SECTION A - BIOACTIVITY STUDIES

CHAPTER 2

ROLE OF CERTAIN MACRONUTRIENTS IN THE TOLERANCE OF MAIZE TO ATRAZINE

A. Deficiencies in N, P, K, Ca and Mg

Introduction

Reports of damage caused by atrazine in maize in certain areas of South Africa during the 1981/82 and 1982/83 growing seasons had stimulated research on factors affecting the tolerance of maize to atrazine. Investigations on the role of cultivars (Le Court de Billot & Nel, 1985) and certain environmental factors (Nel & Reinhardt, 1984) could not satisfactorily explain the occurrence of apparent atrazine toxicity to maize in the field.

The differential rate of atrazine degradation to non-phytotoxic metabolites is the primary factor accounting for differential responses of plant species to atrazine treatments (Ashton & Crafts, 1981). Shimabukuro, Swanson & Walsh (1970) reported that hydrolysis of the Cl atom at the 2-C position of the triazine ring and conjugation of atrazine with glutathione are the main avenues of atrazine inactivation in maize. According to Sosnovaya & Merezhinskii (1979), regulation of the atrazine degradation rate in maize can be attained through variation of nutrient supply. They suggested that

maize plants supplied with optimal levels of nutrients inactivated atrazine at a faster rate than plants in nutrient-deficient growth media.

Deficiencies in essential nutrients cause far-reaching changes in metabolism and growth (Epstein, 1972). Severe deficiencies may lead to disruption of the total plant metabolic system. It has been reported that triazines influence photosynthesis, protein synthesis, RNA synthesis, and lipid synthesis (Ashton & Crafts, 1981). Phytotoxic amounts of atrazine, as well as deficiencies in essential nutrients, disrupt plant metabolism. A survey of the literature indicated that not much work has been conducted on the interaction between nutrient deficiencies in plants and the level of bioactivity of atrazine in them.

The aim of this study was to determine whether deficiencies in certain macronutrients in the growth medium affect the sensitivity of maize seedlings to atrazine. The rate of loss of atrazine from different nutrient solutions was also monitored to determine whether uptake of the herbicide was impaired for plants showing distinct nutrient deficiency symptoms.

Materials and Methods

Two experiments were conducted in an aqueous medium in a glasshouse. In one experiment, two levels of each of N, P and K was used to make up eight different nutrient solutions. This procedure allowed the effects of deficiencies in N, P and K to be determined individually and in certain combinations. Effects of deficiencies in Ca and Mg, alone and in combination, on maize seedling tolerance to atrazine was

investigated in a subsequent experiment. Macronutrient levels and the composition of nutrient solutions used in the two experiments appear in Tables 2 & 3.

Table 2 Macronutrient content of nutrient solutions used in the NPK-experiment

	Concentration (mg L^{-1})					
NPK-solution[a]	N	P	K	Ca	Mg	S
$N_2P_2K_2$	210	31	234	200	49	64
$N_2P_2K_1$	210	31	29.2	220	49	64
$N_2P_1K_2$	210	3.9	200	200	49	64
$N_2P_1K_1$	210	3.9	29.2	200	49	64
$N_1P_2K_2$	26.2	31	224	102	49	192
$N_1P_2K_1$	26.2	31	29.2	122	49	128
$N_1P_1K_2$	26.2	3.9	230	102	49	128
$N_1P_1K_1$	26.2	3.9	29.2	102	49	128

[a] $_2$ = concentration of macronutrient in solution of Hoagland & Arnon (1938).
$_1$ = one eighth the above concentration - values are underlined.

Table 3 Macronutrient content of nutrient solutions used in the CaMg-experiment

	Concentration (mg L^{-1})					
CaMg-solution[a]	N	P	K	Ca	Mg	S
Ca_2Mg_2	210	31	234	200	49	64
Ca_2Mg_1	210	31	234	220	6.1	40
Ca_1Mg_2	210	31	234	25	49	64
Ca_1Mg_1	210	31	234	25	6.1	40

[a] $_2$ = concentration in nutrient solution of Hoagland & Arnon (1938).
$_1$ = one eighth the above concentration - values are underlined.

Relatively large differences in the amounts of Ca and S between certain solutions (Tables 2 & 3) were unavoidable due to the limited number of suitable salts that can be used for establishment in aqueous medium of deficiencies in particular nutrients (Hewitt, 1966). However, the Ca and S concentrations were within the limits regarded by Hewitt (1966) as being acceptable for sustaining normal growth. In addition, the total anion and cation concentrations balanced in each nutrient solution.

The complete nutrient solution of Hoagland & Arnon (1938) was modified and used throughout as the control nutrient treatment. The modification involved substitution of ferri citrate in the nutrient solution of Hoagland & Arnon (1938) with iron sulphate [$Fe_2(SO_4)_3$] plus disodium-ethylenediamine tetra-acetic acid (Na_2EDTA) as source of Fe. This modification alleviated the problem of Fe deficiency symptoms that was experienced with plants growing in the original Hoagland solution. The pH of all nutrient solutions was kept constant at 5.8 with either 0.05 M H_2SO_4 or 0.1 M NaOH. The nutrient solutions were not replaced with fresh solutions since plants in the control solution exhibited no nutrient deficiency symptoms for the duration of the trial period (28 days), and because the aim was to simulate the field situation where atrazine and nutrient levels would be subject to depletion. Water loss was replenished daily with distilled water.

