online through our website www.rirdc.gov.au.

Craig Burns
Managing Director
Rural Industries Research and Development Corporation
Acknowledgments

I acknowledge the technical assistance provided by David Wilson, Matthew Gregory and Caroline Claye during the course of this investigation. Field sample analysis provided by Essential Oils of Tasmania through Sue Hinton added greatly to the application of this project to field production. Thanks are also due to Dr Chris Cooper who helped with document presentation.

I thank the Rural Industries Research and Development Corporation and the Natural Plants Extract Cooperative for funding the research and individual peppermint growers for the cooperation and assistance.

Abbreviations

ANOVA Analysis of Variance
GC / MS Gas Chromatography / Mass Spectrometry
HPLC High Performance Liquid Chromatography
ICPAES Inductively Coupled Plasma Atomic Emission Spectroscopy
LAI Leaf Area Index
S Sulfur
B Boron
Cu Copper
Mo Molybdenum
Zn Zinc
N Nitrogen
P Phosphorous
K Potassium
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Executive Summary

What the report is about - Aims/Objectives

The project was undertaken in two stages, namely a first year to assess the possibility of micronutrient deficiencies occurring in the field. If a nutritional problem was identified then there would be a further two year programme to meet the second and third objectives. The first year of the program was reported and published as a separate publication (Menary, 2005).

Three objectives were identified in relation to stage 2 of this project. They were as follows:

- Survey commercial fields, collect samples and analyse soil and plants;
- Established critical levels for deficiencies already identified;
- Collect analytical data and recommend corrective action.

Who is the report targeted at

The findings of this report will provide a nutritional guide for peppermint producers in Southern Australia. They will form the basis of field recommendations made by Essential Oils of Tasmania and other independent growers in Tasmania.

Background

Peppermint yields in Southern Australia are highly variable and this is impacting on the long term viability of the industry. Yields as high as 140 kg / ha have been achieved in Tasmania using a double cut system (Clark and Menary, 1984). However, in the last 10-15 years there has been a general decline in vigour of crops and a double cut has not been possible. 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.

Methods used

Observations on crop health during the growing season have indicated that deficiencies of sulfur, boron, copper, and molybdenum occur in peppermint crops. This was further supported by soil and plant analysis, calculations of nutrient removal each year at harvest and the lack of replacement through standard fertiliser applications.

To confirm the presence of sulfur, molybdenum, boron and copper deficiencies greenhouse and field trials were conducted. Peppermint was grown in pots in the greenhouse in soil from peppermint fields that were deficient in these elements. Nutrients were added to the same soil at 5 different rates to establish nutrient levels in tissues at which maximum growth occurs. In a field trial all four nutrients were either present or absent and applied in all combinations. Responses were obtained with all four nutrients as expressed by leaf analysis, oil yield, oil content of herb and chemical composition of oil. An oil yield of 130 kg / ha was achieved.

A fertiliser mixture containing granulated forms of micro nutrients was prepared in consultation with a major fertiliser company. This formulation was also recommended for use by peppermint growers. Grower’s trials were monitored for oil yield and leaf composition to ascertain an ideal fertiliser regimen for each production site.
**Results/Key findings**

A complex deficiency involving sulfur, copper, boron and molybdenum was identified in peppermint crops in Tasmania. Critical levels in plant tissues were determined for each of the elements involved. A fertiliser mixture was formulated to correct deficiencies in field grown crops and achieve high oil yields.

**Implications and Recommendations**

1. A field trial demonstrated that the deficiencies of sulfur, copper, boron and molybdenum can be corrected through fertiliser applications. Furthermore it was shown that increased growth through improved nutrition will enable two harvests to be made in the one season. The trial also demonstrated that tissue analysis was an effective method to assess nutrient status and fertiliser recommendation. An oil yield of 130 kg / ha was achieved in the first year and 103 kg / ha for the second year.

2. A granular form of fertiliser should be formulated to guarantee an even distribution of micronutrients to the soil surface. The mixture detailed below is recommended at 200 kg / ha.

<table>
<thead>
<tr>
<th>Element (kg in 200 kg mixture)</th>
<th>Element (kg in 200 kg mixture)</th>
<th>Composition (% in mixture)</th>
<th>Fertiliser Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen (N)</td>
<td></td>
<td>19</td>
<td>urea</td>
</tr>
<tr>
<td>Molybdenum (Mo)</td>
<td>Sub-perfect (superphosphate) containing 0.04 % Mo</td>
<td>25</td>
<td>Sub-perfect (superphosphate) containing 0.04 % Mo</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>zinc hydrate</td>
<td>1</td>
<td>zinc hydrate</td>
</tr>
<tr>
<td>Copper (Cu)</td>
<td>copper granules</td>
<td>2.5</td>
<td>copper granules</td>
</tr>
<tr>
<td>Boron (B)</td>
<td>boron as Granubor®</td>
<td>6.5</td>
<td>boron as Granubor®</td>
</tr>
<tr>
<td>Sulfur (S)</td>
<td>potassium sulphate</td>
<td>30</td>
<td>potassium sulphate</td>
</tr>
<tr>
<td>Sulfur (S)</td>
<td>calcium sulphate</td>
<td>25</td>
<td>calcium sulphate</td>
</tr>
</tbody>
</table>

3. Analysis of soil samples should form the basis of fertiliser recommendations (including micronutrients) for basal applications.

