IMPLICATIONS OF RESIDUAL ATRAZINE FOR WHEAT

by

KHATHUTSHELO EDWARD TSHIPALA

Submitted in partial fulfilment of the requirements for the degree M Inst (Agrar): Plant Protection (Weed Science) in the Department of Plant Pathology and Microbiology

Faculty of Biological and Agricultural Sciences

University of Pretoria

PRETORIA

SUPERVISOR: PROF. C. F. REINHARDT

2000

TSHIPALA, KHATHUTSHELO EDWARD

IMPLICATIONS OF RESIDUAL ATRAZINE FOR WHEAT

M Inst Agrar **UP** **2000**

ACKNOWLEDGEMENTS

This study would not have been possible without the help of several people from the University of Pretoria.

Firstly I would like to express my sincere gratitude and respect towards my study-leader, Prof. Charlie F. Reinhardt, for his valued criticism and encouragement, making this goal in my life possible.

More than anything else I would like to thank my God for his unfailing love during this research project.

I am grateful towards the University of Pretoria for the financial support received. A special word of thanks to Mr. Beyers for his technical support at the phytotron.

A special word of thanks to my friends and colleagues, Mr. Norman Maiwashe, Murembiwa Manyaga, Livhuwani Nngwekhulu, Isac Bok, for their endless support throughout this study.

My mother Tshinakaho Avhapfani Tshipala, my brother – Calvin Tshipala, My sisters – Tshilidzi and Rosinah.

Last, but definitely not the least, I would like to thank my love Mashudu Ilse Booi for her endless support during the last and difficult part of my studies.

ABSTRACT

Reports on the occurrence of atrazine residues in many South African surface waters, prompted this study on the effects of low levels of atrazine on sensitive crops. Residues of atrazine detected were in the range of 3–20 μg l^{-1} for surface water and 0.29–4.36 μg l^{-1} for groundwater. Since these low levels of atrazine may cause injury to sensitive crops, and because little is known about the effects of low (residual) concentrations of atrazine on sensitive crops, such as oats (*Avena sativa* L.), sunflower (*Helianthus annuus* L.), dry beans (*Phaseolus vulgaris* L.) and wheat (*Triticum aestivum* L.), this research was initiated. Wheat (*Triticum aestivum* L.) was selected as an indicator species for testing the response of a sensitive crop to low atrazine levels, which conceivably occur in water used for irrigation. The response of wheat to atrazine was evaluated in a hydroponic system as well as on soil. The specific aim of the study was to determine the critical level at which phytotoxic effects are manifested on the sensitive crop. Plants were exposed to atrazine concentrations in water culture and soil at concentrations, which conceivably, could occur in surface water and groundwater. Atrazine concentrations varied with different experiments conducted, ranging from 2–40 μg l^{-1}. Growth parameters measured included fresh/dry mass of shoot, root, spikes and number of tillers, spikes, spiklets. Injury to winter wheat (cv. Carina PD 402, cv. Caritha, cv. SST 86, cv. Kariega) caused by atrazine levels similar to those detected in local surface water and groundwater was measurable after a single treatment on wheat grown in water culture. Averaged across herbicide rate, cv. Carina PD 402 was significantly more sensetive than cv. SST 86, but the magnitude of growth

reductions was relatively small (6-16%). For cultivar Caritha the injury threshold concentration in water medium was 10 μg l^{-1}. Results suggest that differences in sensitivity to atrazine exist between wheat cultivars. It is plausible that cultivars which are particularly sensitive might be at risk when irrigated with atrazine-contaminated water. No measurable damage was caused by atrazine on wheat (cv. SST 825 and cv. SST 55) grown on the soil, probably partly due to soil properties that reduced herbicide availability for uptake. It is possible that the risk of atrazine damage to wheat will be high for soils with the following properties: high pH, low organic matter and clay content. Conditions that favor the accumulation of atrazine or retardation of its dissipation in soil would exacerbate the problem.

TABLE OF CONTENTS

ABBREVIATIONS AND SYMBOLS USED

The following abbreviations and symbols will be encountered in this manuscript:

Explanation	Abbreviation or symbol
Acidity or alkalinity	pH
Active ingredient	a.i.
Agricultural	Agr.
And	&
And others	*et al.*
Analysis of variance	ANOVA
Analysis of Variance F-test values	F
Australia	Aust.
Coefficient of Variation	CV
Cultivar	Cv
Degrees of freedom	DF
For example	e.g.
Gram	G
Greater than	>
Hectare	Ha
Journal	J.
Kilogram	Kg
Least significant difference	LSD
Less than	<
Litre	L
Microgram	µg
Millimetre	Mm
Not significant	NS
Note	NB.
Percent	%
Probability	P
Proceedings	Proc.
Research	Res.
South African	S.Afr.

CHAPTER ONE

INTRODUCTION

Atrazine belongs to the chloro-triazine group of herbicides. Reinhardt (1993; 1996) reported that atrazine at low residual concentrations in both soil and contaminated water affect sensitive crops. This herbicide is absorbed by the roots and leaves, but most is absorbed by the roots (Hugo, 1994 & Ahrens, 1994). Atrazine is the pesticide which is commonly found in groundwater and surface water (Hugo, 1994). The reason for this could be that atrazine is the most studied pesticide. This combined with very tonnages of atrazine that is applied internationally and locally results in atrazine being chosen for monitoring (Weaver & Reinhardt, 1996). In South Africa, large tonnages of atrazine are applied, with about 2 609 700 kg of active ingredient used every year (Hugo, 1994).

Atrazine has been involved in many cases of damage to cereal crops and other crops as well. According to Eagle (1980), herbicide injury to crop plants occurs in several ways. The common cause of damage is accidental overdosing to treated crops, and due to a carry-over effect on rotational crops. The presence of atrazine residues in soil and irrigation water may cause injury to the follow-up crop and this is most likely after overdosing. Moyer & Blackshaw (1993) reported cases where cereal crops in South Alberta were injured by atrazine.

Among these cereal crops were wheat (*Triticum aestivum* L.), Barley (*Hordeum vulgare* L.), and oat (*Avena sativa* L.).

Wheat (*T. aestivum* L.) is one of the most important crops in South Africa, and possibly the rest of the world. Thus any form of growth abnormalities associated with this crop should be investigated. Damage to wheat by residual atrazine has been reported by many researchers (Saghir & Choudhary, 1967; Moyer & Blackshaw, 1993; Reinhardt, 1996).

However, atrazine damage to crops is governed by various factors, *inter alia*. plant, soil and climatological soil factors. These factors include soil colloids, (organic matter & clay content), soil pH, temperature, soil moisture (Nel & Reinhardt, 1984). Adams (1973); Holford, Haigh & Ferris (1989) and Seta & Karathanasis (1997), further reported factors which influence bioactivity of pesticides. These factors are molecular structure, charge characteristics, water solubility, clay colloids, amorphous minerals, cation saturation, pH, organic matter, and soil microclimate. All these factors govern toxicity of atrazine to sensitive crops, because they determine availability of the herbicide for uptake by plants. Soil water content and temperature have been implicated (Reinhardt & Nel, 1993). Furthermore, atrazine damage to sensitive crops is also determined by plant species, cultivars and growth stage of the plant (Reinhardt, 1996).

Hugo (1992) reported that concentrations of atrazine in surface water (South African rivers and dams) in 1992 were approximately 3-20 $\mu g\ l^{-1}$. It was

reported that before 1992, no atrazine residues were detected in groundwater in South Africa (Hugo, 1994). Despite these findings, Pick *et al.* (1992) found atrazine residue concentrations ranging from 0.49-3.89 $\mu g\ l^{-1}$ in four groundwater samples collected during September 1991 to February 1992 in the eastern Transvaal (Hugo, 1992).

In irrigation agriculture, surface waters may be contaminated by atrazine through aerial spraying of crops, and run-off from land treated with atrazine (Weaver & Reinhardt, 1996). Atrazine is the most detected herbicide in both surface water and groundwater, probably this is not because atrazine move faster down the soil profile than other group of herbicides, but likely due to the relative amounts of active ingredient applied (Ellis, 1992). The potential of herbicide (atrazine) to leach and to contaminate groundwater depends on: 1) chemical properties of the herbicide, 2) soil properties, 3) application conditions, and 4) climatic conditions (Weber, 1991b).

While every weed scientist would acknowledge that the judicious use of herbicide is essential if we are to maintain, or improve the world's standard of living, there is an increasing public awareness with regard the possible damaging effects of herbicides on sensitive parts of the environment. Although most of these herbicides are described as being safe to human health, atrazine in particular, has been found to pose potential toxic effects from atrazine metabolites particularly from the adducts of nitroso derivatives that may be

produced in mammalia systems (Singh, Shea, Hundal, Comfort, Zhang & Hage, 1998).

The availability of monitoring systems for herbicides (especially atrazine), particularly in flowing waters such as irrigation canals, is essential so that the potential damage to the environment and sensitive crop plants may be estimated or assessed. The total herbicides residues in soil (adsorbed and available) obtained by extraction with organic solvents and measured using instruments is not enough information since not all these residues are available for plant uptake. The use of bioassay method offers will be appropriate in finding the exact amount of residues in both soil and irrigation water. This method offers an opportunity to find the exact amount of residues that cause damage to a particular plant. In the present study, a hydroponics medium (water) and soil were used to examine the effects of atrazine on the growth of wheat. The primary aim of this study was to identify the minimum level of atrazine concentration in irrigation water that might cause damage to sensitive crops.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Atrazine is widely used for weed control in maize in the summer grain-producing region of South Africa (Nel & Reinhardt, 1984). According to Ahrens (1994), atrazine belongs to chemical group, triazines, which include prometryne, simazine, terbuthylazine and others. These herbicides, are soil-applied, inhibitors of photosynthesis, and crop injury symptoms resulting from this group is chlorosis and later necrosis.

In general, s-triazine herbicides are soil applied, and are adsorbed readily to the soil colloids. After root absorption, these herbicides are readily translocated upward to the leaves through the transpiration stream. Their mode of action is the inhibition of photosynthesis, which is achieved by interference with carbon dioxide fixation and electrons released by the splitting of water into oxygen and hydrogen (Audus, 1976; Fuerst & Norman, 1991).

According to Rehaman & Matthews (1979), phytotoxicity of s-triazine herbicides towards plants is normally affected by factors such as soil organic matter and other soil colloids. Phytotoxicity of herbicides is also influenced by the ability of

the plant to metabolize that particular herbicide. Shimabukuro & Swanson (1969), indicated that the ability of plants to metabolize atrazine does not render a plant resistant to atrazine. Resistance is determined by the different rate of detoxification .

