Nitrate effects on mobilisation of seed reserves in temperate cereals

M. Andrews, M. Lieffering and B. A. McKenzie

Department of Plant Science, Lincoln University, P.O. Box 84, Canterbury, New Zealand.

Abstract

Effects of additional nitrate (20 mol m^{-3} NO$_3^-$) on growth of barley (*Hordeum vulgare* L. cv. Triumph), oat (*Avena sativa* L. cv. Amuri), rye (*Secale cereale* L. cv. Rapaki), triticale (*x Triticosecale Wittm.* cv. Aranui) and wheat (*Triticum aestivum* L. cv. Otane) prior to emergence from a sowing depth of 70 mm were examined under controlled environment conditions. For all five cereals, additional NO$_3^-$ caused an increase in shoot dry weight (DW) of 17-40% and a decrease in residual seed DW but it did not affect shoot length. In a second experiment in which wheat was supplied with either 0 or 10 mol m^{-3} NO$_3^-$, growth and NO$_3^-$ content of root and shoot were measured at 3 to 4 d intervals prior to emergence. Nitrate did not affect shoot length or root DW. Root and shoot fresh weight (FW) and shoot DW were greater with additional NO$_3^-$ 10 d after sowing (DAS) when shoot length was approximately 15 mm. Twenty one DAS (shoot length = 55 mm approximately), shoot FW and DW were respectively 30% and 17% greater with additional NO$_3^-$. For plants grown without NO$_3^-$, NO$_3^-$ levels were low in both root and shoot throughout the experiment. With NO$_3^-$, root and shoot NO$_3^-$ content increased with increased harvest date to concentrations substantially greater than that applied. It is concluded that NO$_3^-$ stimulated mobilisation of seed reserves is likely to be of common occurrence with temperate cereals. Advantages and disadvantages of increased rate of mobilisation of seed reserves are discussed and areas for further research are considered.

Additional key words: germination, seedling growth.

Introduction

Additional nitrate (NO$_3^-$) has been shown to increase the area of leaf 1 of at least one cultivar of the five main temperate cereals (Dale, 1972; Andrews *et al.*, 1991a). In the case of barley (*Hordeum vulgare* L.), additional NO$_3^-$ was found to increase the dry weight (DW) of leaf 1 also (Dale, 1972; Metivier and Dale, 1977). This effect was greater with seedlings grown from low protein seed. Increased shoot DW was associated with greater allocation of seed reserves to the shoot at the expense of the root and to greater photosynthesis. Also, in some but not all cases, increased shoot DW in part resulted from a more rapid utilisation of seed reserves (Dale *et al.*, 1974; Metivier and Dale, 1977). For barley cultivated in darkness, additional NO$_3^-$ (Calcium nitrate + Potassium nitrate) increased shoot DW within 7 d (Nátr, 1988). Increased shoot DW with NO$_3^-$ or the complete nutrient medium, was due to a greater allocation of seed reserves to the shoot and a greater rate of depletion of seed reserves.

This paper examines NO$_3^-$ effects on pre-emergence growth of an important New Zealand cultivar of each of the five main temperate cereals. It also summarizes data obtained in recent studies of NO$_3^-$ effects on early growth of wheat (*Triticum aestivum* L. cv. Otane) under controlled environment and field conditions (Andrews *et al.*, 1990, 1991b).

Methods

Source of seed

In experiment 1, seed of oat (*Avena sativa* L. cv. Amuri), triticale (*x Triticosecale Wittm.* cv. Aranui) and wheat (*Triticum aestivum* L. cv. Otane) were obtained from Hodder and Tolley Ltd., Christchurch, New Zealand while barley (*Hordeum vulgare* L. cv. Triumph) and rye (*Secale cereale* L. cv. Rapaki) seed were obtained from the Canterbury Malting Company, Christchurch, New Zealand and Crop Research Division of the Department of Science and Industrial Research (now Crop and Food Research), Lincoln, Canterbury, New Zealand, respectively. Wheat seed used in experiment 2 was obtained from Pyne, Gould Guinness, Christchurch, New Zealand. Seed used in both experiments were of mean

seed weight ± 5%, for the respective cultivars. All seed were untreated.

Experimental procedures

In experiment 1, barley, oat, rye, triticale and wheat were supplied with either 0 or 20 mol m^{-3} NO$_3$. Seed were placed at 70 mm depth in 100 mm diameter, 200 mm tall pots containing a vermiculite/perlite (1:1) mixture soaked with basal nutrient solution (Andrews *et al.*, 1989) containing the appropriate NO$_3^-$ (potassium nitrate) concentration. Potassium concentration in the zero NO$_3^-$ treatment was made equal to that at 20 mol m^{-3} NO$_3^-$ by the addition of potassium sulphate. Plants were grown in the dark in a controlled environment chamber. The temperature ranged from 5 - 10°C during each 24 h period. All pots were flushed twice weekly with the appropriate nutrient solution. Plants were harvested 21 d after sowing (DAS) and shoot length and shoot and root fresh weight (FW) were determined for all plants. Shoots, roots and residual seed weights were then dried for 96 h at 70°C and re-weighed.

