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Nitrogen and Water Relations in BORONIA

A report for the Rural Industries Research and Development Corporation

by RC Menary & N Roberts

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Foreword

Since 1975 work has been undertaken in Tasmania to develop a new floral extract from Boronia megastigma Nees under an intensive management system. Preliminary investigations included, micro and macro propagation, clonal selection, flowering physiology and mechanical harvesting, extraction and purification technology.

A lack of understanding of nitrogen nutrition and water relations was an impediment to reliable production which was a key element in the marketing strategy. The project was designed to address nitrogen and water relations under field conditions. Aspects which received specific attention were:

• source, rate and timing of nitrogen
• the establishment of critical levels in plant tissue.
• the effect of nitrogen on oil yield and composition
• the effect of nitrogen on canopy architecture and harvestability
• the effect on clone type on the above
• the interaction between nitrogen and irrigation

The report covers four major experiments, two field trials on nitrogen, one glasshouse experiment on water relations and a field trial to verify the physiological parameters established in this glasshouse trial.

This project was funded from RIRDC Core Funds which are provided by the Federal Government.

This report is an addition to RIRDC's diverse range of over 450 research publications and forms part of our Essential Oils and Plant Extracts R&D program, which aims to support the growth of a profitable and sustainable essential oils and natural plant extracts industry in Australia.

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Peter Core
Managing Director
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Contents

Executive Summary

Objectives

- To determine the source and rate of nitrogen which will give high yields of quality extract.
- To obtain canopies which will meet the requirements of flowering, pest control and mechanical harvesting.
- To establish a predictive test to implement a reliable management strategy for nitrogen nutrition.
- To establish guidelines for water use in relation to nitrogen nutrition.

Background

Boronia produces an exotic flavour and perfume extract which has strong appeal for its berry, apricot and floral characters. It is a high value low volume product which is ideally suited to the Tasmanian environment. Areas most suited to the production of boronia are low fertility, acid, sandy soils which support open eucalypt and heath vegetation. The particular characteristics of the soil pose some special problems of nitrogen nutrition and water relations which have been the subject of this investigation.

Research

The research emphasis was on field production and recommendation with close involvement of industry partners.

Outcomes

Boronia prefers a balance between ammonium and nitrate sources of nitrogen. Ammonium nitrate was shown to be the most effective source compared with calcium nitrate, urea and IBDU (isobutylidene diurea). The two commercial clones tested did vary in their response to nitrogen. A maximum yield for clone 250 being achieved at 60 Kg N/ha and clone 5, 15 Kg N/ha. In the latter case, excess nitrogen caused toxicity symptoms which may be either ammonium toxicity or nitrate toxicity due to reduced nitrate reductase activity associated with molybdenum deficiency.

High levels of nitrogen were shown to increase vegetative growth and decrease mechanical harvesting efficiency. For example at 80 Kg N/ha on clone 250 only 27% of the crop could be harvested as compared with 50% at 40 Kg/ha.

A critical level of total nitrogen from September to February in young leaf tissue was established at 1.5%.

The trial site had plant rows with an east-west orientation. There was a significant difference in maturity between north and south facing sides of the plant row. It was estimated that the southern side of canopies was 7 days later than the north facing side. This accentuated the poor harvest efficiency as the harvester did not remove small unopened flower buds.

Evidence from glasshouse trials established that boronia has resistance to drought. An attempt was made to test this observation under field conditions. Unfortunately the trial period was associated with high rainfall and water stress conditions did not prevail during the production period.

Implications

Controlled nitrogen nutrition is important in controlling shoot extension and ease of mechanical harvesting. Tissue analysis for total nitrogen appears to be an effective method of diagnosis to establish plant requirements for efficient production. While clones have the same physiological requirement for nitrogen they vary in their ability to accumulate nitrogen from the soil solution.

Introduction

Each section of the report has an introduction that is specific to that section.

Nitrogen fertiliser is known to increase herb and oil yield in essential oil crops. This has been shown for Eucalyptus, *Mentha arvensis*, *Mentha citrata*, *Mentha spicata*, *Cymbopogon winterianus*, *Mentha piperita*, *Boronia megastigma*, *Olearia phlogopappa* and Meadow Foam (*Limnanthes alba*) (Menary 1994).

Oil composition can also be affected by nitrogen and this has been demonstrated in Eucalyptus, *Palmarosa*, Fennel, *Mentha arvensis*, Chamomile, *Salvia* and *Leptospermum* (Menary 1994).

Nitrogen nutrition of Boronia had been investigated under glasshouse and field conditions by Reddy & Menary (1989). Preference was shown for slow release ammonium forms of nitrogen. This work also demonstrated a feedback mechanism of nitrate toxicity and inhibition of nitrate reductase. Thompson (1993) demonstrated that Mo deficiency in Boronia occurred under highly acid soil conditions which is a common occurrence in production areas.

Work on flowering physiology (Roberts & Menary, 1989) showed that the flowering potential of boronia was related to the amount of vegetative growth (ie. potential nodes) at the time of flower initiation. Flowering occurs in leaf axils in response to reduced temperatures during the late autumn with a maximum potential of 3 flowers per axil and their continued development being related to total light integral.

Given the known information on the effect of nitrogen on oil yield and composition in essential oil crops and the complex aspects of nitrogen assimilation and potential Mo deficiency, it became apparent that reliable field recommendations for nitrogen were the key to the production of controlled vegetative shoots and hence mature flowers for oil accumulation.

The series of experiments that follow represent a progression through the resolution of the objective outlined above.

2. Effect of Nitrogen on growth, flower yield and oil composition and yield in *Boronia megastigma* Nees

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2.1 Abstract

Four forms of nitrogen, isobutylidene diurea (IBDU), urea, NH_4NO_3 and $Ca(NO_3)_2$, were applied to boronia plants at three rates of application (25, 50, and 100 kg/ha) either as a single application in late October or in two equal applications, one in October and the other in early January. Two sites and two clones were investigated, growth measurements were taken on one site on a regular basis throughout the year, flowers were harvested in September.

Leaf nitrogen, flower nitrogen, number of nodes, flower and oil yields as well as the % volatiles and β-ionone content of the oil were positively correlated with increased rates of nitrogen application. NH_4NO_3 and $Ca(NO_3)_2$ resulted in the highest yields in both clones. IBDU and urea were partially toxic to Clone 5 at 100 kg/ha, this was thought to be due to an inability to detoxify NH_4^+ rather than a low NO_3^- reductase activity. This results of this experiment provides guide lines for nitrogen management in the future.

2.2 Introduction

Boronia megastigma Nees. is an evergreen woody shrub which bears a profusion of yellow-purple flowers in early spring. The flowers are extracted for oil which is highly prized in the flavour and fragrance industry. Since 1990 the size of the commercial plantings in Tasmania (Australia) have increased by approximately 400 % with further significant increases planned over the next two years.

The importance of applying nitrogen to improve the flower yield of boronia was highlighted by Reddy and Menary (1989a &b). This study was designed to provide a more comprehensive evaluation of nitrogen nutrition in relation to oil yield and composition.

2.3 Materials and Methods

Field Design and Growth Measurements
The experiment was conducted at two sites in Tasmania; at Site 1 on gravely sand with a pH of 4.5, and Site 2 on sand with a pH of 4.2. Clone 250 was used at Site 1 whilst Clone 5 was used at Site 2.

The experiment at each site was a 4 x 3 x 2 factorial, with four forms of nitrogen (N), three rates of application, and two dates of application layed out in three complete randomised blocks (each block was confined to a row of plantings). The layout of the boronia plantations consisted of double rows with diagonal spacing having 0.5 m between each plant in the row and 0.5 m between rows which were spaced at 2 m centres. At Site 1 each block consisted of 25 plots of 20 plants separated by four guard plants at the end of each plot. The plant spacings and block design at Site 1 were the same as Site 2, however, only 5 plants were used per plot and plots were separated by two guard plants. The forms of N fertilizer used were isobutylidene diurea (IBDU), urea, NH_4NO_3 and $Ca(NO_3)_2$. Application rates were 25, 50 and 100 kg/ha with a single control plot of 0 kg N/ha for each block. Nitrogen was applied as a single application in late October or in two equal applications, one in October and the other in early January.

Prior to beginning the experiment all plants were pruned to a height of approximately 29 cm. New vegetative growth occurs from one year old wood and flower buds initiate from this new growth in mid to late autumn. Ten plants of Clone 250 in each plot were chosen for monitoring growth throughout the year. On each plant, one lateral of current season's growth (below the pruning level) was marked with paint at the base of the lateral. Measurements taken were plant height, length of the lateral and number of nodes on the lateral. These laterals were removed at harvest and the number of mature flowers and flower buds were counted and expressed as percentage of leaf axils with a mature flower or flower bud. After flower harvest each plant was pruned to approximately 33 cm and the fresh weight of vegetative material was measured and a representative subsample was taken to estimate the dry weight.

The plant spacing used gave a density of 19, 500 plants/ha ; hence the amount of N required for a single plant was calculated. This was applied to each plant by hand spreading in a 10 cm radius around the base of the plant. To minimise any loss of N from urea by volatilization each site was irrigated with 10 mm of water by overhead spray immediately after fertilization.

A base level of additional nutrients was applied as a mixture to both sites at the following rate: 100 kg/ha potassium as (KH_2PO_4), 79 kg/ha phosphorus as (KH_2PO_4), 37 kg/ha sulfur as ($CaSO_4$), 5 kg/ha zinc as ($ZnSO_4.7H_2O$), 3.2 kg/ha copper as ($CuSO_4.5H_2O$), 0.5 kg/ha boron as (H_3BO_3) and 0.05 kg/ha molybdenum as ($Na_2MoO_4.2H_2O$).

Tissue Analysis

The 10 plants of Clone 250 in each plot which were not used for monitoring growth were used to provide leaf material for tissue analysis. Five to 10 laterals of current season's growth were collected from each plant in October (prior to pruning and fertilising), in late March (at the end of the growth period just prior to flower bud initiation) and in mid September (just after harvest). Flowers were also sampled from each plot for N determination. Plant material within each plot was dried at 70 °C for 48 hr. Stem material was removed and the leaves or flowers were ground in a hammer mill, and stored at -18 °C until analysis commenced.

Total N was determined by a modified semimicro Kjeldahl method (Reddy and Menary 1989a), analysis was carried out in duplicate.

Flower Harvest

Flowers were harvested from Clone 250 in mid September and Clone 5 at the end of September. Flowers were hand combed from each plot, weighed then frozen in a blast freezer. A representative sample was taken to estimate the dry weight and the percentage of vegetative material harvested with the flowers. Plants of Clone 250 which were used for tissue analysis were not used to estimate flower yield since vegetative material had been removed during the year.

Oil Analysis

Flowers of Clone 250 were extracted in a mixture of hexane and petroleum ether. Care was taken to accurately weigh the flowers prior to extraction; the extraction time, temperature and number of washes was consistent for all samples. Solvent was removed using a rotary vacuum evaporator and the concrete was weighed to calculate oil yield. C_{18} standard was added to the concrete as well as hexane containing 10 % dichloromethane, a sample of this was transferred to a Gas Chromatograph (GC) vial. The GC configuration and the method used for the analysis are listed in Table 1. The percentage of volatiles in the concrete were calculated from the weight of concrete and the combined peak areas relative to the C_{18} standard; volatiles were considered to be those compounds that eluted prior to the C_{18} standard. Changes within the volatile fraction were monitored by expressing β-ionone as a percentage of the total volatiles as well as a percentage of the concrete. β-ionone is one of

the major volatile components of boronia oil (Legget and Menary, 1980). All samples were extracted and analysed in duplicate.

Statistical Analysis
Results were analysed by Analysis of Variance (ANOVA) using Statistical Applications for Students (SAS), Least significant Differences (LSD) were calculated at the 5% probability level using the Student's 't' test. Regression curves were calculated using the least squares method.

2.4 Results

The years averaged growth data for Clone 250 can be seen in Table 2. Node production increased steadily reaching a peak between January and February; comparatively little vegetative growth occurred between April to September. Cessation of vegetative growth correlated with the end of summer and the beginning of flower initiation.

There was a strong positive correlation (adjusted R^2>0.5) between the rate of N applied and the % N in the leaf and flower and the flower and oil yields per plant (Table 3). The flower yield per plant was increased by the production of more sites for flowering with no change on the % leaf axils with flowers (Table 3). However, increased oil yield per plant was not simply due to a greater flower yield per plant since the percent of oil extracted per gram of flowers was also positively correlated with the rate of N applied, as was the percent volatiles of the concrete. The ratio of volatile components with respect to each other did not appear to change since β-ionone as a percentage of the volatiles was unchanged. High N also significantly increased the percentage of leaf axils with immature flower buds as well as the amount of leaf material in the harvest. This may have been due to reduced sclerophylly and/or cuticle and wax production which was noted by the softer and more flexible foliage in plants receiving high N.

Increasing the N rate in Clone 5 also resulted in a significant increase in flower yield per plant, in comparison there was no significant effect on the amount of leaf material found in the flowers, which was on average four times higher than Clone 250.

There was no significant difference between NH_4NO_3 , $Ca(NO_3)_2$ and urea on the vegetative growth, flower and oil yield per plant on Clone 250, however, urea tended to have the lower values (Table 4). In comparison with the rapidly available forms of N, IBDU resulted in significantly lower percent N in the leaf and flower, as well as producing fewer potential flowering sites, consequently there was a lower flower yield and oil yield per plant. IBDU treated plants also had a significantly lower percent volatile and β-ionone content of the concrete. The effect of N form on Clone 5 was different to Clone 250 (Table 4), whereby both IBDU and urea both had significantly lower flower yields per plant than either NH_4NO_3 or $Ca(NO_3)_2$. This was probably due to toxicity of IBDU and urea when applied at higher rates, seen as yellowing and reddening of new growth, which resulted in a significant interaction between rate and form on flower yield per plant (Table 5).