2.2 Atrazine metabolism and selectivity towards crops

Audus (1976) reported that some plants, especially grasses such as maize, sorghum and sugarcane are not affected by atrazine. He further indicated that s-triazines herbicides are readily absorbed and translocated through the plant. Degradation of s-triazines is accomplished by hydrolysis, N-dealkylation and peptide conjugation (Shimabukuro & Swanson, 1969; Shimabukuro, Swanson & Walsh, 1970, and Audus, 1976). There is a great probability that all higher plants metabolize atrazine by N-dealkylation to some extent, while species such as wheat and maize, which contain benzoxazinone utilize hydroxylation as well (Shimabukuro & Swanson, 1969).

Shimambukuro & Swanson (1969), and Audus (1976), stated that selectivity seem to depend on the extent to which a herbicide remains in the toxic form in the plant. Therefore the metabolism and detoxification of the herbicides in an intact plant may directly affect the persistence in the plant and thus efficacy of kill.

Audus (1976), further stated that besides the rate of absorption and translocation which render plants susceptible or not to herbicides, sensitivity of cultivated crops to herbicides also depends on the stage of their development. It is generally accepted that a young plant growing under optimum conditions is most susceptible to herbicides. Factors such as dosage rate, and formulation may also have an influence on the level of crop susceptibility to that particular herbicide (Audus, 1976), and cultivar differences in susceptibility to herbicides are probably due to variability in their genetic make-up (Nel & Reinhardt,1984).

2.3 Mechanism of action

Atrazine is one of the herbicides that inhibit photosynthesis, and an obvious result of photosynthesis inhibition is the lack of carbohydrate formation leading to starvation of treated plants (Audus, 1976; Fuerst & Norman, 1991). According to Audus (1976), photosynthesis inhibition can be detected within one to two hours, however, complete inhibition is normally achieved within six to twenty four hours.

The primary site of action for this herbicide is the chloroplast, which is the only organelle for photosynthesis processes. Many studies have suggested that herbicides that inhibit photosynthesis can be classified as : a) electron transport inhibitors, b) energy transfer inhibitors, c) uncouplers, d) inhibitory uncouplers, or e) electron acceptors (Fuerst & Norman, 1991). The primary sites of herbicide action in photosynthetic electron transport are the inhibition of Photosystem II

(PS II) electron transport and diversion of the electron flow through Photosystem I (PS I). Photosystem II electron transport inhibitors bind to the D_1 protein of the

PS II reaction center, which results in blockage of electron transfer to plastoquinone. This inhibition of PS II electron transport prevents the conversion of absorbed light energy into electrochemical energy and results in the production of triplet chlorophyll and singlet oxygen which cause the peroxidation of membrane lipids. For PS I electron acceptors accept electrons from the iron-sulfur protein, F_a/F_b. The free radical form of the herbicides results in the production of the hydroxyl radicals, which cause the peroxidation of lipids. Eventually, herbicide-induced lipid peroxidation destroys membrane integrity, leading to cellular disorganization and phytotoxic effects (Fuerst & Norman, 1991).

2.3.1 Toxicity after inhibition of Photosystem II

Generally, yellowing of the leaf veins and necrosis at the leaf margins and interveinal areas are observed (Ahrens, 1994). The pattern may be explained by the distribution of the herbicides in the leaf after absorption and translocation. The herbicide is transported in the leaf along the veins and tends to be concentrated at the margins. When the herbicides act slowly and the supply to the leaf is slow, then effects become gradually visible along the leaf veins. When the herbicide acts rapidly and its concentration in the leaf is constant and high, then toxicity is observed in the interveinal areas where photosynthesis rates are

high. Slow action may produce green to yellow or even white tissue indicating chlorophyll destruction, whereas rapid herbicide action is normally accompanied by wilting and necrosis which gives leaves little time for total chlorophyll destruction. Early experiments have revealed that lack of assimilates in the inhibited plants does not cause observed damage, i.e. the plant does not die of starvation (Audus, 1976)

2.4 Factors which determine the phytotoxicity of chloro-triazine herbicides (atrazine in particular)

The response of a plant to a herbicide is a function of three components: a genetic component, and stage of growth and environmental components (Audus,1976).

2.4.1 Plant factors

Plant factors that determine the toxicity of atrazine to cultivated crops represent the genetic component of the plant (Nel, Reinhardt, 1984). The differential inactivation of the atrazine to non-toxic metabolites is primarily responsible for the differences in the plant. Different plant species have different metabolic pathways for inactivation of atrazine, and this is a function of the genotype of that particular plant (Nel & Reinhardt, 1984). The extent of detoxification and induced biological resistance are dominant determining factors. Nel & Reinhardt (1984), further indicated that differences in susceptibility of crops towards

atrazine are not encountered among species only, differences between cultivars have also been reported, amongst others, in maize.

There are three metabolic pathways for the inactivation of atrazine to non-phytoxic atrazine, *viz.* : a) hydroxylation of C-2 of the triazine ring - the chlorine substitute is replaced by an OH, b) N-dealkylation of alkylamino groups at positions four and six, c) conjugation with tripeptide gluthione at position two of the triazine ring, while splitting of the triazine molecule may also occur. In maize all these metabolic pathways are encountered, in wheat the relative activity of these metabolic pathways is very low (Shimabukuro & Swanson, 1969).

2.4.2 Environmental factors

Environmental factors found to be important in the determination of atrazine phytotoxicity include: soil (clay minerals, organic matter, etc.), and climatological factors (temperature, soil moisture, light, etc.) (Audus, 1976; Nel & Reinhardt, 1984).

2.4.2.1 *Soil factors*

According to Weber, (1991a), fixation and degradation influence the availability and biological activity of atrazine in the soil. Soil colloid characteristics play a vital role in the distribution of atrazine between the adsorbed and dissolved phases. The colloidal surface is the site of hydrolysis of atrazine to inactivate

hydroxyl form, therefore inactivation of atrazine will be enhanced by conditions that promote adsorption. These conditions include high organic matter and soils with high clay content, and *vice versa*. Well-decomposed organic matter has functional groups and ring structures that may carry either positive, negative or no charge, thus allowing the binding of both anions and cations of pesticides on a single organic matter molecule.

According to Weber, Swain, Strek, Sartori (1986) humified substances (humus) make up to 17-97% of the total organic carbon in the soil. Humus consists predominantly of humic acids which are high molecular mass with functional groups and aromatic rings which are lipophylic in nature and which poses

numerous ionizable carboxyl and hydroxyl groups that give the polymer pH-dependent exchange properties. Clay minerals also affect the activity of atrazine in the soil. Koskinen & Harper (1990) and Weber (1991a,b) mentioned two major types of clay minerals present in soils, these are 1:1 and 2:1 (silica:alumina). Kaolinite (1:1 clay mineral) does not exhibit high-intensity colloidal properties because of limited adsorptive capacity for cations and relatively low surface area. In the 2:1 clay mineral, montmorillonite, there is a very large internal surface which far exceeds its external surface area. Negative charge predominates on the clay mineral surfaces, thus affording many adsorption sites for positively charged ions (cations) of pesticides.

Soil pH has an effect on the phytotoxicity of atrazine, at low pH there is a decrease in the availability of atrazine. This decrease is due to protonization of the neutral atrazine molecules and adsorption to the clay fraction by means of cation exchange (Adams, 1973; Rahman & Matthews, 1979; Rahman, 1984; Nel & Reinhardt, 1984). Best & Weber (1974) found that the total amount of atrazine and prometryn applied was absorbed by plants over a five-month period ranged from 0.6 to 4.3% and was closely linked to the pH of the soil, with higher herbicide concentrations occurring in the plants at the higher pH values. Phytotoxicity of atrazine in some soils was increased by increase in the soil pH from below 5 to about pH 6 (Holford, Haigh & Ferris, 1989). They attributed this effect to increased availability of atrazine for uptake by plants, and also to increased chemical stability of atrazine molecules at higher pH values.

According to Bollang & Liu (1990), microbial decomposition has always played a role in determining biological activity of atrazine. These microorganisms are known to use organic chemicals as their energy source - they feed on them, and in the process they inactivate them. Kaufman & Kearnery (1970) listed a number of microorganisms known to have the ability to degrade atrazine in pure culture, most of these are fungi. There are, however, reports of bacteria including *Arthrobacter* sp., *Bacillus* sp. And *Pseudomonas* sp. (Cain & Head, 1991). They also indicated that environmental conditions, which favour the growth of microorganism, hasten the inactivation of herbicides in soil. Atrazine is lost more rapidly from moist than dry soils, and more rapidly from soils at high temperatures than during low temperatures. Kaufman & Kearnery (1970),

reported that oxidative dealkylation appears to be the major mechanism by which microorganisms degrade atrazine, but inactivation can also occur under anaerobic conditions.

2.4.2.2 *Climatological factors*

In addition to plant and soil factors, climatological factors can also play a role in determining the activity of atrazine. Nel & Reinhardt (1984) stated that both temperature and moisture influence the availability of atrazine in soil and its fate in the plant.

2.4.2.2.1 Temperature

Temperature affects the activity of herbicides in various ways, often interrelated with other factors responsible for herbicide inactivation, and *vice versa*. It affects the bioactivity of herbicides by influencing their adsorption in soil, the rates of absorption by organisms, and their fate in those organisms. According to Audus (1976) an increase in ambient temperature from 10°C to 30°C enhances the phytotoxicity of s-triazine herbicides. Nel and Reinhardt (1984) indicted that adsorption of atrazine in the soil increases with an increase in temperature. Increased phytotoxicity of atrazine with increasing temperature is probably due to increased absorption and translocation in the plant. Since temperature is believed to exert an indirect influence on the desorption process through its effect on herbicide solubility. Solubility of atrazine is only 22 mgl^{-1} at 0°C, while

it is 70 mgl^{-1} and 320 mgl^{-1} at 27°C and 85°C, respectively (McGlamery & Slife, 1965).

2.4.2.2.2 Soil moisture

The availability of herbicides for uptake by the roots or underground parts is influenced directly by soil water content, since water is the medium in which herbicides are transported in soil, and from which is either adsorbed or absorbed. Atrazine activity is correlated with soil moisture tension. Appleby (1985) found an inverse relationship between atrazine adsorption and soil water content. Indications have been made that adsorption of atrazine increases with a decrease in the soil moisture content owing to the increase of atrazine concentration. With increased atrazine concentration few polar atrazine molecules can compete more effectively with fewer water molecules for sorption positions. The transport of s-triazines through the soil to the absorption surfaces of the plant roots and hypocotyls occurs by means of mass flow and molecular diffusion (Nel & Reinhardt, 1984). Several studies showed that atrazine phytotoxicity was linked to soil water content, with increases in bioactivity as soil water content increased (Dao & Lavy, 1978; Nel & Reinhardt, 1984). More severe injury to maize from atrazine occurred when plants were kept wet and cold for 48 hours after spraying (Audus, 1976).