Seedlings of the maize cultivar SSM 2041 were transferred from vermiculite to the nutrient solutions eight days after they emerged. Plants were allowed five days to adapt to the aqueous growth medium before atrazine was added. At that stage seedlings displayed active secondary root growth, and those visual symptoms (Epstein, 1972)

usually associated with deficiencies in specific macronutrients. Three atrazine rates were used, namely 0, 6 and 12 mg L^{-1}. Two plants were grown in each 2-L polyethylene pot. Plants were suspended through polystyrene lids by means of foam rubber strips wound around the base of stems. The nutrient solutions from two of the four replicates of the NPK-experiment, as well as from three of the five replicates of the CaMg-experiment, were sampled 14 and 28 days after atrazine application for determination of atrazine with high pressure liquid chromatography (HPLC) according to the technique described by Apostolides, Vermeulen, Potgieter, Smit & Nel (1982).

Each experiment was arranged as a completely randomized design with treatments replicated four and five times in the NPK- and CaMg-experiments, respectively. Plants were maintained in a glasshouse at a maximum day/minimum night temperature of 30/18°C. Leaf diffusive resistance (LDR) of plants was determined 14 days after atrazine was applied. By that stage symptoms of atrazine damage (i.e. veinal chlorosis) had developed on all plants treated with the herbicide. A *Li-Cor Steady State Porometer model LI-1600* was used to measure LDR on a 7.5 cm^2 area on the adaxial side of the youngest, fully unfolded leaf of both plants in each pot. Two measurements were taken as near the centre of each leaf as possible. The mean of the four measurements thus taken at each treatment combination were subjected to statistical analysis. Le Court de Billot & Nel (1981) found that LDR reflects atrazine and cyanazine activity in maize. Plants were harvested 28 days after transfer to the nutrient solutions. Root and shoot dry mass were measured and expressed as total dry mass for statistical analysis using standard procedures (Steel & Torrie, 1980).

Results and Discussion

NPK-experiment

The Atrazine rate x Nutrient solution interaction was significant for dry matter yield (roots plus shoots), and the main effects were significant for data expressed as percent reduction in dry matter (Table 4). Atrazine largely eliminated differential growth which was caused by nutrient deficiencies, especially at the highest rate where growth in all the nutrient solutions was reduced to virtually the same threshold value (Table 4). Consequently, better growth of plants not treated with atrazine in the more complete nutrient solutions resulted in greater percentages reduction in growth being recorded at a particular herbicide rate. As a result, significantly greater percentages reduction in growth averaged across atrazine rate was calculated for the complete solution and the one deficient in K only, compared to the rest of the nutrient solutions (Table 4). No plants died, but typical nutrient deficiency symptoms as described by Epstein (1972) were observed. Atrazine (6 and 12 mg L^{-1}) caused veinal chlorosis in the three oldest leaves of all plants treated with the herbicide.

The main effects for atrazine rate and NPK-level were significant for leaf diffusive resistance (LDR) data (Table 5). LDR averaged across nutrient levels increased significantly with each increase in atrazine rate. The LDR of plants grown in the $N_2P_2K_1$ and $N_1P_2K_2$ nutrient solutions was significantly higher than the LDR for plants in the $N_2P_2K_2$ and $N_2P_1K_1$ solutions. Le Court de Billot & Nel (1981) contended that LDR was strongly and negatively correlated with the photosynthetic activity of maize.

Table 4 Effect of atrazine on total dry matter yield (roots + shoots) of maize seedlings grown in aqueous medium containing different combinations of N, P, and K concentrations [Analysis of variance (ANOVA) for dry mass in Table 1A, Appendix A; and for percent damage in Table 2A]

	Atrazine rate (A) mg L^{-1}						
NPK-level(Ntr)	0	6		12		Mean	
	g $plant^{-1}$	g $plant^{-1}$	% damage	g $plant^{-1}$	% damage	g $plant^{-1}$	% damage
$N_2P_2K_2$	14.6	4.1	72	1.9	85	6.9	79
$N_2P_2K_1$	10.4	6.3	38	1.9	79	6.2	59
$N_2P_1K_2$	6.0	4.3	28	3.5	40	4.6	34
$N_2P_1K_1$	5.8	4.2	27	3.6	37	4.6	32
$N_1P_2K_2$	5.1	3.7	26	1.7	64	3.5	45
$N_1P_2K_1$	5.4	2.9	45	1.7	66	3.4	56
$N_1P_1K_2$	4.5	3.2	28	2.2	49	3.3	39
$N_1P_1K_1$	4.4	2.9	32	2.4	45	3.2	39
Mean	7.0	4.0	37	2.4	58		
LSD_T (P=0.05) g $plant^{-1}$				A x Ntr = 3.1			
LSD_T (P=0.05) % damage		A = 7.4		A x Ntr = ns		Ntr = 23.5	

Table 5 Leaf diffusive resistance of plants exposed to atrazine in aqueous medium containing different combinations of N, P and K concentrations (ANOVA in Table 3A)

