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EFFECTS OF ATRAZINE ON THE ASSIMILATION OF INORGANIC NITROGEN IN CEREALS

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Key Word Index—*Zea mays*; *Hordeum vulgare*; Gramineae; maize; barley; atrazine; nitrate uptake; amino compounds in xylem sap; nitrate assimilating enzymes; nitrate and amino acid incorporation into proteins.

Abstract—The inclusion of sub-lethal amounts of the herbicide atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] in the nutrient solution supplied to maize and barley increased the growth of the root and shoot and the uptake of nitrate. The activities of nitrate and nitrite reductases, glutamine synthetase and glutamate synthase were enhanced and the amino acid and nitrate contents of the xylem sap increased. All these effects of atrazine were found only in plants grown with nitrate as the nitrogen source. The uptake of $^{15}NO_3^-$ and its incorporation into protein in the root and shoot of maize and barley seedlings was significantly greater in the atrazine treated plants. However, a stimulation in the incorporation of leucine-[^{14}C] into TCA-precipitable protein of detached leaves from 7-day-old barley seedlings was obtained only in the absence of a supply of combined nitrogen either in the culture medium or in the *in vitro* incubation mixture containing the labelled amino acid.

INTRODUCTION

There are several reports that *s*-triazine herbicides applied in sub-lethal amounts to a variety of plants increase growth and nitrogen contents [1–11]. These effects were obtained in plants grown with nitrate but not with ammonium salts as a source of nitrogen [3]. A stimulation of RNA synthesis in isolated chromatin [12], an enhanced protein synthesis [11], a decrease in sugar content [5] and a delayed senescence [6] have also been associated with atrazine treatments of plants. Thus the action of these herbicides on nitrate assimilation may be indirect and their initial effect may be on protein synthesis or on carbohydrate metabolism. Studies with maize and barley reported in this paper are concerned with the action of the *s*-triazines on nitrate assimilation.

RESULTS

Growth

Maize seedlings grown with 0.5 mM NO_3^- had significantly greater fresh weight yield than those grown with an equivalent amount of ammonium chloride (Table 1). Atrazine (0.1 μM) supplied in the culture solutions enhanced the fr. wt of nitrate grown plants by 26% in roots and 31% in shoots. It had no effect on seedlings grown without nitrogen and the yields of roots and shoots of ammonium grown seedlings were slightly depressed. Atrazine also stimulated growth in maize plants after 40 days growth in sand cultures supplied with 4.5 mM NO_3^- in 0.5 strength Hoagland's solution supplemented with 1 μM of the herbicide, the increase in fr. wt of roots and shoots being 32 and 21% respectively. Although the fr. wt of the nitrate grown plants was increased by atrazine treatment, the dry wts were unchanged. In barley plants grown in sand culture with 4.5 mM nitrate and given one application of atrazine (1 μM) when the plants were 1-week-old, increased growth was again observed. The application of 1 μM atrazine daily in the nutrient solution of barley plants proved toxic but maize plants in sand culture tolerated 10 μM of the herbicide and 100 μM suppressed growth. There was delayed senescence in the older leaves of plants supplied with either concentration.

Table 1. Effects of atrazine on the fr. wt of maize seedlings grown with nitrate and ammonium

Nitrogen source	Fresh weight (g)					
	Omit atrazine			Atrazine		
	Roots	Shoots	Total	Roots	Shoots	Total
None	0.71	0.67	1.38	0.68	0.65	1.33
Nitrate (0.5 mM)	0.84	0.85	1.69	1.06	1.11	2.17
Ammonium (0.5 mM)	0.64	0.78	1.42	0.58	0.71	1.29

7-day-old maize seedlings were grown in liquid culture containing 0.1 strength Hoagland's soln without a source of nitrogen as described in Experimental. From the 8th day onwards 10 seedlings in each tray of liquid culture were supplied with either nitrate [0.15 mM $Ca(NO_3)_2$ + 0.2 mM KNO_3] or NH_4Cl in 0.1 strength Hoagland's soln and 0.1 μM atrazine as indicated. The seedlings were harvested after a subsequent 7 days growth. Least significant difference $LSD_{95\%}$ for roots = 0.093 and $LSD_{95\%}$ for shoots = 0.068.