4. Herb samples should be taken in early December and March to assess the effectiveness of basal fertiliser and subsequent recommendations for side-dressings or foliar application of nitrogen and micronutrients.

5. Field officers should use critical level data to interpret plant analysis and formulate fertiliser recommendations.

6. Further fieldwork should be undertaken to establish the critical balance between sulfur, boron, copper and molybdenum to achieve maximum oil yield.
1. Introduction

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 that provides the necessary throughput to sustain the viability of field distillation units.

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

Boron is the one micro nutrient which produces a range of development and physiological effects on peppermint. Fischer and Bussler (1984) have shown that boron 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). In addition, Boichenko (1970) has demonstrated field responses in peppermint to applications of boron (B).

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

Peppermint yields as high as 140 kg / ha have been achieved in Tasmania using a double cut system (Clark and 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.

A preliminary study was undertaken to establish the presence of nutritional disorders in peppermint crops in Tasmania (Menary, 2005). Soil and plant analysis surveys were undertaken in all production areas to measure macro and micro nutrient levels. The survey indicated that deficiencies of sulfur (S), molybdenum (Mo), Boron (B), and Copper (Cu) could occur. Plant symptoms expressed were also consistent with those described in the literature for peppermint and related crops (Weir and Creswell, 1993). Based on these preliminary observations a fertiliser mixture containing granulated forms of micronutrients was formulated in consultation with a major fertiliser company and made available to growers in 2005 (Menary, 2005).

During the 2006 / 2007 seasons greenhouse trials were undertaken to establish critical levels of nutrients in peppermint shoots. As well, a field trial was conducted over two seasons to assess and monitor nutrient responses in terms of oil yield and composition and dry matter production. The soil at the field site was a clay loam which was deficient in sulfur (S), boron (B), copper (Cu), molybdenum (Mo) and zinc (Zn). This combination of greenhouse and field trials was considered adequate to establish a diagnosis and fertiliser recommendation system for peppermint production.
2. Objectives

Three objectives were identified in relation to this project. They were as follows:

- Survey commercial fields, collect samples and analyse soil and plants.
- Established critical levels for deficiencies already identified.
- Collect analytical data and recommend corrective action.

The project was undertaken in two stages, namely a first year to assess the possibility of micronutrient deficiencies occurring in the field. If a nutritional problem was identified then there would be a further two year programme to meet the second and third objectives. The first year of the program was reported and published as a separate publication (Menary, 2005).
3. Methodology

3.1 Plant and soil analysis

3.1.1 Nitrogen and sulfur in plant tissue
Nitrogen and sulfur 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 was carried out in a tin cap in O₂ @ 1000°C. The oxide produced was analysed using a thermal conductivity detector.

3.1.2 Metals in plant tissue
Homogenous samples (1 – 2 g) were weighed into a digestion vessel and 10 mL 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 an inductively coupled plasma atomic emission spectrophotometer (ICPAES).

3.1.3 Homogenous total elements in soil
A sub-sample of 1-2 g was accurately weighed into a digestion vessel and 5 mL of aqua-regia (3:1 conc. HCl:HN0₃) was added. 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.

3.1.4 Exchangeable bases
Exchangeable bases were extracted using Soil:1 M NH₄0Ac (1:10). Exchangeable cations were performed as per Method 15D3 in the Australian Soil and Land Survey Handbook (Rayment and Higginson, 1992). Accordingly a sub-sample of soil was equilibrated with 1M NH₄0ac at pH 7.0 for 30 min. The suspension was clarified prior to analysis by ICPAES.

3.1.5 Phosphorus and potassium in soil
Phosphorus and potassium in soil were extracted using Soil: 0.5 M bicarbonate (1:100). This is a modification of the bicarbonate procedure of Olsen et al. (1954). A homogenized sub-sample of 1.0 g was equilibrated in 100 mL of 0.5 M N₄HCO₃ at pH 8.5 for 16 hours. The suspension was clarified prior to analysis via ICPAES.

3.1.6 Calcium phosphate – extractable sulfur
Soil was extracted with 0.01 M Ca(H₂PO₄), using method 10B2 in the Australian Soil and Land Survey Handbook (Raymond and Higginson, 1992). Sulfur was measured via ICPAES.