	Atrazine rate (A) mg L^{-1}			
NPK-level (Ntr)	0	6	12	Mean
	cm s^{-1}	cm s^{-1}	cm s^{-1}	cm s^{-1}
$N_2P_2K_2$	6.3	8.0	10.9	8.4
$N_2P_2K_1$	5.5	7.0	8.8	7.1
$N_2P_1K_2$	5.9	7.9	9.3	7.7
$N_2P_1K_1$	6.4	8.6	10.4	8.4
$N_1P_2K_2$	5.8	6.8	8.8	7.1
$N_1P_2K_1$	6.0	8.1	9.7	7.9
$N_1P_1K_2$	6.1	8.2	9.3	7.9
$N_1P_1K_1$	6.0	7.9	9.5	7.8
Mean	6.0	7.8	9.6	
LSD_T (P=0.05)	A = 0.6	Ntr = 1.2	A x Ntr = ns	

The rates of loss of atrazine from the nutrient solutions used in the NPK experiment are illustrated in Figure 1. Absorption by plants and chemical degradation would conceivably be the principal factors responsible for the progressive loss of atrazine over time. Differences in atrazine concentrations between solutions could be ascribed to differential uptake by plants. The concentration of atrazine had already declined significantly in all eight nutrient solutions 14 days after application (Figure 1). After 28 days there were no significant differences between atrazine concentrations in nutrient solutions of the NPK-experiment.

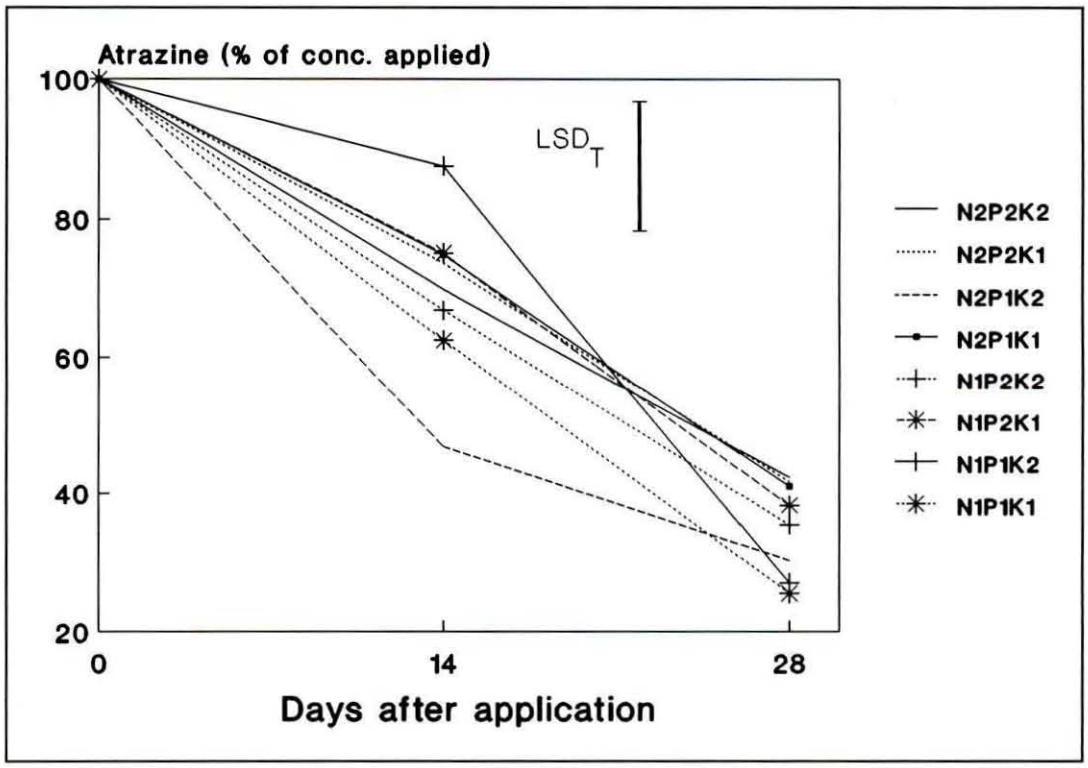

Figure 1 Percent atrazine remaining in NPK-solutions at 14 and 28 days after application (ANOVA in Table 4A)

Ca Mg-experiment

The Atrazine rate x Nutrient solution interaction was significant for dry matter yield, and the main effects were significant for data expressed as percent reduction in dry matter yield (Table 6). Despite having no effect on dry matter yield of plants not treated with atrazine, the low Mg treatment (Ca_2Mg_1) caused the biggest reduction in growth at the 6 mg L^{-1} herbicide rate. The cardinal role of Mg in the chlorophyll molecule (Clarkson, 1980) might explain this reaction to atrazine, a known inhibitor of

photosynthesis. Except at the low Ca treatment (Ca_1Mg_2), growth was reduced to virtually the same threshold value by 12 mg atrazine L^{-1}.

Averaged across atrazine rates, percentages reduction in growth were significantly smaller in the Ca_1Mg_2 and Ca_1Mg_1 nutrient combinations which contained low Ca levels (Table 6). At both those nutrient treatments the rate of atrazine uptake was apparently restricted by the debilitating effect of the low Ca level on plants growing in these nutrient solutions (Figure 2).