In experiment 2, wheat sown at 60 mm depth was supplied with either 0 or 10 mol m^{-3} NO$_3^-$. Growth conditions were as in experiment 1. Plants from each treatment were harvested at 3 to 4 d intervals until 21 DAS. Shoot length and shoot and root FW and DW were determined for all plants. From the second harvest onward, the dried material was ground and an aqueous extract of a 10-30 mg sample analysed for NO$_3^-$ content by the method described in MacKereth *et al.* (1978).

Analysis of results

In both experiments, there were six replicates per treatment, each replicate comprising one pot of twenty (experiment 1) or five (experiment 2) plants. Growth measurements were carried out on all six replicates. In experiment 2, NO$_3^-$ content was determined on pooled material from pairs of replicates. Experiments were carried out twice. Analysis of variance was carried out on all data at each harvest. All effects discussed have a F ratio with a probability $P<0.01$ and were obtained in repeat experiments.

Results

For all cereals in experiment 1, additional NO$_3^-$ caused an increase in shoot DW and a decrease in residual seed weight, but it did not affect shoot length (Table 1). The increase in shoot DW with additional NO$_3^-$ ranged from 17% for barley to 40% for oat. The effect of NO$_3^-$ on root DW was dependent on species. With additional NO$_3^-$, root DW increased in the case of

rye, changed little with oat and wheat and decreased with barley and triticale.

In experiment 2, which examined NO$_3^-$ effects on growth of wheat, shoot length was not affected by NO$_3^-$ (Fig. 1a). Root and shoot FW were greater (17-36%) with additional NO$_3^-$ at all harvests from 10 DAS onwards (Fig. 1b). Shoot length was approximately 15 mm, 10 DAS. Shoot DW was also greater with additional NO$_3^-$ from 10 DAS onwards but root DW changed little (Fig. 1c). The increase in shoot DW (11% - 22%) was less than the increase in shoot FW with additional NO$_3^-$ (19-33%). Additional NO$_3^-$ caused an increase in percentage water of both root and shoot from 10 DAS onwards. Without NO$_3^-$, NO$_3^-$ content of root and shoot was low (<0.2 µmol g^{-1} FW) throughout the experiment (Fig. 1d). With NO$_3^-$, root and shoot NO$_3^-$ content increased with increased harvest date. Nitrate content was greater in root than shoot until final harvest

Table 1. Effect of nitrate (NO$_3^-$) on shoot length and dry weight (DW) of the component parts of cereal seedlings 21 d after sowing at a depth of 70 mm.

Applied NO$_3^-$ (mol m^{-3})	Shoot length (mm)	Shoot DW (mg)	Residual seed weight (mg)	Root DW (mg)
Barley				
0	59	8.7	14.1	6.4
20	65	10.2	12.1	5.6
s.e.m.	2.6	0.23	0.40	0.16
Oat				
0	52	6.2	19.8	1.8
20	57	8.7	17.6	1.7
s.e. mean	3.5	0.54	0.57	0.02
Rye				
0	43	6.4	21.1	2.2
20	40	8.4	17.6	2.6
s.e.m.	2.8	0.37	0.58	0.11
Triticale				
0	62	13.5	20.0	5.3
20	67	16.1	17.9	4.7
s.e.m.	4.1	0.38	0.28	0.13
Wheat				
0	51	8.9	21.5	4.8
20	56	11.2	18.7	4.7
s.e.m.	2.3	0.41	0.59	0.09

when levels in the two plant parts were similar. At final harvest, NO_3^- content of root and shoot was 30 and 24 μmol g^{-1} FW respectively.

Discussion

Nitrate effects on seedlings

It has been shown here that for at least one cultivar of the five main temperate cereals, additional NO_3^- can cause increased rate of mobilisation of seed reserves prior to emergence from the substrate (Table 1). In the case of rye, this resulted in greater shoot and root DW but for all other cereals, root DW did not change or decreased while shoot DW increased. For all cereals, shoot to root DW ratio increased with additional NO_3^- as commonly occurs with plants exposed to light (Andrews, 1986; Andrews et al., 1989). The magnitude of the increase in shoot DW (17% - 40%) was similar to that found for the NO_3^- effect on area and DW of leaf 1 of temperate cereals, post-emergence (Dale, 1972; Andrews et al., 1991a). Thus carbon derived from seed reserves as opposed to current photosynthesis may be the main cause of increased growth of leaf 1 of cereals post-emergence.