3.1.7 Soil pH
Soil pH was measured on a 10 g sub-sample shaken for 1 hour in 50 mL of deionised water. The suspension was then tested for pH using a calibrated pH probe.
3.2 Sample collection

In early spring of 2004 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 200 mm 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. 200 mm in length were harvested, dried at 65°C, ground in a stainless steel hammer mill and stored at 2°C until analysed.

One square meter quadrats were sampled from all treatments and replicates in Dec 05, March 06, December 06 and March 07. Fresh samples were stored in plastic bags at -20°C. Samples were subsequently thawed and distilled. The oil was then analysed for major components. Shoot tips 4 nodes in length were harvested at each of the dates above and dried at 50°C before grinding and being stored at 4°C prior to analysis for inorganic components.

3.3 General laboratory methods

Several general analytical techniques are common to the trials conducted and the details of these are provided here.

3.3.1 Laboratory steam distillation

Laboratory samples were distilled in bench-top cohabation stills, comprised of a vat attached to a glass, water-cooled condenser unit. The oil was collected over water in a glass separator. The vats were of one of two sizes. Larger samples of herb (~1 kg) were distilled in 18 L stainless-steel vats. Small samples (less than 200 g) were distilled in 5 L glass, round-bottom flasks. The condenser/separator units were interchangeable between vats.

For distillation of herb material, the entire sample was weighed and then chopped into approximately 10 cm lengths and mixed well. Duplicate samples were taken for determination of dry matter. Sufficient of the remaining material was used to fill the appropriate vat, containing 2 L of warm water for the stainless–steel vats or 1 L water for the glass units, and the vat sealed. The vat was heated using an electric hotplate or heating mantle. Timing of the distillation commenced at breakthrough and the duration of distillation was two hours.

3.3.1.1 Oil yield determination

Oil yield was determined by weight and presented as a w/w percentage of dry plant weight.

3.3.1.2 Oil composition

Oil composition was determined by gas chromatography. Accordingly oil samples were dried using anhydrous Na2SO4 and a sample of 5 μL of oil was dissolved in 1 ml of HPLC grade hexane. Oil composition was determined using a Hewlett Packard 5890 Series II gas chromatograph, fitted with a flame ionisation detector.

The column was a 30m HP-INNOWax column (phase ratio 160), 0.32 mm ID, 0.5 μm film thickness. The carrier gas was high purity nitrogen with a split vent flow of 100 mL/min, a column flow of 2 mL/min (split ratio 50:1), a purge flow of 3 mL/min and a head pressure of 7.5 psi. The GC injector temperature was 250°C and the detector temperature 280°C. The oven temperature was programmed from 50°C (1 min) rising at 7.0°C/min to 210°C. For steam distilled oil the oven remained at the final temperature of 210°C for 6.14 min. For solvent extracted oils the oven temperature programme was
altered to rise at 7.5°C per minute a final temperature of 240°C and to maintain this final temperature for 10.7 min.

Peak identification was initially based on GC/MS and subsequently determined by retention time. Approximate retention times of the components to be reported upon are presented in Table 2.

<table>
<thead>
<tr>
<th>Peak number</th>
<th>Component</th>
<th>Retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>α-pinene</td>
<td>4.97</td>
</tr>
<tr>
<td>2</td>
<td>limonene</td>
<td>8.25</td>
</tr>
<tr>
<td>3</td>
<td>1,8-cineole</td>
<td>8.49</td>
</tr>
<tr>
<td>4</td>
<td>trans-sabinene hydrate</td>
<td>13.43</td>
</tr>
<tr>
<td>5</td>
<td>menthone</td>
<td>13.70</td>
</tr>
<tr>
<td>6</td>
<td>menthofuran</td>
<td>13.97</td>
</tr>
<tr>
<td>7</td>
<td>iso-menthone</td>
<td>14.24</td>
</tr>
<tr>
<td>8</td>
<td>menthyl acetate</td>
<td>15.35</td>
</tr>
<tr>
<td>9</td>
<td>neo-menthol</td>
<td>15.87</td>
</tr>
<tr>
<td>10</td>
<td>menthol</td>
<td>16.65</td>
</tr>
</tbody>
</table>

### 3.4 Greenhouse pot trials

A pot trial was established in the greenhouse using soil from a site adjacent to the peppermint field trial. The soil had not been fertilised for 5 years and the chemical analysis verified a deficiency of S, B, Cu, Mo and Zn. Each pot contained 1.5 kg of air dry soil. For each nutrient under test there were five levels used. Nitrogen, P and K were applied to all pots at 460, 350 and 450 ppm respectively. Zinc was marginally deficient in this soil and was applied to all pots at 4 ppm. The trial for each element was laid out as a $5 \times 5$ latin square design for each of the four nutrients being investigated. The five levels for each nutrient are detailed in table 3.