The atrazine phytotoxicity symptom of veinal chlorosis manifested in all plants treated with 6 and 12 mg L^{-1}. Necrosis of parts of leaves was observed only at control plants (0 mg atrazine L^{-1}) in the Ca deficient nutrient solution (Ca_1Mg_2). Typical deficiency symptoms as described by Epstein (1972) were exhibited by plants growing in nutrient solutions containing low levels of Ca and Mg.

Table 6 Effect of atrazine on total dry matter yield (roots + shoots) of maize seedlings grown in aqueous medium containing different combinations of Ca and Mg concentrations (ANOVA's for dry mass and percent damage in Tables 5A and 6A, respectively)

	Atrazine rate (A) mg L^{-1}						
CaMg-level (Ntr)	0	6		12		Mean	
	g $plant^{-1}$	g $plant^{-1}$	% damage	g $plant^{-1}$	% damage	g $plant^{-1}$	% damage
Ca_2Mg_2	10.4	6.2	38	2.0	81	6.2	59
Ca_2Mg_1	10.5	3.8	60	2.1	78	5.5	69
Ca_1Mg_2	7.6	5.4	25	4.2	41	5.8	33
Ca_1Mg_1	5.5	4.7	10	2.7	49	4.3	30
Mean	8.5	5.0	33	2.7	62		
LSD_T (P=0.05) g $plant^{-1}$				A x Ntr = 3.5			
LSD_T (P=0.05) % damage		A = 14.7		A x Ntr = ns		Ntr = 22.9	

Only the main effect for atrazine rate was significant for LDR data in Table 7, indicating that plants responded appreciably to atrazine only. The 12 mg atrazine L^{-1} rate had a significantly bigger effect on LDR than 6 mg atrazine L^{-1}, but the latter rate did not affect LDR significantly (Table 7).

Table 7 Leaf diffusive resistance of maize seedlings exposed to atrazine in aqueous medium containing different combinations of Ca and Mg concentrations (ANOVA in Table 7A)

	Atrazine rate (A) mg L^{-1}			
CaMg-level (Ntr)	0	6	12	Mean
	cm s^{-1}	cm s^{-1}	cm s^{-1}	
Ca_2Mg_2	6.7	7.7	8.4	7.6
Ca_2Mg_1	5.0	7.6	10.2	7.6
Ca_1Mg_2	5.1	5.9	9.8	7.0
Ca_1Mg_1	5.9	6.3	13.7	8.7
Mean	5.7	6.9	10.5	
LSD_T (P=0.05)	A = 2.1	A x Ntr = ns	Ntr = ns	

Atrazine concentrations in all the nutrient solutions of the CaMg-experiment were reduced by at least 50% at day 14 after application (Figure 2). At that stage and also at day 28, the atrazine content of the complete nutrient solution (Ca_2Mg_2) was significantly lower than that of the nutrient solution containing low levels of both Ca and Mg (Ca_1Mg_1). Thus, dry matter yield differences between aforementioned two nutrient treatments might have resulted from the differential uptake of atrazine. It is to be expected that plants which were not subjected to nutrient stress would absorb atrazine more efficiently. It is also known that Ca is crucial for the maintenance of cell

membrane integrity (Clarkson, 1980), which is of vital importance for normal root uptake, and therefore plants deficient in Ca might have absorbed less atrazine than those growing in the complete nutrient solution.