In Clone 250 significant interactions occurred between rate and form affecting the percent N in the leaf and flower as well as the percentage of volatiles in the concrete for Clone 250, the regression equations for each form is shown in Table 5. The greatest responses to increased nitrogen occurred (in descending order) with NH_4NO_3, $Ca(NO_3)_2$, urea and IBDU, this may be seen by the slope and adjusted R^2 for each regression equation.

The time of application did not affect growth or the N content of leaves or flowers. A non-split application resulted in a marginally significant greater flower yield per plant in Clone 250. However there was no effect on the oil yield per plant. Application date had no significant effect on Clone 5 (Table 6).

The strong correlation between rate of N application and flower yield, oil yield and percent N in the leaf and flower indicate that yield may be influenced by leaf N. This is demonstrated in Figures 1 and 2 where the flower yield per plant and oil yield per plant respectively are plotted against percent N in the leaf in both April and September. Calculating quadratic v's linear regression resulted in only a small increase in the correlation coefficient, nevertheless the curves show that there is little yield to be gained from leaf N contents greater than approximately 1.7% in April and 1.5% in September. The small decline in flower yield seen in plants with a leaf N greater than 1.7% in April indicates that further increases N may result in yield losses.

The percent N in the leaf was closely correlated with the percent N in the flower which was in-turn positively correlated with the percentage of volatiles and percentage of β-ionone of the concrete (Table 7). In comparison with flower yield per plant the percentage of volatiles and percentage of β-ionone of the concrete were still rising at the highest percent N in the flower (not shown).

2.5 Discussion

The application of 100 kg/ha of N to Clone 250 resulted in strong vegetative growth over the summer (seen as the number of nodes per lateral). The high number of nodes produced eventually translated to a proportionally higher number of flowers, and therefore oil yield per plant. High rates of N application, even as a split application, did not result in competition between vegetative growth and flowering. Promotion of vegetative growth by application of N has been reported in *Boronia serrulata* by Lamont (1985) and *B. megastigma* by Reddy and Menary (1989b) and Thomas (1981). Reddy and Menary (1989b) also reported that high levels of N resulted in continuous vegetative growth and a decrease in the percent of leaf axils with mature flowers as well as smaller flowers at anthesis. These earlier studies were carried out under glasshouse conditions with elevated winter temperatures which allow continued vegetative vigour. Under these conditions flower bud initiation will still occur, however, they will generally abort or revert to vegetative differentiation before reaching anthesis (Richards, 1985; Roberts and Menary, 1989a & b, 1990).

Despite the similar experimental parameters between the current trial and that reported by Reddy and Menary (1989a), they found that the highest flower yield was achieved with IBDU at 50 or 100 kg/ha. However, their experiment was carried out on a different clone of much smaller plants with slower growth rates (Menary pers obs) and this was reflected by the lower flower yield, 10-20% of the current yield at comparative rates of N application. The small plants were probably not able to utilise such high levels of leaf N which reached 3 % with the readily available forms in comparison with 1.7% in the present experiment. Hence the gradual release of N from IBDU allowed the smaller plants to absorb and utilise the higher amount of available nitrogen.

In the current experiment IBDU did not provide as much N as the other forms to Clone 250, this was due to lower mineralisation where granuals were still seen on the ground at harvest. Consequently these plants were smaller and had lower leaf N contents; since the percent leaf axils with mature flowers was unchanged there was a correspondingly lower flower and oil yield per plant. IBDU also resulted in reduced N content in the flowers and percent volatile and β-ionone of the concrete. Of the readily available forms of N, urea resulted in the lowest vegetative mass, flower and oil yield as well as percent volatiles; this may have been due to ammonium toxicity (discussed below).

Nitrogen application rate had the same effect on flower yield in Clone 5 as for Clone 250, however, toxicity effects were seen with high levels of urea and IBDU (both of which release NH_4^+). Due to the relatively low pH (≤4.5) of both sites it is assumed that little if any nitrification took place, levels of nitrate in the plant were not measured however. Therefore in the field it appears that either NO_3^- or equal amounts of NH_4^+ and NO_3^- (previously untested) provide the best source of N for maximum yield in both clones. This would suggest that in boronia the low nitrate reductase activity reported by Reddy and Menary (1990) is not a limiting factor in the field; instead it may indicate that

detoxification of NH_4^+ is not occurring sufficiently quickly under field conditions. Reddy and Menary (1989b) found that boronia grown in the glasshouse in composted eucalyptus bark:sand (2:1 v/v) supplied with 25 mM NH_4^+ gave superior growth in comparison to NO_3^- and the most favourable response was to a combination of NH_4^+ and NO_3^-. The lack of toxicity to NH_4^+ could have been due to the adsorption of NH_4^+ by compost, the ability of plants to detoxify ammonium when grown in peat and sand but not in sand alone was reported by Magalhaes and Wilcox (1984). Ammonium toxicity has also been related to insufficient carbohydrates available for ammonium assimilation (Givan, 1979). In nutrient deficient environments, carbon is produced in excess, since any further growth would require additional nutrients (Schulze, 1982). Whilst this may occur in the wild, the plants used here were supplied with adequate amounts of nutrients which stimulated extra growth thus reducing available carbohydrates for ammonium assimilation. Hence the toxicity of urea and IBDU to Clone 5 compared with Clone 250 may have been due to soil type or the clones different abilities to provide sufficient carbohydrates and requires further investigation.

Increasing the rate of N application resulted in more oil per gram of flowers and a higher volatile (lower wax) component of the oil , probably as a result of reduced sclerophylly and/or wax production. The volatile components of the oil did not appear to change with respect to each other. A higher volatile content of the oil was also seen for *B. megastigma* plants grown under 30% full sunlight (Brussel and Considine pers comm). These conditions result in plants having a much softer leaf texture in a similar fashion to those plants supplied with high levels of N (Roberts pers obs). Nitrogen applications also increased the oil content of *Foeniculum vulgare* (Bhati et al., 1989) and *Mentha piperita* (Bhardwaj and Kaushal, 1989). In terms of oil content, higher rates of N have been reported to increase the free menthol content of *Mentha piperita* (Bhardwaj and Kaushal, 1989) and the menthone content of *Mentha arvensis* L. (Singh, K. et al., 1989), whilst the form of N affected the sesquiterpene percentage of *Ocimum basilicum* L. (Alder et al., 1989).

Maximum potential yield was not achieved in this experiment as seen from the continued increase in yield with higher levels of N application. However, in Clone 250 the flower and oil yields per plant did appear to be reaching a maximum when the leaf N level was approximately 1.7% in April and 1.5% in September. In comparison the volatile and β-ionone content of the concrete was still increasing with the highest N levels in the flower. Since the N levels in the leaf and flower are so closely correlated then there must be some compromise between achieving maximum oil yield per plant and maximum percentage volatiles. Therefore, the ideal N fertilisation would maximise oil yield and volatile content whilst still allowing for mechanical harvesting and minium immature bud and leaf material in the product, although the latter can be relatively simply screened out. In boronia, however, as seen above this is complicated both by plant age, clonal variety, growing conditions and soil type. Small plants are quite susceptible to N toxicity and should be treated accordingly. Mature plants produced their highest yields at 100 kg N/ha and whilst the yield was still rising at this level of application there were a number of problems. These included difficulty of mechanical harvesting due to vigorous spring growth, particularly in Clone 250 (Roberts pers obs). Susceptibility to wind damage was also noticed in Clone 250 with the larger canopies (Roberts pers obs). Hence, 50 kg N/ha would be a more realistic application rate given the current harvester design and the openness of some plantations to strong winds. The form of N to apply to mature plants appears to depend upon the Clone, however, both NH_4NO_3 and $Ca(NO_3)_2$ appear to be quite suitable with the added advantage that they are considerably cheaper than IBDU. The time of application appeared to make little difference to the oil yield, however, a split application (immediately after pruning and again in early January) would provide ample N for the production of flowering sites whilst giving a safe guard against N loss due to leaching if heavy rains followed application.

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Table 1. Gas Chromatograph Configuration and Operating Conditions for Boronia Concrete Analysis.

Gas Chromatograph	Hewlett-Packard 5890-II
Injector Temperature	250°C
Split Vent Flow	50.0 ml/min
Column Head Pressure	8 psi
Column	SGE 15 m BP1
Internal Diameter	0.2 mm
Film Thickness	0.33 µm non polar methyl silicone
Carrier Gas	N_2
Column Flow	1.0 ml/min
Detector	Flame Ionization Detector (FID)
Detector Temperature	280°C
Detector H_2 Flow	30 ml/min
Detector Air Flow	300 ml/min
Auxillary Gas Flow (N_2)	30 ml min^{-1} (1 ml/min from column, 29 ml/min from detector)
Oven Start Temperature	50°C
Equilibration Time	3 min
Initial Time	1 min
Initial Rate	10.0°C/min
Final Temperature	250°C
Final Time	14 min

Table 2. **Average Growth Data for Clone 250 Including Change in Plant Height/Day, Increase in Lateral Length/Day and Increase in Number of Nodes/Day Over Each Growth Period.**

Sample Date	Days From Start	Height (mm)	Δ Height (mm/day) Since Last Period	Lateral Length (mm)	Δ Lateral Length (mm/day) Since Last Period	Number of New Nodes per Lateral Initiated After Day 0	Change in Node Number per Day Since Last Period
28-10-92	0	293	0	51	0	0	0
30-11-92	33	351	1.76	95	1.33	3.6	0.11
05-01-93	69	468	3.25	190	2.64	18.2	0.41
22-02-93	116	594	2.68	288	2.09	46.4	0.60
21-04-93	174	708	1.97	335	0.81	73.6	0.47
28-07-93	272	748	0.41	352	0.17	78.6	0.05
14-09-93	320	764	0.33	na	na	80.7	0.04

Table 3. The Effect of Nitrogen Rate on the Growth, % N in the Leaf and Flower and the Flower and Oil Yields Per Plant.

Parameter Measured	Nitrogen Rate (kg/ha)				Slope ($x10^{-3}$) of Parameter v's Nitrogen Rate	Y Intercept	Adjusted R^2	Probability>F
	25	50	100	LSD				
(Clone 250)								
Height (mm)	746.9	775.0	783.0	25.6	63.00	728.3	0.1486	0.0004
Lateral Length (mm)	330.5	353.2	389.9	41.627	990.08	296.7	0.1605	0.0002
Number of Nodes/Lateral	61.9	74.7	113.1	19.9	741.97	39.2	0.3197	0.0001
Dry Leaf Weight/Plant (g)	69.9	92.1	114.6	12.8	639.67	53.8	0.3365	0.0001
% N in Leaf (Oct '92) (w/w)	1.63	1.57	1.65	ns	6.41	1.58	0.0107	0.1842
% N in Leaf (Apr '93) (w/w)	1.24	1.37	1.61	.06	4.87	1.12	0.5691	0.0001
% N in Leaf (Sep '93) (w/w)	0.96	1.08	1.39	.06	5.57	0.82	0.6685	0.0001
Number of Flowers/Lateral	36.1	41.2	59.2	13.5	343.10	25.0	0.2092	0.0001
% Leaf Axils with a Flower	29.6	27.2	27.6	ns	-34.62	30.3	0.0124	0.1694
Dry Flower Weight/Plant (g)	47.1	57.7	73.0	4.7	374.08	36.9	0.5505	0.0001
% Leaf Material in Harvest (w/w)	2.9	3.2	4.4	0.6	18.77	2.4	0.1817	0.0001
% N in Flower (w/w)	0.90	0.96	1.16	0.04	3.56	0.80	0.6647	0.0001
Number of Flower Buds/Lateral	1.2	2.0	5.7	2.1	59.73	-0.5	0.1747	0.0001
% Leaf Axils with a Flower Bud	0.9	1.1	2.7	1.0	25.30	0.1	0.1352	0.0007
% Oil Yield (w/w)	1.47	1.46	1.55	0.05	1.16	1.43	0.0404	0.0461
Oil Yield/Plant (g)	0.69	0.84	1.13	0.08	6.28	0.51	0.5412	0.0001
% Volatiles (w/w)	7.90	8.73	10.56	0.58	35.16	7.02	0.4594	0.0001
% β-ionone of Volatiles (w/w)	34.53	34.74	34.95	ns	5.24	34.37	-0.0038	0.4000
% β-ionone of Concrete (w/w)	2.73	3.06	3.67	0.23	12.61	2.42	0.4033	0.0001
(Clone 5)								
Dry Flower Weight/Plant (g)	21.5	23.6	29.1	4.7	100.23	18.9	0.0889	0.0054
% Leaf Material in Harvest (w/w)	14.5	13.1	15.3	2.3	13.93	13.5	-0.0020	0.3581

Table 4. The Effect of Nitrogen Form on the Growth, % N in the Leaf and Flower and the Flower and Oil Yields Per Plant.

