Ms. 4207

THE DEVELOPMENT OF WATERLOGGING DAMAGE IN WHEAT SEEDLINGS (*TRITICUM AESTIVUM* L.)

II. ACCUMULATION AND REDISTRIBUTION OF NUTRIENTS BY THE SHOOT

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KEY WORDS

Aeration Anaerobic Flooding Inorganic nutrients Mineral nutrition Nutrient accumulation *Triticum aestivum* Urea Waterlogging

SUMMARY

Decreases in the concentrations of nitrogen, phosphorus, potassium, calcium and magnesium in the shoots of wheat seedlings soon after the start of waterlogging were mainly attributed to an inhibition of ion uptake and transport by roots in the oxygen deficient soil. There was a small net accumulation of nitrogen, phosphorus and potassium by the aerial tissues, principally the tillers rather than the main shoot. By contrast, calcium and magnesium accumulated in both tillers and main shoot. With waterlogging, nitrogen, phosphorus and potassium were translocated from the older leaves to the younger growing leaves, and in the case of nitrogen this was associated with the onset of premature senescence. Calcium and magnesium were not translocated from the older leaves, the younger leaves acquiring these cations from the waterlogged soil. The promotion of leaf senescence by waterlogging was counteracted by applications of nitrate or ammonium to the soil surface, or by spraying the shoots with solutions of urea, but the beneficial effects on shoot growth were small.

The role of mineral nutrition in relation to waterlogging damage to young cereal plants is discussed.

INTRODUCTION

In an earlier paper[19], the responses of the shoots and roots of young wheat plants to the onset of waterlogging were compared with the changes simultaneously taking place in the soil. Early symptoms of plant damage were found to be closely associated with the rapid decrease in the concentration of oxygen in the soil-water. A much longer time was required for various solutes in the soil-water to accumulate to potentially harmful concentrations.

In the present paper, we consider whether waterlogging leads to interference in

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the accumulation of inorganic nutrients by the shoot, contributing to symptoms of damage. Experiments in nutrient solution have established that the uptake and translocation of ions by roots are inhibited when oxygen supplies are inadequate for aerobic respiration [8, 9, 23]. Shoot concentrations of nitrogen, phosphorus and potassium decrease when soil becomes waterlogged [4, 11, 12, 13, 15]. This suggests that ion uptake is inhibited more than dry matter accumulation by shoots, but in few experiments has any attempt been made to determine whether simultaneous changes in the concentrations of ions in the anaerobic soil-water contribute to this effect. Denitrification causes loss of nitrate, and under our experimental conditions this was accompanied by decreases in the concentrations of calcium and potassium [19]. We investigated in detail the nitrogen status of plants in waterlogged soil because additions of inorganic nitrogen to the soil can sometimes alleviate the adverse effects of waterlogging on shoot growth [6, 17, 20, 21]. Furthermore, nitrogen compounds are mobile in the plant, and are readily withdrawn from leaves during senescence [3, 24], a condition typically promoted in cereals by waterlogging.

MATERIALS AND METHODS

Winter wheat (*Triticum aestivum* L., cv. Capelle Desprez) was grown in a sandy soil, in a controlled environment cabinet at 14°C. After 11 days growth, the entire volume of soil was waterlogged to the surface and plants were sampled at various times from the start of waterlogging (0, 2, 4, 8 and 15 days). A full description of the experimental procedure is given elsewhere [19].

Chlorophyll concentration was measured in a 1 cm length of leaf lamina by extracting the freshly excised tissue in 5 ml of 80% acetone [1]; the absorbance was measured using a Unicam spectrophotometer at 625 nm. The remainder of the shoot was dried at 80°C, ground and used for nutrient analysis. Subsamples of the shoots were, (i) analysed for free nitrate and total nitrogen [5]; (ii) wet ashed [18] and analysed for phosphorus, calcium and potassium [19]; (iii) dry ashed at 600°C, dissolved in hydrochloric acid and used for iron and manganese determinations [19].