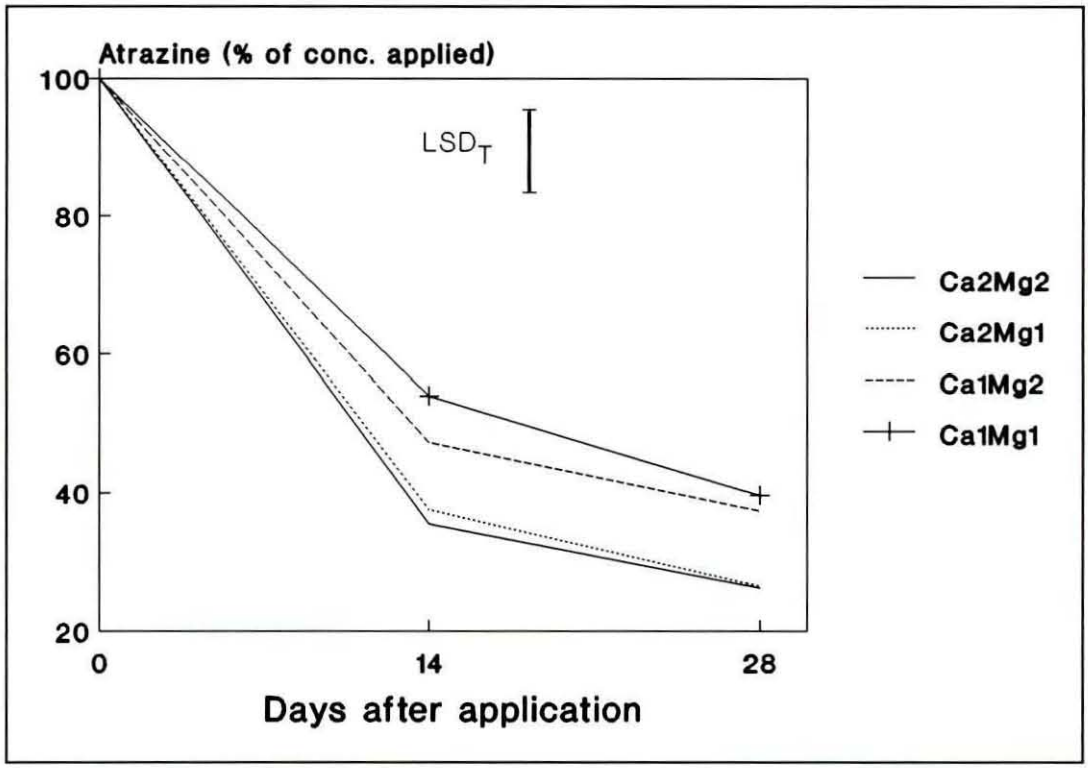

Figure 2 Percent atrazine remaining in CaMg-solutions at 14 and 28 days after application (ANOVA in Table 8A)

In general, the growth of plants least affected by nutrition was reduced most by atrazine, probably because these plants absorbed more atrazine than those which suffered nutrient deficiencies. There was a tendency towards low tolerance to atrazine in nutrient solutions containing low levels of Mg. This finding is the only one which

appears to correspond with those of Sosnovaya & Merezhinski (1979) who stated that the tolerance to atrazine of maize grown with an adequate supply of N, P, K, Ca and Mg was greater than the tolerance shown by plants which received poor nutrition. The same conclusion can not be drawn from the present study. Generally, results suggest that growth-retarding low levels of N, P, K, Ca and Mg in the growth medium of maize seedlings do not significantly influence their sensitivity to atrazine. Nutrient deficiencies on the scale evoked in these experiments were unlikely to have occurred simultaneously on the many farms on which atrazine damage was reported during the 1981/82 and 1982/83 growing seasons, and therefore stress due to deficiencies in key macronutrients is unlikely to have played a role in the field.

B. Phosphorus and combinations of phosphorus and NH_4^+:NO_3^- -N ratios

Introduction

Physiological and biochemical disorders may result from both insufficient and excess amounts of essential nutrients in the plant system. Disruption of normal metabolic processes may impact negatively on the ability of a plant to degrade phytotoxic amounts of herbicide to harmlessly low levels. Since band-placing of up to 300 kg 3:2:1 (25 %) fertilizer ha^{-1} at plant row widths of 2.1 m was common practice where maize damage was reported in the 1981/82 and 1982/83 growing seasons, high levels of P could conceivably have been available for uptake by young seedlings exposed to atrazine. Therefore high P concentrations could have rendered the plants more sensitive to

atrazine, since both toxic P levels and the herbicide reportedly inhibit photosynthesis.

Stolp & Penner (1973) found that the growth of maize seedlings was reduced by exposure to combinations of high atrazine and P concentrations in solution due to increased respiration and reduced net photosynthesis. Claassens & Fölscher (1985) reported that P concentration in shoots was positively correlated with reduced growth of wheat (*Triticum aestivum* spp *vulgare* McKey) cv Inia. They suggested that the detrimental effect of the high P level on growth might be due to phosphatase inhibition in photosynthesis. Loneragen, Grunes, Welch, Aduayi, Tengah, Lazar & Carey (1982) stated that high concentrations of P cause osmotic damage to leaf cells.

The present study was undertaken to investigate the effect of high P application in the root zone or relatively high P concentrations in shoots on photosynthesis and growth of maize seedlings treated with atrazine, in order to assess the potential impact of this factor on the tolerance of maize to atrazine.

Materials and Methods

Glasshouse experiments - general procedure

Two experiments were conducted in a glasshouse; one in an aqueous culture and the other in a sand culture. The maize cultivar SSM 2041 was used as a test plant because it was one of the cultivars reportedly damaged by atrazine in the field (Le Court de Billot & Nel, 1985). The full-strength nutrient solution of Hoagland & Arnon (1938) was employed as the control solution in all experiments. The concentrations of

macronutrients in all nutrient solutions used are shown in Table 8. Micronutrients and their levels (mg L^{-1}) in all the nutrient solutions were the following: B (0.5), Cu (0.02), Fe (1.1), Mn (0.5), Mo (0.01) and Zn (0.05). The pH of nutrient solutions was adjusted weekly to 6.5 with either 0.05 M H_2SO_4 or 0.1 M NaOH. Pots were arranged according to the completely randomized design.

Table 8 Nutrient solution treatments used in the glasshouse experiments using both an aqueous and sand culture

Nutrient treatment	Macronutrient elements						
	Ca	Mg	K	NH_4-N	NO_3-N	H_2PO_4-P	SO_4-S
Phosphorus level	me L^{-1}						
Hoagland solution	10	4	6	0	15	1.0	4
54 mg P L^{-1}	10	4	6	0.7	14.3	1.7	4
108	10	4	6	1.4	13.6	3.5	4
162	10	4	6	2.5	12.5	5.2	4
217	12	4	6	2.5	12.5	7.0	5
310	10	4	6	4.5	10.5	10.0	4
$P/NH_4^+:NO_3^-$ -N ratio							
Hoagland solution	10	4	6	0	15	1.0	4
310/12:3[a]	9	4	4	12	3	10.0	16
310/3:12	11	5	6	3	12	10.0	3
403/12:3	10	4	6	12	3	13.0	16
403/3:12	12	6	7	3	12	13.0	3

[a] Phosphorus concentration (mg L^{-1}) $NH_4^+:NO_3^-$ -N ratio (mass basis).