Parameter Measured	Nitrogen Form				
	IBDU	**UREA**	**CALCIUM NITRATE**	**AMMONIUM NITRATE**	**LSD**
(Clone 250)					
Height (mm)	743.4	776.9	773.8	778.9	ns
Lateral Length (mm)	339.9	355.5	356.3	379.8	ns
Number of Nodes/Lateral	59.5	90.6	88.9	93.9	23.0
Dry Leaf Weight/Plant (g)	63.8	95.0	105.0	105.0	14.8
% N in Leaf (Oct '92) (w/w)	1.65	1.64	1.57	1.61	ns
% N in Leaf (Apr '93) (w/w)	1.27	1.39	1.50	1.46	0.07
% N in Leaf (Sep '93) (w/w)	1.07	1.13	1.18	1.20	0.07
Number of Flowers/Lateral	37.7	48.2	44.7	51.3	ns
% Leaf Axils with a Flower	31.3	26.6	26.5	28.1	ns
Dry Flower Weight/Plant (g)	48.4	61.3	62.6	64.8	5.5
% Leaf Material in Harvest (w/w)	3.0	3.5	3.8	3.7	ns
% N in Flower (w/w)	0.94	1.00	1.03	1.05	0.04
Number of Flower Buds/Lateral	1.0	3.6	3.1	4.2	2.4
% Leaf Axils with a Flower Bud	0.8	2.1	1.6	1.9	ns
% Oil Yield (w/w)	1.46	1.50	1.52	1.50	ns
Oil Yield/Plant (g)	0.71	0.92	0.95	0.98	0.09
% Volatiles (w/w)	8.26	8.89	9.23	9.87	0.67
% β-ionone of Volatiles (w/w)	34.48	34.87	35.06	34.87	ns
% β-ionone of Concrete (w/w)	2.86	3.10	3.24	3.42	0.26
(Clone 5)					
Dry Flower Weight/Plant (g)	19.9	18.9	30.3	29.9	5.4
% Leaf Material in Harvest (w/w)	13.6	15.7	13.8	14.0	ns

Table 5. Regression Equations Describing the Interaction Between Nitrogen Rate and Form on the % Nitrogen in the Leaf and Flower and % Volatiles of the Concrete in Clone 250 and Flower Yield Per Plant in Clone 5.

Parameter Measured	Nitrogen Form	Slope ($x10^{-3}$) of Parameter v's Nitrogen Rate	Y Intercept	Adjusted R^2	Probability>F
% N in Leaf (Apr) (Clone 250)	NH_4NO_3	6.10	1.11	0.8255	0.0001
	$Ca(NO_3)_2$	6.01	1.15	0.8276	0.0001
	UREA	4.41	1.14	0.6895	0.0001
	IBDU	2.39	1.13	0.5135	0.0002
% N in Leaf (Sep) (Clone 250)	NH_4NO_3	6.94	0.82	0.8127	0.0001
	$Ca(NO_3)_2$	6.31	0.83	0.7954	0.0001
	UREA	4.47	0.88	0.7344	0.0001
	IBDU	2.43	0.93	0.5530	0.0001
% N in Flower (Clone 250)	NH_4NO_3	4.26	0.81	0.8691	0.0001
	$Ca(NO_3)_2$	4.28	0.79	0.8254	0.0001
	UREA	3.58	0.80	0.8291	0.0001
	IBDU	1.75	0.84	0.5114	0.0002
% Volatiles (Clone 250)	NH_4NO_3	51.76	0.05	0.7991	0.0001
	$Ca(NO_3)_2$	39.31	0.04	0.5921	0.0001
	UREA	29.31	0.03	0.6719	0.0001
	IBDU	17.38	0.02	0.2168	0.0193
Flower Yield per Plant (Clone 5)	NH_4NO_3	212.13	17.8	0.5247	0.0001
	$Ca(NO_3)_2$	160.50	20.7	0.2736	0.0088
	UREA	45.77	16.7	0.0209	0.2470
	IBDU	-26.39	18.3	-0.0373	0.6018

Table 6. The Effect of N Application Date on the Growth, % N in the Leaf and Flower and the Flower and Oil Yields Per Plant.

		Application Date	
Parameter Measured	**Spring Only (Non Split)**	**Spring & Summer (Split)**	**LSD**
(Clone 250)			
Height (mm)	76.7	76.9	ns
Lateral Length (mm)	348.9	366.8	ns
Number of Nodes/Lateral	84.4	82.1	ns
Dry Leaf Weight/Plant (g)	93.4	91.0	ns
% N in Leaf (Oct '92) (w/w)	1.63	1.60	ns
% N in Leaf (Apr '93) (w/w)	1.40	1.41	ns
% N in Leaf (Sep '93) (w/w)	1.16	1.12	ns
Number of Flowers/Lateral	44.0	47.0	ns
% Leaf Axils with a Flower	26.8	29.4	ns
Dry Flower Weight/Plant (g)	61.6	57.0	3.9
% Leaf Material in Harvest (w/w)	3.6	3.4	ns
% N in Flower (w/w)	1.02	1.00	ns
Number of Flower Buds/Lateral	3.5	2.4	ns
% Leaf Axils with a Flower Bud	1.8	1.3	ns
% Oil Yield (w/w)	1.49	1.50	ns
Oil Yield/Plant (g)	0.92	0.86	ns
% Volatiles (w/w)	9.25	8.87	ns
% β-ionone of Volatiles (w/w)	34.60	34.88	ns
% β-ionone of Concrete (w/w)	3.21	3.10	ns
(Clone 5)			
Dry Flower Weight/Plant (g)	23.8	25.6	ns
% Leaf Material in Harvest (w/w)	14.7	13.9	ns

Table 7. Regression Equations Describing the Correlations Between % N in the Flower and % N in the Leaf (September), % Volatiles in the Concrete and % N in the Flower, % β-ionone in the Concrete and % N in the Flower.

Parameters Correlated	Regression Equation	Adjusted R^2	Probability>F
% N flower with % N leaf (September)	y = 0.0630017x + 0.28568	0.952439	0.0001
% Volatiles in Concrete with % N flower	y = 9.62463x - 0.64216	0.851291	0.0001
% β-ionone in Concrete with % N flower	y = 3.30578x - 0.47438	0.72125	0.0001

3. Effect of Nitrogen Fertilisation on Growth, Flower Yield Oil Composition and Yield of Two Clones of *Boronia megastima Nees*

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3.1 Introduction

Roberts and Menary (1994) have tested four sources and three rates of nitrogen on two boronia cultivars, clones 250 and 5. In these trials, ammonium nitrate and calcium nitrate gave the highest yields. Previous work by Reddy and Menary (1989) have shown the importance of balanced NH_4+ and $N0_3$- nutrition for optimum growth and flower production. A trial was proposed which combined the benefits of balanced ammonium and nitrate as a source of nitrogen with two clones under commercial conditions where mechanical harvesting was practised.

3.2 Materials and Methods

The experiment was conducted on four year old plants grown on a gravely sand ph 4 .5. The experiment was a 3 x 3 randomised block design with 600 plants per plot with inter-row buffer and buffers between plots. The plant spacing was 0.5 metres between plants in the row and 0.5 metres between rows which were spaced at 2 metre centres. Two clones were used namely 250 and 5 and rates of application of nitrogen as NH_4 $N0_3$ were 40, 60 and 80 kg/ha for clone 250 and 15, 30 and 50 kg/ha for clone 5. The range of nitrogen levels used reflected the responses obtained from Roberts and Menary (1994). Basal nutrients were applied according to Roberts and Menary (1994). Fertiliser treatments were applied in late November.

Tissue analysis
50 laterals were selected at random from 600 plants in each plot in November, February and May for total nitrogen determinations. Plant material was dried at 70°C for 48 hours ground in a hammermill and stored at -18°C. Total nitrogen was determined by semi micro kjeldahl method (Reddy and Menary, 1989). The analysis was carried out in duplicate.

Plant Growth
Plant height was measured at the commencement of the trial in November and again before harvest. The difference was defined as extension growth. 50 primary extension growths were sampled at random. The number of laterals, their length and the number of nodes were recorded.

Flower Harvest
Flowers were harvested from clone 250 at the beginning of September and clone 5 mid September. Flowers were then hand combed from clone 250 after mechanical harvesting to obtain an estimate of harvest efficiency. A representative sample of mechanically harvested flowers from each plot were stored at -20° for oil yield and oil composition.

Oil Analysis
Flowers of clone 250 and clone 5 were extracted in a mixture of hexane and petroleum ether. Care was taken to accurately weigh the flowers prior to extraction; the extraction time, temperature, and number of washes was consistent for all samples. The solvent was removed using a rotary vacuum evaporator and the concrete was weighed to calculate oil yield. A C18 standard was added to the concrete as well

as hexane containing 10% dichioromethane, and a sample of this was transferred to a Gas Chromatograph (GC) vial. The GC configuration and the method used for the analysis are listed in Table A. The percentage of volatiles in the concrete were calculated from the weight of concrete and the combined peak areas relative to the C18 standard. Volatiles were considered to be those compounds that were eluted prior to the C18 standard. Changes within the volatile fraction were monitored by expressing ß-ionone as a percentage of the total volatiles as well as a percentage of the concrete. ß-ionone is one of the major volatile components of boronia oil (Leggett and Menary, 1980). All samples were extracted and analysed in duplicate.

Table A. Gas Ghromatograph Configuration and Operating Conditions for Boronia Concrete Analysis.

Gas Chromatograph	Hewlett-Packard 5890-II
Injector Temperature	250°C
Split Vent Flow	50.0 ml/min
Column Head Pressure	8 psi
Column	SGE 15 m BP1
Internal Diameter	0.2 mm
Film Thickness	0.33 µm non polar methyl silicone
Carrier Gas	N_2
Column Flow	1.0 ml/min
Detector	Flame Ionization Detector (FID)
Detector Temperature	280°C
Detector N_2 Flow	30 ml/min
Detector Air Flow	300 ml/min
Auxillary Gas Flow (N_2)	30 ml min^{-1} (1 ml/min from column, 29 ml/min from detector)
Oven Start Temperature	50°C
Equilibration Time	3 min
Initial Time	1 min
Initial Rate	10.0°C/min
Final Temperature	250°C
Final Time	14 min

Statistical Analysis
Results were analysed by Analysis of Variance (ANOVA) using Statistical Applications for Students (SAS).

3.3 Results

The average extension growth, lateral growth, number of laterals and node numbers in relation to nitrogen treatment for clone 250 are given Table 1. Mean extension growth was significantly higher at 80 kg nitrogen per hectare than at 40 and 60 respectively. Whilst there was no significant effect of nitrogen on lateral length, number of laterals and the number of nodes per lateral were significantly increased with increasing levels of nitrogen.

This effect is shown in Plate 1. In this case there is an increased extension growth at 80 kg compared with 40 kg/ha and the absence of flowers on tip growth. It is also obvious that flowers are more advanced on the northern edge of rows (right hand side) which had an East West orientation. At 40 kg nitrogen per hectare there is less extension growth, fewer laterals and more even flowering.

On clone 5, nitrogen increased extension growth with increasing nitrogen levels applied. Table 2. There was no significant effect on lateral length and number of nodes but a significant increase in the number of laterals. The increase in extension growth and number of laterals can be seen in Plate 2.

Total yield and percentage harvestable flowers per plant for clone 250 is given in Table 3. Increasing nitrogen level from 40 to 60 kg per hectare significantly increased flower yield, but increasing nitrogen to 80 kg per hectare showed no significant response. The effect of the 80 kg nitrogen per hectare was to decrease harvestability of flowers from 49% at 40 kg per hectare to 27% at 80 kg per hectare.

Total yield of harvestable flowers in clone 5 are given Table 2. Nitrogen has had no significant effect of flower yield.

The yield of concrete, % volatiles and % ß-ionone in volatiles in clone 250 is given in Table 4. At 80 kg nitrogen there was a significant increase in yield of concrete over 40 kg and 60 kg nitrogen per hectare. % volatiles were significantly higher at 80 kg per hectare than at 40 and 60 kg per hectare.

For clone 5 there was a significant increase in yield oil from 15 to 30 kg per hectare and a significant decrease at 50 kg nitrogen per hectare. Table 5. Nitrogen had no significant effect on % volatiles and % ß-ionone in volatiles.

Total nitrogen levels in plant tissues were monitored three times, namely November, February and May. The results for clone 250 and clone 5 are given in Figures 1 and 2 respectively. In the case of clone 250 the levels of nitrogen increased from approximately 1% to a maximum of 1.75% in May. Samples which were taken after the application of fertiliser, mainly February and May, showed a direct relationship between nitrogen in plant tissue and nitrogen applied. In the case of clone 5, Figure 2, the level of nitrogen in November was approximately 1% as for clone 250. However, in the case of clone 5 the level was highest in February rising to approximately 1.6%, there being no significant difference between treatments. However in the May sampling there was a significant increase in nitrogen levels as the rate of nitrogen increased. The fact that the nitrogen levels were similar in February irrespective of the rate of application is probably the explanation for the lack of flower response to nitrogen.

The extension growth which occurred in September prior to harvest did show a significant increase in height between application rates of 15 and 50 kg N per hectare. This reflected the levels of nitrogen which prevailed during the later stages of vegetative growth prior to harvest.

3.4 Discussion

Maximum flower yield was associated with a tissue level of 1.5% nitrogen. This corresponds with levels recorded by Roberts and Menary (1994) for maximum flower yield and oil yield in the same clone. Roberts and Menary (1994) also reported a nitrogen toxicity effect in clone 5 at 100 kg nitrogen per hectare. In the current trial there was no flower yield response to nitrogen and perhaps adequate nitrogen was available during the flower initiation period in early autumn. Samples taken in February showed that a level of 1.5% was recorded.

The plots containing clones 250 and 5 were adjacent to each other in a uniform field as shown in Plates 1 and 2. Although direct statistical comparisons cannot be made between clones it is clear that they have different nitrogen response curves even though the critical value of nitrogen appears to be similar in both cases.

Table 1 . Extension growth, length of laterals, number of laterals and nodes on clone 250 prior to harvest.

Treatment	Extension	Length of	No of	No of

(kg N/ha)	Growth (cm)	Laterals (cm)	Laterals	Nodes
40	31	49	12	18
60	31	48	13	21
80	35	55	16	22
LSD	3.8	8.9	3.6	3.3

Table 2 . Flower yield, extension growth, length of laterals, number of laterals on clone 5 prior to harvest.

Treatment (kg N/ha)	Flower yield (g/plant)	Extension growth (cm)	Length of Laterals (cm)	No of Laterals	No of Nodes
15	87	26	58	10	21
30	98	29	57	14	22
50	90	31	62	19	22
LSD	3.1	3.7	12.0	3.5	2.7

Table 3 . Flower yield and harvestability of clone 250.