For wheat, increases in shoot FW and DW were detectable 10 DAS when coleoptile length was 15 mm (Fig. 1a-c). The increase in shoot FW was greater than that for shoot DW indicating greater levels of osmoticum in cells (Andrews et al., 1989). This is likely to have been at least in part due to increased solute derived from increased rate of mobilisation of seed reserves. Nitrate can also act as osmoticum (Sprent and Thomas, 1984; Steingröver et al., 1986). For plants supplied NO_3^- in experiment 2, NO_3^- concentration averaged over the entire shoot reached 27 mol m^{-3}. This NO_3^-, in conjunction with counter ions, would contribute to the osmotic potential of cells. Also, if some NO_3^- taken up was assimilated, it is possible that amino acids and
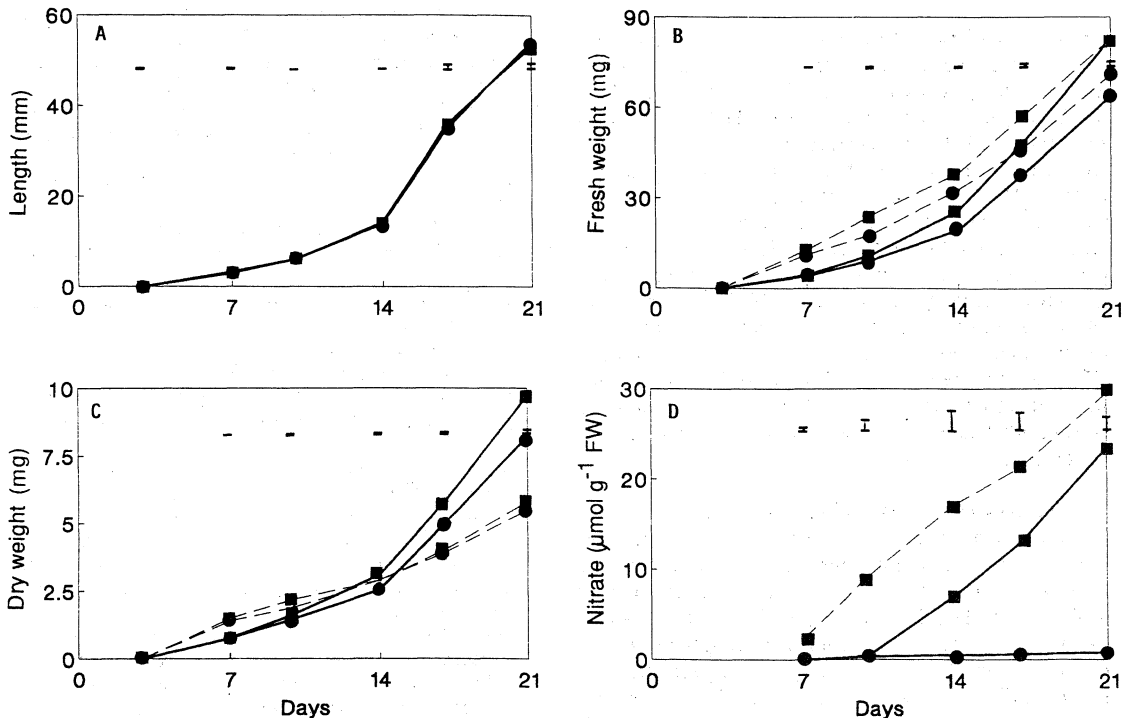

Figure 1. Growth and NO_3^- content of shoots (———) and roots (----) of *Triticum aestivum* L. cv. Otane supplied 0 (●) or 10 (■) mol m^{-3} NO_3^-. Seed (dry weight = 46 ± 2 mg) was sown at 60 mm depth. Vertical lines indicate s.e.m. Data taken from Andrews et al. (1991b).

organic acids produced in the assimilation process would also contribute to osmoticum (Raven, 1985). Nitrate reductase activity is detectable in both roots and shoots of wheat (cv. Otane) prior to emergence and there is little doubt that NO_3^- assimilation occurs at this time (Andrews *et al.*, 1991b). However, the extent of NO_3^- assimilation in cereals prior to emergence has yet to be determined.

With regard to the field situation, NO_3^- concentration in the interstitial water of non-fertilised soil in temperate regions is likely to be in the range 1 to 10 mol m^{-3} after cultivation and hence during seedling development (Barber, 1984; Haynes *et al.*, 1986). For wheat (cv. Otane), the major part of the NO_3^- effect on seedlings occurred with increased concentration up to 1 mol m^{-3} (Andrews *et al.*, 1991b). Under field conditions, NO_3^- accumulation in shoots of wheat in unfertilised soil was as great as that obtained here at 10 mol m^{-3} NO_3^- (Andrews *et al.*, 1990). Thus, endogenous soil NO_3^- concentration is likely to be high enough to cause the effects described here.