2.4.2.2.3 Light

It should be noted that excessive activation by sunlight of electrons in herbicides might cause loss of structural integrity, which results in biologically inactive residues. Although this route of degradation is only applicable to pesticides which remain on the soil surface for long period, it also affect those pesticides that are exposed to sunlight in clear water (Weaver & Reinhardt, 1996). According to Audus (1976) high light intensities increase the degree and rapidity of injury from triazine herbicides, however, light also acts directly to inactivate many triazines.

2.5 Atrazine occurrence in South African surface and groundwater

Although one should acknowledge the fact that this topic does not fit very well in this manuscript, it is very imperative to give an overview of the occurrence of atrazine, as well as residual atrazine in both surface water and groundwater of most South African areas where these sensitive crops are grown. The implications of the presence of atrazine residues in all types of water sources are equitable, since water for household use, as well as for irrigation in South Africa come from both surface water and ground water (Weaver & Reinhardt, 1996).

2.5.1 Atrazine occurrence in dam water

Reports by Weaver & Reinhardt (1996), indicates that the presence of atrazine in five dams were monitored, and these dams were in major maize-producing areas of South Africa, *viz.* the Wentzel dam (Schweizer-Reneke), Bloemhof dam (Bloemhof), Koppies dam (Koppies), Loskop dam (Middelburg), Strydom dam (Kroonstad), and Vaal dam (Deneysville and Oranjeville). Atrazine was found in all dams monitored (Table 1). The occurrence of atrazine in the dams follows a seasonal tendency, as atrazine is used as pre-germination herbicide and is applied at the start of the planting season (Weaver & Reinhardt, 1996). For the ranking of atrazine occurrence in all dams examined, see Table 1.

Table 1. Atrazine concentration in water (µg l^{-1}) detected at regular intervals in different dams from August 1990 until February 1993

Date	*Bloemhof dam*	*Vaal dam at Deneysville*	*Vaal dam At Oranjeville*	*Koppie dam*	*Loskop dam*	*Wenzel dam*	*Strydom dam*
Aug 1990	0.05	0.05	0.05	-	0.08	0.05	0.05
Sep 1990	0.05	0.05	0.05	-	0.05	-	-
Oct 1990	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Nov 1990	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Dec 1990	0.05	0.05	1.20	0.05	0.05	0.05	0.05
Jan 1991	1.35	1.64	2.22	3.33	2.82	0.05	11.48
Feb 1991	4.55	2.71	0.39	1.22	3.89	0.55	1.69
Mar 1991	2.67	0.84	0.39	0.46	1.24	1.46	1.09
Apr 1991	1.86	0.43	0.39	0.30	0.46	0.56	0.20
May 1991	1.09	0.48	0.33	0.26	0.38	0.44	0.22
Jun 1991	0.98	0.46	0.35	0.19	0.34	0.43	0.31
Jul 1991	1.95	0.89	0.52	1.10	0.41	1.93	2.35
Aug 1991	1.81	-	0.64	2.25	0.51	1.81	1.99
Sep 1991	1.70	0.05	0.05	0.92	0.05	1.57	2.12
Oct 1991	0.05	2.22	0.05	0.05	2.12	0.05	-
Nov 1991	0.05	0.89	6.6	2.26	2.20	0.05	-
Dec 1991	2.13	3.57	3.22	1.71	3.86	1.57	-
Jan 1992	2.90	4.73	0.05	2.56	-	3.81	-
Feb 1992	6.18	0.05	0.05	4.82	-	3.61	-
Mar 1992	3.17	1.49	0.05	2.72	0.05	3.75	-
Apr 1992	-	4.77	-	0.86	0.05	-	-
May 1992	-	1.15	-	0.05	0.05	-	-
Jun 1992	-	0.05	-	0.05	0.05	-	-
Jul 1992	-	0.07	-	0.05	0.05	-	-
Aug 1992	-	0.05	-	0.05	0.05	-	-
Sep 1992	-	0.05	-	0.05	0.05	-	-
Oct 1992	-	0.05	-	0.05	0.05	-	-
Nov 1992	-	0.41	-	0.98	0.53	-	-
Dec 1992	-	0.55	-	-	0.56	-	-
Jan 1993	-	1.87	-	-	0.47	-	-
Feb 1993	-	-	-	0.30	0.43	-	-

Although these concentrations are generally low, concentrations as high as 6.18 $\mu g\ l^{-1}$ of atrazine was detected at Bloemhof dam during Feb'92 month. Hugo (1994) reported that the atrazine concentrations detected in the rivers could range from 3 – 20 $\mu g\ l^{-1}$, which according to preliminary research studies of the implication of atrazine residues in irrigation water on winter wheat, had significantly inhibited the growth of wheat (Reinhardt, 1996).

2.5.2 The monitoring for chloro-triazine (atrzine) residues in South Africa

Evaluation of atrazine residues in both surface water and groundwater in South Africa was done from 1989 onwards, due to large amounts of atrazine, which is used, for weed control in maize in South Africa (Weaver & Reinhardt, 1996).

2.5.2.1 *Atrazine occurrence in surface water*

Dams monitored or examined were Bronkhorstspruit-, Vaal-, Bloemhof- and Hartbeespoort. Results have been summarized in Table2.

Table 2. Residual atrazine in surface water of most South African dams ($\mu g\ l^{-1}$) (Weaver & Reinhardt, 1996)

Sampling dates	Bronkhorst-spruit Dam	Vaal River	Bloemhof Dam	Hartbeespoort Dam
January 1989	1.0	1.3	0.7	0.8
June 1989	1.0	1.5	0.7	0.3
March 1990	0.9	0.1	0.44	0.2
August 1990	0.4	1.6	0.3	0.1
September 1994	0.45	2.09	1.07	0.86
September 1995	0.1	-	0.05	<0.05

2.5.2.2 *Atrazine occurrence in groundwater*

Atrazine residues occurrence in groundwater was also found in samples taken from various regions in South Africa. These areas include farms in Kroonstsd, Lichtenburg, Bethlehem and Bethal (Weaver & Reinhardt, 1996) (see Table 3).

Table 3. Atrazine residues in South African groundwater ($\mu g\ l^{-1}$)

Sampling dates	Bethlehem	Kroonstad	Bethal	Lichtenburg
August 1992	<0.05	0.33	1.15	<0.05
November 1992	<0.05	0.25	0.92	<0.05
January 1993	<0.05	0.21	0.07	<0.05
March 1993	0.46	0.30	0.15	<0.05
June 1993	<0.05	0.33	<0.05	<0.05
September 1993	<0.05	0.08	<0.05	<0.05
December 1993	<0.05	0.30	0.56	<0.05
July 1994	<0.05	0.30	1.00	<0.05
October 1994	<0.05	0.16	0.95	<0.05
September 1995	<0.05	<0.05	0.06	<0.05

CHAPTER THREE

GENERAL EXPERIMENTAL PROCEDURE

3.1 General experimental design

In all of the experiments, a completely randomized design (CRD) was used. The completely randomised design was used simply because of the following reasons:

- there is no other design that could provide more error of degrees of freedom (df) than this;
- and of more importance, this design minimises chances of complication due to missing data (Petersen, 1994).

To provide an estimate for the experimental error for future experiments, a minimum of three replicates were used in all experiments conducted for this study.

3.2 Statistical analysis

Statistical analysis was done using the computer programme of the Statistical Analysis System (SAS, 1992). Data for different growth parameters were either analysed in original form or first expressed as "% of control" or "% growth reduction", whichever was more appropriate.

Analysis of variance was conducted to determine if significant differences existed between the means of treatments (Petersen, 1994). The Tukey test was used

for pairwise comparisons of means. Means were compared at the 5% level of significance ($p = 0.05$).

3.3 Method used

Much work has been done to investigate the phytotoxic effect of residual atrazine in soil on sensitive crops such as oats, soya-beans and wheat (Reinhardt, 1993 & 1996). However, bioassays using hydroponic systems for the above-mentioned purpose are limited. Bioassays using hydroponic systems can render reliable estimates of the damage threshold of sensitive crops. These methods (bioassays) are not expensive and are sufficiently accurate (Tchan *et al.*, 1975).

The trial was conducted in the glasshouse at the University of Pretoria Experimental farm, Hatfield, Pretoria. The lighting in the glasshouse was not supplemented and the average daily minimum/maximum temperature was 12/23^{0}C (May to August, 1997 & 1998). Wheat seedlings for use in the experiments were grown on germination media (quartz sand) to make them washable during transplanting (first three experiments). For the last experiment refer to Chapter five for materials and methods. Thereafter, the seedlings were transplanted into pots. The pots used had a water-holding capacity at two to three litres. These pots were lined with plastic bags. The plants were suspended in the water through holes in polystyrene lids (see Slate 1 & 2). After transplanting, plants were allowed to grow for 7 to 10 days before treatments were applied. The herbicide used was atrazine (as atrazine 500SC, 495 g a.i l^{-1}, Sanachem). Atrazine was applied with a calibrated dispenser and atrazine rates (rate of atrazine

applied) varied with experiments. In the first experiment, the following rates were applied: 0, 2, 4 & 8 $\mu g\ l^{-1}$. In the second experiment, the rates were: 0, 10, 15, 20 and 25 $\mu g\ l^{-1}$. In the third experiment, rates tested were: 0, 10, 20, and 40 $\mu g\ l^{-1}$. For the last experiments rates tested were: 0, 2, 4 and 8 $\mu g\ l^{-1}$ (applied twice a week). Wheat plants were allowed to grow for a period of one to three months before they were harvested. Harvesting was done by cutting shoots at approximately three millimetres (3mm) above the polystyrene lids (first three experiments) and soil (last experiment). Dry mass was obtained by drying plant material for 24 hours at 65°C.

Figure 1. Photograph representation of the seedling trays and pots used

Figure 2. Photograph representation of two leaf stage seedlings immediately after transplanting

CHAPTER FOUR

EFFECT OF LOW LEVELS OF ATRAZINE ON DIFFERENT WHEAT CULTIVARS IN WATER MEDIUM

4.1 Introduction

Atrazine has been cited in many reports on damage to sensitive rotational crops, these include the report by Wood, Harold, Johnson & Hance (1991). During the 1980's the presence of pesticide residues in groundwater became an issue in Europe and the USA. The presence of triazine herbicides and particularly atrazine became a matter of concern for people in Europe especially. As a pro-active measure, Novartis implemented use-reduction programmes and started water-monitoring studies at various localities (Weaver & Reinhardt, 1996). Since large quantities of atrazine and terbuthylazine are used for weed control in maize in South Africa, it was decided to evaluate the situation in this country. Surface water, groundwater and tap water were monitored for residues of atrazine from 1989 onwards.

