SOME ASPECTS OF NITROGEN NUTRITION OF *BORONIA MEGASTIGMA* NEES

by

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This thesis contains no material which has been accepted for the award of any other degree or diploma in any university and to the best of my knowledge, contains no material previously published or written by another person except where due reference is made in the text of the thesis.

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ABSTRACT

Boronia is a native plant with potential as a new essential oil crop. Various growth and metabolic responses of boronia to N were investigated to improve N nutrition of boronia.

When N was supplied to boronia at 50 or 100 kg ha^{-1}, two fast-release N sources (ammonium sulfate and calcium nitrate) caused toxicity while a slow-release N source (IBDU) did not cause toxicity. When N was supplied in a single dose in October during the active vegetative growth phase, highest flower yield was obtained and when the same amount of N was supplied in split doses at different phases of plant growth, the yield decreased.

Increasing N levels from 0 to 25 mM in the nutrient solution increased the plant's production of nodes, lateral shoots from these nodes and further nodes on these lateral shoots. With the same level of N, production of nodes and lateral shoots was in the order: NH_4^++NO_3^- > NH_4^+ > NO_3^-. The increase in the number of nodes subsequently translated into increased number of axils initiating flower buds and then into fully developed flowers. However, increasing N levels decreased the percentage of total flower buds that developed to anthesis and the individual flower weight. Increasing N levels increased the leaf N concentration, with the concentration in the order: NH_4^++NO_3^- > NH_4^+ > NO_3^-. N form did not affect the leaf tissue concentrations of P, K, Ca and Mg. At lower N levels, the concentrations of these nutrients in the leaf tissue were higher and may have reached toxic levels and caused the toxicity symptoms on the leaves. At higher N levels, the

concentrations of these nutrients were diluted due to increased growth and no toxicity symptoms were produced.

At decontrolled as well as controlled pH (4.5 and 6.5) of the nutrient solution, uptake of NH_4^+-N by the plant was higher than that of NO_3^--N. With NH_4^+ nutrition, concentrations of amides (namely, asparagine and glutamine) in plant tissues were higher than with NO_3^- nutrition.

When a low NO_3^- level (15 mmol per plant) was given, NO_3^- entering the plant was reduced without any accumulation and without nitrate reductase (NR) activity reaching its maximum capacity. When higher NO_3^- levels ($\geq$25 mmol per plant) were given, NR activity increased to a maximum of only ca. 500 nmol NO_2^- g^{-1} fresh weight h^{-1} both in the roots and leaves irrespective of 6-fold difference in the NO_3^- supply while NO_3^- continued to accumulate in proportion to the level of NO_3^- supplied. Consequently, high levels of NO_3^- accumulated in the plant tissue and at ca. 32 µmol NO_3^- g^{-1} fresh weight, toxicity symptoms appeared on the leaves. The low level of NR in boronia was not due to limited NO_3^- or electron donor availability, but it seems to be genetically tuned to slow growth in low NO_3^- producing native soils.

These responses of boronia to N are discussed in terms of the plant's adaptations to survive in native soils and their manipulation in commercial cultivation of boronia.

I. INTRODUCTION

Agriculture should be enriched and diversified with new crops so that farmers locked into the production of a single traditional crop (such as wheat) can turn to the new crops and be less vulnerable to the price instabilities of a single crop. For economic reasons, those plants that yield products having industrial applications offer good potential as new crops.

The fragrance and flavour industry has a sales volume of ca. $1.7 billion and is growing at an average annual rate of 10%. Of this volume, raw materials account for 40-50% and of these raw materials, about half is natural essential oils. A growth in this industry then means that the increased demand for fragrance and flavour compounds will generate an increased demand for natural essential oils (Menary, 1985). Thus there exists a potential for expansion of agriculture into the field of essential oil crops. Furthermore, the products of essential oil crops have an advantage of high price and low volume making them suitable for distant markets.

The Australian flora contain an abundance of essential oil bearing members which yield commercially useful and structurally interesting isolates (Lassak and Southwell, 1977). Boronia megastigma Nees (a member of the family Rutaceae; hereafter called boronia) is an evergreen woody shrub endemic to the southwestern region of Western Australia (WA). Because of its highly scented flowers, boronia is prized as an ornamental plant and has considerable demand as a cut flower. An extract of boronia flowers is highly priced ($3 million per tonne) and is used in high class

chypre and fougère perfumes. The major components of boronia flower extract are dodecanol, dodecyl acetate, tetradecyl acetate and β-ionone: the last compound being widely used in the perfumery (Leggett and Menary, 1980).

In WA, flowers of boronia are collected from the plants growing in the natural locations. However the natural abundance of these plants would be restricted. Further, the natural plant populations are subject to disease, intensive harvesting of flowers by pickers and clearing for other uses of land. Beard (1984) estimated that 54% of the original native vegetation in the southwestern portion of WA has already been alienated for alternate land uses. The yield of flowers from the wild plants would fluctuate due to the uncultivated conditions. All these factors affect the availability of essential oil of boronia. Therefore, to ensure a stable supply of high quality essential oil as well as cut flowers, there exists a commercial incentive for systematic domestication and cultivation of boronia.

Successful establishment of boronia plantations depends on the knowledge of suitable cultural practices. There is little published information as to boronia's cultural requirements. Most earlier attempts to establish boronia plantations were not successful (Matheson, 1979). This stresses the need for systematic research to overcome the constraints and establish the conditions that will make boronia a commercial crop.

Furthermore, information gained from the research on boronia may perhaps be applied to other Australian native plants, most of which are attracting much attention on the international cut flower

markets.

Generally fertilizers as percentage of farm costs are one of the largest single expense the farmer has (23%, Ozanne, 1982). For this reason, plant nutrition has received considerable attention in the production of crops to improve the efficiency of fertilizers.

Chapin (1980) pointed out that the rules worked out for the crop plants which have been selected and bred for high yield with high fertilization are not simply repeated by the wild plants which have evolved under the pressure of low nutrient supplies.

Many Australian soils are low in essential plant nutrients such as N and P. Consequently a large number of the Australian flora can be expected to have adapted to cope with these low levels of nutrients. Much attention has been focussed on P as a limiting nutrient for Australian flora (Beadle, 1954, 1966; Specht and Groves, 1966), but Bowen (1981) pointed out that low N is also extremely common in the Australian soils.

Of the four factors limiting plant growth, _viz_. carbon, light, water and nitrogen, N is likely to be the first in limiting plant growth (Ågren, 1985). In the WA jarrah forest where boronia occurs naturally, N is the nutrient likely to be limiting plant growth (Kimber in Hingston _et al_., 1982). However, as Pate (1980) remarked, very little is known of the patterns of uptake and assimilation of N in the woody plants associated with the natural vegetation where N present in the soils is in very low amounts especially as nitrate. This knowledge is important because any existing constraints in the utilization of N by these plants may be manipulated in the production of these plants. Epstein (1983)

also commented that more attention should be paid to the inorganic nutrition of wild plants to extend the present narrow focus of plant nutritional knowledge in terms of the experimental material.

The present study was conducted to form an information base on which N management practices for boronia could be built to improve N efficiency in the production of the crop. This study compared the effects of different levels, forms, sources and times of application of N on growth, flower production and physiology and biochemsitry of boronia. An attempt is made to understand how the observed effects are brought about and how they could be used in the N management of commercial plantations of boronia.

II. REVIEW OF LITERATURE

There is very little information on the N nutrition of boronia (in fact only one paper: Thomas, 1981). There is a large body of literature on the N nutrition of other plants, most of which is on the cultivated plants for economic reasons. However this is not exhaustively reviewed here. Instead, from the general background of this literature, an approach is made to relate some aspects of N nutrition of other plants to the probable N requirements of boronia, thereby forming hypotheses for the research work on boronia. Wherever possible specific examples are given.

1. N AND YIELD

A method of establishing the importance of N in boronia production is by an application of N to the plant. If such an application results in an increase in boronia yield then it demonstrates the importance of N as a limiting factor in boronia production.

There are numerous experiments that have established that plant generally respond to N with the increased yields, both biological (total plant material) and economic (those plant organs for which the plant is cultivated). However, biological and economic yield responses to N vary. In some instances N increases the biological yield but not the economic yield and in other instances N increases biological as well as economic yields.

Thomas (1981) found that N fertilization increased the dry weight of boronia plant. However there is no information on the yield of flowers which is the economic yield in boronia.

2. N AND YIELD COMPONENTS

In boronia, flowers are produced laterally in the axils of leaves (i.e. at nodes). It is logical to assume that the number of nodes on a plant will have a bearing on the number of flowers formed on that plant. Thus the number of nodes is an important yield component in boronia.

The relation between the number of nodes and the number of flowers on a plant will be the same in other axillary flowering plants such as apricot, peach, blackberry, guava, etc. In Pinaceae also, reproductive structures (cones) are borne laterally on the shoots. Despite the importance of the number of nodes in the flowering of these plants, surprisingly there have been very few studies that recorded the number of nodes in relation to the number of flowers. Only in apricot, Jackson (in Jackson and Sweet, 1972) showed a positive correlation between the number of nodes and the number of flowers produced.

When the relationship between the number of nodes and the number of flowers is positive, the number of flowers on a plant can be increased by increasing the number of nodes. In apricot, Jackson (1970) further found that the number of nodes can be increased by N application. Sweet and Hong (1978) suggested that N may increase the number of sites in the crown where cones are initiated in Pinus. Thus if the relationship between the number of nodes and the number of flowers is positive in boronia, its flower yield can be increased through increased production of nodes by N application.

Once nodes are produced other factors such as hormones, temperature, photoperiod, water stress as well as nutrition may

determine the number of flowers produced at each node. In apricot the number of flowers per node is also increased by N application (Jackson, 1970).

In considering application of N to boronia, the form of N is an important factor as there are two major forms of N for the majority of plants: ammonium (NH_4^+) and nitrate (NO_3^-) (Haynes and Goh, 1978).

3. UTILIZATION OF NH_4^+ AND NO_3^- BY PLANTS

Responses of plants to NH_4^+ and NO_3^- have been investigated by several workers. A survey of literature showed that utilization of NH_4^+ or NO_3^- as a source of N by a plant involves several plant and environmental factors interacting in a complex way. These factors are considered here.

3.1. Differences between plant species

There are differences between plant species in the utilization of NH_4^+ or NO_3^-. The ability of a plant to utilize NH_4^+ or NO_3^- is related to its relative soil and environmental adaptations.

In most arable soils the predominant form of N is considered to be NO_3^- and therefore most cultivated plants grow better with NO_3^-. NH_4^+ nutrition of these plants is considered to cause toxicity as it injures roots and tops of several plants. Some examples of the cultivated plants that utilize NO_3^- better than NH_4^+ are cited here. Because of the number of papers reporting this finding, the results are tabulated (Table 1).

Table 1. Plants that utilize NO_3^- better than NH_4^+ as a source of N.

Plant species	Observation: increase in	Reference
Tomato	DW[a] of leaves, stems, roots; leaf area	Woolhouse and Hardwick (1966)
	DW of leaves, petioles, stems, roots	Kirkby and Mengel (1967)
	FW[b]; leaf area	Pill and Lambeth (1977)
	DW of whole plant	Harada et al. (1968)
	DW of whole plant	Kirkby (1969)
	FW and DW of shoots and roots	Wilcox et al. (1973)
	FW and DW of whole plant	Magalhaes and Wilcox (1984)
White mustard	DW of leaves and stems	Kirkby (1968)
	DW of whole plant	Kirkby (1969)
Sugarbeet	FW of whole plant	Harada et al. (1968)
Rye	DW of whole plant	Kirkby (1969)
Oats	DW of whole plant	Kirkby (1969)
Buckwheat	DW of whole plant	Kirkby (1969)
Chenopodium album	DW of whole plant	Kirkby (1969)
Cucumber	FW and DW of whole plant	Barker and Maynard (1972)
Pea	FW and DW of whole plant	Barker and Maynard (1972)
Potato	FW of shoots, roots, tubers	Polizotto et al. (1975)
	Root and shoot growth	Davis et al. (1986)

Table 1 (continued).

Plant species	Observation: increase in	Reference
Lima bean	DW of shoots, stems, roots, pods	McElhannon and Mills (1978)
Radish	FW of whole plant	Goyal et al. (1982)
White bean	DW of leaves, stems, root; leaf area	MacLeod and Ormrod (1985)
Muskmelon	DW of shoots and roots; Shoot ht, root length, root surface area	Elamin and Wilcox (1986)
Bean	FW of whole plant	Chaillou et al. (1986)

[a]Dry weight; [b]fresh weight.

Plants originating from the soils where NH_4^+ is the major source of N utilize NH_4^+ in preference to NO_3^-. A large number of plants belonging to the family Ericaceae grow predominantly in acid soils where NH_4^+ is considered to be the main source of N. These are the best examples of the plants showing a preference for NH_4^+ (Table 2).

Table 2. Species of Ericaceae showing a preference for NH_4^+ than for NO_3^-.

Plant species	Observation: increase in	Reference
Vaccinium corymbosum	Linear growth	Cain (1952)
	FW[a] of shoots, roots; no. of branches	Townsend (1967)
V. angustifolium	Growth	Townsend (1966)
	Shoot growth, Root wt	Townsend (1969)
V. macrocarpon	DW of whole plant	Greidanus et al. (1972)

Table 2 (continued).

Plant species	Observation: increase in	Reference
V. vitis idaea	Growth rate	Ingestad (1973)
	Growth rate	Ingestad (1976)
V. myrtillus	Growth rate	Ingestad (1976)
V. ashei	Shoot length	Spiers (1978)
Rhododendron obtusum	FW of whole plant	Colgrove and Roberts (1956)

[a]Abbreviations as in Table 1.

Such favorable effects of NH_4^+ have been ascertained with some members of Coniferae which grow in the soils where the conditions are considered to be unfavorable for nitrification leading to NH_4^+ becoming the predominant form of N. Krajina et al. (1973) tested growth responses of four species (which grow naturally in different habitats) to NH_4^+ or NO_3^-. Their results show that Pinus contorta and Tsuga heterophylla plants supplied with NH_4^+ grew larger in terms of dry weight than those supplied with NO_3^-. Krajina (1969) earlier found that these species grow naturally in habitats where nitrification does not actively occur. Pseduotsuga meniessi and Thuja plicata plants grow naturally where nitrification takes place and these species grew larger when NO_3^- was supplied. Results of Bigg and Daniel (1978) also show that Pinus contorta (and Picea englemanni) made better growth with NH_4^+ and Pseudotsuga menziesii with NO_3^-. Pinus radiata and Picea glacua showed greater growth with NH_4^+ than with NO_3^- (McFee and Stone, 1968). Data of Ingestad (1979) indicate a somewhat lower growth of Pinus silvestris and Picea

abies with NO_3^- than with NH_4^+.

Some species of grasses also respond according to their ecological distribution. Deschampsia flexuosa and Nardus stricta grow naturally in the soils in which NH_4^+ predominates and these grew better in terms of increase in dry weight when N was available as NH_4^+. Scabiosa columbaria and Seslaria albicans grow naturally in the soils with predominating NO_3^- and these grew better when NO_3^- was available (Gigon and Rorison, 1972). Recently Atkinson (1985) also found that D. flexuosa, N. stricta and other species from the same habitat, viz. Festuca ovina and Juncus squarrosus show greater growth rates with NH_4^+ than with NO_3^-. Wiltshire (1973) found that climax perennial grasses yield more with NH_4^+ with than with NO_3^-. He suggested that succession in high altitude is towards plant species adapted to NH_4^+ nutrition.

3.1.1. NH_4^+ plus NO_3^- as N source. Some plants grow better when supplied with a mixture of both NH_4^+ and NO_3^- than with either form of N separately. These plants include cultivated plants as well as the plants belonging to the localities where NH_4^+ is predominant (Table 3).

Table 3. Plants that utilize $NH_4^+ + NO_3^-$ better than either form of alone.

Plant species	Observation: increase in	Reference
	Preference: $NH_4^+ + NO_3^- > NO_3^- > NH_4^+$	
Sunflower	DW[b] of whole plant	Weissman (1964)
Picea glauca	DW of whole plant	van den Driessche (1971)

Table 3 (continued).

Plant species	Observation: increase in	Reference
Corn	FW and DW of shoots and roots	Schrader et al. (1972)
	FW and DW of whole plant	Handa et al. (1985)
Wheat	FW of shoots and roots; leaf extension rate	Cox and Reisenauer (1973)
	DW	Gashaw and Mugwira (1981)
Spinach	FW	Mills et al. (1976)
Tomato	FW and DW of shoots and roots	Ganmore-Neumann and Kafkafi (1980)
	RGR[a]	Ikeda and Yamada (1986)
	DW of shoots and roots	Hartman et al. (1986)
Triticale	DW	Gashaw and Mugwira (1981)
Rye	DW	Gashaw and Mugwira (1981)
Peach	DW of shoots and roots; terminal length; no. of laterals; trunk sectional area; root volume	Edwards and Horton (1982)
Asparagus	FW and DW of shoots and roots	Precheur and Maynard (1983)
Strawberry	DW of shoots and roots	Ganmore-Neumann and Kafkafi (1985)
	$NH_4^+ + NO_3^- > NH_4^+ > NO_3^-$	
Vaccinium corymosum	FW of shoots and roots; no. of branches	Townsend (1967)
Psedstsuga menziesii	DW	van den Driessche (1971)

Table 3 (continued).

Plant species	Observation: increase in	Reference
Picea sitchensis	DW	van den Driessche (1971)
Eucalyptus agglomerata	DW of shoots and roots; stem ht.	Moore and Keraitis (1971)
E. macrohyncha	DW of shoots and roots; stem ht.	Moore and Keraitis (1971)
Pinus contorta	DW of shoots and roots	Bigg and Daniel (1978)
Picea engelmanni	DW of shoots and roots	Bigg and Daniel (1978)

[a]Relative growth rate; [b]other abbreviations as in Table 1.

Most of these studies employed a NH_4^+:NO_3^- ratio of 1:1.

It is evident from all the above findings that there are differences in the plant species in their ability to utilize NH_4^+ or NO_3^- as a source of N. As this ability is related to the soil environmental adaptations of the plant species, the availability of form of N in the soils of natural habitat of boronia is speculated here.

3.2. N form in soils of natural habitat of boronia

Boronia occurs naturally in the forest areas of Warren and Stirling districts of the southwestern province in Western Australia. Rainfall is high in these areas and thereby the sites are wet or seasonally wet. The soils are sandy and are slightly acidic (Christensen and Skinner, 1978). The availability of possible form of N under such conditions is considered here.

Runge (1983) described the major N transformations in the forest soils. These transformations and other steps relevant to the availability of N form in the natural soils of boronia are schematically presented in Fig. 1.

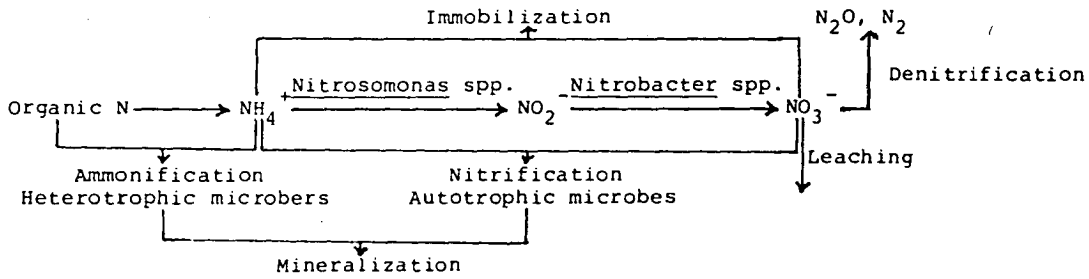


Fig. 1. N transformations in soil.

A number of factors differently influence the ammonification and nitrification processes, consequently determining the supply of NH_4^+ and NO_3^- to the plants. However, only those factors that are related to the natural habitat of boronia are discussed here.

3.2.1. Moisture and aeration. In wet soils O_2 availability will be low. By low O_2, ammonification is less affected (Haynes and Goh, 1978) but the rate of nitrification is reduced (Amer and Bartholomew, 1951). In wet soils, denitrification is also increased as Arnold (1954) showed that soils saturated with water rapidly release large amounts of N_2O.

3.2.2. Temperature. Wet soils are likely to be cold in the mediterranean-type temperatures of Western Australia. At low

temperatures, nitrification is retarded more than ammonification (Haynes and Goh, 1978). Thus Flint and Gersper (1974) found very low concentrations of NO_3^- as compared to NH_4^+ in a wet meadow tundra.

3.2.3. Acidity. Ammonification is less sensitive to acidity (Haynes and Goh, 1978) but laboratory experiments used to assess nitrification indicated sensitivity of nitrifying bacteria to acidity (Wallace and Nicholus, 1969). Thus nitrification decreased with increasing soil pH (Nyborg and Hoyt, 1978) and was minimal below pH 5.0 (Haynes and Goh, 1978). Although increasing acidity leads to a tendency towards the predominance of NH_4^+, production of NO_3^- is not impossible in the acid soils. Runge (1974) recorded that NO_3^- can constitute a proportion of total N in some acid forest soils.

NO_3^- leaches through the sandy soils when rainfall is high because most temperate soils possess an overall net negative charge on their colloids when repel NO_3^- ion (Haynes and Goh, 1978).

Nitrification is also influenced by the type of vegetation. Christensen and Skinner's (1978) description of boronia sites indicates a climax type of plant community. In a climax type of plant community, Rice and Pancholy (1972) found a low quantity of NO_3^- as compared to NH_4^+ and a low number of nitrifying bacteria. They invoked allelopathy: that is, the plant species present in a climax plant community inhibit nitrification because NH_4^+ is adsorbed on to colloids in the soil and thus is not lost as easily as NO_3^-.

Large areas of forest in Australia occur in the soils that

contain low phosphate (<2 µg HCO_3^--extractable phosphate per g soil, Bowen, 1981). In P-deficient soils nitrification is restricted because nitrifying bacteria are sensitive to P deficiency (Purchase, 1974).

Because of the dependency of N transformations on moisture and temperature, one would expect that seasonal variations in these factors will influence the transformation processes. Thus a combination of factors such as wet soils in winter months may further restrict nitrification.

All this evidence suggests the presence of predominantly NH_4^+ in the natural soils of boronia. However, NO_3^- may never be totally absent from such soils. Boronia growing under such conditions may adapt to some extent to NH_4^+ based N nutrition. Therefore a question arises whether boronia prefers or even requires the particular N form and the possible implications of this question have to be considered in the N nutrition of boronia.

Aside from the differences between the plant species, there are other factors that affect the utilization of NH_4^+ and NO_3^- by the plants. They are also discussed here.

3.3. N form and pH interactions

Utilization of NH_4^+ and NO_3^- by a plant is affected by pH of the growth medium. This effect is also dependent on the plant species. Interaction of N form X pH in some plant species is discussed here.

Usually the medium containing growing plants with NH_4^+ drifts towards acidity while that with NO_3^- drifts towards alkalinity. This observation has been make in a variety of plants (e.g. in rough lemon by Wander and Sites, 1956; in rice by Karim and Vlamis, 1962

and by Kirkby and Hughes, 1970). However, in contrast to these findings, Asher (Asher and Edwards, 1983) observed a decrease in pH of the medium even when NO_3^- was supplied to nonnodulated jackbean. Therefore, it seems that there are differences between plant species with regard to changes in the growth medium pH.

Changes in pH of the growth medium generated due to NH_4^+ nutrition of plants may be considerable. Kirkby and Hughes (1970) mentioned that changes of ca. 0.5 units can take place within an hour. Data of Gigon and Rorison (1972) show that pH of 500 ml NH_4^+ nutrient solution containing one Rumex acetosa plant drifted from 7.2 to 3.3 within 48 h, but with Deschampsia flexuosa, pH change was not so striking. Glass et al. (1983) reported a decrease in pH of the medium from 5.2 to 4.9 in 40 min during NH_4^+ uptake by barley roots. With corn, pH may fall as low as 2.8 with NH_4^+ in 14 days (Maynard and Barker, 1969). Because of such pH changes, monitoring and maintenance of growth medium pH is important in the experiments with boronia, as these changes in pH affect the uptake of NH_4^+ and NO_3^-.

Generally NH_4^+ uptake occurs more readily at lower pH whereas NO_3^- uptake occurs more rapidly at higher pH. Chen (in Kirkby and Hughes, 1970) reported that in rice maximum NH_4^+ absorption occurred at pH 8.0 while maximum absorption of NO_3^- occurred at pH 4.0. In barley, NO_3^- absorption decreased with increasing pH (Rao and Rains, 1976). However, it is interesting to note that pH did not influence the trends of NO_3^- and NH_4^+ absorption by lima bean (McElhannon and Mills, 1978), because 100% NO_3^- absorption occurred with pHs ranging from 3.5 to 7.5 and NH_4^+ absorption showed different trends at

similar pHs. These results suggest differences between plant species in the uptake of NH_4^+ and NO_3^-.

When the pH of the growth medium was controlled, it sometimes resulted in elimination of the adverse responses shown by most cultivated plants that had been attributed to the utilization of NH_4^+. Barker et al. (1966) partially alleviated the detrimental effects of NH_4^+ in bean by controlling the acidity which results from NH_4^+ nutrition. Similar results were obtained with sweet corn, cucumber and pea (Maynard and Barker, 1969). Breteler (1973) grew sugarbeet by eliminating the changes in the medium pH and Breteler's data show that sugarbeet dry matter production between NH_4^+ and NO_3^- nutrition differs by only 12%. Without the control of pH in other experiments, sugarbeet yielded less on NH_4^+ than on NO_3^-. In Pseudotsuga menziesii, Krajina et al. (1973) as well as Bigg and Daniel (1978) ascertained less growth with NH_4^+ than with NO_3^-, but when van den Driessche (1978) controlled acidity, NH_4^+ also produced good growth.

On the other hand, Cox and Seeley (1984) found that when pH of the growth media was controlled, it enhances NH_4^+ injury in poinsettia.

The differential effects of NH_4^+ or NO_3^- on the growth of different plant species were observed even after the control of pH in some plant species. Bogner (1968) observed better growth of the species from NH_4^+ predominant habitats on NH_4^+ at low pH and poor or no growth at all on NO_3^- at high pH. Results of McFee and Stone (1968) indicated that Pinus radiata and Picea glauca gave greater dry weights with NH_4^+ than with NO_3^- at all the pHs tested, viz. 3.6,

5.0, 5.9 and 6.2. Townsend (1969) separated the effect of form of N from that of pH to measure the influence of each on the growth of Vaccinium angustifolium. This plant made better growth at pH 4.5 than at pH 6.0 and with NH_4^+ than with NO_3^-. Absence of pH X form of N interaction in Townsend's data suggest that the effects of pH and form of N were independent in this species. Quite similar results were reported by Gigon and Rorison (1972). Their results showed that the growth of Deschampsia flexuosa (which grows in NH_4^+ predominant soils) with NH_4^+ was about the same at all pH levels viz. 4.2, 5.8 and 7.2 and was better than with NO_3^-, with which the growth decreased with increasing pH. Scabiosa columbaria (which prefers NO_3^-) failed to survive with NH_4^+ at pHs 4.2 and 5.8. Similarly in the study by Poilzotto et al. (1975), increasing pH and preventing pH changes of the growth medium showed little effect of preventing the detrimental effects of NH_4^+ on potato.

Thus the interrelationships between N form and pH appear to be variable with different plant species. Therefore, an investigation on the role of pH in the utilization of N form by boronia is significant.

3.4. N form and uptake of other ions by plants

The form of N is known to affect the concentrations of other ions in plants. Generally, plants grown with NH_4^+ contain lower concentrations of inorganic cations and higher concentrations of organic anions than those grown with NO_3^-. This has been found in many plants (Table 4).

Table 4. Plants that had higher concentrations of cations and lower concentrations of anions when supplied with NO_3^- and *vice versa* with NH_4^+.

Plant species	Ions increase (>) decrease (<) no effect (=) with NO_3^-	Reference
Tomato	> K, Ca, Mg < P, S, Cl	Kirkby and Mengel (1967)
	> K, Ca, Mg < P	Harada *et al.* (1968)
	> K, Ca, Mg	Kirkby (1969)
	> K, Ca, Mg	Wilcox *et al.* (1973)
	> K, Ca, Mg	Wilcox *et al.* (1977)
	> P, K, Ca, Mg	Pill and Lambeth (1977)
	> Ca, Mg, K < P	Ikeda and Yamada (1984)
	> K, Ca, Mg	Hartman *et al.* (1986)
White mustard	> K, Ca, Mg = P, S, Cl	Kirkby (1968)
Sugarbeet	> K, Ca, Mg < P	Harada *et al.* (1968)
	> K, Ca, Mg, Na < P, S, Cl	Breteler (1973) (1973)
Rye	> K, Ca, Mg	Kirkby (1969)
	> Ca, Mg, Mn < P, Fe	Gashaw and Mugwira (1981)
Chenopodium album	> K, Ca, Mg	Kirkby (1969)
Corn	> Ca, Mg < P, S	Blair *et al.* (1970)
Cucumber	> K, Ca, Mg	Barker and Maynard (1972)
Sweet corn	> K, Ca, Mg	Wilcox *et al.* (1973)

Table 4 (continued).

Plant species	Ions increase (>) decrease (<) no effect (=) with NO_3^-	Reference
Wheat	> K, Ca, Mg, Mn, Zn < P, S	Cox and Reisenauer (1973)
	> Ca, Mg, Mn < P, Fe	Gashaw and Mugwira (1981)
Potato	> Ca, Mg < P = K	Polizotto *et al.* (1975)
	> Ca, Mg = K	Davis *et al.* (1986)
Triticale	> Ca, Mg, Mn < P, Fe	Gashaw and Mugwira (1981)
White bean	> K, Ca < Mg, P	MacLeod and Ormrod (1985)
Muskmelon	> K, Ca, Mg, Mn < P	Elamin and Wilcox (1986)
Bean	> K, Ca, Mg, Na = P	Chaillou *et al.* (1986)

All the above findings are in the cultivated plants most of which generally prefer NO_3^-. On the other hand, in wild plants, results of Gigon and Rorison (1972) show very small differences in K concentration between *Deschampsia flexuosa* plants grown with either NH_4^+ or NO_3^-. *D. flexuosa* grows in soils where NH_4^+ occurs predominantly. In contrast, *Rumex acetosa* and *Scabiosa columbaria* (which grow where NO_3^- predominates) had only half the concentration of K with NH_4^+ as with NO_3^-. Similarly, when supplied with NH_4^+, *Pinus contorta* (which prefers NH_4^+) showed no deficiencies while *Pseudotsuga meniesii* and *Thuja plicata* (which prefer NO_3^-) developed

Ca and Mg deficiency symptoms (Krajina et al., 1973). In cultivated Vaccinium ashei (which grows better with NH_4^+), Spiers (1978) found no difference in the concentration of K between NH_4^+ and NO_3^- and that the concentrations of P, Ca and Mg were even higher with NH_4^+. In woody ornamentals Cotoneaster dammeri, Pyracantha coccinea and Weigela florida, Ca, Mg and K concentrations were higher when NH_4^+ was supplied although P concentration was unaffected by N form (Gilliam et al., 1980). In Eucalyptus rossii, E. sideroxylon and E. polyanthemos, which respond better to NH_4^+, Ca uptake was higher with NH_4^+ than with NO_3^- (Moore and Keraitis, 1971).

Therefore, it appears that some plants which normally grow where the conditions are unfavorable for nitrification, thus developing under the conditions of NH_4^+ nutrition, have an effective ability to take up cations. Otherwise, deficiency of these nutrients can develop because of the antagonistic effect of NH_4^+ on the uptake of other cations which occurs in most cultivated plants.

The variability in the uptake of other ions resulting from the form of N can in turn affect the plant growth and productivity. Therefore, study of concentrations of other ions resulting from NH_4^+ or NO_3^- nutrition of boronia is important.

In addition to the effect on the uptake of other ions, the form of N seems to have an effect on the assimilation pattern of N itself.

3.5. N form and amino acids in plants

NH_4^+ and NO_3^- are assimilated in plants into amino acids. Generally, concentrations of amino acids in the plant increase with NH_4^+

nutrition compared to NO_3^- nutrition. The pattern of increase seems to be characteristic of the plant species although the common increase is in the amides, glutamine and asparagine and the amino acids closely related to them.

In white mustard, Kirkby (1968) found that its leaves contain higher concentrations of amino acids when supplied with NH_4^+ than with NO_3^-. Kirkby's results show that the concentrations of the amino acids and amide already present in the highest amounts (viz. aspartic acid, glutamic acid, alanine, proline and glutamine) increased.

In tomato, NH_4^+ nutrition compared to NO_3^- nutrition increased the concentrations of amides, asparagine and glutamine and amino acids, asparagine, glutamine, aspartic acid, glutamic acid, arginine and lysine (Harada et al., 1968; Hoff et al., 1974; Lorenz, 1975; Magalhaes and Wilcox, 1984)

Sugarbeet supplied with NH_4^+ contained higher concentrations of amides as well as amino acids (Harada et al., 1968; Breteler, 1973). In barley fed with NH_4^+, aspartate increased (Richter et al., 1975). Data from Yoneyama and Kumazawa (1975) compared to that from Yoneyama and Kumazawa (1975) show that rice treated with NH_4^+ contained higher levels of asparagine and glutamine than that treated with NO_3^-.

In bean, Chaillou et al. (1976) found higher concentrations of amides, glutamine and asparagine and amino acid, serine with NH_4^+ nutrition than with NO_3^- nutrition.

All the above findings are in the plants for which NH_4^+ is considered to be toxic. The increases in amides and amino acids in

NH_4^+ fed plants is considered to be a detoxification of NH_4^+ by the synthesis of nontoxic amides and amino acids with organic acids as sources of carbon. There appear to be no information as to the changes in amino acids in the plants that prefer NH_4^+.

3.6. N form and flowering

NH_4^+ and NO_3^- seem to affect flowering differentially in different plants. As the flower is the desirable product in boronia, literature on the effect of N form on flowering is considered here. Although the effect of N form on flowering has been reported in some plants, any generalizations do not seem to be possible.

With lima bean, a preference for NO_3^- was observed during the reproductive development (McElhannon and Mills, 1978) and a similar trend was observed with southernpea (Sasseville and Mills, 1979).

In strawberry, Ganmore-Neumann and Kafkafi (1985) found a preference for NO_3^- during flowering and fruiting. In apple, Grasmanis and colleagues (1967, 1974) found that NH_4^+ caused initiation of a higher proportion of flower buds. In rabbiteye blueberry, Spiers (1978) observed about 4 times as many flower buds with NH_4^+ as with NO_3^-.

The preference for NH_4^+ or NO_3^- seems to change between the initiation and the development of reproductive part. In sweet corn, NO_3^- absorption was greater than NH_4^+ absorption during tasseling whereas during ear development NH_4^+ uptake was higher than NO_3^- uptake (Mills and McElhannon, 1972). In tomato, NO_3^-:NH_4^+ ratio did not influence the number of fruits formed within each flower cluster but increasing NH_4^+ reduced the fruit weight (Hartman et

al., 1986).

4. APPLICATION OF N

A major factor in considering application of N to boronia is to determine the amount of N to be used. The amount of N applied to plants depends largely on the particular crop and the prevalent soil conditions. Excessive amount of N may become detrimental to plants and may reduce their yield.

Native habitat of plants can influence their utilization of N. There is little experimental information but much speculation on the N requirements of Australian native plants.

Generally some popular publications recommend no N for the native plants (e.g. Lord, 1948). It might be based on the belief that as the native plants grow well in their native soil, they do not require any additional fertilizers and further when N was applied, some native plants including boronia (Fairall, 1970) died.

An analysis of literature has shown that the native plants died when N was applied at the levels that are normal for cultivated plants. Although it is difficult to compare the levels of N applied in different experiments because of the differences in growing media and sources of fertilizer, nevertheless the experiments are considered here to obtain a general idea.

Specht (1963) observed that even ca. 40 kg N (as $NaNO_3$) ha^{-1} killed many native plants. Higgs (1970) observed severe chlorosis in Grivellea rosmarinifolia within few weeks of application of ca. 20 g N (as KNO_3) per m^3 of sand/peat media. Thomas (1979) also observed very severe damage and death of G. rosmarinifolia after 3 months after supplying the plant with more than 450 g N (from Osmocote)

per m^3. With the same amount of N, Hakea laurina showed severe toxicity symptoms after 11 months. Groves and Keraitis (1976) found that Banksia serrata and Eucalyptus pilularis did not survive at 250 mg N (9:1 NO_3^-:NH_4^+) l^{-1} solution but survived at 25 mg N l^{-1}.

Many Australian soils are low in N. Chemical analysis showed that the sandplain soils of southern half of Australia have 0.12% N on a dry weight basis (Groves et al., 1983). Introduced agricultural species are unable to grow successfully in these soils without the addition of N. Thus most Australian plants are adapted to grow in low N soils. Application of high levels of N to such plants results in an excess availability of N causing toxic reaction. Thus these plants may tolerate and respond to only low levels of N.

More indications that to a low level supply of N, native plants do respond can be drawn from the literature. Beadle (1966) grew Angophora, Eucalyptus, Leptospermum, Melaleuca, Banksia, Hakea, Lambertia and Acacia in their own native soil with and without the addition of Hoagland (15 mM N) solution. He found that all the plants produced more leaves when nutrients were added. It indicates that nutrient deficiency occurs in the native soils although the native plants are well adapted to grow in such soils. Similarly, Moore and Keraitis (1966) obtained significant response from Grevillea robusta with increasing levels of N up to 13.5 mM. Other Australian shrubs Callistemon citrinus and Hakea laurina also showed strong responses to N from controlled release fertilizers (Thomas, 1982). Of course, there are differences in the N requirement between the species within the group of Australian native plants. For example, Grevillea robusta showed a high

requirement (120 g N per m^3 per month) whereas Hakea laurina showed a low need (50 g N per m^3 per month) (Thomas, 1979).

To avoid the adverse effects of excess N, a limited supply of N is important. Therefore, while considering the supply of N to boronia the source of N fertilizer is an important factor.

4.1. Source of N fertilizer. In addition to the two sources of fast acting conventional fertilizers which are common in Australia, viz. $(NH_4)_2SO_4$ and $Ca(NO_3)_2$, slow release N (SRN) fertilizers have been commercially available for some time. SRN fertilizers release only small quantities of N into the soil solution. Control of the solubility of fertilizer materials is commonly achieved by the use of compounds that have limited water-solubility or by altering the soluble materials to reduce their nutrient release into soil solution.

Allen (1984) listed theoretical benefits of SRN fertilizers including a lower fertilizer toxicity. The danger of causing toxicity in plants by a large single application of conventional fertilizers may be reduced by a single application of SRN or by split applications of conventional fertilizers.

The relative cost per unit of N from SRN is higher (Allen, 1984). However, boronia is a high value crop and the use of high cost fertilizers is therefore justified.

There are several SRN fertilizers: urea formaldehyde, isobytylidene diurea, Formolene and sulphur coated urea. Isobutylidene diurea (IBDU) is a popular SRN and is briefly considered here.

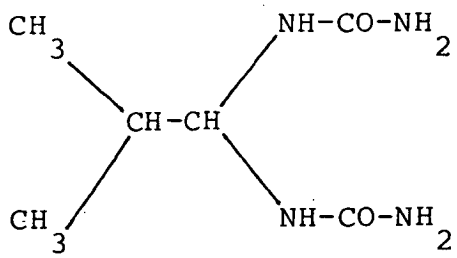


Fig. 2. Structural formula for IBDU.

Fig. 2 shows the structural formula for IBDU. IBDU is prepared by reaction of urea and isobutyraldehyde. IBDU contains 31% N. IBDU is very insoluble in water but once dissolution begins, hydrolysis proceeds rapidly with the regeneration of original reactants. Soil transformations of the product of IBDU hydrolysis, viz. urea, are identical to the transformations of urea from any other source (Allen, 1984).

The determination of whether to use a SRN or a conventional fertilizer in single or split applications should take into consideration that N requirement of plants may be greater at certain times during their growth cycle. A restriction on N availability at a growth stage when the plant requires N may have an adverse effect on the yield. Therefore the time of application of N is an important factor.

4.2. Time of N application. Greater efficiency of the utilization of N can be achieved by timely application of N, i.e. applying it when the plant needs it. In boronia, two distinct phases of growth and development that may be influenced by N are vegetative growth and flowering. The time of application of N has been found to affect flowering, in addition to vegetative growth, in some woody

plants.

In apple, Delap (1967) found that compared to an application of N in spring, application in summer or autumn increased the flower bud production. Hill-Cottingham and Williams (1967) also noted that a summer application of N induced more flowers in the following spring and if applied in spring, N promoted shoot elongation which competed with the development of flower buds. Although apple is deciduous, similar knowledge of the influence of N on flowering in evergreen boronia will raise a possibility of developing methods for increased flower yield.

To aid in predicting N requirements at different stages of plant growth, plant analysis is a useful technique.

5. DIAGNOSIS OF N DEFICIENCY AND SUFFICIENCY

To know if a plant is receiving an optimal supply of N, tests have been devised to assess the plant N status. The physiological basis of these tests is that if all the environmental factors except the supply of N are optimal, then the plant growth will be a function of the supply of N. Increased supply of N is accompanied by an increase in the uptake and concentration of N in the plant tissue and usually results in an increased plant growth (Bouma, 1983).

To use plant analysis tests in assessing the N requirement of a plant, first a relationship between N concentration in the plant tissue and the yield is established and then this relationship is used for the comparison. The relationship between the yield and the N concentration in the plant tissue is often curvilinear and a generalized curve is shown in Fig. 3.

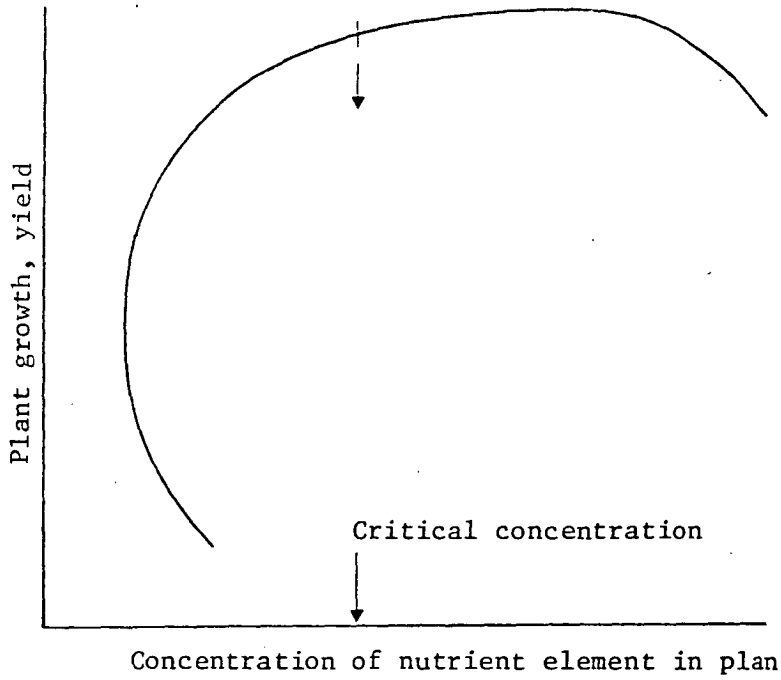


Fig. 3. Generalized curve showing the relationship between yield concentration of N in plant tissue (adapted from Bouma, 1983).

The concentration of N which is just sufficient or just deficient for maximum yield is defined as the critical concentration.

Each crop requires an extensive study in order to establish the critical values of N for that crop. Critical values of N have been widely published for many different crops (e.g. Walsh and Beaton, 1973; Jones, 1985) and these values are used to assess N requirement of the crops. There is a need to establish the critical values of N for boronia. To establish the critical values of N for a crop, generally glasshouse or field experiments are conducted in which increasing levels of N are applied to the crop and the concentration of N in the plant tissue is determined at different stages of plant growth and the subsequent crop yields are related to these concentrations of N.

In addition to the time of sampling (which varies with the crop), the plant part sampled for diagnosis of N status of the crop is

also important. Many plant tissues are used for diagnosis including roots, stem, leaves (lamina, petiole or midrib), seed and fruit. In general, the changes in N concentration are greater in the leaves than in the other organs as the leaf is the principle site of metabolism. Leaves are also easy to sample.

6. N ASSIMILATION

An application of N at 100 kg ha^{-1} caused toxicity in boronia (Menary, R. C., personal communication). Such levels of N are not high for agricultural plants. Toxicity due to the supply of moderate levels of N has been reported for other Australian native plants also (as discussed previously). However the physiological basis of this toxicity has not been examined.

Because of the application of NO_3^- itself or oxidation of NH_4^+ to NO_3^- in the cultivated soils over time even when NH_4^+ is applied (without adding nitrification inhibitors), the major form of N available to the plants will be NO_3^-. Therefore it is important to understand the mechanisms of NO_3^- assimilation in boronia to help identify the limiting factor that is involved in N toxicity.

To gain an appreciation of the processes in NO_3^- assimilation, the relevant literature on higher plants is considered here.

After absorption, utilization of NO_3^- by plant is influenced by

NO_3^-
1. accumulation in roots
2. reduction in roots
3. transport to leaves -----> 1. accumulation in leaves
2. reduction in leaves

(Huffaker and Rains, 1978).

The pathway for reduction of NO_3^- is as follows (Beevers and Hageman, 1973).

$$\underset{NADH}{NO_3^-} \xrightarrow{NR} \underset{NAD\ 6Fd_{red}}{NO_2^-} \xrightarrow{NiR} \underset{6Fd_{ox}}{NH_3}$$

The two enzymes involved are nitrate reductase (NR) and nitrite reductase (NiR). NR catalyzes the reduction of NO_3^- to NO_2^- by reduced pyridine nucleotide. NR is emphasized here, with differences in NO_3^- assimilation between plant species.

6.1. Kinetic characteristics of NR

One enzyme characteristic which may be of particular importance is enzyme-substrate affinity or K_m. Kinetic properties of NR enzyme from many cultivated plants indicate a K_m for NO_3^- of 200 µM (Beevers and Hageman, 1983).

Lee and Stewart (1978) determined the kinetic properties of NR from a range of plants that included those from NO_3^- deficient habitats (e.g. Elymus arenaria, Deschampsia flexuosa) and those from NO_3^- rich habitats (e.g. Poa annua, Chenopodium album). From their results it is evident that K_m values of NR from the plants from NO_3^- poor habitat are not markedly different from those reported for other plants. They all fall in the range of 100 to 300 µM.

Thus it seems that plants from contrasting habitats possess NR enzyme with similar kinetic characteristics. Therefore it is likely that NR in boronia has similar kinetic characteristics.

However, the quantitative level of the enzyme is another factor which will determine the capacity of a plant to assimilate NO_3^-.

6.2. Level of NR activity

There are major differences between plant species as regards the maximum possible level of NR activity. However a comparison of absolute values of NR activity can only be made with certain reservations, because (1) the activity is dependent on the experimental methods, (2) the activity is influenced by the site environmental factors such as temperature and light and (3) the enzyme is a substrate induced, so the extent of induction must be noted. Nevertheless the levels of NR activity in different species are used here for a general survey, so that the result will give an indication of the potential of the different plants to utilize NO_3^-.

From the levels of NR activity, a generalized relationship can be made between the NR capacity of plants and the availability of NO_3^- in the regime where they naturally occur.

High levels of NR activity are found in cultivated plants such as barley (>7 µmol NO_2^- g^{-1} fresh weight h^{-1}, Barneix et al., 1985), which are bred for high utilization of NO_3^- fertilizers.

Equally high levels of NR activity are found in plants characteristic of wasteland soils in which NO_3^- supply is high. In these plants, levels of activity of 15 µmol NO_2^- g^{-1} fresh weight h^{-1} can be found. These plants include Chenopodium album (Austenfeld, 1972; Al Gharbi and Hipkin, 1984), Anthriscus silvestris (Janiesch, 1973), Urtica dioica (Havill et al., 1974; Al Gharbi and Hipkin, 1984), Arabidopsis thaliana, Calystegia sepium, Galinsoga ciliata, Solanum nigrum (Al Gharbi and Hipkin, 1984).

Very low levels of NR activity are found in some plants whose natural occurrence is restricted to soils with low NO_3^- production. Typical representatives of this group are certain species of Ericaceae (Routley, 1972; Smirnoff *et al.*, 1984). In several species (*Vaccinium myrtillus*, *V. vitis-idaea*, *V. oxycoccos*, *Erica tetralix*, *E. cinerea*, *Andromeda polifolia*), Havill *et al.* (1974) measured no NR activity in the field before and only negligible activity (<0.1 µmol NO_2^- g^{-1} fresh weight h^{-1}) after supplying NO_3^- to the plants. Plants from grassland (*viz.* *Deschampsia flexuosa*, *Festuca ovina*, *Juncus squarrosus* and *Nardus stricta*) where NO_3^- is present in small quantities showed low NR activities (Atkinson, 1985). Smirnoff *et al.* (1984) found consistently low NR activities in Proteaceae also (generally less than 0.2 µmol NO_2^- g^{-1} fresh weight h^{-1}). These species also include Australian native plants *viz.* *Banksia collina*, *B. erucifolia*, *Grevillea alpina* and *Hakea epiglottis*.

As the level of NR activity seems to reflect the native habitat of the plants, a low level of NR activity may be expected in boronia because the natural occurrence of boronia is on the soils with probably little NO_3^- production (as discussed previously).

All the above mentioned results of NR levels are for leaf tissue. Care should be taken in interpretation of the results on NR activity data for the leaf tissue alone because a low level of leaf NR activity by itself is not a proof of a low capacity of the plant to utilize NO_3^- as roots might have a greater NR activity. Therefore the site of NO_3^- reduction in plants is considered here.

6.3. Sites of NO_3^- reduction

Reduction of NO_3^- can occur in both the roots and the shoots of plants. NO_3^- reduction has been examined in the roots and leaves of several species and there are differences between plants as to where NO_3^- is reduced: whether in the roots or in the shoots or in both the roots and the shoots. Thus, three groups of plants are recognized.

In some plants, NR is largely restricted to the shoot, i.e. leaves: Borago, Xanthium pensylvanicum (Wallace and Pate, 1967), cucumber (Olday et al., 1976), cotton (Radin, 1977). Therefore, all these plants transport most of the absorbed NO_3^- to the shoot.

Most annual and perennial herbaceous plants reduce NO_3^- both in their leaves and roots, e.g. barley (Aslam and Huffaker, 1982).

Some plants have high root NR activity, e.g. Raphanus (Pate, 1973), Lupinus (Atkins et al., 1979).

Compared to the information on these herbaceous plants, only limited information is available about partitioning of NO_3^- reduction between the roots and the leaves of woody plants (like boronia). In xylem sap studies on some woody plants such as apple and other Rosaceous species, very little NO_3^- was found in their xylem sap and therefore woody plants are considered to reduce most of NO_3^- in their roots (Bollard, 1957).

Further, almost all the studies on the distribution of NO_3^- reduction between the root and the shoot have been carried out on cultivated plants. For plants growing under natural or seminatural conditions, data are available on NR activity in the leaf alone. In the leaves of several woody plants, Smirnoff et al. (1984)

measured NR activity and therefore they suggested that NO_3^- reduction can occur in the leaves of woody plants also. However, because of the consistently low NR activities in the leaves of Ericaceae, Proteaceae and Gymnosperms (which occur naturally in NO_3^- poor soils), Smirnoff et al. suggested that NO_3^- reduction may predominate in the roots in these plants. Therefore it is important to know the extent of NR activity in the roots of boronia while studying its NO_3^- reduction capacity.

Some work has shown that the extent to which the plant parts reduce NO_3^- is variable and is dependent on the external NO_3^- concentration. Recently Andrews (1986) correlated the predominance of root or shoot NO_3^- reduction to the environment. Temperate plants carry out most of their NO_3^- assimilation in the roots when growing in the low external NO_3^- concentrations that are likely to occur under the natural conditions or in the nonagricultural soils, but as the external NO_3^- concentration increases to the range found in the fertilized agricultural soils, shoot assimilation becomes important. In the case of tropical/subtropical plants, shoot is the major site of NO_3^- assimilation and the partitioning between root and shoot remains constant regardless of the external NO_3^- concentration.

It is worth noting that most data for NR activity are available for external NO_3^- applied at the concentrations of 1 to 20 mM or up to 40 mM (data from Andrews, 1986). In the natural soils, NO_3^- commonly occurs at the concentrations of 1 mM or less (Russell, 1973). The general agricultural practice of NO_3^- supply is by making a single application of fertilizer. Under such conditions the roots

of plants can be exposed to NO_3^- concentrations extending from 0.00143 to 1430 mM (Clement et al., 1978). The roots experience the upper end of the concentration when the fertilizer is applied to the soil and this concentration declines over time due to plant uptake.

6.4. NiR enzyme

NiR converts the 6 electron reduction of NO_2^- to NH_3. Compared to the work on NR, much less work has been done on NiR. NiR from the leaves has been extensively purified and shown to be dependent upon reduced ferredoxin as reductant (Beevers and Hageman, 1983). Measurement of NiR activity is important to see whether this enzyme is limiting NO_3^- assimilation in boronia. It is reported that generally NiR is not a rate limiting enzyme and also NO_2^- does not accumulate in the plant tissues unlike NO_3^- which accumulates under the conditions of excess NO_3^-.

7. RESPONSES TO NO_3^- EXCESS

Application of NO_3^- fertilizers to cultivated soils results in a temporary oversupply of NO_3^- to levels in excess of that in natural environments. In general, tolerance of most plants to an oversupply of NO_3^- seems to be high. Many herbaceous plants accumulate NO_3^- without any toxic effect (Maynard and Barker, 1971). Nonetheless, high levels NO_3^- can be toxic to the plants although the exact mechanism of toxicity is unknown as pointed out by Barker and Mills (1980). The level at which NO_3^- may become excessive depends on the plant species (e.g. 2 mg NO_3^--N g^{-1} dry weight in leaf lettuce vs. 4 mg NO_3^--N g^{-1} in spinach, Maynard and Barker, 1971). Further, in

contrast to herbaceous plants, no report on accumulation of NO_3^- in woody plants has been found and therefore woody plants usually do not seem to store NO_3^-.

III. GENERAL MATERIALS AND METHODS

The methods that are common to more than one experiment are described in this chapter to prevent repetition whereas the methods that are specific to a particular experiment are described in the methods section of the relevant chapter.

CHEMICAL ANALYSES

The analytical procedures for inorganic elements were adapted from Allen (1974) and AOAC Methods (1980) to suit the requirements and facilities on hand. The main procedures are described here. AR grade reagents were used in all the chemical analyses.

pH. pH was measured by a T.P.S. Auto pH meter or a Corning pH meter 155.

Analysis of N in nutrient solutions

Samples of the nutrient solutions were usually analyzed immediately after their collection. However, sometimes the samples were stored at 5^{0}C before the analysis.

NH_4^+. A 20 ml sample was transferred to a Tecator Kjeltec digestion tube and ca. 0.2 g of an alkaline reagent, MgO was added. NH_3 was distilled in a Tecator Kjeltec System 1002 Distilling Unit. A 50 ml distillate was collected in a flask containing 10 ml of 4% H_3BO_3 combined with bromocresol green-methyl red indicators and titrated with 0.1 N HCl to a pale neutral end point using a piston burette. NH_4^+ was estimated as 1 ml 0.1 HCl = 1.4 mg NH_4^+.

NO_3^-. After collecting the distillate for NH_4^+ determination, ca. 0.4 g Devarda's alloy was added to the digestion tube to reduce NO_3^- to NH_3. NH_3 was distilled and titrated in the same way as

described for NH_4^+ analysis.

Elemental analysis of plant material

Preparation of leaf samples

Fresh leaf samples were rinsed in distilled water to remove any surface contamination, dried in a forced-draft electrical oven at 65^{0}C for 48 h to stop enzymatic changes and ground in a hammer mill to ensure greater uniformity. The ground material was placed in a sealed screw-capped bottle and stored in a cold room at 5^{0}C until the chemical analysis was carried out. Prior to analysis the powder was dried at 65^{0}C for 2 h to remove any moisture in it.

Determination of N. Total N was estimated by a semimicro Kjeldahl method. 100 mg of dry ground sample was weighed into a Tecator Kjeltec digestion tube and a Kjeldahl catalyst tablet (Na_2SO_4, Se) and 5 ml conc. N free H_2SO_4 (with salicylic acid) were added. The tube was placed in an Al block (similar to that of Faithfull, 1969) and heated gently by a hot plate until frothing subsided. Then a Kjeldahl flask was placed neck downward in the mouth of digestion tube to aid the acid to reflux down the tube walls and then the heat was increased. The digestion was continued until the solution became clear and then 30 min longer. On completion of the digestion, the digestion tube was cooled outside the block until it was just warm and then diluted with 50 ml water to avoid the precipitation of sulphate.

To the aliquot of the sample digest, 20 ml of 40% NaOH was added to make the contents strongly alkaline and NH_3 in the solution was steam distilled in a Kjeltec System in the same way as for NH_4^+ determination in the nutrient solution samples.

Determination of P, K, Ca and Mg. For determination of P, K, Ca and Mg, plant samples were wet ashed.

Wet ashing. For rapid analyses, small samples were wet digested in test tubes on Al blocks in a way similar to that of Smith and Johnson (1974). 0.2 g of the prepared leaf sample was weighed into a test tube and 7 ml of 5:1:1 mix of conc. H_2SO_4:70% $HClO_4$ was added. The tubes were placed in the holes in the Al block and after letting them stand overnight (to avoid excessive frothing during the subsequent digestion), the block was heated by a hot plate to oxidize the organic matter leaving the inorganic ions in the solution. After completion of the digestion, the tubes were removed from the block and cooled. The digest was diluted to 20 ml with water, shaken and allowed to settle overnight. 5 ml of the solution from the top was transferred to a test tube and diluted to 25 ml with water. The diluted solution was analyzed for individual elements as described below.

Phosphorus. Colorimetry was used for the determination of P. A suitable aliquot (normally 5 ml) of the digest solution was taken and diluted to 15 ml, 2.5% ammonium molybdate in 28% H_2SO_4 was added to it and mixed for the formation of heteropolyphosphomolybdate complex. 2 ml of $SnCl_2$ in 2% HCl was added, mixed for reduction of the complex (which gives blue color) and diluted to 25 ml. It was left for 30 min and the absorbance was read at 700 nm using a Hitachi 101 spectrophotometer fitted with a sequential sampler. The amount of P in the sample solution was determined from a curve made of the standards ranging from 0 to 30 µg P.

Because of the low P concentration in boronia leaf tissue, the

molybdovanadophosphate method of P determination was found to be less sensitive in a preliminary determination.

Calcium. Ca was determined by atomic absorption spectrophotometry using a Unicam SP1900 AAS. Normally 10 ml of the digest solution was diluted to 20 ml and aspirated. The dilution also included a releasing agent, 400 mg La in 1% H_2SO_4 l^{-1} to control potential P and Al interference. The concentration of Ca in the sample solution was determined from a calibration curve prepared by using standards from 0 to 10 mg Ca l^{-1}. The standards also contained same concentrations of La and H_2SO_4 as used in the sample solutions.