To examine the effect of nitrogen additions to the soil surface on plant response to waterlogging, 1.5 mg N/plant/day was supplied as calcium nitrate or ammonium salts (carbonate, sulphate or chloride) dissolved in 2 ml of water. These additions commenced at the start of the waterlogging treatments. The water-table in the waterlogged treatment was maintained either at the soil surface or 10 cm below. For foliar applications of nitrogen, a 0.1 *M* solution of urea was sprayed until leaf surfaces were saturated, on three consecutive days commencing either at the start of waterlogging, or when senescence of the first leaf was initially observed (after 6 days). Urea is readily absorbed through the leaves of plants [7], but spraying the solution for more than three days caused scorch of the leaf tips.

RESULTS

Effect of waterlogging on nutrient accumulation and redistribution by the shoot

Accumulation of nitrogen, phosphorus, potassium, calcium and magnesium by the shoot had already slowed 2 days after the start of waterlogging (Fig. 1).

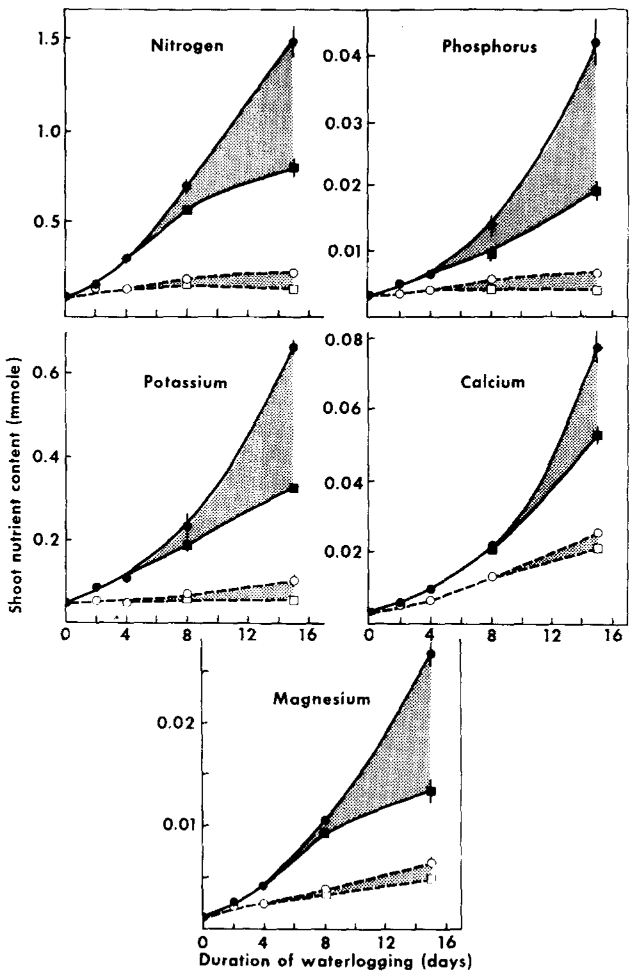
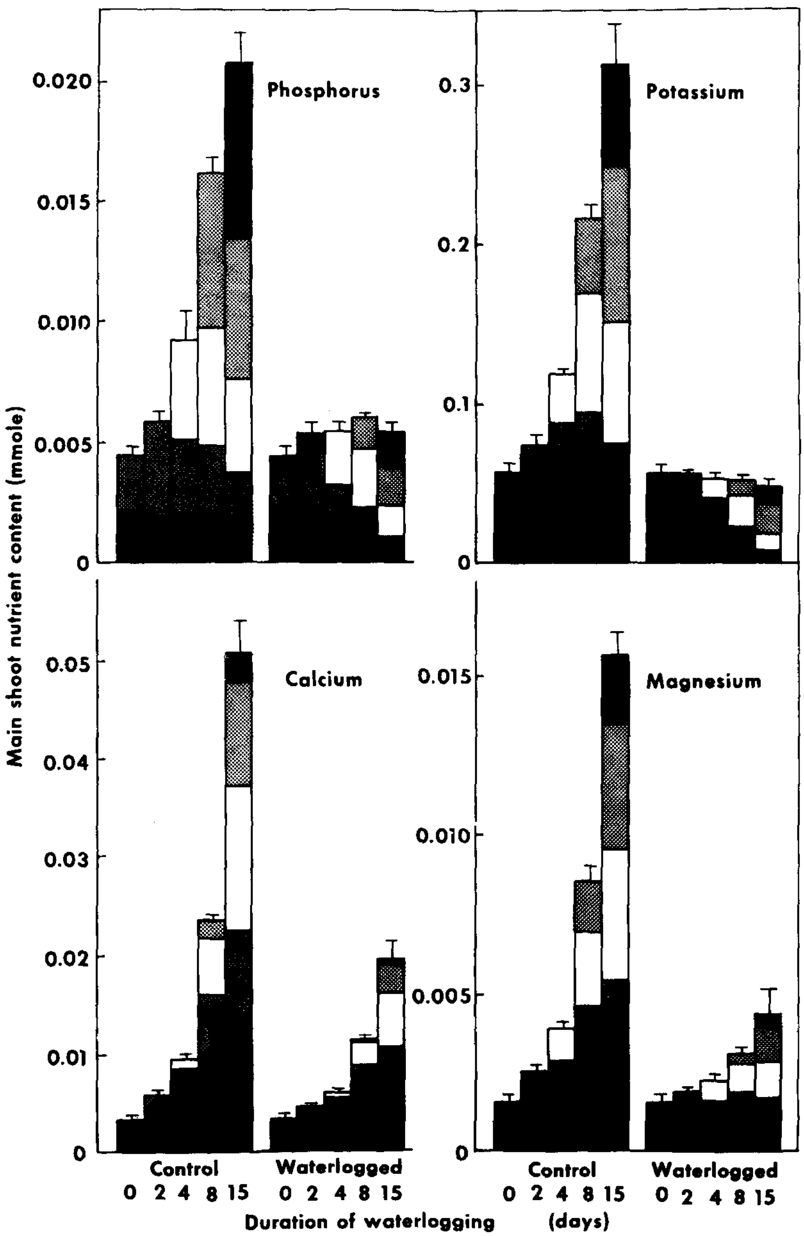