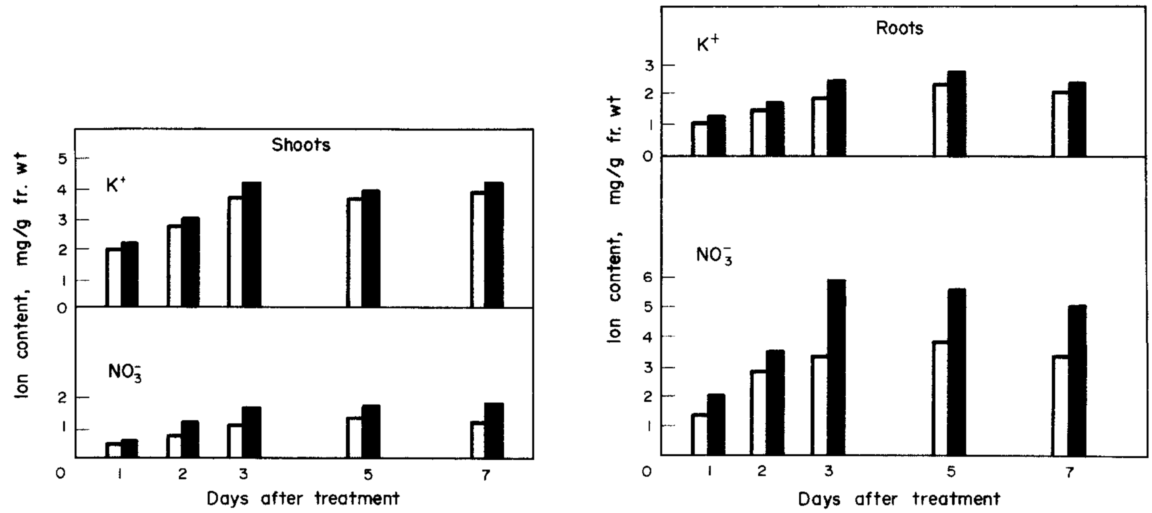

Fig. 1(a and b). Effects of atrazine on the uptake of NO_3^- and K^+ in maize seedlings. The 7-day-old seedlings were grown without nitrogen as described in Experimental. From the 8th day onwards, they were supplied with 4.5 mM NO_3^- [1.5 mM $Ca(NO_3)_2$ + 1.5 mM KNO_3] and 0.1 µM atrazine in 0.5 strength Hoagland's soln. The seedlings were harvested at various times after the application of nitrate and atrazine on the 8th day, as indicated. Open histogram, without atrazine, solid histogram, with atrazine.

Nutrient uptake

When 7-day-old maize seedlings were transferred into 0.5 strength Hoagland's solution containing 1.5 mM $Ca(NO_3)_2$ and 1.5 mM KNO_3, the nitrate content per g fr. wt in the roots and shoots respectively increased up to the 3rd day and then remained relatively constant for the next 4 days (Fig. 1a and b). A similar pattern was observed for the uptake of K^+, Ca^{2+} and Mg^{2+} whereas PO_4^{3-} accumulation in roots and shoots remained relatively constant at *ca* 0.4 mg PO_4^{3-} per g fr. wt. The

Table 2. Effects of atrazine on the amino acid and amide contents of xylem sap collected from maize plants supplied with nitrate and ammonium nitrogen

	nmol amino compound per ml of xylem sap			
	Nitrate grown plants		Ammonium grown plants	
Amino compounds	Omit atrazine	Atrazine	Omit atrazine	Atrazine
Aspartic acid	232	350	240	344
Threonine	259	410	442	398
Serine	1310	2050	2150	1790
Asparagine	475	1180	1350	1330
Glutamine	6400	12000	20000	17800
Proline	40	59	37	40
Glutamic acid	202	307	505	476
Glycine	103	192	148	116
Alanine	504	1020	723	606
Valine	303	439	541	511
Half cystine	77	72	78	70
Methionine	9	14	16	15
Isoleucine	71	103	108	121
Leucine	63	110	116	119
Tyrosine	31	43	77	75
Phenylalanine	15	28	70	58
γ-Aminobutyric acid	24	29	55	79
Ornithine	49	53	150	98
Lysine	235	450	501	430
Histidine	85	155	134	109
Tryptophan	25	21	39	34
Arginine	175	371	315	273
Total	10700	19500	27800	24900

Maize plants were grown in sand cultures as described in Experimental. From the 15th day onwards the plants were supplied daily with 0.5 strength Hoagland's soln containing either 4.5 mM nitrate [1.5 mM $Ca(NO_3)_2$ + 1.5 mM KNO_3] or 4.5 mM NH_4Cl, supplemented with 1 µM atrazine as indicated. After 40 days' growth xylem sap was collected and analysed for amino compounds as described in Experimental.

uptake of nitrate was always enhanced by atrazine and maximum increases in both roots and shoots (76 and 62 % respectively) were recorded at 3 days (Fig. 1 a and b). A similar pattern was observed for K^+ accumulation but the effect of atrazine was less than for nitrate, i.e. 30 % for roots and 13 % for shoots on the 3rd day (Fig. 1a and b). Increases in Ca^{2+} and Mg^{2+} were observed in roots only on the 3rd day but at a later stage the herbicide was without effect. The uptake of Ca^{2+} and Mg^{2+} into shoots and PO_4^{3-} into roots and shoots was not affected by the atrazine treatment. During the period of this experiment, the growth rate increased in atrazine treated plants by 38 % in roots and 43 % in shoots.

