Nitrogen and the Leaf Growth of Temperate Cereals

A thesis submitted in fulfilment
of the requirements for the
Degree of Doctor of
Philosophy

by Mark Lieffering

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1995

Certificate

I hereby certify that the experimental work contained in this thesis was planned, executed and described by Mark Lieffering, under the supervision of Dr. M.H.G. Andrews and me.

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Abstract of a thesis submitted in fulfilment of the requirements for the Degree of Doctor of Philosophy

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Under agricultural conditions where soil moisture is adequate, low nitrogen (N) availability is usually the main soil factor limiting the growth and yield of temperate cereals. A major part of the positive response of plant growth to additional N is a result of greater leaf area, an important determinant of plant photosynthetic capacity. This thesis investigated various aspects of the influence of N on the leaf growth of temperate cereals.

Data were presented in Chapter 2 which investigated the influence of additional N as nitrate (NO_3^-) or ammonium (NH_4^+) on reserve mobilisation and seedling growth prior to emergence from the substrate. The amount of N assimilated was similar with either form of N, but as a result of enhanced endosperm mobilization, seedling dry weight (d.wt) was greater with NO_3^-. When seedlings were supplied chloride, reserve mobilisation and seedling growth were as great as with NO_3^-. It was concluded that the increased rate of mobilisation of seed reserves and subsequently greater seedling growth with additional NO_3^- were due to greater seedling water uptake, probably acting via increased seed water content. A similar mechanism, but acting directly via the seed, was suggested for enhanced reserve mobilisation with increased levels of endogenous seed N.

Chapter 3 investigated the influence of N form and availability on the growth of individual main stem and tiller leaves. With increasing external N concentrations over the range 0 to 2.5 - 5 mol m^{-3} leaf growth characteristics and maximum leaf area attained were similar with N supplied as NO_3^-, NH_4^+ or glutamine. Leaf area increased further with increasing external concentrations of NO_3^- or glutamine to 20 mol m^{-3} but with NH_4^+ it usually declined substantially. As leaf growth was similar with NO_3^- or glutamine over a wide range of external N concentrations, it was suggested that the site of N assimilation is probably not a major factor in determining the extent of individual leaf area development. However, it is possible that factors associated with NH_4^+ toxicity influence the growth of leaves.

It was demonstrated in Chapter 4 that greater individual leaf area with additional NO_3^- was associated with an increase in both cell number and size. Increased cell division was thought to be due to increased availability of both photosynthate and N. It was proposed that greater cell size with additional NO_3^- was due to an increase in the availability of osmoticum, primarily sucrose. Also, it was suggested that at higher external NO_3^- concentrations, additional types of osmotica, such as NO_3^-, counter ions and organic acids, are also available as a result of assimilation and storage of NO_3^- in the leaves.

The influence of N availability and form on shoot to root d.wt ratio (S:R) and leaf d.wt as a fraction of total plant d.wt (LWR) were investigated in Chapter 5. It was shown that regardless of whether N was supplied as NO_3^-, NH_4^+ or glutamine, increasing external N concentration resulted in an increase in plant reduced-N content and S:R or LWR, though at any given total plant d.wt, all three parameters were greater for plants supplied NH_4^+ or glutamine. Hence, at any given plant N content, S:R or LWR were similar, regardless of N form supplied. These results were discussed in terms of a proposed mechanism for the control of S:R by N. It was also shown that leaf area produced per unit leaf N was greater for plants supplied NO_3^- compared to NH_4^+ or glutamine; this does not appear to have been reported previously.

In Chapter 6 it was demonstrated that despite relatively high initial levels of soil N, fertilizer N applied at sowing had positive effects on the grain yield of all the temperate cereals investigated. The reason for the increase was similar for all species: additional N increased the fraction of available photosynthetically active radiation (PAR) intercepted by increasing the rate of canopy development. As a result, crop dry matter (DM) production increased and as harvest index (HI) was not affected, grain yield was greater with additional N. Differences between species in the amount of grain produced were not associated with the amounts of PAR intercepted or DM produced, but were related to differences in HI.

Additional key words: nitrate, ammonium, glutamine, seed reserve mobilization, leaf expansion, cell number and size, dry matter partitioning, canopy development, grain yield.

Table of Contents

Chapter page

1 **General Introduction** 1
- 1.1 Importance of Cereals 2
- 1.2 Characteristics of Cereal Plant Growth 3
- 1.3 Plant Growth and Carbon Assimilation 5
- 1.4 Nitrogen Assimilation 7
- 1.5 Nitrogen Effects on Plant Growth 8
- 1.6 Previous Research on Cereals Relevant to the Study 9
- 1.7 Objectives of the Study 11

2 **Nitrogen Effects on the Mobilisation of Seed Reserves** 12
- 2.1 Introduction 13
- 2.2 Materials and Methods 14
- 2.3 Results 18
- 2.4 Discussion 25
- 2.5 Conclusions 28

3 **Nitrogen and the Growth of Individual Leaves** 33
- 3.1 Introduction 34
- 3.2 Materials and Methods 36
- 3.3 Results 38
- 3.4 Discussion 52
- 3.5 Conclusions 54

4 **Nitrogen and the Cellular Aspects of Individual Leaf Area Development** 56
- 4.1 Introduction 57
- 4.2 Materials and Methods 58
- 4.3 Results 61
- 4.4 Discussion 67
- 4.5 Conclusions 70

5 **Nitrogen and the Partitioning of Dry Matter** 72
5.1 Introduction 73
5.2 Materials and Methods 75
5.3 Results 76
5.4 Discussion 83
5.5 Conclusions 85

6 **Nitrogen, Canopy Development and Grain Yield and Quality** 87
6.1 Introduction 88
6.2 Materials and Methods 90
6.3 Results 93
6.4 Discussion 104
6.5 Conclusions 109

7 **General Discussion** 111
7.1 Introduction 112
7.2 Seed Reserve Mobilisation 114
7.3 Leaf Growth 115
7.4 Partitioning of Dry Matter 119
7.5 Crop Growth and Grain Yield 121
7.6 Conclusions 123

Acknowledgements 125
References 126

List of Figures

page

Fig. 1.1 Some growth stages representative of the development of spring sown *Hordeum vulgare* L. 4

Fig. 2.1 Effects of different applied nitrate (NO_3^-) concentrations on fresh weight, dry weight (d.wt) and NO_3^- content of root and shoot and d.wt of the residual seed of *Avena sativa* L., *Hordeum vulgare* L., *Secale cereale* L., X *Triticosecale* Wittmack and *Triticum aestivum* L. 19

Fig. 2.2 Change in shoot, root and residual seed dry weight (d.wt) and residual seed fresh weight:d.wt and nitrate (NO_3^-) content of *Hordeum vulgare* L. seedlings supplied basal nutrient solution only or with 5 mol m^{-3} NO_3^- added 23

Fig. 3.1 The effects of different concentrations of nitrate, ammonium or glutamine on the absolute maximum extension rate, weighted mean extension rate and duration of extension of main stem leaves 2 - 4 and tiller 1 leaf 2 of *Hordeum vulgare* L. 40

Fig. 3.2 The effects of different concentrations of nitrate, ammonium or glutamine on the absolute maximum extension rate, weighted mean extension rate and duration of extension of main stem leaves 2 - 4 and tiller 1 leaf 2 of *Triticum aestivum* L. 41

Fig. 3.3 The effects of different concentrations of nitrate, ammonium or glutamine on the absolute maximum extension rate, weighted mean extension rate and duration of extension of main stem leaves 2 - 4 and tiller 1 leaf 2 of *Secale cereale* L. 42

Fig. 3.4 The effects of different concentrations of nitrate, ammonium or glutamine on the absolute maximum extension rate, weighted mean extension rate and duration of extension of main stem leaves 2 - 4 and tiller 1 leaf 2 of X *Triticosecale* Wittmack 43

Fig. 3.5 The effects of different concentrations of nitrate, ammonium or glutamine on the absolute maximum extension rate, weighted mean extension rate and duration of extension of main stem leaves 2 - 4 and tiller 1 leaf 2 of *Avena sativa* L. 44

Fig. 3.6 The effects of different concentrations of nitrate, ammonium or glutamine on the final length, width and area of main stem leaves 2 - 4 and tiller 1 leaf 2 of *Hordeum vulgare* L. 45

Fig. 3.7 The effects of different concentrations of nitrate, ammonium or glutamine on the final length, width and area of main stem leaves 2 - 4 and tiller 1 leaf 2 of *Triticum aestivum* L. 46

Fig. 3.8 The effects of different concentrations of nitrate, ammonium or glutamine on the final length, width and area of main stem leaves 2 - 4 and tiller 1 leaf 2 of *Secale cereale* L. 47

Fig. 3.9 The effects of different concentrations of nitrate, ammonium or glutamine on the final length, width and area of main stem leaves 2 - 4 and tiller 1 leaf 2 of X *Triticosecale* Wittmack 48

Fig. 3.10 The effects of different concentrations of nitrate, ammonium or glutamine on the final length, width and area of main stem leaves 2 - 4 and tiller 1 leaf 2 of *Avena sativa* L. 49

Fig. 3.11 The effects of different concentrations of nitrate, ammonium or glutamine on the absolute maximum extension rate, weighted mean extension rate and duration of extension of main stem leaves 2 - 4 and tiller 1 leaf 2 of *Hordeum vulgare* L. (repeat experiment) 50

Fig. 3.12 The effects of different concentrations of nitrate, ammonium or glutamine on the final length, width and area of main stem leaves 2 - 4 and tiller 1 leaf 2 of *Hordeum vulgare* L. (repeat experiment) 51

Fig. 4.1 Relationships between epidermal cell area or number and the area of main stem leaves 1 - 6 of *Hordeum vulgare* L. supplied 0.5, 2 or 5 mol m^{-3} nitrate 64

Fig. 4.2 Relationships between fresh weight per unit DNA and leaf DNA content and the area of main stem leaves 1, 3 and 6 of *Hordeum vulgare* L. supplied 0.5, 2 or 5 mol m^{-3} nitrate 65

Fig. 5.1 Effects of different concentrations of nitrate, ammonium or glutamine on total plant dry weight and leaf area of *Hordeum vulgare* L. 79

Fig. 5.2 Effects of different concentrations of nitrate, ammonium or glutamine on shoot to root dry weight (d.wt) ratio, leaf d.wt as a fraction of total plant d.wt and plant nitrogen content of *Hordeum vulgare* L. 80

Fig. 5.3 The relationships between shoot to root dry weight (d.wt) ratio or leaf d.wt as a fraction of total plant d.wt versus plant d.wt or nitrogen content of *Hordeum vulgare* L. supplied different concentrations of nitrate, ammonium or glutamine 81

Fig. 5.4 The relationships between total plant reduced nitrogen (N) and plant dry weight and total leaf reduced-N and plant leaf area of *Hordeum vulgare* L. supplied different concentrations of nitrate, ammonium or glutamine 82

Fig. 6.1 The effects of 0, 100 or 200 kg nitrogen ha^{-1} applied at sowing on the predicted (lines) and actual (symbols) canopy leaf area index during the development of spring sown crops of *Triticum aestivum* L., *Hordeum vulgare* L., *Secale cereale* L., X *Triticosecale* Wittmack and *Avena sativa* L. 95

Fig. 6.2 The relationship between leaf area index and diffuse interception of spring sown *Triticum aestivum* L., *Hordeum vulgare* L., *Secale cereale* L., X *Triticosecale* Wittmack and *Avena sativa* L. 97

Fig. 6.3 The relationship between final crop dry matter and grain yield of spring sown *Triticum aestivum* L., *Hordeum vulgare* L., *Secale cereale* L., X *Triticosecale* Wittmack and *Avena sativa* L. supplied 0, 100 or 200 kg nitrogen ha^{-1} at sowing 100

List of Tables

page

Table 1.1 Area and production of cereals harvested worldwide, 1992 2

Table 1.2 Area and production of cereals harvested in New Zealand, 1992 3

Table 1.3 Range of elemental composition of healthy, growing plants, including cereals, expressed as % of dry matter (DM) or part per million (ppm) DM 6

Table 2.1 Effect of additional ammonium and nitrate on dry weight and water content of shoot, residual seed and root; shoot and root *in vivo* nitrate reductase activity (NRA) and total plant nitrogen of *Avena sativa* L., *Hordeum vulgare* L., *Secale cereale* L., X *Triticosecale* Wittmack and *Triticum aestivum* L., Values in parentheses are *in vitro* NRA. 20

Table 2.2a Effects of basal nutrient solution alone or with added 5 or 20 mol m^{-3} ammonium, nitrate (NO_3^-), NO_3^- with tungsten in place of molybdenum, urea, thiourea, chloride or chlorate on shoot and root fresh weight and dry weight (d.wt) and residual seed d.wt of *Hordeum vulgare* L. prior to emergence from the substrate. 21

Table 2.2b The effect of basal nutrient only or with 5 mol m^{-3} nitrate (NO_3^-) or 5 mol m^{-3} NO_3^- plus tungsten added on shoot and root *in vivo* nitrate reductase activity of *Hordeum vulgare* L. 21

Table 2.3a Effects of additional nitrate or chloride on dry weight, and water content of shoot, residual seed and root of *Hordeum vulgare* L. prior to emergence from the substrate 22

Table 2.3b Nitrate (NO_3^-), chloride (Cl^-), sulphate and phosphate content of the shoot, residual seed and root of *Hordeum vulgare* L. seedlings prior to emergence supplied basal nutrient solution only or with 5 mol m^{-3} NO_3^- or Cl^- added 22

Table 2.4 Effect of seed nitrogen content and additional nitrate on dry weight of shoot plus root and dry weight and water content of residual seed of *Hordeum vulgare* L. seedlings 10 and 14 days after sowing 24

Table 4.1 The effects of additional nitrate on the area, length, width and apparent thickness of main stem leaves 1 to 6 of *Hordeum vulgare* L. 62

Table 4.2 The effects of additional nitrate on abaxial epidermal cell length, width and area; percentage of the surface occupied by non-veinal cells (NVC), total number of cells per leaf, cells per leaf length and width of main stem leaves 1 - 6 of *Hordeum vulgare* L 63

Table 4.3 The effects of additional nitrate on total DNA content, fresh weight to DNA ratio and leaf area to DNA ratio of leaves 1, 3, and 6 of *Hordeum vulgare* L. 66

Table 5.1 Effect of additional ammonium (NH_4^+) or nitrate (NO_3^-) on shoot, root and residual seed dry weight (d.wt), shoot:root d.wt and total seedling NO_3^-, NH_4^+ and reduced nitrogen content of *Hordeum vulgare* L. prior to emergence from the substrate 78

Table 5.2 Effect of additional chloride or nitrate on shoot, root and residual seed (RS) dry weight (d.wt), shoot:root d.wt of *Hordeum vulgare* L. prior to emergence from the substrate 78

Table 6.1 The effects of 0, 100 or 200 kg nitrogen ha^{-1} applied at sowing on the derived parameters of canopy development: maximum canopy growth rate, weighted mean average canopy growth rate and duration of growth and actual canopy leaf area index at anthesis of spring sown crops of *Triticum aestivum* L., *Hordeum vulgare* L., *Secale cereale* L., X *Triticosecale* Wittmack and *Avena sativa* L. 94

Table 6.2a Interception of diffuse solar radiation at a leaf area index of 1 and the value of critical leaf area of spring sown crops of *Triticum aestivum* L., *Hordeum vulgare* L., *Secale cereale* L., X *Triticosecale* Wittmack and *Avena sativa* L. 96

Table 6.2b Effects of 0, 100 or 200 kg nitrogen ha^{-1} applied at sowing on the time taken to reach critical leaf area index and on the amount of photosynthetically active radiation intercepted from sowing up to anthesis by spring sown crops of *Triticum aestivum* L., *Hordeum vulgare* L., *Secale cereale* L., X *Triticosecale* Wittmack and *Avena sativa* L. 96

Table 6.3 Effect of 0, 100 or 200 kg nitrogen ha^{-1} applied at sowing on total dry matter production, grain yield and harvest index of spring sown *Triticum aestivum* L., *Hordeum vulgare* L., *Secale cereale* L., X *Triticosecale* Wittmack and *Avena sativa* L. 98

Table 6.4 Effect of 0, 100 or 200 kg nitrogen ha^{-1} applied at sowing on the coefficient of conversion of spring sown *Triticum aestivum* L., *Hordeum vulgare* L., *Secale cereale* L., X *Triticosecale* Wittmack and *Avena sativa* L. 99

Table 6.5 Effects of 0, 100 or 200 kg nitrogen ha^{-1} applied at sowing on tiller number, head number, grains per head and individual grain weight of spring sown *Triticum aestivum* L. and *Hordeum vulgare* L. 101

Table 6.6 Effect of additional nitrogen (N) applied at either sowing or anthesis on the grain %N of spring sown *Triticum aestivum* L., *Hordeum vulgare* L., *Secale cereale* L., X *Triticosecale* Wittmack and *Avena sativa* L. 102

List of Appendices

page

Appendix 2.1: Basal nutrient solution (adapted from Andrews, Love and Sprent (1989)) 29

Appendix 2.2: Calibration graph relating initial concentration of nitrate reduced to nitrite using spongy cadmium to the absorbance of the resultant red-azo dye at 543 nm obtained via the method described of Mackereth, Heron and Talling (1978) 30

Appendix 2.3: Calibration graph relating the concentration of nitrite to the absorbance of the resultant red-azo dye at 543 nm obtained via the method described by Mackereth, Heron and Talling (1978) 31

Appendix 2.4: Calibration graph relating the concentration of ammonium to absorbance at 650 nm obtained via the method described by Baethgen and Alley (1989) 32

Appendix 4.1: Leaf DNA content - sample calculation 71

Appendix 6.1: Mean daily temperature and monthly incident solar radiation and precipitation between September 1991 and February 1992 110

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Table of Contents - Chapter 1

General Introduction

page

1.1 Importance of Cereals 2

1.2 Characteristics of Cereal Plant Growth 3

1.3 Plant Growth and Carbon Assimilation 5

1.4 Nitrogen Assimilation 7

1.5 Nitrogen Effects on Plant Growth 8

1.6 Previous Research on Cereals Relevant to the Study 9

1.7 Objectives of the Study 11

1.1 Importance of Cereals

The cereal species are members of a large monocotyledonous family, the Gramineae, which account for a large proportion of worldwide human dietary needs. Their food value lies in having relatively large edible grains which contain carbohydrates, proteins, minerals and fibre in variable amounts, depending on species, cultivar and growing conditions (Kent, 1983). Cereals grow in a wide range of climatic conditions, from the humid tropics to the cold sub-arctic regions. In this thesis, the term 'cereal(s)', unless otherwise stated, refers to those species that normally grow only in the temperate climatic zones. Worldwide, in terms of both area grown and tonnage harvested, wheat (*Triticum aestivum* L.) is the most important cereal species sown, followed by barley (*Hordeum vulgare* L.), rye (*Secale cereale* L.) and oats (*Avena sativa* L.) (Table 1.1). In New Zealand, barley is the most important cereal grown (Table 1.2). All species are grown for both direct human consumption and animal feed. Also, barley is grown extensively to produce malt for use in beer brewing while in regions where rye and oats are forage crops, a substantial proportion of the grain is used for reseeding purposes. In addition to the main cereal species, triticale (X *Triticosecale* Wittmack), an artificially bred intergeneric hybrid, is being grown on an increasing scale. Triticale was originally bred to combine the advantages of superior grain quality, yield and disease resistance of wheat with the hardiness of rye. However, a number of factors, primarily low baking quality, have meant that triticale has not yet developed into a major crop.

Table 1.1 Area and production of cereals harvested worldwide, 1992

	Area cultivated ($x10^6$ ha)	Production ($x10^6$ t)	Yield (t ha^{-1})
wheat	220.6	563.6	2.55
barley	73.4	160.1	2.18
oats	20.5	33.9	1.65
rye	13.4	29.2	2.18
triticale	n.a.	n.a.	n.a.

Source: FAO (1993)

Table 1.2 Area and production of cereals harvested in New Zealand, 1992

	Area cultivated (ha)	Production (t)	Yield (t ha^{-1})
wheat	37,797	191,039	5.05
barley	67,380	318,787	4.73
oats	14,033	57,625	4.10
rye and triticale	1367	4871	3.56

Source: Statistics N.Z. (1994)

1.2 Characteristics of Cereal Plant Growth

Cereals are herbaceous annuals whose development show a distinct vegetative phase followed by a reproductive phase. Some important stages in the development of a typical cereal plant are illustrated in Figure 1.1. Under normal agricultural conditions, where cereal seeds are sown into a prepared seed bed of adequate soil moisture content, plant growth commences with water uptake by the seed (imbibition) which initiates the process of germination and the growth of the embryo. Embryo growth results in the emergence of the primary roots from the caryopsis, followed by the coleoptile (Fig. 1.1a). The coleoptile, the bladeless sheath which surrounds the rest of the shoot, elongates and penetrates the soil to the surface. The first leaf then expands and grows up through the coleoptile. Cereal leaves consist of a tubular structure called the sheath and a planar structure called the lamina with the latter being the main site of photosynthesis (Section 1.3). After the first leaf emerges from the coleoptile, cereal plant growth is characterised by the development of successive leaves from buds on the compressed main stem at the base of the seedling (Fig. 1.1b). These leaves, referred to as the main stem leaves, expand within and then above the sheath of the previous leaf. As the number of main stem leaves increases, tillering, the development of shoot buds in the axils of the main stem leaves, commences (Fig 1.1c). Secondary tillers may form in the axils of these primary tillers. Tillers develop in a similar manner to the main stem, with successive leaves expanding within the sheaths of the previous leaves. Cereals normally produce from seven to 14 main stem leaves and depending on the species, cultivar and environmental conditions, from zero to over 20 tillers, with each tiller having approximately five leaves. Cereal plants also usually have between five

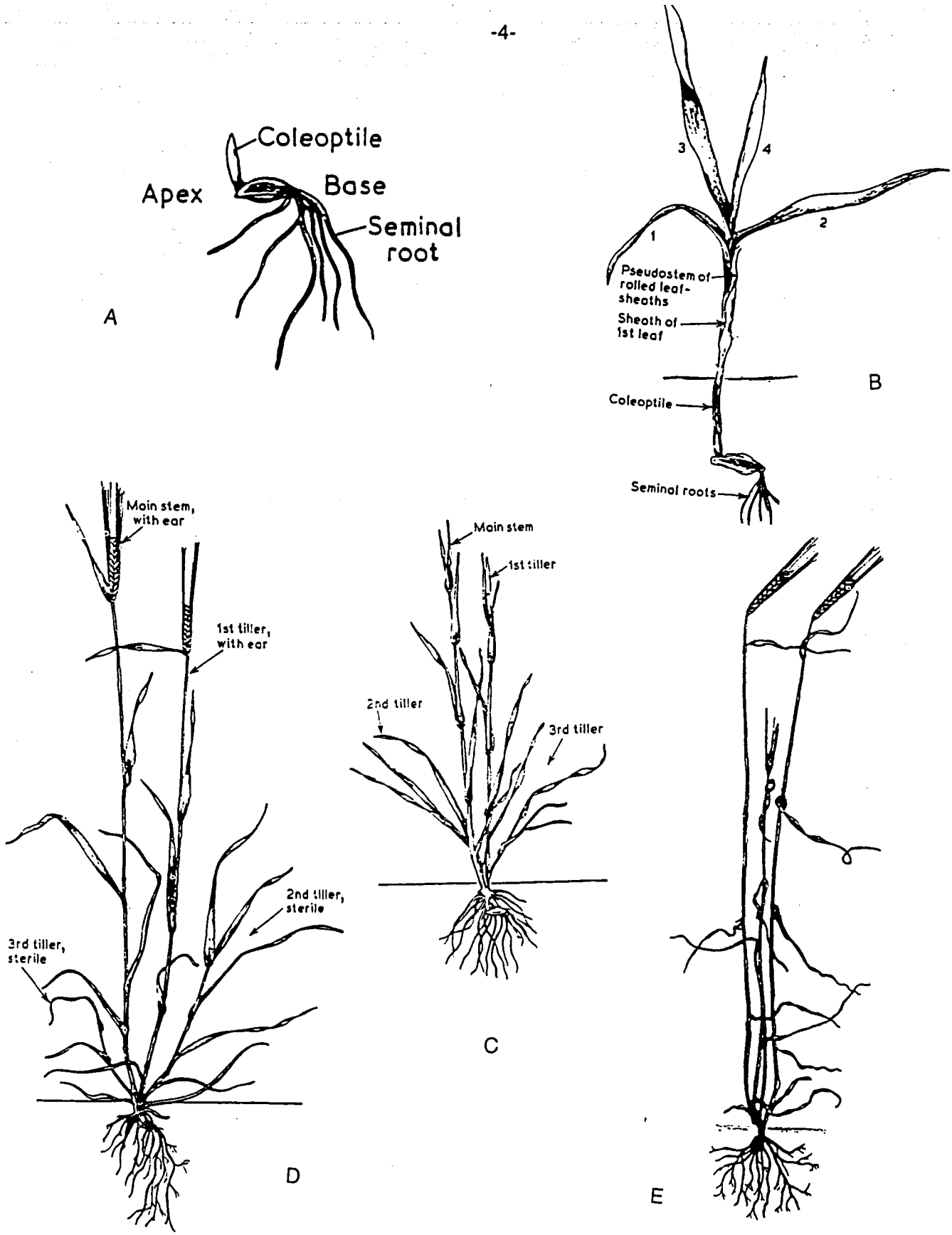


Fig. 1.1 Some growth stages representative of the development of spring sown barley (*Hordeum vulgare* L.): a) seedling prior to prior to emergence; b) young seedling with four main stem leaves; c) plant just after booting with main stem and three tillers; d) plant just after anthesis with fertile main stem, one fertile tiller and two sterile tillers; e) senescent plant with two fully developed heads (from Briggs, 1978; drawings not to scale).

and ten primary (seminal) roots, depending on species, cultivar and growing conditions. These primary roots branch as the plant grows, forming a fibrous mass. In addition, the plant may develop secondary (adventitious) roots which tend to be thicker and less branched than the primary roots. The roots are important for water and nutrient uptake, and also provide support and anchorage for the plant.

During the vegetative phase of cereal development both the main stem and tiller apices are situated near ground level. With the initiation of the reproductive phase, which commences some time before tiller production ceases, the apices develop into inflorescences. As the internodes of the main and tiller stems elongate, the developing inflorescences are raised within and above the surrounding sheaths (Fig. 1.1d). Once above the surrounding leaves, the developed flower opens up, pollination occurs and the grain develops. With the development of the grain, carbohydrates, proteins and other plant constituents are withdrawn from the leaves, stems and roots, leading to senescence of the parent plant (Fig 1.1e).

1.3 **Plant Growth and Carbon Assimilation**

The development of annual plants, such as that described for cereals in Section 1.2, generally involves an increase in total plant dry weight (d.wt) over time; this is usually referred to as growth. As approximately 45% of plant d.wt is carbon (C)(Table 1.3), the amount of C assimilated is an important determinant of plant growth. For terrestrial plants the major source of C is atmospheric carbon dioxide (CO_2), which is fixed via photosynthesis into simple carbohydrates. The following account of photosynthesis has been summarised from Goodwin and Mercer (1983) and Salisbury and Ross (1985). Photosynthesis involves two phases - firstly, the capture and utilization of light energy and secondly, the reduction of CO_2 into carbohydrates. The first phase consists of a series of reactions involving several pigment/protein complexes. Light energy is used to produce chemical energy in the form of reductant such as nicotinamide adenine dinucleotide phosphate-reduced form (NADPH), made by reducing nicotinamide adenine dinucleotide phosphate ($NADP^+$) using electrons from water molecules. Light energy is also used to generate adenosine 5'-triphosphate (ATP) from adenosine 5'-diphosphate (ADP) and $H_2PO_4^-$. The photosynthetic reactions are also used to manufacture lipids which are used in cell membranes.

Table 1.3 Range of elemental composition of healthy, growing plants, including cereals, expressed as % of dry matter (DM) or part per million (ppm) DM. Values obtained from Mengel and Kirkby (1987). Note that composition depends on species, cultivar, element availability, growth stage, plant part and environmental conditions.

Element	Concentration (% DM)
C	40 - 50
O	40 - 50
H	5 - 7
N	1 - 6
K	1.5 - 5
Ca	1 - 3
P	0.2 - 0.4
Mg	0.2 - 0.5
S	0.1 - 0.3
Cl	0.5

Element	Concentration (ppm DM)
Fe	50 - 150
B	20 - 100
Mn	20 - 100
Zn	20 - 100
Cu	5 - 20
Mo	5 - 20
Na	0.5
Co	0.5
Si	0.3

The reducing power of NADPH and the energy contained in ATP are used in the second phase of photosynthesis - the reduction of CO_2 into carbohydrates. This cycle, sometimes referred to as the Calvin or C_3 cycle, uses a number of enzymes, including ribulose 1,5-bisphosphate carboxylase (RUBISCO), to incorporate CO_2 into triose phosphates; these are eventually used to produce sucrose and starch. Fixed C is transported around the plant via the phloem mainly as sucrose. During periods of high photosynthetic activity starch is formed as a temporary storage form of fixed C and it accumulates within the chloroplasts as starch grains. This starch is converted into sucrose during periods of no or low photosynthetic activity and translocated around the plant. In addition, sucrose can be transported to specialized storage sites such as tubers or developing seeds and stored as starch for later use.

Fixed C has two main uses - as a component of plant compounds and as an energy source for plant processes. Carbon is the major component of compounds used in plant structure and biochemical functions. Structural compounds, which frequently make up the major proportion of plant d.wt, include polysaccharides like cellulose which are used to construct cell walls, and lipids, used in cell membranes. Compounds important in biochemical function include nucleic acids, proteins and enzymes (eg. RUBISCO), reductants (eg. NADPH) and energy carriers (eg. ATP).

The other main use of fixed C is as an energy source for cellular/plant processes. This energy is generated through respiration. Respiration is an oxidative process in which complex C containing molecules like carbohydrates are broken down by enzymes into CO_2, water and energy. A high proportion of the liberated energy is conserved via generation of ATP from ADP and $H_2PO_4^-$. The energy stored in ATP is used to maintain plant function and structure (maintenance respiration) and in the building of new structural material (growth respiration).

Because such a large proportion of plant d.wt is C, plant growth is related primarily to the difference between the amount of C fixed in photosynthesis and that used in maintenance and growth respiration. Carbon which is left over is incorporated into structural material or stored and is evident as new growth and a gain in d.wt.

1.4 Nitrogen Assimilation

Plant dry matter usually contains 1 - 6% N, depending on species, age, plant organ and environmental conditions (Table 1.3; Beevers and Hageman, 1983; Haynes *et al.*, 1986; Mengel and Kirkby, 1987). Most terrestrial plants acquire the majority of their N from the soil via the roots. The dominant forms of mineral N available to and taken up by cereals under agricultural conditions are nitrate (NO_3^-) and ammonium (NH_4^+), though the former usually predominates under temperate agricultural conditions (Haynes *et al.*, 1986). Nitrate, once taken up, is reduced in either the root or shoot. Reduction is carried out by the enzymes nitrate reductase ($NO_3^- \rightarrow NO_2^-$) and nitrite reductase ($NO_2^- \rightarrow NH_4^+$)(Layzell, 1990). Nitrate taken up in excess of the plant's NO_3^- reduction capacity can be stored in the vacuoles of either the root or shoot cells (Granstedt and Huffaker, 1982). Ammonium, both that resulting from the reduction of NO_3^- and that taken up from the soil, is converted into the amino acid glutamate via the coupled reactions involving the amino acid glutamine and two enzymes: glutamine synthetase (GS) and glutamine(amide):2-oxoglutarate amino transferase (commonly known as GOGAT or glutamate synthase)(Goodwin and Mercer, 1983; Layzell, 1990). The glutamate produced by the GS/GOGAT cycle is transformed by various aminotransferase enzymes into different amino acids and used to construct N containing compounds (Layzell, 1990). Ammonium, in contrast to NO_3^-, does not normally accumulate in plant tissues and is rapidly assimilated through the GS/GOGAT pathway (Layzell, 1990). Accumulation of NH_4^+ in plant tissue can cause damage and a decrease in plant growth (Mehrer and Mohr, 1989).

Increasing availability of external N, either as NO_3^- or NH_4^+, generally leads to increased N uptake and assimilation by the plant. Assimilation of exogenous NH_4^+ nearly always occurs in the root while the site of NO_3^- assimilation depends on the species, external NO_3^- concentration and other environmental conditions (Andrews, 1986). For cereals, at low external concentrations (around 1 mol m^{-3}) nearly all the NO_3^- taken is up is assimilated in the root; with increasing external concentrations of NO_3^- in the range likely to occur in agricultural soils (1 - 20 mol m^{-3}), a greater proportion of the assimilation occurs in the shoot (Andrews *et al.*, 1992). Increased N assimilation generally leads to higher plant N levels which, as discussed below, can have marked effects on plant growth.

1.5 Nitrogen Effects on Plant Growth

Though N comprises a relatively small fraction of plant d.wt, its availability to plants has a large influence on their growth. This is mainly due to N being a component of most constituents vital for plant function such as nucleotides, amino acids, pigments, proteins, cell membranes and cell walls. Therefore the rate and/or extent of processes that utilize these compounds, which includes most plant activities, will be affected by the plant N status. Notable among these processes is photosynthesis, which, as the major input of C into plants, largely determines the extent of their growth (Section 1.3). Plant photosynthetic capacity is determined by two factors: the rate of photosynthesis per unit leaf area and the amount of photosynthetically active radiation (PAR) intercepted. Most compounds important in photosynthesis, such as chlorophyll and RUBISCO, contain significant amounts of N (Salisbury and Ross, 1985) and increased uptake and assimilation of N by the plant enables greater production of these compounds. This generally increases their concentration in plant tissues and usually results in a greater rate of photosynthesis per unit leaf area, with the magnitude of the response to additional N depending on the N status of the plant.

The other determinant of plant photosynthetic capacity is the fraction of incident PAR intercepted by the plant's leaves. Total plant leaf area depends on both the size of individual leaves and the total number of leaves. Over a period of time, production of new leaf material is a function of the amount of C fixed per unit existing leaf area, the fraction of this C that is used for maintenance and growth respiration and the extent to which the C left over for growth is partitioned to the leaves relative to other plant parts. Hence, if both the rate of respiration and the fraction of dry matter partitioned to the leaves remain constant, greater rates of photosynthesis with increasing

levels of external and plant N generally increases the amount of leaf produced. This in turn increases the fraction of available PAR intercepted, leading to greater photosynthetic capacity and increased overall plant growth.

1.6 Previous Research on Cereals Relevant to the Study

The influence of N availability on the leaf area of cereals is the general theme of this study. In this section, previous relevant work on N effects on cereals is discussed briefly prior to stating the objectives of the thesis. Greater discussion of the literature is given in the relevant chapters.

Nitrogen availability can affect the leaf area and hence growth of cereals at most stages of their development. Carbon for the growth of leaf 1 and the rest of the seedling prior to its emergence from the substrate and commencement of photosynthetic activity is derived primarily from starch contained in the endosperm of the seed. Previous studies have shown that before and after emergence, the level of endogenous seed N, as well as the availability of exogenous N, can affect the growth of leaf 1 of cereals. For example, wheat seedlings grown from high N seed had greater total plant d.wt and area of main stem leaves 1 - 3 than those from low N seed (Lowe and Ries, 1972; 1973). For barley harvested 6 d after sowing, seedlings from high N seed had a greater rate of reserve mobilization, total plant d.wt, area of leaf 1, leaf protein concentration and photosynthetic rate (Metivier and Dale, 1977a;b). In addition, exogenous N supplied as NO_3^- has been shown to increase the rate of mobilization of seed reserves in barley and wheat grown in darkness or prior to emergence from the substrate, resulting in seedlings with greater shoot and total d.wt (Nátr, 1988; Andrews, Scott and M^{c}Kenzie; 1991). However, the mechanism(s) underlying the effects of seed N content and additional NO_3^- on enhanced mobilisation of seed reserves and greater seedling growth are not known and need further investigation.

A review of the available data on the effects of N on the growth of individual leaves of cereals indicates that, generally, additional N increases leaf area, though the magnitude of the response can depend on species, leaf position and environmental conditions. For example, in a recent study, Andrews, M^{c}Kenzie and Jones (1991) found that for main stem leaves 1 - 4 of a range of cereals, including wheat and barley, additional N as NO_3^- increased mean extension rate 75 - 120%, final length 80 - 100% and area 50 - 150%. Most studies investigating the effects of N on leaf growth supplied N as NO_3^-, this being the dominant form of N available to and taken up by cereals under temperate agricultural conditions (Section 1.4). However, cereals can also take up

and assimilate NH_4^+. Levels of NH_4^+ in the soil can be significant and when rates of nitrification are low NH_4^+ may be the main form of N available. Depending on external N concentration, the processes of translocation, storage and assimilation of NO_3^- and NH_4^+ can be different. For example, NH_4^+ is almost exclusively assimilated in the root, whereas the site of NO_3^- assimilation depends on the external concentration. Also, if the uptake of NO_3^- is greater than the assimilation capacity, NO_3^- can be stored while in healthy plants NH_4^+ rarely accumulates. Though factors such as these could possibly affect leaf growth, no reports were found which compared NO_3^- and NH_4^+ with respect to the effects on individual leaf growth characteristics. However, in a comparison of the effects of NO_3^- and NH_4^+ supply on plant growth, Lips *et al.* (1990) suggested that the form of N supplied to plants affected the rates of leaf expansion more than the photosynthetic rate of their chloroplasts.

At the cellular level, greater individual leaf area with additional N is generally associated with increases in both cell size and cell number per leaf (eg. Morton and Watson, 1948; Lawlor, Kontturi and Young, 1989). However, the magnitude of the response seems to depend on the level of N addition, the species being investigated, cell type examined and leaf position. The latter is especially important as leaves tend to get larger with increasing main stem leaf position. This effect is usually associated with a greater cell number (eg. Milthorpe and Newton, 1963; Steer, 1971). Also, there is generally a positive interaction between the increase in area with leaf position and the availability of N with the increase in area of successive leaves being larger with additional N (Puckeridge, 1956; cited in Bunting and Drennan, 1966). However, as no studies were found which investigated cell size and number with increasing leaf position and various levels of N, the cellular basis for this interaction has not been established.

Nitrogen availability can affect the partitioning of dry matter to the leaf, stem and root of cereals from the seedling stage through to maturity (Hocking and Meyer, 1991). Usually both shoot to root d.wt ratio (S:R) and leaf weight ratio (leaf d.wt as a fraction of total plant d.wt) increase with NO_3^- supply, regardless of its effect on growth (Andrews, 1993 and references therein). However, the mechanism of the N effect on dry matter partitioning is not known. For a range of species supplied NO_3^-, S:R was positively correlated with tissue N content (eg. Hirose, 1986; Ingestad and Agren, 1991; Boot, Schildwacht and Lambers, 1992). Also, several reports indicate that for a similar total plant d.wt, both S:R and plant reduced N content are greater with NH_4^+ than with NO_3^- (Cox and Reisenhauer, 1973; Bowman and Paul, 1988; Troelstra, Wagenaar and Smant; 1992). Thus it is possible that the relationships between S:R and tissue N content hold regardless whether N is supplied as NO_3^- or NH_4^+.

Under field conditions where soil moisture is adequate, low N availability is usually the soil factor limiting the yield of cereals and it is common practice to use fertilizer N to achieve greater grain production and profits. Grains are composed mainly of carbohydrates (Kent, 1983). As the main products of photosynthesis are carbohydrates (Section 1.3), the overall effect of additional N on yield is to increase the level of crop photosynthesis, mainly through greater interception of available PAR by the canopy (Monteith, 1977). Many studies have established the relationships between N availability, canopy development, interception of PAR and dry matter accumulation. However, these relationships have been established only for a limited number of species, usually wheat and barley and hence it is difficult to assess whether the crop response to additional N is similar for all cereal species.

1.7 Objectives of the Study

This thesis had five main objectives:

1. To gain a greater understanding of the mechanism(s) of the NO_3^- and seed N effects on the mobilisation of seed reserves in cereals.

2. To determine whether the characteristics of individual leaf growth are similar with different N forms.

3. To assess the changes in the cellular aspects of individual leaf area with additional NO_3^- and for successive main stem leaves.

4. To determine whether the form of applied N affects the relationship between plant reduced N content and the partitioning of dry matter between shoot and root.

5. To establish whether different cereal species, grown under comparable environmental conditions, behave similarly with regard to canopy development, dry matter accumulation and final grain yield and quality in response to additional N.

Table of Contents - Chapter 2

Nitrogen Effects on the Mobilisation of Seed Reserves in Temperate Cereals

page

2.1 Introduction 13

2.2 Materials and Methods 14

2.3 Results 18

2.4 Discussion 25

2.5 Conclusions 28

Appendix 2.1 29

Appendix 2.2 30

Appendix 2.3 31

Appendix 2.4 32

2.1 Introduction

Studies have shown that for both monocotyledonous and dicotyledonous species, seed nitrogen (N) content and the availability of external N are positively related to early seedling growth (eg. Schweizer and Ries, 1969; Dale, 1972; Lowe and Ries, 1973; Rahman and Goodman, 1983; Tremblay and Senecal, 1988; Fenner and Lee, 1989; Wood, 1990). For *Triticum aestivum* L. (wheat) seedlings 21 d after sowing (DAS), total plant dry weight (d.wt) and area of main stem leaves 1 - 3 were greater for high N seed than for low N seed (Lowe and Ries, 1972, 1973). For *Hordeum vulgare* L. (barley) harvested 6 d after sowing, seedlings from high N seed, in comparison with those from low N seed, had greater reserve mobilization, total plant d.wt, area of leaf 1, leaf protein concentration and photosynthetic rate (Metivier and Dale, 1977a,b). Additional N as nitrate (NO_3^-) had little effect on seedlings from high N seed, but increased the growth rate of seedlings from low N seed and, if applied early (2 d after sowing), resulted in similar growth rates for the two seed lines (Metivier and Dale, 1977b). It was proposed that additional NO_3^- resulted in increased levels of organic N which compensated in some way (probably via photosynthesis) for low levels of endogenous N in low N seed. In a related experiment (Dale, Felippe and Marriott, 1974) barley seedlings were supplied N as ammonium (NH_4^+) and though it was concluded that NH_4^+ was similar to NO_3^- in its effects on growth, root damage due to NH_4^+ toxicity makes these results difficult to interpret.

Recently, additional NO_3^- has been shown to increase the rate of mobilization of seed reserves of barley and wheat grown in darkness or prior to emergence from the substrate (Nátr, 1988; Andrews, Scott and M^{c}Kenzie, 1991). As photosynthesis was probably negligible under these experimental conditions, the positive effects of NO_3^- on seedling growth could not be related to enhanced photosynthetic activity. For wheat seedlings, shoot NO_3^- reductase activity (NRA) was approximately 80% lower in darkness than in light, indicating that their ability to assimilate NO_3^- was low but reduced N was not determined to confirm this (Andrews, Scott and M^{c}Kenzie, 1991).

The primary objective of the present study was to gain greater understanding of the mechanism of the NO_3^- effect on mobilisation of seed reserves in temperate cereals prior to emergence. Initially, in order to determine the relationships between N supply, uptake and assimilation and the rate of reserve mobilisation, the growth and N content of seedlings supplied no N, NO_3^- or NH_4^+ were compared. Seedlings supplied either NO_3^- or NH_4^+ took up and assimilated similar amounts of N but increased mobilisation of seed reserves occurred with NO_3^- only. As seedling water content was greater with NO_3^-, further experiments were conducted to examine the effects

of other N forms and inorganic ions and seed N content on seedling water uptake and reserve mobilisation. Data from these experiments were used to develop an hypothesis setting out a possible mechanism by which NO_3^- enhances seed reserve mobilisation.

2.2 Materials and Methods

a) Plant material and growth conditions

Seed of oats (*Avena sativa* L. cv. Amuri; mean seed weight, 32 mg), triticale (X *Triticosecale* Wittmack cv. Aranui, 54 mg) and wheat (cv. Otane; 51 mg) was obtained from Hodder and Tolley Ltd. Christchurch, New Zealand. Barley (cv. Triumph, 44 mg) and rye (*Secale cereale* L. cv. Rapaki, 28 mg) seed was obtained from the New Zealand Institute for Crop and Food Research Ltd., Lincoln, New Zealand. For all species in experiments 1 and 2 and barley in experiments 3, 4 and 5 individual seed weight used was mean seed weight ±1 mg. In experiments 6 and 7, two seed lines of barley with the same range of individual seed weights (46 to 48 mg) but different mean seed N contents (1.4 and 1.9%) were used. These low and high N seed lines were obtained from the field experiment reported in Chapter 6 in which fertilizer N was varied. Seed of all species and lines showed >95% germination and was not chemically treated. All experiments were carried out in the dark at 10±1°C in controlled environment chambers.