Treatment (kg N/ha)	Yield Concrete (g/plant)	Machine harvested (g/plant)	% harvestable
40	164.3	80	49
60	225.6	90	39
80	208.2	56	27
LSD	34.6		21.7

Table 4 . Oil yield of volatiles and % ß-ionone in volatiles, clone 250.

Treatment (kg N/ha)	Yield Concrete (% FW)	% Volatiles	% ß-ionone volatiles
40	0.43	20.8	21.6
60	0.42	19.6	19.8
80	0.54	17.0	23.2
LSD	0.1	3.8	6.9

Table 5 . Oil yield of volatiles and % ß-ionone in volatiles, clone 5.

Treatment (kg N/ha)	Oil Yield (% FW)	% Volatiles	% ß-ionone volatiles
15	0.35	17.6	32.4

30	0.38	21.7	30.0
50	0.36	18.3	34.5
LSD	.014	9.9	14.7

3.5 References

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Plate 1. Clone 250 before harvest with fertilizer applications of 40 and 80 kg N/ha respectively.

Plate 1. Clone 250 before harvest with fertilizer applications of 40 and 80 kg N/ha respectively.

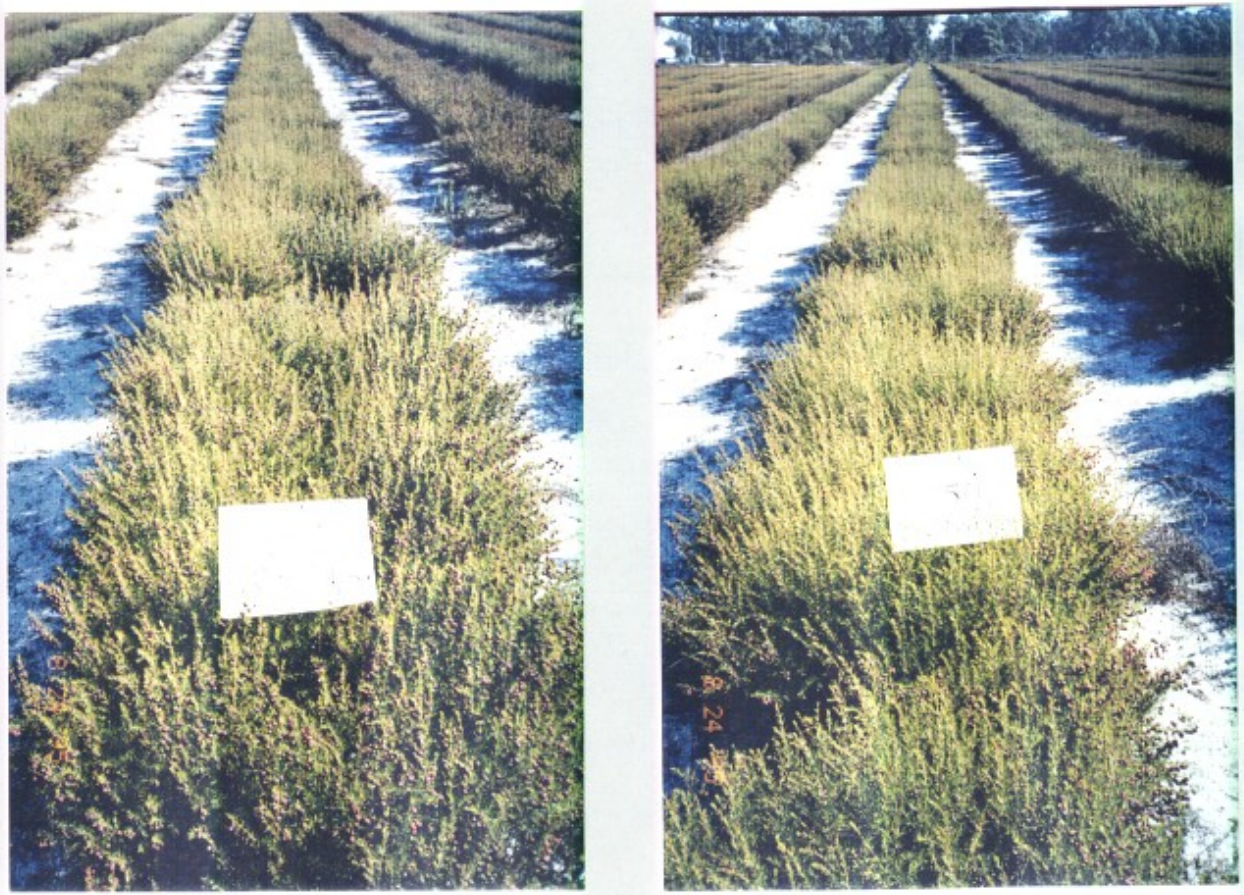

Plate 2. Clone 5 before harvest with fertiliser applications of 15 and 50 kg N/ha respectively.

Plate 2. Clone 5 before harvest with fertiliser applications of 15 and 50 kg N/ha respectively.

Figure 1
Levels of total nitrogen in leaves at 3 dates of harvest, clone 250.

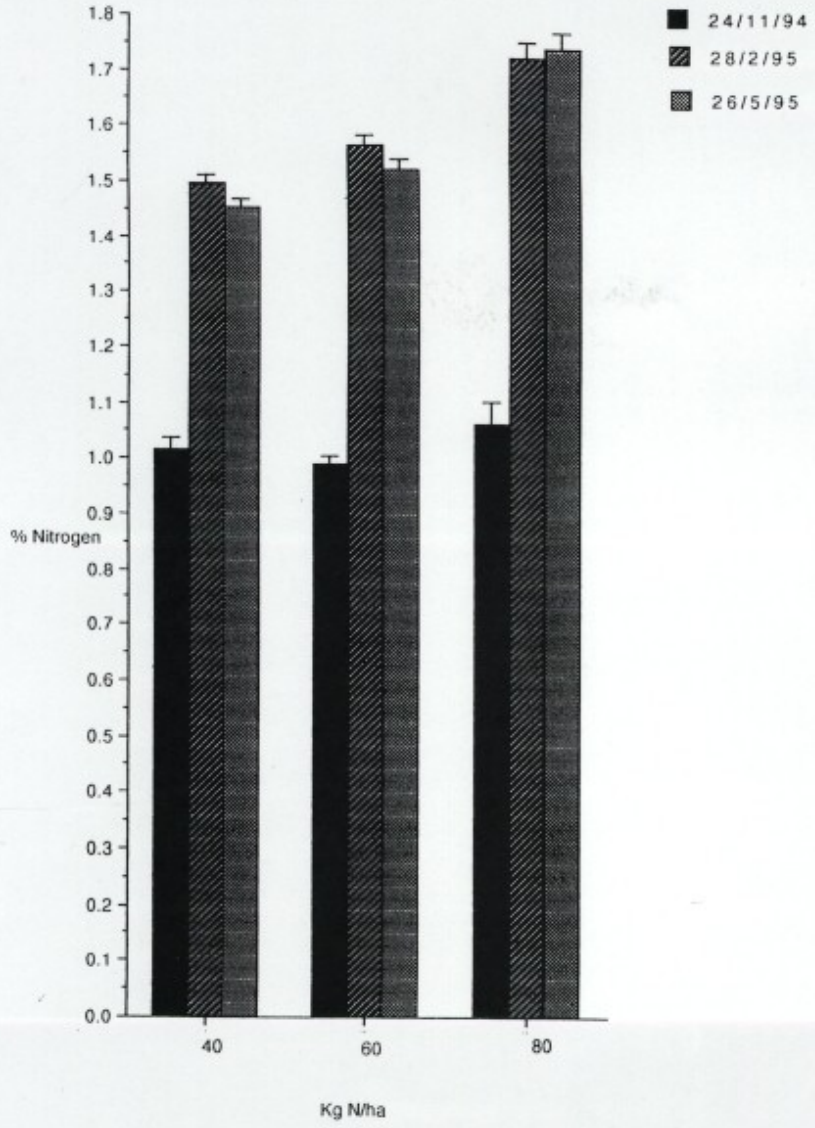

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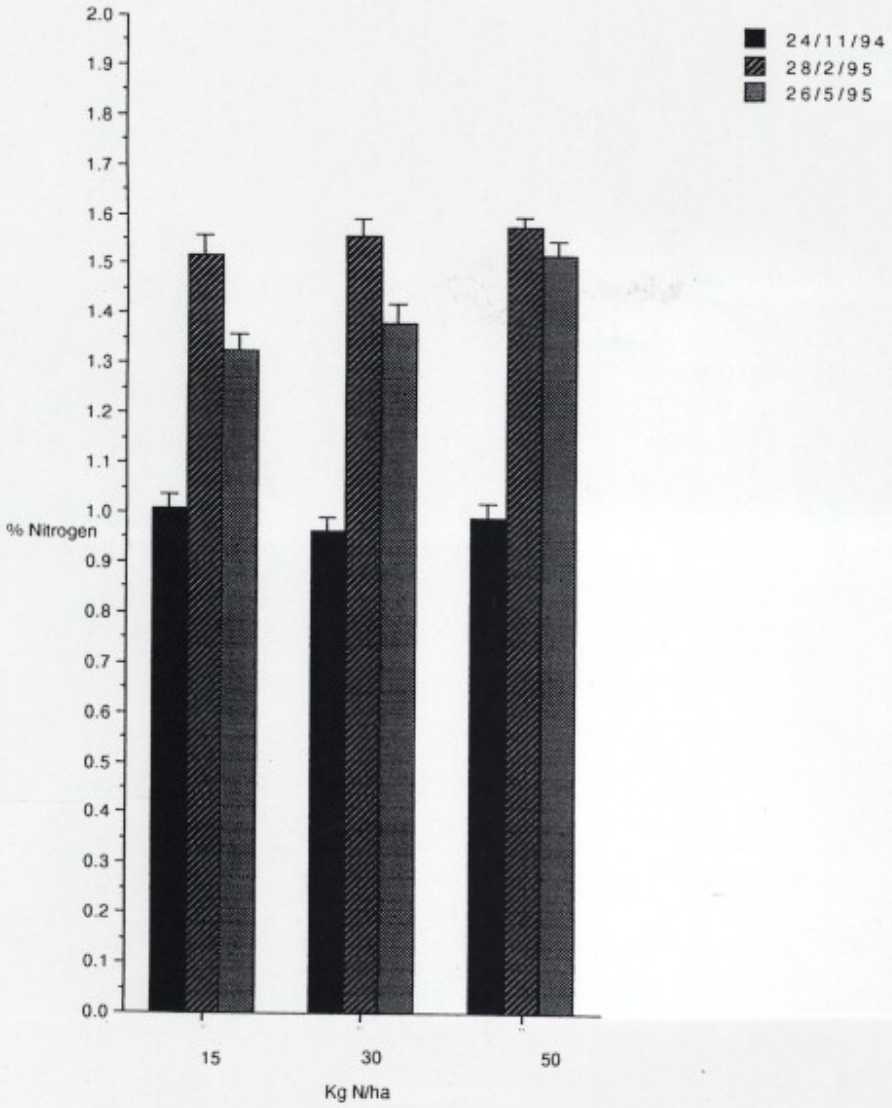
Figure 2
Levels of total nitrogen in leaves at 3 dates of harvest, clone 5.



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4. Water Relations in *Boronia megastigma* Nees

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4.1 Abstract

Measurements of gas exchange and water content were carried out on fully expanded and apical non fully expanded leaves in *Boronia megastigma* Nees. during a drying cycle. The relationships between the following were described: $1/\Psi_L$ & 100-RWC, Ψ_3 & RWC, gg & A, gg & E, gg & c_i, RWC & A, RWC & E, RWC & gg, where Ψ_L , Ψ_3, RWC, A, E, gg, & c_i are leaf water potential, soil water potential, leaf relative water content, assimilation, transpiration, total leaf conductance to diffusion of water vapour and intercellular CO_2 concentration respectively. The rates of A and E in fully expanded leaves had curvilinear relationships with gg, whereas the relationships were linear in the apical leaves. This was thought to be due to the relatively low gg attained by the latter leaves. Leaf conductance to diffusion did not appear to be the limiting factor for A since c_i was relatively constant in both leaf types. Assimilation, E and gg showed threshold responses in relation to the mid afternoon RWC. Assimilation ceased in the apical leaves when they reached visible wilting; at this stage their mid afternoon RWC was 91-92 %. In comparison, the fully expanded leaves were still assimilating and their mid afternoon RWC was approximately 87 %. The turgor loss point of the fully expanded leaves occurred at -1.7 MPa, which corresponded to a RWC of 88 %. Decreasing Ψ_3 from -0.1 to -1.4 MPa resulted in a decrease in pre-dawn RWC of fully expanded leaves from 99 to 93 %. A further decline in Ψ_3 to -2.5 MPa resulted in a drop of RWC from 93 to 60 %. The first signs of visible wilting occurred in the apical leaves when the Ψ_3 was between -0.7 to -1.0 MPa.

KEY WORK INDEX: *Boronia megastigma* Nees., water relations, desiccation.

4.2 Introduction

Boronia megastigma Nees. (Brown boronia) is native to Australia where it is restricted to the far south western corner of Western Australia. It grows as a woody under story shrub one to two metres in height on moist or seasonally wet sandy soils of near neutral pH. Flowers are initiated in autumn and continue to differentiate and develop during the winter bearing a profusion of strongly scented brown, purple and yellow flowers in early spring (1). The flowers are extracted for oil which is highly prized in the flavour and fragrance industry. Since 1990 commercial plantings in Tasmania (Australia) have increased by approximately 400 % with further significant increases planned.