Nitrogen content of xylem sap

Fourteen-day-old maize seedlings grown in liquid cultures with 0.5 mM nitrate as a source of nitrogen secreted more xylem sap (60 µl/plant/hr) than did those grown with 0.5 mM ammonium chloride (42 µl/plant/hr). A relatively small volume of exudate was recorded in seedlings grown without added nitrogen (6 µl/plant/hr). Atrazine increased the rate of xylem sap secretion in nitrate grown seedlings by 70 % while it had no effect on ammonium grown plants. Similar results were obtained with older plants grown in sand cultures after 40 days growth.

There was a larger amount of amino compounds (27.8 µmol/ml) in the sap of 40-day-old ammonium grown plants compared with those grown with nitrate (10.7 µmol/ml) Table 2. The dominant compound was glutamine (60 and 72 % of the total amino compounds in plants grown with nitrate and ammonium chloride respectively). The composition of other amino compounds in plants grown with nitrate and ammonium chloride respectively are as follows (% of total amino compounds), serine (12 and 8 %), asparagine (4.5 and 5 %) and alanine (5 and 3 %). In ammonium grown plants glutamine makes up the greater proportion (72 %) of the total amino compounds of the xylem sap and is 213 % higher than in exudates of plants grown with nitrate only. Other amino compounds showing significant increases in the ammonium plants compared with nitrate grown plants were asparagine (184 %), glutamic acid (150 %), tyrosine (148 %), phenylalanine (367 %), γ-aminobutyric acid (129 %), ornithine (206 %) and lysine (113 %). Similar amounts of aspartic acid, proline and half cystine were recorded in the sap of plants grown with either ammonium or nitrate nitrogen. Atrazine treatment resulted in an 82 % increase in the total amino compounds of the exudates of plants grown with nitrate but in a corresponding treatment of plants grown with ammonium salts, there was a 10 % decrease. Atrazine treatment increased asparagine by about 149 %. In addition to the other amide, glutamine (88 %) the following amino acids were also increased as a result of atrazine treatment, alanine (102 %), arginine (112 %), lysine (91 %), histidine (82 %), phenylalanine (87 %), leucine (75 %) and aspartic acid (51 %). Even in ammonium grown plants where atrazine treatment decreased most of the amino acids the level of aspartic acid was increased by 43 %. Maize plants grown for 40 days with 2 mM ammonium chloride had a lower amino nitrogen content in the xylem sap (5.1 µmol/ml) than those grown with 4.5 mM ammonium chloride but atrazine again had no effect.

The effects of atrazine on the organic and inorganic nitrogen fractions of xylem sap are presented in Table 3. Organic nitrogen was the main component transported in the xylem sap of plants grown with ammonium chloride whereas in nitrate grown plants, both organic nitrogen (91.6 % of the total nitrogen) and inorganic nitrogen (8.4 %) were detected. In nitrate grown plants atrazine increased the organic nitrogen and nitrate nitrogen transported through xylem sap by 86 and 63 % respectively. Nitrate was not detected in the xylem sap of maize plants grown with ammonium salts and atrazine decreased the organic nitrogen content of these plants by 10 %. Neither nitrite nor protein was detected in the xylem sap.

The total content of soluble amino nitrogen in the 80 % ethanol extracts of roots and shoots was not affected by atrazine treatment (*ca* 80 µmol/g fr. wt in roots and shoots respectively irrespective of atrazine treatment).

Enzyme activities

The application of atrazine increased the activities of nitrate and nitrite reductases, glutamine and glutamate

Table 3. Effects of atrazine on various nitrogen fractions in the xylem sap

	µg N per ml of xylem sap			
	Nitrate grown plants		Ammonium grown plants	
Types of nitrogen	Omit atrazine	Atrazine	Omit atrazine	Atrazine
Organic nitrogen				
(a) ninhydrin positive amino nitrogen	150	272	390	348
(b) amide nitrogen of asparagine and glutamine	96	185	307	274
(c) other nitrogen of amino acid	15	29	22	23
(d) total	261	486	719	645
Inorganic nitrogen				
Nitrate	24	39	0	0
Total nitrogen	285	525	719	645

The xylem sap was obtained from 40-day-old maize plants described in Table 2. The various organic nitrogen fractions were calculated from the data in Table 2.