Magnesium. Mg was determined by atomic absorption spectrophotometry in the same way as Ca determination. Usually 1 ml aliquot of the digest solution was diluted to 20 ml. The calibration curve was prepared using 0 to 500 µg Mg l^{-1} standard solutions.

Potassium. K was determined by flame photometry using an EEL 100. Normally 2 ml of the digest solution was diluted to 20 ml (the dilution included 1% H_2SO_4) and aspirated. A calibration curve of a range from 0 to 10 mg K l^{-1} was prepared to determine the concentration of K in the sample solution.

Determination of NO_3^- in plant tissue

NO_3^- in the plant tissue was determined by Woolley et al. (1960) procedure of using Bray's reagent in which powdered Zn reduces NO_3^- to NO_2^- which reacts first with H_2SO_4 and then with a-naphthylamine subsequently forming an azo-dye, all in the same reaction mixture. The color was measured using a Hitachi 101 spectrophotometer. A standard curve from 0 to 2.5 µmol NO_3^- was made.

Determination of NO_2^- in plant tissue

NO_2^- was determined by the Griess-Ilosvay colorimetric method. To the suitable aliquot or a solution diluted from the aliquot containing a concentration within the range of calibration curve, 1 ml of sulfanilamide (1% w/v in 1.5 HCl) was added, shaken, 1 ml of N-1-naphthyl ethylenediamine diHCl (0.02% w/v) was added and shaken. After 30 min the absorbance of the color produced was measured at 540 nm using a Hitachi 101 spectrophotometer. NO_2^- was determined from a calibration curve ranging from 0 to 60 nmol NO_2^-.

Determination of protein

Protein was determined by the Bio-Rad protein assay based on the principle of protein-dye binding (Bradford, 1976). Bovine plasma albumin was used as a protein standard and the standard assay procedure was used. The absorbance was measured using a Pye Unicam SP 8-200 UV/VIS spectrophotometer.

STATISTICAL ANALYSES

Statistical procedures followed were those of Steel and Torrie (1980) and Gomez and Gomez (1984).

III.1. ANALYSIS OF AMINO ACIDS IN PLANT MATERIAL BY REVERSE-PHASE HPLC

In the course of work on N metabolism in boronia, a method for analysis of free amino acids became necessary. Chromatographic techniques are the basis of most amino acid analyses. A perusal of the literature on the analysis of amino acids indicated that most methods for analysis of amino acids involve automatic amino acid analyzers.

High performance liquid chromatography (HPLC) is used to analyze a variety of biological compounds. However the potential of HPLC for the analysis of amino acids has not been exploited fully. The development of automatic amino acid analyzers seems to have reduced the significance of all other chromatographic techniques that can also be used for analysis of amino acids. Use of HPLC for analysis of amino acids is a promising approach because of the speed, automation and low detection limits possible with it. Therefore, HPLC was used in an attempt to analyze amino acids in boronia plant tissue.

Initial attempts to analyze underivatized free amino acids by reverse-phase HPLC (as reported by Hancock *et al.*, 1979) at a detection wavelength of 214 nm were unsuccessful. It was thought that the problem may have been caused by the low sensitivity at such a wavelength due to the low UV extinction coefficients of most amino acids. To give them higher extinction coefficients which will allow their detection at a higher wavelength and thus with a higher sensitivity, amino acids can be derivatized. The well known derivatizing reagents are dinitrophenyl (DNP), phenylthiohydantoin

(PTH), orthophthaldehyde (OPA) and dansyl chloride (DNS). Among derivatizations with these reagents, DNP-derivatization is attractive because it eliminates the step of cation exchange process generally required to purify the amino acids from sugars and salts which are also present in the amino acid extracts from the plants. DNP-derivatized amino acids are soluble in organic solvents and thus can be extracted from sugars and salts into ether.

Thin-layer chromatography (TLC) was popular for separation of DNP-amino acids. However there have been only two reports on the separation of DNP-amino acids by HPLC. Zimmerman and Pisano (1977) separated DNP-amino acids using a Zorbax-ODS column, but the need to control the temperature (62^0) of column is a drawback in their method when such facilities are not available. Kozukue *et al.* (1982) separated DNP-amino acids using a LiChrosorb RP-18 column at room temperature, but they separated only 14 amino acids, used methyl benzoate as an internal standard and their chromatogram shows that peaks were not resolved completely from neighboring peaks, which will pose a problem in determining peak areas.

Recent developments in HPLC include small columns with small particles to improve the resolution of compounds. In the present analysis of amino acids, a 5 μ C_{18} cartridge was used and a clear separation was obtained. Further, quantitation of the amino acids was done using calibration curves without the use of an internal standard. The method is described here.

MATERIALS AND METHODS

Extraction. Extraction of amino acids from plant tissue was based

on the method of Bieleski and Turner (1966). 500 mg of fresh plant tissue was placed in 10 ml methanol-chloroform-water mix of 12:5:3 v/v (MCW), immersed in liquid N_2 to stop enzymatic activity, removed and stored at -20^{0}C until analyzed.

The tissue was homogenized using an Ultra-Turrax. The homogenate was centrifuged at 1200 X g for 10 min (MSE Super Minor) and the supernatant was collected. To the residue, a further 10 ml of MCW mix was added, mixed (on Vortex), centrifuged and the supernatant was added to the first supernatant. For maximum removal of amino acids, the residue was extracted a further 4 times in the same way with 10 ml portions of 80% v/v aqueous ethanol and the supernatants were combined. To the MCW supernatant, 5 ml chloroform and 7.5 ml water were added, centrifuged and the top water-alcohol fraction was added to the ethanol supernatants and the bottom chloroform fraction (of pigments and lipids) was discarded. The combined extract was dried under vacuum at 35^{0}C on a rotatory evaporator.

Dinitrophenylation. Dinitrophenylation and extraction of DNP-amino acids were done in the same way as for TLC (Pataki, 1969). DNP-amino acids decompose in the light, therefore dinitrophenylation was done in the absence of light and subsequently formed DNP-amino acids were protected from the light by wrapping their glassware with Al foil.

The plant tissue extract was taken in 5 ml of carbonate buffer (8.4 g $NaHCO_3$ + 2.5 ml 1 N NaOH made up to 100 ml with H_2O) and 100 ul 2,4-Dinitroflurobenzene (DNFB, Sigma) was added, shaken at 40^{0}C for 3 h. Then the excess DNFB was removed by extracting it 5 times with a 10 ml portion of diethyl ether each time.

Extraction of DNP-amino acids. The remaining aqueous fraction was carefully acidified by adding 500 µl of 6 N HCl and the DNP-amino acids were extracted with diethyl ether until the ether no longer became colored. This extraction usually required 3 to 5 X 10 ml portions of diethyl ether. The ether portions were combined and the ether was evaporated to dryness in vacuo at 30°C. The residue was taken in 5 ml methanol (LC grade) and was filtered through a Swinny filter using a Millipore FH type filter paper.

A kit of DNP-amino acid standards, except glutamine and threonine, was obtained from Sigma. Each standard amino acid was prepared by dissolving 1 mg in 1 ml of methanol. DNP-glutamine and DNP-threonine standards, which were not in the kit, were prepared. 10 mM of the free amino acid and 2 g of anhydrous Na_2CO_3 in 40 ml water were mixed with 10 mM of DNFB in the form of 10% acetone solution. Procedures for dinitrophenylation and extraction of DNP-amino acids were same as for the plant amino acids. The dried residue was taken in methanol and was diluted to obtain 1 mg ml^{-1}. A standard mixture was prepared by mixing all the amino acids.

Chromatography. The chromatograph used was a Waters ALC-200 Series equipped with two Model 6000A pumps (to generate a solvent gradient), a Model 440 detector and a Model U6K injector. The column was a 5 µ Nova-Pak C_{18} Radial-Pak cartridge, 8 mm ID X 10 cm and radial compression was applied to the cartridge by a RCM-100. Normally a 20 µl sample was injected with a Hamilton microsyringe. The mobile phase was 20% (solvent A) and 75% (solvent B) v/v acetonitrile in 1% v/v glacial acetic acid in water. Glacial acetic acid was added to the solvent because it improves the

separation of DNP-amino acids in TLC (Brenner et al., 1965). Acetonitrile and acetic acid were of LC standard and water was filtered through a 0.45 μm Millipore filter paper (HA type). The mobile phase was degassed by ultrasonication. The DNP-amino acids were eluted in a gradient mode with a Model 680 AGC from 100% solvent A to 100% solvent B over 60 min by curve No. 6 (linear curve) at a flow rate of 1 ml min^{-1}. The amino acids were detected at a wavelength of 254 nm. Output signal from the detector was recorded on a recorder (OmniScribe). The recorder sensitivity was 0.2 and the chart speed was 20 cm h^{-1}. After 60 min the column was flushed with solvent B at a flow rate of 2 ml min^{-1} for 10 min followed by a reversed gradient at 1 ml min^{-1} for 5 min and then equilibrated with solvent A at 1 ml min^{-1} for 15 min. This equilibration prior to next injection was necessary, because otherwise it was observed that some impurities concentrated on the column during a run were eluted in the subsequent run. Sigma 10 B system was used to collect peak area data.

RESULTS AND DISCUSSION

DNFB reacts quantitatively with α-amino groups of amino acids to form DNP-amino acids. The reagent also reacts with ε-amino group of lysine and phenolic hydroxy group of tyrosine and thus lysine and tyrosine are recovered as di-DNP derivatives.

Fig. 4 shows a chromatogram obtained with standard amino acids mixture. The complete mixture was resolved in 60 min. All amino acids showed good separation except leucine and isoleucine which came together. There was sufficient resolution between the amino acids. This resolution was a significant improvement over that of

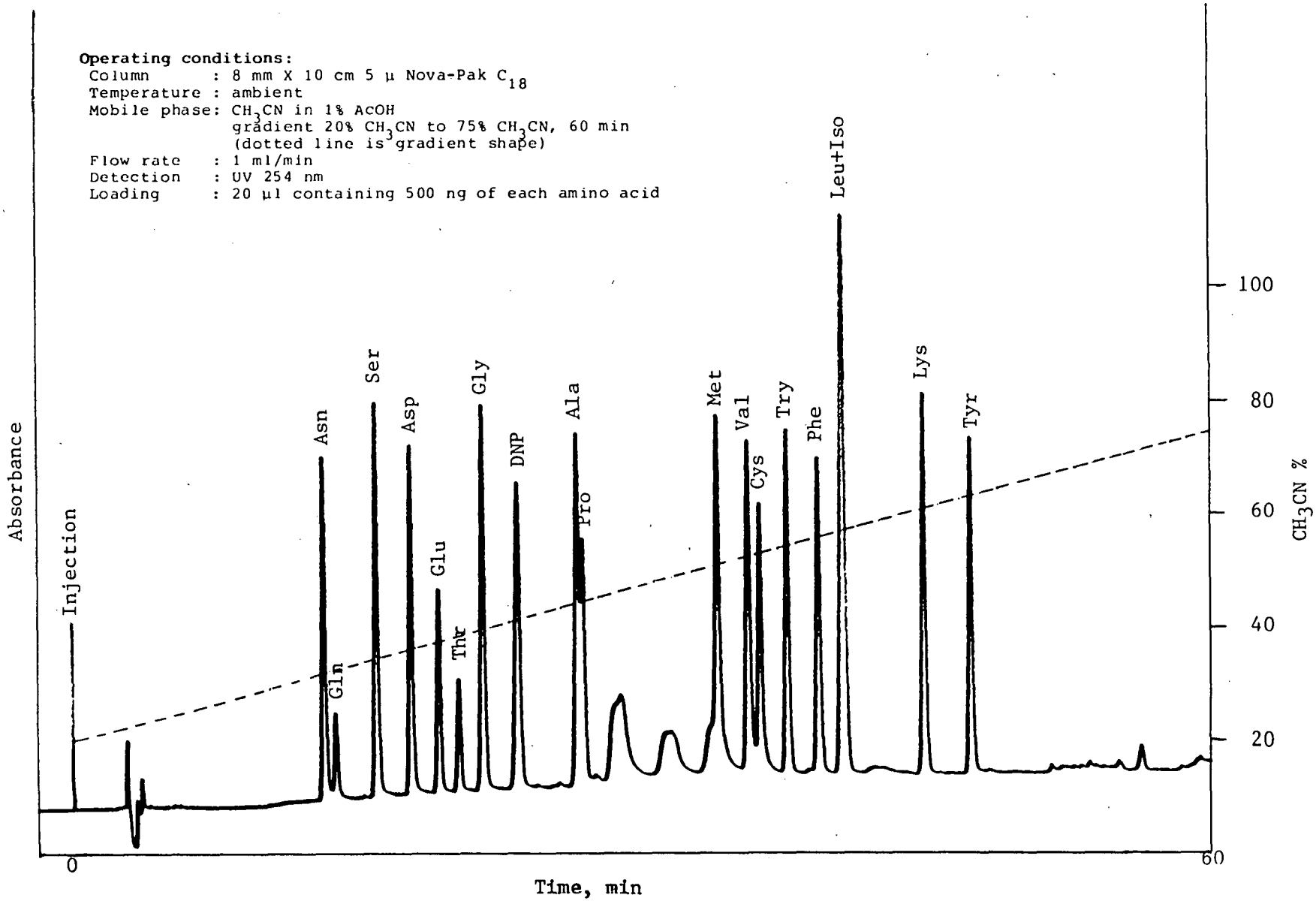


Fig. 4. Chromatogram of standard DNP-amino acids mixture.

Kozukue et al. (1982). This difference may be related to the column performance. They used a LiChrosorb column whereas in the present method a Nova-Pak cartridge was used. There are such examples in the literature as to the better column for the separation of a particular sample. In the separation of amino acids derivatized with OPA, μBondapak fatty acid analysis column gave better results than Altex Ultrasphere ODS column (5 μ) or μBondapak C_{18} (Larsen and West, 1981). Similarly in the separation of DNS-amino acids, only Brownlee RP-300 (10 μ) gave good separation among other columns, viz. Brownlee RP-8 (10 μ), RP-18 (5 & 10 μ); Partisil-10 ODS-2 and ODS-3; Zorbax ODS and Ultrasphere-ODS (5 μ) (DeJong et al., 1982).

The peaks were identified in on-line mode by peak enhancement technique, that is the amount of a known amino acid in the standard amino acid mixture was increased and that particular amino acid peak on the chromatogram was identified by the increased peak.

For quantifying the amino acids, a standard curve of peak area vs. concentration for each amino acid was used. Linear regression analysis over a concentration range of 250 ng to 1 μg gave correlation coefficients (R^2) of >0.93 for all amino acids except for methionine for which R^2 was 0.75 (Table 5).

A chromatogram of the amino acids extracted from the leaf tissue of boronia is shown in Fig. 5. The resolution was good for most amino acids in the plant sample also. There were some unknown peaks in the plant sample.

This method shows that HPLC can be used for efficient analysis of amino acids. Because of the simplicity, analysis of DNP-amino acids by HPLC may gain the same popularity as TLC analysis of DNP-

Table 5. Correlation coefficients of linear regression of peak area *vs.* concentration of standard DNP-amino acids. The concentration range was 250 ng to 1 µg.

Amino acid	Correlation Coefficient (R^2)
Asn	0.97
Gln	0.95
Ser	0.96
Asp	0.95
Glu	0.95
Thr	0.95
Gly	0.94
Ala	0.97
Pro	0.94
Met	0.75
Val	0.93
Cys	0.94
Try	0.94
Phe	0.93
Leu+Iso	0.94
Lys	0.95
Try	0.93

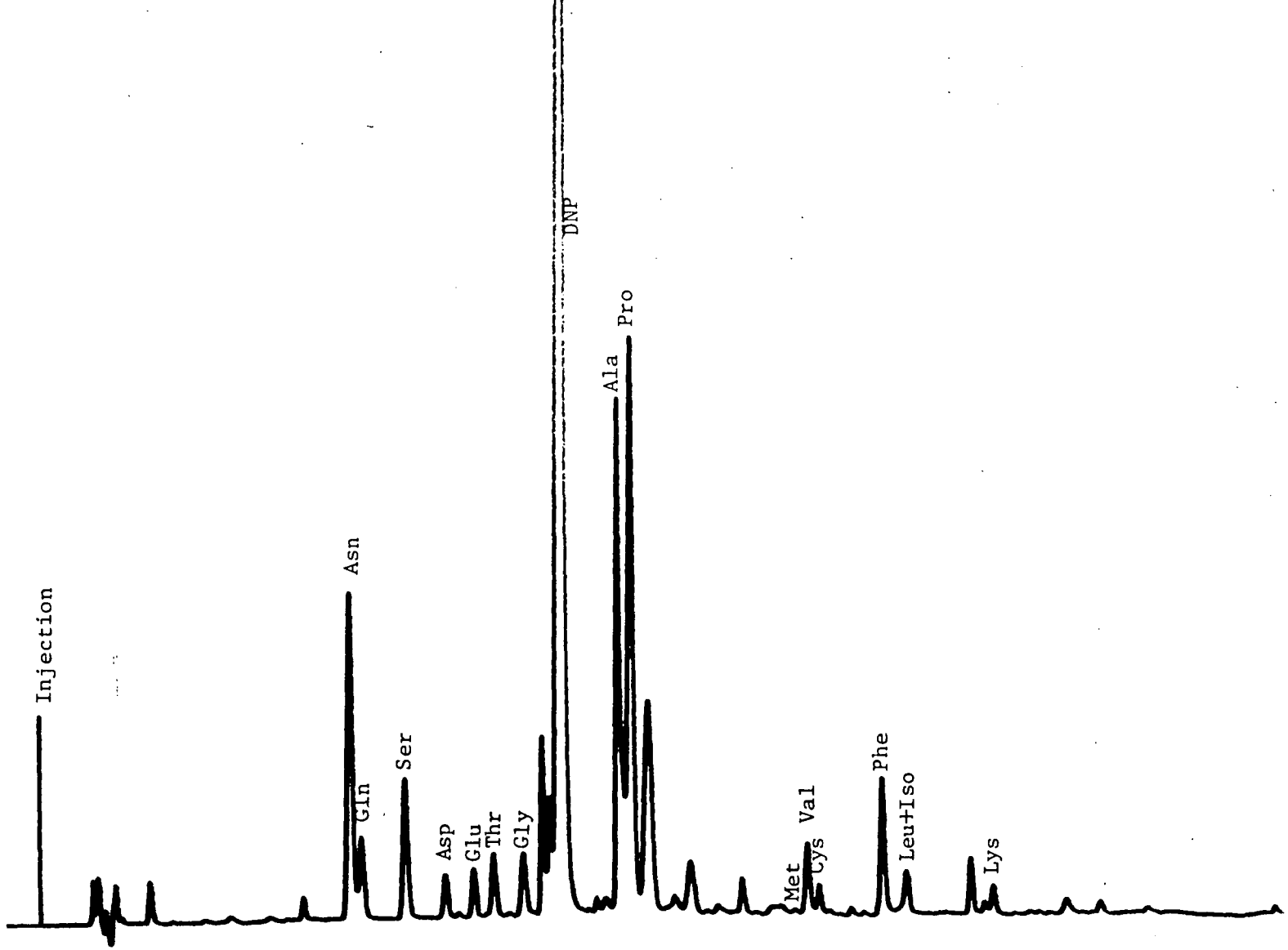


Fig. 5. Chromatogram of amino acids in boronia leaf tissue. Chromatographic conditions were the same as described in Fig. 4.

amino acids had in the past. This method can be adapted to analyze amino acids in other biological samples as well as for analyses of peptides and proteins.

IV. EFFECTS OF SOURCE, RATE AND APPLICATION TIME OF NITROGEN ON FLOWER YIELD AND LEAF NITROGEN CONCENTRATION IN BORONIA

An experiment was conducted to study the effects of fast-acting conventional fertilizers and a slow release N fertilizer on flower yield in boronia. N was applied at different rates either in a complete dose or in split doses at different stages of plant growth and development. Concentration of N in the leaf tissue at different stages of plant growth was also determined. The relationships between the flower yield and the concentration of N in the leaf tissue at different stages of plant growth were established. The experiment was conducted under field conditions to obtain a practical situation of boronia cultivation.

MATERIALS AND METHODS

Site. The experiment was conducted at Kingston in Tasmania (latitude 43^0S). The soil was sandy with a pH of 4.5 (soil:water 1:2). Prior to the experiment, the site was occupied by eucalypti and heath. P at 50 kg (as rock phosphate) and K at 100 kg (as K_2SO_4) per ha were applied.

Layout. The experiment layout was in a randomized block design. The planting rows were 1 m apart. Within each row there were treatment plots with 1 m between two plots and in each plot there were 3 plants spaced at 0.5 m. Thus there were 3 plants per m^2 equal to a density of 15,000 plants per ha^{-1}. A treatment was applied to all 3 plants in a plot but measurements were taken on the center plant only and the plants on either side of it were considered as guard plants.

2-yr old boronia plants were planted in September 1984. 3 clones viz. HC-X, HC-3 and HC-2 were used. Each clone was assigned to a separate block (replication) so that clonal variation became a part of the block variation and thus excluded from the experimental error.

Treatments. The sources of N were: two conventional fertilizers, $(NH_4)_2SO_4$ (21% N; obtained from EZ Co.) and $Ca(NO_3)_2$ (15.5% N; obtained from Hoechst) and a SRN fertilizer, IBDU (31% N; obtained from Fertool). The application rates were: 25, 50 and 100 kg ha^{-1}. The times of application were: early October (spring), mid-June (early winter) and mid-August (late winter) which correspond to the periods of vegetative growth, flower bud initiation and flower bud development respectively in boronia under local conditions. Each rate was applied either in one dose in October or split into 2 or 3 equal doses. The two doses were applied in October and June or October and August. The three doses were applied in October, June and August. The fertilizer was placed under the canopy of each plant and mixed into the soil.

Maintenance. The plants were drip irrigated with an emitter at each plant. The irrigation was given once a week from October 1984 to February 1985 and from March till September 1985, the plants were irrigated once a fortnight plus whenever it was a hot day. Weeds were controlled by wick-wiping with glyphosate and psyllids which were noticed on some plants were controlled with demeton-S-methyl.

Measurements. Fully developed leaves just below the apex of the shoots were sampled in late October 1984, mid-January, mid-July and mid-September 1985 and their total N was determined by

semimicro Kjeldahl method (General Materials and Methods, p. 40).

During the time of flowering, flowers were picked as they developed and their fresh weights were taken. These weights were combined and the yields are reported as g per plant.

After the harvest of flowers, the plants were lightly pruned and the treatments were repeated in 1985-86. The maintenance and measurements in 1985-86 were the same as in 1984-85. However, leaf N concentration values in October 1985 were not obtained.

The effects of the treatments on leaf N concentration in each sampled month and on flower yield were statistically analyzed using ANOVA and the F value was tested at P = 0.05 and 0.01. The differences between the treatment means were compared using the LSD test. Regression analysis was used to examine the relationships between the leaf N concentration in different months and the final yield. For regression analysis, the treatment means were used so that the variation between the replications did not enter into the analysis. When the R^2 increment from linear to quadratic regression was significant, a quadratic regression was fitted.

RESULTS

Within a week after the application of $(NH_4)_2SO_4$ or $Ca(NO_3)_2$ at 50 or 100 kg N ha^{-1} in October, tips of most leaves on the plants became chlorotic. Compared to the plants supplied with N, plants not given N had sparse growth and had very few lateral shoots with pale green leaves. However the characteristic leaf yellowing symptoms of N deficiency were not observed on the plants given no N.

Leaf N concentration. Leaf N concentration in different treatments

in various sampling months during 1984-85 is shown in Table 6. The leaf N concentration varied over the months, mainly due to the application of N. At each time of application there were various amounts of N, viz. 0, 8.3, 12.5, 16.7, 25, 33.3, 50 and 100 kg ha^{-1} (the last rate only in October) as a consequence of complete and split applications of N rates. These amounts were positively reflected in the leaf N concentration which was analyzed within a month of application (except in January). This shows that the leaf N concentration indicated the increased N availability in the soil. The trends were similar in 1985-86 (Table 7).

In addition to the differences in leaf N concentration in each sampling month between treatments, the pattern of change in leaf N concentration over the time is also discussed. Because of the quantitative nature of N rate treatments and a significant interaction between the source X rate of N, comparisons are made between the sources of N applied at the same rate only.

From 0 to 100 kg N ha^{-1}, the leaf N more than doubled with $(NH_4)_2SO_4$ or $Ca(NO_3)_2$ whereas it increased about 1.5 times with IBDU. When the rates of N applied were up to 25 kg ha^{-1}, there were no significant differences in the leaf N concentration in the following month between the sources of N. At higher rates there were no significant differences between $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ but the leaf N with IBDU was about 1.5 times lower. Therefore, in the month after an application, availability of N from the conventional sources was higher only at rates higher than 25 kg ha^{-1}.

The time of application also influenced the availability of N. When $(NH_4)_2SO_4$ or $Ca(NO_3)_2$ was applied in a complete dose in October,

Table 6. Leaf N concentration in different months as affected by rate, time of application and source of N in 1984-85.

N applied			Leaf N, % dry wt.			
Rate[a] kg ha^{-1}	Time of application	Source	Oct.	Jan.	July	Sept.
0			1.47	1.59	1.66	1.66
25	Oct.	$(NH_4)_2SO_4$	1.68	1.68	1.66	1.66
		$Ca(NO_3)_2$	1.82	1.56	1.61	1.56
		IBDU	1.70	1.73	1.56	1.59
	Oct.&June	$(NH_4)_2SO_4$	1.84	1.61	1.75	1.82
		$Ca(NO_3)_2$	1.54	1.61	1.70	1.61
		IBDU	1.61	1.61	1.77	1.59
	Oct.&Aug.	$(NH_4)_2SO_4$	1.87	1.61	1.66	1.89
		$Ca(NO_3)_2$	1.52	1.63	1.59	1.82
		IBDU	1.68	1.70	1.49	1.75
	Oct,June&Aug.	$(NH_4)_2SO_4$	1.52	1.63	1.70	1.75
		$Ca(NO_3)_2$	1.54	1.61	1.73	1.75
		IBDU	1.52	1.52	1.75	1.77
50	Oct.	$(NH_4)_2SO_4$	2.92	2.82	2.26	2.15
		$Ca(NO_3)_2$	2.57	2.47	2.17	2.10
		IBDU	1.98	2.12	1.96	1.87
	Oct.&June	$(NH_4)_2SO_4$	1.91	1.68	2.15	1.77
		$Ca(NO_3)_2$	1.84	1.63	2.15	1.84
		IBDU	1.80	1.80	2.03	1.94
	Oct.&Aug.	$(NH_4)_2SO_4$	1.91	1.63	1.70	2.31
		$Ca(NO_3)_2$	1.94	1.56	1.75	2.26
		IBDU	1.75	1.77	1.94	2.05
	Oct,June&Aug.	$(NH_4)_2SO_4$	1.66	1.61	2.08	2.17
		$Ca(NO_3)_2$	1.56	1.68	2.22	2.19
		IBDU	1.70	1.87	2.10	2.12
100	Oct.	$(NH_4)_2SO_4$	3.45	3.31	2.68	2.66
		$Ca(NO_3)_2$	3.34	3.10	2.64	2.50
		IBDU	2.59	2.71	2.52	2.64
	Oct.&June	$(NH_4)_2SO_4$	2.96	2.68	3.08	2.99
		$Ca(NO_3)_2$	2.71	2.47	3.15	2.99
		IBDU	1.89	2.15	2.19	2.24
	Oct.&Aug.	$(NH_4)_2SO_4$	2.80	2.75	2.12	3.08
		$Ca(NO_3)_2$	2.66	2.47	2.17	3.08
		IBDU	1.94	2.08	2.10	2.29
	Oct,June&Aug.	$(NH_4)_2SO_4$	2.33	2.05	3.00	3.13
		$Ca(NO_3)_2$	2.33	2.00	3.13	3.13
		IBDU	1.91	1.91	2.12	2.14
					LSD[b]	
		P = 0.05	0.17	0.10	0.14	0.15
		0.01	0.23	0.14	0.18	0.19

[a] Each rate was applied completely and split into 2 and 3 equal doses and applied

[b] LSD is for comparison between sources of N applied at the same rate at the same time.

Table 7. Leaf N concentration in different months as affected by rate, time of application and source of N in 1985-86.

N applied			Leaf N, % dry wt.		
Rate[a] kg ha^{-1}	Time of application	Scurce	Jan.	July	Sept.
0			1.59	1.63	1.61
25	Oct.	$(NH_4)_2SO_4$	1.75	1.66	1.63
		$Ca(NO_3)_2$	1.73	1.61	1.56
		IBDU	1.77	1.61	1.61
	Oct.&June	$(NH_4)_2SO_4$	1.66	1.80	1.80
		$Ca(NO_3)_2$	1.70	1.70	1.63
		IBDU	1.68	1.77	1.63
	Oct.&Aug.	$(NH_4)_2SO_4$	1.68	1.70	1.89
		$Ca(NO_3)_2$	1.68	1.63	1.87
		IBDU	1.68	1.54	1.82
	Oct,June&Aug.	$(NH_4)_2SO_4$	1.61	1.77	1.87
		$Ca(NO_3)_2$	1.61	1.77	1.87
		IBDU	1.56	1.80	1.77
50	Oct.	$(NH_4)_2SO_4$	2.85	2.26	2.10
		$Ca(NO_3)_2$	2.80	2.17	2.10
		IBDU	2.15	2.03	1.84
	Oct.&June	$(NH_4)_2SO_4$	1.75	2.29	1.94
		$Ca(NO_3)_2$	1.75	2.26	1.91
		IBDU	1.87	2.08	1.96
	Oct.&Aug.	$(NH_4)_2SO_4$	1.68	1.82	2.36
		$Ca(NO_3)_2$	1.75	1.77	2.26
		IBDU	1.75	1.91	2.10
	Oct,June&Aug.	$(NH_4)_2SO_4$	1.63	2.12	2.17
		$Ca(NO_3)_2$	1.61	2.19	2.19
		IBDU	1.82	2.08	2.15
100	Oct.	$(NH_4)_2SO_4$	3.24	2.73	2.64
		$Ca(NO_3)_2$	3.17	2.73	2.50
		IBDU	2.71	2.54	2.59
	Oct.&June	$(NH_4)_2SO_4$	2.75	3.08	2.99
		$Ca(NO_3)_2$	2.73	3.13	2.94
		IBDU	2.19	2.24	2.19
	Oct.&Aug.	$(NH_4)_2SO_4$	2.73	2.15	3.10
		$Ca(NO_3)_2$	2.75	2.19	3.10
		IBDU	2.22	2.10	2.26
	Oct,June&Aug.	$(NH_4)_2SO_4$	2.08	3.00	3.17
		$Ca(NO_3)_2$	2.08	3.00	3.17
		IBDU	1.91	2.19	2.29
				LSD[b]	
		P = 0.05	0.13	0.13	0.15
		0.01	0.17	0.17	0.19

a&b Same as in Table 6.

the leaf N decreased in the following months while when IBDU was applied, the leaf N concentration increased in the earlier months (January) and then decreased in the later months (July and September). These changes were more gradual with IBDU. Also with split doses applied in October, the leaf N, before the application of next split dose, decreased with $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ but increased with IBDU. However, with split doses applied in June or August the leaf N decreased in all the following months irrespective of the source of N. These results suggest that when applied in October, availability of N from the conventional sources decreased over the time but its availability increased in the earlier months and decreased in the later months. However, when applied in June or August, availability of N even from IBDU decreased in the following months.

When N was applied only in October without any further application, there were differences in the availability of N in the following months due to the source of N. By January, there were no significant differences in the leaf N concentration between the sources of N applied at 8.3 or 12.5 kg N ha^{-1} (these rates were a consequence of split doses). When applied at 16.7 or 25 kg N ha^{-1}, the leaf N differences were not significant. When applied at 50 or 100 kg N ha^{-1} in October, however, the leaf N was higher with $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ in both January and July. These results suggest that when N was applied up to 25 kg ha^{-1} in October, its availability later in January and July was higher from IBDU but at

higher rates of application, N availability was higher from the conventional sources.

Flower yield. The normal flowering period in boronia is August-September. However, the experimental plants flowered in January 1985 probably due to low temperatures that occurred in the earlier month (Appendix Fig. II.1). Nevertheless, these flowers were harvested and the data analyzed. By January the only treatment variables were different rates of different sources of N applied in October 1984. Regression analysis was done for each source of N separately. The relationships between the yield and the rates of N were quadratic with all sources of N (Fig. 6). However the N rate at which the yield was maximum differed with the source of N. It was about 1.5 times lower with $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ indicating that at lower rates of N the yield reduction was higher with the conventional sources of N.

The normal flower yield in September was affected by the source, rate and application time of N and there were interactions between source X rate, source X time and rate X time. The results of 1984-85 and 1985-86 experiments are shown in Figs. 7 and 8 respectively. The trends in both the years were similar although the yields were about 1.2 times higher in 1985-86 because the plants in the second year of the experiment were larger. Since the same rates of N were applied in both the years, residue of N applied in the first year would have contributed to the requirements of the increased plant growth in the second year.

The flower yield increased with increasing N rates (Fig.7a)

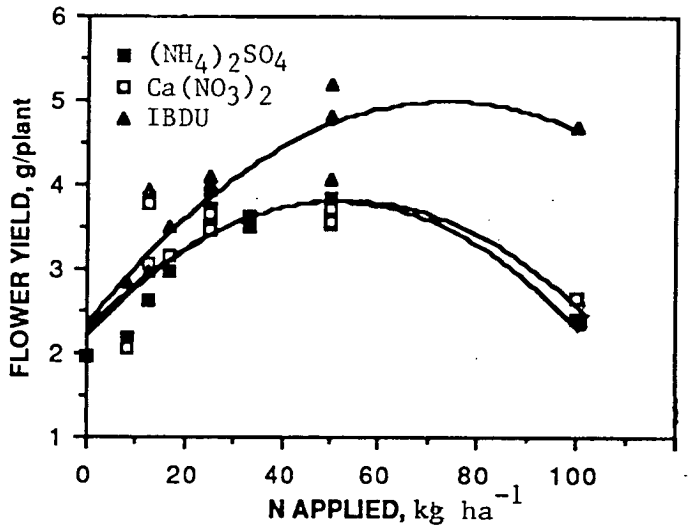
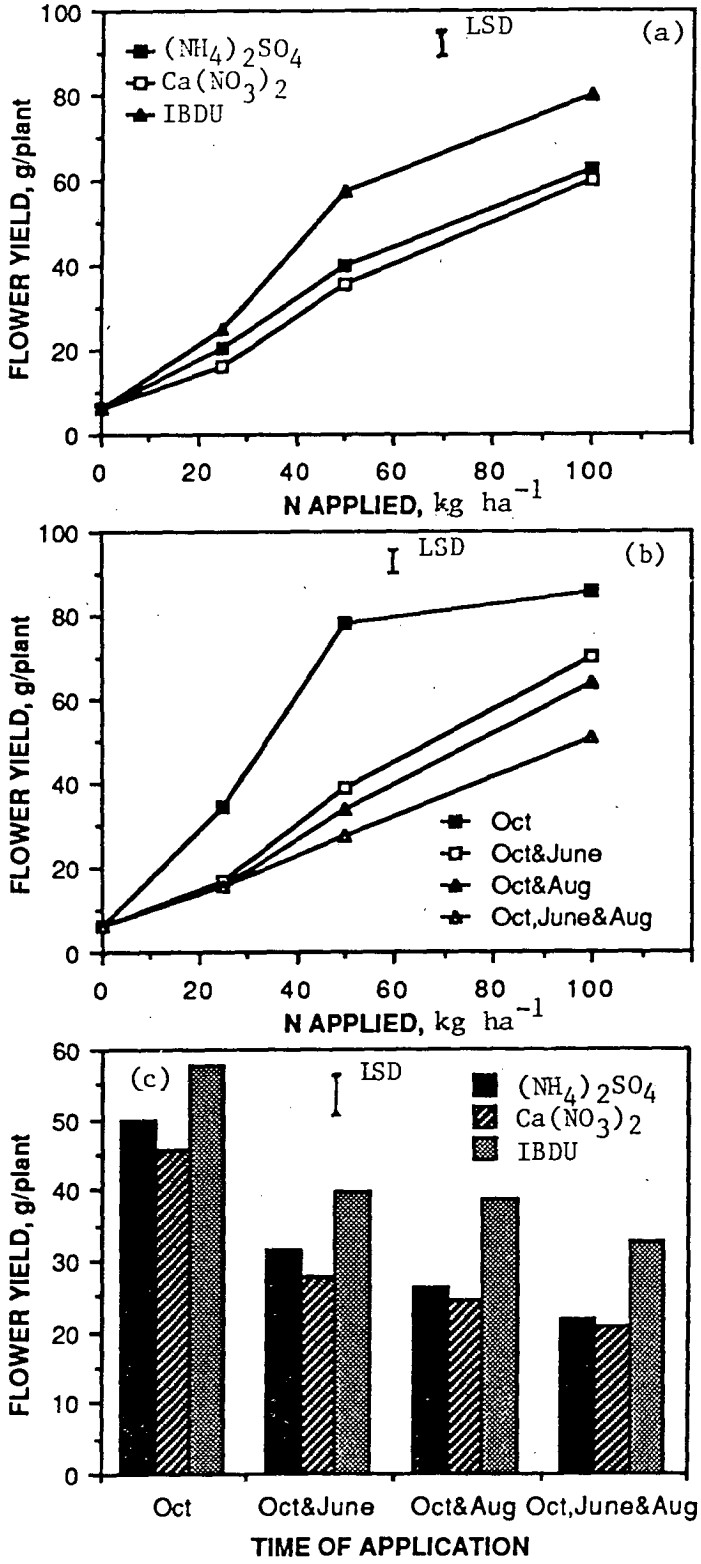


Fig. 6. Relationship between the flower yield in January 1985 and the rate of N from different sources applied in October 1984. The fitted regression equations are $(NH_4)_2SO_4$: $Y = 2.23+0.061X-0.0006X^2$ ($R^2 = 0.68$), $Ca(NO_3)_2$: $Y = 2.33+0.057X-0.0005X^2$ ($R^2 = 0.66$) and IBDU : $Y = 2.36+0.072X-0.0005X^2$ ($R^2 = 0.80$). Linear and quadratic regression coefficients in each equation are significant at $P = 0.01$. R^2 for each equation is significant at $P = 0.01$.


(a)
LSD
$(NH_4)_2SO_4$
$Ca(NO_3)_2$
IBDU
FLOWER YIELD, g/plant
N APPLIED, kg ha^{-1}
(b)
LSD
Oct
Oct&June
Oct&Aug
Oct,June&Aug
FLOWER YIELD, g/plant
N APPLIED, kg ha^{-1}
(c)
LSD
$(NH_4)_2SO_4$
$Ca(NO_3)_2$
IBDU
FLOWER YIELD, g/plant
Oct
Oct&June
Oct&Aug
Oct,June&Aug
TIME OF APPLICATION

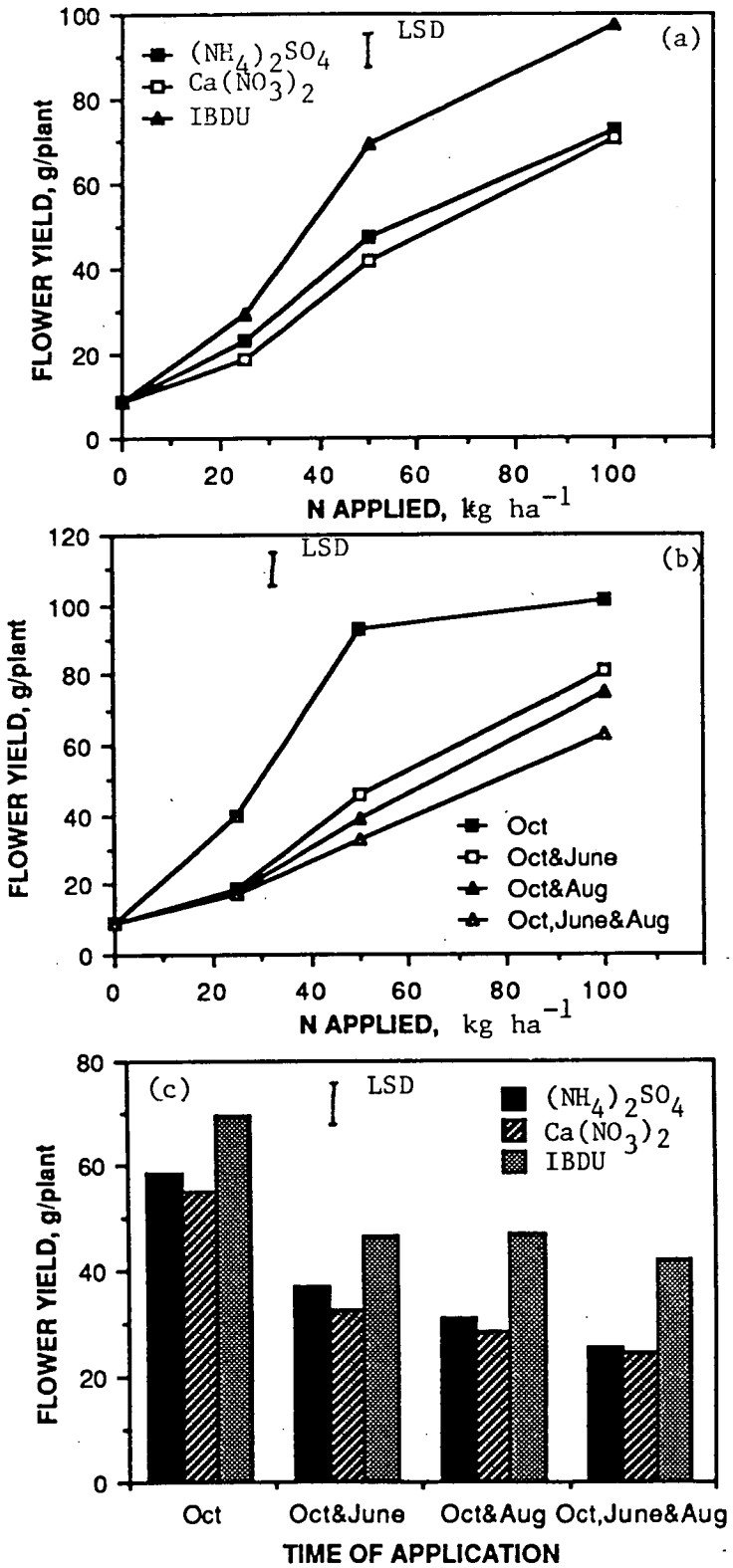


Fig. 8. Yield of flowers in September 1986 as affected by interaction between (a) source X rate, (b) time of application X rate and (c) source X time of application of N. Other details are the same as in Fig. 7.

although the increase in the yield was not proportional to the rate of N. The yield more than doubled from 25 to 50 kg N ha^{-1} but the yield increase was only 1.5 times from 50 to 100 kg N ha^{-1}. The yields with $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ did not differ significantly but the yield with IBDU was about 1.4 times higher than that with the conventional sources.

Complete doses of all N rates which were applied in October gave the highest yields and split doses of the same N rates applied at different times of the season gave lower yields (Fig. 7b). At 25 and 50 kg ha^{-1} the yields with N applied in a complete dose in October were more than double the yields with N applied in split doses. Between 2 and 3 split doses there were no significant differences in the yields at 25 kg N ha^{-1} but at 50 or 100 kg N ha^{-1} the yields were significantly lower with 3 split doses than with 2 split doses.

At all times of application, IBDU gave the highest yield and the differences in the yield between $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ were not significant (Fig. 7c). The difference in the yields between the conventional sources of N and IBDU increased at the later application times.

Relationship between yield and leaf N concentration. The January 1985 yield was related to the leaf N concentration in October 1984 and January 1985 and shown in Fig. 9. The relationships were quadratic indicating a declining yield at higher concentration of leaf N although the correlation coefficients (R^2) were poor. The concentration of leaf N at which maximum yields in January 1985 were obtained were estimated as 2.37% in October 1984 and 2.31% in

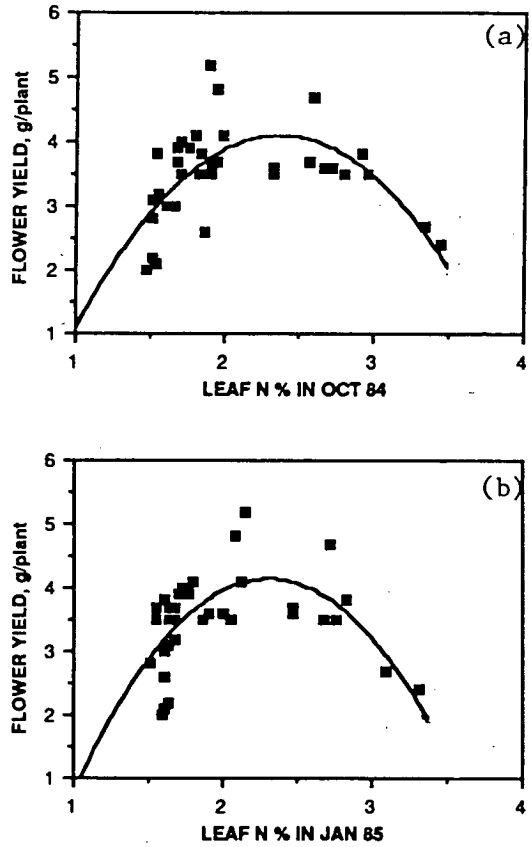


Fig. 9. Relationships between the yield of flowers in January 1985 and the concentration of leaf N in (a) October 1984 and (b) January 1985. The fitted regression equations are October: $Y = -4.91+7.63X-1.61X^2$ ($R^2 = 0.42$) and January: $Y = -6.29+9.05X-1.96X^2$ ($R^2 = 0.39$). Linear and quadratic regression coefficients in each equation are significant at $P = 0.01$. R^2 for each equation is significant at $P = 0.01$.

January.

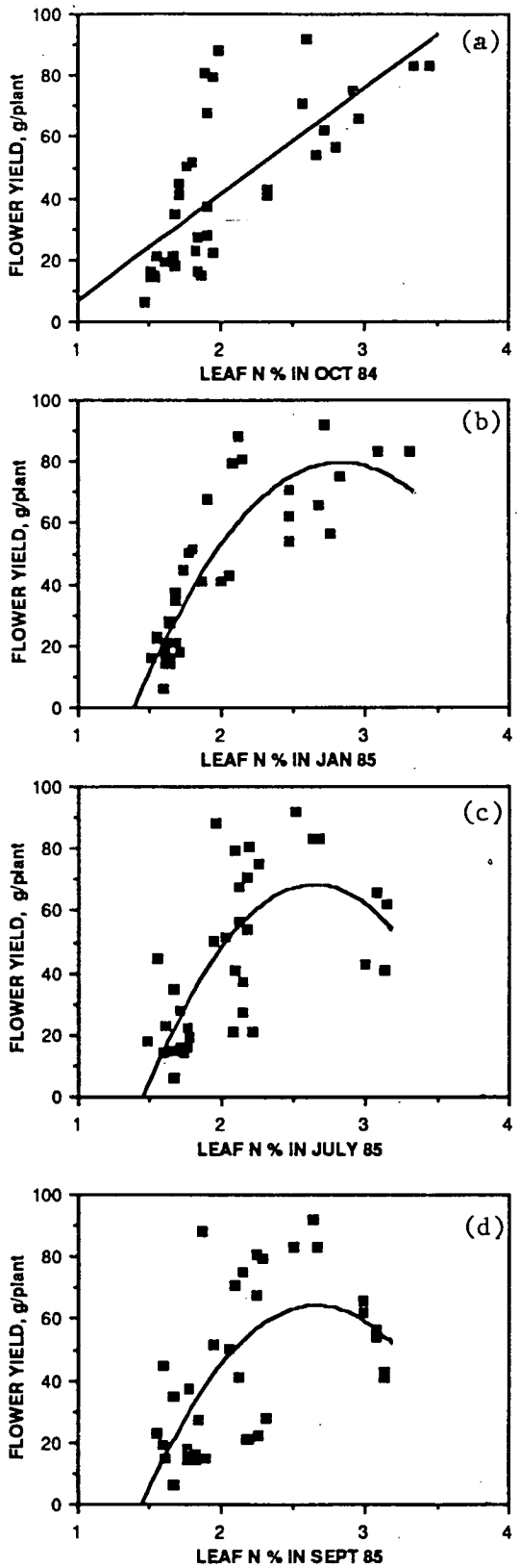
The relationships between the September yields and the concentration of leaf N in October, January, July and September in 1984-85 and 1985-86 are shown in Figs. 10 and 11 respectively. The relationships were linear with the leaf N in October (Fig. 10a) and quadratic with the leaf N in January (Fig. 10b), July (Fig. 10c) and September (Fig. 10d). The R^2 values were marginal for all the months except for January which was high (0.77). These relationships suggest that the yield increased with higher concentration of leaf N in October but declined with higher concentration of leaf N in January, July and September.

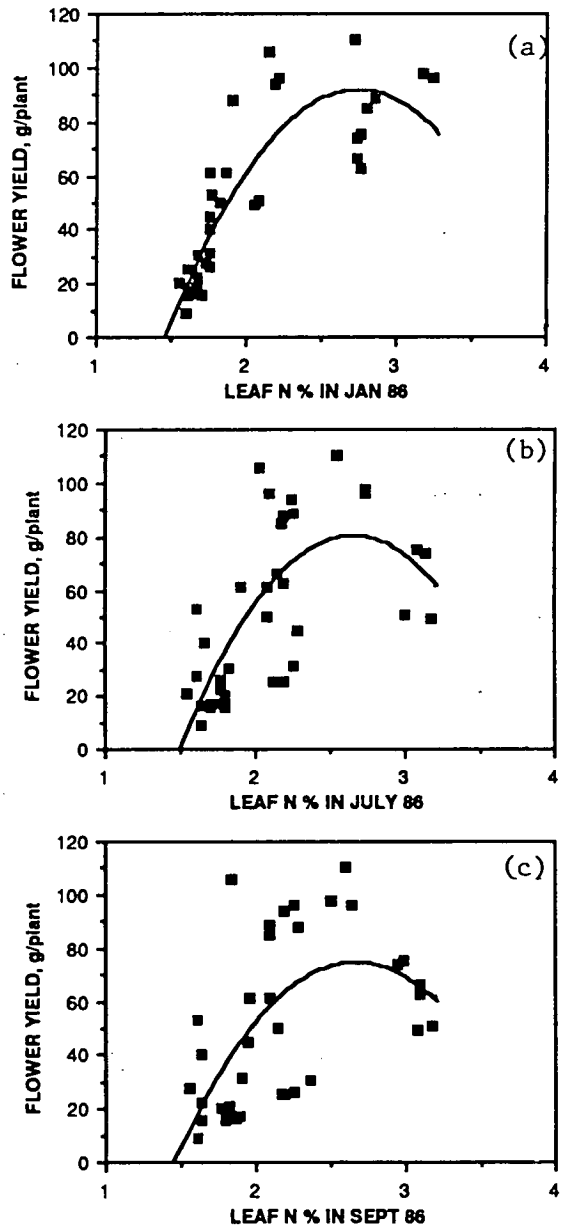
DISCUSSION

The characteristic leaf yellowing symptoms of N deficiency were absent in boronia plants given no N. As no N was applied to the soil even prior to the experiment, the available N in the soil may have been very low. The plants may have adjusted to this low N by reduced growth and reduced lateral branching which indeed were observed on the plants given no N. N deficiency symptoms vary in appearance with plant species (Tucker, 1984).

It was assumed that the concentration of N in the leaf indicated the availability of N from the soil to the plant. Although the concentration of N in the leaf tissue is also subject to the plant growth and the factors limiting the growth, the reflection of rates of N in the leaf N concentration (Tables 6 and 7) supports the view that the assumption is valid.

Higher leaf N in the month following the application of N from the conventional sources (Table 6) indicates that more N was


(a)
FLOWER YIELD, g/plant
LEAF N % IN OCT 84
(b)
FLOWER YIELD, g/plant
LEAF N % IN JAN 85
(c)
FLOWER YIELD, g/plant
LEAF N % IN JULY 85
(d)
FLOWER YIELD, g/plant
LEAF N % IN SEPT 85


(a)
FLOWER YIELD, g/plant
LEAF N % IN JAN 86
(b)
FLOWER YIELD, g/plant
LEAF N % IN JULY 86
(c)
FLOWER YIELD, g/plant
LEAF N % IN SEPT 86

available immediately after their application. Higher leaf N in January than in October with IBDU indicates that the availability of N from IBDU was lower immediately after the application but increased later. It might be due to the fact that the dissolution of IBDU may be slow in the beginning (after application) and rapid later. Allen (1984) stated that once dissolution of IBDU begins, hydrolysis proceeds rapidly. However, absence of such an increase in the leaf N when IBDU was applied in June or August may be related to low winter soil temperatures during that period which may affect the dissolution of IBDU.

The maintenance of leaf N over the season with IBDU as compared with other sources, without any further application, indicates that N from this sources was released over a longer time. At such rates, NO_3^- from conventional sources may have leached beyond the root zone of the plant, while N from IBDU is released slowly, therefore reducing the leaching losses. However, at higher rates, availability of N was highest from the conventional sources but decreased with time, while N availability from IBDU was lower due to the limited release of N from this source.

N promotes the growth of foliage in boronia (Thomas, 1981). Thus, in axillary-flowering boronia, increasing N levels increase the production of axils (associated with leaves) which translate into an increased number of flowers. The highest flower yield with single doses of N rates (Fig. 7b) were therefore due to higher N available early in the season (during spring and summer when temperatures were higher, Appendix Fig. II.I) for a greater

production of axils. When a rate was split, N available early in the season was lower resulting in a lower production of axils. In addition, application of N during the flowering time further decreased the yield. For example, the plants supplied with 50 kg N ha^{-1} per ha in October either as a complete dose of 50 kg N ha^{-1} or as a first split part of 100 kg ha^{-1} dose had the same quantity of N and hence should have similar number of axils until the time of second split part of 100 kg ha^{-1} dose (which was during the flowering). However the plants supplied with 50 kg N ha^{-1} during the flowering as a second split dose yielded less than the plants not supplied with additional N (in Fig. 7b: 50 kg in October line vs. 100 kg in October & June and October & August lines). The decreased in the yield due to the application of N during the flowering time was greater with higher rates of N (Fig. 7b) and less with IBDU (Fig. 7c) indicating that a higher availability of N during the flowering decreased the yield. This effect was relatively high when N was applied in August, i.e. during flower bud development, when compared to N applied in August, i.e. during flower bud initiation (Fig. 1c). In Boronia heterophylla, Richards (1985) observed that an increase in the vegetative growth was associated with the abortion of flower buds. High availability of N during the flowering in the present experiment may have promoted the vegetative growth resulting in the abortion of flower buds and thereby decreasing the flower yields. Similarly, Gutschick (1981) mentioned a case of salvia which dropped flowers after an application of excessive levels of N.

Although the correlations between the flower yield and the

concentration of leaf N in different months (except January) were marginal, the concentrations that maximized the yield were estimated from the regression equations. The values are 3.23%, 2.83%, 2.64% and 2.65% in October, January, July and September 1984-85 respectively; 2.74%, 2.65% and 2.67% in January, July and September 1985-86 respectively. Thus the values in each sampled month in both the years are similar. The computed leaf N concentration values in each month for maximum yield in 1984-85 are graphically presented in Fig. 12. The pattern indicates that a declining leaf N concentration over the season was associated with an increased flower yield. Examination of the data from this point of view shows that the plants that gave higher yields had such a declining pattern of leaf N (compare Table 1 and Fig. 2b). For example, the plants that were given N rates in complete doses in October.

Although, at higher N rates, the plants supplied with conventional sources had higher leaf N than those supplied with IBDU (Table 6), the flower yields with the conventional sources were lower than with IBDU (Fig. 6a). This may be due to luxury consumption when N was highly available from the conventional sources. Indeed the appearance of chlorotic leaf tips on the plants applied with 50 or 100 kg N ha^{-1} as $(NH_4)_2SO_4$ or $Ca(NO_3)_2$ in a complete dose may indicate the deleterious effect of luxury consumption affecting the plant growth. Similar symptoms were observed by Specht (1963) on some Australian native plants supplied with ca. 40 kg N ha^{-1}. Many Australian native plants seem to have adapted to assimilate only low levels of N available under

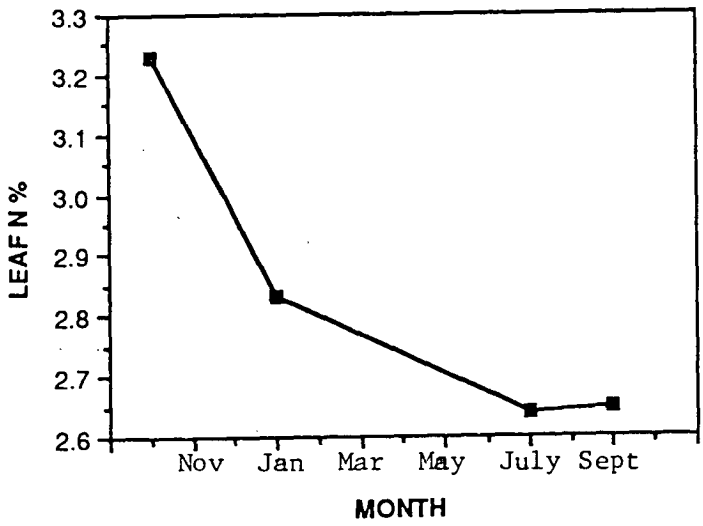


Fig. 12. Pattern of desirable leaf N concentration during the year for maximum flower yield. The leaf N values in different months which maximized the flower yield were estimated from the regression equations given in Fig. 10.

naturally nutrient poor Australian soils. In boronia, high N fertilization levels above the assimilation capacity of the plant led to high concentrations of unreduced N in the leaf tissue causing toxicity (Chapter VIII). When total N in the leaf tissue was analyzed by the method used in this experiment, unreduced N also contributed to the total N concentration. This would give a high total N value in the tissue while the plant may be suffering from the luxury consumption and the consequent impaired growth (and flower yield as observed). Thus, under excess N conditions, total N analysis as such without the analysis of unreduced N may not be an accurate method of determining the critical leaf N concentrations in boronia. This may be a reason for the marginal R^2 values observed for the relationships between the flower yield and leaf N concentration (Fig. 10). With IBDU applied at the same rates (50 or 100 kg N ha^{-1}), the toxicity did not occur because the N amount from IBDU solubilized and available to the plant at any given time would be small and thus would have been within the assimilation capacity of the plants.

There were generally no significant differences between $(NH_4)_2SO_4$ and $Ca(NO_3)_2$. However, it is difficult to resolve the effects of NH_4^+ and NO_3^- on plant growth under field conditions where temperature, aeration, pH and other factors affecting the nitrification may fluctuate during the growing season. Under these conditions some NH_4^+ would have been converted to NO_3^- raising a possibility of a supply of NH_4^+ plus NO_3^- mixture.

In conclusion, for boronia the availability of N should be high early in the season (during the active vegetative growth period)

and should decline as the flowering time approaches. N released from a single application of IBDU early in the season seems to maintain leaf N to meet the growth requirements of boronia without ~~any~~ luxury consumption.