Most of the established commercial plantings are on well drained sandy soils, however, the seasonal and annual rainfall varies considerably between the sites. In general the literature reporting on the investigation of moisture relations in essential oils plants is empirical in nature; there is a complete absence of published literature on water relations in boronia. Brussell and Considine (pers. comm.) suggested that irrigation did not influence oil yield or composition in the field. However, these observations were not supported by physiological measurements during the experiment. This study was designed to provide a physiological basis for measuring the effects of water stress in boronia.

4.3 Experimental

Plant Material- All work was performed on a single clone of boronia (Clone 5). Plants were grown from cuttings for two years in a shade tunnel, utilising the natural daylength and temperatures of Hobart (Tasmania), in 15 cm diameter pots in a mixture of composted bark and sand (50:50 v/v). Nutrient solution (N. Hoaglands) was applied weekly. Drying cycles reported here utilised between 8-16 plants, allowing between 120-130 individual data points per cycle.

Estimation of Leaf Area- Boronia has opposing pairs of trilobed leaves arranged in a decussate fashion on a lateral. Laterals are the current seasons node-bearing structures from which flower buds or vegetative buds are initiated (9). Each plant has many laterals with actively growing apices which includes approximately three pairs of apical non fully expanded leaves located at nodes 0.5-2 mm apart. These leaves are 1-5 mm long and are almost parallel with the stem. Below this there is a rapid expansion in both the leaf size (10-20 mm long) and the angle between the leaf and lateral is increased as is the internode length (10-20 mm).

Given the shape, size and phyllotaxis of boronia leaves it was not possible to completely cover the surface area of any commercial Infra Red Gas Analyser (IRGA) leaf chamber, hence an accurate calculation of leaf area had to be made after any gas exchange measurements. To this end, the fully turgid leaf weight was used to calculate leaf area. A preliminary study showed that boronia leaves reached their fully turgid weight after floating on distilled water for 5-7.5 hours. The relationship between fully turgid weight and leaf area was calculated by weighing individual leaves (after blot drying) and measuring their surface area with a digital image processor. This was carried out on a range of leaf sizes and the following equation was subsequently derived:

$$\text{leaf area } (mm^2) = 3847.21 \times \text{fully turgid leaf weight } (g) + 5.22$$
$$(\text{adjusted } r^2 = 0.9746,\ p<0.0001)$$

Relationship between Soil Water Potential on Leaf Relative Water Content and Estimation of Turgor Loss Point- Tinklin and Weatherley (11) proposed that when transpiration (E) = 0 then $\Psi_L = \Psi_3$, where Ψ_L and Ψ_3 are total water potential of the leaf and soil respectively. In order for this to be valid then equilibrium conditions must have been attained during the night (7). By sealing boronia plants in a plastic bag and allowing them to equilibrate for 8 hours in darkness it was assumed that E = 0. After this time one lateral was removed and the Ψ_3 at the root zone was calculated by measuring Ψ_L in a pressure chamber. Pressure was increased at a rate of 0.01 MPa s^{-1}. Immediately following the measurement of Ψ_L the opposite lateral was removed and leaf Relative Water Content (RWC) was calculated as described by Turner (12).

Leaf water potential and RWC were plotted as a composite pressure volume curve ($1/\Psi_L$ against 100 - RWC) for the estimation of the turgor loss point (TLP). This was calculated from the point at which the curve deviates from the linear portion of the curve. The linear phase describes the osmotic behaviour of the tissue after turgor has been reduced to zero (3).

Changes in Leaf Relative Water Content, Total Leaf Conductance to Diffusion of H_2O, CO_2 Assimilation and Intercellular CO_2 Concentration during a drying cycle- An Analytical Development Company type LCA-3 IRGA with a PLC3-C leaf chamber was used for all gas exchange measurements. The IRGA and leaf chamber were calibrated for CO_2 and humidity respectively at the start of each day. Leaf boundary layer resistance was calculated separately for fully expanded leaves and apical leaves using the method described by Parkinson (6).

Gas exchange measurements were performed at 22°C with a photon flux density of 800 μmol m^{-2} s^{-1}. Maximum net CO_2 fixation occurs at approximately 20°C in boronia whereas maximum "true" photosynthesis (net CO_2 fixation minus both photorespiration and dark respiration) occurs at approximately 25°C (8). Light saturation for boronia occurs between 400-600 μmol m^{-2} s^{-1} (8). The

leaf chamber was supplied with compressed air (351 vpm CO_2) at 110 ml min^{-1}, approximately 50% relative humidity. Readings were made when the leaves had reached steady assimilation (A) and E rates; this took approximately fifteen minutes. The maximum net CO_2 assimilation depleted the total CO_2 concentration by approximately 9%. Total leaf conductance to diffusion of H_2O (gg), intercellular CO_2 concentration (c_i), as well as A and E were calculated using the formulae shown in Table 1 (4). Immediately after gas exchange measurements were taken the leaves were removed from the plant for estimation of RWC and for calculation of leaf area.

Preliminary experiments showed that maximum A and E rates were achieved less than 15 minutes after day break and that there was little variation during the photosynthetic period. On the other hand, RWC dropped over the first four hours of daylight by approximately 4%; our measurements of gas exchange and RWC were taken 10 hours after sunrise (mid afternoon).

Fully expanded leaves were measured for A, E, gg, c_i and RWC on plants during the course of a drying cycle to the visible wilting point (VWP) of the apical leaves since they are the first to show signs of wilting. A similar analysis was performed on the apical leaves. Further measurements were made on fully expanded leaves 24-72 hours after rewatering.

RESULTS AND DISCUSSION: Decreasing Ψ_L from -0.1 to -1.4 MPa corresponded to a drop in pre-dawn RWC of the fully expanded leaves from 99 % to approximately 93 % (Figure 1). The first signs of visible wilting occurred in the apical leaves when the Ψ_3 was between -0.7 to -1.0 MPa. A further decrease in Ψ_3 from -1.4 to -2.5 coincided with a much larger decrease in pre-dawn RWC, from 93 % to 60 %. The TLP of the fully expanded leaves occurred at -1.7 MPa, which corresponded to a RWC of 88 % (Figure 2).

In the fully expanded leaves both A and E had curvilinear responses to gg, where there was little increase in either A or E when gg increased above approximately 300 mmol H_2O $m^{-2}s^{-1}$ (Figure 3). The maximum gg of the apical leaves was approximately half that of the fully expanded leaves (Figure 4). With the reduced maximum gg the relationship to A and E was relatively linear.

Despite the decrease in A and gg in similar proportions during water stress the c_i concentration remained nearly constant in both fully expanded and apical leaves (Figure 5). Hence, there was no direct link between the decline in gg and the inhibition of photosynthesis; this is in agreement with Farquhar and Sharkey (4). In a review on water stress Cowan (2) suggested that the decline in gg may have been the result of inhibition of photosynthesis. However, not all species behave in the same fashion since stomatal closure is sometimes sufficient to restrict the supply of CO_2 thus reducing c_i and decreasing A (2).

Rates of A, E and gg in the apical leaves were relatively variable compared to the fully expanded leaves. This can be attributed to the increased variance in the estimation of leaf area and in the IRGA output which was working at its limit of detection for apical leaves. Also there was a greater variation in leaf maturity within the apical leaves than fully expanded leaves as seen by the differences in leaf colour, i.e. dark green versus light green.

A decrease in the mid afternoon RWC of the fully expanded leaves from a maximum of 94-95 % to 87-88 % corresponded to large decreases in A, E and gg (Figure 6). As noted above, the TLP (-1.7 MPa) also occurred when the RWC was 88%. Reducing the RWC still further to 80 % correlated with relatively smaller reductions in A, E and gg; A continued until the mid afternoon RWC was approximately 78 % (Figure 6). Reducing the mid afternoon RWC of the apical leaves from a maximum of 95-96 % to only 91-92 % (Figure 7) corresponded to a similar sharp decline of A, E and gg seen in the fully expanded leaves. In comparison however, there was on net assimilation of CO_2 when the RWC was below approximately 91 % (zero assimilation points not shown). Under natural

conditions it is more common to observe a progressive rather than a threshold response of gg to declining leaf RWC (10). It would be useful to see if the responses for field boronia in both fully expanded and apical leaves were different to those found above.

The VWP of the apical leaves occurred when their mid afternoon RWC dropped below approximately 91 %. At this stage the fully expanded leaves were not showing signs of wilting and their mid afternoon RWC was approximately 87 %, i.e. at TLP. The ability of the fully expanded leaves to carry out A (albeit at a relatively low rate) below the TLP, well after the VWP of the apical leaves, is probably attributed to the differences in leaf morphology.

When plants were rewatered after a drying cycle to VWP (of apical leaves) full recovery of A, E and gg in the fully expanded leaves had not occurred within 72 hours after rewatering (Figures 3, 5a & 6). No recovery data was taken on the apical leaves.

The results of this work are consistent with the conclusions of both Turner (13) and Lösch (5) and have shown that by measuring Ψ_3 at the root zone, pre-dawn and mid afternoon RWC of the mature leaves it is possible to calculate the effects of water stress on gg, A and E. However, it must be remembered that these measurements were taken under controlled temperature, light and humidity conditions and it is only by studying water stress under natural conditions that the integrated effect can be clarified (10).

ACKNOWLEDGMENTS: We gratefully acknowledge the Rural Industries Research and Development Corporation (RIRDC) for funding for this project and Essential Oils of Tasmania (EOT) for providing logistical support. We thank S. Wilson (University of Tasmania) for his assistance with the pressure chamber and in the preparation of this manuscript. We thank D. Sheriff (CSIRO, South Australia) for his assistance with the calculations involving gas exchange.

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Table 1. Equations for calculating assimilation, transpiration, total conductance to water vapour, and intercellular CO_2 concentration.

$f = (f_V/1000) \times (1/22.4) \times [273.15/(273.15 + T)] \times (p/101.3) \times (1/60)$

$E = (f/s) \times (X_o - X_e) \times [(1 - X_e)/(1 - X_o)]$

$A = (f/s) \times \Delta c[(1 - X_e)/1 - X_o)]$

$gg = E/(X_{sTI} - X_o)$

X_{sTI} = wt (g) of a m^3 of satd aq vap @ T $\quad 1000 \times 22.4/18 \times [273.15/(273.15 + T)] \times (p/101.3)$

$X_o = H_o \times$ wt (g) of a m^3 of satd aq vap @ T $\quad 1000 \times 22.4/18 \times [273.15/(273.15 + T)] \times (p/101.3)$

$X_e = H_i \times$ wt (g) of a m^3 of satd aq vap @ T $\quad 1000 \times 22.4/18 \times [273.15/(273.15 + T)] \times (p/101.3)$

Including the boundary layer resistance, the total gas phase conductance to diffusion of CO_2 is calculated as follows: $1/gg = 1/g_s + 1/g_b$ where g_b is determined independently using a paper replica.
Then: $1/g_s = 1/gg - 1/g_b$ and g_c is calculated as follows: $1/g_c = 1.61/g_s + 1.37/g_b$
$c_i = [(c_e - \Delta c) \times (g_c - E/2) - A]/[g_c + E/2)]$

Symbol	Explanation
A	Assimilation rate (mol m^{-2} s^{-1})
c_e	CO_2 concentration at inlet (mol mol^{-1})
c_i	Internal CO_2 concentration (mol mol^{-1})
Δc	CO_2 difference between inlet and outlet (mol mol^{-1} or vpm)
E	Transpiration rate (mol m^{-2} s^{-1})
f	Mole fraction of air (mol s^{-1})
f_v	Volumetric flow of air (cm^3 min^{-1})
g_b	Boundary layer conductance to diffusion of water vapour (mol m^{-2} s^{-1})
g_c	Total gas phase conductance to diffusion of CO_2 (mol m^{-2} s^{-1})
gg	Total gas phase conductance to diffusion of water vapour (mol m^{-2} s^{-1})
g_s	Stomatal conductance to diffusion of water vapour (mol m^{-2} s^{-1})
H_i	Inlet relative humidity
H_o	Outlet relative humidity
p	Atmospheric pressure during measurement (kPa)
s	Leaf area (m^2)
T	Temperature (°C)
X_e	Water vapour at inlet (mol mol^{-1})
X_o	Water vapour at outlet (mol mol^{-1})
X_{sTI}	Water vapour at saturation (mol mol^{-1})

Figure 1. Pre-dawn relative water content of fully expanded leaves plotted against soil water potential at the roots.

Figure 2. Plot of $1/\Psi_L$ against 100 - RWC giving a composite pressure volume curve for fully expanded leaves.

Figure 3. a. Assimilation rate of CO_2 by fully expanded leaves plotted against total leaf conductance to diffusion of water vapour, λ during desiccation, μ during recovery.

b. Transpiration rate of H_2O by fully expanded leaves plotted against total leaf conductance to diffusion of water vapour, λ during desiccation, μ during recovery.

Figure 4. a. Assimilation rate of CO_2 by apical leaves plotted against total leaf conductance to diffusion of water vapour.

b. Transpiration rate of H_2O by apical leaves plotted against total leaf conductance to diffusion of water vapour.

Figure 5. a. Intercellular CO_2 concentration in fully expanded leaves plotted against total leaf conductance to diffusion of water vapour, λ during desiccation, μ during recovery.

b. Intercellular CO_2 concentration in apical leaves plotted against total leaf conductance to diffusion of water vapour.

Figure 6. a. Assimilation rate of CO_2 by fully expanded leaves plotted against mid afternoon relative water content of fully expanded leaves,λ during desiccation, μ during recovery.

b. Transpiration rate of H_2O by fully expanded leaves plotted against mid afternoon relative water content of fully expanded leaves, λ during desiccation, μ during recovery.

c. Total leaf conductance to diffusion of water vapour of fully expanded leaves plotted against mid afternoon relative water content of fully expanded leaves, λ during desiccation, μ during recovery.