Table 4. Effects of atrazine on the activities of nitrate and nitrite reductases, glutamine synthetase and glutamate synthase in maize seedlings grown with nitrate

Enzymes	Enzyme activities Roots Omit atrazine	 Atrazine	 Shoots Omit atrazine	 Atrazine
Nitrate reductase (μmol NO_2^- produced/g fr. wt/hr)	1.1	2.3	1.8	2.9
Nitrite reductase (μmol NO_2^- utilized/g fr. wt/hr)	17.9	25.5	19.9	29.6
Glutamine synthetase (μmol glutamyl hydroxamate produced/g fr. wt/hr)	80.0	112.0	96.0	144 0
Glutamate synthase (μmol glutamate produced/g fr. wt/hr)	53.2	67.8	147 0	172.2
Average fr. wt six plants (g)	1.17	1.60	1.14	1.73

Maize seedlings grown with nitrate and atrazine in the nutrient solutions as described in Fig. 1 were harvested after a 7-day treatment. Enzymes were determined in cell-free extracts as described in Experimental.

synthetases in roots and shoots of 14-day-old maize seedlings grown with nitrate (Table 4). The activity of nitrate reductase was increased most markedly by atrazine in roots (109 %) and to a lesser extent in shoots (61 %). The activities of other enzymes were increased by the herbicide by about 40–50 % in both roots and shoots except for glutamate synthase where the increases were less, viz. 27 and 17 % for roots and shoots respectively. Seven-day-old barley seedlings grown without added nitrogen as described in the Experimental were supplied with nitrate or ammonium chloride as a source of nitrogen together with atrazine for the next 3 days (Table 5). The activities of these enzymes in nitrate grown barley leaves were enhanced by atrazine treatment and the results are similar to those of the nitrate grown maize seedlings described earlier. When ammonium was the nitrogen source, the activities of nitrate and nitrite reductases were very low as expected and atrazine did not stimulate them nor the activities of glutamine synthetase and glutamate synthase.

Assimilation of $^{15}NO_3^-$

Seven-day-old barley seedlings were supplied with 3 mM $K^{15}NO_3$ and 1 μM atrazine in the nutrient solu-

Table 5. Effects of atrazine on nitrate and nitrite reductases, glutamine synthetase and glutamate synthase in leaves of barley seedlings grown with either nitrate or ammonium chloride as a source of nitrogen

Enzymes	Enzyme activities Nitrate treated plants Omit atrazine	 Atrazine	 Ammonium treated plants Omit atrazine	 Atrazine
Nitrate reductase (μmol NO_2^- produced/g fr. wt/hr)	4.47	8.1	0.34	0 26
Nitrite reductase (μmol NO_2^- utilized/g fr wt/hr)	11.0	17.2	5.84	4.12
Glutamine synthetase (μmol glutamyl hydroxamate produced/g fr. wt/hr)	136.0	192.0	120.0	116.0
Glutamate synthase (μmol glutamate produced/g fr. wt/hr)	73.4	99.9	65.3	61.2

7-day-old barley seedlings grown in sand cultures (without the supply of nitrogen compound as described in Experimental) were treated daily with either [1.5 mM $Ca(NO_3)_2$ + 1.5 mM KNO_3] and 1 μM atrazine or 3 mM NH_4Cl and 1 μM atrazine both in 0.5 strength Hoagland's soln. Leaves were collected at 72 hr after the application of nitrogen and atrazine. Enzymes were assayed in cell-free extracts as given in Experimental.

Table 6. $^{15}NO_3^-$ uptake and incorporation into TCA-precipitable protein and into total nitrogen in barley

Plant materials	No. of days of $^{15}NO_3^-$ treatment	μg $^{15}NO_3^-$ (N/g fr. wt) TCA-precipitable protein Omit atrazine	TCA-precipitable protein Atrazine	Total nitrogen Omit atrazine	Total nitrogen Atrazine
Roots	3	33	39	62	72
	5	57	84	103	159
Shoots	3	69	81	220	265
	5	214	457	400	771
Total	3	102	120	282	337
	5	271	541	503	930

7-day-old barley seedlings were grown in sand cultures without added nitrogen in the nutrient solutions as described in Experimental. These were treated with 3 mM $K^{15}NO_3$ and 1 μM atrazine for the next 3 days and for a further 2 days with $K^{15}NO_3$ only. Roots and shoots were harvested separately on the days indicated and analysed for $^{15}NO_3^-$ incorporation into the TCA-precipitable protein and into total nitrogen as described in Experimental.