<table>
<thead>
<tr>
<th>Analyte Level ppm</th>
<th>Sulfur</th>
<th>Boron</th>
<th>Copper</th>
<th>Molybdenum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>7</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>400</td>
<td>14</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>600</td>
<td>21</td>
<td>21</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>800</td>
<td>28</td>
<td>28</td>
<td>4</td>
</tr>
</tbody>
</table>
Fine sand was added to nutrients to make 20 g of fertiliser mixture in order to aid in the distribution of nutrients over the soil surface. Nutrients were mixed with dry soil on a plastic sheet by rolling the soil mixture 10 times in one direction by alternatively raising each edge of the sheet and then similarly 10 times at a right angle. This ensured even mixing of soil and nutrients. The dry soil was added to the pot and compressed to a standard volume and wet to field capacity. Each pot had a saucer to catch leachate which was returned to the pot as required.

Cuttings of a single clone of cultivar Black Mitcham that were 5 nodes in length were planted directly into each pot. A light mist was used for two days until the cuttings produced roots. Thereafter watering was carefully gauged to avoid soil leaching. Shoots were harvested above ground level at 12 weeks, dried at 65 °C and ground for chemical analysis.

3.5 Field trial

In 2004, a peppermint field was established on a soil deficient in S, B, Cu, Mo and Zn. The soil levels of these elements were 9.84, 0.62, 0.66, < 1 and 1.6 ppm respectively. A pre planting fertiliser was applied at a rate shown in table 4. Tip cuttings were planted direct into the field under a pivot irrigation system. In 2005 a basal fertiliser was applied in October at a rate given in table 3. Nitrogen, P and K were applied to the whole area while sulfur and micronutrients were applied to treatments only at the rates specified in table 4.

This was followed by two equal side dressings totalling 100 units of nitrogen supplied as urea through the irrigation system. In October 2006 the same basal levels of N, P and K were applied as in 2005. The only micronutrient added was B which was applied as a foliar spray of Solubor® at 0.24 Kg / ha.

As in 2005, 100 units of nitrogen were applied as side dressings.
### Table 4: Fertiliser application rates for field trial

<table>
<thead>
<tr>
<th>Analyte</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>S</th>
<th>Zn</th>
<th>Cu</th>
<th>Mo</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal 2004</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kg/ha</td>
<td>10.6</td>
<td>4.5</td>
<td>36</td>
<td>27</td>
<td>0.7</td>
<td>1.2</td>
<td>0.2</td>
<td>2.0</td>
</tr>
<tr>
<td>Basal 2005</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kg/ha</td>
<td>22</td>
<td>25</td>
<td>52</td>
<td>30</td>
<td>3.0</td>
<td>5.4</td>
<td>2.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Side 2005</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kg/ha</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal 2006</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kg/ha</td>
<td>22</td>
<td>25</td>
<td>52</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.24</td>
</tr>
<tr>
<td>Side 2006</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kg/ha</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### 3.5.1 Treatments and design

Four elements (S, B, Cu and Mo) were either present or absent and arranged in all possible combinations giving a $2^4$ factorial set out in randomised blocks with 4 replicates. The 16 treatments were applied to plots (2 m × 4 m) within the treatment row with a buffer of 2 m between plots. Between rows or replications the buffer was 5 m. Nitrogen and phosphorus were added as diammonium phosphate and potassium chloride. Other nutrients used in treatments were applied as calcium sulphate, borax, copper, sulphate and sodium molybdate.

#### 3.5.2 Statistical analysis

Statistical analysis were carried out using procedures of the SAS statistical package version 6.12, 1989–96, SAS Inc., Cary, NC, USA.
4. Greenhouse Trials

4.1 Results

A plot of dry matter against chemical composition of shoots for S, B, Cu and Mo are given in figures 1 to 4.

Figure 1: Effect of Sulfur levels in tissue on peppermint dry matter production

\[ y = -310.85x^2 + 242.6x - 19.103 \]

\[ R^2 = 0.98 \]

Critical level 0.39%

Figure 2: Effect of Boron levels in tissue on peppermint dry matter production

\[ y = -0.0483x^2 + 2.6233x - 3.2965 \]

\[ R^2 = 0.97 \]

Critical level 27 ppm
Figure 3: Effect of Molybdenum levels in peppermint tissue on dry matter production

$y = -0.0951x^2 + 2.3508x + 19.004$

$R^2 = 0.85$

Critical level 12 ppm

ANOVA Critical level 4ppm

Figure 4: Effect of Copper levels in peppermint tissue on dry matter production

$y = -0.4406x^2 + 8.2387x - 5.9188$

$R^2 = 0.99$

Critical level 9 ppm
The polynomials which are shown in figures 1, 2 and 4 describe the relationship between nutrient concentration of S, B and Cu in shoot tissues and dry matter yields. The corresponding $R^2$ values are 0.98, 0.97 and 0.99 respectively. The point on the curve at which the rate of change of dry matter with respect to nutrient concentration is zero represents the critical level (i.e. no further growth increase occurs with increasing tissue concentration of nutrient). The critical values are 0.39% for S, 27 ppm for B and 9 ppm for Cu.