In the study by Pestemer and Ghinea (1983) plant available atrazine residues were estimated by extracting soil samples with water, and it was found that between 30 and 120 $\mu g\ l^{-1}$ of atrazine was potentially available for the use by the plants. These atrazine levels resulted in significant wheat growth reduction. The effect of atrazine to wheat was determined in hydroponics culture using herbicide concentrations corresponding with plant-available fractions measured in different soils, results

showed that winter wheat ($ED_{50,}$ 20-30 $\mu g\ l^{-1}$) was sensitive. In South Africa, atrazine has been monitored, and most surveys showed that atrazine concentrations in surface water (rivers) were considerable (3-20 $\mu g\ l^{-1}$), and atrazine residue concentrations in groundwater range from 0.29-4.36 $\mu g\ l^{-1}$ for most locations surveyed (Hugo, 1994). Approximately five dams were monitored for atrazine residues, and the results showed that atrazine is found in all dams examined (Reinhardt & Weaver, 1996). Although the residual concentrations of atrazine in all dams were low, i.e. highest concentration of 6.to 8 $\mu g\ l^{-1}$ of atrazine detected at Bloemhof dam.

According to Hugo (1994), atrazine residues in surface waters can reach a concentration of approximately 20 $\mu g\ l^{-1}$. Atrazine levels of 20 $\mu g\ l^{-1}$ under favourable conditions have been found to reduce growth of wheat by approximately 20%, particularly on susceptible wheat cultivars such as cv. Carina PD 402 (Reinhardt, 1996). It is likely that the atrazine present in irrigation water can be doubled to 40 $\mu g\ l^{-1}$, depending on irrigation frequency, which might reduce the growth by a reasonable percentage. Generally, it is assumed that low herbicide concentration will not affect the growth of the plant. Increasing in herbicide level beyond a certain critical concentration "no-effect level" (NOEL) will result in crop visible damage.

4.2 Experiment 1 & 2

4.2.1 Materials and Methods

See chapter 3 for general procedures used. The study was carried out at Hatfield experimental farm, in a controlled environment. Three-liter pots lined with plastic bags to prevent leaching were used. Two cultivars were used to investigate the response of winter wheat to low residual concentrations in irrigation water, *viz.* Cv. Caritha and cv. Carina PD 402. Plants were transplanted from seedling trays during two-leaf stage and planted in three-liter pots filled with nutrient solution to their capacity (3-liter).

Treatments were applied nine days after transplanting to give plants time to overcome all stress due to transplanting. Four treatments were applied, *viz.* 0, 2, 4 & 8 $\mu g\ l^{-1}$, and each replicated five times. The growth period for this experiment was approximately four weeks (27 days) from the date of planting. After the date of treatment application, only nutrient solution was used when irrigation was necessary till date of harvesting. Three growth parameters were used to investigate the phytotoxic of atrazine on winter wheat, *viz.* Top growth fresh mass, dry mass and root dry weight for both cultivars.

4.2.2 Results

Effect on growth

The top growth fresh mass data are presented in Table 4. The two wheat cultivars did not respond differently to atrazine concentration from 0 – 8 μg l^{-1} ($p < 0,05$). Atrazine concentration of 8 μg l^{-1} did not have any detectable damage or significant effect on the growth of winter wheat.

Table 4. Effect of different atrazine concentrations on top growth of two winter wheat cultivars, *viz.* cv. Carina PD 402 and cv. Caritha (Fresh mass/pot)

(ANOVA in Table 1A & 4A)

Treatment (Atrazine rates)	Exp.1 Cv. Carina PD 402	Exp.2 cv. Caritha
	Fresh mass/pot(grams)	
0 $\mu g\ l^{-1}$	18.08 a	13.89 a
2 $\mu g\ l^{-1}$	25.18 a	16.91 a
4 $\mu g\ l^{-1}$	19.85 a	21.22a
8 $\mu g\ l^{-1}$	15.31 a	11.29 a

Means with same letter do not differ significantly at $p = 0.05$.

Dry matter production

As for growth, dry matter yields obtained from these two winter wheat cultivars were not significantly different ($P<0.05$). The dry matter yields are presented in Table 5. Atrazine at all concentrations (0, 2, 4, & 8 $\mu g\ l^{-1}$) did not affect dry matter production of all cultivars, significantly (Table 5).

Table 5. Effect of atrazine treatments on dry matter production of wheat (ANOVA in Table 2A & 5A)

Treatment (Atrazine rates)	Exp 1 cv. Carina PD 402	Exp.2 cv. Caritha
	Dry matter yield (g/pot)	
0 $\mu g\ l^{-1}$	3.20 a	2.02 a
2 $\mu g\ l^{-1}$	4.00 a	3.17 a
4 $\mu g\ l^{-1}$	3.01 a	3.16 a
8 $\mu g\ l^{-1}$	2.54 a	2.49 a

Means with the same letter do not differ significantly at $p = 0.05$.

Effect on root dry mass

Effect of residual atrazine on root storage capacity was also investigated, results are presented in Table 6. The root dry weight of all treatments was not significantly affected by varying atrazine residue levels ($p>0.05$).

Table 6. Effect of atrazine on wheat root dry weight

(ANOVA in Table 3A & 6A)

Treatments (Atrazine rates)	Exp. 1 cv. Carina PD 402	Exp. 2 cv. Caritha
0 $\mu g\ l^{-1}$	0.62 a	0.61 a
2 $\mu g\ l^{-1}$	0.92 a	0.88 a
4 $\mu g\ l^{-1}$	0.74 a	0.73 a
8 $\mu g\ l^{-1}$	0.61 a	0.65 a

Means with the same letter do not differ significantly at $p=0.05$.

4.2.3 Discussion

Atrazine concentration of 10 μg l^{-1} has been found to affect the growth of wheat, especially under conditions which favour accumulation and availability of atrazine to plants (Reinhardt, 1996). Although all parameters measured in this experiment did not reflect any significant phytoxic effects of atrazine on wheat, the damage can be expected under those conditions, which make atrazine more available in high concentrations to plants. These factors include high frequency of irrigation with atrazine contaminated water at significant levels, light textured soil, in which most of the atrazine in soil is available for plants use. With regard to susceptible cultivar (cv.Carina PD 402), there has been a progressive reduction in growth with increasing atrazine rate, from 2 to 8μg l^{-1}. Although a once off application of atrazine did not have an effect on the crop, crop injury may occur at the levels tested due to frequency of irrigation, soil type and many other prevailing conditions which favour availability of atrazine to crop. Although there was no significant growth reduction at atrazine levels tested, crop injury may occur at those levels of atrazine, due to factors such as frequency of irrigation with atrazine contaminated water at those low levels and soil type. Atrazine damage to wheat could be expected in light-textured soil, low organic matter, and with high frequency of irrigation with atrazine contaminated water.

Atrazine, at all three concentrations did not have a significant effect on wheat growth . The reason for no growth reduction effects could be due to low rates of atrazine which were applied. To investigate the possibility of low atrazine rates

for having no effect on wheat growth, the second experiment was set, in which much higher atrazine rates (5-25 μgl^{-1}) were used.

4.3 Experiment 3

4.3.1 Materials and Methods

Bioassays using hydroponics were used, *i.e.* growing wheat in an aqueous nutrient solution. Two winter wheat cultivars (cv. SST 86 and cv. Carina PD 402) were used to determine their sensitivity towards atrazine concentrations. Atrazine concentrations used were: 0, 5, 10, 15, 20 and 25 μgl^{-1}. A 2 x 6 factorial experiment was used, with each treatment replicated three times. Treatments were applied seven days after transplanting. Seedlings were transplanted during two leaf- stage. Water lost due to evapo-transpiration was replenished on alternate days with nutrient solution. Plants were allowed to grow for three months before they were harvested. The following growth measurements were made: number of tillers and dry matter of top growth.

4.3.2 Results

Response of two wheat cultivars to atrazine

The susceptibility difference between the two wheat cultivars tested, towards atrazine was very distinctive. The difference in terms of their sensitivity towards

atrazine in water culture was significant (Table 7). The cultivar Carina PD 402 was more susceptible to residual atrazine, whereas cultivar SST 86 was less susceptible. The differences in their sensitivity to atrazine may be attributed to their differences in their uptake, and genetic composition. In fact, the herbicide injury to cultivar SST 86 was very negligible at all residual atrazine rates as compared to cultivar Carina PD 402.

Table 7. Differential sensitivity of two wheat cultivars exposed to atrazine
(Data averaged across atrazine rates)
(ANOVA in Table 7A & 8A)

Cultivar	Dry matter yield	Number of tillers
	% of control	
cv.SST 86	107.3a	100.8a
cv.Carina PD 402	93.6b	83.7b

Means with the same letters are not significantly different at p = 0.05.

Dry matter production

The effect of atrazine residues on wheat was not significant (Table 8). However atrazine damage of about 20% growth reduction was detected at the highest atrazine rate on cultivar Carina PD 402, which was more susceptible to atrazine. Although atrazine damage amounting to about 20% growth reduction was measured, no physical (obvious) symptoms were manifested due to these residues, even at the highest dosage rate of atrazine.

Table 8. Effect of different atrazine rates on wheat growth, mean dry matter yield (% of control)
(ANOVA in Table 7A)

Atrazine rates	Dry matter yield cv.SST 86	Dry matter yield cv.Carina PD 402
5 $\mu g\ l^{-1}$	102.00a	102.00a
10 $\mu g\ l^{-1}$	104.10a	106.40a
15 $\mu g\ l^{-1}$	135.11a	87.10a
20 $\mu g\ l^{-1}$	95.00a	92.00a
25 $\mu g\ l^{-1}$	101.00a	81.00a

Means with the same letters are not significantly different at $p = 0.05$.

Tillering

The tillering of all cultivars was not significantly affected by different atrazine concentration applied as referred in Table 9. However, a tendency was observed with the number tillers decreasing with increasing atrazine rates.

Table 9. Wheat response in terms of tillering to different atrazine rates (% of control)
(ANOVA in Table 8A)

Atrazine rates	Number of tillers cv.SST 86	Number of tillers cv.Carina PD 402
5 $\mu g\ l^{-1}$	101.01a	88.32a
10 $\mu g\ l^{-1}$	102.02a	88.00a
15 $\mu g\ l^{-1}$	112.00a	82.00a
20 $\mu g\ l^{-1}$	94.00a	84.00a
25 $\mu g\ l^{-1}$	96.00a	77.30a

Means with the same letter are not significantly different at $p = 0.05$.