V. EFFECTS OF DIFFERENT LEVELS OF NITROGEN AS NH_4^+, NO_3^- OR NH_4^+ PLUS NO_3^- ON VEGETATIVE GROWTH, FLOWERING AND NUTRIENT CONCENTRATION IN LEAF TISSUE IN BORONIA

To study in detail the effects of different levels of different forms of N, viz. NH_4^+, NO_3^- and NH_4^+ plus NO_3^- on vegetative growth and flowering in boronia, an experiment was conducted under greenhouse conditions. Observations were made on the plants for a long term (full year) to determine the differences in vegetative growth and flowering occurring over time as a result of the treatments. In addition to the observations on vegetative growth and flowering, concentrations of N, P, K, Ca and Mg in the leaf tissue were determined to study the changes in these nutrients with changes in the form and level of N supplied. The relationships between the vegetative growth, flowering and concentrations of nutrients are also discussed here.

MATERIALS AND METHODS

Application of nutrient solutions. It has been noted (in the Review of Literature) that most Australian native plants are sensitive to high levels of N. It was therefore necessary to develop methods for N nutrition of boronia which would enable the plant's growth and development to proceed without the adverse effect. Further, as the experiment was to be for a long time, it was also necessary to save the time and labor involved in the supply of nutrient solutions to the plants. For these reasons, an automatic system was designed and operated for application of nutrient solutions to boronia plants.

The system is shown in Fig. 13. The essential component of the system was a device to elevate a platform which had reservoirs on it. The reservoirs held the nutrient solutions. The platform was lifted to above the level of plant containers which were on benches. This lift was achieved through two vertically mounted shafts connected by a chain and sprocket at their base and driven by a 1 HP single-phase reversible motor. Provisions existed for manual operation in case of a failure of electric power. The platform could be held in either an elevated position (above the benches) or a lowered position (floor level). The height in each case was determined by limit switches. The timing of lift was controlled by an electronic programmable timer capable of 1 to 4 lifts per 24 h.

Each of the nutrient solution reservoirs fed its own 20 mm (internal diameter) polytene pipe 'main' which circled the greenhouse. These mains were fitted with drainage outlets. Each main fed 8 supply hoses of 12 mm (ID), each leading to the base of a 9-l plastic bucket. The bucket contained a layer of blue metal on which rested the plant pot.

When activated by the timer, the platform was lifted to above the height of buckets (Fig. 14). Then the nutrient solution entered the buckets and seeped through the bottom of pots towards the surface of growth medium. After a chosen time, the platform was descended to the floor level (Fig. 15) and the nutrient solution drained back into its reservoir. The chosen time was ca. 10 min so that the plant roots were exposed to the nutrient ions for only a short period. The system was operated once daily in the beginning

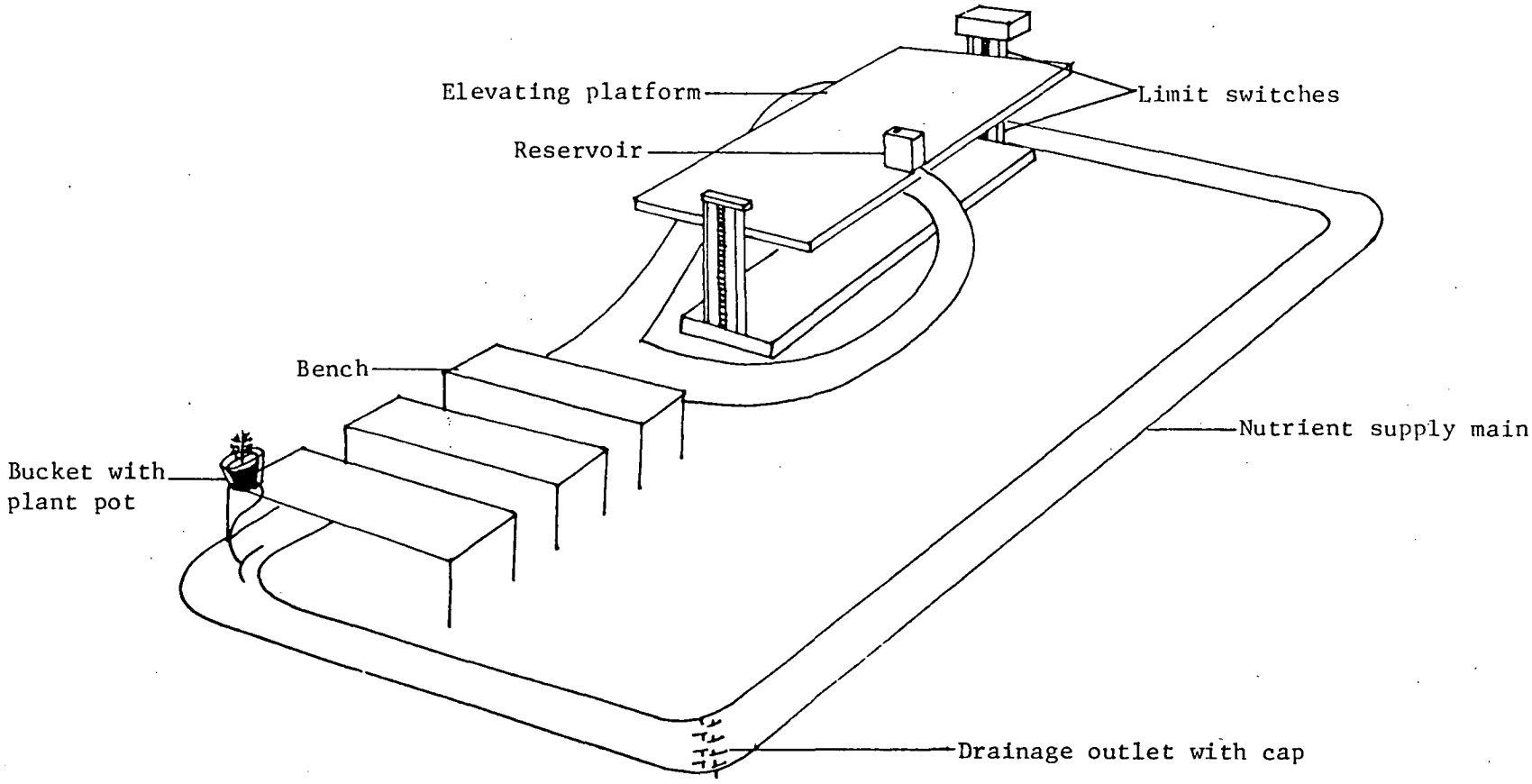


Fig. 13. Diagrammatic layout of the automatic system used to supply nutrient solutions.

Fig. 14. View of automatic nutrient supply system showing the platform in elevated position. Plant pots are kept in benches.

Fig. 15. View of automatic nutrient supply system with the platform in lowered position (at the floor level).

of the experiment and as the plants became bigger it was operated 2-3 times daily.

Large reservoirs were used to employ large volume of solutions (25 l for 4 plants) to minimize the changes in concentration and pH of the solutions due to absorption by the plants.

Plant culture. A clone designated HC-1 was used in this experiment. 2-yr old plants were potted in 200 mm plastic shrub pots. The growth medium used was 2 parts composted eucalyptus bark and 1 part sand by volume. At the bottom of the pot, vinyl with an overlying layer of blue metal stones was placed to obtain free drainage and a layer of blue metal was spread over surface of the medium to reduce evaporation and to prevent algal growth.

Growth conditions. The automatic system for application of nutrient solutions to the plants was set up in a greenhouse under natural light conditions. Maximum temperature was held at ca. $25^{o}C$ by evaporative coolers and temperatures below $25^{o}C$ varied which were slightly higher than those outside the glasshouse (the temperatures outside the greenhouse are shown in Appendix Figs. III.1 and III.2).

Before commencing the treatments, the growth medium was leached with water daily for one week to wash out any salts in it. The leachate had a pH of 6.0 and contained no NH_4^+ or NO_3^-. At this time, plant analysis indicated a leaf N concentration of 1.54% + or - 0.06 (n = 10).

Composition of nutrient solutions. The nutrient solutions used were modified from Long Ashton solution. 3 types of N, *viz*. NH_4^+, NO_3^- or NH_4^+ plus NO_3^- each at 5, 10, 15, 20 and 25 mM were used. To obtain

these treatments, combinations of salts as shown in Table 8 were used.

Table 8. Composition of nutrient solutions.

Salt	Level of N, mM				
	5	10	15	20	25
	Concentration in mM				
NH_4^+-type					
$(NH_4)_2SO_4$	2.25	5.00	7.50	10.00	10.00
$CaCl_2$ $2H_2O$	8.27	8.23	8.20	8.27	8.33
K_2SO_4	4.14	4.13	4.13	4.14	4.13
Na_2HPO_4 $12H_2O$	1.33	1.33	1.33	1.33	1.33
NO_3^--type					
$Ca(NO_3)_2$	1.67	3.33	5.00	6.67	8.33
KNO_3	1.67	3.33	5.00	6.67	8.33
$CaCl_2$ $2H_2O$	6.60	4.90	3.20	1.60	0
K_2SO_4	3.30	2.45	1.60	0.80	0
NaH_2PO_4 $2H_2O$	1.33	1.33	1.33	1.33	0
NH_4^++NO_3^--type					
NH_4NO_3	2.50	5.00	7.50	10.00	12.50
$CaCl_2$ $2H_2O$	8.27	8.23	8.20	8.27	8.33
K_2SO_4	4.14	4.13	4.13	4.14	4.13
Na_2HPO_4 $12H_2O$	0.67	0.67	0.67	0.67	0.67
NaH_2PO_4 $2H_2O$	0.67	0.67	0.67	0.67	0.67

In addition, there was a no N treatment in which 8.27 mM $CaCl_2$ $2H_2O$; 4.14 mM K_2SO_4; 0.67 mM Na_2HPO_4 $12H_2O$ and 0.67 mM NaH_2PO_4 $2H_2O$ were used. Thus, there were 16 treatments.

The following salts were added to all the nutrient solutions at the concentrations stated (in mM): $MgSO_4$ $7H_2O$ 1.5; $MnSO_4$ $4H_2O$ 0.01; $CuSO_4$ $5H_2O$ and $ZnSO_4$ $7H_2O$) 0.001; H_3BO_3 0.05; Na_2MoO_4 $2H_2O$ 0.0005. Iron was supplied as a chelate Fe EDTA, Na salt at 0.05 mM.

The major differences in the composition of nutrient solutions (because a change in a given nutrient ion is necessarily accompanied by a change in an ion of the opposite sign) were only in SO_4^{2-} concentration. Because of the large volume of solutions

required, laboratory grade salts and tap water were used to prepare the nutrient solutions. After preparation the nutrient solutions were adjusted for pH 6.5 by the addition of requisite amounts of 0.1 M H_2SO_4 or NaOH. The pH varied less than 0.5 units during a month.

Once a month, the nutrient solutions were changed to restore the concentrations of depleted nutrients. At the same time, the growth medium in the plant pots was leached with water to wash out any accumulated salts. With regard to possible nitrification, NH_4^+ type solutions were tested occasionally for the presence of NO_3^- by the Kjeldahl method (General Materials and Methods, p. 39) and no NO_3^- was detected anytime.

The 16 plants (treatments) were set randomly in a block and there were 4 blocks (replications). Once a month, plant pots in one block were rearranged in another block to randomize any effect of the position in glasshouse.

Measurement of responses. Growth parameters were measured monthly as follows. Plant stem diameter near the surface of the potting medium at a standard marked location was measured using Vernier calipers. On each plant, two shoots (termed main shoots, see Fig. 16) were selected. On each main shoot, each month, the number of nodes, the number of axillary shoots (termed lateral shoots) and the number of nodes on these lateral shoots were counted. During flowering, on both main and lateral shoots, the number of axils initiating flower buds, the number of flower buds and the number of fully developed flowers were counted. The weights of total yield of fully developed flowers of each plant and 10 of these fully

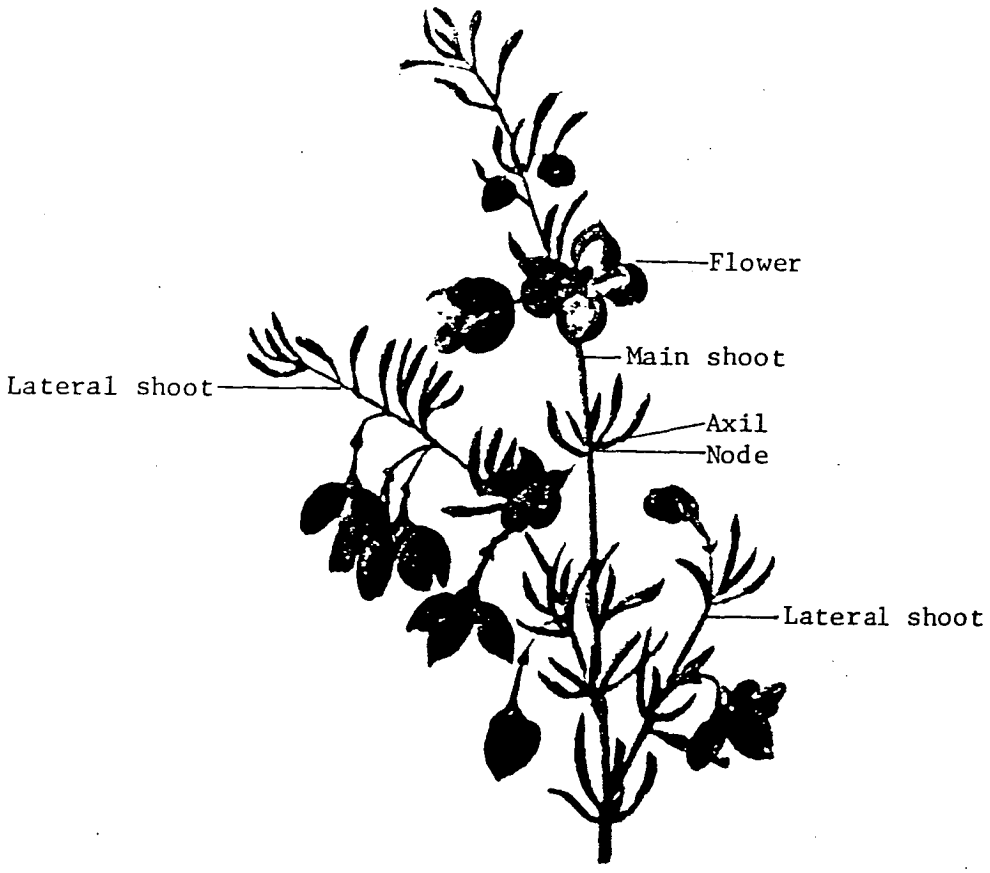


Fig. 16. Branch of Boronia megastigma

developed flowers were taken.

For analysis of leaf nutrient concentration (in 1984-85 experiment only), fully developed mature leaves were taken from main and lateral shoots in November (a month after commencing the supply of nutrient solutions); in February (when growth was rapid) and in June (at the time of flower initiation). The samples were prepared (General Materials and Methods, p. 40) and analyzed for the nutrients. Total N was estimated by a semimicro Kjeldahl method (p. 40). Wet ashing was used to analyze P, K, Ca and Mg (p. 41). P was determined by colorimetry (p. 41), Ca and Mg were determined by atomic absorption spectrophotometry (p. 42) and K was determined by flame photometry (p.42)

The experiment was conducted from March to September in 1984. In 1984-85, the experiment was repeated with a new set of plants from September 1984 to September 1985 to obtain a full year's results.

The primary data of measurements collected for each parameter in

each month were statistically analyzed. Where measurements of a parameter were made on two main shoots in each plant, the values were averaged and the analysis performed on the means. ANOVA was employed to examine the effects of the treatments. When the F value for a treatment effect was significant at P = 0.01 or 0.05, LSD test was applied to determine differences between the treatment means. The data derived from the primary data were examined using regression lines.

RESULTS

The automatic system used in this experiment for supplying nutrient solutions to boronia plants proved reliable, convenient and effective.

The effects of treatments on different growth parameters started to become apparent as time progressed and the magnitude of the differences between treatments increased with time.

The plants in the 1984-85 experiment were larger as compared to the 1984 experimental plants, because the 1984-85 experimental period included the time of the year during which maximum growth occurred (i.e. October to April). Consequently the magnitude of the differences between treatments were larger in the 1984-85 experiment than in the 1984 experiment. However, the trends were similar in both the experiments. Therefore, the results of the 1984 experiment are briefly presented and the results of the 1984-85 experiment are discussed in detail.

General growth and morphology

Within a month after commencing the supply of nutrient solutions, tips of older leaves on the plants supplied with 0, 5, and 10 mM

N began to become chlorotic. However, these symptoms did not resemble the characteristic yellowing of N deficiency. The newly developing leaves on these plants were normal green, though pale when compared to the leaves on the plants supplied with 15, 20 and 25 mM N.

In B. megastigma, there are normally 3 leaflets. However, in the 1984-85 experiment the plants supplied with N produced 5 leaflets, more frequently at 20 and 25 mM N and during the summer months. The normal one central leaflet was divided into 3 leaflets (Fig. 17).

During the experimental period the plants shed very few leaves.

1984 experiment

The increase in stem diameter in each month was not significantly affected by form of N, level of N or their interaction (Appendix Table III.1). Formation of nodes on the main shoot was significantly affected by level of N (Appendix Table III.2) and the effect of form of N on the formation of nodes showed up from May and that of interaction between form X level of N from August. The trends of these effects were similar to those in the 1984-85 experiment (described later). During the period of the 1984 experiment, lateral shoots were not initiated on the plants supplied with 0, 5 and 10 mM N. From 15 to 25 mM, increasing levels of N increased the initiation of lateral shoots (Appendix Table III.3). Form of N did not significantly affect this initiation of lateral shoots. Formation of nodes in each month on these lateral shoots increased with increasing levels of N (Appendix Table III.4) and the effects of form of N and interaction between form X level of N became significant in September.

Fig. 17. Normal (left) and variation (right) in the number of leaflets.

Number of axils with flower buds was significantly affected by level and form of N (Appendix Table III.5). The number increased with increasing levels of N and the plants supplied with NH_4^+ plus NO_3^- had the most number of axils with flower buds. Total number of flower buds was significantly affected by form and level of N (Appendix Table III.6). The number increased up to a level of 20 mM and then declined slightly at 25 mM. Highest number of flower buds were on the plants supplied with NH_4^+ plus NO_3^-. The number of developed flowers was significantly affected by form and level of N (Appendix Table III.7). The number of developed flowers increased with N level up to 15 mM N and highest number of flowers were on the plants supplied with NH_4^+ plus NO_3^-.

1984-85 experiment

Stem diameter. There was no significant effect of interaction between level X form of N on the increase in stem diameter in any month, therefore the means of N levels averaged over the forms of N and <u>vice versa</u> are shown in Fig. 18. The effect of N level was not significant during October to December 1984 and from January 1985, the effect became significant (Fig. 18a). From January 1985, stem diameter increased with increasing levels of N and the magnitude of the differences in stem diameter between different levels of N increased with time. For example the difference in stem diameter between 0 and 25 mM N levels increased 2.5-fold by September.

From June, the effect of N form also became significant (Fig. 18b). Stem diameter was greatest with NH_4^+ plus NO_3^- followed by NH_4^+ and NO_3^-. Generally stem diameter increased rapidly from October

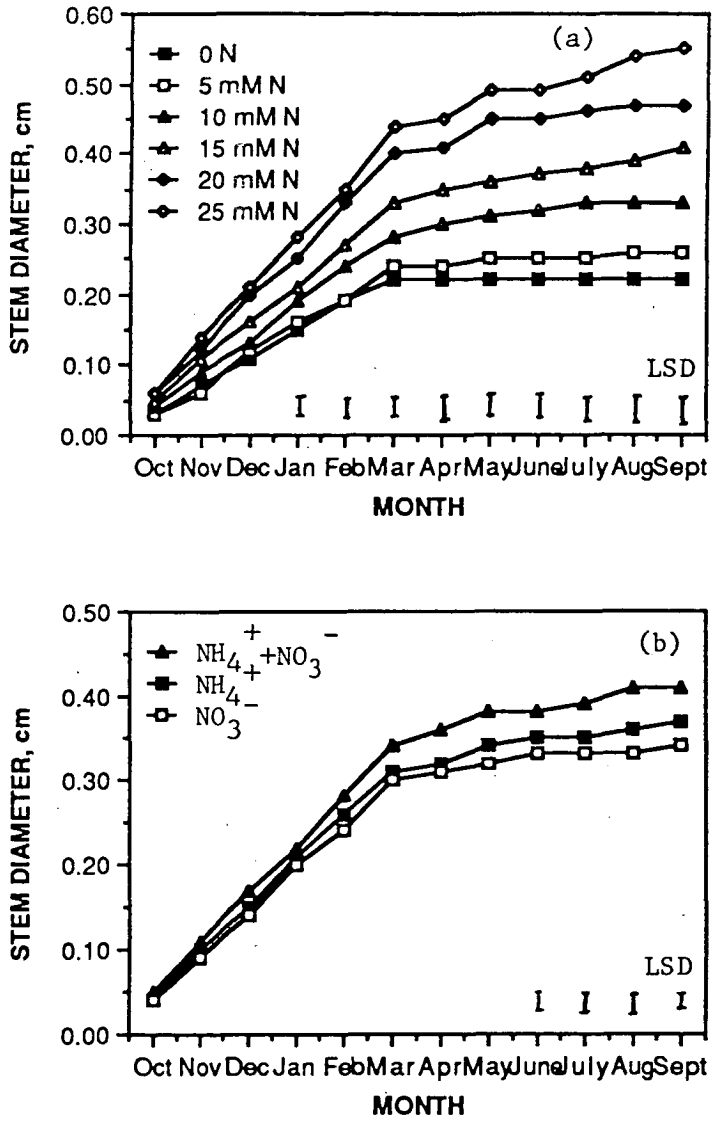


Fig. 18. Increase in stem diameter during the year as affected by (a) level of N and (b) form of N. The effect of level of N was not significant during October to December and the effect of form of N was not significant up to May. LSD at P = 0.01.

to March; by March the stem diameter of 25 mM N plant was double that of 0 N plant. Thereafter, increase in stem diameter ceased with 0 N while it slowly increased in the later months with increasing levels of N.

Nodes on the main shoot. Production of nodes on the main shoot during the year is shown in Fig. 19. The effect of level of N became significant within a month of supply of N solutions and from the next month (November), the effect of form of N became significant. From January, there was a significant interaction between level X form of N indicating that the differences between forms of N were not same at different levels of N. In each month, generally increasing levels of N increased the production of nodes and at the same level of N, highest number of nodes were produced by NH_4^+ plus NO_3^- followed by NH_4^+ and NO_3^-. As the plants grew larger over time, the differences between the treatments increased. By June (when the plants started initiating flower buds), the differences in the number of nodes between 0 and 25 mM N were 3.3-fold (higher) with NH_4^+ plus NO_3^-, 3-fold with NH_4^+ and 2.8-fold with NO_3^-.

From March (when the mean temperature reached ca. 15^{0}C), 0, 5 and 15 mM N plants almost ceased producing nodes on the main shoot while 15, 20 and 25 mM plants continued to produce the nodes.

Nodes (axils) produced on the main shoot are the potential sites for the initiation of lateral shoots.

Lateral shoots. Fig. 20 shows the production of lateral shoots during the year. Form and level of N significantly affected the initiation of lateral shoots from the beginning of the supply of

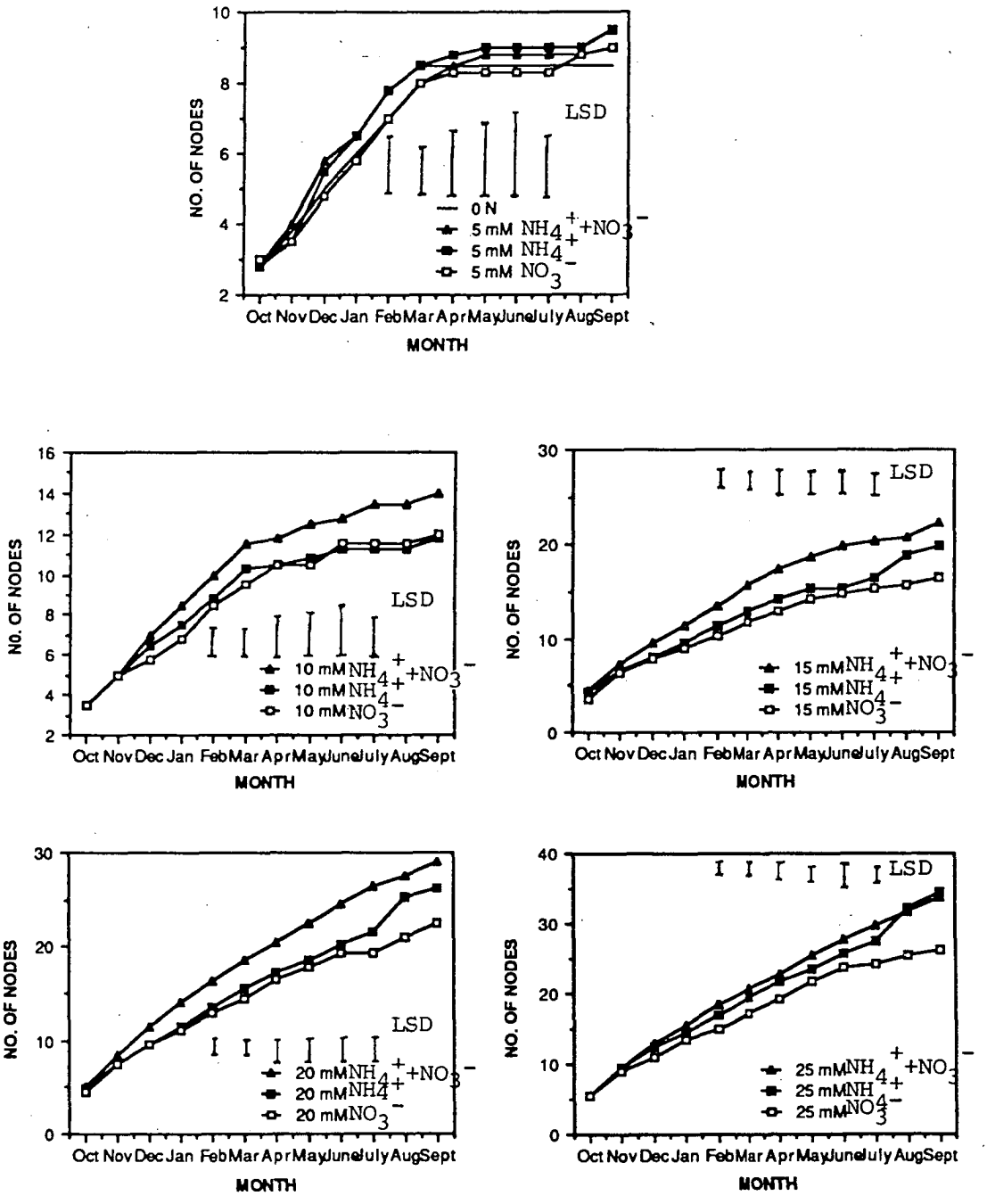


Fig. 19. Production of nodes on the main shoot during the year as affected by level and form of N. For clarity of curves all the treatments are not drawn in one graph. LSD at P = 0.01.

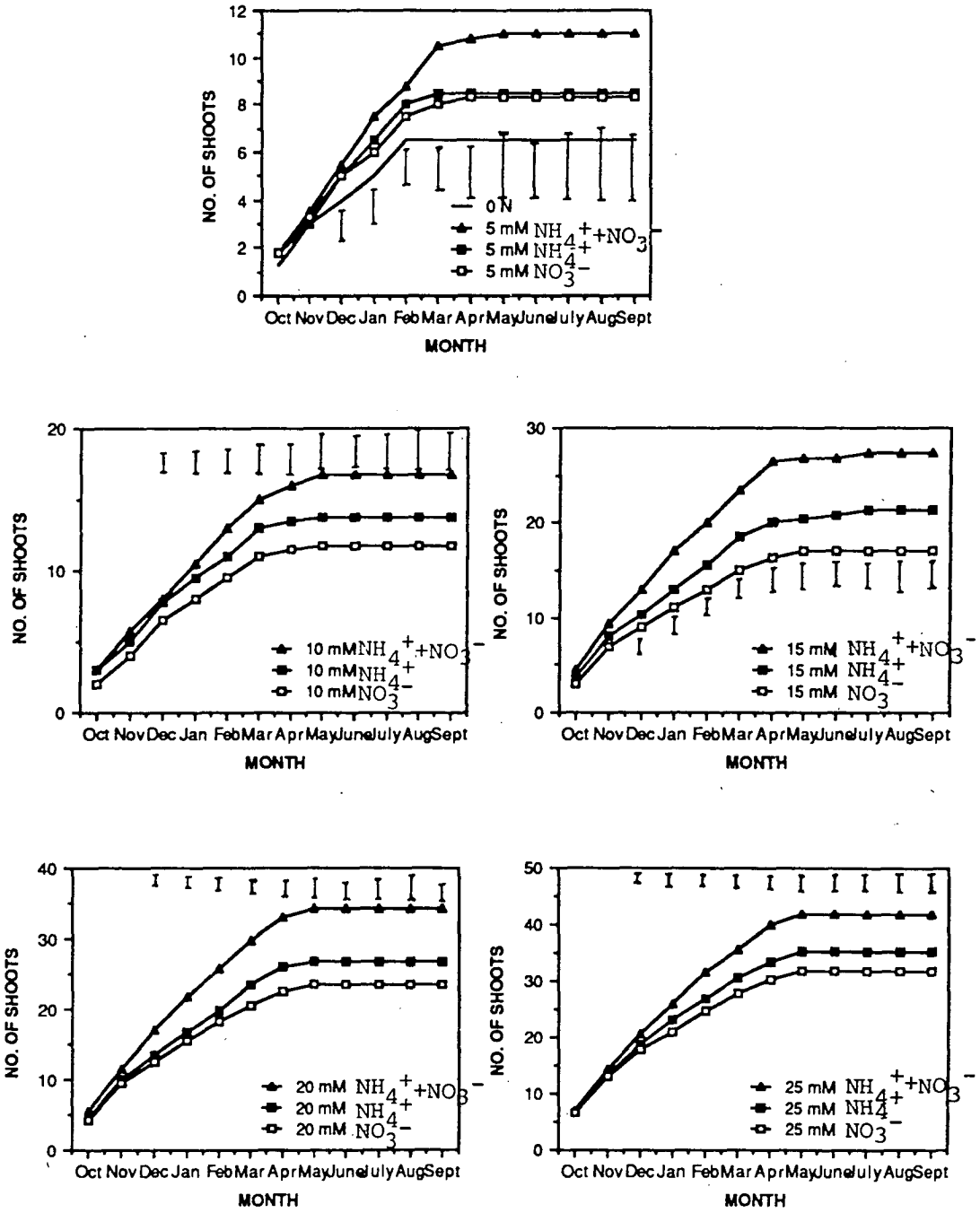


Fig. 20. Production of lateral shoots during the year as affected by level and form of N. LSD at P = 0.01.

N solutions and from December, there was a significant interaction between form X level of N. In a manner similar to that of the production of nodes on the main shoot, increasing levels of N increased the initiation of lateral shoots and at the same level of N, NH_4^+ plus NO_3^- produced the maximum number of lateral shoots followed by NH_4^+ and NO_3^-. More than 95% of the lateral shoots were initiated by April. By then they were 6.2-fold higher (over 0 N) with 25 mM N as NH_4^+ plus NO_3^-, 5.2-fold higher with 25 mM N as NH_4^+ and 4.7-fold higher with 25 mM N as NO_3^-. In the later months, lateral shoots did not arise even on 15, 20 and 25 mM N plants which continued to produce the potential sites (i.e. nodes on the main shoot) for the production of lateral shoots.

As the number of axils on the main shoot largely determine the production of lateral shoots, the production of lateral shoots as related to the total number of axils on the main shoot is shown in Fig. 21. The percentage of axils producing the shoots increased rapidly in the first two months of the experiment and gradually thereafter up to March. From April, lateral shoots were not initiated despite the formation of nodes on the main shoot (on 15, 20 and 25 mM N plants). Therefore the percentage of axils on the main shoot initiating lateral shoots decreased. The trends in the percentage of axils initiating lateral shoots in response to level and form of N were similar to those of the production of absolute number of lateral shoots. Thus N particularly NH_4^+ plus NO_3^- enhanced the initiation of the lateral shoots from a higher number of available axils on the main shoot.

Nodes on lateral shoots. Production of nodes on lateral shoots

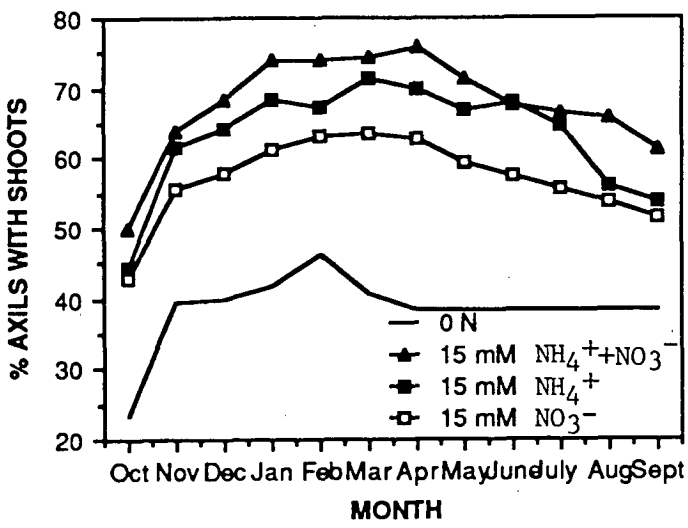


Fig. 21. Percentage of axils on the main shoot producing lateral shoots during the year as affected by level and form of N. Percentage data are derived from the ratio of number of lateral shoots (Fig. 20) to total number of axils on main shoot (Fig. 19). Results at only 15 mM are shown; patterns at other levels of N were similar.

Fig. 22. Example of effect of N on the production of nodes (sites for flower buds): branch from nil N plant (left) as compared to that from 25 mM NH_4^++NO_3^- plant (right).

during the year as affected by form and level of N is shown in Fig. 23. Form and level of N significantly affected the production of nodes on lateral shoots even from the first month of the supply of N solutions and from the second month, there was a significant interaction between form X level of N. The trends in the production of nodes on lateral shoots were similar to those of the production of nodes on the main shoot. By April, 85% of the total number of nodes on 0. 5, 10 mM N plants were produced and with increasing levels of N, the production of nodes continued into later months though at a declining rate. By June, from 0 to 25 mM N the nodes increased 18.7-fold with NH_4^+ plus NO_3^-, 15.8-fold with NH_4^+ and 13.8-fold with NO_3^-.

These results show that the potential sites for flower buds (axils) could be enhanced by N supply and at a similar level of N, NH_4^+ plus NO_3^- was most effective followed by NH_4^+ and NO_3^-.

Flowering

Axils with flower buds. Flower buds began to appear in June when the mean monthly temperature decreased to ca. 8.5^{0}C and the mean light hours were ca. 9. Formation of flower buds continued into July although >95% of the axils initiated flower buds in June (Appendix Table III.12). Flower buds were formed in the leaf axils on both lateral and main shoots including in some of those axils on the main shoot from which lateral shoots were initiated earlier.

The number of axils with flower buds counted in both June and July was significantly affected by form, level and interaction of form X level of N (Appendix Table III.12). The cumulative number of axils with flower buds in July is shown in Fig. 24a. The number

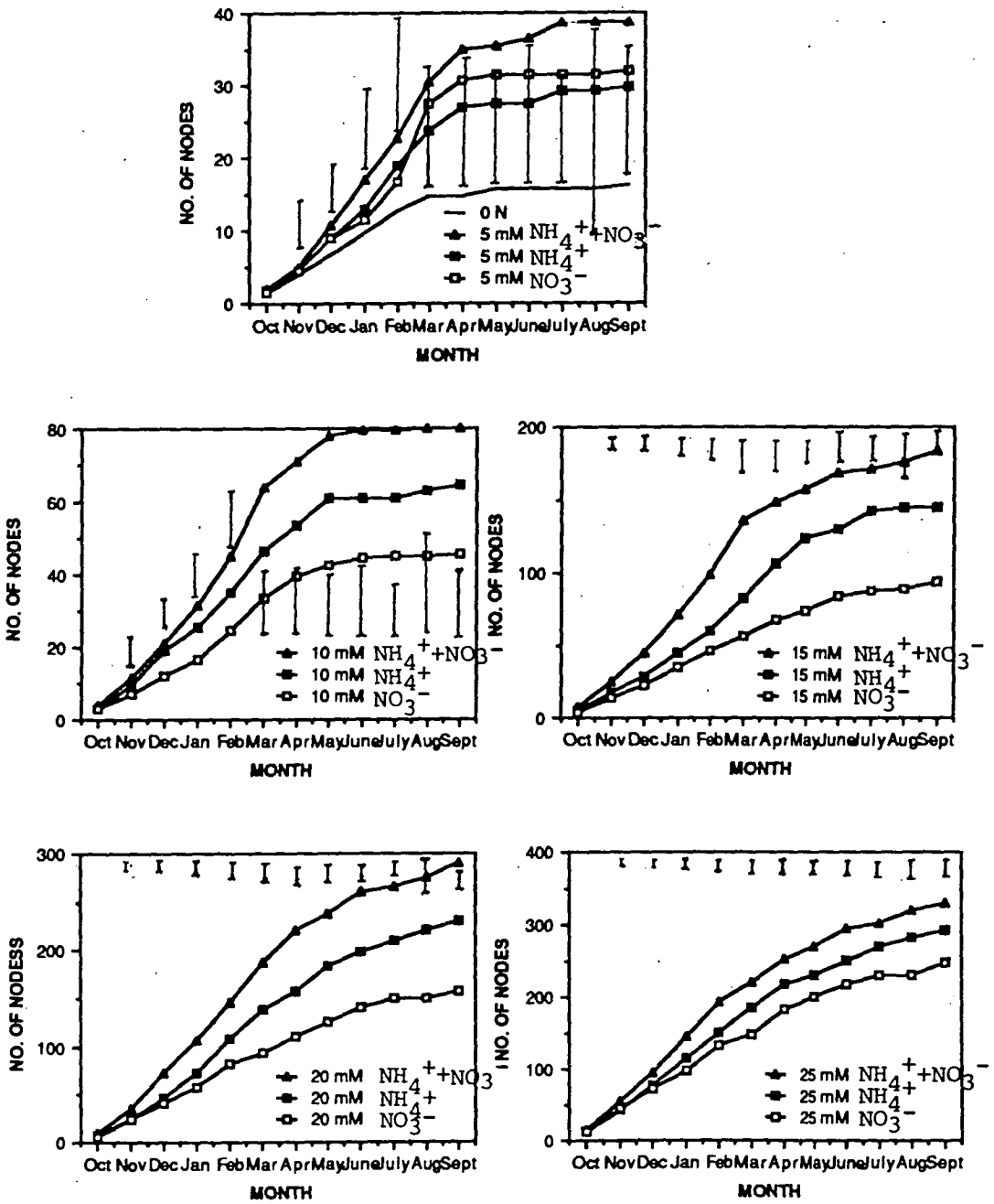


Fig. 23. Production of nodes on lateral shoots during the year as affected by level and form of N. P = 0.01.

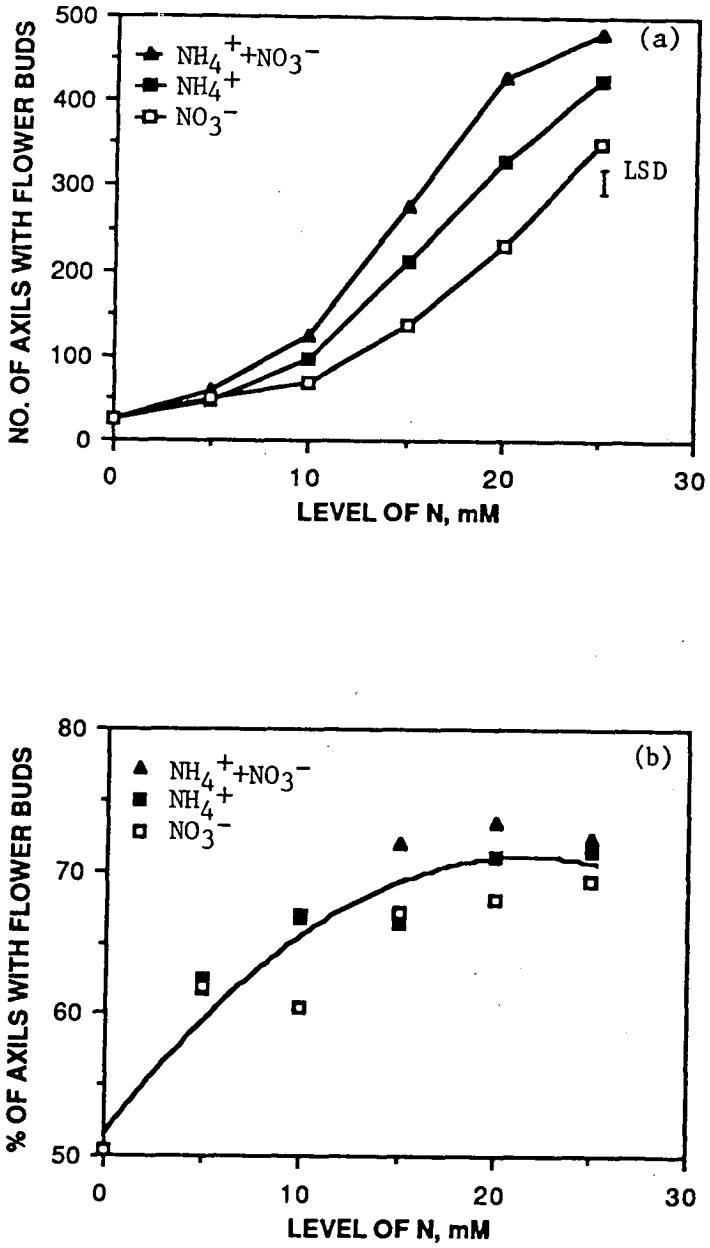


Fig. 24. (a) Number of axils with flower buds in July as affected by level and form of N. N was supplied from Oct. LSD bar (P = 0.01) is for comparison between forms of N at the same level.
(b) Percentage of axils with flower buds in July as affected by level and form of N. Percentage data are derived from the ratio of number of axils with flower buds (Fig. 24a) to total number of axils on main shoot (Fig. 19) and lateral shoots (Fig. 23).

of axils with flower buds increased with increasing N level. At 5 mM N, there were no differences between forms of N but at higher N levels, NH_4^+ plus NO_3^- plants had the highest number of axils with flower buds. For example, at 25 mM N the number of axils with flower buds were 19.7-fold higher with NH_4^+ plus NO_3^-, 17.3-fold higher with NH_4^+ and 14.3-fold higher with NO_3^- as compared to 0 N.

Although the increase in the number of axils initiating flower buds was mainly due to the increased number of axils that were available at flowering for initiation of flower buds, not all available axils initiated flower buds. In general the percentage of axils initiating flower buds showed a curvilinear trend (Fig. 24b). The percentage of axils initiating flower buds increased with the level of N up to 15 mM and began to decline at higher levels of N. Most of the axils that did not initiate flower buds were observed to be the ones towards the apical side of the shoots which were produced late (after May, particularly in 20 and 25 mM N plants, Fig. 19 and 23), although some of the axils that were produced early also did not have flower buds. However, though the axils produced in later months were more on NH_4^+ plus NO_3^- plants (Figs. 19 and 23), the percentage of axils with flower buds was also higher on these plants (Fig. 24b). This suggests that the form of N may have affected the initiation of flower buds. The trends of NH_4^+ and NO_3^- were not so clear as that of NH_4^+ plus NO_3^-: there were sharp declines in the percentage of axils with flower buds at 15 mM NH_4^+ and at 10 mM NO_3^-.

Total number of flower buds. Each axil had 0 to 4 flower buds. 85% of the total flower buds were initiated in June (Appendix Table

III.13).

The trends of total number of flower buds in response to level and form of N (Fig. 25a) were similar to those of the number of axils initiating flower buds. However the actual differences in the total number of flower buds in July between 0 and 25 mM N were 17.1-fold with NH_4^+ plus NO_3^-, 15-fold with NH_4^+ and 12.8-fold with NO_3^-. Although the total number of flower buds increased with an increase in the number of axils initiating flower buds, the average number of flower buds in each axil increased slightly from 0 to 15 mM and then declined at higher N levels (Fig. 25b). However, the trends between forms of N were not clear.

Number of developed flowers. Flower buds developed rapidly during August and September. Since all flowers on a plant did not develop simultaneously, they were picked as they developed and combined.

The number of developed flowers in each treatment is shown in Fig. 26a. The number of developed flowers was significantly affected by form and level of N and their interaction. With NH_4^+ plus NO_3^- the number of developed flowers increased up to 20 mM N and then declined at 25 mM N. However, with NH_4^+ and NO_3^- there was no decline. The differences between forms of N were not significant at 5 mM N. Because of the decline with NH_4^+ plus NO_3^- at 25 mM N, the difference between NH_4^+ plus NO_3^- and NH_4^+ was not significant and the difference between NH_4^+ and NO_3^- was also not significant. These trends were not similar to those of the total number of flower buds, because some of the initiated flower buds did not develop to anthesis and aborted. In general the percentage of flower buds that developed to anthesis decreased with increasing levels of N (Fig.

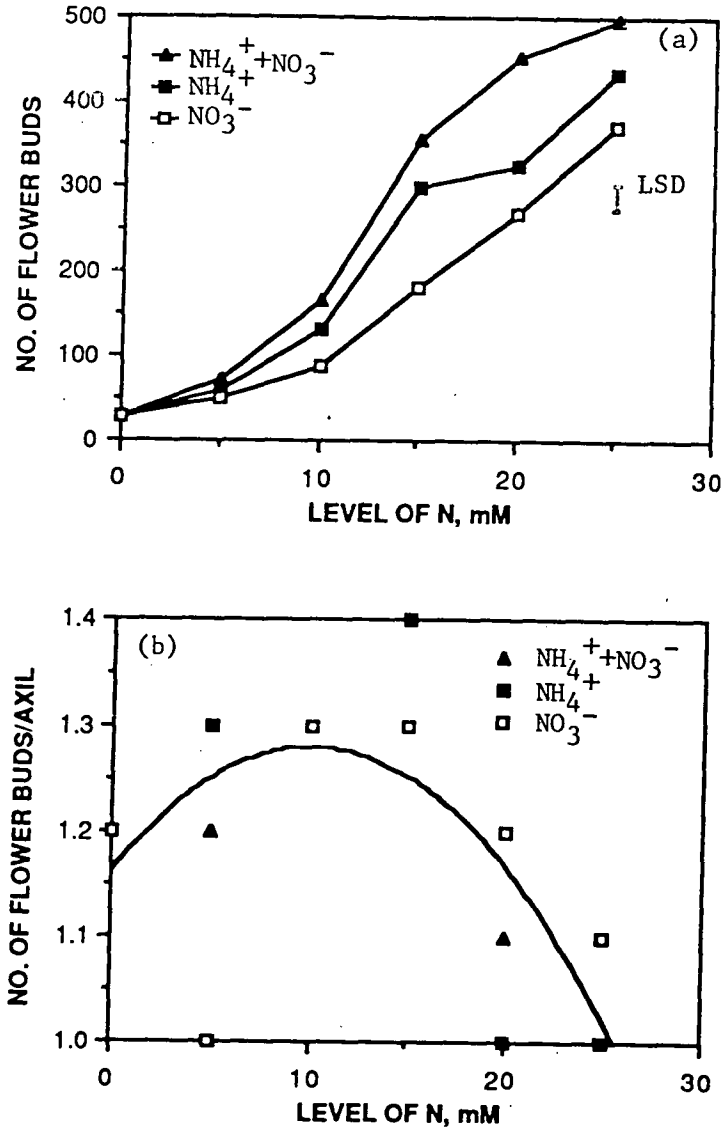


Fig. 25. (a) Number of flower buds in July as affected by level and form of N. N was supplied from Oct. LSD bar (P = 0.01) is for comparison between forms of N at the same level. (b) Average number of flower buds/axil in July as affected by level and form of N. Average data are derived from the ratio of number of flower buds (Fig. 25a) to total number of axils with flower buds (Fig. 24a).

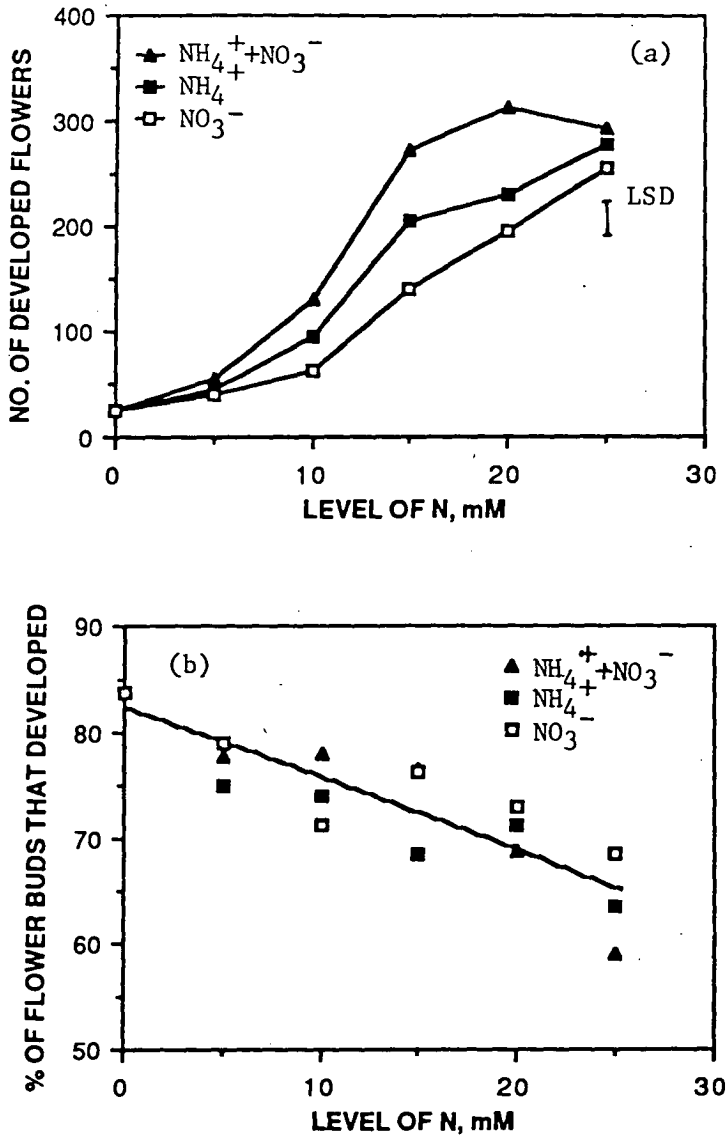


Fig. 26. (a) Number of developed flowers as affected by level and form of N. LSD bar (P = 0.01) is for comparison between forms of N at the same level.
(b) Percentage of flower buds developing to anthesis as affected by level and form of N. Percentage data are derived from the ratio of number of developed flowers (Fig. 26a) to total number of flower buds (Fig. 25a).

26b). At 15 mM and lower levels of N the differences between forms of N were not clear whereas at higher levels of N the percentage of flower buds that did not develop to anthesis was highest with NH_4^+ plus NO_3^- followed by NH_4^+ and NO_3^-. The effect of N on this failure of some flower buds to develop fully may have been influenced by the vegetative growth (as discussed in the Discussion section).

10-flower weight. It was not significantly affected by form of N and interaction between form and level of N. Therefore the means of N level averaged over the forms of N are shown in Fig. 27. The individual flower weight decreased with increasing levels of N up to 20 mM and leveled off.

Nutrient concentration in leaves

Nitrogen. Form of N supplied did not affect the concentration of N in the leaves in November but the level of N supplied significantly affected the concentration of N in the leaves (Fig. 28a). Increasing the level of N supply increased the concentration of leaf N although the difference between two adjacent levels of N (except 15 and 20 mM) were not significant.

The leaf N concentration in February increased significantly with an increase in each level of N (Fig. 28b). Form of N also significantly affected the leaf N concentration: the concentration was significantly higher with NH_4^+ plus NO_3^- (Fig. 28c).

Form of N, level of N and their interaction significantly affected the leaf N concentration in June. Increased levels of N increased the leaf N concentration (Fig. 28d). At lower levels of N, viz. 5, 10 and 15 mM, the differences in the concentrations of

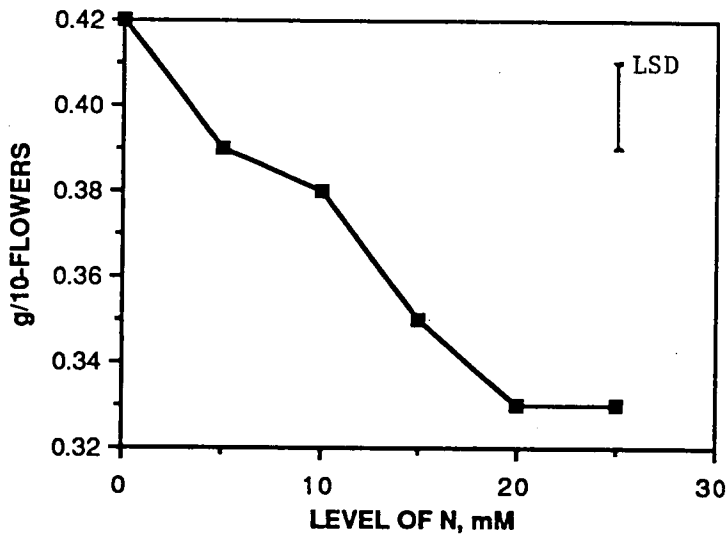


Fig. 27. 10-flower (fresh) weight as affected by level of N. LSD at P = 0.01.

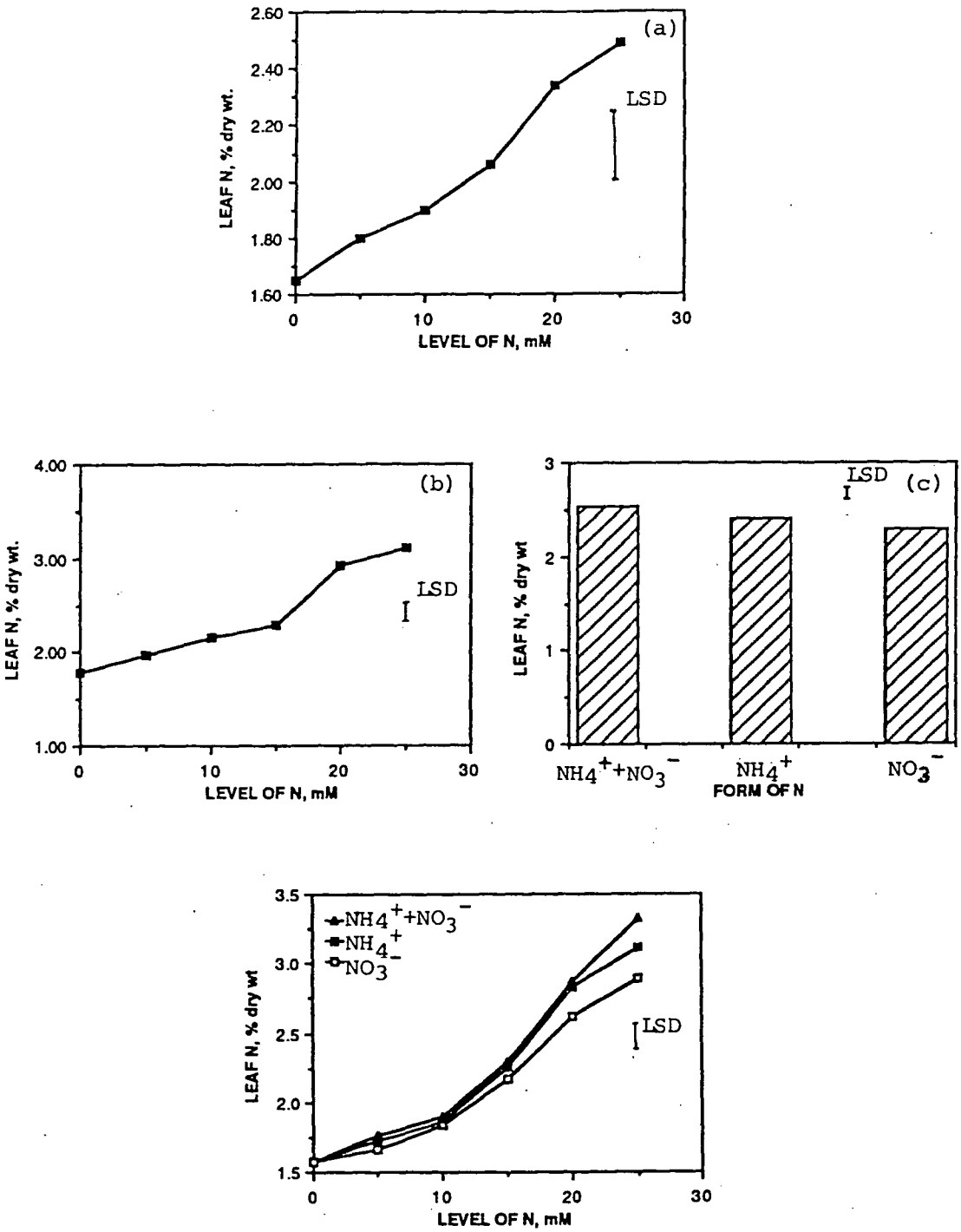


Fig. 28. Concentration of N in leaf in (a) November, (b) & (c) February and (c) June as affected by level and form of N supplied. In November, the effect of form of N was not significant; in February, the effect of form X level of N was not significant. In June, LSD bar is for comparison between forms of N at the same N level.

leaf N between forms of N were not significant. At 20 mM the leaf N concentration was lower with NO_3^- than with NH_4^+ plus NO_3^- and NH_4^+ and at 25 mM N the concentration was highest with NH_4^+ plus NO_3^- followed by NH_4^+ and NO_3^-.

The concentration of leaf N in general was higher in February when the plants were actively growing and lower in June when the growth was slowing down.

While the concentration of N in the leaf tissue increased, the concentrations of P, K, Ca and Mg decreased (Fig. 29).

Phosphorus. Form of N supplied did not significantly affect the concentration of P in the leaves in any of the months in which leaf tissue was sampled but the concentration of leaf P significantly decreased with increasing levels of N. The concentration of leaf P in June is shown in Fig. 29a and the trends in P concentration in the other months were similar (Appendix Table III.18). The leaf P concentration was slightly lower in November and in the other two months the concentrations were almost similar.

Potassium. Form of N did not significantly affect the concentration of K in the leaf in any month but level of N significantly affected the concentration of leaf K. The leaf K concentration decreased with increasing levels of N (in June, Fig. 29a). However the differences in the concentration between some two adjacent high N levels were not significant (10 & 15 and 20 & 25 in November; 15 & 20 in February; 10 & 15 and 15 & 20 in June). The leaf K concentration was slightly higher in November as compared to the other two months (Appendix Table III.19).