In all experiments except experiment 6, seeds were placed at 70 mm depth in 80 mm diameter, 180 mm tall pots (20 per pot) filled with a vermiculite/perlite (1:1, v/v) mixture soaked in basal nutrient solution (Appendix 2.1) containing the appropriate treatment. Pots were flushed with the appropriate nutrient solution every 2 d. Experiment 6 was carried out in petri dishes. Seeds were placed on filter paper and kept moist with the addition of the appropriate solution every 2 - 3 d. For all treatments potassium (K^+) was maintained at 23.6 mol m^{-3} for experiment 1 and 8.6 mol m^{-3} for all other experiments by the addition of potassium sulphate where necessary.

b) Experiment 1

All five species were supplied basal nutrient solution only or with 1.0, 5.0 or 20.0 mol m^{-3} NO_3^- as potassium nitrate (KNO_3) added. At harvest, 21 DAS, plants were separated into shoot, root and residual seed. Shoot and root fresh weight (f.wt) were determined and all plant parts were dried separately at 70°C for 4 d and weighed. Dried shoot and root material was ground and an

aqueous extract of a 10-30 mg sample was analysed for NO_3^- content as described by Mackereth, Heron and Talling (1978). Briefly, the method involved the reduction of NO_3^- to nitrite (NO_2^-) using spongy cadmium (Cd); the NO_2^- was then determined spectrophotometrically. Cadmium metal was generated electrochemically by placing zinc (Zn) rods in a 20% w/v solution of cadmium sulphate; after standing overnight, the resulting Cd metal was scraped off the Zn rods and divided into small particles using a spatula. The spongy Cd was washed using 2% v/v hydrochloric acid (HCl - diluted from concentrated acid), then rinsed several times with distilled water. To a 5 ml sample of the aqueous plant material extract contained in a cappable 25 ml vial, 3 ml of a 2.6% w/v aqueous solution of ammonium chloride and 1 ml of a 2.1% w/v aqueous solution of sodium tetraborate were added, followed by approximately 0.6 g of spongy Cd. The vial was capped and shaken for 1 hr on an orbital shaker. The concentration of NO_2^- in the sample was determined by taking a 5 ml subsample of the mixture, transferring it to a 12 ml polypropylene test tube and adding 1 ml of a 1% w/v (10% v/v HCl) solution of sulphanilamide. After mixing by swirling and then standing for 5 min, 1 ml of 0.1% w/v aqueous solution of N-1-naphthylethylenediamine dihydrochloride was added and mixed. The test tube was spun at 5000 rpm for 5 min and left to stand for 10 min. The resultant red azo-dye was determined spectrophotometrically at 543 nm. A calibration graph (Appendix 2.2) was prepared using a dilution series from a standard NO_3^- solution and a mean factor relating NO_2^- concentration in the cuvette to absorbance was determined.

c) Experiment 2

All species were supplied either basal nutrient solution alone, or with 5 mol m^{-3} NO_3^- as KNO_3 or 5 mol m^{-3} NH_4^+ as ammonium sulphate ($(NH_4)_2SO_4$) added. Twenty-one DAS, plants were harvested and divided into root, shoot and residual seed for f.wt determination. A sub-sample of fresh root and shoot was then analysed for NRA using an *in vivo* assay (Andrews *et al.*, 1992). Briefly, a known weight of approximately 0.5 g f.wt of the appropriate plant part was vacuum infiltrated for 10 min with 5 ml of a 100 mol m^{-3} sodium phosphate buffer (pH 7.6) containing 4% v/v propan-1-ol and 50 mol m^{-3} KNO_3. After removal of a time zero sample (1 ml), the mixture was incubated at 30°C for 30 min in the dark and then a final 1 ml sample was taken. Both the time zero and final samples were analysed for NO_2^- as described for the NO_3^- assay. A calibration graph (Appendix 2.3) was prepared using a dilution series from a standard NO_2^- solution and a mean factor relating NO_2^- concentration in the cuvette to absorbance was determined. The difference between the zero time and final NO_2^- concentrations was taken as NRA, which was expressed as µmol NO_2^- g $f.wt^{-1}$ hr^{-1}.

For plants supplied NO_3^- an *in vitro* assay was also carried out (Wallace, 1986). Shoot and root material (0.5 g f.wt) was ground in liquid N and resuspended in 5 ml of an extraction buffer containing 200 mol m^{-3} Tris-HCl (pH 8.5), 50 mol m^{-3} potassium phosphate (pH 8.5), 5 mol m^{-3} EDTA, 1 mol m^{-3} cysteine, 10 mmol m^{-3} flavin adenine dinucleotide, 2.5% w/v Polycar AT and 1% w/v casein. After centrifuging (5000 rpm for 10 min), 0.1 ml of the supernatant was added to an assay mixture containing 0.5 ml 100 mol m^{-3} potassium phosphate buffer (pH 7.5), 0.1 ml 50 mol m^{-3} KNO_3, 0.2 ml distilled water and 0.1 ml 2 mol m^{-3} nicotinamide adenine dinucleotide phosphate in a phosphate buffer. The mixture was incubated at 25°C for 15 min. The reaction was stopped by adding 1 ml of a 1% w/v (10% v/v HCl) solution of sulphanilamide. Nitrite was determined as in the NO_3^- assay.

The remaining plant material was dried, weighed and ground and an aqueous extract of a 10-30 mg sample analysed for NO_3^- (as described above) and NH_4^+ content. Ammonium was determined by adapting the method of Baethgen and Alley (1989). Briefly, to 1 ml of the aqueous extract contained in a 50 ml test tube, 5 ml of a buffer solution containing 2.68% w/v sodium di-hydrogen phosphate, 5% w/v sodium-potassium tartrate and 5.40% w/v sodium hydroxide were added and mixed. To this, 4 ml of a mixture containing 15% w/v sodium salicylate and 0.03% w/v sodium nitroprusside were added and mixed. Lastly, 2 ml of 0.32% v/v sodium hypochlorite solution (prepared by diluting 6 ml of a 5.25% v/v sodium hypochlorite solution to 100 ml) was added and mixed. The mixture was allowed to stand for 45 min at 25°C and the absorbance at 650 nm of the resultant colour determined using a spectrophotometer. A series of standard NH_4^+ solutions were assayed with every batch of determinations and a mean factor relating NH_4^+ concentration in the cuvette to absorbance was determined. A representative calibration graph, prepared using a dilution series from a standard NH_4^+ solution, is presented in Appendix 2.4. Also, a 30-60 mg sample of dried material was analysed for total N content using a Europa Scientific (U.K.) N analyser. Assimilated N was assumed to be the difference between total N and NO_3^- plus NH_4^+-N.

d) Experiment 3

Barley was supplied basal nutrient solution alone or with 5 or 20 mol m^{-3} NO_3^- as KNO_3, 5 or 20 mol m^{-3} NH_4^+ as $(NH_4)_2SO_4$, 5 or 20 mol m^{-3} urea, 5 or 20 mol m^{-3} thiourea, 5 or 20 mol m^{-3} chloride (Cl^-) as potassium chloride (KCl), 5 or 20 mol m^{-3} chlorate (ClO_3^-) as potassium chlorate added. In addition, a nutrient solution containing 5 mol m^{-3} NO_3^- as KNO_3 and 50 mmol m^{-3} tungsten (W) added as $Na_2WO_4 \cdot 2H_2O$ in place of the micronutrient molybdenum (Mo) (Appendix

2.1) was used. Tungsten can compete with Mo for incorporation into the nitrate reductase enzyme complex and results in enzyme inactivation (Deng, Moureaux and Caboche, 1989). At harvest, 21 DAS, plants were divided into shoot, root and residual seed and f.wt and d.wt of the parts determined. Also, a sub-sample of the shoot and root of freshly harvested seedlings supplied either basal nutrient solution, 5 mol m^{-3} NO_3^- or 5 mol m^{-3} NO_3^- plus W was assayed for *in vivo* NRA as described in experiment 2.

e) Experiment 4

Barley was supplied basal nutrient solution alone or with 5 mol m^{-3} NO_3^- as KNO_3 or 5 mol m^{-3} Cl^- as KCl added. Plants were harvested 21 DAS and shoot, root and residual seed f.wt and d.wt were determined. Aqueous extracts of 10 - 30 mg d.wt samples were analysed for NO_3^-, Cl^-, phosphate and sulphate content using a Waters (Massachusetts, U.S.A) 712 WISP ion exchange column. Standards containing known concentrations of the measured anions were used.

f) Experiment 5

Barley was supplied either a basal nutrient solution alone or with 5 mol m^{-3} NO_3^- as KNO_3 added. Seedlings were harvested 7, 12, 16, 19, 21, and 24 DAS. Shoot, root and residual seed f.wt and d.wt were determined at each harvest. In addition, the NO_3^- content of the residual seed was also determined for each harvest date.

g) Experiment 6

Experiment 6 was carried out in petri dishes (10 seeds per dish). Low and high N content barley seed lines were supplied a basal nutrient solution only. Plants were harvested at 2, 4, 8, 12 and 16 DAS and residual seed f.wt and d.wt were determined.

h) Experiment 7

Low and high N content barley seed was grown in pots as in experiments 1 - 5 and supplied either a basal nutrient solution alone or with 5 mol m^{-3} NO_3^- as KNO_3 added. Plants were harvested 10 and 14 DAS and residual seed and shoot plus root f.wt and d.wt were determined.

i) Experimental design and analyses

All experiments were of a randomised complete block design. In experiment 1 all treatments were replicated five times, experiments 2, 3 and 4 six times while in experiments 5, 6 and 7 all treatments had six replicates per harvest. An analysis of variance was carried out on all data using "Statistix" (Analytical Software, St.Paul, MN, U.S.A). All effects discussed have a probability of $P<0.05$ and were obtained in repeat experiments. Means stated as significantly different are on the basis of an LSD ($P<0.05$) test.

2.3 Results

For all species at harvest (21 DAS) in experiment 1, shoot plus root f.wt increased similarly with increased applied NO_3^- concentration from 0 to 5 mol m^{-3} then either increased further or changed little with additional NO_3^- to 20 mol m^{-3} (Fig. 2.1). Increased shoot plus root d.wt was associated with a corresponding decrease in residual seed d.wt. Maximum increases in shoot plus root d.wt with additional NO_3^- were around 10% for triticale, 15% for wheat and barley and 20% for rye and oats. For all species, shoot and root NO_3^- content increased 50 - 100 fold with increased NO_3^- supply to 20 mol m^{-3} (Fig. 2.1).

In experiment 2, the effects of additional NO_3^- or NH_4^+ on mobilisation of seed reserves and water content, NRA and N content of seedlings were similar for all species (Table 2.1). Addition of 5 mol m^{-3} NO_3^- caused an increase in root plus shoot d.wt and a decrease in residual seed d.wt but 5 mol m^{-3} NH_4^+ did not affect the rate of mobilisation of seed reserves. Also, additional NO_3^- but not NH_4^+ often caused an increase in root and shoot water content (%water) and always caused an increase in residual seed water content. *In vivo* NRA in shoots increased with additional NO_3^- but was not affected by NH_4^+. At 5 mol m^{-3} NO_3^-, *in vitro* NRA was 2-3 times greater than *in vivo* NRA for oats and rye but for other species activity was similar with the two assays. Total N uptake and assimilation were as great with NH_4^+ as with NO_3^-. Depending on species, NO_3^--N constituted 8 - 19% of total plant N in NO_3^- fed plants but was always around 1% or less of total plant N in NH_4^+ fed plants. Ammonium-N was around 0.1% of total plant N in all treatments.

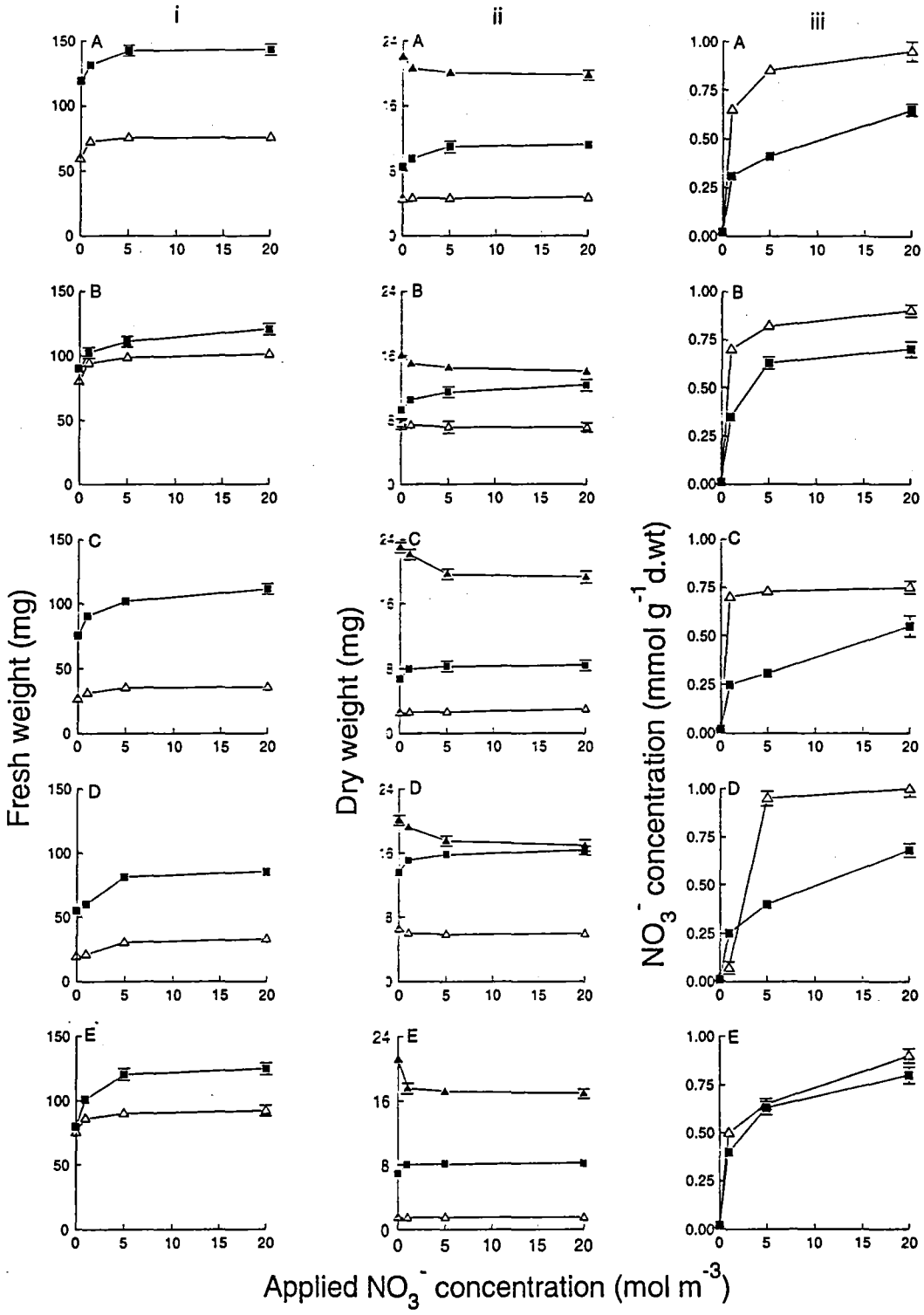


Fig. 2.1 Effect of different applied nitrate (NO_3^-) concentrations on the fresh weight, dry weight (d.wt) and NO_3^- content of the root (Δ) and shoot (■) and d.wt of the residual seed (▲) of 21 d old seedlings of *Avena sativa* L. (A), *Hordeum vulgare* L. (B), *Secale cereale* L. (C), *Triticosecale* Wittmack (D) and *Triticum aestivum* L. (E) seedlings prior to emergence from the substrate. Error bars indicate ± standard error of mean where larger than symbol.

Table 2.1 Effect of additional ammonium (NH_4^+) or nitrate (NO_3^-) on the dry weight and water content of shoot (S), residual seed (RS) and root (R); shoot and root *in vivo* nitrate reductase activity (NRA) and total plant nitrogen (N) of 21 d old seedlings of *Avena sativa* L. (oat), *Hordeum vulgare* L. (barley), *Secale cereale* L. (rye), *Triticosecale* Wittmack (triticale) and *Triticum aestivum* L. (wheat) prior to emergence from the substrate. Values in parentheses are *in vitro* NRA; SE = standard error of mean.

	Dry weight (mg)			Water (%)			NRA (µmol NO_2^- g^{-1} d.wt h^{-1})				Nitrogen (µg $seedling^{-1}$)		
Oat	S	RS	R	S	RS	R	S		R		Total	NO_3^--N	NH_4^+-N
basal	10.2	13.4	2.3	90.6	76.6	92.7	0.27		0.90		807	7.2	0.5
NH_4^+	10.2	13.2	2.6	90.8	78.1	92.9	0.35		0.74		992	7.6	1.5
NO_3^-	12.4	11.2	2.6	91.4	80.8	92.9	0.59	(1.88)	0.71	(1.96)	1002	165.4	0.5
SE	0.34	0.33	0.09	0.11	0.35	0.13	0.10	(0.16)	0.42	(0.08)	32	4.5	0.1
Barley													
basal	8.7	14.1	6.4	90.8	76.1	93.5	3.51		2.98		611	5.1	0.5
NH_4^+	8.4	14.5	6.0	91.6	76.0	93.3	5.14		3.11		920	4.6	1.4
NO_3^-	10.3	12.5	6.3	92.6	78.1	94.1	23.4	(19.55)	5.21	(7.60)	977	186.9	0.7
SE	0.29	0.41	0.14	0.13	0.34	0.15	2.01	(0.68)	0.32	(0.78)	15	10.2	0.5
Rye													
basal	6.4	21.1	2.1	91.6	67.2	93.8	0.34		4.45		821	9.2	0.3
NH_4^+	6.3	21.5	2.2	91.2	65.7	93.7	0.52		4.68		940	8.9	0.9
NO_3^-	7.9	18.8	2.4	91.8	68.8	94.4	1.06	(4.11)	6.45	(13.22)	976	75.4	0.4
SE	0.25	0.45	0.11	0.17	0.25	0.12	0.21	(0.79)	0.80	(0.96)	18	2.7	0.3
Triticale													
basal	13.5	17.7	5.3	88.2	76.3	93.4	1.25		5.51		834	11.8	0.4
NH_4^+	13.0	18.1	5.4	88.5	76.4	93.7	1.32		3.21		1167	10.0	1.6
NO_3^-	15.7	15.9	5.4	89.1	78.0	93.7	3.71	(3.15)	4.02	(3.97)	1177	191.3	0.8
SE	0.31	0.50	0.17	0.07	0.31	0.10	0.68	(0.19)	0.60	(0.60)	13	8.4	0.4
Wheat													
basal	9.5	20.2	4.7	88.6	67.4	92.8	0.31		1.31		933	14.7	0.9
NH_4^+	9.0	20.9	4.8	89.0	65.8	93.2	0.51		1.52		1175	14.5	1.3
NO_3^-	11.8	17.9	4.8	89.6	69.4	94.1	3.08	(3.10)	5.14	(4.85)	1181	163.2	0.7
SE	0.24	0.66	0.39	0.12	0.29	0.16	0.75	(0.30)	0.97	(0.16)	11	6.5	0.1

Table 2.2a Effects of basal nutrient solution alone or with added 5 or 20 mol m^{-3} ammonium (NH_4^+), nitrate (NO_3^-), NO_3^- with tungsten in place of molybdenum (NO_3^- (W); see text), urea (CH_4N_2O), thiourea (CH_4N_2S), chloride (Cl^-) or chlorate (ClO_3^-) on shoot (S) and root (R) fresh weight (f.wt) and d.wt and residual seed (RS) d.wt of 21 d old seedlings of *Hordeum vulgare* L. prior to emergence from the substrate. Standard error of mean (SE) is given.

Applied treatment	F.wt (mg)		D.wt (mg)		
	S	R	S	RS	R
basal	106.2	134.0	10.7	17.6	8.09
5 NH_4^+	108.6	128.5	10.8	17.8	8.01
20 NH_4^+	99.2	119.3	10.1	18.1	7.92
5 NO_3^-	163.6	126.9	14.8	13.7	7.91
20 NO_3^-	168.8	132.6	14.9	13.9	8.00
5 NO_3^- (W)	148.4	125.7	14.5	14.2	8.12
20 NO_3^- (W)	162.5	130.4	15.2	13.6	8.05
5 CH_4N_2O	105.6	119.5	11.2	16.9	7.96
20 CH_4N_2O	108.6	125.6	11.5	17.2	8.12
5 CH_4N_2S	112.9	101.9	11.9	17.8	7.19
20 CH_4N_2S	90.1	58.5	9.2	21.9	4.64
5 Cl^-	133.6	139.2	12.5	14.6	8.21
20 Cl^-	133.0	142.6	12.1	14.7	8.28
5 ClO_3^-	23.7	8.5	2.5	29.9	0.83
20 ClO_3^-	18.9	7.5	1.8	30.1	0.75
SE	4.4	3.2	0.32	0.48	0.20

Table 2.2b The effect of basal nutrient only or with 5 mol m^{-3} nitrate (NO_3^-) or 5 mol m^{-3} NO_3^- plus tungsten in place of molybdenum (NO_3^- plus W; see text) added on shoot (S) and root (R) *in vivo* NO_3^- reductase activity (NRA) of 21 d old seedlings of *Hordeum vulgare* L. seedlings prior to emergence from the substrate. Standard error (SE) of mean is given.

	NRA (µmol NO_2^- g $d.wt^{-1}$ h^{-1})	
	S	R
basal	1.63	2.03
NO_3^- plus W	5.63	4.36
NO_3^-	15.22	8.52
SE	0.25	0.32

Table 2.3a Effect of additional nitrate (NO_3^-) or chloride (Cl^-) on dry weight, and water content of shoot (S), residual seed (RS) and root (R) of 21 d old seedlings of *Hordeum vulgare* L. prior to emergence from the substrate. Standard error of mean (SE) is given.

	Dry weight (mg)			Water (%)		
Treatment	S	RS	R	S	RS	R
basal	11.1	12.8	5.8	91.0	69.5	94.1
NO_3^-	13.5	9.7	5.2	91.8	72.4	94.4
Cl^-	12.5	10.5	5.7	91.7	72.4	94.4
SE	0.41	0.51	0.35	0.15	0.24	0.12

Table 2.3b Nitrate (NO_3^-), chloride (Cl^-), sulphate (SO_4^{-2}) and phosphate (PO_4^{-3}) content of the shoot, residual seed and root of 21 d old *Hordeum vulgare* L. seedlings prior to emergence supplied basal nutrient solution only or with 5 mol m^{-3} NO_3^- or Cl^- added. Standard error of mean (SE) is given.

		Anion content (µmol g $d.wt^{-1}$)				
		NO_3^-	Cl^-	SO_4^{-2}	PO_4^{-3}	Total
Shoot	basal	2.3	115.4	434.8	363.4	916.0
	NO_3^-	958.8	134.2	208.7	379.0	1680.5
	Cl^-	4.5	994.3	162.9	314.8	1476.5
	SE	10.7	25.3	31.5	36.5	25.2
Residual seed	basal	0.5	14.4	26.5	26.5	89.9
	NO_3^-	55.3	14.2	9.9	23.5	134.1
	Cl^-	1.1	63.1	23.5	9.9	122.2
	SE	6.1	3.2	2.3	5.6	5.3
Root	basal	17.6	103.4	180	422.7	1044.7
	NO_3^-	1779.0	38.1	87	159.6	2231.8
	Cl^-	28.5	968.9	104	137.7	1531.6
	SE	22.1	37.5	12.6	18.5	23.5

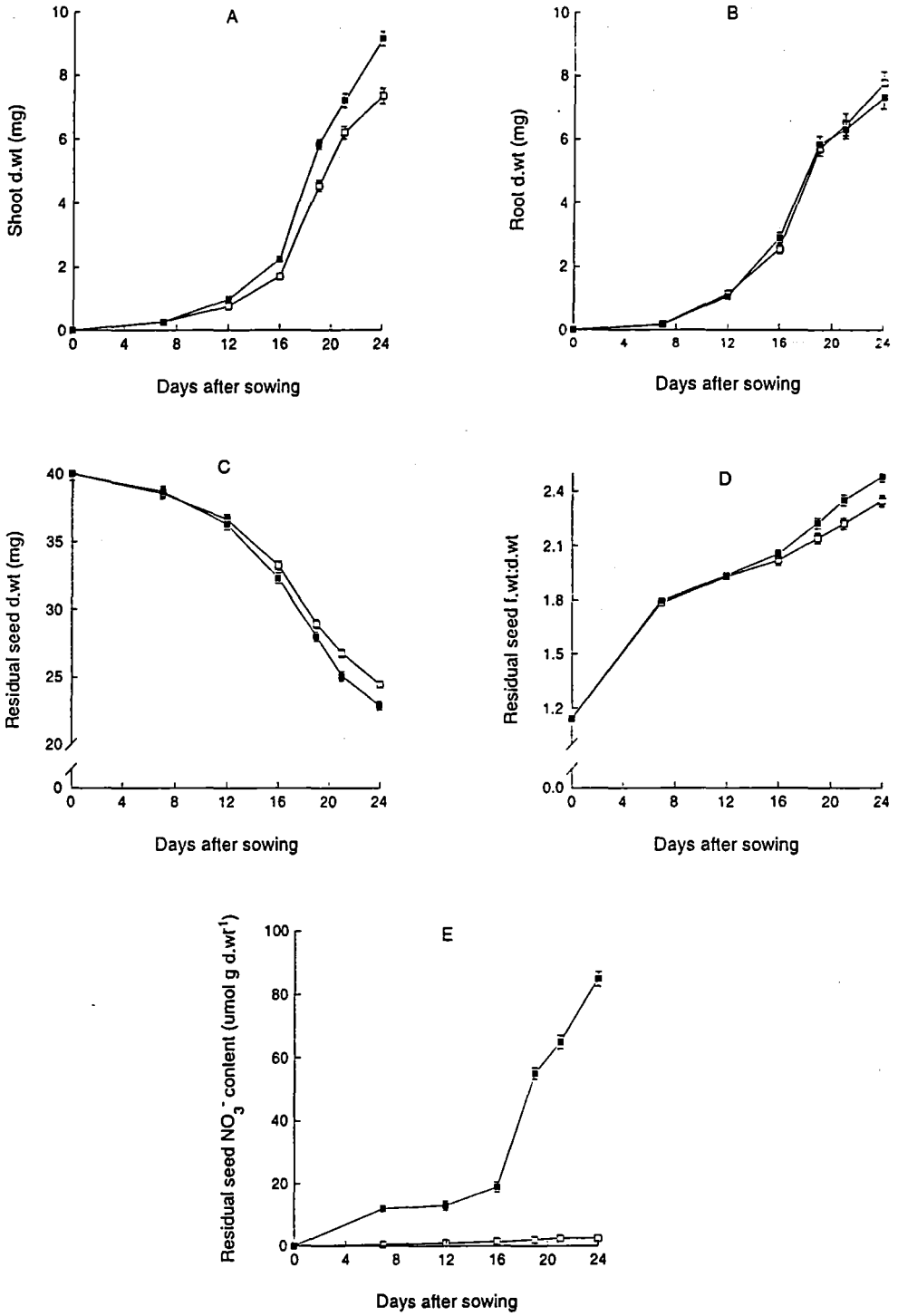


Fig. 2.2 Change in shoot (a), root (b) and residual seed (c) dry weight (d.wt) and residual seed fresh weight:d.wt (d) and nitrate (NO_3^-) content (e) of 21 d old *Hordeum vulgare* L. seedlings prior to emergence supplied basal nutrient solution only (□) or with 5 mol m^{-3} NO_3^- added (■). Error bars indicate ± standard error of mean where larger than symbol.

Table 2.4 Effect of seed nitrogen (N) content and additional nitrate (NO_3^-) on the dry weight (d.wt) of shoot plus root (S+R) and d.wt and water content of the residual seed (RS) of pre-emergent *Hordeum vulgare* L. seedlings 10 and 14 DAS after sowing (DAS).

Treatment		10 DAS			14 DAS		
		Dry weight (g)		Water (%)	Dry weight (g)		Water (%)
Seed N	NO_3^-	S+R	RS	RS	S+R	RS	RS
Low	-	3.12	37.7	48.7	14.1	22.6	69.3
Low	+	3.21	37.5	48.9	16.8	18.9	72.2
High	-	4.08	35.3	57.5	17.1	18.7	72.2
High	+	4.13	35.6	57.2	17.7	17.7	73.9
SE		0.09	0.54	0.75	0.24	0.44	0.51

Experiment 3 examined the effects of a range of chemicals on the mobilization of seed reserves of barley. Addition of 5 or 20 mol m^{-3} NO_3^-, Cl^- or NO_3^- plus W resulted in greater shoot d.wt, decreased residual seed d.wt and little difference in root d.wt compared to seedlings supplied only basal nutrient solution (Table 2.2a). In contrast, 5 or 20 mol m^{-3} NH_4^+, urea, thiourea or ClO_3^- had no effect or a negative effect on seedling growth and reserve mobilisation. *In vivo* NRA of the shoot and root of seedlings supplied NO_3^- plus W was lower than that of seedlings supplied NO_3^- plus Mo, though not as low as those supplied basal nutrient solution only (Table 2.2b).

Potassium chloride at concentrations of 5 mol m^{-3} consistently increased the rate of mobilisation of seed reserves of barley (Table 2.3a). Chloride was similar to NO_3^- in that it caused increases in total seedling water and residual seed water content. Tissue content of the measured anions was always higher in seedlings supplied NO_3^- or Cl^-. Nitrate and Cl^- comprised from 40 - 80% of the total anions measured in the plant parts of seedlings supplied NO_3^- and Cl^- respectively (Table 2.3b).

From planting to 12 DAS, no differences in growth were detected for barley seedlings supplied either basal nutrient solution alone or with NO_3^- added (Fig. 2.2a,b). However, from 12 DAS onwards, seedlings supplied additional NO_3^- had a greater shoot d.wt. Root d.wt continued to be similar for both treatments. Also, residual seed d.wt was less and percent water was greater with NO_3^- compared to those supplied basal nutrient solution only (Fig. 2.2c,d). Levels of NO_3^- in the shoot and root only increased significantly with time where NO_3^- was supplied; at final harvest levels were similar to that found in experiments 1 and 2 (data not shown). Residual seed NO_3^-

content was low (>2 μmol g d.wt^{-1}) at all harvest times with basal nutrient solution alone but with additional NO_3^- it increased steadily over time to approximately 85 μmol g d.wt^{-1} (Fig. 2.2e).

Two DAS in experiment 6, residual seed water content was 25.8±0.2 and 27.5±0.3% for low and high N seed respectively (data not shown). This difference in water content between low and high N seed increased with harvest date until 12 DAS then decreased. At 12 DAS seed d.wt was less for high N seed than for low N seed (34.8±0.3 and 37.1±0.3 mg respectively). Similarly at 16 DAS, seed d.wt for high and low N content seed was 22.8±0.7 and 28.8±0.6 mg respectively.

In experiment 7, seed water content and shoot plus root d.wt were greater for high N seed 10 DAS but were not affected by additional NO_3^- until 14 DAS (Table 2.4).

2.4 Discussion

Previously, additional NO_3^- was shown to increase the rate of mobilisation of seed reserves of barley and wheat grown in darkness (Nátr, 1988; Andrews, Scott and M^{c}Kenzie, 1991). Data obtained in the present study show that this is also the case with oat, triticale and rye (Fig. 2.1; Table 2.1) and that for barley, the effect is similar for seed of low or high N content (Table 2.4). It has also been shown that the rate of mobilisation of seed reserves increases with applied NO_3^- concentration from 0.5 to 5.0 mol m^{-3}, the range likely to occur in agricultural soils (Barber, 1984; Haynes *et al.*, 1986). The magnitude of the increase in shoot d.wt with additional NO_3^- (20 - 40%), was similar to that found for the NO_3^- effect on area and d.wt of leaf 1 of temperate cereals, post emergence (Dale, 1972; Andrews, M^{c}Kenzie and Jones, 1991). Thus, carbon derived from seed reserves as opposed to current photosynthesis is likely to be the main cause of increased growth of leaf 1 of cereals with additional NO_3^- (cf. Dale, Felippe and Marriott, 1974; Metivier and Dale, 1977a,b).

For all cereals, shoot and root NO_3^- content increased with increased applied NO_3^- concentration up to 20 mol m^{-3} (Fig. 2.1). Values for NO_3^- content of shoot and root in the present study were greater than those obtained in mature plants on similar NO_3^- supply in a previous study (Andrews *et al.*, 1992). Nitrate reductase activity (*in vivo* and *in vitro* assays) was also lower in seedlings than in mature plants and this may have been at least part cause of the high NO_3^- accumulation (Oakes, 1983). Despite all seedlings having relatively low NRA, total plant reduced N increased by at least 20% with additional NO_3^- in experiment 2 (Table 2.1). In this experiment, additional

NO_3^- caused a decrease in residual seed d.wt and an increase in shoot plus root d.wt, but additional NH_4^+ did not affect seedling growth (Table 2.1). However, with NH_4^+ N uptake was as great as with NO_3^-. Also, as NH_4^+-N constituted around 0.1% of total N in plants of all treatments, then N assimilation was as great with NH_4^+ as with NO_3^-. The N containing products of NO_3^- and NH_4^+ assimilation are likely to be the same (Layzell, 1990; Andrews, 1993). Thus, although NO_3^- effects on temperate cereal seedlings are dependent on the concentration of NO_3^- supplied (Fig. 2.1), they do not appear to be related to the products of NO_3^- assimilation such as proteins/enzymes, as is the case with mature plants (Khamis and Lamaze, 1990; Zhen and Leigh, 1990). Further evidence for the lack of involvement of the products of NO_3^- assimilation in the process of reserve mobilisation is provided by experiment 3. Seedlings supplied NO_3^- plus W had lower root and shoot *in vivo* NRA compared to those supplied NO_3^- with the standard basal nutrient solution but reserve mobilisation was similar in the two treatments (Table 2.2b). Also in experiment 3, as with NH_4^+, the other N containing compounds urea and thiourea did not affect the rate of mobilisation of seed reserves.

The rate of germination is dependent on the rate of water uptake by the seed and seedling (Jones, 1969; Cardwell, 1984). In the present study, the increase in shoot plus root d.wt with additional NO_3^- was matched by a proportionally similar increase in shoot plus root f.wt (Fig. 2.1). Indeed, shoot and root %water were often slightly greater with additional NO_3^- while residual seed water content was consistently greater with NO_3^- (Tables 2.1,2.2a; Fig. 2.2). In contrast, additional NH_4^+ did not affect total plant water or %water of root, shoot or residual seed. A substantial increase in seedling water indicates a substantial increase in total seedling osmoticum. Increased osmoticum was almost certainly at least partly due to increased availability of solutes, primarily sugars, derived from increased rate of mobilisation of seed reserves (Andrews, Scott and M^{c}Kenzie, 1991). However, in cases where additional NO_3^- stimulated the mobilisation of seed reserves, NO_3^- accumulated to levels which could contribute substantially to the osmotic potential of cells. For example, soluble sugar (primarily glucose) concentrations of up to 180 mol m^{-3} have been measured in oat coleoptile cell sap (Kamisaka *et al.*, 1988). This would generate around 0.5 MPa of osmotic potential (Wyn Jones and Gorham, 1983). For oat in experiment 2, NO_3^- concentration averaged over the root and shoot was approximately 90 mol m^{-3} (Fig. 2.1; Table 2.1). This NO_3^-, together with counter ions would generate around 0.4 MPa of osmotic potential. It is proposed that the NO_3^- effect on mobilisation of seed reserves is due to increased water uptake/retention caused by NO_3^- accumulation in tissues.

If the NO_3^- effect on reserve mobilisation is related to water uptake/retention, then accumulation of other ions to concentrations similar to that of NO_3^- should result in a comparable increase in total plant water and a similar increase in the rate of mobilisation of seed reserves, as long as the ion does not damage the plant. In experiment 3 a range of ions, including N containing compounds, as well as ClO_3^- and Cl^-, were supplied to barley seedlings and their effects on reserve mobilisation compared to that of NO_3^-. Chlorate has frequently been used as an analogue of NO_3^- in screening for the presence or absence of NRA; in plants with NRA, ClO_3^- is reduced to the toxic compound chlorite (ClO_2^-) and the plant is damaged and/or dies (eg. Hofstra, 1977). Chloride is an ion that is readily taken up by plants but which is not assimilated and can result in substantial increases in water uptake/retention by plants (Clarkson and Hanson, 1980; Andrews, Love and Sprent, 1989). Ammonium, urea and thiourea did not affect reserve mobilisation while seedlings supplied ClO_3^- showed extensive damage, possibly due to the production of ClO_2^-. Only seedlings supplied Cl^- showed increased reserve mobilisation to a similar extent as NO_3^- (Table 2.2a). In experiment 4, NO_3^- and Cl^- caused similar increases in total anion content, total water per plant, seed water content and rate of mobilisation of seed reserves (Table 2.3a,b). These findings, in conjunction with the lack of correlation between mobilisation of seed reserves and seedling reduced-N content (Table 2.1) or NRA (Table 2.2b), provide strong evidence that the NO_3^- effect on mobilisation of seed reserves in temperate cereals is an osmotic effect.

For all species, a 20 - 40% increase in total seedling reduced N with additional NH_4^+ did not affect seedling growth (Table 2.1). In contrast, a 20% increase in total barley seed reduced N resulted in an increased rate of mobilisation of seed reserves (Table 2.4). Increased seed but not seedling reduced N also resulted in an increase in seed water content. Both rate and degree of seed imbibition are closely related to the colloidal properties of the seed. Proteins are the main form of seed N and represent the major colloidal constituent of seeds (Cardwell, 1984). Rates of water uptake have been reported to be greater for high N content barley and wheat seed (Lopez and Grabe, 1971). In experiment 6 this was found to be the case for barley within 2 d of sowing. It is proposed that increased seed water content is the cause of the seed N effect on mobilisation of seed reserves. With regard to the NO_3^- effect, it is possible that increased seed water content resulting from increased seedling water content is the cause of increased rate of mobilisation of seed reserves. If greater water uptake into the seed is the cause of increased rate of mobilisation of seed reserves with high protein seed and additional NO_3^-, then the seed N effect should occur before the NO_3^- effect. This was found to be the case in experiment 7 where additional NO_3^- did not affect seed water content until 12 - 14 DAS. It is concluded that evidence is strong that increased rate of mobilisation of seed reserves with additional NO_3^- is due to

increased seedling water uptake probably acting via increased seed water content. Further investigations need to be carried out to determine the pathways of water into the endosperm reserves after the emergence of the seminal roots.

2.5 Conclusions

In this chapter, the nature of the NO_3^- and seed N effects on the mobilisation of seed reserves in temperate cereals prior to emergence were investigated. It is concluded that evidence is strong that increased rate of mobilisation of seed reserves with additional NO_3^- is due to increased seedling water uptake which results in increased water entering the endosperm reserves and hence leading to a greater rate of mobilization. It was proposed that the seed N effect is specifically due to increased seed water content.

Appendix 2.1: Basal nutrient solution (adapted from Andrews, Love and Sprent (1989).

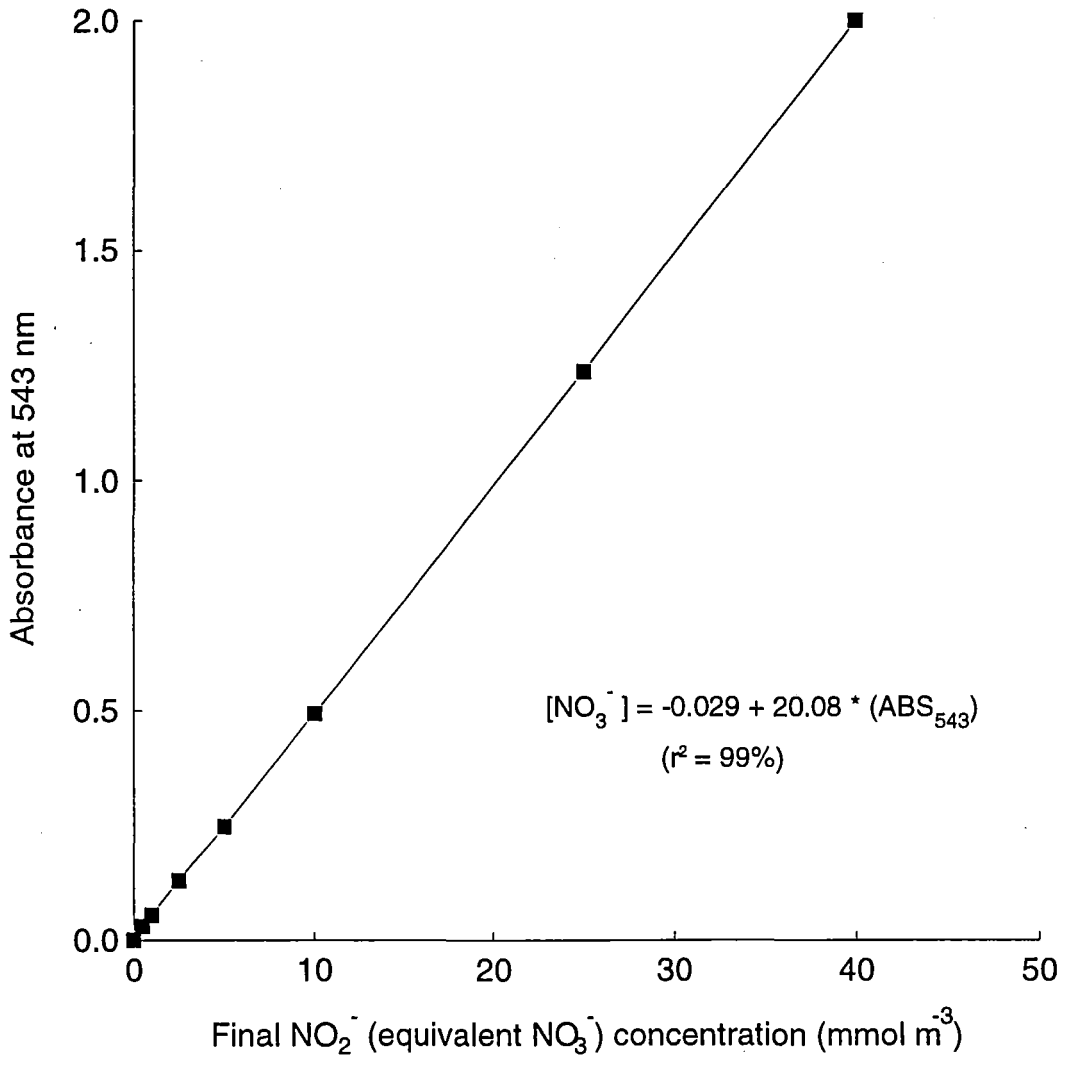
Macronutrients

1. calcium - 3 mol m^{-3} - $CaSO_4 \cdot 2H_2O$
2. magnesium - 3 mol m^{-3} - $MgSO_4 \cdot H_2O$
3. phosphorus - 3 mol m^{-3} - KH_2PO_4 } pH 5.7 buffer
   0.6 mol m^{-3} - K_2HPO_4 }
4. potassium - maintained at the appropriate concentration using K_2SO_4 when necessary
5. sulphur - ≈6 mol m^{-3} - added as SO_4^{-2} in macro/micronutrient salts

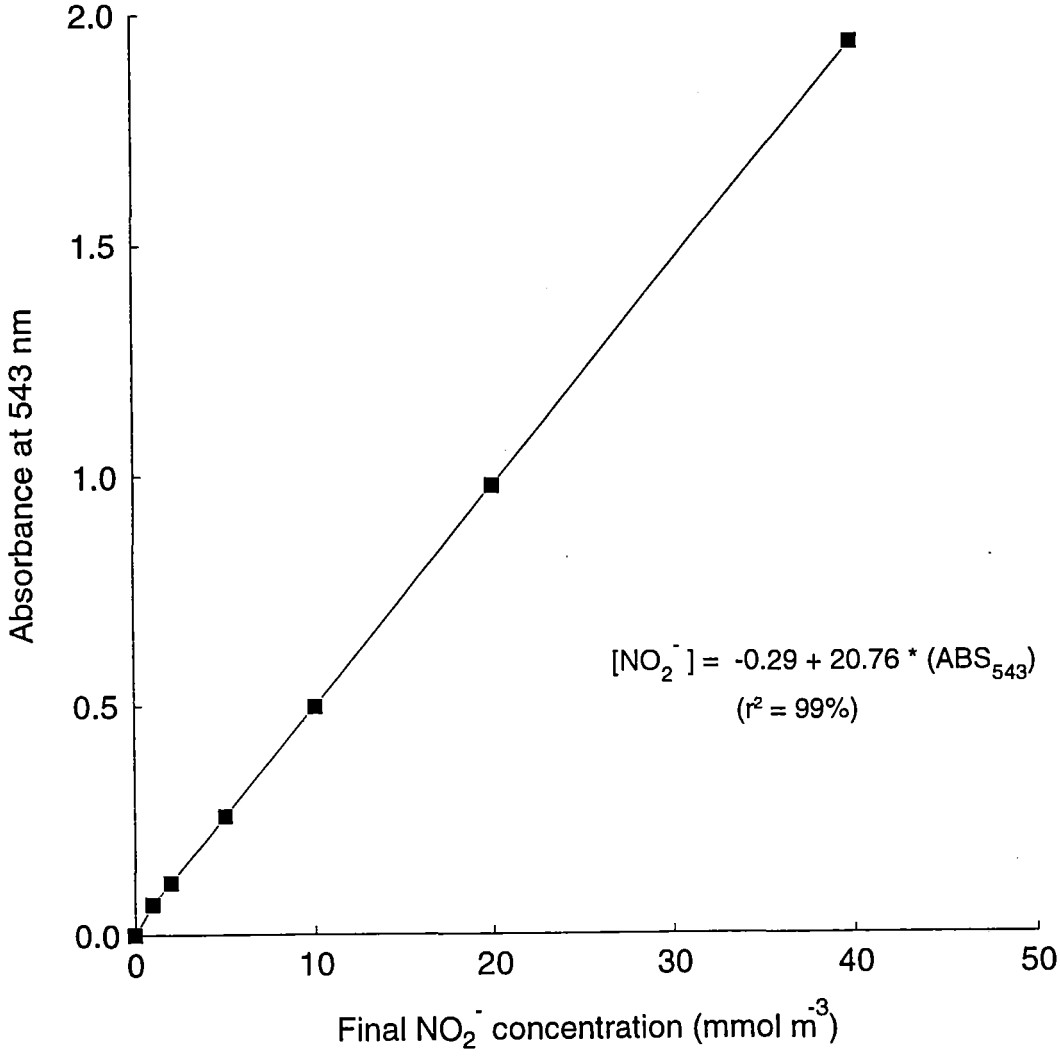
Micronutrients:

6. boron - 5 mmol m^{-3} - H_3BO_3
7. chlorine - 10 mmol m^{-3} - NaCl
8. cobalt - 0.02 mmol m^{-3} - $CoSO_4$
9. copper - 0.1 mmol m^{-3} - $CuSO_4 \cdot 5H_2O$
10. iron - 5 mmol m^{-3} - $C_6H_5O_7Fe \cdot 5H_2O$
11. manganese - 1 mmol m^{-3} - $MnSO_4 \cdot 4H_2O$
12. molybdenum - 0.5 mmol m^{-3} - $Na_2MoO_4 \cdot 4H_2O$
13. zinc - 0.1 mmol m^{-3} - $ZnSO_4 \cdot 7H_2O$

Appendix 2.2: Calibration graph relating initial concentration of NO_3^- reduced to NO_2^- using spongy Cd to the absorbance of the resultant red-azo dye at 543 nm obtained via the method described of Mackereth, Heron and Talling (1978).