Fig. 1. Nutrient accumulation in the aerial tissues (main shoot and tillers) of wheat seedlings growing in waterlogged and non-waterlogged soil.

Mean values ± one standard error of 6 (8 and 15 days) and 8 plants are given.

Legend:	Waterlogged	non-waterlogged
Total shoot	- - - ○ - - -	——●——
Main shoot	- - - □ - - -	——■——

The shaded area is the accumulation by the tillers.


Phosphorus
Potassium
Calcium
Magnesium
Main shoot nutrient content (mmole)
0.020
0.015
0.010
0.005
0
0.3
0.2
0.1
0
0.05
0.04
0.03
0.02
0.01
0
0.015
0.010
0.005
0
Control
Waterlogged
0 2 4 8 15
0 2 4 8 15
Control
Waterlogged
0 2 4 8 15
0 2 4 8 15
Duration of waterlogging (days)

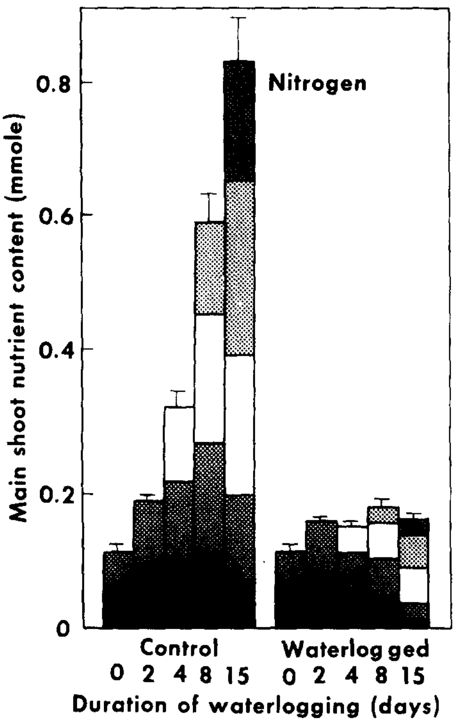


Fig. 2. (*Continued*)

Fig. 2. Nutrient content of individual leaves on the main shoot of wheat seedlings growing in waterlogged or non-waterlogged soil.