P level - glasshouse experiment

This experiment was conducted in nutrient solution. In addition to the Hoagland solution, five other nutrient solutions were prepared by varying, as far as possible, only

the P concentration (Table 8). The P levels in the nutrient solutions were 31 (as in the Hoagland solution), 54, 108, 162, 217 and 310 mg L^{-1}. This range of P concentrations was dictated by specific combinations of salts that had to be used in order to balance the levels of the other nutrients in the different solutions. The P sources were KH_2PO_4 and $Ca(H_2PO_4)_2$. Three atrazine levels were used, namely 0, 5 and 15 mg L^{-1}. Treatments were replicated six times.

Seedlings were transferred from quartz sand to the nutrient solutions eight days after they emerged. Plants were allowed five days to adapt to the aqueous growth medium before atrazine was added. Two plants were grown in each 2-L polyethylene pot. Plants were suspended through lids by means of foam rubber strips. Compressed air was used to aerate the nutrient solutions. Water loss through evapotranspiration was replenished daily with deionized water. Nutrient solutions were not used to replace water lost since the aim was to simulate the field situation where atrazine and P levels would be subject to depletion. Fe (source: $FeNa_2EDTA$) at 1.1 mg L^{-1} was added at 5-day intervals to maintain healthy plants.

The experiment was conducted at a mean day/night temperature of 21/18°C. Leaf diffusive resistance (LDR) was determined on the abaxial side of the youngest, fully-unfolded leaves 14 days after atrazine application, with a LI-COR 1600 Steady State Porometer. Seedlings were harvested 20 days after atrazine application and total leaf area and dry mass (shoots and roots) measured.

P and NH_4^+:NO_3^- -N ratio - glasshouse experiment

This experiment was conducted in quartz sand (mean particle diameter 0.5 mm). Five seeds were planted per pot and upon emergence plants were thinned to three seedlings per pot. The control nutrient solution (Hoagland) and four other nutrient treatments which resulted from the combination of two NH_4^+:NO_3^- -N ratios (12:3 and 3:12 on a N equivalent basis) with two P levels (310 and 403 mg L^{-1}) were used. Each nutrient solution was used in the preparation of atrazine levels of 0, 5 and 15 mg L^{-1}. Treatment commenced immediately after planting ungerminated seed. Thereafter the atrazine/nutrient solution mixtures were applied (0.5 L per pot) on alternate days. Each pot contained 1.5 kg sand and was allowed to drain freely. Failure to induce significant atrazine damage in the initial glasshouse experiment prompted the use of sustained high atrazine and P concentrations for the duration of this experiment. Treatments were replicated eight times.

The minimum and maximum temperature ranges over the trial period were 16-20°C and 25-30°C, respectively. Plants were harvested 21 days after treatment commenced. The leaf area of the live parts (blades and sheaths) of leaves and dry mass of shoots were measured. The photosynthetic CO_2 fixation rate and leaf diffusive resistance of plants were determined with a LI-COR 6000 Portable Photosynthesis System two days before harvesting. These measurements were taken on a 7.4 cm^2 central section of the youngest, fully unfolded leaf of intact plants from four replicates. The P status in shoots from two replicates was also determined (Technicon Auto Analyzer II, 1972) and

presented as a percentage of shoot dry mass. All other results are expressed on a per plant basis.

Field experiment

The trial was conducted on the Hatfield Experimental Farm (Pretoria) on soil of the Hutton form with 15% clay in the 0-100 mm zone. Fertilizer [3:2:1 (25%) + Zn] was band-placed at levels of 150, 200, 300, 405 and 600 kg ha^{-1} 50 mm to the side and 50 mm below the seed. In this way P rates of 18.75, 25, 37.5, and 50.6 kg ha^{-1} were applied in close proximity to seed. Band placement of up to 300 kg ha^{-1} of the same fertilizer was common practice in the areas where atrazine damage was reported. The maize cultivar SSM 2041 was planted with row widths of 910 mm. All plants received a side-dressing of 40 kg N ha^{-1} (LAN, 28% N) six weeks after planting. Broadcast application of atrazine was made at levels of 0, 2.5, 5.0, 7.5, 10, 12.5 and 15 kg ai ha^{-1} with a CO_2 field sprayer which delivered 200 L ha^{-1} at 300 kPa. Although the recommended rate of the herbicide for the trial site was 1.625 kg ai ha^{-1}, excessive amounts of atrazine were used to simulate conditions of high herbicide availability.

Plants were monitored visually once a week for symptoms of P and atrazine phytotoxicity. Plant stand and plant height measurements were made at 30 day intervals throughout growth and seed yield was determined at seed maturity. A split-plot design was used, with whole plots laid out according to the randomized complete block design. Atrazine rates were assigned to whole plots and fertilizer levels to sub-plots in strips

across whole plots. The strip treatments were randomized. All treatments were replicated three times. Standard analysis of variance was performed on the data.