Appendix 2.3: Calibration graph relating the concentration of NO_2^- to the absorbance of the resultant red-azo dye at 543 nm obtained via the method described by Mackereth, Heron and Talling (1978).



Appendix 2.4: Calibration graph relating the concentration of NH_4^+ to absorbance at 650 nm obtained via the method described by Baethgen and Alley (1989).

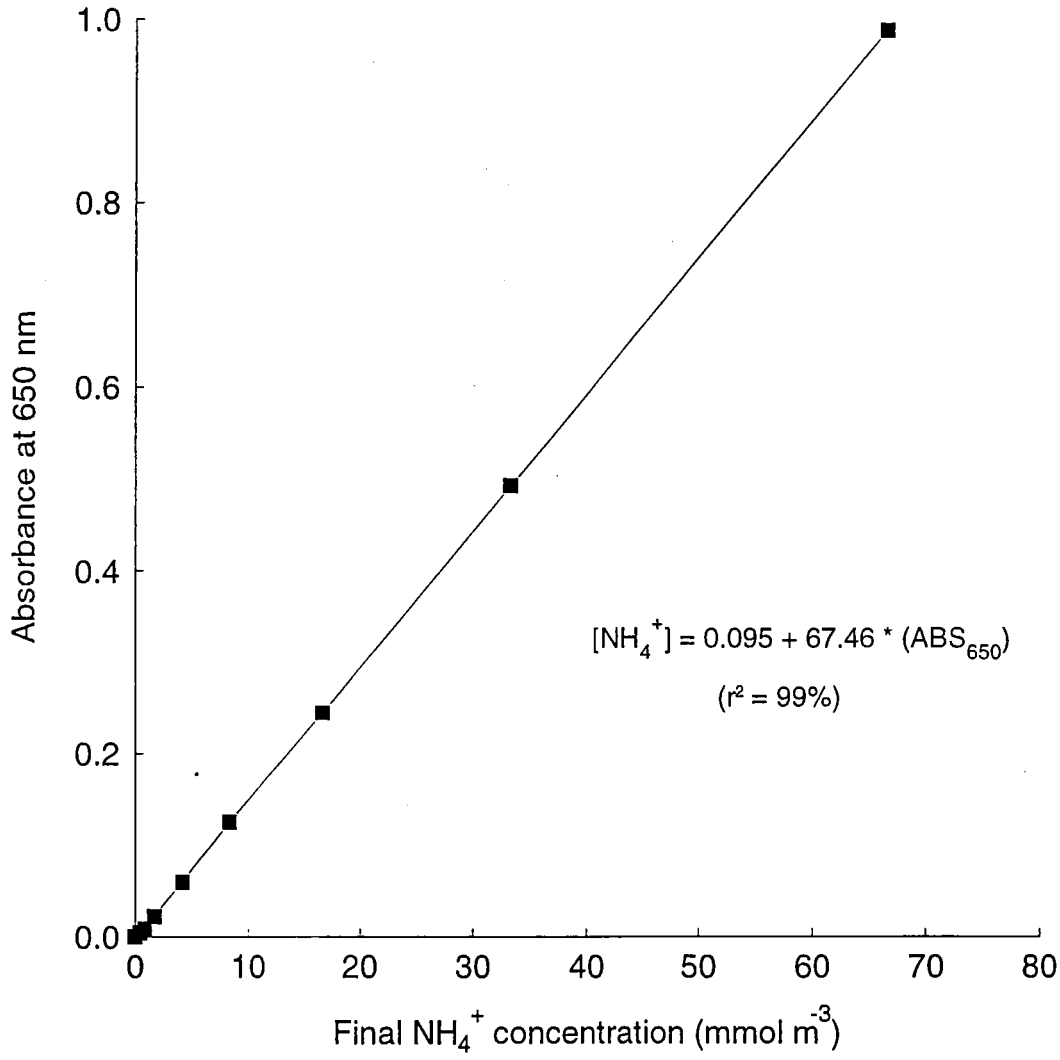


Table of Contents - Chapter 3

Nitrogen and the Growth of Individual Leaves

page

3.1 Introduction 34

3.2 Materials and Methods 36

3.3 Results 38

3.4 Discussion 52

3.5 Conclusions 54

3.1 Introduction

The extent of plant growth depends primarily on the amount of carbon acquired through photosynthesis (Section 1.3). Nitrogen (N) availability can influence plant photosynthetic capacity in two main ways. Firstly, N supply can affect the photosynthetic rate per unit leaf area, primarily as a result of changes in the concentrations of photosynthetic pigments or enzymes (eg. Lawlor *et al.*, 1987). Secondly, N availability can affect plant leaf area and therefore the proportion of incident photosynthetically active radiation intercepted (Novoa and Loomis, 1981). The determinants of plant leaf area which for some species have been shown to be influenced by N availability include the rate of leaf appearance, rate and duration of leaf expansion, individual leaf area, leaf longevity and total number of leaves per plant (Hay and Walker, 1989).

The size of individual leaves has an important bearing on whole plant leaf area. The available data on the effects of N availability on the growth of individual leaves of cereals indicate that there are substantial differences between species. Also, for some species, there is considerable inconsistency between the results of different workers. Some studies have found little or no effect of additional N on individual leaf growth. For example, in the study of Radin (1983), the extension rate of main stem leaf 3 of *Hordeum vulgare* L. (barley) and *Triticum aestivum* L. (wheat) increased only 15 and 19% respectively with 5 mol m^{-3} nitrate (NO_3^-) compared to 0.5 mol m^{-3} NO_3^-. Also, for wheat, extension rate and final area of main stem leaves 1 - 4 were similar for low and high N treatments (Lawlor *et al.*, 1988), while for barley, increasing external NO_3^- concentration from 2.8 to 23 mol m^{-3} did not affect the growth of main stem leaves 1 - 3 (Maan, Wright and Alcock, 1989). The proposed reason for the lack of response to N in the latter two studies was that seed reserves were adequate for growth of these leaves. In contrast to these results, most workers have found that increased N availability has a large, positive effect on individual leaf growth. For example, under field conditions, addition of N (form unspecified) nearly doubled the extension rate and increased by nearly 50% the area of main stem leaf 4 of wheat (Bunting and Drennan, 1966; Kemp and Blacklow, 1982). Similar responses to additional N were obtained for *Avena sativa* L. (oats) under both controlled environment and field conditions (Andrews, Love and Sprent, 1989; Dickson *et al.*, 1990). For main stem leaves 4 - 6 of barley, additional NO_3^- increased extension rate 70 - 100% and final area 100 - 200% (Maan, Wright and Alcock, 1989). Recently, Andrews, M^{c}Kenzie and Jones (1991) found that for main stem leaves 2 - 4 of a range of temperate cereals, additional NO_3^- over the range 0.1 - 5 mol m^{-3} increased mean extension rate 75 - 120%, final length 80 - 100% and area 50 - 150%. Duration of expansion decreased 25 - 30%. For all species except *Secale cereale* L. (rye) the major part of

the responses occurred with additional NO_3^- over the range 0.1 - 5 mol m^{-3} and little or no change occurred with further increase in NO_3^- supply. For rye, leaf size continued to increase with increased NO_3^- concentration to 20 mol m^{-3} (Andrews, M^{c}Kenzie and Jones, 1991).

Most of the studies reviewed above supplied N as NO_3^-, this being the dominant form of N available to and taken up by cereals under temperate agricultural conditions (Section 1.4). However, cereals can also take up and assimilate ammonium (NH_4^+). Levels of NH_4^+ in the soil can be significant and when rates of nitrification are low, such as in cold, damp conditions, NH_4^+ may be the main form of N available to plants (Haynes *et al.*, 1986). No reports were found which compared different forms of N with respect to effects on individual leaf growth characteristics. However, many studies have compared plant growth and determined shoot dry weight (d.wt) with N supplied as NO_3^- or NH_4^+. As shoot d.wt in many species, including cereals, is usually related to total leaf area (Chapter 5), these studies can give some indication of the effects of N form on overall leaf growth. For seedlings harvested prior to emergence, NO_3^- but not NH_4^+ increased the shoot d.wt of a range of cereal species (Chapter 2). Increased shoot growth with NO_3^- was a result of both greater mobilisation of seed reserves and increased allocation of dry matter to the shoot. Greater reserve mobilization was hypothesised to be due to increased water uptake by the seedling. In contrast, in a series of experiments using barley exposed to light, it was concluded that addition of N as NO_3^- or NH_4^+ increased shoot d.wt and area of leaf 1 to a similar extent (Dale, 1972; Dale, Felippe and Marriott, 1974; Metivier and Dale, 1977a,b). It was proposed that additional N (NO_3^- or NH_4^+) probably increased the level of photosynthesis, leading to greater growth. However, the results with NH_4^+ are difficult to interpret as there was evidence of NH_4^+ toxicity in the seedlings (Dale, Felippe and Marriott, 1974). In more mature plants the effects of N form on shoot d.wt appear to depend partly on the species being investigated and the experimental conditions, particularly the pH of the rooting medium. A review of the available literature indicates that NO_3^- has usually been found to be superior for shoot growth though occasionally NH_4^+ has been found to be better, while in some cases no differences are evident (Cox and Reisenauer, 1973; Gashaw and Mugwira, 1981; Troelstra and Blacquière, 1986; Vessey *et al.*, 1990; Cramer and Lewis, 1993). These data indicate that different forms of N may not necessarily have a similar effect on plant leaf growth. In a comparison of the effects of NO_3^- and NH_4^+ supply on plant growth, Lips *et al.* (1990) suggested that the form of N supplied to plants affected the rates of leaf expansion more than the photosynthetic of their chloroplasts.

In the present study, the effects of availability and form of N on the extension growth and final length and area of main stem leaves 2 - 4 of five cereals were measured under controlled environment conditions. Nitrogen was supplied as three forms, NO_3^-, NH_4^+ and glutamine. There are important differences in the assimilation patterns of NO_3^- and NH_4^+ which may affect leaf growth. For cereals, exogenous NH_4^+ is assimilated almost exclusively in the root while the site of NO_3^- assimilation depends on the external NO_3^- concentration (Section 1.4; Andrews *et al.*, 1992). At low external concentrations nearly all the NO_3^- taken up is assimilated in the root; with increasing external concentration of NO_3^- a greater proportion of the assimilation occurs in the shoot. Also, NO_3^- taken up in excess of that able to be assimilated can be stored in cell vacuoles (Granstedt and Huffaker, 1982). In contrast, NH_4^+ does not usually accumulate in plant tissues (Mehrer and Mohr, 1989). Exogenous NO_3^- and NH_4^+ are both assimilated into amino acids such as glutamine. Amino acids such as glutamine are found in the interstitial soil solution (Bremner, 1965) and are taken up and utilized by plants (Jones and Darrah, 1993). In the present experiment, glutamine was supplied via the nutrient solution to simulate the exclusive root assimilation of NO_3^- and NH_4^+, thus providing a valuable tool to assess the influence of site of N assimilation on plant growth.

Though main stem leaves are the major site of photosynthesis in the early stages of cereal development, during latter growth tiller leaves can provide a substantial proportion of total plant leaf area, at least in species and cultivars capable of tiller production. Despite the importance of tillers for plant leaf area, no reports were found in the literature on the effects of N availability on the growth characteristics of individual tiller leaves. Hence, in this study, the growth characteristics of leaf 2 of tiller 1 were also determined. The primary objective of the study was to determine, for a range of temperate cereals, if individual leaf growth characteristics are dependent on the form of N supplied.

3.2 Materials and Methods

a) Plant material and growth conditions

Seed of wheat (cv. Otane; mean seed weight, 50 mg), oats (cv. Amuri, 34 mg) and X *Triticosecale* Wittmack (triticale) (cv. Aranui, 55 mg) was obtained from Hodder and Tolley Ltd. Christchurch, New Zealand. Rye (cv. Rapaki, 30 mg) and barley (cv. Triumph, 45 mg) seed was

obtained from the New Zealand Institute for Crop and Food Research Ltd., Lincoln, New Zealand and the Canterbury Malting Company, Christchurch, New Zealand respectively.

Seeds were germinated on paper towels moistened with distilled water. After 4 d, seedlings with a coleoptile length of approximately 20 mm were transplanted to 80 mm diameter, 180 mm tall pots (one per pot) containing a vermiculite/perlite (1:1 v/v) mixture soaked in basal nutrient solution (Appendix 2.1) containing the appropriate N treatment. There were six rates of N (0, 0.5, 1.0, 2.5, 5.0, 20.0 mol m^{-3}) supplied as one of three forms: NO_3^- as potassium nitrate, NH_4^+ as ammonium sulphate, or glutamine. For all treatments, potassium was maintained at 23.6 mol m^{-3} using potassium sulphate. Pots were flushed every 2 - 3 days with the appropriate nutrient solution. Plants were grown in a glasshouse with natural spring/summer light. The photoperiod was approximately 15 h and the temperature ranged from 14 - 30°C.

b) Measurement of leaf length and area

In this chapter the term "leaf" refers to the blade portion (lamina) of the cereal leaf proper (Section 1.2). As transplanted seedlings were used, length and final area of leaf 1 were not measured. The lengths of main stem leaves 2 - 4 and leaf 2 of tiller 1 were measured daily until full extension was reached. Leaf length was taken as the leaf tip to point of leaf emergence from the coleoptile for leaf 2 and leaf tip to where the leaf subtended the leaf sheath for leaves 3 and 4 and leaf 2 of tiller 1. Leaves were considered fully extended when three successive measurements were identical. Plants were harvested 31 d after planting. Final width was determined at the widest part of the leaf and area was measured using a CI-201 leaf area meter (CID Inc. Moscow, ID, U.S.A).

c) Growth analysis and experimental design

Leaf extension over time was analysed using variates derived from a generalised logistic curve. This curve, a type of Richards function, was chosen because the parameters are accepted to have biological meaning (Venus and Causton, 1979; Causton and Venus, 1981; Hunt, 1982). The generalised logistic curve has the equation: $y=C/(1+T \exp(-b(x-m)))^{1/T}$ where 'C' is the final leaf length and 'T', 'b' and 'm' are constants. All curves were fitted using the Maximum Likelihood Programme (Ross *et al.*, 1979). The leaf extension variates absolute maximum extension rate $(bC/(T+1)^{T+1/T})$, weighted mean extension rate (bC/2(T+2)) and the time required for the majority of extension to occur (2(T+2)/b), were derived from the curve parameters as described by

Dennett, Auld and Elston (1978). The experiment was of a completely randomised design with five replicates for all treatments. The extension variates were analysed by analysis of variance using "Statistix" (Analytical Software, St.Paul, MN, U.S.A). All effects discussed have an F ratio with a probability $P<0.05$.

f) Repeat experiment

The main effects of N concentration and form on leaf growth characteristics were similar for all species and because of this the repeat experiment was carried out on barley only. Experimental conditions were similar to those in the main experiment except the three forms of N (NO_3^-, NH_4^+ and glutamine) were applied at seven rates (0, 0.5, 1, 2, 4, 6, 10 mol m^{-3}). Potassium was maintained at 13.6 mol m^{-3}. Leaf growth measurements, analyses and harvesting methods used were the same as the main experiment.

3.3 Results

For all species, regardless of N form supplied, the absolute maximum and weighted mean extension rates of all leaves increased with increasing N concentration over the range 0 to 1 - 2.5 mol m^{-3} (Figs. 3.1 - 3.5). Depending on leaf position and species, extension rates increased 50 - 300%. For any species, the maximum extension rate achieved for a given leaf was similar with the different N forms. Also for all species, the magnitude of the increase in extension rate with additional N over the range 0 - 2.5 mol m^{-3} N increased with successive leaves. For all species, the extension rate of all leaves either changed little or increased further with additional NO_3^- or glutamine over the range 2.5 - 20 mol m^{-3} N. The extension rates of main stem leaf 2 and leaf 2 of tiller 1 of all species changed little with addition of NH_4^+ over the range 2.5 - 20 mol m^{-3}. However, for most species, extension rates of leaves 3 and 4 decreased with increased applied NH_4^+ concentrations from 2.5 - 20 mol m^{-3} while leaf 2 of tiller 1 of plants supplied 5 and 20 mol m^{-3} NH_4^+ did not survive to harvest. Regardless of N form applied, for all species duration of extension of all leaves decreased from 12 - 14 d to approximately 7 - 8 d with additional N over the range 0 - 2.5 mol m^{-3} but changed little with additional N from 2.5 to 20 mol m^{-3} (Figs. 3.1 - 3.5).

For all species, regardless of N form supplied, final length, width and area of all leaves increased with increased N concentration over the range 0 to 1 - 2.5 mol m^{-3} (Figs. 3.6 - 3.10). Depending

on leaf number and species, length and width increased 50-100% and area 100-400%. The maximum length, width and area achieved for a given leaf of any species were similar regardless of N form supplied. In general, for any species, the magnitude of the increases in length, width and area with additional N increased with successive leaves. Also, for any species and N treatment, the final area of successive main stem leaves increased. Regardless of N form, for leaf 2 of most species, final length, width and area changed little with additional N over the range 2.5 - 20 mol m^{-3}. For all species, the length, width and area of leaves 3 and 4 and leaf 2 of tiller 1 changed little or increased further with additional NO_3^- or glutamine over the range 2.5 - 20 mol m^{-3} N (Fig. 3.6 - 3.10). Generally, width increased to a greater extent than length. However, for most species, there was a substantial decrease in the final length of leaves 3 and 4 of plants supplied NH_4^+ over the range 2.5 - 20 mol m^{-3}, though width was not affected. The decrease in final length resulted in a marked decrease in the area of these leaves.

There were differences in the leaf growth of the species. At external N concentrations over the range 0 - 2.5 mol m^{-3}, for a given leaf, extension rate and final length tended to be higher for rye and oats while width was greatest for oats and barley. Especially for main stem leaves 3 and 4, oats had the greatest final leaf area. Rye responded differently to high external concentrations of N. The extension rate and final area of leaves 3 and 4 and leaf 2 of tiller 1 of rye increased with external NO_3^- or glutamine concentrations over the range 2.5 - 20 mol m^{-3} while for the other species growth increased little or not at all. Also for rye, leaf growth decreased only slightly with additional NH_4^+ over the range 2.5 - 20 mol m^{-3} whereas for the other species the decrease in growth was substantial.

In the repeat experiment using barley only, the growth responses of all leaves to additional NO_3^- or glutamine were similar to those in the main experiment (cf. Figs. 3.11; 3.12 and 3.1; 3.6). With additional NO_3^- or glutamine over the range 0 - 2 - 5 mol m^{-3} N, extension rate and final leaf length, width and area of all leaves increased while duration of extension decreased. There was little further change in the growth of these leaves with additional NO_3^- or glutamine over the range 5 - 10 mol m^{-3} N. With additional NH_4^+ over the range 0 - 2 mol m^{-3}, extension rate and final length, width and area of all leaves where similar to when NO_3^- or glutamine were supplied. However, in contrast to the main experiment, with additional NH_4^+ over the range 2 - 10 mol m^{-3}, the extension rate and final length, width and area of all leaves were considerably less than with NO_3^- or glutamine. In general, for a given leaf, maximum extension rate and final length, width and area were less with NH_4^+ compared to NO_3^- or glutamine.

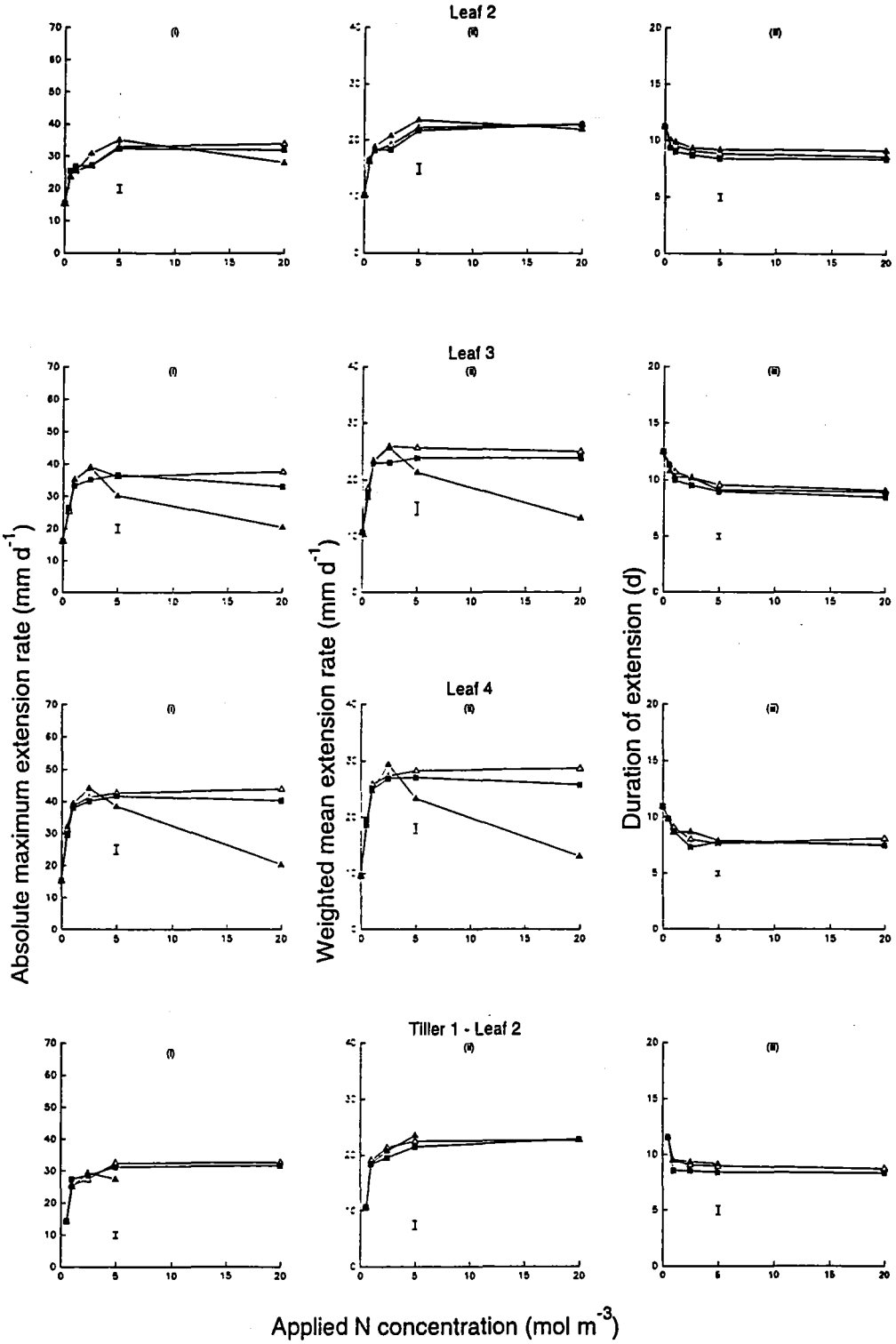


Fig. 3.1 The effects of different concentrations of nitrate (■), ammonium (▲) or glutamine (Δ) on the absolute maximum extension rate (i), weighted mean extension rate (ii) and duration of extension (iii) of main stem leaves 2 - 4 and tiller 1 leaf 2 of *Hordeum vulgare* L. Where larger than the symbol, error bars indicate ± standard error of the mean.

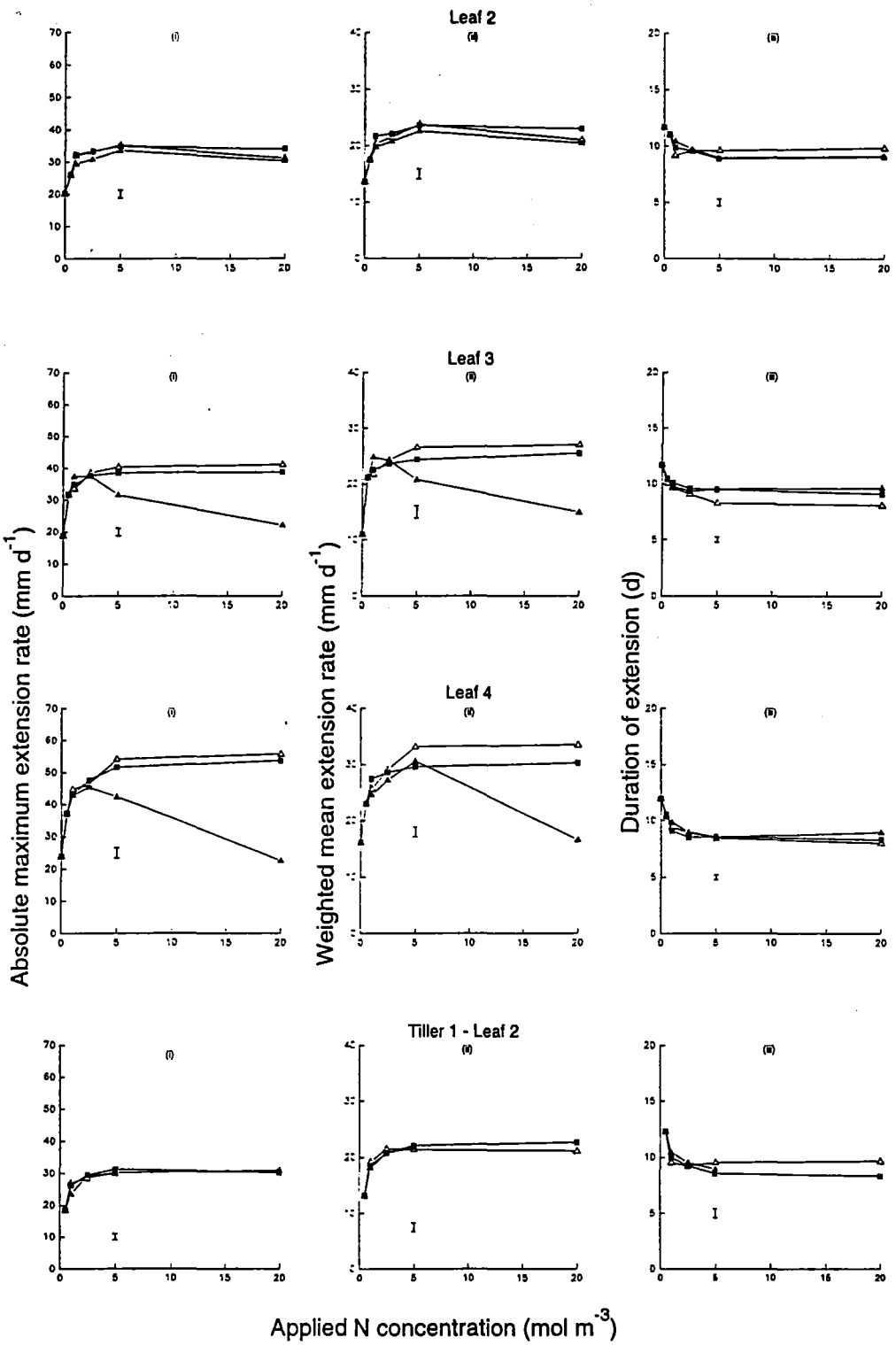


Fig. 3.2 The effects of different concentrations of nitrate (■), ammonium (▲) or glutamine (Δ) on the absolute maximum extension rate (i), weighted mean extension rate (ii) and duration of extension (iii) of main stem leaves 2 - 4 and tiller 1 leaf 2 of *Triticum aestivum* L. Where larger than the symbol, error bars indicate ± standard error of the mean.

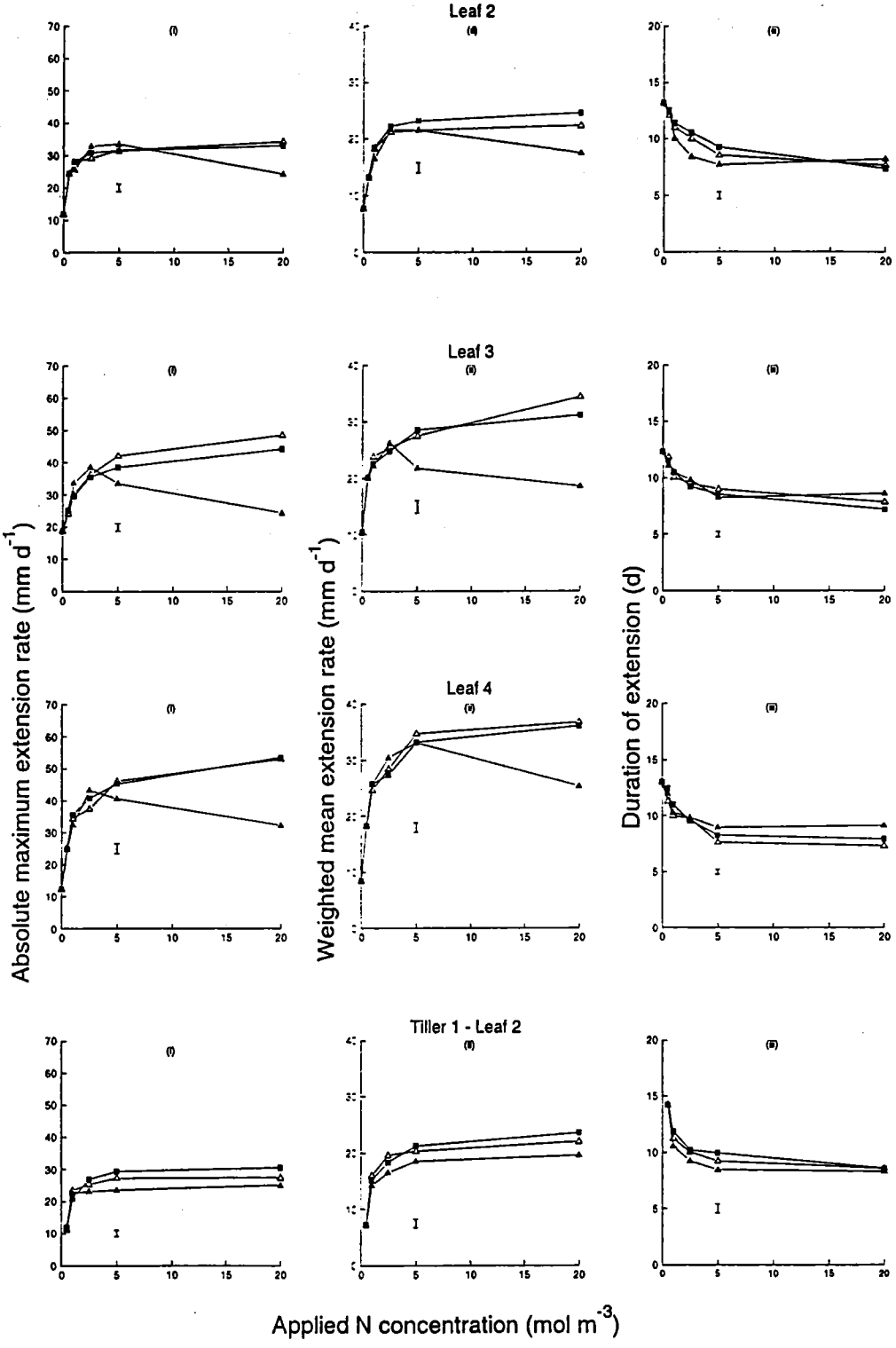


Fig. 3.3 The effects of different concentrations of nitrate (■), ammonium (▲) or glutamine (Δ) on the absolute maximum extension rate (i), weighted mean extension rate (ii) and duration of extension (iii) of main stem leaves 2 - 4 and tiller 1 leaf 2 of *Secale cereale* L. Where larger than the symbol, error bars indicate ± standard error of the mean.

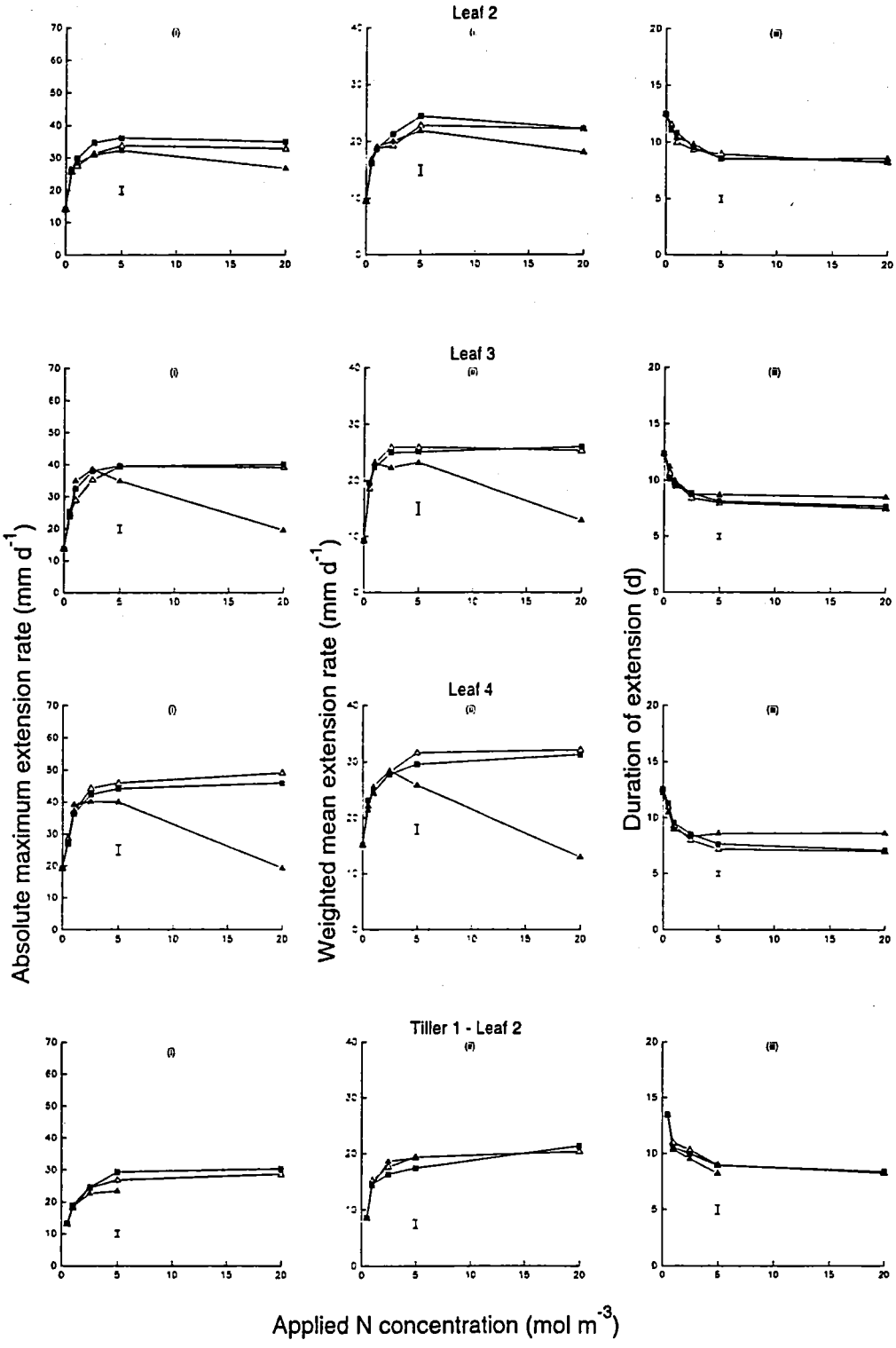


Fig. 3.4 The effects of different concentrations of nitrate (■), ammonium (▲) or glutamine (Δ) on the absolute maximum extension rate (i), weighted mean extension rate (ii) and duration of extension (iii) of main stem leaves 2 - 4 and tiller 1 leaf 2 of X *Triticosecale* Wittmack. Where larger than the symbol, error bars indicate ± standard error of the mean.

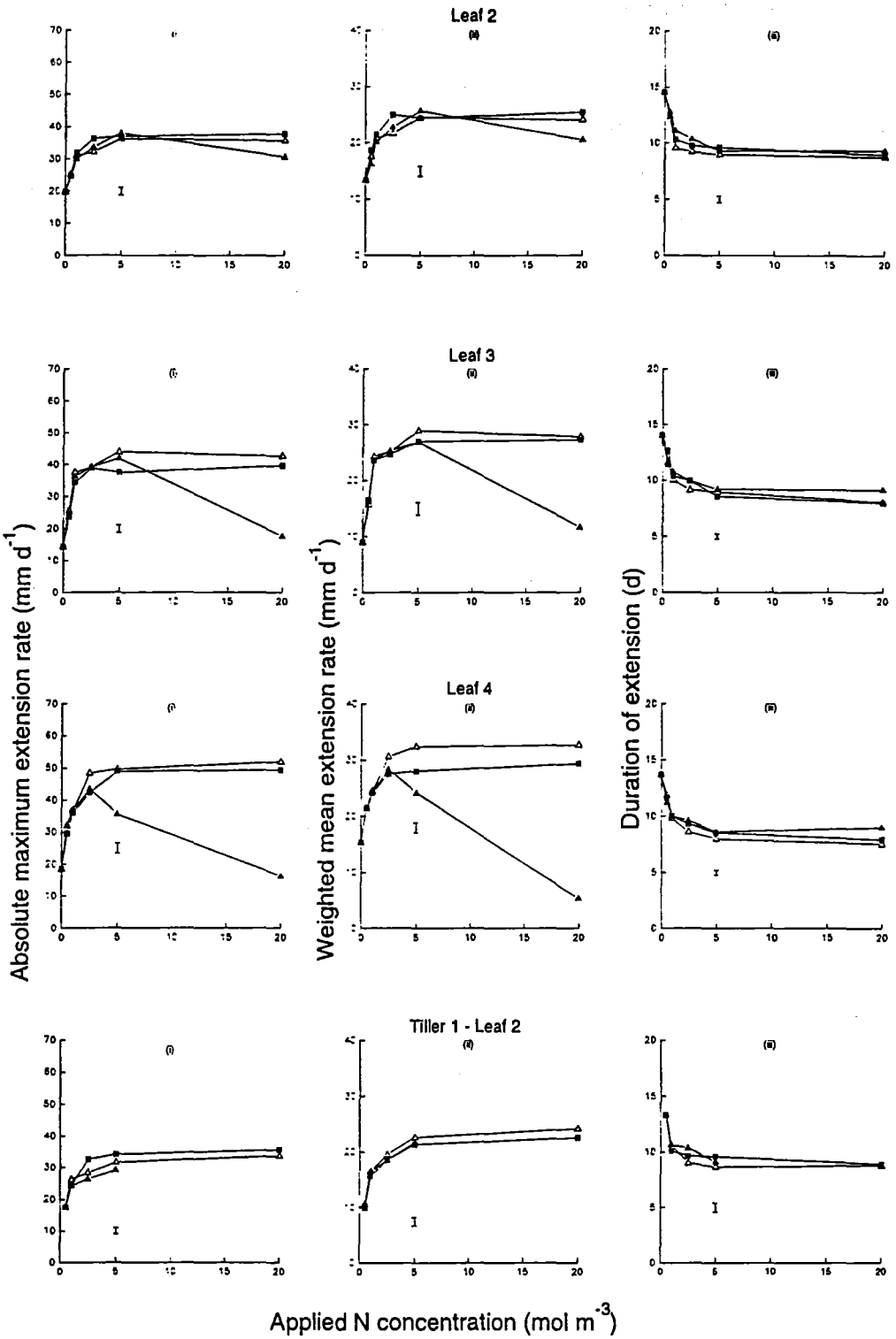


Fig. 3.5 The effects of different concentrations of nitrate (■), ammonium (▲) or glutamine (Δ) on the absolute maximum extension rate (i), weighted mean extension rate (ii) and duration of extension (iii) of main stem leaves 2 - 4 and tiller 1 leaf 2 of *Avena sativa* L. Where larger than the symbol, error bars indicate ± standard error of the mean.

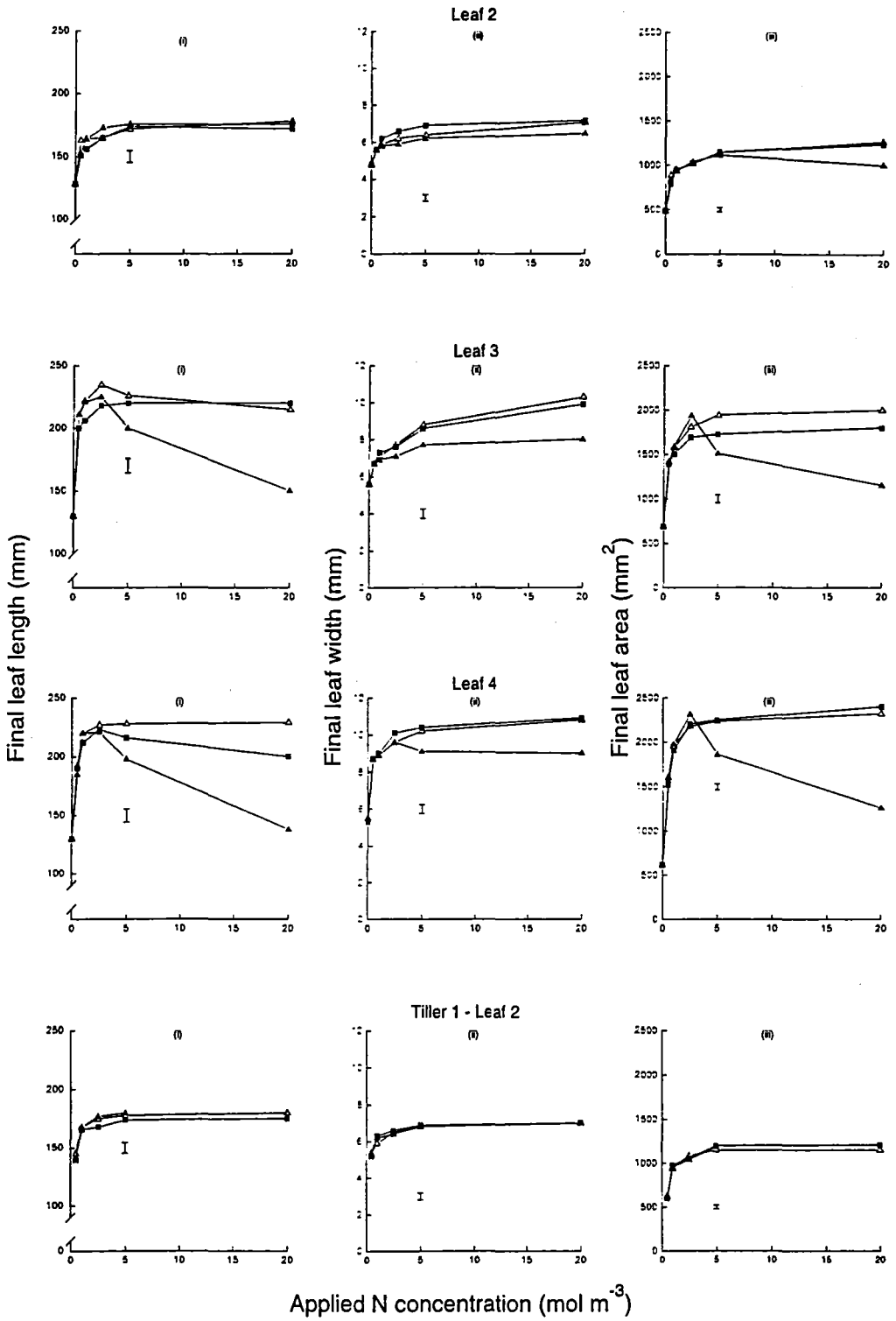


Fig. 3.6 The effects of different concentrations of nitrate (■), ammonium (▲) or glutamine (Δ) on the final length (i), width (ii) and area (iii) of main stem leaves 2 - 4 and tiller 1 leaf 2 of *Hordeum vulgare* L. Where larger than the symbol, error bars indicate ± standard error of the mean.