Mean values ± one standard error of 6 (8 and 15 days) and 8 plants are given. The first leaf (shaded black) is the oldest leaf on the main shoot. The younger leaves, where these have emerged, are indicated by differently shaded areas in sequence up each column. The total height of each column represents the content per main shoot.

Table 1. Concentration of nutrients in the shoot of wheat seedlings growing in waterlogged and non-waterlogged soil

Days from the start of waterlogging	Nutrient concentration (μmoles g^{-1} dry weight)			
	2[a]		15[b]	
Treatment	Control	Waterlogged	Control	Waterlogged
Total nitrogen	2910	2014*	2016	621*
Nitrate-nitrogen[c]	307	36*	107	21*
Phosphorus	94	84*	52	23*
Potassium	1339	823*	864	259*
Calcium	118	95*	95	68*
Manganese	0.71	0.40[ns]	0.16	0.26*
Iron	2.34	2.13[ns]	0.34	0.45[ns]

Mean values of 8(a), 6(b) or 3(c) plants are shown.

* Significantly different from the control, $P < 0.05$: ns not significant.

However there was a small but continuous accumulation of all nutrients by the aerial tissues throughout the waterlogging period. Potassium uptake initially was most severely restricted but recovered somewhat between 8 and 15 days when compared to nitrogen and phosphorus. Calcium and magnesium uptake were least affected by waterlogging. There was only a small net accumulation of nitrogen, phosphorus and potassium by the main shoot during the waterlogging treatment, and any net accumulation by the aerial tissues was largely by the tillers (Fig. 1). By contrast, calcium and magnesium accumulated in both the main shoot and the tillers.

Waterlogging resulted in a rapid decline in the average concentration of most nutrients in the shoot (Table 1), because of interference in nutrient accumulation together with the initial increase in shoot dry weight above that of the controls[19]. The concentration of nitrate in the shoot was particularly sensitive to waterlogging and fell to about 12 per cent of the controls at 2 days. The concentrations of iron and manganese were slightly greater than in the corresponding controls after 15 days but were less than at 2 days. Since these concentrations were much lower than those associated with toxicity in cereals[2], it is unlikely that they contributed to waterlogging damage in our experiment.

There was a smaller content of nitrogen, phosphorus and potassium in individual leaves on the *main* shoot of waterlogged plants (Fig. 2). The youngest leaves continued to accumulate these ions, apparently at the expense of the older leaves, since there was little increase in the content in the main shoot. The contents of phosphorus and potassium in the first (oldest) leaf fell at 2 days waterlogging (Fig. 2), and that of nitrogen at 4 days. The fall in nitrogen content coincided with the onset of premature leaf senescence in the tip of this first leaf (Table 2). The content of nitrogen, phosphorus and potassium in the first leaf

Table 2. Chlorophyll concentration in the first leaf of wheat seedlings growing in waterlogged and non-waterlogged soil

Days from the start of waterlogging	Chlorophyll concentration g^{-1} leaf fresh weight (arbitrary units)			
	0	2	4	8
Control	27.3	26.9	25.0	20.7
Waterlogged	–	25.6^{ns}	18.6*	2.1*

The chlorophyll concentration was measured in a 1 cm section of leaf blade from the tip of the first leaf, this being the region where leaf senescence was first observed.

Mean values of 8 (days 0–4) or 6 (day 8) plants are given.

* significantly different from the control, $P < 0.05$.