The results for Mo are presented in figure 3. The $R^2$ value for the Mo response is 0.85 which is a relatively poor fit. Furthermore, there was no significant dry matter response after tissues reached a level of 4 ppm. In this case, the critical level was 4 ppm. However, using the polynomial the critical level is 12 ppm. Therefore the actual critical level may be between 4 and 12 ppm depending on the interpretation of the statistical approach being used.

### 4.2 Discussion

A preliminary report on this project (Menary, 2005) gave nutrient levels in shoots from peppermint grown in the presence and absence of the applied nutrients S, B, Cu and Mo. The soil used was deficient in all nutrients mentioned above. Where nutrients were applied, it was expected that they would have been adequate for normal growth. All values reported for treated soil are higher than the critical values reported here. This means that the nutrients applied in the preliminary trials were in the luxury consumption range. The critical levels for S, B and Cu reported here are in accord with values published for vegetable crops such as cauliflower, brussel sprouts, broccoli, cabbage, carrot, cucurbits onion and pea (Weir and Cresswell, 1993).

The level of Mo in tissue in the absence of added Mo was 0.3 ppm. With the addition of 1 ppm to soil the level rose to 4.0 ppm. The critical level from the polynomial was 12.0 ppm. An analysis of variance on the data indicated that no significant increase in dry matter occurred after a tissue level of 4.0 ppm was reached. The original analysis of the soil used in the pot experiment was 0.5 ppm Mo. This means that the level in the soil at the commencement of the trial was 1.5 ppm and the measured tissue level was 4 ppm (Tisdale et al., 1984). To raise the level of Mo in soils in the USA is in the range 1.2 to 1.3 ppm. To raise the level of Mo in soil (in a field situation) by 1 ppm would require 0.5 kg / ha of Mo as fertiliser (assuming a bulk density of 1.1 and a root exploitation depth of 20 cm). Therefore the addition of 0.5 kg / ha of Mo to the field trial would be a reasonable level of applied fertiliser to correct this soil deficiency. It is further likely that 4 ppm is a reasonable estimate for the critical value of Mo in peppermint.
5. Field Trials

5.1 Results

5.1.1 First harvest Dec 05
There was a significant decrease in oil yield per ha in treatments receiving Cu. The mean oil yield in the absence of Cu being 64.2 kg / ha compared with 57.3 kg / ha in the presence of Cu. The decrease in yield with added Cu was associated with damage caused to shoots after the application of copper sulphate crystals. This toxicity caused a delay in shoot extension and appeared to encourage the growth of lateral shoots. This resulted in a decrease in the physiological age of vegetative growth at harvest. This was reflected in the significantly higher levels of menthone namely 28.8 % versus 26.5 % in the absence of Cu. Further evidence of delayed maturity was the significantly lower level of menthyl acetate in the presence of Cu namely 1.6% versus 1.8% in the absence of Cu. The oil yield and compositional changes were significant at the 1% level.

In the case of boron, there was a significant decrease in yield of herb/plot being 3.1 kg for B treated versus 3.7 kg for untreated. The toxicity was expressed morphologically as marginal yellowing which persisted for the whole season. Although there was a reduction in herb yield resulting from apparent B toxicity, there was a significant increase in the percentage of oil yield on a dry matter basis. Percentage oil yield increased from 1.0 % in untreated plots to 1.4 % in treated plots. This increase in oil yield was able to compensate for the loss of oil through decreased herb production. The mean yield over all treatments was 60.8 kg / ha.

5.1.2 Second harvest Mar 06
A second harvest of the trial was made in March 06. There was a significant decrease (7.3%) in oil yield in the presence of Mo. This decrease could be explained through a decrease of 9% in oil content of herb. On the other hand, oil content in herb from B treated plots was 9.4 % higher than for untreated plots. This compensated for the loss in oil yield due to decreased dry matter production in B treated plots. The mean yield over all treatments was 69.8 kg / ha. This meant that the total yield from harvests 1 and 2 of the 05 / 06 season was 130 kg / ha. This yield is comparable with the highest recorded yield of 140 kg / ha which was achieved in the early stages of the development of the peppermint industry (Clark and Menary, 1984).

At the second harvest the composition of the 5 major components of the oil did not vary significantly in relation to treatment.

5.1.3 First harvest Dec 06
In the second year of the trial, B was the only treatment applied as a foliar spray (see table 3). There was a significant increase in oil yield in response to previously applied Mo and S. The increases were 8 and 12% respectively. There were also highly significant interactions between B and S, B and Cu, and Mo and S. In the case of B×S interaction there was a 10 % increase above boron alone and a 3 % increase above S alone. For B×Cu the increase was 8% above Cu alone and 6% above B alone. For Mo×S the combination gave a 7% increase in yield over Mo and S alone. The mean yield over all plots was 52.1 kg / ha.