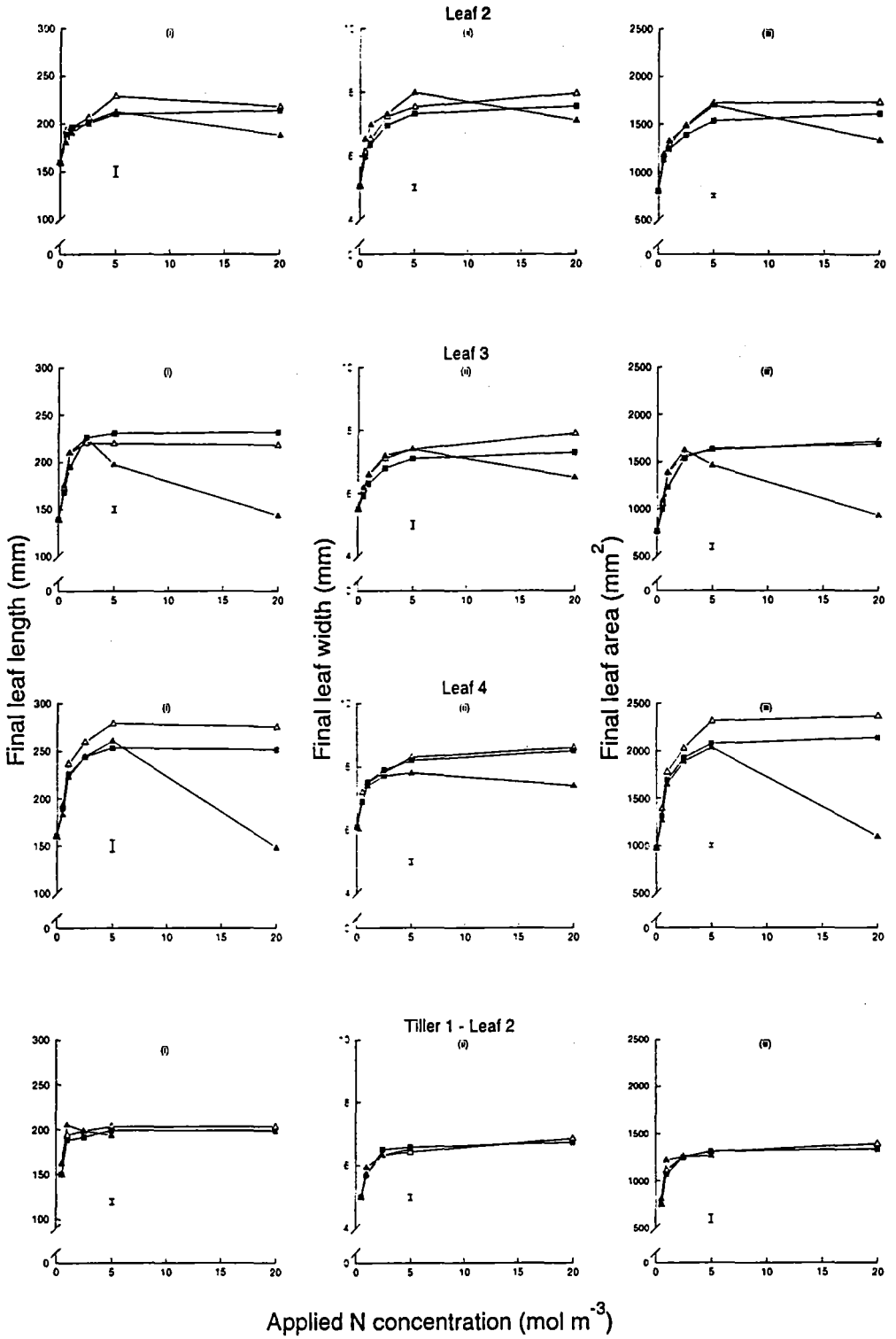


Fig. 3.7 The effects of different concentrations of nitrate (■), ammonium (▲) or glutamine (Δ) on the final length (i), width (ii) and area (iii) of main stem leaves 2 - 4 and tiller 1 leaf 2 of *Triticum aestivum* L. Where larger than the symbol, error bars indicate ± standard error of the mean.

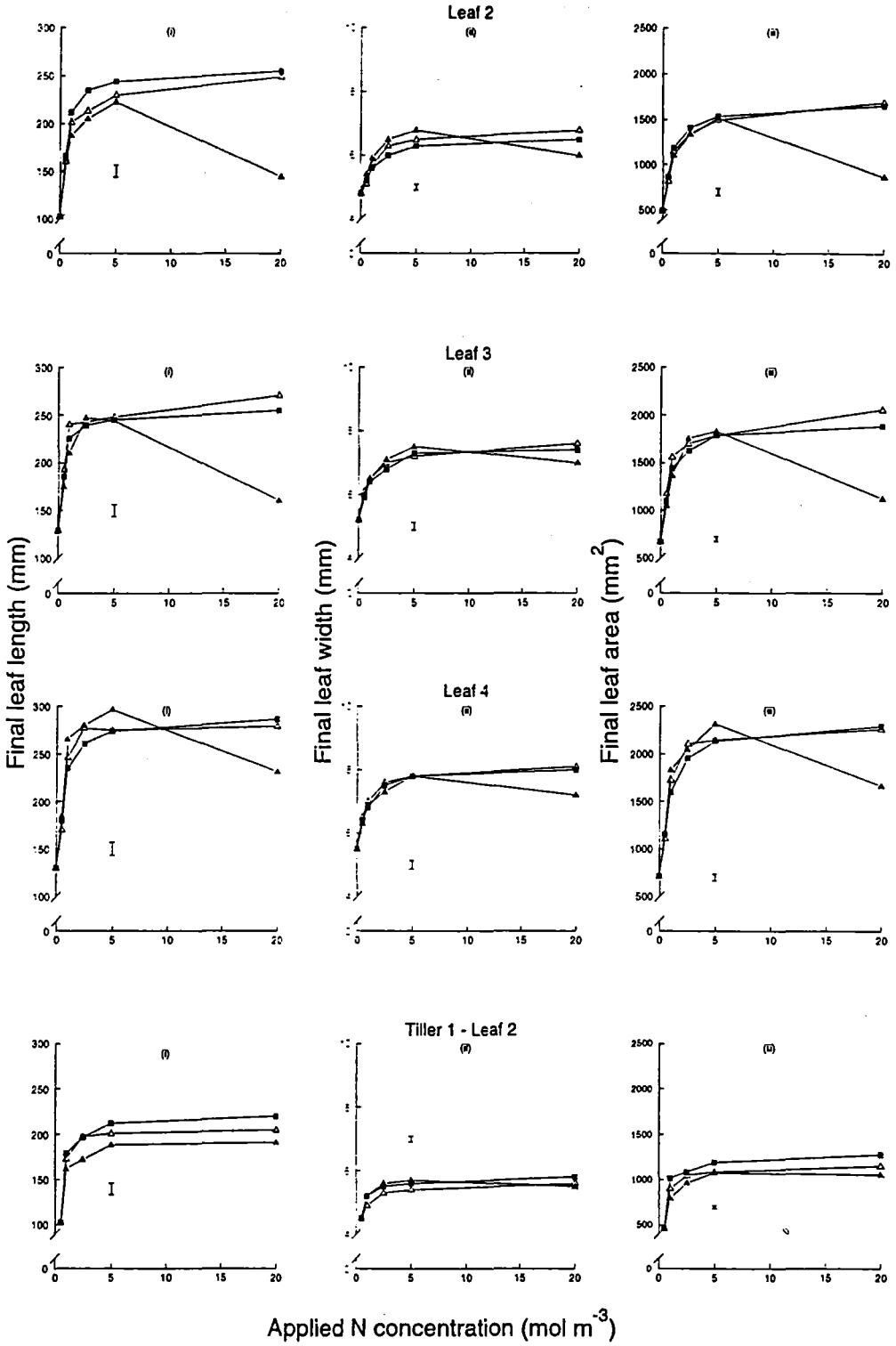


Fig. 3.8 The effects of different concentrations of nitrate (■), ammonium (▲) or glutamine (Δ) on the final length (i), width (ii) and area (iii) of main stem leaves 2 - 4 and tiller 1 leaf 2 of *Secale cereale* L. Where larger than the symbol, error bars indicate ± standard error of the mean.

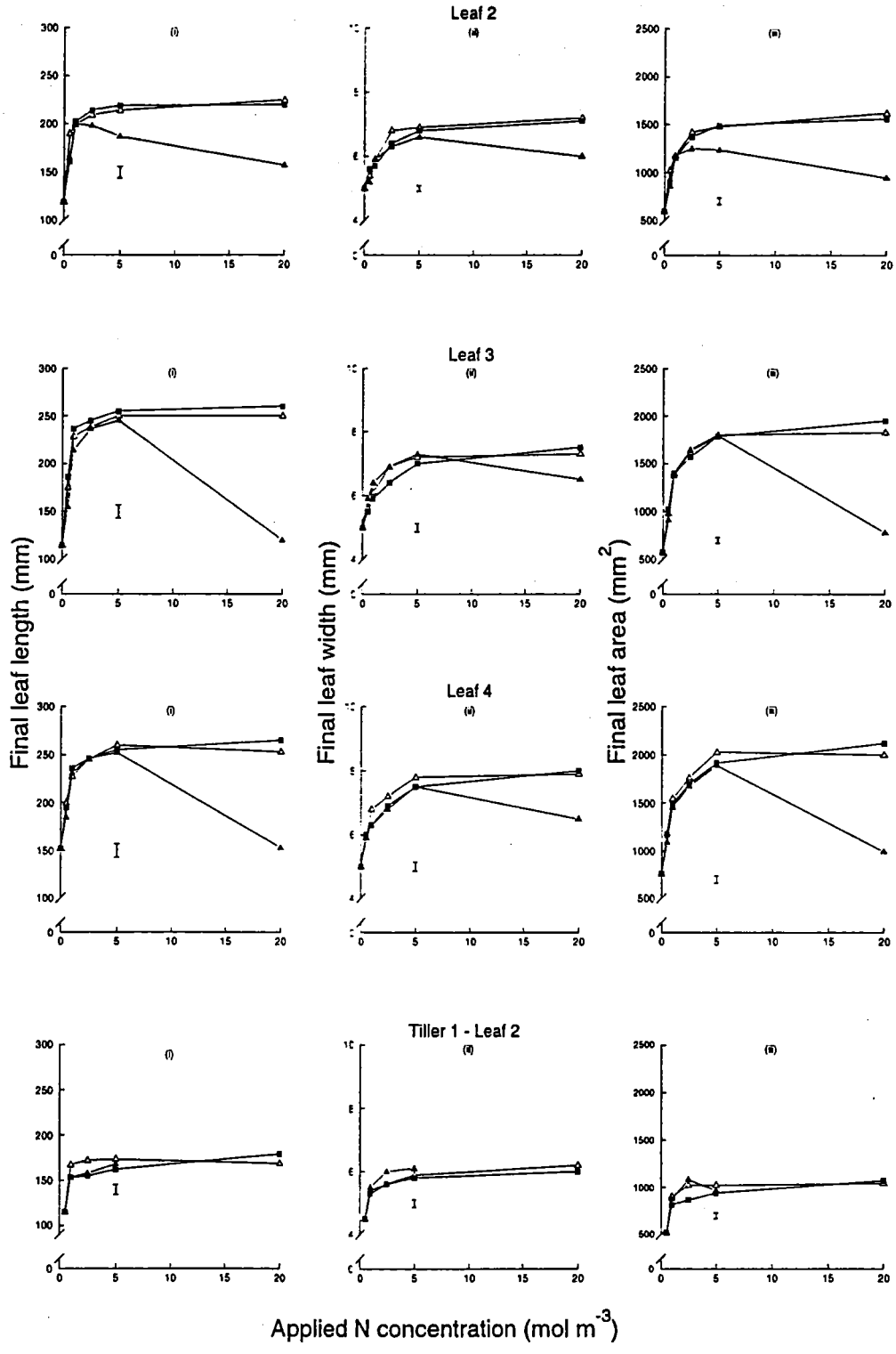


Fig. 3.9 The effects of different concentrations of nitrate (■), ammonium (▲) or glutamine (Δ) on the final length (i), width (ii) and area (iii) of main stem leaves 2 - 4 and tiller 1 leaf 2 of X *Triticosecale* Wittmack. Where larger than the symbol, error bars indicate ± standard error of the mean.

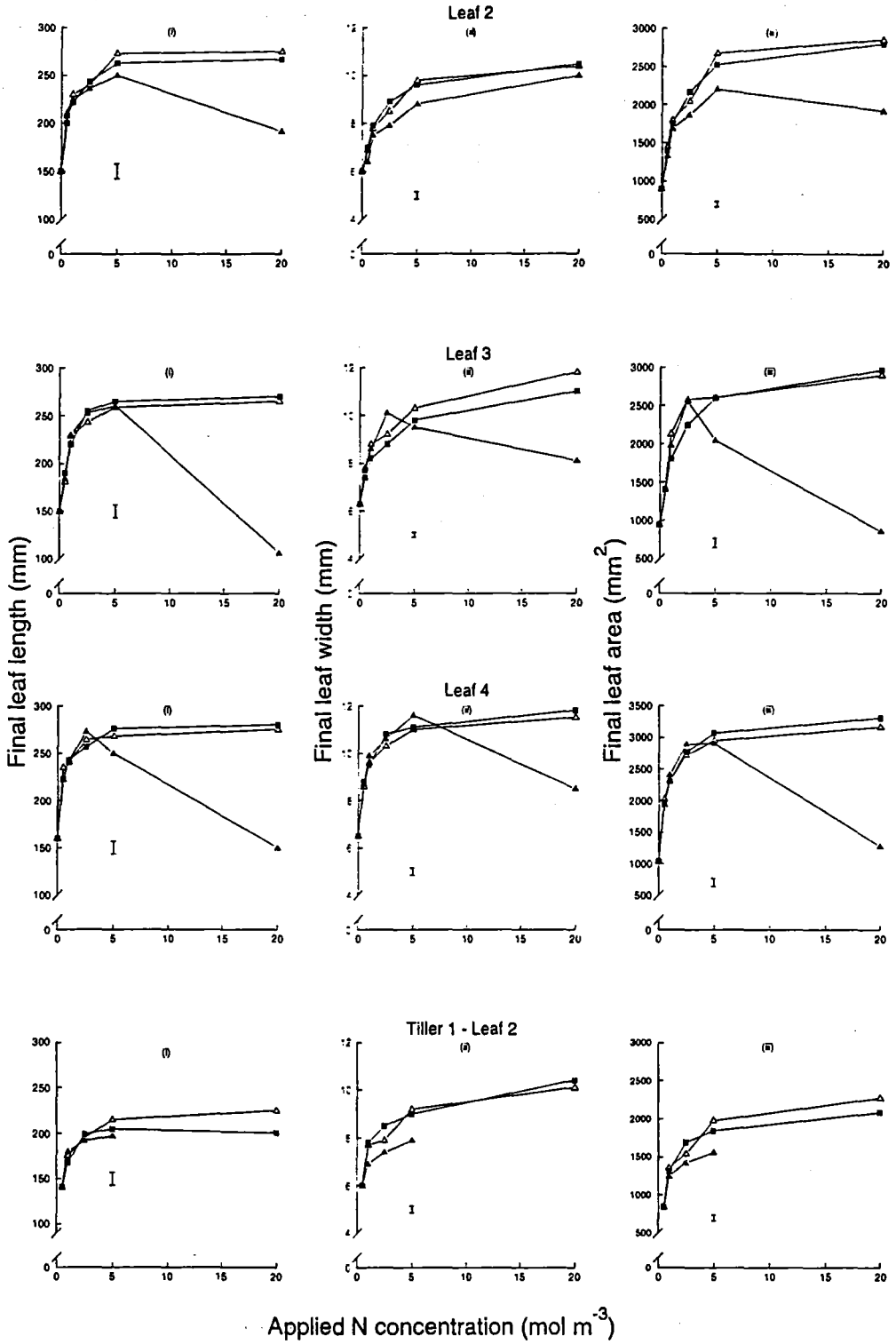


Fig. 3.10 The effects of different concentrations of nitrate (■), ammonium (▲) or glutamine (Δ) on the final length (i), width (ii) and area (iii) of main stem leaves 2 - 4 and tiller 1 leaf 2 of *Avena sativa* L. Where larger than the symbol, error bars indicate ± standard error of the mean.

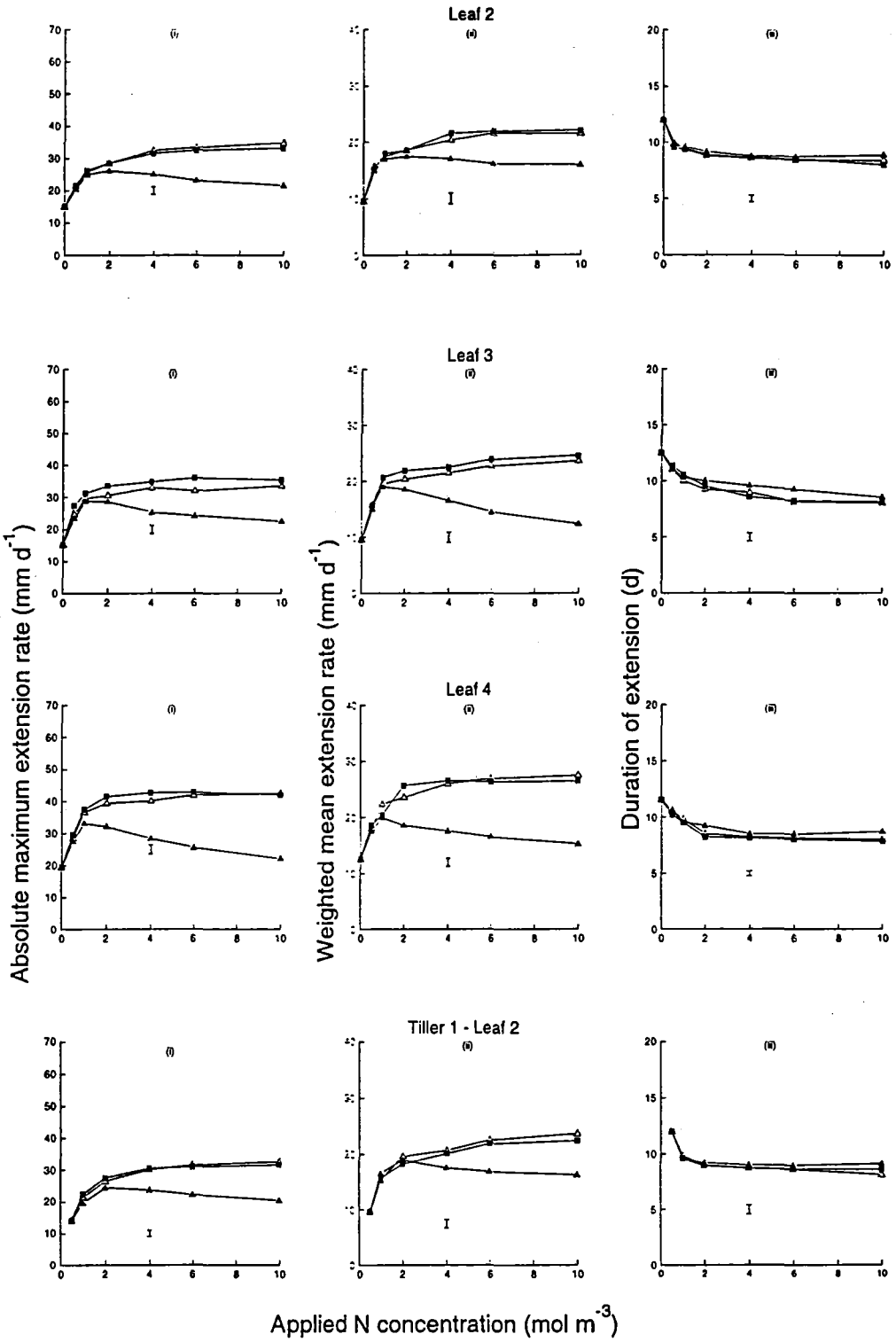


Fig. 3.11 Repeat Experiment. The effects of different concentrations of nitrate (■), ammonium (▲) or glutamine (Δ) on the absolute maximum extension rate (i), weighted mean extension rate (ii) and duration of extension (iii) of main stem leaves 2 - 4 and tiller 1 leaf 2 of *Hordeum vulgare* L. Where larger than the symbol, error bars indicate ± standard error of the mean.

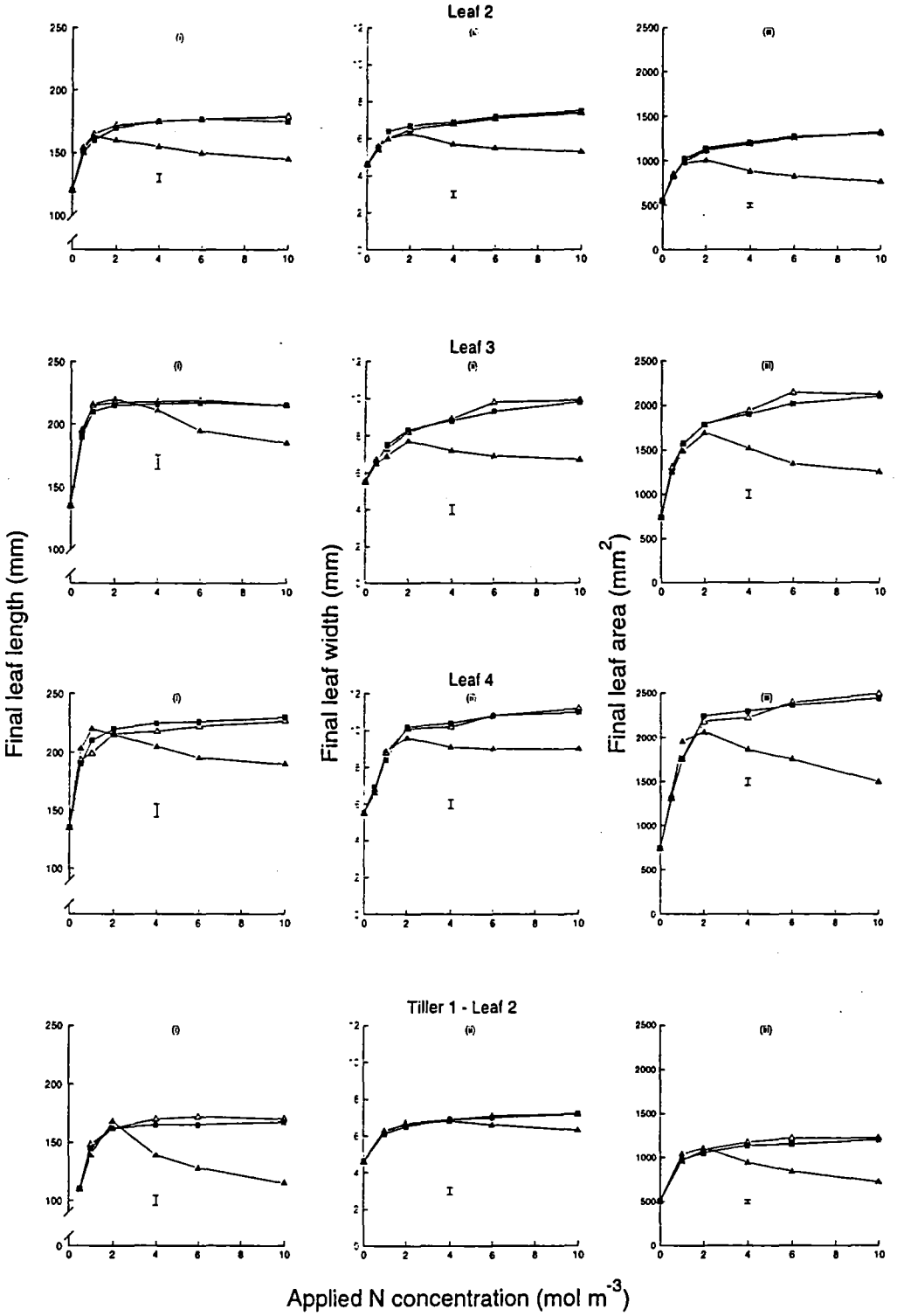


Fig. 3.12 Repeat Experiment. The effects of different concentrations of nitrate (■), ammonium (▲) or glutamine (Δ) on the final length (i), width (ii) and area (iii) of main stem leaves 2 - 4 and tiller 1 leaf 2 of *Hordeum vulgare* L. Where larger than the symbol, error bars indicate ± standard error of the mean.

3.4 Discussion

A review of the available data on the effects of NO_3^- on the growth of individual leaves indicates that for the cereal species there are major inconsistencies between researchers. Data of some workers have shown little or no increase in main stem leaf area with additional NO_3^- (eg. Radin, 1983; Lawlor *et al.*, 1988) while that of others have demonstrated substantial increases in growth (eg. Andrews, M^{c}Kenzie and Jones, 1991). In the present study, the effects of additional NO_3^- on leaf growth characteristics were similar to those found by Andrews, M^{c}Kenzie and Jones (1991). For all species additional NO_3^- over the range 0 - 2.5 mol m^{-3} substantially increased the extension rate of main stem leaves 2 - 4 while duration of extension was reduced (Figs. 3.1 - 3.5). The magnitude of the increase in extension rate was larger than the decrease in duration and hence final leaf length increased. Final width was also greater and as a consequence leaf area increased with additional NO_3^- over the range 0 - 2.5 mol m^{-3} (Figs. 3.6 - 3.10).

Not only did additional NO_3^- increase the area of individual leaves, but the magnitude of the response to additional NO_3^- increased with successive leaves. Also, for a given concentration of external NO_3^-, the maximum area attained usually increased with successive leaves. The development of individual leaf area is a function of the rate and extent of cell division and/or expansion. However, the available literature provides little information on the cellular basis for the increase in leaf area with additional NO_3^- and the increase in leaf size with leaf position. These aspects of leaf growth are investigated in Chapter 4. Differences in cell number and size may also have been at least partially responsible for the differences between species in leaf growth with additional NO_3^- over the range 0 - 2.5 mol m^{-3}. Further work needs to be carried out to determine the differences in the cellular aspects of leaf growth of different cereal species.

For all species except rye, additional NO_3^- over the range 2.5 - 20 mol m^{-3} caused little further increase in extension rate (Fig. 3.1 - 3.5). As duration of extension did not decrease further, final leaf length also changed little (Fig. 3.6 - 3.10). In contrast, width continued to increase with additional NO_3^- from 2.5 - 20 mol m^{-3}, leading to greater final area of main stem leaves 2 - 4. Compared to length, leaf width increased over a greater range of external NO_3^- concentrations, possibly due to differences in cell division/expansion in the longitudinal and transverse directions. These data highlight the importance of width as a factor in determining leaf area, especially at higher external NO_3^- concentrations.

In contrast to the other species, the extension rate and final length of main stem leaves 2 - 4 of rye increased substantially with additional NO_3^- over the range 2.5 - 20 mol m^{-3} (Figs. 3.3, 3.8). Width was also greater, resulting in a large increase in leaf area. These data demonstrate the ability of individual leaves of rye to respond over a larger range of external NO_3^- concentrations compared to other temperate cereals, as was also found by Andrews, M^{c}Kenzie and Jones (1991). However, from the available data it is difficult to establish why rye responds differently. It is possible that the ability of rye to produce more tillers, leaf area and dry matter compared with other temperate cereal species (Andrews *et al.*, 1992, unpublished data), serves to dilute excessive amounts of NO_3^- which may otherwise somehow curtail individual leaf growth.

In species or cultivars capable of tiller production, tiller leaves frequently make up a substantial proportion of the leaf area of cereals. However, few studies have investigated the growth of individual tiller leaves with additional N. In the present study, for all species, the effects of additional NO_3^- on the growth of leaf 2 of tiller 1 was similar to that of main stem leaves. Leaf 2 of tiller 1 was generally smaller than the largest main stem leaf measured, though later tiller leaves can be as large or larger than main stem leaves (pers. obs).

Nitrate is usually the dominant form of N available to cereals under temperate agricultural conditions and concentrations in the interstitial soil solution of cultivated, unfertilized soils are typically around 2 mol m^{-3} (Haynes *et al.*, 1986; Mengel and Kirkby, 1987; Chapter 6). With application of fertilizer N, the concentration of NO_3^- can be as high as 20 mol m^{-3} (Mengel and Kirkby, 1987). In the present study, extension rates and final lengths increased with additional NO_3^- over the range 0 - 2.5 mol m^{-3}, though the major part of the responses occurred with applied NO_3^- from 0 - 1 mol m^{-3}. Hence, under agricultural conditions, little response in leaf extension rate or final length could be expected with the addition of fertilizer N. However, in the present study, leaf width and area increased with NO_3^- to 20 mol m^{-3}, and it is likely that under field conditions individual leaf area would increase as a result of N applied at sowing (Andrews, M^{c}Kenzie and Jones, 1991).

The overall objective of the present study was to determine if individual leaf growth characteristics are dependent on whether N is supplied as NO_3^- or NH_4^+. Plants were also supplied glutamine to simulate exclusive root assimilation of NO_3^-. For all species, at all external N concentrations used, plants supplied either NO_3^- or glutamine showed similar responses in terms of maximum and mean extension rates, duration of growth and final leaf length, width and area attained.

Therefore, under the conditions of the present experiment, the site of N assimilation does not appear to influence the development of individual leaf area.

At external N concentrations over the range 0.5 to 2.5 mol m^{-3}, plants supplied NH_4^+ showed similar individual leaf growth characteristics to those of plants supplied NO_3^-. Also, for a given leaf the maximum final leaf area attained with NO_3^- or NH_4^+ were similar. However, with increasing applied NH_4^+ over the range 2.5 - 20 mol m^{-3}, leaf extension rates and final length and area decreased substantially for most species. Plants grown at high external NH_4^+ concentrations displayed symptoms consistent with those reported in the literature as being the result of NH_4^+ toxicity (Mehrer and Mohr, 1989). Leaves tended to dry out or were chlorotic, overall plant health was poor and tillers were very small or did not form. The exact cause of the depression in growth with NH_4^+ toxicity is not known, though one of the reasons frequently cited is the decrease in the pH of the rooting medium (Mehrer and Mohr, 1989). In the present experiments frequent replacement of the nutrient solution should have prevented this. Hence, though high levels of external NH_4^+ decreased individual leaf growth, it is not clear whether this was a direct result of factors associated with NH_4^+ toxicity syndrome. Also, the decrease in leaf growth and the severity of the symptoms of NH_4^+ toxicity were different in the main and repeat experiments. In the former, with 5 or 20 mol m^{-3} NH_4^+, leaves exhibited various degrees of acute damage and overall plant health was poor. In contrast, in the repeat experiment, even with 10 mol m^{-3} NH_4^+, plants remained healthy, though leaves tended to be smaller than where NO_3^- or glutamine were supplied. This suggests that other environmental factors may influence the effects of high levels of external NH_4^+ on leaf growth and/or the development and expression of the NH_4^+ toxicity syndrome. For example, it has been reported that plants supplied NH_4^+ transpire nearly twice as much water as those supplied NO_3^- (Lips *et al.*, 1990). Further, more definitive work, needs to be carried out on the biochemical aspects of NH_4^+ toxicity. As rye did not appear to be affected by high external NH_4^+ concentrations to the same extent as the other species, this may prove a valuable tool in these investigations.

3.5 Conclusions

The data presented in this chapter have demonstrated that the area of individual leaves of cereals increases with increasing external concentrations of N as NO_3^-, NH_4^+ or glutamine over the range 0 - 2.5 mol m^{-3}. Over this range of external N concentrations growth characteristics were similar for all forms of N, as was the leaf area attained. Leaf area increased further with

increasing external concentrations of NO_3^- or glutamine to 20 mol m^{-3} but with NH_4^+ it usually declined substantially. It is possible that factors associated with NH_4^+ toxicity influence the growth of leaves. Similar maximum leaf growth was achieved with NO_3^-, NH_4^+ or glutamine as N sources, indicating that the extent of leaf growth does not depend on the site of N assimilation. However, as shown in Chapter 5, plants supplied NH_4^+ or glutamine usually have a higher leaf N content than those supplied NO_3^-, especially at higher external N concentrations, and hence it is likely that the amount of leaf area produced per unit leaf N may depend on site of N assimilation.

Table of Contents - Chapter 4

Nitrogen and the Cellular Aspects of Individual Leaf Area Development

page

4.1 Introduction 57

4.2 Materials and Methods 58

4.3 Results 61

4.4 Discussion 67

4.5 Conclusions 70

Appendix 4.1 71

Introduction

Total leaf area, an important determinant of plant photosynthetic capacity, is a function of the number of leaves per plant and average individual leaf area. Numerous studies have shown that additional N usually has a large, positive effect on the area of single cereal leaves (Section 3.1). For example, the final area of main stem leaves 2 - 4 of five cereal species increased between 20 - 150% with increasing applied nitrate (NO_3^-), ammonium (NH_4^+) or glutamine over the range 0 to 1 - 2.5 mol m^{-3} N (Chapter 3). Greater final area was due primarily to increased length, though width also increased. Leaf area increased further with additional NO_3^- or glutamine over the range 2.5 to 20 mol m^{-3} N, mainly as a result of greater width.

As has been shown in a limited number of studies, greater individual leaf area with additional N can be associated with increases in average cell size and/or number or changes in aspects of leaf architecture. Cell number and size are determined by the rate and duration of cell division and expansion respectively while leaf architecture is characterised by both the internal structure of the leaf, particularly the extent of air-spaces, and the relative number of the different cell types. For *Beta vulgaris*, greater leaf area with additional NO_3^- was a result of increases in both cell number per leaf and apparent cell size (Morton and Watson, 1948). Greater area of *Gossypium hirsutum* (cotton) and *Salix viminalis* leaves with additional NO_3^- were associated with increases in the average area of individual epidermal cells (Radin and Parker, 1979; McDonald, 1989). In contrast, additional N as NH_4NO_3 had little effect on the mesophyll cell size of two *Panicum* species and *Festuca arundinacae* (tall fescue), though for all three species leaves were thicker, average inter-veinal distances were greater and the extent of internal airspaces increased (Bolton and Brown, 1980). The effects of additional N on individual leaf area were not reported in this paper. Also for tall fescue, leaf elongation rate (LER) increased 90% with additional N, but the length of epidermal cells was not affected, suggesting that much of the increase in LER was a result of greater cell number (Volenec and Nelson, 1983). In another study using tall fescue, additional N increased mesophyll cell number more than epidermal cell number, and it was suggested that the process of division in these cell types may be differentially sensitive to N availability (MacAdam, Volenec and Nelson, 1989). For cereals, the area of leaf 1 of *Hordeum vulgare* (barley) was greater with additional NO_3^- but cell number was not affected and it was inferred that cell size increased (Dale, 1972). In contrast, a more than doubling in the flag leaf area of *Triticum aestivum* (wheat) with additional NO_3^- was associated with a 85% increase in cell number and calculated average cell volume increased 30% (Lawlor, Kontturi and Young, 1989).

A number of methods can be used to assess leaf cell size and number. The simplest technique involves determining epidermal cell characteristics from surface casts obtained by using a suitable impression substance. While this method was used in the present study, it suffers from some possible drawbacks. Though final leaf area and internal cell size and number must be constrained by the size of the epidermis, extrapolating data obtained from epidermal cell characteristics to other leaf cells must be done with some caution. Epidermal cells only make up around 10% of total leaf cells and their rate and duration of division and/or expansion can differ considerably from other cells (Avery, 1933; Jellings and Leech, 1982; MacAdam, Volenec and Nelson, 1989). Hence, it is possible that epidermal cell characteristics may not accurately reflect the size and number of cells in other leaf tissues. Also, palisade and mesophyll cells, the most numerous types of leaf cells, are the major sites of photosynthesis (Pyke, Jelling and Leech, 1990) and it is therefore important to assess the changes in their number and size relative to changes in leaf area. Usually, total cell number has been determined by digesting leaf material with chromic acid and assessing the number of cells in a subsample (eg. Sunderland, 1960). In the present study, an alternative method has been used in which the DNA content of a leaf was determined using a fluorescent dye (Baer *et al.*, 1985). For a given species, the amount of nuclear DNA is usually constant and hence the quantity of DNA extracted from a leaf gives an indication of cell number.

The overall aim of the study described in this chapter was to gain a better understanding of the cellular basis of leaf growth in temperate cereals. It is difficult to establish from the literature in what way changes in cell number are related to changes in cell size. Also, the magnitude of the responses to additional N appear to possibly depend on species, ontogeny, cell types examined and environment. Therefore, the primary objective of the study was to estimate, for the first six main stem leaves of barley, the relative contributions of changes in cell size and number in determining the increase of individual leaf area with additional NO_3^-.

4.2 Materials and Methods

Barley (cv. Triumph) seed, obtained from the Canterbury Malting Company, Christchurch, New Zealand, was used in the present experiment. Seeds (mean weight - 45 mg) were germinated on paper towels moistened with the appropriate treatment (see below). After 4 d, seedlings with a coleoptile length of approximately 10 mm were transferred to 80 mm diameter, 180 mm tall pots (one per pot) filled with a vermiculite/perlite (1:1 v/v) mixture soaked in basal nutrient solution

(Appendix 2.1) containing the appropriate NO_3^- concentration. Three rates of NO_3^- (0.5, 2, 5 mol m^{-3}) were supplied as potassium nitrate. In all treatments potassium (K^+) was maintained at 13.6 mol m^{-3} using potassium sulphate. Pots were flushed every 2 d with the appropriate nutrient solution. Plants were grown under glasshouse conditions with a photoperiod of approximately 12 h, maximum light levels of around 1500 μmol photons m^{-2} s^{-1} and day/night temperatures of approximately 20/10°C.

For each treatment, leaves 1 - 6 were harvested when fully expanded. Separate plants were used for the harvest of each leaf position. In this chapter the term "leaf" refers only to the lamina portion of the cereal leaf (Section 1.2). The leaves were detached at the ligule and fresh weight (f.wt) determined. Length, width and area were measured using a CI-201 leaf area meter (CID Inc. Moscow, ID, U.S.A). Apparent leaf thickness was calculated as the ratio of leaf f.wt to area (Dijkstra, 1990). Casts of the abaxial leaf surface were obtained by applying clear cosmetic nail varnish ("Cutex", Rexona NZ Ltd, Petone, N.Z.) to the leaf surface at three positions (⅓,½ and ⅔ of the way along the leaf). The impression cast was allowed to dry and then removed by affixing it to clear cellotape (Tiki-Tape, Christchurch, N.Z.) and gently peeling both off the leaf. The casts and adhering tape were then mounted on a glass microscope slide.

Using a light microscope at 100X magnification and a calibrated 1 cm graticule, the average length and width of 100 randomly selected cells were determined for each of the three impressions from each leaf. Average cell area for that cast was calculated from the average cell length and width. Veinal cells were not assessed. For each leaf, average cell length, width and area were calculated as the mean of the values from the three impressions. At 10 random locations on each cast, the number of cells (excluding veins) per calibrated 1 cm^2 grid was assessed. Multiplying this number by average cell area for that cast enabled the proportion of leaf surface occupied by non-veinal cells to be calculated. Taking this factor (hereafter termed NVC) into account, non-veinal epidermal cells per leaf was calculated as (leaf area * NVC)/average cell area.

For the harvests of leaves 1, 3 and 6 extra plants were grown for DNA determination. The method of Baer *et al.* (1985), which uses the DNA-complexing fluorescent dye 4',6'-diamidino-2-phenylindole (DAPI), was adapted and optimized for barley leaves. Fresh weight and area of the leaves were determined. A known amount of leaf material (0.1 - 0.3 g) was homogenized in 5 ml buffer (2000 mol m^{-3} NaCl, 10 mol m^{-3} EDTA, 10 mol m^{-3} Tris-HCl [pH 7.0]) using a mortar and pestle. Chloroform (1.5 volumes) was added to the homogenate and mixed vigorously. After

centrifugation at 1,000 g for 10 minutes, the aqueous supernatant was used for fluorometric measurements. Fluorescence was measured with excitation and emission wavelengths set at 350 and 450 nm respectively using a Shimadzu (Kyoto, Japan) RF540 spectrofluorophotometer. Because of the quenching of fluorescence of the DAPI-DNA complex by substances in the leaf homogenates, using a standard curve based on the fluorescence of purified DNA to determine the sample DNA content is not satisfactory. To overcome this problem Procedure 'A' of Baer *et al.* (1985) was followed. This involved successive addition and mixing of four 10 µl aliquots of homogenate and four 10µl aliquots of DNA standard solution (calf thymus DNA: 25 µg ml^{-1} buffer - 100 mol m^{-3} NaCl, 10 mol m^{-3} EDTA, 10 mol m^{-3} Tris-HCl [pH 7.0]) into a single cuvette (1 cm pathlength; U.V. quartz) containing 3 ml of DAPI solution (final concentration 100 ng ml^{-1} buffer - 100 mol m^{-3} NaCl, 10 mol m^{-3} EDTA, 10 mol m^{-3} Tris-HCl [pH 7.0]). Fluorescence was measured at the beginning of the assay and after addition of each aliquot. When fluorescence units were plotted against the cumulative volume of aliquots, two joined, straight lines were obtained (one for the homogenate and one for the DNA standard) and their slopes calculated. The concentration of DNA in the homogenate was calculated by multiplying the concentration of the DNA in the standard solution by the ratio of slopes of the increase in fluorescence for the homogenate to the increase in fluorescence for the standard solution. For clarification, a calculated example is presented in Appendix 4.1. Values of DNA content of these leaves were used to calculate the DNA content of the equivalent leaf from which epidermal cell characteristics were assessed.

The accurate determination of DNA using DAPI requires the use of a standard - the present experiment used calf thymus DNA. However, because of possible differences in the base composition of the standard and samples, the use of animal DNA as a standard for plant DNA determination has been questioned (Price *et al.*, 1980). Hence, DNA values from the assay used in this study have not been converted into absolute cell numbers and only DNA content per leaf is presented, rather than inferred cell number. Similarly, derived values like cell volume are presented on a DNA basis. However, it is recognized that leaf DNA content is related to cell number and relative differences in DNA content are taken as evidence for differences in cell number.

The experiment was a completely randomized block design with six replicates. Data for each leaf were analysed separately. Analyses were carried out using the "Statistix" (Analytical Software, St.Paul, MN, U.S.A) package. Means stated as being different are based on an $LSD_{0.05}$ and were obtained in a repeat experiment.

4.3 Results

For plants receiving 0.5 mol m^{-3} NO_3^-, the area of successive leaves increased up to leaf 4 but then changed little for leaf 5 and decreased for leaf 6 (Table 4.1). In contrast, for plants receiving 5 mol m^{-3} the area of leaves 1 - 6 increased steadily. Within any leaf position, growth was affected by the level of applied NO_3^-. For most parameters the major part of the response occurred with increasing external NO_3^- from 0.5 to 2 mol m^{-3} with smaller changes with additional NO_3^- to 5 mol m^{-3}. Leaf area increased with additional NO_3^- from 34% for leaf 1 to nearly 190% for leaf 6. Except for leaf 1, greater area was due to increases in both length and width. Only width increased for leaf 1. The length of leaves 2, 3 and 4 increased approximately 20% while that of leaves 5 and 6 increased substantially more (approximately 50 and 100% respectively). Leaf width increased between 14 and 40%, generally more so with increasing leaf position. For leaves 1 - 5 apparent leaf thickness was not affected by additional NO_3^- but for leaf 6 it increased 30%.

Leaf epidermal cell characteristics changed with increasing leaf position. For all three levels of NO_3^-, with increasing leaf position, cell length, width and area generally decreased while total cell number increased. However, the decreases in cell length, width and area were substantially larger for plants supplied 0.5 mol m^{-3} NO_3^- compared to those supplied 2 and 5 mol m^{-3}. Within any leaf position, epidermal cell characteristics were markedly affected by applied NO_3^- and like leaf area, the magnitude of the responses tended to increase with successive leaves (Table 4.2). For most measurements and leaves the major part of the increase occurred with additional NO_3^- from 0.5 to 2 mol m^{-3}. With additional NO_3^-, cell area increased nearly 40% for leaf 1, 60 - 70% for leaves 2, 3, 4 and 5 and over 130% for leaf 6. Greater cell area was a result of increased cell length and width. The increase in cell length ranged from 8% for leaf 1 to 54% for leaf 6 while the increase in width varied between 25 and 50%, though not consistently with leaf position. For all leaves NVC increased from around 25% for plants supplied 0.5 mol m^{-3} up to 50% for those supplied 5 mol m^{-3}. Taking NVC into account, for all leaves cell number increased with additional NO_3^-. The magnitude of the increase varied from 15% for leaf 1 to nearly 130% for leaf 6.

The relationships between applied NO_3^- concentration, leaf position and epidermal cell characteristics are shown in Figs. 4.1a,b. For a given leaf, greater average epidermal cell area with additional NO_3^- was associated with an increase in leaf area (Fig. 4.1a). For all leaves, the relationship was always nearly linear though the slope of the response tended to increase for successive leaves. For example, for leaf 2, an increase of 1 x 10^{-5} cm^2 in average epidermal cell

Table 4.1 The effects of additional nitrate (NO_3^-) on the area, length, width and apparent thickness of main stem leaves 1 to 6 of *Hordeum vulgare* L. The standard error of the mean (SE) is given.