Results and Discussion

Visual symptoms

Interveinal chlorosis and leaf tip necrosis were exhibited by control plants (0 mg atrazine L^{-1}) supplied with 310 and 403 mg P L^{-1} in both glasshouse experiments. The intensity of the symptoms was higher at the 12:3 than at the 3:12 NH_4^+:NO_3^- -N ratio. Similar symptoms have been described by other researchers. Loneragen *et al.* (1982) and Claassens & Fölscher (1985) observed that the symptoms associated with accumulation of P to toxic levels in top growth of wheat are interveinal chlorosis and leaf tip necrosis. Nel & Reinhardt (1984) reported that high levels of atrazine induce veinal chlorosis in the lower leaves of young maize plants. Ultrastructural investigations (Malan *et al.*, 1985) revealed changes in mesophyll chloroplasts of such plants, while bundle sheath chloroplasts were virtually unaffected. Green *et al.*, (1973) reported leaf tip necrosis on barley (*Hordeum vulgare* L. cv Conquest) treated with high P levels and ascribed it to high osmotic pressure caused by the accumulation of phosphorus.

Veinal chlorosis was induced in the lower three to four leaves of all plants treated with 5 and 15 mg atrazine L^{-1} in the glasshouse. This symptom was also observed in the field on plants treated with 10, 12.5 and 15 kg atrazine ha^{-1}. Treatment with atrazine did not cause necrosis of leaf tissue. In an ultrastructural study on maize exhibiting

chlorosis of the main veins of the leaf after treatment with high atrazine levels, Malan *et al.* (1985) found that the organisation and integrity of mesophyll chloroplasts were severely impaired. The debilitating effect of atrazine on mesophyll chloroplasts would conceivably reduce growth through inhibition of photosynthesis. Assuming that high P levels also have a detrimental effect on photosynthesis, the combination of phytotoxic atrazine and P concentrations may have a compounded effect on sensitive plants.

Phosphorus level - glasshouse experiment

The main effect for atrazine rate was significant for dry mass data, and the Atrazine rate x Phosphorus rate interaction was significant for leaf diffusive resistance (LDR) data (Table 9). The dry mass of plants was significantly reduced with each increase in atrazine rate. The presence of 15 mg atrazine L^{-1} largely eliminated differential growth in different nutrient solutions (Table 9). Phosphorus alone did not affect LDR, but the presence of atrazine caused significant increases in LDR of plants grown in certain nutrient solutions. The highest LDR values were reached at the maximum P level (310 mg L^{-1}) in the presence of 5 and 15 mg atrazine L^{-1}. Shoot dry mass was strongly correlated ($r = -0.82$) with LDR.

Table 9 Effect of P application on dry mass and leaf diffusive resistance of maize seedlings exposed to atrazine in aqueous culture (ANOVA for dry mass and LDR in Tables 9A & 10A, respectively)

P concentration (mg L^{-1})	Atrazine (mg L^{-1})					
	0		5		15	
	g $plant^{-1}$	s cm^{-1}	g $plant^{-1}$	s cm^{-1}	g $plant^{-1}$	s cm^{-1}
Hoagland solution	3.8	8	1.3	15	0.7	30
54	3.5	7	1.4	13	0.6	35
108	3.5	11	1.5	18	0.7	28
162	3.5	10	1.4	18	0.8	31
217	3.3	11	1.3	22	0.7	42
310	3.2	11	1.2	23	0.7	46
Mean	3.5	9	1.3	18	0.7	35
LSD_T (P=0.05) g $plant^{-1}$		Atrazine = 0.1	Atrazine x P = ns		P = ns	
LSD_T (P=0.05) s cm^{-1}			Atrazine x P = 11			

P and NH_4^+:NO_3^- -N - glasshouse experiment

The Atrazine rate x Nutrient solution interaction was significant for plant dry mass data (Table 10). At all atrazine concentrations and P levels, dry mass was significantly lower at the high NH_4^+:NO_3^- -N ratio (12:3), than at the low ratio of 3:12. Growth in the Hoagland solution was virtually the same as that observed at the combinations of both 310 and 403 mg P L^{-1} with a low NH_4^+:NO_3^- -N ratio. In contrast, the high NH_4^+:NO_3^- -N ratio significantly reduced growth, irrespective of the rate of phosphorus or whether the seedlings were exposed to atrazine or not. Growth in all the nutrient solutions decreased significantly as the atrazine concentration increased to 5 mg L^{-1}. A further increase in herbicide concentration to 15 mg atrazine L^{-1} caused significant growth reduction in all nutrient solutions except those with the high NH_4^+:NO_3^- ratio.

Table 10 Effect of high P application and NH_4^+:$N0_3^-$ -N ratio on total dry mass (roots + shoots) of maize seedlings exposed to atrazine in sand culture (ANOVA in Table 11A)

	Atrazine (mg L^{-1})		
Nutrient trtm. (Ntr)	0	5	15
	g $plant^{-1}$		
Hoagland solution	1.9	1.4	1.0
310/12:3[a]	1.4	0.8	0.6
310/3:12	1.7	1.4	1.0
403/12:3	1.2	0.9	0.7
403/3:12	1.8	1.5	1.1
LSD_T (P=0.05)	Atrazine x Ntr = 0.3		

[a] Phosphorus concentration (mg L^{-1})/NH_4^+:$N0_3^-$ -N ratio.