tions. Roots accumulated about one-fifth of the labelled nitrogen on the 3rd and 5th day after the application of $^{15}NO_3^-$ (Table 6). The inclusion of atrazine in the nutrient solutions enhanced the total uptake of $^{15}NO_3^-$ by 20% after 3 days and by 85% after 5 days. On the 5th day the incroporation of $^{15}NO_3^-$ into TCA-precipitable protein and into total nitrogen was increased by atrazine treatment in roots by 47 and 54% respectively as well as in shoots by 114 and 93% respectively. The increased incorporation of $^{15}NO_3^-$ into TCA-precipitable protein and into total nitrogen was detectable in leaves of barley within 2 days of adding the herbicide and $K^{15}NO_3$ (Fig. 2). Unlike barley, maize accumulated a higher proportion of the labelled nitrate nitrogen in the roots as shown in Table 7. On the 1st day, 67% of the nitrate nitrogen accumulated in roots and on the 3rd day 50%. Here again, the application of 0.1 μM atrazine increased the uptake of $^{15}NO_3^-$ as well as the incorporation of labelled nitrate into TCA-precipitable protein and into total nitrogen in both roots and shoots. On the 3rd day, the uptake of $^{15}NO_3^-$ was increased (23%) by atrazine as was the incorporation into protein in roots and shoots (28 and 18% respectively).

Incorporation of L-*leucine*-[U-^{14}C]

Detached leaves from 7-day-old barley seedlings grown without added nitrogen were inserted into vials containing leucine-[^{14}C] and atrazine in phosphate buffer (pH 7.5) for 12 hr, as described in Experimental. Atrazine increased the incorporation of leucine-[^{14}C] into TCA-precipitable protein of these leaves by *ca* 47% (Table

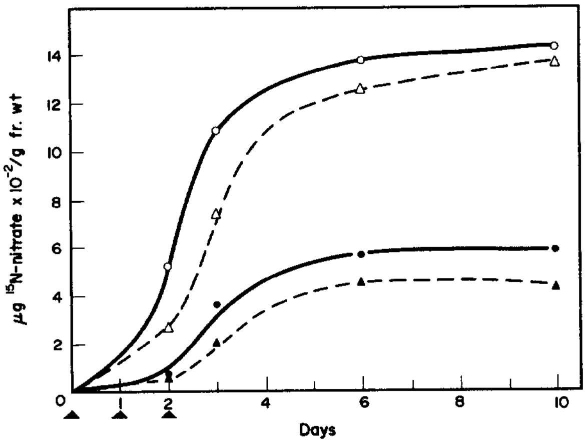

Fig. 2. Effect of atrazine on the incorporation of $^{15}NO_3^-$ into TCA-precipitable protein and into total nitrogen. 7-day-old barley seedlings grown in sand cultures (without the supply of nitrogen compounds as described in Experimental) were treated for the next three days with 3 mM $K^{15}NO_3$ in half-strength Hoagland's soln containing 1 μM atrazine (as indicated by the arrows). They were then maintained on unlabelled nitrate [1.5 mM $Ca(NO_3)_2$ + 1.5 mM KNO_3] in half-strength Hoagland's soln without atrazine. Leaves were analysed at the 2nd, 3rd, 6th and 10th day respectively after the first application of the tracer and atrazine; the incorporation of $^{15}NO_3^-$ into protein and total nitrogen was determined. $^{15}NO_3^-$ incorporated into total nitrogen, ●——● omit atrazine and ○——○ atrazine; $^{15}NO_3^-$ incorporated into TCA-precipitable protein, ▲-----▲ omit atrazine and △-----△ atrazine.

Table 7. $^{15}NO_3^-$ uptake and incorporation into TCA-precipitable protein and into total nitrogen in maize

Plant materials	No. of days after $^{15}NO_3^-$ treatment	μg $^{15}NO_3^-$ (N/g fr. wt) TCA-precipitable protein Omit atrazine	TCA-precipitable protein Atrazine	Total nitrogen Omit atrazine	Total nitrogen Atrazine
Roots	1	123	135	265	293
	3	341	435	581	747
Shoots	1	71	84	129	140
	3	375	443	586	685
Total	1	194	219	394	433
	3	716	878	1170	1430

11-day-old maize seedlings were grown in liquid cultures without nitrogen as described in Experimental. From the 12th day onwards, 3 mM $K^{15}NO_3$and 0.1 μM atrazine were added to the nutrient solutions. Roots and shoots were harvested on the days indicated and analysed for $^{15}NO_3^-$ incorporation into the TCA-preciptable protein and into total nitrogen.