	Applied NO_3^- (mol m^{-3})	Leaf area (cm^2)	Leaf length (cm)	Leaf width (cm)	Thickness ($x10^{-2}$ cm)
Leaf 1	0.5	2.70	8.58	0.47	2.03
	2	3.61	9.58	0.57	2.05
	5	3.63	8.88	0.60	2.08
	SE	0.19	0.25	0.017	0.09
Leaf 2	0.5	3.51	14.31	0.45	2.46
	2	5.85	17.65	0.55	2.48
	5	6.44	17.55	0.59	2.45
	SE	0.42	0.67	0.016	0.19
Leaf 3	0.5	6.73	17.76	0.54	2.32
	2	9.91	21.03	0.66	2.33
	5	11.09	20.23	0.68	2.45
	SE	0.56	0.76	0.017	0.11
Leaf 4	0.5	7.96	18.78	0.63	2.18
	2	12.35	23.75	0.71	2.22
	5	12.58	22.98	0.72	2.29
	SE	1.5	0.88	0.021	0.12
Leaf 5	0.5	7.98	17.55	0.72	2.30
	2	14.30	24.23	0.83	2.50
	5	17.06	26.78	0.91	2.48
	SE	0.91	0.53	0.022	0.10
Leaf 6	0.5	6.50	14.32	0.71	1.94
	2	13.40	24.61	0.86	2.28
	5	18.71	29.13	1.01	2.58
	SE	0.48	0.85	0.036	0.09

Table 4.2 The effects of additional nitrate (NO_3^-) on abaxial epidermal cell length, width and area; percentage of the surface occupied by non-veinal cells (NVC), total number of cells per leaf, cells per leaf length and width of main stem leaves 1 - 6 of *Hordeum vulgare* L. The standard error of the mean (SE) is given.

	Applied NO_3^- (mol m^{-3})	Cell area ($x10^{-5}$ cm^2)	Cell length ($x10^{-2}$ cm)	Cell width ($x10^{-3}$ cm)	NVC (%)	Total cell number ($x10^4$)
Leaf 1	0.5	2.81	1.55	1.81	28.8	2.77
	2	3.52	1.65	2.11	38.7	3.97
	5	3.86	1.67	2.26	39.0	3.67
	SE	0.15	0.04	0.05	1.5	0.20
Leaf 2	0.5	2.76	1.51	1.78	33.9	4.32
	2	4.50	1.75	2.56	47.9	6.23
	5	4.88	1.76	2.75	47.9	6.33
	SE	0.21	0.08	0.06	2.1	0.41
Leaf 3	0.5	2.87	1.49	1.85	32.8	7.71
	2	4.12	1.64	2.51	37.1	8.93
	5	4.65	1.70	2.72	43.7	10.43
	SE	0.14	0.06	0.06	1.8	0.53
Leaf 4	0.5	2.37	1.20	1.93	27.3	9.17
	2	3.44	1.58	2.18	31.0	11.13
	5	4.03	1.62	2.46	39.2	12.24
	SE	0.22	0.07	0.06	1.6	1.63
Leaf 5	0.5	2.19	1.13	1.82	29.4	10.72
	2	2.92	1.31	2.23	30.2	14.79
	5	3.60	1.47	2.48	32.7	15.53
	SE	0.11	0.06	0.04	1.5	0.97
Leaf 6	0.5	1.53	1.00	1.54	19.9	8.46
	2	2.36	1.20	1.88	27.5	15.59
	5	3.62	1.54	2.30	37.1	19.22
	SE	0.37	0.11	0.09	2.3	0.43

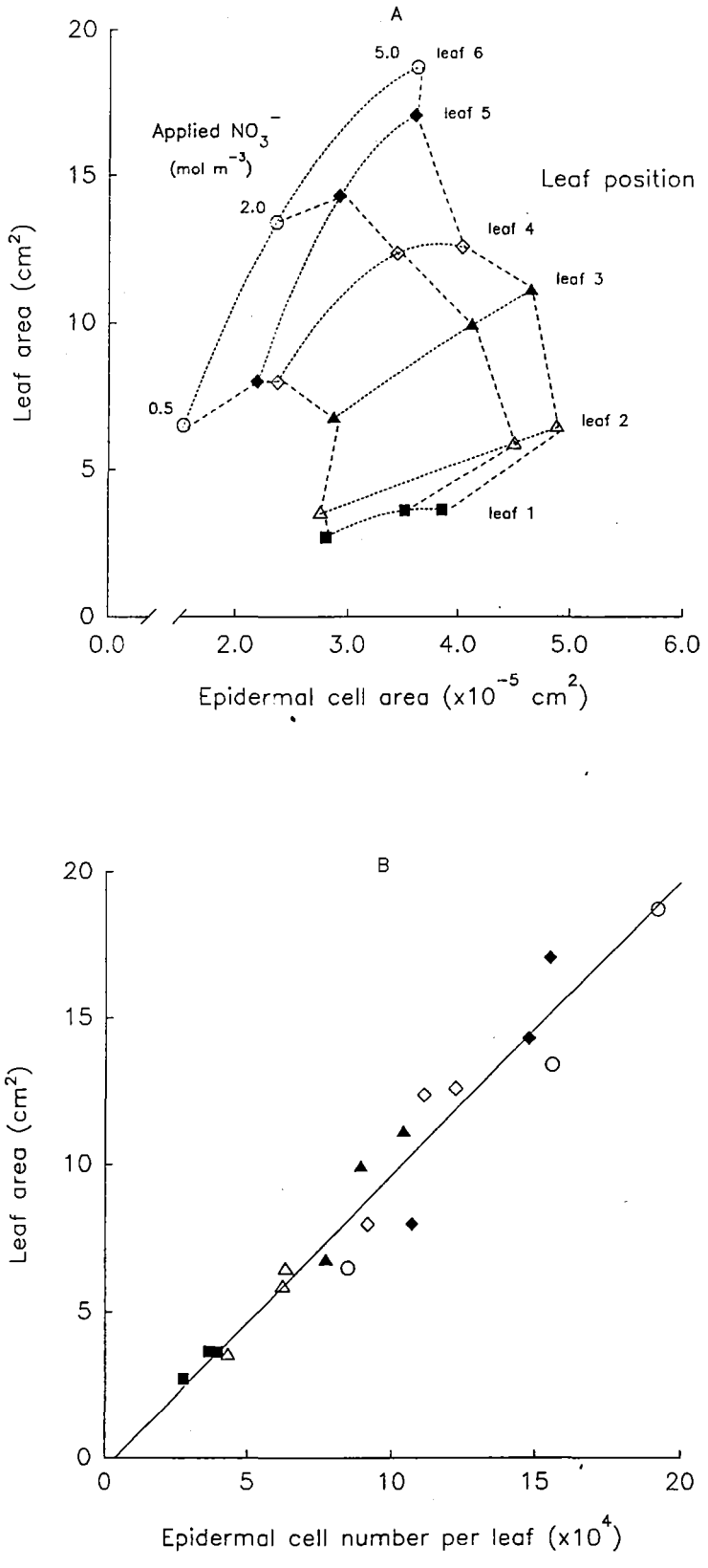


Fig. 4.1 Relationships between epidermal cell area (A) and number (B) and the area of main stem leaves 1 - 6 (■, Δ, ▲, ◊, ♦ and ○ respectively) of *Hordeum vulgare* L. supplied 0.5, 2 or 5 mol m^{-3} nitrate (NO_3^-). Lines are drawn for clarity and were fitted by eye.

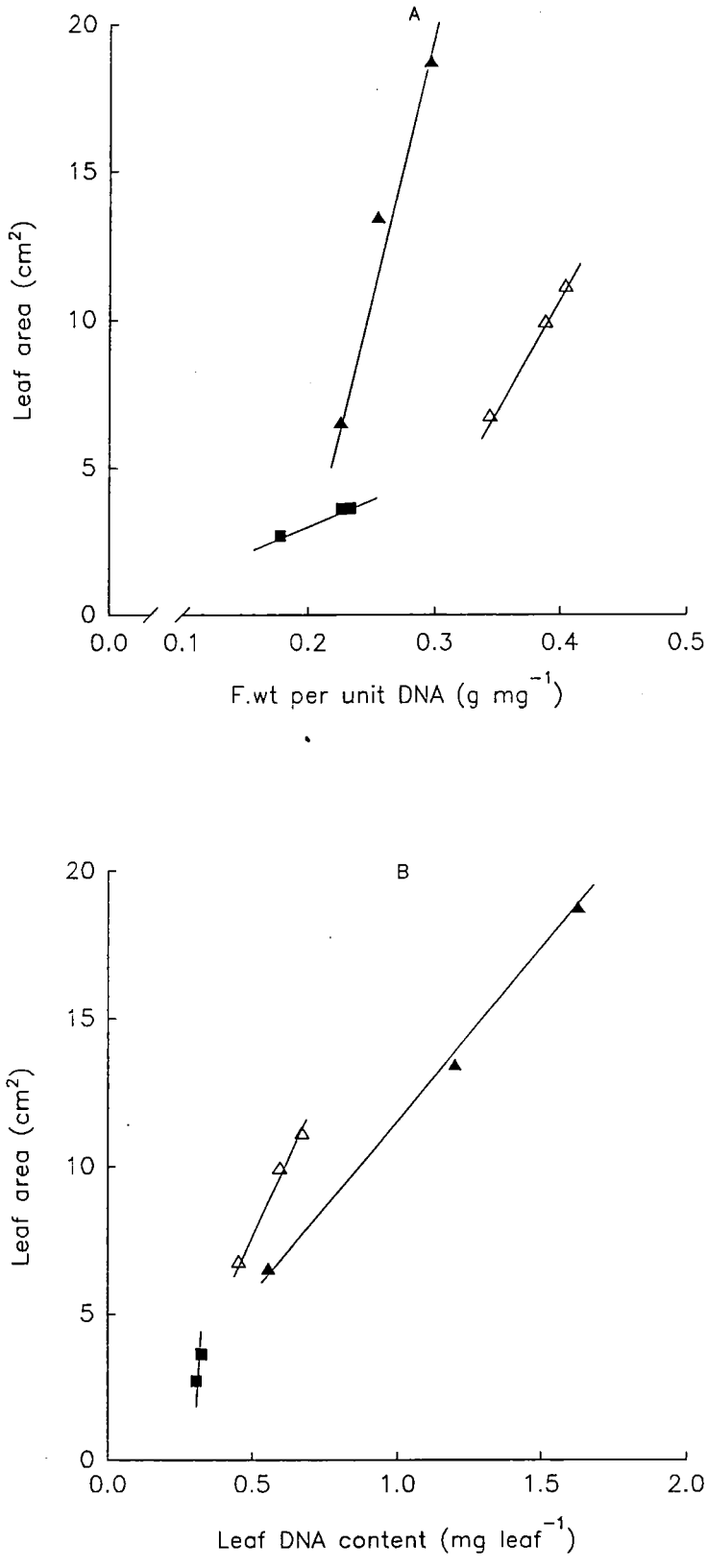


Fig. 4.2 Relationships between f.wt per unit DNA (A) and leaf DNA content (B) and the area of main stem leaves 1, 3 and 6 (■, Δ and ▲ respectively) of *Hordeum vulgare* L. supplied 0.5, 2 or 5 mol m^{-3} nitrate (NO_3^-). Lines are drawn for clarity and were fitted by eye.

Table 4.3 The effects of additional nitrate (NO_3^-) on the total DNA content, fresh weight (f.wt) to DNA ratio and leaf area to DNA ratio of leaves 1, 3, and 6 of *Hordeum vulgare* L. The standard error of the mean (SE) is given.

	Applied NO_3^- (mol m^{-3})	DNA content (mg $leaf^{-1}$)	F.wt per DNA (g mg^{-1})	Area per DNA (cm^2 mg^{-1})
Leaf 1	0.5	0.307	0.178	8.79
	2	0.327	0.226	11.03
	5	0.324	0.233	11.20
	SE	0.02	0.011	0.46
Leaf 3	0.5	0.453	0.344	14.85
	2	0.595	0.388	16.65
	5	0.672	0.404	16.48
	SE	0.03	0.021	0.32
Leaf 6	0.5	0.558	0.225	11.64
	2	1.202	0.254	11.14
	5	1.630	0.296	11.47
	SE	0.12	0.03	0.12

area with additional NO_3^- was associated with a leaf area increase of 1.3 cm^2. In contrast, for leaf 5 a similar increase in cell area resulted in an leaf area increase of 7.7 cm^2. This indicated that for successive leaves other factors, most likely cell number, must have become increasingly important in determining leaf area. Indeed, across all leaves and NO_3^- treatments, there was a positive, linear relationship between epidermal cell number and leaf area (Fig. 4.1b).

For leaves 1, 3 and 6 DNA content was determined. Additional NO_3^- increased the DNA content of leaf 1 by only 6%, leaf 3 over 30% and leaf 6 nearly 200% (Table 4.3). For all three leaves, f.wt per unit DNA, an indication of cell volume, increased from 20 to 30% with additional NO_3^-. Apparent cell volume increased from leaf 1 to 3 but then decreased for leaf 6 at a given applied NO_3^- concentration. Leaf area per unit DNA increased over 30% with increased NO_3^- supply for leaf 1, only 10% for leaf 3 but was not affected by NO_3^- in leaf 6. For all three leaves, an increase in f.wt per unit DNA was associated with an increase in leaf area, though like epidermal cell area, the slope of the relationship increased with leaf position, indicating that other factors had a greater influence on leaf area (Fig. 4.2a). When all leaves and NO_3^- treatments are plotted together, there was a positive correlation between leaf DNA content and leaf area (Fig. 4.2b).

4.4 Discussion

Data presented in this chapter have shown that for all leaves, greater leaf area with additional NO_3^- (Table 4.1) was associated with increased cell size, expressed as either epidermal cell area (Table 4.2) or f.wt per unit DNA (average cell volume) (Table 4.3). Cell area and volume are largely determined by the degree of cell expansion, a process which depends on the movement of water into the cell (Tomos, 1985). Water influx is a result of the lowering of cell water potential, brought about by the accumulation of solutes, the most common of which is usually sucrose (Morgan, 1984). With low external NO_3^- concentrations, leaf N levels are usually less than optimal for maximum photosynthetic rates, and the supply of sucrose for cell expansion may be limited (Section 1.4). Sucrose availability for leaf cell expansion may be further limited because at low external NO_3^- concentrations temperate cereals assimilate NO_3^- in the root (Andrews *et al.*, 1992) and the energy, reductants and C skeletons used in assimilation must be derived from the respiration of sucrose translocated from the leaves (Layzell, 1990). As the concentration of external NO_3^- increases, plant N content and photosynthetic rate usually also increase (Section 1.4) and the supply of sucrose able to be used as osmoticum for cell expansion is probably greater. Further gains in sucrose availability are possible because a greater proportion of the NO_3^- is assimilated in the leaves at high external NO_3^- concentrations (Andrews *et al.*, 1992) and the assimilation reactions can be supported directly by the photosynthetic processes (Layzell, 1990).

Assimilation of NO_3^- in the leaves has other advantages in terms of osmotica generation. Nitrate assimilation results in the generation of excess hydroxide ions, which in the leaves are usually neutralized by the production of organic acids (Raven, 1985). These acids accumulate in the vacuoles and can contribute to the osmotic potential of cells. In addition, NO_3^- taken up in excess of that able to be assimilated is usually also stored in the vacuoles (Granstedt and Huffaker, 1982) and together with counter ions, particularly K^+, can also contribute significantly to the osmotic potential of cells (Blom-Zandstra and Lampe, 1985; Steingröver, Woldendorp and Sijtsma, 1986). It is suggested that for barley in the present study greater cell size with increasing concentrations of external NO_3^- may have been the result of increased availability of osmotica - principally sucrose but also other ions such as organic acids, NO_3^- and K^+. This is similar to a suggestion by Sprent and Thomas (1984), who proposed that for some legume seedlings it was theoretically possible for osmotically driven leaf expansion to be partly dependent on NO_3^- transported to the leaves. However, further work is needed to determine the relative contribution of the various forms of osmotica to increases in cell size with additional N.

Though greater leaf area with additional NO_3^- was associated with increases in cell size, the slope of the relationships between leaf area and epidermal cell area and f.wt per unit DNA increased for successive leaves (Figs. 4.1a, 4.2a). This indicated that with increasing leaf position cell size became less important in determining final leaf area and other factors, most likely cell number, became more influential. For all leaves, cell number increased with additional NO_3^- (Tables 4.2, 4.3) and across all leaves and NO_3^- treatments both epidermal cell number (Fig. 4.1b) and whole leaf DNA content (Fig. 4.2b) were closely related to final leaf area. Cell number is determined by the rate and extent of cell division. Additional N has been shown to increase cell number in a range of species (eg. Morton and Watson, 1948; Volenec and Nelson, 1983; Lawlor, Kontturi and Young, 1989). However, the mechanism by which additional N enhances cell division is not known. As the manufacture of new cells involves the building of cell walls and membranes, which contain significant amounts of C, increased photosynthesis as a result of higher plant N content could be expected to support a greater rate of cell division. For *Cucumis sativus* (cucumber), plants grown under low light conditions had lower rates of C fixation per unit leaf area and smaller leaves, the latter being a result of lower number of cells per leaf (Milthorpe and Newton, 1963). Nitrogen is also an important constituent of many of the compounds vital for cell formation and function, and therefore enhanced N supply would also be expected to support a greater rate of cell division.

Nitrate supply also affected leaf parameters other than cell number and size. For all leaves, increasing external NO_3^- concentration increased NVC, an indicator of the fraction of the leaf surface occupied by non-veinal cells. Increased NVC appeared to be related to inter-veinal distance. Observations indicated that the number of veins per leaf stayed constant with varying NO_3^- supply, and therefore increased inter-veinal distance was probably a result of increased average cell width. As discussed above, cell width, an important component of cell size, would increase with an increased supply of osmotica. Additional N has been reported to increase inter-veinal distance in two *Panicum* species and tall fescue (Bolton and Brown, 1980). Most of the chloroplasts in leaves are likely to be found in cells below non-veinal cells and hence greater inter-veinal distance would result in an increase in the fraction of the leaf surface that would be able to utilize incident photosynthetically active radiation (PAR). This could possibly lead to greater rates of C fixation per unit leaf area.

For some leaves, thickness, as reflected by the ratio of f.wt to leaf area (Dijkstra, 1990), increased with additional NO_3^- supply (Table 4.1). This is supported by the changes in leaf area per unit DNA (Table 4.3). For leaf 1, area per unit DNA increased markedly while leaf DNA

content increased little. In contrast, for leaf 6, the substantial increase in DNA content did not result in a change in the amount of leaf area produced per unit DNA. This could be expected if leaf thickness increased, with more layers of cells leading to more DNA per unit leaf area. Thicker leaves with more layers of cells and hence chloroplasts per unit leaf area would also increase the proportion of incident PAR being utilized, leading to greater leaf photosynthetic capacity. Similar reasoning was proposed by Bolton and Brown (1980), who argued that thicker leaves with additional N increased apparent photosynthesis per unit leaf area in *Panicum* and tall fescue was possibly because of greater PAR absorption and increases in the amounts of photosynthetic enzymes per unit leaf area.

In this chapter data have been presented which was based on the determination of leaf DNA content. However, the quantification of DNA to assess changes in leaf cellular characteristics has possible limitations, the most important of which is the contribution of extranuclear DNA (predominantly chloroplastic) to total DNA. As additional N usually increases leaf chlorophyll content (Section 1.4), it is likely that chloroplastic DNA per cell also increases, though few studies have investigated the magnitude of the response. For *Spinacia oleraceae* seedlings, additional N (form and rate unspecified) increased the contribution of chloroplastic DNA to total DNA from 10 to 16% (Scott and Possingham, 1983). In the present study, the increase in the DNA content of leaf 6 with additional NO_3^- was substantially greater than this (200%) and it is likely that most of the increase in leaf DNA content was due to an increase in nuclear DNA and therefore reflected an increase in cell number per leaf.

Data presented in this chapter have demonstrated that the increase in leaf area with additional NO_3^- was associated with increases in both cell size and number. However, there was a large interaction between leaf position and NO_3^- supply with regard to both leaf area and cell size/number. Hence, the relative contribution of changes in cell size and number to the increased leaf area with additional NO_3^- depended on the position of the leaf being investigated. For a given level of external NO_3^-, the area of successive leaves increased. However, average cell size generally decreased and the increase in area was due to greater cell number per leaf. These changes in cell size and number with increasingly larger successive leaves have been reported for the monocotyledon *Lolium temulentum* (Borrill, 1961) and the dicotyledons cucumber (Milthorpe and Newton, 1963), *Capsicum frutescens* (Steer, 1971) and cotton (Radin and Parker, 1979). The factors affecting this pattern of decreasing cell size and increasing cell number in of successive leaves do not appear to have been investigated. It is possible that the availability of C for new cell wall production and osmotica for cell expansion changes with leaf number per

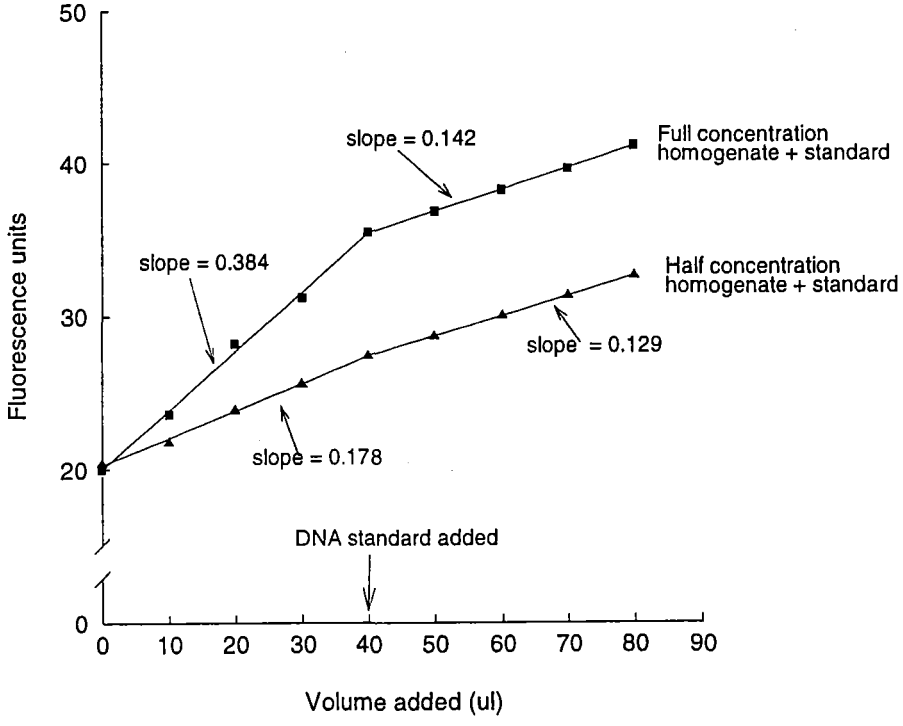
plant and hence influences the cellular characteristics of newly formed leaves. This suggestion is supported by the interaction between the supply of NO_3^- and leaf position, with the increase in the area of successive leaves being greater with additional NO_3^-. Successive leaves of plants supplied NO_3^- had greater increases in cell number and smaller decreases in average cell size. The greater increase in the area of successive leaves with additional N has been described before for wheat (Puckeridge, 1956; cited in Bunting and Drennan, 1966), though the cellular aspects of this interaction do not appear to have been reported. As discussed above, it is possible that greater availability of photosynthate with additional NO_3^- may increase the rate and extent of cell division and expansion and hence result in an increase in the area of successive leaves.

4.5 Conclusions

Data from the experiment conducted for this chapter have shown that greater individual leaf area with additional NO_3^- was associated with an increase in both cell size and number, though the latter increased in importance with leaf position. It was proposed that greater cell size with additional NO_3^- was due to an increase in the availability of osmoticum, primarily sucrose, but also other solutes such as free NO_3^-. Increased cell number was thought to be related to greater levels of photosynthate and N increasing the rate or duration of cell division.

Appendix 4.1: Leaf DNA content - sample calculation.

A known fresh weight of leaf tissue was ground in 5 ml homogenizing buffer (Section 4.2). A 2 ml subsample was diluted with 2 ml homogenizing buffer to give a sample with half the concentration of DNA of the original homogenate. The DNA content of both full and half concentration homogenates was then determined using Procedure A of Baer *et al.* (1985) as described in Section 4.2. A graph of fluorescence units versus cumulative volume of aliquots added for the two samples is presented below:



Calculations of the DNA content of the two homogenates are as follows:

	Full concentration	Half concentration
slope with homogenate only	0.384	0.178
slope with homogenate and standard	0.142	0.129
ratio of slopes	2.70	1.37
DNA concentration of standard (µg ml^{-1} buffer)	60	
DNA concentration of homogenate (µg ml^{-1} buffer)	162.0	82.8
ratio of DNA content of the full to half concentration homogenates	1.96	
actual ratio of DNA content in full and half concentration homogenates	2.00	

Table of Contents - Chapter 5

Nitrogen and the Partitioning of Dry Matter

page

5.1 Introduction 73

5.2 Materials and Methods 75

5.3 Results 76

5.4 Discussion 83

5.5 Conclusions 85

5.1 Introduction

For many plant species grown under a wide range of environmental conditions, shoot to root dry weight (d.wt) ratio (S:R) has been found to increase with increased external nitrate (NO_3^-) concentration over part of the range 0.1 to 20 mol m^{-3} (Andrews, 1993). For temperate cereals, NO_3^- availability has been found to affect the S:R from the seedling stage through to maturity. For example, addition of 5 mol m^{-3} potassium nitrate (KNO_3) plus 5 mol m^{-3} calcium nitrate instead of distilled water increased the S:R of dark grown *Hordeum vulgare* L. (barley) from 1.6 to 2.7 within 19 days of planting (Nátr, 1988). For more mature plants, the S:R of five species of temperate cereals increased from around 1 to nearly 2 with increased NO_3^- over the range 0.1 to 5 mol m^{-3} (Andrews *et al.*, 1992). Commonly associated with the increase in S:R with additional NO_3^- are two other plant growth responses. Firstly, plant or shoot N content also increases with additional NO_3^- (Section 1.4; Andrews, 1993). Data indicate that for many species grown under NO_3^- nutrition, S:R increases linearly with increasing shoot or total plant N content (Hirose, 1986; Ingestad and Agren, 1991; Boot, Schildwacht and Lambers 1992). Secondly, plant d.wt usually also increases with additional NO_3^- as a result of increased plant photosynthetic capacity (Novoa and Loomis, 1981; Section 1.4). Conversely, with low external NO_3^- concentrations plant growth is usually curtailed and S:R is less than at high external NO_3^- concentrations. It has been argued that variation in the partitioning of dry matter between the shoot and root at different external N concentrations is an adaptive response which maintains balanced activity between the root (site of N uptake) and the shoot (site of photosynthesis) such that growth rate is optimized or maximized (Troughton, 1956;1960; Brower, 1962; Davidson, 1969; Agren and Ingestad, 1987; Hilbert, 1990; Gleeson, 1993). However, in some situations, additional N has been shown to increase S:R with no increase in plant d.wt. For example, Andrews (1993) reported that with additional NO_3^- over the range that total plant d.wt does not change, the S:R of mature *Triticum aestivum* (wheat) continued to increase. Also, at external concentration of NO_3^- that are toxic to plants, S:R frequently continues to increase (Andrews, 1993).

There are also several reports that for herbaceous species, S:R increases with increased growth/development independent of N supply (Foth, 1962; Rufty, Raper and Huber, 1984; Caloin, 1987; Andrews, Scott and M^{c}Kenzie, 1991; Larsson *et al.*, 1991). Therefore, it is possible that part of the observed N effect on S:R may be related to plant development (ontogeny). This is also likely to be the case where S:R changes with seedling development, at least where growth is reliant on seed reserves as opposed to current photosynthate and N assimilation.

Associated with S:R is the fraction of plant d.wt that is leaf, commonly referred to as the leaf weight ratio (LWR). Though LWR is related to S:R, it is probably a more useful parameter than S:R when describing changes in plant leaf area. However, few data are available for the effect of NO_3^- supply on LWR. On the basis of the data available, LWR appears to increase with increased NO_3^- supply in the range which total plant d.wt increases, then either changes little or increases further with increased NO_3^- thereafter (Hocking and Meyer, 1991; Andrews *et al.*, 1992). For the five main temperate cereals in the vegetative phase, LWR increased from around 0.3 to 0.4 with increased NO_3^- supply from 0.5 to 5 mol m^{-3}, the range usually found in agricultural soils (Andrews *et al.*, 1992). In a separate study, LWR of reproductive wheat plants increased from around 0.2 to 0.3 with increased NO_3^- concentration from 0.5 to 12 mol m^{-3} (Hocking and Meyer, 1991).

In comparison with NO_3^-, fewer studies have been carried out on the effects of ammonium (NH_4^+) availability on S:R. There are several reports that as for NO_3^-, S:R increases with increased NH_4^+ supply but that at a similar total plant d.wt, S:R is greater with NH_4^+ as an N source (Cox and Reisenhauer, 1973; Timpo and Neyra, 1983; Bowman and Paul, 1988; Troelstra, Wagenaar and Smant 1992; Andrews, 1993). Also, there are reports that for plants of similar d.wt, tissue N content can be greater with NH_4^+ than with NO_3^- as an N source (Cox and Reisenhauer, 1973; Bowman and Paul, 1988; Raven, Wollenweber and Handley 1992; Troelstra, Wagenaar and Smant 1992). Thus it is possible that the relationship between S:R and tissue N content is the same regardless of whether N is supplied as NO_3^- or NH_4^+.

A number of explanations for the N effect on S:R have been proposed (reviewed by Andrews, 1993). Andrews concluded that none of these proposals fully explained all the data available. The overall aim of the experiments conducted in this chapter were to further investigate the relationships between N supply, plant reduced-N content and growth and S:R. Firstly, data are presented from experiments carried out on barley in Chapter 2 which are used to assess the relative importance of growth and tissue N content in determining S:R at the seedling stage. Secondly, the effects of form and availability of N on S:R and LWR of vegetative barley are examined. Nitrogen was supplied as NO_3^-, NH_4^+ or glutamine. Amino acids such as glutamine are found in the interstitial soil solution (Bremner, 1965) and can be taken up and utilized by plants (Jones and Darrah, 1993). When N is supplied solely as glutamine, this mimics exclusive root assimilation of N (Section 1.4). The primary objective of the study was to determine if the relationship between S:R and tissue N content holds regardless of N form supplied.

5.2 Materials and Methods

Data from three experiments are reported in this chapter. For all experiments seed of barley (cv. Triumph, mean seed weight - 45 mg) was obtained from the Canterbury Malting Company, Christchurch, New Zealand. Seed was not chemically treated and showed 95% germination.

The first two experiments in this chapter are fully described in Chapter 2 as Experiments 2 and 4. Briefly, for both these experiments, seeds were placed at 70 mm depth in 80 mm diameter, 180 mm tall pots (20 per pot) filled with a vermiculite/perlite (1:1 v/v) mixture soaked in basal nutrient solution (Appendix 2.1) containing the appropriate N treatment. In Experiment 1 seedlings were supplied either basal nutrient solution alone, or with 5 mol m^{-3} NO_3^- as KNO_3 or 5 mol m^{-3} NH_4^+ as ammonium sulphate ($(NH_4)_2SO_4$) added. In Experiment 2 seedlings were supplied basal nutrient solution alone or with 5 mol m^{-3} NO_3^- as KNO_3 or 5 mol m^{-3} chloride (Cl^-) as potassium chloride added. For all treatments potassium (K^+) was maintained at 8.6 mol m^{-3} by the addition of potassium sulphate (K_2SO_4) where necessary. Pots were flushed with the appropriate nutrient solution every 2 d. Both experiments were carried out in the dark at 10±1°C in controlled environment chambers. Seedlings were harvested 21 days after sowing (DAS), separated into shoot, root and residual seed and dried at 70°C for 72 h for d.wt determination. In Experiment 1 the NO_3^- and NH_4^+ contents of the seedlings were determined as described by Mackereth, Heron and Talling (1978) and Baethgen and Alley (1989) respectively. Also, seedlings were analysed for total N content using a Europa Scientific (U.K.) N analyser. Assimilated N was assumed to be the difference between total N and NO_3^- plus NH_4^+-N.

For Experiment 3, seeds were germinated on paper towels moistened with distilled water. After 4 d seedlings with a coleoptile length of approximately 10 mm were transferred to 80 mm diameter, 180 mm tall pots (one per pot) filled with a vermiculite/perlite (1:1 v/v) mixture soaked in basal nutrient solution (Appendix 2.1) containing the appropriate N treatment. There were nine rates of N (0, 0.5, 1, 2, 3, 4, 5, 6 or 10 mol m^{-3}) supplied as one of three forms: NO_3^- as KNO_3, NH_4^+ as $(NH_4)_2SO_4$, or glutamine. For all treatments, K^+ was maintained at 13.6 mol m^{-3} using K_2SO_4 where necessary. Pots were flushed every 2 - 3 d with the appropriate nutrient solution. Plants were grown under controlled environment conditions with a photoperiod of 14 h, at a light level of approximately 400 µmol photons m^{-2} s^{-1} and with day/night temperatures of 20/15±2°C.

Plants were harvested 35 DAS, separated into leaf (lamina), stem (leaf sheaths) and root. Leaf area was determined using a Model 3100 leaf area meter (Lambda Instrument Corp., NE,

U.S.A.). Plant material was dried at 70°C for 72 h for d.wt determination. The dried material was finely ground and analysed for reduced-N using a "Kjeltec Autosampler System 1035 analyser" (Tecator; Höganäs, Sweden).

All experiments were of a randomised complete block design with five replicates. An analysis of variance was carried out on all data using the computer package "Statistix" (Analytical Software, St.Paul, MN, U.S.A). All effects discussed have a probability of $P<0.05$ and were obtained in repeat experiments. Means stated as significantly different are on the basis of an LSD ($P<0.05$) test. Where regression lines were fitted ("Sigmaplot Scientific Graphing System v. 5.01; Jandel Corp., CA, U.S.A), 95% confidence intervals are also plotted. Lines were considered dissimilar where confidence intervals did not overlap.

5.3 Results

In Experiment 1, addition of 5 mol m^{-3} NO_3^- but not NH_4^+ caused an increase in root plus shoot d.wt and a decrease in residual seed d.wt (Table 5.1). There were no signs of NH_4^+ toxicity in seedlings supplied NH_4^+. Nitrogen uptake was slightly greater with NO_3^--N than with NH_4^+ as an N source. Ammonium-N and NO_3^--N constituted only a small proportion (<1%) of total N in seedlings supplied NH_4^+ whereas NO_3^--N constituted almost 20% of N where NO_3^- was supplied. Nitrogen assimilation (calculated as total N minus NH_4^+ and NO_3^--N) was greater when N was supplied as NH_4^+ compared to NO_3^-. In Experiment 2, both Cl^- and NO_3^- caused decreases in residual seed d.wt and increases in shoot plus root d.wt and S:R, though the magnitude of the response was greater with NO_3^-.

In Experiment 3, with increasing external N over the range 0 - 4 mol m^{-3}, total plant d.wt increased similarly for all three N forms, from less than 0.1 g to approximately 3 g (Fig. 5.1a). Dry weight increased further with additional N to 10 mol m^{-3} as NO_3^- or glutamine but with NH_4^+ it changed little and finally decreased. Over the range 0 - 4 mol m^{-3} external N, total plant leaf area increased from less than 10 cm^2 to approximately 200 cm^2 and was similar for all three forms of N (Fig. 5.1b). Leaf area increased further with additional NO_3^- or glutamine to 10 mol m^{-3} but changed little with additional NH_4^+. At higher external N concentrations leaf area was greater with NO_3^-.

With increasing external N concentrations over the range 0 - 10 mol m^{-3}, S:R increased steadily from under 1 to over 2 with NO_3^- and to nearly 3 with NH_4^+ or glutamine supplied (Fig. 5.2a). Similarly, LWR increased steadily from about 0.28 to 0.35 for plants supplied NO_3^- and to approximately 0.4 with NH_4^+ or glutamine (Fig. 5.2b). For any given external N concentration both S:R and LWR were smaller for plants supplied NO_3^-, especially at higher external N concentrations.

Whole plant reduced-N content increased from less than 1% to over 2% with additional N over the range 0.5 - 4 mol m^{-3}, regardless of N form applied (Fig. 5.2c). With further additions of N as NO_3^- to 10 mol m^{-3} it increased to nearly 3% while with NH_4^+ and glutamine it increased to approximately 3.5 and 4% respectively.

For all three forms of N supplied, S:R and LWR showed linear relationships between and both plant d.wt and plant %N (Fig. 5.3). The regression equations of the fitted lines for the three N forms in Figs. 5.3a-d are as follows (GLN = glutamine):

Fig. 5.3a	NO_3^- -	S:R = (d.wt * 0.245) + 0.829	$r^2 = 0.948$
	NH_4^+ -	S:R = (d.wt * 0.570) + 0.720	$r^2 = 0.974$
	GLN -	S:R = (d.wt * 0.475) + 0.835	$r^2 = 0.914$
Fig. 5.3b	NO_3^- -	LWR = (d.wt * 0.021) + 0.262	$r^2 = 0.963$
	NH_4^+ -	LWR = (d.wt * 0.051) + 0.246	$r^2 = 0.906$
	GLN -	LWR = (d.wt * 0.035) + 0.260	$r^2 = 0.883$
Fig. 5.3c	NO_3^- -	S:R = (%N * 0.595) + 0.446	$r^2 = 0.989$
	NH_4^+ -	S:R = (%N * 0.574) + 0.722	$r^2 = 0.915$
	GLN -	S:R = (%N * 0.773) + 0.420	$r^2 = 0.964$
Fig. 5.3d	NO_3^- -	LWR = (%N * 0.049) + 0.233	$r^2 = 0.959$
	NH_4^+ -	LWR = (%N * 0.060) + 0.228	$r^2 = 0.988$
	GLN -	LWR = (%N * 0.060) + 0.226	$r^2 = 0.996$

Especially at greater plant d.wts, both S:R and LWR were lower for plants supplied NO_3^- compared to NH_4^+ or glutamine (Fig. 5.3a,b). However, if S:R or LWR are plotted against plant %N, the differences between N forms become less pronounced (Fig 5.2c,d) and .

Plotting total plant and leaf reduced-N against total plant d.wt and leaf area respectively shows distinct differences between the three forms of N supplied (Fig. 5.4). Regression equations for the relationships in Fig. 5.4a,b are presented below (PN = total plant reduced-N; GLN = glutamine; LA = leaf area; LN = leaf N):

Fig. 5.4a NO_3^- - $d.wt = (-3 \times 10^{-4} * PN^2) + (0.068 * PN) + 0.180$ $r^2 = 0.99$
NH_4^+ - $d.wt = (-3 \times 10^{-4} * PN^2) + (0.064 * PN) + 0.194$ $r^2 = 0.99$
GLN - $d.wt = (-3 \times 10^{-4} * PN^2) + (0.066 * PN) + 0.217$ $r^2 = 0.99$

Fig. 5.4b NO_3^- - $d.wt = (-0.061 * LN^2) + (9.345 * LN) - 3.860$ $r^2 = 0.99$
NH_4^+ - $d.wt = (-0.066 * LN^2) + (8.206 * LN) + 0.890$ $r^2 = 0.99$
GLN - $d.wt = (-0.053 * LN^2) + (7.982 * LN) + 1.587$ $r^2 = 0.99$

With increasing amounts of plant reduced-N up to about 60 mg, total plant d.wt increased similarly for all forms of N. However, at higher levels of plant reduced-N, d.wt produced per unit plant reduced-N was greatest where NO_3^- was supplied, intermediate with glutamine and lowest with NH_4^+. Leaf area produced per unit leaf N was similar for all forms of N supplied up to about 30 mg leaf N. However, with higher leaf N levels, leaf area produced was greatest with NO_3^-, intermediate with glutamine and lowest with NH_4^+. For example, with 60 mg of leaf N, plants supplied NO_3^- produced 30% more leaf area than those supplied NH_4^+ (337 vs 256 cm^2 respectively).

Table 5.1 Effect of additional ammonium (NH_4^+) or nitrate (NO_3^-) on shoot (S), root (R) and residual seed (RS) dry weight (d.wt), shoot:root d.wt (S:R) and total seedling NO_3^-, NH_4^+ and reduced nitrogen (N) content of *Hordeum vulgare* L. prior to emergence from the substrate. The standard error of the mean (SE) is given.

	Dry weight (mg)				Nitrogen (μg seedling^{-1})		
Treatment	S	RS	R	S:R	Total	NO_3^--N	NH_4^+-N
basal	8.7	14.1	6.4	1.35	611	5.1	0.5
NH_4^+	8.4	14.5	6.0	1.31	920	4.6	1.4
NO_3^-	10.3	12.5	6.3	1.63	977	186.9	0.7
SE	0.29	0.41	0.14	0.06	15	10.2	0.5

Table 5.2 Effect of additional chloride (Cl^-) or nitrate (NO_3^-) on shoot (S), root (R) and residual seed (RS) dry weight (d.wt), shoot:root d.wt (S:R) of *Hordeum vulgare* L. prior to emergence from the substrate. The standard error of the mean (SE) is given.

	Dry weight(mg)			S:R
	S	RS	R	S
basal	11.1	12.8	5.8	1.91
NO_3^-	13.5	9.7	5.2	2.59
Cl^-	12.5	10.5	5.7	2.19
SE	0.41	0.51	0.35	0.05

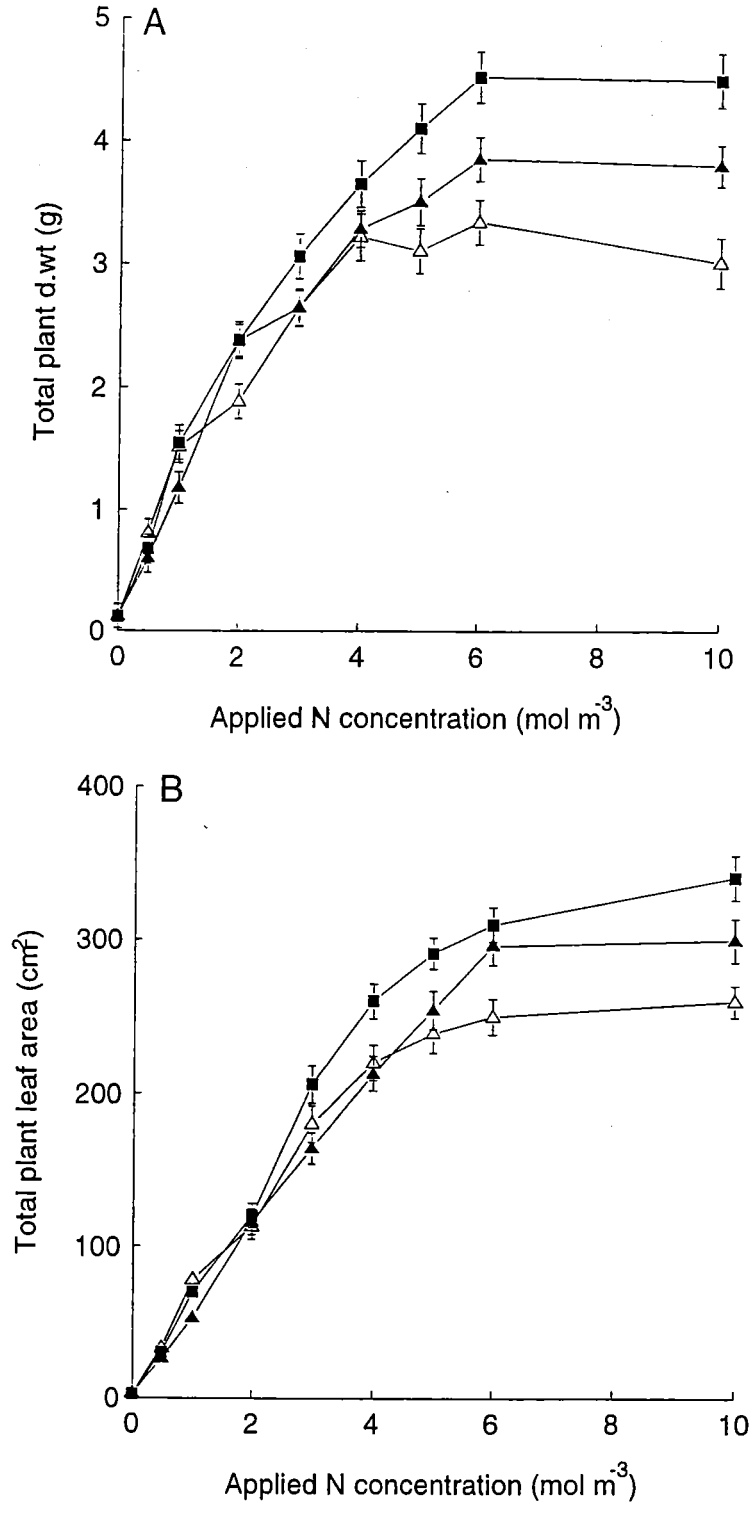


Fig. 5.1 Effects of different concentrations of nitrate (■), ammonium (Δ) or glutamine (▲) on total plant dry weight (d.wt) (A) and leaf area (B) of *Hordeum vulgare* L. cv. Triumph. Error bars indicate ± standard error of mean where larger than symbol.