Table 3. Percent redistribution of N, P, K, Ca, and Mg from the lower leaves of the main shoot during waterlogging for 15 days

Leaf number	N	P	K	Ca	Mg
1	80.2$^{(2)}$	82.5$^{(1)}$	76.1$^{(1)}$	x	x
2	70.9$^{(2)}$	80.5$^{(2)}$	88.1$^{(2)}$	x	26.6$^{(2)}$
3	8.3$^{(3)}$	49.7$^{(3)}$	52.5$^{(3)}$	x	x

The maximum content was measured (1) at the start of waterlogging, (2) after 2 days, or (3) after 8 days. The minimum was that measured after 15 days for N, P, and K. For Ca and Mg, x indicates that maximum contents were attained on day 15.

$$\% \text{ redistribution/plant part} = \frac{\text{maximum nutrient content} - \text{minimum nutrient content}}{\text{maximum nutrient content}} \times 100$$

continued to decrease throughout the waterlogging treatment, and at 15 days about 80 per cent of its nutrients had been retranslocated to other parts of the plant (Table 3). Nitrogen, phosphorus and potassium accumulated in the second and third leaves of the main shoot with maxima at 2 and 8 days respectively (Table 3). There was little retranslocation of these nutrients from the lower leaves of the control plants (Fig. 2).

Calcium continued to accumulate in all leaves of the plants growing in waterlogged soil throughout the experiment, even where leaf growth (extension, fresh weight and dry weight) had ceased[19], and where leaves had begun to senesce prematurely. Magnesium accumulation by the first leaf ceased when plants were waterlogged, and there was little net change in content during the experiment (Fig. 2), the youngest leaves apparently acquiring magnesium from the soil.

Effect of nitrogen fertilization on the response of wheat to waterlogging

Nitrogen applications to the soil surface resulted in small and non-significant increases in shoot fresh and dry weights at all water-table depths (Table 4). The total fresh and dry weights were least when soil was waterlogged to the surface. By itself, shoot dry weight was an unreliable measure of waterlogging damage, for as reported earlier[19] the dry matter increased to about 20 per cent when the soil was waterlogged to the surface or to 10 cm (Table 4). This increase was associated with an accumulation of starch in the lower leaves.

Nitrogen applications also had little effect on root growth when the soil was waterlogged to the surface. When the water-table was maintained at 10 cm, appreciable growth of seminal roots occurred in the surface layers. Nitrate

Table 4. Effect of nitrogen applications to the soil, and water-table depths, on the response of wheat to waterlogging

	Non-waterlogged controls		Water-table depth: At surface		Water-table depth: 10 cm below the surface		
Nitrogen additions	0	NO_3^-	0	NO_3^-	0	NO_3^-	NH_4^+
Shoot fresh weight (g)	4.3a	4.7a	1.3c	1.6c	2.1b	2.3b	2.2b
Shoot dry weight (mg)	508 a	533 a	305 c	350 c	441 b	475 b	440 b
Percent shoot dry matter	11.9a	11.5a	23.1c	21.9b	21.1b	21.2b	20.5b
Root dry weight (mg)	214 a	208 a	59 c	60 c	85 b	86 b	87 b
Chlorophyll concentration (g^{-1} shoot f.wt., arbitrary units)	8.8a	9.0a	2.2e	3.0cd	2.6de	3.4c	5.3b
Total shoot nitrogen (μmoles)	1564 a	1721 a	200 e	330 d	343 d	500 c	700 b
*Shoot nutrient concentration (μmoles g^{-1} shoot dry weight)**							
Nitrogen	3079 a	3229 a	571 e	843 d	779 d	1050 c	1621 b
Phosphorus	179 a	172 a	113 b	89 c	120 b	89 c	117 b
Potassium	1495 a	1636 a	272 e	331 d	377 cd	415 bc	467 b
Calcium	118 a	125 a	62 c	78 bc	64 c	84 b	80 b
Magnesium	48 a	57 a	18 cd	18 cd	17 d	21 bc	23 b