Calcium. Form of N did not significantly affect the concentration

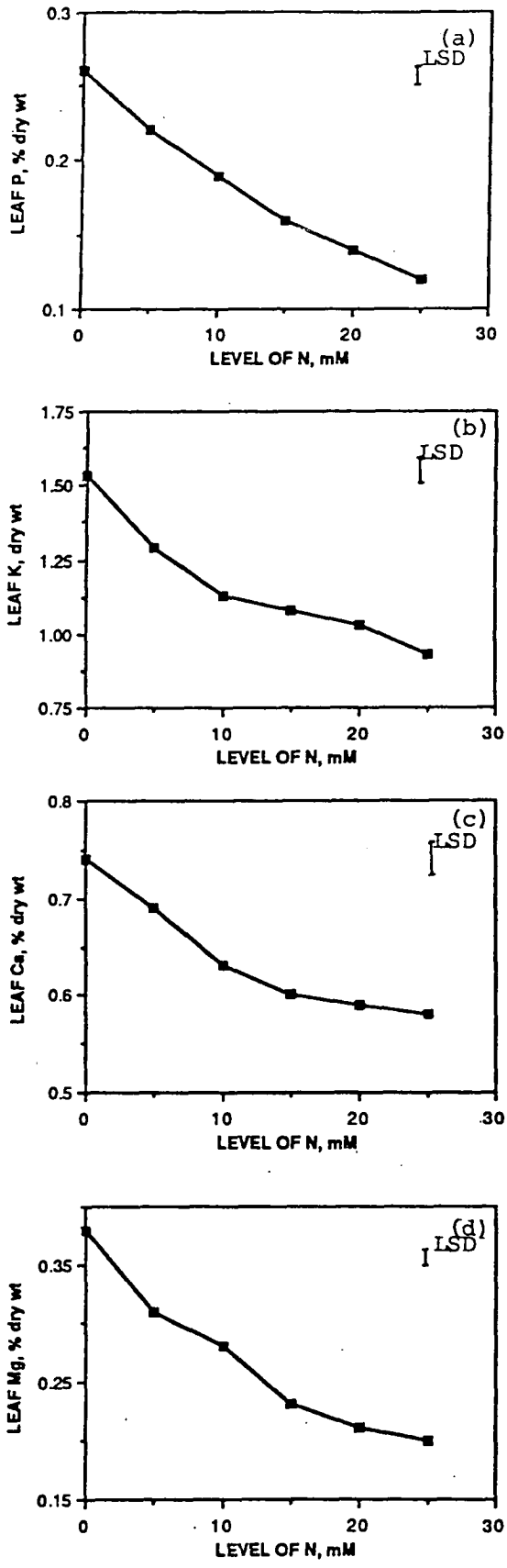


Fig. 29. Concentrations of P, K, Ca and Mg in June as affected by level of N. Trends in concentrations in November and February were similar.

of Ca in the leaf in any month but the leaf Ca concentration decreased with increasing levels of N (in June, Fig. 29c). However the differences between 15, 20 and 25 mM were not significant. The concentrations of Ca were lower in November than in the other months (Appendix Table III.20).

Magnesium. Form of N did to significantly affect the concentration of Mg in the leaf in any month but the leaf Mg concentration significantly decreased with increasing levels of N (in June, Fig. 27d). The concentration of leaf Mg was slightly lower in November than in the other two months (Appendix Table III.21).

DISCUSSION

Boronia probably has a capacity for producing 5 leaflets. Under sufficient N supply when other environmental conditions particularly temperature (during summer) were favorable, the plant may have realized its full potential and formed 5 leaflets (Fig. 17).

In the present experiment the characteristic leaf yellowing symptoms of N deficiency did not appear on boronia plants even when N was not supplied. As the leachate of the growth medium collected prior to commencing the treatments contained no N, the availability of N to 0 N plants may have been only from any N impurity in the reagents that were used for preparing the solution containing the nutrients other than N. Beadle (1966) observed that some Australian native plants have the capacity to pass into a static condition (but not dormant) when N supply was withheld and could remain in this condition for even more than 2 years. In the present experiment there was a very small growth increment in the nil N

plants. N for this growth could have come from older leaves. In nature, native plants do not seem to show visible deficiency symptoms even in low nutrient soils. They seem to adapt to the changes in the levels of nutrient availability by changing their growth rate accordingly and remain healthy. Thus with N supply, growth of boronia increased according to the level of N. Therefore the degree of N limitation existing in a boronia plant can only be recognized by its growth response after N is supplied. Since there was no depression of vegetative growth it was apparent that boronia would have responded to higher levels of N than those supplied.

The effects of treatments on different parameters showed up after different periods of time depending on the growth rate of each parameter.

Stem diameter is a measurement of cambial growth which contributes to boronia shrub volume. The cambial growth was responsive to N (Fig. 18). The cambial growth pattern during the year was similar to that of shoot growth although the magnitude of cambial growth response was less obvious than that of shoot growth response. Cambial growth is largely affected by the activity of shoots through the downward flow of growth regulating metabolites (Kozlowski, 1971). Increasing levels of N increased the shoot activity in boronia which may have resulted in an increased production and flow of metabolites leading to the increased cambial growth.

Shoot growth pattern is controlled by internal (genetic) and environmental factors. Generally, shoot growth of woody plants occurs in several flushes during the growing season (Kramer and

Kozlowski, 1979). Shoot growth pattern in boronia has not been investigated before. In this experiment, boronia did not show flushes of shoot growth as indicated by the continuous production of nodes and lateral shoots. Probably with continuous daily N supply, shoot growth was continuous. However other environmental factors, viz. temperature and light period also influenced the shoot growth in boronia. When the mean monthly temperature reached ca. 15^{0}C and the light period reached ca. 12 h (in March-April), shoot growth almost ceased with low levels of N supply. In many woody plants, low temperatures and short days cause cessation of shoot growth (Kramer and Kozlowski, 1979). Growth in a variety of Australian woody plants was severely restricted when daily mean temperatures fell below 16-18^{0}C (Groves, 1965). Similarly, Paton (1978) found that a 12 h photoperiod is optimal for growth in 12 species of Eucalyptus. However high levels of N promoted, though slowly, shoot growth in boronia even at low temperatures and short days. Temperature and light seem to have differentially affected the production of nodes and lateral shoots. Lower temperature and shorter days stopped the initiation of lateral shoots (Fig. 20) while only slowing the production of nodes (Figs. 19 and 23).

During the active growing period, increasing N levels led to increased number of lateral shoots (Fig. 20) and nodes (Figs. 19 and 23) in boronia. Similarly, Jackson (1970) found that high N supply increased the number of branches and nodes in apricot. McIntyre (1977) found that increased N supply made lateral buds grow in flax (Linum usitatissimum). Production of lateral shoots and nodes is a result of the growth activity of axillary and apical

meristems. Thus at low levels of N the growth activity was only in few meristems while at high N levels, most meristems were active. Therefore there would have been an internal competition for N for growth activity in meristems and N supply reduced the competition and permitted the growth according to the level of N. Recently Trewavas (1985) suggested that the phenomenon of correlative influence of a growing apical mersitem inhibiting the development of axillary meristems is a form of competition between the growing leaves and the buds mediated by N.

The stimulating effect of N on the initiation of lateral shoots (Figs. 20 and 21) is similar to the effect of phytohormone cytokinin. Richards (1985) reported that an application of cytokinin BAP induced lateral shoots in a related species B. heterophylla. N supply may have increased the endogenous cytokinins in boronia. N supply induced the formation of cytokinins in the roots and their export to the shoots in sunflower plant (Salama and Wareing, 1979) and in apple the increase in cytokinins was greater with NH_4^+ as compared with NO_3^- nutrition (Buban et al., 1978). In boronia the stimulation of production of lateral shoots was maximum with NH_4^+ plus NO_3^-.

One may anticipate that plants that inhabit similar ecological situation as that of boronia may show similar growth responses to the addition of N. Other Australian plants Grevillea robusta (Moore and Keraitis, 1966), Callistemon citrinus and Hakea laurina (Thomas, 1982) showed strong responses to N with increased foliage and dry weight.

In boronia, increasing N supply led to a faster production of

more nodes (Fig. 19 and 23; see also Figs. 22 and 30) before the initiation of flowers and thus more sites for flower bud initiation. The nodes so obtained by N supply would have little effect on flower yield unless they produce flowers. Indeed most of these nodes produced flowers.

Because of the different sizes of the plants in different treatments at the time of flowering (e.g. Fig. 30), factors (other than nutrients) within the plants and in the microevironment of the plants may have also changed and affected the flowering. In boronia, flowers started to appear at a time when extension growth markedly slowed down. Therefore it is reasonable to assume that a reduction in vegetative growth was associated with flowering in boronia.

In boronia the percentage of axils initiating flower buds was low at low N levels and was diminished at high N levels (Fig. 24b). At low N levels, N may not have met the basic requirement of the initiation. At high N levels the shoots on the plants were usually enclosed within canopy and were subjected to a low light condition which may have affected the initiation of flower buds. In apricot, low light intensity greatly suppressed the flower bud initiation and decreased the number of flower buds initiated at each node (Jackson, 1969).

Form of N seemed to have regulated the initiation of flower buds in boronia though the effects were not clear (Figs. 24b and 25b). Grasmanis and colleagues (1967 and 1974) showed that NH_4^+ added to NO_3^- promoted flower bud initiation in apple. This promotion may have resulted from changes in the levels of nitrogenous compounds.

Fig. 30. Variation in the size of plants due to the effect of N: nil N plant (left) and 20 mM NH_4^++NO_3^- plant.

Grasmanis and Leeper (1965) recorded higher concentrations of asparagine and arginine in apple trees supplied with NH_4^+ compared to the trees supplied with NO_3^-. Perhaps these or some other amino acids may be involved in the initiation of flower buds in boronia. Phytohormones play an important role in the flower bud initiation, therefore interactions between N nutrition and phytohormones may also be involved in these effects evoked by N on the initiation of flower buds in boronia.

Increasing levels of N negatively affected the development of flower buds in boronia (Fig. 26a). With increasing levels of N the nodes and associated leaves continued to grow during flowering. The percentage of flower buds that developed to anthesis is plotted against the number of nodes increased during August-September (the time of flower bud development) (Fig. 31). It shows an inverse relationship. Thus the developing leaves may have competed with the developing flowers for similar metabolites (not necessarily only for N). In Bougainvillea, Sachs and colleagues (in Kinet et al., 1985) showed that leaves developing at the same time as flowers act as competing sinks although this may also be interpreted in terms of production of inhibitors by the developing leaves.

The developing leaves may have also hastened up the development of flower buds towards anthesis. Thus the duration of the development of flowers would have been shorter reducing the flower weight (Fig. 27). At low N levels vegetative growth did not resume until September, so without any competition of developing leaves the duration of flower development would be longer resulting in increased weight of flowers at anthesis. Flower weight is an

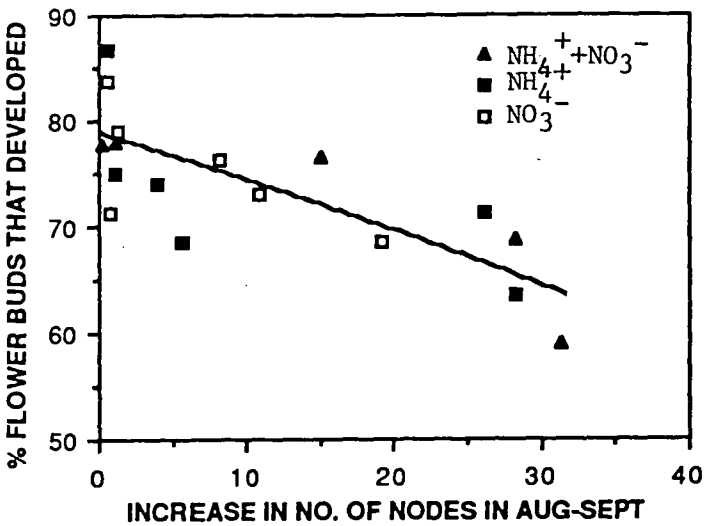


Fig. 31. Relationship between the percentage of flower buds developed to anthesis and the increase in growth during August-September (time of development of flower buds). Percentage data are derived from Fig. 26b and number of nodes data are derived from Figs. 19 and 23.

important trait to assess the flower quality in boronia.

Nutrient concentration in leaves

The ability of a plant to utilize NH_4^+ or NO_3^- has been found to be related to its relative soil environmental adaptations (Haynes and Goh, 1978). The present results of increased growth by boronia with NH_4^+ plus NO_3^- followed by NH_4^+ and NO_3^- can be related to the natural habitat of boronia. Boronia naturally occurs in the areas where it seems ammonification is not greatly affected but nitrification is reduced (Chap. II. 3.2, p. 13). It leads to NH_4^+ as the predominant form of N although not without small quantities of NO_3^-. Boronia growing under such conditions may have adapted to mixed assimilation of NH_4^+ plus NO_3^- as well as predominantly NH_4^+-based N nutrition. Similar observations of higher growth with NH_4^+ plus NO_3^- or NH_4^+ than with NO_3^- were made on the plants that naturally occur where nitrification is reduced (Table 2, p. 9).

However, higher yields of plants that prefer NO_3^- were also obtained with a NH_4^+ plus NO_3^- mixture than with NO_3^- solely (Table 3, p. 11). The reasons for this effect are not clear. Cox and Reisenauer (1973) ascribed the growth stimulation by NH_4^+ plus NO_3^- to a decreased energy requirement for the assimilation of NH_4^+ compared to the assimilation of NO_3^- (which has to be reduced to NH_4^+ before assimilation while NH_4^+ is directly incorporated) in the plant. However if this were the case then sole NH_4^+ grown plants should yield higher than NH_4^+ plus NO_3^- plants.

The increased growth with NH_4^+ plus NO_3^- seems to be the result of an increased uptake of N. Higher concentration of N in the

leaves of boronia grown with NH_4^+ plus NO_3^- (Figs. 28c and 28d) showed that N uptake was higher with NH_4^+ plus NO_3^- than with NH_4^+ or NO_3^-. Results with other plants also show higher N in plants grown on NH_4^+ plus NO_3^- (e.g. Schrader et al., 1972; Cox and Reisenauer, 1973). It may be that plants are able to absorb more N from NH_4^+ plus NO_3^- than from either source alone.

Since total N in the leaves increased with increasing levels of N supply (Fig. 28), organic N may have also increased. Organic N is likely to be critical in supporting the growth and development of shoot meristems (Elliott and Nelson, 1983). Increased total N, hence increased organic N may have stimulated the development of nodes and lateral shoots in boronia (Figs. 19, 20 and 23).

Although there were significant differences in the growth of boronia with different forms of N the differences in the concentrations of P, K, Ca and Mg in the leaf tissue were not significant. This suggests that to some degree, variability in the growth did not affect the concentration of these ions.

It has been ascertained in many investigations that increased NO_3^- uptake as opposed to NH_4^+ uptake led to increased uptake of cations while anion uptake is diminished (Table 4, p. 20). However the differences in the concentrations of cations K, Ca, Mg and anion P in boronia leaves with different forms of N were not significant. A hypothesis to explain the increased uptake of cations associated with NO_3^- uptake is that to balance the negative charges taken up in the form of NO_3^-, cations are taken up along with NO_3^- and translocated to the leaves where NO_3^- is reduced (Kirkby and Knight, 1977). The same rationale is applied to the

uptake of NH_4^+: to maintain an electrical neutrality the uptake of NH_4^+ would be accompanied by the uptake of an anion, e.g. $H_2PO_4^-$ (Hageman, 1984). Though with the increasing levels of N supply there was an increase in the uptake of NO_3^- (as indicated by an increase in the concentration of N in the leaves), there was no increase in the uptake of cations. Thus the hypothesis is probably true when NO_3^- is reduced in the leaves. If NO_3^- is reduced in the roots and translocated to the shoots in a reduced form such as amino acids, cation concentration should not increase in the leaves. NO_3^- reduction site varies with plant species and external concentration of NO_3^- (Pate, 1980). In the present experiment, with the exposure of roots to the nutrient solutions for a short time, the absorbed NO_3^- may have been reduced in the roots. Similarly, Barker and Maynard (1972) found that NH_4^+ relative to NO_3^- did not affect the cation concentration in pea shoots. The different behavior of plants in this respect of cation accumulation may be an adaptation for efficient utilization of N for growth in their natural habitats.

Cox and Reisenauer (1973) attributed the decrease in cation uptake during NH_4^+ nutrition to ionic competition with NH_4^+ ions at the site of intake and the increase in cation uptake with NO_3^- nutrition to reduced competition in the absorption process. Such antagonistic effect of NH_4^+ on the uptake of cations may lead to deficiency of cations in the plants growing under the conditions where NH_4^+ is the predominant form of N. Therefore, boronia and other plants adapted to better utilization of NH_4^+ may have an effective ability to take up cations even in the presence of NH_4^+.

Other plants adapted to NH_4^+ uptake did not show Ca or Mg deficiency when grown on NH_4^+ (Pinus contorta, Krajina et al., 1973) or any significant differences in P and K concentrations between NH_4^+ or NO_3^- nutrition (Deschmpsia flexuosa, Gigon and Rorison, 1972). Moore and Keraitis (1971) found that in Eucalyptus rossi, E. sideroxylon and E. polyanthemos which grow better with NH_4^+, Ca uptake was high with NH_4^+. Ingestad (1976) found that the cation uptake capacity of Vaccinium plants, which occur in localities with NH_4^+ as the main source of N, is strong and independent of NO_3^-. Such efficient ion uptake mechanism may be a significant property in the plants that are able to grow in habitats where availability of nutrient ions is low.

Symptoms similar to those that occurred in boronia at lower N levels (chlorotic leaf tips) were observed on some Australian native Wallum plants by Grundon (1972) who attributed the symptoms to P toxicity. Nutrients other than N were supplied at similar levels to all boronia plants. Apparently all the plants took up similar amounts of other nutrients regardless of their growth differences. Thus at lower N levels, due to reduced growth the concentrations of other nutrients including P in the leaves were higher (Fig. 29) and may have reached toxic levels in the leaf tips causing the symptoms. The P content of a Wallum plant Leptospermum liversidgii showing slight chlorosis was 0.76% (Grundon, 1972). The P concentration in boronia was lower (did not exceed 0.26%). However, bulked shoot (leaf and stem) tissue was analyzed in L. liversidgii where as in boronia only the leaf tissue was analyzed and further, the symptoms in boronia were very slight. There may

also be species differences in the concentration of P causing the toxicity.

With increasing N levels due to increased growth, the concentrations of other nutrients were diluted (Fig. 29) and no toxicity symptoms were produced. Negative relations between N and P were observed in other Australian native plants also: Acacia verticillata (Thomas, 1981), Hakea laurina (Thomas, 1982). Such a dilution by increased growth with increased N levels may eventually cause deficiency of other nutrients although in boronia no familiar deficiency symptoms of other nutrients were produced. Moore and Keraitis (1966) observed K deficiency symptoms on an Australian native plant Grevillea robusta at high N levels due to increased growth.

Low concentration of other nutrients may also affect flower development. In tomato, P deficiency reduced the number of flowers that develop to anthesis (Menary and van Staden, 1976) and insufficient K produced smaller than normal roses (Seeley, 1950).

Practical implications

Since N deficiency symptoms did not readily occur in boronia, care should be taken to identify N stress. Reduction in shoot growth and in the production of lateral shoots were associated with N stress. Although high N at the time of flowering seemed to have a negative effect on the development of flower buds, it was the number of nodes at the time of flowering that largely determined the number of flowers per plant. Therefore boronia should be free of N stress so as to produce as many nodes as possible during the early vegetative growth period (Sept.-Mar.). Care should be taken to time

N application so that it does not encourage vegetative growth at the time of flowering. Amount of shoot growth and branching determine the form of plant which is important in mechanical harvesting. This factor should also be considered in application of N as apparently branching can be controlled by N nutrition. The N fertilizer applied should contain both NH_4^+ and NO_3^- forms. A positive balance of nutrients especially between N and P is important to avoid deficiency or toxicity of P and other nutrients. Overall the present results show that with careful N application flower yields can be improved in boronia.

VI. EFFECTS OF NITROGEN FORM AND pH ON UPTAKE AND ASSIMILATION OF NITROGEN IN BORONIA

As noted in the Review of Literature, generally rhizosphere pH decreases with NH_4^+ nutrition and increases with NO_3^- nutrition. Further, maximum absorption of NH_4^+ by plants occurs at higher pH while maximum absorption of NO_3^- occurs at lower pH though there are species differences in this effect. Thus generally the effects of NH_4^+ nutrition and NO_3^- nutrition on rhizosphere pH have detrimental effects on the absorption of the respective ions.

An experiment was conducted to study the effects of NH_4^+ and NO_3^- on the direction and magnitude of changes in pH of the growth medium and the uptake of NH_4^+ and NO_3^- by boronia under such changing pH conditions. Further, the uptake of NH_4^+ and NO_3^- by boronia under the conditions where pH was held constant (at lower and higher pH) was also studied. In addition, assimilation pattern of NH_4^+ and NO_3^- absorbed at low and high pH into amides and amino acids in root and leaf tissue was also studied.

A water culture technique was used in this experiment to monitor the pH changes closely and for easy recovery of roots for the analysis.

MATERIALS AND METHODS

Plant material. 1-yr old well rooted cuttings of boronia clone HC-1 growing in vermiculite were taken. These plants were being supplied with 10 mM NH_4^+ plus NO_3^- type Long Ashton nutrient solution once a week. The vermiculite was washed off the roots gently in running water by a hand sprinkler and the plants were transferred

to a solution containing 10 mM NH_4^+ plus NO_3^- type nutrient solution.

Culture technique. The nutrient solution was held in a 9-l brown plastic bucket wrapped with Al foil. The top of the bucket was covered with a white painted hardwood (Masonite) lid in which holes were made. A plant was placed in a hole and wrapped with crimped Terylene fibre for support. In another hole a tube was inserted for supplying roots with O_2. Compressed air was bubbled through this tube into the nutrient solution (for 10 min every 30 min). This aeration stirred the solution which would have prevented any nutrient depletion at the root surface.

Growth conditions. The plants were placed in a growth cabinet (Controlled Environments). Light hours were set for 15 and light intensity was 165 uE m^{-2} s^{-1} at the top of the plant. Day temperature was set at 20°C and night temperature was set at 15°C. Such conditions which naturally occur locally during summer promote vegetative growth in boronia.

Composition of nutrient solutions. The nutrient solutions used as the treatments were 10 mM NH_4^+ or NO_3^- type solutions as given in Table 8 (p. 84). The nutrient solutions were prepared with deionized water. During the experimental period the volume of nutrient solution in a bucket was kept constant by adding deionized water to make up the loss by evapotranspiration.

Treatments. The treatments were: 2 N forms (NH_4^+ and NO_3^-) X 3 pHs (uncontrolled and controlled at 4.5 and 6.5). Each treatment had 3 replications. Because of the restricted availability of space under growth cabinet the replications were done consecutively. The experimental design was factorial in a CRD.

The pH of nutrient solutions was adjusted by adding the required amounts of 0.1 M HCl to make them acidic or 0.1 M NaOH to make them alkaline. Where pH was controlled daily, the acid or alkali was added daily.

Measurements. The pH of nutrient solutions was recorded daily. Every 5 days the solutions were sampled and NH_4^+ or NO_3^- in the respective solutions were analyzed by Kjeldahl method using MgO or Devarda's alloy (General Materials and Methods, p. 39). The NH_4^+ solutions were analyzed for NO_3^- and no NO_3^- was detected anytime, therefore the NH_4^+ solutions were free of nitrification effect. Every 5 days on each plant on two selected shoots, the number of nodes produced and the number of laterals produced were counted. After 30 days the plants under constant pH conditions were harvested, roots and leaves were washed with distilled water and analyzed for amides and amino acids by HPLC (Chap. III.1, p. 44).

The data of measurements were analyzed statistically. Where the measurements were made on two selected shoots on each plant the values were averaged and the analysis performed on the means. Data of measurements made every 5 days were analyzed by treating the days as a factor and analyzing as a split-plot design. ANOVA was computed and when the F test for a treatment effect was significant at $P = 0.05$ or 0.01, LSD test was used for comparison of the treatment means.

RESULTS

After transferring the plants from vermiculite to the solution culture, roots began to become dark brown and a few leaves abscised. After 10-15 days, new roots began to emerge. Compared to

numerous, long, thin and brown old roots, the new roots were few, short, thick and white. Within a week after the initiation of new roots, shoot nodes started to elongate. The original roots blackened and died. The new rooting occurred on <1/3 of the plants that were transferred to the nutrient solutions and on the other 2/3 of the plants the new roots were not initiated and the plants died. The successfully rooted plants were transferred to the treatment solutions and then the measurements were started (see Fig. 32).

pH. When pH of the nutrient solution was controlled, the daily deviations were generally <0.2 unit from the desired pH values (Fig. 33) and thus the daily adjustment of pH was adequate to maintain a satisfactory stability of the pH treatments.

The changes in pH of NH_4^+ and NO_3^- solutions when pH was not controlled are shown in Fig. 34. With NO_3^- solution, pH rose from 6.5 to 7.0 while in contrast NH_4^+ solution pH dropped from 6.5 to 3.0. Thus the magnitude of change in pH was very low with NO_3^- (only 0.5 unit in 30 days) while the decrease in pH with NH_4^+ was >0.1 unit a day.

N depletion from nutrient solutions. The concentration of N in the nutrient solutions significantly decreased over the days (Table 9) indicating an uptake of N by the plants. The nutrient solution pH did not significantly affect the uptake of N. Irrespective of pH, significantly more N was depleted from NH_4^+ solutions than form NO_3^- solutions.

Nodes. Production of nodes significantly increased over the days (Table 10) but the effect of N form or pH was not significant.

Fig. 32. New roots initiated by water culture.

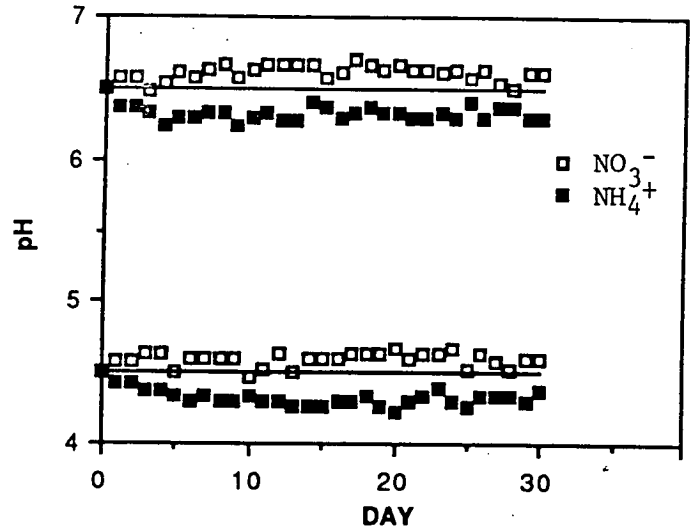


Fig. 33. Daily variation in pH of the solutions containing NH_4^+ or NO_3^-. Each day pH was measured and adjusted back to 4.5 or 6.5. The lines indicate desirable pHs.

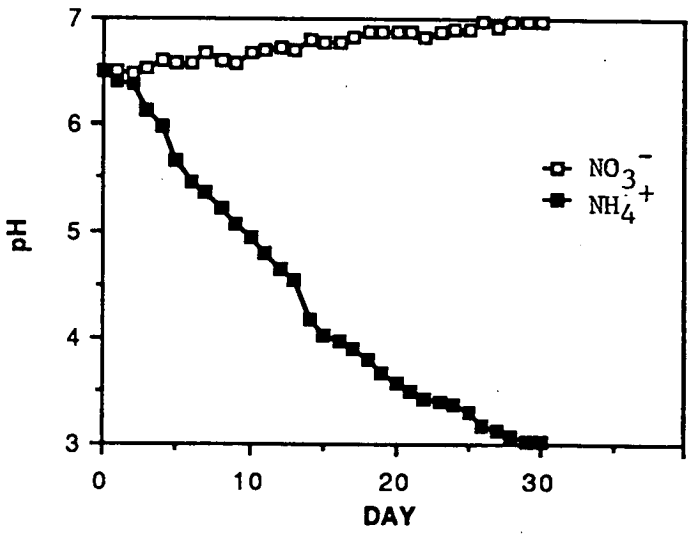


Fig. 34. Changes in pH of the solutions containing NH_4^+ or NO_3^- when pH was not adjusted.

Table 9. Depletion of N from solutions containing NH_4^+ or NO_3^- at different pHs.

Form of N	pH	Days:	N in solution, mM 0	5	10	15	20	25	30	Mean
NH_4^+	changing[a]		10.00	9.30	9.23	9.07	8.93	8.93	8.93	9.20
	4.5		10.00	9.57	9.47	9.07	9.17	9.13	8.93	9.33
	6.5		10.00	9.63	9.47	9.40	9.23	9.07	9.00	9.40
										9.31
NO_3^-	changing		10.00	9.63	9.47	9.20	9.13	9.13	9.13	9.39
	4.5		10.00	9.70	9.47	9.57	9.37	9.20	9.23	9.50
	6.5		10.00	9.57	9.40	9.40	9.40	9.20	9.30	9.47
										9.45
	Mean		10.00	9.57	9.42	9.29	9.21	9.11	9.09	

Significance	P	LSD
Form of N	0.05	0.11
	0.01	ns
pH	0.05	ns
Form of N X pH	0.05	ns
Days	0.05	0.17
	0.01	0.13
Form X Days	0.05	ns
pH X Days	0.05	ns
Form of N X pH X Days	0.05	ns

[a] pH adjusted only in the beginning to 6.5.

Table 10. Production of lateral shoots as affected by NH_4^+ or NO_3^- at different pHs.

Form of N	pH	Days:	No. of nodes, cumulative 0	5	10	15	20	25	30	Mean
NH_4^+	changing		0	0.5	1.3	1.7	2.0	2.8	3.3	1.67
	4.5		0	0.3	1.3	1.7	2.0	2.7	3.2	1.60
	6.5		0	0.2	1.0	1.3	1.7	2.2	2.7	1.29
										1.52
NO_3^-	changing		0	0	0.7	1.2	1.8	2.5	3.0	1.31
	4.5		0	0.3	1.2	1.5	1.7	2.3	2.8	1.40
	6.5		0	0.3	0.8	1.3	1.5	1.8	2.2	1.14
										1.28
	Mean		0	0.28	1.06	1.45	1.78	2.39	2.87	

Significance	P	LSD
Form of N	0.05	ns
pH	0.05	ns
Form of N X pH	0.05	ns
Days	0.05	0.04
	0.01	0.03
Form X Days	0.05	ns
pH X Days	0.05	ns
Form of N X pH X Days	0.05	ns

Lateral shoots. Lateral shoots were apparent at the nodes only after 25 days after the experiment began. After 5 more days there was a significant increase in the initiation of lateral shoots (Table 11) but the effect of form of N or pH was not significant.

Amides and amino acids in plant tissues. The concentrations of amides and amino acids in root and leaf tissues as affected by form of N and pH are shown in Table 12. Form of N had a pronounced effect on the concentration of amides, asparagine and glutamine. Within the plants, concentration of asparagine was significantly higher in the roots than in the leaves. In both plant parts, concentration of asparagine was higher (3.5 times in the roots and 2.2 times in the leaves) when supplied with NH_4^+ than with NO_3^-. Concentration of glutamine was also significantly higher with NH_4^+ than with NO_3^-; however, there were no significant differences between the roots and the leaves. There was no significant effect of pH on the concentration of amides. There were no significant differences in any of the amino acids due to form of N or pH or plant part or their interactions.

Generally in the decreasing order of concentration the amides and amino acids were:

Asn>Pro>Gln>Thr>Glu>Ala>Ser>Asp>Gly>Val>Met>Phe>Leu+Iso>Cys>Lys.

DISCUSSION

The contrasting changes in the nutrient solution pH (Fig. 34) may be due to the excretion of H^+ or OH^- by the plants. Raven and Smith (1976) proposed that when N is assimilated in the cytoplasm of plant root cell, one H^+ per NH_4^+ or one OH^- per NO_3^- which are generated in the cytoplasm are removed to maintain a constant

Table 11. Production of lateral shoots as affected by NH_4^+ or NO_3^- at different pHs.

Form of N	pH	No. of lateral shoots, cumulative							
	Days:	0	5	10	15	20	25	30	Mean
NH_4^+	changing	0	0	0	0	0	0.5	0.7	0.17
	4.5	0	0	0	0	0	0.2	0.5	0.10
	6.5	0	0	0	0	0	0	0.2	0.02
									0.10
NO_3^-	changing	0	0	0	0	0	0.2	0.3	0.07
	4.5	0	0	0	0	0	0.2	0.3	0.07
	6.5	0	0	0	0	0	0	0.2	0.02
									0.05
	Mean	0	0	0	0	0	0.17	0.36	

Significance	P	LSD
Form of N	0.05	ns
pH	0.05	ns
Form of N X pH	0.05	ns
Days	0.05	0.01
	0.01	0.01
Form X Days	0.05	ns
pH X Days	0.05	ns
Form of N X pH X Days	0.05	ns

Table 12. Concentrations of free amino acids in roots and leaves as affected by N form at different pHs.

Form of N	pH	Plant part	Amino acid, nmol g^{-1} fresh wt														
			Asn	Gln	Ser	Asp	Glu	Thr	Gly	Ala	Pro	Met	Val	Cys	Phe	Leu+ Iso	Lys
NH_4^+	4.5	Root	16087	2691	653	585	870	1252	427	729	3361	184	307	154	169	168	138
		Leaf	9557	2610	693	608	835	1442	452	705	3954	181	350	145	175	172	138
	6.5	Root	16942	2812	708	618	850	1360	435	713	3546	178	325	148	169	172	138
		Leaf	8940	2853	678	625	852	1370	448	733	3677	178	332	148	175	170	136
NO_3^-	4.5	Root	4695	2414	682	588	842	1307	427	697	3311	181	350	147	172	172	140
		Leaf	4306	2188	656	625	823	1268	448	725	3432	181	339	148	172	173	138
	6.5	Root	4854	2234	715	615	852	1373	448	741	3503	178	343	148	166	175	140
		Leaf	4027	2110	693	665	825	1320	452	674	3666	178	350	150	178	173	138

Significance	P	LSD	
Form of N	0.05	1445	283
	0.01	1046	205
pH	0.05	ns	ns
Form of N X pH	0.05	ns	ns
Plant part	0.05	1445	ns
	0.01	1046	
Form of N X Plant part	0.05	2042	ns
	0.01	1482	
pH X Plant part	0.05	ns	ns
Form of N X pH X Plant part	0.05	ns	ns

The effects were not significant with regard to all other amino acids.

cytoplasmic pH favorable for protein production. Reading the changes in pH of the nutrient solutions in the present experiment as reflections of the relative NH_4^+ influx/H^+ efflux and NO_3^- influx/OH^- efflux would allow a conclusion that boronia plants absorbed more NH_4^+. However when NH_4^+ is assimilated, H^+ is excreted to the external medium and when NO_3^- is assimilated, OH^- is partly excreted to the external medium and partly neutralized *via* the 'biochemical pH stat' which restores a balance of pH by producing organic acids whenever the intercellular pH rises above a certain limit (Raven and Smith, 1976). Also, the changes in pH of the nutrient solutions may be caused by the difference in the uptake of cations and anions.

However, the higher pH changes in NH_4^+ solutions corresponded with higher depletion of N from these solutions as compared to NO_3^- solutions (Table 9). NH_4^+ and NO_3^- uptake by boronia was independent of pH: 4.5, 6.5 as well as changing pH. Generally more NH_4^+ uptake occurs at 6.0 to 7.0 while more NO_3^- uptake occurs at 4.5 to 6.0 (e.g. Hewitt, 1966). However, dependence of uptake of NH_4^+ or NO_3^- on pH seems to be dependent on the plant species. Favorable effect on growth with NH_4^+ at all pHs have been reported for plants that grow naturally in the soils where NH_4^+ occurs predominantly (*Pinus radiata* and *Picea glauca*, McFee and Stone, 1968; *Vaccinium angustifolium*, Townsend, 1969; *Deschampsia flexuosa*, Gigon and Rorison, 1972). The conditions that occur in the natural habitat of boronia indicate an availability of more NH_4^+ than NO_3^- (Chap. II.3.2, p. 13). Thus the plants adapted to such conditions may have better uptake capacity for NH_4^+ than for NO_3^- independent of pH.

Recently Atkinson (1985) found that NH_4^+ uptake was higher in 4 plants that co-exist in an upland acidic grassland, *viz*. *Deschampsia flexuosa*, *Festuca ovina*, *Juncus squarrosus* and *Nardus stricta*.

The absorbed NH_4^+ and NO_3^- are assimilated to form amino acids in the plants. Current theory suggests GS-GOGAT system (Oaks and Hirel, 1985) according to which glutamine synthetase (GS) catalyzes incorporation of NH_3 to give glutamine and glutamine synthase (GOGAT) catalyzes glutamine to glutamate.

Most of the N in the tracheal sap is present as amino acids in citrus (Moreno and Garcia-Martinez, 1980), apple and other woody perennial plants (Bollard, 1957) suggesting that incoming inorganic N is transformed and amino acids are synthesized in the roots and translocated to the aerial parts under normal conditions of N supply in the woody perennial plants. Thus the amino acids detected in the leaves of boronia may have root originated and those in the roots may have been synthesized there itself. However whether amino acids are recycled in the phloem from the leaves to the roots in woody plants is not known.

Each plant species seems to have a characteristic spectrum of the amides and amino acids, possibly as a consequence of differences in metabolism. In boronia, high levels of asparagine were found (Table 12). In citrus, Kato (1980) using ^{15}N established that newly taken up N was assimilated into glutamine and then into glutamate followed by asparagine which became predominant. Asparagine is synthesized by the transfer of amide from glutamine to aspartate by asparagine synthetase. Moreno and Garcia-Martinez

(1980) reported asparagine to be the major nitrogenous compound in the tracheal sap of citrus. Therefore in these plants considering its large pool, probably asparagine acts as an amino acid storage pool and is the main transport form of N upward from the roots leading to higher asparagine in the leaves.

Higher levels of amides were found in boronia plants supplied with NH_4^+ than NO_3^- (Table 12). The pattern of NO_3^- assimilation is similar to that of NH_4^+ assimilation (Yoneyama and Kimazava, 1975) indicating that the incorporation of NO_3^- into amino acids takes place after the reduction of NO_3^- to NH_4^+. Therefore, synthesis of amino acids in NO_3^- plants may have occurred at a slower rate due to the low NO_3^- reduction in boronia (Chap. VIII) resulting in lower levels of amino acids in NO_3^- supplied plants.

Higher levels of amino acids were found in many plants with NH_4^+ nutrition (Chap. II. 3.5, p. 22). However all these plants exhibit adverse effect when grown with NH_4^+. An explanation for this effect is that absorbed NH_4^+ within the plant is rapidly detoxified by the synthesis of amides and amino acids. This necessitates a high level of demand on carbon skeletons at the expense of other necessary carbon compounds resulting in the reduced growth of plant. No adverse effect on the growth was apparent in boronia plants grown with NH_4^+. In sclerophyllous plants such as boronia carbon is produced in excess (and deposited in cell walls or cuticle) (Schulze, 1982), therefore such plants may not be limited by the carbon supply. Similarly Rufty et al. (1983) observed that NH_4^+ did not depress the growth in soybean when exposed to NH_4^+ during the steady exponential growth during which the uptake of N was balanced

with the flux of carbohydrate to the roots. Therefore they concluded that plants can utilize NH_4^+ as long as a balance is maintained between the carbohydrate availability and acquisition of NH_4^+.

The differences in growth (production of nodes and lateral shoots) were not significant although the trend showed a higher growth with NH_4^+ than with NO_3^- (Tables 10 and 11). The increase in plant growth during this experiment was low resulting in a low response to the treatments. A longer experimental period may have yielded higher growth and greater response to the treatments.

VII. OPTIMIZATION OF ASSAY CONDITIONS FOR NITRATE REDUCTASE AND NITRITE REDUCTASE ENZYMES FROM BORONIA

Nitrate reductase (NR) activity is measured *in vivo* and *in vitro* (Hageman and Reed, 1980). The *in vivo* assay is also carried out in laboratory vessels using excised plant tissue and not on intact tissue on plant itself.

It is usual in the enzyme work to aim at conditions that give maximum activity of the enzyme being studied. Factors affecting the assays of NR activity have been reported for many plants (e.g. Havill *et al.*, 1974; Jones and Sheard, 1977; Lillo, 1983; Shivshanker and Ramdasan, 1983; Davies and Ross, 1985). From these reports it is evident that the assay conditions that are optimal for one plant species are not necessarily the same for other plant species. Therefore it is necessary to establish optimum conditions for the enzyme from particular species that is being examined.

Like NR activity, nitrite reductase (NiR) activity is also measured *in vivo* (Vega *et al.*, 1980). However, NiR has not been characterized to the same extent as NR has been. Similarly there are very few reports (Ferrari and Varner, 1971; Pierson and Elliott, 1981) and some indications (Klepper, 1974, 1975, 1976, 1979; Finke *et al.*, 1977) characterizing NiR activity *in vivo*. It may be due to the fact that NiR is not considered to be a limiting enzyme in most plants.

Before investigating the role of NR and NiR enzymes in N toxicity in boronia, a series of experiments were conducted with an objective of obtaining the optimal assay conditions for determination of:

NR activity *in vivo* in roots,

NR activity *in vivo* in leaves.

NR activity *in vitro* in leaves,

NiR activity *in vivo* in leaves and

NiR activity *in vitro* in leaves.

MATERIALS AND METHODS

The methods common to all the experiments are described in this section and any variations in these methods (for testing specific treatments) are given in the next section along with the Results and Discussion of each experiment. The optimum factor established in one experiment was used while testing the other factors in the subsequent experiments.

Plant culture. 3-yr old plants of clone HC-1 were grown in 2:1 v/v composted eucalyptus bark:sand in 15-cm plastic pots. The plants were grown in a greenhouse under natural daylight (mean daily temperature ca. 22^{o}C). The plants were supplied with 10 mM NH_4^+ type Long Ashton nutrient solution (Table 8) once a week and with water once a day.

One day before the analyses of plant tissue, each plant was supplied with 25 mmol NO_3^- as $Ca(NO_3)_2$ and the growth medium was watered till it was saturated.

NR exhibits a diurnal variation in its activity (Janiesch, 1973; Lillo, 1983). Therefore to reduce these light dependent fluctuations in NR activity, the leaves and roots were always sampled about noon. Fine roots and fully developed middle leaves on shoots were sampled. The roots and leaves were washed in deionized water to remove any exogenous NO_3^-, blotted with tissue

and weighed.

The basic procedures for the enzyme assays were those of Hageman and Reed (1980) for NR and of Vega et al. (1980) for NiR.

NR activity in vivo in roots and leaves. 300 mg of roots or leaves was placed in a vial containing 5 ml of cold (refrigerated) assay medium which in all the cases was composed of 100 mM K/HPO_4 buffer, pH 7.5. Details of NO_3^- concentration and other additives in the assay medium are given in the description of each experiment in the next section. The vial was wrapped in Al foil to exclude light and incubated in an incubator at 30°C. At the end of incubation period (usually 1 h) an aliquot of the assay medium was tested to determine the NO_2^- produced. There was a zero time control.

NiR activity in vivo in leaves. NiR activity in vivo was determined in an assay similar to NR activity in vivo assay but as the disappearance of NO_2^- from the assay medium in a photoreduction method.

300 mg of leaves were placed in a vial containing 3 ml of the assay medium which was composed of K/HPO_4 buffer and NO_2^-. The vial was then placed in a shaking water bath at 30°C and illuminated by fluorescent bulbs that gave >230 $\mu E\ m^{-2}\ s^{-1}$ at the leaf surface. At the end of incubation period (usually 1 h) an aliquot of the assay medium was taken for the estimation of NO_2^- reduced by the enzyme.

Preparation of cell-free enzyme extract from leaves. The extraction medium contained 100 mM K/HPO_4 buffer, pH 7.5; 1 mM EDTA (to minimize a possibility of inhibition of the enzymes by metal ions) and other additives as given in the description of the experiments. The leaves were homogenized in the extraction medium at 0°C at a

leaf tissue:extraction medium ratio of 1:6 w/v. The homogenate was squeezed through 4 layers of cheesecloth. The filtrate was centrifuged at 30,000 X g for 15 min at 0^0C (MSE High Speed 18 centrifuge) and the supernatant was used for the assays.

NR is very unstable (Hageman and Reed, 1980), therefore the extraction and assay were done at 0^0C within 2 h after sampling the leaves.

NR activity in vitro assay. NR activity was measured by the NO_2^- formed by the enzyme. Usually the assay mixture contained 50 µmol (500 µl of 100 mM) K/HPO_4 buffer, pH 7.5; 800 nmol (400 µl of 2 mM) NADH (Serva); 20 µmol (200 µl of 100 mM) KNO_3; the enzyme extract and distilled water to make a final volume of 2 ml. The reaction was started by adding the enzyme. The incubation was at 30^0C in a water bath for a period of usually 30 min. A zero time was used for the control.

Residual NADH in the assay medium at the end of reaction period is known to interfere in full development of color for the determination of NO_2^- (Hageman and Reed, 1980) and this interference can be overcome by the removal of residual NADH. Residual NADH was precipitated with Zn salts by adding 100 µmol (100 µl of 1 M) zinc acetate and mixing on a Vortex. The precipitate was clarified by centrifugation at 5000 X g for 15 min. An aliquot of the supernatant was used for NO_2^- determination.

NiR activity in vitro assay. NiR activity was measured by the dithionite assay (Vega et al., 1980) which involves sodium dithionite as the reductant and an artificial substitute of ferredoxin, methyl viologen as the electron donor. The enzyme

activity was measured by the disappearance of NO_2^- from the assay medium.

The assay mixture consisted of 150 µmol (300 µl of 500 mM) Tris HCl buffer, pH 8.0; 15 µmol (300 µl of 5 mM) methyl viologen (Aldrich); usually 400 µmol (200 µl of 2 mM) $NaNO_2$; 300 µl of fresh sodium dithionite solution; the enzyme extract and distilled water to give a final volume of 2 ml. Just prior to its use, 25 mg sodium dithionite was dissolved in 1 ml of 0.29 M $NaHCO_3$. The reaction was started by the addition of sodium dithionite. After incubation at 30°C (usually for 20 min) in a water bath the reaction was stopped by mixing the test tubes on a Vortex until dithionite was oxidized (until the blue dye became colorless). The aliquot was diluted and NO_2^- was determined.

NO_2^- was determined by the Griess-Ilosvay colorimetric method (General Materials and Methods, p. 43). The calibration curves were prepared in the presence of respective treatment additives.

Protein was determined by Bio-Rad protein assay (p. 43).

The activity of the enzymes is expressed on a fresh weight basis for *in vivo* assays and on both fresh weight and protein bases for *in vitro* assays.

For each treatment, assays were run on two samples from each of three plants which were supplied with NO_3^- fertilizer. The values of two analyses were averaged. Statistical analysis to examine the significance of the difference between the treatments was done by ANOVA (and LSD) when more than two treatments were examined and by t-test when only two treatments were examined.

RESULTS AND DISCUSSION

NR activity *in vivo* assay in roots and leaves

Effect of NO_3^- concentration. The concentration of NO_3^- in the assay medium was varied to determine the concentration for maximum NR activity *in vivo* in the roots and leaves.

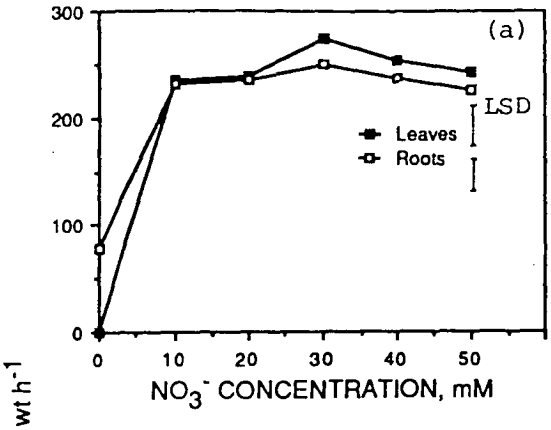
For both the roots and leaves the NR activity was maximum at a NO_3^- concentration of 30 mM (Fig, 35a). Without any NO_3^- in the assay medium, leaves did not show any NR activity while roots had a NR activity of 78 nmol NO_2^- g^{-1} fresh weight h^{-1}. This indicates that, with the given level of NO_3^- supply to the plant (25 mmol), leaves did not accumulate NO_3^- while roots accumulated NO_3^- and reduced it during the assay.

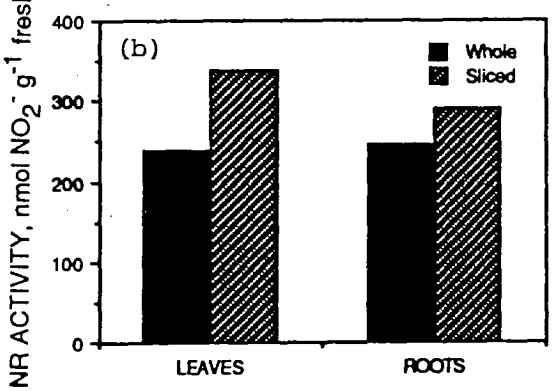
In the *in vivo* assay, prior to incubation the tissue is generally subjected to different treatments mainly to increase the tissue permeability to metabolites. These treatments were tested for boronia tissue as below.

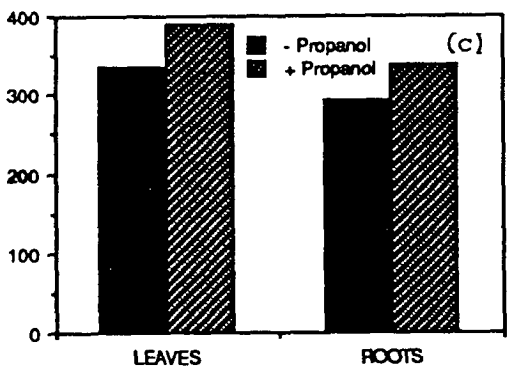
Effect of slicing the tissue. Generally for *in vivo* assay, leaf discs are punched from whole leaves with a cork borer (Jones, 1973). However, boronia has needle-like leaves and therefore they were sliced. Leaves as well as roots were cut with a razor blade (moistened for easier cutting) into 3-4 mm fragments.

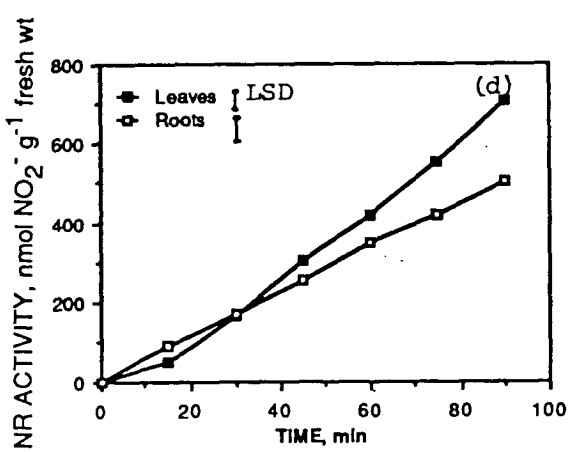
Slicing increased the NR activity by 41% in the leaves; however there was no significant increase in the enzyme activity in the roots (Fig. 35b). The increase in NR activity due to slicing as compared to whole leaves shows that the cut edges would have caused more rapid diffusion of NO_3^- into the tissue and NO_2^- out of the tissue.

Fig. 35. Effect of different assay conditions on NR activity *in vivo*.
(a) NO_3^- concentration in assay medium. LSD at P = 0.01.
(b) Slicing of tissue. Leaves and roots were cut into 3-4 mm fragments. Difference between treatments was significant in leaves at P = 0.01 and not significant in roots, t-test.
(c) Propanol in assay medium (at 1% v/v). Difference between treatments was significant in both leaves and roots at P = 0.05, t-test.
(d) Time course. After a given incubation time NO_2^- produced was measured. LSD at P = 0.01.


(a)
LSD
Leaves
Roots
300
200
100
0
0
10
20
30
40
50
NO_3^- CONCENTRATION, mM


(b)
Whole
Sliced
NR ACTIVITY, nmol NO_2^- g^{-1} fresh wt h^{-1}
400
300
200
100
0
LEAVES
ROOTS


(c)
- Propanol
+ Propanol
400
300
200
100
0
LEAVES
ROOTS


(d)
Leaves
Roots
LSD
NR ACTIVITY, nmol NO_2^- g^{-1} fresh wt
800
600
400
200
0
0
20
40
60
80
100
TIME, min

Effect of propanol. Propanol was included in the assay medium at 1% v/v. This inclusion of propanol enhanced the NR activity by 15% in the roots and by 16% in the leaves (Fig. 35c).

As to the enhancement of NR activity by propanol, Menary and Jones (1972) suggested that alcohols enhance the membrane permeability and thus increase the availability of substrate NO_3^- to the enzyme. Aryan and Wallace (1983) explained the stimulatory effect of propanol as a result of the generation of NADH for NR *via* alcohol dehydrogenase (ADH). Propanol may also lower the surface tension of the assay solution and thus increase the transfer of NO_3^- to the enzyme.

Effect of anaerobiosis with air or N_2. Anaerobic conditions are essential for measuring NR activity *in vivo* (Canvin and Woo, 1979). Therefore, anaerobic conditions were created for boronia tissue samples. The vial containing the tissue and assay medium was placed in a vacuum desiccator and the air was evacuated and released for 2-3 times. By then the tissue sank in the assay medium. In the case of N_2 treatment the gas was bubbled through the assay medium in the vial after the air evacuation.

There was no significant enhancement when N_2 was bubbled (Table 13) indicating that high anaerobiosis was achieved by the vacuum infiltration with air only.

Table 13. Effect of anaerobiosis of tissue with air and N_2 on NR activity *in vivo* in roots and leaves.

	NR activity, nmol NO_2^- g^{-1} fresh h^{-1}	
Treatment	Leaves	Roots
Air	392	322
N_2	397	325

Difference between treatments was not significant, t-test.

Time course of NR activity. To establish that the enzyme assays were linear with time, the tissue was incubated for various periods at 15 min intervals up to 90 min.

NO_2^- production was linear with time in the roots and leaves except during the initial 15 min period of incubation in the case of leaves (Fig. 35d). This lag phase may have been due to residual air in the leaf tissue after air evacuation which may cause low permeability to the movement of external NO_3^- and internal NO_2^- as suggested by Klepper et al. (1971).

NiR activity in vivo assay in leaves

The treatments that increased NR activity in vivo, viz. leaf slicing and propanol in the assay medium were adopted for NiR activity in vivo assay also. From pilot experiments it was found that the NiR activity in vivo could be measured only when both pH and NO_2^- concentration in the assay medium were low.

Effect of buffer pH. The buffer pH was varied from 4.5 to 7.5 (for pH 4.5, the buffer was adjusted with orthophosphoric acid).

The NiR activity decreased with increasing pH (Fig. 36a). This is in agreement with the method of Ferrari and Varner (1971) for measuring NiR activity in barley aleurone layers. However the high NiR activity at low pH in boronia is in contrast to the method of Pierson and Elliott (1981) who used a buffer with a pH of 7.5 for bean leaf tissue. As NiR enzyme was active in vitro even in pH 8.0 buffer it is suspected that the lack of NR activity in vivo at higher pH may be due to the inability of NO_2^- to enter the boronia leaf tissue at higher pH.

Effect of NO_2^- concentration. The concentration of NO_2^- in the assay

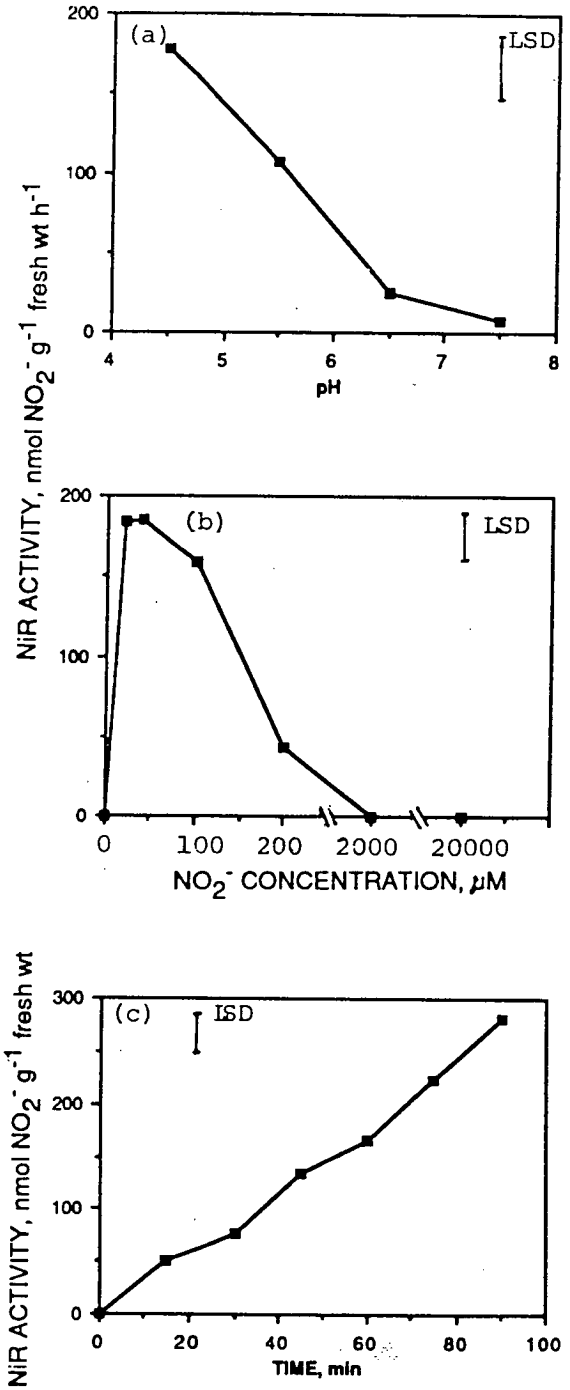


Fig. 36. Effect of different assay conditions on NR activity <u>in vivo</u>. LSD at P = 0.01.
(a) Assay medium pH. The buffer was 100 mM K/HPO_4; pH was adjusted to 4.5 with orthophosphoric acid.
(b) NO_2^- concentration in assay medium.
(c) Time course. After a given incubation time NO_2^- disappeared was measured.

medium was varied. Six concentrations of NO_2^- were used, viz. 20, 40, 100, 200 µM, 2 and 3 mM.

A low concentration (20 or 40 µM) was adequate for maximum NiR activity (Fig. 36b) and the enzyme activity decreased with increasing concentration of NO_2^-. However the results at higher concentrations should be viewed with caution because at higher concentrations the amount of NO_2^- disappearing from the assay solution would be small compared to the amount present in the solution and dilution factors (to bring the aliquot into the spectrophotometric detection range) would be extreme, thus reducing the sensitivity.