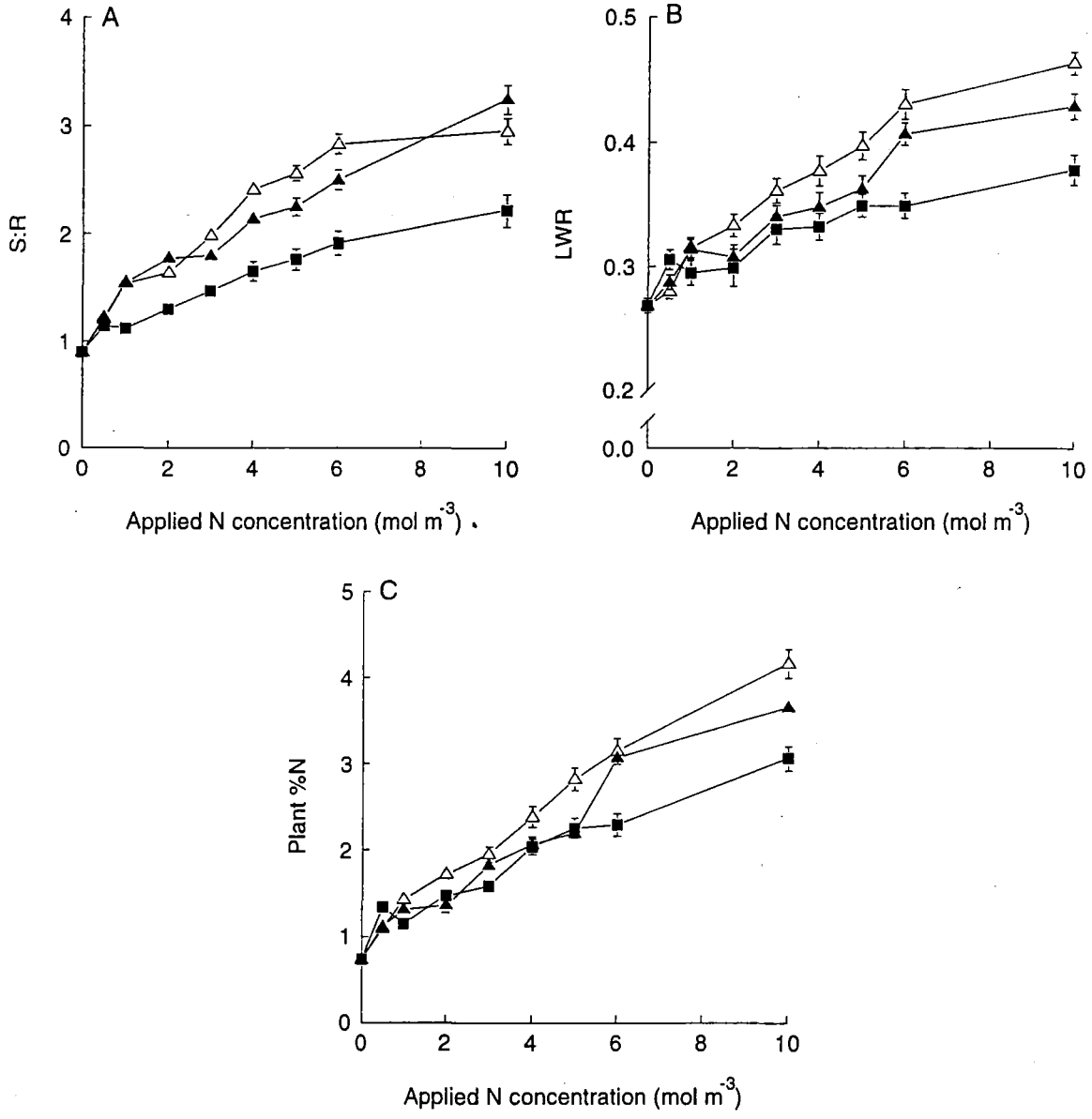


Fig. 5.2 Effects of different concentrations of nitrate (■), ammonium (Δ) or glutamine (▲) on shoot to root dry weight (d.wt) ratio (S:R) (A), leaf d.wt as a fraction of total plant d.wt (LWR) (B) and plant %N (C) of *Hordeum vulgare* L. cv. Triumph. Error bars indicate ± standard error of mean where larger than symbol.

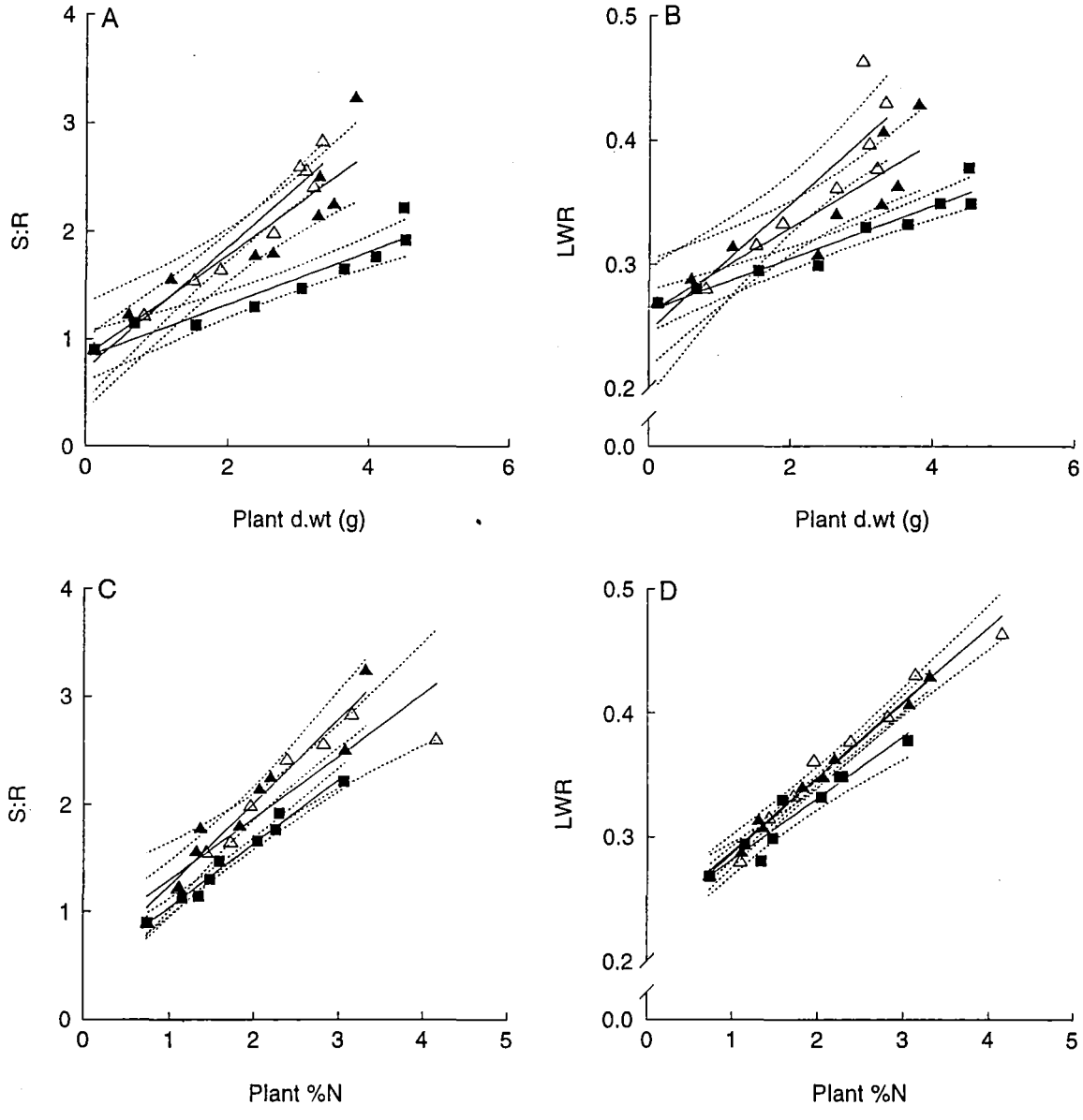


Fig. 5.3 The relationships between shoot to root dry weight (d.wt) ratio (S:R) (A,C) or leaf d.wt as a fraction of total plant d.wt (LWR) (B,D) versus plant d.wt or %N of *Hordeum vulgare* L. cv. Triumph supplied different concentrations of nitrate (■), ammonium (Δ) or glutamine (▲). Regression equations reported in Section 5.3; 95% confidence intervals shown.

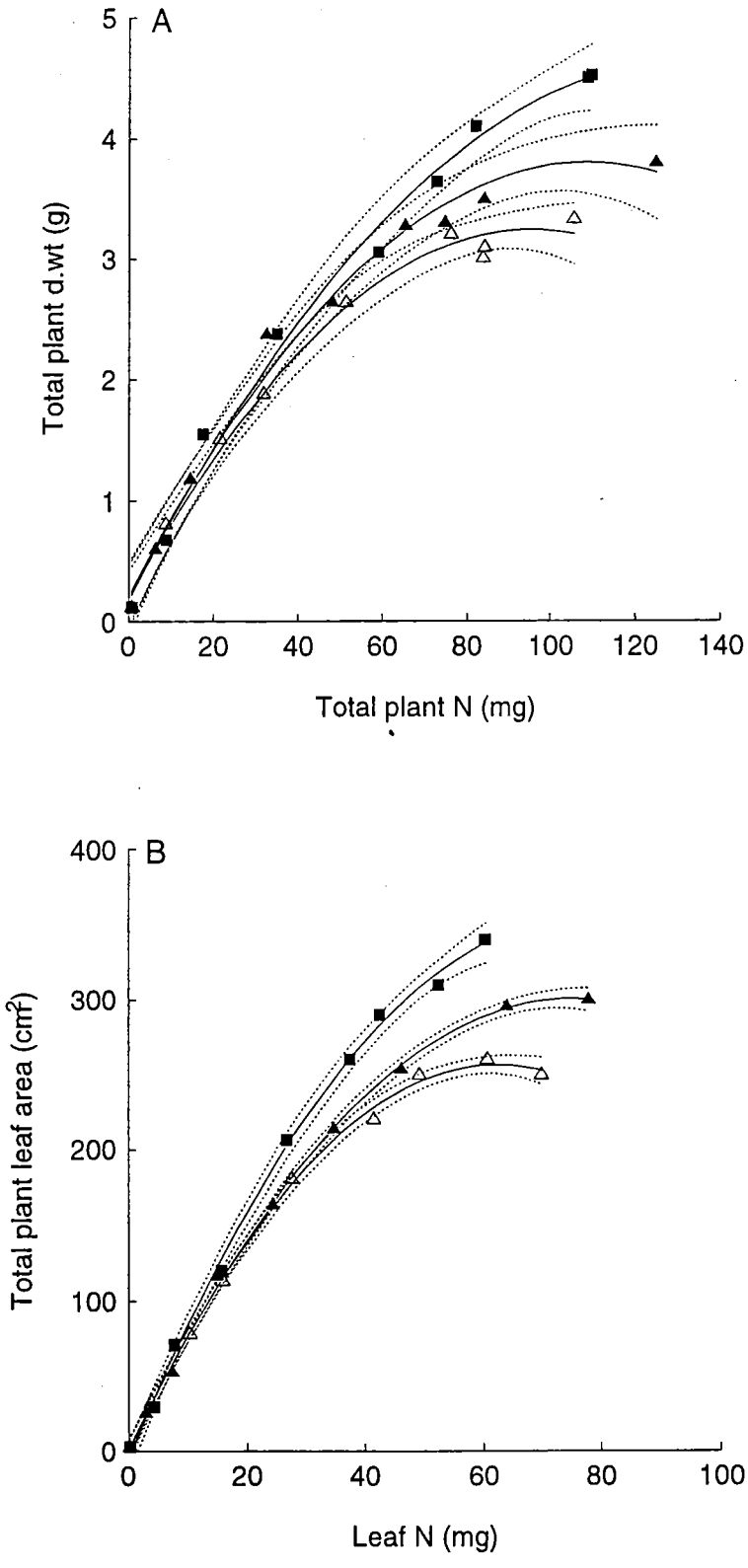


Fig. 5.4 The relationships between total plant reduced-N and plant dry weight (A) and total leaf N and plant leaf area (B) of *Hordeum vulgare* L. cv. Triumph supplied different concentrations of nitrate (■), ammonium (Δ) or glutamine (▲). Regression equations reported in Section 5.3; 95% confidence intervals shown.

5.4 Discussion

For many plant species grown under a wide range of environmental conditions, S:R has been found to increase with increasing external NO_3^- over part of the range 0.1 to 20 mol m^{-3}. Also associated with increasing NO_3^- supply is greater plant d.wt. It has been argued that the change in S:R with additional NO_3^- is an adaptive mechanism which maintains balanced activity between the root (site of N uptake) and shoot (site of photosynthesis) such that plant growth is increased or maximised (Troughton, 1956;1960; Brower, 1962; Davidson, 1969; Hilbert, 1990; Gleeson, 1993). Together with the increase in S:R with increasing external NO_3^- is greater plant or shoot N content and there is frequently a near linear relationship between plant reduced-N content and S:R (Andrews, 1993). This association between S:R and plant reduced-N content has been used as the basis for various proposals which attempt to explain the controlling mechanism for the N effects on S:R (reviewed by Andrews, 1993). However, Andrews argued that none of the hypotheses fully explain all the data available and he put forward an alternative hypothesis.

Andrews (1993) proposed that the N effect on S:R can be explained by the effect of increased N assimilation and protein synthesis on photosynthesis, and hence growth, and by competition between the N assimilation/protein synthesis processes and growth for energy/carbon (C) derived from photosynthesis. Andrews argued that increased N assimilation/protein synthesis results in an increased proportion of energy from photosynthesis being utilised in processing N at the expense of growth, and that this is reflected in a higher tissue reduced-N content. Over part of the external N concentration range 0.1 to 20 mol m^{-3}, the effect of increased N assimilation/protein synthesis on photosynthesis is so great that there is a net increase in photosynthate available for growth. This was found to be so in Experiment 3 where total plant d.wt increased with applied NO_3^-, NH_4^+ or glutamine concentration over the range 0 - 6 mol m^{-3} (Fig. 5.1a). Shoot to root d.wt ratio also increased over this range of external N concentrations (Fig. 5.2a) and Andrews (1993) proposed that this was a due to the proximity of the shoot to the C source and increased N availability for growth.

In Experiment 3, at external N concentrations over the range 6 - 10 mol m^{-3}, d.wt changed little (Fig. 5.1a) but S:R continued to increase (Fig. 5.2a). Andrews (1993) suggested that when N assimilation/protein synthesis increases to a point where photosynthate available for dry matter production decreases, S:R will still increase as the shoot will realise a greater proportion of its growth potential due to its proximity to the source of C and the availability of reduced N for growth.

In the present study, as has been found in previous studies, there was a linear relationship between plant d.wt and S:R or LWR within each N form. However, at a similar total plant d.wt, S:R or LWR tended to be greater with NH_4^+ or glutamine compared to NO_3^-, especially at high external N concentrations (Fig. 5.3a,b). Also at high external N concentrations, for plants of similar d.wt, tissue reduced-N content was greater with NH_4^+ or glutamine. Hence, if S:R and LWR are plotted against plant reduced-N content, then there are no significant differences between the three forms of N (Figs. 5.3c,d). Therefore, it is proposed that the relationships between S:R and tissue reduced-N content are similar regardless of N form supplied or site of N assimilation. It is possible that if the relationship between S:R and plant reduced-N is expressed on a plant protein rather than reduced-N basis that the regression lines for the different N forms match even better than those in Fig. 5.3c,d.

The proposal of Andrews (1993) can also be used to explain the greater S:R of plants supplied NH_4^+ or glutamine compared to NO_3^-. Especially at higher external N concentrations, plants supplied NH_4^+ and glutamine had greater plant reduced-N content. If the demands for energy/C for N assimilation and protein synthesis are greater for these plants, an increased proportion of energy from photosynthesis will be utilised in processing N, compared to when NO_3^- is supplied. Hence, based on the proximity premise, with the shoot being closer to the source of C, S:R be greater with NH_4^+ and glutamine.

For several herbaceous species, S:R has been reported to increase with increased growth or development, independent of N supply (Foth, 1962; Rufty, Raper and Huber, 1984; Caloin, 1987; Andrews, Scott and M^{c}Kenzie, 1991; Larsson *et al.*, 1991). It is therefore possible that part of the effect of N on S:R may be an ontogenetic one, though the extent and relative importance of this has yet to be determined. However, as plant development is usually associated with increasing d.wt, it may be difficult to separate the effects of growth associated with the effects of additional N and those associated with development. Increases in S:R independent of N supplied were also found in this chapter for barley seedlings prior to emergence. In Experiment 1, uptake of N was similar with either NO_3^- or NH_4^+ while N assimilation was greater with NH_4^+. However, only seedlings supplied NO_3^- had greater S:R and d.wt, the latter associated with an increase in reserve mobilization. The association between S:R and growth or d.wt, rather than N assimilation, is supported by data from Experiment 2 where, compared to seedlings supplied only basal nutrient solution, those supplied either NO_3^- or Cl^- showed enhanced reserve mobilisation, greater growth and increased S:R. It is proposed that at least for seedlings prior to emergence, increased S:R with additional N as NO_3^- is not related to N supply or assimilation but rather to

greater growth. In Chapter 2 it was suggested that the NO_3^- effect on the growth of seedlings prior to emergence is probably an osmotic effect, rather than an increase in the products of N assimilation, with greater water uptake increasing the rate of seed reserve mobilization.

There are reports in the literature that dry matter production per unit N is greater with NO_3^- compared to NH_4^+ as an N source (Cox and Reisenhauer, 1973; Bowman and Paul, 1988; Troelstra, Wagenaar and Smant 1992). This was found to be the case in Experiment 3, where for a given amount of plant reduced-N, total plant d.wt was greater with NO_3^- compared to NH_4^+ or glutamine (Fig 5.4a). Especially at higher levels of external N (6 to 10 mol m^{-3}), d.wt did not increase appreciably (Fig. 5.1a), though plant reduced-N content continued to increase (Fig. 5.2c) and hence the amount of d.wt produced per unit plant reduced-N decreased (Fig. 5.4a). Theoretically, N use 'efficiency' should be greater with NH_4^+ compared to when NO_3^- is supplied because of the reduction of NO_3^- to NH_4^+ requires energy to generate reductants and produce and maintain the nitrate and nitrite reductase and enzyme systems. However, this is often not the case (Raven, 1985). Andrews (1993) argued that because at a given plant d.wt both S:R and plant reduced-N content were greater with NH_4^+ and glutamine compared to NO_3^-, the energy demands associated with extra protein synthesis from amino acids is greater than that associated with the production of amino acids from inorganic N. As plants supplied NH_4^+ or glutamine had higher reduced-N contents, a greater fraction of the total energy derived from photosynthesis is used for assimilating N and hence N use efficiency would be greater with NO_3^-. A similar reasoning could be used to explain the greater leaf area per unit leaf N when N was supplied as NO_3^- compared to NH_4^+ or glutamine (Fig. 5.4b). This effect does not appear to have been reported before, though not totally unexpected. As there was a close relationship between S:R and LWR, a decrease in N use 'efficiency' with NH_4^+ or glutamine in terms of dry matter production should be reflected in a corresponding decrease in the amounts of leaf area produced per unit leaf N.

5.5 Conclusions

Data presented in this chapter have shown that over the range of external N concentrations from 0 to 10 mol m^{-3}, there was a near-linear relationship between plant d.wt and S:R, regardless of whether N was supplied as NO_3^-, NH_4^+ or glutamine, though for a given d.wt, S:R tended to be lower with NO_3^-. Hence, for a given amount of plant reduced-N content, S:R was similar for all N forms and it was concluded that the linear relationship between S:R and plant reduced-N content

holds regardless of the N form supplied. The data presented appear to support the hypothesis of Andrews (1993) which explains the effects of N on the S:R as competition for photosynthetically derived energy or C between N assimilation/protein synthesis and growth. It was also shown that leaf area produced per unit leaf N was greater where N was supplied as NO_3^- rather than NH_4^+ or glutamine and this was related to the decrease in the 'efficiency' of dry matter production with these forms of N.

Table of Contents - Chapter 6

Nitrogen, Canopy Development and Grain Yield and Quality

page

6.1 Introduction 88

6.2 Materials and Methods 90

6.3 Results 93

6.4 Discussion 104

6.5 Conclusions 109

Appendix 6.1 110

6.1 Introduction.

The monetary return from a cereal crop depends primarily on the quantity of grain produced (yield), though often the quality of the grain also has a large influence on final crop value. Cereal grains are composed mainly of carbohydrates (Kent, 1983), which are produced through the photosynthetic activity of the crop. The process of photosynthesis depends on the interception of photosynthetically active radiation (PAR) and involves the conversion of this energy into carbohydrates via a series of reactions (Section 1.4). During most stages of cereal crop development PAR is intercepted primarily by the leaves, which collectively make up the canopy. The extent of a crop canopy is usually expressed as the leaf area index (LAI - leaf area per unit ground area; Hay and Walker, 1989).

Grain yield is related to the net amount of photosynthate or dry matter (DM) produced during crop development, and the proportion of this DM that is partitioned to the grains relative to non-harvested parts of the crop (Evans, Wardlaw and Fischer, 1975). Using a variety of crop species, previous studies have established the general relationships between canopy development, light interception, crop DM production and harvestable yield (Biscoe and Gallagher, 1977; Monteith, 1977). These relationships can be expressed as follows:

$$\text{yield} = Q * I * \varepsilon * H$$

(Equation 6.1)
(Hay and Walker, 1989)

Parameter Q is the quantity of incident PAR per unit land area during the time of crop development, I is the fraction of Q that is intercepted by the crop and ε is the coefficient by which intercepted PAR is converted to DM. The product of Q, I, and ε is the quantity of DM produced by the crop and is a reflection of the magnitude of net crop photosynthesis. In field studies usually only above ground crop DM production is measured, as complete root recovery is often difficult. Parameter H (sometimes referred to as the harvest index - HI) is the proportion of this above ground DM that is partitioned to the grain.

In most cropping situations, where soil moisture is adequate, low nitrogen (N) availability is the soil factor limiting the yield of cereals and it is common practice to use fertilizer N to increase

grain production (Wibberley, 1989). Increased grain yield as a result of the addition of fertilizer N must be due to changes in one or more of the parameters in Equation 6.1. Parameter *Q* depends primarily on environmental factors such as latitude, daylength, time of year and weather conditions and is not affected by the application of N. The coefficient of conversion (ε) is largely determined by the difference between crop photosynthesis and respiration and is also not generally affected by additional N (van Keulen and Stol, 1991). However, this may not always be the case (Chapter 5). The influence of N availability on HI is poorly understood. For single plants HI depends the partitioning of assimilate between the plant and the developing grains and is influenced by the relative strengths of the sinks 'competing' for available carbohydrates (Hay and Walker, 1989). For cereal crops the effects of N supply on the partitioning of DM to the grains do not appear to be consistent with reports of HI increasing, decreasing or not changing with additional N (Evans, Wardlaw and Fischer, 1975; Hay and Walker, 1989).

Available data indicate that for most crop species, under normal growing conditions, greater DM production with additional N is due primarily to increases in *I* as a result of greater canopy development (see Hay and Walker, 1989). Numerous studies have shown an increase in LAI with additional N at various stages of crop development. For example, additional N (50 - 100 kg ha^{-1} at sowing) more than doubled the LAI of spring sown *Triticum aestivum* L. (wheat) prior to anthesis (Power and Alessi, 1978; Morgan, 1988). If LAI is not already optimum for PAR interception, an increase in the extent of the canopy increases the fraction of available PAR intercepted, usually resulting in a greater production of DM. Also, as well as increasing the extent of the canopy, additional N can increase the time that leaves remain photosynthetically active, further increasing *I*. The importance of *I* and canopy development in determining crop DM is illustrated by the fact that notwithstanding severe stresses, for a given species, over many seasons and sites, there is a linear relationship between the amount of PAR intercepted and DM production (Biscoe and Gallagher, 1977; Monteith, 1977).

Though yield is usually the main determinant of the monetary return from a crop, grain quality can also be important. For both wheat and *Hordeum vulgare* L. (barley), a common parameter of grain quality is the %N of the grain. High %N is desirable for wheat because it increases the flour baking quality while for barley, low grain %N leads to better malting and brewing characteristics (Kent, 1983). Numerous experiments have shown that grain %N is influenced by N availability. However, the magnitude and direction of the response depends on factors like species, cultivar, soil N status, amount of N applied, and time of application (Martin *et al.*, 1989). In general, increased rates of fertilizer N, especially if applied at or near anthesis, will increase

the grain %N. However, if soil N levels are low and N is applied early, increased tillering will result in more grains per plant and the N may be diluted.

The value of the parameters that determine grain yield (Equation 6.1) depend on factors like species, cultivar and environment. Species may have different leaf growth rates (Chapter 3) or morphologies which may affect canopy development. Nitrogen availability is an environmental factor that is relatively easy to manipulate and through its effects on canopy development, can have a large influence on crop DM and grain production. No reports were found in which the five main temperate cereals were grown in the same experiment and crop growth varied through the application of fertilizer N. The primary aim of the experiment described in this chapter was to determine and compare the influence of N availability on the canopy development and grain yield of the main temperate cereals. In addition, as no reports were found where grain %N of the temperate cereals was determined from crops growing in the same experiment, the influence of N supply and time of application was also determined.

6.2 Materials and Methods.

The experiment was carried out during the spring/summer of 1991-1992 at the Henley Block of the Lincoln University Research Farm, Canterbury New Zealand. For the previous 3 years the experimental site was in ryegrass grown for seed. Over the duration of the experiment both the daily mean temperature and total rainfall were less than the long term mean but incident solar radiation receipts were slightly above average (Appendix 6.1). Irrigation (80 mm) was only applied to aid incorporation of fertilizer applied at anthesis. The soil type used was a Templeton silt loam, a typical Canterbury cropping soil. Soil samples to 30 cm depth were collected immediately after cultivation but before application of fertilizer N. Samples were divided in two subsamples of known weights; one was dried to determine soil moisture content and total N while the other was used to determine interstitial soil solution nitrate (NO_3^-) and ammonium (NH_4^+) contents. Total N was determined on finely ground dried soil samples using a modified Kjeldahl digestion and a "Kjeltec Autosampler System 1035 analyser" (Tecator; Höganäs, Sweden). Nitrate and NH_4^+ were determined using 2 M potassium chloride as an extractant and the methods described in Chapter 2 were followed. Final NO_3^- and NH_4^+ concentrations were expressed by using the calculated soil moisture content of the other subsample.

The experiment was sown on September 24th 1991 into a conventionally cultivated weed free seed bed using an Ojord precision cone seeder. Row spacing was 15 cm. The experiment was a split-split plot design with species as the main plots, applied N rate as the subplots and time of N application as the sub-sub plots. Sub-plots were 8 m long by 2.1 m wide and there were 4 replicates. Five species were used: wheat (cv. Otane; mean seed weight, 54 mg), oats (*Avena sativa* L. cv. Amuri, 29 mg) and triticale (X *Triticosecale* Wittmack cv. Aranui, 46 mg) seed was obtained from Hodder and Tolley Ltd. Christchurch, New Zealand; while rye (*Secale cereale* L. cv. Rapaki, 27 mg) and barley (cv. Triumph, 42 mg) seed was obtained from the New Zealand Institute for Crop and Food Research Ltd., Lincoln, New Zealand and the Canterbury Malting Company, Christchurch, New Zealand respectively. Seeds were not chemically treated. Sowing rates were adjusted for seed size and germination percentage to achieve plant populations of approximately 350 plants m^{-2}. Nitrogen was supplied as urea at three rates: 0, 100 and 200 kg ha^{-1}, and applied at either sowing or near anthesis, approximately 85 days after sowing (DAS). Weed control was achieved through two applications of "Salvo" (4 litres ha^{-1})(Shell Chemicals; active ingredients, all as dimethyl salts: MCPA - 107 g l^{-1}, mecoprop - 210 g l^{-1}, dichlorprop - 233 g l^{-1}, dicamba - 17 g l^{-1}) at 40 and 60 DAS while fungal pathogens were controlled by spraying "Tilt" (0.5 litres ha^{-1})(CIBA-GEIGY; active ingredient: propiconazole - 250 g l^{-1}) at 70 DAS.

The extent of the canopy was measured only on plots where N was applied at sowing. Crop canopy LAI measurements were made 42, 50, 57, 67, 76 and 90 DAS using a LI-COR 2000 canopy analyser (LI-COR, Lincoln, NE, U.S.A). The last measurement was made so to be as near as possible to anthesis for all species. The means of four LAI measurements per plot were used and analysed using the Maximum Likelihood Program as in Section 3.2. The curve parameters obtained were used to derive equations describing the response of LAI to additional N with time. The curve parameters were also used to obtain values of maximum and weighted mean average canopy growth rate (MCGR and WMACGR respectively - both in LAI d^{-1}) and duration of canopy growth (DUR - days)(Section 3.2). The LI-COR 2000 analyser also measured the "diffuse non-interception" (DNI) of the canopy. The value of (1 - DNI) or diffuse interception is an indicator of the amount of light absorbed by the crop and depends on both the extent and architecture of the canopy. The mean value of diffuse interception for each species and level of N addition at each measuring date was integrated with the daily PAR receipts between measurement dates (Appendix 6.1) using the locally developed program "PAR" (Lincoln University) to obtain the total amount of PAR intercepted up to anthesis.

For plots supplied N at sowing, final harvesting was carried out from 130 - 145 DAS, depending on the grain maturity of the different species. In each plot all above ground plant material was cut from a randomly placed 1 m^2 quadrat using hand shears. The material was air dried and mass of total DM determined. The stalks and grain were separated and total quadrat grain weight determined. A sample of the grain was retained and reduced N determined using a modified Kjeldahl digestion and a "Kjeltec Autosampler System 1035 analyser" (Tecator; Höganäs, Sweden). Grain samples were also taken from plots where N was applied near anthesis and these were also analysed for grain %N. In addition, for wheat and barley only, a representative sample of approximately 10% by weight of the DM cut from the 1 m^2 quadrat was taken for yield components analyses. Total number of tillers and ears were counted and 25 randomly selected ears were retained to determine mean number of grains per ear and mean grain weight.

All data were analysed using the "Statistix" (Analytical Software, St.Paul, MN, U.S.A) statistical package. All means discussed are different at $P<0.05$ and were obtained in a repeat experiment sown approximately 2 weeks after the one reported here. In Section 6.3 the analysis standard error of the mean (SE) is presented, while for grain %N values, where species and time of N application comparisons can be made, the least significant differences (LSD) for the split-split plot design have been calculated.

6.3 Results.

For all species, the rate of canopy development increased with the addition of 100 kg N ha^{-1} at sowing (Table 6.1). Both the derived parameters MCGR and WMACGR increased between 20 and nearly 90%, depending on species. In general, additional N to 200 kg ha^{-1} did not result in further increases in rate of canopy development. Maximum canopy growth rate and WMAGR were greatest for barley, rye and oats, intermediate for triticale and smallest for wheat. Differences in duration of canopy growth with added N were not detected. For most species the development of LAI over time showed a sigmoidal response and predicted LAI values matched those actually obtained (Fig. 6.1). For all species additional N increased LAI at anthesis (Table 6.1). The magnitude of the response varied from between 22 to 40% and there were significant differences between species in the final LAI attained with oats > barley = rye > triticale > wheat.

The relationships between LAI and diffuse interception (DI) are plotted in Fig. 6.2. Regression equations for the species are:

wheat: $DI = 0.3038 + (0.3044 * LAI) - (0.03633 * LAI^2)$, $r^2 = 97.7\%$;
barley: $DI = 0.4657 + (0.1876 * LAI) - (0.01684 * LAI^2)$, $r^2 = 97.5\%$;
rye: $DI = 0.4880 + (0.1784 * LAI) - (0.01602 * LAI^2)$, $r^2 = 98.6\%$;
triticale: $DI = 0.3738 + (0.2441 * LAI) - (0.02508 * LAI^2)$, $r^2 = 98.3\%$;
oats: $DI = 0.5635 + (0.1418 * LAI) - (0.01153 * LAI^2)$, $r^2 = 98.1\%$.

Regression lines of all five species shown together in Fig. 6.2f (data points obtained from repeat experiment are also included). For all species except wheat, with increasing LAI diffuse interception increased to over 95% and then changed little with further increases in LAI. Wheat did not intercept >95% of the available PAR. The regression curves of diffuse interception versus LAI for all species are plotted together in Fig. 6.2f and show a similar overall pattern. However, at low leaf area indices (eg. LAI=1) wheat and triticale intercepted less than 60% of the available PAR compared to around 65% for the other species (Table 6.2a). The LAI at which 95% of the available PAR was intercepted (frequently termed critical LAI - LAI_{crit}) was similar for all species except wheat (Table 6.2a). For plots of wheat, rye and triticale with no N added LAI_{crit} was not achieved while for barley and oats the addition of N substantially decreased the time taken to reach LAI_{crit} (Table 6.2b). Increases in the extent of the canopy and time in which LAI_{crit} was reached with additional N resulted in a greater amount of PAR being intercepted up to anthesis for all species (Table 6.2b). The increase with additional N was around 5% for all species.

Table 6.1 The effects of 0, 100 or 200 kg ha^{-1} N applied at sowing on the derived parameters of canopy development: maximum canopy growth rate (MCGR), weighted mean average canopy growth rate (WMACGR) and duration of growth (DUR) and actual canopy leaf area index at anthesis (FINLAI) of spring sown *Triticum aestivum* L. (wheat), *Hordeum vulgare* L. (barley), *Secale cereale* L. (rye), X *Triticosecale* Wittmack (triticale) and *Avena sativa* L. (oats). Standard error of the mean (SE) is given.

Species	Applied N (kg ha^{-1})	MCGR (LAI d^{-1})	WMACGR (LAI d^{-1})	DUR (d)	FINLAI
Wheat	0	0.055	0.037	65	3.2
	100	0.059	0.040	66	3.8
	200	0.066	0.046	67	4.0
Barley	0	0.116	0.077	52	4.4
	100	0.180	0.121	53	5.5
	200	0.182	0.128	53	6.0
Rye	0	0.114	0.071	53	4.1
	100	0.177	0.107	55	4.7
	200	0.187	0.113	56	5.4
Triticale	0	0.098	0.067	49	3.0
	100	0.124	0.078	55	4.1
	200	0.143	0.091	56	4.2
Oats	0	0.134	0.094	57	5.3
	100	0.166	0.112	55	6.2
	200	0.174	0.118	59	6.7
	SE	0.006	0.005	3.5	0.2

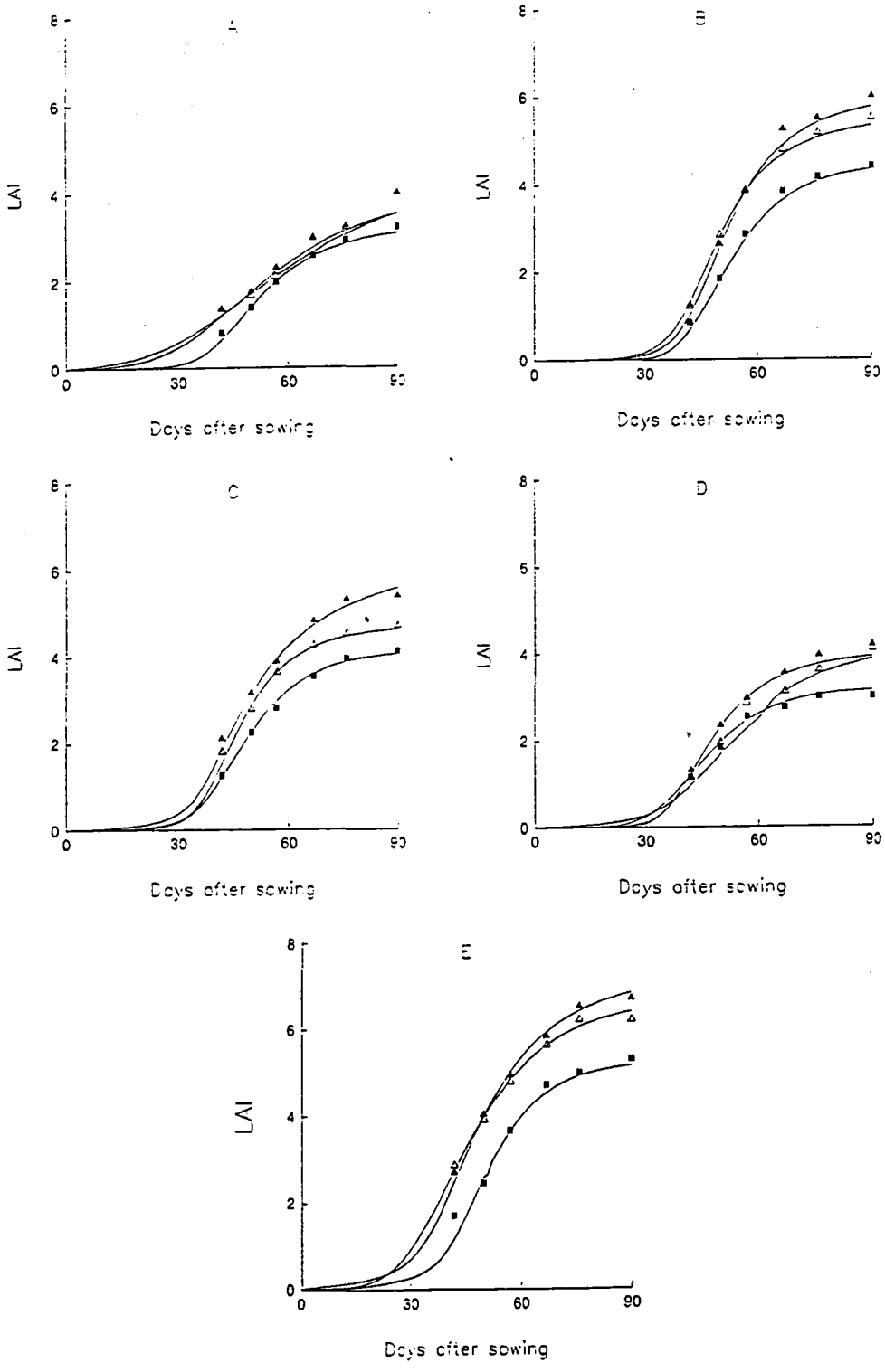


Fig. 6.1 The effects of 0 (■), 100 (Δ) or 200 (▲) kg N ha^{-1} applied at sowing on the predicted (lines) and actual (symbols) canopy leaf area index (LAI) over the development of spring sown *Triticum aestivum* L. (A), *Hordeum vulgare* L. (B), *Secale cereale* L. (C), X *Triticosecale* Wittmack (D) and *Avena sativa* L. (E).

Table 6.2a Fraction of full sunlight intercepted (diffuse interception) at leaf area index (LAI)=1 and the value of the critical LAI (interception of 95% PAR) of spring sown *Triticum aestivum* L. (wheat), *Hordeum vulgare* L. (barley), *Secale cereale* L. (rye), X *Triticosecale* Wittmack (triticale) and *Avena sativa* L. (oats).

Species	Diffuse interception[1]	Critical LAI[2]
wheat	0.571	N.R.
barley	0.636	4.06
rye	0.650	4.09
triticale	0.593	4.09
oats	0.686	4.07

Notes:
[1] - values obtained from regression equations in Fig. 6.2
[2] - N.R. - not reached

Table 6.2b Effects of 0, 100 or 200 kg ha^{-1} N applied at sowing on the time taken to reach critical leaf area index (LAI) (95% interception of full sunlight) and on the amount of photosynthetically active radiation (PAR) intercepted from sowing up to anthesis by spring sown *Triticum aestivum* L. cv. Otane (wheat), *Hordeum vulgare* L. cv. Triumph (barley), *Secale cereale* L. cv. Rapaki (rye), X *Triticosecale* Wittmack cv. Aranui (triticale) and *Avena sativa* L. cv. Amuri (oats).

Species	Applied N (kg ha^{-1})	Days after sowing to attain critical LAI[1]	PAR intercepted (MJ m^{-2})
Wheat	0	N.R.	447.5
	100	81	476.8
	200	76	478.5
Barley	0	76	488.2
	100	59	511.0
	200	59	511.7
Rye	0	N.R.	498.5
	100	64	520.1
	200	59	525.2
Triticale	0	N.R.	471.2
	100	85	484.1
	200	82	496.0
Oats	0	63	502.1
	100	52	537.9
	200	52	542.4

Note:
[1] - wheat did not intercept 95% of PAR - time values are based on interception of 90% PAR.

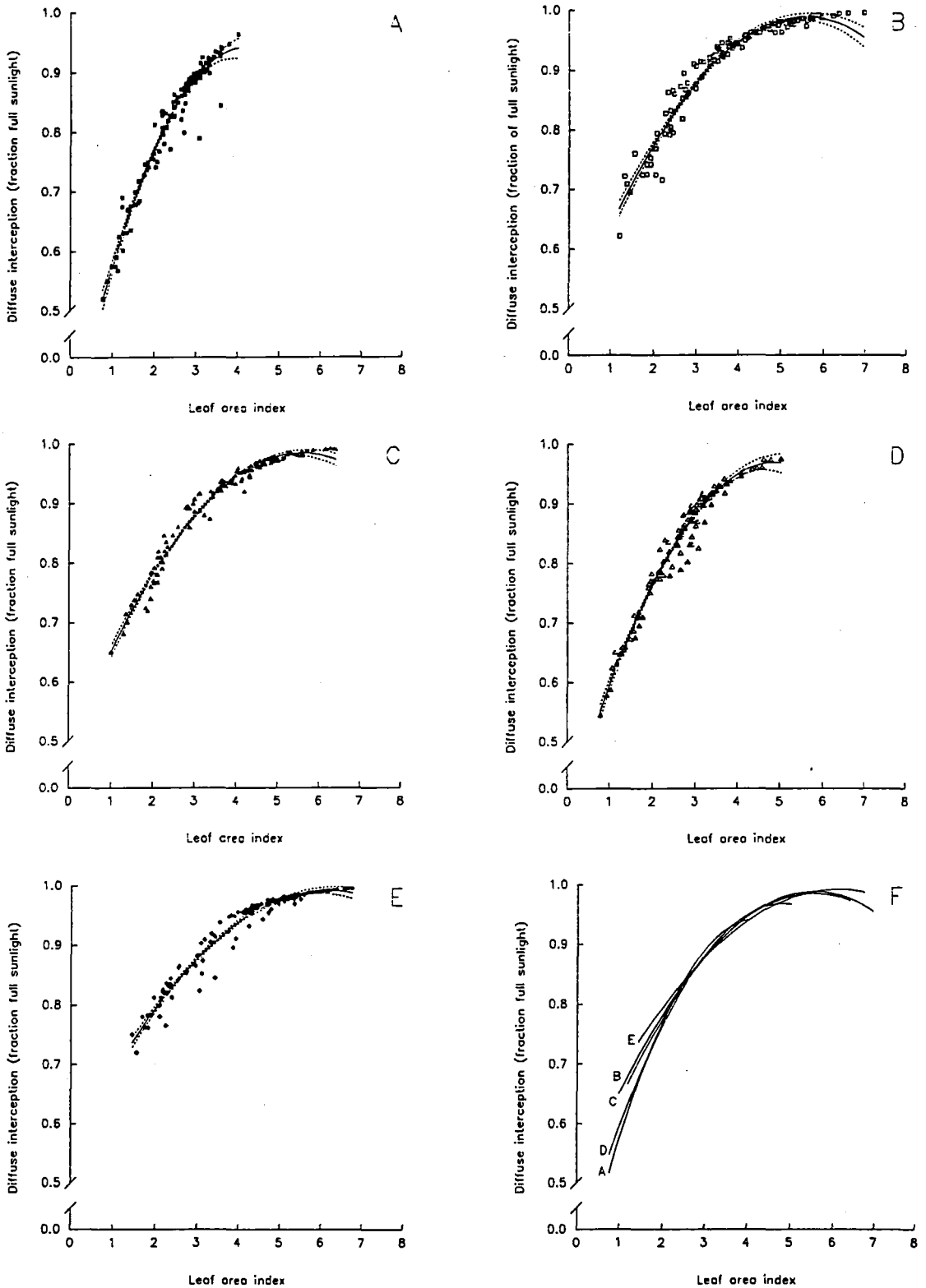


Fig. 6.2 The relationships between leaf area index and the fraction of full sunlight intercepted (diffuse interception) of spring sown *Triticum aestivum* L. (A), *Hordeum vulgare* L. (B), *Secale cereale* L. (C), X *Triticosecale* Wittmack (D) and *Avena sativa* L. (E). All species are plotted together in (F). Data points are derived from measurements taken at regular intervals from 42 to 90 days after sowing on plots supplied 0, 100 or 200 kg N ha^{-1}. Regression equations are reported in Section 6.3. Confidence intervals (95%) shown.

Table 6.3 Effects of 0, 100 or 200 kg ha^{-1} N applied at sowing on total dry matter production, grain yield and harvest index of spring sown *Triticum aestivum* L. (wheat), *Hordeum vulgare* L. (barley), *Secale cereale* L. (rye), X *Triticosecale* Wittmack (triticale) and *Avena sativa* L. (oats). Table of least significant differences ($LSD_{0.05}$) reported at the bottom of the main table.

Species	Applied N (kg ha^{-1})	Total dry matter (g m^{-2})	Grain yield (g m^{-2})	Harvest Index (%)
Wheat	0	1283	580	45.2
	100	1466	655	44.7
	200	1475	664	45.0
Barley	0	1525	670	43.9
	100	1695	767	45.3
	200	1759	786	44.7
Rye	0	1606	404	25.2
	100	1720	464	27.3
	200	1769	483	27.3
Triticale	0	1526	640	41.9
	100	1753	720	41.0
	200	1773	730	41.1
Oats	0	1524	533	35.0
	100	1665	604	36.2
	200	1690	614	36.3

	LSD		
Means to compare	Total Dry matter	Yield	Harvest Index
a) species at same or different N rate	122.6	54.8	2.62
b) N rate for same species	104.5	52.7	2.51

Table 6.4 Effect of 0, 100 or 200 kg ha^{-1} N applied at sowing on the coefficient of conversion of spring sown *Triticum aestivum* L. (wheat), *Hordeum vulgare* L. (barley), *Secale cereale* L. (rye), X *Triticosecale* Wittmack (triticale) and *Avena sativa* L. (oats).