Table 8. L-Leucine-[U-^{14}C] incorporation by detached leaves.
(a) Nitrogen supply in culture medium

Source of nitrogen	Leucine-[^{14}C] incorporation into protein (dpm/mg protein) Atrazine μM 0	25	% Increase
Omit N	5070	7590	49
4.5 mM NO_3^-	8060	8450	5
15 mM NO_3^-	7210	7880	9
4.5 mM NH_4^+	7240	7300	1

Barley seedlings were grown for 5 days in 4 trays supplied daily with 0.1 strength Hoagland's soln without nitrogen. On the 6th and 7th day, the seedlings were supplied with either equimolar KNO_3 and $Ca(NO_3)_2$ or NH_4Cl as indicated. Leaves were detached on the 8th day and the cut ends immersed in incubation solns containing 2 μCi leucine-[^{14}C] and 25 μM atrazine in 0.05 M Pi buffer (pH 7.5). The vials containing the cut leaves were kept in a phytotron at 22° and 350 μ*E*/m^2/sec. The incorporation of leucine-[^{14}C] into protein was determined in leaves after a 12 hr incubation as described in Experimental.

(b) Nitrogen supply in leucine-[^{14}C] incubation medium

Source of nitrogen	Leucine-[^{14}C] incorporation into protein (dpm/mg protein) Atrazine μM 0	25	% Increase or decrease
Omit N	6160	8940	+45
4.5 mM NO_3^-	10700	8960	−16
4.5 mM NH_4^+	8240	7020	−15

Barley seedlings were grown for 7 days in sand cultures supplied with 0.1 strength Hoagland's soln without nitrogen Leaves were detached on the 8th day and the cut ends immersed in incubation solns containing 2 μCi leucine-[^{14}C], 25 μM atrazine in 0.05 M Pi buffer (pH 7.5) and a nitrogen supply as indicated. Samples were incubated for 12 hr as described above.

8a and b). The leucine-[^{14}C] incorporation into leaves of plants grown without nitrogen and atrazine was about 5–6 × 10^3 dpm/mg protein. The uptake of labelled leucine into protein was higher in leaves of similar seedlings grown with nitrate or ammonium but without atrazine as in Table 8a (7.2–8 × 10^3 dpm/mg protein) and in detached leaves of plants grown without a nitrogen source but supplied with either nitrate or ammonium in the incubation solution as in Table 8b (8.2–10.7 × 10^3 dpm/mg protein). This increased incorporation of leucine-[^{14}C] was comparable to that in atrazine treated leaves of barley seedlings grown without added nitrogen (7.5–9 × 10^3 dpm/mg protein). Atrazine did not stimulate the incorporation of leucine-[^{14}C] into leaves of seedlings grown with a nitrogen source (Table 8a) or in detached leaves of seedlings grown without a nitrogen source but supplied with either nitrate or ammonium chloride in the incubation solution (Table 8b).

DISCUSSION

Atrazine stimulated the growth of maize and barley in agreement with previous reports on a variety of plants [1–5]. The growth response was observed in plants supplied with nitrate but not with those given ammonium salts or no combined nitrogen. Ries *et al.* [3] have shown that simazine increased protein in rye plants grown with nitrate but not when ammonium was the sole nitrogen source. In experiments described herein, increased growth resulting from atrazine treatment was correlated with an increased nitrate content and enhanced level of its assimilation in roots and shoots. This effect was confirmed by the uptake and incorporation of $^{15}NO_3^-$ into protein and total nitrogen of roots and shoots respectively.

In addition to the increased accumulation of nitrate, it has been reported that the uptake of K^+ [6, 8], Ca^{2+} [6], Mg^{2+} [8] and PO_4^{3-} [6, 8] are also stimulated by triazines. In the present study it is shown that the effect of atrazine on the uptake of K^+ by maize was less marked than for nitrate. Atrazine however had no effect on the accumulation of PO_4^{3-}. Wray *et al.* [13] also concluded that simazine had a specific effect in stimulating nitrate uptake in barley since it did not affect SO_4^{2-} and PO_4^{3-}. The stimulation of nitrate uptake by atrazine reported herein appears to be a specific effect so that the increased accumuation of other ions e.g. K^+ is probably secondary. It has also been shown that the uptake of nitrate in barley seedlings resulted in an augmented accumulation of K^+ [14].

The differential effect of atrazine on the growth of maize plants supplied with nitrate compared to those given ammonium may be explained in terms of the uptake and utilization of these compounds in plants. Nitrate and other major anions are probably actively accumulated, but cations like ammonium may be passively absorbed [15]. In recent studies with maize roots Yoneyama *et al.* showed that the uptake of ^{15}N labelled ammonium was more rapid than ^{15}N labelled nitrate [16] and the uptake of the anion was more temperature sensitive [17]. There is evidence in several plant cells for the induction by nitrate of a nitrate transport system which involves protein synthesis [18]. It is also known that the absorption of nitrate by barley roots was decreased by inhibitors of respiration and oxidative phosphorylation [19]. The enhancement of nitrate uptake by the triazines could be due to their effects on the nitrate carrier system and its associated metabolic activity.