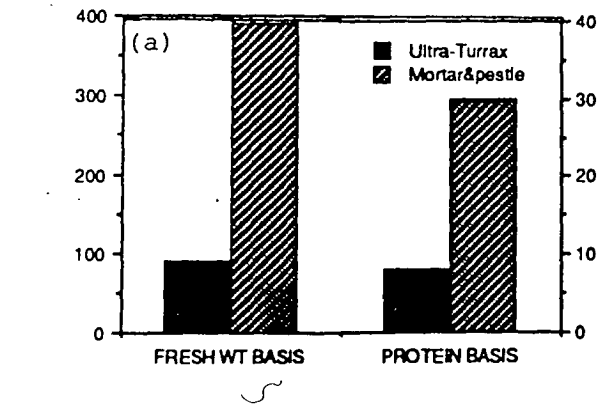
Time course of NiR activity. Leaf tissue was incubated for 15, 30, 45, 60, 75 and 90 min. The NiR activity was linear with time (Fig. 36c).

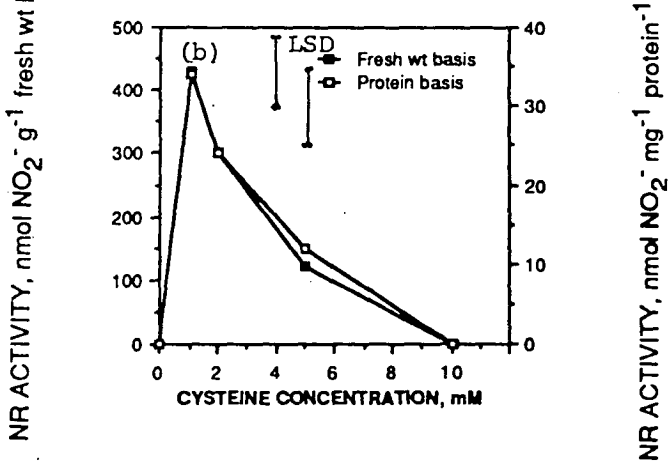
Preparation of cell-free enzyme extract from leaves.

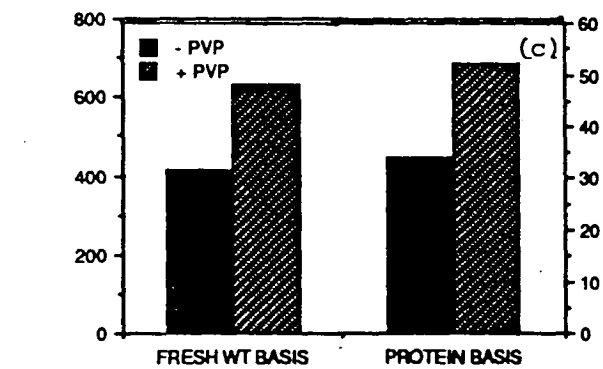
Effect of homogenization method. The leaves were homogenized in two ways. The leaves and extraction medium were placed in a tube which was immersed in ice and homogenized with an Ultra-Turrax homogenizer or the leaves were placed in an ice cold mortar, frozen with liquid N_2 and rapidly ground into powder with a pestle while still in a frozen state. Then the ground powder was taken in the extraction medium.

Compared to the homogenization by Ultra-Turrax, homogenization with pestle and mortar increased the NR activity by 333% on a fresh weight basis and by 275% on a protein basis (Fig. 37a). Very low NR activity by homogenization with Ultra-Turrax may be due to failure of the homogenizer to rupture all cells as boronia leaves

Fig. 37. Effect of different conditions in the preparation of cell-free enzyme extract on NR activity *in vitro* in leaves.
(a) Homogenization method. Leaves were homogenized by an Ultra-Turrax or frozen with liquid N_2 and ground in mortar and pestle. Difference between treatments was significant on both fresh weight and protein bases at P = 0.01, t-test.
(b) Cysteine concentration in external medium. LSD at P = 0.01.
(c) PVP in extraction medium at 1 g per g fresh weight. Difference between treatments was significant on both fresh weight and protein bases at P = 0.01, t-test.


(a)
Ultra-Turrax
Mortar&pestle
400
300
200
100
0
40
30
20
10
0
FRESH WT BASIS
PROTEIN BASIS


NR ACTIVITY, nmol NO_2^- g^{-1} fresh wt h^{-1}
NR ACTIVITY, nmol NO_2^- mg^{-1} protein^{-1} h^{-1}
(b)
LSD
Fresh wt basis
Protein basis
500
400
300
200
100
0
40
30
20
10
0
0
2
4
6
8
10
CYSTEINE CONCENTRATION, mM


- PVP
+ PVP
(c)
800
600
400
200
0
60
50
40
30
20
10
0
FRESH WT BASIS
PROTEIN BASIS

are sclerophyllous with thick cell walls. Mechanical homogenization may have also caused some physical denaturation of the enzyme protein.

Various protectants are generally added to the extraction medium to increase the stability of NR. They were tested for boronia NR as below.

Effect of cysteine. Hageman and Reed (1980) suggested that the optimum concentration of cysteine in the extraction medium must be established for each plant species that is being examined. Cysteine protects NR against the oxidation of sulphydryl (-SH) groups on the enzyme. The concentration of cysteine (Sigma) in the extraction medium was varied.

There was no NR activity without cysteine in the extraction medium (Fig. 37b). 1 mM cysteine gave maximum enzyme activity and higher concentrations lowered or inhibited the activity.

NR activity due to the presence of cysteine suggests the existence of active -SH groups in boronia enzyme molecules. The optimum level of cysteine in the extraction medium varies from plant species to plant species, e.g. 1 mM for tomato (Hageman et al., 1962) and 10 mM for corn (Hageman and Hucklesby, 1971). Plants requiring a lower concentration of cysteine may have an endogenous substance functioning as an enzyme stabilizer instead of cysteine.

Effect of bovine serum albumin (BSA). Addition of protein to the extraction medium prolongs NR activity in oat, tobacco (Schrader et al., 1974) and wheat (Sherrard and Dalling, 1978). It has been suggested that the exogenous protein protects NR from the action of inactivating or proteolytic enzymes during the extraction.

Therefore, BSA was included in the extraction medium at 1% v/v for boronia tissue.

BSA did not significantly increase the NR activity in boronia (Table 14). Therefore, such proteolytic enzymes may not be active during the extraction of NR from boronia.

Table 14. Effect of BSA in extraction medium on NR activity *in vitro* in leaves.

Treatment	NR activity, nmol NO_2^- h^{-1}	
	g^{-1} fresh wt	mg^{-1} protein
+BSA	420	33
-BSA	410	33

Difference between treatments was not significant, t-test.

Effect of extraction buffer system. A comparison was made between K/HPO_4 and Tris-HCl buffers. There was no significant difference in NR activity between the use of these buffers (Table 15).

Table 15. Effect of extraction buffer on NR activity *in vitro* in leaves.

Buffer	NR activity, nmol NO_2^- h^{-1}	
	g^{-1} fresh wt	mg^{-1} protein
K/HPO4	400	34
Tris-HCl	390	33

Differences between treatments was not significant, t-test.

Effect of polyvinylpyrrolidone (PVP). When leaves are homogenized their cells are broken and phenols and other compounds from vacuole are mixed with the cytoplasmic matrix. Phenols inactivate NR during the extraction, thus leading to a failure in detecting the enzyme (Klepper and Hageman, 1969). Insoluble PVP (obtained from Calibochem or Sigma) which forms complexes with polyphenols and

tannins was added to the extraction medium at 1 g per 1 g of leaves.

PVP was found to contain NO_2^-. To remove this NO_2^-, PVP was placed in water, stirred on a magnetic stirrer and filtered through a filter paper. This procedure was repeated until the water was free of NO_2^- (checked by the colorimetric method, p. 43).

Addition of PVP to the extraction medium increased NR activity by 54% on a fresh weight basis and by 53% on a protein basis (Fig. 37c) indicating that PVP complexed phenolics in boronia leaves and protected NR.

NR activity in vitro assay in leaves.

Effect of NO_3^- concentration. The concentration of NO_3^- in the assay medium was varied and the enzyme was assayed. The maximum activity was at 100 mM NO_3^- (Fig. 38a).

Effect of enzyme concentration. The quantity of the enzyme extract was varied up to 500 µl. In each case the reaction mixture was made up to a final volume of 2 ml by the addition of distilled water as required.

The NR activity was proportional to the quantity of the enzyme extract (Fig. 38b).

Time course of NR activity. The reaction was stopped at various intervals of 15 min up to 1 h. The reaction was linear with time (Fig. 38c).

NiR activity in vitro in leaves

Effect of NO_2^- concentration. The concentration of NO_2^- in the assay medium was varied up to 20 mM. The results showed that NO_2^- above 5 mM was inhibitory to the NiR activity (Fig. 39a). However, at

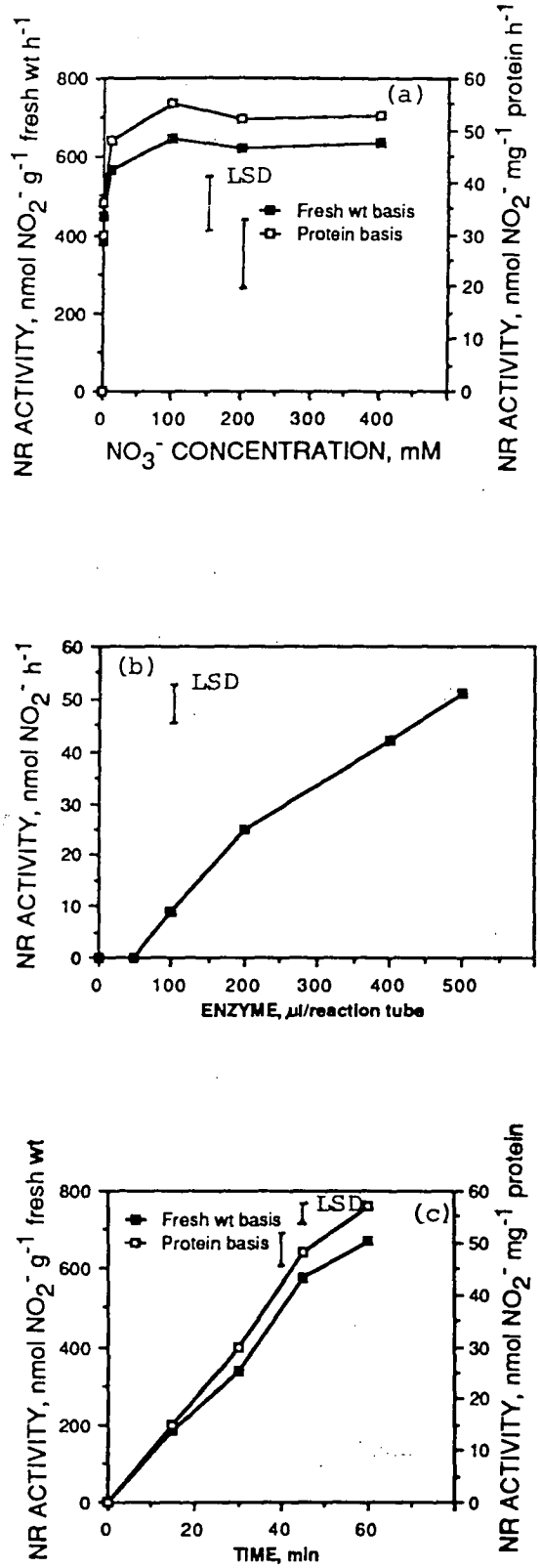


Fig. 38. Effect of different assay conditions on NR activity <u>in vitro</u> in leaves. LSD at P = 0.01.
(a) NO_3^- concentration in assay medium.
(b) Enzyme concentration. 1 g leaves were extracted in 6 ml extraction medium.
(c) Time course. After a given incubation time NO_2^- produced was measured.

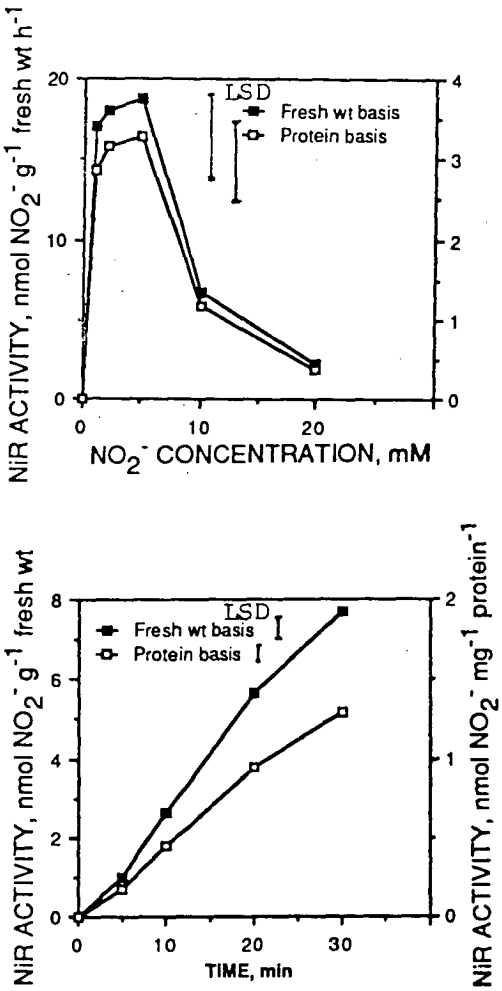


Fig. 39. Effect of different conditions on NiR activity <u>in vitro</u> in leaves. LSD at P = 0.01.
(a) NO_2^- concentration in assay medium.
(b) Time course. After a given incubation time NO_2^- disappeared was measured.

higher concentrations the dilution factors (as indicated for NiR assay *in vivo*) may have affected the sensitivity of NO_2^- measurement.

Time course of NiR activity. The reaction was stopped at timed intervals up to 30 min. The reaction was linear with time (Fig. 39b).

CONCLUSIONS

In general the activities of NR and NiR were higher *in vitro* than *in vivo*. Compared to the cell-free extract of an enzyme *in vitro*, *in vivo* assay would be somewhat limited by the movement of substrate to the enzyme site. However there was close correspondence in the trends between *in vitro* and *in vivo* assays.

From the above findings the following optimum factors were added to the routine procedures of NR and NiR enzyme assays for investigating the NO_3^- reducing system in relation to N toxicity in boronia.

NR activity *in vivo* assay. The leaves (as well as the roots) were sliced into fragments. The concentration of NO_3^- in the assay medium was 30 mM. Propanol at 1% was included in the assay medium. Anaerobic conditions were created by submerging the tissue in the assay solution by vacuum infiltration. The incubation period was varied between 30-90 min (usually 60 min) to obtain sufficient NO_2^- for measurement.

NiR activity *in vivo* assay. Procedures similar to those of NR activity *in vivo* assay were followed for NiR activity *in vivo* assay except with the following modifications: the buffer pH was 4.5; as there was no significant difference between 20 and 40 µM NO_2^- in the

assay medium, 20 mM was used to increase the sensitivity of NO_2^- measurement.

Preparation of cell-free enzyme extracts from leaves. Homogenate was prepared by freezing with liquid N_2 and grinding into powder using a pestle and mortar. The extraction medium contained 100 mM K/HPO_4, pH 7.5; 1 mM cysteine and 1 g PVP.

NR activity <u>in vitro</u> assay. The concentration of NO_3^- in the assay medium was 100 mM. The quantity of the enzyme extract and the reaction time were varied up to 500 µl and 1 h respectively to obtain sufficient NO_2^- for measurement.

NiR activity <u>in vitro</u> assay. As there was no significant difference between 2 and 5 mM NO_2^- in the assay medium, 2 mM was chosen for increased sensitivity of NO_2^- measurement. The reaction time was varied up to 30 min.

VIII. NITRATE UPTAKE, REDUCTION AND ACCUMULATION IN RELATION TO NITRATE TOXICITY IN BORONIA

As indicated in the Review of Literature, N fertilizer applied at rates considered moderate for many agricultural plants (100 kg ha^{-1}) caused toxicity in boronia. Similar observations were made on some other Australian native plants also (Specht, 1963; Higgs, 1970; Groves and Keraitis, 1976). However the physiological basis of this toxicity has not been studied.

Regardless of the form of N fertilizer applied, generally NO_3^- becomes the major form of N available for plants in normal cultivated soils due to nitrification. Therefore an understanding of the mechanism of NO_3^- assimilation by the plant would allow a more efficient use of N fertilizer and may minimize the detrimental effects.

After its uptake by plant, NO_3^- is reduced by NR to NO_2^- which in turn is reduced by NiR to NH_3.

To understand the physiological basis of NO_3^- toxicity in boronia, an experiment was conducted to study the patterns of NO_3^- uptake, reduction and accumulation in the roots and leaves with changes in external NO_3^- levels.

The *in vivo* NR assay used for measuring NO_3^- reduction by plant is usually carried out with added NO_3^- in the assay medium but with only endogenous level of reductant in the tissue. NR activity thus measured in the presence of added NO_3^- is often thought to be an overestimated level of actual *in situ* activity in the tissue. For example Timpo and Neyra (1983) observed substantial NR activity *in*

vivo with NO_3^- in the assay medium in the leaves of *Phaseolus vulgaris* plants that were grown in the absence of NO_3^-. Therefore NR *in vivo* assay with added NO_3^- in the assay medium is considered to indicate the potential capacity of the enzyme to reduce NO_3^- when NO_3^- is not limiting.

To provide a measure of the actual reduction of NO_3^- within the tissues of the plant, an *in vivo* NR assay in the absence of NO_3^- in the assay medium was developed which relies on endogenous levels of both NO_3^- and reductant. It is considered that this assay gives *in situ* NO_3^- reduction rates in view of its close correlation to the actual reduced N accumulation (Radin *et al.*, 1975; Breteler *et al.*, 1979; Breteler and Hanisch ten Cate, 1980).

The *in vitro* NR assay is carried out with NO_3^- and reductant NADH at nonlimiting (saturating) levels in the assay medium and thus it is considered to indicate the capacity of the enzyme when neither substrate nor reductant is limiting.

In the present work, NR activity was assayed under all the three conditions, *viz.* *in vivo* without NO_3^- in the assay medium, *in vivo* with NO_3^- in the assay medium and *in vitro*. Any difference in NR activity *in vivo* between plus and minus NO_3^- in the assay medium will reveal a limitation to the enzyme activity by NO_3^- availability. The *in vitro* NR activity will indicate the capacity of the enzyme when neither NO_3^- nor NADH is limiting.

MATERIALS AND METHODS

Plant culture. Plant culture conditions were the same as those described in the earlier chapter on the optimization of assay

conditions for NR and NiR.

Treatments. The treatments were applied in a manner that simulates the situation encountered by the plants in the field when fertilizer is applied. That is, a given level of NO_3^- was surface applied to the growth medium and the medium was watered daily. In this situation the NO_3^- concentration that plant roots experience will be high initially when NO_3^- is applied and will decline with time due to uptake by the plant and due to leaching.

From preliminary experiments, treatment levels were chosen to cover a range from zero to toxic level, *viz*. 0, 15, 25, 50, 100 and 150 mmol NO_3^- per plant. NO_3^- was supplied in the form of $Ca(NO_3)_2$. In the preliminary experiments, it was found that KNO_3 also caused similar toxicity symptoms. NO_3^- was applied at 9:00 AM.

Observations. In the preliminary experiments, it was found that while boronia leaves showed toxicity symptoms, its roots had no apparent injury. Further, NO_3^- was found in the leaves showing the toxicity symptoms while healthy leaves had no NO_3^- suggesting that NO_3^- reduction was limiting. Therefore analyses in this study were focussed on the leaves and NR enzyme.

As indicated previously, NR was assayed under 3 conditions: (i) *in vivo* without NO_3^- in the assay medium, i.e. with merely endogenous NO_3^- (termed actual activity), (ii) *in vivo* with added NO_3^- in the incubation medium (termed potential activity, i.e. in relation to NO_3^- availability) and (iii) *in vitro*.

After the supply of NO_3^- to the plants, fresh root and leaf samples were taken and analyzed at 0, 1, 5 and 10 h on day 1 and at noon every day 2 to day 7.

The samples were analyzed for the following:

roots: NO_3^- concentration

NR activity *in vivo* without and with NO_3^- in the assay medium

leaves: NO_3^- concentration

NR activity *in vivo* without and with NO_3^- in the assay medium

NR activity *in vitro*

NO_2^- concentration

NiR activity *in vivo*

NiR activity *in vitro*

It was found that the plants sampled once were not suitable for another sampling, because under these conditions NO_3^- accumulation was detected at the time of second sampling in the leaves of plants that were supplied with even the lowest level of NO_3^-. This accumulation of NO_3^- was not found when the same plants were sampled directly at the second sampling time without any sampling at the first time. Possibly a decreased leaf area following the first sampling may have caused an increased NO_3^- accumulation in the remaining leaves by the second sampling time. Therefore, each treatment was applied to 10 plants and a plant was used at one sampling time only and then discarded. A similarly treated fresh plant was used at the next sampling time.

Because of the number of analyses involved at each time of sampling, instead of having all the replications at one time, the treatments were repeated five times and each time was considered as a replication.

Analyses. For NR and NiR enzyme assays the optimum conditions that were established previously (described in the previous chapter) were used. In the case of NR activity *in vivo* assay without added NO_3^- in the assay medium, the enzyme activity was obtained by following the procedure indicated for NR assay *in vivo* but omitting NO_3^- from the assay medium.

For NO_3^- and NO_2^- extraction from the plant tissue, normally 1 g of fresh roots or leaves was rinsed, weighed and frozen in liquid N_2 in a mortar and then ground into powder. The powder was transferred to a test tube, hot distilled water was added and the mixture was stirred. The tube was placed in a boiling water bath for 10 min, cooled to room temperature and centrifuged at 5000 X g for 10 min. The supernatant was used for NO_3^- and NO_2^- determination.

NO_3^- was determined according to the procedure of Woolley *et al.* (1960). NO_2^- was determined by Griess-Ilosvay colorimetric method. Protein was determined by Bio-Rad protein assay.

The data on each measurement were statistically analyzed by ANOVA.

RESULTS

There was no apparent increase in the growth of plants over the duration of experiment. The plants supplied with 15, 25 or 50 mmol NO_3^- were healthy but the plants supplied with 100 or 150 mmol NO_3^- started showing the toxicity symptoms on day 4 (see Fig. 40). First, chlorosis appeared at the tip of the leaf, then the chlorosis gradually enlarged and occupied the whole leaf. However during the experimental period, majority of the leaves were less than 3/4 chlorotic or at the most up to 1/2 chlorotic. After the

Fig. 40. Leaves showing toxicity symptoms.

experiment, some of the leaves became completely chlorotic and abscised resulting in the death of some plants.

NO_3^- content in roots. NO_3^- was not present in the roots (nor in the leaves) of boronia that was grown in the absence of NO_3^-. Even when supplied with 15 mmol NO_3^- also, NO_3^- was not detected in the plants. Supply of higher levels of NO_3^-, however, caused NO_3^- accumulation in the plants.

With higher levels ($\geq$25 mmol) of NO_3^- supply also, NO_3^- was not detected in the roots in 1 h after the supply. After 1 h, NO_3^- started to accumulate in the roots. The accumulation of NO_3^- in the roots was earlier when supplied with higher levels of NO_3^- (Fig. 41): after only 1 h with 150 or 100 mmol while after 5 h with 50 or 25 mmol NO_3^-. The concentration of NO_3^- in the roots at any given time was a function of the level of NO_3^- supplied. Over the time, the maximum concentration of NO_3^- in the roots was lower with lower levels of NO_3^- supply. Also the concentration of NO_3^- in the roots began to decline earlier with lower levels of NO_3^- supply. Thus when supplied with 25 mmol NO_3^-, the concentration of NO_3^- in the roots attained a maximum of 9 µmol NO_3^- g^{-1} fresh weight on day 2 and returned to nil NO_3^- in 6 days. But when supplied with higher levels ($\geq$50 mmol) of NO_3^-, although the concentration of NO_3^- in the roots decreased, it was not completely depleted even up to day 7.

NO_2^- in roots. NO_2^- was not found in the roots in any treatment at any time.

Actual NR activity in roots (<u>in vivo</u> minus NO_3^- assay). NR was not present in the roots (as well as in the leaves) of boronia when grown without NO_3^-. Supply of NO_3^- to the plants induced NR in the

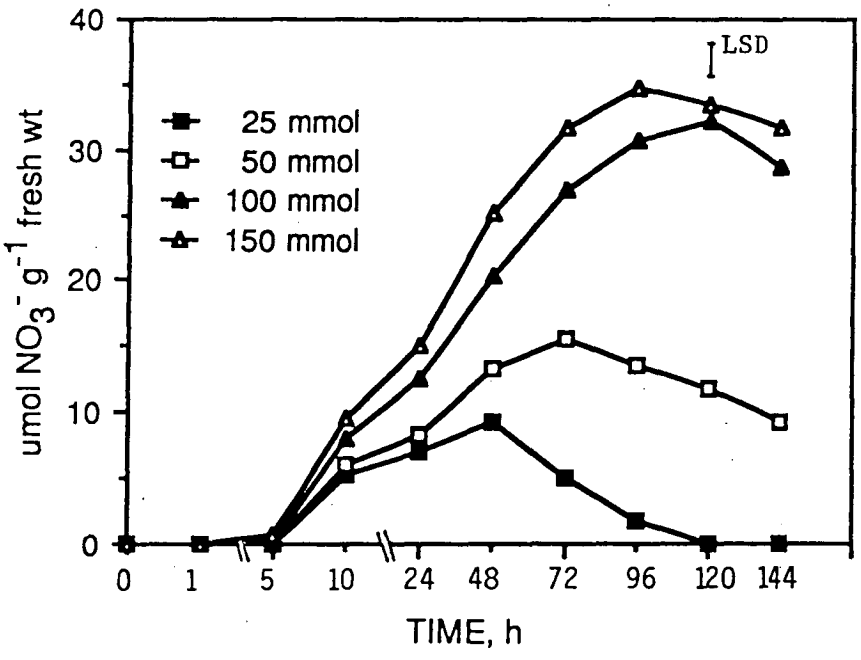


Fig. 41. Time course of NO_3^- concentration in roots after supply of different levels of NO_3^- to plants. The given level of NO_3^- was supplied in a single application at zero hour. LSD bar (P = 0.01) is for comparison at the same time between levels of NO_3^- supplied.

roots within an hour. With the lowest level of NO_3^- (15 mmol) supply, NR activity increased only slightly (to a maximum of only 126 nmol NO_2^- g^{-1} fresh weight h^{-1} on day 2) and declined to nil within 4 days (Fig. 42). As the actual NR activity is generated with endogenous NO_3^-, this slight enzyme activity in the roots of 15 mmol NO_3^- plants in which NO_3^- was not detected indicates that endogenous NO_3^- was present in these roots and was reduced by the enzyme during the assay but the NO_3^- concentration was low (beyond the sensitivity of the method used for NO_3^- determination).

With higher levels of NO_3^- ($\geq$25 mmol) supply, the actual NR activity increased markedly in the beginning for 2 days. The rate of this increase was a function of the level of NO_3^- supplied. Thus the actual NR activity in the roots reached a maximum level earlier with a higher level of NO_3^- supply. However with 6 fold difference in NO_3^- supply, the difference in the maximum levels of the enzyme activity was only + or - 7% (of 422 nmol NO_2^- g^{-1} fresh weight h^{-1}).

Subsequent to attaining the maximum level, the actual NR activity in the roots of plants supplied with 25 mmol NO_3^- dropped to nil in 7 days while the activity in 50 mmol plants continued in a more or less steady maximum range. However in 100 and 150 mmol NO_3^- plants, the enzyme activity began to decline from day 2.

Although the endogenous NO_3^- content in the roots found even in the beginning (e.g. 5 µmol NO_3^- g^{-1} fresh weight, 10 h after the supply of 25 mmol NO_3^-, Fig. 41) was in excess of that required for the maximum NR activity observed (449 mmol NO_2^- g^{-1} fresh weight h^{-1}), the pattern of increase and decrease in the actual NR activity over the time resembled the pattern of NO_3^- concentration in the roots

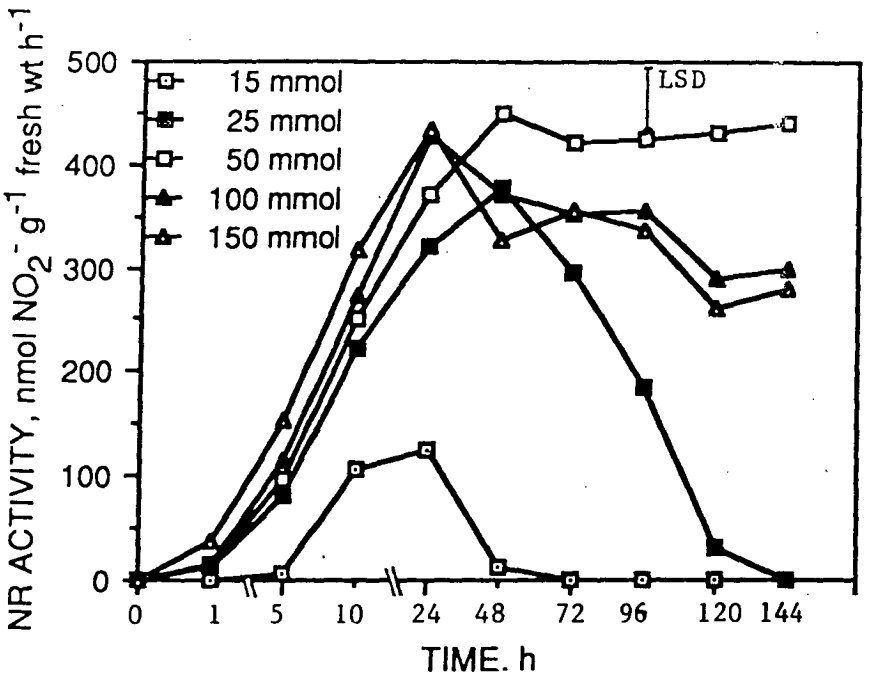


Fig. 42. Time course of actual NR activity (<u>in vivo</u> $-NO_3^-$ assay) in roots after supply of different levels of NO_3^- to plants. Other details as in Fig. 41.

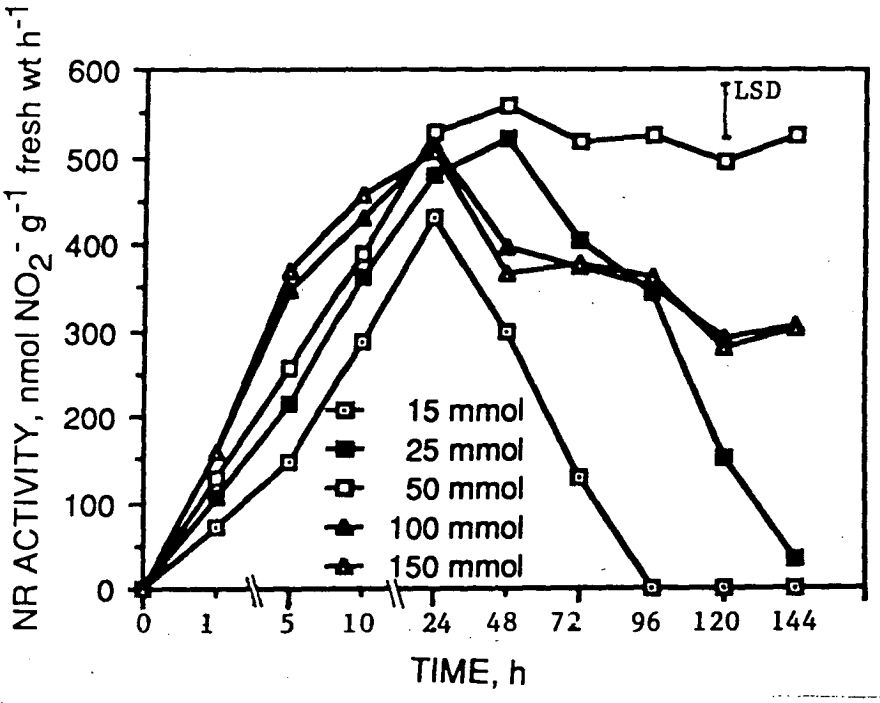


Fig. 43. Time course of potential NR activity (<u>in vivo</u> $+NO_3^-$ assay) in roots after supply of different levels of NO_3^- to plants. Other details as in Fig. 41.

over the time (compare Figs, 42 and 41). Thus the increase (or decrease) in NR activity seems to be depended more on the NO_3^- that was coming into the roots (and accumulating) than on the actual NO_3^- concentration in the roots. However once NR activity reached the maximum level (at 13 µmol NO_3^- g^{-1} fresh weight), further NO_3^- influx did not cause an increase in the enzyme activity and above 20 µmol NO_3^- g^{-1} fresh root weight, the enzyme activity even declined. Thus the relationship between NR activity and endogenous NO_3^- concentration in the roots was curvilinear (Fig. 44).

Potential NR activity in roots (<u>in vivo</u> plus NO_3^- assay). Even with added NO_3^- in the assay medium, NR was not observed in the roots and leaves of boronia plants that were grown without NO_3^-. Following the induction by the supply of NO_3^- to the plants, however, a low NR activity was detected earlier with added NO_3^- in the assay medium (1 h after supplying 15 mmol NO_3^-, Fig. 43) than without added NO_3^- in the assay medium. Similarly, before its decline, NR activity was detected for longer with added NO_3^- in the assay medium (on day 4 in 15 mmol and on day 7 in 25 mmol NO_3^- plants). These results indicate a potential of the enzyme to reduce NO_3^- but lack of available endogenous NO_3^-. At these times, it appears that whatever low NO_3^- that was coming into the roots was being reduced immediately without any accumulation.

At other times the potential NR activity (measured with added NO_3^- in the assay medium) was higher than the actual NR activity (measured without added NO_3^-) (compare Figs. 42 and 43). However even with nonlimiting NO_3^-, the potential NR activity did not reach the same maximum level all the time indicating different levels of

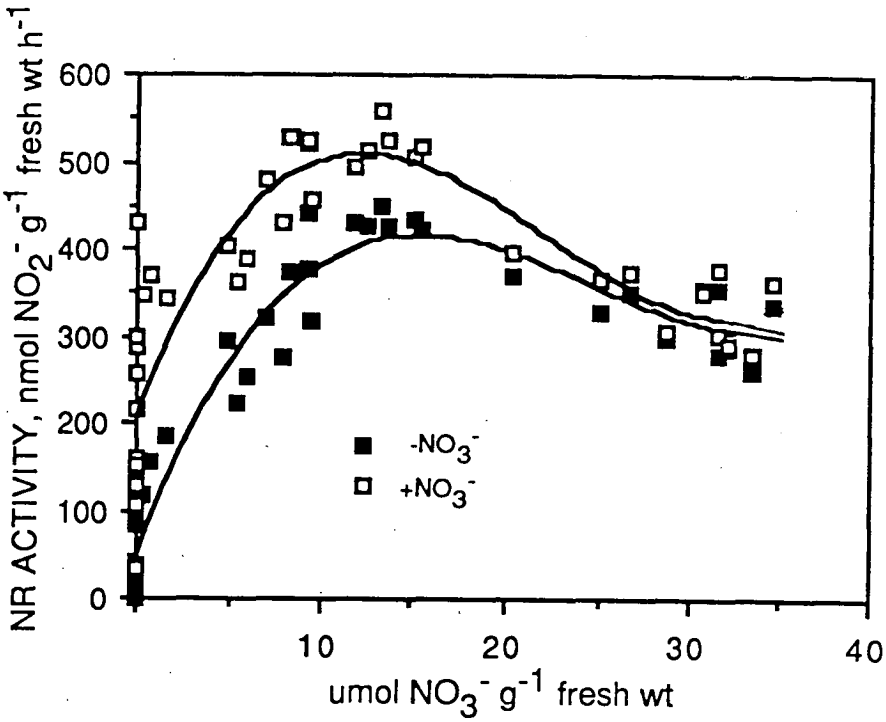


Fig. 44. Relation between NR activity <u>in vivo</u> (minus and plus NO_3^- assays) and NO_3^- concentration in roots. The values are obtained from Figs. 42 and 43 <u>vs</u>. 41.

induction of the enzyme at different times. Thus in general the time course pattern of rise and fall of the potential NR activity was similar to the pattern of actual NR activity. However the extent of increase in the potential activity was large when the actual activity was lower. When the actual activity was higher, the potential activity did not increase considerably. As pointed out earlier, despite sufficient endogenous NO_3^- for the maximum level of NR activity, the increase in NR activity by the addition of NO_3^- to the assay medium indicates that some of the endogenous NO_3^- was unavailable to the enzyme. As endogenous NO_3^- increased, the availability of NO_3^- to the enzyme also appears to have been increased as indicated by an increase in its activity, which eventually reached the maximum level. Therefore, addition of NO_3^- to the assay medium had least effect on the increase in the potential NR activity (over that of the actual NR activity) when the actual NR activity was higher (Fig. 44). Thus the maximum potential NR activity observed in the roots, 521 nmol NO_2^- g^{-1} fresh weight h^{-1}, was only 16% higher than the maximum NR activity.

NO_3^- content in leaves. As was in the roots, NO_3^- began to accumulate in the leaves earlier and in higher concentrations as the level of NO_3^- supply increased (Fig. 45). However compared to the roots (cf. Fig. 41), the accumulation of NO_3^- in the leaves began later (e.g. 5 h after in the roots vs. 10 h after in the leaves after supplying 25 mmol NO_3^-). Conversely the NO_3^- concentration in the plants supplied with 25 or 50 mmol NO_3^- began to decline a day earlier in the leaves than in the roots (and became nil 2 d earlier in the leaves than in the roots of 25 mmol plants). These trends suggest

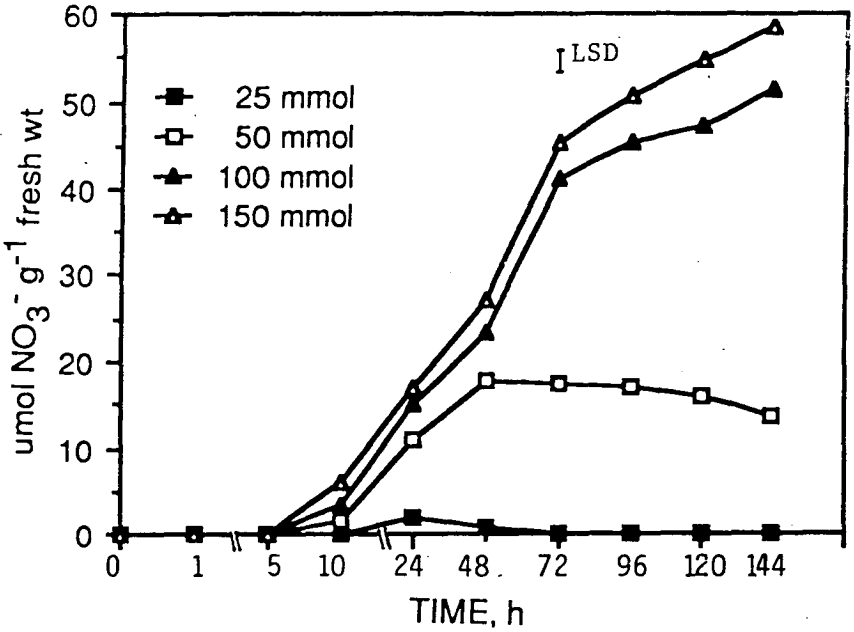


Fig. 45. Time course of NO_3^- concentration in leaves after supply of different levels of NO_3^- to plants. Other details in Fig. 41.

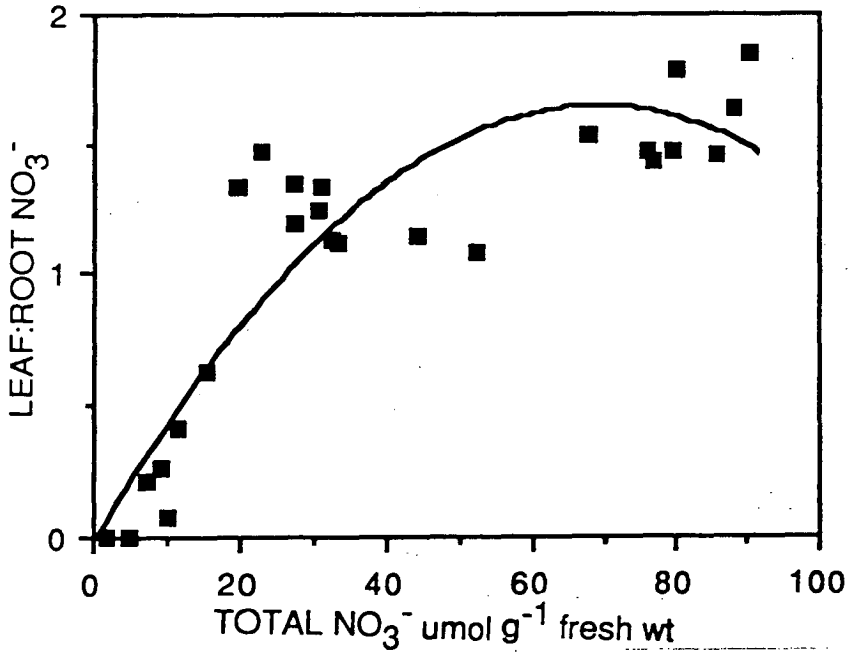


Fig. 46. Relation between leaf:root ratio of NO_3^- concentration to total NO_3^- concentration on per g basis. The values are derived from Figs. 41 and 45.

that as the amount of NO_3^- absorbed by the roots increased, NO_3^- was transported to the shoot and as the amount of NO_3^- absorbed decreased, NO_3^- transport to the shoot also decreased.

However when the plants were supplied with higher levels of NO_3^- (100 or 150 mmol), the NO_3^- concentration in the leaves continued to increase. By this time, however, the toxicity symptoms started appearing on the leaves.

On a per g fresh weight basis, the ratio of leaf:root NO_3^- concentration increased with increasing total endogenous (leaf plus root) NO_3^- concentration (Fig. 46), again suggesting that as the influx of NO_3^- into the root increased, a higher proportion of it was transported to the shoot.

NO_2^- in leaves. NO_2^- was not found in the leaves in any treatment at any time.

Actual NR activity in leaves (<u>in vivo</u> minus NO_3^- assay). When 15 mmol NO_3^- was supplied to boronia, NR activity could not be detected in the leaves without added NO_3^- in the assay medium. Only with higher levels ($\geq$25 mmol) of NO_3^- supply the actual NR activity was observed in the leaves. Increasing levels of NO_3^- supply caused earlier appearance and higher levels of NR activity in the leaves (Fig. 47). As compared to the roots (cf. Fig. 42), the time of appearance of NR was later in the leaves (never within an hour after the supply of any level of NO_3^-). This was in accordance with the late arrival of the enzyme substrate NO_3^- in the leaves (cf. Fig. 45).

Low levels of the actual NR activity were found in the leaves during the initial hours after the supply of NO_3^- (5 h and also 10

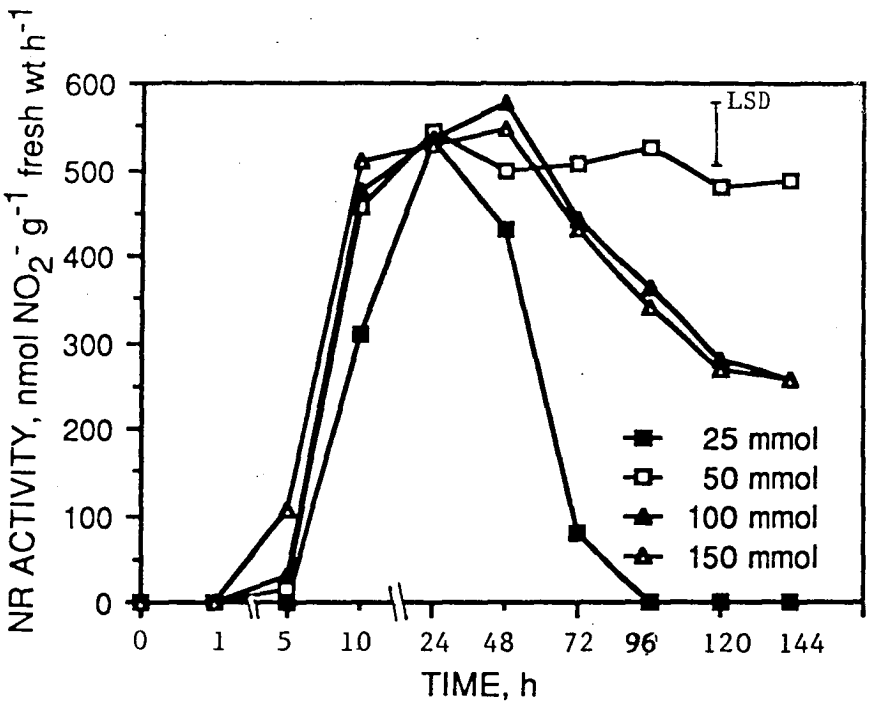


Fig. 47. Time course of actual NR activity (<u>in vivo</u> $-NO_3^-$ assay) in leaves after supply of different levels of NO_3^- to plants. Other details in for Fig. 41.

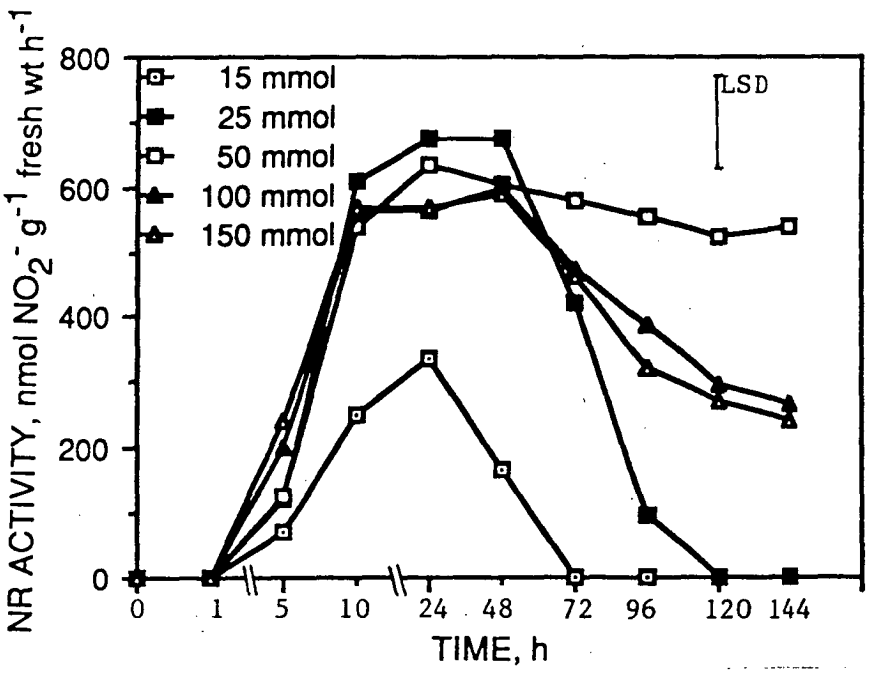


Fig. 48. Time course of potential NR activity (<u>in vivo</u> $+NO_3^-$ assay) in leaves after supply of different levels of NO_3^- to plants. Other details in for Fig. 41.

h in the case of 25 mmol NO_3^- plants) even when endogenous NO_3^- was not detected. This indicates that NO_3^- was just beginning to accumulate in the leaves in low concentrations and this NO_3^- was reduced during the assay.

Similar low levels of the actual NR activity found in the leaves (on day 4 in 25 mmol NO_3^- plants) before its decline also indicates the presence of low NO_3^- concentration beyond the sensitivity of the NO_3^- determination method used in this experiment.

Irrespective of 6 fold difference in the level of NO_3^- supply, the actual NR activity in the leaves increased rapidly in the beginning attaining an average maximum level of 534 + or - only 7 nmol NO_2^- g^{-1} fresh weight h^{-1} in 2 days. The maximum actual NR activity in the leaves (577 nmol NO_2^- g^{-1} fresh weight h^{-1}) was 29% higher than that in the roots. As was in the roots, despite sufficient NO_3^- concentration in the leaves in the beginning (e.g. 1 µmol NO_3^- g^{-1} fresh weight after 10 h in 25 mmol NO_3^- plants, Fig. 45) for the maximum level of NR activity observed, the enzyme activity seems to have increased depending on the further influx of NO_3^- into the leaves (compare Figs. 47 and 45).

The actual NR activity in the leaves of 25 mmol NO_3^- plants dropped to nil on day 5, that is 2 days earlier than in the roots (cf. Fig. 42). This was in accordance with the depletion in endogenous concentration of the enzyme substrate NO_3^-. While NR activity in the leaves on 50 mmol NO_3^- plants continued in the maximum range, the enzyme activity on 100 or 150 mmol plants began to decline (at >24 umol NO_3^- g^{-1} fresh weight). Thus the relationship between NR activity and endogenous NO_3^- concentration was

curvilinear in the leaves (Fig. 49). However the concentration of NO_3^- at which NR activity started to decline in the leaf tissue was ca. 20% higher than the concentration in the root tissue.

On a per g fresh weight basis the ratio of leaf:root NR activity increased with increasing total (leaf plus root) NR activity (Fig. 50) in accordance with the partitioning of the enzyme substrate NO_3^-.

Potential NR activity in leaves (<u>in vivo</u> plus NO_3^- assay). In the leaves of the plants supplied with 15 mmol NO_3^-, a low potential NR activity was observed after 1 and up to day 4 after the supply of NO_3^- (Fig. 48). But neither the actual NR activity nor NO_3^- was detected in the leaves of these plants. This suggests that NO_3^- reached these leaves and induced NR but the amount of NO_3^- entering the leaves was low and was being reduced immediately. Similar low potential NR activity existing in the leaves of 25 mmol NO_3^- plants was found on day 5 just before the disappearance of the enzyme.

The time course trends in the potential NR activity were similar to the trends of the actual NR activity. But as was in the roots, the difference between potential and actual NR activity decreased with increasing NO_3^- content (Fig. 49). The maximum potential NR activity observed in the leaves was 674 nmol NO_3^- g^{-1} fresh weight h^{-1}.

NR activity <u>in vitro</u> in leaves. The time course trends of NR activity <u>in vitro</u> in the leaves were more closely related to those of the actual NR activity (such as not detecting the enzyme activity in 15 mmol NO_3^- plants) than to the trends of the potential NR activity. Further, NR activity <u>in vitro</u> could be detected only

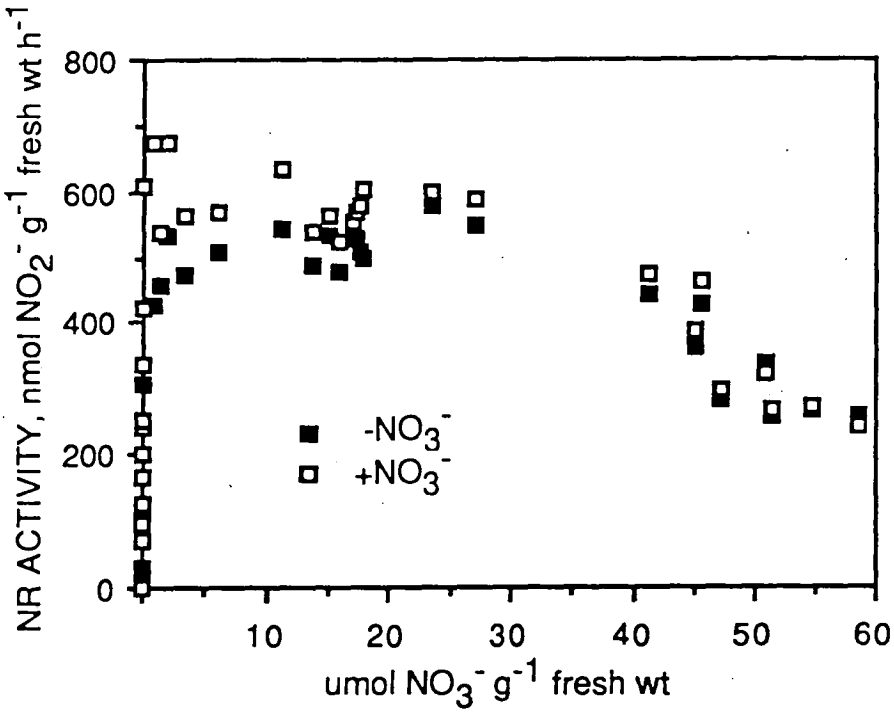


Fig. 49. Relation between NR activity <u>in vitro</u> (minus and plus NO_3^- assays) and NO_3^- concentration in leaves. The values are obtained from Figs. 47 and 48 <u>vs</u>. 45.

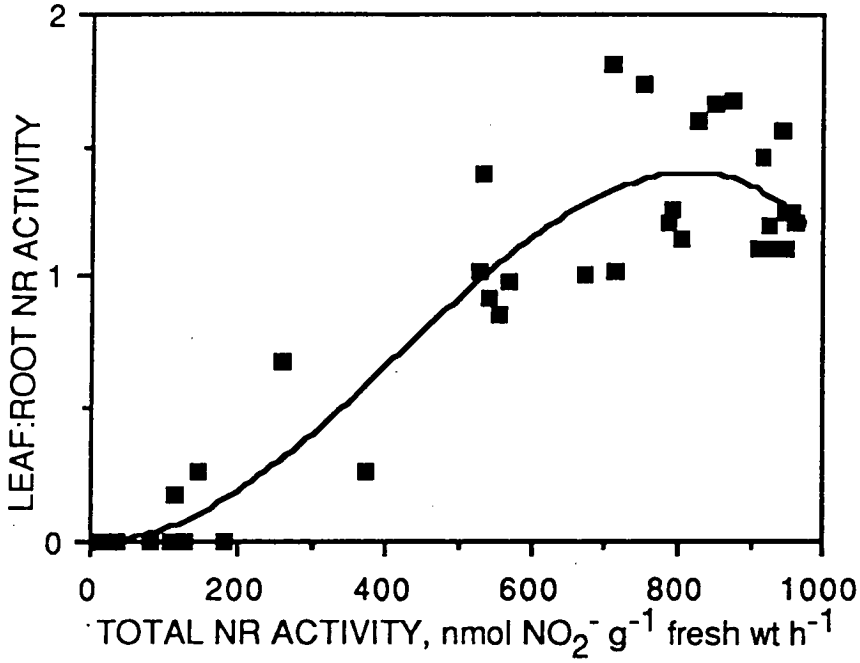


Fig. 50. Relation between leaf:root ratio of NR activity to total NR activity (actual), on per g basis. The values are derived from Figs. 42 and 47.

when the actual NR activity was rather high (ca. 309 nmol NO_2^- g^{-1} fresh weight h^{-1}). As high actual NR activity was observed at high endogenous NO_3^- concentration and as NR is highly unstable, it may be possible that the endogenous NO_3^- was acting as a stabilizer during the extraction of the enzyme for *in vitro* assay.

NR activity *in vitro* was lower than *in vivo* in the initial hours (10 h) after the supply of NO_3^- to the plants but was slightly higher at other times. The difference between NR activity *in vitro* and NR activity *in vivo* was relatively lower with the potential activity than with the actual activity, probably due to nonlimiting NO_3^- in both *in vitro* and potential activity *in vivo* assays.

However even when NO_3^- as well the reducing energy NADH were not limiting, NR activity *in vitro* did not attain the maximum level all the time. This suggests that the level of the enzyme synthesized was different at different times.

NR activity *in vitro* was greatly lower that *in vivo* on days 6 and 7 in 100 and 150 mmol NO_3^- plants when the endogenous NO_3^- concentration was very high (47 µmol NO_3^- g^{-1} fresh weight). As a decrease in NR activity was observed at high NO_3^- content in the leaves (Fig. 47), a high endogenous NO_3^- plus NO_3^- in the assay medium may have aggravated the decrease in the enzyme activity.

The maximum NR activity observed *in vitro* (671 nmol NO_2^- g^{-1} fresh weight h^{-1}) was comparable to that of the potential activity.

The trends in NR activity *in vitro* expressed on a protein basis (Fig. 51b) were similar to those expressed on a fresh weight basis. Therefore the different levels of the enzyme observed at different times do not seem to be due to changes in the protein content.

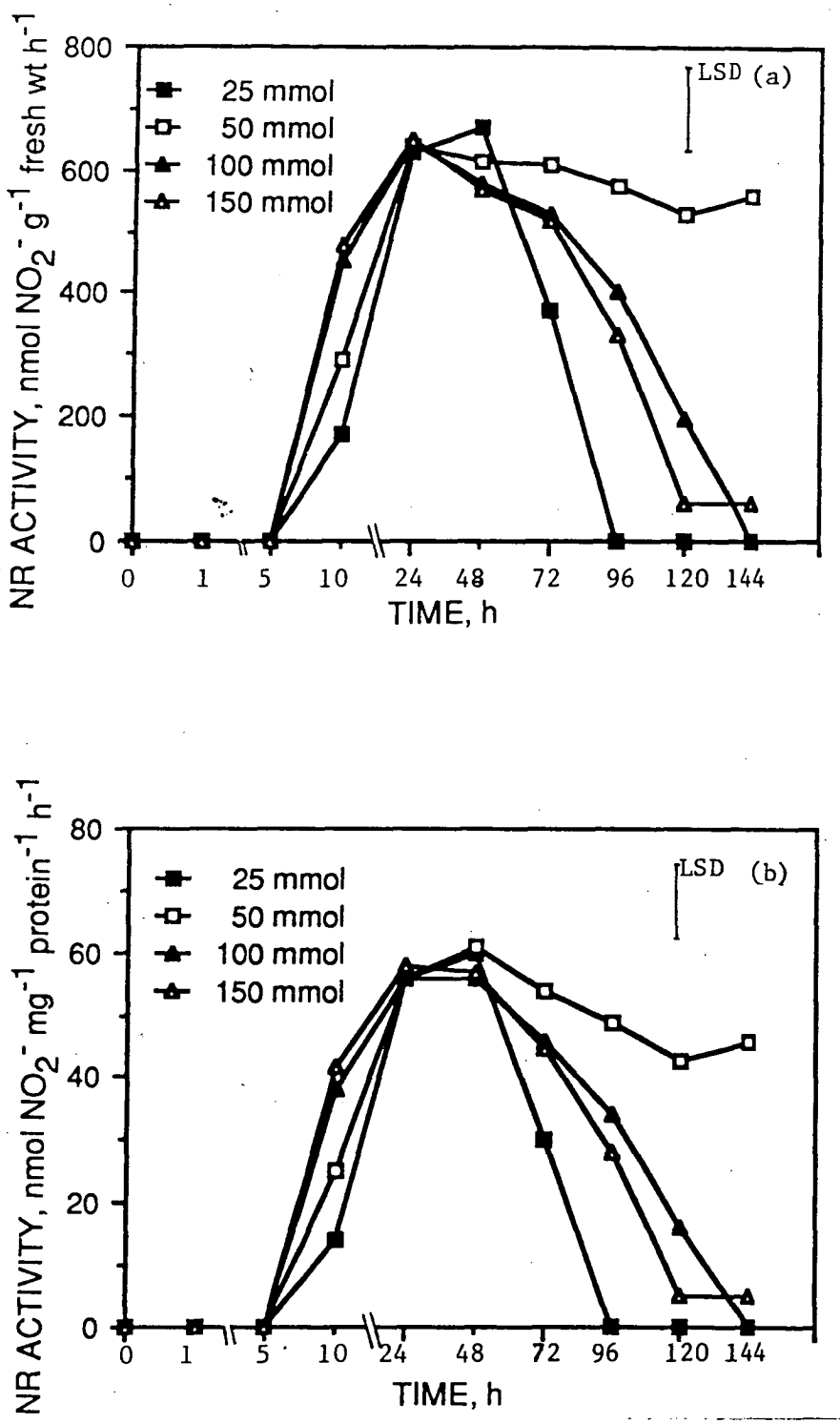


Fig. 51. Time course of NR activity <u>in vitro</u> in leaves on (a) fresh weight and (b) protein bases after supply of different levels of NO_3^- to plants. Other details as in Fig. 41.