Species	Applied N (kg ha^{-1})	Coefficient of conversion (g DM MJ^{-1} PAR intercepted)[1]
Wheat	0	2.86
	100	3.07
	200	3.08
Barley	0	3.12
	100	3.31
	200	3.43
Rye	0	3.22
	100	3.31
	200	3.36
Triticale	0	3.23
	100	3.62
	200	3.57
Oats	0	3.03
	100	3.09
	200	3.11

[1] calculated as final DM produced divided by PAR intercepted up to anthesis.

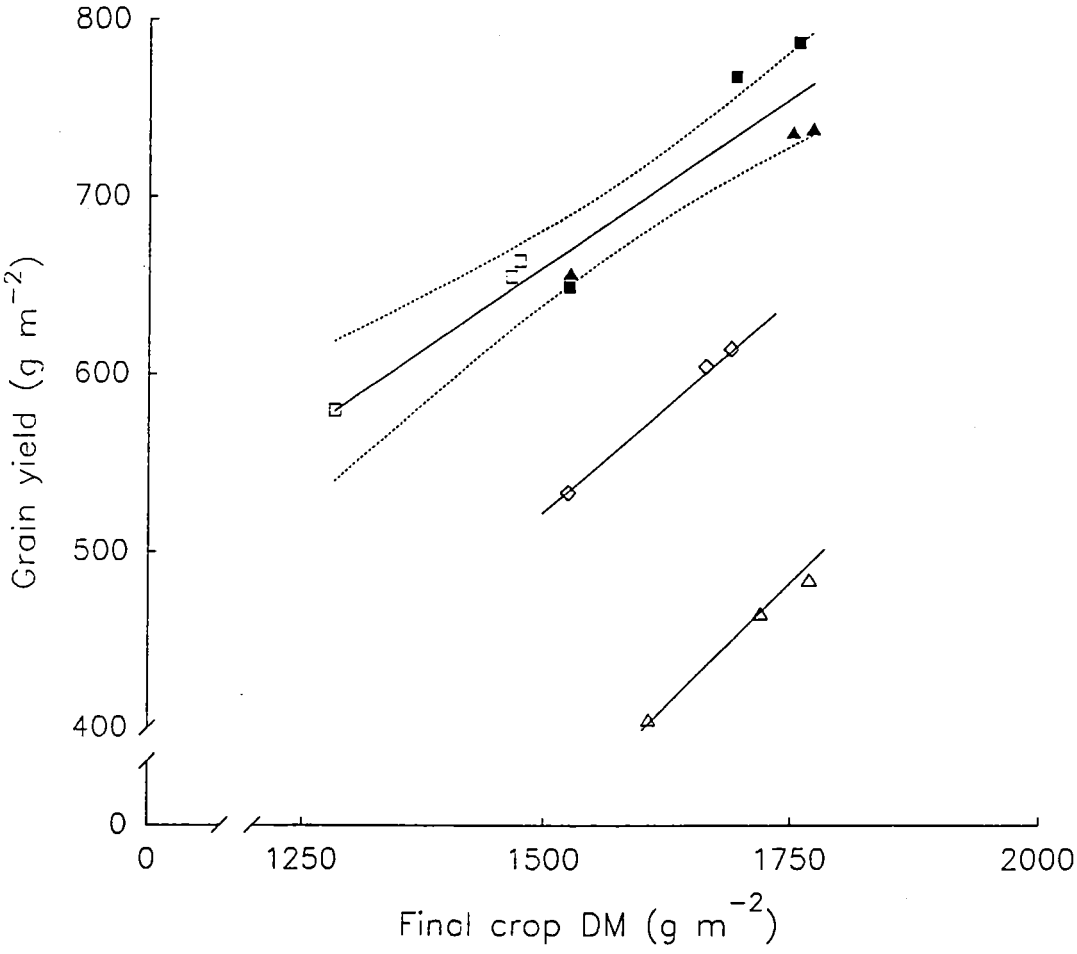


Fig. 6.3 The relationship between final crop dry matter (DM) and grain yield (GY) of spring sown *Triticum aestivum* L. (□), *Hordeum vulgare* L. (■), *Secale cereale* L. (Δ), X *Triticosecale* Wittmack (▲) and *Avena sativa* L. (◊) supplied 0, 100 or 200 kg N ha^{-1} at sowing. Confidence intervals (95%) shown.

Table 6.5 Effects of 0, 100 or 200 kg ha^{-1} N applied at sowing on tiller number, head number, grains per head and individual grain weight of spring sown *Triticum aestivum* L. (wheat) and *Hordeum vulgare* L. (barley).

Parameter	Applied N (kg ha^{-1})	Wheat	Barley
Tiller number (m^{-2})	0	464	867
	100	523	1128
	200	532	1099
	s.e.m	12.8	19.4
Head number (m^{-2})	0	427	821
	100	493	1028
	200	483	1049
	s.e.m	12.1	13.8
Grains per head	0	35.1	25.0
	100	37.5	27.5
	200	38.6	27.2
	s.e.m	0.74	0.41
Individual grain weight (mg)	0	53.4	51.0
	100	51.9	46.1
	200	51.7	44.7
	s.e.m	1.09	1.40

Table 6.6 Effect of additional N applied at either sowing or anthesis on the grain nitrogen content (%N) of spring sown *Triticum aestivum* L. (wheat), *Hordeum vulgare* L. (barley), *Secale cereale* L. (rye), X *Triticosecale* Wittmack (triticale) and *Avena sativa* L. (oats). Table of least significant differences ($LSD_{0.05}$) reported at the bottom of the main table.

		Grain %N	
		Time of N application	
		Sowing	Anthesis
Wheat	0	2.060	
	100	2.334	2.374
	200	2.385	2.410
Barley	0	1.529	
	100	1.931	2.006
	200	2.213	2.337
Rye	0	2.015	
	100	2.220	2.570
	200	2.322	2.526
Triticale	0	1.999	
	100	2.213	2.346
	200	2.287	2.332
Oats	0	2.072	
	100	2.959	3.101
	200	2.831	3.226

Means to compare	LSD
a) species at same or different N rate and time of application	0.1966
b) N rate for same species and same or different time of application	0.1956
a) time of application for same species and N rate	0.1851

For all species, 100 kg N ha^{-1} supplied at sowing increased both final DM and grain yield (Table 6.3). Addition of 200 kg N ha^{-1} did not result in further increases. Additional N increased DM from 10% for wheat to nearly 30% for rye. Grain yield increased from 10% (wheat) to 30% (barley) with additional N applied at sowing. For all species, N did not affect HI. However, rye and oats had lower harvest indices compared to the other species. The differences in HI between the species are illustrated in Fig. 6.3. Three distinct groupings are evident - one containing wheat, triticale and barley and the others containing oats and rye separately. These groupings correspond to harvest indices of approximately 45, 35 and 25% respectively. The regression equations for the three groupings are as follows (GY - grain yield (g m^{-2}); DM - dry matter (g m^{-2})):

wheat, barley and triticale: GY = 81.56 + (0.382 * DM), r^2 = 94.5%;
rye: GY = -345.30 + (0.470 * DM), r^2 = 98.3%;
oats: GY = -216.84 + (0.492 * DM), r^2 = 98.9%.

For all species, the coefficient of conversion increased with additional N, with the magnitude of the increases ranging from 3% (oats) to 12% (triticale) (Table 6.4). For most species, the main part of the increase occurred with additional N to 100 kg ha^{-1} and little further increase with 200 kg ha^{-1} N. Taking the average of the values for the three N treatments for each species indicates that wheat and oats had lower coefficients (≈3 g DM MJ^{-1} PAR) than barley and rye (≈3.3 g DM MJ^{-1} PAR), with triticale having the highest (3.5 g DM MJ^{-1} PAR).

For both wheat and barley, N applied at sowing increased tiller number 14 and 26% respectively and head number 13 and 27% respectively (Table 6.5). Nitrogen did not affect the percentage of tillers producing heads. For wheat, additional N increased the number of grains per head by 10% but individual grain weight decreased 3%. For barley, additional N increased grains per head 9% but decreased grain weight 12%.

For all species, N applied at either sowing or anthesis increased grain %N (Table 6.6). For rye and oats, N applied at anthesis resulted in a greater grain %N than N applied at sowing. In contrast, for wheat, barley and triticale the increase in grain %N was similar whether N was applied at sowing or anthesis. Increases in grain %N with additional N ranged from 10% (triticale) to over 50% (barley and oats).

6.4 Discussion

Additional N applied at sowing increased the grain yield of all species examined (Table 6.3). As expressed in Equation 6.1, yield is determined by final crop DM (the product of *Q*, *I* and ε) and HI. Harvest index was not affected by N supply (Table 6.3), and thus for all species greater yield with additional N must have been due to increases in either *I* or ε. The fraction of available PAR intercepted (*I*) depends primarily on the extent of the canopy over the course of crop development. Addition of N increased canopy growth rates (Table 6.1), resulting in greater leaf area indices at all measuring dates up to anthesis (Fig. 6.1). Notwithstanding possible changes in plant population, the positive effects of N availability on LAI must have been a result of differences in the leaf area of individual plants. Plant characteristics that affect LAI and which have been shown to be influenced by N availability, include average individual leaf area and the number of leaves per plant. In Chapter 3 it was shown that under controlled environment conditions, the major part of the individual leaf area response to additional NO_3^- was over the range 0 - 2.5 mol m^{-3} and there was only small increases with higher concentrations of NO_3^-. The concentrations of NO_3^- in the interstitial soil solution of unfertilized but cultivated soils are usually in the range 1 - 5 mol m^{-3} (Russell, 1973; Haynes *et al.*, 1986; this chapter), hence it is unlikely that addition of fertilizer N would markedly increase individual leaf areas. In contrast, whole plant leaf area, which is largely determined by the number of leaves per plant and is therefore a function of tiller number (Section 1.2), usually increases over a wide range of external N concentrations (Chapter 5). Previous studies have established that under field conditions additional N usually increases tiller (and hence leaf) number (eg. Aspinall, 1961; Pearman, Thomas and Thorne, 1975). Therefore it is likely that the major part of the response of LAI to additional N in the present experiment was also due to increased tiller number. Observations indicated that over the course of crop development, plants supplied additional N had a greater number of tillers. This was confirmed for wheat and barley, where additional N increased tiller number at final harvest (Table 6.5).

There were major differences between species in the rate and extent of canopy formation. The greater rates of canopy development and LAI at anthesis of barley, rye and oats compared to wheat and triticale (Tables 6.1) were likely a result of differences in the leaf area of individual plants. Observations indicated that wheat and triticale had a smaller number of tillers per plant than the other species. Also, at early stages of crop development these two species intercepted a smaller fraction of the available PAR at a given LAI (Table 6.2a). This was possibly a result of differences in canopy architecture and/or the surface characteristics, thickness, orientation, angle

and size of individual leaves. Interception of a lower fraction of available PAR may have led to decreased crop photosynthesis, contributing to the lower rates of canopy expansion of wheat and triticale (Table 6.1).

A major aspect of canopy growth is whether canopy closure (LAI_{crit}) is attained and if so, the time taken to reach this stage of development. For wheat, triticale and rye, LAI_{crit} was not reached when no N was applied and for all species additional N decreased the time taken to reach canopy closure (Table 6.2b). Faster canopy closure increases the duration of maximum available PAR interception, resulting in a greater total amount of PAR being intercepted (Table 6.2b). With the exception of wheat, LAI_{crit} was similar for all species (Table 6.2a). Also, over a wide range of leaf area indices, the relationship between LAI and diffuse interception were comparable for all species (Fig. 6.2f), indicating that despite contrasts in the final LAI achieved, factors which influence diffuse interception and development of LAI_{crit}, were similar for all species.

From Equation 6.1, the extent to which intercepted PAR is converted to DM depends on the value of ε, the coefficient of conversion. Differences between the rates of photosynthesis and respiration per unit leaf area largely determine the value of ε. Under field conditions ε is difficult to determine directly and it is generally considered to be little affected by additional N (Hay and Walker, 1989; van Keulen and Stol, 1991). However, in the present experiment, calculating the coefficients from the means of total DM accumulated (Table 6.3) and PAR intercepted up to anthesis (Table 6.2b) indicates that for all species ε increased with additional N (Table 6.4). These values of ε are slightly higher than the commonly accepted value of approximately 2.8 g DM MJ^{-1} PAR (Monteith, 1977; Gallagher and Biscoe, 1978). This was probably due to interception of PAR only being assessed up to anthesis while DM was determined at final harvest. A substantial amount of PAR is intercepted after anthesis and the photosynthate produced is used for DM production (Evans, Wardlaw and Fischer, 1975). Well fertilized canopies usually have a longer green area duration than where no N is applied and hence produce more DM over the growing season (Hay and Walker, 1989). In the present experiment, had post-anthesis PAR interception been measured, it is likely that the increases in total DM with additional N would have been matched by increases in PAR interception. Values of ε would then have been closer to published values and the apparent increase in ε with additional N may have been non-evident.

In the present experiment there were substantial differences between species in the value of ε across all N treatments. Recently, it was reported that under cropping conditions, rye had a lower rate of canopy respiration than wheat, which was suggested to be due to rye's lower rate of

maintenance respiration (McCullough and Hunt, 1993). This was associated with higher rates of DM accumulation for rye. However, the importance of differences in rates of photosynthesis and respiration between species in terms of differences in the value of ε and hence rates of DM accumulation need to be further assessed. It is possible that the differences in ε between species noted in the present study may have been a consequence PAR interception not being measured up to final harvest, as discussed above.

The amounts of PAR intercepted and DM produced by the different species were not directly related to the quantities of grain produced. For example, wheat, which had both the lowest rate of canopy growth and LAI at anthesis and did not reach LAI_{crit} (even with additional N), had a higher grain yield than rye and nearly the same as that of oats. Compared to wheat, both rye and oats had faster rates of canopy development and larger leaf area indices at anthesis. This lack of correlation between PAR interception, and grain yield was mainly a result of variation in the HI of the species (Table 6.3, Fig. 6.3). Rye and oats allocated only 25 and 35% respectively of the total DM produced to grain as opposed to approximately 45% for wheat, barley and triticale. It is suggested that in the present experiment, the differences between species in HI was the major reason for the differences in grain yield. Though HI is an important determinant of grain yield in cereals, the factors influencing it are poorly understood. Gifford *et al.* (1984) presented data showing that increased grain yields of winter wheat cultivars introduced over the last 80 years have not been due to greater above ground dry matter production but rather a result of increases in HI. In terms of both worldwide area sown and harvested yield, wheat and barley are the most important temperate cereals grown (Table 1.1) and these species have therefore been selected over the years for mostly for high yields. Triticale, an artificially bred intergeneric hybrid, has probably also been developed with yield as the main selection criterion. In contrast, rye and oats, which are currently not important for grain production, have probably not been developed for grain yield to the same extent. In fact, the cultivars of rye and oats used in the present experiment are also used as forage crops. Therefore, in the present experiment, the higher HI of wheat, barley and triticale may have been due to a more intensive selection and breeding history of these species compared to rye and oats.

The influence of N availability on grain yield was also investigated by examining the components of yield of wheat and barley (Table 6.5). Cereal grain yield can be expressed as follows:

grain yield ($g\ m^{-2}$)	=	plants per m^2	*	fertile tillers (heads) per plant	*	grains per head	*	mean grain weight (g)

(Equation 6.2)

For both wheat and barley, the main effect of additional N was to increase the number of heads per m^2 by increasing the number of tillers per plant (Table 6.5). Greater tiller number usually increases the potential number of grain bearing ears per plant (Darwinkel, 1978). However, only the first 2 - 3 formed tillers produce viable seed bearing heads, with later formed tillers being 'parasitic' on the plant and not contributing to grain yield (Thorne and Wood, 1988). In this experiment, tiller numbers per plant were generally low, with wheat and barley having a maximum of only 1.5 and 3 tillers per plant respectively. This was possibly due to the high plant population restricting tiller production. As a consequence, the proportion of tillers producing a viable head was relatively high and not affected by N supply. Increased tiller number also increases LAI and crop photosynthesis (see above). A greater supply of photosynthate can affect the initiation and development of the grains, influencing both the number of grains per ear and mean grain weight. For both wheat and barley, addition of N resulted in increases in the number of grains per head, though this was offset by a decrease in average grain weight. The factors determining grain weight are complex, depending on interactions between the sources and availability of assimilate and the number of grains that are to be filled. In previous studies additional N has been reported to increase (Whingwiri and Kemp, 1980), decrease (Pearman, Thomas and Thorne, 1977) or not affect (Pearman, Thomas and Thorne, 1977) individual grain weight. In the present study the substantial increase in grain number per plant, primarily a result of greater tiller number, was likely to have been larger than the increase in the supply of material for available for grain filling and hence mean grain weight decreased.

Numerous experiments have demonstrated the positive influence of additional N on the growth and yield of cereal crops. However, the magnitude of a crop's response to fertilizer N can depend, amongst other factors, on the amount of mineral N made available from indigenous soil sources over the course of crop development. In some experiments reported in the literature, the levels of soil mineral N have been high, and the addition of fertilizer N resulted in little or no increase in final grain yield (eg. Pearman, Thomas and Thorne, 1977). In the present

experiment, the effects of additional N on canopy development and yields were not as large as anticipated, probably because growth without additional N was already large. For example, the grain yields from plots of wheat and barley supplied no additional N were 5.8 and 6.5 t ha^{-1} respectively. Yields such as this are above the average for New Zealand farms (Table 1.1) and considered acceptable for well managed, fertilized crops (D. Jack, Lincoln University, pers. comm.). The total N content of the soil to 30 cm depth was approximately 3 mg g^{-1} dry soil. After cultivation, the concentrations of NO_3^- and NH_4^+ in the interstitial soil solution of plots receiving no fertilizer N were 2.7 and 0.2 mol m^{-3} respectively. These amounts of total and mineral N are typical of medium to high fertility agricultural soils (Mengel and Kirkby, 1987; Selvarajah, Cameron and Swift, 1989) and may have been a reason for the small response of crop growth to fertilizer N.

The final aspect of temperate cereal grain production investigated in this study was the influence of N supply and time of application on the N content of the grain. Grain N is derived from two sources: that taken up prior to anthesis and stored in the parent crop and then remobilized to the developing grain; and that taken up from the soil after anthesis and translocated directly to the grain. Additional N applied at sowing increased the grain %N of all species (Table 6.6), despite the already high grain %N of crops where no N was added. For example, with no additional N the grain N content of barley was 1.5%, which is considered near the maximum acceptable for high quality malting grain (Kent, 1983). These high levels of grain %N where no N was applied were probably due to the elevated background levels of soil N. However, for wheat used for bread flour or barley sold for feed grain, high seed N content is desirable. Applications of N at or near anthesis are usually expected to increase grain %N to a greater extent than N applied at sowing (Wibberley, 1989). Therefore, fertilizer N is frequently applied near anthesis in order to maximise grain %N. However, in the present experiment only for rye and oats did later applications of N increase grain %N to a greater extent than when it was applied at sowing. For wheat, barley and triticale grain %N changed little with late additions of N. However, it is suggested that for wheat, barley and triticale, applications of N at anthesis increased the duration of the canopy and the supply of photosynthate increased, leading to increased grain size and yield, and hence no increase in grain %N was evident. In contrast, for rye and oats, with lower potential grain yields, an increase in the duration of the canopy may not have resulted in increased yields and hence grain %N increased. However, further work would need to be carried out to verify these ideas. It is also possible that the grain %N of rye and oats are more responsive to increases in available N than wheat, barley and triticale or that the latter species had reached a plateau in their response to additional N. The factors and mechanisms which

determine seed N content are complex and need further investigating, especially the differences between species in the response to additional N noted in the present study.

6.5 Conclusions.

The experiment reported in this chapter has demonstrated that despite relatively high initial levels of soil N, fertilizer N applied at sowing had positive effects on the grain yield of all the temperate cereals investigated. The reason for the increase was similar for all species: additional N increased the fraction of available PAR intercepted and hence the total amount of PAR intercepted by the canopy. Greater interception of PAR, which was a result of increased rates of canopy development and corresponding reductions in the time taken to reach LAI_{crit}, was associated with increased DM production at final harvest. Additional N did not affect HI, hence grain yield increased for all species. However, differences between species in the amount of grain produced were not directly related to the levels of PAR intercepted and DM produced, but rather to differences in HI. It is suggested that although all cereals respond in a similar manner to additional N in terms of canopy development and grain yield, the magnitude the parameters in Equation 6.1, particularly HI, can be different. Finally, N applied at either sowing or anthesis increased the %N of the grain, though only for rye and oats was the increase in grain %N greater with late N.

Appendix 6.1 Mean daily temperature and monthly incident solar radiation and precipitation between September 1991 and February 1992. Long term means (LTM) and differences (Δ) between LTM and 1991-92 also given.

	Mean daily temperature (°C)			Monthly solar radiation (MJ m^{-2})			Monthly precipitation (mm)		
Month	1991-92	LTM	Δ	1991-92	LTM	Δ	1991-92	LTM	Δ
Sept.	9.5	9.4	-0.1	359	408	-49	21	47	-26
Oct.	11.3	11.7	-0.4	532	558	-26	10	49	-39
Nov.	11.7	13.6	-0.9	600	618	-18	71	53	+18
Dec.	13.3	15.4	-2.1	664	651	+13	80	57	+23
Jan.	16.6	16.4	+0.2	807	666	+141	28	60	-32
Feb.	15.4	16.2	-0.8	574	562	+12	35	54	-19
Total or mean	12.9	13.8	-0.9	3536	3463	+73	245	320	-75

Source: Lincoln University meteorology data (held on computer)

Table of Contents - Chapter 7

General Discussion

page

7.1 Introduction 112

7.2 Seed Reserve Mobilisation 114

7.3 Leaf Growth 115

7.4 Partitioning of Dry Matter 119

7.5 Crop Growth and Grain Yield 121

7.6 Conclusions 123

7.1 Introduction

Where soil moisture is adequate, low nitrogen (N) availability is usually the main soil factor limiting the growth of many plant species. Growth can be defined as an increase in total plant dry weight (d.wt) over time. As 40 to 50% of plant d.wt is carbon (C) (Table 1.3), the amount of C assimilated is an important determinant of growth. Most terrestrial plants acquire nearly all their C by converting atmospheric carbon dioxide into simple carbohydrates via photosynthesis. The fraction of the newly produced photosynthate that is able to be used in the building of new plant material (growth) depends on the amount of C respired. Respiration consists of a series of reactions which synthesize energy-rich compounds such as adenosine 5'-triphosphate and reductants like nicotinamide adenine dinucleotide phosphate-reduced form which are used in the processes that maintain existing plant function (maintenance respiration) and those that build new plant material (growth respiration). As long as C inputs are greater than respiration, excess C is available for growth and plant d.wt increases (Section 1.4).

When levels of soil mineral N are low, greater N availability usually increases plant growth by increasing plant photosynthetic capacity (Novoa and Loomis, 1981). For most plants the leaves are the main site of photosynthesis. Greater photosynthetic capacity is achieved by increasing either the rate of photosynthesis per unit leaf area or the amount of photosynthetically active radiation (PAR) intercepted by the leaves. Nitrogen is an important component of many of the compounds involved in photosynthesis, such as chlorophyll and ribulose 1,5-bisphosphate carboxylase (Section 1.5). Generally, when external levels of available N are low, the concentrations of these compounds are less than optimal for maximum photosynthetic rates. With increased external mineral N concentrations over the range commonly found in soils, plant N content usually also increases, and the ability of plants to manufacture the compounds involved in photosynthesis is greater, increasing the rate of C fixation per unit leaf area.

The second way additional N can increase photosynthetic capacity is by increasing plant leaf area and hence the amounts of PAR intercepted. Increased photosynthetic rate per unit leaf area with additional N leads to an increase in the availability of C for the construction of new leaf material. Frequently, the increase in leaf area with additional N is the main reason for greater levels of C fixation and increased plant growth. At any time, plant leaf area is a function of the average area of individual leaves and the total number of leaves per plant while the extent of new leaf development depends on the availability of C for new leaf production. The overall aim of this thesis was to investigate the influence of N availability on aspects of plant leaf area development. Chapter 2 looked at the effects of N on seed reserve

mobilisation and seedling growth prior to emergence while Chapters 3 and 4 examined aspects of the effects of N supply on the size of individual leaves. Chapter 5 investigated the effects of N form and availability on the partitioning of dry matter between the leaves and other plant parts while Chapter 6 looked at the influence of additional N on crop leaf area under field conditions. Each chapter's results were discussed in detail. This General Discussion will review and integrate the concepts and ideas developed in the individual chapters and assess the overall implications for plant and crop growth.

All experiments in this thesis were conducted using one or more of the temperate cereal species. As cereals provide a large proportion of the world's dietary needs (Section 1.1), many aspects of their growth have been studied extensively. Cereals grow relatively fast and their response to additional N is usually large. Also, the importance of cereals to world agriculture has meant that they have been bred extensively, resulting in the availability of cultivars with low genetic X environment interactions. These cultivars usually show consistent and repeatable responses to changing environmental variables, making them suitable for studies such as those described in this thesis. However, the processes, relationships and concepts described and discussed in this thesis are relevant to other species, including non-agricultural ones.

Most plants are capable of taking up and assimilating nitrate (NO_3^-) and ammonium (NH_4^+), though under temperate agricultural conditions the former is frequently the dominant form of N available (Haynes *et al.*, 1986) Under agricultural cropping conditions, levels of mineral N increase when nitrogenous fertilizers are applied or crop residues with a high N content are incorporated into the soil and the organic N mineralized. In pastures and natural communities, elevated mineral N levels occur where animal urine or faeces are deposited or high N content plants decompose. In addition to NO_3^- and NH_4^+, plants can also take up amino acids through the roots (Jones and Darrah, 1993). Although amino acids occur in the interstitial soil solution of both agricultural and natural communities (Bremner, 1965), the importance of this form of soil N to plants is not known. Under controlled environment conditions, if an amino acid such as glutamine is supplied in nutrient solution as the only source of N (Section 1.5), this effectively mimics the exclusive root assimilation of N, providing a useful tool in studying the effects of site of N assimilation on the growth of plants.

7.2 Seed Reserve Mobilisation

For some time after emergence from the soil, leaf 1 is the seedling's main site of photosynthesis and therefore its ability to fix C plays an important role in determining the extent of the growth of subsequent leaves and other plant parts. Studies have shown that for cereals and other species, the area of leaf 1 and the overall growth of seedlings after emergence are positively related to both seed N content and the level of available exogenous N (Section 2.1). In most cases these N effects have been attributed to increased photosynthetic rates per unit leaf area. However, experiments in Chapter 2 demonstrated that for a range of temperate cereals harvested prior to emergence, when photosynthesis is negligible, additional N supplied as NO_3^- increased shoot growth to a similar extent as in above-ground studies. Greater shoot growth was a result of enhanced endosperm reserve mobilisation and an increase in the partitioning of reserves to the shoot relative to the roots. A series of experiments demonstrated that the NO_3^- effect was not related to the amounts of NO_3^- assimilated but rather that it was an osmotic effect, with accumulated NO_3^- increasing water uptake by the seedling. A similar mechanism was proposed for the greater reserve mobilisation and d.wt of seedlings grown from high N content *Hordeum vulgare* L. (barley) seed compared to low N content seed. As most seed N is contained in proteins and proteins are the major colloidal components of seeds, it was proposed that greater water uptake occurred with high N seed. It was suggested that for both the NO_3^- and seed N effects on reserve mobilisation, increased water uptake could stimulate the activity of α-amylase, the main enzyme regulating the breakdown of starch. Further work needs to be carried out to determine the relationships between seed N content, NO_3^- uptake, water uptake, α-amylase activity and reserve mobilization.

From an ecological perspective, the ability of the seedlings of some species but not others to increase growth through enhanced reserve mobilisation could have consequences for the species composition of both agricultural and natural plant communities. External NO_3^- concentrations which enhance mobilisation occur in both agricultural and non-agricultural soils. A greater rate of mobilisation would increase the extent and rate of seedling growth, leading to plants that emerged faster, and also had greater leaf area and bigger root systems. Compared to seedlings that did not have the capacity for enhanced mobilisation, these seedlings would be better able to compete for light, water and nutrients, and therefore grow faster once emerged and establish more successfully. A similar situation would arise if seedlings from high and low N seed germinated and grew together. Development of high N content seed is usually a result of parent plants growing in soil with elevated levels of available soil N (Chapter 6). If seedlings from species capable of increasing seed N content

establish more successfully than seedlings of species that did not have this capacity, the latter species could have difficulty establishing or maintaining a population at high fertility sites. In agricultural situations the differences between species in seedling growth rates prior to and after emergence may have implications for the establishment and persistence of weed populations in crops. However, the ability of species other than the cereals examined here to show enhanced mobilisation of reserves needs further investigation.

7.3 Leaf Growth

Once seed N reserves are exhausted, plants must take up and assimilate N from the soil in order to grow and develop normally to maturation. Above ground cereal growth consists of the production of laminae (hereafter referred to as leaves), sheaths and stems. Depending on species, cultivar and environmental conditions, cereals produce approximately seven to 14 main stem leaves and from zero to over 20 tillers, with each tiller having approximately five leaves. An important component of total plant leaf area is individual leaf area which, as been shown in many studies, can be markedly influenced by the level of external N available to the plant. In Chapter 3 it was demonstrated that for all cereal species, with increasing external concentrations of NO_3^-, NH_4^+ or glutamine over the range 0 to 2.5 - 5 mol m^{-3}, the area of individual main stem leaves increased. Over this range of external N concentrations, leaf N content increased similarly for all three N forms (Chapter 5), probably reflecting greater concentrations of photosynthetic pigments and enzymes (Lawlor *et al.*, 1988). Therefore, it is likely that photosynthetic rate per unit leaf area increased markedly and greater individual leaf area was a result of increased availability of both C and N. For barley, at the cellular level greater main stem leaf area with additional NO_3^- over the range 0.5 - 2 mol m^{-3} was associated with both more cells and larger cells (Chapter 4). It was proposed that due to higher rates of photosynthesis, greater cell expansion was a result of higher levels of sucrose, the main form of osmoticum in plant cells. Greater availability of C and N was thought to increase the extent of cell division, leading to more cells per leaf, although the mechanism for this response is not known. At lower external N concentrations, the amounts, forms and concentrations of N compounds transported to the leaves are probably similar regardless of whether NO_3^-, NH_4^+ or glutamine are supplied. Hence, the cellular basis of increased leaf area at lower external N concentrations proposed for additional NO_3^- in Chapter 4 are probably also valid where other forms of N are supplied.

With increasing concentrations of external NO_3^- or glutamine over the range 5 to 20 mol m^{-3}, the growth of individual leaves usually increased further. At higher external NO_3^-

concentrations, an increasing proportion of the NO_3^- taken up is transported to the leaves and assimilated there (Andrews *et al.*, 1992). In the leaves, the NO_3^- assimilation reactions can be coupled to the photosynthetic pathways and sucrose does not need to be respired to provide energy and reductants (Layzell, 1990). Also, if the supply of NO_3^- is greater than the assimilation capacity, NO_3^-, together with counter ions like potassium (K^+), can accumulate in the vacuoles and contribute substantially to the osmotic potential of the cells. The assimilation of NO_3^- results in the production of excess hydroxide ions which, in order to maintain cellular pH, are neutralized via the production of organic acids. These acids also accumulate in the vacuoles and contribute to cellular osmotic potential. In Chapter 4 increased leaf area with additional NO_3^- from 2 to 5 mol m^{-3} was associated with greater cell size. It was proposed that at higher external NO_3^- concentrations the additional solutes mentioned above could contribute significantly to cell expansion. In addition to the increase in cell size, the number of cells per leaf increased. It was suggested that additional N increased the rate and extent of cell division, though, as mentioned above, the exact mechanism for this response is not known.

Supplying glutamine in nutrient solution to the roots effectively mimics the exclusive root assimilation of NO_3^-. However, in contrast to NO_3^-, at high external concentrations of glutamine, 'alternative' forms of osmotica such as organic acids or K^+ would not accumulate to high levels in the leaves. However, in Chapter 3 it was shown that at all concentrations supplied, individual leaf growth was similar with N supplied as NO_3^- or glutamine. There are a number of reasons why this could be possible. Firstly, cell expansion may be similar because glutamine itself, if present in amounts greater than that required for plant function, can accumulate in the vacuoles and contribute to the osmotic potential of cells (Morgan, 1984). It is also possible that because there are no costs associated with assimilation when glutamine is the source of N, that there is more sucrose available for cell expansion. In addition, C is a significant component of glutamine and further sucrose savings can be made this way.

The development of similarly sized leaves when N is assimilated in either the root or shoot can also be explained if the morphology of cereal leaf growth is considered. The main stem leaves of cereals originate from primordia situated on the central axis which are encased by the sheaths of the surrounding leaves. Primordia develop into a zone of dividing cells, the intercalary meristem, from which a ligule forms, separating the meristem into two parts: the upper part developing into the lamina and the lower into the sheath (Williams, 1975). Both the lamina and the sheath expand up between the central axis and the surrounding sheaths by a combination of cell division and expansion. When the lamina emerges from between the central axis and the sheaths of the previously formed leaves, growth of the exposed portion of the leaf stops. Elongation of the whole lamina ceases when the ligule, raised up by the

growth of the sheath, emerges from the surrounding leaf sheaths. However, cell division and expansion continues in the sheath for a short while after that in the blade meristem ceases, raising the whole lamina above the previous leaf (Langer, 1979). The length of a fully extended leaf lamina is determined by the distance it has to travel up between the central axis and the surrounding sheaths. The proportion of this length that is achieved through cell expansion or division probably depends on the extent of cell division and the availability of osmoticum for cell expansion. If osmoticum is not readily available and cells are not able to expand to full size, then, in order to emerge, the leaf must extend as a result of an increase in cell number. Hence, it is possible to have leaves of a similar length and possibly area, made up of either many small cells or less but larger cells. It is proposed that though leaf growth is similar with N supplied as NO_3^- or glutamine, there might be differences at the cellular level, with plants supplied NO_3^- having larger cells and those supplied glutamine having more cells for a given leaf area. This suggestion has yet to be tested, though its relevance may be questioned when considering data from Chapter 4. As the area of main stem leaves tends to increase with leaf position, differences, if any, between N forms in leaf area should become more evident for later formed leaves. However, it was shown that relative to cell area, cell number became increasingly more important in determining leaf area for successive leaves. Therefore, for later formed leaves at least, differences in the availability of osmoticum for cell expansion would probably not be important in determining area.

In contrast to NO_3^- and glutamine, with additional NH_4^+ over the range 5 to 20 mol m^{-3} the area of individual main stem leaves generally decreased and evidence was strong that plants displayed signs of NH_4^+ toxicity. Decreased leaf area must be a result of either less cells, smaller cells or a combination of both. In healthy plants, regardless of external N concentration, assimilation of exogenous NH_4^+ takes place exclusively in the roots and all energy and reductants used must ultimately be provided from the respiration of photosynthate. Possibly to avoid toxicity problems, NH_4^+ is not stored and all of that taken up must be assimilated. However, assimilation uses photosynthate which may be needed for other plant functions, including cell division and expansion. Other aspects of plant growth may be affected by NH_4^+. Plants taking up NH_4^+ tend to have lower concentrations of other cations such as K^+ (Lips *et al.*, 1990) - this may affect the extent of cell expansion. However, as the mechanisms of NH_4^+ toxicity are not known, it is difficult to speculate why high levels of NH_4^+ decrease the area of individual leaves. Also, the extent or severity of the symptoms of NH_4^+ toxicity and the consequential decrease in growth appear to depend on factors other than just the level of external NH_4^+. Firstly, different species appear to have different levels of tolerance to high levels of NH_4^+. In particular, *Secale cereale* L. (rye) did not exhibit the symptoms of NH_4^+ toxicity to the same degree as other species. Rye has the capacity to produce more

tillers than other species (pers. obs.) and this may serve to dilute any excess NH_4^+ taken up. Also, in a repeat experiment, conducted under slightly different environmental conditions, barley showed markedly fewer symptoms of NH_4^+ toxicity, and leaf and total plant growth was not decreased to the same extent as in the main experiment. It was thought that factors such as degree of water loss may contribute to the expression of NH_4^+ toxicity.

In addition to main stem leaves, cereals can also develop tillers, each having from one to approximately five leaves. The total number of tillers and hence leaves per plant depends on species, cultivar, plant developmental stage and environmental conditions. Especially for species where many tillers are produced, tiller leaves can contribute substantially to the total leaf area of plants. Many studies have shown that total tiller area usually increases with the addition of N. However, as with main stem leaves, no reports were found which investigated the influence of N form on the growth of individual tiller leaves. Chapter 3 demonstrated that tiller leaf growth characteristics were generally similar to those of main stem leaves: with additional N over the range 0 to 2.5 - 5 mol m^{-3} growth was similar for all forms of N supplied. Also, observations indicated that tiller number increased from no tillers with 0.5 mol m^{-3} N to as many as four tillers with 5 mol m^{-3} N, with the final number of tillers usually depending on species. With additional NO_3^- or glutamine over the range 5 to 20 mol m^{-3} individual tiller leaf areas changed little but the number of tillers increased further, substantially increasing total plant leaf area. With high levels of NH_4^+ tillers did not emerge or were very stunted, though the severity of this reduction was not consistent between experiments.

Though the cellular basis for tiller leaf area development was not investigated in Chapter 4, it is likely that as the morphological development of tiller leaves is similar to that of main stem leaves, changes in cell size and number with additional N will also be similar. Tillers develop from shoot buds in the axils of the main stem leaves and the physical constraint experienced in relation to cell expansion would be similar to that of main stem leaves. Williams (1975) proposed that whether or not a given tiller bud will emerge from the surrounding sheaths and become an independent shoot system depends on whether its 'potential for growth' can match the constraints of its physical surroundings. Williams did not expand on the concept of tiller growth potential. However, it is possible that if overcoming physical restraint depends on the ability of tiller to produce cells and degree of expansion of these cells, then additional N could, through increased rates of photosynthesis, provide additional C for enhanced cell division or greater cell expansion. Total tiller leaf area would increase if more tiller buds were able to emerge from the surrounding sheaths and the tiller laminae attain a greater size.

This thesis has investigated leaf growth in cereals, which are monocotyledonous species. However, the process of leaf development and expansion in dicotyledons is different from that in cereals (Radin, 1983), leading to the possibility that the relationships between cell division and expansion in determining final leaf area may also be dissimilar. For example, leaves of dicotyledons, whether arising from the main stem or branches, are not physically constrained to the same extent as those of monocotyledons and hence cell expansion may play a bigger role in determining final leaf area. Also, the capacity for transverse expansion is greater for the leaves of dicotyledons, increasing the potential size of the leaves. These possible differences between species need further investigation, both at the whole leaf and cellular levels.

7.4 Partitioning of Dry Matter

The dominant effect of additional N on plant growth is to increase total leaf area and hence plant photosynthetic capacity. The extent of the increase in leaf area depends on the amount of C available for growth (photosynthesis minus respiration) and the fraction of this C that is partitioned to the leaves relative to other plant parts such as the roots and stems. The ratio of shoot (leaves plus stem) d.wt to root d.wt (S:R) is frequently used to describe the partitioning of dry matter between plant parts. Associated with S:R is the leaf weight ratio (leaf d.wt as a fraction of total plant d.wt; LWR). Depending on plant developmental stage, leaves can make up a considerable fraction of shoot d.wt and an investigation of S:R can be useful in examining the effects of N supply on changes in plant leaf area. However, though S:R and LWR usually change similarly, the latter is probably a more useful parameter to consider than S:R as it is more directly related to changes in plant leaf area. Also, especially at later stages of cereal development, stem material makes up a considerable fraction of shoot d.wt and LWR may more accuratley reflect the photosynthetic capacity of the plant.

For many species of higher plant growing under a range of environmental conditions, both S:R and LWR increase with increasing external N over the range of concentrations commonly found under natural and agricultural conditions (Section 5.1). Based on the commonly observed linear relationship between plant N content and S:R, a number of hypotheses have been put forward which try to account for the increase in S:R with additional N. However, in a review, Andrews (1993) argued that none completely explained the available data. In putting forward an alternative hypothesis Andrews proposed that the influence of N availability on S:R could be explained by the effect of increased N assimilation and protein synthesis on photosynthesis, and hence growth, and by competition between the N assimilation/protein

synthesis processes and growth for C (energy) derived from photosynthesis. To more fully understand the relationships between S:R and N supply, Chapter 5 investigated the effects of not only N availability but also N form on dry matter partitioning. It was demonstrated that with additional N over the range 0 - 6 mol m^{-3}, regardless of form applied, plant d.wt increased. Maximum d.wt attained was greatest with NO_3^-, intermediate with glutamine and lowest with NH_4^+. Both plant N content and S:R increased almost linearly with increasing external N, though at a given d.wt, S:R tended to be lower with NO_3^-. However, if S:R was plotted against plant N content, differences between N forms were not detected and it was concluded that the relationship between plant N content and S:R holds regardless of form of N applied.

Greater N availability usually increases plant d.wt and S:R/LWR concurrently and at a similar rate. However, though increases in S:R/LWR, when combined with greater plant d.wt, result in an increase in leaf area, increased plant growth is not a direct result of greater S:R/LWR. Rather, both increased growth and S:R/LWR can be seen as consequences of increases in plant N. With greater N availability, plant, and especially leaf, N content usually increases, reflecting an increase in photosynthetic pigments and enzymes. This generally leads to greater rates of photosynthesis per unit leaf area, total plant leaf area and hence plant photosynthetic capacity. As long as photosynthetic C inputs are greater than respiration, plant d.wt usually increases. However, in terms of C/energy, proteins and enzymes are expensive to make and maintain (Penning de Vries, 1975). The leaves, with a higher N content and being closer to the source of C, will realise a greater proportion of the newly produced C, resulting in an increase in both S:R and LWR. This relationship should hold, regardless of the form of N supplied, because it depends on plant N content rather than d.wt. If NH_4^+ or glutamine are supplied N content will be greater and S:R correspondingly larger, even though d.wt may be similar or less compared to with NO_3^-. This was shown to be so in Chapter 5.

A possible complicating factor in the study of the N effects on dry matter partitioning is the change in S:R with plant development independent of N supply. Experiments in Chapter 5 demonstrated that seedlings supplied N as NO_3^- or NH_4^+ prior to emergence had similar reduced N contents but those supplied NO_3^- had a greater S:R. This increase in S:R was associated with greater seedling d.wt. The association between plant d.wt and S:R rather than N content in seedlings prior to emergence was reinforced in a further experiment where seedlings were supplied chloride: as with NO_3^-, increased S:R was associated with increased d.wt. Some of the increase in S:R may therefore be related to plant ontogeny rather than exclusively to plant N content. However, the proposed explanation of Andrews (1993) for the N effects on S:R of photosynthesising plants could also hold for seedlings prior to emergence

if the roots obtain a substantial proportion of their seed derived C via the shoot. However, the pathways of C translocation from the seed to the shoots and roots still have to be determined. For mature plants previous studies have shown increases in S:R independent of N supply (Section 5.1). It is therefore possible that where N effects on S:R are associated with increased growth, at least part of the N effect may have been ontogenetic. The reason for this shift in S:R with development is not know, though it may be related to increased demands for C/energy by the reproductive structures of the plant. Further work is required to differentiate between ontogenetic and N effects on S:R.

7.5 Crop Growth and Grain Yield

The aim of most non-subsistence temperate cereal growing systems is to harvest grain at a financial profit. The monetary return from a crop usually depends primarily on the quantity of grain produced, though sometimes the quality of the grain is also important. Under agricultural conditions where soil moisture is adequate, low N availability is usually the main soil factor limiting the yield of temperate cereal crops. The response to additional N is generally substantial, and hence the strategic application of fertilizer N is frequently an important management tool used to grow profitable crops.

Cereal grains are composed mainly of carbohydrates, which are derived from the parent crop either as stored dry matter or current photosynthate produced after anthesis. The availability of stored carbohydrates depends on the quantity of dry matter produced prior to anthesis while current photosynthate supply is determined by crop photosynthetic capacity during grain filling. Final grain yield depends on the proportion of the carbohydrates produced during the season that are partitioned to the grains relative to non-harvested parts of the crop. The positive effects of additional N on grain yield must therefore be a result of either greater carbohydrate production or an increase in the allocation of carbohydrates to the grain.

Crops are made up of individual plants and like single plants, the extent of crop growth and carbohydrate production should be related to the difference between photosynthesis and respiration (Section 1.3). Crop respiration is difficult to measure under field conditions and is generally not thought to be affected markedly by N supply (Hay and Walker, 1989), though recent work has suggested that there may be differences between cereal species in respiration rate per unit d.wt (McCullough and Hunt, 1993). Crop photosynthetic capacity is dependent on the leaf area of the crop, the distribution and arrangement of the leaves down through the canopy and the average rate of photosynthesis per unit leaf area. The leaves of

the crop collectively make up the canopy, the extent of which is frequently quantified as the leaf area per unit ground area (leaf area index; LAI). Leaf area index is determined by the number of plants per unit area, the number of leaves per plant and the average individual leaf area.