4.3.3 Discussion

Despite the lack of obvious symptoms of damage on wheat plants at these low levels of atrazine applied in irrigation water, growth reduction was measurable after three months of once off treatment of atrazine, which could be more worse when measured early during the growth stage, as in the past experiments. Even though this results cannot be extrapolated to all sensitive crops, it is possible that damage can occur to other sensitive crops since wheat was damaged at these low amounts of atrazine, and wheat is the least sensitive crop as compared to other crops such as, oats; sunflower and groundnut crops. The damage to the crop at this concentration will be governed by the amount of atrazine in the soil, which is determined by the soil texture (organic matter). High frequency of irrigation with atrazine contaminated water, in light textured soil, more atrazine will be available to the crop, and the more likely the damage. The only significant difference declared was for cultivars. Atrazine had no significant effect on wheat growth. However a tendency was observed in which increasing rates of atrazine resulted in wheat growth reduction. It is believed that this effect will increase with application of higher concentrations and/or increased irrigation frequency with atrazine contaminated water. This is sought to be worse at much higher rates of atrazine with high frequency of irrigation. To validate these assumptions an experiment was set, in which higher atrazine rates (10-40 μgl^{-1}) were investigated.

4.4 Experiment 4 & 5

4.4.1 Materials and Methods

To investigate toxicity effects of these high atrazine residues, two cultivars were used in this study i.e. cv. Caritha and cv. Kariega. Wheat plants were transplanted from the seedling trays during two-leaf stage and planted in two litre (2l) pots. The growth response of wheat to four atrazine dosage rates was investigated in single-factor experiment. Treatments were replicated ten times in this experiment.

4.4.2 Results

None of the plants used for this experiment were killed as results of atrazine toxicity. However, plants in this investigation developed sublethal phytotoxic responses to atrazine. The severity of these sublethal phytotoxic responses to atrazine depended largely on the level of atrazine applied. This was indicated by the increase in severity with increasing atrazine concentration. No visual injury symptoms were observed on wheat.

Atrazine effect on growth

In both cultivars grown on hydroponics systems containing atrazine residues, growth reduction was observed as shown in Table 10 & 11. Even though there were no development of visual symptoms of atrazine phytotoxicity on plants, measurable plant

growth responses (fresh and dry weight) have been used to develop a diagnosis base for the determination of atrazine damage to wheat. Growth damage for cultivar cv. Caritha was significant, amounting to about 25% at 40 $\mu g\ l^{-1}$ against the control. The other cultivar had 10% growth reduction at the highest atrazine dosage rate.

Table 10. Wheat growth response to atrazine residues in both irrigation and soil water (ANOVA in Table 12A & 14A)

Atrazine rate	Exp. 4 cv. Caritha	Exp.5 cv. Kariega
	Fresh mass reduction	
0 $\mu g\ l^{-1}$	0 a	0.0a
10 $\mu g\ l^{-1}$	12b	8.0a
20 $\mu g\ l^{-1}$	14b	10.2a
40 $\mu g\ l^{-1}$	25c	10.3a

Means with same letter are not significantly different at $p = 0.05$.

Reduction in dry matter accumulation increased with increasing atrazine dosage rate. For cultivar cv. Caritha dry mass was reduced by 14% at the lowest dosage rate and by 16% at the highest dosage rate; and in cultivar cv. Kariega growth was not significantly reduced even at the highest dosage rate of 40 $\mu g\ l^{-1}$ (See Table 10).

Table 11. Percentage reduction in dry matter production of wheat exposed to atrazine (ANOVA in Table 13A & 15A)

Atrazine rates	Exp. 4 cv. Caritha	Exp. 5 cv. Kariega
	% reduction in dry mass	
0 $\mu g\ l^{-1}$	0 a	0a
10 $\mu g\ l^{-1}$	14b	6a
20 $\mu g\ l^{-1}$	15b	7a
40 $\mu g\ l^{-1}$	24c	9a

Means with the same letter are not significantly different at p = 0.05

4.4.3 Discussion and conclusion

Damage to wheat was reported decades ago by Saghir & Ghoudhary (1967), Eagle (1980), and Moyer & Blackshaw (1993). However, crop injury was due to high residual concentration in soil. Reinhardt (1996) confirmed wheat injury at low concentration of approximately 10 $\mu g\ l^{-1}$ and beyond. The use of protected LSD test relates that the difference among the means was significantly different for cultivar cv. Caritha, and no significant difference was found for cultivar cv. Kariega. However tendency was observed in cultivar cv. Kariega where growth was reduced with

increasing atrazine dosage rates. Wheat growth was reduced by 25% in cultivar cv. Caritha and 10% in cultivar cv. Kariega. This clearly shows that cv. Caritha is more susceptible to atrazine than cv. Kariega. This experiment showed that atrazine concentration exceeding 10 $\mu g\ l^{-1}$ may inhibit the growth of wheat, if applied only once, depending on cultivars, and various wheat cultivars vary in their susceptibility to atrazine toxicity.

Atrazine has been cited in many reports on damage to sensitive rotational crops, these include the report by Wood, Harold, Johnson & Hance (1991). During the 1980's the presence of pesticide residues in groundwater became an issue in Europe and the USA. The presence of triazine herbicides and particularly atrazine became a matter of concern for people in Europe especially. As a pro-active measure, Novartis implemented use-reduction programmes and started water-monitoring studies at various localities (Weaver & Reinhardt, 1996). Since large quantities of atrazine and terbuthylazine are used for weed control in maize in South Africa, it was decided to evaluate the situation in this country . Surface water, groundwater and tap water were monitored for residues of atrazine from 1989 onwards and the findings were positive with significant amount of residues being detected in various dams surrounding the maize growing or summer -crop growing areas in South Africa. The damage to sensitive crops may occur on soils with very low adsorptive capacity and high soil pH and high frequency of irrigation with atrazine-contaminated water.

CHAPTER FIVE

GROWTH RESPONSES OF WHEAT IRRIGATED WITH ATRAZINE-CONTAMINATED WATER

5.1 Introduction

In general, water-monitoring programs clearly show that much higher residues of triazines occur in surface water than in groundwater (Weaver & Reinhardt, 1996). Restrictions on the use of atrazine are in place in many countries. Future use of atrazine may depend on innovation in formulation, application and management methods that prevent excessive carry-over and contamination of water resources.

It has been reported that residual effects of atrazine vary between field sites, as well as its persistence over relatively short distances at a particular site, probably due to variation in soil properties such as pH and the nature and adsorptive capacity of colloids (Reinhardt, Ehlers & Nel, 1990). Ehlers, Reinhardt & Nel (1988); Nel, Mennega & Reinhardt (1992) identified a number of other factors, which determine the phytotoxicity of atrazine residues, *inter alia*. the plant species.

Much outstanding work has been done to predict atrazine persistence based on key environmental variables that determine its dissipation rate. The ability to link amounts of residual herbicides, which are present in both irrigation water and the soil solution,

to the tolerance of crops is of paramount importance. This chapter further reports on a study aimed at assessing the susceptibility of wheat to high concentrations of atrazine, which is known to be highly intolerant to atrazine, to low atrazine levels similar to those detected in both groundwater and surface water.

5.2 Materials and methods

The sensitivity of two wheat cultivars (cv. SST 825 and cv. SST 55) to atrazine was assessed in two separate bioassays. Selected properties of soil for both experiments appear in Table 12. Each experiment had four atrazine rates (0, 2, 4, & 8 $\mu g\ l^{-1}$) with three replications for cv. SST 55 and four replications for cv. SST 825. Four atrazine rates were used in each experiment. Stocks of each rate were prepared by a mixing pre-determined volume atrazine product with five litres of complete nutrient solution in each 45x45x45 cm container. The controls involved nutrient solution only. Soil that was to be irrigated with these stock solutions was contained in plastic bags in pots to prevent leaching of herbicide out of the soil. The herbicide was foliar applied by placing pots in a demarcated $1m^2$ area and spraying (5 mm) over the top with a hand-held sprayer. This was done twice every week to simulate irrigation frequency.

Experimental conditions in the glasshouse were similar for the two experiments described above. Water content of the soils on which the two cultivars were grown was adjusted to 75% of the total water content per pot at field capacity level through weighing of pots on alternate days. Lighting in the glasshouse was not supplemented. The fresh and dry weight of the wheat plant top growth, ears and the number of

spikes and spikelets were measured 90 days after seeding. The plants were cut 3 mm above the soil. The fresh and dry mass yield in each pot was calculated on a per pot basis (three plants per pot cv. SST 55 and six plants per pot cv. SST 825). Pots were arranged according to a completely randomised design both experiments. Data were expressed as percentage reduction in growth compared to the untreated controls.

Table 12. Soil properties of the soil selected (Soil analysis data)

pH value	Clay%	Silt%	Sand%	C%
5.8	18.1	8.2	70.5	0.6

5.3 Results

Results for cv. SST 825 and cv. SST 55 are shown in Table 13 to 15. The growth responses of wheat to all atrazine rates were not significant. This can be explained by relatively low atrazine bioactivity (0, 2, 4, & 8 $\mu g\ l^{-1}$). From all parameters measured no real damage was found. Dry matter accumulation, which is strongly correlated to photosynthesis, with atrazine known to be photosynthetic inhibitor, was also not significant for both cultivars. These results suggest either tolerance of these two cultivars to atrazine or low bioactivity of these atrazine residues as effected by soil properties.

Table 13. Growth response of two wheat cultivars exposed to atrazine in irrigation water (ANOVA in Table 16A – 19A)

Cultivar	Atrazine ($\mu g\ l^{-1}$)	Fresh mass of shoots (g/pot)	Fresh mass of ears (g/pot)
Exp.1:SST 825	0	41.87a	15.43a
	2	42.76a	14.22a
	4	47.60a	13.39a
	6	47.40a	11.58a
Exp.2:SST 55			
	0	11.38a	10.42a
	2	19.77a	12.19a
	4	12.06a	8.43 a
	6	14.67a	10.38a

Table 14. Dry matter yield of two wheat cultivars exposed to atrazine in irrigation Water (ANOVA in Table 20A – 23A)

Cultivar	Atrazine ($\mu g\ l^{-1}$)	Dry matter yield of shoots (g/pot))	Dry matter yield (ears/pot)
Exp.1: SST 825	0	10.06a	3.01a
	2	10.38a	6.10a
	4	10.84a	3.04a
	6	10.28a	3.51a
Exp.2: SST 55			
	0	4.26a	2.63a
	2	4.02a	2.90a
	4	3.87a	2.31a
	6	3.21a	2.39a

Table 15. Yields of two wheat cultivars exposed to atrazine in irrigation water (ANOVA in Table 24A – 27A)

Cultivar	Atrazine ($\mu g\ l^{-1}$)	Number of ears/spikes	Total number of spikelets/pot	Average number of spikelets/pot
Exp.1: SST 825	0	10a	138.50a	15.75a
	2	10a	149.75a	15.75a
	4	9a	148.00a	15.25a
	6	9a	134.25a	15.00a
Exp. 2: SST 55				
	0	3a	50.00a	14.00a
	2	5a	58.00a	14.23a
	4	3a	39.33a	13.66a
	6	4a	57.66a	13.66a

5.4 DISCUSSION

In these experiments, the frequent application of low levels of atrazine did not injure wheat. In these experiments in which plants were grown in soil, soil properties had a major role to play by determining the amount of atrazine available for uptake by the plants. Also, in these experiments, cultivars were different from those used in previous experiments, which might also suggest relatively greater tolerance of these cultivars towards atrazine.