Plants were grown for 11 days, and then the soil was waterlogged to surface, or to 10 cm below the surface for 14 days. Nitrogen additions were made daily to the soil during the waterlogging period, either as calcium nitrate, or as ammonium salts (carbonate, sulphate or chloride); 0.1 mmole nitrogen per plant per day, added in 2 ml of water. The chlorophyll concentrations were measured in a 1 cm section of leaf blade from the base of the second leaf. Mean values of 6 or 3(*) plants are given. Values in rows followed by different letter are statistically significantly different $P < 0.05$.

applications encouraged root growth in the zone 2–4 cm below the soil surface, while ammonium applications caused surface rooting, the roots showing negative geotropism, and emerging from the soil. The form of the ammonia addition (CO_3^{2-}, SO_4^{2-}, Cl^-) had little effect on the plant response. The nitrogen applications had no significant effect on the depth reached by nodal roots in the soil. When the water-table was maintained at the soil surface, or at 10 cm, nodal root depth was restricted to 20 cm and 22 cm respectively, compared to 37 cm for the non-waterlogged control plants.

The nitrogen additions influenced the nutrient accumulation and senescence of leaves (Table 4). The final nitrogen concentrations and contents were greater in shoots where nitrogen had been added to the soil surface, and this was reflected in an increase in the chlorophyll concentrations. Nitrogen additions also increased the concentrations in shoots of all nutrients, except for phosphorus which was usually depressed.

Foliar sprays of urea-N (Table 5) had a similar effect on the response of plants to waterlogging as had additions of nitrate and ammonium to the soil surface. The urea sprays slightly increased the fresh weight of the shoot and the chlorophyll concentration, when compared to the unsprayed plant. The earlier spraying (from the start of waterlogging) proved to be most beneficial, but again the effect of the nitrogen addition was small when compared to the effect of waterlogging.

DISCUSSION

Only 2 days waterlogging inhibited markedly the uptake and translocation to shoots of nitrogen, phosphorus, potassium and to a lesser extent magnesium and

Table 5. Effect of urea sprays on shoot fresh weight and chlorophyll levels of wheat plants grown in waterlogged soil

Timing of urea spray (days from the start of waterlogging)	Control			Waterlogged		
	None	0–3	6–9	None	0–3	6–9
Shoot fresh weight (g)	2.76ab	3.21a	3.07a	1.62d	2.20bc	1.89cd
Chlorophyll concentration (g^{-1} fresh wt. arbitrary units)	0.54a	0.58a	0.54a	0.13c	0.21b	0.18b

Plants were grown for 11 days and then the soil was waterlogged to the surface. The shoots of some plants were then sprayed with a 0.1 *M* solution of urea for the first 3 days of waterlogging, or when visible signs of yellowing were first observed (6–9 days). After 14 days waterlogging the plants were harvested and chlorophyll was extracted from the entire shoot in 50 ml 80% acetone.
The mean values of 6 plants were shown. Values in rows followed by different letter are statistically significantly different ($P < 0.05$).

calcium (Fig. 1). As there were only slow changes in the concentration of these nutrients in the soil solution during the experiment[19], the results indicate that interference in ion transport by roots under oxygen deficient conditions was a major factor. The concomitant redistribution of mobile nutrients within the shoot, from the older to the younger leaves (Fig. 2), may partially alleviate the development of nutrient stress at the shoot apex once ion uptake by the roots was arrested. We suggest that this retranslocation from older leaves was an important contributory factor in promoting their premature senescence.

Although the young leaves were able to appropriate nitrogen, phosphorus and potassium at the expense of the older leaves, the amounts accumulated in these young leaves were 2 to 3 times less than in the controls. The calcium content of the older leaves continued to increase throughout the waterlogging treatment, and despite the onset of premature leaf senescence, previously accumulated calcium and magnesium were not retranslocated to the younger leaves. In general, previously absorbed calcium is not retranslocated within the shoot, in contrast to nitrogen, phosphorus and potassium[22]. Magnesium is known to be mobile in the phloem[16] and to be intermediate between potassium and calcium in the extent to which it is redistributed within the plant[10]. It is interesting to note that the patterns of retranslocation of all nutrients out of the lower leaves of our wheat plants in waterlogged soil were similar to those observed in nutrient stressed maize[10]. Because of the immobility of calcium once it has been accumulated in the shoot, the young leaves and the apical meristem require a continuous supply of calcium from the roots, regardless of the average shoot concentration[14]. However it is not known whether the interference in calcium and magnesium accumulation caused by waterlogging in our experiments led to a nutrient stress at the apex that disrupted growth.