Figure 7. a. Assimilation rate of CO_2 by apical leaves plotted against mid afternoon relative water content of apical leaves.

b. Transpiration rate of H_2O by apical leaves plotted against mid afternoon relative water content of apical leaves.

c. Total leaf conductance to diffusion of water vapour of apical leaves plotted against mid afternoon relative water vapour content of apical leaves.

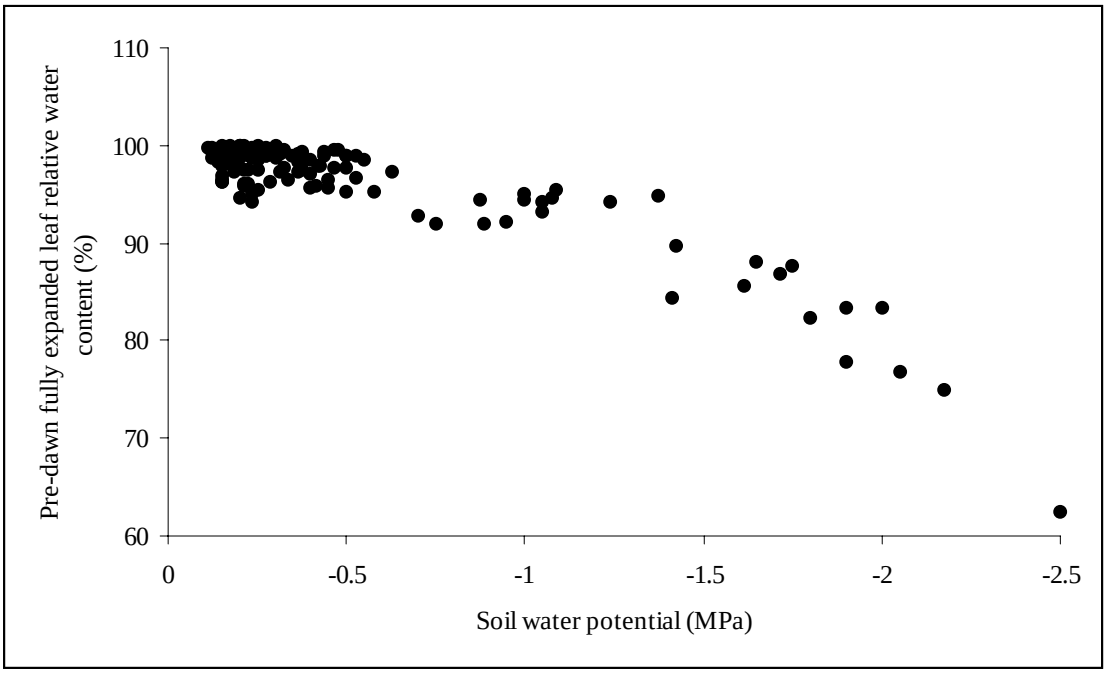

Figure 1. Pre-dawn relative water content of fully expanded leaves plotted against soil water potential at the roots.

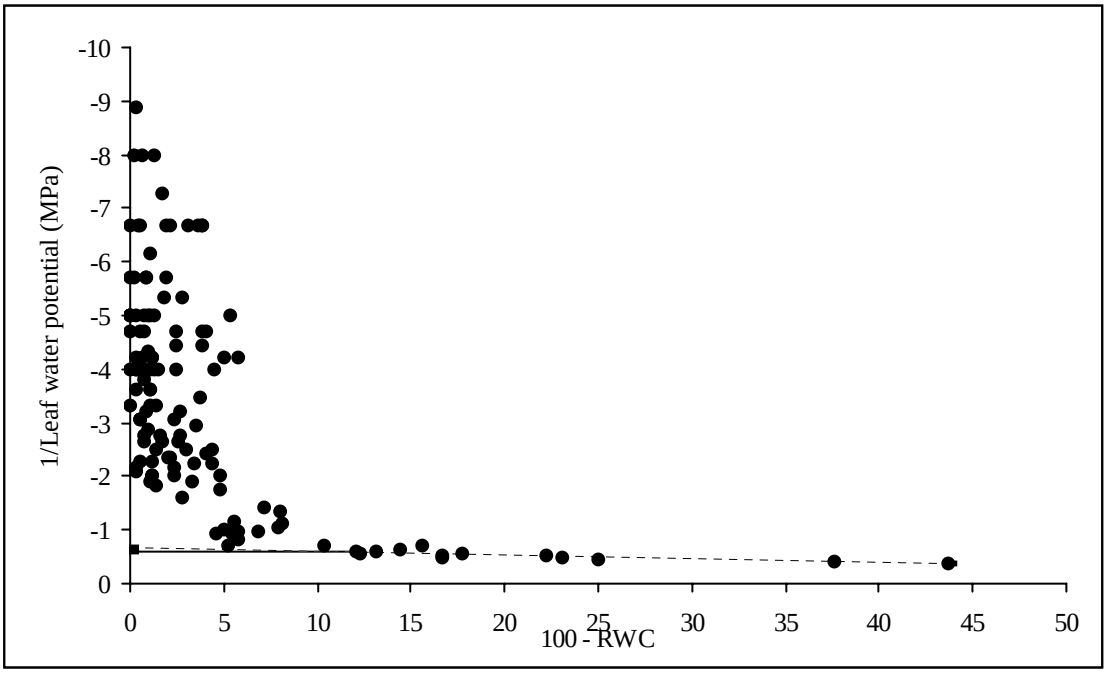

Figure 2. Plot of 1/yL against 100 - RWC giving a composite pressure volume curve for fully expanded leaves.

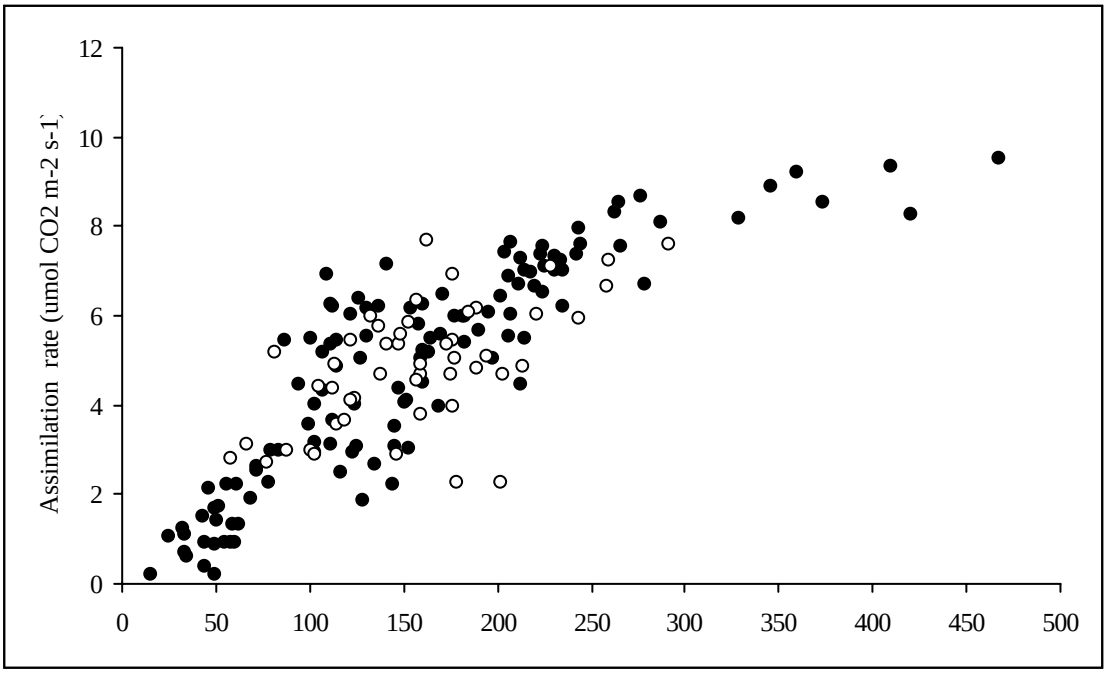

Figure 3a. Assimilation rate of CO2 by fully expanded leaves plotted against total leaf conductance
to diffusion of water vapour, • during desiccation, ° during recovery.

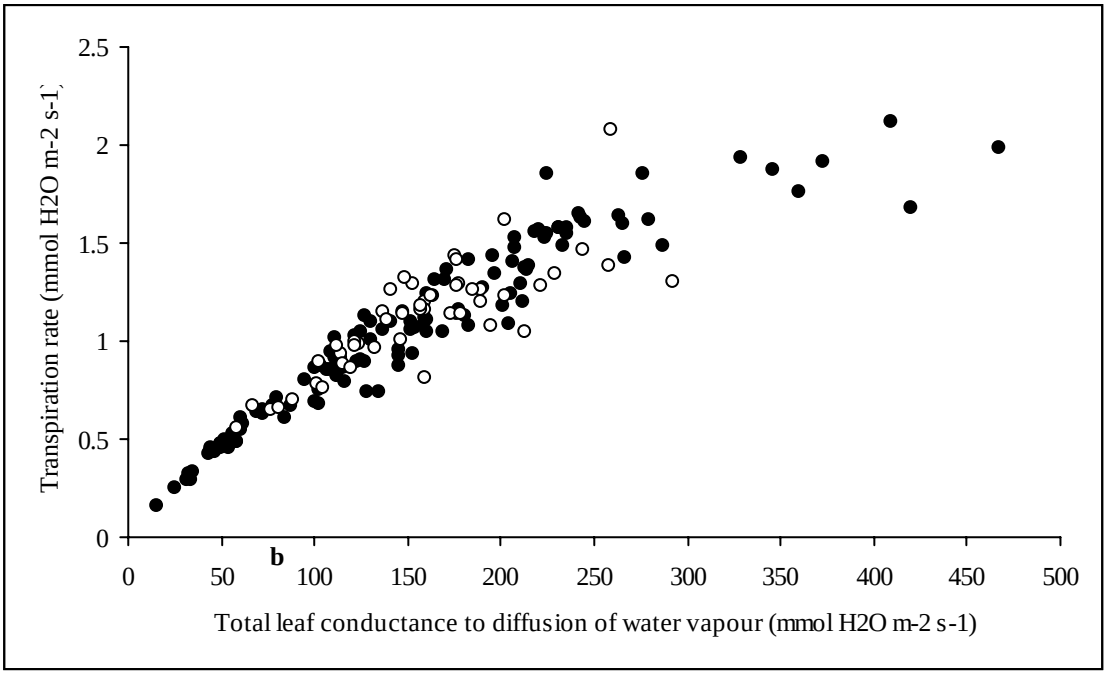

Figure 3b. Transpiration rate of H2O by fully expanded leaves plotted against total leaf conductance
to diffusion of water vapour, • during desiccation, ° during recovery.

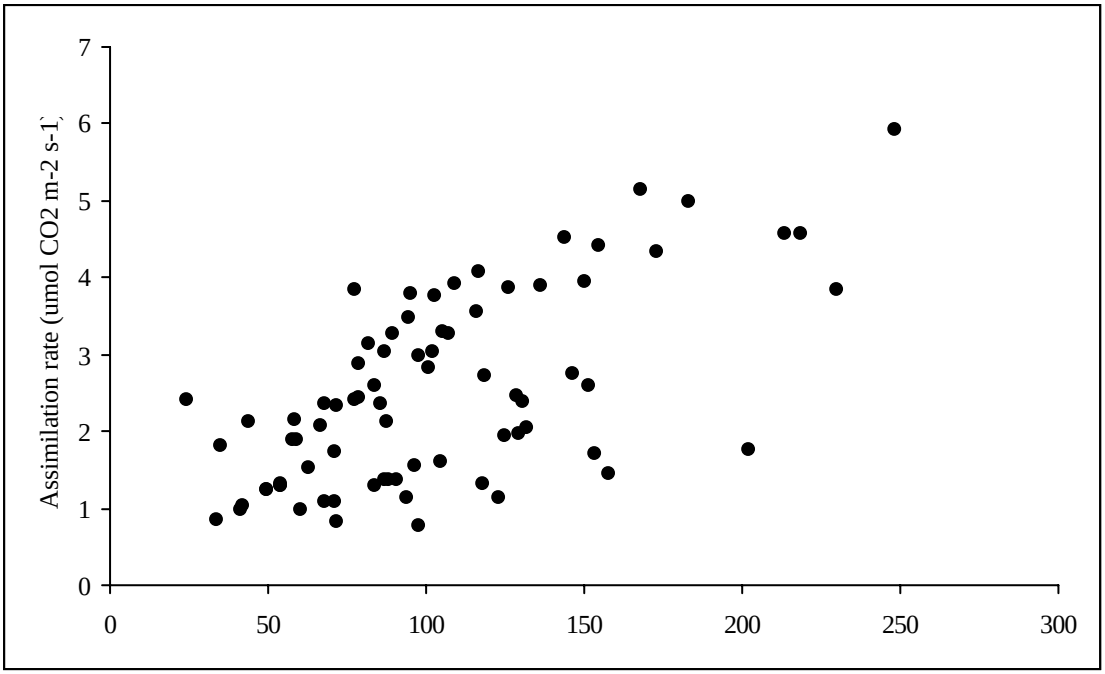

Figure 4a. Assimilation rate of CO2 by apical leaves plotted against total leaf conductance
to diffusion of water vapour.