Atrazine is known to be readily absorbed through roots from the soil and translocated to shoots via the apoplast (essentially in the xylem). Not much absorbed into leaves from the post-emergence application with essentially no basipetal translocation out of treated leaves, unless surface-active adjuvants are added to enhance absorption into foliage (Ahrens, 1994). In these experiments, atrazine was foliar-applied, which might have resulted in less atrazine being absorbed due to spray drift(sprays falling on the ground surface not in pot).

The properties of the soil used in these experiments are reflected in Table 13. Residual effects of atrazine vary between field sites, as well as its persistence over relatively short distances at a particular site, probably due to variation in soil properties (Nel, & Reinhardt, 1984). The reason for no real atrazine effect on wheat plants might be due to atrazine adsorption. Holford, Haigh & Ferris (1989) found that wheat growing in a rotation experiment on an alkaline black earth soil was severely damaged by atrazine residues whereas wheat on slightly acid red clay was not

damaged. They also found that atrazine concentration was positively correlated with pH and negatively correlated with organic carbon. Atrazine persistence and phytotoxicity should increase as the pH increases, and decrease with organic carbon increases.

In these experiments, fairly high organic carbon (0.6%) might have adsorbed some of the herbicide molecules leaving very little for absorption by the plants. Most probably the degradation of this herbicide to hydroxyatrazine was enhanced through catalysation by adsorption to organic matter (Reinhardt & Nel, 1993). High levels of organic matter in soil irrigated with atrazine-contaminated water will probably result in less phytotoxicity to sensitive species. According to Walker (1987), the organic matter content of soil might be expected to influence the degradation rate of pesticides, since it is the most important variable controlling adsorption, and hence their distribution between the solid and solution phases.

Atrazine is hydrolysed to hydroxyatrazine under acid-soil conditions. Organic matter and clay content are correlated negatively with atrazine availability in the soil solution. Ehlers, *et al.*, (1988), and soil pH correlate positively with atrazine availability in the soil solution, and the stability of atrazine against hydrolysis to inactive hydroxyatrazine increases progressively as soil pH increases to around neutral (Reinhardt & Nel, 1993).

To conclude, atrazine at the concentrations tested, which also occur in surface waters, might have significant effects on wheat growth under field conditions, especially for

soils with very low adsorptive capacity and high soil pH. Frequent irrigation with atrazine-contaminated water could result in significant amounts of atrazine accumulation in the soil.

CHAPTER SIX

6.1 IMPLICATIONS OF FINDINGS FOR RESOURCE-POOR FARMERS

The beneficial effects of herbicides are sometimes mitigated by their persistence in the environment. Water may be contaminated with herbicides from aerial spraying or through runoff from land treated with herbicides. Persistence of residual herbicides in soils has undesirable impacts on sensitive crops subsequently grown on the same soil. The total herbicide residues in soil (adsorbed and available) determined by extraction with organic solvents and measured instrumentally may be of secondary interest to small-scale farmers and the method is also expensive. Bioassay methods offer the opportunity to detect the presence of toxic compounds at biologically active doses and also real or practical effects (symptoms) which poor-resource farmers are likely to see.

In general, bioassays do not require expensive equipment, which of course poor-resource farmers do not have or cannot afford. Since atrazine is used extensively in maize, many small-scale farmers have been afforded this weed control technology through their extension officers, who offer them assistance on when to apply, how to apply and which equipment to use, etc. These extension officers, however, often do not have adequate knowledge of chemical weed control, herbicide usage and the behaviour of herbicides in the environment. Although agrochemical companies provide the information on how to handle, apply and store, as well as waiting periods for most crops, they do not provide information on all sensitive crops, for example "maraho" which small-scale farmers grow mainly for household consumption.

Any amount of atrazine residues in both soil and irrigation water above the injury threshold for sensitive crops will have an effect on sensitive follow-up crops. Bioassays to assess the bioactivity of atrazine in soil towards wheat or any other any sensitive crop are easy to conduct and very demonstrable to small-scale farmers, unlike laboratory tests, which are very expensive and even make them very sceptic about the analysis report, since they want something they can see and draw conclusions from.

Farmers who use atrazine-contaminated water, their livelyhoods are at stake, and only this practise can minimise the effects on sensitive crops, i.e. by growing less sensitive or tolerant crops.

6.2 GENERAL DISCUSSION

This study was initiated against the background of the detection of atrazine residues in ground and surface waters in South Africa. The specific aim of this study was to determine the critical level at which phytotoxic effects are manifested on sensitive crops. Wheat was selected as an indicator for testing the response of a sensitive crop to such low levels of atrazine, which may conceivably occur in water used for irrigation.

It was found that cultivar differences in sensitivity exist among wheat cultivars as expected, probably due to genetic variation. Chaplin & Alban, (1960); Grogan, Eastin & Palmer, (1963) and Anderson, (1964), also found that differential tolerance towards atrazine is not encountered among species only; differences between cultivars have also been reported, amongst others in maize. In light of this, it could be recommended that for areas where soil or water sources are known to be contaminated by atrazine, less sensitive cultivars be grown to minimise the risk of crop damage.

Wheat injury by atrazine was measurable after a single treatment on wheat grown in water. This growth medium was chosen in order to simulate severe conditions, in terms of bioactivity of atrazine. Only cultivar Caritha was significantly damaged. Damage could also be expected under conditions that favour accumulation of atrazine, and enhance its bioactivity such as, temperature. pH. Soil type etc. Coarse-textured soil (sandy) with low organic matter will promote phytoxicity of atrazine to

crops (Rahman & Matthews, 1979). More often the lower the pH, and the higher clay and organic matter content, the lower the availability of atrazine in the soil, and hence, the lower the risk of dang to crops (Adams, 1973).

The study showed that atrazine, when available for uptake at concentrations in the range 10 -40 $\mu g\ l^{-1}$ is likely to cause appreciable damage to wheat. Although significant atrazine effects were only observed in cultivar Caritha, tendencies for growth reduction were observed in other cultivars.

In the experiment where soil was used as growth medium, wheat plants were not damaged by atrazine in water used for repeated irrigation. It is believed that soil properties might have influenced the availability of atrazine to wheat plants. These properties include, soil texture, soil pH, clay content, carbon content and the microbial composition of soil. All these soil properties determine the availability of atrazine or bioactivity of atrazine to wheat plants. Normally, at very low atrazine levels, high organic matter, high microbial activity, low pH and high clay content will decrease the activity of many s-triazines.

Although, few significant effects were observed in these experiments, 10 $\mu g\ l^{-1}$ appear to be the injury threshold concentration for wheat. Any atrazine levels below 10 $\mu g\ l^{-1}$ might be considered as "no effect level", but 10 $\mu g\ l^{-1}$ is the critical level above which atrazine may negatively affect wheat. It was found that for cultivar Caritha the injury threshold concentration in water medium was 10 $\mu g\ l^{-1}$. This indicates that other crops (oats, sunflower, soyabean, and tomato), which are probably

more sensitive to atrazine than wheat might be affected more by these low levels of atrazine.

Therefore, atrazine may cause injury to crops at very low concentrations depending on conditions prevailing at the time of irrigation with atrazine contaminated-water. Conditions that will increase phytotoxicity of atrazine on sensitive crops are: coarse-textured soil, low organic matter, and low microbial activity. Other factors, which directly affect the toxicity of atrazine to sensitive crops are: I) atrazine levels in contaminated water, ii) frequency of irrigation. Knowledge on the extent of contamination of waters with atrazine, or any other herbicide for that matter, plus information on the relative sensitivity of crops or cultivars, would reduce the risks to crops in general.

Because, with this study it is not possible to extrapolate directly the effects of low residual atrazine on wheat to field situations, further studies that also involve field trials should be considered. In the course of this research valuable information regarding the effects of residual atrazine in irrigation water on sensitive crops was gained.

6.3 SUMMARY

South African surface waters are contaminated with atrazine (Weaver & Reinhardt, 1996). Residues of atrazine detected were in the range of 3 – 20 $\mu g\ l^{-1}$ for surface water and 0.29-4.36 $\mu g\ l^{-1}$ for groundwater. Atrazine in tolerant crops is readily metabolised, and in sensitive crops, unaltered atrazine is known to accumulate and results in chlorosis and death in more sensitive crops. In light of this, it was necessary to investigate the effects of these low levels of atrazine on sensitive crops. Since, atrazine-contaminated water may be used for irrigation purposes, which might pose a threat to sensitive crops, such as oats, sunflower, dry beans and wheat. This study also made it possible to identified the "no-observable effect level" (NOEL) for atrazine on wheat, which can help us make inferences on the impact of low residual atrazine on sensitive crops.

Experiments designed to investigate the effects of atrazine on wheat were conducted during the 1997, 1998 and 1999 growing season. Pot experiments were conducted under the glasshouse conditions. Bioassay techniques using hydroponic systems were used, where wheat plants were grown in pots containing water medium. Further more, wheat plants were also grown on soil in three litre pots. In total, six cultivars were tested, *viz.* cv. Carina PD 402, cv. Caritha, cv. Kariega, cv. SST 55, cv. SST 825 and cv. SST 86. Atrazine was tested on different cultivars at in concentrations ranging from 0-40 $\mu g\ l^{-1}$. Growth parameters measured included fresh/dry mass of spikes, shoots, roots and the number of tillers, spikes, spikelets.