Pulver and Ries [10] have reported an increased incorporation of leucine-[^{14}C] in detached barley leaves resulting from simazine treatment and suggest that the herbicide stimulated protein synthesis which then increased nitrate uptake. In the present study with detached barley leaves atrazine was found to enhance the incorporation of leucine-[^{14}C] into TCA-precipitable protein but only in the absence of a nitrogen supply. The inclusion of nitrate or ammonium in the plant culture solutions or in the leucine-[^{14}C] incubation mixtures *in vitro* however gave a higher rate of incorporation of the isotope which was not further increased by atrazine. It does not appear to us that the primary action of atrazine is on protein synthesis. The stimulation of the rate of leucine-[^{14}C] incorporation into the nitrogen deficient leaves may be due to some alteration in the availability of the endogenous amino acids.

The triazines are non-polar molecules and are rapidly taken up by plant roots [20] and transported to the shoot [21]. Because of their lipophilic nature, they could exert some effect on membrane permeability. In addition to the increase in total nitrate uptake, they could favour a higher proportion of nitrate in the active (cytoplasm) vs storage (vacuole) pool in the cell. Glucose and light have been shown to mediate similar changes in the distribution of nitrate in barley leaves [22]. Ammonium unlike nitrate is not accumulated in the plant since it is rapidly metabolised. Thus its assimilation would not be expected to be altered by triazine affecting the localisation of ammonium ions in the cell.

A striking effect of atrazine on maize plants grown with nitrate, described in this paper, is the increase in the amino acid content of the xylem sap. It is stimulated almost to the level of that in the ammonium grown plants. This suggests that as for plants grown with ammonium, a relatively high rate of amino acid synthesis occurs in roots of plants grown with nitrate and atrazine and the higher rate of amino acid synthesis is correlated with the enhanced uptake of nitrate by the herbicide. Glutamine was the dominant amino compound in the xylem sap of nitrate and ammonium grown plants but asparagine was the compound most affected by atrazine treatment of maize plants grown with nitrate and atrazine (149% increase). The maize plants used in this work were grown at a sub-optimal temperature and with a low nitrate regime. This may be the reason for the relatively high ratio of amino nitrogen to nitrate nitrogen in the xylem sap, the reverse is usually the case in maize plants grown in more normal conditions [23].

It is well established in several plants [3–5] that the activity of nitrate reductase is increased in those treated with triazines. Aslam and Huffaker [7] however reported that while the activity of nitrate reductase in detached barley leaves was increased, its *in vivo* activity was decreased. In their experiments however they used a relatively high concentration of herbicide (0.1 mM) which would inhibit the growth of barley. In a more recent study [24] with an *in vivo* assay for nitrate and nitrite reductases, atrazine at *ca* 0.05 mM inhibited nitrite reduction. At these relatively high levels of herbicide photosynthesis was severely inhibited and therefore the supply of reducing equivalents (ferredoxin) for the nitrite reductase impaired. In the current work with sublethal amounts of atrazine, the enhanced activities of the nitrate assimilatory enzymes have been correlated with increased incorporation of ^{15}N labelled nitrate into protein and into total nitrogen.

One other important characteristic of the triazine effect is that the increase in fr. wt and protein content is not correlated with an increased dry wt. Wu *et al.* [5] have shown that triazine treated plants had decreased starch and soluble carbohydrate levels which would explain why the dry wts are not increased by the herbicide treatment. It is quite likely that even at sub-lethal concentrations of the herbicides, there is some inhibition of photosynthesis and the plants suffer some carbon stress. It is interesting that such conditions are associated with a higher rate of nitrate assimilation and growth in maize and barley plants treated with the herbicide.