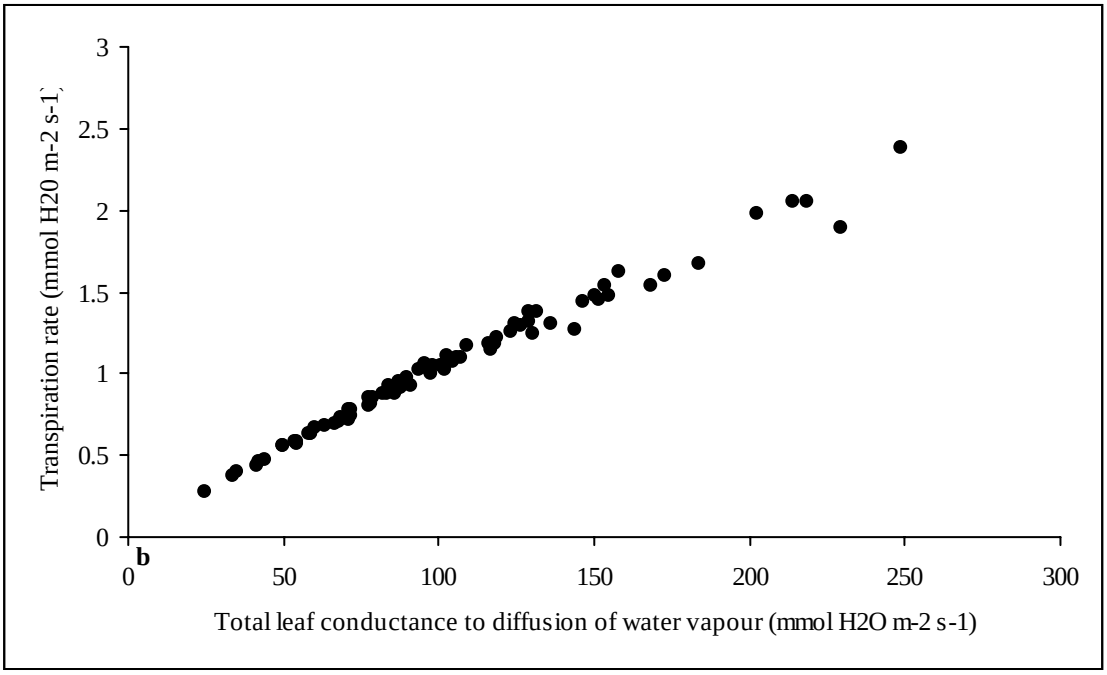

Figure 4b. Transpiration rate of H2O by apical leaves plotted against total leaf conductance to diffusion of water vapour.

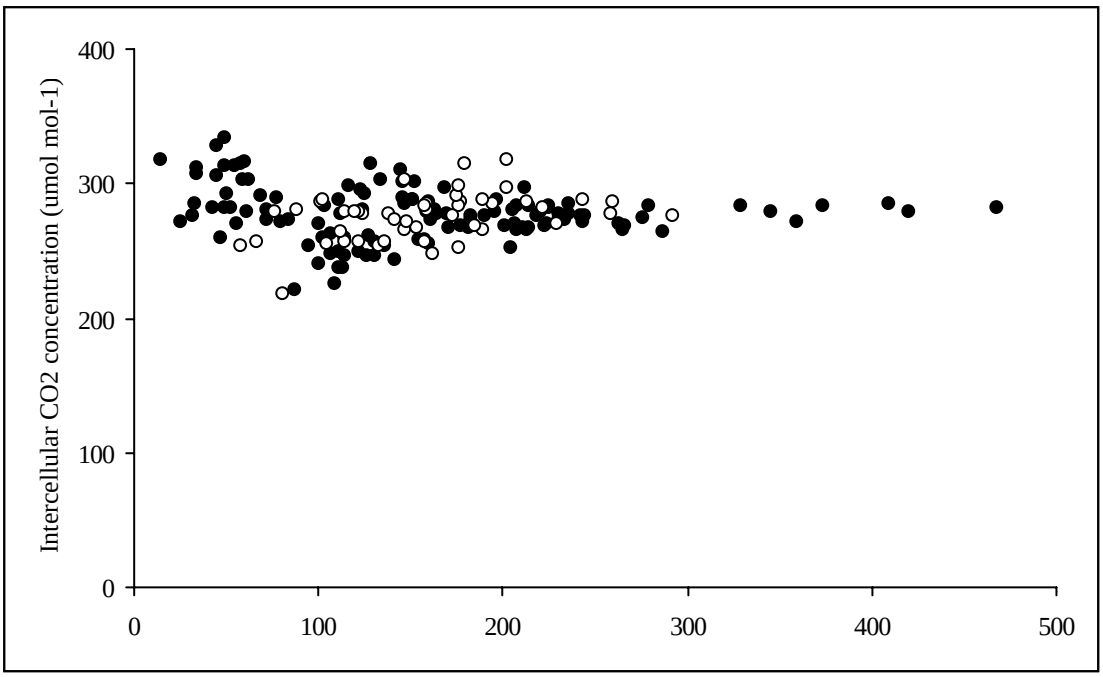

Figure 5a. Intercellular CO2 concentration in fully expanded leaves plotted against total leaf conductance to diffusion of water vapour, • during desiccation, ° during recovery.

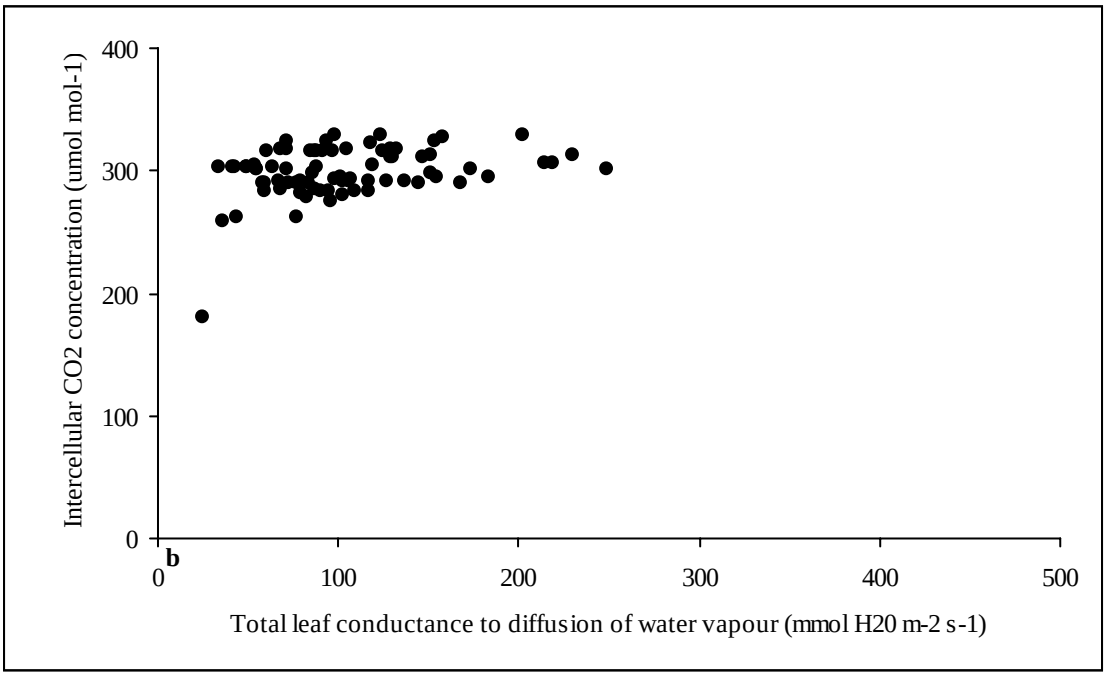

Figure 5b. Intercellular CO2 concentration in apical leaves plotted against total leaf conductance to diffusion of water vapour.

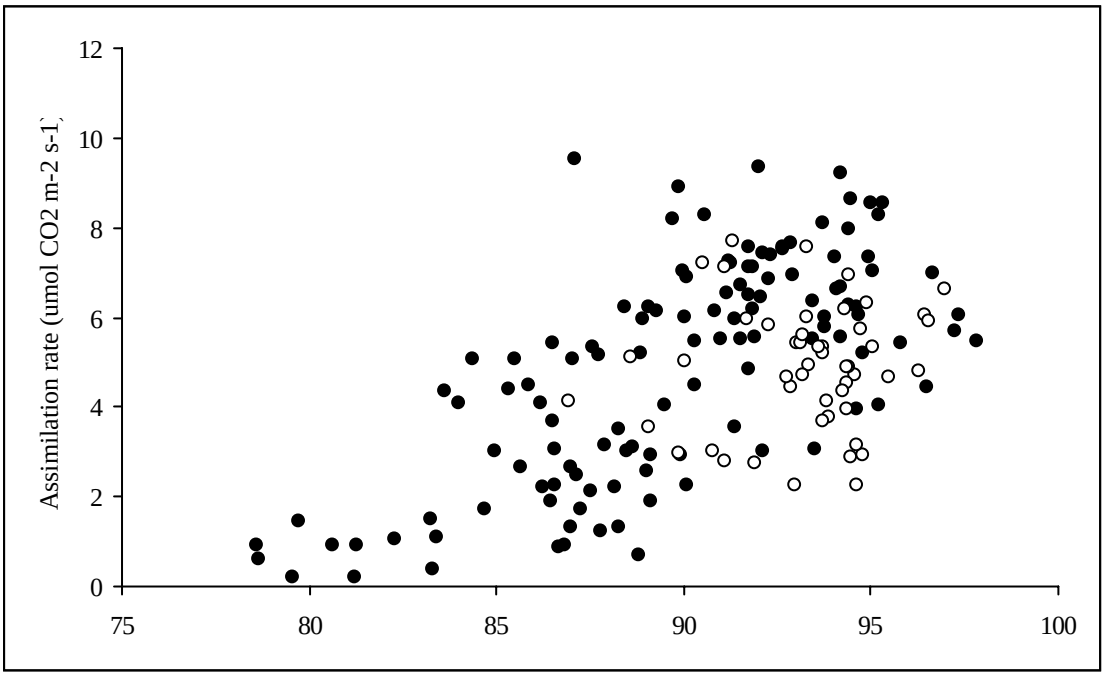

Figure 6a. Assimilation rate of CO2 by fully expanded leaves plotted against mid afternoon relative water content
of fully expanded leaves, • during desiccation, ° during recovery.

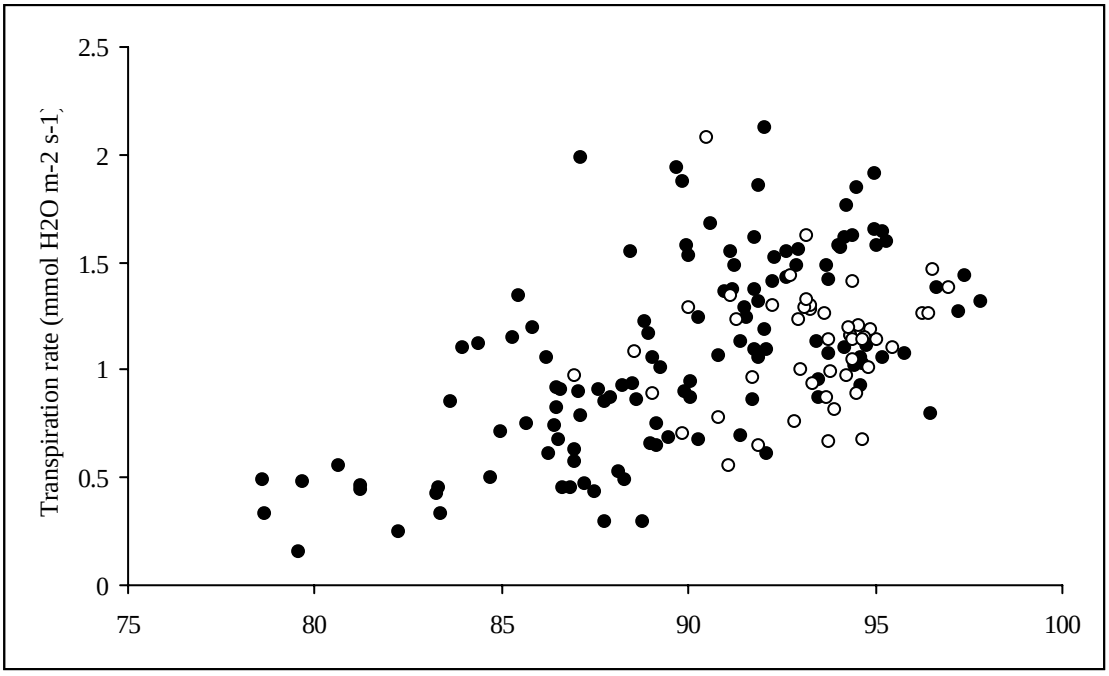

Figure 6b. Transpiration rate of H2O by fully expanded leaves plotted against mid afternoon relative water content
of fully expanded leaves, • during desiccation, ° during recovery.

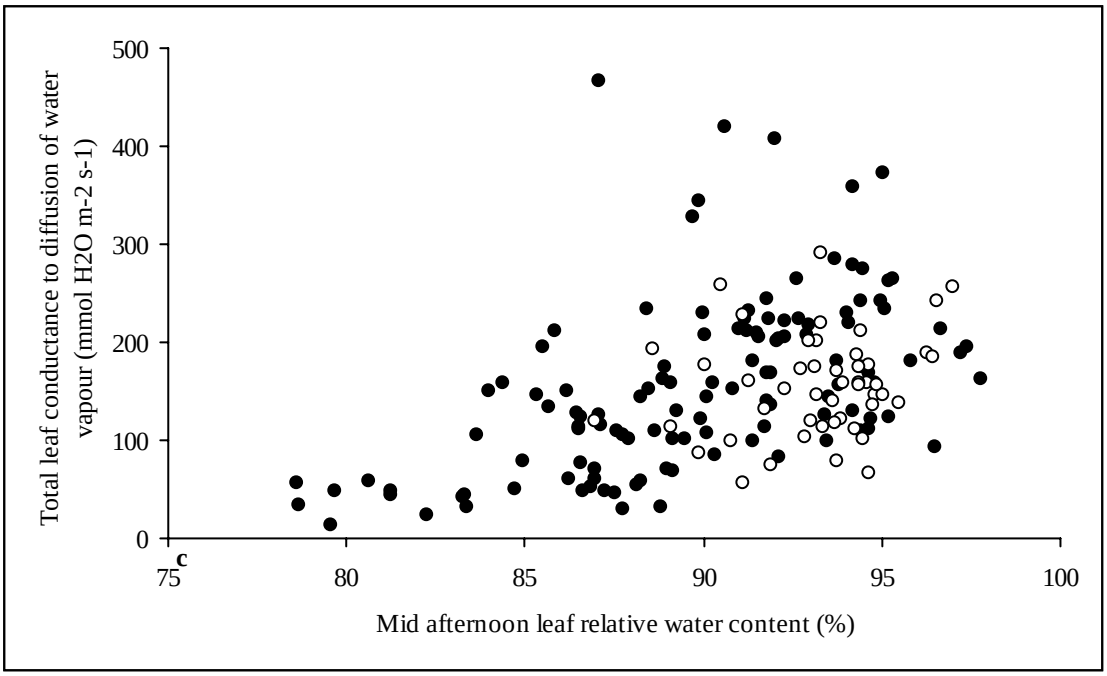

Figure 6c. Total leaf conductance to diffusion of water vapour of fully expanded leaves plotted against mid afternoon relative water content of fully expanded leaves, • during desiccation, ° during recovery.