Injury to wheat (cv. Carina PD 402, cv. Caritha, cv. SST 86, cv. Kariega) caused by atrazine levels similar to those detected in local surface water and groundwater was measurable after a single treatment on wheat grown in water culture. This was probably due to total availability of atrazine to plants, since soil properties were negated in these experiments. The cultivar Caritha was significantly injured by atrazine at 10 $\mu g\ l^{-1}$. This suggests that 10 $\mu g\ l^{-1}$ is the injury threshold for this cultivar. Under field conditions the same level of injury can be expected only atrazine concentration in soil solution is 10 $\mu g\ l^{-1}$. Results of these experiments suggest that atrazine may also be toxic to crops that are more sensitive than wheat. Below 10 $\mu g\ l^{-1}$ no significant injury was observed at any of the cultivars, therefore these levels appear to represent the "no observable effect level" (NOEL) or critical level beyond which atrazine will negatively affect wheat growth.

In a subsequent experiment (Exp. 3), cultivar responses were investigated to establish whether wheat cultivars exhibit differential sensitivity. It was demonstrated that the cultivars tested respond differently to atrazine. The cultivar Carina PD 402 was significantly more sensitive to atrazine than cultivar SST 86.

In the experiment where soil was used as growth medium, no measurable damage was caused by atrazine on wheat (cv. SST 825 and cv. SST 55). This was probably due to soil properties that reduced the herbicide availability for uptake. The adsorption of atrazine to soil colloids probably prevented enough atrazine from being available in the soil solution for wheat to be injured. Results of Exp.1, Exp. 2 & Exp. 3 indicate,

at least for cultivar cv. Caritha, that the critical atrazine concentration for significant injury is 10 $\mu g\ l^{-1}$ in the soil solution.

It is plausible that crops, which are sensitive to atrazine, might be at risk when irrigated with atrazine-contaminated water. Conditions that favour the accumulation of atrazine or retardation of its dissipation in soil would increase the problem.

6.4 REFERENCES

ADAMS, R.S., 1973. Factors influencing soil adsorption and bioactivity of pesticides. *Residues Reviews.* 47, 1-54.

AHRENS, W.A., 1994. Herbicide handbook. 7th Edition. WSSA. U.S.A

ANDERSON, R.N., 1964. Differential response of corn inbreds to simazine and atrazine. *Weed Sci.* 12, 60-61.

AUDUS, L.J., 1976. Herbicide: Physiology, Biochemistry, Ecology. Academic press. London

APPLEBY, A.P., 1985. Factors in examining fate of herbicide in soil with bioassays. *Weed Sci.* 33, 2-6.

BAILEY, G.W. & WHITE, J.L., 1970. Factors influencing the adsorption,desorption and movement of pesticides in soil. In: F.A. Gunther (ed). The triazine herbicides. *Residue Reviews*. 32, 29-92.

BEST, J.A & WEBER, J.B., 1974. Disappearance of s-triazines as affected by soil pH using a balance sheet approach. *Weed Sci.* 22, 364-373.

BOESTEN, J.J.T.I. & VAN DER LINDEN, A.M.A., 1991. Modelling the influence of sorption and transformation of pesticide leaching and persistence. *Journal of Environmental Quality*. 20, 425-435.

BOLLANG, J-M. & LIU S-Y., 1990. Biological transformation of pesticides. In: H.H Cheng (ed). Pesticides in the soil environment: Processes, impacts and modelling. Book no.2 in soil Science Society of America Book Series. SSA Inc., Madison, Wisconsin, USA.

CAESL, R.F., MULKEY, L.A., LORBER, M.N. & BASKIN, L.B., 1985. The pesticide root zone Model (PRZM): A procedure for evaluating leaching threats to groundwater. Ecological Model. 30, 150.

CAIN, R.B. & HEAD, I.M., 1991. Enhanced degradation of pesticides: In: biochemical and molecular biological basis. In: A Walker (ED). Pesticides in soil and water: Current Perspective. *BCPC Mono*. No. 47,23-40.

CHAPLIN, D. & ALBAN, E.K., 1960. Varietal response of sweet corn to simazine. *Proc. N. Central Weed Contr. Conf.* 17, 52-53.

DAO, T.H. & LAVY, T.L., 1978. Atrazine adsorption on soil as influenced by temperature, moisture content and electrolyte concentration. *Weed Sci.* 26, 303-308.

EAGLE, D.J., 1980. Herbicide injury to crop plants . Proceedings of conference March to 2nd April. 63-65.

EAGLE, D.J., CAVERLY, D.J. & HOLLY, K., 1981. Diagnosis of herbicide damage to crops. Ministry of Agriculture, Fisheries and Food. Ref. Book221. England.

EHLERS, J.G., REINHARDT, C.F., & NEL, P.C., 1988. Effect of certain soil factors on the activity of atrazine. *S. Afr. J. Plant Soil*. 1, 84-87.

ELLIS, J.F., 1992. Herbicide development and marketing of weed control in the United States of America. *Pro. First International Weed Control C.onference*. 1, 74-82.

FRANK, R., SIRONS, G.J., & ANDERSON, G.W., 1983. Atrazine: The impact of persistent residues in soil on susceptible crop species. *Canadian J. of Soil Science*. 63, 315-325.

FUERST, C.P. & Norman, M.A., 1991. Introduction of herbicides with photosynthesis electron Transport. *Weed Sci.* 39, 458-464.

GROGAN, C.O., EASTIN, E.F. & PALMER, R.D., 1963. Inheritance of susceptibility of a line of maize to simazine and atrazine. *Crop Sci.* 3, 451.

HOLFORD, I.C., HAIGH, B.M. & FERRIS, I.G., 1989. Atrazine persistence and phytotoxicity on wheat as affected by nitrogen and rotation-induced change in soil properties. *Aust. J. Agric. Res.* 40, 1143-1153.

HUGO, K.J., 1994. Leaching of atrazine and terbuthylazine in some South African soils. PhD Thesis. University of Pretoria.

KAUFMAN, D.D. & KEARNERY, P.C., 1970. Microbial degradation of s-traizines herbicides. *Residue Reviews*. 32, 235-265.

KOSKIEN, W.C & HARPER, S.S., 1990. The retention process: Mechanism. In: C C Cheng (ed). Pesticides in the soil environment: Processes, impacts and modelling. Book no.2 in soil Science Society of America Book Series. SSSA Inc., Madison, Wisconsin, USA.

McCORMICK, L.I. & HILTBOLD, A.E., 1996. Microbiological decomposition of atrazine and diuron in soil. *J. of Weed Society of America*. 14, 77-82.

McGLAMERY, M.D. & SLIFE, F.W., 1965. The adsorption and desorption of atrazine as affected by pH, temperature, and concentration. *Weeds*. 14, 237-239.

MOYER, S.R. & BLACKSHAW, R.E., 1993. Effect of soil moisture on atrazine and cynazine persistence and injury on subsequent crops in Southern Alberta. *Weed Technology*. 7, 988-994.

NEL, P.C. & REINHARDT, C.F., 1984. Factors affecting the activity of atrazine in plants and soil. *S. Afr. J. Plant Soil. 1*, 67-72.

NEL, P.C., MENNEGA, R., REINHARDT, C.F., 1992. Evaluations of techniques used in conventional bioassays for determining atrazine activity in soil. *Proc. 1st International Weed Control Congress*. 2, 359 –361.

NICHOLLS, P.H., WALKER, A. & BAKER, R.J., 1982. Measurement and simulation of the movement and degradation of atrazine and matribuzin in a fallow soil. *Pesticide Sci.* 12, 484-494.

PESTEMER, W., GHINEA, L. & RADULESLU, V., 1993. Residual effects of chlorotriazine herbicides in soil at three Rumanian sites. II. Prediction of the phytotoxicity of atrazine residues to following crops. *Weed Res.*. 24, 371-377.

PETERSEN, R.G., 1994. Agricultural field experiments. Marcel Dekker. Inc. New York.

PICK, F.E., VAN DYK, L.P. & BOTHA, E., 1992. Atrazine in ground and surface water in maize production areas of the Transvaal, South Africa. *Chemosphere* 25 (3), 335 – 341.

RAHMAN, A., 1984. Influence of particle size and type of formulation on phytotoxicity and persistence of atrazine. *Weed Res.* 24, 255-260.

RAHMAN, A. & MATTHEWS, L.J., 1979. Effect of soil organic matter on the phytotoxicity of Thirteen s-Triazine Herbicides. *Weed Sci.* 27, 158-161.

REINHARDT, C.F., 1993. Biological activity and persistence of atrazine . PhD Thesis. University of Pretoria.

REINHARDT, C.F., EHLERS, J.G. & NEL, P.C., 1990. Persistence as affected by soil properties. *S.Afr. Journal of Plant Soil.* 7, 182-187.

REINHARDT, C.F & NEL, P.C., 1993. The influence of soil type, soil moisture content band temperature on atrazine persistence. *S. Afr. Journal of Plant Soil.* 61, 612-617.

REINHARDT, C.F., 1996. Implication of residual atrazine occurring in soil and in water for sensitive crops. *Proceedings of the 2nd International Weed Congress.* Denmark. 25-28 June 1996. 1-4, 355-360.

SAGHIR, A.R. & CHOUDHARY, A.H., 1967. Triazine herbicides on maize and their residual effects following crops. *Weed Res.* 7, 272-280.

SAS – Statistical Analysis Systems, 1982. User's guide. Statistics, SAS Institute Inc. Cary, North Carolina.

SETA, A.K. & KARATHANASIS, A.D., 1997. Atrazine adsorption by soil colloids and co-transport through subsurface environment. *Soil Sci. Am. J.* 61, 612-617.

SMIT, N.S.H; NEL, P.C. & FOLSCHER, W.J., 1979. Effect of pH on availability, residual activity and desorption of atrazine in soils with the same clay content. *Crop Prod.* 8, 125-129.

SMIT, N.S.H; NEL, P.C. & FOLSCHER, W.J., 1980. Factors affecting the sorption and degradation of atrazine in soil. *Crop Prod.* 9, 135-139.

SHIMABUKORO, R.H. & SWANSON, H.R., 1969. Atrazine metabolism, selectivity, and mode of action. *J. Agr. Food Chemistry*. 17, 198-205.

SHIMABUKURO, R.H, SWANSON, H.R, WALSH, W.C., 1970. Glutathione conjugation. *Plant Physiology*. 46, 103-107.

SINGH, J., SHEA, P.J., HUNDAL, L.D., COMFORT, S.D., ZHANG, T.C. & HAGE, D.S., 1998. Iron-enhanced remediation of water and soil containing atrazine. *Weed Sci.* 46, 381-388.

TCHAN, Y.T., ROSEBY, J.E., & FUNNEL, G.R., 1974. A new rapid bioassay for photosynthesis inhibiting herbicides. *Soil Biological Biochemistry*.7, 39-44.

WAGENET, R.J. & HUSTSON, J.L., 1992. LEACM leaching estimation and chemistry Model. Continuum Vol. 2, Water Resources Institute, Cornel University, Ithaca, NY. 148.