NiR activity in leaves. NiR activity was observed in the plants grown without NO_3^- (Appendix Tables VI.9, VI.10 and VI.11). Its activity was lower in vivo than in vitro. It may have been due to the limitation of substrate NO_2^- entry into the tissue in the in vivo assay. NiR activity did not significantly change due to the supply of NO_3^- the plants. However, NiR activity in vitro was considerably higher than NR activity suggesting that NO_2^- would not accumulate in the leaves. This agrees with the finding that no NO_2^- was found in the plants.

DISCUSSION

NO_3^- was absent in boronia plants that were grown without NO_3^-. When supplied with NO_3^-, the rapidity with which boronia responded to NO_3^- by the changes in endogenous NO_3^- concentration and NR activity shows the plant's sensitivity to NO_3^-.

NO_3^- in the plant tissue will be in a dynamic state since its concentration in a given plant part at a given time depends on that part's uptake, reduction and translocation to other plant parts. After the supply of NO_3^- to the plants, there was very low NO_3^- concentration in the roots up to 1 h (as indicated by a low NR activity, Figs. 42 and 43), then followed by a marked accumulation (Fig. 41). Considering that NR activity was very low in the first hour and NO_3^- did not reach the shoot in the first hour, the uptake of NO_3^- may have been restricted during this time. This low NO_3^- uptake may have been caused by a limitation to the movement of NO_3^- ions through the media to the root surface or there may have been a limitation to the entry of NO_3^- ions into the roots during the

initial period. Slow initial rates of NO_3^- uptake have been observed in other plant species even when grown in solution culture and such an initial lag period has been interpreted (first by Jackson et al., 1972 and recently by Goyal and Huffaker, 1986 in wheat) as a period during which there would be induction of NO_3^- transporter by NO_3^- itself.

Only following the supply of its substrate NO_3^-, NR was induced in boronia plants. NR activity in the plants increased or decreased significantly within hours depending on the changes in endogenous NO_3^- which in turn are brought about by the changes in NO_3^- concentration in the growth medium. Such modulations in barley NR levels by NO_3^- are regulated by a protein synthesized de novo and protein degradation and not by reversible activation-inactivation of some inert precursor of NR (Somers et al., 1983).

After the induction, the extent of increase in NR activity in boronia seemed to be dependent on the influx of NO_3^- into the tissue rather than on the NO_3^- concentration that was already in the tissue. Shaner and Boyer (1976) showed that NR in maize leaves is regulated by the influx of NO_3^- from the roots via the transpiration stream than on the actual amount of NO_3^- in the leaves. It has been proposed that NO_3^- in the cell exists in two compartments: in vacuole as storage pool and in cytoplasm as metabolic pool (Ferrari et al., 1973). NR is located in the cytoplasm (Hewitt et al., 1976). As NO_3^- entered into a cell, some NO_3^- may have been partitioned into the vacuole and would not have been available immediately for NR. Thus the level of NR activity may have been regulated by the NO_3^- in the cytoplasm. When the tissue NO_3^- was

measured, both cytoplasmic and vacuolar NO_3^- contribute to the NO_3^- value. Thus, even though the endogenous NO_3^- concentration found at times was sufficient for the maximum level of NR activity, the level of NR activity at those times was in fact lower.

NO_3^- concentration in the tissue declined to nil before NR activity did. Hence it seems that as the cytoplasmic NO_3^- was depleted after the influx of NO_3^- into the cell ceased, NO_3^- from the vacuole readily flowed to the cytoplasm to maintain NR activity until NO_3^- is exhausted.

The level of NR observed in boronia was similar to the levels in some other Australian native plants (<0.5 µmol NO_2^- g^{-1} fresh weight h^{-1}, Smirnoff et al., 1984) and several species of Vaccinium (e.g. <0.1 µmol NO_2^- g^{-1} fresh weight h^{-1}, Havill et al., 1974). These levels, however, are very low relative to those of cultivated plants (e.g. >4.0 µmol NO_2^- g^{-1} fresh weight h^{-1} in sorghum, Scott and Neyra, 1979; >2.0 µmol NO_2^- g^{-1} fresh weight h^{-1} in tomato, Mills and Lips, 1984). Among the wild plants, ruderal plants have high levels of NR (e.g. >6.0 µmol NO_2^- g^{-1} fresh weight h^{-1} in Chenopodium album, Al Gharbi and Hipkin, 1986). These differences in the level of NR activity between the plant species seem to be related to their growth rates. Grundon (1972) recorded RGRs between 0.03 and 0.06 g g^{-1} d^{-1} for the Australian native Wallum plants compared with 0.24 for sorghum and 0.18 for tomato. Similarly Ericaceae (Vaccinium) species have slow growth rates and ruderal species have high growth rates. Rapidly growing plants require high NR activity to meet their metabolic requirements of reduced N. On the other hand, low levels of NR activity in slow growing plants may be sufficient to

sustain themselves in the soils where NO_3^- availability is low. They also seem to utilize NH_4^+ preferentially.

Plants differ as to where majority of NO_3^- is reduced (Pate, 1980). The extent to which root or shoot acts as the main center for NO_3^- reduction is influenced by the concentration of NO_3^- in the root medium. With low concentrations of NO_3^- that occur in the naturally NO3- poor soils, it is likely that all of the NO_3^- may normally be reduced in the roots. Thus in several species of Vaccinium in their natural site, Havill et al. (1974) detected no NR activity in the leaves. Addition of NO_3^- fertilizer increases the NO_3^- concentration in the soil. With the increasing levels of NO_3^- supply, a higher proportion of NO_3^- was found in the leaves of boronia (Fig. 46) and relatively a higher level of NR activity was observed in the leaves (Fig. 50).

With increasing NO_3^- influx, NR activity in boronia increased up to a maximum level and further increase in NO_3^- influx caused a decrease in the enzyme activity (Fig. 44 and 49). Though NR repression by the substrate NO_3^- has not been reported, decreased NR activity at higher NO_3^- concentrations has been observed, the inhibiting concentration differing with plant species. Melzer et al. (1984) observed an inhibition of NR above ca. 500 µmol NO_3^- g^{-1} dry weight in the leaves of Rumex obtusifolius. Woodin et al. (1985) found that repeated application of high NO_3^- concentration led to a decline in NR activity in ombrotrophic Sphagnum species. Similarly a decrease in NR activity has been observed in in vivo assays when the NO_3^- concentration in the incubation medium is beyond a certain level (50 mM in corn-- Klepper et al., 1971, Jones

and Sheard, 1977; 200 mM in pigweed-- Klepper et al., 1971; 50 mM in pea, wheat, barley, Gomphrena and 100 mM in marrow-- Jones and Sheard, 1977; 100 mM in wheat-- Baer and Collet, 1981; 200 mM in potato-- Davies and Ross, 1985; 70 mM in Raphanus-- Schulze et al., 1985). Thus there is a possibility of an inhibition of NR by excess substrate. The inhibition of NR activity at high concentration of NO_3^- at least in boronia could not be due to an accumulation of the products of NO_3^- reduction (NH_4^+ or amino acids) because the rate of NO_3^- reduction in 50 mmol and 100 or 150 mmol NO_3^- plants was in a similar range before the inhibition (Fig. 47). However the inhibition of NR occurred only in 100 and 150 mmol NO_3^- plants in which NO_3^- accumulation increased (cf. Fig. 45).

Thus when a lower level (15 mmol) of NO_3^- was supplied to boronia, the absorbed NO_3^- is reduced without any accumulation and without even NR activity reaching its maximum capacity. When higher levels of NO_3^- were supplied, with an increase in the NO_3^- absorption, NR activity increased up to a maximum level. Beyond this level, the increase in NO_3^- uptake was not followed by an increased NR activity. As a consequence, NO_3^- accumulated, presumably in the vacuole. NO_3^- accumulation in the vacuole will be limited by vacuole capacity. After this capacity is full, further influx of NO_3^- into the cell may lead to an accumulation in the cytoplasm. Because NR is in the cytoplasm, the excess cytoplasmic NO_3^- may inhibit NR aggravating the situation of NO_3^- accumulation in the cytoplasm. This will lead to extracellular NO_3^- accumulation which may reduce turgor pressure and affect metabolism and growth.

From the appearance of the toxicity symptoms, it seems that

excess extracellular NO_3^- build up was in the leaf tip in the beginning and moved progressively inward. The leaf abscission observed in some plants after the experiment may be due to saturation of the entire leaf with NO_3^-. This leaf shedding may be a protective reaction of the plant to remove excess NO_3^-.

The endogenous concentration of NO_3^- at which toxicity symptoms started appearing on boronia leaves was 32 µmol g^{-1} fresh weight. Much higher NO_3^- concentrations are found in herbaceous plants such as spinach, beet and radish without any injury to the plants (e.g. 478 µmol NO_3^- g^{-1} fresh weight in spinach, Barker et al., 1971; 600 µmol NO_3^- g^{-1} dry weight in Urtica dioica, Rosnitschek-Schimmel, 1982). This NO_3^- accumulation is not associated with a low NR activity (the NR levels are indeed high in these plants, as pointed out earlier). These plants are characteristic of NO_3^- rich habitats and therefore it may be an evolutionary tendency of these plants to cope with high NO_3^- availability. On the other hand, woody plants such as boronia that are characteristic of NO_3^- poor habitats do not seem to have a large capacity for NO_3^- storage. Ingestad (1973) also found that high external concentrations of NO_3^- are toxic to Vaccinium species which are characteristic of NO_3^- poor habitats.

Why does NO_3^- uptake occurs to toxic levels in boronia? Generally NO_3^- uptake is thought to be subject to negative feedback from high levels of endogenous NO_3^- (in barley, Smith, 1973) or some product of NO_3^- assimilation (NH_4^+ or amino acids in Arabidopsis thaliana, Doddema et al., 1978). Such feedback processes against luxury consumption may be expected in the plants that grow naturally in NO_3^- abundant soils. As excess NO_3^- circumstances would rarely if

ever occur in the native soils of boronia, its feedback controls may not be efficient.

NiR was present in boronia plants that were grown without NO_3^-. Warner et al. (1977) found that barley NR mutants still possessed NiR. Pierson and Elliott (1981) found that Phaseolus vulgaris maintained its NiR even when it lost NR. As in boronia, a higher activity of NR compared to that of NiR was also observed in apple (Klepper and Hageman, 1969) and mustard (Rajasekhar and Mohr, 1986). NiR in boronia was not subjected to turnover even under toxic conditions. NiR was found to be much less sensitive to stress than NR (Heuer et al., 1979). The different responses of NR and NiR may be due to their different locations in the cell: NR is located in the cytoplasm and NiR is located in the chloroplast (Beevers and Hageman, 1983).

The phenomenon of NO_3^- toxicity in boronia agrees well with the behavior of the plants from nutrient poor soils as proposed by Chapin (1980). He proposed that these plants have difficulty in growing fast under the conditions of high nutrient availability and therefore accumulate toxic nutrient levels. With respect to slow growth in boronia, the control point is low NR activity and the consequent low production of metabolites for growth. This low level of NR activity in boronia is genetically controlled and tuned to slow growth in low NO_3^- producing native soils.

Practical implications. An idea of the NO_3^- reducing capacity of boronia can be obtained by NR activity X weight of plant tissue X time. Assuming that the estimated in vivo NR activity is equal to in situ NR activity, a gram of fresh boronia leaves can reduce ca.

500 nmol NO_3^- in an hour. In addition to the leaves and roots, consideration should also be given to other parts of the plant as NR activity was observed in stems (Andrews et al., 1984), branch bark, inner tissues of branch and trunk xylem tissues (in Alnus glutinosa, Pizelle and Thierry, 1986). NR activity also depends on diurnal variations (Lillo, 1983).

One way of preventing the toxic accumulation of NO_3^- in boronia during its cultivation is to restrict NO_3^- availability to the plants through application of fertilizers at a rate matching the plant's NO_3^- reduction capacity. Indeed it was observed that boronia tolerates and responds well to a slow release N fertilizer, IBDU (Chapter IV) which perhaps simulates NO_3^- production in the native soil of the plant.

IX. GENERAL DISCUSSION

The present study was concerned with the efficient use of N in the production and culture of boronia. In order to gain a better understanding of the various aspects of N nutrition of boronia, several different responses such as yield responses, morphological responses, physiological and enzymological responses of the plant to N were investigated. Results of these different investigations have already been elaborated in the earlier chapters. In this chapter an attempt is made to integrate and summarize the highlights of the information obtained, with consideration to their conceivable ecological importance and their implications in the cultivation of boronia. Since these processes are not fully understood, some speculations are made which may lead to hypotheses for further research.

A point that became apparent from the present study is that, although some findings in boronia have close parallels to the findings in cultivated plants, some findings are unique to boronia. The nutrient traits exhibited by boronia seem to relate more to the traits of other plants that are also evolved under similar environmental conditions (infertile soils) than to the traits of phylogenetically related plants. Apparently these different traits enable the plants to survive and exploit their characteristic environments. Therefore N nutrition of commercial boronia plantations would differ from that of most cultivated plants and requires special attention.

Boronia has an ability to maintain its metabolism effectively under N stress as indicated by the absence of characteristic N

deficiency symptoms when no N was supplied to the plants (Chapters IV and V). Such an adaptation would enable boronia to survive and remain healthy in the N-limited native soils. Although boronia can similarly remain in the field without any supplemental N when commercially cultivated, N stress reduces the production of nodes at which flowers are borne (Chapters IV and V). The production of nodes and in turn the production of flowers by boronia can, however, be increased with increased supply of N (Chapters IV and V). Therefore in the commercial cultivation of boronia, N should be supplied to the plants.

Between NH_4^+ and NO_3^- forms of N, uptake of NH_4^+ by boronia was higher (Chapter VI). This confirms the speculation made (in Chapter II, Section 3.2) that NH_4^+ rather than NO_3^- may be the predominant form of N in the native soils of boronia. Having adapted to such soils, boronia preferred NH_4^+. Although NH_4^+ is less mobile than NO_3^- in the soil, boronia plants can enlarge their root surface through vesicular-arbuscular mycorrhiza (Appendix I) and thus increase their access to NH_4^+. Further, native soils of boronia are sandy with little cation-exchange capacity and therefore NH_4^+ would also be mobile in these soils.

Uptake of NH_4^+ by boronia decreased pH of the growing medium (Chapter VI). A decrease in pH increases the ratio of $H_2PO_4^-$ to HPO_4^{2-} (Soon and Miller, 1977). $H_2PO_4^-$ is absorbed by the plants several times faster than HPO_4^{2-} and HPO_4^{2-} has a tendency to precipitate at the root-soil surface (Miller et al., 1970). Thus with lowered pH, there is an increase in the availability of P from the soil (Riley and Barber, 1971). Therefore by absorbing NH_4^+,

boronia may be maximizing the uptake of P in the P-deficient native Australian soils. Under low pH conditions, high concentrations of Al (Huett, 1979) and Mn (Jones, 1973) which are potentially toxic to the plants may also exist. However, ambient NH_4^+ inhibits the plant uptake of Al (Rorison, 1985) and Mn (McGrath and Rorison, 1982).

It may be seldom exclusively NH_4^+ exists in the native soils of boronia, as nitrification which is dependent on many factors (Chapter II, Section 3.2) may not always be inhibited. The general situation would be the presence of predominant NH_4^+ plus a small proportion of NO_3^-. Thus with NH_4^+ plus NO_3^-, generally the growth by boronia was highest (Chapter V). Therefore in the commercial cultivation, boronia should be provided with both NH_4^+ and NO_3^- forms of N.

In contrast to the native soils of boronia where N is scarce, provision of N fertilizers to the cultivated soils would result in an excess availability of N to boronia. In most agricultural soils where nitrification occurs, NH_4^+ applied would be oxidized resulting in NO_3^-.

At low external NO_3^- concentration, NR activity in boronia leaves was either absent or low (Chapter VIII). Therefore, at normally low NO_3^- concentrations that are habitually available, boronia may reduce NO_3^- in the roots. Nonetheless, significant NR capacity was observed in the leaves of boronia. In the natural environment this capacity may be to utilize occasional NO_3^- flushes that occur seasonally or after fire. Due to the application of fertilizer NO_3^- also, NO_3^- reduction will occur in the leaves (Chapter VIII).

When the available NO_3^- becomes too high, however, the leaf NR level also becomes insufficient to allow rapid reduction of NO_3^-. Boronia lacks the ability to effectively reduce or store large amounts of NO_3^- (Chapter VIII).

One possible reason (in addition to the reason of adaptation to low NO_3^- soils) for a low level of NR activity in boronia may be the deficiency of Mo in the native Australian soils (Bowen, 1981). Mo is a constituent of NR enzyme (Notton and Hewitt, 1971) and Mo deficient plants produce aberrant NR which possesses only partial NR activity (Notton et al., 1974). In fact whiptail of cauliflower, a Mo deficiency symptom is caused by the accumulation of high concentrations of NO_3^- in the leaves (Agarwala, 1952). Boronia plants may have adapted to cope with a low availability of Mo in their native soils by absorbing NH_4^+ and minimizing the need for a high level of NR.

Although assimilation capacity of NH_4^+ by boronia seems to be higher relative to that of NO_3^-, the overall N (either form) assimilation capacity of boronia may still be lower when compared to that of crop plants. Generally plants from low nutrient habitats grow slowly when compared to the plants from high nutrient habitats (Chapin, 1980) and their nutrient requirements can be expected to be low.

Plants adapted to low nutrient soils seem to absorb nutrients efficiently under low nutrient conditions and continue to absorb them efficiently under high nutrient conditions also but their genetically controlled slow growth leads to high and toxic levels of nutrients in the tissues (Chapin, 1980). Groves and Keraitis

(1976) observed that high levels of fertilizers such as those commonly used in agriculture were detrimental to some Australian native plants. This was also observed with boronia in the present study.

The detrimental effects of excess N fertilizer on boronia and other Australian native plants are similar to the effects of excess pollutant N on the forests. R. H. Waring (personal communication, Oregon State University, Corvallis) pointed out that many coniferous forests are declining as a result of atmospheric N pollution. Conifers, like boronia, have a low capacity to synthesize NR and are unable to store excess NO_3^- leading to their death.

In the cultivation of boronia, therefore, the detrimental effect of high levels of N should be counteracted. Ideally N should be supplied to a boronia plant at a rate corresponding only to the assimilation potential of the plant and not to the actual absorption potential of the plant.

A slow release N fertilizer IBDU seems to meet the N requirements of boronia without causing toxicity (Chapter IV). The slow availability of N from this fertilizer may be emulating the native habitat of the plant. Similarly when the roots were exposed to N for a short time, the amount of N absorbed by the plants would have been within the assimilation capacity of the plants and thus the plants grew without any N toxicity (Chapter V).

Measured in terms of vegetative growth (Chapter V), boronia is a late spring and summer growing species. Therefore the plants should be not be fertilized during early spring because such a

fertilization would give weeds which grow in early spring a competitive advantage over boronia. As extension of vegetative growth at the time of flowering seems to have a counteraction on the development of flower buds in boronia (Chapters IV and V), availability of high levels of N to the plants at the time of flowering should be avoided.

Since N responses of a boronia plant would depend on the assimilatory potential of that particular plant, the amounts and concentrations of N used in this study should be extrapolated to other situations with caution. However, the information obtained in this study can be used to estimate the permissible N input rates to commercial boronia plantations (e.g. p. 188).

In conclusion, the present study extended the previous limited information on the N nutrition of boronia and has explained the nature of the adaptations of boronia to its native environmental conditions and how they can be manipulated to achieve improved N nutrition and yields of boronia in commercial plantations.

X. REFERENCES

Agarwala, S. C. (1952). Relation of nitrogen supply to the molybdenum requirement of cauliflower grown in sand culture. Nature. 169:1099.

Agren, G. I. (1985). Limits to plant production. J. theor. Biol. 113:89-92.

Al Gharbi, A. and Hipkin, C. R. (1984). Studies on nitrate reductase in British angiosperms. I. A comparison of nitrate reductase activity in ruderal, woodland-edge and woody species. New Phytol. 97:629-639.

Allen, S. E. (1974). Chemical analysis of ecological materials. Blackwell Scientific Publications, Oxford. p. 565.

Allen, S. E. (1984). Slow-release N fertilizers. In: Nitrogen in crop production. pp. 195-206. ASA-CSSA-SSSA, WI.

Amer, F. M. and Bartholomew, W. V. (1951). Influence of oxygen concentration in soil air on nitrification losses of nitrous oxide from soil. Soil Sci. 71:215-219.

Andrews, M. (1986). The partitioning of nitrate assimilation between root and shoot of higher plants. Plant Cell Env. 9:511-519.

Andrews, M., Sutherland, J. M., Thomas, R. J. and Sprent, J. I. (1984). Distribution of nitrate reductase activity in six legumes: the importance of stem. New Phytol. 98:301-310.

Arnold, P. W. (1954). Losses of nitrous oxide from soil. J. Soil Sci. 5:116-128.

Aryan, A. P. and Wallace, W. (1983). Involvement of alcohol dehydrogenase in the enhancement of the in vivo nitrate reductase activity of root tissue by propanol. Plant Sci. Lett. 30:25-32.

Asher, C. J. and Edwards, D. G. (1983). Modern solution culture techniques. In: Lauchli, A. and Bieleski, R. L., eds. Inorganic plant nutrition (Encyclopedia of plant physiology, new ser., v. 15). pp. 94-119. Springer-Verlag, Berlin.

Aslam, M. and Huffaker, R. C. (1982). In vivo nitrate reduction in roots and shoots of barley (Hordeum vulgare L.) seedlings in light and darkness. Plant Physiol. 70:1009-1003.

Association of Official Analytical Chemists. (1980). Official methods of analysis of the Association of Official Analytical Chemists. The Association of Official Analytical Chemists, Washington. p. 1018.

Atkins, C. A., Pate, J. S. and Layzell, D. B. (1979). Assimilation and transport of nitrogen in non-nodulated (NO_3^--grown) Lupinus albus L. Plant Physiol. 64:1078-1082.

Atkinson, C. J. (1985). Nitrogen acquisition in four co-existing species from an upland acidic grassland. Physiol. Plant. 63:375-387.

*Austenfeld, F. A. (1972). Untersuchungen zur Physiologie der Nitratspeicherung und NItratassimilation von Chempodium album L. Pflanzenphysiol. 61:271-281.

Baer, G. R. and Collet, G. F. (1981). In vivo determination of parameters of nitrate utilization in wheat (Triticum aestivum L.) seedlings grown with low concentration of nitrate in the nutrient solution. Plant Physiol. 68:1237-1243.

Barker, A. V. and Mills, H. A. (1980). Ammonium and nitrate nutrition of horticultural crops. Hort. Rev. 2:395-423.

Barker, A. V. and Maynard, D. N. (1972). Cation and nitrate accumulation in pea and cucumber plants as influenced by nitrogen nutrition. J. Amer. Soc. Hort. Sci. 97:27-30.

Barker, A. V., Peck, N. H. and McDonald, G. E. (1971). Nitrate accumulation in vegetables. I. Spinach grown in upland soils. Agron. J. 63:126.

Barker, A. V., Volk, R. J. and Jackson, W. A. (1966). Growth and nitrogen distribution patterns in bean plants (Phaseolus vulgaris L.) subjected to ammonium nutrition. I. Effect of carbohydrate and acidity control. Soil Sci. Soc. Am. Proc. 30:228-232.

Barneix, A. J., James, D. M., Watson, E. F. and Hewitt, E. J. (1985). Nitrate assimilation and growth of Steptoe barley and one of its nitrate reductase deficient mutants. Ann. Bot. 56:711-718.

Beadle, N. C. W. (1954). Soil phosphate and the delimitation of plant communities in eastern Australia. Ecology. 43:281-288.

Beadle, N. C. W. (1966). Soil phosphate and its role in molding segments of the Australian flora and vegetation, with special reference to xeromorphy and sclerophylly. Ecology. 47:992-1007.

Beard, J. S. (1984). Biogeography of the kwongan. In: Pate, J. S. and Beard, J. S., eds. Kwongan, plant life of the sandplain, pp. 1-26. University of Western Australia Press, WA.

Beevers, L. and Hageman, R. H. (1983). Uptake and reduction of nitrate: bacteria and higher plants. In: Lauchli, A. and Bieleski, R. L., eds. Inorganic plant nutrition (Encyclopedia of plant physiology, new. ser., v. 15). pp. 351-375. Springer-Verlag, Berlin.

Bieleski, R. L. and Turner, N. A. (1966). Separation and estimation of amino acids in crude plant extracts by thin-layer chromatography. Anal. Biochem. 17:278-293.

Bigg, W. L. and Daniel, T. W. (1978). Effects of nitrate, ammonium and pH on the growth of conifer seedlings and their production of nitrate reductase. Plant Soil. 50:371-385.

Blair, G. J., Miller, M. H. and Mitchell, W. A. (1970). Nitrate and ammonium as sources of nitrogen for corn and their influence on the uptake of other ions. Agron. J. 62:530-532.

*Bogner, W. (1968). Experimentelle Prufung von Waldbodenpflanzen auf ihre Anspruche an die Form der Stickstoff-Ernahrung, Mitt. Ver. Forstl. Standortsk. Forstpflanzenzucht. 18:3-45.

Bollard, E. G. (1957). Nitrogenous compounds in tracheal sap of woody members of the family Rosaceae. Aust. J. Biol. Sci. 10:288-291.

Bouma, D. (1983). Diagnosis of mineral deficiencies using plant tests. In: Lauchli, A. and Bieleski, R. L., eds. Inorganic plant nutrition (Encyclopedia of plant physiology, new ser., v. 15). pp. 120-146. Springer-Verlag, Berlin.

Bowen, G. D. (1981). Coping with low nutrients. In: Pate, J. S. and McComb, A. J., eds. The biology of Australian plants. pp. 32-64. University of Western Australia Press, WA.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

Brenner, M., Niederwieser, A. and Pataki, G. (1965). Amino acids and derivatives. In: Stahl, E., ed. Thin-layer Chromatography, a laboratory handbook. pp. 391-440. Springer-Verlag, Berlin.

Breteler, H. (1973). A comparison between ammonium and nitrate nutrition of young sugar beet plants grown in nutrient solution at constant acidity. 1. Production of dry matter, ionic balance and chemical composition. Neth. J. agric. Sci. 21:227-244.

Breteler, H. and Hanisch ten Cate, Ch. H. (1980). Fate of nitrate during initial nitrate utilization by nitrogen-depleted dwarf bean. Physiol. Plant. 48:292-296.

Breteler, H., Hanisch ten Cate, Ch. H. and Nissen, P. (1979). Time-course of nitrate uptake and nitrate reductase activity in nitrogen-depleted dwarf bean. Physiol. Plant. 47:49-55.

Buban, T., Varga, A., Tromp, J., Knegt, E. and Bruinsma, J. (1978). Effect of ammonium and nitrate nutrition on the levels of zeatin and amino nitrogen in xylem sap of apple rootstocks. Z. Pflanzenphysiol. 89:289-295.

Cain, J. C. (1952). A comparison of ammonium and nitrate nitrogen for blueberries. Proc. Amer. Soc. Hort. Sci. 59:161-166.

Canvin, D. T. and Woo, K. C. (1979). The regulation of nitrate reduction in spinach leaves. Can. J. Bot. 57:1155-1160.

Chaillou, S., Morot-Gaudry, J. F., Salsac, L., Lesaint, C. and Jolivet, E. (1986). Compared effects of NO_3^- and NH_4^+ on growth and metabolism of French bean. Physiol. Veg. 24:679-687.

Chapin, F. S. III. (1980). The mineral nutrition of wild plants. Ann. Rev. Ecol. Syst. 11:233-260.

Christensen, P. and Skinner, P. (1978). The ecology of Boronia megastigma (Nees) in Western Australian forest trees. Research Paper 38. Forests Department of Western Australia.

Clement, C. R., Hooper, M. J. and Jones, L. H. P. (1978). The uptake of nitrate Lolium perenne from flowing nutrient solution. I. Effect of NO_3^- concentration. J. Exp. Bot. 29:453-464.

Colgrove, M. S. Jr. and Roberts, A. N. (1956). Growth of the azalea as influenced by ammonium and nitrate nitrogen. Proc. Amer. Soc. Hort. Sci. 68:522-536.

Cox, D. A. and Seeley, J. G. (1984). Ammonium injury to poinsettia: effects of NH_4^+-N:NO_3^--N ratio and pH control in solution culture on growth, N absorption, and N utilization. J. Amer. Soc. Hort. Sci. 109:57-62.

Cox, W. J. and Reisenauer, H. M. (1973). Growth and ion uptake by wheat supplied nitrogen as nitrate, or ammonium, or both. Plant Soil. 38:363-380.

Davies, H. W. and Ross, H. A. (1985). Factors affecting in vivo and in vitro determination of nitrate reductase activity in potato leaves. J. Plant Physiol. 119:1-17.

Davis, J. M., Loescher, W. H., Hammond, M. W. and Thornton, R. E. (1986). Response of potatoes to nitrogen form and to change in nitrogen form at tuber initiation. J. Amer. Soc. Hort. Sci. 111:70-72.

DeJong, C., Hughes, G. J., van Weirnigen, E. and Wilson, K. J. (1982). Amino acid analyses by high-performance liquid chromatography. An evaluation of the usefulness of pre-column Dns derivatization. J. Chromatogr. 241:345-359.

Delap, A. V. (1967). The effect of supplying nitrate at different seasons on the growth, blossoming and nitrogen content of young apple trees in sand culture. J. hortic. Sci. 42:149-167.

Doddema, H. Hofstra, J. J. and Fenstra, W. J. (1971). Uptake of nitrate by mutants of Arabidopsis thaliana, disturbed in uptake

or reduction of nitrate. I. Effect of nitrogen source during growth on uptake of nitrate and chlorate. Physiol. Plant. 43:343-350.

Edwards, J. H. and Horton, B. D. (1982). Interaction of peach seedlings to NO_3:NH_4 ratios in nutrient solutions. J. Amer. Soc. Hort. Sci. 107:142-147.

Elamin, O. M. and Wilcox, G. E. (1986). Nitrogen form ratio influence on muskmelon growth, composition and manganese toxicity. J. Amer. Soc. Hort. Sci. 111:320-322.

Elliott, G. C. and Nelson, P. V. (1983). Relationship among nitrogen accumulation, nitrogen assimilation and plant growth in chrysanthemums. Physiol. Plant. 57:250-259.

Epstein, E. (1983). Foreword. In: Lauchli, A. and Bieleski, R. L., eds. Inorganic plant nutrition (Encyclopedia of plant physiology, new ser., v. 15). pp. V-IX. Springer-Verlag, Berlin.

Fairall, A. R. (1970). West Australian native plants in cultivation. Pergamon Press, NSW. p. 253.

Faithfull, N. T. (1969). Multiple Kjeldahl digestion unit. Lab. Pract. 18:1302.

Ferrari, T. E. and Varner, J. E. (1971). Intact tissue assay for nitrite reductase in barley aleurone layers. Plant Physiol. 47:790-794.

Ferrari, T. E., Yoder, O. C. and Filner, P. (1973). Anaerobic nitrite production by plant cells and tissues: evidence for two nitrate pools. Plant Physiol. 51:423-431.

Finke, R. L., Warner, R. L. and Muzik, T. J. (1977). Effect of herbicides on in vivo nitrate and nitrite reduction. Weed Sci. 25:18-22.

Flint, P. S. and Gersper, P. L. (1974). Nitrogen nutrient levels in arctic tundra soils. In: Holding, A. J., Heal, O. W., Maclean, S. F. and Flanagen, P. W., eds. Soil organisms and decomposition in tundra. pp. 375-387. Tundra Biome Steerinf Committee, Stockholm.

Ganmore-Neumann, R. and Kafkafi, U. (1980) Root temperature and percentage NO_3^-/NH_4^+ effect on tomato plant development. I. Morphology and growth. Agron. J. 72:758-761.

Ganmore-Neumann, R. Kafkafi, U. (1985). The effect of root temperature and nitrate/ammonium ratio on strawberry plants. II. Nitrogen uptake, mineral ions, and carboxylate concentrations. Agron. J. 77:835-840.

Gashaw, L. and Mugwira, L. M. (1981). Ammonium-N and nitrate-N effects on the growth and mineral composition of triticale, wheat, rye. Agron. J. 73:47-51.

Gigon, A. and Rorison, R. H. (1972). The response of some ecologically distinct plant species to nitrate- and to ammonium-nitrogen. J. Ecol. 60:93-102.

Gilliam, C. H., Fretz, T. A. and Sheppard, W. J. (1980). Effect of nitrogen form and rate on elemental content and growth of pyracantha, cotoneaster and weigela. Scientia. Hortic. 13:173-179.

Glass, A. D. M., Siddiqi, M. Y. and Dean-Drummond, C. E. (1983). A multichannel microcomputer-based system for continuously measuring and recording ion activities for uptake solutions during ion absorption by roots of intact plants. Plant Cell Env. 6:247-253.

Gomez, K. A. and Gomez, K. A. (1984). Statistical procedures for agricultural research. John Wiley & Sons, New York.

Goyal, S. S. and Huffaker, R. C. (1986). The uptake of NO_3^-, NO_2^-, and NH_4^+ by intact wheat (Triticum aestivum) seedlings. I. Induction and kinetics of transport systems. Plant Physiol. 82:1051-1056.

Goyal, S. S., Lorenz, O. A. and Huffaker, R. C. (1982). Inhibitory effects of ammoniacal nitrogen on growth of radish plants. I. Characterization of toxic effects of NH_4^+ on growth and its alleviation by NO_3^-. J. Amer. Soc. Hort. Sci. 107:125-129.

Grasmanis, V. O. and Edwards, G. R. (1974). Promotion of flower initiation in apple trees by short exposure to the ammonium ion. Aust. J. Plant Physiol. 1:99-105.

*Grasmanis, V. O. and Leeper, G. W. (1965). Ammonium versus nitrate in culture solutions. Agrochemica. 10:54-63.

Grasmanis, V. O. and Leeper, G. W. (1967). Ammonium nutrition and flowering of apple trees. Aust. J. bio. Sci. 20:761-767.

Greidanus, T., Peterson, L. A., Schrader, L. E. and Dana. M. N. (1972). Essentiality of ammonium for cranberry nutrition. J. Amer. Soc. Hort. Sci. 97:272-277.

Groves, R. H. (1965). Growth of heath vegetation. II. The seasonal growth of a heath on ground-water podzol at Wilson's Promontory, Victoria. Aust. J. Bot. 13:281-289.

*Groves, R. H. (1983). Nutrient cycling in Australian heath and South African fynbos. In: Kruger, F. J., Mitchell, D. T. and Jarvis, J. U. M., eds. Mediterranean-type ecosystems: the role of nutrients. pp. 74-85. Springer-Verlag, Berlin.

Groves, R. H. and Keraitis, K. (1976). Survival and growth of seedlings of three sclerophyll species at high levels of phosphorus and nitrogen. Aust. J. Bot. 24:681-690.

Grundon, N. J. (1972). Mineral nutrition of some Queensland heath plants. J. Ecol. 60:171-181.

Gutschick, V. P. (1981). Evolved strategies in nitrogen acquisition by plants. Am. Nat. 117:607-637.

Hageman, R. H. (1984). Ammonium versus nitrate nutrition of higher plants. In:Nitrogen in crop production. pp. 67-85. ASA-CSSA-SSSA, WI.

Hageman, R. H., Cresswell, C. F. and Hewitt, E. J. (1962). Reduction of nitrate, nitrite and hydroxylamine extracted from higher plants. Nature. 193:247-250.

Hageman, R. H. and Hucklesby, D. P. (1971). Nitrate reductase from higher plants. In: San Pietro, A. ed. Methods in enzymology, volume XXIII. pp. 491-503. Academic Press, New York.

Hageman, R. H. and Reed, A. J. (1980). Nitrate reductase from higher plants. In: San Pietro, A. ed. Methods in enzymology, volume 69. pp. 270-280. Academic Press, New York.

Hancock, W. S., Bishop, C. A. and Hearm, M. T. W. (1979). The analysis of nanogram levels of free amino acids by reverse-phase high-pressure liquid chromatography. Anal. Biochem. 92:170-173.

Handa, S., Huber, D. M., Warren, H. L. and Tsai, C. Y. (1985). Nitrogen nutrition and N-assimilation in maize seedlings. Can. J. Plant Sci. 65:87-93.

Harada, T., Takaki, H. and Yamada, Y. (1968). Effect of nitrogen sources on the chemical components in young plants. Soil Sci.

Plant Nutr. 14:47-55.
Hartman, P. L., Mills, H. A. and Jones, J. B. Jr. (1986). The influence of nitrate:ammonium ratios on growth, fruit development, and elemental concentration in 'Floradel' tomato plants. J. Amer. Soc. Hort. Sci. 111:487-490.
Havill, D. C., Lee, J. A. and Stewart, G R. (1984). Nitrate utilization by species from acidic and calcareous soils. New Phytol. 73:1221-1231.
Haynes, R. J. and Goh, K. M. (1978). Ammonium and nitrate nutrition of plants. Biol. Rev. 53:465-510.
Heuer, B., Plaut, Z. and Federman, E. (1979). Nitrate and nitrite reduction in wheat as affected by different types of water stress. Physiol. Plant. 46:318-323.
Hewitt, E. J. (1966). Sand and water culture methods used in the study of plant nutrition. Commonwealth Agricultural Bureaux, England. p. 547.
Hewitt, E. J., Hucklesby, D P. and Noton, B. A. (1976). Nitrate metabolism. In: Bonner, J. and Varner, J. E., eds. Plant biochemistry. pp. 633-681. Academic Press, New York.
Higgs, A. L. (1970). The effect of planting depth and fertilizer on survival and growth of Grevillea and Leptospermum rooted cuttings. Aust. Plants. 6:23-25.
Hill-Cottingham, D G. and Williams, R. R. (1967). Effect of time of application of fertilizer nitrogen on the growth, flower development and first set of maiden apple trees, var. Lord Lambourne, on distribution of total nitrogen within the trees. J. hortic. Sci. 42:319-338.
Hingston, F. J., Malajczuk, N., Grove, T. S. (1982). Acetylene reduction (N_2-fixation) by jarrah forest legumes following fire and phosphate application. J. Applied Ecol. 19:631-645.
Hoff, J. E., Wilcox, G E. and Jones, C M. (1974). The effect of nitrate and ammonium on the free amino acid composition of tomato plants and tomato fruit. J. Amer. Soc. Hort. Sci. 99:27-30.
Huffaker, R. C. and Rains, D. W. (1978). Factors influencing nitrate acquisition and fate of reduced nitrogen. In: Nielsen, D. R. and MacDonald, J. G., eds. Nitrogen in the environment. volume 2. pp. 1-43. Academic Press, New York.
Huett, D. O. (1979). A study of some factors associated with aluminum uptake by three plant species. Ph. D. thesis. University of Tasmania, Hobart.
Ikeda, M. and Yamada, Y, (1984). Palliative effect of nitrate supply on ammonium injury of tomato plants: growth and chemical composition. Soil Sci. Plant Nutr. 30:485-493.
Ingestad, T. (1973). Mineral nutrient requirements of Vaccinium vitis idaea and V. myrtillus. Physiol. Plant. 29:239-246.
Ingestad, T. (1976). Nitrogen and cation nutrition of three ecologically different plant species. Physiol. Plant. 38:29-34.
Ingestad, T. (1979). Mineral nutrient requirements of Pinus silvestris and Picea abies seedlings. Physiol. Plant. 45:373-380.
Jackson, D. I. (1969). Effects of water, light, and nutrition on flower-bud initiation in apricots. Aust. J. biol. Sci. 22:69-75.
Jackson, D. I. (1970). Effects of temperature and nutrition on growth and flower-bud initiation in apricots. N. Z. J. agric. Res. 13:726-734.
Jackson, W. A., Volk, R. J. and Tucker, T. C. (1972). Apparent

induction of nitrate uptake in nitrate-depleted plants. Agron. J. 64:518-521.
*Janiesch, P. (1973). Beitrag zur Physiologie der Nitrophyten. Nitratspeicherung und Nitratassimilation bei Anthuisucs sylvestris Hoff. Flora. 162:479-491.
Jones, J. B. Jr. (1985). Soil testing and plant analysis: guides to the fertilization of horticultural crops. Hort. Rev. 7:1-68.
Jones, R. H. (1973). The effect of excess manganese on nitrate assimilation in lettuce. Ph.D. thesis. Universtity of Tasmania, Hobart.
Jones, R. W. and Sheard, R W. (1977). Conditions affecting in vivo nitrate reductase activity in chlorophyllous tissues. Can. J. Bot. 55:896-901.
Karim, A. Q. M. B. and Vlamis, J. (1962). Comparative study of the effects of ammonium and nitrate nitrogen in the nutrition of rice. Plant Soil. 16:32-41.
Kato, T. (1980). Nitrogen assimilation in citrus trees. 1. Ammonium and nitrate assimilation by intact roots, leaves and fruits. Physiol. Plant. 48:416-420.
Kinet, J. M., Sachs, R. M. and Bernier, G. (1985). The physiology of flowering, volume 3. CRC Press, Inc., Florida. p. 274.
Kirkby, E. A. (1968). Influence of ammonium and nitrate nutrition on the cation-anion balance and nitrogen and carbohydrate metabolism of white mustard plants in dilute nutrient solutions. Soil Sci. 105:133-141.
Kirkby, E. A. (1969). Ion uptake and ionic balance in plants in relation to the form of nitrogen nutrition. In: Rorison, R. H., ed. Ecological aspects of the mineral nutrition of plants. pp. 215-235. Blackwell Scientific Publication, Oxford.
Kirkby, E. A. and Hughes, D. A. (1970). Some aspects of ammonium and nitrate nutrition in plant metabolism. In: Kirkby, E. A., ed. Nitrogen nutrition of the plant. pp. 69-77. The University of Leeds, Leeds.
Kirkby, E. A. and Knight, A. H. (1977). The influence of the level of nitrate nutrition on ion uptake and assimilation, organic acid accumulation and cation-anion balance in whole tomato plants. Plant Physiol. 60:349-353.
Kirkby, E. A. and Mengel, K. (1967). Ionic balance in different tissues of the tomato plant in relation to nitrate, urea, or ammonium nutrition. Plant Physiol. 42:6-14.
Klepper, L. A. (1974). A mode of action of herbicides: inhibition of the normal process of nitrite reduction. Nebr. Agric. Exp. Stn. Bull. 259. p. 42.
Klepper, L. A. (1975). Inhibition of nitrite reduction by photosynthetic inhibitors. Weed Sci. 23:188-190.
Klepper, L. A. (1976). Nitrite accumulation within herbicide-treated leaves. Weed Sci. 24:533-535.
Klepper, L. A. (1979). Effects of certain herbicides and their combinations on nitrite reduction. Plant Physiol. 64:273-275.
Klepper, L., Fletcher, D. and Hageman, R. H. (1971). Generation of reduced nicotinamide adenine dinucleotide for nitrate reduction in green leaves. Plant Physiol. 48:580-590.
Klepper, L. A. and Hageman, R. H. (1969). The occurrence of nitrate reductase in apple leaves. Plant Physiol. 44:110-114.
Kozlowski, T. T. (1971). Growth and development of trees, volume

II. Academic Press, New York. p. 514.
Kozukue, N., Kozukue, E., Terai, H., Mizuni, S. (1982). HPLC analyses of amino acids in plant materials as DNP-derivatives by use of methyl benzoate as the internal standard. J. Food. Sci. 47:1584-1586.
*Krajina, V. J. (1969). Ecology of forest trees in British Columbia. Ecol. West North Am. 2:1-146.
Krajina, V. J., Madoc-Jones, G. and Mellor, G. (1973). Ammonium and nitrate in the economy of some conifers growing in Douglas-fir communities of the Pacific North-West of America. Soil Biol. Biochem. 5:143-147.
Kramer, P. J. and Kozlowski, T. T. (1979). Physiology of woody plants. Academic Press, New York. p. 811.
Larsen, B. R. and West, F. G. (1981). A method for quantitative amino acid analysis using precolumn o-phthalaldehyde derivatization and high performance liquid chromatography. J. Chromatogr. Sci. 19:259-269.
Lassak, E. V. and Southwell, I. A. (1977). Essential oil isolates from Australian flora. IFFA. 8:126-132.
Lee, J. A. and Stewart, G. R. (1978). Ecological aspects of nitrogen assimilation. Adv. Bot. Res. 6:1-43.
Legett, G. W. and Menary, R. C. (1980). Boronia production-its growth and oil characteristics. VIIIth Int. Cong. of Essential Oils. Paper No. 36.
Lillo, C. (1983). Studies of diurnal variations of nitrate reductase activity in barley leaves using various assay methods. Physiol. Plant. 57:357-362.
Lord, E. E. (1948). Shrubs and trees for Australian gardens. Lothian Publishing Co. Pty. Ltd., Melbourne. p. 453.
Lorenz, H. (1975). Free amino acids in tomato in relation to form and concentration of nitrogen in the rooting medium. Plant Soil. 45:163-168.
MacLeod, K. C. and Ormrod, D. P. (1985). Response of white bean to ammonium or nitrate nutrition at three temperatures. Can J. Plant Sci. 65:201-205.
Magalhaes, J. R. and Wilcox, G. E. (1984). Ammonium toxicity development in tomato plants relative to nitrogen form and light intensity. J. Plant Nutr. 7:1477-1496.
Matheson, E. M. (1979). Other crops of potential importance. In: Lovett, J, V. and Lazenby, A. eds. Australian field crops, volume 2. pp. 263-286. Angus & Robertson Publishers, Aust.
Maynard, D. N. and Barker, A. V. (1969). Studies on the tolerance of plants to ammonium nutrition. J. Amer. Soc. Hort. Sci. 94:235-239.
Maynard, D. N. and Barker, A. V. (1971). Critical nitrate levels for leaf lettuce, radish, and spinach plants. Commun. in Soil Sci. Plant Anal. 2:461-470.
McElhannon, W. S. and Mills, H. A. (1978). Influence of percent NO_3^-/NH_3^+ on growth, N absorption, and assimilation by lima beans in solution culture. Agron. J. 70:1027-1032.
McFee, W. W. and Stone, E. L. Jr. (1968). Ammonium and nitrate as nitrogen sources for Pinus radiata and Picea glauca. Soil Sci. Soc. Amer. Proc. 32:879-884.
McGrath, S. P. and Rorison, I. H. (1982). The influence of

nitrogen source on the tolerance of Holcus lanatus L. and Bromus erectus Huds. to manganese. New Phytol. 91:443-452.
McIntyre, G. I. (1977). The role of nutrition in apical dominance. Symposia of the Society for Experimental Biology. 31:251-273.
Melzer, A., Gebauer, G. and Rehder, H. (1984). Nitrate content and nitrate reductase activity in Rumex obtusifolius L. Oecologia (Berlin). 63:380-385.
Menary, R. C. and Jones, R. H. (1974). Accumulation of nitrate in papaw fruits. In: Bieleski, R. L., Ferguson, A. R. and Cresswell, M. M., eds. Mechanism of regulation of plant growth. Bulletin 12, The Royal Society of New Zealand, Wellington. pp. 77-83.
Menary, R. C. (1985). Essential oils and natural chemicals. In: Proceedings of the 3rd Australian Agronomy Conference. pp. 86-89. Australian Society of Agronomy, Victoria.
Menary, R. C. and van Staden, J. (1976). Effect of phosphorus nutrition and cytokinins on flowering in the tomato, Lycopersicon esculentum Mill. Aust. J. Plant Physiol. 3:201-205.
Miller, M. H., Mammaril, C. P. and Blair, G. J. (1970). Ammonium effects on phosphorus absorption through pH changes and phosphorus precipitation at the soil-root interface. Agron. J. 62:524-527.
Mills, H. A., Barker, A. V. and Maynard, D. N. (1976). Effect of nitrapyrin on nitrate accumulation in spinach. J. Amer. Soc. Hort. Sci. 101:202-204.
Mills, D. and Lips, S. H. (1984). Nitrate reduction and compartmentation in tomato leaves. I. Nitrate accumulation in leaf sections in aqueous and gaseous medium. Physiol. Plant. 61:141-148.
Mills, H. A. and McElhannon, W. S. (1982). Nitrate uptake by sweet corn. HortScience. 17:743-744.
Moore, C. W. E. and Keraitis, K. (1966). Nutrition of Grevillea robusta. Aust. J. Bot. 14:141-163.
Moore, C. W. E. and Keraitis, K. (1971). Effect of nitrogen source on growth of eucalyptus in sand culture. Aust. J. Bot. 19:125-141.
Moreno, J. and Garcia-Martinez, J. L. (1980). Extraction of tracheal sap from citrus and analysis of its nitrogenous compounds. Physiolo. Plant. 50:298-303.
Nichols, D. G., Jones, D. L. and Beardsell, D. V. (1979). The effect of phosphorus on the growth of Grevillea 'Poorinda Firebird' in soil-less potting-mixtures. Scientia Hortic. 11:197-205.
Notton, B. A., Graf, L., Hewitt, E. J. and Povey, R. C. (1974). The role of molybdenum in the synthesis of nitrate reductase in cauliflower (Brassica oleracea L.). Biochem. Biophys. Res. Commun. 44:702-710.
Nyborg, M. and Hoyt, P. B. (1978). Effects of soil acidity and liming on mineralization of soils nitrogen. Can. J. Soil. Sci. 58:331-338.
Oaks, A. and Hirel, B. (1985). Nitrogen metabolism i roots. Ann. Rev. Plant Physiol. 36:345-365.
Olday, F C., Barker, A. V. and Maynard, D. N. (1976). A physiological basis for different patterns of nitrate accumulation in cucumber and pea. J. Amer. Soc. Hort. Sci.

101:219-221.
Pataki, G. (1969). Techniques of thin-layer chromatography in amino acid and peptide chemistry. Ann Arbor-Humphrey Science Publishers, Inc., Ann Arbor.
Pate, J. S. (1973). Uptake, assimilation and transport of nitrogen compounds by plants. Soil Biol. Biochem. 5:109-119.
Pate, J. S. (1980). Transport and partitioning of nitrogenous solutes. Ann. Rev. Plant Physiol. 31:313-340.
Paton, D. M. (1978). Eucalyptus physiology. I. Photoperiodic responses. Aust. J. Bot. 26:633-642.
Peirson, D. R. and Elliott, J. R. (1981). In vivo nitrite reduction in leaf tissue of Phaseolus vulgaris L. Plant Physiol. 68:1068-1072.
Pill, W. G. and Lambeth, V. N. (1977). Effects of NH_4 and NO_3 nutrition with and without pH adjustment on tomato growth, ion composition, and water relations. J. Amer. Soc. Hort. Sci. 102:78-81.
Pizelle, G. and Thiery, G. (1986) Reduction of nitrate in the perennial tissues of aerial parts of Alunus glutinosa. Physiol. Plant. 68:347-352.
Polizotto, K. R., Wilcox, G. E. and Jones, C. M. (1975). Response of growth and mineral composition of potato to nitrate and ammonium nitrogen. J. Amer. Soc. Hort. Sci. 100:165-168.
Precheur, R. J. and Maynard, D N. (1982). Growth of asparagus plants as influenced by nitrogen form and lime. J. Amer. Soc. Hort. Sci. 108:169-172.
Purchase, B. S. (1974). The influence of phosphate deficiency on nitrification. Plant Soil. 41:541-547.
Radin, J. W. (1975). Physiological significance of the in vivo assay for nitrate reductase in cotton seedlings. Crop Sci. 15:710-713.
Radin, J. W. (1977). Contribution of the root system to nitrate assimilation in whole cotton seedlings. Aust. J. Plant Physiol. 4:811-819.
Rajasekhar, V. K. and Mohr, H. (1986). Effect of ammonium and nitrate on growth and appearance of nitrate reductase and nitrite reductase in dark- and light-grown seedlings. Planta. 169:594-599.
Rao, K. P. and Rains, D. W. (1976). Nitrate absorption by barley. 1. Kinetics and energetics. Plant Physiol. 57:55-58.
Raven, J. A. and Smith, F. A. (1976). Nitrogen assimilation and transport in vascular land plants in relation to intracellular pH regulation. New Phytol. 76:415-431.
Rice, E. L. and Pancholy, S. K. (1972). Inhibition of nitrification by climax ecosystem. Amer. J. Bot. 59:1033-1040.
Richards, D. (1985). Effect of cytokinin in application and environment on growth and flowering in Boronia heterophylla F. Muell. Scientia Hortic. 27:325-334.
Richter, R., Dijkshoon, W. and Vonk, C. R. (1975). Amino acids of barley in relation to nitrate, urea or ammonium nutrition. Plant Soil. 42:601-618.
Riley, D. and Barber, S. A. (1971). Effect of ammonium and nitrate fertilization on phosphorus uptake as related to root-induced pH changes at the root-soil interface. Soil Sci. Soc. Amer. Proc. 35:301-306.

Rorison, I. H. (1985). Nitrogen source and the tolerance of Deschampsia flexuosa, Holcus lanatus and Bromus erectus to aluminum during seedling growth. J. Ecol. 73:83-90.

Rosnitschek-Schimmel, I. (1982). Effect of ammonium and nitrate supply on dry matter production and nitrogen distribution in Urtica dioica. Z. Pflanzenphysiol. 108:329-341.

Routley, D. G. (1972). Nitrate reductase in leaves of Ericaceae. HortScience. 7:85-87.

Rufty, T. W. Jr., Raper, C. D. Jr. and Jackson, W. A. (1983). Growth and nitrogen assimilation of soybeans in response to ammonium and nitrate nutrition. Bot. Gaz. 144:466-470.

*Runge, M. (1974). Die Stickstoff-Mineralization im Boden eines Sauerhumus-Buchenwaldes. II. Die Nitraproduction. Oecol. Plant. 9:219-230.

Runge, M. (1983). Physiology and ecology of nitrogen nutrition. In: Lange, O. L., Nobel, P. S., Osmond, C. B. and Ziegler, H. eds. Physiological plant ecology III (Encyclopedia of plant physiology; new ser. 12C). pp. 163-200. Springer-Verlag, Berlin.

*Russell, E. W. (1973). Soil conditions and plant growth. Longman, London. p. 849.

Salama, El-D. A. M. S. and Wareing, P. F. (1979). Effect of mineral nutrition on endogenous cytokinins in plants of sunflowers (Helianthus annuus L.). J. Exp. Bot. 30:971-981.

Sanderson, G. W. and Cocking, E. C. (1964). Enzymic assimilation of nitrate in tomato plants. I. Reduction of nitrate t nitrite. Plant Physiol. 39:416-422.

Sassville, D. N. and Mills, H. A. (1979). N form and concentration: effects on reproductive development growth and N content of southern peas. J. Plant Nutr. 1:241-254.

Schrader, L. E., Domska, D., Jung, P. E. Jr. and Peterson, L. A. (1972). Uptake and assimilation of ammonium-N and nitrate-N and their influence on the growth of corn (Zea mays L.). Agron. J. 64:690-695.

Schrader, L. E., Cataldo, D. A. and Peterson, D. M. (1974). Use of protein in extraction and stabilization of nitrate reductase. Plant Physiol. 53:688-690.

Schulze, E.-D, (1982). Plant life forms and their carbon, water and nutrient relations. In: Lange, O. L., Nobel, P. S., Osmond, C. B. and Ziegler, H. eds. Physiological plant ecology II (Encyclopedia of plant physiology; new ser., v. 12B). pp. 615-676. Springer-Verlag, Berlin.

Schulze, E.-D., Koch, G., Percival, F., Mooney, H. A. and Chu, C. (1985). The nitrogen balance of Raphanus sativus X raphanisitrum plants. I. Daily nitrogen use under high nitrate supply. Plant Cell Env. 8:713-720.

Scott, D. B. and Neyra, C A. (1979). Glutamine synthetase and nitrate assimilation in sorghum (Sorghum vlugare) leaves. Can. J. Bot. 57:754-758.

Seeley, J. G. (1950). Potassium deficiency of greenhouse roses. Proc. Amer. Soc. Hort. Sci. 56:466-474.

Shaner, D. L. and Boyer, J. S. (1976). Nitrate reductase activity in maize (Zea mays L.) leaves. I. Regulation by nitrate flux. Plant Physiol. 58:499-504.