Dry matter yield was significantly increased by applications of Mo and S and in both cases the increase was 10 %. There was also a highly significant interaction between Cu and S. Copper and S together gave an increase of 13% over Cu alone and 8 % over S alone.

Fertiliser treatments had no significant effect on the level of the five major components in the oil.
5.1.4 Second harvest March 07

In the 2\textsuperscript{nd} harvest for the season there were no significant oil yield responses to fertiliser treatment. The mean oil yield over all treatments was 50.7 kg / ha giving a total of 102.8 kg / ha for two harvests. However the previously applied Cu (in 2006) caused a 2.7 \% increase in menthol and a 14 \% increase in menthone above untreated plots in the 2007 season.

5.1.5 Plant analysis

Plant analysis was undertaken on samples collected in March 2006 and 2007. The trial had received a basal fertiliser of all nutrients in the 2005 season as previously described in table 3. All plots received their specific fertiliser treatments at the commencement of the 2005 / 2006 season. In 2006 / 2007 the only treatment applied was a foliar spray of B to boron treated plots. The results are listed in table 4. Each mean in the table was derived from 32 samples.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>S (%)</th>
<th>B (ppm)</th>
<th>Cu (ppm)</th>
<th>Mo (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertiliser absent 06</td>
<td>0.29</td>
<td>16.2</td>
<td>10.1</td>
<td>3.2</td>
</tr>
<tr>
<td>Fertiliser present 06</td>
<td>0.33</td>
<td>24.5</td>
<td>11.6</td>
<td>52.0</td>
</tr>
<tr>
<td>Fertiliser absent 07</td>
<td>0.27</td>
<td>18.6</td>
<td>6.66</td>
<td>2.07</td>
</tr>
<tr>
<td>Fertiliser present 07</td>
<td>0.28</td>
<td>28.2</td>
<td>9.17</td>
<td>15.4</td>
</tr>
<tr>
<td>Critical level</td>
<td>0.39</td>
<td>27</td>
<td>9.3</td>
<td>4 - 12</td>
</tr>
</tbody>
</table>

Tissue samples taken at the end of each growing season gave an indication of the uptake of applied nutrients and dilution effects due to herb removal and soil factors such as leaching and fixation. At the end of the first season, sulfur was slightly below the critical level and by the end of the second season was in the poverty adjustment range with the level of fertilised plots being the same as unfertilised treatments. Boron levels in unfertilised plots were in the poverty adjustment range despite a basal application of boron at the commencement of the 2005 / 2006 season. Since boron gave a response in the first season and is known to be beneficial at high levels (Nandi and Chatterjee, 1986) a foliar spray was applied at the commencement of the 2006 / 2007 season. This is reflected in the increase in B to 28.2 \% from 24.5 \% at the end of the 2006 season. Again the effect of the basal B in 2005 is not evident in the unfertilised 2006 sampling.

Tissue Cu levels were higher in fertilised plots than unfertilised and the effect of basal fertiliser disappeared after 2 years. Copper applied in 2005 gave 10.1 ppm in tissues at the end of 2005 / 2006 season but one year later the level had decreased to 6.66 ppm (i.e. into the poverty adjustment range). Likewise Cu applied in the 2005 / 2006 season gave 11.6 ppm at the end of the season but just below the critical level at the end of the 2006 / 2007 season.

Fertiliser Mo applied in 2005 (2 kg / ha) appears to be too high and resulted in a tissue level of 52 ppm. However by the end of the 2nd season the level was almost in the critical level range. For unfertilised plots, the level was in the poverty adjustment range for both seasons namely 3.2 and 2.07 ppm respectively.
5.1.6 Growers samples

Table 6: Range of element concentrations in shoot tissue from 10 commercial peppermint fields

<table>
<thead>
<tr>
<th>Sulfur</th>
<th>Boron</th>
<th>Copper</th>
<th>Molybdenum</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.28-0.46</td>
<td>16-44</td>
<td>5.6-13.0</td>
<td>0.2-1.2</td>
</tr>
</tbody>
</table>

In all cases basal fertiliser containing S, B, Cu and Mo had been applied at the levels specified in table 3 (as basal 2005). In some cases, deficiency levels were still being recorded for S, B and Cu. In the case of Mo the deficiency was not corrected in any of the fields.

5.2 Discussion

In the season preceding the establishment of the fertiliser trial, the elements S, B, Cu, Mo and Zn were applied to the newly established crop as all elements were deficient according to the soil analysis. This treatment was then supplemented with a treatment schedule of all four elements (excluding Zn) either present or absent in all possible combinations. Although no visible symptoms of Cu toxicity were apparent in the second harvest of the 2006 / 2007 season, there was still a significant increase in menthone and a decrease in menthol. This result suggested that high Cu in tissue may have had delaying effect on maturation of plants and/or oil.