WALKER, A., 1987. Herbicide persistence in soil. *Rev. Weed Sci.* 3, 1-17.

WEBER, J.B., 1991a. Fate and behaviour of herbicide in the soil. *Appl. Plant Sci.* 5, 28-41.

WEBER, J.B., 1991b. Potential for ground water contamination from selected herbicides: a herbicide/soil ranking system. *Proc. Southern Weed Sci. Soc.* 44, 45-57.

WEAVER, J. & REINHARDT, C.F., 1996. Section 5: Groundwater contamination by pesticide. C.S.I.R. and University of Pretoria.

WIEDMAN, S.J. & APPLEBY, A.P., 1976. Plant growth stimulation by sublethal concentrations of herbicides. *Weed Res.* 12, 65-74.

WOOD, V. K., HAROLD, J., JOHNSON, A & HANCE, R J (1991). The potential for atrazine degradation in aquifer. *BCPC Mono.* No. 47, pp 175-182.

6.5 APPENDIX

Table 1A. ANOVA for top growth fresh mass data of cv. Caritha

Source	DF	SS	MS	F-value	Pr>F
Herbicide rate	3	259.657	86.552	1.75	0.197
Error	16	792.063	49.503		
Corrected total	19	1051.720			
	R-square	CV	Root MSE		VMean
	0.246888	35	7.035		19.605

Table 2A. ANOVA for top growth dry mass data of cv. Carina

Source	DF	SS	MS	F-value	Pr>F
Herbicide rate	3	5.545	1.8485	0.76	0.533
Error	16	30.946	2.434		
Corrected total	19	44.492			
	R-square	CV	Root MSE		VMean
	0.124	48.86	1.5601		3.193

Table 3A. ANOVA for root dry mass data of cv. Carina

Source	DF	SS	MS	F-value	Pr>F
Herbicide rate	3	0.3045	0.1015	1.04	0.185
Error	14	0.7706	0.0550		
Corrected total	17	1.0752			
	R-Square	CV	Root MSE		VMean
	0.283	32.28	0.2346		0.7266

Table 4A. ANOVA for top growth fresh mass data of cv. Caritha

Source	DF	SS	MS	F-value	Pr>F
Herbicide rate	3	239.182	79.727	2.36	0.1151
Error	14	472.247	33.721		
Corrected total	17	711.430			
	R-Square	CV	Root MSE		VMean
	0.336	37.58	5.807		15.452

Table 5A. ANOVA for top growth dry mass data of cv. Caritha

Source	DF	SS	MS	F-value	Pr>F
Herbicide rate	3	4.629	1.543	1.00	0.4197
Error	16	24.775	1.540		
Corrected total	19	29.405			
	R-Square	CV	Root MSE		VMean
	0.157	45.90	1.244		2.710

Table 6A. ANOVA for root dry mass of cv. Caritha

Source	DF	SS	MS	F-value	Pr>F
Herbicide rate	3	0.2237	0.075	2.08	0.542
Error	15	1.5184	0.121		
Corrected total	18	1.7442			
	R-Square	CV	Root MSE		VMean
	0.129	44.46	0.318		0.7154

Table 7A. ANOVA for dry mass data of two wheat cultivars (cv. Carina PD 402 & cv. SST 86)

Source	DF	SS	MS	F-value	Pr>F
Herbicide rate	9	5790.71	643.413	3.08	0.0185
Error	19	3963.79	208.6205		
Corrected total	28	9754.50			
	R-Square	CV	Root MSE		VMean
	0.593	14.38	14.4437		100.4167
Cultivar	1	1463.8458	1463.8458	7.02	0.0158
Atrazine Rate	4	1667.2368	416.8092	2.00	0.1357
cv.*Rate	4	2659.6351	664.9087	3.19	0.0367

Table 8A. ANOVA for percentage of control tillers of two wheat cultivars (cv. SST 86 & cv. Carina PD 402)

Source	DF	SS	Ms	F-value	Pr>F
Model	9	3023.89	335.98	5.67	0.0007
Error	19	1125.97	59.26		
Corrected total	28	4149.82			
	R-Square	CV	Root MSE		VMean
	0.728	8.3553	7.6981		92.134
cv.	1	2219.74	2219.74	37.46	0.0001
Atrazine Rate	4	437.00	109.25	1.84	0.162
cv.*Rate	4	367.14	91.78	1.55	0.228

Table 9A. Atrazine rate main effect: Least square means for % of control data for two wheat culitivars

Atrazine Rate	Dosage	LSMean Dry mass	LSMean Tillers
5 $\mu g\ l^{-1}$		101.791	94.668
10 $\mu g\ l^{-1}$		105.214	94.872
15 $\mu g\ l^{-1}$		111.098	96.542
20 $\mu g\ l^{-1}$		93.315	88.786
25 $\mu g\ l^{-1}$		90.728	86.353

Table 10A. Atrazine rate main effect: Least square means for % of control data for interaction (cv. SST 86 x atrazine)

Atrazine Rate	Dosage	LSMean Dry mass	LSMean Tillers
5 $\mu g\ l^{-1}$		101.77	101.01
10 $\mu g\ l^{-1}$		104.06	102.02
15 $\mu g\ l^{-1}$		135.11	111.61
20 $\mu g\ l^{-1}$		94.77	93.93

Table 11A. Atrazine rate main effect: Least square means for % of control data for interaction (cv. Carina PD 402 x atrazine)

Atrazine Rate	Dosage	LSMean Dry mass	LSMean Tillers
5 $\mu g\ l^{-1}$		101.81	88.32
10 $\mu g\ l^{-1}$		106.36	87.72
15 $\mu g\ l^{-1}$		87.07	81.47
20 $\mu g\ l^{-1}$		91.85	83.63
25 $\mu g\ l^{-1}$		80.65	77.26

Table 12A. ANOVA for top growth fresh mass data of cv. Caritha

Source	DF	SS	MS	F-value	Pr>F
Herbicide rate	3	4898.18	1632.72	10.77	0.0001
Error	34	5153.63	151.63		
Corrected total	37	10053.82			
	R-Square	CV.	Root MSE		VMean
	0.487196	11.38	12.3140		108.166

Table 13A. ANOVA for top growth dry mass data of cv. Caritha

Source	DF	SS	MS	F-value	Pr>F
Herbicide rate	3	43.608	14.536	3.49	0.026
Error	34	141.553	4.163		
Corrected total	37	185.161			
	R-Square	CV	Root MSE		VMean
	0.2355	14.179	2.040		14.389

Table 14A. ANOVA for top growth fresh mass data of cv. Kariega

Source	DF	SS	MS	F-value	Pr>F
Herbicide rate	5	294.781	58.956	2.28	0.172
Error	6	155.317	25.886		
Corrected total	11	450.098			
	R-Square	CV.	Root MSE		VMean
	0.654	4.8	5.087		105.27

Table 15A. ANOVA for top growth dry mass data of cv. Kariega

Source	DF	SS	MS	F-value	Pr>F
Herbicide rate	5	2.969	0.593	0.75	0.617
Error	6	4.776	0.796		
Corrected total	11	7.746			
	R-square 0.383	CV 6.537	Root MSE 0.8922		

Table 16A. ANOVA for shoot fresh mass data of cv. SST 825

Source	DF	SS	MS	F-value	Pr>F
Herbicide rate	3	108.794	36.264	0.55	0.656
Error	12	787.206	65.600		
Corrected total	15	896.001			
	R-Square	CV	Root MSE		Vmean
	0.121	18.03	8.099		44.916

Table 17A. ANOVA for spike fresh mass data of cv. SST 825

Source	DF	SS	MS	F-value	Pr>F
Herbicide rate	3	31.242	10.41	2.03	0.1629
Error	12	61.450	5.12		
Corrected total	15	92.693			
	R-Square	CV	Root MSE		Vmean
	0.0337	16.56	2.262		13.658

Table 18A. ANOVA for shoot fresh mass data of cv. SST 55

Source	DF	SS	MS	F-value	Pr>F
Herbicide rate	3	130.586	43.528	3.13	0.0877
Error	8	111.42	13.928		
Corrected total	11	242.013			
	R-Square	CV	Root MSE		Vmean
	0.539	25.78	3.732		14.473

Table 19A. ANOVA for spike fresh mass data of cv. SST 55

Source	DF	SS	MS	F-value	Pr>F
Herbicide rate	3	21.193	7.0644	2.49	0.134
Error	8	22.652	2.831		
Corrected total	11	43.845			
	R-Square	CV	Root MSE		Vmean
	0.483	16.24	1.682		10.358

Table 20A. ANOVA for shoot dry matter yield data of cv. SST 825

Source	DF	SS	MS	F-value	Pr>F
Herbicide rate	3	1.287	0.429	0.13	0.9422
Error	12	40.519	3.376		
Corrected total	15	41.806			
	R-Square	CV	Root MSE		Vmean
	0.030	17.68	1.837		10.388

Table 21A. ANOVA for spike dry matter mass data cv. SST 825

Source	DF	SS	MS	F-value	Pr>F
Herbicide rate	3	2.421	0.807	2.56	0.104
Error	12	3.788	0.315		
Corrected total	15	6.21			
	R-Square	CV	Root MSE		Vmean
	0.389	14.62	0.561		3.842

Table 22A. ANOVA for shoot dry matter yield data of cv. SST 55

Source	DF	SS	MS	F-value	Pr>F
Herbicide rate	3	5.27	1.75	0.60	0.64
Error	4	11.69	2.92		
Corrected total	7	16.97			
	R-Square	CV	Root MSE		Vmean
	0.31	15.96	1.71		10.711

Table 23A. ANOVA for shoot dry matter yield data of cv. SST 55

Source	DF	SS	MS	F-value	Pr>F
Herbicide rate	3	1.633	0.544	2.32	0.216
Error	4	0.937	0.234		
Corrected total	7	2. 570			
	R-Square	CV	Root MSE		Vmean
	0.635	13.473	0.484		3. 592

Table 24A. ANOVA for number of spikes data of cv. SST 825

Source	DF	SS	MS	F-value	Pr>F
Herbicide rate	3	1.000	0.333	0.08	0.969
Error	12	50.000	4.166		
Corrected total	15	51.000			
	R-Square	CV	Root MSE		Vmean
	0.019	22.067	2.041		9.250

Table 25A. ANOVA for number of spikes data of cv. SST 55

Source	DF	SS	MS	F-value	Pr>F
Herbicide rate	3	6.916	2.305	3.46	0.071
Error	8	5.33	0.666		
Corrected total	11				
	R-Square	CV	Root MSE		Vmean
	0.56	21.77	0.816		3.750