Sherrard, J. H. and Dalling, M. J. (1978). Effect of casein on the extractability and stability of nitrate reductase from wheat

leaves. Ann. Bot. 42:1421-1427.
Shivshankar, S. and Ramdasan, A. (1980). Nitrate reductase activity in coconut leaves. J. Sci. Food Agric. 34:1179-1184.
Smirnoff, N., Todd, P. and Stewart, G R. (1984). The occurrence of nitrate reduction in the leaves of woody plants. Ann. Bot. 54:363-374.
Smith, F. A. (1973). The internal control of nitrate uptake into excised barley roots with differing salt contents. New Phytol. 72:769-782.
Smith, F. W. and Johnson, A. D. (1974). Rapid semi-micro digestion technique for major element analysis of plant material. Lab. Pract. 23:51-52.
Somers, D. A., Kuo, T. M., Kleinhofs, A., Warner, R. L. and Oaks, A. (1983). Synthesis and degradation of barley nitrate reductase. Plant Physiol. 72:949-952.
Soon, Y. K. and Miller, M. H. (1977). Changes in rhizosphere due to NH_4^+ and NO_3^- fertilization and phosphorus uptake by corn seedlings (Zea mays L.). Soil Sci. Soc. Am. J. 41:77-80.
Specht, R. L. (1963). Dark Island heath (Ninety-Mile Plain, South Australia). VII. The effect of fertilizers on composition and growth, 1950-60. Aust. J. Bot. 11:64-67.
Specht, R. L. and Groves, R. H. (1966). A comparison of the phosphorus nutrition of Australian heath plants and introduced economic plants. Aust. J. Bot. 14:201-221.
Spiers, J. M. (1978). Effects of pH level and nitrogen source on elemental leaf content of 'Tifblue' rabbiteye blueberry. J. Amer. Soc. Hort. Sci. 103:705-708.
Steel, R. G. D. and Torrie, J. H. (1980). Principles and procedures of statistics: a biometrical approach. McGraw-Hill, New York. p. 633.
*Sweet, G B. and Hong, S. O. (1978). The role of nitrogen in relation to cone production in Pinus radiata. N. Z. J. For. Sci. 8:225-238.
Sym, G J. (1984). Optimization of the in-vivo assay conditions for nitrate reductase in barley (Hordeum vlugare L. cv. Igri). J. Sci. Food Agric. 35:725-730.
Thomas, M. B. (1979). Nitrogen response of proteaceous shrubs and other nursery plants grown in containers. Int. Plant Propag. Soc. Comb. Proc. 29:143-157.
Thomas, M. B. (1981). Nutrition of five species of container-grown acacia, boronia, choisya and eucalyptus. Scientia Hortic. 14:55-68.
Thomas, M. B. (1982). Nutrition of container-grown Callistemon citronus and Hakea laurina. Scientia Hortic. 17:159-167.
Tempo, E. A. and Neyra, C. A. (1983). Expression of nitrate and nitrite reductase activities under various forms of nitrogen nutrition in Phaseolus vulgaris L. Plant Physiol. 72:71-75.
Townsend, L. R. (1966). Effect of nitrate and ammonium nitrogen on the growth of the lowbush blueberry. Can. J. Plant. Sci. 46:209-210.
Townsend, L. R. (1967). Effect of ammonium nitrogen and nitrate nitrogen, separately and in combination, on the growth of the highbush blueberry. Can. J. Plant Sci. 47:555-562.
Townsend, L. R. (1969). Influence of form of nitrogen and pH on growth and nutrient levels in the leaves and roots of the lowbush

blueberry. Can. J. Plant Sci. 49:333-338.
Trewavas, T. (1985). A pivotal role for nitrate and leaf growth in plant development. In: Barker, N. R., Davies, W. J. and Ong, C. K., eds. Control of leaf growth. pp. 77-91. Cambridge University Press, Cambridge.
Tucker, T. C. (1984). Diagnosis of nitrogen deficiency in plants. In: Nitrogen in crop production. pp. 249-262. ASA-CSSA-SSSA, WI.
van den Driessche, R. (1971). Response of conifer seedlings to nitrate and ammonium sources of nitrogen. Plant Soil. 34:421-439.
van den Driessche, R. (1978). Response of Douglas-fir seedlings to nitrate and ammonium sources of nitrogen. Plant Soil. 49:607-623.
Vega, J. M., Cardenas, J., Losada, M. (1980). Ferredoxin-nitrite reductase. In: San Pietro, A. Methods in enzymology, volume 69. pp. 255-270. Academic Press, New York.
Wallace, W. and Nicholus, D. J. D. (1969). The biochemistry of nitrifying microorganisms. Biol. Rev. 44:359-391.
Wallace, W. and Pate, J. S. (1967). Nitrate assimilation in higher plants with special reference to cocklebur (Xanthium pennsylvanicum Wallr.). Ann. Bot. 31:213-218.
Walsh, L. M. and Beaton, J. D. (1973). Soil testing and plant analysis. Soil Science Society of America, Inc., WI. p. 491.
Wander, I. W. and Sites, J. W. (1956). The effects of ammonium and nitrate nitrogen with and without pH control on the growth of rough lemon seedlings. Proc. Amer. Soc. Hort. Sci. 68:211-226.
Weissman, G. S. (1964). Effect of ammonium and nitrate nutrition on protein level and exudate composition. Plant. Physiol. 39:947-952.
Wilcox, G. E., Hoff, J. E. and Jones, C. M. (1973). Ammonium reduction of calcium and magnesium content of tomato and sweet corn leaf tissue and influence of blossom end rot of tomato fruit. J. Amer. Soc. Hort. Sci. 98:86-89.
Wiltshire, G. H. (1956). Responses of grasses to nitrogen sources. J. appl. Ecol. 10:429-435.
Woodin, S. J., Press, M. C. and Lee, J. A. (1985). Nitrate reductase activity in Sphagnum fuscum in relation to wet deposition of nitrate from the atmosphere. New. Phytol. 99:381-388.
Woolhouse, H. W. and Hardwick, K. (1966). The growth of tomato seedlings in relation to the form of the nitrogen supply. New. Phytol. 65:518-525.
Woolley, J. T., Hicks, G. P. and Hageman, R. H. (1960). Rapid determination of nitrate and nitrite in plant material. Agric. Food Chem. 8:481-482.
Yoneyama, T. and Kumazawa, K. (1974). A kinetic study of the assimilation of ^{15}N-labelled ammonium in rice seedling roots. Plant & Cell Physiol. 15:655-661.
Yoneyama, T. and Kumazawa, K. (1975). A kinetic study of the assimilation of ^{15}N-labelled nitrate in rice seedlings. Plant & Cell Physiol. 16:21-26.
Zimmerman, C. L. and Pisano, J. J. (1977). High-performance liquid chromatography of amino acid derivatives. In: Hirs, C. H. W. and Timaseff, S. N., eds. Methods in enzymology, vol. XLVII. pp. 45-51. Academic Press, New York.

*Not seen in original.

Appendix 1. MYCORRHIZAL STATUS OF BORONIA

During the course of study it was decided to determine the mycorrhizal status of Boronia megastigma.

Therefore, root samples were collected from boronia plants growing in Bruny Island (Tasmania) and were examined. There were no changes in the external root morphology. The roots were cleared and stained using the method of Trappe et al. (1973) and examined under a compound microscope.

Arbuscules were observed, but vesicles were not seen. Gigaspora marginata does not form vesicles (Bonfante-Fasolo, 1984). Whether infection is caused by this species is to be confirmed. Lamont (1982) also found Boronia to be VA mycorrhizal in the native jarrah forest (in WA).

After completion of the greenhouse experiments, the experimental plants were examined randomly. However there were no mycorrhizas. This absence may be due to the supply of P (a nontreatment factor) to the plants, as high levels of P in the growth medium are known to inhibit root colonization by mycorrhizal fungi (Hetrick, 1984).

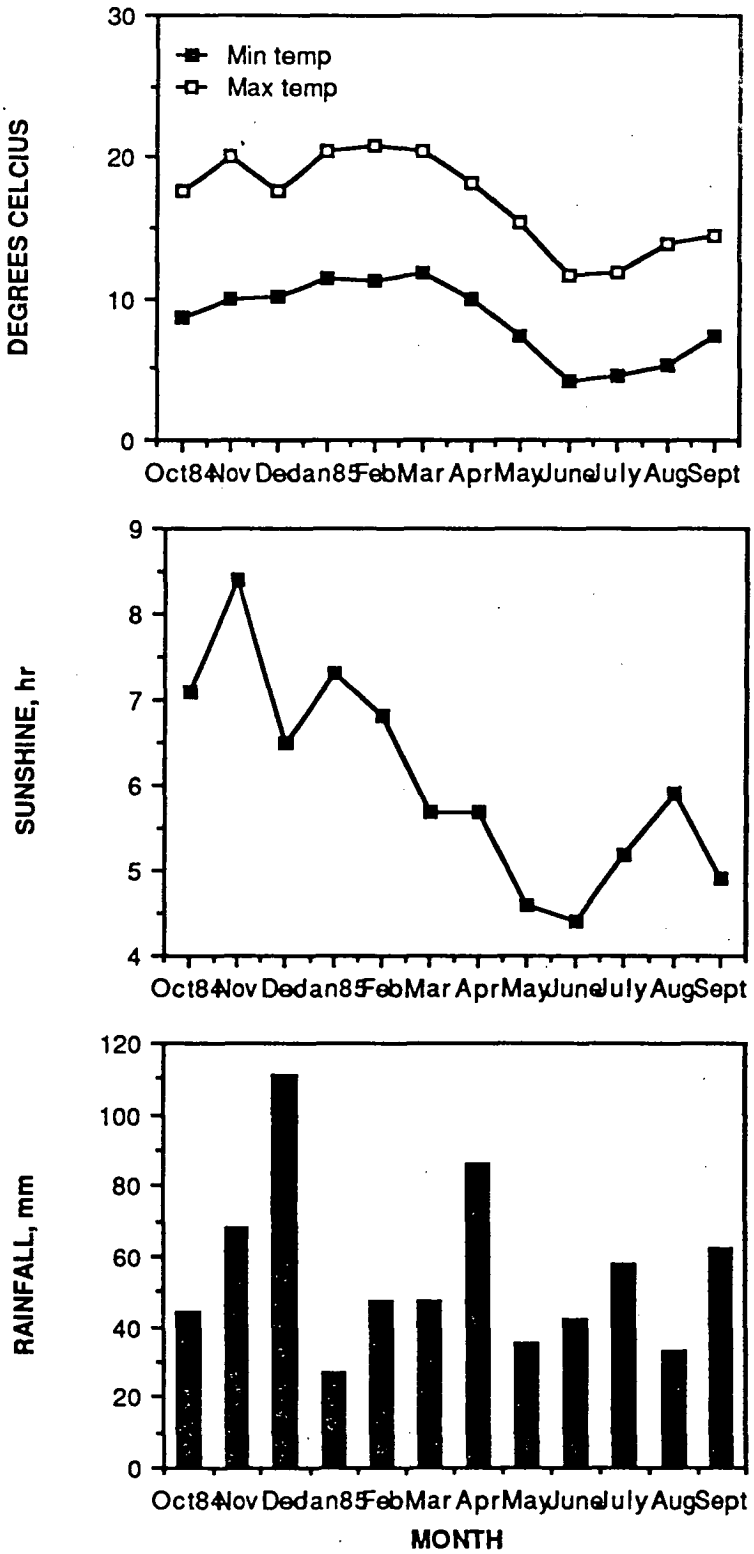
References

Bonfante-Fasolo, P. (1984). Anatomy and morphology of VA mycorrhizae. In: Powell, C. L. and Bagyaraj, D. J., eds. VA mycorrhiza. pp. 5-33. CRC Press, Inc., Florida.

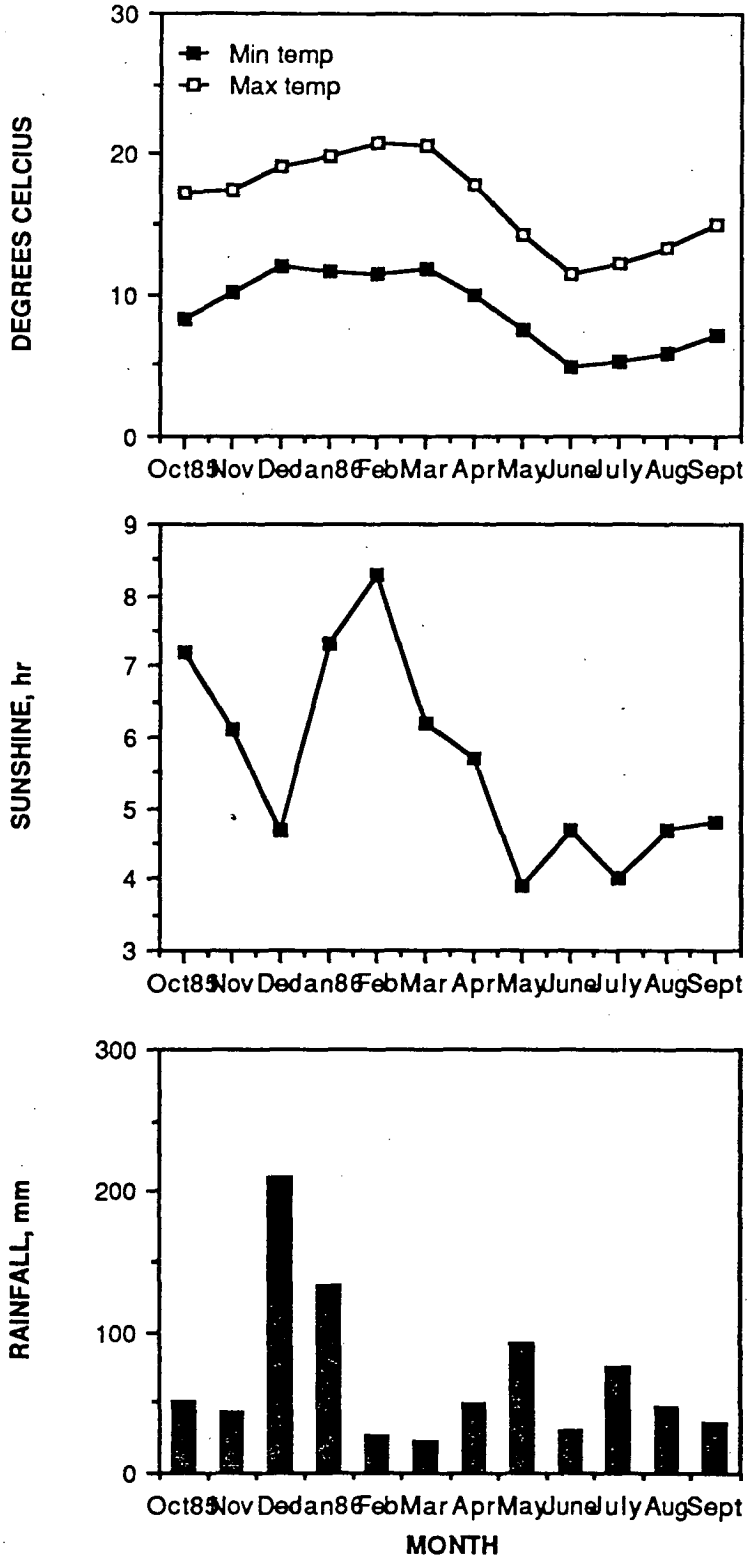
Hetrick, B. A. D. (1984). Ecology of VA mycorrhizal fungi. In: Powell, C. L. and Bagyaraj, D. J., eds. VA mycorrhiza. pp. 35-55. CRC Press, Inc., Florida.

Lamont, B. (1982). Mechanisms for enhancing nutrient uptake in plants, with particular reference to mediterranean South Africa and Western Australia. Bot. Rev. 48:597-689.

Trappe, J. M., Stahly, E. L., Benson, N. R. and Duff, D. M. (1973). Mycorrhizal deficiency of apple trees in high arsenic soils. HortScience. 8:52-53.



Appendix Fig. II.1. Temperature, solar radiation and rainfall during 1984-85. Temperature and solar radiation were recorded at Hobart (42°.53'S) and rainfall was recorded at Kingston (Blackman's Bay, 43°.01'S).



Appendix Fig. II.2. Temperature, solar radiation and rainfall during 1985-86. Temperature and solar radiation were recorded at Hobart and rainfall was recorded at Kingston upto Feb. 86 and from Mar. 86 at Hobart.

Appendix Table II.1. Leaf N concentration in October 1984 (data on which part of Table 6 is based).

Source of N	Rate of N kg ha^{-1}	Leaf N, % dry wt. — Time of N application: Oct 84	Oct&June 84 85	Oct&Aug 84 85	Oct 84,June Aug 85	Mean
	0	1.47 (0.07)				
$(NH_4)_2SO_4$	25	1.68 (0.07)	1.84 (0.11)	1.87 (0.04)	1.52 (0.04)	1.73
	50	2.92 (0.28)	1.91 (0.18)	1.91 (0.15)	1.66 (0.11)	2.10
	100	3.45 (0.04)	2.96 (0.18)	2.80 (0.14)	2.33 (0.18)	2.89
Mean		2.38	2.05	2.00	1.74	
$Ca(NO_3)_2$	25	1.82 (0.19)	1.54 (0.07)	1.52 (0.04)	1.54 (0.07)	1.60
	50	2.57 (0.15)	1.84 (0.23)	1.94 (0.16)	1.56 (0.04)	1.98
	100	3.34 (0.18)	2.71 (0.18)	2.66 (0.07)	2.33 (0.15)	2.76
Mean		2.30	1.89	1.90	1.73	
IBDU	25	1.70 (0.15)	1.61 (0.07)	1.68 (0.07)	1.52 (0.11)	1.63
	50	1.98 (0.18)	1.80 (0.04)	1.75 (0.07)	1.70 (0.04)	1.81
	100	2.59 (0.25)	1.89 (0.14)	1.94 (0.11)	1.91 (0.15)	2.08
Mean		1.94	1.69	1.71	1.65	

Significance	LSD P = 0.05	0.01
Source	0.04	0.06
Rate	0.05	0.07
Time	0.05	0.07
Source X Rate	0.09	0.11
Source X Time	0.09	0.11
Rate X Time	0.10	0.13
Source X Rate X Time	0.17	0.23

Appendix Table II.2. Leaf N concentration in January 1985 (data on which part of Table 6 is based).

Source of N	Rate of N kg ha^{-1}	Leaf N, % dry wt. — Time of N application: Oct 84	Oct&June 84 85	Oct&Aug 84 85	Oct 84,June Aug 85	Mean
	0	1.59 (0.11)				
$(NH_4)_2SO_4$	25	1.68 (0.07)	1.61 (0.07)	1.61 (0.07)	1.63 (0.11)	1.63
	50	2.82 (0.23)	1.68 (0.07)	1.63 (0.11)	1.61 (0.07)	1.94
	100	3.31 (0.11)	2.68 (0.11)	2.75 (0.11)	2.05 (0.11)	2.70
Mean		2.35	1.89	1.90	1.72	
$Ca(NO_3)_2$	25	1.56 (0.08)	1.61 (0.07)	1.63 (0.08)	1.61 (0.07)	1.60
	50	2.47 (0.11)	1.63 (0.11)	1.56 (0.11)	1.68 (0.07)	1.84
	100	3.10 (0.11)	2.47 (0.08)	2.47 (0.11)	2.00 (0.15)	2.51
Mean		2.18	1.83	1.81	1.72	
IBDU	25	1.73 (0.11)	1.61 (0.07)	1.70 (0.15)	1.52 (0.04)	1.64
	50	2.12 (0.08)	1.80 (0.11)	1.77 (0.04)	1.87 (0.11)	1.89
	100	2.71 (0.18)	2.15 (0.15)	2.08 (0.11)	1.91 (0.04)	2.21
Mean		2.04	1.79	1.79	1.72	

Significance	LSD P = 0.05	0.01
Source	0.03	0.03
Rate	0.03	0.04
Time	0.03	0.04
Source X Rate	0.05	0.07
Source X Time	0.05	0.07
Rate X Time	0.06	0.08
Source X Rate X Time	0.10	0.14

Appendix Table II.3. Leaf N concentration in July 1985 (data on which part of Table 6 is based).

Source of N	Rate of N kg ha^{-1}	Leaf N, % dry wt. — Time of N application: Oct 84	Oct&June 84 85	Oct&Aug 84 85	Oct 84, June Aug 85	Mean
	0	1.66 (0.04)				
$(NH_4)_2SO_4$	25	1.66 (0.11)	1.75 (0.07)	1.66 (0.08)	1.70 (0.04)	1.69
	50	2.26 (0.11)	2.15 (0.15)	1.70 (0.11)	2.08 (0.15)	2.05
	100	2.68 (0.28)	3.08 (0.14)	2.12 (0.11)	3.00 (0.07)	2.72
Mean		2.07	2.16	1.79	2.11	
$Ca(NO_3)_2$	25	1.61 (0.07)	1.70 (0.04)	1.59 (0.04)	1.73 (0.08)	1.66
	50	2.17 (0.19)	2.15 (0.11)	1.75 (0.07)	2.22 (0.08)	2.07
	100	2.64 (0.25)	3.15 (0.14)	2.17 (0.21)	3.13 (0.11)	2.77
Mean		2.02	2.16	1.79	2.18	
IBDU	25	1.56 (0.11)	1.77 (0.04)	1.49 (0.08)	1.75 (0.07)	1.65
	50	1.96 (0.07)	2.03 (0.14)	1.94 (0.04)	2.10 (0.14)	2.00
	100	2.52 (0.25)	2.19 (0.04)	2.10 (0.12)	2.12 (0.08)	2.23
Mean		1.93	1.91	1.80	1.91	

Significance	LSD P = 0.05	0.01
Source	0.03	0.05
Rate	0.04	0.05
Time	0.04	0.05
Source X Rate	0.07	0.09
Source X Time	0.07	0.09
Rate X Time	0.08	0.10
Source X Rate X Time	0.14	0.18

Appendix Table II.4. Leaf N concentration in September 1985 (data on which part of Table 6 is based).

Source of N	Rate of N kg ha^{-1}	Leaf N, % dry wt. — Time of N application: Oct 84	Oct&June 84 85	Oct&Aug 84 85	Oct 84, June Aug 85	Mean
	0	1.66 (0.11)				
$(NH_4)_2SO_4$	25	1.66 (0.11)	1.82 (0.07)	1.89 (0.07)	1.75 (0.07)	1.78
	50	2.15 (0.11)	1.77 (0.15)	2.31 (0.11)	2.17 (0.15)	2.10
	100	2.66 (0.21)	2.99 (0.11)	3.08 (0.12)	3.13 (0.11)	2.96
Mean		2.03	2.06	2.23	2.18	
$Ca(NO_3)_2$	25	1.56 (0.04)	1.61 (0.07)	1.82 (0.07)	1.75 (0.07)	1.69
	50	2.10 (0.12)	1.84 (0.11)	2.26 (0.18)	2.19 (0.18)	2.10
	100	2.50 (0.18)	2.99 (0.15)	3.08 (0.12)	3.13 (0.11)	2.92
Mean		1.95	2.02	2.21	2.18	
IBDU	25	1.59 (0.08)	1.59 (0.04)	1.75 (0.07)	1.77 (0.04)	1.67
	50	1.87 (0.08)	1.94 (0.11)	2.05 (0.11)	2.12 (0.11)	2.00
	100	2.64 (0.11)	2.24 (0.07)	2.29 (0.11)	2.24 (0.07)	2.35
Mean		1.94	1.86	1.94	1.95	

Significance	LSD P = 0.05	0.01
Source	0.04	0.05
Rate	0.04	0.06
Time	0.04	0.06
Source X Rate	0.07	0.10
Source X Time	0.07	0.10
Rate X Time	0.08	0.11
Source X Rate X Time	0.15	0.19

Appendix Table II.5. Leaf N concentration in January 1986 (data on which part of Table 7 is based).

Source of N	Rate of N_{-1} kg ha	Leaf N, % dry wt. — Time of N application: Oct 85	Oct&June 85	June 86	Oct&Aug 85	Aug 86	Oct 84,June Aug 86	Mean
	0	1.59 (0.04)						
$(NH_4)_2SO_4$	25	1.75 (0.07)	1.66 (0.04)		1.68 (0.07)	1.61 (0.07)		1.67
	50	2.85 (0.18)	1.75 (0.14)		1.68 (0.14)	1.63 (0.15)		1.98
	100	3.24 (0.11)	2.75 (0.18)		2.73 (0.07)	2.08 (0.11)		2.70
Mean		2.36	1.94		1.92	1.73		
$Ca(NO_3)_2$	25	1.73 (0.04)	1.70 (0.11)		1.68 (0.07)	1.61 (0.07)		1.68
	50	2.80 (0.04)	1.75 (0.07)		1.75 (0.14)	1.61 (0.07)		1.68
	100	3.17 (0.11)	2.73 (0.07)		2.75 (0.11)	2.05 (0.15)		2.68
Mean		2.32	1.94		1.94	1.72		
IBDU	25	1.77 (0.11)	1.68 (0.12)		1.68 (0.07)	1.56 (0.04)		1.67
	50	2.15 (0.11)	1.87 (0.11)		1.75 (0.07)	1.82 (0.07)		1.90
	100	2.71 (0.11)	2.19 (0.11)		2.22 (0.18)	1.91 (0.15)		2.26
Mean		2.05	1.83		1.81	1.72		

Significance	LSD P = 0.05	0.01
Source	0.03	0.04
Rate	0.04	0.05
Time	0.04	0.05
Source X Rate	0.06	0.08
Source X Time	0.06	0.08
Rate X Time	0.07	0.10
Source X Rate X Time	0.13	0.17

Appendix Table II.6. Leaf N concentration in July 1986 (data on which part of Table 7 is based).

Source of N	Rate of N_{-1} kg ha	Leaf N, % dry wt. — Time of N application: Oct 85	Oct&June 85	June 86	Oct&Aug 85	Aug 86	Oct 84,June Aug 86	Mean
	0	1.63 (0.08)						
$(NH_4)_2SO_4$	25	1.66 (0.11)	1.80 (0.11)		1.70 (0.11)	1.77 (0.04)		1.73
	50	2.26 (0.18)	2.29 (0.08)		1.82 (0.07)	2.12 (0.11)		2.12
	100	2.73 (0.14)	3.08 (0.19)		2.15 (0.11)	3.00 (0.14)		2.74
Mean		2.07	2.20		1.83	2.14		
$Ca(NO_3)_2$	25	1.61 (0.07)	1.70 (0.11)		1.63 (0.04)	1.80 (0.11)		1.69
	50	2.17 (0.07)	2.26 (0.11)		1.77 (0.04)	2.19 (0.04)		2.10
	100	2.73 (0.25)	3.13 (0.15)		2.19 (0.23)	3.17 (0.11)		2.81
Mean		2.04	2.18		1.81	2.20		
IBDU	25	1.61 (0.07)	1.77 (0.04)		1.54 (0.07)	1.80 (0.04)		1.68
	50	2.03 (0.07)	2.08 (0.08)		1.91 (0.08)	2.08 (0.11)		2.02
	100	2.54 (0.21)	2.24 (0.07)		2.10 (0.07)	2.19 (0.04)		2.27
Mean		1.95	1.93		1.80	1.93		

Significance	LSD P = 0.05	0.01
Source	0.03	0.04
Rate	0.04	0.05
Time	0.04	0.05
Source X Rate	0.06	0.08
Source X Time	0.06	0.08
Rate X Time	0.07	0.10
Source X Rate X Time	0.13	0.17

Appendix Table II.7. Leaf N concentration in September 1986 (data on which part of Table 7 is based).

Source of N	Rate of N, kg ha^{-1}	Leaf N, % dry wt.				
		Time of N application				
		Oct 85	Oct&June 85 86	Oct&Aug 85 86	Oct 84,June Aug 86	Mean
	0	1.61 (0.07)				
$(NH_4)_2SO_4$	25	1.63 (0.04)	1.80 (0.08)	1.89 (0.12)	1.87 (0.04)	1.80
	50	2.10 (0.07)	1.94 (0.27)	2.36 (0.08)	2.17 (0.07)	2.14
	100	2.64 (0.11)	2.99 (0.04)	3.10 (0.11)	3.17 (0.04)	2.98
Mean		2.00	2.08	2.24	2.21	
$Ca(NO_3)_2$	25	1.56 (0.11)	1.63 (0.04)	1.87 (0.11)	1.80 (0.04)	1.72
	50	2.10 (0.07)	1.91 (0.11)	2.26 (0.11)	2.19 (0.11)	2.12
	100	2.50 (0.15)	2.94 (0.14)	3.10 (0.15)	3.08 (0.12)	2.91
Mean		1.94	2.02	2.21	2.17	
IBDU	25	1.61 (0.07)	1.63 (0.08)	1.82 (0.07)	1.77 (0.11)	1.71
	50	1.84 (0.11)	1.96 (0.07)	2.10 (0.07)	2.15 (0.04)	2.00
	100	2.59 (0.07)	2.19 (0.15)	2.26 (0.11)	2.29 (0.11)	2.33
Mean		1.91	1.85	1.95	1.95	

Significance	LSD P = 0.05	0.01
Source	0.04	0.05
Rate	0.04	0.06
Time	0.04	0.06
Source X Rate	0.07	0.10
Source X Time	0.07	0.10
Rate X Time	0.08	0.11
Source X Rate X Time	0.15	0.19

Appendix Table II. 8. Flower yield in January 1985 (data on which Fig. 6 is based).

Amount of N applied in October^{-1} 84[a] kg ha^{-1}	Flower yield, g fresh weight plant^{-1}					
	Source of N					
	$(NH_4)_2SO_4$		$Ca(NO_3)_2$		IBDU	
0			1.97	(0.67)		
8.3 (25/Oct,June&Aug)	2.20	(0.30)	2.07	(0.15)	2.83	(0.45)
12.5 (25/Oct&June)	3.83	(0.59)	3.77	(0.74)	2.97	(0.32)
(25/Oct&Aug)	2.63	(0.78)	3.07	(0.61)	3.93	(0.31)
16.8 (50/Oct,June&Aug)	2.97	(0.35)	3.17	(0.06)	3.50	(0.40)
25 (25/Oct)	3.70	(1.35)	3.47	(1.58)	4.00	(0.61)
(50/Oct&June)	3.50	(0.56)	3.47	(0.90)	4.10	(1.00)
(50/Oct&Aug)	3.73	(0.42)	3.67	(0.47)	3.93	(0.21)
33.3 (100/Oct,June&Aug)	3.50	(0.70)	3.63	(0.75)	3.63	(0.45)
50 (50/Oct)	3.83	(0.93)	3.73	(0.76)	4.07	(0.55)
(100/Oct&June)	3.53	(0.90)	3.57	(0.86)	5.20	(0.50)
(100/Oct&Aug)	3.53	(0.59)	3.57	(0.47)	4.80	(1.15)
100 (100/Oct)	2.40	(0.36)	2.67	(0.51)	4.70	(0.70)

[a] As a result of complete or split application as given in brackets.

Appendix Table II.9. Flower yield in September 1985 (data on which Fig. 7 is based).

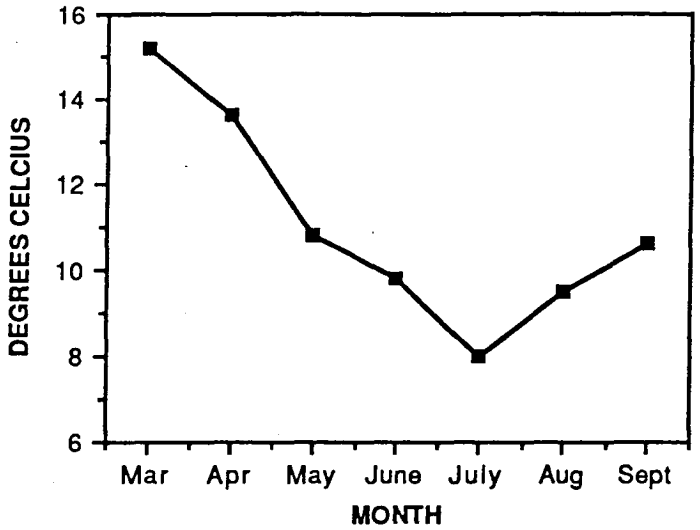
Source of N	Rate of N kg ha^{-1}	Flower yield, g fresh weight plant-1				
		Time of N application				
		Oct 84	Oct&June 84 85	Oct&Aug 84 85	Oct 84,June Aug 85	Mean
	0	6.3 (1.5)				
$(NH_4)_2SO_4$	25	34.7 (7.5)	16.3 (5.5)	14.7 (1.5)	16.0 (2.7)	20.4
	50	75.0 (8.7)	37.0 (8.5)	28.0 (4.6)	21.0 (3.6)	40.3
	100	83.0 (8.5)	66.0 (6.2)	56.7 (7.6)	43.0 (9.6)	62.2
Mean		49.8	31.4	26.4	21.6	
$Ca(NO_3)_2$	25	23.0 (1.7)	14.7 (3.1)	14.0 (4.0)	14.0 (4.6)	16.4
	50	71.0 (9.2)	27.3 (5.5)	22.3 (4.5)	21.0 (4.6)	35.4
	100	83.0 (6.6)	62.0 (5.0)	54.3 (11.6)	41.0 (6.6)	60.1
Mean		45.8	27.6	24.3	20.6	
IBDU	25	45.0 (7.2)	19.3 (3.1)	18.3 (2.5)	16.3 (4.0)	24.8
	50	88.3 (15.8)	51.7 (4.7)	50.3 (10.5)	41.0 (3.6)	57.8
	100	91.7 (19.0)	81.0 (11.1)	79.7 (10.0)	67.7 (6.0)	80.0
Mean		57.8	39.6	38.7	32.8	

Significance	LSD P = 0.05	0.01
Source	2.5	3.3
Rate	2.9	3.9
Time	2.9	3.9
Source X Rate	5.0	6.7
Source X Time	5.0	6.7
Rate X Time	5.8	7.7
Source X Rate X Time	ns	

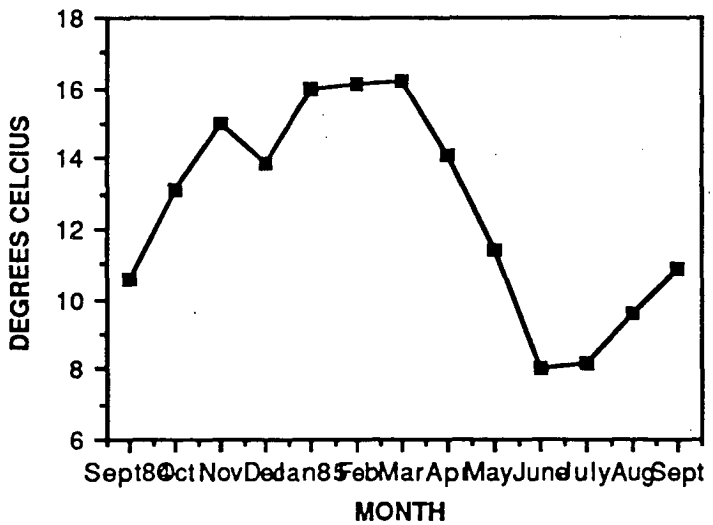
Appendix Table II.10. Flower yield in September 1986 (data on which Fig. 8 is based).

Source of N	Rate of N kg ha^{-1}	Flower yield, g fresh weight plant^{-1}				
		Time of N application				
		Oct 85	Oct&June 85 86	Oct&Aug 85 86	Oct 85,June Aug 86	Mean
	0	9.0 (1.0)				
$(NH_4)_2SO_4$	25	40.3 (8.0)	18.3 (6.1)	17.0 (2.7)	17.3 (4.5)	23.3
	50	89.0 (8.9)	44.7 (8.1)	30.3 (7.6)	25.0 (4.4)	47.3
	100	96.0 (13.5)	75.0 (2.0)	66.7 (11.9)	51.0 (9.9)	72.2
Mean		58.6	36.8	30.8	25.6	
$Ca(NO_3)_2$	25	27.3 (4.0)	16.0 (3.0)	16.7 (5.1)	15.3 (5.8)	18.8
	50	85.0 (10.2)	31.0 (5.3)	26.3 (5.5)	25.0 (4.6)	41.8
	100	97.7 (4.5)	73.7 (5.5)	62.3 (13.1)	49.3 (8.1)	70.8
Mean		54.8	32.4	28.6	24.7	
IBDU	25	52.7 (8.6)	22.7 (2.1)	21.0 (2.0)	20.3 (6.5)	29.2
	50	105.7 (19.9)	61.0 (6.2)	61.3 (16.0)	49.7 (3.8)	69.4
	100	110.0 (24.1)	94.0 (12.0)	96.3 (11.0)	88.3 (20.1)	97.2
Mean		69.3	46.7	46.9	41.8	

Significance	LSD P = 0.05	0.01
Source	3.3	4.3
Rate	3.8	5.0
Time	3.8	5.0
Source X Rate	6.5	8.6
Source X Time	6.5	8.6
Rate X Time	7.5	10.0
Source X Rate X Time	ns	



Appendix Fig. III.1. Mean daily temperature during 1984.



Appendix Fig. III.2. Mean daily temperature during 1984-85.

Appendix Table III.1. Stem diameter in 1984 experiment.

N Form	Level, mM	Stem diameter, cm Apr	May	June	July	Aug	Sept
	0	0	0	0	0	0	0
$NH_4^+ + NO_3^-$	5	0	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)
	10	0	0.03 (0.01)	0.03 (0.01)	0.03 (0.01)	0.03 (0.01)	0.03 (0.01)
	15	0	0.03 (0.01)	0.03 (0.01)	0.03 (0.01)	0.03 (0.01)	0.04 (0.01)
	20	0.02 (0.10)	0.04 (0)	0.04 (0.02)	0.05 (0.01)	0.05 (0.01)	0.06 (0.01)
	25	0.03 (0.01)	0.04 (0)	0.05 (0.02)	0.05 (0)	0.05 (0.01)	0.08 (0.02)
NH_4^+	5	0	0	0	0	0	0.02 (0.01)
	10	0	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)	0.03 (0.01)
	15	0	0.03 (0.01)	0.03 (0.01)	0.03 (0.01)	0.03 (0.01)	0.04 (0.01)
	20	0.02 (0.01)	0.04 (0.01)	0.04 (0.01)	0.04 (0.02)	0.05 (0.01)	0.05 (0.01)
	25	0.02 (0.01)	0.04 (0.01)	0.05 (0)	0.05 (0)	0.05 (0.01)	0.07 (0.02)
NO_3^-	5	0	0	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)
	10	0	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)	0.03 (0.01)
	15	0	0.03 (0.01)	0.03 (0.01)	0.03 (0.01)	0.03 (0.01)	0.04 (0)
	20	0.02 (0.01)	0.04 (0.01)	0.04 (0.01)	0.04 (0)	0.04 (0.01)	0.04 (0)
	25	0.02 (0.01)	0.04 (0.01)	0.04 (0.01)	0.04 (0.01)	0.04 (0.01)	0.06 (0)

Effect of form, level and their interaction in each month are not significant at P = 0.05.

Appendix Table III.2. Number of nodes on main shoot in 1984 experiment.

N Form	Level, mM	No. of nodes Apr	May	June	July	Aug	Sept
	0	1.0 (0.8)	1.8 (1.3)	1.8 (1.3)	1.8 (1.3)	1.8 (1.3)	1.8 (1.3)
$NH_4^+ + NO_3^-$	5	1.8 (0.5)	3.5 (0.6)	4.0 (0.8)	4.0 (0.8)	4.0 (0.8)	4.5 (1.3)
	10	2.8 (0.5)	5.0 (0.8)	6.0 (1.8)	6.0 (0.8)	6.0 (0.8)	7.3 (1.0)
	15	2.5 (0.6)	5.3 (0.5)	6.5 (0.6)	6.8 (1.0)	7.8 (1.5)	7.8 (1.7)
	20	3.3 (1.3)	5.8 (1.0)	7.8 (1.0)	9.5 (1.3)	9.5 (1.3)	10.5 (1.3)
	25	3.5 (1.3)	5.5 (1.3)	8.5 (1.7)	9.3 (1.7)	11.0 (2.2)	13.0 (2.2)
NH_4^+	5	1.5 (0.6)	2.8 (1.0)	3.3 (1.5)	3.3 (1.5)	3.3 (1.5)	4.0 (1.8)
	10	2.5 (0.6)	4.5 (1.3)	5.3 (1.7)	5.3 (1.7)	5.3 (1.7)	5.3 (1.7)
	15	2.0 (0.8)	4.3 (1.0)	5.3 (1.7)	5.3 (1.7)	5.5 (2.1)	6.5 (2.9)
	20	3.0 (1.4)	5.0 (1.6)	7.3 (2.1)	8.0 (2.9)	8.0 (1.7)	8.3 (1.7)
	25	3.5 (1.3)	5.8 (1.0)	7.8 (1.0)	9.3 (1.5)	10.0 (1.7)	11.5 (2.1)
NO_3^-	5	0.8 (0.5)	1.5 (0.6)	2.0 (1.2)	2.0 (1.2)	2.0 (1.2)	2.3 (1.5)
	10	2.0 (0.8)	3.5 (1.3)	4.0 (1.8)	4.0 (1.8)	4.0 (1.8)	4.0 (1.8)
	15	2.0 (0.8)	3.5 (1.3)	4.0 (1.8)	4.3 (2.2)	4.3 (2.2)	4.0 (1.8)
	20	2.5 (0.6)	4.5 (0.6)	6.5 (0.6)	7.8 (1.0)	8.5 (1.3)	10.3 (1.5)
	25	3.5 (1.3)	5.5 (1.3)	7.5 (1.3)	8.8 (1.5)	10.3 (1.7)	11.3 (1.7)

Significance	P	LSD					
Form	0.05	ns	0.4	0.4	0.5	0.3	0.5
	0.01		0.5	0.6	0.7	0.4	0.7
Level	0.01	0.6	0.5	0.6	0.7	0.5	0.7
	0.01	0.8	0.7	0.8	1.0	0.6	0.9
FormXLevel	0.05	ns	ns	ns	ns	0.8	1.2
	0.01					1.1	1.6

Appendix Table III.3. Number of lateral shoots as in 1984 experiment.

N Form	Level, mM	No. of lateral shoots Apr	May	June	July	Aug	Sept
$NH_4^+ + NO_3^-$	0	0	0	0	0	0	0
	5	0	0	0	0	0	0
	10	0	0	0	0	0	0
	15	1.8 (0.5)	1.8 (0.5)	1.8 (0.5)	1.8 (0.5)	1.8 (0.5)	1.8 (0.5)
	20	2.0 (0)	2.3 (1.3)	2.8 (1.5)	2.8 (1.5)	2.8 (1.5)	2.8 (1.5)
	25	2.2 (0.5)	3.3 (1.0)	3.5 (1.3)	3.5 (1.3)	3.5 (1.3)	3.5 (1.3)
NH_4^+	5	0	0	0	0	0	0
	10	0	0	0	0	0	0
	15	1.8 (0.5)	1.8 (0.5)	1.8 (0.5)	1.8 (0.5)	1.8 (0.5)	1.8 (0.5)
	20	2.0 (0)	2.8 (1.0)	3.3 (1.0)	3.3 (1.0)	3.3 (1.0)	3.3 (1.0)
	25	2.0 (0)	3.3 (1.7)	3.3 (1.7)	3.3 (1.7)	3.3 (1.7)	3.3 (1.7)
NO_3^-	5	0	0	0	0	0	0
	10	0	0	0	0	0	0
	15	1.5 (1.0)	1.5 (1.0)	1.5 (1.0)	1.5 (1.0)	1.5 (1.0)	1.5 (1.0)
	20	2.0 (0)	3.0 (0.8)	3.0 (0.8)	3.0 (0.8)	3.0 (0.8)	3.0 (0.8)
	25	2.0 (0.8)	2.5 (1.3)	2.5 (1.3)	2.5 (1.3)	2.5 (1.3)	2.5 (1.3)
Significance	P			LSD			
Level	0.05	0.3	0.6	0.6	0.6	0.5	0.5
	0.01	0.4	0.7	0.8	0.8	0.7	0.7

Effects of form and form X level interaction in each month were not significant at P = 0.05.

Appendix Table III.4. Number of nodes on lateral shoots in 1984 experiment.

N Form	Level, mM	No. of lateral shoots Apr.	May	June	July	Aug.	Sept.
$NH_4^+ + NO_3^-$	0	0	0	0	0	0	0
	5	0	0	0	0	0	0
	10	0	0	0	0	0	0
	15	1.8 (0.5)	3.5 (1.3)	3.8 (1.7)	3.8 (1.7)	3.8 (1.7)	4.8 (1.5)
	20	2.8 (1.0)	3.3 (1.7)	3.3 (1.7)	4.3 (1.7)	4.5 (1.3)	7.8 (3.1)
	25	3.5 (1.9)	4.3 (1.7)	4.3 (1.7)	5.8 (1.7)	7.5 (1.9)	12.0 (3.9)
NH_4^+	5	0	0	0	0	0	0
	10	0	0	0	0	0	0
	15	1.8 (0.5)	3.0 (2.2)	4.0 (1.4)	4.3 (1.3)	4.3 (1.3)	5.3 (2.2)
	20	2.8 (1.0)	3.5 (1.3)	3.5 (1.3)	4.3 (1.7)	5.5 (1.3)	6.5 (1.3)
	25	3.3 (9.5)	4.3 (1.7)	4.3 (1.7)	5.3 (2.2)	6.8 (2.8)	6.8 (2.5)
NO_3^-	5	0	0	0	0	0	0
	10	0	0	0	0	0	0
	15	1.5 (1.0)	3.0 (2.0)	3.0 (2.0)	3.0 (2.0)	3.0 (2.0)	3.8 (2.6)
	20	2.5 (1.0)	3.8 (1.3)	3.8 (1.3)	4.3 (1.7)	5.8 (2.6)	7.0 (2.9)
	25	2.5 (0.6)	3.0 (1.8)	4.5 (2.1)	5.3 (2.2)	5.5 (1.3)	6.5 (2.1)
Significance	P				LSD		
Form	0.05	ns	ns	ns	ns	ns	0.8
	0.01						ns
Level	0.05	0.6	0.8	0.8	0.8	0.9	1.2
	0.01	0.8	1.0	1.0	1.1	1.2	1.6
FormXLevel	0.05	ns	ns	ns	ns	ns	2.1
	0.01						2.8

Appendix Table III.5. Number of axils with flower buds in 1984 experiment.

N Form	Level, mM	No. of axils with flower buds June	July
$NH_4^+ + NO_3^-$	0	2.5 (1.0)	2.5 (1.0)
	5	6.0 (1.6)	6.5 (1.9)
	10	10.0 (1.6)	11.5 (1.9)
	15	10.5 (3.4)	10.5 (3.4)
	20	12.5 (3.4)	12.5 (5.3)
	25	11.0 (2.6)	16.0 (4.9)
NH_4^+	5	5.0 (2.6)	5.5 (3.0)
	10	8.5 (3.4)	8.5 (3.4)
	15	9.0 (4.2)	9.5 (3.4)
	20	10.0 (1.6)	12.5 (2.5)
	25	10.5 (3.4)	10.5 (5.5)
NO_3^-	5	3.0 (2.0)	3.0 (2.0)
	10	5.5 (3.0)	7.0 (3.5)
	15	6.5 (4.4)	7.5 (6.2)
	20	11.0 (1.2)	11.0 (2.6)
	25	10.0 (1.6)	11.0 (2.6)

Significance

	P	LSD	
Form	0.05	1.5	1.9
	0.01	ns	ns
Level	0.05	2.1	2.7
	0.01	2.8	3.6
FormXLevel	0.05	ns	ns

Appendix Table III.6. Total number of flower buds in 1984 experiment.

N Form	Level, mM	No. of flower buds June	July
$NH_4^+ + NO_3^-$	0	2.5 (1.0)	2.5 (1.0)
	5	6.5 (1.9)	8.2 (2.4)
	10	11.0 (1.2)	14.3 (1.7)
	15	11.8 (5.1)	18.5 (6.0)
	20	12.8 (4.6)	15.8 (4.4)
	25	13.8 (4.8)	17.8 (5.1)
NH_4^+	5	5.0 (2.6)	6.5 (3.1)
	10	8.5 (3.4)	11.3 (3.6)
	15	11.3 (4.3)	14.5 (5.5)
	20	11.8 (3.5)	15.8 (3.5)
	25	11.5 (4.4)	15.0 (6.7)
NO_3^-	5	3.0 (2.0)	4.5 (3.4)
	10	6.0 (3.6)	9.5 (4.5)
	15	7.5 (4.8)	11.8 (5.4)
	20	10.5 (1.3)	15.3 (2.5)
	25	11.0 (2.6)	12.3 (3.3)

Significance

	P	LSD	
Form	0.05	1.4	2.0
	0.01	1.8	2.7
Level	0.05	1.9	2.8
	0.01	2.6	3.8
FormXLevel	0.05	ns	ns

Appendix Table III.7. Number of developed flowers N in 1984 experiment.

N Form	Level, mM	No. of flowers
$NH_4^+ + NO_3^-$	0	2.5 (0.6)
	5	8.0 (2.2)
	10	13.8 (1.7)
	15	15.8 (5.0)
	20	12.0 (2.9)
	25	11.8 (2.8)
NH_4^+	5	6.3 (3.3)
	10	9.8 (3.4)
	15	13.5 (4.9)
	20	12.0 (3.7)
	25	11.8 (2.6)
NO_3^-	5	4.3 (3.1)
	10	9.0 (2.9)
	15	13.8 (6.9)
	20	9.3 (3.6)
	25	10.0 (2.2)

Significance

	P	LSD
Form	0.05	1.4
	0.01	2.0
Level	0.05	2.2
	0.01	2.9
FormXLevel	0.05	ns

Appendix Table III.8. Stem diameter in 1984-85 experiment (data on which Fig. 18 is based).

N Form	Level, mM	Stem diameter, cm											
		Oct 84	Nov	Dec	Jan 85	Feb	Mar	Apr	May	June	July	Aug	Sept
	0	0.03	0.07	0.11	0.15	0.19	0.22	0.22	0.22	0.22	0.22	0.22	0.22
		(0.01)	(0)	(0.01)	(0.01)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
$NH_4^+ + NO_3^-$	5	0.04	0.08	0.12	0.17	0.21	0.26	0.27	0.28	0.28	0.28	0.29	0.29
		(0.01)	(0.02)	(0.02)	(0.03)	(0.02)	(0.03)	(0.02)	(0.02)	(0.02)	(0.02)	(0)	(0.02)
	10	0.04	0.09	0.14	1.20	0.25	0.30	0.34	0.35	0.36	0.36	0.36	0.36
		(0.01)	(0.01)	(0.01)	(0.02)	(0.02)	(0.02)	(0.02)	(0.04)	(0.03)	(0.03)	(0.04)	(0.03)
	15	0.06	0.13	0.19	0.25	0.31	0.36	0.41	0.41	0.41	0.43	0.46	0.48
		(0.01)	(0)	(0)	(0.03)	(0.01)	(0.04)	(0.02)	(0)	(0.04)	(0.03)	(0.03)	(0.02)
	20	0.06	0.14	0.22	0.28	0.36	0.42	0.43	0.49	0.49	0.51	0.51	0.51
		(0.01)	(0.01)	(0.02)	(0.04)	(0.02)	(0.02)	(0.03)	(0.03)	(0.05)	(0.02)	(0.02)	(0.06)
	25	0.07	0.16	0.24	0.28	0.37	0.49	0.49	0.54	0.54	0.54	0.61	0.61
		(0.02)	(0.02)	(0.02)	(0.03)	(0.03)	(0.02)	(0.03)	(0.04)	(0.04)	(0.04)	(0.02)	(0.02)
NH_4^+	5	0.03	0.06	0.12	0.15	0.19	0.23	0.23	0.23	0.25	0.25	0.25	0.25
		(0.01)	(0)	(0.01)	(0.02)	(0)	(0.02)	(0.12)	(0.03)	(0.03)	(0.04)	(0.04)	(0.03)
	10	0.04	0.09	0.14	0.19	0.23	0.27	0.30	0.30	0.30	0.31	0.30	0.30
		(0.01)	(0)	(0)	(0)	(0.02)	(0.03)	(0.02)	(0)	(0.02)	(0.02)	(0.02)	(0.02)
	15	0.05	0.12	0.16	0.20	0.27	0.32	0.33	0.35	0.37	0.38	0.39	0.39
		(0)	(0.05)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.04)	(0.02)	(0.03)	(0.03)	(0.02)
	20	0.07	0.11	0.20	0.26	0.32	0.39	0.41	0.47	0.47	0.47	0.47	0.48
		(0)	(0.02)	(0)	(0)	(0.03)	(0.02)	(0.02)	(0.03)	(0.05)	(0.04)	(0.03)	(0.03)
	25	0.06	0.13	0.20	0.29	0.36	0.43	0.46	0.47	0.47	0.49	0.52	0.54
		(0.02)	(0.02)	(0)	(0)	(0.03)	(0.02)	(0.03)	(0.03)	(0.04)	(0.04)	(0.05)	(0.05)
NO_3^-	5	0.03	0.05	0.12	0.15	0.19	0.22	0.22	0.23	0.23	0.23	0.23	0.24
		(0.01)	(0)	(0)	(0.01)	(0)	(0.02)	(0.03)	(0.03)	(0)	(0.02)	(0.03)	(0)
	10	0.04	0.09	0.14	0.20	0.23	0.29	0.28	0.30	0.31	0.31	0.31	0.31
		(0.01)	(0.02)	(0)	(0.02)	(0.02)	(0.03)	(0.03)	(0.02)	(0)	(0.02)	(0.02)	(0)
	15	0.04	0.09	0.14	0.20	0.23	0.29	0.32	0.32	0.33	0.33	0.33	0.35
		(0.01)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.03)	(0.02)	(0.02)	(0.03)	(0.03)	(0.04)
	20	0.06	0.11	0.18	0.22	0.30	0.40	0.40	0.40	0.40	0.40	0.42	0.42
		(0)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.03)	(0)	(0.02)	(0.03)	(0.04)	(0.02)
	25	0.06	0.12	0.20	0.28	0.31	0.40	0.40	0.47	0.47	0.49	0.49	0.49
		(0)	(0.02)	(0)	(0.02)	(0.02)	(0.02)	(0.04)	(0.02)	(0.03)	(0.03)	(0.02)	(0.02)
Significance													
										LSD			
Form	0.05	ns	ns	ns	ns	ns	ns	ns	ns	0.02	0.02	0.02	0.01
	0.01									0.02	0.02	0.02	0.01
Level	0.05	ns	ns	ns	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
	0.01				0.02	0.02	0.02	0.03	0.02	0.03	0.03	0.03	0.03
FormXLevel	0.05	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Appendix Table III.9. Number of nodes on main shoot in 1984-85 experiment (data on which Fig. 19 is based).

N Form	Level, mM	No. of nodes on main shoot: Oct 84	Nov	Dec	Jan 85	Feb	Mar	Apr	May	June	July	Aug	Sept
	0	2.8	3.8	5.0	6.0	7.0	8.0	8.5	8.5	8.5	8.5	8.5	8.5
		(0.5)	(1.0)	(0.8)	(0.8)	(0.8)	(0.8)	(1.3)	(1.3)	(1.3)	(1.3)	(1.3)	(1.3)
$NH_4^+ + NO_3^-$	5	2.8	4.0	5.8	6.5	7.8	8.5	8.5	8.8	8.8	8.8	8.8	9.0
		(0.5)	(0.8)	(1.0)	(1.3)	(1.7)	(1.3)	(1.3)	(1.7)	(1.7)	(1.7)	(1.7)	(2.2)
	10	3.5	5.0	7.0	8.5	10.0	11.5	11.8	12.5	12.8	13.5	13.5	14.0
		(0.6)	(0.8)	(0.8)	(0.6)	(0.8)	(0.6)	(1.0)	(1.3)	(1.7)	(2.4)	(2.4)	(2.9)
	15	4.5	7.3	9.5	11.5	13.5	15.8	17.5	18.8	19.8	20.5	20.8	22.3
		(0.6)	(0.5)	(0.6)	(0.6)	(0.6)	(1.0)	(1.3)	(1.7)	(1.7)	(1.3)	(1.7)	(2.8)
	20	5.0	8.5	11.5	14.0	16.3	18.5	20.5	22.5	24.5	26.5	27.5	29.0
		(0.8)	(0.6)	(0.6)	(0.8)	(1.0)	(1.3)	(1.3)	(1.3)	(1.3)	(1.3)	(1.3)	(1.4)
	25	5.5	9.5	13.0	15.5	18.5	20.8	22.8	25.5	27.8	29.8	31.8	33.8
		(0.6)	(0.6)	(0.8)	(0.6)	(0.6)	(1.0)	(1.0)	(1.3)	(1.7)	(1.7)	(1.7)	(2.2)
NH_4^+	5	2.8	3.5	5.5	6.5	7.8	8.5	8.8	9.0	9.0	9.0	9.0	9.5
		(0.5)	(1.7)	(0.6)	(0.6)	(1.0)	(1.3)	(1.7)	(2.2)	(2.2)	(2.2)	(2.2)	(2.1)
	10	3.5	5.0	6.5	7.5	8.8	10.3	10.5	10.8	11.3	11.3	11.3	11.8
		(0.6)	(0.8)	(1.3)	(1.3)	(1.0)	(1.0)	(1.3)	(1.7)	(2.2)	(2.2)	(2.2)	(2.8)
	15	4.3	6.5	8.0	9.5	11.5	13.0	14.3	15.3	15.3	16.5	19.0	19.8
		(0.5)	(0.6)	(0.8)	(0.6)	(0.6)	(0.8)	(1.7)	(2.5)	(3.2)	(2.7)	(4.7)	(4.8)
	20	4.8	7.5	9.5	11.5	13.5	15.5	17.3	18.5	20.3	21.5	25.3	26.3
		(0.5)	(0.6)	(0.6)	(0.6)	(0.6)	(0.6)	(1.0)	(1.3)	(1.7)	(1.3)	(4.6)	(4.6)
	25	5.5	9.5	12.5	14.5	17.0	19.5	21.8	23.5	25.8	27.5	32.3	34.5
		(0.6)	(0.6)	(0.6)	(0.6)	(0.8)	(1.3)	(1.0)	(1.3)	(1.7)	(1.3)	(7.3)	(7.1)
NO_3^-	5	3.0	3.5	4.8	5.8	7.0	8.0	8.3	8.3	8.3	8.3	8.8	9.0
		(0)	(1.7)	(1.3)	(1.3)	(0.8)	(0.8)	(1.0)	(1.0)	(1.0)	(1.0)	(1.7)	(2.2)
	10	3.5	5.0	5.8	6.8	8.5	9.5	10.5	10.5	11.5	11.5	11.5	12.0
		(0.6)	(0.8)	(1.0)	(1.4)	(1.3)	(1.3)	(1.3)	(1.9)	(2.4)	(2.4)	(2.4)	(2.9)
	15	3.5	6.3	7.8	9.0	10.3	11.8	13.0	14.3	14.8	15.3	15.8	16.5
		(0.6)	(1.3)	(1.5)	(1.4)	(1.3)	(1.7)	(1.8)	(2.5)	(2.8)	(3.3)	(2.8)	(3.1)
	20	4.5	7.5	9.5	11.0	13.0	14.5	16.5	17.8	19.3	19.8	21.0	22.5
		(0.6)	(0.6)	(0.6)	(0.8)	(0.8)	(1.3)	(1.3)	(1.0)	(1.7)	(1.7)	(1.4)	(2.1)
	25	5.5	9.0	11.0	13.5	15.0	17.3	19.3	21.8	23.8	24.5	25.5	26.3
		(0.6)	(0.8)	(0.8)	(1.3)	(0.8)	(1.0)	(1.3)	(1.7)	(1.5)	(1.7)	(1.3)	(1.7)

Significance	P	LSD											
Form	0.05	ns	0.4	0.4	0.5	0.5	0.4	0.6	0.7	0.8	0.6	1.5	1.8
	0.01		ns	0.5	0.6	0.7	0.6	0.8	0.9	1.0	0.8	2.0	2.4
Level	0.05	0.3	0.6	0.5	0.6	0.7	0.6	0.8	0.9	1.1	0.8	2.1	2.5
	0.01	0.4	0.8	0.7	0.9	0.9	0.8	1.1	1.2	1.5	1.1	2.8	3.4
FormXLevel	0.05	ns	ns	ns	1.1	1.2	1.0	1.4	1.6	1.9	1.4	ns	ns
	0.01				ns	1.6	1.4	1.9	2.1	2.5	1.9		

Appendix Table III.10. Number of lateral shoots in 1984-85experiment (dataon which Fig. 20 is based).