Although visible symptoms of Cu toxicity occurred as a moderate to severe necrosis of emerging shoots, the level of Cu in shoot tissue at the end of the season was 11.6 ppm. According to Zeinali et al. (2003) this is not outside the normal range and they report 21.5 ppm as a maximum level. Also the rate of Cu used in the first year was 1.2 kg / ha and in the second 5.4 kg / ha. These rates are not regarded as excessive (Tisdale et al., 1990). It is likely that direct contact between the copper sulphate and the leaves caused the damage delaying plant growth for a few weeks. Hence the harvested herb was immature when the other treatment plots were harvested. This is supported through the observations that menthone was significantly higher and menthol lower, which is typical of an oil derived from immature plants (Clark and Menary, 1984).

Boron treatment caused an increase in oil content of herb for both the first and second harvests in the first year. The increase of 20% in the first harvest and 9% in the second was in accord with observations by Boichenko (1968) who reported an increase of 11% in the presence of high soil B. Since the level of B recorded at the end of the season was 24.5 ppm (i.e. in the normal range as stated by Weir and Creswell, 1993) then toxicity cannot be postulated. It seems likely that B is slowing vegetative growth in favour of secondary metabolite biosynthesis. Furthermore, the total amount of B applied over the 2nd year period was 9 kg / ha which is not regarded as high (Tisdale et al., 1990).

The rate of Mo used in the first year was 0.2 kg / ha and in the second 2 kg / ha. The latter application rate was high but this was thought to be necessary in response to the low level of Mo recorded in shoot tissue after the first year. However 2 kg / ha was excessive and a level of 52 ppm was recorded in tissue at the end of the first season .The toxicity was shown through its effect on oil accumulation with no decrease in herb production. By the end of the second season the level of Mo had fallen to around the critical level. The response to Mo at this level compared to unfertilised treatments at 2.07 ppm in the second season is therefore not surprising.

Sulfur fertiliser rates used amounted to 70 kg / ha over two years. This gave a tissue level of 0.33 % at the end of the 2006 season which approximated to the critical level. It is likely that there was a carry-over effect from the 2004 application of S resulting in adequate S being available in the unfertilised plots in 2005. Thus there was no response to S in the first season.
In the second year of production there was an oil yield and a dry matter increase due to Mo and S. By the end of the second season the levels of Mo had fallen to around the critical level. The response to Mo at this level compared to the unfertilised treatments at 2.07 ppm in the second season is therefore not surprising. At the commencement of the season the tissue level in treatments which had received S would have been around 0.33 % (i.e. about 12% higher than unfertilised). The oil yield was further complicated by the positive interaction with B and Cu. Dry matter production on the other hand showed a positive interaction between Cu and S.

The interaction between Mo and S, B and Cu, B and S and Cu and S indicated the complex nature of this nutritional problem. Further investigations of these factors would be necessary to optimise oil yields.

Samples taken from commercial production sites all show Mo levels in the poverty adjustment range. This occurred despite an application of 0.2 kg / ha thus emphasising the need to increase Mo application rates to at least 0.5 kg / ha.
6. General Discussion

The soil and plant analyses from commercial peppermint fields showed deficiencies of S, B, Cu and Mo. All four elements were not deficient at all sites and this was related to soil type, previous fertiliser history and the age of the crop in years. This age factor is related to the removal of approximately 3.5 tonnes of dry matter annually which corresponds to an annual loss of 0.3 kg Mo, 0.6 kg Cu, 1.0 kg B and 10.5 kg S. These annual losses were not being taken into account in the fertiliser management practice.

The level of Mo fertiliser added to soil in the greenhouse trial ranged from 0 to 4 ppm. The poverty adjustment range appeared to be 0-1 ppm of added Mo based on dry matter production. However at 1 ppm the tissue level was 4 ppm. In a solution culture trial (Menary, 2005) a deficiency of Mo was demonstrated as evidenced by the presence of free nitrate in leaves and interveinal chlorosis at 4 ppm Mo in tissue samples. The increments of 1 ppm between treatments were too great for precision and a further refinement would be required over the range 0-2 ppm applied Mo to achieve a more accurate estimate of the critical level. In a soil system it is difficult to achieve an even mixing of small quantities of added chemical. To overcome this it would be preferable to use solution culture with large volumes of solution to buffer against concentration changes with time (Asher et al., 1965). In this report the critical level is given as a range 4-12 ppm. Until a more detailed study is undertaken these results should act as a guide to diagnosis of Mo deficiency.

In the field trial, a highly significantly oil yield and dry matter response was obtained when the Mo level in tissue was 2.07 ppm. This level was at least double the value reported in the literature (Weir and Creswell, 1993). Hence it appears that peppermint has a high requirement for Mo and the responses are also related to the level of B, Cu, and S. This high requirement might be associated with the high turnover of nitrogen in the final stages of growth when Leaf Area Index (LAI) is increasing exponentially prior to harvest. It also appears that the high levels of Mo (52 ppm) did not affect nitrogen assimilation but did have an effect on oil biosynthesis.