The most important factor determining the extent of crop photosynthesis is the proportion of available PAR that is intercepted by the leaves. This was shown to be so for a range of temperate cereals in Chapter 6, where greater grain yield with additional N was primarily a result of increased crop dry matter (ie. stored carbohydrates) brought about by an increase in the amount of PAR intercepted up to anthesis as a result of greater LAI. Though post-anthesis LAI was not assessed, it was assumed that the positive response of LAI to additional N continued up to crop senescence, increasing the amount of current photosynthate produced. It is also probable that additional N increased the duration that the canopy was photosynthetically active after anthesis, further increasing the amount of PAR intercepted.

There are some important points to consider when investigating the causes of the positive effects of additional N on crop growth. Under agricultural conditions, levels of NO_3^-, the dominant form of N taken up and utilized by temperate cereal crops, in the interstitial solution of unfertilized soils are in the range 1 - 5 mol m^{-3}. For some of the components of plant photosynthetic capacity, this level of external NO_3^- is already near optimum. For example, photosynthetic rate per unit leaf area usually changes little under field conditions (Hay and Walker, 1989). Similarly, in Chapter 3 it was shown that the largest part of the individual leaf area response to N supply was over the range 0 to 2.5 - 5 mol m^{-3} additional N. Hence, with the addition of N as fertilizer, when levels of NO_3^- in the interstitial soil solution can be as high as 20 mol m^{-3}, increases, if any, in these components of plant photosynthetic capacity would be expected to contribute little to the increase in crop photosynthetic capacity. Therefore, under field conditions, the main reason for the increase in crop growth with additional N is usually a result of increased total leaf area, largely determined by leaf number which in turn is largely influenced by plant tiller number.

Another possible consequence of increased N availability under field conditions with regard to greater photosynthetic capacity and growth are increases in S:R and LWR. As discussed above, increased S:R and LWR are not direct causes of greater plant growth, but rather responses that occur concurrently with increased plant N content when N availability is increased. Because root d.wt is usually difficult to accurately assess under field conditions, the influence of N availability on S:R under field conditions has not been extensively studied. However, as changes in S:R occur over the range of external N concentrations found in

agricultural soils, it is likely that the response found under controlled environment conditions also occur in crops. It is therefore possible that greater S:R increases the availability of dry matter able to be allocated to the grains. However, this suggestion is difficult to investigate using existing data as HI is usually based the partitioning of dry matter between the grains and other above ground plant material, rather than the whole plant. Also, the response of HI to additional N appears to be very variable, sometimes increasing, other times decreasing and sometimes not changing and other factors may influence HI more.

7.6 Conclusions

The chapters in this thesis have investigated some of the wide-ranging effects of N on the growth of temperate cereals. As set out in the individual chapters, all the objectives of the thesis were achieved. In general, the effects of N supply on plant growth were similar for all species investigated. Though all the experiments were conducted using one or more of the cereal species, it is suggested that the processes and mechanisms discussed generally apply to other plant species, though in some cases, such as the cellular basis of increased leaf area, further work needs to be carried out to determine possible differences between monocotyledons and dicotyledons. Overall, greater plant growth with additional N is a result of increased photosynthetic capacity through either increased photosynthetic rate per unit leaf area, larger average individual leaf area or more leaves per plant. It was shown that for all processes investigated, the amount of N supplied affected the magnitude of the measured response, while for some responses the form of N supplied was important. For example, in Chapter 2, NO_3^- but not NH_4^+ increased reserve mobilisation, leading to greater seedling growth with NO_3^-. For more mature plants, at lower external N concentrations there were generally no differences between N forms in responses such as leaf area or dry matter production, though with increasing N concentrations differences became apparent. In Chapter 3 it was shown that as maximum leaf area was similar with NO_3^- and glutamine, individual leaf area development did not depend on the site of N assimilation. However, there was evidence that high external NH_4^+ concentrations were detrimental to individual leaf area development, possibly due to toxicity effects. Chapter 4 demonstrated that increased leaf area with additional NO_3^- was a result of both bigger cells and more cells, though with increasing leaf position the latter became more important. It was suggested that at lower levels of external N the cellular basis for leaf area is probably similar regardless of N form supplied, though this may not necessarily be so at higher external N concentrations. In Chapter 5 it was demonstrated that there were differences between N forms in the partitioning of dry matter between plant parts, with plants supplied NH_4^+ or glutamine having a greater S:R at a given

d.wt compared to those supplied NO_3^-. Based on an hypothesis put forward by Andrews (1993) on the control of S:R by N, this was thought to be related to plants supplied NO_3^- having a lower reduced N content. Hence, there is a linear relationship between plant reduced N content and S:R, regardless of N form supplied. Finally, under field conditions, it was proposed that the component of plant photosynthetic capacity that was most affected by additional N the increase in the number of leaves per plant brought about by an increase in tiller number. This increased canopy leaf area over the duration of crop development and hence the amount of PAR intercepted. All species investigated showed this general response to additional N, though there were differences in grain yield which were attributed to variations in HI.

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Seed reserve mobilization and the partitioning of dry matter in barley seedlings prior to emergence.

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Abstract

The effects of nitrate (NO_3^-), ammonium (NH_4^+) and chloride (Cl^-) on rate of endosperm reserve mobilization and shoot to root dry weight ratio (S:R) were examined in barley (*Hordeum vulgare*) prior to emergence. Caryopsis dry weight (d.wt) decreased while shoot d.wt, S:R and shoot and root NO_3^- content increased with increased NO_3^- applied over the range 0 - 20 mol m^{-3}. At an external concentration of 5 mol m^{-3}, nitrogen uptake and assimilation were as great with NH_4^+ as with NO_3^- but NH_4^+ did not affect the rate of reserve mobilization or S:R. Addition of 5 mol m^{-3} Cl^- increased the rate of reserve mobilization and S:R. Shoot fresh weight and percentage water of shoot and root increased with additional NO_3^- or Cl^- but did not change with additional NH_4^+. It is proposed that NO_3^- or Cl^- causes increased water uptake by seedlings which results in increased water entering the caryopsis hence a greater rate of reserve mobilization. Increased S:R with NO_3^- or Cl^- appears to be related to increased rate of mobilization of endosperm reserves.

Additional key words: Hordeum vulgare *L., nitrate, ammonium, chloride, shoot:root*

Introduction

For barley (*Hordeum vulgare* L.) cultivated in darkness, application of either a full nutrient solution or 5 mol m^{-3} potassium nitrate (KNO_3) plus 5 mol m^{-3} calcium nitrate instead of distilled water caused a 45 to 65% increase in shoot dry weight (d.wt) within 7 days of planting (Nátr, 1988a,b). Increased shoot growth was due to a greater rate of endosperm reserve mobilization and to a greater allocation of reserves to the shoot at the expense of the root. For barley sown at 70 mm depth, addition of 20 mol m^{-3} nitrate (NO_3^-) as KNO_3 to an otherwise complete nutrient solution caused increases in endosperm reserve mobilization and the proportion of reserves allocated to the shoot prior to emergence from the substrate (Andrews, Lieffering and McKenzie, 1991). The NO_3^- concentrations used in these studies are at the upper end of the range found in agricultural soils (Barber, 1984; Haynes *et al.*, 1986; Wild, 1988). In the present study, relationships between applied NO_3^- concentration, rate of reserve mobilization and the partitioning of dry matter between shoot and root were examined in barley prior to emergence from the substrate. In addition, NO_3^-, ammonium (NH_4^+) and chloride (Cl^-) were compared with regard to their effect on seedling growth.

Materials and Methods

Seed of barley (*Hordeum vulgare* L. cv. Triumph) was obtained from the Crop Research Division of the Department of Scientific and Industrial Research, Lincoln, New Zealand. Individual seed weight was 44±1, 46±1 and 48±1 mg in Experiments 1, 2, and 3 respectively. Seed showed 98% germination and was not chemically treated.

All experiments were carried out in the dark at 10±1°C in a controlled environment chamber. In all experiments, seed was placed at 70 mm depth in 80 mm diameter, 180 mm tall pots (20 per pot) filled with a vermiculite/perlite (1:1) mixture soaked in basal nutrient solution (Andrews, Love and Sprent, 1989) containing the appropriate treatment. In all treatments, potassium was maintained at 23.6 mol m^{-3} using potassium sulphate as necessary. Pots were flushed with the appropriate nutrient solution every 2 days. Seedlings were harvested 21 days after sowing and fresh weight (f.wt) of the shoot and root determined. The shoot, root and caryopsis were then dried at 70°C for 4 days for d.wt determination.

In Experiment 1, plants were supplied 0, 1.0, 5.0 or 20.0 mol m^{-3} NO_3^- as KNO_3. Dried shoot and root material was ground and an aqueous extract of a 10 - 30 mg sample was analysed for NO_3^- content as described

by Mackereth, Heron and Talling (1978). There were three nitrogen (N) treatments in Experiment 2: 0 N, 5.0 mol m^{-3} NO_3^- and 5.0 mol m^{-3} NH_4^+ added as ammonium sulphate. Nitrate, NH_4^+ (Mackereth *et al.*, 1978) and total N (Europa Scientific CN analyser) content of all plant parts were determined. In Experiment 3, plants were supplied 0 N, 5.0 mol m^{-3} NO_3^- or 5.0 mol m^{-3} Cl^- as potassium chloride.

Each experiment was a randomised complete block design. Experiment 1 had five replicates while Experiments 2 and 3 had six replicates. An analysis of variance was carried out on all data. All effects discussed have an F ratio with a probability $P<0.05$ and were obtained in repeat experiments. Means stated as significantly different are on a basis of an LSD ($P<0.05$) test.

Results and Discussion

Previously, application of 20 mol m^{-3} NO_3^- was shown to increase the rate of mobilization of endosperm reserves and the shoot to root d.wt ratio (S:R) of barley seedlings prior to emergence from the substrate (Andrews *et al.*, 1991). In Experiment 1, the magnitude of the NO_3^- effect on mobilization of seed reserves was shown to be dependent on external NO_3^- concentration as caryopsis d.wt decreased with increased applied NO_3^- over the entire range used (Fig. 1a). Also, shoot d.wt increased with decreases in caryopsis d.wt but root d.wt changed little thus S:R increased with increased applied NO_3^- throughout. Shoot and root NO_3^- content increased with increased applied NO_3^- concentration over the entire range used (Fig. 1b). At applied NO_3^- concentrations of 1 - 20 mol m^{-3}, NO_3^- content was greater in root than in shoot. Values for NO_3^- content of shoot and root in the present study were greater than those obtained for mature plants grown on comparable NO_3^- supply in a previous study (Andrews *et al.*, 1992).

No report was found of the extent of NO_3^- assimilation in temperate cereals prior to emergence from the substrate. Barley seedlings grown in the dark have been shown to have nitrate reductase activity (Aslam and Huffaker, 1982) and therefore may assimilate NO_3^- prior to emergence. In Experiment 2, the effect of NH_4^+ on seedling growth and the relationships between N uptake, N assimilation, mobilization of endosperm reserves and S:R were examined. Additional NO_3^- caused a decrease in caryopsis d.wt and increases in shoot d.wt and S:R as in Experiment 1, but additional NH_4^+ did not affect d.wt of shoot, root or caryopsis (Table 1). However, N uptake was as great with NH_4^+ as with NO_3^-. Also, as NH_4^+-N and NO_3^--N constituted only a small proportion (<1%) of total N in seedlings supplied NH_4^+, then N assimilation was as great with NH_4^+ as with NO_3^-. The N containing products of NO_3^- and NH_4^+ assimilation are likely to be the same (Layzell, 1990). Thus, although NO_3^- effects

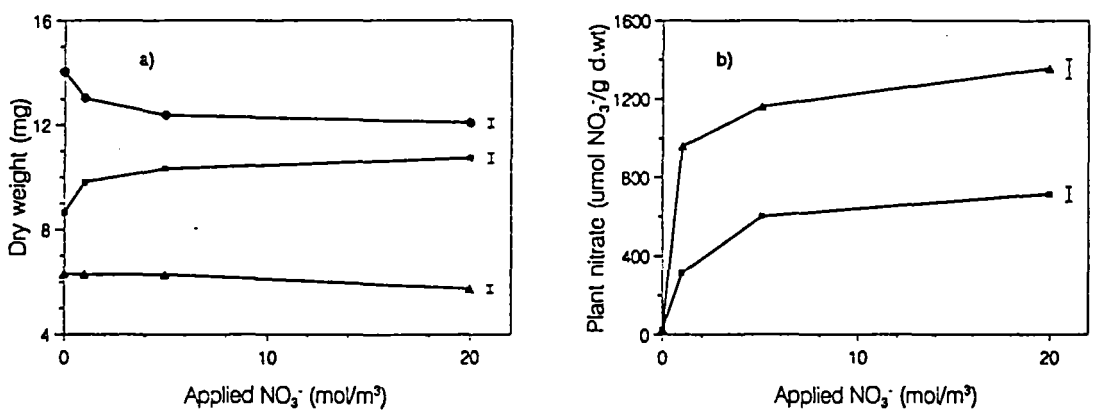

Figure 1. Effect of different concentrations of applied NO_3^- on a) shoot (■), caryopsis (●) and root (▲) d.wt and b) NO_3^- content of the shoot and root of barley prior to emergence from the substrate. Vertical lines indicate SEM.

Table 1. Effect of 5 mol m^{-3} applied NO_3^- or NH_4^+ on shoot (S) and root (R) f.wt and d.wt, caryopsis (C) d.wt, S:R, shoot and total N, NO_3^--N and NH_4^+-N content of barley prior to emergence from the substrate.

	D.wt (mg)				F.wt (mg)		N (μg seedling^{-1})		
Applied N	S	C	R	S:R	S	R	Total N	NO_3^--N	NH_4^+-N
nil	8.67	14.06	6.38	1.36	94.4	97.6	610.5	5.1	0.5
NH_4^+	8.36	14.51	6.02	1.39	99.1	90.3	920.2	4.6	1.4
NO_3^-	10.31	12.46	6.33	1.63	138.5	107.8	976.9	185.2	0.7
SEM	0.37	0.41	0.14	0.06	4.3	5.2	15.4	10.2	0.5

on barley seedlings appear to be related to the amount of NO_3^- taken up (Fig. 1a,b), they do not appear to be related to products of NO_3^- assimilation such as proteins/enzymes, as is the case with mature plants (Khamis and Lamaze, 1990; Zhen and Leigh, 1990).

In Experiment 2, shoot f.wt and percentage water of shoot and root increased with additional NO_3^- but did not change with NH_4^+ (Table 1). It is possible that the NO_3^- effects on reserve mobilization and S:R ratio are related to water uptake. Chloride is an ion which is readily taken up by plants but which is not assimilated (Clarkson and Hanson, 1980). Addition of Cl^- at concentrations of 5 or 20 mol m^{-3} can result in substantial increases in percentage water of shoots (Andrews *et al.*, 1989). In Experiment 3, addition of Cl^- caused increases in shoot f.wt and percentage water in shoot and root (Table 2). Chloride also caused increases in the rate of mobilization of endosperm reserves and S:R. These data, in conjunction with those obtained in Experiments 1 and 2, indicate that NO_3^- effects on seedlings prior to emergence are osmotic effects. It is proposed that NO_3^- causes increased water uptake by seedlings which results in increased water entering the caryopsis and hence a greater rate of reserve mobilization. If NO_3^- accumulates in the endosperm reserves then this would have a more direct effect on water uptake by the caryopsis. The increase in S:R with additional NO_3^- or Cl^- appears to be related to the increased rate of reserve mobilization. Studies are currently under way to determine the relationships between rate of reserve mobilization, and NO_3^- and water content of the caryopsis.

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Table 2. Effect of 5 mol m^{-3} applied NO_3^- or Cl^- on shoot (S) and root (R) f.wt and d.wt, caryopsis (C) d.wt and S:R of barley prior to emergence from the substrate.

	D.wt (mg)				F.wt (mg)	
Treatment	S	C	R	S:R	S	R
nil	9.98	17.57	6.41	1.55	105.6	121.5
NO_3^-	12.67	14.37	6.34	1.99	148.5	127.5
Cl^-	11.93	15.36	6.24	1.91	139.5	128.9
SEM	0.52	0.25	0.20	0.07	4.8	4.5

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Effects of nitrogen on leaf growth of temperate cereals: A review

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Abstract

Under agricultural conditions where soil moisture is adequate, low nitrogen (N) availability is usually the main soil factor limiting the growth and yield of temperate cereals. As the response to additional N is generally substantial, the strategic application of fertilizer N is an important management tool used to increase yields. Nitrogen availability can affect photosynthetic rate per unit leaf area but often the main reason for the large effect of additional N on crop growth is that it increases leaf area per plant and consequently increases leaf area index (leaf area per unit land area): this results in increased crop photosynthesis. This paper reviews recent work on the influence of N on leaf growth of temperate cereals from seed germination through to maturity. New data are presented in order to provide greater understanding of the mechanism of the nitrate effects on 1) mobilization of seed reserves; 2) partitioning of dry matter to leaf, stem and root and 3) expansion of leaves. The effects of additional N on leaf and plant growth are discussed in relation to crop growth in terms of canopy development and grain yield. Areas where further research is required are highlighted.

Additional key words: seed reserve mobilization, nitrate, dry matter partitioning, leaf expansion, cell size, canopy development, grain yield.

Introduction

Under agricultural conditions where soil moisture is adequate, low nitrogen (N) availability is usually the main soil factor limiting the growth and yield of temperate cereals. As the response to additional N is generally substantial, the strategic application of fertilizer N is frequently an important management tool used to increase yields. In New Zealand, barley (*Hordeum vulgare* L.) is the most important cereal in terms of area sown (96,000 ha) and tonnage harvested (435,000 T) (Department of Statistics, 1991). Second to barley is wheat (*Triticum aestivum* L.) which is grown on approximately 40,000 ha with 188,000 T being harvested. Recommended N fertilizer rates for cereal crops in New Zealand depend on the species sown, the N status of the soil and the end use of the crop. Usually, 50 kg N ha^{-1} applied at sowing is recommended for malting barleys, while up to 100 kg N ha^{-1}, applied at sowing and anthesis, is recommended for high protein bread wheats (Montgomery, 1986a,b). N fertilizer can be added in a range of forms such as urea, calcium ammonium nitrate, ammonium sulphate or a mixture like 'calurea' (calcium nitrate plus urea). However, under temperate agricultural conditions, rates of nitrification are usually rapid and nitrate (NO_3^-) is likely to be the dominant form of N available to, and taken up by, temperate cereals in most soils (Haynes *et al.*, 1986). Overall N fertilizer usage in New Zealand is low compared to that in Western Europe, but it has increased steadily over the past decade with nearly 46,000 T being applied in 1991 (FAO, 1992).

Plant dry matter usually contains 1 - 6% N, depending on species, age, plant organ and environmental conditions (Haynes *et al.*, 1986; Mengel and Kirkby, 1987). N is a constituent of many cellular components such as nucleic acids, chlorophyll, proteins, enzymes, cell membranes and cell walls which are vital to the function and growth of plants. Therefore the rate and/or extent of processes that utilise these compounds will be affected by plant N status. Such processes include photosynthesis. Additional N can increase the rate of photosynthesis per unit leaf area by, for example, increasing the concentrations of photosynthetic pigments and enzymes (Lawlor *et al.*, 1987). However, the major influence of additional N on crop growth under agricultural conditions appears to be due to increased total leaf area (Andrews *et al.*, 1991b and references therein). This paper reviews recent work on the

influence of N on leaf growth of temperate cereals. New data are presented to provide greater understanding of the mechanism of the NO_3^- effect on 1) mobilization of seed reserves; 2) partitioning of dry matter to leaf, stem and root and 3) expansion of leaves. The effects of additional N on leaf and plant growth are discussed in relation to crop growth in terms of canopy development and grain yield. Areas where further research is required are highlighted.

Materials and Methods

The new data on the effects of N availability on dry matter partitioning, plant leaf area development, canopy development and crop growth presented in this review were obtained in experiments carried out recently by the authors.

i) Partitioning of dry matter (experiment 1)

Seeds of barley (cv. Triumph; mean weight - 45 mg), obtained from the Canterbury Malting Company, Christchurch, New Zealand, were germinated on paper towels moistened with distilled water. After 4 d, seedlings with a coleoptile length of approximately 10 mm were transferred to 80 mm diameter, 180 mm tall pots (one per pot) containing a vermiculite/perlite (1:1 v/v) mixture soaked in basal nutrient solution (Andrews, Love and Sprent, 1989) containing the appropriate N concentration. There were 9 rates of N (0, 0.5, 1, 2, 3, 4, 5, 6 or 10 mol m^{-3}) supplied as either NO_3^- (KNO_3) or ammonium (NH_4^+)($(NH_4)_2SO_4$). In all treatments, potassium was maintained at 13.6 mol m^{-3} using potassium sulphate where appropriate. Pots were flushed every 3 d with the appropriate nutrient solution. Plants were grown under controlled environment conditions with a photoperiod of 14 h, a light level of approximately 400 µmol photons m^{-2} s^{-1} and day/night temperatures of 20/15±2°C. Plants were harvested 30 d after sowing (DAS) and separated into leaf, stem and root. Total leaf area was measured using a LI-COR model 3100 area analyser (LI-COR, Lincoln, NE, U.S.A) and the plant parts dried separately for dry weight (d.wt) determination. Reduced-N content of the plant parts was determined using Kjeldahl digestion and a "Kjeltec Autosampler System 1035 analyser" (Tecator; Höganäs, Sweden).

ii) Leaf area development (experiments 2a and 2b)

The same lot of barley seed and environmental conditions as in experiment 1 were used in experiments 2a and 2b. In experiment 2a, 7 rates (0, 0.5, 1, 2, 4, 6 and 10 mol m^{-3}) of NO_3^- or NH_4^+ were used. The lengths of individual main stem leaves 2 - 4 were measured daily until full extension was reached. Leaf length was taken as the leaf tip to point of leaf emergence from the coleoptile for leaf 2 and leaf tip to where the leaf subtended the leaf sheath for leaves 3 and 4. Leaves were considered fully extended when three successive measurements were identical. Plants were harvested 30 DAS and the final area of individual leaves determined. Leaf extension over time was analysed using variates derived from a generalised logistic curve, as described by Andrews *et al.* (1991b). In a similar experiment (experiment 2b) epidermal impressions using nail varnish were taken from three positions on leaf 3 of plants supplied 0.5 and 5 mol m^{-3} NO_3^-. Impressions were mounted on a glass slide and average cell size (non-veinal cells only) determined using a microscope. By compensating for the area estimated to be taken up by veins, cell number was calculated as leaf area divided by average cell area.

iii) Canopy development (experiment 3)

In a field experiment, wheat (cv. Otane) and barley (cv. Triumph) were sown into a conventionally cultivated weed free seed bed. The experiment was sited near Lincoln, New Zealand on a Templeton silt loam. Over the course of the experiment (September 1991 to February 1992) solar radiation receipts and temperatures were close to the long term average but it was slightly wetter than normal. N (0, 100 and 200 kg ha^{-1}) was applied as urea at sowing. The extent of the canopy was measured at approximately 1 week intervals until anthesis using a LI-COR 2100 canopy analyser. Final dry matter and grain yield were determined by hand-harvesting all plants from a 1 m^2 quadrat. Prior to threshing, a subsample (approximately 10% by weight) was kept to evaluate components of yield. N content of the grain was determined as in experiment 1.

Seed Reserve Mobilization and Leaf Growth

The growth of cereal seedlings depends on seed N content (specifically endosperm N) and external N supply. Increased seed N content often results in greater seedling growth. For wheat seedlings 21 DAS, total plant d.wt and area of main stem leaves 1 - 3 were greater for high N seed than for low N seed (Lowe and Ries, 1972, 1973). Also, for barley harvested 6 DAS, seedlings from high N seed had greater reserve mobilization, total plant d.wt, area of leaf 1, leaf protein concentration and photosynthetic rate (Metivier and Dale, 1977a,b; Rahman and Goodman, 1983). Additional NO_3^-

starch breakdown and which is sensitive to seed water potentials (Jones, 1969; Jones and Armstrong. 1971; Wilson, 1971). Increased reserve mobilization and greater early growth of seedlings with high N seed may also be due to increased water uptake. The rate and degree of imbibition, the physical process of water absorption by the seed, are closely related to the colloidal properties of the seed (Cardwell. 1984). Proteins are the dominant form of seed N and represent the major colloidal constituent of seeds (Arnott and Jones. 1971). For wheat and barley seeds, rates of water uptake increased as a result of higher seed N (Lopez and Grabe, 1971). Also, α-amylase activity has been found to be higher in wheat seedlings grown from high %N seed (Ching and Rynd, 1978). Further work needs to be carried out to determine the relationships between seed N content, NO_3^- uptake, water uptake, α-amylase activity and reserve mobilization.

Partitioning of Dry Matter to Leaves

Nitrogen availability can affect the partitioning of dry matter to the leaf, stem and root of temperate cereals from the seedling stage through to maturity (Table 1; Hocking and Meyer, 1991; Andrews *et al.*. 1992). Usually shoot to root d.wt ratio (S:R) increases with increased N supply regardless of form supplied or of its effects on growth (Andrews, 1992 and references therein) although during seedling development this need not be the case (Tables 1,2). At this stage, plant (shoot+root) d.wt, in comparison with plant N, shows a better correlation with S:R. Leaf weight ratio (LWR; leaf d.wt as a fraction of total plant d.wt) appears to increase with NO_3^- supply over the range in which total plant d.wt increases. then either changes little or increases further with increasing NO_3^- supply thereafter (Hocking and Meyer. 1991; Andrews *et al.*, 1992). Little information is available with regard to N effects on LWR through the different stages of plant development. For the five main temperate cereals. in the vegetative phase. LWR increased from around 0.3 to 0.4 with increased NO_3^- supply from 0.5 to 5 mol m^{-3}, the range normally found in agricultural soils (Andrews *et al.*, 1992). In a separate study. using reproductive wheat plants. LWR increased from around 0.2 to 0.3 with increased NO_3^- concentration from 0.5 to 12 mol m^{-3} (Hocking and Meyer, 1991). The mechanism of the N effect on dry matter partitioning is not known. For a range of species supplied NO_3^-. S:R was positively correlated with tissue N content (eg. Hirose. 1986; Ingestad and Agren. 1991; Boot *et al.*, 1992). Several reports indicate that for a similar total plant d.wt. S:R is greater with NH_4^+ than with NO_3^- as an N supply (Andrews, 1992;1993). Experiment 1 compared the effects of NO_3^- and NH_4^+ on S:R, LWR and tissue reduced-N content of barley in the vegetative phase. Total plant d.wt increased with applied NO_3^- or NH_4^+ concentration over the range 0 - 6 mol m^{-3}, then changed little with increasing N supply thereafter (Fig. 1a). Leaf area increased with applied N over the range 0 - 10 mol m^{-3} (Fig. 1b). At higher external N concentrations both d.wt and total leaf area were greater for plants supplied NO_3^-. Shoot to root ratio and LWR increased with increasing total plant d.wt up to 6 mol m^{-3} applied N (Figs. 1c,d) but for any given d.wt both parameters were greater for plants supplied NH_4^+. as has been found previously (Cox and Reisenhauer. 1973; Timpo and Neyra, 1983; Bowman and Paul. 1988; Troelstra *et al.*, 1992). However, for a given plant d.wt, tissue reduced-N content was greater with NH_4^+ than with NO_3^- and if S:R and LWR are plotted against plant reduced-N content, then there are no significant differences between the two forms of N (Figs. 1e,f).

Andrews (1992) proposed that the NO_3^- effect on S:R can be explained by the effect of increased NO_3^- assimilation and protein synthesis on photosynthesis, and hence growth, and by competition between the NO_3^- assimilation/protein synthesis processes and growth for energy derived from photosynthesis. It was argued that increased NO_3^- assimilation/protein synthesis results in an increased proportion of energy from photosynthesis being utilised in processing N at the expense of growth, and that this is reflected in a higher tissue reduced-N content. Over part of the external NO_3^- concentration range 0.1 to 20 mol m^{-3}, the effect of increased NO_3^- assimilation/protein synthesis on photosynthesis is so great that increased photosynthate is available for growth. It was proposed that the increase in shoot d.wt relative to root d.wt over this range is due to proximity of the shoot to the carbon (C) source and increased N availability for growth. As NO_3^- assimilation/protein synthesis increases, N use efficiency decreases. When NO_3^- assimilation/protein synthesis increases to a point where photosynthate available for dry matter production decreases. S:R will still increase as the shoot will realise a greater proportion of its growth potential due to its proximity to the source of C and the availability of reduced N for growth.

There are reports in the literature that dry matter production per unit N is greater for NO_3^- than for NH_4^+ (Cox and Reisenhauer, 1973; Bowman and Paul. 1988; Troelstra *et al.*, 1992). This was found to be the case in experiment 1 (Fig. 2a). It was also found that leaf area per unit leaf N was greater for plants supplied NO_3^- (Fig. 2b). This effect does not appear to have been reported

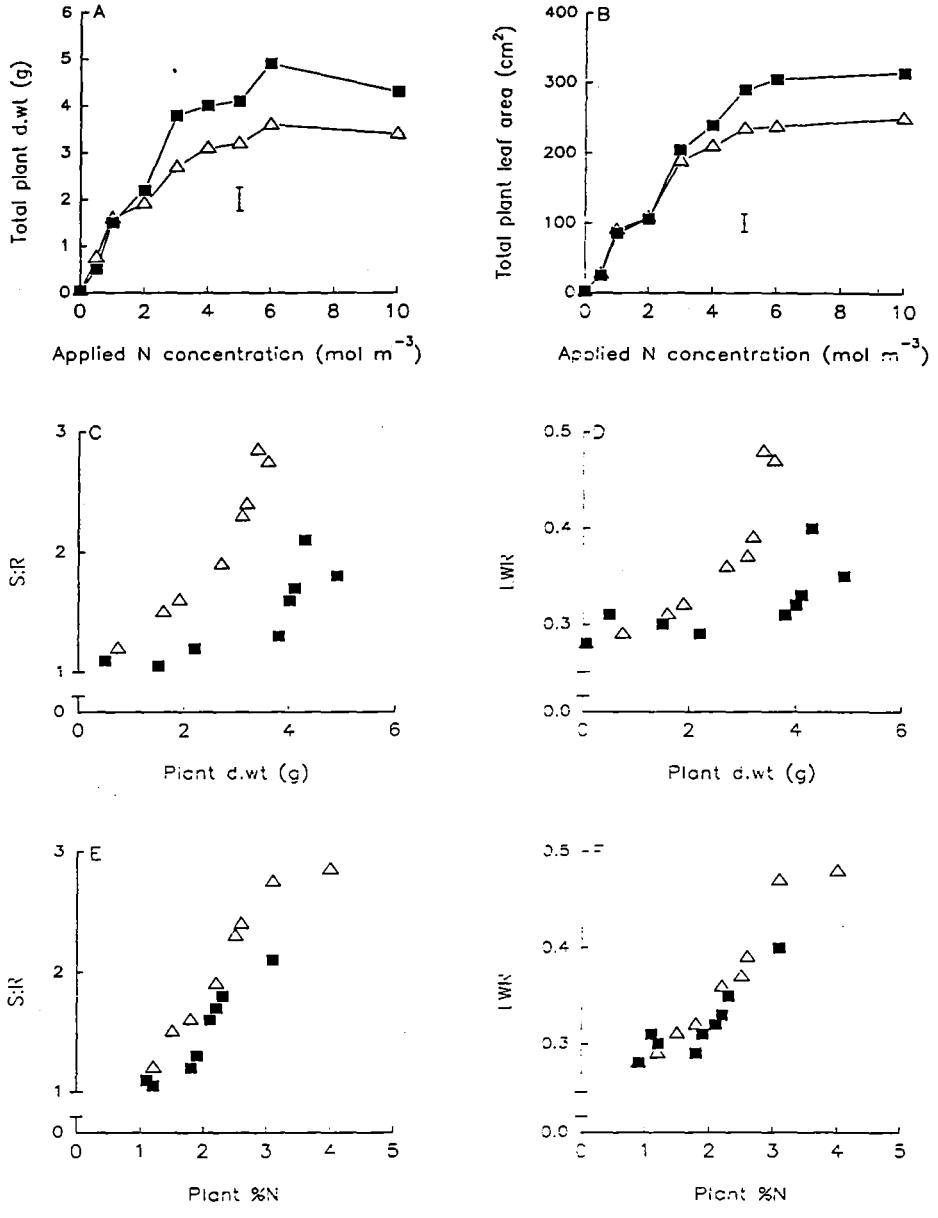

Figure 1. The effects of different concentrations of applied nitrate (■) or ammonium (Δ) on total plant dry weight (d.wt)(A) and leaf area (B) and the relationships between shoot to root d.wt ratio (S:R) and plant d.wt (C), leaf weight ratio (LWR) and plant d.wt (D), S:R and plant %N (E) and LWR and plant %N (F) for the two N forms. Error bars indicate $LSD_{0.05}$.

before. Possible reasons for greater efficiency in leaf area production with NO_3^- in comparison with NH_4^+ are discussed below.

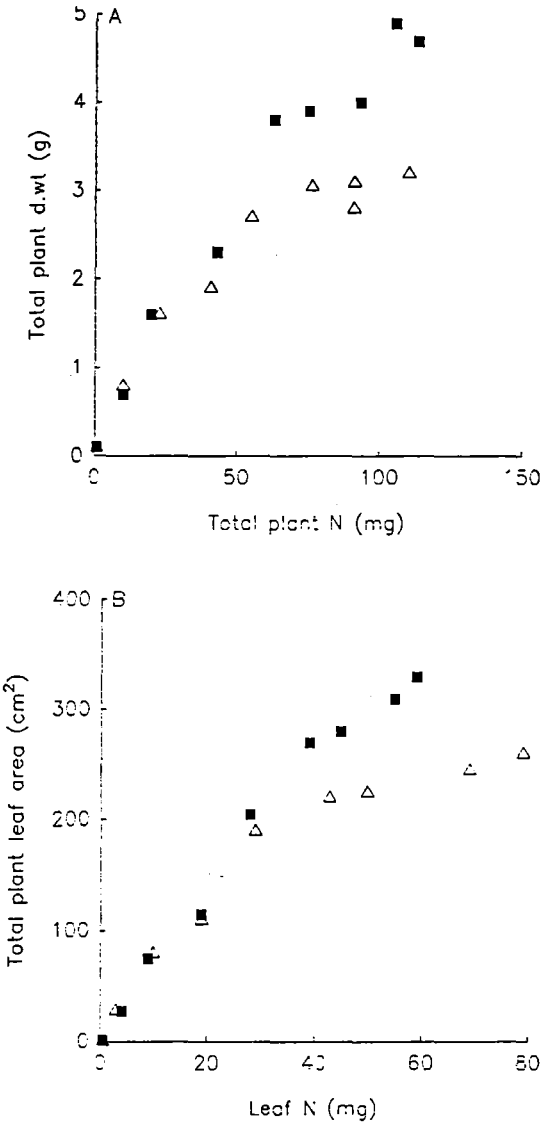

Figure 2. The relationships between total plant dry weight (d.wt) and total plant N (A) and total plant leaf area and leaf N (B) of barley supplied various concentrations of nitrate (■) or ammonium (Δ).

Leaf Area Development

Nitrogen availability strongly influences the growth characteristics of leaves of the five main temperate cereals (Andrews *et al.*, 1991b). Specifically, additional N as NO_3^- over the range 0.1 - 5 mol m^{-3} caused a decrease in duration of extension growth but increased maximum and mean extension rate and final length of main stem leaves 2 - 4. In general, the greater part of these responses occurred with increased applied NO_3^- from 0.1 - 1.0 mol m^{-3}. The magnitude of the response to NO_3^- was considerable and increased with increased leaf number 1 - 4. For example, increased applied NO_3^- from 0.1 to 1.0 mol m^{-3} caused a two to threefold increase in maximum and mean extension rates and at least a twofold increase in final length of leaf 3 of all cereals. Nitrate also had effects on area of leaves 1 - 4 of all cereals. As with final length, final area increased substantially with increased applied NO_3^- from 0.1 - 1.0 mol m^{-3}. In contrast to leaf length, leaf area for all species increased substantially with increased applied NO_3^- from 1.0 - 5.0 mol m^{-3}. These data emphasise that even in cases where rate of leaf extension and final leaf length are unaffected by NO_3^- supply, leaf area can be affected greatly.

Increased individual leaf area with additional N must be due to increased cell size, increased cell number and/or changes in leaf architecture. The main effect of additional N has usually been attributed to increased total cell number, although cell size has also been found to increase (Humphries and Wheeler, 1963; Dale, 1972; Dale and Milthorpe, 1983; Hay and Walker, 1989). We have found that NH_4^+ is similar to NO_3^- with respect to its effects on duration of growth, extension rate, and final length and area of main stem leaves 2 - 5 of barley (experiment 2a - data for leaf 4 are shown in Fig. 3). The major part of the response occurred over the range 0 - 2 mol m^{-3}. In experiment 2b, individual area of leaf 3 was twice as great at 5.0 mol m^{-3} NO_3^- compared to 0.5 mol m^{-3} (Table 3). Increased leaf area with additional NO_3^- was associated with an increase in both epidermal

Figure 3. (opposite) The effects of different concentrations of applied nitrate (■) or ammonium (Δ) on the duration of growth (A), mean extension rate (B) and final leaf area (C) of leaf 4 of barley (*Hordeum vulgare* L. cv. Triumph). Error bars indicate $LSD_{0.05}$.

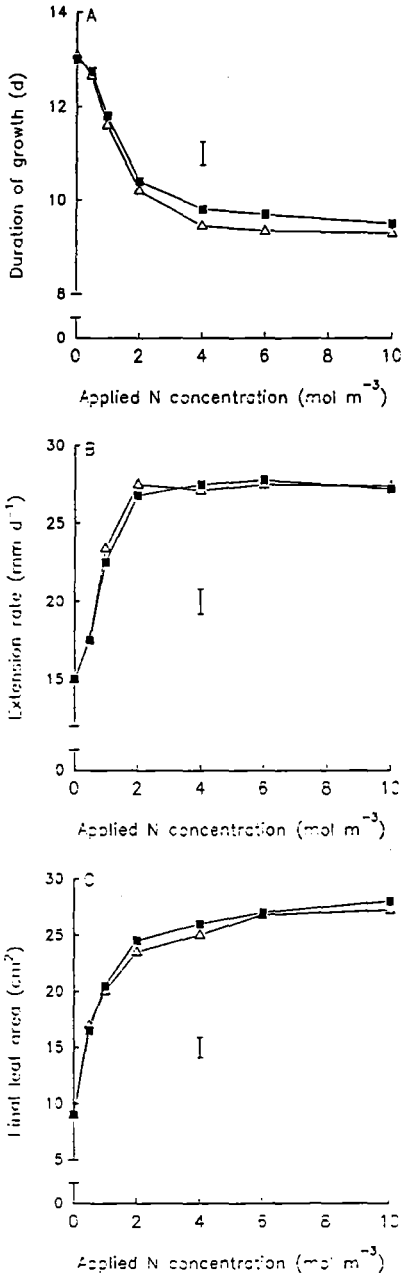

Table 3. The effects of 0.5 or 5.0 mol m^{-3} nitrate (NO_3) on area, non-veinal epidermal cell number and cell area (see text) of leaf 3 of barley (*Hordeum vulgare* L. cv. Triumph).

Appplied NO_3^- (mol m^{-3})	Leaf area (cm^2)	Cell number (x10^7 leaf^{-1})	Cell area (x10^{-7} cm^2)
0.5	5.63	1.07	2.34
5.0	12.70	1.42	4.00
s.e.m.	0.28	0.04	0.06

cell number and size. However, for this leaf at least, increased leaf area with additional NO_3^- was due more to increased cell size than to increased cell number.

Cell expansion requires the influx of water into the cell. This occurs in part as a result of the lowering of the cell water potential through the accumulation of solutes. Final cell size is determined by the availability of solutes and water, the extensibility of the existing cell wall and the availability of C for new cell wall material. The most common solute in plant cells appears to be sucrose, produced by photosynthesis (Morgan, 1984). Nitrogen availability strongly influences photosynthetic rate, hence greater cell size with additional NO_3^- could be due to increased C assimilation resulting in greater sucrose availability for osmoticum and cell wall production. Greater leaf area per unit N with NO_3^- compared to NH_4^+ (Fig. 2) could be due to increased levels of osmoticum and hence differences in cell size. In this case differences in site and pathway of NO_3^- and NH_4^+ assimilation may be important. In plants, NH_4^+-N is converted into amino acid-N primarily in the root; if NH_4^+ is transported to the shoot it can be toxic (Mehrer and Mohr, 1989). In contrast, at high external NO_3^- concentrations, a substantial, if not major, proportion of NO_3^- assimilation in cereals occurs in the shoot (Andrews *et al.*, 1992). Nitrate can accumulate to substantial levels in cereal leaves (Andrews *et al.*, 1992) and together with counter ions such as potassium, can contribute to the osmotic potential of the cell (Blom-Zandstra and Lampe, 1985; Steingrover *et al.*, 1986). In addition, the assimilation of NO_3^- leads to the generation of hydroxyl ions. Hydroxyl ions are neutralized by organic acids which are also osmotically active (Raven, 1985). Hence, at higher external N concentrations, increased leaf area per unit N with NO_3^- compared to NH_4^+ could at least in part be due to increased cell size caused by greater levels of osmoticum. Further work is required to determine the nature and concentration of solutes in the leaves of plants

supplied different levels of NO_3^- and NH_4^+ to assess the importance of site of NO_3^- assimilation in determining leaf area.

At the plant level, additional N can increase total plant leaf area in cereals by increasing leaf number. Under field conditions, additional N does not normally have a strong effect on rate of development of the main stem (Langer and Liew, 1973) and increased leaf number with additional N is likely to be due to increased tiller production and/or more leaves per tiller. The capacity to produce tillers varies considerably in temperate cereals. For example, uniculm barleys have little capacity for tiller production while some cultivars of rye can have over 20 tillers. Tillering capacity appears to be an important factor determining the ability of cereals to respond to N and hence, to some extent determines the overall growth potential of the plant (Andrews *et al.*, 1992). Most commercial cultivars have some tillering capacity and an increase in leaf number via increased tiller number is likely to contribute to increased plant leaf area with additional N. For example, it has been shown that additional N at sowing can result in a 40% increase in tiller number of Otane wheat by the 5th leaf stage under conditions where the rate of development of the main stem is unaffected by additional N (Andrews *et al.*, 1990).

Table 4. The effects of 0, 100 or 200 kg ha^{-1} N applied at sowing to spring barley (*Hordeum vulgare* L. cv. Triumph) and wheat (*Triticum aestivum* L. cv. Otane) grown at Lincoln, Canterbury. Mean canopy growth rate (MGR), maximum canopy leaf area index (MAXLAI) attained and final quadrat grain yield are presented.

	Applied N (kg ha^{-1})	Barley	Wheat
MGR (LAI d^{-1})			
	0	0.077	0.037
	100	0.121	0.040
	200	0.128	0.046
	s.e.m.	0.007	0.002
MAXLAI			
	0	4.4	3.2
	100	5.5	3.8
	200	6.0	4.0
	s.e.m.	0.25	0.15
Grain yield (T ha^{-1}) (quadrat)			
	0	6.70	5.80
	100	7.67	6.55
	200	7.86	6.64
	s.e.m.	0.15	0.10

Canopy Development

At the crop level, individual plants form a canopy. The extent of canopy development is usually quantified in terms of the leaf area index (LAI, leaf area per unit ground area). LAI determines the fraction of available photosynthetically active radiation intercepted by the canopy and hence crop dry matter production (Hay and Walker, 1989). For cereals, crop dry matter production is usually positively correlated with grain yield (Biscoe and Gallagher, 1977).

The effects of additional N on individual leaf and total plant leaf area are reflected at the crop level by increases in rate of canopy development, maximum LAI

Table 5. Effects of N application at sowing on seed head number, grains per head, individual grain weight and grain %N of spring sown barley (*Hordeum vulgare* L. cv. Triumph) and wheat (*Triticum aestivum* L. cv. Otane).

	Applied N (kg ha^{-1})	Barley	Wheat
Head number (m^{-2})			
	0	821	427
	100	1028	493
	200	1049	483
	s.e.m.	13.8	12.1
Grains per head			
	0	25.0	35.1
	100	27.5	37.5
	200	27.2	38.6
	s.e.m.	0.41	0.74
Individual grain weight (mg)			
	0	51.0	53.4
	100	46.1	51.9
	200	44.7	51.7
	s.e.m.	1.4	1.09
Grain %N			
	0	1.52	2.06
	100	1.93	2.33
	200	2.21	2.38
	s.e.m.	0.12	0.09

achieved and final grain yield (experiment 3 - Table 4). Often, the component of yield most affected by additional N is head number, which usually reflects an increase in the tiller number (Table 5, Hay and Walker, 1989; Wibberley, 1989). Nitrogen availability can also affect grain quality. In experiment 3, N applied at sowing increased the grain N content of both species (Table 5). For wheat, high grain N content is desirable as it increases baking quality while for barley low grain N results in better malting characteristics (Wibberley, 1989).

Conclusions

This paper reviews the effects of N on leaf growth of temperate cereals. It is concluded that:

1) Nitrogen availability affects leaf growth from the seedling stage to maturity.
2) Increased rate of mobilization of seed reserves with additional NO_3^- is related to increased water uptake.
3) An important factor determining partitioning of dry matter to leaf, stem and root is plant N content.
4) Increased individual leaf area with additional N is due to greater cell number and greater cell size.
5) For most cultivars, increased leaf number due to increased tillering is likely to contribute substantially to increased leaf area with additional N.

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