Differences between nutrients in the pattern of translocation out of individual leaves may also be influenced by the 'demand' of the younger leaves and the shoot apex, but this does not explain readily why nitrogen, phosphorus and potassium accumulated in the tillers, but not in the main shoot, in contrast to calcium and magnesium. These differences are not a reflection of changes in the nutrient concentration in the soil solution during waterlogging[19]. One possibility is that calcium transport into the main shoot and tillers was largely by passive processes through the dead or injured roots, calcium moving by mass flow in the xylem stream into the most rapidly transpiring aerial tissues. The small amounts of nitrogen, phosphorus and potassium accumulating in the tillers may have originated mainly from uptake by the emerging nodal roots that were adapted to oxygen deficiency and preferentially supplied the tillers. Measurements made of transpiration[19], and the concentration of ions in the soil water suggest, nonethe-

Table 6. Calculated nutrient concentration in the xylem sap of wheat seedings growing in waterlogged and non-waterlogged soil

Days from the start of waterlogging		Nutrient concentration in the xylem sap (mM)		
		0–4	4–8	8–15
Nitrogen	Control	17.5	13.0	12.2
	Waterlogged	5.3	3.9	1.5
		[8.7]	[6.6]	[3.6]
Phosphorus	Control	0.57	0.34	0.29
	Waterlogged	0.20	0.10	0.10
		[0.004]	[0.004]	[0.004]
Potassium	Control	8.51	5.72	5.10
	Waterlogged	0.66	0.97	1.62
		[1.11]	[1.07]	[1.02]
Calcium	Control	0.87	0.62	0.75
	Waterlogged	0.38	0.60	0.75
		[4.03]	[3.43]	[2.61]

Xylem sap concentration estimated by dividing the net nutrient accumulation by the shoot by the weight of water transpired during the same period. Values in parenthesis are the average nutrient concentration in the soil solution (waterlogged treatment) for the time periods given, as described earlier[19]. The nitrogen concentration in the soil solution was entirely in the form of nitrate.

less, that the amounts of nitrogen, potassium and calcium (but not phosphorus) accumulated by the shoots *could* have been transported passively through damaged roots in the transpiration stream (Table 6).

Nitrogen compounds in leaves are mobile, and readily withdrawn during leaf senescence[3,24]. The onset of premature senescence of the first leaf was associated with a net movement of nitrogen out of that leaf. Additions of nitrate or ammonium to the soil surface delayed the onset of premature senescence, and there was a correlation between the concentrations in the shoot of nitrogen and chlorophyll. However these higher concentrations of chlorophyll and nitrogen were not accompanied by marked improvements in shoot growth. Foliar sprays of urea had effects similar to those from additions of nitrate or ammonium.

Our results differ from those of experiments where additions of nitrogen fertilizer to waterlogged soil led to an appreciable increase in plant growth[6,17,20,21]. The reason for this difference is a matter for speculation. In some earlier experiments[17,20,21] the additional nitrogen fertilizer was added at sowing, so that the nitrogen status of the seedlings at the start of waterlogging may have been high, perhaps conferring a greater tolerance to any nutrient stress induced at a later stage. Alternatively, growth could have been limited by the availability of another nutrient in the soil, *e.g.* phosphorus, particularly in the narrow zone near the soil surface. In the present work the concentration of phosphorus was consistently smaller in the shoot in the fertilized treatments than in the non-fertilized treatments (Table 4), and phosphorus was translocated out of the third leaf between 8 and 15 days (Table 3). This suggests that there was a large demand for this nutrient in other parts of the plant. Effects of the concentration of nutrients in the rootingmedium on the response of plants to waterlogging were investigated in experiments to be reported elsewhere.

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