It should also be noted that levels of Mo in herb above 5.0 ppm may cause molybdenosis in ruminants (Tisdale et al., 1984). Therefore growers should exercise care when ruminants are allowed to graze on peppermint crops.

Copper fertiliser application caused damage to young growth even though these levels were not in the toxicity range. The toxic effect was due to direct contact between leaves and the copper sulphate crystals. To prevent this problem in the future, basal applications should be applied before shoot growth occurs. Additionally, there was evidence that high Cu levels in tissue may reduce the rate of oil maturation as well as plant development. Care should be taken to ensure that application of Cu does not result in tissue exceeding the critical level of 9 ppm.

Boron has a promotive effect on oil accumulation and annual applications are necessary to maintain tissue levels above the critical level. Foliar spraying with Solubor® was an effective method of achieving absorption as highlighted through the observation that a foliar spray of 0.24 kg / ha was as effective as a soil dressing of 7 kg / ha in raising tissue level to the critical value.

Sulfur levels in soil are depleted by oxidation and leaching etc (Tisdale et al., 1990). Crops that use moderate levels of S have recommended rates of application in the range 20 - 40 kg / ha. This study demonstrated that an annual application will be necessary in peppermint to maintain tissue levels above the critical level of 39 ppm. In this work 57 kg / ha applied over 2 years met the requirement. However with the absence of an application in 2006 the level decreased to 28.2 ppm within 12 months. It could be argued that this low level of sulfur was the reason why there was no response to Mo, B or Cu which were observed to be slightly above critical levels.
An annual application of 20 kg / ha of S is recommended. However, where soils are low in sulfur this should be supplemented with another 20 kg / ha of S applied as calcium sulphate. This recommendation is supported from tissue level measurements in samples taken from commercial properties where 20 kg / ha of sulfur gave levels between 0.28 and 0.46 % S.

The trial described here demonstrates significant interaction between S, Mo, Cu and B. These interactions act in different ways on dry matter production and oil accumulation. Further work is required to optimise the levels required to achieve maximum oil yield.
7. Implications

A complex deficiency involving S, Cu, B and Mo has been demonstrated. A field trial demonstrated that deficiencies can be corrected through fertiliser applications. Furthermore it was shown that increased growth through improved nutrition will enable two harvests to be made in the one season. The trial also demonstrated that tissue analysis was an effective method to assess nutrient status and fertiliser recommendation. An oil yield of 130 kg / ha was achieved in the first year and 103 kg / ha for the second year.
8. Recommendations

This project has identified those nutrients which had impacted on the economic production of peppermint oil. Several steps should now be taken to capture the economic benefits of a revised fertiliser program. They are as follows:

1. A granular form of fertiliser should be formulated to guarantee an even distribution of micronutrients to the soil surface. The mixture as detailed in table 6 is recommended at 200 kg / ha:

Table 7: Peppermint fertiliser mixture containing micronutrients

<table>
<thead>
<tr>
<th>Element</th>
<th>Fertiliser Type</th>
<th>Composition (% in mixture)</th>
<th>Element (kg) (in 200 kg / ha mixture)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen (N)</td>
<td>urea</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td>Molybdenum (Mo)</td>
<td>Su-perfect (superphosphate) containing 0.04 % Mo</td>
<td>25</td>
<td>2</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>zinc hydrate</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>Copper (Cu)</td>
<td>copper granules</td>
<td>2.5</td>
<td>0.66</td>
</tr>
<tr>
<td>Boron (B)</td>
<td>boron as Granubor®</td>
<td>6.5</td>
<td>2</td>
</tr>
<tr>
<td>Sulfur (S)</td>
<td>potassium sulphate</td>
<td>30</td>
<td>11</td>
</tr>
<tr>
<td>Sulfur (S)</td>
<td>calcium sulphate</td>
<td>25</td>
<td>9</td>
</tr>
</tbody>
</table>

2. Analysis of soil samples should form the basis of fertiliser recommendations including micronutrients for basal applications.
3. Herb samples should be taken in early December and March to assess the effectiveness of basal fertiliser and subsequent recommendations for side-dressings or foliar application of nitrogen and micronutrients.
4. Field officers should use critical level data to interpret plant analysis and formulate fertiliser recommendations.
5. Further field work should be undertaken to establish the critical balance between S, B, Cu and Mo to achieve maximum oil yield.
9. References


Increased Yields from Peppermint Crops through Improved Micro nutrient Nutrition (Stage 2)

By Professor Robert Menary

Pub. No. 12/096

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 follows on from a preliminary study which identified elements which were in the deficiency range for peppermint. The deficient elements identified were sulfur, copper, boron and molybdenum.

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