EXPERIMENTAL

Liquid cultures of maize seedlings. Seeds of *Zea mays* L. (Hybrid variety DSC1) supplied by the De Kalb Shand Seed Co., Tamworth, N.S.W., Australia, were surface sterilised as described previously [25]. They were germinated on 1% (w/v) agar at 28° in the dark. At the 4-day-old stage, the seedlings were transferred into 0.1 strength Hoagland's soln without added nitrogen in 30 × 20 × 6 cm trays. 30 seedlings were supported in holes in the lid of the container with pieces of sponge. The solns were aerated and the plants grown with 16 hr light periods at an intensity of 350 µE/m^2/sec at 22° followed by 8 hr dark periods at 17°. 7-day-old seedlings grown in pretreatment culture solns as described above were supplied with either NO_3^- or NH_4Cl in dil. Hoagland's soln. When nitrogen compounds viz. 0.5 mM of either NO_3^- [0.15 mM $Ca(NO_3)_2$ + 0.2 mM KNO_3] or NH_4Cl was supplied in 0.1 strength Hoagland's soln, the liquid cultures were replenished daily. When higher concns of nitrogen were used viz. 4.5 mM NO_3^- [1.5 mM $Ca(NO_3)_2$ + 1.5 mM KNO_3] or NH_4Cl, the liquid cultures were changed every 3 days. Hoagland's soln was supplemented with $NaMoO_4$ (0.01 µg Mo/ml) and $FeSO_4$ (1 µg Fe/ml). The pH of the NO_3^- culture solns was adjusted daily to 4 with N H_2SO_4 and with N KOH to 6 when NH_4Cl was the nitrogen source. During one day, the pH did not alter by more than 1 unit.

Sand culture expts. Surface sterilised seeds of maize were planted in black plastic pots (15 × 16 cm) containing washed sand and watered daily for 14 days with 0·5 strength Hoagland's soln without added nitrogen. These plants were thinned to 6 per pot and 0.5 strength Hoagland's soln containing 4.5 mM NO_3^- [1.5 mM $Ca(NO_3)_2$ + 1.5 mM KNO_3] or 4.5 mM NH_4Cl and 1 µM atrazine was supplied daily. The plants were grown as described previously. In expts with barley (*Hordeum vulgare* L.) the seeds were soaked in 0.2% (w/v) $HgCl_2$ for 3 min followed by 5 rinses with sterile H_2O. They were then planted in sand in plastic trays (21 × 15 × 5 cm). One-tenth Hoagland's soln (without nitrogen) ws added daily for the first 7 days. From the 8th day onwards the seedlings were supplied with a nitrogen source and atrazine as indicated in the tables. The atrazine sample (Gesaprim, 80% Atrazine) was provided by Ciba-Geigy Australia Ltd.

Xylem sap. Plants were cut at the base of the shoots and the bleeding sap was collected into test tubes using Pasteur pipettes. The first drop of the xylem sap was discarded and the sap was collected over the first hr. The amino nitrogen in the sap was determined by the ninhydrin reaction [26] and individual amino acids in a Beckman Amino acid Analyser, Model 119 with a lithium citrate buffer system to allow direct measurement of glutamine and asparagine.

Measurement of nitrate and other ions. Nitrate was determined by the procedure of ref. [27] using a nitrate reductase enzyme prepared from *E. coli*, strain B. Phosphate was determined by the molybdenum blue method of ref. [28]. An atomic absorption spectrophotometer, was used to determine K^+, Ca^{2+} and Mg^{2+}.

Enzyme assays. Cell-free extracts of roots and shoots respectively were prepared in an extraction medium containing 0.5 mM Na-EDTA, and 3% (w/v) casein in 50 mM Pi buffer (pH 7.5). In addition cysteine (either 5 mM for roots or 20 mM for shoots) was included in the extraction medium. Extracts were analysed for *in vitro* nitrate reductase activity by the method of ref. [25], nitrite reductase activity by the procedure of ref. [29], glutamine synthetase activity by the method of ref. [30] and the glutamate synthase activity as described in ref. [31].

Incorporation of $^{15}NO_3^-$. The seedlings were supplied with 3 mM $K^{15}NO_3$ (31.25% enrichment, Office Industrial De L'Azote, France) in 0.5 strength Hoagland's soln as described in each expt (see Tables). The roots and shoots were harvested separately and the incorporation of $^{15}NO_3^-$ into total nitrogen (Kjeldahl) and into TCA-precipitable protein was determined by MS.

Incorporation of leucine-[U-^{14}C] (330 mCi/mmol, obtained from the Radiochemical Centre, Amersham, U.K.), into TCA-precipitable protein was studied in detached leaves of 7-day-old barley seedlings. The supply of nitrogen compounds either to the barley seedlings or to detached leaves in vials is described in Table 8a and b. The detached leaves were immersed in incubation solns in the vials (25 µM atrazine was included because this amount produced maximum stimulation of nitrate reductase activity in detached barley leaves) and these were placed in a phytotron at 22° and at a light intensity of 350 µE/m^2/sec. The leaves collected after a 12 hr incubation were rinsed × 3 with

H_2O. Soluble proteins were extracted by the method of ref. [10]. The pptd protein was collected on a 0.22 µm millipore filter, washed with 5% TCA and 95% EtOH and then air dried. The dried filters were then placed in vials containing 5 ml of scintillation fluor [0.8% (w/v) butyl-PBD in toluene] and ^{14}C incorporation was determined in a liquid scintillation spectrometer.

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