N Form	Level, mM	No. oflateral shoots Oct.84	Nov.	Dec.	Jan.85	Feb.	Mar.	Apr	May	June	July	Aug	Sept
	0	1.3	3.0	4.0	5.0	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
		(1.0)	(0.8)	(0)	(0.8)	(1.3)	(1.3)	(1.3)	(1.3)	(1.3)	(1.3)	(1.3)	(1.3)
$NH_4^+ + NO_3^-$	5	1.8	3.5	5.5	7.5	8.8	10.5	10.8	11.0	11.0	11.0	11.0	11.0
		(0.5)	(1.3)	(1.3)	(1.3)	(1.0)	(1.3)	(1.7)	(2.2)	(2.2)	(2.2)	(2.2)	(2.2)
	10	3.0	5.8	8.0	10.5	13.0	15.0	16.0	16.8	16.8	16.8	16.8	16.8
		(0.8)	(1.7)	(0.8)	(1.3)	(0.8)	(0.8)	(0.8)	(1.7)	(1.7)	(1.7)	(1.7)	(1.7)
	15	4.5	9.3	13.0	17.0	20.0	23.5	26.5	26.8	26.8	27.3	27.3	27.3
		(1.3)	(1.3)	(0.8)	(0.8)	(0.8)	(0.6)	(1.3)	(1.7)	(1.7)	(1.7)	(1.7)	(1.7)
	20	5.5	11.5	17.0	21.8	25.8	29.8	33.0	34.3	34.3	34.3	34.3	34.3
		(1.3)	(0.6)	(0.8)	(1.0)	(1.0)	(1.0)	(0.8)	(1.3)	(1.3)	(1.3)	(1.3)	(1.3)
	25	6.8	14.5	20.5	26.0	31.5	35.5	40.0	41.8	42.0	42.0	42.0	42.0
		(1.0)	(1.3)	(1.3)	(1.2)	(1.3)	(1.3)	(0.8)	(1.0)	(0.8)	(0.8)	(0.8)	(0.8)
NH_4^+	5	1.8	3.0	5.0	6.5	8.0	8.5	8.5	8.5	8.5	8.5	8.5	8.5
		(0.5)	(0.8)	(0.8)	(0.6)	(0.8)	(1.3)	(1.3)	(1.3)	(1.3)	(1.3)	(1.3)	(1.3)
	10	3.0	5.0	7.8	9.5	11.0	13.0	13.5	13.8	13.8	13.8	13.8	13.8
		(0.8)	(0.8)	(1.0)	(0.6)	(0.8)	(0.8)	(1.3)	(1.7)	(1.7)	(1.7)	(1.7)	(1.7)
	15	3.8	8.0	10.3	13.0	15.5	18.5	20.0	20.5	20.8	21.3	21.3	21.3
		(1.0)	(0.8)	(1.0)	(0.8)	(0.6)	(1.3)	(1.4)	(2.7)	(2.8)	(2.5)	(2.7)	(2.6)
	20	4.5	10.0	13.5	16.8	19.8	23.5	26.0	26.8	26.8	26.8	26.8	26.8
		(1.3)	(0.8)	(1.3)	(1.0)	(1.0)	(1.3)	(1.8)	(2.5)	(2.5)	(2.5)	(2.5)	(2.5)
	25	6.5	13.5	18.8	23.0	27.0	30.5	33.5	35.3	35.3	35.3	35.3	35.3
		(1.3)	(0.6)	(1.0)	(0.8)	(0.8)	(1.3)	(1.0)	(1.3)	(1.3)	(1.3)	(1.4)	(1.3)
NO_3^-	5	1.8	3.3	5.0	6.0	7.5	8.0	8.3	8.3	8.3	8.3	8.3	8.3
		(0.5)	(1.0)	(0.8)	(0.8)	(1.3)	(0.8)	(1.0)	(1.0)	(1.0)	(1.0)	(0.8)	(0.8)
	10	2.0	4.0	6.5	8.0	9.5	11.0	11.5	11.8	11.8	11.8	11.8	11.8
		(0.8)	(0.8)	(0.6)	(0.8)	(0.6)	(0.8)	(1.3)	(1.7)	(1.7)	(1.7)	(1.7)	(1.7)
	15	3.0	7.0	9.0	11.0	13.0	15.0	16.3	17.0	17.0	17.0	17.0	17.0
		(0.8)	(0.8)	(0.8)	(0.8)	(0.8)	(1.4)	(2.2)	(2.6)	(2.6)	(2.8)	(2.8)	(2.6)
	20	4.3	9.5	12.5	15.5	18.3	20.5	22.5	23.5	23.5	23.5	23.5	23.5
		(1.0)	(0.6)	(0.6)	(0.6)	(0.5)	(0.6)	(0.6)	(1.3)	(1.3)	(1.3)	(1.3)	(1.3)
	25	6.5	13.0	17.8	21.0	24.8	27.8	30.3	32.0	32.0	32.0	32.0	32.0
		(1.3)	(0.8)	(1.0)	(0.8)	(1.0)	(1.0)	(1.5)	(1.8)	(1.8)	(1.8)	(1.8)	(1.8)
Significance	P						LSD						
Form	0.05	0.4	0.4	0.4	0.4	0.5	0.6	0.6	0.8	0.7	0.8	0.9	0.8
	0.01	0.6	0.6	0.5	0.6	0.6	0.8	0.8	1.0	0.9	1.0	1.2	1.1
Level	0.05	0.6	0.6	0.5	0.6	0.6	0.8	0.9	1.1	1.0	1.1	1.2	1.1
	0.01	0.8	0.8	0.7	0.8	0.8	1.1	1.1	1.4	1.3	1.4	1.7	1.5
FormXLevel	0.05	ns	ns	0.9	1.0	1.1	1.4	1.5	1.9	1.6	1.9	2.1	1.9
	0.01			1.2	1.4	1.5	1.8	2.0	2.5	2.2	2.5	2.9	2.6

Appendix Table III.11. Number of nodes on lateral shoots in 1984-85 experiment (data on which Fig. 23 is based).

N Form	Level, mM	Oct 84	Nov	Dec	Jan 85	Feb	Mar	Apr	May	June	July	Aug	Sept
		No. ofnodes											
$NH_4^+ + NO_3^-$	0	1.5	4.0	6.8	9.8	12.8	14.8	14.8	15.8	15.8	15.8	15.8	16.3
		(0.6)	(1.5)	(1.0)	(1.7)	(3.0)	(3.0)	(2.6)	(3.3)	(2.6)	(3.0)	(3.0)	(3.1)
	5	2.0	5.0	10.8	17.0	22.8	30.5	35.0	35.5	36.5	38.8	38.8	38.8
		(0.8)	(2.6)	(4.3)	(5.3)	(4.9)	(6.2)	(8.8)	(9.7)	(9.0)	(7.3)	(9.0)	(9.3)
	10	4.0	11.5	21.0	31.3	45.0	63.8	71.0	77.8	79.3	79.3	79.8	79.8
		(1.8)	(5.5)	(4.2)	(7.2)	(7.0)	(6.2)	(5.7)	(6.5)	(7.0)	(4.7)	(6.3)	(7.6)
	15	7.3	24.8	45.5	71.8	98.8	135.8	149.3	158.0	169.3	171.0	175.8	184.3
		(3.3)	(5.1)	(6.6)	(9.0)	(12.6)	(7.9)	(8.2)	(11.5)	(9.2)	(9.4)	(7.5)	(9.6)
	20	9.0	35.3	72.3	106.8	146.3	187.5	221.3	238.8	259.5	265.3	276.0	291.0
		(3.4)	(3.3)	(7.9)	(8.7)	(11.0)	(10.7)	(12.5)	(14.5)	(14.0)	(8.3)	(8.6)	(12.0)
	25	13.8	56.0	95.3	144.3	191.3	220.5	253.3	268.8	295.0	303.5	319.8	330.8
		(3.5)	(8.8)	(12.4)	(11.2)	(23.0)	(18.5)	(20.4)	(21.6)	(20.3)	(18.1)	(22.2)	(23.0)
NH_4^+	5	1.8	4.5	9.0	13.0	19.0	23.8	27.0	27.5	27.5	29.3	29.3	29.8
		(0.5)	(1.9)	(2.5)	(2.2)	(3.7)	(5.4)	(5.3)	(5.8)	(5.3)	(3.3)	(5.7)	(3.9)
	10	2.8	9.3	18.8	25.5	35.0	46.5	53.5	61.0	61.0	61.0	63.0	64.5
		(1.0)	(2.8)	(4.1)	(2.4)	(4.6)	(5.8)	(8.8)	(5.9)	(6.0)	(5.5)	(5.0)	(5.7)
	15	5.3	17.5	29.3	45.3	59.5	82.0	106.3	124.3	130.5	142.5	144.8	144.8
		(2.2)	(3.4)	(4.1)	(5.6)	(3.7)	(13.9)	(18.7)	(9.7)	(12.4)	(5.8)	(9.2)	(14.7)
	20	7.3	24.3	47.8	73.0	109.3	138.5	157.3	184.0	198.5	209.5	222.0	230.8
		(3.3)	(3.6)	(9.0)	(8.4)	(11.4)	(13.5)	(15.3)	(16.4)	(16.2)	(13.9)	(10.1)	(11.4)
	25	12.8	42.5	77.3	114.5	151.0	186.0	217.3	229.5	250.3	270.0	281.3	291.3
		(4.0)	(5.7)	(9.2)	(8.7)	(9.9)	(15.6)	(13.2)	(11.5)	(18.6)	(13.3)	(13.2)	(15.2)
NO_3^-	5	1.5	4.5	9.0	11.5	16.8	27.5	30.8	31.5	31.5	31.5	31.5	32.0
		(0.6)	(1.9)	(2.5)	(2.7)	(4.7)	(2.4)	(4.1)	(4.2)	(4.6)	(4.2)	(4.2)	(4.7)
	10	2.8	7.0	11.8	16.3	24.5	33.3	39.5	42.5	44.3	45.0	45.0	45.3
		(9.0)	(2.5)	(1.7)	(3.6)	(2.4)	(4.7)	(6.1)	(7.9)	(6.3)	(6.2)	(6.4)	(8.2)
	15	3.8	13.3	23.0	35.0	45.8	55.8	67.0	73.8	83.5	87.0	89.3	94.0
		(1.7)	(3.3)	(4.2)	(5.6)	(5.4)	(9.5)	(7.5)	(7.6)	(8.4)	(9.1)	(8.5)	(5.9)
	20	6.3	23.8	41.0	58.0	82.3	93.0	109.8	124.8	140.8	149.8	150.5	158.0
		(2.6)	(2.8)	(2.9)	(3.4)	(4.0)	(6.5)	(8.4)	(10.1)	(7.0)	(6.2)	(11.2)	(9.9)
	25	11.8	45.5	73.5	97.8	131.5	146.8	183.3	200.0	217.8	229.3	229.3	246.5
		(3.3)	(6.4)	(8.2)	(8.7)	(8.5)	(15.5)	(11.5)	(12.8)	(11.0)	(10.3)	(55.1)	(16.3)
Significance	P	LSD											
Form	0.05	1.3	2.2	2.3	3.5	5.0	5.4	5.5	4.9	6.0	4.5	8.8	5.5
	0.01	ns	2.9	3.1	4.7	6.6	7.2	7.3	6.6	8.0	6.0	11.7	7.3
Level	0.05	1.8	3.1	3.3	5.0	7.0	7.7	7.8	7.0	8.5	6.4	12.4	7.8
	0.01	2.4	4.1	4.4	6.7	9.4	10.2	10.4	9.3	11.3	8.5	16.6	10.4
FormXLevel	0.05	ns	5.3	5.7	8.7	12.0	13.2	13.5	12.0	14.7	11.0	21.6	13.5
	0.01		7.1	7.6	11.5	16.2	17.7	17.9	16.0	19.6	14.7	28.7	18.0

Appendix Table III.12. Number of axils with flower buds in 1984-85 experiment (Fig. 24a is based on part of these data).

N Form	Level, mM	No. of axils with flower buds: June 85	July
$NH_4^+ + NO_3^-$	0	23.8 (3.5)	24.5 (4.9)
	5	58.3 (14.1)	58.8 (13.7)
	10	123.0 (9.8)	123.8 (9.0)
	15	263.0 (17.9)	275.3 (18.8)
	20	416.3 (24.0)	429.0 (21.8)
	25	472.3 (36.6)	481.8 (24.8)
NH_4^+	5	47.8 (4.8)	47.8 (7.0)
	10	94.0 (12.3)	96.8 (7.5)
	15	203.3 (27.5)	211.3 (24.1)
	20	316.8 (26.6)	328.0 (13.8)
	25	401.8 (28.0)	424.8 (23.8)
NO_3^-	5	48.0 (7.8)	49.3 (7.1)
	10	68.3 (13.0)	68.3 (11.7)
	15	129.8 (14.9)	137.5 (13.3)
	20	219.8 (17.8)	230.5 (17.5)
	25	333.3 (19.6)	351.3 (23.8)

Significance	P	LSD	
Form	0.05	10.2	9.0
	0.01	13.6	11.9
Level	0.01	14.5	12.7
	0.01	19.3	16.9
FormXLevel	0.05	25.1	21.9
	0.01	33.4	29.2

Appendix Table III.13. Total number of flower buds in 1984-85 experiment (Fig. 25a is based on part of these data).

N Form	Level, mM	No. of flower buds: June 85	July
$NH_4^+ + NO_3^-$	0	23.3 (4.1)	29.0 (5.5)
	5	60.3 (14.7)	70.8 (13.8)
	10	139.3 (12.6)	165.3 (10.1)
	15	342.0 (21.3)	355.3 (9.6)
	20	423.8 (25.7)	453.0 (17.7)
	25	457.5 (22.5)	495.8 (15.1)
NH_4^+	5	48.0 (10.1)	60.0 (7.0)
	10	107.5 (12.1)	129.8 (8.1)
	15	271.0 (25.6)	298.8 (21.8)
	20	288.5 (18.7)	323.8 (13.0)
	25	402.0 (19.2)	434.5 (18.9)
NO_3^-	5	45.0 (2.9)	49.8 (3.3)
	10	77.3 (6.2)	89.0 (6.3)
	15	167.3 (9.1)	182.8 (11.5)
	20	237.0 (16.7)	268.0 (11.5)
	25	335.8 (20.1)	372.0 (11.1)

Significance	P	LSD	
Form	0.05	9.3	6.9
	0.01	12.3	9.2
Level	0.05	13.1	9.8
	0.01	17.5	13.0
FormXLevel	0.05	22.7	16.9
	0.01	30.2	22.6

Appendix Table III. 14. Number of developed flowers in 1984-85 experiment (data on which (Fig. 26a is based).

N Form	Level, mM	No. of flowers
$NH_4^+ + NO_3^-$	0	24.3 (5.3)
	5	55.0 (9.1)
	10	128.8 (6.9)
	15	272.0 (15.9)
	20	311.5 (24.8)
	25	292.3 (40.0)
NH_4^+	5	45.0 (8.8)
	10	96.0 (7.2)
	15	204.3 (21.2)
	20	231.0 (30.3)
	25	276.3 (15.7)
NO_3^-	5	39.3 (3.0)
	10	63.5 (7.2)
	15	139.3 (6.8)
	20	196.0 (11.8)
	25	254.3 (26.0)

Significance

	P	LSD
Form	0.05	10.0
	0.01	13.4
Level	0.05	14.2
	0.01	18.9
FormXLevel	0.05	25.6
	0.01	32.8

Appendix Table III.15. 10-flower weight in 1984-85 experiment (data on which Fig. 27 is based).

N Form	Level, mM	10-flower fresh wt, g
$NH_4^+ + NO_3^-$	0	0.42 (0.02)
	5	0.39 (0.02)
	10	0.38 (0)
	15	0.35 (0.01)
	20	0.33 (0.01)
	25	0.33 (0.01)
NH_4^+	5	0.40 (0)
	10	0.38 (0.01)
	15	0.35 (0)
	20	0.34 (0)
	25	0.33 (0)
NO_3^-	5	0.39 (0.01)
	10	0.38 (0)
	15	0.35 (0.01)
	20	0.33 (0)
	25	0.33 (0.01)

Significance

	P	LSD
Form	0.05	ns
Level	0.05	0.01
	0.01	0.02
FormXLevel	0.05	ns

Appendix Table III.16. Flower yield/plant in 1984-85 experiment.

N. Form	Level, mM	Flower yield, g fresh wt/plant
$NH_4^+ + NO_3^-$	0	13.3 (2.5)
	5	48.3 (7.0)
	10	113.5 (15.0)
	15	242.3 (17.6)
	20	291.3 (9.9)
	25	259.5 (26.6)
NH_4^+	5	45.0 (5.4)
	10	84.0 (12.1)
	15	185.5 (8.5)
	20	212.5 (8.1)
	25	253.5 (40.8)
NO_3^-	5	37.3 (4.5)
	10	58.8 (4.1)
	15	129.8 (10.8)
	20	192.0 (14.5)
	25	241.8 (16.4)

Significance	P	LSD
Form	0.05	8.8
	0.01	11.7
Level	0.05	12.4
	0.01	16.6
FormXLevel	0.05	21.5
	0.01	28.7

Appendix Table III.17. Leaf N concentration in 1984-85 experiment (data on which Fig. 28 is based).

N suppled Form	Level, mM	Leaf N, % dry wt. Nov.84	Feb.85	June
$NH_4^+ + NO_3^-$	0	1.65 (0.04)	1.79 (0.09)	1.58 (0.04)
	5	1.82 (0.06)	2.00 (0.07)	1.75 (0.06)
	10	2.00 (0.09)	2.22 (0.09)	1.89 (0.06)
	15	2.10 (0.15)	2.34 (0.17)	2.29 (0.12)
	20	2.43 (0.38)	2.87 (0.24)	2.84 (0.17)
	25	2.40 (0.38)	3.24 (0.19)	3.31 (0.12)
NH_4^+	5	1.82 (0.13)	1.99 (0.04)	1.72 (0.04)
	10	1.91 (0.16)	2.19 (0.09)	1.86 (0.09)
	15	2.05 (0.15)	2.28 (0.09)	2.26 (0.07)
	20	2.29 (0.31)	2.96 (0.12)	2.87 (0.13)
	25	2.40 (0.37)	3.06 (0.12)	3.12 (0.04)
NO_3^-	5	1.75 (0.06)	1.89 (0.08)	1.68 (0.06)
	10	1.80 (0.07)	2.08 (0.09)	1.84 (0.07)
	15	2.03 (0.14)	2.22 (0.09)	2.17 (0.06)
	20	2.29 (0.21)	2.92 (0.09)	2.63 (0.22)
	25	2.66 (0.30)	3.03 (0.13)	2.89 (0.12)

Significance	P	Nov.84	LSD Feb.85	June
Form	0.05	ns	0.07	0.06
	0.01		ns	0.08
Level	0.05	0.17	0.10	0.08
	0.01	0.23	0.13	0.11
Form X Level	0.05	ns	ns	0.14
	0.01			0.19

Appendix Table III.18. Leaf P concentration in 1984-85 experiment (Fig. 29a is based on part of these data).

N supplied		Leaf P, % dry wt.		
Form	Level, mM	Nov.84	Feb.85	June
$NH_4^+ + NO_3^-$	0	0.23 (0.02)	0.26 (0)	0.26 (0)
	5	0.19 (0)	0.22 (0)	0.22 (0.02)
	10	0.18 (0.02)	0.18 (0.01)	0.19 (0)
	15	0.16 (0.01)	0.17 (0)	0.17 (0)
	20	0.13 (0.01)	0.14 (0.01)	0.14 (0)
	25	0.11 (0.01)	0.12 (0)	0.12 (0.01)
NH_4^+	5	0.19 (0)	0.22 (0.01)	0.22 (0.02)
	10	0.18 (0.01)	0.19 (0)	0.19 (0)
	15	0.15 (0)	0.16 (0.01)	0.17 (0.01)
	20	0.13 (0.01)	0.14 (0)	0.14 (0)
	25	0.11 (0.01)	0.11 (0.01)	0.12 (0)
NO_3^-	5	0.19 (0.01)	0.21 (0)	0.21 (0.01)
	10	0.17 (0.01)	0.18 (0.01)	0.18 (0.01)
	15	0.15 (0.01)	0.16 (0.01)	0.16 (0)
	20	0.13 (0)	0.14 (0)	0.14 (0)
	25	0.11 (0.01)	0.11 (0.01)	0.11 (0.01)

Significance	P	LSD		
Form	0.05	ns	ns	ns
Level	0.05	0.01	0.01	0.01
	0.01	ns	ns	ns
Form X Level	0.05	ns	ns	ns

Appendix Table III.19. Leaf K concentration in 1984-85 experiment (Fig. 29b is based on part of these data).

N supplied		Leaf K, % dry wt.		
Form	Level, mM	Nov.84	Feb.85	June
$NH_4^+ + NO_3^-$	0	1.40 (0.14)	1.58 (0.10)	1.53 (0.10)
	5	1.13 (0.10)	1.27 (0.10)	1.28 (0.10)
	10	1.00 (0.08)	1.08 (0.10)	1.10 (0.12)
	15	0.95 (0.06)	1.00 (0.08)	1.03 (0.05)
	20	0.80 (0.08)	1.00 (0.14)	1.00 (0.08)
	25	0.80 (0.08)	0.88 (0.10)	0.90 (0.08)
NH_4^+	5	1.18 (0.05)	1.30 (0.08)	1.30 (0.08)
	10	1.00 (0.08)	1.15 (0.13)	1.13 (0.10)
	15	0.98 (0.10)	1.00 (0.08)	1.10 (0.12)
	20	0.83 (0.10)	1.02 (0.10)	1.03 (0.05)
	25	0.80 (0.08)	0.90 (0)	0.93 (0.05)
NO_3^-	5	1.20 (0.08)	1.33 (0.10)	1.30 (0.08)
	10	1.08 (0.10)	1.18 (0.13)	1.15 (0.13)
	15	0.98 (0.10)	1.05 (0.06)	1.10 (0.08)
	20	0.90 (0.08)	1.02 (0.10)	1.05 (0.06)
	25	0.90 (0.08)	0.93 (0.05)	0.98 (0.10)

Significance	P	LSD		
Form	0.05	ns	ns	ns
Level	0.05	0.08	0.08	0.06
	0.01	0.10	0.10	0.08
Form X Level	0.05	ns	ns	ns

Appendix Table III.20. Leaf Ca concentration in 1984-85 experiment (Fig. 29c is based on part of these data).

N supplied		Leaf Ca, % dry wt.		
Form	Level, mM	Nov.84	Feb.85	June
$NH_4^+ + NO_3^-$	0	0.67 (0.03)	0.72 (0.06)	0.74 (0.03)
	5	0.61 (0.02)	0.66 (0.02)	0.66 (0.03)
	10	0.59 (0.02)	0.62 (0.04)	0.61 (0)
	15	0.57 (0.01)	0.59 (0.03)	0.59 (0.02)
	20	0.57 (0)	0.59 (0.02)	0.59 (0)
	25	0.56 (0.01)	0.59 (0.01)	0.58 (0.03)
NH_4^+	5	0.62 (0.02)	0.68 (0.03)	0.70 (0)
	10	0.60 (0)	0.64 (0.04)	0.64 (0.02)
	15	0.58 (0.01)	0.60 (0.05)	0.60 (0.02)
	20	0.58 (0.02)	0.58 (0.02)	0.59 (0.04)
	25	0.56 (0)	0.59 (0.03)	0.59 (0.03)
NO_3^-	5	0.63 (0.03)	0.71 (0.04)	0.71 (0.03)
	10	0.62 (0.01)	0.66 (0)	0.65 (0.02)
	15	0.59 (0.01)	0.62 (0.03)	0.62 (0.02)
	20	0.59 (0)	0.60 (0)	0.59 (0.01)
	25	0.57 (0)	0.61 (0.03)	0.59 (0.02)

Significance	P	LSD		
Form	0.05	ns	ns	ns
Level	0.05	0.03	0.02	0.02
	0.01	0.04	0.02	0.03
Form X Level	0.05	ns	ns	ns

Appendix Table III.21. Leaf Mg concentration in 1984-85 experiment (Fig. 29d is based on part of these data).

N supplied		Leaf Mg, % dry wt.		
Form	Level, mM	Nov.84	Feb.85	June
$NH_4^+ + NO_3^-$	0	0.38 (0.02)	0.37 (0.03)	0.38 (0)
	5	0.30 (0.01)	0.29 (0.02)	0.30 (0.01)
	10	0.27 (0)	0.25 (0.02)	0.27 (0)
	15	0.23 (0)	0.23 (0)	0.22 (0.01)
	20	0.22 (0.02)	0.21 (0.02)	0.21 (0.01)
	25	0.19 (0.01)	0.19 (0.02)	0.20 (0)
NH_4^+	5	0.31 (0.01)	0.31 (0)	0.31 (0)
	10	0.28 (0.02)	0.27 (0)	0.29 (0)
	15	0.24 (0.01)	0.23 (0.02)	0.23 (0)
	20	0.22 (0.01)	0.20 (0.01)	0.20 (0)
	25	0.20 (0)	0.20 (0)	0.20 (0.01)
NO_3^-	5	0.32 (0.01)	0.30 (0)	0.31 (0.01)
	10	0.28 (0.01)	0.28 (0)	0.29 (0.01)
	15	0.25 (0.02)	0.25 (0.02)	0.23 (0.01)
	20	0.22 (0.01)	0.21 (0.01)	0.21 (0.01)
	25	0.21 (0)	0.21 (0.03)	0.20 (0.01)

Significance	P	LSD		
Form	0.05	ns	ns	ns
Level	0.05	0.01	0.01	0.01
	0.01	0.02	0.02	0.01
Form X Level	0.05	ns	ns	ns

Appendix Table IV.1. pH of solutions containing NH_4^+ or NO_3^- when pH was adjusted back (data on which Fig. 33 is based).

	pH			
	4.5		6.5	
Day	NH_4^+	NO_3^-	NH_4^+	NO_3^-
1	4.43 (0.06)	4.57 (0.06)	6.37 (0.06)	6.57 (0.06)
2	4.43 (0.06)	4.57 (0.06)	6.37 (0.06)	6.57 (0.06)
3	4.37 (0.06)	4.63 (0.06)	6.33 (0.06)	6.47 (0.06)
4	4.37 (0.15)	4.63 (0.06)	6.23 (0.15)	6.53 (0.15)
5	4.33 (0.06)	4.50 (0.10)	6.30 (0.10)	6.60 (0.10)
6	4.30 (0.10)	4.60 (0.10)	6.30 (0.10)	6.57 (0.15)
7	4.33 (0.15)	4.60 (0.10)	6.33 (0.06)	6.63 (0.06)
8	4.30 (0.10)	4.60 (0.10)	6.33 (0.15)	6.57 (0.06)
9	4.30 (0.10)	4.60 (0.10)	6.23 (0.15)	6.57 (0.15)
10	4.33 (0.15)	4.47 (0.06)	6.30 (0.10)	6.63 (0.06)
11	4.30 (0.10)	4.53 (0.12)	6.33 (0.06)	6.67 (0.06)
12	4.30 (0.10)	4.63 (0.06)	6.27 (0.21)	6.67 (0.06)
13	4.27 (0.12)	4.50 (0.10)	6.27 (0.12)	6.67 (0.06)
14	4.27 (0.15)	4.60 (0.10)	6.40 (0.10)	6.67 (0.06)
15	4.27 (0.12)	4.60 (0.10)	6.37 (0.15)	6.57 (0.15)
16	4.30 (0.10)	4.60 (0.10)	6.30 (0.10)	6.60 (0.10)
17	4.30 (0.10)	4.63 (0.15)	6.33 (0.12)	6.70 (0.10)
18	4.33 (0.12)	4.63 (0.15)	6.37 (0.15)	6.67 (0.06)
19	4.27 (0.12)	4.63 (0.06)	6.33 (0.12)	6.63 (0.06)
20	4.23 (0.15)	4.67 (0.06)	6.33 (0.12)	6.67 (0.06)
21	4.30 (0.10)	4.60 (0.10)	6.30 (0.10)	6.63 (0.06)
22	4.33 (0.12)	4.63 (0.06)	6.30 (0.10)	6.63 (0.06)
23	4.40 (0.10)	4.63 (0.06)	6.33 (0.15)	6.60 (0.10)
24	4.30 (0.10)	4.67 (0.06)	6.30 (0.10)	6.63 (0.06)
25	4.27 (0.06)	4.53 (0.15)	6.40 (0.10)	6.57 (0.15)
26	4.33 (0.15)	4.63 (0.12)	6.30 (0.10)	6.63 (0.06)
27	4.33 (0.06)	4.57 (0.15)	6.37 (0.06)	6.53 (0.06)
28	4.33 (0.06)	4.53 (0.06)	6.37 (0.06)	6.50 (0.10)
29	4.30 (0.10)	4.60 (0.10)	6.30 (0.10)	6.60 (0.10)
30	4.37 (0.15)	4.60 (0.10)	6.30 (0.10)	6.60 (0.10)

Appendix Table IV.2. pH of solutions containing NH_4^+ or NO_3^- when pH was not adjusted (data on which Fig. 34 is based).

	pH	
Day	NH_4^+	NO_3^-
1	6.40 (0.10)	6.50 (0)
2	6.37 (0.06)	6.47 (0.06)
3	6.13 (0.06)	6.53 (0.15)
4	5.97 (0.12)	6.60 (0.10)
5	5.67 (0.15)	6.57 (0.15)
6	5.47 (0.12)	6.57 (0.06)
7	5.37 (0.15)	6.67 (0.06)
8	5.20 (0.10)	6.60 (0.10)
9	5.07 (0.06)	6.57 (0.06)
10	4.93 (0.06)	6.67 (0.15)
11	4.80 (0.10)	6.70 (0.10)
12	4.63 (0.12)	6.73 (0.12)
13	4.53 (0.15)	6.70 (0.10)
14	4.17 (0.12)	6.80 (0)
15	4.03 (0.15)	6.77 (0.06)
16	3.97 (0.06)	6.77 (0.06)
17	3.90 (0.10)	6.83 (0.06)
18	3.80 (0.10)	6.87 (0.06)
19	3.67 (0.06)	6.87 (0.06)
20	3.57 (0.06)	6.87 (0.06)
21	3.50 (0.10)	6.87 (0.06)
22	3.43 (0.06)	6.83 (0.12)
23	3.40 (0.10)	6.87 (0.06)
24	3.37 (0.06)	6.90 (0.10)
25	3.30 (0.10)	6.90 (0.10)
26	3.17 (0.12)	6.97 (0.06)
27	3.13 (0.06)	6.93 (0.06)
28	3.07 (0.06)	6.97 (0.06)
29	3.03 (0.06)	6.97 (0.06)
30	3.03 (0.06)	6.97 (0.06)

Appendix Table IV.3. N in solution (data on which Table 9 is based).

Form of N	pH/Days:	0	5	10	15	20	25	30	Mean
		N in solution, mM							
NH_4^+	changing	10.00 (0)	9.30 (0.36)	9.23 (0.25)	9.07 (0.12)	8.93 (0.12)	8.93 (0.12)	8.93 (0.12)	9.20
	4.5	10.00 (0)	9.57 (0.12)	9.47 (0.25)	9.07 (0.12)	9.17 (0.35)	9.13 (0.40)	8.93 (0.23)	9.33
	6.5	10.00 (0)	9.63 (0.12)	9.47 (0.25)	9.40 (0.36)	9.23 (0.45)	9.07 (0.23)	9.00 (0.20)	9.40
	Mean	10.00	9.50	9.39	9.18	9.11	9.04	8.96	
NO_3^-	changing	10.00 (0)	9.63 (0.12)	9.47 (0.25)	9.20 (0)	9.13 (0.12)	9.13 (0.12)	9.13 (0.12)	9.39
	4.5	10.00 (0)	9.70 (0)	9.47 (0.25)	9.57 (0.12)	9.37 (0.29)	9.20 (0)	9.23 (0.25)	9.50
	6.5	10.00 (0)	9.57 (0.12)	9.40 (0.36)	9.40 (0.17)	9.40 (0.17)	9.20 (0)	9.30 (0.17)	9.47
	Mean	10.00	9.63	9.44	9.39	9.30	9.18	9.22	

Significance	P	LSD
Form of N	0.05	0.11
	0.01	ns
pH	0.05	ns
Form of N X pH	0.05	ns
Days	0.05	0.17
	0.01	0.13
Form X Days	0.05	ns
pH X Days	0.05	ns
Form of N X pH X Days	0.05	ns

Appendix Table IV.4. Number of nodes (data on which Table 10 is based).

Form of N	pH/Days:	0	5	10	15	20	25	30	Mean
		No. of nodes							
NH_4^+	changing	0	0.5 (0.5)	1.3 (0.3)	1.7 (0.3)	2.0 (0.5)	2.8 (0.8)	3.3 (0.6)	1.70
	4.5	0	0.3 (0.3)	1.3 (0.3)	1.7 (0.3)	2.0 (0.5)	2.7 (0.8)	3.2 (1.3)	1.60
	6.5	0	0.2 (0.3)	1.0 (0.5)	1.3 (0.6)	1.7 (0.8)	2.2 (0.3)	2.7 (0.8)	1.29
	Mean	0	0.33	1.22	1.56	1.89	2.56	3.06	
NO_3^-	changing	0	0	0.7 (0.3)	1.2 (0.8)	1.8 (0.6)	2.5 (0.9)	3.0 (1.0)	1.31
	4.5	0 (0)	0.3 (0.3)	1.2 (0.6)	1.5 (0)	1.7 (0.3)	2.3 (0.6)	2.8 (0.8)	1.40
	6.5	0 (0)	0.3 (0.3)	0.8 (0.3)	1.3 (0.3)	1.5 (0.5)	1.8 (0.6)	2.2 (0.7)	1.14
	Mean	0	0.22	0.89	1.33	1.67	2.22	2.67	

Significance	P	LSD
Form of N	0.05	ns
pH	0.05	ns
Form of N X pH	0.05	ns
Days	0.05	0.04
	0.01	0.03
Form X Days	0.05	ns
pH X Days	0.05	ns
Form of N X pH X Days	0.05	ns

Appendix Table IV.5. Number of lateral shots (data on which Table 11 is based).

Form of N	pH Days:	0	5	10	15	20	25	30	Mean
		No. of lateral shoots							
NH_4^+	changing	0	0	0	0	0	0.5 (0.5)	0.7 (0.3)	0.17
	4.5	0	0	0	0	0	0.2 (0.3)	0.5 (0.5)	0.10
	6.5	0	0	0	0	0	0	0.2 (0.3)	0.02
	Mean	0	0	0	0	0	0.22	0.44	
NO_3^-	changing	0	0	0	0	0	0.2 (0.3)	0.3 (0.3)	0.07
	4.5	0	0	0	0	0	0.2 (0.3)	0.3 (0.3)	0.07
	6.5	0	0	0	0	0	0	0.17 (0.29)	0.02
	Mean	0	0	0	0	0	0.1	0.28	

Significance	P	LSD
Form of N	0.05	ns
pH	0.05	ns
Form of N X pH	0.05	ns
Days	0.05	0.01
	0.01	0.01
Form X Days	0.05	ns
pH X Days	0.05	ns
Form of N X pH X Days	0.05	ns

Appendix Table IV.6. Concentrations of amino acids (data on which Table 12 is based).

Form of N	pH	Plant part	Amino acid, µg g^{-1} fresh wt														
			Asn	Gln	Ser	Asp	Glu	Thr	Gly	Ala	Pro	Met	Val	Cys	Phe	Leu+ Iso	Lys
NH_4^+	4.5	Root	4797	894	177	175	445	382	103	186	945	58	87	88	56	100	66
			(403)	(108)	(21)	(8)	(21)	(17)	(4)	(9)	(31)	(2)	(5)	(2)	(3)	(3)	(2)
		Leaf	2850	867	188	182	427	440	109	180	1112	57	99	83	58	102	66
			(505)	(113)	(7)	(18)	(7)	(39)	(6)	(12)	(29)	(2)	(8)	(6)	(4)	(6)	(2)
	6.5	Root	5052	934	192	185	435	415	105	182	997	56	92	85	56	102	66
			(282)	(75)	(3)	(5)	(6)	(37)	(8)	(11)	(15)	(2)	(6)	(2)	(3)	(4)	(2)
		Leaf	2666	858	184	187	436	418	108	187	1034	56	94	85	58	101	65
			(620)	(72)	(7)	(19)	(9)	(43)	(4)	(7)	(67)	(2)	(4)	(3)	(2)	(5)	(1)
NO_3^-	4.5	Root	1400	802	185	176	431	399	103	178	931	57	99	84	57	102	67
			(253)	(58)	(10)	(6)	(33)	(28)	(10)	(9)	(175)	(4)	(7)	(6)	(1)	(3)	(2)
		Leaf	1284	727	178	187	421	387	108	185	965	57	96	85	57	103	66
			(289)	(60)	(4)	(6)	(2)	(24)	(8)	(10)	(131)	(2)	(1)	(2)	(1)	(4)	(1)
	6.5	Root	1367	742	194	184	436	419	108	189	985	56	97	85	55	104	67
			(115)	(39)	(3)	(7)	(25)	(13)	(1)	(15)	(130)	(2)	(3)	(7)	(1)	(1)	(2)
		Leaf	1201	701	188	199	422	403	109	172	1031	56	99	86	59	103	66
			(23)	(78)	(2)	(7)	(5)	(14)	(6)	(13)	(108)	(3)	(12)	(1)	(0)	(3)	(2)
Significance		P	LSD														
Form of N		0.05	431	94	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		0.01	312	68													
pH		0.05	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Form of N X pH		0.05	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Plant part		0.05	431	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		0.01	312														
Form of N X Plant part		0.05	609	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		0.01	442														
pH X Plant part		0.05	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Form of N X pH X Plant part		0.05	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Appendix Table V.1. NR activity in vivo: NO_3^- concentration (data on which Fig. 35a is based).

NO_3^- concentration mM	NR activity, nmol NO_2^- g^{-1} fresh wt h^{-1}	
	Leaves	Roots
0	0	78 (18)
10	234 (19)	231 (13)
20	239 (17)	234 (9)
30	273 (15)	250 (8)
40	253 (17)	236 (7)
50	243 (13)	225 (8)
Significance	LSD	
P = 0.05	27	19
0.01	37	27

Appendix Table V.2. NR activity in vivo: whole tissue vs. sliced tissue (data on which Fig. 35b is based).

Treatment	NR activity, nmol NO_2^- g^{-1} fresh wt h^{-1}	
	Leaves	Roots
Whole	239 (19)	247 (31)
Sliced	338 (34)	291 (25)
Significance		
t-test	P = 0.01	ns

Appendix Table V.3. NR activity in vivo: without propanol vs. with propanol (data on which Fig. 35c is based).

Treatment	NR activity, nmol NO_2^- g^{-1} fresh wt h^{-1}	
	Leaves	Roots
- Propanol	336 (17)	293 (19)
+ Propanol	389 (23)	338 (21)
Significance		
t-test	P = 0.05	0.05

Appendix Table V.4. NR activity in vivo: vacuum infiltration by air vs. N_2 (data on which Table 13 is based).

Treatment	NR activity, nmol NO_2^- g^{-1} fresh wt h^{-1}	
	Leaves	Roots
Air	392 (25)	322 (18)
N_2	397 (13)	325 (9)

Difference between treatments was not significant, t-test.

Appendix Table V.5. NR activity in vivo: time course (data on which Fig. 35d is based).

Incubation time min	NR activity, nmol NO_2^- g^{-1} fresh wt h^{-1}	
	Leaves	Roots
0	0	0
15	49 (17)	89 (26)
30	163 (21)	167 (22)
45	305 (13)	255 (21)
60	418 (27)	350 (22)
75	550 (8)	417 (9)
90	705 (13)	500 (22)
Significance	LSD	
P = 0.05	28	34
0.01	39	47

Appendix Table V.6. NiR activity *in vivo*: pH (data on which Fig. 36a is based).

pH	NiR activity nmol NO_2^- g^{-1} fresh wt h^{-1}
4.5	178 (15)
5.5	107 (23)
6.5	25 (8)
7.5	7 (5)
Significance	LSD
P = 0.05	27
0.01	39

Appendix Table V.7. NiR activity *in vivo*: NO_2^- concentration (data on which Fig. 36b is based).

NO_2^- concentration	NiR activity nmol NO_2^- g^{-1} fresh wt h^{-1}
0	0
20 µM	184 (8)
40	185 (9)
100	159 (21)
200	43 (28)
2 mM	0
20	0
Significance	LSD
P = 0.05	24
0.01	27

Appendix Table V.8. NiR activity *in vivo*: time course (data on which Fig. 36c is based).

Incubation time min	NiR activity nmol NO_2^- g^{-1} fresh wt h^{-1}
0	0
15	50 (21)
30	76 (20)
45	135 (18)
60	165 (10)
75	224 (10)
90	281 (13)
Significance	LSD
P = 0.05	26
0.01	36

Appendix Table V.9. NR activity *in vitro*: homogenization with Ultra-Turrax *vs.* mortar & pestle (data on which Fig. 37a is based).

Treatment	NR activity, nmol NO_2^- h^{-1}	
	g^{-1} fresh wt	mg^{-1} protein
Ultra-Turrax	90 (79)	8 (7)
Mortar & pestle	390 (30)	30 (2)
Significance		
t-test	P = 0.01	0.01

Appendix Table V.10. NR activity *in vitro*: cysteine concentration (data on which Fig. 37b is based).

Cysteine concentration mM	NR activity, nmol NO_2^- h^{-1}	
	g^{-1} fresh wt	mg^{-1} protein
0	0	0
1	430 (46)	34 (3)
2	300 (60)	24 (4)
5	120 (60)	12 (7)
10	0	0
Significance	LSD	
P = 0.05	78	7
0.01	112	10

Appendix Table V.11. NR activity *in vitro*: without BSA *vs.* with BSA (data on which Table 14 is based).

Treatment	NR activity, nmol NO_2^- h^{-1}	
	g^{-1} fresh wt	mg^{-1} protein
+BSA	420 (79)	33 (4)
-BSA	410 (62)	33 (5)

Difference between treatments was not significant, t-test.

Appendix Table V. 12. NR activity *in vitro*: K/HPO_4 buffer *vs.* Tris-HCl buffer (data on which Table 15 is based).

Buffer	NR activity, nmol NO_2^- h^{-1}	
	g^{-1} fresh wt	mg^{-1} protein
K/HPO_4	400 (92)	34 (7)
Tris-HCl	390 (90)	33 (8)

Difference between treatments was not significant, t-test.

Appendix Table V.13. NR activity *in vitro*: without PVP *vs.* with PVP (data on which Fig. 37c is based).

Treatment	NR activity, nmol NO_2^- h^{-1}	
	g^{-1} fresh wt	mg^{-1} protein
- PVP	410 (75)	34 (6)
+ PVP	630 (90)	52 (8)
Significance		
t-test	P = 0.05	0.05

Appendix Table V. 14. NR activity *in vitro*: NO_3^- concentration (data on which Fig. 38a is based).

NO_3^- concentration mM	NR activity, nmol NO_2^- h^{-1}	
	g^{-1} fresh wt	mg^{-1} protein
0	0	0
0.1	382 (68)	30 (4)
1	448 (50)	36 (5)
10	568 (66)	48 (7)
100	645 (68)	55 (8)
200	623 (19)	52 (4)
400	634 (66)	53 (7)
Significance	LSD	
P = 0.05	95	10
0.01	133	13

Appenidix Table V.15. NR activity *in vitro*: enzyme concentration (data on which Fig. 38b is based).

Enzyme µl/reaction tube	NR activity$_{-1}$ nmol NO_2^- h^{-1}
0	0
50	0
100	9 (3)
200	25 (2)
400	42 (6)
500	51 (2)
Significance	LSD
P = 0.05	5
0.01	7

Appendix Table V.16. NR activity in vitro: time course (data on which Fig. 38c is based).

Time, min	NR activity, nmol NO_2^- g^{-1} fresh wt	NR activity, nmol NO_2^- mg^{-1} protein
0	0	0
15	186 (16)	15 (1)
30	339 (25)	30 (3)
45	574 (25)	48 (2)
60	672 (25)	57 (3)
Significance	LSD	
P = 0.05	38	4
0.01	54	6

Appendix Table V.17. NiR activity in vitro: NO_2^- concentration (data on which Fig. 39a is based).

NO_2^- concentration mM	NiR activity, µmol NO_2^- h^{-1} g^{-1} fresh wt	NiR activity, µmol NO_2^- h^{-1} mg^{-1} protein
0	0	0
1	17.03 (1.28)	2.86 (0.31)
2	18.00 (0.45)	3.15 (0.18)
5	18.75 (1.72)	3.28 (0.36)
10	6.75 (2.25)	1.17 (0.43)
20	2.25 (3.90)	0.37 (0.64)
Significance	LSD	
P = 0.05	3.63	0.67
0.01	5.10	0.94

Appendix Table V.18. NiR activity in vitro: time course (data on which Fig. 39b is based).

Time, min	NiR activity, µmol NO_2^- g^{-1} fresh wt	NiR activity, µmol NO_2^- mg^{-1} protein
0	0	0
5	1.00 (0.28)	0.17 (0.05)
10	2.65 (0.23)	0.45 (0.05)
20	5.65 (0.16)	0.95 (0.07)
30	7.70 (0.26)	1.29 (0.03)
Significance	LSD	
P = 0.05	0.39	0.08
0.01	0.55	0.12

Appendix Table VI.1. NO_3^- concentration in roots (data on which Fig. 41 is based).

NO_3^- supplied mmol	Time: 0 h	1 h	5 h	10 h	2 d	3 d	4 d	5 d	6 d	7 d	Mean
	NO_3^- concentration, nmol g^{-1} fresh wt										
0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	5390 (1540)	7155 (1195)	9239 (1524)	4942 (1019)	1648 (1614)	0	0	2837
50	0	0	0	6030 (1540)	8321 (1485)	13320 (1361)	15542 (1738)	13604 (820)	11754 (2229)	9239 (2689)	7781
100	0	0	275 (614)	8044 (1538)	12545 (2980)	20422 (2551)	26814 (3035)	30697 (2088)	32197 (1611)	28743 (1855)	15973
150	0	0	709 (1033)	9531 (2353)	15104 (3308)	25080 (3909)	31657 (4282)	34629 (2903)	33534 (2350)	31719 (2509)	18196
Mean	0	0	164	4833	7187	11343	13159	13430	12914	11617	

Significance	LSD P = 0.05	0.01
NO_3^-	1007	1364
Time	707	930
NO_3^- X Time	1733	2278

Appendix Table VI.2. NR actvitiy *in vivo* ($-NO_3^-$) in roots (data on which Fig. 42 is based).

NO_3^- supplied mmol	Time: 0 h	1 h	5 h	10 h	2 d	3 d	4 d	5 d	6 d	7 d	Mean
	NR activity, nmol NO_2^- g^{-1} fresh wt h^{-1}										
0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	7 (15)	107 (66)	126 (89)	14 (32)	0	0	0	0	25
25	0	12 (26)	83 (59)	223 (38)	320 (24)	377 (40)	295 (69)	184 (80)	30 (43)	0	152
50	0	15 (33)	96 (29)	252 (31)	372 (25)	449 (47)	421 (75)	425 (48)	430 (36)	440 (37)	290
100	0	16 (36)	116 (55)	274 (37)	427 (43)	370 (24)	351 (44)	354 (28)	288 (40)	299 (25)	249
150	0	37 (51)	154 (56)	318 (21)	435 (57)	328 (17)	356 (27)	335 (26)	261 (27)	281 (38)	251
Mean	0	13	76	196	280	256	237	216	168	170	

Significance	LSD P = 0.05	0.01
NO_3^-	17	23
Time	18	23
NO_3^- X Time	44	57

Appendix Table VI.3. NR actvitiy in vivo ($+NO_3^-$) in roots (data on which Fig. 43 is based).

NO_3^- supplied mmol	NR activity, nmol NO_2^- g^{-1} fresh wt h^{-1}										
Time:	0 h	1 h	5 h	10 h	2 d	3 d	4 d	5 d	6 d	7 d	Mean
0	0	0	0	0	0	0	0	0	0	0	0
15	0	73	147	286	431	297	127	0	0	0	136
		(29)	(28)	(80)	(36)	(68)	(23)				
25	0	107	214	362	481	521	404	344	151	35	262
		(28)	(61)	(20)	(71)	(24)	(47)	(71)	(87)	(48)	
50	0	130	256	390	530	557	518	523	494	523	392
		(46)	(28)	(20)	(36)	(29)	(35)	(43)	(55)	(41)	
100	0	159	349	431	513	398	373	352	290	304	317
		(87)	(40)	(27)	(19)	(31)	(38)	(23)	(28)	(34)	
150	0	160	369	455	507	365	379	363	279	302	318
	0	(72)	(41)	(28)	(39)	(32)	(45)	(17)	(33)	(48)	
Mean	0	105	223	321	410	356	300	264	202	194	

Significance	LSD	
P =	0.05	0.01
NO_3^-	16	30
Time	19	25
NO_3^- X Time	47	62

Appendix Table VI.4. NO_3^- concentration in leaves (data onwhich Fig. 45 is based).

NO_3^- supplied mmol	NO_3^- concentration, nmol g^{-1} fresh wt										
Time:	0 h	1 h	5 h	10 h	2 d	3 d	4 d	5 d	6 d	7 d	Mean
0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	1958	744	0	0	0	0	270
					(1286)	(1020)					
50	0	0	0	1328	11089	17679	17447	16915	15927	13550	9373
				(1247)	(1983)	(1697)	(912)	(1052)	(1309)	(1830)	
100	0	0	0	3296	14917	23554	41054	45133	47252	51303	22650
				(857)	(2098)	(1911)	(2588)	(1807)	(2268)	(3085)	
150	0	0	0	5972	17130	27071	45195	50684	54862	58659	25957
				(1295)	(2719)	(2347)	(2184)	(5092)	(6064)	(3564)	
Mean	0	0	0	1766	7516	11508	17283	18789	19640	20585	

Significance	LSD	
P =	0.05	0.01
NO_3^-	1246	1689
Time	659	866
NO_3^- X Time	1614	2121

Appendix Table VI.5. NR actvitiy *in vivo* ($-NO_3^-$) in leaves (data on which Fig. 47 is based).

NO_3^- supplied mmol	NR activity, nmol NO_2^- g^{-1} fresh wt h^{-1}										
Time:	0 h	1 h	5 h	10 h	2 d	3 d	4 d	5 d	6 d	7 d	Mean
0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	309 (56)	531 (58)	429 (179)	79 (72)	0	0	0	0
50	0	0	16 (35)	457 (73)	544 (85)	499 (60)	506 (56)	526 (49)	479 (76)	486 (31)	351
100	0	0	30 (41)	474 (31)	534 (77)	577 (66)	442 (41)	361 (43)	281 (28)	257 (31)	296
150	0	0	104 (60)	510 (56)	527 (99)	546 (54)	430 (18)	339 (31)	267 (12)	258 (45)	298
Mean	0	0	25	292	356	342	243	204	171	167	

Significance	LSD P = 0.05	0.01
NO_3^-	25	33
Time	21	27
NO_3^- X Time	52	68

Appendix Table VI.6. NR actvitiy *in vivo* ($+NO_3^-$) in leaves (data on which Fig. 48 is based).

NO_3^- supplied mmol	NR activity, nmol NO_2^- g^{-1} fresh wt h^{-1}										
Time:	0 h	1 h	5 h	10 h	2 d	3 d	4 d	5 d	6 d	7 d	Mean
0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	70 (157)	250 (255)	338 (318)	164 (103)	0	0	0	0	82
25	0	0	122 (146)	609 (122)	674 (75)	674 (159)	425 (248)	97 (168)	0	0	260
50	0	0	125 (77)	538 (91)	634 (45)	605 (64)	580 (55)	555 (35)	525 (49)	538 (23)	410
100	0	0	199 (62)	564 (32)	566 (48)	600 (85)	475 (25)	389 (63)	298 (46)	265 (21)	336
150	0	0	241 (42)	569 (26)	570 (114)	587 (34)	462 (27)	324 (27)	273 (22)	243 (40)	327
Mean	0	0	126	422	464	438	324	228	183	174	

Significance	LSD P = 0.05	0.01
NO_3^-	41	56
Time	42	55
NO_3^- X Time	102	134

Appendix Table VI.7. NR actvitiy *in vitro* in leaves (data on which Fig. 51a is based).

NO_3^- supplied mmol	Time:	NR activity, nmol NO_2^- g^{-1} fresh wt h^{-1} 0 h	1 h	5 h	10 h	2 d	3 d	4 d	5 d	6 d	7 d	Mean
0		0	0	0	0	0	0	0	0	0	0	0
15		0	0	0	0	0	0	0	0	0	0	0
25		0	0	0	171 (235)	629 (53)	671 (65)	371 (219)	0	0	0	184
50		0	0	0	291 (268)	638 (76)	613 (39)	610 (47)	576 (41)	526 (18)	556 (36)	381
100		0	0	0	451 (260)	645 (57)	579 (36)	529 (59)	405 (37)	196 (185)	0	280
150		0	0	0	480 (277)	651 (52)	569 (26)	519 (52)	331 (37)	60 (134)	61 (137)	267
Mean		0	0	0	232	427	405	338	219	130	103	

Significance P =	LSD 0.05	0.01
NO_3^-	45	61
Time	41	54
NO_3^- X Time	101	132

Appendix Table VI.8. NR actvitiy *in vitro* in leaves (data on which Fig. 51b is based).

NO_3^- supplied mmol	Time:	NR activity, nmol NO_2^- mg^{-1} protein h^{-1} 0 h	1 h	5 h	10 h	2 d	3 d	4 d	5 d	6 d	7 d	Mean
0		0	0	0	0	0	0	0	0	0	0	0
15		0	0	0	0	0	0	0	0	0	0	0
25		0	0	0	14 (20)	56 (4)	60 (6)	30 (18)	0	0	0	16
50		0	0	0	25 (23)	56 (4)	61 (1)	54 (3)	49 (4)	43 (2)	46 (5)	33
100		0	0	0	38 (22)	56 (4)	56 (6)	46 (3)	34 (3)	16 (15)	0 0	24
150		0	0	0	42 (24)	58 (6)	57 (6)	45 (4)	28 (3)	5 (11)	5 (12)	24
Mean		0	0	0	20	38	39	29	19	11	9	

Significance P =	LSD 0.05	0.01
NO_3^-	3	4
Time	4	5
NO_3^- X Time	9	12

Appendix Table VI.9. NiR actvitiy *in vivo* in leaves.

NO_3^- supplied mmol	Time: 0 h	1 h	5 h	10 h	2 d	3 d	4 d	5 d	6 d	7 d	Mean
	NiR activity, nmol NO_2^- g^{-1} fresh wt h^{-1}										
0	182	181	186	186	178	180	183	181	182	181	182
	(9)	(7)	(7)	(7)	(4)	(6)	(10)	(6)	(11)	(4)	
15	177	179	180	173	180	180	179	179	186	179	179
	(5)	(6)	(6)	(14)	(3)	(5)	(8)	(7)	(7)	(13)	
25	179	188	183	179	182	185	186	186	186	187	184
	(6)	(6)	(5)	(3)	(9)	(9)	(11)	(7)	(5)	(7)	
50	187	183	178	188	190	190	191	179	176	173	184
	(5)	(11)	(6)	(13)	(8)	(5)	(9)	(7)	(10)	(12)	
100	177	176	184	177	171	167	172	176	171	180	175
	(5)	(8)	(14)	(4)	(12)	(20)	(13)	(11)	(8)	(12)	
150	176	172	177	175	178	179	175	176	174	178	176
	(5)	(14)	(15)	(10)	(17)	(10)	(8)	(15)	(10)	(11)	
Mean	180	180	181	180	180	180	181	179	179	180	

Appendix Table VI.10. NiR actvitiy *in vitro* in leaves.

NO_3^- supplied mmol	Time: 0 h	1 h	5 h	10 h	2 d	3 d	4 d	5 d	6 d	7 d	Mean
	NiR activity, nmol NO_2^- g^{-1} fresh wt h^{-1}										
0	17604	17370	17665	17856	17667	17734	17465	17146	17807	17640	17595
	(357)	(513)	(690)	(380)	(363)	(315)	(424)	(739)	(596)	(377)	
15	17280	17595	17266	17663	17478	17630	18000	17640	16834	17240	17463
	(433)	(582)	(700)	(694)	(547)	(646)	(318)	(493)	(437)	(459)	
25	16826	17199	17147	16893	17573	17925	18038	18338	18445	18733	17711
	(293)	(244)	(170)	(253)	(658)	(776)	(1188)	(595)	(569)	(1141)	
50	18483	18140	17755	18302	18552	18813	16785	17205	16796	16750	17758
	(2019)	(2699)	(454)	(926)	(1423)	(1775)	(680)	(1051)	(971)	(1216)	
100	17025	17017	16875	16815	16645	16785	16940	16555	16830	17710	16920
	(1137)	(411)	(574)	(801)	(955)	(867)	(1330)	(1284)	(824)	(1722)	
150	17254	16740	17035	16885	17063	17593	17120	16665	17175	17640	17117
	(1273)	(988)	(1057)	(1168)	(941)	(1210)	(1388)	(1530)	(1020)	(1154)	
Mean	17412	17343	17290	17402	17496	17747	17391	17258	17314	17619	

Appendix Table VI.11. NiR actvitiy *in vitro* in leaves.

NO_3^- supplied mmol	Time:	NiR activity, nmol NO_2^- mg^{-1} protein wt h^{-1}										
		0 h	1 h	5 h	10 h	2 d	3 d	4 d	5 d	6 d	7 d	Mean
0		2982	3039	3002	3123	3086	3072	3046	2999	3007	3003	3036
		(149)	(81)	(136)	(208)	(107)	(160)	(32)	(73)	(223)	(136)	
15		2860	3012	2995	2913	3130	3079	3033	2972	2828	2920	2974
		(110)	(124)	(118)	(258)	(135)	(191)	(142)	(221)	(190)	(123)	
25		2872	3038	2984	2854	2985	3069	3000	3218	3264	3230	3051
		(182)	(159)	(89)	(171)	(143)	(163)	(213)	(83)	(161)	(183)	
50		2847	3046	3055	3010	2931	3147	2150	3045	2861	2951	2984
		(136)	(158)	(180)	(285)	(256)	(275)	(151)	(106)	(212)	(302)	
100		2925	2935	3040	2838	2857	2807	2846	2824	3063	3111	2925
		(312)	(209)	(147)	(161)	(314)	(184)	(373)	(249)	(224)	(299)	
150		2971	2962	2943	2868	3094	3013	2982	2877	2999	3014	2972
		(277)	(259)	(216)	(285)	(138)	(215)	(254)	(188)	(96)	(268)	
Mean		2910	3006	3003	2934	3014	3031	2976	2989	3004	3038	

Publications

Reddy, K. S. and Menary, R. C. Vegetative growth, flowering and leaf nutrient concentration of boronia as affected by nitrogen level and form. Accepted by Scientia Hortic. (1989)

Reddy, K. S. and Menary, R. C. High-performance liquid chromatographic separation of dinitrophenyl-derivatized amino acids. Submitted for J. Chromatogr. (1989)

Reddy, K. S. and Menary, R. C. Effects of nitrogen source, rate and application time on boronia (Boronia megastigma Nees) leaf nitrogen and flower yield. Fert. Res. (1989)

Reddy, K. S. and Menary, R. C. Nitrate reductase and nitrate accumulation in relation to nitrate toxicity in Boronia megastigma Nees. Physiol. Plant. (1989)