Importance of rate of mobilisation of seed reserves

Increased rate of mobilisation of seed reserves into leaves resulting in greater area of leaf 1 is also likely to result in greater photosynthesis per plant at the leaf 1 stage (Dale, 1972). Its occurrence when soil NO_3^- levels are high is likely to allow the plant to make greater use of this NO_3^-. However, at concentrations which cause increased rate of mobilisation of seed reserves, additional NO_3^- has been found to cause decreased emergence percentage of wheat from deep (60 mm) but not shallow (30 mm) sowings (Andrews *et al.*, 1991b). In all cases where seedlings failed to emerge, leaf 1 emerged from the coleoptile within the substrate. It was proposed that NO_3^- caused greater expansion of leaf 1 within the substrate which resulted in a more open leaf structure which folded more easily and was more prone to damage (Andrews *et al.*, 1991b).

The mechanism of NO_3^- effects on seeds

The way by which NO_3^- exerts its effects on cereal seeds is not obvious from the data presented here. In cases where NO_3^- was found to increase area and DW of leaf 1 of barley, it was proposed that exogenously supplied NO_3^- relieved an inadequate supply of N from the endosperm which limited early seedling growth (Dale *et al.*, 1974; Metivier and Dale, 1977). However, additional ammonium or NO_3^- resulted in similar N accumulation in seedlings but root plus shoot DW appears to have been substantially greater with NO_3^- (Dale *et al.*, 1974). These data indicate that increased

rate of mobilisation of seed reserves may not be related to increased N assimilation. Further work is required with different N sources to determine (a) the extent of N assimilation in cereal seedlings prior to emergence and (b) if N assimilation shows a correlation with rate of mobilisation of seed reserves. In addition, work is also required to determine if ions other than NO_3^- can affect the rate of mobilisation of seed reserves.

References

Andrews, M. 1986. The partitioning of nitrate assimilation between root and shoot of higher plants. *Plant, Cell and Environment 9*, 511-519.

Andrews, M., Jones, A.V. and Hines, S.E. 1990. Nitrogen effects on early growth of wheat: possible advantages and disadvantages of adding fertiliser nitrogen at sowing. *Proceedings Agronomy Society of New Zealand 20*, 1-7.

Andrews, M., Love, B.G. and Sprent, J.I. 1989. The effects of different external nitrate concentrations on growth of *Phaseolus vulgaris* cv. Seafarer at chilling temperatures. *Annals of Applied Biology 114*, 195-204.

Andrews, M., McKenzie, B.A. and Jones, A.V. 1991a. Nitrate effects on growth of the first four main-stem leaves of a range of temperate cereals and pasture grasses. *Annals of Botany 67*, 451-457.

Andrews, M., Scott, W.R. and McKenzie, B.A. 1991b. Nitrate effects on pre-emergence growth and emergence percentage of wheat (*Triticum aestivum* L.) from different sowing depths. *Journal of Experimental Botany 42*, 1449-1454.

Barber, S.A. 1984. *Soil nutrient bioavailability. A mechanistic approach.* John Wiley and Sons, New York.

Dale, J.E. 1972. Growth and photosynthesis in the first leaf of barley: the effect of time of application of nitrogen. *Annals of Botany 36*, 967-979.

Dale, J.E., Felippe, G.M. and Marriott, C. 1974. An analysis of the response of young barley seedlings to time of application of nitrogen. *Annals of Botany 38*, 575-588.

Haynes, R.J., Cameron, K.C., Goh, K.M. and Sherlock, R.R. 1986. *Mineral nitrogen in the plant-soil system.* Academic Press Inc, Orlando, Florida.

Mackereth, F.J.H., Heron, J. and Talling, J.F. 1978. Water analysis: some revised methods for limnologists. *Scientific Publications of the Freshwater Biological Association 36*, 1-139.

Metivier, J.R. and Dale, J.E. 1977. The utilisation of endosperm reserves during early growth of barley cultivars and the effect of time of application of nitrogen. *Annals of Botany 41*, 715-728.

Nátr, L. 1988. Shoot/root ratio during the early heterotrophic growth of barley as influenced by mineral nutrition. *Plant and Soil 111*, 237-240.

Raven, J.A. 1985. Regulation of pH and osmolarity generation in vascular land plants: costs and benefits in relation to efficiency of use of energy, nitrogen and water. *The New Phytologist 101*, 25-77.

Sprent, J.I. and Thomas, R.J. 1984. Nitrogen nutrition of seedling grain legumes: some taxonomic, morphological and physiological constraints. *Plant, Cell and Environment 7*, 637-645.

Steingröver, E., Woldendorp, J. and Sijtsma, L. 1986. Nitrate accumulation and its relation to leaf elongation in spinach leaves. *Journal of Experimental Botany 37*, 1093-1102.