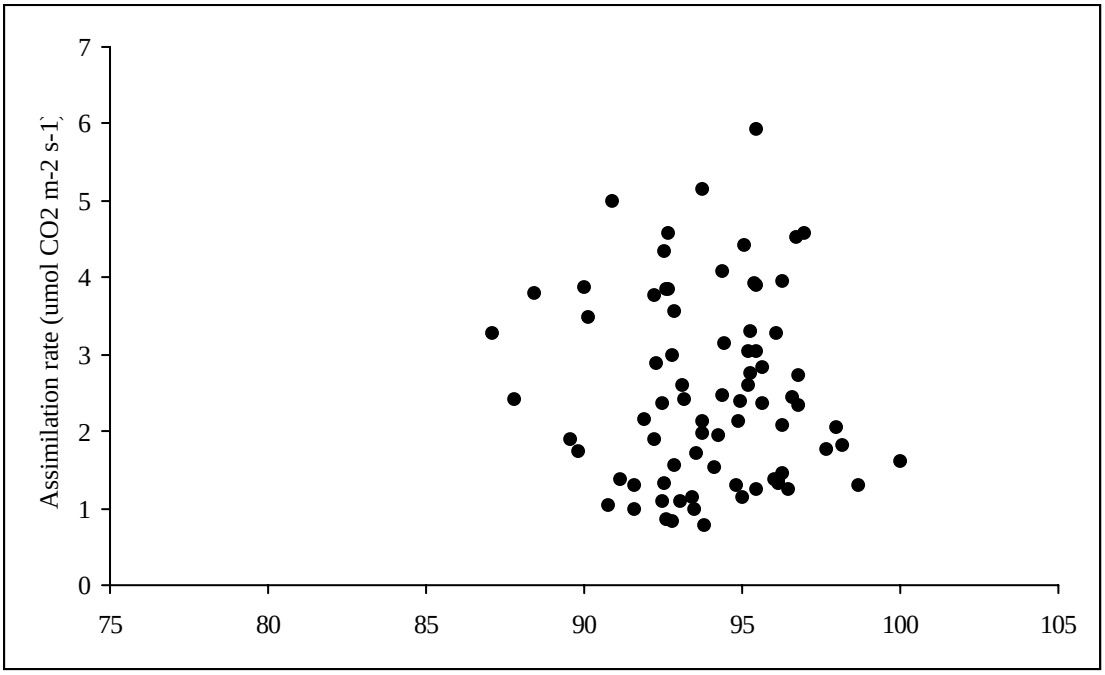

Figure 7a. Assimilation rate of CO2 by apical leaves plotted against mid afternoon relative water content
of apical leaves.

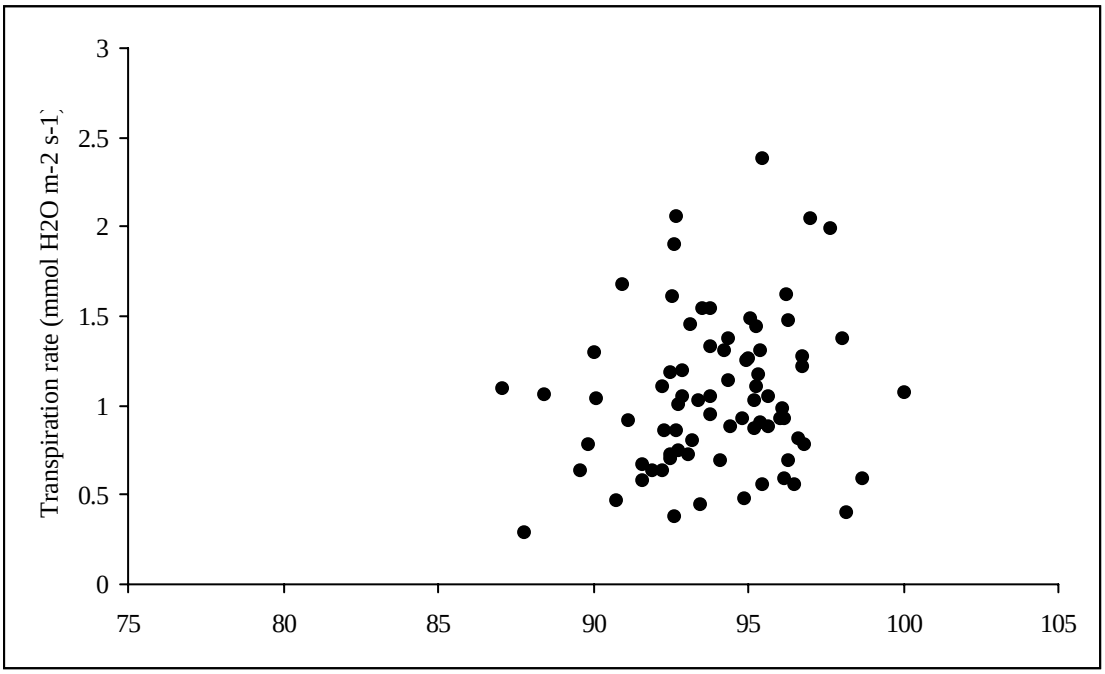

Figure 7b. Transpiration rate of H2O by apical leaves plotted against mid afternoon relative water content of apical leaves.

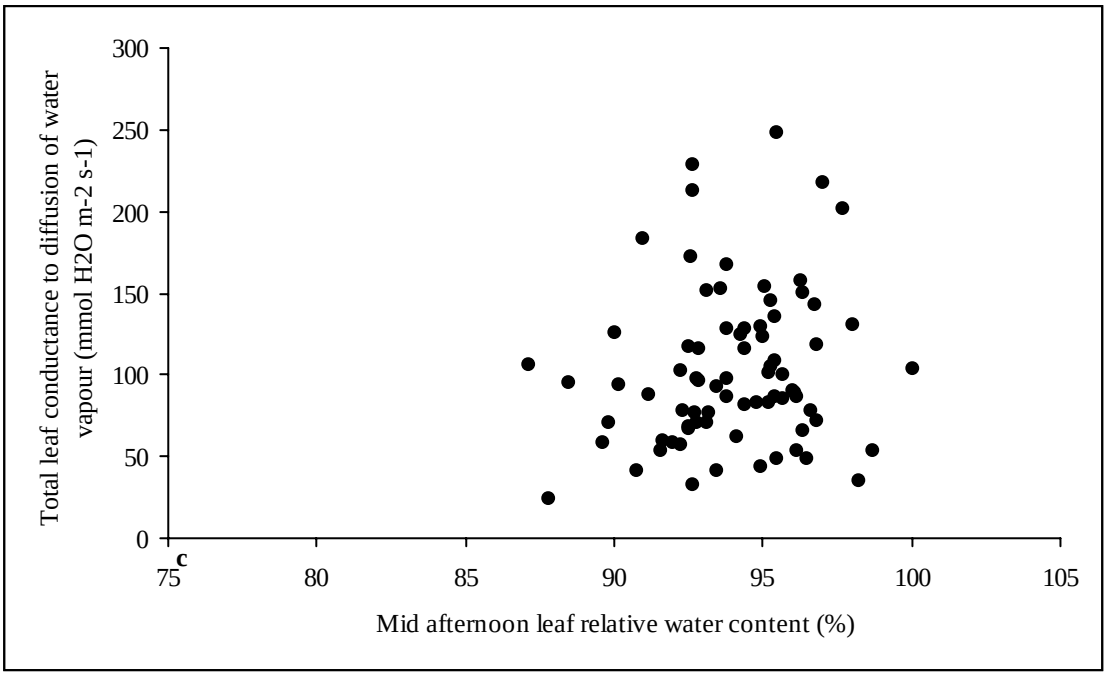

Figure 7c. Total leaf conductance to diffusion of water vapour of apical leaves plotted against mid afternoon relative water vapour content of apical leaves.

5. Effect of nitrogen fertilisation and irrigation on growth and flower yield of Boronia megastigma Nees

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5.1 Introduction

Preliminary work was carried out under glasshouse conditions to establish the effect of water stress on net assimilation rate. Boronia responds to applications of nitrogen Reddy & Menary (1989) and Roberts & Menary (1994). As with other oil bearing species the interaction between nitrogen and irrigation is likely to be significant Clark & Menary (1980).

A field experiment was designed to investigate the effect of nitrogen and irrigation on plant response and flower yield.

5.2 Materials & Methods

The experiment was conducted on 4 year old plants of Clone 250 which were grown on a gravely sand pH 4.5. The experiment was a 6 x 4 randomised block with inter-row buffers and buffers between plots. The plant spacing was 0.5 meters between plants in the row and 0.5 metres between rows which were spaced at 2 meter centres. There were 24 plants per plot. Nitrogen was applied at 30 and 60 Kg N/ha as ammonium nitrate with the following irrigation treatments; no irrigation, 28 mm every 14 days and 60 mm every 30 days.

Plant Growth
Plant height was measured at the commencement of the trial in January and again in August. Extension growth is expressed as the difference between these measurements.

Harvesting
Flowers were harvested with a hand held comb and commenced 4 September when 75% of flowers were open.

Tissue Analysis
Twenty laterals were selected at random from each plot in December and May for total nitrogen determination. Plant material was dried at 70°C for 48 hours, ground in a hammermill and stored at -18°C. Total nitrogen was determined by a semi micro Kjeldahl method (Reddy & Menary 1989).

Statistical Analysis
Results were analysed by Analysis of Variance using Statistical Applications for Students (SAS).

5.3 Results

During the period January to July, rainfall exceeded evaporation and there was no requirement for irrigation. The only irrigation applied was in August when there were two applications of 28 mm and one of 60 mm. The maximum plant water potential reached at 4 pm prior to the irrigation in August was - 0.6 Mpa.

There was no significant difference in extension growth between the six treatments. (Table 1) Likewise there was no significant difference in flower yield per plant.

Total nitrogen content of shoots prior to the commencement of the experiment was 1% and in May, 1.5%. This analysis demonstrated that plants were adequately supplied with nitrogen with no significant difference between the 30 and 60 Kg/ha rates. (Figure 1)

5.4 Discussion

Since only one irrigation was necessary at the end of the season and the plant water potential did not reach a level that would affect net assimilation rate (previous report), the lack of response to water is not surprising. Likewise the previous field trial on nitrogen fertilizer showed that 1.5% N in May was adequate to produce maximum extension growth, however in this case there was no increase in flower yield between 30 and 60 Kg/ha. In the case of the previous fertilizer trials the increase in flower yield was associated with an increase in the number of laterals per primary stem. It is likely that the increased lateral number in response to nitrogen in the irrigation trial did not occur because of unfavorable light conditions during the growing season.

5.5 References

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Roberts N.J. and Menary R.C. (1994). Effect of Nitrogen on growth, flower yield, oil composition and yield in *Boronia megastigma* Nees. Journal of Plant Nutrition 17(12):2035-2052.

Table 1 - Effect of Irrigation and Nitrogen on extension growth and flower yield

Treatment	Extension growth (cm)	Flower yield g/plant
No water, 30 Kg/N/ha	32.9	232.0
No water, 60 Kg/N/ha	36.5	191.7
28 mm water/14 days, 30 Kg/N/ha	36.8	223.1
28 mm water/14 days, 60 Kg/N/ha	39.9	199.0
60 mm water/30 days, 30 Kg/N/ha	39.2	226.1
60 mm water/30 days, 60 Kg/N/ha	39.0	202.8

Figure 1 - Nitrogen content of shoots

6. Implications and Recommendations

Tissue samples should be taken in November to determine the level of nitrogen required to produce balanced growth which will allow light penetration into the canopy for optimum flower production (Roberts & Menary 1989). The ideal length of primary laterals should be approximately 20 cms to allow efficient harvesting by mechanical means.

Boronia appears to be resistant to water stress and wilting occurs in apical leaves when leaf water potential was between -0.7 and -1.0 MPa. No change in net assimilation occurred until wilting was apparent.

Recommendations

- Sample shoot growth in November for total nitrogen analysis.
- A level of 1.5% is the critical level required for extension growth in summer and flowering in autumn.
- Application rates of NH_4 $N0_3$ should be 60 kg N/ha for clone 250 and 15 kg N/ha for clone 5 where soils are low in nitrogen.
- A molybdenum foliar spray should be applied after pruning to ensure adequate nitrate reductase activity.
- Irrigation should be applied when plant water potential reaches -1.0 MPa.

7. Publications

N.J Roberts and R.C. Menary (1994). Effet of Nitrogen on Growth, Flower Yield, Oil Composition, and Yield in *Boronia Megastigma* Nees. Journal of Plant Nutrition, 17(12), 2035-2052.

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