The Atrazine rate x Nutrient solution interaction was significant for photosynthetic CO_2 fixation tempo data (Table 11). In untreated plants (0 mg atrazine L^{-1}), the photosynthetic efficiency of plants exposed to the 403 mg P L^{-1} and high NH_4^+:NO_3^- -N ratio combination was significantly lower than that of plants at the other nutrient treatments. In the presence of atrazine, however, there was a tendency for photosynthesis to be lowered to the same threshold value, irrespective of the nutrient treatment. Because of the relationship between LDR and CO_2 fixation rate (r = -0.73), only the results of the latter parameter are presented in Table 11.

Table 11 Effect of high P supply and NH_4^+:$N0_3^-$ -N ratio on the photosynthesis rate (CO_2 fixation tempo) of maize seedlings exposed to atrazine in sand culture (ANOVA in Table 12A)

	Atrazine (mg L^{-1})		
Nutrient trtm. (Ntr)	0	5	15
		mg m^{-2} s^{-1}	
Hoagland solution	1.5	1.0	0.5
310/12:3[a]	1.6	1.0	0.6
310/3:12	1.5	1.2	0.5
403/12:3	1.0	0.8	0.6
403/3:12	1.5	1.1	0.5
LSD_T (P=0.05)		Atrazine x Ntr = 0.5	

[a] Phosphorus concentration (mg L^{-1})/NH_4^+:$N0_3^-$ -N ratio.

The main effects for atrazine rate and nutrient solution were significant. Averaged across atrazine rates, the P status in plants exposed to 310 and 403 mg P L^{-1} was significantly higher at the high (12:3) NH_4^+:NO_3^- -N ratio than at the low ratio of 3:12

(Table 12). The P status in plants increased significantly as the atrazine level was raised. This high P status probably resulted from suppression of growth by atrazine and high NH_4^+. Percent P in shoots was negatively correlated with growth over the levels used (r = -0.79). Claassens and Fölscher (1985) found that the presence of NH_4^+ in nutrient solution increased the P status in shoots of wheat.

Table 12 Effect of high P application and NH_4^+:NO_3^- -N ratio on the P status (% P) in shoots of maize seedlings exposed to atrazine in sand culture (ANOVA in Table 13A)

	Atrazine (mg L^{-1})			
Nutrient treatment (Ntr)	0	5	15	Mean
	%	%	%	
Hoagland solution	0.4	0.5	0.7	0.5
310/12:3[a]	1.3	1.5	1.8	1.6
310.3:12	0.7	1.0	1.5	1.1
403/12:3	1.0	1.6	1.8	1.5
403/3:12	0.7	1.0	1.4	1.0
Mean	0.8	1.1	1.4	
LSD_T (P=0.05)	Atrazine = 0.1	Ntr = 0.2	Atrazine x Ntr = ns	

Field experiment

The main effect of atrazine was significant for yield data presented in Table 13. Significant yield reductions were observed at certain atrazine rates above the recommended rate. Symptoms of atrazine toxicity (veinal chlorosis) on the two lower leaves of maize treated with 10, 12.5 and 15 kg ai atrazine ha^{-1} were noted. The amount of fertilizer did not have a synergistic effect on atrazine phytotoxicity. There

were no significant differences in plant stand or plant height, and therefore these data are not presented here. Failure of the crop to respond positively to fertilizer rates could be explained by adequate P and K reserves in the soil at the onset of the trial (P=60 mg kg^{-1}; K=120 mg kg^{-1} ; Mg=159 mg kg^{-1}; Ca=275 mg kg^{-1}) and the application of 40 kg N ha^{-1} as a side-dressing.

Table 13 Influence of a P-containing fertilizer (3:2:1 25% + Zn) on the grain yield (ton ha^{-1}) of maize treated with atrazine in the field (ANOVA in Table 14A)

	Atrazine (A) kg ai ha^{-1}						
Fertilizer (F) kg ha^{-1}	0	2.5	5.0	7.5	10	12.5	15
	ton ha^{-1}						
150	6.5	5.8	5.5	4.6	5.0	5.6	5.5
200	6.8	6.3	5.6	5.2	5.8	6.2	6.2
300	6.6	5.9	5.0	5.8	5.4	5.6	5.0
405	6.3	6.0	5.5	5.8	6.0	6.1	5.8
600	6.1	5.7	5.8	4.9	5.0	5.5	5.3
Mean	6.4	5.9	5.5	5.2	5.4	5.8	5.5
LSD_T (P=0.05)	A = 0.7 F = ns A x F = ns						

Results show that high P concentrations in the root zone of maize seedlings can cause symptoms of P phytotoxicity without seriously inhibiting growth. The tolerance to atrazine of plants showing these symptoms was not lowered. Research on the tolerance of maize to atrazine was not pursued further. (*The work reported in Study A and Study B have been published: Reinhardt, Nel, Vermeulen, Apostolides & Potgieter, 1986; Reinhardt & Nel, 1992*).